Tropical Grasses and Legumes: Optimizing genetic diversity for multipurpose use

Annual Report 2005
IP5 Project

Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture

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ANNUAL REPORT 2005

PROJECT IP-5

Centro Internacional de Agricultura Tropical
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Tropical Grasses and Legumes: Optimizing Genetic Diversity for Multipurpose Use (Project IP5)

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Cover photo: Multiple use of tropical forages

Tropical Grasses and Legumes: Optimizing genetic diversity for multipurpose use
(Project IP5). 2006. Annual Report 2005. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. 266 p.

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Project Description and Log Frame

IP5: Tropical Grasses and Legumes: Optimizing Genetic Diversity for Multipurpose use (Project Manager: Carlos E. Lascano)

Goal: To improve the livelihoods of poor rural livestock communities and contribute to greater access of poor urban consumers to high and safe quality animal products by strengthening forage-based feeding systems while taking advantage of the potential of forages to enhance natural resource management and provide environmental services

Objective: To develop and disseminate improved forage-based technologies to enhance productivity, profitability and sustainability of smallholder crop/livestock systems in tropical areas of both low and high potential

External Conditions: Livestock development is key for alleviating poverty given that livestock are an important part of the livelihoods of many small farmers and that high growth in demand for animal products in developing countries is expected. However, a large proportion of the land in the humid and sub-humid tropics that supports smallholder crop/livestock systems is in different stages of degradation, which leads to low productivity, deforestation and poverty. In addition, resource-poor farmers in these regions have shortage of labor to collect feed from forest or wastelands and as a consequence livestock intensification is severely limited. Thus, restricted access to feed resources is a growing constraint for many poor livestock producers in tropical regions. Development and dissemination of improved forage technologies can improve livelihoods of small livestock producers while contributing to forest conservation, to the restoration of degraded agricultural land and to more abundant, cheaper, and safer high quality animal products for rural and poor urban consumers.

Important Assumptions: 1) International and national policies as well as livestock services are directed to favor small-scale producers to allow them to link to commodity markets; 2) Resources (unrestricted and restricted core) are identified to support Forage R&D in LAC, SE Asia and Africa; 3) The alliance with private forage seed companies continues to be strong to facilitate diffusion and adoption of improved forage cultivars.

Target Ecoregions: Tropical grasses and legumes being developed at CIAT are targeted to three main agroecological zones in the tropics: Savannas, Forest Margins and Hillsides/Uplands. These agro-ecosystems are characterized by low fertility soils and variable rainfall, ranging from sub-humid (600-1500 mm/year rainfall and 4-8 months dry season) to humid (2,000 to 4,500 mm/year rainfall and limited or no dry season stress). A common constraint across the three targeted agro-ecosystems is low quantity and quality of forage biomass available to feed livestock (ruminants and non-ruminants); as a result animal production and productivity is low and environmental degradation is high.

Beneficiaries and End Users: Researchers from NARS, governmental and non-governmental development programs, private seed companies and small and large farmers throughout the sub-humid and humid tropics who need additional grass and legume genetic resources with high potential to intensify and sustain productivity of agricultural and livestock systems.

Collaborators: Australia: QDPI, CSIRO and Curtin University; **Brazil:** EMBRAPA; **Cambodia:** NAHPIC; **China:** South China University and CATAS; **Colombia:** Universidad de Sucre, REVERDECER, Universidad Nacional de Colombia-Palmira, Universidad de la Amazonía, CORPOICA, FIDAR, Universidad Nacional de Colombia-Bogotá, Universidad de los Llanos and Fundación Universitaria San Martin; **Costa Rica:** UCR, MAG, Corporación de Fomento Ganadero, ECAG and CATIE; **Uganda:** African Highland Initiative and National Agricultural Research Organisation (NARO); **Ethiopia:** Areka Agricultural Research Institute, FARM Africa, International Livestock Research Institute (ILRI), Land-O-Lakes, Melkassa Agricultural Research Center and EARO; **Germany:** University of Goettingen, University of Hohenheim and University of Hannover; **Guatemala:** Asociación de Criaderos de Ganado Brahman and MAGA; **Honduras:** DICTA, FENAGH, GTZ and SERTEDESO; **India:** ILRI and ICRISAT; **Japan:** JIRCAS, Jokkaido University and National Grassland Research Institute; **Kenya:** ILRI, IFDC-CIMMYT and ICRAF; **Lao, PDR:** NAFRI and NAFE; **Malawi:** Department of Agricultural Research Service (DARS); **Mexico:** Semillas Papalotla and INIFAP; **Nicaragua:** INTA, MAG-FOR, FAGANIC, and Asociación Campos Verdes; **Nigeria:** IITA; **Philippines:** PCARRD and ViSCA; **Switzerland:** ETH; **Thailand:** DLD, PCANRC; **United States:** University of Kentucky and Rutgers University; **Vietnam:** NIAH and DARD

Project Changes: Since the last MTP 2004-2007, the Tropical Forage Project modified the goal and purpose.

Previous Goal: Contribute to the improved welfare of small farmers and urban poor by increasing milk and beef production while conserving and enhancing the natural resource base

Comment: As written the goal placed too much emphasis on milk and beef when increasingly we are working on other kinds of livestock (such as monogastrics) and other kinds of benefits (such as freeing up labor) that improve poor farmers welfare and contribute to lower price of animal products for poor rural and urban consumers.

New Goal: To improve the livelihoods of poor rural livestock communities and contribute to greater access of poor urban consumers to high and safe quality animal products by strengthening forage-based feeding systems while taking advantage of the potential of forages to enhance natural resource management and provide environmental services

Previous Objective*:* To develop and deliver to farmers superior gene pools of grasses and legumes for sustainable agriculture systems in sub-humid and humid tropics.

Comment: The objective as written implied that we are only involved in development and transfer of new forage cultivars. Increasingly we are working on the development of forage- based technologies that benefit livestock farmers (such as seed delivery system, or adding value to forage through processing) in areas of low and high potential for development of small holder livestock enterprises.

New Objective: To develop and disseminate improved forage-based technologies to enhance productivity, profitability and sustainability of smallholder crop/livestock systems in tropical areas of both low and high potential.

Project IP5 Log-Frame (MTP 2005-2007)

Area: Genetic Resources Research
Project: Tropical Grasses and Legumes: Optimizing Genetic Diversity for Multipurpose Use
Project Manager: Carlos E. Lascano

NARRATIVE SUMMARY	MEASURABLE INDICATORS	MEANS OF VERIFICATION	IMPORTANT ASSUMPTIONS
Goal To contribute to the improved welfare of small farmers and urban poor by increasing milk and beef production while conserving and enhancing the natural resource base	• New cultivars of grasses and legumes used by farmers. • Raised productivity of livestock and crops while protecting biodiversity and land in savannas, forest margins and hillsides	Statistics and case studies on socio-economic benefits and natural resource conservation in smallholder livestock farms in the subhumid and humid tropics	Policies are put in place by governments to favor sustainable livestock and forage development in marginal areas occupied by small farmers
Purpose To identify and deliver to farmers superior gene pools of grasses and legumes for sustainable crops-livestock systems in subhumid and humid tropics.	• Demonstrated economical and ecological benefits of multipurpose grasses and legumes to livestock and crop farmers in tropical regions of Latin America, Africa and South East Asia	• Range of genetic variation in desirable plant traits • Performance of forage components in systems	• Support from traditional and nontraditional donors • Effective collaboration: • CIAT's Projects • ARO's, partners and farmers, NGOs
Outputs 1. Grass and legume genotypes with high forage quality attributes are developed.	• Determined the utility of legume mixtures for increasing protein supply in ruminants while reducing methane emissions 20% by 2005 • Selected at least 10 Brach aria hybrids (sexual) with high digestibility (>60%) and crude protein (> 10%) by 2006 • The little bag silage technology with selected forage species adopted by at least 100 small farmers in Honduras and Nicaragua, results in 20-30%% milk yield increase in the dry season by 2007	• Demonstrated differences under field conditions • Scientific publications • Annual Reports • Theses	• Effective collaboration with CIAT Projects (PE-2), AROs, partners and farmer groups
2. Grass and legume genotypes with superior adaptation to edaphic and climatic constraints are developed.	• Two improved accessions of Vigna and one of Lablab multiplied (500 or 100 kg of seed produced, respectively) and distributed to two national partners (DICTA, INTA), one NGO (SERTEDESO), one farmer organization (Campos Verdes) one development project (GTZ), in Honduras and Nicaragua by 2005 • A new *Brachiaria* hybrid (CIAT 36087, cv. Mulato-II) with better adaptation to acid soils and tolerance to dry season (50% higher dry season forage yield on acid soils	• Demonstrated differences under field conditions • Scientific publications • Annual Reports • Theses	• Effective collaboration with CIAT Projects (SB-1, PE-2, PE-4), AROs, partners, NGOs and farmer groups

NARRATIVE SUMMARY	MEASURABLE INDICATORS	MEANS OF VERIFICATION	IMPORTANT ASSUMPTIONS
	in Central/South America by 2007.		

3. Grass and legume genotypes with superior adaptation to edaphic and climatic constraints are developed.	• Two improved accessions of Vigna and one of Lablab multiplied (500 or 100 kg of seed produced, respectively) and distributed to two national partners (DICTA, INTA), one NGO (SERTEDESO), one farmer organization (Campos Verdes) one development project (GTZ), in Honduras and Nicaragua by 2005
	• A new *Brachiaria* hybrid (CIAT 36087, cv. Mulato-II) with better adaptation to acid soils and tolerance to dry season (50% higher dry season forage yield on acid soils than the current hybrid cultivar), and resistance/tolerance to at least three Colombian species of spittlebugs, and with 2-3 times higher seed yield available for release (50 tons of commercial seed available) by 2006
	• Defined the genetic variability for nitrification inhibition in at least 500 *Brachiaria* hybrids by 2007
		• Demonstrated differences under field conditions
		  • Scientific publications
		  • Annual Reports
		  • Theses
			• Effective collaboration with CIAT Projects (SB-1, PE-2, PE-4), AROs, partners, NGOs and farmer groups

4. In partnership with NARS, superior and diverse grasses and legumes are evaluated and disseminated through participatory research.	• New market opportunities for processed forages assessed trough surveys to at least 100 farmers with and without livestock in Honduras and Nicaragua by 2005
	• *Brachiaria brizantha* cv. Toledo seed produced (500 kg to 1 t) by one farmer enterprise (PRASEFOR). in Honduras by 2006
	• A forage production systems established with >5000 farmers in 4 countries of SE Asia supported by >50 experienced staff and key technical information about forage technologies and their development by 2006
	• At least 5,000 ha of Brachiaria hybrid (Mulato II) planted in Colombia, Honduras, Nicaragua and Mexico by 2007
	• Improved multipurpose grasses and legumes result in 20% more on-milk, and in 30% reduced labor requirements in benchmark sites in SE Asia by 2007.
		• Promotional publication
		  • Newsletters
		  • Journal
		  • Extension booklets
		• Surveys on adoption impact of new grasses and legumes:
		  • Seed sold
		  • Area planted
		  • Production parameters
		  • Environmental/socioeconomic indicators
			• Effective collaboration with CIAT Projects (PE-2, SN-1, SN-2, SN-3, BP-1 and Ecoregional Program), partners, NGOs and farmer groups

Output Targets reported to the CGIAR-Science Council in 2005

Output	Output Targets 2005	Category of Output Targets	Achieved?
Grass and legume genotypes with high forage quality attributes are developed	• Developed and validated a methodology to screen *Brachiaria* hybrids for forage quality using NIRS (near-infrared reflectance spectroscopy) • The use of mixtures of legumes with and without tannins decrease apparent ruminal protein degradation by 50-60% and increase 3- 4 fold the proportion of dietary crude protein digested by acid-pepsin incubation	Practice Practice	**Achieved**
Grass and legume genotypes with known reaction to pests and diseases and interaction with symbiont organisms are developed	• Developed a screening method that combines laboratory and field selection with capacity to evaluate 1000 genotypes for resistance to Rhizoctonia in *Brachiaria*. Selected hybrids (4) with high levels of resistance • Selected apomictic *Brachiaria* hybrids (6) with resistance to 5 species of spittlebug present in Colombia	Practice Material	**Achieved**
Grass and legume genotypes with superior adaptation to edaphic and climatic constraints are developed	• Developed and validated a high-throughput method to screen *Brachiaria* hybrids for adaptation to low fertility acid soils • Selected accessions (2) of *Desmodium velutinum* with high forage yield and quality in the wet and dry season	Practice Material	**Achieved**
In partnership with NARS, superior and diverse grasses and legumes are evaluated and disseminated through participatory research	• Household impact demonstrated through improved forages in Lao PDR. More than 1300 farmers in 106 villages (covering 5 districts in 2 provinces) adopt planted forages for livestock feeding. A total of 900 farmers benefit from improved forages: labor saving, improved animal production and increased household income • Expert system for targeting forages -Selection of forages for the tropics (SoFT)- is released	Practice Material	**Achieved**

Research Highlights 2005

Output 1: Grass and legume genotypes with high forage quality are developed

- **Validated a methodology to screen for quality large number of *Brachiaria* hybrids using NIRS**

Results from this year confirm that it is possible to detect with NIRS differences in crude protein (CP) and in vitro dry matter digestibility (IVDMD) among entries in a population of *Brachiaria* hybrids. Significant genetic variation among hybrid clones was detected for both IVDMD and CP. Large variation among sampling dates was also detected (for IVDMD, mean square = 7.9 vs. 878.8 for genotypes or sampling dates, respectively; for CP, mean square = 6.9 vs. 1,716.0 for genotypes or sampling dates, respectively). However, it is encouraging that despite the large effect of sampling date on both quality parameters, the interaction of genotype with sampling, while statistically significant, was less than that for genotypes (for IVDMD, mean square = 7.9 vs. 1.8 for genotypes or genotype-sampling date interaction, respectively; for CP, mean square = 6.9 vs. 2.6 for genotypes or genotype-sampling date interaction, respectively). Hence, it appears that sampling field-grown plants even on only a single date should be effective in identifying *Brachiaria* genotypes superior for quality traits.

- **Demonstrated the potential benefits of mixtures of legumes with and without tannins on nitrogen utilization by ruminants using an *in vitro* fermentation system**

Results from *in vitro* studies using a rumen simulation technique had shown that supplementation of low quality grasses with tannin-rich legumes decreases ruminal protein degradation. However, it is likely that such a decrease would result in a greater flow of dietary N to the lower digestive tract of ruminants feed tannin-rich legumes. This year, our work focused on the assessment of the potential acid-pepsin digestibility of rumen-undegradable protein from grass-based diets supplemented with legumes with and without tannins. Results showed that in mixed diets based on a low-quality grass, the replacement of *Cratylia argentea*, a legume without detectable amounts of tannins, by the tanniniferous *C. calothyrsus* (CIAT 22310) decreased apparent ruminal crude protein degradation by 65% (from 884 to 576 mg/g) and increased almost 4 fold (from 70 to 272 mg/g of N supplied) the proportion of dietary crude protein digested by acid-pepsin incubation. If these results are validated *in vivo*, the use of mixtures of legumes with and without tannins could effectively improve the supply of available protein in ruminants fed low quality grasses.

Output 2: Grass and legume genotypes with known reaction to pests and diseases and to interaction with symbiont organisms are developed

- **Selected apomictic and sexual *Brachiaria* hybrids with multiple resistance to spittlebug**

We continued to make significant progress in incorporating resistance to spittlebug in the *Brachiaria* improvement program using a recurrent selection scheme. In 2004 we reported varying levels of resistance to *Prosapia simulans* (one of the most important species affecting *Brachiaria* in Mexico) in 34 apomictic hybrids. These hybrids had been pre-selected in Mexico for good adaptation and desirable agronomic characteristics. A series of replicated tests were carried out in 2005 to evaluate the resistance of these genotypes to *Prosapia simulans* and to four major species of spittlebug present in Colombia (*A. varia*, *A. reducta*, *Z. carbonaria*, and *Mahanarva trifissa)*. A total of 6 (18%) apomictic hybrids were selected for having resistance to all 5 species of spittlebug. In addition, this year 565 new sexual hybrids were tested for resistance to three spittlebug species and results showed that 96.2%, 94,7% and 93.9% were rated as resistant to *A. varia*, *A. reducta*, and *Z. carbonaria*, respectively.

- **Validated a field method to screen *Brachiaria* hybrids for *Rhizoctonia* foliar blight and found hybrids with high levels of resistance**

Rhizoctonia foliar blight is a disease of increasing importance in *Brachiaria* pastures in humid areas. The disease can be very destructive when environmental conditions are particularly conducive (high relative humidity, dense foliar growth, high nitrogen fertilization, and extended wet periods). A field experiment was carried out in 2005 to screen 137 *Brachiaria* genotypes for resistance to *Rhizoctonia*. Six plants of each of the *Brachiaria* genotypes were transplanted from a CIAT glasshouse to a field site in the Amazon of Colombia. Plants were inoculated one month after transplanting and evaluated for disease reaction using a 0 – 5 visual scale. Disease symptoms developed fully in susceptible genotypes 10-15 days after inoculations. There was a high degree of correlation in disease scoring among the various evaluation dates. The resistant control CIAT 16320 and four *Brachiaria* hybrids **(**BR04- 2577, BR04-2557, BR04-2983, and BR04-1214) showed less than 6% overall plant tissue damage, and thus, a high-level of resistance to the disease.

Output 3: Grass and legume genotypes with superior adaptation to edaphic and climatic constraints are developed

- **Developed and validated a high-throughput method to screen *Brachiaria* hybrids for adaptation to low fertility acid soils**

A hydroponic screening method using stem cuttings was developed to quantify two key traits associated with adaptation to acid-infertile soils: root vigor and Al resistance. The vigor of root growth under nutrient deprivation influences plant adaptation to a range of nutrient deficiencies given that that nutrient acquisition relies on soil foraging, particularly in the case of immobile nutrients such a phosphorous. Al toxicity was targeted for selection because previous research showed genetic variability for this trait among *Brachiaria* species. Using the hydroponic screening method, 9 *Brachiaria* hybrids (out of a preselected population of 139 apomictic/sexual) were superior to *B. decumbens* cv Basilisk (parent in the breeding program and selected for superior adaptation to acid-infertile soils) in terms of root length in the presence of toxic level of Al. Among the 9 selected hybrids, one (BR04NO2681) was superior to *B. decumbens* cv Basilisk in terms of fine root development (lower root diameter), which is an indication of superior adaptation to low nutrient availability in soil.

- **Developed and tested greenhouse methodologies to screen *Brachiaria* hybrids for drought tolerance and adaptation to poorly drained soils**

A major objective of the *Brachiaria* improvement program is to develop genotypes that combine resistance to biotic constraints with adaptation to abiotic stresses such as drought and poorly drained soils. This year progress was made in the development of a greenhouse method to screen for drought tolerance based on root development and leaf expansion. Contrasting genotypes (16) of *Brachiaria* planted in transparent plastic cylinders filled with a mix of sandy-loam Oxisol with sand (2:1 w/w) were subjected to two levels of water supply (50% and 100% field capacity). The method used allowed to observe after 45 days of growth significant variation among checks and hybrids of *Brachiaria* in dry matter distribution, specific leaf area and root attributes due to drought stress (50% field capacity). Progress was also made in developing a method to screen *Brachiaria* genotypes for adaptation to waterlogging using small pots. Contrasting genotypes (23) were subjected to two treatments: control at field capacity and excessive water supply (5 cm over soil surface). The test allowed the selection based on green leaf biomass of genotypes that are known to perform well in poorly drained soils (*B. humidicola* CIAT 679 and 6133 and *B. brizantha* cv Toledo). One *Brachiaria* hybrid (BR02NO1245) was markedly superior in its tolerance to waterlogging than the other hybrids, suggesting that there is scope to select for this trait in the *Brachiaria* improvement program.

- **Characterized diversity in the shrub legume *Desmodium velutinum***

Selection of tropical shrub legumes adapted to acid soils and with high forage quality is of high priority for development of improved feeding systems. Species such as *Desmodium velutinum* may be an alternative feed resource for intensive dairy system given its high nutritive value. However, most of the information available on *D. velutinum* is restricted to few accessions. A total of 137 accessions classified in three groups based on growth habit (erect, semi-erect and prostrate) are being evaluated in an acid low fertility site. Large variation in forage yield in the wet season was observed for accessions in the erect (142-297 g DM/plant) and semi-erect (112-246 g DM/plant) groups and less for accession on the prostrate (104- 130 g DM/plant) group. Variation in forage yield was also observed in the dry season. Forage quality in the high yielding accessions varied (IVDMD: 64- 73% and CP: 19- 22%) among accessions. These results indicate that there is scope for selecting high yielding and high quality accessions of *D .velutinum* for use in intensive livestock systems.

Output 4: Superior and diverse grasses and legumes delivered to NARS partners are evaluated and released

- **Significant household impact was achieved through improved forages in Lao PDR**

The Forages and Livestock Systems Project (FLSP) in Laos, funded by the Australian Agency for International Development (AusAID) was completed in June 2005. The project achieved its targets of developing and disseminating forage technologies to smallholder farmers, resulting in significant household impacts. More than 1300 farmers in 106 villages (covering 5 districts in 2 provinces) had adopted planted forages for livestock feeding. A total of 900 farmers were benefiting from significant impacts such as labor saving, improved animal production and increased household income. More than 150 farmers report they have been able to reduce or stop shifting cultivation as a direct result of intensifying their livestock production. More than 200 farmers report that intensifying their livestock production systems has allowed their children to attend school. The technologies deployed by this project and the approaches of working with farmers to achieve adoption has attracted considerable interest by large development projects, NGOs and the donor community in Laos, and have been incorporated into several project as a major component.

- **Demand and benefits of forage conservation technologies in Central America was documented as part of a pilot study**

Feed shortage during 5-6 months dry season severely limits livestock production and farm income in the subhumid areas of Central America. The Forage Program of CIAT has developed and promoted improved grass and legume species suitable for grazing, cut/carry systems and silage and hay production. In addition we have been working on adapting silage technologies to smallholder systems. Through independent surveys we documented this year the potential demand and benefits to smallholders of silage and hay for dry season feeding. Results indicate that feeding silage or hay to milking cows is profitable and that the benefit can be greater for small scale farmers than for large scale farmers. Plastic bag silage offers a low cost opportunity for small scale farmers, but the non-availability and high cost of suitable bags are seen as constraints for further uptake. An emerging market for hay was found in Costa Rica and to a lesser extent in Honduras. Farmers also saw a market opportunity for plastic bag silage.

- **Expert system for targeting forages -Selection of forages for the tropics (SoFT)- was released**

Forage research over the last 50 years has identified many tropical grasses and legumes that have a role in farming systems in developed and developing countries. Information on the adaptation and use of these species resides in peer-reviewed literature and research reports with limited distribution and, often most importantly, in the memories of forage agronomists with decades of experience of working with a wide range of forages in diverse farming systems. To address these deficiencies in knowledge sharing an inter- institutional (CIAT, CSIRO, QDPI, ILRI and the U of Hohenheim) project funded by ACIAR, Australia was setup to develop a knowledge system for the identification of forages suitable for specified niches within smallholder farming systems in the tropics and subtropics. The main features of the data base are: a) information in fact sheets on the adaptation, uses and management of forage species, cultivars and elite accessions, b) a selection tool built on LUCID™ that enables easy identification of best-bet species, c) bibliography of more than 6,000 references and abstracts on forage diversity, management and use which enable users with poor library facilities to access summaries of some of the key literature, d) global maps of climate adaptation for each species and e) a collection of photographs and images of species to help in their identification and use. The tool was officially released at the XXth International Grassland Congress in Ireland in June 2005. The database is now freely available on the Internet (www.tropicalforages.info) and on CD. Since the release there has been a steady increase in the number of visits in the internet site from 249 in June to 4810 in October.

Output 1: Grass and legume genotypes with high forage quality attributes are developed

1.1 Development of *Brachiaria* hybrids with high quality

Highlights

- Using NIRS we detected differences in CP and IVDMD in a selected population of sexual *Brachiaria* hybrids planted in pots. The variability observed in CP and IVDMD in the sexual population examined indicates that there is considerable scope for genetic improvement of quality in *Brachiaria*.

- The magnitude of genetic variation for key nutritional quality parameters in a set of *Brachiaria* hybrids was greater than the interaction of genotypes with sampling dates.

- Milk yield of cows with limited milking genetic potential was similar in well managed pastures of *B. brizantha* cv Toledo as compared to pastures with Br*achiaria* hybrids

- Breeding *Brachiaria* for adaptation to acid soils has resulted in cultivars (i.e. cv Mulato II) with mineral concentrations (i.e. P) in leaf tissue similar to what is recorded in commercial cultivars with adaptation to edaphic constraints (i.e. cv Toledo).

1.1.1 Methodology development for screening *Brachiaria* hybrids for high digestibility and protein

Contributors: P. Avila, G. Ramirez, C. Lascano and J.W. Miles (CIAT)

Rationale

In 2004 we reported that with a small set of *Brachiaria* hybrids grown in the field there was a significant sampling period and genotype effect for crude protein and in vitro digestibility and that sampling x genotype interaction was not significant for both quality variable measured. This year we wanted to reconfirm results from last year, but using a larger population of *Brachiaria* hybrids transplanted in pots rather than in the field.

Materials and Methods

A total of 119 *Brachiaria* sexual hybrids (selected in 2003) were transplanted in replicated (3) pots. Initially the plants were cut at a uniform height and after 6 weeks of growth, leaves were harvested in three successive samplings. After each harvest 1 g of urea was applied per plant. All samples (leaves) were analyzed in CIAT's Forage Quality Laboratory for crude protein (CP) and in vitro digestibility (IVDMD) using NIRS. Results were subject to an analysis of variance using SAS version 9.1.3.

Results and Discussion

Results showed that average CP and IVDMD values recorded in the sexual hybrids differed between entries and sampling periods, but that the interaction hybrid x sampling period was not significant (Table 1).

Variation in quality of the hybrids across the three sampling periods was 14.4 to 22.9 % for CP and 57.9 to 68.1 % for IVDMD. The mean CP (19 %) and IVDMD (64 %) observed were high for a tropical grass probably related to genetic factors as well as management factors (i.e. N fertilization and age of leaf at harvest).

These results agree with results from last year that showed that in a population of 50 hybrids

Table 1. Analysis of Variance associated with crude protein (CP) and in vitro digestibility (IVDMD) measured in three successive samplings in a population of *Brachiaria* sexual hybrids.

Variable	Source of Variation (Significance)		
	Hybrid	Sampling	Hybrid x Sampling
CP	0.0001	0.0024	0.8364
IVDMD	0.0001	0.0008	0.1125

from the cross *B. ruziziensis* x *B. brizantha* cv. Marandu there were significant sampling (P < 0.0001) and genotype (P< 0.002 –0.006) effects for both CP and IVDMD, but a non significant sampling date x genotype interaction. Variation in CP (12.1-21.8%) and IVDMD (63.5-74.1%) measured in the hybrids last year was similar to the variation observed in the sexual population evaluated this year.

Last year we indicated that to screen for quality *Brachiaria* hybrids coming out of the improvement program we needed to standardize a protocol for plants grown in pots in the greenhouse. Results from this year confirm that it is possible to detect with NIRS differences in CP and IVDMD among entries in a population of *Brachiaria* hybrids planted in pots. The variability observed in CP and IVDMD in leaf tissue from the sexual population examined indicates that there is scope for genetic improvement of quality in *Brachiaria*. Screening for quality in the latest population of sexual hybrids selected by the breeder is currently underway.

1.1.2 Nutritional quality parameters (dry matter digestibility, crude protein content) of *Brachiaria* hybrids (series BR04), preselected at Quilichao and Matazul in 2004

Contributors: C. Lascano; J.W. Miles; P. Ávila (CIAT)

Rationale

Inherent nutritional quality (dry matter digestibility [IVDMD] and crude protein content [CP]) will be important in determining the commercial success of new hybrid *Brachiaria* cultivars. It is clear from past results that ample genetic variation for quality parameters exists in the breeding population, probably owing to the heavy contribution of high quality *B. ruziziensis* germplasm in the synthesis of the original population. Reliable selection to maintain and improve quality attributes will depend on quantification of the stability of expression of quality traits over environmental variation so as to design effective and efficient evaluation and selection protocols.

Materials and Methods

Three hundred thirty-nine preselected BR04, hybrid *Brachiaria* clones were delivered to the forage quality lab (as small, rooted cuttings, in 4-in pots) early in the year. These plants were propagated and a thrice-replicated field trial established at CIAT-Quilichao. Plants were sampled three times during the season and IVDMD and CP determined by NIRS (Near Infra-Red Spectroscopy).

Results and Discussion

Significant genetic variation over sampling dates among hybrid clones was detected for both IVDMD and CP. Large variation among sampling dates was detected (For IVDMD, mean square = 7.9 vs. 878.8 for genotypes or sampling dates, respectively; for CP, mean square = 6.9 vs. 1,716.0 for genotypes or sampling dates, respectively). However, it is encouraging that despite the large effect of sampling date on both quality parameters, the interaction of genotype with sampling, while statistically significant, was less than that for genotypes (For IVDMD, mean square = 7.9 vs. 1.8 for genotypes or genotype-sampling date interaction, respectively; for CP, mean square = 6.9 vs. 2.6 for genotypes or genotype-sampling date interaction, respectively).

Hence, it appears that sampling field-grown plants even on only a single date should be effective in identifying genotypes superior for quality traits. Given the significant genotype-sampling date interaction, prudence would suggest sampling in multiple environments (dates or locations). A strategy aimed at efficient use of laboratory resources might be to cull on the results of a single sampling and then assess the remaining genotypes more intensively to identify those with consistently superior nutritional quality.

1.1.3 Milk yield of cows grazing *Brachiaria* pastures managed with high grazing pressure

Contributors: M. Betancourth (U. Nacional), P. Avila, G. Ramirez, and C. Lascano (CIAT)

Rationale

We had reported that milk yield of cows grazing *Brachiaria* hybrid cv Mulato and the newly released *Brachiaria* hybrid cv Mulato II was higher than in *B. brizantha* cv Toledo when no N was applied. In both hybrids MUN (Milk Urea Nitrogen) was greater as compared with *B. brizantha* cv Toledo and this was associated with higher crude protein in the leaf tissue. Thus we concluded that the potential to produce milk in the absence of N fertilization was similar in the two *Brachiaria* hybrids and superior to *B. brizantha* cv Toledo, which is an accession selected from the collection held by CIAT.

An observation made when grazing *B. brizantha* cv Toledo in several on-farm trials was that it had a very fast regrowth after grazing or cutting and that as a result if not properly managed (i.e. adequate stocking rate and grazing frequency) it tended to mature and loose quality (lower crude protein) very fast. Thus this year we were interested in comparing milk production in the two *Brachiaria* hybrids with cv Toledo in N fertilized pastures managed with similar grazing pressure in two contrasting seasons of the year.

In addition, we wanted to document that breeding *Brachiaria* for adaptation to acid soils with low fertility (i.e. low bases and low P) did not result in cultivars with lower quality and concentration of minerals as compared to commercial cultivars.

Material and Methods

Two grazing trials were carried out in the Quilichao research station in a rainy period (March 21 to May 3, 2005) and a dry period (July 4 to August 16, 2005) to measure milk production in pastures of *B. brizantha* cv Toledo (control), *Brachiaria* cv Mulato *and Brachiaria* cv Mulato II arranged in reversible 3 x 3 LSD. Each period was of 14 days of which 7 were for adjustment and 7 for measurements. Total rainfall in the wet and dry periods was 242 and 27 mm, respectively.

In the two experiments, pastures were mowed, fertilized with 50 kg of N/ha and allowed to grow for 6 weeks before being grazed by 6 cows (crossbreds and Holstein) with similar days of lactation and initial milk production (5 liters/day). The average stocking rate used in both seasons of the year was 3 cows/ha (each cow averaged 450 kg liveweight).

Measurement in the pastures included forage on offer, quality (CP and IVDMD) in pluck samples and mineral composition in leaf tissue. Milk yield was measured AM and PM and milks samples were analyzed for fat, non-fat solids and MUN (Milk Urea Nitrogen). Results from the vegetation and from the animals were subject to analysis of variance using SAS program package version 9.1.3 and the Ryan- Einot- Gabriel-Welsch Multiple Range Test was used to separate means.

Table 2. Quality and forage availability in pastures of *Brachiaria* grazed by milking cows in two contrasting seasons of the year

Pastures	Forage on offer (kg DM/ha)	CP (%)	IVDMD (%)
	Maximum rainfall		
Toledo (control)	2905 (58)*	9.1 b	66.6
Mulato	2666 (60)	9.7 b	67.2
Mulato II	3042 (58)	11.4 a	66.3
Significance	NS	P <0.05	NS
	Minimum rainfall		
Toledo (control)	3082 (78)*	7.4 b	57.9
Mulato	2815 (48)	7.5 b	61.1
Mulato II	3269 (52)	8.4 a	61.0
Significance	NS	P <0.05	NS

* Values in parenthesis are the proportion of green leaf in the forage on offer

Results and Discussion

The amount of forage on offer did not vary among pasture treatments in both seasons of the year (Table 2). As expected the proportion of green leaf was higher in the wet season (58 to 60%) than in the dry season (48 to 52 %) with the exception of *B. brizantha* cv Toledo, which had a very high proportion of leaf (78%) in the dryer period (Table 2), which is an indication of the high adaptation of this grass to the dry season.

As expected, CP and IVDMD in the three grasses were lower in the dry period as compared to the wet period (Table 2). On the other hand, IVDMD did not vary among grasses in the wet or dry periods. In contrast the level of CP was higher in Mulato II as compared to the other grasses in both seasons of the year. The fact that all pastures were managed with a high grazing pressure undoubtedly controlled the growth rate of Toledo which in turn contributed to maintain a relatively high leaf proportion and high CP content on the forage on offer.

In this study we were also interested in determining variation in mineral concentration of the three grasses grown in acid soil with low fertility and only fertilized with N. Results presented in Table 3 are consistent with what is expected for a tropical grass. For example, the concentration of P, which is a key mineral in the nutrition of livestock, averaged 0.22% which is similar to the concentration recorded in grasses grown in acid-low fertility soils. It was also

Table 3. Mineral composition of leaf tissue in pastures of *Brachiaria* grazed by milking cows in two contrasting seasons of the year

Pastures	Ca (%)	P (%)	S (%)	K (%)	Mg (%)
			Maximum rainfall		
Toledo (control)	0.33 b	0.22	0.14	1.68	0.34 b
Mulato	0.49 a	0.19	0.11	1.82	0.37 b
Mulato II	0.54 a	0.24	0.14	1.56	0.44 a
Significance	P <0.05	NS	NS	NS	P < 0.05
			Minimum rainfall		
Toledo (control)	0.39 c	0.17 b	0.11	1.57	0.32
Mulato	0.47 b	0.20 a, b	0.10	2.24	0.35
Mulato II	0.52 a	0.25 a	0.13	1.62	0.43
Significance	P <0.05	P <0.05	NS	NS	NS

Table 4. Milk yield and composition of cows grazing *Brachiaria* pastures in two contrasting seasons of the year

Pastures	Milk yield (l/cow/d)	Fat (%)	Non fat solids (%)	MUN (Mg/dL)
		Maximum rainfall		
Toledo (control)	4.7	4.4 a	9.4	6.1 a
Mulato	4.7	4.2 b	9.4	5.2 b
Mulato II	4.4	4.0 c	9.5	7.8 a
Significance	NS	P <0.05	NS	P <0.05
		Minimum rainfall		
Toledo (control)	5.4	4.2	8.8	3.0
Mulato	5.5	4.2	8.8	3.0
Mulato II	5.2	3.9	8.9	3.6
Significance	NS	NS	NS	NS

interesting to observe that with the exception of Ca and Mg (higher in the two *Brachiaria* hybrids) the concentration of other minerals measured was not affected by grass cultivar. Results presented in Table 4 indicate that milk yield was similar in pastures sown with Toledo, in spite of the higher CP level in Mulato II in both seasons of the years.

The finding that milk yield in *Brachiaria* hybrids Mulato and Mulato II was similar than in Toledo could be the result of: a) using cows with limited ability to respond to improvements in quality of the forage on offer and/or b) using high grazing pressure to manage the pastures that prevented Toledo from becoming mature as result of its fast growth rate. In general, results indicate that milk yield of cows with limited ability to produce milk can be as high in pastures of *B. brizantha* cv Toledo as compared to *Brachiaria* hybrids provided they are well managed in terms of grazing pressure and fertilization with nitrogen. In addition, results suggest that breeding *Brachiaria* for adaptation to acid soils has resulted in cultivars (i.e. cv Mulato II) with concentration of minerals (i.e. P) in leaf tissues similar to commercial cultivars with adaptation to edaphic constraints (i.e. cv Toledo).

1.2 Conservation of forages for dry season feeding in smallholder systems

Highlights

- The addition of small quantities of molasses to good quality grasses ensiled in plastic bags is an insurance to obtain good quality silage for use in the farm or for sale to other farmers.

- Supplementing (1% DM of BW) medium quality grass hay (60-65% IVDMD and 9-10% CP) to cows grazing pastures with limited forage availability but adequate CP resulted in ½ liter more milk per day.

1.2.1 Quality of different grass silages with and without additive

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Rationale

A major constraint of small milk producers is lack of high quality feed in the dry season, which results in large economic losses. One known alternative to overcome the shortage of feed in the dry season is forage conservation in the form of silage. However, the adoption of forage conservation technologies by smallholders has been very limited in part due to lack of information and of machinery to make good quality silage.

One alternative to the traditional “bunker” type silage is the “bag silage” technology which has several advantage for smallholders: (a) forage can be harvested and ensiled anytime during the year when labor is available either in small or large quantities (a person can make about 1 bag of 30-35 kg per hour), (b) machinery is not needed to harvest the forage material to be conserved; (c) silage produced can be used in the farm or sold to other farmers to obtain additional income; and (d) silage can be produced by either livestock or non-livestock owners. Generally two bags are used: one made of any material to put the forage biomass, and then this bag is inserted into the plastic bag that will avoid the penetration of oxygen to allow the fermentation process to take place.

As part of two special projects (CIAT led BMZ and ILRI led CFC) operating in Central America we are testing with smallholders with and without cattle the option of producing silage in plastics bags. Several farmer training courses have been carried out with practical demonstrations on principles for making good quality “bag silage” using grasses and legumes. One concern expressed by some farmers in Honduras and Nicaragua is the need for using molasses as an additive when ensiling grasses given that most of the local experience is ensiling maize or sorghum with adequate sugar content. Thus this year we evaluated the quality of grass silage with and without molasses in order to support on –farm work on forage conservation through feedback to extensionists and farmers in Central America.

Material and Methods

Material to make the silage was hand- harvested after 6 weeks regrowth from pastures sown with different *Brachiaria* cultivars (the hybrids cv Mulato and Mulato II and *B. brizantha* cv Toledo) in Quilichao and fertilized with 50 kilos of N/ha. The material was cut (3 cm) and one kilogram (fresh material with 25% DM) was placed in small plastic bags (23 x 32 cm). Molasses (5% on DM basis) was applied and mixed with the forage in bags prior to compaction.

Treatments were arranged in a randomized design with two factors (grasses and molasses) and 4 replications. The process of anaerobic fermentation was allowed to proceed for 40 days before opening the bags for chemical analysis. All silage samples were analyzed for crude protein, fiber content, and pH. In addition, we measured fermentation parameters (extent and rate of gas production) using the gas transducer

Table 5. Effect of adding molasses on quality of silage made from three *Brachiaria* grasses

Treatments	CP (%)	NDF (%)	ADF (%)	pH
Mulato + Molasses	9.7	58.4	25.6	3.8
Mulato - Molasses	9.5	64.4	33.9	5.4
Mulato II + Molasses	9.3	56.7	25.1	3.9
Mulato II - Molasses	10.6	62.4	31.5	5.6
Toledo + Molasses	8.9	59.9	30.1	3.9
Toledo - Molasses	9.4	72.9	37.5	5.5
Significance (P)				
Grass	0.002	0.0001	0.0001	NS
Molasses	0.003	0.0001	0.0001	0.001
Grass x Molasses	0.002	0.0001	0.009	NS

technique. Results were subject to analysis of variance using the SAS program package version 9.1.3.

Results and Discussion

The effect on quality of adding molasses to the three grasses ensiled is shown in Table 5. The protein content of the two *Brachiaria* hybrids was higher than in Toledo and was slightly lower when molasses was added. In contrast, both fiber components measured (NDF and ADF) were lower with the addition of molasses.

The pH of the ensiled material, a good indicator of quality in silages, was not affected by the material being conserved but varied significantly with addition of molasses. A good quality silage should have a pH of around 4 or less, which was the case with the grasses ensiled with molasses. Silage with a low pH is associated with fermentation producing high lactic acid whereas a high pH (5.5 or greater) is associated with inadequate fermentation and as a result with low quality silage.

The results on extent and rates of degradation by ruminal microorganisms of the three grass silages are presented in Table 3. Adding molasses to the material had a greater effect on fermentation parameters than cultivar of the grass. With all three grasses the extent and rate of gas production was greater when 5% (DM) molasses was added. However, we did observe that the two *Brachiaria* hybrids had a slightly higher digestibility than Toledo, which is consistent with having lower cell wall content (Table 6).

The addition of low levels of molasses when ensiling tropical grasses with low sugar content provides insurance for making good silage. Thus small farmers making silage in plastic bags for their own use or for sale should be advised on the advantages of adding molasses or other sources of fermentable sugars to the material being ensiled, particularly if they are planning to conserve grasses and legumes with low sugar contents. It is also important that farmers keep in mind that silage with high pH does not only have poor quality in terms of nutrient composition but that it also consumed in low quantities.

Table 6. Fermentation parameters of *Brachiaria* silage with and without additives.

Treatments	Maximum gas production (ml)	Rate of gas production (ml/h)
Mulato + Molasses	224	0.0602
Mulato - Molasses	200	0.0533
Mulato II + Molasses	232	0.0622
Mulato II - Molasses	210	0.0555
Toledo + Molasses	232	0.0518
Toledo - Molasses	219	0.0466
Signficance (P)		
Grass	0.0001	0.0001
Molasses	0.0001	0.0001
Grass x Molasses	0.03	NS

One final comment has to do with the process of reducing humidity of the forage before ensiling in small plastic bags through wilting. As part of the work for adjusting appropriate technologies for conservation of grasses as silage by smallholders, we investigated wilting vs. no wilting of grasses (6 weeks regrowth and 25% DM).

Results (not shown) indicated that wilting had a negative effect on the quality of the silage made with the three grasses which is contrary to what is expected. One explanation was that the forage was wilted for 24 hours under shade which favored growth of fungi. Thus if wilting of the forage is not done properly it can result in poor quality silage.

1.2.2 Milk yield of cows supplemented with grass hay

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Rationale

The Forage Program of CIAT is developing/ adjusting forage technologies to feed livestock in the dry season that are appropriate for smallholders. Farmers in dry hillsides of CA are rapidly adopting improved grasses such as *B. brizantha* cv Toledo and *Brachiaria* hybrid cv Mulato, which have shown to be drought tolerant. In addition, there is growing interest among small farmers in conserving forages as hay or silage for dry season feeding and/ or for sale. One option is to harvest excess forage from improved pastures in the wet season to make high quality silage or hay for the dry season.

There is abundant literature on production and utilization of silage and hay in different livestock systems, but in most cases the technology available is not useful to small farmers given that it relies on machinery (i.e. tractors and mechanical forage harvesters) out of the reach of these farmers. Thus we have been investigating alternative technologies such as the "bag silage" (see previous activity) and "bag hay" that could be more appropriate for livestock systems operated by smallholders.

In this section we report the results of feeding grass hay to milking cows grazing a native pasture with limited forage availability but with adequate level of protein.

Materials and Methods

Pastures of *B. brizantha* cv Toledo, *Brachiaria* hybrid cv Mulato and *Brachiaria* hybrid cv Mulato II were used to make hay for supplementing milking cows grazing a *Paspalum notatum* pasture in the Quilichao Research Station. The *Brachiaria* pastures were mowed and fertilized with 50 kg N/ha and allowed to grow for 6 weeks. The regrowth was cut with a mower, collected manually and then sun –dried for three days. After drying the hay was stored in plastic bags and subsequently utilized to feed cross bred cows in late lactation.

A total of 8 cows were assigned to one of the following treatments arranged in 4 x 4 reversible LSD: T1*: Paspalum notatum* (control), T2: *Paspalum notatum* + Toledo hay, T3: *Paspalum notatum* + Mulato hay and T4: *Paspalum notatum* + Mulato II hay. Cows milked twice a day were offered hay of the three grasses at a level of 1% DM of BW. Each experimental period was of 14 days of which 7 days was for adjustment and 7 days for measurements. Measurements in the experimental pasture included forage on offer and quality parameters. To assess quality of the three hays we measured extent and rate of gas production, in vitro digestibility and crude protein. Hay consumption and milk yield and composition were measured daily.

Table 7. Quality of three hays made from *Brachiaria* pastures of similar regrowth age*

Hay	Maximum gas production (ml)	Rate of gas production (ml/h)	IVDMD %	CP %
Toledo	228 b	0.061 a	64.0 b	9.1
Mulato	230 b	0.048 c	64.8 b	9.2
Mulato II	241 a	0.055 b	74.8 a	10.8
Significance	P <0.05	P <0.05	P <0.05	

* Six weeks regrowth fertilized with 50 kg of N/ha

Table 8. Milk production and composition of cows grazing a *Paspalum notatum* pasture and supplemented with grass hay.

Hay	Milk yield (l/cow/d)	Fat (%)	Non fat solids (%)	MUN (mg/dL)
Toledo *	5.3	4.8 b	8.4	3.8 b
Mulato **	5.2	5.3 a	8.2	3.7 b
Mulato II ***	5.5	4.8 b	8.4	3.0 b
Control	4.8	5.0 b	8.3	4.2 a
Significance	P<0.06	P<0.005	NS	P <0.001

*Intake 0.74% DM of BW
** Intake 0.63% DM of BW
*** Intake 0.79% DM of BW

Results were subject to analysis of variance using the SAS -GLM program package version 9.1.3, given that one cow was declared as missing cell due to abnormal behavior.

Results and Discussion

Forage availability in the experimental pasture was low (750 kg DM/ha), with medium digestibility (55%) but with high CP content (11%). The quality of the three hays (Table 7) was different since Mulato II hay had higher digestibility (gas production and IVDMD) and CP, and this was associated with slightly higher intake (7%) relative to the other two hays.

Supplementing hay to cows grazing a pasture with low forage availability resulted in 8 to 15% (0.4 to 0.7 liters/cow/d) more milk than unsupplemented cows (Table 8). Milk fat varied among treatments, but no consistent trend was observed with hay supplementation.

In a previous study we had evaluated the utility of supplementing legume hay (cowpea) and legume silage (Cratylia) to milking cows grazing *B. decumbens* pastures in the dry season. Results from those studies indicated that supplementation of high quality legume- based silage and hay resulted in 11 to 18% more milk yield when compared with milk production of the control cows, which is similar to the increments recorded with grass hays in the present experiment.

In summary, results indicate that supplementing relatively small quantities (1% DM of BW) of medium quality grass hay (60-65% IVDMD and 9-10% CP) to cows grazing pastures with limited forage availability but adequate CP resulted in ½ liter more milk per day. These results need to be confirmed in on -farm experiments in order to assess the economics of grass hay production for feeding dairy cows with different genetic potential in the wet and dry season.

1.3 Assessment of the potential of tanniniferous legumes to improve ruminant nutrition in the tropics

Highlights

- Biomass production of selected shrub legumes was affected by soil fertility in planting site and by fertilization, but the extent of response varied widely among species. *Leucaena leucocephala* was by far the most affected species, while *Flemingia macrophylla* was not affected.

- Tannins from *Calliandra calothyrsus* 22310 showed higher solubility in ethanol than those from *Calliandra calothyrsus* 22316. In addition, results revealed that *C. calothyrsus* 22310 had a larger proportion of tannins of low polarity than *C. calothyrsus* 22316.

- A major part of the tannin-bound protein in shrub legumes, which is protected from microbial degradation in the rumen, was digested with acid-pepsin in an in vitro system.

- The PEG: tannin ratio required to maximize ruminal fermentation varied among shrub legume species and is probably influenced by the type of tannins present in the plants.

- Tannins from *Leucaena leucocephala* and *Flemingia macrophylla* were less effective in decreasing ruminal nutrient degradation and VFA production than tannins from *Calliandra calothyrsus*. Between two accessions of *Calliandra*, tannins from the accession 22310, were more reactive than those from the accession 22316.

- Of three protocols evaluated for extraction of DNA from ruminal fluid from tanniniferous diets the best results were obtained with the protocol recommended by IAEA**.**

1.3.1 Assessment of the effects of plant nutritional status on forage yield, quality, concentration and chemical properties of condensed tannins of selected shrub legume species

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Rationale

Type and concentration of secondary metabolites in plants may be affected by environmental factors. Results from experiments carried out during 2004 indicated that forage quality of tanniniferous shrub legumes could be improved by the selection of appropriate planting sites or by improving the growing conditions (e.g. by fertilization). Therefore the present activity concentrates on defining the environmental factors (i.e. soil type and fertility) responsible for differences in forage quality and type and concentration of condensed tannins of a range of tanniniferous shrub legumes. For this purpose, experimental plots were established at two contrasting sites in Colombia. The aim of this activity is to identify growing conditions which could contribute to an improved forage quality of tanniniferous legumes.

Material and Methods

In Experiment 1, the shrub legume species *Cratylia argentea* (CIAT 18516), *Calliandra calothyrsus* (CIAT 22310), *Desmodium velutinum* (CIAT 33443), *Flemingia macrophylla* (CIAT17403) and *Leucaena leucocephala* (CIAT 17502) were raised in Jiffy™ pots and after 6-7 weeks transplanted to the plot in May 2004 at the beginning of the rainy season on a Mollisol (fine silty, mixed, isohypothermic, Aquic Hapludoll, pH: 7.5, rainfall is bimodal, average annual precipitation: 896 mm; mean annual temperature: 24.3°C) at CIAT's headquarters in Palmira and on an Oxisol in Matazul (typic isohipertermic Caolinitic Haplustox, pH 4.5, low natural fertility (in particular deficient in P, basic compounds and organic matter); rainfall is unimodal, annual total: 2251 mm (April-November); annual mean temperature: 26°C) in the Eastern Plains of Colombia, and two levels of fertilization were applied (low fertilization level [kg/ha]: 20 P, 20 K, 500 lime, 10 S; high level: 50 P, 60 K, 1000 dolomite, 40 S) in order to assess the effect of fertilization as affected by soil type.

In Experiment 2, the same legume species were established in Matazul and four fertilizer treatments were applied in order to assess the effect of S and P fertilization: (i) no fertilizer, (ii) lime, P, K, S, Ca, and Mg applied in a water soluble form in a quantity equivalent to the amount of elements that can be taken up by the plant within 18 months, (iii) the same amounts of nutrients without S and (iv) the same amounts of nutrients without P. The five legume species were transplanted in three replicates per site and fertilizer treatment, and arranged in a randomized complete block design. One year after transplanting the first standardization cut was carried out, with cutting heights of 100 cm for *Leucaena leucocephala* and of 50 cm for the remaining species. Since then the plants have been cut every 9 weeks and one sampling has been carried out. Prior to sampling, the number of surviving plants and their growth performance were assessed visually. In order to determine the feeding value, edible forage (branches up to 1cm diameter) from actively growing shoots was collected from five randomly chosen plants from every legume species per fertilizer level and replicate.

To assess dry matter production, plants were cut as described above and the harvested material was separated into leaves and stems. Samples for quality analyses were packed in plastic bags stored on ice and transported to the laboratory. Subsequently the samples were kept frozen at –20°C until freeze-drying. Dried samples were ground in a laboratory mill to pass a 1-mm screen and stored in air-tight containers at –20°C. Plant tissues will be analyzed for C, P, N, K, S, Ca and Mg, and the concentration, astringency, monomer composition and molecular weight of condensed tannins will be determined. Furthermore, biological N fixation by the legumes will be estimated by comparing the natural ^{15}N abundance in legumes and in weedy grasses growing in the same plot. For this purpose one grass and one herbal weed species from the borders of the plots were harvested.

Results and Discussion

In Experiment 1, on the acid soil in Matazul, *L. leucocephala* showed the lowest survival rate and performance in all parameters. Given that it did not reach the minimum size for harvesting, it was discarded. The establishment of the remaining legume species in Matazul was successful and survival rates ranged between 94 and 100%, with no significant differences (P>0.05) between species and fertilization level.

On the more fertile and less acidic soil in Palmira, *L. leucocephala* and *D. velutinum* showed the best survival rates of 100% followed by *C. calothyrsus* with 97.5%. *F. macrophylla* showed an intermediate survival rate of 89% and *C. argentea* presented the lowest survival rate. Fertilization increased survival rate of *C. argentea* from 77% to 88% but had no effect of the survival rates of the remaining legume species.

In Matazul, fertilizer application increased (P<0.001) biomass production from 88 to 147 g DM per plant on average among all legume species. The highest increase was observed in *C. argentea* (from 26 to 112 g DM per plant) and the lowest in *F. macrophylla* (from 166 to 179 g DM). Independent of fertilizer application *F. macrophylla* (173 g) and *C. calothyrsus* (139 g) showed higher (P<0.05) DM yields than *D. velutinum* (90 g) and *C. argentea* (69 g). *L. leucocephala* did not reach the minimum height for the standardization cut and no evaluation could be conducted in Matazul.

With exception of *F. macrophylla*, which did not show any differences due to planting site, biomass production was two to three times higher in Palmira than in Matazul (interaction planting site legume species P<0.001). Independent of planting site and fertilizer treatment, *F. macrophylla* produced between 138 and 180 g of dry matter per plant within nine weeks. Biomass production of the remaining species tended to increase with fertilizer addition in Palmira although the increase was much less pronounced than in Matazul. The largest increase was observed with *L. leucocephala* (+50%) in Palmira. The increase in yield in *C. calothyrsus*, *C. argentea* and *D. velutinum* varied between 5 and 20%.

Fertilization also increased the ratio of leafy biomass to total biomass. Averaged over sites and fertilizer treatments, dry matter production was higher (P<0.05) in *C. calothyrsus* (200 g per plant) than in the remaining species (between 133 and 162 g per plant. *L. leucocephala* was not included in this overall comparison because no data were available from Matazul. However, in Palmira, *L. leucocephala* produced considerably more dry matter (>35% with fertilization and >85% without) than the other species.

In Experiment 2, both P and S fertilization showed clear effects on biomass production but seemed to have little influence on the initial establishment and the survival rate of the experimental species. Although both elements affected biomass production ($P<0.05$), a lack of P resulted in lower dry matter yields than a lack of S ($P<0.05$). Dry matter production among all species averaged 168 g per plant when all elements were supplied. DM production averaged 132 g with no S and 99 g per plant with no P. With *C. calothyrsus* and *C. argentea,* dry matter production decreased by 50% without P and by 36% without S. *D. velutinum* and *F. macrophylla* seemed to be more tolerant to deficiencies in P or S. Without P, biomass production of these species decreased by 35% and without S, by 6 and 10%, respectively.

In summary, these results show that biomass production of most shrub legumes was affected by planting site and fertilization, but the extent of response may vary widely among species. *L. leucocephala* was by far the most affected species, while *F. macrophylla* was not affected at all. On average for both sites, *C. calothyrsus* showed higher biomass production than the other shrub legumes, underlining the good adaptation and high agronomic potential of this species. For production systems on acidic low-fertility soils (e.g. in the Eastern Plains of Colombia) where no fertilizers are applied the most promising species in respect of survival rates, biomass production and development would be *F. macrophylla* and *C. calothyrsus*.

1.3.2 Extraction and characterization of tannins from different legumes

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Rationale

Previous studies conducted at CIAT revealed that differences may exist in the chemical characteristics and the biological activity among condensed tannins from different legume species but also among accessions within a species. In particular, the condensed tannins from *Calliandra calothyrsus* var. Patulul (CIAT 22316) and San Ramon (CIAT 22310) have been shown to differ in their monomer composition and their protein binding capacity. The aim of this activity was to better characterize the chemical properties of the condensed tannins of these two *C. calothyrsus* accessions.

Materials and Methods

Condensed tannins from two accessions of *Calliandra calothyrsus* (CIAT 22310 and CIAT 22316) were extracted using ethyl alcohol (70%) with 0.5% formic acid and 0.05% ascorbic acid as solvent. For that purpose, 40 g of dried and ground leaves were mixed with 400 ml of extraction solution and incubated for 30 minutes, subsequently filtered and centrifuged at 1000g for 15 min. The supernatant was transferred to glass flasks and the alcohol was removed in a rotary evaporator at 40°C. The resulting aqueous solution was filtrated through a Sephadex LH-20 gel column and the different fractions were washed out with ethanol. Subsequently condensed tannins were removed from the column with aqueous acetone (50%), concentrated by evaporation, frozen and lyophilized. For thin layer chromatography (TLC) silica gel was used as stationary phase and butanol: acetic acid: water mixtures or mixtures of ethyl acetate: methyl ethyl ketone: formic acid: water (5:3:1:1) in combination with chloroform: methanol: water (64:33:15) as mobile phase.

Results and Discussion

Tannins from *Calliandra calothyrsus* 22310 showed higher solubility in ethanol than those from *Calliandra calothyrsus* 22316. Tannins did not show displacement in TLC with chloroform: methanol (93:7) which indicates that the components are polar. With the mixture of ethyl acetate: methyl ethyl ketone: formic acid: water (5:3:1:1), *C. calothyrsus* 22310 showed two components of minor polarity with rf 0.86 and 0.58, while *C. calothyrsus* 22316 presented one component with rf 0.86 and one with rf 0.47. Double exposure revealed that *C. calothyrsus* 22310 had a larger proportion of tannins of low polarity than *C. calothyrsus* 22316.

Exposure on bi-dimensional plaques of cellulose with 2-butanol:acetic acid: water (3:1:1) in one direction and acetic acid (30%) in the other, showed that tannins from *C. calothyrsus* 22310 in general have a lower polarity than those of *C. calothyrsus* 22316, probably due to differences in size of the different fractions. In crude extracts, both accessions showed similar proportions of components of low polarity (rf>0.7) but differences in medium polarity between rf 0.7 and 0.5. In *C. calothyrsus* 22310 the 54 detected fractions were run on a polarity gradient silica gel with isopropyl acetate petrol ether, resulting in melting them to 22 new fractions depending on their composition. Two of the major components have been isolated and will be purified for identification as next step.

1.3.3 In vitro assessment of ruminal protein degradability and protein release under conditions resembling acid-pepsin digestion in the abomasum

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Rationale

The inclusion of tannin-free legumes into low-quality grass diets generally increases the ruminal degradation of crude protein. On the other hand, results from in vitro studies conducted last year using a rumen simulation technique demonstrated that supplementation of tannin-rich legumes decreases ruminal protein degradation. It is likely that such a decrease would result in a greater flow of dietary N to the lower digestive tract of ruminants feed tannin-rich legumes. However, given that in previous study only ruminal fermentation processes were considered, the effects of tannin-rich legumes on postruminal protein digestion are unknown. This year, our work focused on the assessment of the potential acid-pepsin digestibility of rumen-undegradable protein from grass-based diets supplemented with contrasting legumes.

Materials and Methods

One grass-alone and 13 legume supplemented diets were incubated for 48 h in vitro in fermentation liquid (McDougall buffer: rumen liquid, 1:4) under CO_2 gasification. *Brachiaria humidicola* (CIAT 6133) was used as grass species. The legume supplement (1/3 of dietary dry matter) consisted either of a single legume (*Cratylia argentea* CIAT 18516, *Calliandra calothyrsus* CIAT 22310 and 22316, *Flemingia macrophylla* CIAT 17403 and *Leucaena leucocephala* CIAT 734) or combinations of *C. argentea* with the other legumes in proportions of 2:1 and 1:2. A total of nine samples were incubated per treatment. Subsequently three samples of every treatment were filtered and the solid residue was analyzed for dry matter (DM) and organic matter (OM) to determine apparent ruminal DM and OM degradabilities. Three samples were filtered, and the solid residue

Table 9. Degradation parameters (mg/g of supply) measured under conditions simulating the rumen and the abomasum (values with same letters in one column are not significantly different).

Treatments		Apparent ruminal degradability		True ruminal degradability	Ruminal + acid-pepsin degradability	True ruminal and total degradability of N	
		OM	DM	DM	DM	Ruminal	Total
B. humidicola 100%		666a	655a	759a	641a	995ab	935ab
C. argentea 18516		576b	565bc	710bc	646a	884abc	954a
C.calothyrsus 22310	3:0	431e	426e	614f	511c	576d	848de
C.argentea	2:1	506cd	499d	654de	581b	n.a.	900abcd
	1:2	538bc	528cd	677cd	625°	787abcd	931abc
C.calothyrsus 22316	3:0	482de	485d	688bcd	467d	677bcd	793e
C.argentea	2:1	497cd	491d	693bc	514c	727abcd	884cbd
	1:2	589b	583b	709bc	557b	789abcd	920abc
F.macrophylla 17403	3:0	500cd	499d	625ef	501cd	605d	870bcd
C.argentea	2:1	540bc	538bcd	656de	499cd	621cd	868cd
	1:2	566b	560bc	682bcd	559b	752abcd	928abc
L. leucocephala 734	3:0	542bc	534bcd	715b	575b	n.a.	906abcd
C.argentea	2:1	572b	557bc	711bc	582b	736abcd	910abcd
	1:2	583b	567bc	711b	584b	801abcd	930abc

a, b, c, d, e means in the same column different (P <0.05)
n.a., data not available due to technical problems

treated with NDF solution and analyzed for DM and N to determine true ruminal DM and N degradability. The remaining three samples were further incubated for 24 h with HCl-pepsin to simulate abomasal digestion. After incubation samples were filtered and the solid residues analyzed for DM and N. Ammonium concentration in the fermentation fluid was measured at 0, 6, 12, 24 and 48 h of incubation using the method of Kjeldahl.

Results and Discussion

The rate of ammonia production during incubation in ruminal fluid was affected by the type of legume supplement and decreased with increasing proportion of tanniniferous legume in the diet. Over all, diets with high proportion of *C. calothyrsus* resulted in the lowest ammonia production. Apparent and true ruminal degradation of DM and of total DM degradation (ruminal + acid-pepsin) as well as nitrogen degradation was inversely related to the proportion of tanniniferous legume in the mixture. This effect varied with species and was not as clear as the relationship between ammonium concentration and legume proportion. Nitrogen degradation was notably improved by incubation with acid-pepsin solution.

Results indicate that the supplementation with tanniniferous legumes increases the proportion of acid-pepsin digestible protein of dietary origin given that the proportion of acid-pepsin digested N (difference between ruminal and total N degradability) was lowest with *C. argentea* alone and increased with the proportion of tanniniferous legume in the mixture (Table 9).

In general, our results show that in mixed diets based on a low-quality grass, the replacement of *Cratylia argentea*, a legume without detectable amounts of tannins, by the tanniniferous

C. calothyrsus (CIAT 22310) decreased apparent ruminal crude protein degradation by 65% (from 884 to 576 mg/g) and increased almost 4 fold (from 70 to 272 mg/g of N supplied) the proportion of dietary crude protein digested by acid-pepsin incubation. This suggests that a high proportion of the tannin-bound protein, which is protected from microbial degradation in the rumen, could be available for acid-pepsin digestion in the abomasums. If these results are validated in vivo the use of mixtures of legumes with and without tannins could effectively improve the supply of available protein in ruminants fed low quality grasses.

The lower ruminal degradation of protein in diets containing a high proportion of *C. calothyrsus* CIAT 22310 as compared with *C. calothyrsus* CIAT 22316 is in good agreement with the results of a feeding trial conducted at CIAT and with observations made in activity 1.3.4 (see below), both of which indicated that tannins from *C. calothyrsus* CIAT 22310 have a higher protein-binding capacity (i.e lower degradability by rumen microbes) than those from *C. callothyrsus* CIAT 22316. This difference in protein-binding capacity between provenances of *Calliandra* has been associated in previous studies with the monomer composition of the extractable CT (ECT) fraction. The ECT from CIAT 22310 have more delphinidin units than the ECT from CIAT 22316, which have more cyanidin units. In temperate legume it has also been shown that tannins from legumes with more delphinidin units (*Lotus pedunculatus*) are more reactive with Rubisco than tannins from legumes with more cyanidin units (*Lotus corniculatus*).

1.3.4 In vitro ruminal fermentation of grass-legume mixtures as affected by PEG and by increasing proportions of purified tannins from different legumes

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Rationale

Low quality of ruminant diets, in conjunction with low feed conversion efficiency leads to low levels of animal production in tropical regions. Protein deficiency is by far the most important cause of low performance of ruminants maintained on low-quality forages, and ensuring adequate ammonia levels in the rumen for microbial growth is the first priority in optimizing fermentative digestion of forage. Promising forage legume species have been identified to overcome these limitations. Many of these legumes contain tannins that could be either advantageous or disadvantageous in terms of feed efficiency and metabolizable protein supply to the animal. The three multipurpose legume species, *Flemingia macrophylla*, *Calliandra calothyrsus* and *Leucaena leucocephala*, have shown good growth performance and could serve as forage supplements for ruminants fed tropical grass-based diets. Results from previous studies showed large differences in the tannin content among these legumes and suggested that there could also be differences in the type of tannins. However, little is known about the relevance of these differences and the effect of different tannins on digestion in ruminants.

Material and Methods

In Experiment 1, seven contrasting grass-legume mixtures were tested using the gas transducer technique. A low quality hay of the tropical grass *Brachiaria humidicola* (CIAT 6133; formerly called *B. dictyoneura*), cut after a growing period of 12 weeks, was used as basal substrate in all treatments. As supplements (1/3 of dry matter), leaves of the shrub legume species *Calliandra calothyrsus* (CIAT 22310 and 22316), *Cratylia argentea* (CIAT 18516), *Flemingia macrophylla* (CIAT 17403), *Leucaena leucocephala* (CIAT 734) and *Desmodium velutinum* (CIAT 33443) and the herbaceous legume *Vigna unguiculata* (cowpea; CIAT 391) were used. Leaves of shrub legumes were harvested manually eight weeks after the last cut. The material was immediately stored at –20°C and subsequently freeze dried.

The foliage of cowpea (the whole plant, before flowering) was harvested eight weeks after establishment and sun dried for three days. The dried plant material of all species was ground in a laboratory mill to pass a 1 mm screen. All mixtures were incubated without and with polyethylene glycol (PEG), to bind and inactivate soluble condensed tannins. The levels of PEG addition were determined according to the species specific tannin content and were equivalent to 0.33, 0.66 and 1 of the analyzed tannin content. Additionally, all mixtures, including those without any detectable amounts of condensed tannins, were incubated with 240 mg of PEG/g of legume dry matter. This level corresponded to the highest condensed tannin content in legume dry matter observed in the experiment. The experiment was conducted for 144 h with measurements of gas pressure and volume after 3, 6, 9, 12, 24, 36, 48, 60, 72, 96, 120 and 144 h. The solid fermentation residues were analyzed for dry matter and nitrogen content and the fermentation fluid was analyzed for volatile fatty acids.

In Experiment 2, a mixture of *Brachiaria humidicola* (CIAT 6133 and cowpea (2:1) was incubated without or with the addition of increasing levels (25, 50, 75 and 100 mg/g DM) of purified tannins extracted from *C. calothyrsus* (CIAT 22310 and 22316), *F. macrophylla* (CIAT 17403) and *L. leucocephala* (CIAT 734). The measurement of gas production was conducted as described above, but the incubation period lasted for 168 h, to assure that the plateau phase was reached in all treatments.

Results and Discussion

In Experiment 1, PEG addition increased ($P<0.05$) the apparent in vitro dry matter degradation (IVDMD) of the diet containing *C. calothyrsus* 22310, tended to increase ($P<0.1$) IVDMD of the diet containing *F. macrophylla*, had no effect on diets with *C. calothyrus* 22316, *C. argentea* or *L. leucocephala*, and tended to decrease ($P<0.1$) IVDMD of diets containing Desmodium or cowpea. However, it has to be acknowledged that the determination of IVDMD in the presence of PEG is not always free of error, because part of the PEG added may be bound in the solid residue and this could have resulted in an underestimation of the IVDMD in the treatments with PEG. In all mixtures containing tanniniferous legumes, the percentage of crude protein (CP) apparently degraded was increased ($P<0.0001$) by PEG addition. In contrast, in the mixtures with *C. argentea* or *D. velutinum*, the amount of CP degraded tended ($P<0.1$) to decrease, and in the mixture with cowpea significantly less CP was degraded in the presence of PEG ($P<0.05$). The reasons for this unexpected decrease in the amount of CP degraded due to PEG addition are unknown.

The gas production parameters agreed well with the data on IVDMD. Gas production increased with PEG addition in all treatments with tanniniferous legumes. Without PEG addition, treatments with *C. calothyrsus* 22316 and *F. macrophylla* showed a lower initial gas production than the remaining mixtures. This could be related to an inhibition of the initial microbial activity (colonization of forage particles) due to the presence of tannins.

The effects of PEG addition on the concentration of individual volatile fatty acids (VFA) were very variable. In the treatments with *C. calothyrsus* 22310, PEG addition had no effect ($P>0.05$) on the proportion of acetate or propionate but increased ($P<0.05$) the proportion of butyrate. In treatments with *C. calothyrsus* 22316, the total concentration of VFA and the proportion of individual VFA did not show any change due to PEG addition. In the treatments with *F. macrophylla* or *L. leucocephala*, concentration of VFA was not affected ($P>0.05$) by the addition of low or intermediate levels of PEG, but increased with the addition of high levels of PEG.

Overall, there seemed to be a trend for higher VFA concentrations when high levels of PEG were added to mixtures containing tanniniferous

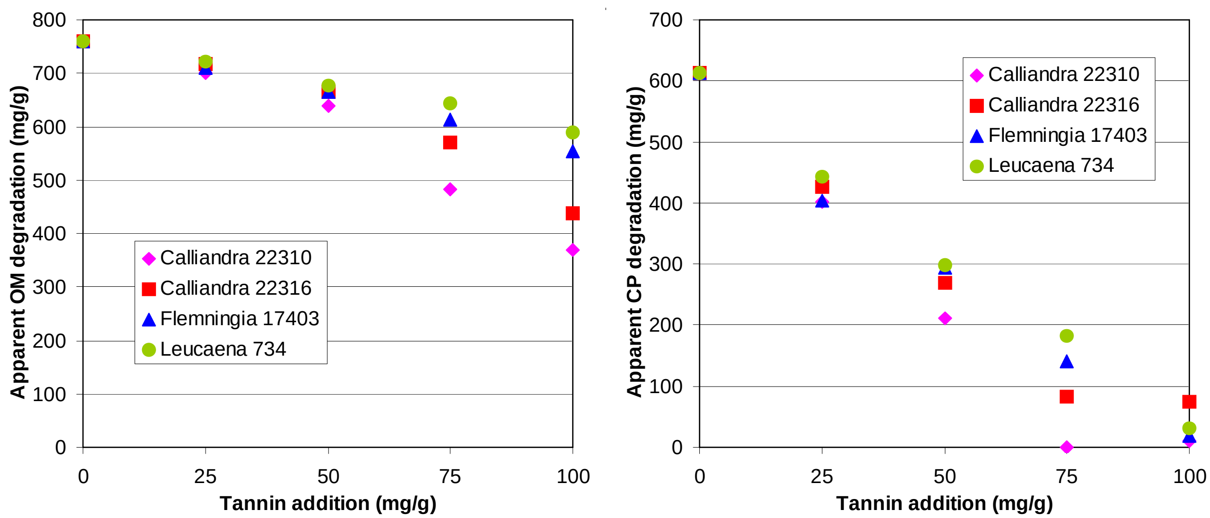

Figure 1. Effect of extracted tannins from different shrub legumes on in vitro apparent organic matter (OM) and crude protein (CP) degradation.

legumes. This trend was more pronounced with butyrate than with acetate or propionate concentrations. In treatments with legumes without detectable amounts of condensed tannins, PEG addition had no effect (P>0.05) on the concentrations of VFA except in the treatment with *C. argentea*, where PEG addition tended to increase (P>0.1) total concentration of VFA.

A comparison of the treatments with tanniniferous legumes incubated with the highest level of PEG (240 mg/g legume dry matter), which is supposed to bind and inactivate all soluble condensed tannins, showed differences in fermentation characteristics of the various diets. The apparent IVDMD was highest with *L. leucocephala*, intermediate with *C. calothyrsus* 22310, and lowest with *C. calothyrsus* 22316 and with *F. macrophylla* (P<0.05). The apparent CP degradation was highest with *C.calothyrsus* 22310 (67%), intermediate with *L. leucocephala* (55%), and lowest with *C. calothyrsus* 22316 (45%) and with *F. macrophylla* (43%) (P<0.05). The concentration of acetate on the fermentation fluid was higher (P<0.05) with *L. leucocephala* than with *C. calothyrsus* 22316, but did not differ significantly with the other two legumes. Concentrations of propionate and butyrate were higher (P<0.05) with *L. leucocephala* than with any other legume. Based on these results, the feeding value of the tested legumes could be ranked as follows: *Leucaena leucocephala* 734 > *Calliandra calothyrsus* 22310 > *Flemingia macrophylla* 17403 > *Calliandra calothyrsus* 22316.

Results from this experiment indicate that a PEG: tannin ratio of 1:1 is not always sufficient to bind and inactivate all soluble tannins. While in mixtures with *C. calothyrsus* 22310 a ratio of 2:3 was adequate to maximize apparent CP degradation, in mixtures with *C. Calothyrsus* 22316 or *F. macrophylla*, CP degradation increased throughout the range of PEG addition tested. This suggests that the PEG: tannin ratio required to maximize ruminal fermentation varies among species and is probably influenced by the type of tannins present in the legumes. To assure a complete inhibition of condensed tannins, the amount of PEG added to the diet should therefore always exceed the estimated amount of tannins in the diet.

Results from Experiment 2 showed that apparent IVDMD decreased with increasing addition of tannins regardless of the origin of the tannins. However, the extent of the reduction varied (P<0.05) among tannins (Figure 1). The largest decrease was observed with tannins from *C. calothyrsus* 22310, followed by tannins from *C. calothyrsus* 22316 and *F. macrphylla*. The smallest decrease was found with tannins from *L. leucocephala*. The apparent CP degradation was drastically decreased with increasing levels of tannins (61% without tannins, 40-45% with 25 mg, 20-30% with 50 mg, less than 18% with 75 mg and less than 7.5% with 100 mg tannins) ($P<0.001$). With the highest tannin level, CP degradation was almost completely inhibited. Although the effect of tannins on CP degradation was more pronounced than the effect on IVDMD, the differences among types of tannins were smaller. The decrease in CP degradation was larger ($P<0.05$) with tannins from *C. calothyrsus* 22310 than with tannins from the remaining legumes.

Concentrations of acetate, propionate and butyrate in the fermentation fluid decreased ($P<0.001$) with increasing tannin addition and the decrease varied with the origin of the tannins. Acetate concentration was less affected by tannins from *F. macrophylla* than by any other tannins ($P<0.05$). Propionate concentration was higher ($P<0.05$) with tannins from *F. macrophylla* or *L. leucocephala* than with tannins from *C. calothyrsus* 22310 or 22316. Butyrate concentration was highest ($P<0.05$) with tannins from *F. macrophylla*, intermediate with tannins from *L. leucocephala* and lowest ($P<0.05$) with tannins from *C. calothyrsus*.

These results confirm that large differences may exist in the effects of tannins from different legume species on ruminal fermentation. The results further suggest that tannins from *L. leucocephala* and *F. macrophylla* were less effective in decreasing ruminal nutrient degradation and VFA production than tannins from *C. calothyrsus*. Among the two accessions of *C. calothyrsus*, tannins from the accession 22310 were more effective in reducing ruminal nutrient fermentation than those from the accession 22316.

1.3.5 Evaluation of the effects of legume mixtures on rumen microbial populations

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Rationale

The rumen is a complex ecosystem and the population dynamics of ruminal microbes are affected by multiple factors. Recent studies showed that manipulation of ruminal fermentation or a change of the diet may alter the population composition of the whole ecosystem. In respect to the low quality of tropical forages and the increasing demand for more food of high quality, strategies to improve animal nutrition and to increment productivity are required. One possibility could be to raise the proportion of feed protein that escapes rumen fermentation by the inclusion of tanniniferous plants in the diet. However, up to now, relatively little is known about the effects of tanniniferous diets on microbial population dynamics in the rumen. Several modern techniques, including polymerase chain reaction (PCR) and real time PCR (rtPCR), are available to monitor microbial populations, but it is unknown whether these techniques give reliable results with ruminal fluid samples containing tannins. To test the reliability of these techniques, several protocols were applied and compared, and samples of ruminal fluid with and without tannins were analyzed.

Material and Methods

For DNA extraction, three protocols were tested: (i) the protocol recommended by the International Atomic Energy Agency (IAEA) for ruminal fluid; (ii) a protocol for extraction of DNA from gram negative bacteria, adapted for rumen fluid samples; and (iii) a protocol from BioTechniques recommended for ruminal fluid containing tannins. The primers used were made available by Chris McSweeney (CSIRO, Australia) in accordance with IAEA and were specifically designed for this purpose to detect *Archaea*, *Bacteria*, *Fungi*, *Fibrobacter succinogenes* and

Ruminococcus flavefaciens. For conventional PCR, oligonucleotid primers from IAEA and PCR chemicals from Boehringer Mannheim, Germany were used according to manufacturer's recommendations. The PCR-products were separated by agarose-gel electrophoresis (2% gels), stained with ethidium bromide (EB) and visualized under UV. For rtPCR the instructions of the iCycler IQ real-time manual (Biorad, CA) were followed. All reactions were carried out as recommended by the IAEA.

To establish the protocol for rtPCR, DNA was extracted from rumen fluid of a fistulated Holstein bull by the protocol of the IAEA. Concentration and A260:A280 DNA ratios were determined by photospectroscopy, resulting in 250 ng/ì l at a ratio of 1.84. The ratio is based on the fact, that some proteins and phenolic compounds show a peak at 280 nm. This may affect the spectrometric results of DNA measurement at 260 nm. As reference of the impact of such contaminations the ratio allows to calculate the true DNA content resulting from the obtained peak. Based on this DNA, dilutions were prepared of 10, 1, 0.1, 0.01, 0.001 and 0.0001 ng/ì l which were then used to generate a calibration curve using the primer for total bacteria. Subsequently, the amplification of DNA extracted from tannin rich samples was carried out, applying the same protocol as mentioned above.

Results and Discussion

The three protocols evaluated for extraction of DNA from ruminal fluid showed differences in efficiency and manageability (Table 10). The best results were obtained with the protocol recommended by IAEA. Although the quality of the extracted DNA, i.e. purity, was equal with that obtained by the protocol for gram negative bacteria, the efficiency was much higher, yielding the double amount of DNA. The control by electrophoresis showed very little background for samples extracted according to the protocol of IAEA.

Over all, the protocol for gram negative bacteria yielded comparable results. However, a big disadvantage of this technique is the resulting high risk waste of chloroform. Furthermore, it is not known if DNA of all microbial groups of interest was extracted because this technique was specifically developed to extract DNA of gram negative bacteria. Both techniques showed good reproducibility.

The protocol of BioTechniques was less suitable than the other two and yielded a slightly brownish extract instead of a transparent one. This was also reflected by a low ratio value and a higher standard deviation in the concentration of DNA obtained, and resulted in a lower repeatability.

The primers provided by IAEA amplified well for conventional PCR and for rtPCR, and with samples extracted according to the protocol of IAEA and the protocol for gram negative bacteria (Figures 2 and 3).

These results indicate that the method for DNA extraction and amplification was successfully established and suggest that rtPCR could be a reliable tool to monitor microbial populations in ruminal fluid of animals feed tanniniferous diets.

Table 10. Comparison of the three evaluated methods for DNA extraction

Method	Concentration [ng/µl]	STD [ng/µl]	Ratio (260/280nm)	STD (260/280nm)	µl DNA
IAEA	45.5	8.2	1.89	0.34	100
Gram negative	46.8	14.2	1.96	0.69	50
BioTechniques	61.3	72.9	0.98	0.48	50

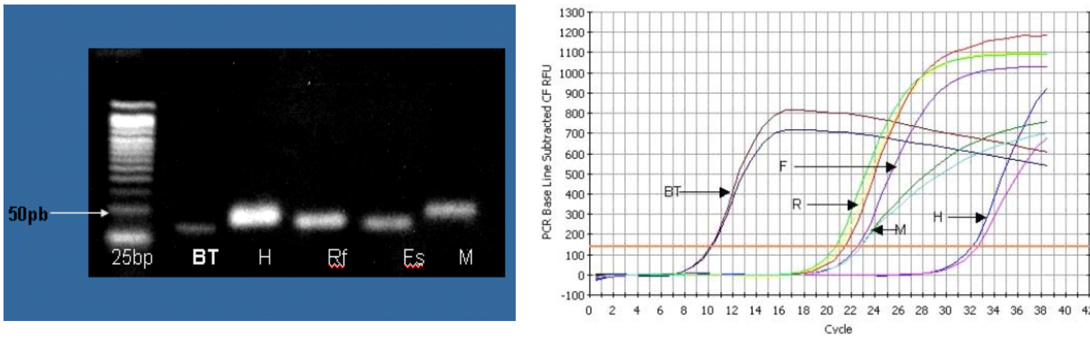

Figure 2. Amplification of PCR products with specific primers for conventional PCR and real time PCR (BT, total bacteria; H, fungi; R, *Ruminococcus flavefaciens*; F, *Fibrobacter succinogenes*; *M. archaea*).

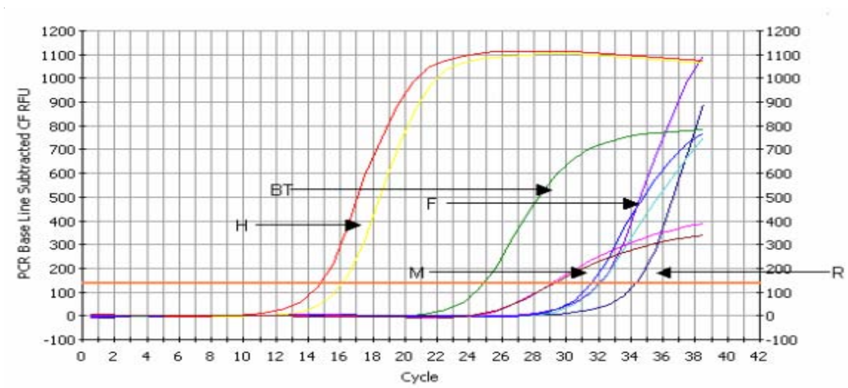

Figure 3. Amplification of DNA of rumen fluid samples rich in tannins (BT, total bacteria; H, fungi; R, *Ruminococcus flavefaciens***;** F**,** *Fibrobacter succinogenes***;** M**,** *Archaea***).**

Output 2: Grass and legume genotypes with known reaction to pests and diseases and to interaction with symbiont organisms are developed

2.1 Breeding *Brachiaria* for resistant to biotic and abiotic stresses

Highlights

- Implementation of a scheme of selection on testcross progeny performance has begun in the *Brachiaria* grass breeding program. This year was year 1 of the first, 3-year selection cycle.
- A total of 1,419 *Brachiaria* hybrid clones (series BR05) were established in space-planted field trials at CIAT-Quilichao and at the Matazul research field in the Llanos.
- Seed of 16 selections (14 BR02's and 2 MX02's) was delivered to Semillas Papalotla early this year and distributed for agronomic trials in Asia (Thailand and Lao) and Central America and Mexico.
- One hundred thirty-seven preselections (series BR04) were assessed for resistance to three Colombian spittlebug species. Thirteen promising resistant hybrids were identified.
- Promising new *Brachiaria* hybrids are being progeny tested to identify apomicts, i.e., candidates for cultivar release.

2.1.1 Screening and selection of sexual clones (SX05)

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Rationale

A broad-based, synthetic tetraploid sexual *Brachiaria* population is the core of the breeding program. This population has been cyclically selected over six cycles (12 years) on "per se" performance, mainly on spittlebug resistance and general agronomic merit. Significant progress in spittlebug resistance has been achieved (Miles et al., In press).

Recently, selection pressure on Al tolerance, Rhizoctonia resistance, and nutritional quality is being incorporated in the selection process as screening protocols for these additional traits improve in capacity and speed.

A proposal to implement selection based on combining ability (rather than on "per se" performance) in the sexual synthetic *Brachiaria* grass breeding population was made a decade ago (Miles 1995).

This scheme requires increasing cycle time from two years to three. We expect that the greater accuracy in identifying sexual clones that combine well with our chosen tester (cv. Basilisk) will more than offset the longer cycle time.

Effective implementation has been delayed owing to logistical complications and to the fact that important genetic gains in spittlebug resistance continued to be achieved by the simpler "per se" selection.

However, the moment has been reached, now that very high levels of multiple spittlebug resistance have been achieved, to modify the selection scheme to take into account hybrid performance (i.e., combining ability).

Materials and Methods

A total of 565 sexual clones (508 SX05's produced by recombining 34 highly resistant SX03 clones, plus 57 highly spittlebug-resistant SX03 clones that were identified too late for inclusion in SX03 recombination block) were established as widely-spaced plants (approx. 5x5 m) in a field of *B. decumbens*, CIAT 606 at Popayán in April 2005. Seed harvest (OP seed) began last August, and will be terminated by 30NOV05. OP seed (resulting from crosses to CIAT 606) will be sown in 2006 to generate 200-300 testcross progenies for field evaluation.

Results and Discussion

All 565 clones were screened for reaction to three spittlebug species and 67 clones were culled on susceptibility to one or more species. Further clones will be culled on seed fill (information from testcross crossing block). Full, apparently viable seed harvested on the individual sexual plants ranges from zero up to 1,864.

We anticipate producing on the order of 200-300 testcross families (of 10 individuals each: 2,000 to 3,000 plants total) for field evaluation in 2006. Sexual clones selected on the basis of testcross performance will be recombined in 2007, and a new selection cycle initiated in 2008.

2.1.2 Screening a population of hybrid clones (BR05) resulting from crosses with *Brachiaria decumbens*

Contributors: J.W. Miles; A. Betancourt; J. Muñoz; C. Plazas; and D. Vergara (CIAT)

Rationale

The *Brachiaria* breeding project seeks to create, identify and propagate useful apomictic hybrids. Each cycle of selection in the synthetic sexual population identifies a small number of elite sexual clones. These are recombined and also are crossed (as females) to selected apomictic accessions to generate novel apomictic hybrids.

Materials and Methods

Thirty-four highly spittlebug-resistant SX03 clones were identified in mid-2004. These were propagated and established as widely-spaced (approx. 5x5 m) plants in a field of *B. decumbens* cv. Basilisk in August 2004. Open-pollinated seeds of these sexual plants (crosses with Basilisk) were scarified and germinated in early 2005.

A total of 1,419 hybrid clones (series BR05) were effectively established in space-planted field trials at CIAT-Quilichao and at the Matazul ranch in May.

Results and Discussion

Two hundred fourteen preselections (cultivar candidates) have been identified (as of late October) and successfully propagated to CIAT for evaluation of reactions to spittlebug, Rhizoctonia, and aluminum, and for quality assessment. Seed is being harvested at CIAT-Quilichao on these 214 plants for a progeny test, to be established during second semester, 2006 (to assess reproductive mode). Small quantities of seed of apomictic final selections may be available for distribution in early 2007, or in 2008.

2.1.3 Screening a population of hybrid clones (RZ05) resulting from crosses with *Brachiaria brizantha* CIAT 16320

Contributors: J.W. Miles; S. Kelemu; A. Betancourt; J. Muñoz; C. Plazas; and D. Vergara (CIAT)

Rationale

Our mainstream tetraploid sexual breeding population is generally very susceptible to Rhizoctonia foliar blight. The accession of *B. brizantha* CIAT 16320 shows exceptional resistance to Rhizoctonia foliar blight. In 2001, several selected clones from the sexual population were crossed with CIAT 16320. Several hundred of these hybrids were assessed for Rhizoctonia resistance by the detached-leaf assay. Seven apparently sexual hybrids with an intermediate level of resistance were identified. These were recombined to create a large segregating population in which we expected to identify tetraploid sexual clones with higher levels of resistance so that this resistance (originally derived from CIAT 16320) can be introgressed into the mainstream tetraploid sexual breeding population.

Materials and Methods

Seven apparently sexual (on progeny test), apparently more or less resistant to Rhizoctonia foliar blight (on detached-leaf assay) hybrids with the highly Rhizoctonia-resistant *B. brizantha* accession CIAT 16320 were identified in 2003, from crosses made in 2001. These seven clones were propagated vegetatively and a small isolated recombination block established early in 2004. Open pollinated seed harvested from this recombination block were germinated in early 2005, and 498 seedlings obtained. These were propagated and transplanted to two, space-planted field trials (Quilichao and Matazul).

Results and Discussion

Field observations revealed that one of the seven parental clones was in fact a facultative apomict (produced mostly uniform progeny). Hence, the population is “contaminated” with apomixis and not completely sexual as anticipated: This is a temporary, but not fatal, setback.

One hundred seventeen preselections were made, in the progenies of the six sexual parental clones. These preselections will need careful progeny testing in 2006 to discard any facultative or fully apomictic individuals. Fully sexual clones will be assessed for reaction to Rhizoctonia and spittlebugs. Depending on the levels of resistance to Rhizoctonia identified in fully sexual plants, these will be either introgressed directly into the main sexual brachiariagrass population (if high levels of resistance are found), or subjected to further selection and recombination to develop levels of Rhizoctonia resistance in sexual tetraploid germplasm adequate to be introgressed into the main sexual population.

2.1.4 Spittlebug reaction of selected *Brachiaria* hybrids (series BR04)

Contributors: J.W. Miles; C. Cardona; G. Sotelo; and J. Muñoz (CIAT)

Rationale

The current Brachiaria breeding scheme generates a cohort of sexual-by-apomictic hybrids every selection cycle. In 2003, two spittlebug-resistant sexual clones were identified, one of which was exceptionally resistant (antibiotic) to three Colombian species. The resistance of hybrids of these resistant sexual clones crossed with susceptible, but apomictic, pollen parents needs to be determined.

Materials and Methods

Two spittlebug-resistant sexual clones were identified in 2002 from the cohort of sexual plants produced in 2001. One of the two clones was exceptionally resistant (strong antibiosis) to three Colombian species. These two sexual clones were each crossed to four apomictic accessions of *B. brizantha*. Hybrid progenies were assessed in field trials during 2004, and preselections (137) tested for spittlebug reaction in replicated trials in the CIAT forage entomology glasshouse, using standard procedures.

Results and Discussion

Thirteen hybrids were considered "resistant" to the three Colombian spittlebug species used in glasshouse tests. For only five of the thirteen were enough progeny obtained for a progeny trial; observations to date (04NOV05) suggest that at least two of these five are apomicts. Additional open-pollinated seed of the remaining eight hybrids is being generated currently for progeny a trial in 2006.

2.1.5 Reproductive mode (by progeny test) of selected *Brachiaria* hybrids (series BR04)

Contributors: J.W. Miles; A. Betancourt; and J. Muñoz (CIAT)

Rationale

Individuals in sexual-by-apomictic hybrid populations segregate approximately 1:1 for reproductive mode (sexual:apomictic). When plants with promising attributes [vigor, leafiness, seed set, spittlebug resistance (where required), etc.] are identified, their reproductive behavior must be determined, as only apomicts are viable candidates for commercial release.

A simple progeny test is the most straightforward means to determine actual reproductive behavior while simultaneously achieving an initial seed multiplication of the apomicts that are identified, by harvesting seed from the (uniform) progeny rows.

Materials and Methods

We attempted to harvest open pollinated seed at Quilichao from individual spaced plants of over 300 BR04 hybrid clones preselected during the 2004 season. On the basis of seed yield and seed fill (caryopsis formation), the cohort of preselections was culled to 137.

Results and Discussion

Sufficient progeny seedlings to be included in the progeny test (at least 10) were obtained for only 42 of the 137 preselected hybrids. Seed of eight additional promising BR04 hybrids (spittlebug resistance) is being multiplied for progeny testing next year.

Preliminary results suggest that 23 of the 42 preselections are probably apomicts, the remainder appear to be either sexuals or facultative apomicts.

2.1.6 Fingerprinting selected *Brachiaria* hybrids with appropriate molecular markers (BR02 and MX02)

Contributors: J.W. Miles and J. Tohme (CIAT)

Rationale

With each selection cycle a new cohort of sexual-by-apomictic hybrids is formed. As these are assessed for a series of attributes, most are culled and a small group of promising apomictic pre-selections identified for distribution for wider evaluation. As a means of positive identification of these advanced hybrids (and potential cultivars) they need to be "fingerprinted" by reliable molecular markers prior to (or at least simultaneous with) wider distribution outside of CIAT, so as to avoid its unauthorized "escape" prior to formal release.

Materials and Methods

Greenhouse-grown plants of 42 selected genotypes were sampled in early December 2005. We anticipate having these plants "fingerprinted" for 25 microsatellite markers by the end of January 2006.

2.1.7 Distribution of seed of promising apomictic (BR02 and MX02) hybrids to SE Asia, Mexico, Costa Rica, and Brazil

Contributors: J.W. Miles (CIAT); E. Stern (Semillas Papalotla); M. Hare (Univ. Ubon Ratchathani, Thailand); and P. Horne (CIAT, Lao)

Rationale

Selected apomictic hybrids require regional evaluation before being considered for commercial release. This function is being coordinated and fully financed by Semillas Papalotla through a network of employees and collaborators throughout the tropical world.

Materials and Methods

Small quantities of seed of nine apomictic hybrids was produced on progeny test plots at CIAT during 2003, and delivered to CIAT-Lao in early 2004. These hybrids and seven more were multiplied in larger plots, at CIAT-Popayán during 2004, so that larger quantities of seed were available for distribution this year. Seed was sent to Semillas Papalotla, and from there distributed to collaborators in Asia (Thailand and Lao), Central America, Mexico, and Brazil. Agronomic trials are in progress.

Results and Discussion

Dr. Michael Hare has already tentatively identified a couple of "promising" hybrids, based on general vigor, upright growth habit (preferred for cut-and-carry systems in Asia), and regrowth following cutting. We have noticed two hybrids with growth habit very similar to cv. Basilisk. At least one of these prostrate lines has excellent aluminum tolerance and is more resistant to spittlebugs than cv. Basilisk.

2.2 Screening and selection of *Brachiaria* genotypes for spittlebug resistance

Highlights

- A large number of sexual hybrids (SX03, SX05) with high levels of antibiosis resistance to *Aeneolamia varia*, *A. reducta*, and *Zulia carbonaria* were identified

- High levels of antibiosis resistance to *A. varia*, *A. reducta* and *Z. carbonaria* were detected in 9 apomictic hybrids (series BR04).

- Six apomictic hybrids of the MX02 series, selected for resistance to *Prosapia simulans*, also showed resistance to *A. varia*, *A. reducta, Z. carbonaria*, and *Mahanarva trifissa.*

- Six apomictic hybrids of the series BR02 and 11of the series MX02 were identified as resistant to *A. varia*, *Z. carbonaria*, *Z. pubescens*, and *M. trifissa* under field conditions.

2.2.1 Continuous mass rearing of spittlebug species

Contributors: G. Sotelo and C. Cardona (CIAT)

A permanent supply of insects is essential in the process of evaluating genotypes for resistance to spittlebug. At present, the progress made in mass rearing of nymphs and in obtaining eggs from adults collected in the field allows us to conduct simultaneous screening of large number of *Brachiaria* genotypes for resistance to all major spittlebug species present in Colombia. Insects produced in our mass rearing facilities are used for greenhouse evaluations in Palmira and field evaluations in Caquetá. Our mass rearing and mass screening techniques have proved to be successful in Brazil and Mexico.

2.2.2 Greenhouse screening of *Brachiaria* accessions and hybrids for resistance to four spittlebug species

Contributors: C. Cardona, G. Sotelo, J. W. Miles, and A. Pabón (CIAT)

Rationale

Assessment of resistance to spittlebugs is an essential step in the process of breeding superior *Brachiaria* cultivars at CIAT. In 2005, intensive screening of selected hybrids was conducted under greenhouse and field conditions. All available genotypes were evaluated.

Materials and Methods

Screenings for resistance in the greenhouse were conducted with *Aeneolamia varia, A. reducta, Zulia carbonaria, Z. pubescens, Mahanarva trifissa* and *Prosapia simulans*. Test materials were usually compared with five checks fully characterized for resistance or susceptibility to *A. varia*. Plants were infested with six eggs per plant of the respective spittlebug species and the infestation was allowed to proceed without interference until all nymphs were mature (fifth instar stage) or adult emergence occurred. Plants (usually 5-10 per genotype) were scored for symptoms using a damage score scale (1, no visible damage; 5, plant dead) developed in previous years. Percentage nymph survival was calculated. Materials were selected on the basis of low damage scores (<2.0 in a 1-5 scale) and reduced percentage nymph survival (<30%). All those rated as resistant or intermediate were reconfirmed. All susceptible hybrids were discarded.

Results and Discussion

In 2005, 119 pre-selected sexual (SX03) hybrids were simultaneously screened for resistance to *A. varia*, *A. reducta*, and *Z. carbonaria*. We used five replications per hybrid per insect species. For comparison, we used five well-known checks replicated 10 times per insect species. All but one of the hybrids were resistant to all three test species. To the extent that mean percentage nymph survival in the population did not differ from percentage survival in our most resistant check, the hybrid SX01NO/0102 (Table 11). These results clearly indicate that a very significant progress has been made in incorporating antibiosis resistance to all of the three test species in a relatively short period of time.

Table 11. Levels of resistance to three spittlebug species in 119 sexual *Brachiaria* hybrids and checks

Genotype	Spittlebug species					
	Aeneolamia varia		*Aeneolamia reducta*		*Zulia carbonaria*	
	Damage scores[1]	Percentage nymph survival	Damage scores	Percentage nymph survival	Damage scores	Percentage nymph survival
BRX-44-02[2]	5.0a	86.6a	4.9a	83.3a	4.5a	79.6a
CIAT 0606[2]	4.8a	93.3a	4.4a	85.2a	4.4a	69.9a
CIAT 6294[3]	1.9bc	36.7bc	3.1b	52.4b	2.4b	50.0b
CIAT 36062[3]	2.2b	26.7bc	1.7cd	18.5c	2.2b	38.3b
CIAT 36087[4]	2.3b	48.1b	2.0c	18.3c	1.2c	1.7c
Mean 119 SX03 hybrids	1.4c	13.1c	1.2d	2.9d	1.3c	6.5c
SX01NO/0102[3]	1.2c	0c	1.1d	3.3d	1.0c	0c

[1] On a 1 – 5 damage score scale (1, no visible damage; 5, severe damage, plant killed)
[2] Susceptible check
[3] Resistant check
[4] 'Mulato 2'; commercial check.
Means of 5 reps per genotype per insect species. Means within a column followed by the same letter are not significantly different at the 5% level according to Scheffe's multiple range test for arbitrary comparisons. Each species analyzed separately.

Further proof of the rapid progress made in incorporating resistance to spittlebug was obtained when 565 new hybrids (SX05 series) were tested for resistance to three spittlebug species. As shown in Figure 4, 96.2%, 94,7% and 93.9% were rated as resistant to *A. varia*, *A. reducta*, and *Z. carbonaria*, respectively. 468 hybrids (82.8%) were classified as highly resistant to all three species tested. Progress was also detected when resistance reactions in two consecutive cycles were compared (Figure 5) It is valid to conclude that there has been a steady increase in the frequency of resistant genotypes as a result of recurrent selection through cycles.

In support of continuous breeding activities we screened a set of 141 apomictic BR04 hybrids. Most were susceptible but a handful of them showed acceptable levels of antibiosis resistance to all three tests species (Table 12). As in previous occasions, correlations between damage

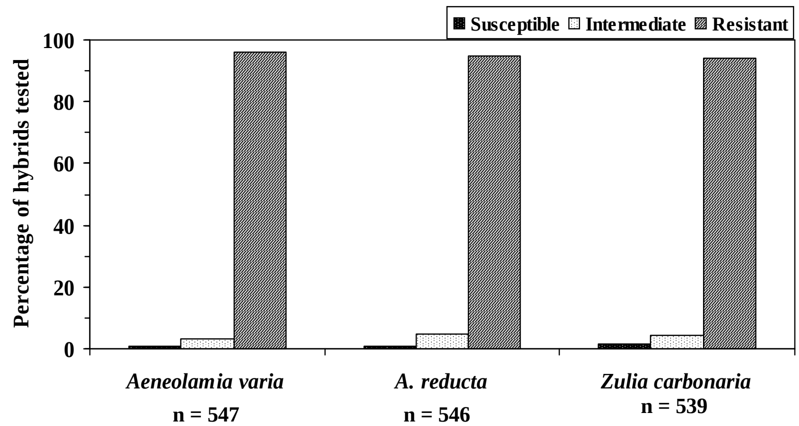

Figure 4. Frequency distribution of resistance reactions in a population of 565 sexual *Brachiaria* hybrids (SX05 series) tested for resistance to three major spittlebug species.

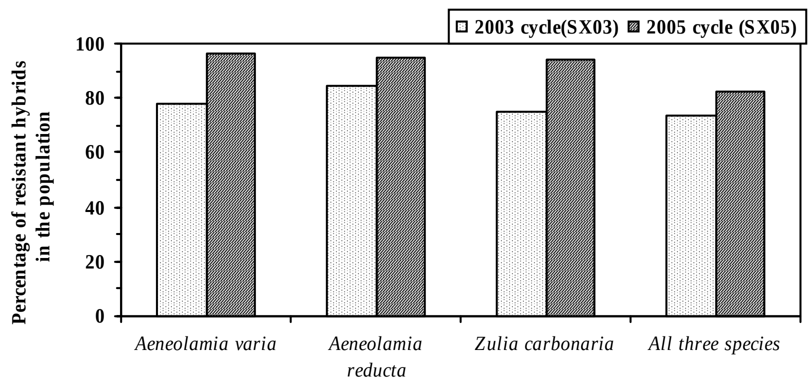

Figure 5. Frequency distribution of resistant reactions in two consecutive cycles of selection in *Brachiaria* for resistance to three major spittlebug species.

Table 12. Percentage nymph survival in selected *Brachiaria* genotypes screened for resistance to three major spittlebug species.

Genotype	Spittlebug species		
	Aeneolamia varia	*Aeneolamia reducta*	*Zulia carbonaria*
BR04NO/1751	6.7	13.3	3.3
BR04NO/1819	13.3	0	0
BR04NO/1889	10.0	-	3.3
BR04NO/2007	26.7	0	13.3
BR04NO/2405	23.3	23.3	16.7
BR04NO/2455	-	0	3.3
BR04NO/2515	33.3	6.7	10.0
BR04NO/2557	33.3	20.0	0
BR04NO/2793	-	0	16.7
BRX-44-02[1]	93.3	91.7	90.0
CIAT 0606[1]	91.7	93.3	81.7
CIAT 6294[2]	58.3	56.7	38.3
CIAT 36062[2]	12.5	13.3	25.0
CIAT 36087[3]	81.7	28.3	20.0
SX01NO/0102[2]	5.0	0	0

[1] Susceptible check
[2] Resistant check
[3] Commercial check
Means of 5 reps per genotype per species.

scores and percentage nymph survival were highly significant: 0.802** for *A. varia*, 0.924** for *A. reducta* and 0.840** for *Z. carbonaria*. In 2004 we reported on varying levels of resistance to *Prosapia simulans* (one of the most important species affecting *Brachiaria* in Mexico) in 34 apomictic hybrids (coded MX).

These hybrids had been pre-selected in Mexico for good adaptation and desirable agronomic characteristics. In 2005 we conducted a series of replicated tests to evaluate the resistance of these genotypes to four major species present in Colombia. Those showing multiple resistances are listed in Table 13.

Table 13. Percentage nymph survival in selected *Brachiaria* apomictic hybrids tested for resistance to five spittlebug species. Means ± SEM of five replications per genotype.

Genotype	Spittlebug species				
	Aeneolamia varia	*Aeneolamia reducta*	*Zulia carbonaria*	*Mahanarva trifissa*	*Prosapia simulans*
MX02NO/1809	41.7 ± 9.7	45.0 ± 9.3	40.0 ± 8.7	0	16.7 ± 1.8
MX02NO/1905	25.0 ± 7.1	23.3 ± 8.3	13.3 ± 6.9	3.3 ± 2.4	3.3 ± 0.7
MX02NO/2273	3.3 ± 3.3	0	1.7 ± 1.7	13.3 ± 5.7	6.2 ± 1.9
MX02NO/2552	30.0 ± 6.5	16.7 ± 7.8	48.3 ± 10.7	0	33.3 ± 2.3
MX02NO/3056	8.3 ± 5.1	1.7 ± 1.7	13.3 ± 6.9	26.7 ± 6.2	1.7 ± 0.5
MX02NO/3213	5.0 ± 2.5	13.3 ± 7.8	43.3 ± 9.4	10.0 ± 7.1	9.2 ± 1.3
BRX-44-02[1]	88.3 ± 2.6	90.4 ± 3.7	68.3 ± 7.2	80.0 ± 5.7	68.3 ± 2.4
CIAT 0606[1]	80.0 ± 9.6	91.7 ± 3.5	75.9 ± 4.6	76.7 ± 4.7	49.9 ± 2.0
CIAT 6294[2]	38.3 ± 8.2	55.0 ± 11.1	57.0 ± 8.8	3.3 ± 2.4	6.7 ± 1.2
CIAT 36062[2]	8.3 ± 3.7	11.1 ± 7.0	30.0 ± 6.2	3.3 ± 2.4	0
SX01NO/0102[2]	5.0 ± 3.5	1.7 ± 1.7	18.3 ± 7.6	0	-
CIAT 36087[3]	63.3 ± 11.0	30.0 ± 7.8	1.7 ± 1.7	30.0 ± 8.7	1.7 ± 0.5

[1] Susceptible check
[2] Resistant check
[3] Commercial check.

2.2.3 Field screening of *Brachiaria* accessions and hybrids for resistance to four spittlebug species

Contributors: C. Cardona, G. Sotelo, and J. W. Miles (CIAT)

Rationale

Assessment of spittlebug resistance under natural levels of infestation in the field is very difficult due to the focal, unpredictable occurrence of the insect. This problem has been overcome since 1998 when we developed a technique that allows us to properly identify resistance under field conditions. Evaluating for resistance under field conditions is important because it allows us to reconfirm levels of resistance identified under greenhouse conditions.

Materials and Methods

Using the experimental unit described in our 1998 Annual Report, the genotypes (usually 10 replicates) are initially infested in the greenhouse with an average of 10 eggs per stem. Once the infestation is well established, with all nymphs feeding on the roots, the units are transferred to the field and transplanted 10-15 days after infestation. The infestation is then allowed to proceed without interference until all nymphs have developed and adults emerge some 30-35 days thereafter. The plants are then scored for damage by means of the 1-5 visual scale utilized in greenhouse screenings. The number of stems per clump is counted before and after infestation and a tiller ratio (tillers per plant at the end of the infestation process/tillers per plant at the beginning of the infestation process) is then calculated. Using this methodology, 20 major screening trials (seven with *A. varia*, six with *Zulia carbonaria,* five with *Z. pubescens*, and two with *Mahanarva trifissa*) were conducted in Caquetá in 2005. The main purpose of these trials was to reconfirm resistance in 36 apomictic hybrids (BR02) and 34 apomictic hybrids (MX) that had been previously evaluated in Palmira under greenhouse conditions.

Results and Discussion

Using tiller ratios (the ratio between tillers per plant at the beginning of the infestation process and tillers per plant at the end of the infestation

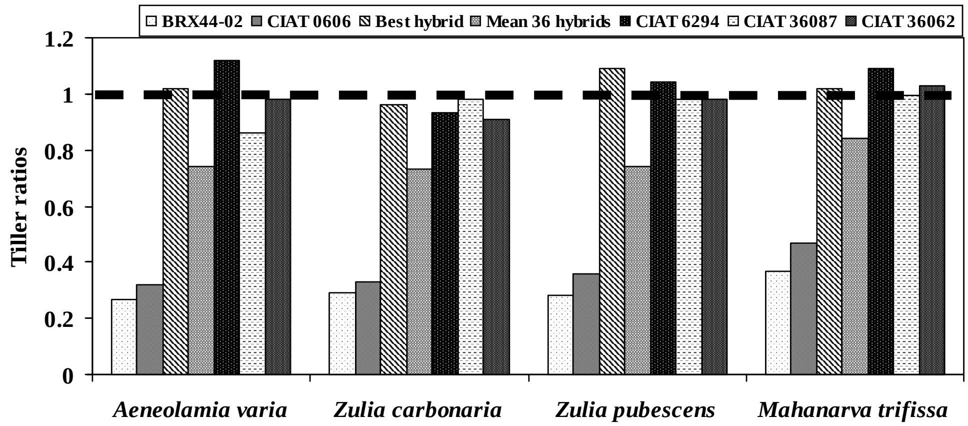

Figure 6. Resistance to four spittlebug species in selected *Brachiaria* apomictic (BR02) hybrids and checks tested under field conditions. The dotted line represents the cut-off point for resistance rating and selection.

process) as the main selection criterion, we found that most of the BR02 hybrids tested were susceptible to spittlebug (Figure 6). A handful, listed in Table 14, showed a more or less acceptable level of field resistance due to the relatively high levels of antibiosis resistance present in these hybrids. The mechanism protected the plants from intense insect damage, allowing the plants to grow and lose less tillers than the susceptible checks. One of the commercial checks (CIAT 36087, 'Mulato 2') was resistant. Better results were obtained when 33 apomictic hybrids coded MX were tested for resistance to *A. varia*, *Z. carbonaria* and *M. trifissa*. Most of the genotypes were

Table 14. Tiller ratios (tillers per plant at the end of the infestation process/tillers per plant at the beginning of the infestation process) in selected *Brachiaria* genotypes tested for resistance to four spittlebug species under field conditions in Caquetá, Colombia.

Genotype	Spittlebug species			
	Aeneolamia varia	*Zulia carbonaria*	*Zulia pubescens*	*Mahanarva trifissa*
Selected hybrids				
BR02NO/1487	0.92	0.95	0.90	0.91
BR02NO/1912	0.73	0.96	0.83	0.98
BR02NO/1245	0.80	0.85	0.79	0.91
BR02NO/0638	1.01	0.65	0.69	1.02
BR02NO/0892	0.96	0.83	0.81	0.87
BR02NO/1747	0.72	0.76	1.01	0.88
Mean selected hybrids	0.86b	0.83c	0.84b	0.93b
Resistant checks				
CIAT 6294	1.12	0.93	1.05	1.09
CIAT 36062	0.98	0.91	0.98	1.03
Mean resistant checks	1.05a	0.92b	1.01a	1.06a
Commercial check				
CIAT 36087	0.86b	0.98a	0.98a	0.99a
Susceptible checks				
CIAT 0606	0.32	0.33	0.36	0.47
BRX44-02	0.26	0.29	0.28	0.36
Mean susceptible checks	0.29c	0.31d	0.32c	0.41c

Means of 10 reps per genotype per species per trial; 4 trials in the case of *A. varia*, 3 trials with *Z. carbonaria* and *M. trifissa*, and 2 trials with *Z. pubescens*. Means within a column followed by the same letter are not significantly different at the 5% level according to Scheffe's multiple range test for arbitrary comparisons. Each species analyzed separately.

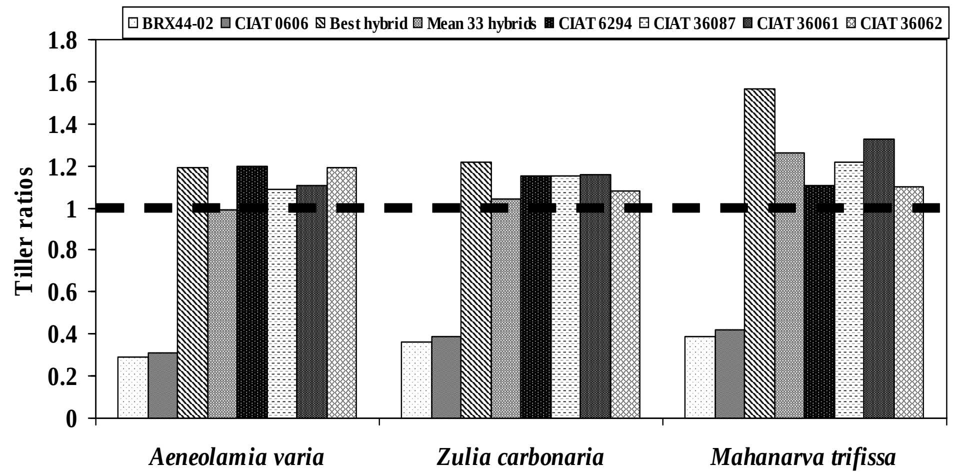

Figure 7. Resistance to four spittlebug species in selected *Brachiaria* apomictic (MX02) hybrids and checks tested under field conditions. The dotted line represents the cut-off point for resistance rating and selection.

classified as resistant both in terms of damage scores (data not shown) and tiller ratios (Figure 7). The mean of selected genotypes did not differ from the mean of the resistant checks. The two commercial checks, 'Mulato' and 'Mulato II' showed a high level of field resistance (Table 15).

Table 15. Tiller ratios (tillers per plant at the end of the infestation process/tiller per plant at the beginning of the infestation process) in selected *Brachiaria* genotypes tested for resistance to three spittlebug species under field conditions in Caquetá, Colombia.

Genotype	Spittlebug species		
	Aeneolamia varia	*Zulia carbonaria*	*Mahanarva trifissa*
Selected hybrids			
MX02/2273	1.19	1.22	1.57
MX02/3861	1.14	1.16	1.35
MX02/3056	1.10	1.07	1.64
MX02/3213	1.14	1.10	1.38
MX02/2531	1.09	1.15	1.32
MX02/1809	1.03	1.14	1.40
MX02/1942	1.12	1.06	1.32
MX02/3567	1.06	1.14	1.20
MX02/1769	1.12	1.07	1.13
MX02/1660	1.09	1.14	1.09
MX02/3426	1.03	1.05	1.39
Mean selected hybrids	1.10a	1.11a	1.34a
Resistant checks			
CIAT 6294	1.20	1.15	1.11
CIAT 36062	1.19	1.08	1.10
Mean resistant checks	1.19a	1.11a	1.10c
Commercial checks			
CIAT 36061	1.11	1.16	1.33
CIAT 36087	1.09	1.15	1.22
Mean commercial checks	1.10a	1.15a	1.27b
Susceptible checks			
CIAT 0606	0.31	0.39	0.42
BRX44-02	0.29	0.36	0.39
Mean susceptible checks	0.30b	0.37b	0.40d

Means of 10 reps per genotype per species per trial; 3 trials with *A. varia* and *Z. carbonaria*, 2 trials with *M. trifissa*. Means within a column followed by the same letter are not significantly different at the 5% level according to Scheffe's multiple range test for arbitrary comparisons. Each species analyzed separately.

2.3 Host mechanisms for spittlebug resistance in *Brachiaria*

Highlights

- Antibiosis resistance in a Brachiaria hybrid had a significant effect on the demography of *Zulia carbonaria*.
- No correlation was found between amino acid content in xylem of Brachiaria hybrids and resistance ratings to spittlebug measured as nymph survival.
- No signs of antibiosis to adults of spittlebug (*A. varia*) has been detected.

2.3.1 Effect of host plant resistance on the demography of *Zulia carbonaria*

Contributors: M. F. Miller, C. Cardona, and G. Sotelo (CIAT)

Rationale

Varying levels of antibiosis resistance to nymphs of several spittlebug species have been well characterized in a number of resistant *Brachiaria* genotypes. The effects of antibiosis on the biology of nymphs have also been studied. Not much was known about possible direct effects of antibiotic genotypes on the biology of adults. Even less was known about sub-lethal effects (i. e., reduced oviposition rates, reduced longevity, prolonged generation times, reduced rates of growth, etc.) on adults resulting from nymphs feeding on antibiotic genotypes. In 2004 we initiated a series of studies aimed at measuring how antibiotic genotypes may directly or indirectly (through sub-lethal effects) affect the biology of adults of *A. varia*.

In 2005, similar studies were conducted with another major species, *Z. carbonaria*. We used the life-table technique, which is widely recognized as one of the most effective means of teasing apart the subtle, interrelated aspects of changes in population density. Longevity, age-specific fecundity, sex ratio and generation time can be examined and compared among treatments as they relate to the most important demographic parameter, the intrinsic rate of natural increase.

Materials and Methods

A comprehensive series of experiments aimed at determining whether antibiosis to nymphs has an adverse effect on the demography of *Z. carbonaria* were conducted. For this, 8 life tables (four fecundity, four complete) were constructed. Treatment combinations are shown in Table 16.

For each of these treatments we established cohorts of 105 pairs of spittlebug and the fate and reproductive rate of individuals were recorded until death occurred. From these data the following life-table statistics were derived: net reproductive rate (R_o) [net contribution per female to the next generation]; mean generation time (T) [mean time span between the birth of individuals of a generation and that of the next generation]; doubling time (D) [time span necessary to double the initial population]; finite rate of population increase (ë) [multiplication factor of the original population at each time period]; and intrinsic rate of natural increase (r_m) [innate capacity of the population to increase in numbers]. Life-table statistics were analyzed using the SAS program based on jackknife estimates of demographic parameters. Other variables recorded were sex ratios, percentage egg fertility and adult dry weights. These data were submitted to analysis of variance and when the *F* test was significant, we performed mean separation by LSD.

Table 16. Treatment combinations to study possible sub-lethal effects of high levels of nymphal antibiosis on adults of *Zulia carbonaria.*

Nymphs reared on:	Resulting adults feeding on:	Null hypothesis
CIAT 0654[a]	CIAT 0654	Absolute check
CIAT 0654	SX01NO/0102	A genotype that is highly antibiotic to nymphs does not affect adults
SX01NO/0102	CIAT 0654	High antibiosis to nymphs does not affect resulting adults
SX01NO/0102	SX01NO/0102	High antibiosis to nymphs does not affect resulting adults even when these are feeding on a highly antibiotic genotype

[a] CIAT 0654 is a highly susceptible accession; SX01NO/0102 (a resistant hybrid) possesses high levels of antibiosis resistance to nymphs of *Z. carbonaria.*

Results and Discussion

A. Sub-lethal effects of resistance on adults of *Zulia carbonaria*: The impact of antibiosis to nymphs on the reproductive biology of resulting adults

The resistant genotype SX01NO/0102 caused significant effects on the demography of *Z. carbonaria*. In general, rearing of nymphs of *Z. carbonaria* on the resistant genotype had a deleterious effect on the weight of resulting males and on the number and fertility of eggs laid per female (Table 17).

Age-specific survival and age-specific fecundity curves for *Z. carbonaria* adults are presented in Figure 8. Mean survival times for the four treatment combinations did not differ at the 5% level, meaning that there was not a major impact of nymphal antibiosis on the survival of resulting males or females. On the contrary, rearing of the insect on the resistant genotype SX01NO/0102 did have a pronounced effect on the ability of resulting females to lay eggs. Independently of the food substrate used to feed the adults, females obtained from rearing the nymphs on the resistant genotype laid fewer eggs, for a slightly shorter period of time, than those obtained from rearing the insect on the susceptible genotype. This can be interpreted as a sub-lethal effect of nymphal antibiosis on the reproductive capacity of the insect.

All demographic parameters of *Z. carbonaria* adults were significantly affected by the antibiotic effect of SX01NO/0102 on the nymphs (Table 18). Females originating from nymphs reared on the resistant genotype had lower net reproductive rates, lower intrinsic rates of natural increase, and lower finite rates of increase than those obtained from rearing the insect on the susceptible genotype. We conclude that antibiosis to nymphs in the resistant *Brachiaria* hybrid SX01NO/0102 causes significant sub-lethal effects on the reproductive biology of resulting adults.

Table 17. Life history parameters of *Zulia carbonaria* as affected by all possible combinations of rearing immature stages and feeding resulting adults on susceptible (CIAT 0654) or resistant (SX01NO/0102) *Brachiaria* genotypes.

Treatment[a]		Adult dry weight (g x 10^{-3})		Eggs per female	Percentage egg fertility
Nymphs reared on:	Resulting adults feeding on:	Females	Males		
CIAT 0654 (S)	CIAT 0654 (S)	1.52a	0.81a	451.4a	97.4a
CIAT 0654 (S)	SX01NO/0102 (R)	1.48b	0.78ab	440.0a	96.8a
SX01NO/0102 (R)	CIAT 0654 (S)	1.47b	0.75bc	353.7b	88.1b
SX01NO/0102 (R)	SX01NO/0102 (R)	1.43c	0.73c	286.9c	85.2c

[a] S, susceptible; R, resistant.
Within a column, means followed by the same letter are not significantly different at the 5% level by LSD.

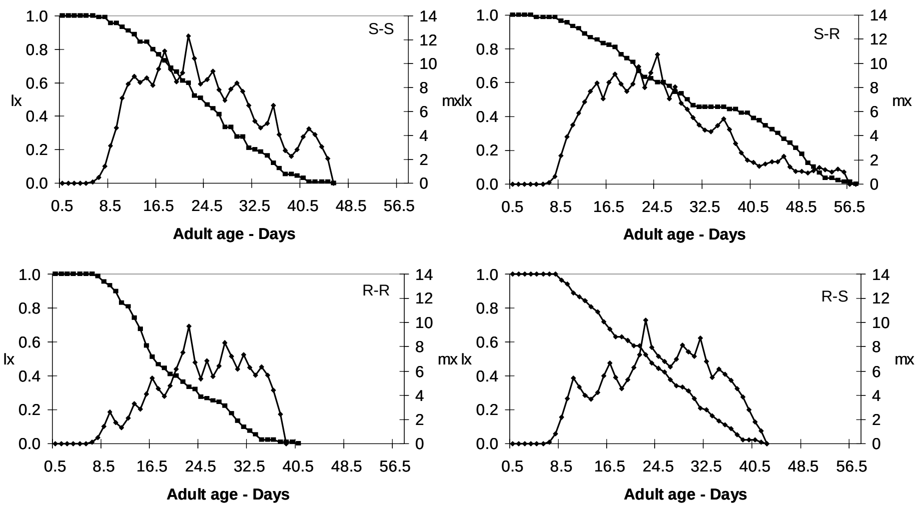

Figure 8. Age-specific survival (l_x) (%) and age-specific fecundity (m_x) (f&) curves for adults of *Zulia carbonaria* as affected by all possible combinations of food substrate for adults and nymphs. First initial in letter combinations indicates the food substrate for nymphs followed by the initial for the food substrate for resulting adults. S, susceptible genotype (CIAT 0654); R, resistant genotype (SX01NO/0102).

Table 18. Fecundity life-table statistics for *Zulia carbonaria* adults as affected by all possible combinations of rearing immature stages and feeding resulting adults on susceptible (CIAT 0654) or resistant (SX01NO/0102) *Brachiaria* genotypes.

Treatment[a]		Demographic parameters		
Nymphs reared on:	Resulting adults feeding on:	Net reproductive rate (R_o)	Intrinsic rate of natural increase (r_m)	Finite rate of increase (λ)
CIAT 0654 (S)	CIAT 0654 (S)	229,8a	0.295a	1.344a
CIAT 0654 (S)	SX01NO/0102 (R)	230.1a	0.267a	1.306b
SX01NO/0102 (R)	CIAT 0654 (S)	184.3b	0.260b	1.297b
SX01NO/0102 (R)	SX01NO/0102 (R)	140.6c	0.248c	1.282c

[a] S, susceptible; R, resistant
Within a column, means followed by the same letter are not significantly different at the 5% level by LSD
Jackknife estimates of the intrinsic rate of increase (per capita rate of population growth).

B. Total effects of resistance on the demography of *Zulia carbonaria*

To measure the total impact of antibiosis resistance on the demography of *Z. carbonaria*, we took into account the rates of immature mortality caused by both the resistant and the susceptible genotypes. Age-specific survival curves for nymphs and adults, as well as age-specific fecundity curves for *Z. carbonaria* adults are presented in Figure 9. The antibiosis to nymphs present in the resistant genotype SX01NO/0102 had a significant deleterious effect on the biology of the insect, which reflected in very high levels of immature mortality. As a result, survival curves were very low as compared to those obtained with the susceptible genotype. Rearing of the insect on the resistant genotype caused a delay in the emergence of adults. Antibiosis also had a significant effect on the ability of resulting females to lay eggs. Independently of the food substrate used to feed the adults, females obtained from rearing the nymphs on the resistant genotype laid less eggs than those obtained from rearing the insect on the susceptible genotype.

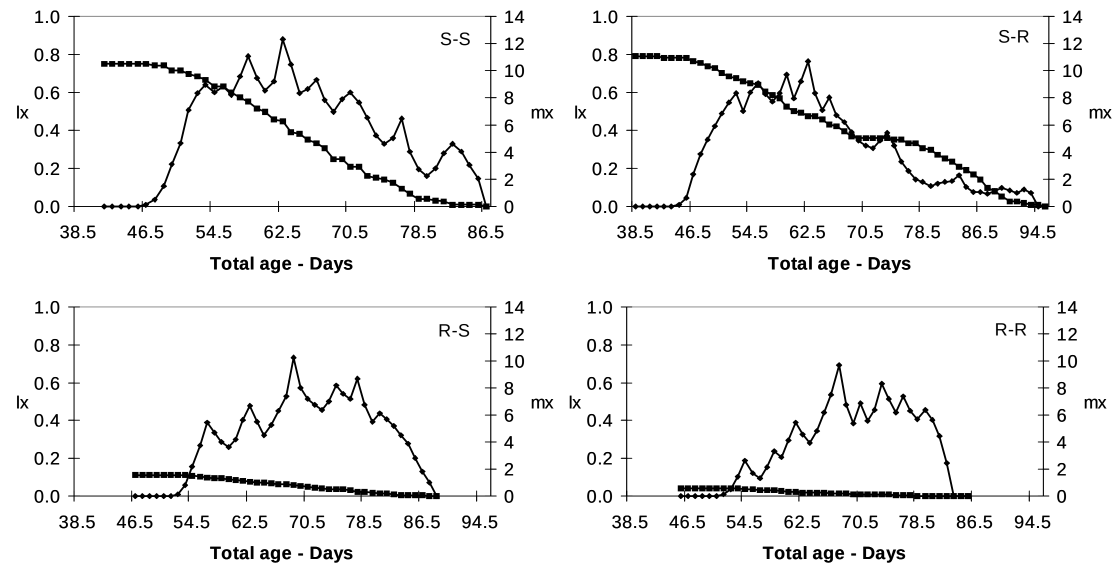

Figure 9. Age-specific survival (l_x) (%) and age-specific fecundity (m_x) (f&) curves for *Zulia carbonaria* as affected by all possible combinations of food substrate for adults and nymphs. First initial in letter combinations indicates the food substrate for nymphs followed by the initial for the food substrate for resulting adults. S, susceptible genotype (CIAT 0654); R, resistant genotype (SX01NO/0102).

As a result of high immature mortality and sub-lethal effects on resulting adults, all demographic statistics of the *Z. carbonaria* population tested were significantly affected by the antibiosis present in SX01NO/0102 (Table 19). Populations derived from the resistant genotype had lower net reproductive rates, lower intrinsic rates of natural increase, lower finite rates of increase and longer generation times than those obtained from rearing the insect on the susceptible genotype. The finite rate of increase is a parameter that describes deleterious effects on a given population. It is defined as a multiplication factor of the original population at each time period. The decimal part of the finite rate of increase corresponds to the daily rate of increase expressed as a percentage. This means that populations reared on the susceptible genotype would grow by 8% whereas those on the resistant genotype would grow by 2.3-4.2% (Table 19). We conclude that high immature mortality caused by the resistant *Brachiaria* hybrid SX01NO/0102 and sub-lethal effects of antibiosis on resulting adults have a very major impact on the demography of *Z. carbonaria*.

Table 19. Life-table statistics for *Zulia carbonaria* as affected by all possible combinations of rearing immature stages and feeding resulting adults on susceptible (CIAT 0654) or resistant (SX01NO/0102) *Brachiaria* genotypes.

Treatment[a]		Demographic parameters				
Nymphs reared on:	Adults feeding on:	Net reproductive rate (R_o)	Intrinsic rate of natural increase (r_m)	Mean generation time (T)	Doubling time (Dt)	Finite rate of increase (λ)
CIAT 0654 (S)	CIAT 0654 (S)	172.3a	0.077a	66.7b	9.0c	1.080a
CIAT 0654 (S)	SX01NO/0102 (R)	181.8a	0.071a	66.5b	8.9c	1.081a
SX01NO/0102 (R)	CIAT 0654 (S)	20.3b	0.041b	73.8a	17.0b	1.042b
SX01NO/0102 (R)	SX01NO/0102 (R)	5.6c	0.023c	74.8a	29.9a	1.023c

[a] S, susceptible; R, resistant

Within a column, means followed by the same letter are not significantly different at the 5% level by LSD Jackknife estimates of the intrinsic rate of increase (per capita rate of population growth).

2.3.2 Studies on possible biochemical factors associated with antibiosis resistance to spittlebug

Contributors: C. Cardona, G. Sotelo, J. Miles (CIAT) and Brent Brodbeck (University of Florida)

Rationale

As stated before, high levels of antibiosis resistance to nymphs of several spittlebug species have been well characterized in numerous resistant *Brachiaria* genotypes. Identification of the biochemical basis of spittlebug resistance, and development of rapid and precise biochemical assays for resistance would provide a valuable addition to breeding efforts to introgress spittlebug resistance into adapted *Brachiaria* germplasm. Scientists at the University of Florida have long proposed that changes in xylem-feeders development may be related to differences in xylem nutrient profiles (i. e. subtle differences in xylem nutrients may result in varying developmental success of the insect). To test this possibility we approached Drs. Brent V. Brodbeck and Peter C. Andersen who kindly accepted our request to analyze xylem samples taken from resistant and susceptible *Brachiaria* genotypes.

Materials and Methods

We used an array of genotypes well characterized for resistance or susceptibility to *A. varia*: 18 sexual hybrids (SX03), two susceptible checks (accessions BRX44-02 and CIAT 0606) and three resistant checks (accessions CIAT 36062, CIAT 6294 and the sexual hybrid SX01NO/0102). Plants were grown in large pots in the greenhouse (24^{o} C, 75% R.H.) and infested with 100 mature eggs each. Infestation was then allowed to proceed without interference. When nymphs reached the fourth instar stage, the plants were cut off at approx. 3 cm from the soil surface. Several stems of approx. 4- to 5-mm diameter were wrapped with tape to increase their effective diameter. The 8-mm interior diameter nozzle of a plastic, disposable syringe was fitted over the entire cut end of the stem wrapped with tape to make a tight connection. Taping externally further sealed the union of the nozzle of the syringe and the cut stem. Suction was applied by withdrawing the syringe plunger, which was held in the withdrawn position until the desired volume of liquid accumulated within the syringe. Xylem samples thus obtained were immediately frozen and shipped to the University of Florida where they were analyzed for contents of 19 different amino acids.

Results and Discussion

There was not a significant correlation between amino acid contents and resistance ratings based on percentage nymph survival. In spite of these disappointing preliminary results, we intend to continue this line of research using a small grant from the USAID-University linkage fund.

2.3.3 Studies on tolerance to adult feeding damage as a component of resistance to spittlebug

Contributors: F. López, C. Cardona, and G. Sotelo (CIAT)

Rationale

Our studies have clearly identified nymphal antibiosis as the main mechanism of resistance to several species of spittlebug in many different *Brachiaria* genotypes. In fact, we have also been able to document rapid progress in the incorporation of antibiosis resistance to nymphs in sexual and apomictic hybrids developed through a recurrent selection-breeding scheme. Given that adults can be as damaging as the nymphs, it is widely accepted that antibiosis to nymphs should be combined with an acceptable level of tolerance to adult feeding damage. However, nothing is known about mechanisms of resistance to adult feeding damage in *Brachiaria*. For this reason, and for the first time, in 2005 we initiated a series of studies aimed at characterizing tolerance as a possible component of resistance to spittlebug.

Materials and Methods

To study tolerance to adult feeding we initially compared the response of the susceptible accession CIAT 0654 and the resistant hybrid SX01NO/0102 to increasing levels of infestation with adults of *A. varia*. Thirty-day old plants of CIAT 0654 and SX01NO/0102 were exposed to 0, 2, 3, 5, 7, 9, 12, and 15 adults per plant. The 16 host genotype-infestation level treatment combinations were randomly assigned to single-plant experimental units with 10 replications per treatment combination. Plants were infested with neonate adults and the infestation was allowed to proceed until all adults died. Percentage adult survival was calculated. Damage scores in a 1-5 visual damage score scale were taken 5 and 10 days after infestation. To measure chlorophyll loss as a result of adult feeding, we used a SPAD-502 chlorophyll meter 5 and 10 days after infestation. Four representative readings per plant were taken and their averages were recorded. SPAD index values were then calculated with respect to the uninfested checks. At the end of the trial, when all insects had died, plants were cut at soil level and dried in an oven at 40^{o} C. Percentage biomass losses were calculated with respect to the uninfested checks. Damage scores and percentage biomass losses were used to calculate functional plan loss indices.

Results and Discussion

Adult survival was not affected by the genotype when plants were infested with 2, 3 or 5 adults per plant. At higher infestation levels (7, 9, 12, and 15 adults per plant) adult survival on the susceptible genotype was significantly lower possibly due to depletion of food and increased competition among insects. This means that a 5-6 level of infestation could be used in future studies. The resistant sexual hybrid SX01NO/0102 plants suffered significantly less damage than susceptible (CIAT 0654) plants at all levels of infestation (Figure 10). In addition, at all levels of infestation, SX01NO/0102 plants suffered significantly less damage (expressed as percentage chlorophyll loss and percentage biomass loss) than susceptible CIAT 0654 plants (Figure 11).

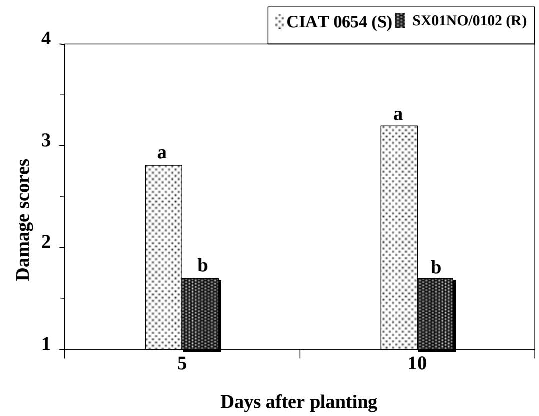

Figure 10. Response of susceptible (CIAT 0654) or resistant (SX01NO/0102) *Brachiaria* genotypes to attack by adults of *Aeneolamia varia*. Means of eight levels of infestation. For each scoring date, bars with the same letter do not differ at the 5% level of significance by LSD.

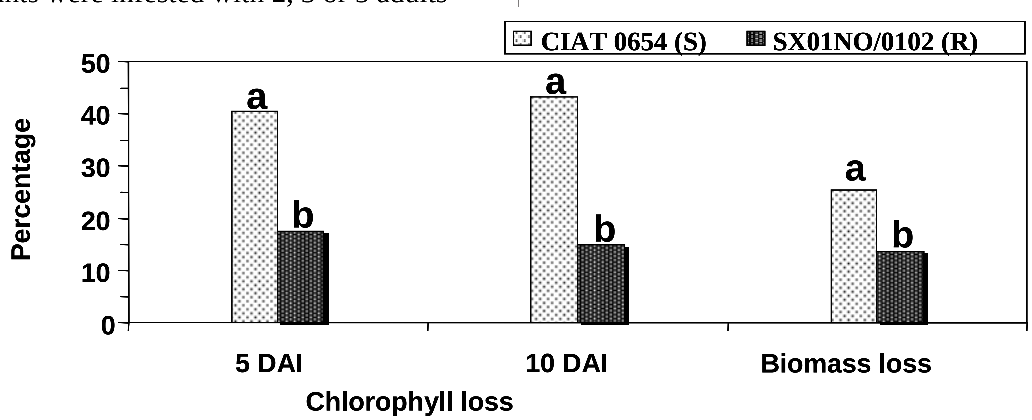

Figure 11. Chlorophyll and biomass losses due to adult *Aeneolamia varia* feeding on susceptible (CIAT 0654) or resistant SX01NO/0102 *Brachiaria* genotypes. DAI, days after infestation. Means of eight levels of infestation. For each variable, bars with the same letter do not differ at the 5% level of significance by LSD.

Significant correlations were found between damage scores and percentage chlorophyll losses ($r = 0.858$; $P<0.001$), between damage scores and percentage biomass losses (0.473; $P<0.001$) and between percentage chlorophyll losses and percentage biomass losses ($r = 0.891$; $P<0.001$) indicating that damage scores are useful in predicting losses and that SPAD units are useful in measuring insect damage. Furthermore, when a Functional Plant Loss Index (combining damage scores and percentage biomass losses) was calculated, we found that at all levels of infestation losses were highest for the susceptible genotype CIAT 0654 (Figure 12). Since no obvious signs of antibiosis to adults were found in this experiment, we interpret lower damage scores, lower chlorophyll and biomass losses, and lower functional plant losses as the expression of tolerance to adult feeding damage in the resistant genotype. Further results on this line of research, aimed at developing a mass screening procedure for adult spittlebug damage, will be reported in 2006.

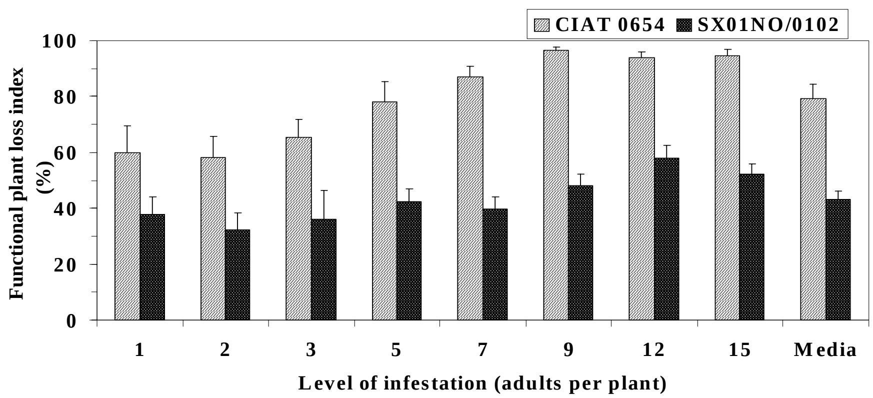

Figure 12. Functional plant loss indices (percentage) for susceptible (CIAT 0654) or resistant (SX01NO/0102) *Brachiaria* genotypes exposed to eight levels of infestation with adults of *Aeneolamia varia*.

2.4 Define interactions between host and pathogen in *Brachiaria*

Highlight

- The resistant accession (*B. brizantha* 16320) and four *Brachiaria* hybrids showed high levels of resistance to Rhizoctonia foliar blight under field conditions.

2.4.1 Evaluation of *Brachiaria* hybrids for resistance to *Rhizoctonia solani* under field conditions in Caqueta

Contributors: G. Segura, W., X. Bonilla, J. Miles, and S. Kelemu (CIAT)

Rationale

Rhizoctonia foliar blight, caused by *Rhizoctonia solani* Kühn, is a disease of increasing importance on a number of crops. The disease can be very destructive when environmental conditions are particularly conducive (high relative humidity, dense foliar growth, high nitrogen fertilization, and extended wet periods).

Rhizoctonia solani is the most widely known species of *Rhizoctonia* with a wide host range. In nature *R. solani* reproduces mainly asexually and exists as vegetative mycelia and/or dense sclerotia. These sclerotia can survive in soil and on plant debris for several years, and can germinate and produce hyphae that can infect a wide range of host plants. The pathogen primarily infects below ground plant parts in a number of plant species, but can also infect above ground plant parts such as pods, fruits, and leaves and stems as is the case with *Brachiaria*. In *Brachiaria*, infected leaves first appear water-soaked, then darken, and finally turn to a light brown color. Lesions may coalesce quickly during periods of prolonged leaf wetness and temperatures between 21 and 32°C.

Disease management through the use of host resistance, when available, remains to be the most practical and environmentally friendly strategy. Differences in reaction to *R. solani* exist in genotypes of *Brachiaria.* The ability to uniformly induce disease and measure resistance accurately is crucial in a breeding program for developing resistant cultivars. The objectives of this study were to: (1) artificially inoculate and induce uniform disease development in selected *Brachiaria* genotypes generated by CIAT's tropical forages project, and to (2) accurately measure resistance and identify resistant materials among these *Brachiaria* genotypes.

Materials and Methods

Plant materials: 137 *Brachiaria* genotypes with BR04 series and provided by the breeding program were planted in the field at Macagual ICA/CORPOICA Research Station in Florencia, Caquetá. CIAT 16320, CIAT 36061 and CIAT 36087 were included as controls. The field location is highly conducive to the development of the disease, with mean annual relative humidity of 84 %, an average temperature of 25.5°C and an annual rainfall of 3793 mm.

Field layout, artificial inoculations and disease evaluations: Six plants (that were generated from the same mother plant) of each of the *Brachiaria* genotypes were transplanted from a CIAT glasshouse to the field site in Caquetá. The space between plants was 80 cm, and 1 m between blocks. The entries were replicated 3 times in a randomized complete block design. Plants were inoculated one month after transplanting by placing 0.7 g dry sclerotia of *R. solani* isolate 36061 on the soil surface at the base of each plant. Plants were evaluated for disease reaction 15, 20, 34 and 38 days after inoculations, using the 0 – 5 (0 = no visible infection; 5 = 20 -100% of the aerial portion of the plant infected) scale that we developed earlier and reported in the 2004 Annual Report.

Results and Discussion

Disease symptoms developed fully in susceptible genotypes 10-15 days after inoculations. Plants were evaluated for disease reaction 15, 20, 34 and 38 days after inoculations. There was a high degree of correlation in disease evaluation data among the various evaluation dates (Table 20).

The resistant control CIAT 16320 was consistently evaluated at scale 2. Four genotypes, BR04-2577, BR04-2557, BR04-2983, and BR04-1214 were evaluated at an average between 2.0 and 2.5. Twenty-four others, 1685, 1950, 1963, 3077, 1119, 1252, 1347, 1349, 1824, 1886, 1896, 2060, 2200, 2201, 2265, 3025, 3207, CIAT 36087, 1928, 1941, 2040, 2069, 3066, 3217, scored with an average rating scale of 3.0-3.3 (in the rating scale, this corresponds to a 6% - 9% overall plant tissue damage). The remaining 111 materials, 2429, 2518, 2539, 3051, 3175, 1061, 1219, 1796, 1845, 2774, 2793, 2874, 3214, 1021, 1073, 1141, 1819, 2404, 2405, 2457, 2475, 2515, 2532, 2841, 2940, 3056, 222, 1265, 1311, 1358, 1377, 1592, 1648, 1956, 2110, 2118, 2128, 2179, 2208, 2275, 2403, 2938, 2987, 3069, 3119, 3221, 36061, 1081, 1097, 1113, 1197, 1281, 1296, 1503, 1633, 1697, 2007, 2226, 2235, 2389, 2414, 2969, 1018, 1026, 1060, 1273, 1309, 1360, 1374, 1570, 1629, 1883, 1889, 2093, 2163, 2290, 2338, 2346, 2670, 2681, 2792, 2833, 2849, 2872, 2954, 3068, 3128, 1003, 1058, 1494, 1754, 2109, 2285, 2344, 1428, 1596, 1601, 1751, 1846, 1900, 2156, 2166, 2455, 2863, 2871, 3058, 3134, 2360, 2396, 1552, 3130, scored between 3.5- 5.0.

Table 20. Correlations between disease reaction data collected at various days after inoculations using Pearson's Correlation.

Days	**15**	**20**	**34**	**38**
15	1.00	0.82	0.69	0.60
20	0.82	1.00	0.85	0.78
34	0.69	0.85	1.00	0.94
38	0.60	0.78	0.94	1.00

Figure 13 shows a graphical representation of the results using data from representative genotypes from each of these three groups.

The disease evaluation data taken 38 days after inoculations represented well-developed disease symptoms. The resistant control CIAT 16320 and the four *Brachiaria* hybrids BR04- 2577, BR04-2557, BR04-2983, BR04-1214 showed less than 6% overall plant tissue damage, and thus, a high-level of resistance (Photo 1). The second group of 24 genotypes including CIAT 36087 listed above still had an acceptable level of resistance. All the plants in this trial will be maintained in the field to further observe the level of disease at an extended period of time.

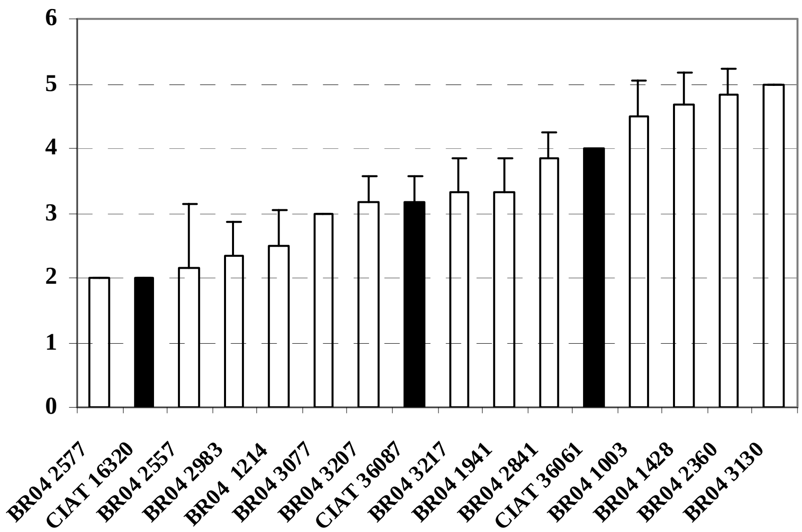

Figure 13. Ratings of *Brachiaria* genotypes for foliar blight disease reaction on a 1-5 scale 38 days after inoculations with sclerotia of *Rhizoctonia solani* under field conditions, Caquetá, Colombia. Bars indicate standard deviation.

Photo 1. Rhizoctonia foliar blight disease symptoms 34 days after inoculations under field conditions in Caquetá, Colombia. **A**: BR04-1214; **B**: BR04-2577: **C**: CIAT 36061; **D**: BR04-1754

2.5 Elucidate the role of endophytes in tropical grasses

Highlights

- The fungal endophyte *Acremonium implicatum* forms strong association with *Brachiaria* genotypes, demonstrated by its re-establishment within plants after elimination using systemic fungicides.

- An efficient transformation system was developed for *Acremonium implicatum* for the first time.

2.5.1 Drought tolerance in endophyte-infected plants under field conditions

Contributors: S. Kelemu, G. Segura, C. Plazas, J. Ricaurte, R. García and I. M. Rao (CIAT)

Rationale

Earlier experiments with potted plants in the greenhouse demonstrated that endophyte-infected plants under severe drought stress conditions could maintain better leaf expansion and produce significantly greater leaf biomass (IP-5 Annual Report, 1999, 2000). In order to validate the greenhouse results, in 2002 we initiated a field study in the Llanos of Colombia to quantify the impact of endophytes in improving drought tolerance and persistence in *Brachiaria*. In 2003, we reported preliminary results from this field trial that indicated that endophyte infection could improve dry season performance by improving the uptake of nutrients by two accessions of *Brachiaria brizantha*. In 2004, we conducted further field evaluations to confirm the role of endophytes in improving dry season tolerance of *Brachiaria* grasses. This on-going field study indicated beneficial effects of endophyte infection on drought tolerance in the first year (2003) and almost no effect in the second year (2004). We speculated that this might be due to the re-growth of the endophyte in the endophyte-free plants. There is evidence for this possibility in greenhouse grown plants. This year, we have tested the persistence of the endophytes in these plants in order to see if the lack of effects was due to the re-appearance of the endophytes in the endophyte-free plants.

Materials and Methods

Field set up: A field trial consisting of 2 accessions of *Brachiaria brizantha* (CIAT 6780 and CIAT 26110) was established at Matazul farm in Colombia in May, 2002. Genetically identical endopyte-free and endophyte-infected plants were generated from an original mother plant containing the endophyte *Acremonium implicatum* (J. Gilman and E. V. Abbott) W. Gams, using methods described in Kelemu et al. 2001. Canadian Journal of Microbiology 47:55-62.The trial was established as a randomized block in split-plot arrangement with the presence or absence of endophytes as main plots and two accessions as subplots with 3 replications. Each plot included 3 rows with 8 plants per row (24 plants/plot). The plot size was 5 x 1.5 m. The trial was established with low levels of initial fertilizer application (kg/ha: 20 P, 20 K, 33 Ca, 14 Mg, 10 S) that are recommended for establishment of grass alone pastures. A number of plant attributes including forage yield, green leaf production, dry matter distribution and green forage nutrient composition, leaf and stem total nonstructural carbohydrate (TNC) content, leaf and stem ash (mineral) content, and shoot nutrient uptake were measured at the end of wet season (November 2003) and dry season (March 2004).

DNA isolation: Leaf blades were collected from *Brachiaria* hybrids and known endophyte-infected or endophyte-free plants and macerated separately in liquid nitrogen for genomic DNA isolation. DNA was extracted using an improved CTAB (Hexadecyltrimethylammonium bromide) method. Extraction buffer [2% CTAB, 100mM Tris-HCl (pH8.0), 20mM EDTA (pH8.0), 1.4mM

NaCl and 1% PVP40) and 1/50 volume of Rnase A (10 mg / ml)] was added to macerated plant tissue, and incubated at 65^{o}C for 30 min. An equal volume of Chloroform: Isoamylalcohol (24:1) was added and mixed well by vortexing. The mixture was then centrifuged at 13,200 rpm for 10 min. The supernatant was transferred to a new tube. About 0.8 ~ 1 volume of ice-cold isopropanol was added to the supernatant and kept at room temperature for 15 minutes to precipitate the DNA. DNA pellet was generated after centrifugation at 13,200 rpm for 20 min. The pellet was washed with 70 % ethanol and re-suspended in 50-ìl Tris-EDTA (TE) buffer (10 mM Tris-HCl (pH7.5), 1 mM EDTA (pH8.0)).

Polymerase chain reaction (PCR) analysis: PCR was carried out using the *A. implicatum*-specific primer pairs,
P1 (5-TTCGAATGATAAGGCAGATC-3')
and P4 (5'-ACGCATCCACTGTATGCTAC-3'). The PCR reaction volume was 20-ìl, and composition was as follow: 1x PCR buffer (QIAGEN); 3mM $MgCl_2$; 0.26mM each deoxynucleotide triphosphate (dNTPs); 1.25-ìM each olygonucleotide primer; 1 units Taq DNA polymerase (Invitrogen) and 30ng template DNA. Amplification cycles were programmed in a Programmable Thermal Controller (MJ Research, Inc.) as follows: step 1, 94°C 3min; step 2, 94°C 30 sec; step 3, 53°C 40 seconds; step 4, 72°C for 45 seconds; step 5, go to step 2 for 35 cycles; then 72°C 4 min. The amplification products were separated by electrophoresis in a 1.2% agarose gel (Invitrogen), stained with ethidium bromide and photographed under UV lighting.

Results and Discussion

The presence of *A. implicatum* in *Brachiaria* tissues was determined by the presence of a diagnostic 500-bp amplification product (Figure 14).

In the Annual Report 2004, we reported that at 18 months after establishment, i.e., at the end of rainy season, the endophyte-infected plants (E+) showed greater values of stem biomass in both

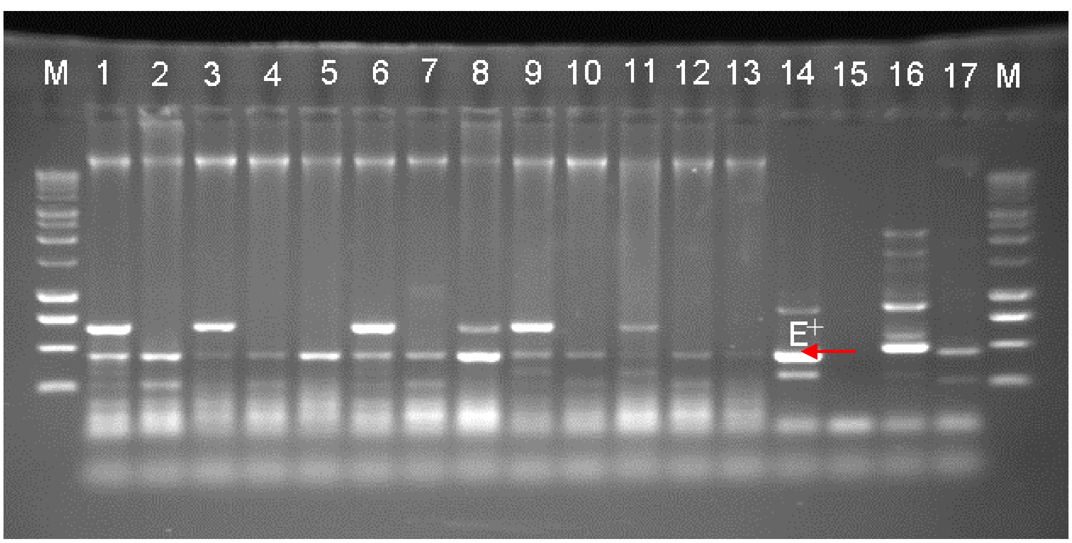

Figure 14. Specific detection of *Acremonium implicatum* in gentypes of *Brachiaria*.
Genomic DNA isolated from tissues of *Brachiaria* plants established in a field trial consisting of 2 accessions of *Brachiaria brizantha* (CIAT 6780 and CIAT 26110) at Matazul farm in Colombia, May, 2002. Lanes 1, 6, 11, *Brachiaria brizantha* CIAT 6780 initially endophyte-free through the use of a systemic fungicide; lanes 3,8,9, *Brachiaria brizantha* CIAT 6780 with endophyte; lanes 4,7,10, *Brachiaria brizantha* CIAT 26110 initially endophyte-free through the use of a systemic fungicide; lanes 2,5, 12, *Brachiaria brizantha* CIAT 26110 with endophyte; lane 13, cv. Mulato; lanes 14, 15, 16, 17, positive control *A. implicatum* DNA, negative control water, positive control from endopyte-positive plant from greenhouse, positive control from the field, respectively. Lane M = size marker.

accessions of *Brachiaria brizantha* (CIAT 6780 and CIAT 26110). In contrast to the results at the end of rainy season, at 22 months after establishment, i.e., at the end of dry season, the endophyte infected plants showed no significant increase in either green leaf or stem biomass in both accessions of *Brachiaria brizantha* (CIAT 6780 and CIAT 26110).

This observation is not consistent with the results from the greenhouse study where the benefits of endophyte infection were noted under severe drought stress. Results on nutrient uptake at the end of dry season also showed that the uptake of N, P and K was not significantly different between the endophyte-infected plants and the endophyte free plants. This on-going field study indicated beneficial effects of endophyte infection on drought tolerance in the first year, but almost no effect in the second year. We speculated that this might be due to the re-growth of the endophyte in the endophyte free plants. There is evidence for this possibility in greenhouse grown plants.

The results of the PCR test indeed demonstrate that the initially endophyte-free plants tested positive for the diagnostic 500-bp amplified product indicating the re-growth of *A. implicatum* after fungicide treatment intended to eliminate the endophyte. This indicates that although the endophyte was successfully removed to the level that we were able to see differences demonstrating the effect of the endophyte on disease and drought resistance.

However, it is evident now that, with time, the endophyte level in the fungicide-treated plants was high enough to eliminate those differences. Work is in progress to use a biocidal product PPM (plant preservative mixture) trying to completely eliminate the endophyte from selected *Brachiaria* genotypes in order to study the long term effect of endophytes on plant development and important agronomic traits.

2.5.2 Endophyte transformation and use as gene delivery system

Contributors: J. Abello and S. Kelemu (CIAT)

Rationale

Acremonium implicatum is an endophytic fungus that forms symbiotic association with species of *Brachiaria*. The green fluorescent protein (GFP) gene, isolated from the jellyfish *Aequorea Victoria*, or its derivatives have been expressed in a wide array of organisms including plants and microbes. The practical implication of seed transmission of endophytes in *Brachiaria* is significant. Once associated with the plant, the fungus can perpetuate itself through seed, especially in apomictic genotypes of *Brachiaria*, for as long as seed storage conditions do not diminish the survival of the fungus.

Several *Brachiaria* hybrids obtained from CIAT's forage breeding program were shown to harbor *A. implicatum*. Therefore, we may be able to exploit this association and its high seed transmission [Dongyi, H. and Kelemu, S. 2004. *Acremonium implicatum*, a seed-transmitted endophytic fungus in *Brachiaria* grasses. *Plant Disease* 88:1252-1254) by using a transgenic *A. implicatum* as a vehicle for production and delivery of gene products of agronomic interest into the host plant to enhance protective benefits and other traits, and thus improve livestock production. In addition, we want to exploit the qualities of GFP as a reporter and study the interactions between *A. implicatum* and its host *Brachiaria*.

This work describes the establishment of a transformation protocol and expression of the green fluorescent protein (GFP) gene in an isolate of *Acremonium implicatum*. In this study, we used a GFP expression vector, pSK1019, to transform *A. implicatum*.

Materials and Methods

Plasmid: Plasmid pSK1019 kindly provided by Dr. Seogchan Kang of the Department of Plant Pathology, University of Pennsylvania, was used. The plasmid contains the *egfp* gene under the promoter of a gene encoding glyceraldehyde-3-phosphate dehydrogenase (GPD) isolated from *Cochliobolus heterostrophus*. It also contains a hygromycin B resistance gene hph, controlled by the *Aspergillus nidulans* trpC promoter, as well as the Kan gene for kanamycin resistance. Hygromycin B, is an aminoglycosidic antibiotic produced by *Streptomyces hygroscopicus*, and is used for the selection and maintenance of prokaryotic and eukaryotic cells transformed with the hph gene. Vector pCAMBIA 1300 that has CaMV 35S promoter, Kan gene and hph gene was used as control.

Preparation of *A. implicatum* cells: *A. implicatum* isolate 6780-201v isolated from *Brachiaria brizantha* CIAT 6780 was used for transformation of its conidia or mycelia. The fungus was grown on YMG agar (D- glucose 4,0g; malt extract 10,0g; yeast extract 4,0g; agar 10,0g; 1L distilled water) medium for 8 days and incubated at 28ºC. Conidia were collected in a solution of 0.15M NaCl and cleaned by passing through a Whatman #1 filter paper. The conidia were then suspended in YMG liquid medium and incubated with shaking (250 rpm) for 4 hours at 28ºC, in order to induce conidial germination. Subsequently, the conidia were collected by filtration and re-suspended in an induction medium IM+AS, (in 1 litre of distilled water: 2.05g K_2HPO_4; 1.45g KH_2PO_4; 0.15g NaCl; 0.5g $Mg_2SO_4 .7H_2O$; 0.07g $CaCl_2 .2H_2O$; 0.0025g $Fe_2SO4.7H_20$; 0.5g $(NH_4)_2SO_4$; 10 mM D-glucose; 0.5%glycerol; 40mM MES [2-n-morppholino ethanesulfonic acid]; 200µM acetosyringone at a concentration of $1x10^6$ conidia/ml. To obtain mycelia for transformation, the protocol describe above was used, but the incubation was extended to 48 hours instead of only 4 hours, and the concentration was adjusted to OD_{600}= 0.35 in IM+AS.

Transformation of *A. implicatum*: The transformation protocol was based on the methods described by Mullins *et al.* (2001, Phytopathology 91:173-180) for transformation of the pathogenic fungus *Fusarium oxysporum*. Some modifications were introduced. *A. tumefaciens* strains AGL1 and LBA4404 were transformed with vectors pSK1019 or pCAMBIA 1300 using methods described by Den Dulk-Ras and Hooykaas (1995, Methods Mol Biol. 55: 63- 72.). The transformed bacteria were grown in TYNG medium (for 1L medium: 10.0 g tryptone, 5.0g NaCl, 5.0 g yeast extract, 0.5g $MgSO_4 \bullet 7H_2O$, pH 7.5) supplemented with kanamycin [100 µg/ml] and incubated at 28ºC in the dark for 16 hours to optical density (OD_{600}) of 0.75. This bacterial cell concentration was subsequently diluted with the induction medium IM+AS to OD_{600}= 0.1 and further incubated for 4 hours to induce virulence genes. Once the incubations was completed, the bacterial cell concentration was adjusted to OD_{600}= 0.2. The *A. implicatum* preparations described above and these *A. tumefaciens* transformant cells were mixed together in equal volumes. Two hundred-µl of each mixture was placed on a 0.45-µm-pore-size, 45-mm diameter nitrocellulose membrane (Whatman, Hillsboro, OR), and plated on IM+AS agar medium (glucose content reduced to 5mM). These mixtures were incubated for 48, 60 and 72 hours. The membranes were subsequently transferred to Petri plates containing YMG agar media containing hygromycin B (100 µg/ml), and cefotaxime (500 µM), and incubated further at 28 ºC. Putative transformant *A. implicatum* cells became apparent on the selection media after 5 days of incubation. Control *A. implicatum* cells were treated the same way except that they were co-cultivated with strains of *A. tumefaciens* that were not transformed with the plasmid vectors.

PCR amplifications: DNA isolated (Kelemu et al. 2003. Molecular Plant Pathology 4:115-118) from putative transformant bacteria and fungus as well as control ones was analyzed using the polymerase chain reaction (PCR) using primers with sequences of *egfp* and/or *hph*. [primers glGFP3 (5‘-GCCGAGCTCAGATCTC ACTTGTACAGCT CGT-3‘) and glGFP5 (5‘-CC

GGAATTCATGAACAAGGG CGAGGAACTG-3‘)] (Fitzgerald *et al.* 2003) and hph122U (5‘-TC GATGTAGGAGGGCGTGGAT-3‘) and hph725L (5‘-CGCGTCTGCTGCTCCATACAAG-3‘) (Irie et al. 2001)]. Amplifications were carried out in a Programable Thermal Controller (MJ Research, Inc) programmed to 35 cycles comprised of 45 seconds denaturation step at 94^{o}C (4 minutes for the first cycle), followed by 1 min at 60^{o}C, and primer extension for 1.5 minute (10 minutes in the final cycle) at 72^{o}C. The amplification products were separated by electrophoresis in a 1.0% agarose gel (Bio-Rad Laboratories), stained with ethidium bromide, and photographed under UV lighting.

Southern blot analysis: The DNA of 19 randomly selected putative *A. implicatum* transformants were analyzed using Southern blot analysis. The hygromycin B resistance gene hph was used as a probe. Southern hybridization was carried out using standard procedures described in Sambrook, *et al.* (1989, Cold spring Harbour Laboratory Press, Cold Spring Harbour, NY). Labeling and detection were carried out using Dig-high prime DNA labeling and detection Kit II (Roche Applied Science)

Microscope examination: The putative GFP-expressing transformants were examined under a LEICA fluorescence microscope fitted with a Leica D filter with an excitation range between 355 and 425 nm, and an H3 filter with an excitation range between 420 and 490 nm.

Plant inoculations: *Brachiaria* seedlings were inoculated with a few selected *A. implicatum* transformants using the method described earlier (Kelemu et al. 2001, Canadian Journal of Microbiology 47:55-62).

Results and Discussion

The endophytic fungus *A. implicatum* was successfully transformed with e*gfp* (enhanced green fluorescent protein) gene. Enhanced color variants [ECFP (cyan), EGFP (green), EYFP (yellow)] have been generated through mutagenesis and these are some of the most widely used reporters in biological research. They can be used as tags to track proteins in living cells, as reporters to monitor promoter activity, and as labels to visualize specific tissues, whole cells or sub-cellular organelles. They are useful for monitoring gene expression and protein localization.

The GFP protein (27 kDa) is a spontaneously fluorescent protein that absorbs light at maxima of 395 and 475 nm and emits at a maximum of 508 nm. This protein is a success as a reporter because it requires only UV or blue light and oxygen, but requires no cofactors or substrates as many other reporters do for visualization.

In 2004, we reported the successful transformation of *A. implicatum* with GFP gene in vectors pWGFP20 and pCT74, although the green fluorescence emitting appeared to be weak, and thus the need for more work to be done in order to get tranformants with a more pronounced emission. We report this year on work of a successful transformation of the fungus with intense emissions.

The protocol that we developed for the transformation of this endophytic fungus is based on the protocol described by Mullins *et al.* (2001, Phytopathology 91:173-180)) for the pathogenic fungus *Fusarium oxysporum*. However, modifications were needed for a successful transformation of *A. implicatum*. For example, *A. implicatum* is a slow growing fungus, and thus the recommended concentration of cefotaxime (200 µM) to inhibit the growth of *A. tumefaciens*, was not sufficient enough to prevent bacterial growth from impeding the growth of *A. implicatum*.

Results from the experiments we conducted indicated that cefotaxime concentrations at 500 µM was sufficient to inhibit the growth of *A. tumefaciens* while allowing *A. implicatum* putative transformants to grow on selection media. Introducing TYNG medium instead of MM [for 1L medium: 2.05g K_2HPO_4, 1.45g KH_2PO_4, 0.15g NaCl, 0.5g $MgSO_4$ $7H_2O$, 0.07g $CaCl_2 \bullet 2H_2O$, 0.0025g FeSO4 • $7H_20$, 0.5g $(NH_4)_2SO_4$] has reduced the time needed to reach the required bacterial concentration (OD_{600} = 0.75) from 48 hours to only 16. In addition, the TYNG medium eliminated the cell aggregation problem we encountered with the growth of *A. tumefaciens* (particularly with strain LBA4404) in MM and that interfered with the transformation process.

A better transformation efficiency was obtained with *A. tumefaciens* strain AGL-1 (Table 21; Figure 15). Although *A. implicatum* transformants containing either pSK1019 (*trpC* promoter) or pCAMBIA 1300 (CaMV35S promoter) were obtained, a significantly higher number of transformants were obtained with pSK1019 (Table 21). However, this suggests that the CaMV35S promoter can function in *A. implicatum* although at a much lower efficiency. The colony size of transformants in both cases is similar with an average size of 19-mm after 12 days of incubation at 28^{O}C on the selection medium.

The results indicate that the transformation efficiency is directly influenced by the length of the co-cultivation (*A. tumefaciens* and *A. implicatum*) period (Table 21, Figure 15). As the co-cultivation period increased from 48 h to 72 h, the efficiency increased from 542 transformant colonies to 1084 in the case of mycelial transformation protocol; and from 271 to 542 for conidial transformation (Table 21, Figure 15). Similar results have been reported for transformation of *Magnaporte grisea* (Rho *et al.* 2001. Mol. Cells 3:407-411) and *F. oxysporum* (Mullins *et al.* 2001). The efficiency of transformation also differed depending on whether we used mycelia or conidia for transformation (Figure 15). The best and optimum transformation results were obtained with *A. tumefaciens* strain AGL-1, plasmid pSK1019 under the control of *trp*C promoter either with mycelial or conidial transformation. However, mycelial transformation consistently generated significantly higher number of transformants than when conidia were used to transform (Figure 15).

A. tumefaciens-mediated transformation has long been applied to transfer foreign genes to a wide-range of plants. In recent years, this has also been used to transform a wide range of fungi allowing efficient genetic manipulations of the recipient organisms. The presence of acetosyringone is important for successful *A. tumefaciens*-mediated transformation.

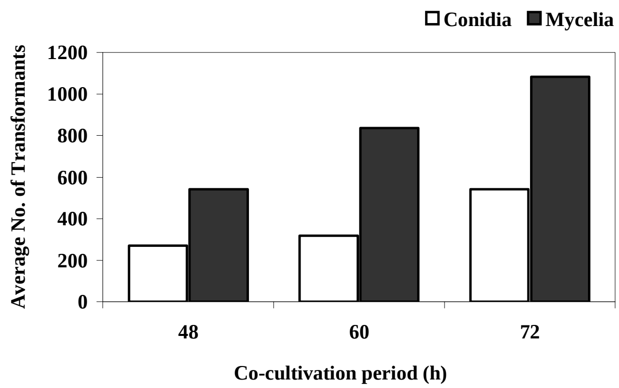

Figure 15. The effect of *Agrobacterium tumefaciens* strain AGL-1 and *Acremonium implicatum* co-cultivation period on transformation efficiency. The *A. tumefaciens* strain contains plasmid pSK1019 that has enhanced green fluorescent protein *(egfp)* gene under the promoter of the gene encoding glyceraldehyde-3-phosphate dehydrogenase (GPD) isolated from the fungus *Cochliobolus heterostrophus*. It also contains a hygromycin B resistance gene hph, controlled by the *Aspergillus nidulans trp*C promoter. The data presented are the average of three plates per treatment.

Table 21. Putative *Acremonium implicatum* transformant colonies per Petri dish of selection medium.

A. tumefaciens strain		AGL-1				LBA4404			
Promoter		***trpC***		CaMV35S		***trpC***		CaMV35S	
Recipient fungal structure		M*	C	M	C	M	C	M	C
Co-cultivation period (Hours)	48	542	271	1,7	2	0,7	0,3	0	0
	60	836	318	1,3	3,3	1	1,0	0	0
	72	108 4	542	0	0	1,3	1,7	0	0

* M = Mycelia, C = Conidia. The values represent the average number of transformants between three plates.

The putative *A. implicatum* transformants selected on hygromycin B containing agar media were further examined using fluorescence microscope, PCR and Southern blot analysis. The PCR method allowed us to quickly examine and further confirm putative transformants that have been selected on antibiotic selection media (Figures 16 and 17). To determine the copy number of the transferred T-DNA, genomic DNA from19 randomly picked transformants from each experimental condition was digested with *Hind*III and anlalyzed with Southern blot. The results exhibited genomes with inserts ranging from a single insert to 5 inserts (data not shown), while the negative control, untransformed *A. implicatum*, showed no hybridization. No correlation existed between the average copy number of T-DNA per genome and the co-cultivation period, the mycelial or conidial transformation or other variables introduced in the experiments.

Microscopic examinations of selected transformants demonstrated strong expression of *egfp* as evidenced by the intense fluorescence emission. All parts of the fungal structure including conidia, mycelia, germinating conidia showed emission. These results demonstrate that the fungal promoter glyceraldehyde-3-phosphate dehydrogenase (GPD) isolated from *Cochliobolus heterostrophus* functions well for expression of genes in the endophytic fungus *A. implicatum* (Photo 2a).

The mitotic stability of the transferred DNA was examined by growing 10 transformants in liquid and agar media for 6 generations without any selection pressure. In all cases, resistance to hygromycin B was maintained indicating that the transferred DNA was stable. They all retained emission of fluorescence as well. The meiotic stability could not be determined because the fungus cannot be crossed.

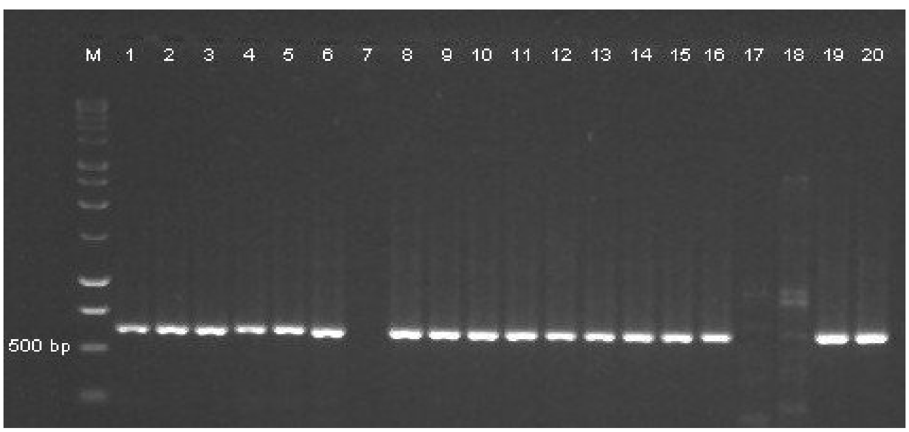

Figure 16. Polymerase chain reaction (PCR) amplifications, with primer specific for sequences of hygromycin B resistance gene (hph), of template DNA isolated from *Acremonium implicatum* transformants. Lanes M = molecular marker; template DNA from: 1 = conidia transformed with pSK1019 in *A. tumefaciens* strain LB4404 co-cultivated for 72 hours, and maintained without antibiotic selection pressure; 2, 3 = conidia transformed with pSK1019 in *A. tumefaciens* strain AGL-1 co-cultivated for 48 hours, 60 hours and maintained without or with antibiotic selection pressure, respectively; 4 = conidia transformed with pCAMBIA1300 in strain AGL-1 co-cultivated for 72 hours, and maintained without antibiotic selection pressure; 5 = mycelia transformed with pSK1019 in strain LB4404 co-cultivated for 60 hours, and maintained without antibiotic selection pressure; 6 = mycelia transformed with pSK1019 in strain AGL-1 co-cultivated for 72 hours, and maintained with antibiotic selection pressure; 7 = negative control (water); 8-11 = mycelia transformed with pSK1019 in strain AGL-1 co-cultivated for 48 hours (lanes 8, 9, and 10) and 72 hours, and maintained with antibiotic selection pressure or without it (lane 10); 12 = conidia transformed with pSK1019 in strain LB4404 co-cultivated for 72 hours, and maintained with antibiotic selection pressure; 13 = conidia transformed with pSK1019 in strain LB4404 co-cultivated for 72 hours, and maintained without antibiotic selection pressure for 4 generations; 14-16 = conidia or mycelia (lane 16) transformed with pSK1019 in strain AGL-1 co-cultivated for 48 hours and 60 hours (lane 16) and maintained with antibiotic selection pressure; 17, 18 = negative controls *Phaeoisariopsis griseola* and *A. implicatum* strain 6780 201V, respectively ; 19, 20 = positive controls pSK1019 and pCAMBIA1300, respectively.

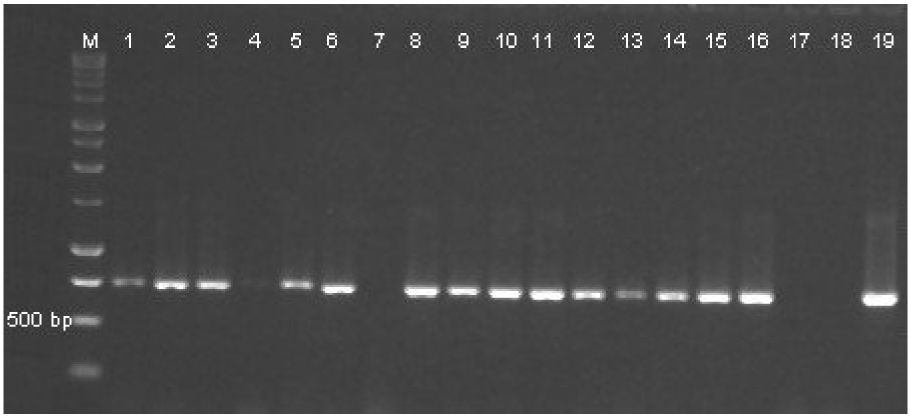

Figure 17. Polymerase chain reaction (PCR) amplifications, with primer specific for sequences of enhanced green fluorescent protein (*egfp*) gene, of template DNA isolated from *Acremonium implicatum* transformants. Lanes M = molecular marker; 1 = conidia transformed with pSK1019 in *A. tumefaciens* strain LB4404 co-cultivated for 72 hours, and maintained without antibiotic selection pressure; 2, 3 = conidia transformed with pSK1019 in *A. tumefaciens* strain AGL-1 co-cultivated for 48 hours, 60 hours and maintained without or with antibiotic selection pressure, respectively; 4 = conidia transformed with pCAMBIA1300 in strain AGL-1 co-cultivated for 72 hours, and maintained without antibiotic selection pressure; 5 = mycelia transformed with pSK1019 in strain LB4404 co-cultivated for 60 hours, and maintained without antibiotic selection pressure; 6 = mycelia transformed with pSK1019 in strain AGL-1 co-cultivated for 72 hours, and maintained with antibiotic selection pressure; 7 = negative control (water); 8-11 = mycelia transformed with pSK1019 in strain AGL-1 co-cultivated for 48 hours (lanes 8, 9, and 10) and 72 hours (lane 11), and maintained with antibiotic selection pressure or without it (lane 10); 12 = conidia transformed with pSK1019 in strain LB4404 co-cultivated for 72 hours, and maintained with antibiotic selection pressure; 13 = conidia transformed with pSK1019 in strain LB4404 co-cultivated for 72 hours, and maintained without antibiotic selection pressure for 4 generations; 14-16 = conidia or mycelia (lane 16) transformed with pSK1019 in strain AGL-1 co-cultivated for 48 hours and 60 hours (lane 16) and maintained with antibiotic selection pressure; 17, 18 = negative controls *Phaeoisariopsis griseola* and *A. implicatum* strain 6780 201V, respectively; 19 = positive control pSK1019.

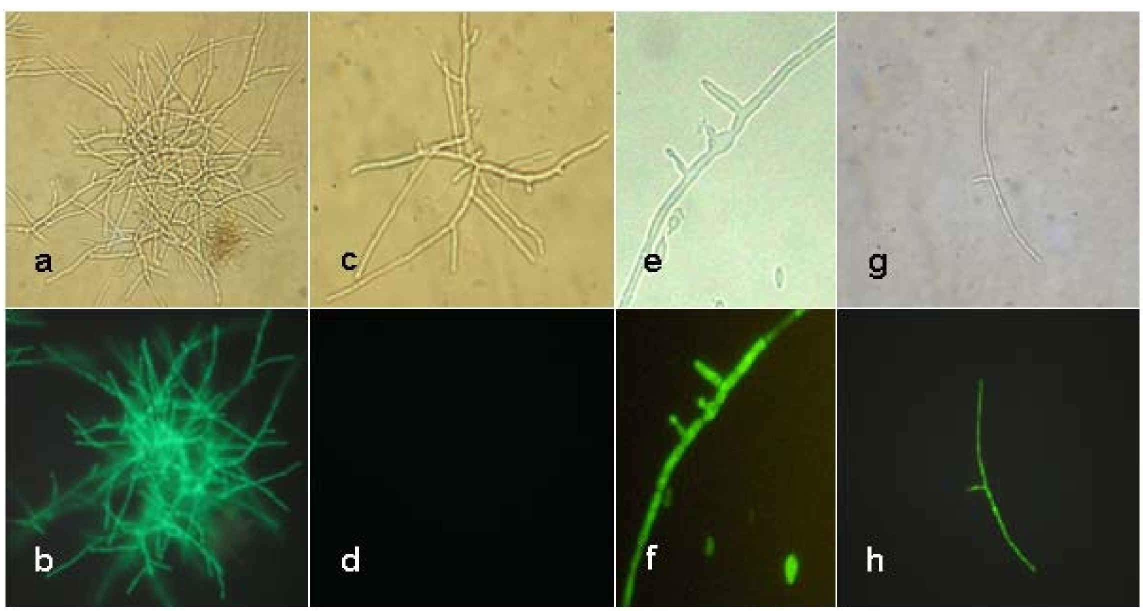

Photo 2. Structures of *Acremonium implicatum* strain 6780 201v transformed with green fluorescent protein gene (*egfp)* and observed microscopically with UV light. Photos a, c, e and g under normal light; b = fluorescence emission from transformed mycelia under Leica D filter (355 and 425 nm); c and d = control untransformed *A. implicatum* strain 6780 201v without and with UV light, respectively; f and h = transformed structures emitting green fluorescence under UV light with H3 filter (420 and 490 nm).

Preliminary data showed that *Brachiaria* tissues taken from plants inoculated with GFP-transformed *A. implicatum* expressed fluorescence emission (Photo 4). Photo 3a shows the gfp-expressing transgenic *A. implicatum* used to inoculate *Brachiaria* plants. This will allow us to study the endophyte-*Brachiaria* interaction, endophyte distribution within the plant tissue, and stability in the seed. This will in turn allow us to examine the potential use of this endophyte as a gene delivery and expression system in plants.

Although various transformation systems have been developed and reported for many fungi, successful application of the technology is still not routine in many species. Furthermore, developing an efficient transformation system for a previously untransformed fungus can be a technical obstacle. This work describes the transformation and expression of the GFP-encoding gene in an isolate of *A. implicatum*, an endophyte in species of *Brachiaria*. We have demonstrated that both the mycelia and conidia of *A. implicatum* can efficiently be transformed using *A. tumefaciens*. To the best of our knowledge, this is the first report on transformation of this endophytic fungus.

The stable integration and expression of the introduced gene into the genome of the recipient fungus indicate that the endophyte may be an excellent tool for delivering and expressing genes of agronomic importance such as disease and

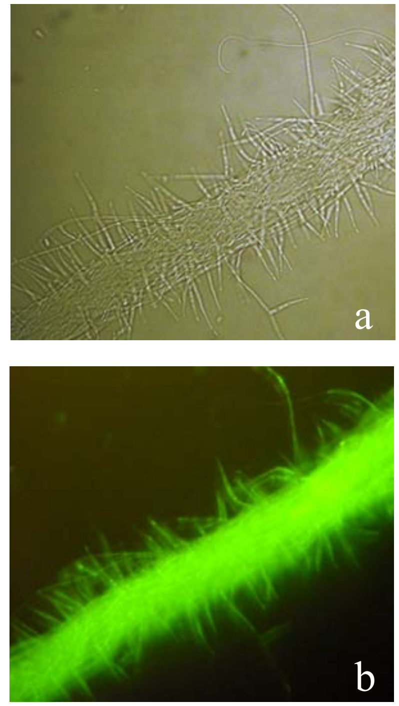

Photo 3. Mycelium of *Acremonium implicatum* transformed with enhanced green fluorescent protein (*egfp)* encoding gene: a) mycelium observed microscopically (40x) under normal lighting, b) the same mycelium observed under UV lighting, and demonstrating fluorescence emission.

insect resistance to host plants. For this to be successful, the practical implication of high seed transmission of *A. implicatum* in *Brachiaria* is significant: once associated with the plant, the fungus can perpetuate itself through seed,

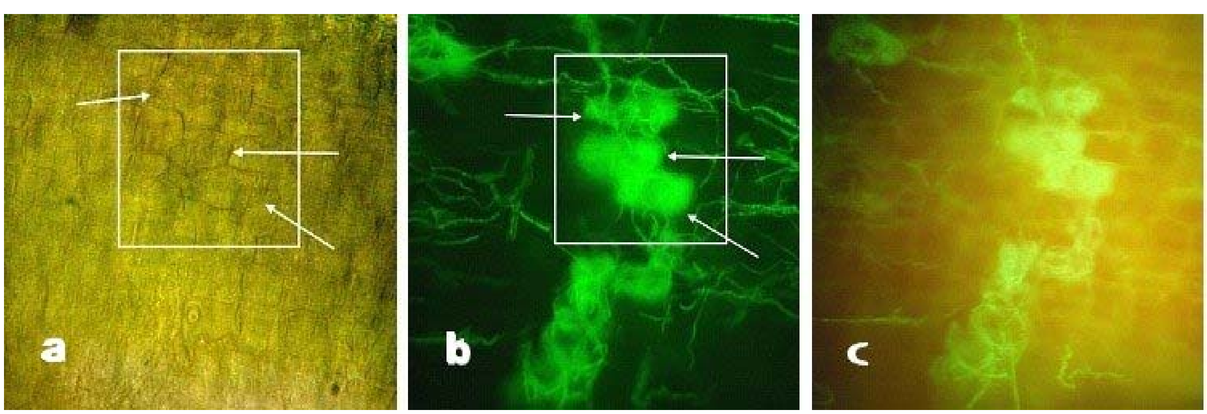

Photo 4. *Brachiaria* tissues from plants inoculated with *Acremonium implicatum* strain 6780 201v transformed with green fluorescent protein gene (*egfp*) [transformed strain shown in Figure 2. (a) under normal lighting, (b) fluorescence emission under UV light with Leica D filter, (c) fluorescence emission under UV light with Leica H3 filter.

especially in apomictic genotypes of *Brachiaria*, for as long as seed storage conditions do not diminish the survival of the fungus (Dongyi and Kelemu, 2004, Plant Disease 88:1252-1254) Several *Brachiaria* hybrids obtained from CIAT's forage breeding program were shown to harbor *A. implicatum*. In addition, we want to exploit the qualities of GFP as a reporter and study the interactions between *A. implicatum* and its host *Brachiaria*.

2.6 Effect of an antifungal protein isolated from seeds of the tropical forage legume *Clitoria ternatea* on disease control

Highlight

- Demonstrated that spraying crude preparation of the antifungal protein "Finotin" was effective in reducing diseases and increasing yield of tomato plants in the field.

2.6.1 Disease control under greenhouse and field conditions of Finotin (antifungal proteins)

Rationale

Contributors: G. Segura, S. Kelemu, and G. Mahuku (CIAT)

Seeds use strategies such as production of antimicrobial and/or insecticidal proteins to germinate and survive in soils that are densely inhabited by a wide range of microfauna and microflora. Antimicrobial proteins and peptides have been isolated from seeds of maize (*Zea mays* L.), radish (*Raphanus sativus* L.) and various other plants. They are believed to play a role in plant defense because of their strong antimicrobial activity. This belief is supported by their ability to confer resistance (to pathogens) to transgenic plants containing genes that encode them.

In a previous study, we examined seeds from several tropical forage legumes, for antifungal properties. Of those examined, we isolated, purified, and characterized a protein, designated 'finotin', from seeds of *Clitoria ternatea* (L.) that exhibited, *in vitro*, strong antifungal activity on the test fungus *Rhizoctonia solani* Kühn (Kelemu et al., 2004. Plant Physiology and Biochemistry 42: 867-873). This protein has antifungal, antibacterial and insecticidal properties.

In this study, we examined the potential use of finotin as a biopesticide for disease control under field and greenhouse conditions.

Materials and Methods

Treatment of P. griseola conidia with the protein finotin: Twenty-µl of a conidial suspension (10^{-4}) was placed on a slide and subsequently covered with a thin layer of potato dextrose agar medium. A 200-µl crude antifungal protein preparation (the same concentration that was used to spray onto bean plants) was applied on the agar. Protein preparation protocols were as described previously (Kelemu et al. 2004. Plant Physiology and Biochemistry 42: 867-873). Control slides had only water. These were placed in Petri dishes containing wet filter paper and incubated at room temperature. Pictures of conidia were taken under the microscope at 0, 32 and 96 hours to observe the development of individual conidia.

Plant inoculation and extract applications: A highly virulent isolate of the pathogen *Phaeoisariopsis griseola*, causal agent of angular leaf spot, was grown on V8 agar at 24°C for 12 days. Conidia were collected and suspended in sterile distilled water at a concentration of 2 x 10^4 conidia per mL. This inoculum was used on *Phaseolus vulgaris* variety Sprite (a susceptible one) bean plants.

Greenhouse testing: Seventeen-day old bean plants (15 plants per treatment) were sprayed with the fungicide benlate (500 ìg/ml), crude antifungal protein preparation, or sterile water. Two hours later all the plants were inoculated with *P. griseola* conidia (2 x 10^4 conidia per mL). The inoculated plants were placed in a humidity chamber for 4 days, then transferred to the greenhouse for symptom development. Treatments with crude antifungal protein, benlate or sterile water continued every 2 days. Disease evaluations were conducted 10 days after inoculation.

Field testing: Thirty days old seedlings of tomato variety Manalucie were transplanted to the field in a randomized design with 3 replications (8 plants per treatment in each replication). Treatments were; 1) control treatment with water alone, 2) spray application (till plants were completely wet) of crude protein preparation once a week, and 3) spray application of crude protein twice a week. Various diseases developed under natural infections.

Results and Discussion

Effect of antifungal protein Finotin on bean angular leaf spot: The crude protein extract from seeds of *C. ternatea* CIAT 20692 showed antifungal activity *in vitro* on the pathogen *P. griseola* (data not shown). Conidia treated with the crude protein failed to germinate 32 or 96 hours after treatment (Photo 5).

Plants treated with the crude antifungal protein preparation consistently developed fewer angular leaf spot disease lesions than the control plants that were treated with sterile distilled water (Figure 18). Had a purified protein been used to control the disease on bean plants, the level of disease control would perhaps have been even higher.

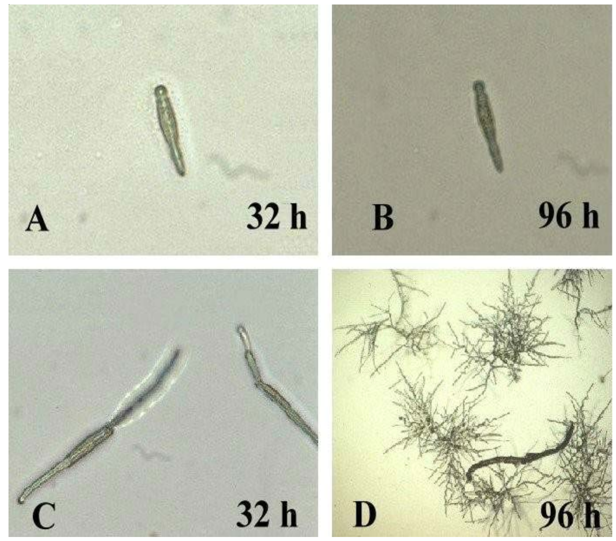

Photo 5. Treatment of *Phaeoisariopsis griseola* conidia with the antifungal protein finotin. Conidia failed to germinate in the presence of the antifungal protein finotin, 32 and 96 hours (A and B) after treatment, whereas those treated with sterile water germinated (C and D). [Annual Report 2004].

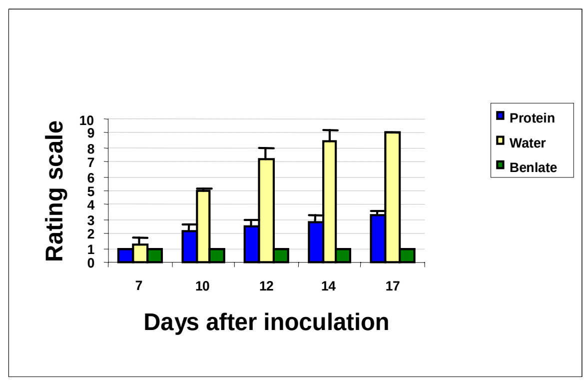

Figure 18. Angular leaf spot disease development in artificially inoculated bean plants following treatment with crude antifungal protein preparations isolated from *C. ternatea* CIAT 20692, the fungicide benlate, or water control (from AR-2004).

Effect of antifungal protein finotin on tomato diseases: Tomatoes are generally susceptible to a number of diseases under natural conditions. The purpose of these experiments is to develop a simple disease control strategy for small producers using this antifungal protein. Plants sprayed with the crude protein preparation once or twice a week developed better, had fewer disease symptoms, had more plant biomass, and produced more tomatoes than control plants (Photo 6 and Figure 19).

Photo 6. Tomato plants sprayed with crude antifungal protein preparations: control-water (1), once (2) and twice (3).

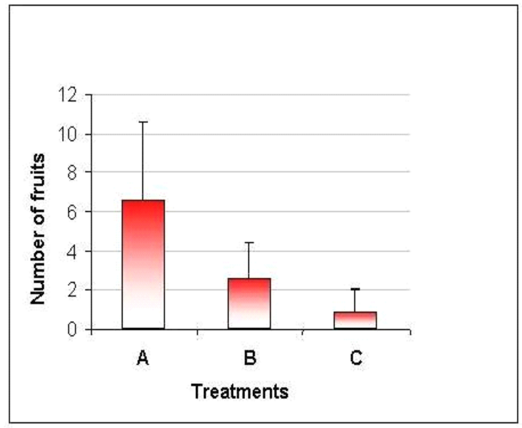

Figure 19. Average tomato fruit yield per tomato plant in plants treated with crude protein preparations twice a week (A), once a week (B) and water only (C).

The protein finotin, is shown to be inhibitory to the growth of a range of important plant pathogenic fungi and at least one important bacterium pathogenic to common bean, as well as two important species of bruchids, *Z.subfasciatus* and *A. obtectus* (Kelemu et al., 2004. Plant Physiology and Biochemistry 42: 867-873). These findings raise the possibility that finotin may contribute to the high level of disease and insect resistance observed in *C. ternatea* in the field. Finotin is released from seeds when the seed coat is mechanically damaged creating a zone of fungal growth inhibition *in vitro*. The antifungal activity of finotin is not affected by high temperatures, which made attractive for the direct use of this protein in disease management under field and greenhouse conditions. The results presented here demonstrate that a disease control strategy can be developed for small producers using this antifungal protein.

2.7 Isolating the gene encoding a biocidal protein named "Finotin"

Highlight

- Progress was made in defining the amino acid sequence of the antifungal protein "Finotin". Data obtained suggest that the cDNA clone generated could be classified as a member of the nsLTP which has been shown to inhibit growth of a number of pathogens.

Contributors: Martin Rodriguez and S. Kelemu (CIAT)

Rationale

Diseases and pests are major biological production constraints in a wide-range of crops. Plants, when attacked by harmful agents, can trigger an array of defense mechanisms. Pathogens and pests, in turn, have an array of matching mechanisms and evolve to overcome and compromise plant defense systems. One type of plant defense mechanism is the synthesis of proteins/peptides or low-molecular weight compounds following mechanical wounding or attack by biological agents. Biocidal or antimicrobial proteins are widely distributed in nature and are synthesized by various organisms. A number of plant-derived proteins that have antimicrobial or insecticidal properties have been isolated and characterized from various plants.

One such example is the isolation, purification and characterization of a highly basic small protein, designated ‘finotin’, from seeds of *Clitoria ternatea* (Kelemu et al. 2004. Plant Physiology and Biochemistry 42: 867-873). This protein has broad and potent antifungal, antibacterial and insecticidal properties, indicating that it may contribute to the high level of disease and insect resistance observed in *C. ternatea* in the field. We have subsequently demonstrated that plants sprayed with the crude protein preparation consistently developed fewer lesions of various diseases than the control plants both in greenhouse and field experiments (Kelemu et al. 2005. Phytopathology 95:S52).

In light of these findings, it is important to isolate the gene encoding finotin for application of non-host resistance in various crops to combat diseases and pests of economic importance. We report here the progress made towards polymerase chain reaction-based cloning of a cDNA corresponding some amino acid sequences of the protein.

Materials and Methods

Plant material: Fully-developed but not dried seeds of *Clitoria ternatea* CIAT accession #20692 were collected directly from the pods and used in this study.

RNA Extraction: Various extraction methods described by several authors were evaluated. Of those evaluated, the methods described by Azevedo et al. 2003 (Plant Mol Biol Reporter 21: 333-338), and Chang et al.1993 (Plant Mol Biol Reporter 11: 113-116) resulted in good quality RNA comparable to that obtained with an RNA isolation kit from Promega. mRNA was isolated from this total RNA using Oligotex® Direct mRNA kit (QIAGEN) according to the manufacturer’s instructions.

Synthesis of cDNA: Although there are various methods for doing so, complementary DNA (cDNA) is often synthesized from mature (i.e. fully spliced) mRNA using reverse transcriptase enzyme, which operates on a single strand of mRNA and generating its complementary DNA based on the pairing of RNA base pairs (A, U, G, C) to their DNA complements (T, A, C, G). In this study, the synthesis was conducted using 200 ng mRNA, 12-µM of BD SMART II™ A oligonucleotide and 12-µM of *Primer* 5’-RACE CDS in a 10-µl volume. This was incubated at 70°C for 2 min, then placed on ice. Two-µl of 5x buffer, 1-µl of DTT (20 mM), 1-µl of 10 mM dNTP and 1-µl of BD PowerScript Reverse Transcriptase were subsequently added to the mixture and incubated at 42°C for 2 h (the reverse transcriptase scans the mature mRNA and synthesizes a sequence of DNA that complements the mRNA template). Fifty-µl of Buffer Tricina-EDTA was added and further incubated at 70 °C for 7 minutes to deactivate the reaction. For synthesis of cDNA for the 3′ end, 500-ng mRNA and 1-µl (100-ng/µl) of primer oligo-$(dT)_{25}$ were mixed and incubated at 70°C for 8 min followed by cooling the mixture on ice. Subsequently, 10-µl of 5X buffer, 1-µl of 25mM dNTP, 2-µl of 100 mM DTT and 1-µl of SuperScript™ III RT (200 U/µl) were added to the mixture and incubated for 1 h at 50°C. At the end the mixture was deactivated by heat treatment at 70°C for 10 min. Information on oligonucleotides used in this study is given in Table 22.

Polymerase chain reaction of cDNA: We contracted Cornell University’s biotechnology unit to sequence finotin. However, the protein (finotin) sequence data obtained from Cornell Biotech was not satisfactory, and as a result we used sequences of an antifungal protein (from Clitoria ternatea) reported by Osborn et al., 1995 (FEBS Lett. 368: 257-262) to generate degenerate primers (Table 22). A 25-µl PCR mixture contained 1.5-mM of $MgCl_2$, 200-ìMof dNTPs, 0.5-ìM of each olgonucleotide (UPM and FINOR5), 200-ng cDNA, 1 unit of Taq DNA polymerase, and 1x PCR buffer. Amplifications were programmed with 35 cycles of a 30 second (3 min for the first cycle) denaturation step at 94 °C, annealing for 45 seconds at 50 °C, and prime extension for 45 seconds at 72 °C.

Table 22. Sequences of primers and adaptors used in this study.

Name	Sequence
Oligo dT	5'-(dT)$_{20}$-VN*-3'
5' RACE CDS-primer A	5'-(T)$_{25}$-VN*-3'
BD SMART II^{TM} A Oligonucleotide	5'AAGCAGTGGTATCAACG- CAGAGTACGCGGG 3'
primer UPM (Universal Primer A Mix)	5'CTAATACGACTCACTATAGGGCAAGCAGTGGTATCA ACG CAGAGT3'
primers NUP (Nested Universal Primer A)	5'AAGCAGTGGTATCAACGCAGAGT3'
MERF 1	5'-TGYGARCGNGCNTCNCTNACNTGG-3'*
MERF 2	5'-GARMGNGCNWSNYTNACNTGGACN-3'*
MERF3	5'-ACNGGNAAYTGYGGNAAYACNGGNCA-3'*
MERF4	5'-AAYYTNTGYGARMGNGCNWSNYT-3' *
MERF5	5'-ACNTGGACNGGNAAYTGY-3' *
FINOR 5	5'-CARTCRAARTARCARAARCAYTT-3'*
FINOR 6	5'-RTTNCCNCKYTTRTGRCANGCNCC-3'*

* N=A, C, G o T; V=A, C o G H=A, C o T D=A, G o T R=A o G Y=C o T M=A o C

Cloning and sequencing: The amplified product was excised and eluted from the agarose gel using a BIO-RAD DNA purification kit. This was cloned in pGEM-T Easy vector (Promega, USA), and sequenced using ABI Prism 377-96 DNA Sequencer. The sequence data were aligned using Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI).

Results and Discussion

Amplified DNA fragments (using cDNA as a template) ranging from 120 to 650 bp were isolated and cloned. Ten combinations of primers, 7 sense and anti-sense orientations and a universal primer, were used on 4 different preparations of cDNAs. A total of 37 clones were generated and of these, 17 have been sequenced so far. The sequence of one such clone is shown in Figure 20. The sequence data demonstrated homology to genes encoding nonspecific lipid transfer proteins (nsLTPs) from plants. These findings may be significant because nsLTPs have been reported to play a role in plant defense systems. A number of peptides (small proteins with sizes ranging 2-10 kDa) including nsLTPs have been reported to be involved in plant defense mechanisms. It is generally believed that seed proteins with antimicrobial activity may play a role in the protection of seeds against harmful microbes. Nonspecific lipid transfer proteins are basic, 9-kDa proteins with conserved cysteines and present in high amounts in plants. One promising clone with homology to nsLTPs is tentatively designated CtLTP. The clone is not complete, but based on sequences from nsLTP of other plants, only a small portion from the 3' end is missing (Figure 21).

The biological function of nsTLPs is still not well understood. However, a number of studies have demonstrated growth inhibition of a range of pathogens by nsTLPs. For example Cammue et al. (1995, Plant Physiol. 109:445-455) isolated a protein from seeds of onions (*Allium cepa* L.) that had sequence homology to nsLTPs and that was a potent groth inhibitory effect against 14 different pathogens.

The data presented in this report indicate that the cDNA clone that we generated may be classified as a member of the nsLTP protein family based on the deduced amino acid sequence. Work is in progress to generate a full length cDNA clone, to complete the sequences of the remaining 20 clones, and to determine the antimicrobial activity of a successful complete clone.

```
1             acgcggggatagtagataagagtaaataaggtagctagcttagtacctggtttaaagtta
61            aggagagtATGGCAAAGTGTAACACAATGGTAATAGCATTAGCAGCAGTAGTAGTAGTGT
1                     M  A  K  C  N  T  M  V  I  A  L  A  A  V  V  V  V
121           TGCTGATTGATGGTGGAGAAAGTTTTGCAATATGTAACGTAGATTCAAGTCAGTTAAGCT
18            L  L  I  D  G  G  E  S  F  A  I  C  N  V  D  S  S  Q  L  S
181           TGTGTCGTGCAGCAGTTAGTGGTGGTAATCCGCCACCACCAGATGAAAAGTGTTGTGCTG
38            L  C  R  A  A  V  S  G  G  N  P  P  P  P  D  E  K  C  C  A
241           TCATTCGTCAGGTCAATCTGCCCTGCCTCTGCCAATACAGGGGATTCCTACTTCGGTTTG
58            V  I  R  Q  V  N  L  P  C  L  C  Q  Y  R  G  F  L  L  R  F
301           GAATCAATCCCAAAAATGCTTTTGCTACTTCGACTG
78            G  I  N  P  K  N  A  F  A  T  S  T
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Figure 20. Sequence analysis of a cDNA clone synthesized from mRNA of seeds of *Clitoria ternatea.* The figure shows nucleotide sequences and the corresponding deduced amino acid sequences The sequence highlighted in green represents a possible TATA box sequence where as the yellow highlighted region represents a sequence commonly present in various LTP genes. The cysteine amino acid sequences are circled. The sequence of primer FINOR5 (anti-sense) is underlined.

```
CtLTP       ..MAKCNT...MVIALAAVVVVLLIDGGE..SFAI.CNVDSSQLSLCRAAVSGGNPPPPDEKCC     56
AtLTP2      ..MGKDNTRILMQFSALAMVLTAAIMVKEATSIPV.CNIDTNDLAKCRPAVTGNNPPPPGPDCC     61
OsLTP2      ........MMKLAVLVLAVAMVAACGGGVVGVAGASCN..AGQLTVCAAAIAGGARPTAA..CC     52
TaLTP2      ...................................ACQ..ASQLAVCASAILSGAKPSGE..CC     25
HvLTP2      MAMAMGMAMRKEAAVAVMMVMVVTLAAGADAGAGAACE..PAQLAVCASAILGGTKPSGE..CC     60
VuLTP2      ...TMKMKMKMSVVCAVVVVALFLIDVGPVAEAVT.CN..PTELSSCVPAITGGSKPSST..CC     56
Consensus                                       c      l  c  a   gg  p      cc
CtLTP       AVIRQVNLPCLCQYRG.FLLRFGINPKNAFATS....................                88
AtLTP2      AVARVANLQCLCPYK......................................                76
OsLTP2      SSLR.AQQGCFCQFAKDPRYGRYVNNPNARKTVSSCGIALPTCH.........                95
TaLTP2      GNLR.AQQGCFCQYAKDPRYGQYIRSPHARDTLTSCGLAVPHC..........                67
HvLTP2      GNLR.AQQGCLCQYVKDPNYGHYVSSPHARDTLNLCGIPVPHC..........               102
VuLTP2      SKLK.VQEPCLCNYIKNPSLKQYVNSPGAKKVLSNCGVTYPNC..........                98
Consensus      r     c c y              a
```

Figure 21. Amino acid sequence comparisons among various plant nsLTPs that are associated with plant defense systems. CtLTP: sequences deduced from this study; AtLTP: nsLTP of *Arabidopsis thaliana*; OsLTP: nsLTP of *Oryza sativa*; TaLTP: nsLTP of *Triticum aestivum*; HvLTP2: nsLTP of *Hordeum vulgare*; VuLTP2: nsLTP of *Vigna unguiculata*.

2.8 Plant growth promoting and nitrogen fixing bacteria associated with *Brachiaria*

Highlights

- Demonstrated that through tissue culture and spraying antibiotics (cefotaxime and vancomycin) we could eliminate endophytic bacteria in *Brachiaria*, which is necessary step to determine their nitrogen fixing properties.

- Showed that through introduction of bacteria isolated from a Brachiaria hybrid (CIAT 36062) to the *Brachiaria* hybrid cv. Mulato (CIAT 36061), exhibited improved growth (more litters and root development) relative to the control (indigenous bacteria only).

- Developed a specific primer useful to detect endophytic bacteria associated with *Brachiaria* using the step PCR instead of nested PCR.

2.8.1 Elimination of bacteria from *Brachiaria* plants using tissue culture methods

Contributors: P. Fory, X. Bonilla and S. Kelemu (CIAT)

Rationale

Endophytic bacteria are known to reside in plant tissues without doing harm to their host. These bacteria are often isolated either from surface-sterilized tissues or extracted from internal plant parts. They can enter plants mainly through the root zone, although other plant parts such as stems, flowers and cotyledons can also be entry points. In general, many of the entry points for pathogenic bacteria can serve the same purpose for the endophytic ones. Several different endophytic bacteria may reside within a single plant. These endophytes may either remain localized at their entry points or spread in other parts of the plant. Various bacterial endophytes have been reported to live within cells, in the intercellular spaces or in the vascular system of various plants. Although variations in the endophyte populations have been reported in various plants depending on a number of factors, generally bacterial populations are higher in roots and decrease in stems and leaves.

Endophytic bacteria that reside in plant tissues without causing any visible harm to the plant have been isolated from surface-sterilized *Brachiaria* tissues. Three bacterial isolates 01-36062-R2, 02-36062-H4, and 03-36062-V2 were isolated from *Brachiaria* CIAT 36062 in roots, leaves and stems, respectively, that tested positive for sequences of the *nif*H gene (the gene that encodes nitrogenase reductase) [IP-5 Annual Report 2003]. Because nitrogen fixation is performed by diverse groups of prokaryotic organisms, detection of a marker gene which is unique and is required for nitrogen fixation may be useful to conduct our studies. The *nifH* gene has been used with a number of PCR primers that amplify the gene from microbes and other samples by a number of researchers.

As stated in the 20003 Annual Report, the fatty acid analysis matched the bacterium coded 03-36062-V2 with *Flavimonas oryzihabitans* at 0.887 similarity index. *F. oryzihabitans* has been described as a plant growth promoting rhizobacterium in graminicolous plants. The analysis matched isolate 02-36062-H4 with *Agrobacterium rubi* at 0.845 similarity index. The name *A. rubi* is synonymous to *Rhizobium rubi*. The match using fatty acid data of the isolate 01-36062-R2, however, was not conclusive, matching it with *Leclercia adecarboxylata*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*, at 0.879, 0.841, and 0.820 similarity index, respectively. Of these, *E.cloacae* has been described as one of the dominant endophytic bacteria isolated from citrus plants (Araújo et al., 2002. Applied and Environmental Microbiology 68:4906-4914). A nitrogen-fixing endophytic strain of *Klebsiella pneumoniae* (Kp342) has been isolated from a nitrogen-efficent line of maize (Chelius and Triplett, 2000. Applied and Environmental Microbiology 66:783-787). This strain has been described to have a very broad host range and is capable of colonizing the interior of many plants with fewer than 10 cells in the inoculum (Dong et al., 2003. Plant Soil 257:49-59). More recently, endophytic colonization and nitrogen fixation in wheat were demonstrated upon inoculation with *Klebsiella pneumoniae* strain Kp342 (Iniguez et al., 2004. Molecular Plant Microbe Interaction 17:1078-1085).

Nitrogen fixation is conducted by phylogenetically diverse groups of prokaryotes. Tropical forage grasses could be ideal for investigating associations with nitrogen fixing bacteria because of their perennial nature and low chemical inputs including fertilizers. *Brachiaria* CIAT 36062 was chosen for these studies because it remains green even under conditions of low nitrogen input. Subsequently, we have determined that other genotypes of *Brachiaria* such as CIAT 36061 (cv. Mulato) also contained endophytic bacteria that tested positive for sequences of the *nif*H gene.

The main objectives of this initiative are to: 1) isolate and characterize indigenous endophytic and rhizospheric bacteria responsible for nitrogen fixation in association with species of *Brachiaria*, and 2) determine their phenotypic properties. In order to achieve the second objective, we have conducted various experiments to create genetically identical plants with or without endophytic bacteria. A key to achieving this is a successful method to eliminate indigenous bacteria. In 2004, we reported that none of the antibiotic or heat treatment procedures successfully eliminated the bacteria that tested positive for *nif*H gene sequences. We also reported that we initiated studies on the possibility of eliminating endophytic bacteria associated with *Brachiaria* CIAT 36062 through tissue culture and regeneration procedures. We report here initial results on elimination of endophytic bacteria.

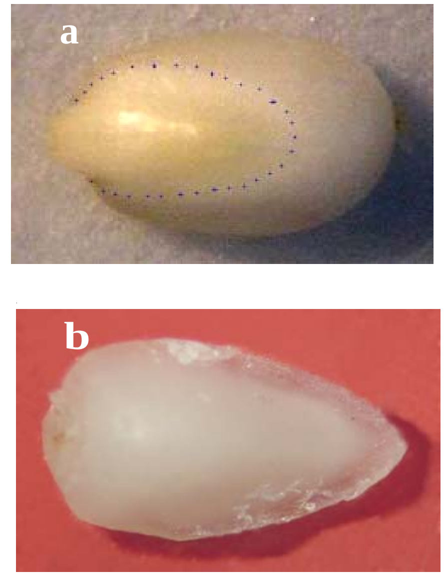

Photo 7. *Brachiaria* seed preparation for the isolation of embryos. (a) seed scarified and marked for isolation of its embryo, (b) embryo isolated free from the endosperm.

Materials and Methods

Plant material: Seeds of *Brachiaria* CIAT 36062 and CIAT 36061 (cv Mulato) were manually scarified and surface sterilized with 70% ethanol for 2 minutes, then with 2.5% NaOCl solution for 10 minutes, and rinsed four times with sterilized distilled water. The seed were then left immersed in sterilized distilled water for an hour in order to soften the endosperm. Longitudinal cuts were made in each seed to separate the embryo from the endosperm as shown in Photos 7a and b.

Plant growth media: The basic medium used was basal MS medium (Murashige and Skoog, 1962. Physiol Plant 15:473-479). This medium was subsequently modified for each sequence of propagation depending on the objective: a) induction medium (IM), b) regeneration medium (RM), and rooting medium (RM).

The isolated embryos were cultured aseptically in petri dishes (8-10 embryos per dish) containing induction medium (IM), with a composition of basal salt mixture of MS medium 4.49 g/L; casein, enzymatic hydrolysate 100 mg/L; 2,4-dichloro-phenoxyacetic acid 2 mg/L, 6-Benzylaminopurine (6-BAP) 0.2 mg/L, and supplemented with 30 g/L sucrose, 5 g/L agar, and incubated at 28°C for 12 weeks, and sub-culturing embryogenic calli every 3 weeks (Photo 8).

Photo 8. Embryogenic calli induction of *Brachiaria* seed embryos on induction medium.

Five embryogenic calli were transferred to each of a wide-mouth bottle (65mm x 45 mm of diameter) containing regeneration medium (basal salt mixture of MS medium 4.49 g/L; vitamin B5, 112.6 mg/L; sucrose 30 g/L; naphthalene-acetic acid 0.1 mg/L; kinetin 0.4 mg/L; gel rite 2.0 g/L; inositol100 mg/L; activated carbon 2.0 g/L; 5 g/L agar with or without antibiotics that inhibit the growth of bacteria (cefotaxime 100 mg/L and vancomycin 20 mg/L) and incubated first for a week under full light condition and then transferred to a growth chamber with a photoperiod of 16/8 hours (light/dark) at 24°C. As early as three weeks of incubation, green shoot formation was observed indicating successful plant development (Photo 9).

Once the plants reached a height of approximately 3 cm, they were transferred to a bigger bottle (130-mm x 75-mm of diameter) containing rooting medium (basal salt mixture of MS medium 2,245 g/L; vitamin B5, 112.6 mg/L; sucrose 30 g/L; casein, enzymatic hydrolysate 100 mg/L; gel rite 2.0 g/L5 g/L agar, with or without antibiotics that inhibit the growth of bacteria (cefotaxime 100 mg/L and vancomycin 20 mg/L) and incubated in a growth chamber under conditions described above for 2 weeks.

Photo 9. *Brachiaria* plant regeneration from embryogenic calli.

Fully developed seedlings were transferred to beakers containing sterile distilled water and maintained at room temperature for 5 days, with a daily change of water. This was done to allow plants adapt to external conditions free from rich culture media. The seedlings were subsequently transplanted onto pots containing sterilized 95% sand and 5% soil, and placed in a growth chamber with 12/12 hours (light/dark) and a temperature of 26±1°C. Each week and for a total of 3 weeks plants that were treated with antibiotics in regeneration and rooting media were also sprayed with the same concentration of antibiotic solutions.

Bacterial isolation from Brachiaria tissues: Isolations were conducted from leaf, stem and root tissues of the seedlings of CIAT 36062 and CIAT 36061 generated from embryogenic calli. Each tissue was sliced to approximately 1-cm size and surface sterilized with sterilized 1% NaOCl solution for 2 minutes, then with 70% ethanol for one minute, and rinsed three times with sterilized distilled water. Each sample was then macerated with mortar and pestle in 1 ml of sterile distilled water. One hundred-ìl of this sample was taken and a dilution series conducted, and a total of 100-ìl of each dilution was spread on plates containing nutrient agar medium (Difco, Detroit, MI). The plates were incubated at 28°C for 24 hours. Independent bacterial colonies were counted.

Results and Discussion

The methods described above were effective in plant development from embroyogenic calli for both *Brachiaria* genotypes: CIAT 36062 and CIAT 36061. No macroscopic plant development differences were observed among control and antibiotic-treated plants. Two evaluations were made for the presence or absence of endophytic bacteria at 2 and 5 weeks after the last spray applications of antiobiotics. Bacterial colonies appeared from leaf, stem and root samples of control plants whereas samples from antibiotic-treated plants largely remained bacterial-free, although a few colonies appeared (Photos 10 and 11). We do not know yet whether these few

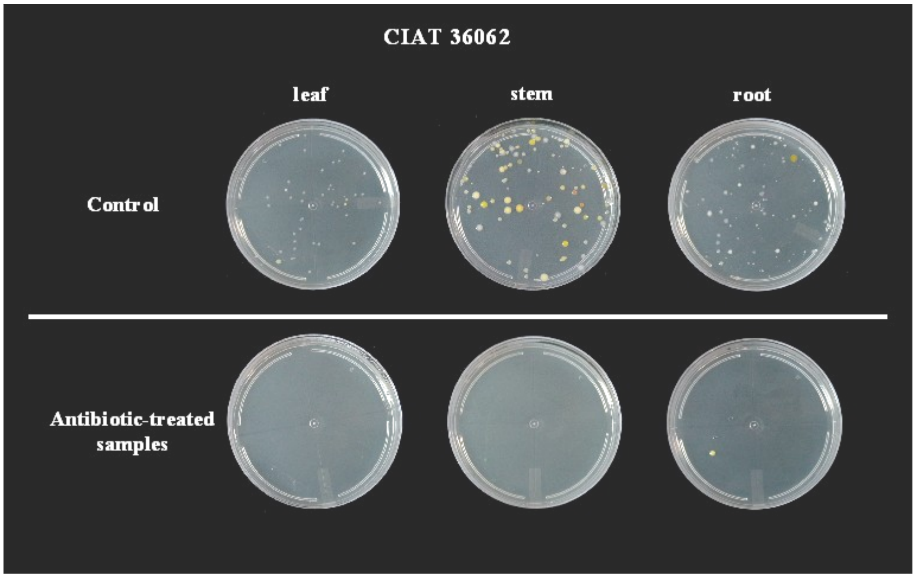

Photo 10. Endophytic bacterial isolation on nutrient agar medium, from leaf, stem and root tissues *Brachiaria* CIAT 36062 samples treated with or without antibiotics, and generated from embryogenic calli.

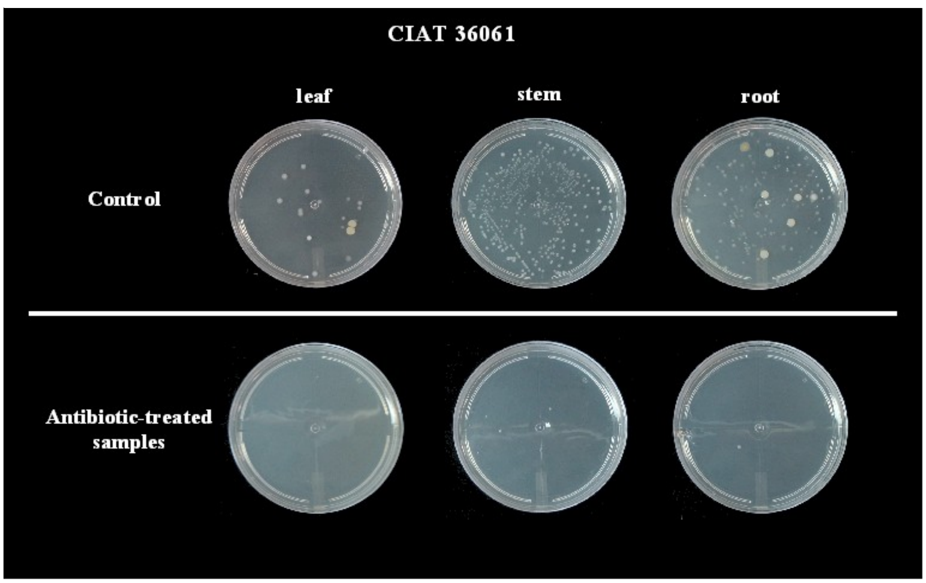

Photo 11. Endophytic bacterial isolation on nutrient agar medium, from leaf, stem and root tissues *Brachiaria* CIAT 36061 samples treated with or without antibiotics, and generated from embryogenic calli.

bacterial colonies from treated plants are nif-H sequence positive ones or whether the bacterial population would increase with time. Therefore, we would need to evaluate the colonies for nif-H sequence using nested PCR and monitor the bacterial population with time in antibiotic-treated plants. In addition, we are currently conducting experiments with a biocidal product PPM (plant preservative mixture) aiming at a total elimination of bacterial endophytes. We are also propagating tillers from treated and control plants for experiments to determine the phenotypic effects of the presence and absence of these bacterial endophytes.

2.8.2 Effects of endophytic bacteria on plant growth and development

Contributors: P. Fory, S. Kelemu, J. Ricaurte, R. Garcia and I. M. Rao (CIAT)

Rationale

In both managed and natural ecosystems, plant-associated bacteria play key roles in host adaptation to changing environments. These interactions between plants and beneficial bacteria can have significant effect on general plant health and soil quality. Associative nitrogen-fixing bacteria may provide benefits to their hosts as nitrogen biofertilizers and plant growth promoters. Several endophytic bacteria have been reported to enhance growth and improve plant health in general (Sharma and Novak, 1998. Can. J. Microbiol. 44:528-536; Stoltzfus et al., 1998. Plant Soil 194:25-36). Many plant-growth-promoting bacteria (PGPB) that include a diverse group of soil bacteria are thought to stimulate plant growth by various mechanisms such as plant protection against pathogens, providing plants with fixed nitrogen, plant hormones, or solubilized iron from the soil.

Brachiaria grasses of African savannas have supported millions of African herbivores over thousands of years. Some of these *Brachiaria* species have many desirable agronomic traits. For example, they are persistent and can grow in a variety of habitats ranging from waterlogged areas to semi-desert. These grasses that are often grown under low-input conditions are likely to harbour unique populations of nitrogen-fixing or plant growth promoting bacteria. The aim of our study is to examine the effects of endophytic bacteria that were isolated from species of *Brachiaria* on plant development.

Materials and Methods

Bacterial inoculum preparation: Three endophytic bacterial isolates 01-36062-R2, 02-36062-H4, and 03-36062-V2 that were originally isolated from *Brachiaria* CIAT 36062 in roots, leaves and stems, respectively, and that tested positive for sequences of the *nif*H gene (the gene that encodes nitrogenase reductase) are maintained at -80°C in 20% glycerol. Bacterial cells were removed from each of the stored samples, plated on nutrient agar medium (Difco, Detroit, MI) and incubated for 24 h at 28°C. The cells from each of the bacterial strains were collected from the plates, suspended in sterile distilled water and adjusted to a concentration of optical density (OD_{600}) = 1.0 with a spectrophotometer.

Plant inoculation: Twenty tillers of about a month old were prepared from a single mother plant of Brachiaria hybrid CIAT 36061 (cv. Mulato), their roots washed with sterile distilled water and made ready for inoculations. The roots of ten of these tillers were immersed in a beaker containing a mixture of equal volumes (50-ml each) of the three strains of endophytic bacterial suspension described above. The remaining ten plants were immersed in a beaker containing the same volume of sterile distilled water. All plants were kept immersed for 48 hours, after which they were removed and rinsed 3 times with sterile distilled water. They were then each transplanted to pots containing sterile sand (95%) and soil (5%) and maintained in the greenhouse under natural day light and at temperatures between 19 and 30°C. No nutrients were applied.

Plant evaluations: Sixty-five days after inoculations the following measurements were taken in control and treated plants: 1) plant growth and development such as plant height, number of tillers, number of leaves, leaf area, 2) leaf chlorophyll content, 3) nitrogen content, and 4) soluble protein content in leaves.

Plant development and other measurements: Plant height was measured in centimeters from stem base to the highest part of the plant. Number of leaves per plant and the number of tillers were determined. Leaf area was determined in cm²/plant and measured using a LI-300 leaf area meter (LI-COR, inc., Lincoln, NE). In addition, dry matter distribution among leaves, stems and roots was determined after drying each tissue separately in an oven at 70ºC for 48 hours. Leaf chlorophyll content was measured with a chlorophyll meter SPAD 502 (Minolta), taken across the third fully developed leaf as an average of 6 measurements. Soluble leaf protein was measured as described by Rao and Terry (Plant Physiol 90: 814-819). Nitrogen content in leaves and stems was determined using methods described by Salinas and García (1985, CIAT, Working document 83 p).

Bacterial population in the roots: Approximately 1 g of root sample was taken from each individual plant, surface sterilized (in 1% NaOCl solution for 2 min, in 70% ethanol for one min, then rinsed 3 times in sterile distilled water) and macerated in mortar and pestle in 1 ml of sterile distilled water. One hundred-ìl of this macerated sample was taken and a dilution series performed. These were plated on nutrient agar medium and incubated for 24 h at 28°C to determine bacterial colony growth, and calculate the number of bacterial cell per gram of root weight.

Experimental design and statistical analysis: The experiment had two treatments (with and without artificial inoculations) each with 10 plants and arranged in a completely randomized design. Analysis of variance was determined using Statistics Analysis System (SAS®). A t-test was conducted.

Results and Discussion

Brachiaria hybrid cv, Mulato (CIAT 36061) have indigenous endophytic bacteria that are difficult to eliminate. Because of the difficulty to eliminate these indigenous bacteria, we set out to introduce three different strains of bacteria, originally isolated from *Brachiaria* hybrid CIAT 36062, into CIAT 36061, in addition to the indigenous bacteria that this hybrid already has. In general, the introduction of these bacteria had a positive effect on plant growth and development in the recipient plant CIAT 36061 (Photo 12). Photo 13 we further demonstrate more tiller and root development in artificially inoculated CIAT 36061 plants than plants containing only indigenous endophytic bacteria.

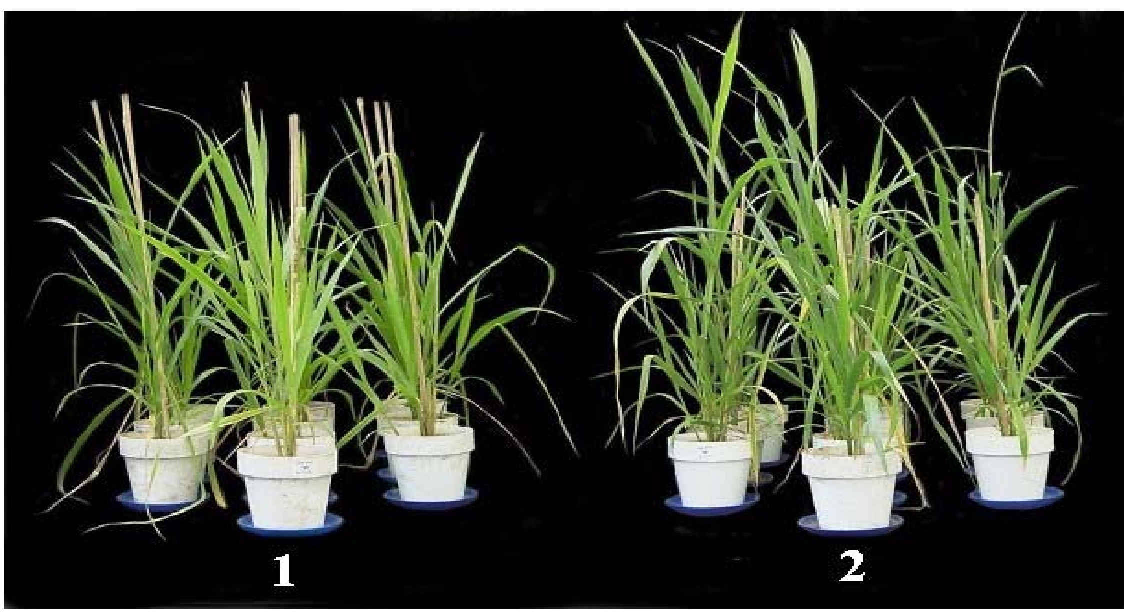

Photo 12. *Brachiaria* hybrid cv. Mulato (CIAT 36061) plants with indigenous endophytic bacteria (1), and inoculated with a mixture of 3 bacterial strains 01-36062-R2, 02-36062-H4, and 03-36062-V2 (originally isolated from *Brachiaria* CIAT 36062) [2], 65 days after inoculations and maintained under greenhouse conditions with no nutrients.

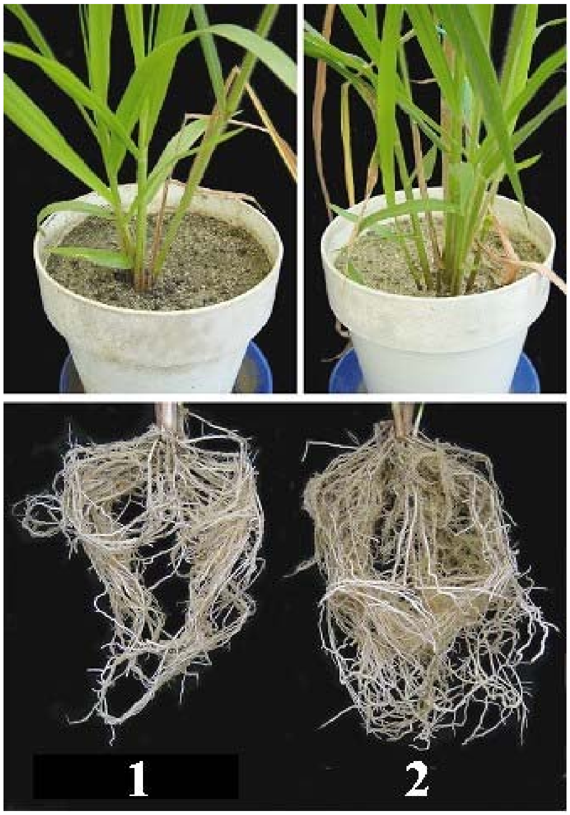

Photo 13. A *Brachiaria* hybrid CIAT 36061 with indigenous endophytic bacteria (1), and inoculated with a mixture of 3 bacterial strains 01-36062-R2, 02-36062-H4, and 03-36062-V2 (originally isolated from *Brachiaria* CIAT 36062) [2], 65 days after inoculations and maintained under greenhouse conditions with no nutrients. Note the difference between the artificially inoculated plant and the one with just indigenous bacteria, in the number of tillers and root growth and development.

Table 23. Average values of various parameters evaluated for endophyte-inoculated and non-inoculated plants of *Brachiaria* hybrid cv. Mulato (CIAT 36061).

Parameters	Control	Inoculated
Plant height (cm)	103.9 b[†]	115.6a**
Leaves/plant	22.5 b	36.9a***
Tillers/plant	4.1 b	7.4a***
Leaf area (cm^2/plant)	993.7 b	1430a***
Chlorophyll SPAD	340.3 b	433.7a***
Soluble Protein (μg/ cm^2 fresh leaf)	928.93 a	1095.42[a]
Stem N content (%)	0.51 b	0.67a**
Green leaf N content (%)	1.0 b	1.3a **
Dead leaf N content (%)	0.44 b	0.66a**

[†] Each value is the mean of values from 10 plants. Data in each row followed by the same letter are not significantly different (P<0.05) according to t-test.

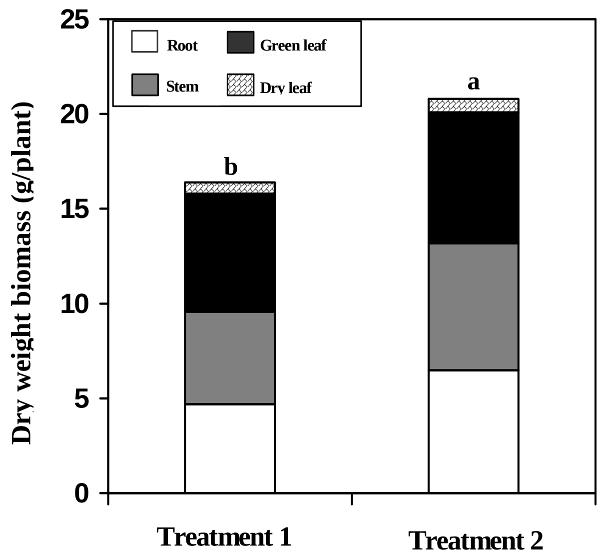

Figure 22. Total tissue biomass production in *Brachiaria* hybrid cv. Mulato (CIAT 36061) control plants with indigenous endophytic bacteria (treatment 1), and inoculated with a mixture of 3 bacterial strains 01-36062-R2, 02-36062-H4, and 03-36062-V2 (originally isolated from *Brachiaria* CIAT 36062) [treatment 2], 65 days after inoculations and maintained under greenhouse conditions with no nutrients. Values are average of 10 plants per treatment.

In nitrogen- and other nutrient-deficient conditions, *Brachiaria* plants inoculated with the three bacterial strains had significantly higher average values in all evaluated parameters (with the exception of soluble proteins in leaves) than those control plants containing just indigenous bacteria Table 23).

Analysis of variance showed that the total biomass production (leaf, stem and root) collected from control Brachiaria CIAT 36061 plants was significantly (P< 0.05) less than that from inoculated ones (Figure 22). The data presented indicate that a close and beneficial interaction existed between the introduced as well as indigenous endophytic bacteria and *Brachiaria* hybrid cv. Mulato (CIAT 36061), resulting possibly in nitrogen fixation and enhancement of plant growth. Had we managed to eliminate the indigenous endophytic bacteria from control CIAT 36061 plants, the difference between bacteria-containing and control plants would probably have been even more dramatic. A high correlation (r= 0.89; P<0.01) was observed between leaf chlorophyll content and %

nitrogen in leaves. Inoculated plants maintained a more profound green color and higher nitrogen content in their leaves than control plants. As expected, bacterial cells were isolated from both control plants containing indigenous bacteria and those inoculated with the 3 bacterial strains with values that are similar (6.56 log_{10} CFU/g of fresh root tissue of inoculated plants vs 6.53 log_{10} CFU/g of fresh root tissue of non-inoculated control plants). These endophytic bacterial population data are very similar to the natural endophyte concentrations in alfalfa, sweet corn, sugar beet, squash, cotton and potato, reported to vary between 2.0 and 6.0 log_{10} CFU/g of tissue (Kobayashi and Palumbo, 2000. *In* Bacon and White, eds. Microbial endophytes. Marcel Dekker, Inc., NY).

These initial results strongly suggest that endophytic bacteria have a direct beneficial effect on plant growth and development, and possibly on associated nitrogen fixation in *Brachiaria*. More work is needed to further verify these findings preferably after completely removing indigenous bacteria from species of *Brachiaria*.

2.8.3 Cloning and characterization of a nitrogen fixation gene *(nif)* sequences from a plant growth-promoting bacterium associated with *Brachiaria* species

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Rationale

A number of prokaryotes are known to be involved in nitrogen fixation as well as enhancement of plant growth. Nif genes which encode the nitrogenase complex (encoded by approximately 20 different nif genes) and other enzymes involved in nitrogen fixation has consensus sequences identical from one nitrogen fixing bacteria to another, but while the structure of the nif genes is similar, the regulation of the nif genes varies between different diazotrophes (nitrogen fixing organisms).

Previously, we have reported the isolation of three strains of bacteria from *Brachiaria* hybrid CIAT 36062 from roots, leaves and stems that were designated 01-36062-R2, 02-36062-H4, and 03-36062-V2, respectively. Using nested PCR and three primers designed for the amplification of the *nifH* gene sequences, amplified products were generated with template DNA from these bacterial strains. We have also reported in 2003 (IP5 Annual Report) that fatty acid analysis conducted on these 3 strains resulted in matching them with various bacteria that are known to be nitrogen fixers and/or plant growth promoters (for example with *Flavimonas oryzihabitans*).

This study focused on strain 01-36062-R2. The fatty acid analysis data of the isolate 01-36062-R2 matched it with *Leclercia adecarboxylata*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*, at 0.879, 0.841, and 0.820 similarity index, respectively (IP-5 Annual Report 2003). *E. cloacae* has been described as one of the dominant endophytic bacteria isolated from citrus plants.

In this study we cloned and sequenced nested PCR amplified products using primers derived from nif-gene sequences. The objective of this work is to develop a specific primer that will allow us to screen (without using nested PCR) *Brachiaria* and other tropical plants-associated bacteria that contain *nif*-gene sequences.

Materials and Methods

Bacterial isolates: Bacterial strain isolated from roots of *Brachiaria* CIAT 36062 and designated 01-36062-R2 was used for this study. For evaluation of the various primers, strains isolated from stems and leaves of the same *Brachiaria* genotype, and designated 02-36062-H4 y 03-36062-V2, *Bradyrhizobium* species CIAT 2469 isolated from the legume *Desmodium* species, and a pathogenic bacterium *Xanthomonas campestris* pv. *graminis* (included as a negative control) were used.

Bacterial DNA extractions: DNA extraction was conducted using a modified protocol based on combinations of standard methods, which includes growing bacterial cells in liquid media LB (tryptone 10g, yeast extract 5g, NaCl 10g, 10 ml of 20% glucose in 1 L of distilled water), treatment of cells with a mixture of lysozyme (10 mg.ml in 25 mM Tris-Hcl, ph 8.0) and RNase A solution, and extraction of DNA with STEP (0.5% SDS, 50 mM Tris-HCl 7.5, 40 mM EDTA, proteinase K to a final concentration of 2mg/ml added just before use. The method involves cleaning with phenol-chloroform and chloroform/isoamyl alcohol and precipitation with ethanol. The quality of DNA was checked on 1 % agarose gel.

Nested PCR Amplification: Three primers were used, which were originally designed by Zehr and McReynolds (1989, Appl. Environ. Microbiol. 55: 2522-2526) and Ueda, et al. (1995, J. Bacteriol. 177: 1414-1417), to amplify fragments of *nifH* genes. Amplification steps described by Widmer et al (1999, Applied and Environmental Microbiology 65:374-380) were adopted. The final product of the nested PCR amplification is about 370 bp in size.

Cloning of amplified DNA fragments: Amplified products were eluted from agarose gel using Wizard ® PCR Preps DNA Purification System (Promega) according to instructions supplied by the manufacturer. The purified fragments were ligated to the cloning vector pGEM- T Easy (Promega) and used to transform *E. coli* DH5á.using standard procedures (Sambrook et al., 1989. Molecular Cloning: a laboratory manual. 2nd ed. Cold spring harbor laboratory, USA)

Plasmid extraction: Plasmids were extracted from transformed *E. coli* DH5á cells using a Wizard® Plus Mini-preps DNA Purification System (Promega) using the protocol supplied by the manufacturer. To confirm whether the transformants contained the desired size of insert (approximately 370 bp), the plasmid DNA was digested to completion with the restriction enzyme *Eco*RI. The digested products were separated by electrophoresis on a 1% agarose gel (Bio-Rad, NJ), stained with ethidium bromide and photographed under UV light.

Amplification of DNA inserts for sequencing: PCR reactions (25-µl) contained 20 ng/µl plasmid DNA, 1 X PCR buffer, 1.5 mM $MgCl_2$, 0.1 mM dNTPs, primers T7 (5'-GTAATACGACTCACTATAGGGC-3') and Sp6 (5' –TATTTAGGTGACACTATAG-3') each at 0.1 µM concentration, 0-5U Taq polymerase and amplified in a programmable thermal controller (MJ Research, Inc, MA) programmed with 35 cycles of a 30 sec (2 min for the first cycle) denaturation step at 94°C, annealing for 30 sec at 50°C, and primer extension for 1 min (4 min in the final cycle) at 72°C. Samples of amplified products were separated on a 2% agarose gel by electrophoresis for further confirmation of the expected size insert.

The ABI prism BigDye terminator Cycle sequencing kit was used to further prepare the samples for sequencing. Sequencing was conducted using ABI PRISM™ 377 DNA sequencer. The sequence data were compared with sequences in databases using the program BLAST version 2.0 or 2.1 (http://www.ncbi.nlm.nih.gov/BLAST/-).. The program compares nucleotide sequences to databases and calculates the statistical significance of matches.

Specific primer construction: Based on the sequence data, primers were designed using the program DNA-MAN (version 4:0), and synthesized by Integrated DNA Technologies, Inc. (Coralville, USA). These primers were tested on bacteria that are confirmed positive and negative controls: strains 01-36062-R2; 02-36062-H4 and 03-36062-V2, *Bradyrhizobium*, and *Xanthomonas campestris* pv. *graminis.*

Results and Discussion

Cloning and sequence analysis: A 371 bp nested PCR amplification product using template DNA isolated from the bacterial strain 01-36062-R2 (a strain isolated from *Brachiaria* CIAT 36062 and that tested positive for *nif*-gene sequences) was successfully cloned in the vector pGEM- T Easy (Promega). Figure 23 shows randomly picked transformants, the majority showing the desired size insert, with the exception of lanes 2 and 7.

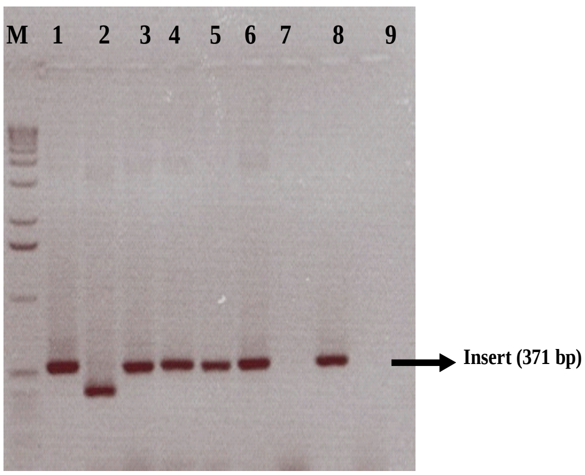

Figure 23. Amplified products of independent clones using template DNA of the bacterial strain 01-36062-R2 associated with *Brachiaria* CIAT 36062. Amplification conditions were as described in the materials and methods section. M = molecular marker. Lanes 1-8 inserts of clones, lane 9 = negative control. The clones that showed the expected 371 bp size insert were sequenced.

The sequence analysis demonstrated the presence of *nif*H gene sequences in these sequenced clones. The deduced amino acid sequence showed a 97% similarity with 120 amino acids that correspond to the *nif*H gene sequence of *Klebsiella pnuemoniae*. These results are in agreement with the fatty acid analysis results of this bacterial strain that matched it with *Klebsiella pnuemoniae* at 0.84 similarity index. Nif genes that encode the nitrogenase complex and other enzymes involved in nitrogen fixation have consensus sequences identical in various nitrogen-fixing bacteria.

Klebsiella pneumoniae is a member of the Enterobacteriaceae that has the ability to fix nitrogen, and possesses a total of 20 *nif* genes that are clustered in a 24 kb region of the chromosome and responsible in nitrogenase synthesis and its regulation. Three of these genes, *nif*HDK, code for the three structural nitrogenase subunits. *K. pnuemoniae* has been reported as an endophytic bacterium associated with various plants and involved in nitrogen fixation, including maize (Chelius and Triplett 2001, Microb. Ecol. 41: 252–263), wheat (Iniguez *et al*., 2004, Molecular Plant-Microbe Interactions 17: 1078–1085) and rice (Dong et al,. 2003, Plant Soil 257:49-59).

The consensus sequences obtained in this study are listed below as deduced amino acid sequences (**1**) and nucleotide sequences (**2**).

1.
GVIQADSTRLILHAKAQNTIMEMAAEVG
SVEDLELEDVLQIGYGGVRCAESGGPEP
GVGCAGRGVITAINLEEEGAYVPDLDFV
FYDVLGDVVCGGFAMPIRENKAQEIYIV
CSGEMMALYA

2.
5’-
TGGGTGTGATCCAAGCCGACTCCACGC
GTTTGATCCTGCATGCGAAAGCGCAGA
ACACCATTATGGAGATGGCCGCCGAAG
TCGGCTCCGTCGAAGACCTGGAATTAG
AAGACGTGCTGCAAATCGGTTACGGCG
GCGTGCGCTGCGCGGAATCCGGTGGCC
CGGAGCCAGGTGTGGGCTGTGCCGGTC
GTGGCGTGATCACCGCGATTAACTTCC
TCGAAGAAGAAGGCGCTTACGTGCCGG
ATCTGGATTTTGTTTTCTACGACGTGCT
GGGCGACGTGGTATGCGGTGGTTTCGC
CATGCCGATTCGTGAAAACAAAGCGCA
GGAGATCTACATCGTTTGCTCTGGCGA
GATGATGGCCCTCTACGCA-3’

Specific primer development: In this study we developed specific primers that would allow us detect endophytic bacteria associated with species of *Brachiaria* just with one step PCR instead of nested PCR. Based on the consensus sequence listed above (in #2) and using the DNA-MAN program, 9 primers were designed and synthesized. Twenty combinations of these primers were tested on selected positive and negative control bacteria. Of these combinations, a pair of primers with sequences 5'-GTTTGATCCTGCATGCAAAAG-3' and 5'-AGAGCAAACGATGTAGATCTCCTG- 3', produced only one amplification product with a size of approximately 344 bp in bacteria that are used as positive controls, where as negative controls resulted in no amplified products (Figure 24). This pair of primers will be tested on various bacteria associated with species of *Brachiaria* and other plants and that are suspected to fix nitrogen. We also want to use this pair of primers to directly detect these bacteria in tissues or soil samples.

± 344 bp

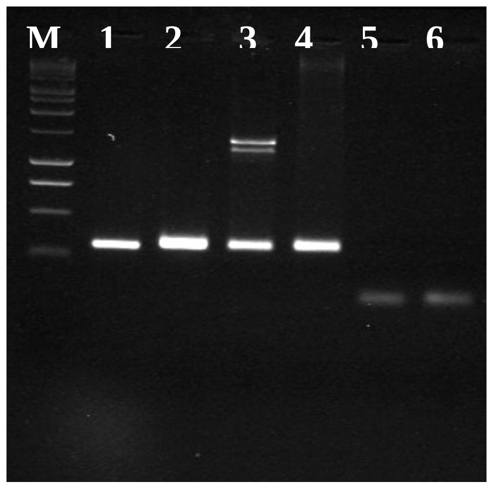

Figure 24. Specific amplifications of template DNA from lanes 1-4, positive control bacteria *Bradyrhizobium* sp., strains 01-36062-R2; 02-36062-H4 and 03-36062-V2 isolated from *Brachiaria*, respectively; lane 5, negative control *Xanthomonas campestris* pv. *graminis*; lane 6: negative control PCR reaction mixture; M, size marker. Primer combination used 5'-GTTTGATCCTGCATGCAAAAG- 3' and 5'-AGAGCAAACGATGTAGATCTCCTG –3. The PCR reaction (20-µl) contained 16 ng/µl template DNA, 1X PCR buffer, 1.5 mM $MgCl_{2,}$ 0.2-µM dNTPs, 0.2-µM each of the primers, 0.2U Taq polymerase. The amplification was carried out in a programmable thermal controller (MJ Research, Inc, MA) programmed as follows: 94°C for 2 min (94°C for 30 sec, 50°C for 45 sec, 72°C for 30 sec), for 35 cycles; 72°C 8 por min. The products were separated on a 1.2% agarose gel by electrophoresis, stained with ethidium bromide and photographed under UV light.

Output 3. Grass and legumes genotypes with superior adaptation to edaphic and climatic constrains are developed

3.1 Genotypes of *Brachiaria* with adaptation to edaphic factors

Highlights

- Through collaborative research with the Yamagata University, Japan, we showed that the high level of aluminum resistance in *Brachiaria decumbens* cv. Basilisk (signalgrass) is associated with less permeability of the plasma membrane of the root-tip portion.

- Confirmed the applicability of a new simple, rapid and reliable technique of methylene blue (MB) staining for the discrimination of aluminum (Al)-resistant protoplasts from a wider range of plant species, cultivars and lines and showed higher level of Al resistance in signalgrass compared with field crops.

- Showed that phosphoenolpyruvate carboxylase is activated in the *Brachiaria* hybrid cv. Mulato under low P supply and low pH conditions and that this increased activity could contribute to its adaptation to tropical acid soils with low P availability.

- Developed and validated a hydroponic screening procedure that uses vegetative propagules (stem cuttings) of *Brachiaria h*ybrids to rapidly evaluate root vigor and aluminum resistance, two key components of edaphic adaptation.

- Screened 139 apomictic/sexual hybrids of *Brachiaria* using a hydroponic screening method and identified 9 hybrids (BR04NO1018, 1552, 1900, 2110, 2128, 2166, 2179, 2201 and 2681) that were superior to *Brachiaria decumbens* parent in terms of aluminum resistance.

- Showed that the *Brachiaria* hybrid, cv. Mulato II performed well into the fourth year after establishment in the Llanos and its superior performance at 42 months after establishment was associated with its ability to acquire greater amounts of nutrients from low fertility soil.

3.1.1 Mechanisms of edaphic adaptation of *Brachiaria*

3.1.1.1 Defining physiological mechanisms and developing screening methods

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Rationale

Within the genus *Brachiaria*, signalgrass was found to be outstandingly resistant to aluminum (Al) as a result of unknown mechanisms independent of secretion of organic acid anions. Previous research by Wagatsuma and coworkers from the Yamagata University, Japan showed direct evidence of the contribution of plasma membrane (PM) lipids to Al resistance, i.e., the reduced permeabilization of plasma membrane as a mechanism of Al resistance in triticale. Here, we report a new feature of Al-resistance based on the PM lipid layer of signalgrass.

Materials and Methods

Al accumulation was observed after hematoxylin staining of root-tip portions treated for 24 h with 20 µM $AlCl_3$ in 0.2 mM $CaCl_2$ (pH 4.9) for other plant species or 100 µM $AlCl_3$ in 0.2 mM $CaCl_2$ (pH 4.2) for the two *Brachiaria* species (*B. decumbens* and *B. ruziziensis*). PM permeability was determined by fluorescein diacetate (FDA)-propidium iodide (PI) fluorescence microscopy (ex 450-490 nm, em 520 nm). PM lipids were obtained using the newly established technique and their composition was analyzed qualitatively by high performance thin layer chromatography (HPTLC). Flavonoid deposition was observed by fluorescence microscopy after staining with Naturstoffreagenz A (NA) (0.1% [w/v] 2-aminoethyl diphenyl borate (ex 390nm, em 450nm). Permeability experiments were carried out by using nylon microcapsule coated with different compositions of lipid molecules or catechin.

Results and Discussion

Root-tips of signalgrass accumulated the least Al among all plant species tested (Photo 14-B_1), indicating their strongest Al exclusion ability. The PM of the root-tip portion of signalgrass was less permeabilized after 9-h Ca treatment following 1-h Al treatment (Photo 15), indicating the strong structure of the PM lipid layer even after elongation of the root-tip portion under normal conditions. More glucocerebrosides and free sterols were observed in the PM lipid of root-tips for Al-tolerant maize cv. Neodent 90 than that in Al-sensitive cv. KD850 (Figure 25), and more free sterols were observed in signalgrass than ruzigrass (data not shown). Since glucocerebrosides and free sterols are, in general, the main lipid components of the PM lipid layer, they can primarily regulate PM physics. Artificially mixed lipids containing larger amounts of glucocerebrosides and free sterols showed less permeability during capsule experiments (data not shown). Glucocerebrosides have longer fatty acyl chains (typically C_{24}) and exist primarily in the outer leaflet of the PM. Several OHs from glucose and hydroxyl-fatty acyl chains, amide and carbonyl groups and other oxygen sites of glucocerebroside have been

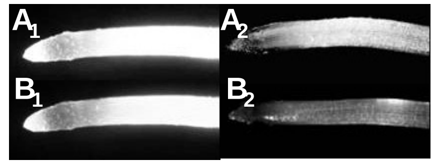

Photo 15. FDA-PI fluorescence microscopy. A: signalgrass, B: ruzigrass. Left: immediately after 1-h Al treatment, right: after 9-h Ca treatment following 1-h Al treatment (100 µM Al). Green fluorescence (white) shows an intact PM and red fluorescence (black) shows permeabilization of the PM.

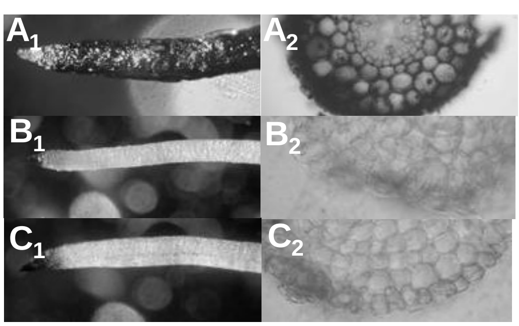

Photo 14. Hematoxylin staining of root-tip portions in A: an Al-sensitive wheat cultivar (Scout), B: signalgrass, C: ruzigrass. Left: portion 1 cm from the tip, right: section 2-3 mm from the tip. Strong purple (black in the picture) shows heavy deposition of Al.

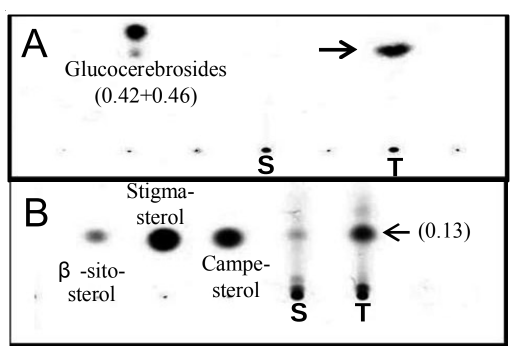

Figure 25. HPTLC analysis of PM lipids isolated from maize root-tips cultured under normal conditions. A: glucocerebrosides, B: free sterols. S: Al-sensitive cultivar (KD850), T: Al-tolerant cultivar (Neodent 90).

considered responsible for hydrogen bonding with neighboring phospholipids and formation of a transbilayer peg using long fatty acyl chains. Free sterols have also been considered responsible for formation of a more rigid lipid layer due to their planer steroid skeleton structure. All these considerations agree with the present capsule experimental data (not shown here).

It is concluded that a plasma membrane (PM) lipid layer containing large amounts of glucocerebrosides, free sterols, and flavonoids has superior resistance against permeabilization by Al ions. This is essential for Al resistance, which has been ascribed to reduced PM permeabilization. No difference was observed in phenylpropa1noid deposition between root-tips of the two *Brachiaria* spp. examined (data not shown). Flavonoids are synthesized in the cytoplasm and thereafter transported, in part, through the PM to the cell wall area. Further experiments are therefore needed in the future, especially to determine the localization of flavonoids within the PM lipid layer.

With all root sections, the outer surface of epidermal cells emitted strong yellowish-green fluorescence (Photo 16). Green fluorescence was recognized in the surface area of entire cortex cells in the root-tip portion of signalgrass. Flavonoids emit green fluorescence with NA staining, and several kinds of flavonoid compounds have been reported in the cell wall and vacuole using this technique. Flavonoids have been shown to penetrate membrane lipid interiors resulting in restriction of membrane fluidity. Many previous studies on animal nutrition, and membrane-rigidifying and tumor cell growth-inhibitory effects have been carried out in connection with the differential penetration activities of flavonoids. We observed reduced permeability in lipid mixture containing catechin (data not shown).

In summary, Al accumulation in the root-tip portion of signalgrass was lowest of all plant species examined in this report. The plasma membrane (PM) of the root-tip portion in signalgrass was less permeabilized than that in the less Al-tolerant ruzigrass after re-elongation of roots following short-term Al treatment. The PM of root-tips in an Al-tolerant maize cultivar was shown to contain larger amounts of glucocerebrosides and free sterols than an Al-sensitive maize cultivar. The permeability of nylon capsules coated with PM lipids isolated from root-tips of the Al-tolerant maize cultivar was lower than that of capsules coated with PM lipids from the Al-sensitive cultivar. Using Naturstoffreagenz A (2-aminoethyl diphenyl borate) fluorescence microscopy, larger amounts of flavonoids were found deposited in the root-tip portion of signalgrass compared to the other portion and species examined. Artificially mixed lipids containing larger amounts of glucocerebrosides, free sterols and catechin showed less permeability in Al medium. We propose a new feature of Al exclusion based on the special composition of the PM lipid layer.

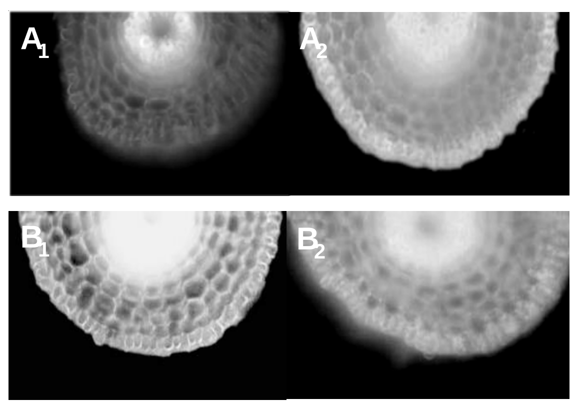

Photo 16. NA-stained fluorescence microscopy of roots grown under normal conditions. A: signalgrass, B: ruzigrass. Left: 2-3mm from the root tip, right: ca. 2cm from the root tip. Green (dark) fluorescence (A_1) shows higher deposition of flavonoids, and whitish blue (white) fluorescence shows autofluorescence by other phenolic compounds.

3.1.1.2 Methylene blue stainability of root-tip protoplasts as an indicator of aluminum resistance in a wide range of plant species, cultivars and lines

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Rationale

Several mechanisms of aluminum (Al) resistance in plants have been reported, (i.e., exudation of organic acid (OA) anions in wheat, cell wall properties in wheat, microtubule properties in cultured tobacco cells, plasma membrane strength (PMS) and root phenolics in woody plants, and plasma membrane (PM) intactness in several plant species). Although exudation of OA anions is accepted as a primary and widely applicable strategy for many plant species, no involvement of OA anions exudation in Al resistance has been reported in rice and sorghum, *Brachiaria* spp., rice, pea, and triticale.

It is generally believed that Al resistance is controlled by multiple genes with different degrees of contribution to Al resistance. Although intensive research work has been carried out using *Arabidopsis* on the identification of Al-tolerant genes and on making transgenic Al-tolerant *Arabidopsi*, Al tolerance of *Arabidopsis* is accepted to be a rather sensitive level lower than that of Al-sensitive wheat line ES3. Contrary to this, *B. decumbens* has been demonstrated to tolerate extraordinarily high concentration of Al ions. Considering no involvement of OA anions release in the extraordinary Al resistance of *B. decumbens*, it is suggested that there may be another higher Al-resistant strategy operated commonly and primarily in a wide range of plant species, cultivars and lines.

We developed a new simple, rapid and reliable technique for the discrimination of Al-tolerant protoplasts: methylene blue (MB) is strongly adsorbed on the plasmalemma of root cells in the tip portion of Al-sensitive plant species among 4 plant species, i.e., rice, oats, maize and pea. The objectives of the present research were to ascertain the applicability of this technique to a wide range of plant species, cultivars and lines and to identify Al-resistance mechanisms operated commonly and primarily in a wide range of germplasm.

Materials and Methods

Seeds of rice (*Oryza sativa* L. cv. Reikoushindan-kurodani and cv. Sasanishiki, *O. glaberrima* L. line W492), maize (*Zea mays* L. cv. Neodnet90 and cv. Golddent KD850), wheat (*Triticum aestivum* L. cv. Kitakami B, cv. Hachiman, cv. Atlas 66, cv. Scout 66, lines ET8 and ES8), barley (*Hordium vulgare* L. cv. Nozomi-nijo), and triticale (*Triticosecale* Wittmark cv. Currency lines ST22 and ST2) were soaked in tap water under aeration for 24 h in the former 2 species and for 12 h in the latter 3 species at 27^{o}C. The seeds of buckwheat (*Fogopyrum esculentum* Moench cv. Shinshu-1) and pea (*Pisum sativum* L. cv. Harunoka) were surface-sterilized for 10 min with 0.5% (v/v) NaClO mixed with 3 drops of Tween 80. The seeds of signalgrass (*Brachiaria decumbens* Stapf cv. Basilisk) and ruzigrass (*B. ruziziensis* Germain and Evrard cv. Common) were surface-sterilized for 1 min with 70% ethanol, and next for 10 min with 2% (v/v) NaClO mixed with 3 drops of Triton X-100. All these seeds were germinated on a nylon screen that was put on a polypropylene container filled with 8 liters of tap water under aeration at 27^{o}C. Seedlings with roots approximately 4 cm in length were used in the experiments. Seedlings were treated with 0.2 mM $CaCl_2$ for 6 h (pH 4.9), and then without (control) or with (+Al) 20 µM $AlCl_3$ in 0.2 mM $CaCl_2$ for 24 h under conditions maintaining pH at 4.9 for the whole duration under aeration at 27^{o}C. Al tolerance was defined as the relative net elongation of the longest root from each 10 seedlings between control and +Al treatments.

The isolation and purification of protoplasts from root-tips were carried out as follows. Briefly, apical 1 cm segments were digested with a medium composed of 0.6 M mannitol, 2% (w/v) Cellulase Onazuka RS, 0.1% Pectolyase Y-23, 0.05% (w/v) BSA, 1 mM $CaCl_2$, and 0.5 mM DTT (pH 5.6). The digested tissues separated by a nylon cloth were gently agitated and washed in suspension medium, and filtered. All filtrates were centrifuged at 200×g. The pellet of crude protoplasts was gently suspended in 30% (w/v) Ficoll in 0.6 M mannitol and 2 mM Tris-MES (pH 6.5). A discontinuous gradient was formed by successive layering of solutions of 8, 5 and 0% Ficoll in 0.6 M mannitol and 2 mM Tris-MES (pH 6.5). Purified protoplasts were collected after centrifugation at 380×g from the interface between the layers of 0% and 5% Ficoll. Isolation and purification was replicated separately in twice or three times.

The purified protoplasts were stained for 3 min with 0.1% (w/v) MB solution in 0.6 M mannitol (pH 4.2). The stained protoplasts were observed under a light microscope and photographed in identical conditions (light intensity: 6.0; fixed exposure; image quality, fine) with digital camera.
Image analysis was carried out by image analysis system. Debris and aggregated protoplasts in each picture were removed by Adobe Photoshop 6.0. File format was converted from Joint Photographic Experts Group (JPEG) to Moving Picture Coding Experts Group (MPEG). The original color pictures were converted to monochrome pictures, and they were treated as binary images. The area at threshold 125, 110, 95 or 85 for each treated image was measured in the conditions of 8-neighbor and minimum pixel of 50. The whole area was expressed as pixel number. For each plant cultivar or line, 5 pictures were selected and analyzed.

Thin section was cut by hand with razor blade at ca. 3 mm from root-tip of 3-4 cm root of maize (cv. Golddent KD850). The section stained preliminarily for 1 h with DAPI (4′,6-diamidino-2-phenylinodol), was put inside of the enclosed area made by cover slip (0.2 mm in thickness) which was adhered with manicure to the slide glass. The enclosed space was filled with digesting medium of the same composition as described above. Digestion process was observed with a fluorescence microscope equipped with a UV filter (Nikon, excitation filter 450-490nm; barrier filter 520 nm) at 15, 45 and 75 min after the start of digestion.

Seedlings other than two *Brachiaria* spp. with roots approximately 4 cm in length were pretreated with 0.2 mM $CaCl_2$ for 6 h (pH 4.9), then treated with 20 µM $AlCl_3$ in 0.2 mM $CaCl_2$ (pH 4.9) for 1 h, and next regrown in 0.2 mM $CaCl_2$ (pH 5.2) for 9h. Two *Brachiaria* spp. were treated with 100 µM $AlCl_3$ in 0.2 mM $CaCl_2$ (pH 4.2) for 2 h. After the Al treatment and regrowth, roots were stained for 10 min with fluorescein diacetate-propidium iodide (FDA-PI). In case of rice, wheat, triticale and *Brachiaria* spp., concentrations were FDA (12.5 mg L^{-1})-PI (5 mg L^{-1}), and in case of pea and maize, these were FDA (12.5 mg L^{-1})-PI (15 mg L^{-1}). After the removal of extra dyes with deionized water, the root-tips were observed under a fluorescence microscope equipped with a UV filter, and photographed by digital camera (Nikon, Coolpix 950). The root-tip portions and their hand sections 2-3 mm from root apices after 24-h treatment with Al were stained with hematoxylin and observed by light microscopy.

Results and Discussion

Under similar stress conditions (20 µM $AlCl_3$, for 24 h), Al tolerance among plant species was in general in the following order: two *Brachiaria spp*.>buckwheat, rice, maize>wheat, pea, triticale>barley. Although there were wide differences in Al tolerance among cultivars or lines within the same plant species, this order agrees with the general tendency: two *Brachiaria spp*. were most tolerant but, on the other hand, barley was most sensitive to Al. Between the two *Brachiaria spp*, signalgrass was more tolerant than ruzigrass. Within *Oryza* spp., *O. glaberrima* was most sensitive and within *O. sativa*, cv. Reikoshindan-kurodani was more tolerant than cv. Sasanishiki. For other plant species, Al tolerance among cultivars or lines was as follows: in wheat, cv. Atlas 66>line ET8, cv. Kitakami B>cv. Hachiman, line ES8, cv. Scout 66; in triticale, line ST2> line ST22.

Although permeabilities of the PM in root-tips of wheat just after 1-h of Al treatment were greater in lines ET8 and ES8, those after re-elongation in 0.2 mM $CaCl_2$ were greater in cv. Scout 66 and line ES8. Among *Brachiaria spp*., rice species (cv. Sasanishiki and *O. glaberrima*), triticale line (ST2 and ST22) and maize cultivars (Neodent 90 and Godldent KD850), the same tendency was observed, i.e., the more PM permeabilization in Al sensitive plant. These results confirm an essential role of the PM intactness of root-tip cells in early stage Al resistance of a variety of plant species.

Al accumulation in root-tips was in the following order: *Brachiaria spp*< rice < wheat, triticale. Among wheat cultivars and lines, Al-sensitive cv. Scout 66 and line ES8 accumulated more amount of Al in root-tips than Al-tolerant cv. Atlas 66 and line ET8. In Al-sensitive wheat cultivar and line, Al was accumulated most heavily in epidermis and also heavily in cortex, but on the contrary, in Al-tolerant wheat cultivar and line, Al was accumulated mainly in epidermis and endodermis. Although Al localization in a cell was recognized mainly in the cell surface area in Al-tolerant plants, higher distribution was also observed in symplastic area in Al-sensitive plants. These results indicate that the permeabilized PM of Al-sensitive plants permits a greater movement of Al into the symplast.

The isolated protoplasts were composed of a variety of protoplasts with differences in MB stainability. Methylene blue [3,7-bis(dimethylamino) phenothiazin-5-ium chloride] has a positive charge with amino and imino groups as auxochromous groups, reacts with sites with negative charges of PM, and assumes a blue color under aerobic conditions. We already reported that MB could be used as a colored analog to non-colored Al ions, and that the more Al sensitive plant species had greater number of heavily-stained protoplasts. The area at threshold 125 almost shows the total area of protoplasts stained with MB, but, on the other hand, the area at threshold 95 shows the total area of protoplasts stained heavily with MB. We defined the proportion of the heavily-stained area at threshold 95 to the whole stained area at threshold 125 as MB stainability (%) of the whole protoplasts. MB stainability was negatively correlated with Al tolerance among wider range of plant species, cultivars and lines (Figure 26, $y= 45.7e^{-0.017x}$, $R^2= 0.577^{**}$). Within the same plant species, negative relationship between Al tolerance and MB stainability was recognized in maize (Al tolerance: cv. Neodent 90>cv. Golddent KD850; MB stainability: in reverse order) and in wheat (Al tolerance: cv. Kitakami B, line ET8>cv. Scout 66, cv. Hachiman>>line ES8; MB stainability: in reverse order). Within rice species and cultivars, the relationship between the above two phenological characteristics was reverse to the former relationship (Al tolerance: cv. Reikoshindan-kurodani>cv. Sasanishiki> *O. glaberrima*; MB stainability: in the same order). Within triticale, Al tolerance was higher in line ST2, though no differences were observed in MB stainiabilities.

There are no clear explanations to these complex relationships among plant species, cultivars and lines. The whole relationship in Figure 26 using 18 plant species, cultivars and lines agree with the former result using only 4 plant species.

The protoplasts used for MB stainability (Figure 26) are expected to be isolated mainly from the outer and center cortices and epidermis and partially from the center of stele. As epidermis, outer and center cortices are the primary cells for Al accumulation in roots, the isolated protoplasts used for MB stainability were accepted to be appropriate for Al research.

MB was observed not only on the protoplast surface but also inside of the protoplast. Surface MB is considered to be the adsorbed MB on the negative sites of PM and inside MB to be permeated MB. This means the similarity in the behaviour of MB to Al ions. Negative correlation between Al tolerance and MB

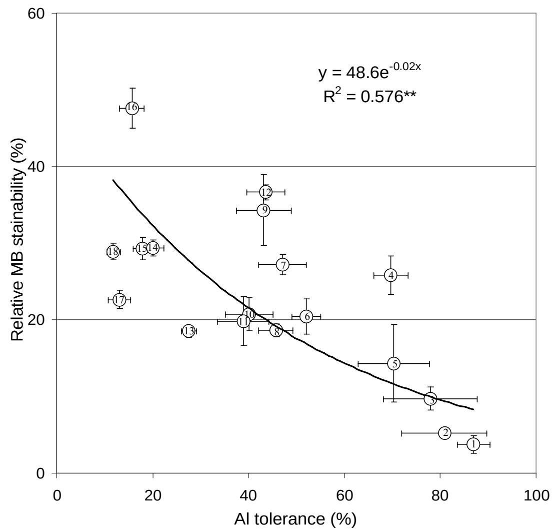

Figure 26. Relationship between Al tolerance among wider range of plant species and MB stainability of protoplasts. Al treatment: 20 μM $AlCl_3$ (pH 4.9) for 24 h. Al tolerance: ratio of the net root elongation of the longest root in Al treatment to that in control. MB stainability of protoplasts (%): ratio of the heavily-stained area at threshold 95 to the whole stained area at threshold 125. 1: signalgrass, 2: ruzigrass, 3: maize (cv. Neodent 90), 4: rice (cv. Reikoushindan-kurodani), 5: buckwheat, 6: rice (cv. Sasanishiki), 7: wheat (cv. Atlas 66), 8: Maize (cv. Golddent KD850), 9: triticale (line ST2), 10: wheat (line ET8), 11: wheat (cv. Kitakami B), 12: pea, 13: *Oryza glaberrima*, 14: Barley, 15: wheat (cv. Hachiman), 16: wheat (line ES8), 17: triticale (line ST22), 18: wheat (cv. Scout 66).

stainability (Figure 26) among a range of plant samples with wider differences in Al resistance finally suggests the importance not only of the less negativity of cell surface but also of the PM strength as has already been defined as the intactness of PM permeability for Al resistance and that this idea can be applicable to a variety of plant samples. Higher significance level found in the exponential regression line (Figure 26) suggests the greater Al permeation in Al-sensitive plants. Al permeation has been suggested to be dependent mainly of the lipid composition of PM.

Although cell wall has been reported as one of the important apparatus for Al tolerance, the negative correlation between Al tolerance and MB stainability among a variety of plant samples strengthens the significant role of the PM strength in Al tolerance of wider range of plant species, cultivars and lines.

There are many known and possible factors that can affect membrane rigidity, fluidity and permeability in association with the composition of lipid molecules (phospholipids, sterols or glucocerebrosides), differences in acyl chains and lipid saturation, and polysaccharides or phenolic environment near membrane.

Further research is needed to clarify the role of lipid composition of PM and its genetic and molecular characteristics as the controlling factors for Al resistance.

In summary, we confirmed the applicability of a new simple, rapid and reliable technique of methylene blue (MB) staining for the discrimination of aluminum (Al)-resistant protoplasts from a wider range of plant species, cultivars and lines and identified a common strategy for Al resistance at an early stage.

A total of 10 plant species, i.e. two *Brachiaria* spp., two *Oryza* spp., buckwheat, maize, pea, triticale, wheat and barley were tested. Al resistance (based on relative net root elongation of the longest root) evaluated at 20 μM $AlCl_3$ (pH 4.9) for 24 h ranged widely from 10 to 88. Among cultivars and lines within the same species, Al accumulation in root-tip portion was greater in Al-sensitive plant, which corresponded to greater permeability of plasma membrane. MB stainability was negatively correlated with Al resistance.

This observation indicates the common importance of PM strength that was already defined as the intactness of PM permeability in addition to PM negativity for Al resistance of a wide range of plant species, cultivars and lines. Defining the role of lipid composition of PM will be an important future task in connection with PM strength.

3.1.1.3 Role of Phospho*enol*pyruvate carboxylase in the adaptation of *Brachiaria* hybrid cv. Mulato to low phosphorus acid soils

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Rationale

Phosphorus (P) deficiency is an important factor limiting forage production in tropical and sub-tropical soils. In these soils, P forms insoluble compounds with a number of di- and tri-valent cations (*e.g.,* Al^{3+}, Fe^{3+}) and it is the least readily available nutrient in the rhizosphere. Correcting P deficiency with application of P fertilizer is not a viable approach for resource-poor farmers in the tropics and sub-tropics, especially on soils with high P-fixing capacity. Under such conditions, the integration of field crops with forage cultivars that can make most efficient use of the P supplied as maintenance fertilizer application represents a key element of sustainable crop-livestock systems in the tropics. Genetic variations in P uptake efficiencies have been widely reported in several food and feed crops. Plant traits responsible for P uptake efficiency include rhizosphere acidification, root exudation of organic acid anions and phosphate mobilizing enzymes, root morphology, uptake kinetics and symbiotic association with mycorrhizal fungi.

Plants have evolved two broad strategies for improving P acquisition and use in nutrient-limiting environments: (1) those aimed at efficient P use; and (2) those directed toward enhanced P acquisition or uptake. Processes that use efficiently the acquired P involve decreased growth rate, increased growth per unit of P uptake, remobilization of internal Pi, modification in internal carbon metabolism that bypass P-requiring steps, and alternative respiratory pathways. By comparison, processes that lead to increased P uptake include enhanced secretion of phosphatases and exudation of organic acids, changes in root morphology and enhanced expression of Pi transporters.

To enhance P uptake, because of their high affinity for divalent and trivalent cations, organic acid anions released from the roots are thought to displace P from insoluble complexes, making it more available for uptake by plants. Organic acid anions also play an important role in detoxification of Al both externally and internally. Enhanced expression and activity of phospho*enol*pyruvate carboxylase (PEPC) has been linked with P deficiency-induced biosynthesis and root exudation of carboxylic acids.

Phospho*enol*pyruvate carboxylase (PEPC) is a cytosolic enzyme widely distributed in most plant tissues, green algae and microorganisms but not in animal cells. PEPC is an important enzyme for the carbon economy of the cell, playing a central role in CO_2 fixation of C_4 and crassulacean acid metabolism (CAM) plants. PEPC activity produces OA (oxaloacetic acid) and malate that replenish the citric acid cycle, the so-called anaplerotic function, providing carbon skeletons for nitrogen assimilation. Recent findings suggest that PEPC may play an important role in the adaptation of plants to environmental stress factors.

Inherent differences in efficiencies of P uptake and utilization exist between tropical forage grass and legume species. Previous research showed that phosphorus use efficiency (PUE) in grass (*e.g., Brachiaria* spp.) is much higher than that of the legumes (*e.g., Stylosanthes* spp.) regardless of P supply. Widespread adoption of forage cultivars depends on efficiently acquiring P from the soil and using them for growth. Plant attributes appear to be linked to different strategies to acquire and use phosphorus. Understanding these linkages is fundamental in integrating plant attributes in a selection index. It is essential to elucidate the mechanisms of plant species conferring superior adaptation to P deficient acidic soils. Although knowledge about the principal mechanisms involved in efficient P acquisition by plants has evolved substantially during recent years, the detailed mechanisms of internal P requirements among different genotypes of crop and forage plants are still not fully understood. In collaboration with the group at Hokkaido University, we have evaluated the role of phospho*enol*pyruvate carboxylase in improving PUE under P deficiency and soil acidity in *Brachiaria* hybrid cv. Mulato comparing with wheat (low P-sensetive) and rice (low P-tolerant).

Materials and Methods

Experiment 1. Effect of low pH and low P in soil. Experiment was conducted in a glasshouse (43^{o} 3' N, 141^{o} 2' E, altitude 17 m; maximum temperature 32^{o} C; minimum temperature 16^{o} C; average photoperiod during experiment = 14.8 h light and 9.2 h darkness; maximum photon flux density = 1550 µmol m^{-2} s^{-1}) of Graduate School of Agriculture of Hokkaido University, Sapporo, Japan. Seeds of *Brachiaria* hybrid cv. Mulato CIAT 36061 (developed from parents of *Brachiaria ruziziensis* clone 44-06 and *Brachiaria brizantha* cv. Marandú and identified as FM9201/1873), wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L. cv. Kitaake) were surface sterilized with 1% of sodium hypochlorite for 10 min. Rice seeds were germinated on a petri dish for 3 days. Seeds (germinated in case of rice) (2-6) were sown in small plastic pots (160 ml) containing soils with two levels of P (+P and -P) and three levels of pH (4.0, 4.5 and 5.0), of which soil were collected from a long-term (25 years-old) experimental field without P fertilizer input, with pH (H_2O) 4.0. Initially, soil was fertilized with 1.87 g N kg^{-1} and 0.8 g K kg^{-1} soils as $(NH_4)_2SO_4$ and K_2SO_4, respectively. In case of +P treatments, $Ca(H_2PO_4)_2$ was added at the rate of 2.22 g kg^{-1} of soil while no P was added in -P treatments. Available P concentration (Bray II, mg/100g soil) was 5.5-5.9 in –P treatment, and 14.0-15.6 in +P treatment, which was higher in lower pH soil. The pH levels of soil were adjusted by adding appropriate amounts of calcium carbonate or 0.1 N H_2SO_4 to the soil. Each treatment was replicated for 6 times arranged in complete randomized block design.

Plants were harvested at 28-50 days of cultivation. Their roots were washed with tap water and then gently washed with deionized water, and plants were separated into root, stem and leaf. Half of each sample was dried in air-forced oven at 80°C for 72 h, then weighed, and ground for nutrient analysis. Another half of each sample was chilled by liquid nitrogen and then stored at -80°C for PEPC and organic acid analyses. Leaves of *Brachiaria* hybrid, wheat and rice were analyzed for PEPC and organic acid analysis. Dried sample was digested with sulfuric acid and hydrogen peroxide. Total nitrogen and P were measured by semi-micro Kjeldahl-method and vanado-molybdate yellow method, respectively.

Lyophilized leaves were homogenized in cold 0.01N HCl sample:HCl = 1:10 in leaves) to determine the organic acid concentrations in the plant. The extract was filtered with a membrane filter (pore size = 0.45 ìm). The organic acid anions were analyzed by a Capillary Ion Analyzer under the following measurement conditions: electrolyte, 2.5% CIA-PAK™ OFM Anion BT in 120 mM $Na_2B_4O_7$; capillary fused silica; and detection, 185 nm. Identification and detection of organic acid anions were done by comparing their retention time and absorption spectra with those of known standards.

For the measurement of PEPC activity under malate inhibition conditions, 200 ìl of the supernatant of the protein extraction solution was mixed with 300 ìl of saturated Na_2CO_3, and set on ice for 10 min. The precipitate was obtained by centrifugation at 17,000 g for 10 min at 4^{0}C, then resuspended with 20 ìl of 50 mM Hepes-NaOH (pH 7.5), 5% (v/v) glycerol, 5 mM $MgCl_2$, 1 mM EDTA, 14 mM 2-mercaptoethanol, 1 mM PMSF, and 10 ìg mL^{-1} chymostatin. After centrifugation at 17,000 g for 5 min at 4^0C, 20 ìl of the supernatant was mixed with 913 ìl of pre-reaction mixture containing 100 mM Hepes-NaOH (pH 7.3), 10 mM $MgCl_2$, 1 mM Na_2CO_3, and 0.2 mM NADH (dissolved in 50 mM Tris-HCl (pH 7.4), and 20 ìl of 50 mM malate and 2 units of MDH. The change in the amount of NADH was monitored after the addition of 40 ìl of 50 mM PEP. To measure the maximum activity of PEPC, 0.2 g of leaf was homogenized with 50 mM Hepes-KOH (pH 7.4), 10 % (v/v) glycerol, 1 mM EDTA, 10 mM $MgCl_2$, 5 mM DTT, 1 mM PMSF, 10 ìM leupeptin, and 5 % (w/v) polyvinyl-polypyrrolidone. 25 ìl of the supernatant was mixed with 908 ìl of pre-mixture, containing 100 mM Hepes-NaOH (pH 7.5), 10 mM $MgCl_2$, 1 mM $NAHCO_3$, 0.2 mM NADH (in 50 mM Tris-HCl), 2 ìl of MDH (diluted to 2 units with 50 % (v/v) glycerol). The amount of NADH was monitored after the addition of 40 ìl of 100 mM PEP. The amount of soluble protein was determined by the Bradford method, using BSA as a standard. This method was applied also in experiment 2.

Experiment 2. PEPC response to low phosphorus in hydroponic solution. *Brachiaria* hybrid cv. Mulato and rice (*Oryza sativa* L. cv. Kitaake) were grown hydroponically under greenhouse conditions. Seedlings were pre-cultured in a 56 L vessel containing 2.12 mM N, 0.77 mM K, 1.25 mM Ca, 0.82 mM Mg, 35.8 µM Fe, 9.1 µM Mn, 46.3 µM B, 3.1 µM Zn, 0.16 µM Cu, 0.05 µM Mo, with 6 µM P. After one week pre-culture, plants were transplanted to 56 L vessels with three levels of P concentration (0 µM, 6 µM and 32 µM, respectively) for two weeks. Phosphorus concentration was measured and adjusted to the respective levels of treatment everyday. The pH of the nutrient solution was adjusted to 5.2 ± 0.1 everyday. The nutrient solution was completely renewed once a week. Three plants were pooled for one replication and each experiment was conducted with three replications. A total of three experiments were conducted. Half of the collected plants was dried in 80ˆC oven for 3 days and weighed. The remaining half was frozen in liquid nitrogen and stored at –80 °C until the analysis of Pi and enzyme activities. All the statistical analyses were done using the SPSS (Windows 10.0) computer program.

Results and Discussion

Experiment 1. Effect of low pH and low P in soil. Dry weights of three crops were lower at -P treatment than those at +P treatment (Table 24). Dry weights of wheat and rice decreased with decrease of soil pH regardless of P treatments.

Table 24. Effect of phosphorus and pH treatments on dry matter production (g/plant).

		-P			+P		
Plant	Organ	pH 4.0	pH 4.5	pH 5.0	pH 4.0	pH 4.5	pH 5.0
Brachiaria	Shoot (S)	0.19±0.03	0.27±0.02	0.20±0.02	0.27±0.03	0.33±0.02	0.32±0.02
	Root (R)	0.06±0.02	0.09±0.01	0.06±0.01	0.08±0.02	0.10±0.01	0.09±0.02
	Total	0.25±0.07c	0.36±0.03b	0.26±0.04c	0.35±0.04b	0.43±0.04a	0.41±0.04a
	R/S ratio	0.32	0.33	0.30	0.30	0.30	0.28
Wheat	Shoot	0.03±0.01	0.03±0.00	0.04±0.01	0.02±0.00	0.04±0.00	0.07±0.01
	Root	0.01±0.00	0.02±0.01	0.03±0.01	0.01±0.00	0.03±0.01	0.05±0.02
	Total	0.04±0.01d	0.05±0.01c	0.07±0.02b	0.03±0.01c	0.07±0.01b	0.12±0.03a
	R/S ratio	0.33	0.66	0.75	0.50	0.75	0.71
Rice	Shoot	2.14±0.25	2.11±0.24	2.44±0.28	2.39±0.27	2.25±0.26	2.81±0.32
	Root	0.41±0.09	0.41±0.09	0.45±0.10	0.40±0.09	0.47±0.11	0.49±0.11
	Total	2.55±0.12c	0.52±0.05c	2.89±0.13b	2.79±0.05bc	2.72±0.14bc	3.30±0.11a
	R/S ratio	0.19	0.19	0.20	0.17	0.21	0.17

Note:Values are the mean of three replicates. Treatment means are different ($P<0.05$) if denoted by different letters (Duncan's multiple range test).

However, dry weight of *Brachiaria* hybrid at -P treatment were higher at pH 4.5 than those of pH 4.0 and 5.0, adapting well to low pH (4.0) even at low levels of soil P, which was more adaptable than the higher pH (5.0). At +P treatment, dry weight of *Brachiaria* hybrid was not much affected by soil pH. Root-to-shoot ratio of *Brachiaria* hybrid increased by P-deficiency, whereas that of wheat and rice was stable or decreased by P-deficiency (Table 24). This ratio remained constant in *Brachiaria* hybrid and rice regardless of decreasing soil pH, however decreased in wheat by a decrease of soil pH. Thus, relative root growth was vigorous in *Brachiaria* hybrid and rice under low pH, also vigorous in rice under low pH, and weak in wheat under low P and low pH. Consequently, root function of Brachiaria hybrid was maintained or stimulated under low pH and low P soils.

Amount of P absorbed by plants was quite similar to dry matter production, indicating that P accumulation and tolerance to low pH were key factors that contributed to plant production in this experiment (Table 25). When relative P absorption ability under acidic low P soil was estimated as (amount of P at -P. pH 4.0/ amount of P at +P. pH 5.0), this ratio was 0.52 in *Brachiaria,* 0.33 in wheat, 0.47 in rice, suggesting that *Brachiaria* hybrid had greater ability to absorb P from acidic low P soil than other crops. Amount of nitrogen absorbed by *Brachiaria* hybrid was less affected by P and pH treatments, whereas response of amount of N absorbed to P and pH treatments was almost similar to those of dry matter production and absorption of P by rice and wheat. It appeared that root activity of *Brachiaria* hybrid was less affected because of constant ability of nitrogen uptake. As expected, available P in soil was affected by P treatment and pH and therefore plant growth was affected by P availability. Root activity was severely depressed in wheat by low P and low soil pH and in rice by low pH.

Phosphorus use efficiency (PUE) was higher in *Brachiaria* hybrid than that of wheat and rice (Table 26). PUE of *Brachiaria* hybrid increased tremendously by P deficient treatment, however, that of rice and wheat was remained constant or slightly increased. In *Brachiaria* hybrid, P use efficiencies were remained constant, or decrease slightly with a decrease of soil pH. Thus *Brachiaria* hybrid seems to utilize absorbed P more efficiently compared to wheat and rice especially under low pH condition.

Fumarate was found as a major organic acid in leaves of *Brachiaria* hybrid regardless of P and pH levels followed by oxalate (Table 27). Oxalate concentrations in leaves of *Brachiaria* hybrid were decreased under P deficient condition. Fumarate was also a major organic acid in wheat leaves followed by malate. A trace amount of oxalate was detected in leaves of wheat. On the other hand, oxalate, a-ketogluterate, malate and

Table 25. Effect of phosphorus and pH treatments on total amount of phosphorus (mg P/plant) accumulated.

Plant	Organ	-P pH 4.0	-P pH 4.5	-P pH 5.0	+P pH 4.0	+P pH 4.5	+P pH 5.0
Brachiaria	Shoot	0.19±0.03	0.27±0.02	0.20±0.02	0.27±0.03	0.33±0.02	0.32±0.02
	Root	0.06±0.02	0.09±0.01	0.06±0.01	0.08±0.02	0.10±0.01	0.09±0.02
	Total	0.25±0.07c	0.36±0.03b	0.26±0.04c	0.35±0.04b	0.43±0.04a	0.41±0.04a
Wheat	Shoot	0.03±0.01	0.03±0.00	0.04±0.01	0.02±0.00	0.04±0.00	0.07±0.01
	Root	0.01±0.00	0.02±0.01	0.03±0.01	0.01±0.00	0.03±0.01	0.05±0.02
	Total	0.04±0.01d	0.05±0.01c	0.07±0.02b	0.03±0.01c	0.07±0.01b	0.12±0.03a
Rice	Shoot	2.14±0.25	2.11±0.24	2.44±0.28	2.39±0.27	2.25±0.26	2.81±0.32
	Root	0.41±0.09	0.41±0.09	0.45±0.10	0.40±0.09	0.47±0.11	0.49±0.11
	Total	2.55±0.12c	0.52±0.05c	2.89±0.13b	2.79±0.05bc	2.72±0.14bc	3.30±0.11[a]

Note:Values are the mean of three replicates. Treatment means are different ($P<0.05$) if denoted by different letters (Duncan's multiple range test).

Table 26. Phosphorus use efficiency*of whole plants grown in soil with phosphorus and pH treatments.

	-P			+P		
Plant	pH 4.0	pH 4.5	pH 5.0	pH 4.0	pH 4.5	pH 5.0
Brachiaria	0.79±0.04ab	0.88±0.05a	0.79±0.01ab	0.53±0.01c	0.52±0.01c	0.72±0.07b
Wheat	0.36±0.01b	0.49±0.01a	0.49±0.05a	0.30±0.02c	0.40±0.01	0.39±0.01b
Rice	0.48±0.01a	0.47±0.01a	0.48±0.01a	0.31±0.01b	0.29±0.01c	0.30±0.01bc

Note: *Phosphorus use efficiency = Total dry weight (g/plant)/Total amount of phosphorus uptake (mg/plant). Values are the means of three replications ±SE. Different letters in each species indicate statistical significance (P<0.05) by Duncan`s multiple range test .

Table 27. Organic acid concentration (m mol/g leaf dry weight) in leaves of plants grown in soil with phosphorus and pH treatments.

Plant	Treatments		------------------------------------ organic acids ---					
	P	pH	Oxalate	Fumarate	a-ketoglutarate	Malate	Citrate	Total
Brachiaria	-P	4.0	12±0.1b	159±7.2b	n.d.	n.d.	n.d.	171±3.7
		4.5	9±0.1d	106±0.7d	n.d.	n.d.	n.d.	115±0.4
		5.0	10±0.3cd	121±0.9c	n.d.	n.d.	n.d.	131±1.1b
	+P	4.0	20±1.2a	156±2.1b	n.d.	n.d.	n.d.	176±1.6
		4.5	11±0.2bc	146±0.5b	n.d.	n.d.	n.d.	157±0.4
		5.0	11±0.1bc	248±2a	n.d.	n.d.	n.d.	259±1.4
Wheat	-P	4.0	2±0.0c	35±0.2e	n.d.	3±0.1e	n.d.	40±0.2
		4.5	3±0.1b	69±0.1c	n.d.	8±0.7ab	n.d.	80±0.3
		5.0	4±0.2a	109±2.1a	n.d.	8±0.3ab	n.d.	121±0.9
	+P	4.0	0.0±0.1d	28±0.9f	n.d.	5±0.9d	n.d.	34±0.6
		4.5	3±0.1b	48±0.2d	n.d.	9±0.2a	n.d.	60±0.2
		5.0	3±0.1b	94±0.7b	n.d.	6±0.3cd	n.d.	103±0.4
Rice	-P	4.5	39±2.9bc	n.d.	6±0.1c	24±5.3ab	7±1.3b	76±0.4
		5.0	42±1.5ab	n.d.	5±0.3c	28±5.6a	13±1.1c	88±1.1c
	+P	4.0	42±1.0cb	n.d.	5±1.3c	17±2.1bc	13±4.0c	77±1.6
		4.5	44±1.3a	n.d.	12±0.5b	11±0.4c	16±0.1a	83±0.4
		5.0	39±0.6bc	n.d.	4±0.1c	20±1.3abc	7±0.4b	70±1.4

Note : n.d. = not detected. Values are the means of three replications ±SE. Different letters in each organic acid in each crop species under various P and pH treatments differ significantly. Treatment means are different (P<0.05) if denoted by different letters (Duncan's multiple range test).

citrate were detected in leaves of rice plant. It appeared that phosphorus and pH treatments had less effect on amount of total organic acid anions in rice plants. Total organic acid level was higher in *Brachiaria* hybrid than in rice and wheat, indicating that in *Brachiaria* hybrid organic acid metabolism and its pool was active and large. PEPC maximum activity in leaves was extremely high in *Brachiaria* hybrid because of its C_4 photosynthetic pathway (Table 28). PEPC maximum activity was higher in –P treatment than in +P treatment, and did not respond to soil

Table 28. PEPC activity and malate inhibition ratio of PEPC in leaves of plants grown in soil with phosphorus and pH treatment.

Plant	Treatments		Malate inhibition ratio*	PEPC activity
	Phosphorus	pH		(μmol/mg protein/min)
Brachiaria	-P	4.0	0.33±0.02	8.46±0.09b
		4.5	0.24±0.06	9.15±0.15a
		5.0	0.58±0.01	9.51±0.23a
	+P	4.0	0.54±0.07	4.40±0.07c
		4.5	0.49±0.04	5.32±0.11d
		5.0	0.43±0.06	5.80±0.11c
Wheat	-P	4.0	0.59±0.02	0.13±0.01b
		4.5	0.61±0.04	0.12±0.02c
		5.0	0.65±0.02	0.15±0.01a
	+P	4.0	0.48±0.05	0.09±0.01d
		4.5	0.68±0.01	0.04±0.00f
		5.0	0.54±0.02	0.05±0.00e
Rice	-P	4.0	0.72±0.09	0.09±0.01d
		4.5	0.69±0.01	0.12±0.02b
		5.0	0.49±0.06	0.10±0.01c
	+P	4.0	0.76±0.01	0.09±0.01d
		4.5	0.69±0.06	0.12±0.02b
		5.0	0.49±0.06	0.10±0.01c

Note: Values are the means of three replications ±SE. Different letters in each species indicate statistically significant ($P<0.05$) by Duncan's test.
*Malate inhibition ratio = [(- malate PEPC activity)-(+ malate PEPC activity)] / [(- malate PEPC activity)].

pH. PEPC maximum activity of rice and wheat was slightly higher in -P treatment than in +P treatment. Malate inhibition ratio of PEPC in *Brachiaria* hybrid was lower in –P treatment than in +P treatment, especially at lower soil pH (Table 28). This ratio in wheat did not respond to P and pH treatment, and in rice did not respond to P treatment, but increased with decrease of soil pH.

Experiment 2. PEPC response to low phosphorus hydroponic medium. Phosphorus concentration in leaf (mg/g) and root of both *Brachiaria* hybrid and rice was extremely low with -P treatment (Table 29). Phosphorus concentration in *Brachiaria* hybrid leaf was 0.67 at –P treatment and 8.33 at +P treatment. Thus in nutrient culture, P treatment was extremely low

Table 29. Phosphorus concentrations (mg g^{-1} DW) in plants grown in hydroponics with different phosphorus treatment.

Plant	Phosphorus		Phosphorus concentration (mg/g DW)	
	Treatments	Leaf	Root	Whole plant
Brachiaria	-P	0.67±0.01b	1.09±0.06b	0.84±0.03b
	+P	8.33±0.12a	7.7±0.06a	8.20±0.08a
Rice	-P	0.37±0.01b	0.70±0.01b	0.72±0.00b
	+P	6.95±0.15a	6.34±0.09a	6.83±0.14[a]

Note : Values are the means of three replications ±SE. Different letters in each organ in each crop species under P treatments differ significantly. Treatment means are different ($P<0.05$) if denoted by different letters (Duncan's multiple range test).

at –P treatment and high at +P treatment, comparing with soil culture experiment. In *Brachiaria* hybrid, oxalate and fumarate were dominant organic acids, and these two decreased by –P treatment (Table 30). In rice, oxalate, a-ketogluterate, malate, and citrate were main organic acids. However, P response was different between oxalate, a-ketogluterate (decreasing by -P treatment) and malate, citrate (increasing by –P treatment).

By -P treatment, PEPC activity of *Brachiaria* hybrid decreased in leaf, and increased in roots (Table 31). Also by –P treatment, PEPC activity of rice remained almost constant in both leaf and roots. Malate inhibition ratio of PEPC in leaf decreased in *Brachiaria* hybrid, but remained constant in rice under –P treatment (Table 31). This ratio was higher in rice than in *Brachiaria* hybrid, indicating that PEPC of rice was mostly inactive.

Brachiaria species are adapted to low-fertility acid soil of the tropics because they are highly tolerant to high Al and low P and Ca. In P deficient condition, they may improve P acquisition by enhancing its root growth, uptake efficiency and ability to utilize poorly available P to plants. It was expected that *Brachiaria* species adapted to low P medium by two strategies; 1) efficient P-uptake, and 2) efficient P utilization in tissue. From acidic low P soil, *Brachiaria* hybrid had relatively high ability to absorb P from soil compared to rice and wheat. This was owing to high root activity in *Brachiaria* than the other two crops. It appeared that in *Brachiaria* hybrid under low P conditions (both soil and hydroponics culture), 1) PEPC activity increased in both leaves and roots, 2) malate inhibition ratio in leaves decreased (results not shown), and 3) organic acid levels decreased. Therefore, it is highly probable that high P uptake depends on high PEPC activity and high rate in organic acid

Table 30. Concentrations of (mmol/g DW) of organic acids in leaves of plants grown in hydroponics with different phosphorus treatments.

Plant	Treatment	--- organic acids ---					
	P treatment	Oxalate	Fumarate	a-ketoglutarate	Malate	Citrate	Total
Brachiaria	-P	10.5±1.1b	90.4±8.0b	n.d.	n.d.	n.d.	100.9
	+P	27.4±5.6a	134.0±16.3a	n.d.	n.d.	n.d.	161.4
Rice	-P	14.1±0.30b	n.d.	9.5.±1.20b	15.3±0.10a	3.98±0.40a	42.9
	+P	69.6±6.80a	n.d.	18.7±1.20a	3.97±0.9b	2.94±0.80b	95.2

Note : n.d. : not detected. Values are the means of three replications ±SE. Different letters in each organic acid in each crop species under P treatments differ significantly. Treatment means are different (P<0.05) if denoted by different letters (Duncan's multiple range test).

Table 31. PEPC activity (m mol/min/mg protein) and malate inhibition ratio in leaves of plants grown hydroponical in different phosphorus treatment.

Plant	P treatment	Malate inhibition ratio* in leaves	PEPC activity	
			Leaves	Roots
Brachiaria hybrid	-P	0.49±0.03	2.01±0.20b	0.31±0.02a
	+P	0.62±0.01	3.11±0.27[a]	0.18±0.02b
Rice	-P	0.84±0.02	0.06±0.01[a]	0.21±0.02a
	+P	0.89±0.01	0.05±0.00b	0.18±0.01b

Note: Values are the means of three replications ±SE. Different letters in each organ in each crop species under P treatments differ significantly. Treatment means are different (P<0.05) if denoted by different letters (Duncan`s multiple range test).

*Malate inhibition ratio = [(- malate PEPC activity)-(+ malate PEPC activity)] / [(- malate PEPC activity)].

metabolism resulting in exudation of organic acids from roots to solubilize relatively less available P in soil.

On the other hand, P use efficiency (PUE) of *Brachiaria* hybrid was extremely higher in -P treatment than +P treatment, comparing with wheat and rice. The PUE of *Brachiaria* hybrid was significantly augmented in response to P-deficiency, which was maximum (0.88) at pH 4.5.

Therefore, *Brachiaria* hybrid has an excellent P utilization mechanism in tissue once P is absorbed. A high P use efficiency is advantageous in low P acid soils, because the plant can then maximize the amount of biomass produced per unit P and thus dominate use of the resources available.

To explain one of the mechanisms of high P use efficiency, it has been suggested that P deficiency induces some glycolytic enzymes. Phospho*enol*pyruvate carboxylase (PEPC) and phospho*enol*pyruvate phosphatase (PEPP) that catalyze bypass reaction of pyruvate kinase (PK), responsible for the regulation of carbon flow from glycolysis to TCA cycle, are induced under P deficiency. This induction is supposed to play an important role in organic acid metabolism and Pi recycling under P deficient condition. In P deficient bean leaves, the increased rate of malate synthesis and enhanced accumulation of aspartate and alanine, the products of PEP metabolism, were observed. It was suggested that the increased activity of PEPC and the utilization of PEP to amino acids synthesis might be the most important response for phosphate recycling in bean leaves at the early stage of P deficiency.

Similar increases in PEPC activity in response to P-deficiency have been noted in the cluster roots of white lupin, in chickpea (*Cicer arietinum*), oilseed rape, and *Sesbania rostrata*. Also, enhanced expression and activity PEPC has been linked with P-deficiency-induced biosynthesis and root exudation of carboxylic acids.

Under P-deficient conditions, the PEPC reaction, which liberates oxaloacetate and Pi, may have a function for Pi recycling in PEP catabolism as a bypass for the ADP- and Pi-depended pyruvate kinase. In general, however, C_4-PEPC in C_4 cycle should not contribute to Pi recycling and large amounts of organic acid production because in C_4 cycle substrates are recycled. In the present experiment, PEPC activity in leaves of *Brachiaria* hybrid, a C_4 plant, was increased up to 3 folds in response to P-deficiency.

It appeared that higher PEPC activity might be related to higher PUE in *Brachiaria* hybrid. In current study, we could not estimate whether C_4- or C_3-PEPC of *Brachiaria* hybrid had a function in responding to P deficiency, suggesting the need for further research to define the precise role of PEPC of *Brachiaria* in adaptation to low P acid soils.

In summary, in *Brachiaria* hybrid under acidic low P soil: 1) P uptake remained high because of high relative P absorption ability and high root activity (estimated from high nitrogen absorption rate), and 2) PUE was significantly high which was appeared to be associated with higher PEPC activity and lower malate inhibition ratio in leaves. Thus, PEPC activity contributed greatly to P uptake/and PUE of *Brachiaria* hybrid and contributed less to rice and wheat (low P use efficient crops) under low P and pH conditions. Consequently, total organic acid level in *Brachiaria* leaf was lower in -P treatment than in +P treatment indicating that organic acids were metabolized actively by increased PEPC activity and decreased malate inhibition ratio in leaves.

Taken together, these results suggest that PEPC activated in *Brachiaria* hybrid under low P supply and low pH conditions could contribute to its greater adaptation to tropical acid soils with low P availability.

3.1.2 Development of a greenhouse method to screen *Brachiaria* genotypes for aluminum resistance and root vigor

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Rationale

Some *Brachiaria* grasses such as *B. decumbens* cv. Basilisk are well-adapted to the soils of Neotropical savannas. *Brachiaria ruziziensis* pastures, by contrast, tend to degrade within a few years after establishment. The acid and infertile savanna soils are characterized by a combination of nutrient deficiencies (most significantly P, but also Ca, Mg, Mo and sometimes N and K) and mineral toxicities (Al; occasionally Mn). Edaphic adaptation presumably is an aggregate trait expressed in mature plants that comprises physiological components conferring adaptation to these stress components.

We are seeking to develop apomictic interspecific hybrids by combining traits of three parental species: acid-soil adaptation and spittlebug resistance of *B. decumbens* and *B. brizantha*, respectively (both tetraploid apomicts), and sexual reproduction of a tetraploidized, sexual biotype of *B. ruziziensis*, which lacks both agronomic traits. Efficient screening methodologies are required to recover the desired traits through stepwise accumulation of favorable alleles in subsequent cycles of recombination and selection. Our aim was to develop a greenhouse-based method to assess edaphic adaptation of large segregating populations.

The main objective of this study was to establish and validate a high-throughput procedure to evaluate the edaphic adaptation of breeding materials using vegetative propagules (stem cuttings) grown in solution culture. The procedure was designed to quantify two key component traits: root vigor and Al resistance. The vigor of root growth under nutrient deprivation is likely to influence a plant's adaptation to a range of nutrient deficiencies because nutrient acquisition relies on soil foraging, particularly in the case of immobile nutrients such as P. Aluminum toxicity was incorporated because previous experiments had confirmed that *Brachiaria* genotypes differ for this trait.

We initially tested the procedure by monitoring, during up to three weeks, the growth of adventitious roots of stem cuttings from the three parental genotypes (*B. decumbens* cv. Basilisk, *B. brizantha* cv. Marandu, and *B. ruziziensis* 44-02). Having established its effectiveness, we evaluated a refined version of the procedure with *B. ruziziensis*, *B. decumbens* and a group of 38 *B. ruziziensis* × *B. decumbens* hybrids, which were expected to segregate for edaphic adaptation because of the typically high heterozygosity level of apomicts such as *B. decumbens*.

Materials and Methods

The three main parents of the *Brachiaria* breeding program (*B. decumbens* cv. Basilisk, *B. brizantha* cv. Marandú, tetraploid *B. ruziziensis* clone 44-02) and a group of 38 *B. ruziziensis* × *B. decumbens* hybrids were propagated in a 40 kg of a 1:3 mixture of sand and soil from an experimental station in Santander de Quilichao (990 m above see level; Oxisol – Plinthic Kandiudox; Cauca Department, Colombia). The sand-soil mixture was fertilized with (milligram of element per kilogram of soil): 20.6 N (urea), 25.8 P (triple superphosphate), 51.6 K (KCl), 34.0 Ca (dolomitic lime) 18.0 Ca (triple superphosphate), 14.6 Mg (dolomitic lime), 10.3 S (elemental sulfur), 1.0 Zn ($ZnCl_2$), 1.0 Cu ($CuCl_2$), 0.05 B (H_3BO_3) and 0.05 Mo ($Na_2MoO_4 \cdot 2\ H_2O$). Soil chemical characteristics before mixing with sand and application of fertilizer were: pH 4.6 at a soil to water ratio of 1:1, 14.7 mg kg^{-1} Bray-II extracted P, 1.8 $cmol_c$ kg^{-1} KCl-extracted Al, 0.15 $cmol_c$ kg^{-1} Bray-II extracted K, 2.3 $cmol_c$ kg^{-1} KCl-extracted Ca, and 1.3 $cmol_c$ kg^{-1} KCl-extracted Mg.

Forty-five days after potting, vegetative propagules were produced from tillers with three to five leaves and not more than three nodes. The tillers were detached below the lowest node above soil level, and all but the youngest three leaves were removed. The remaining leaves were pruned to approximately two centimeters to reduce transpiration. The resulting stem cuttings were used for solution-culture experiments in the greenhouse. The tillers remaining in the pots were pruned and the soil-sand mixture was fertilized with an identical amount of urea and triple superphosphate as had been used for potting. After 23-25 days, a second set of stem cuttings was generated, and the soil-sand mixture was fertilized again with urea and triple superphosphate. After producing a third set of stem cuttings under identical conditions, the remaining tillers were pruned and repotted into a fresh sand-soil mixture that had received a full fertilization. This re-potting cycle was repeated four times in the course of this study.

All growth experiments with nutrient solutions were performed in the greenhouse at CIAT headquarters (3° 30' N, 76° 21' W; 965 m above see level). Typical conditions in the greenhouse were: 19 to 36°C; 48 to 96 % relative humidity and 1,100 μmol m^2 s^{-1} maximum photon-flux density during the day.

The effectiveness of two treatments designed to evaluate adaptation to infertile, acid soils was initially tested using the three parental genotypes. The bases of the stem cuttings produced from tillers of potted plants were inserted into 1.5 cm-thick polyurethane foam discs (diameter: 4 cm) and transplanted to racks floating on a large volume of aerated, low-ionic-strength nutrient solution (Photo 17). This solution, known to support close-to-maximum growth of *Brachiaria* seedlings, contained (in µM): 500 NO_3^--, 50 NH_4^+, 300 K^+, 300 Ca^{2+}, 150 Mg^{2+}, 160 Na^+, 5 H_2PO4^-, 286 SO_4^{2-}, 5 Fe^{3+}, 1 Mn^{2+}, 1 Zn^{2+}, 0.2 Cu^{2+}, 6 H_3BO_3, 5 SiO_3^{2-}, 0.001 MoO_4^{2-}, 5 H_2-$EDTA^{2-}$, 332.4 Cl^- (excluding HCl) and 67.8 HCl to adjust the pH to 4.20.

After nine days, twelve pairs of rooted stem cuttings from each clone were selected for within-pair homogeneity (Photo 17). One propagule of each pair was transferred to solution 1 (200 ìM $CaCl_2$, pH 4.20), the other to solution 2 (200 ìM $CaCl_2$, 200 ìM $AlCl_3$, pH 4.2). The pH of both solutions was adjusted by adding calculated quantities of HCl (64.9 ìM for solution 1; 39.2 ìM for solution 2) and measured with a pH electrode designed for low-ionic-strength solutions. The two groups of 36 stem cuttings (twelve per parent) were grown in two plastic trays wrapped in black polyethylene bags, which held 20 liters of the two solutions (Photo 17). The solutions were continuously aerated and renewed every second day to minimize pH changes (typically, the pH increased by up to 0.15 units when roots were bigger). Root growth was monitored by measuring the length of the longest root every third day, for up to 21 days. The whole experiment was performed twice.

Brachiaria ruziziensis 44-02, *B. decumbens* cv. Basilisk and the 38 *B. ruziziensis* × *B. decumbens* hybrids were included in ten successive, partly overlapping experiments. For each experiment, one to three pairs of stem cuttings of each genotype were prepared. The cuttings were rooted and transplanted to one of the two solutions, as described in the previous section. At harvest, after 21 days of growth, roots were separated from aerial parts. The roots were stained for 24 hours in an aqueous solution containing 0.1 % (w/v) methylene blue and 0.1 % (w/v) neutral red, washed, submersed in a thin layer of water and scanned on a flatbed scanner at 300 dpi (Photo 17). The images were analyzed with WinRHIZO software (Régent Instruments Inc., Québec, Canada) to measure total root length (RL) and the average root diameter (RD) for each individual root system. The aerial parts were dried at 60°C for 48 hours and their dry weights record.

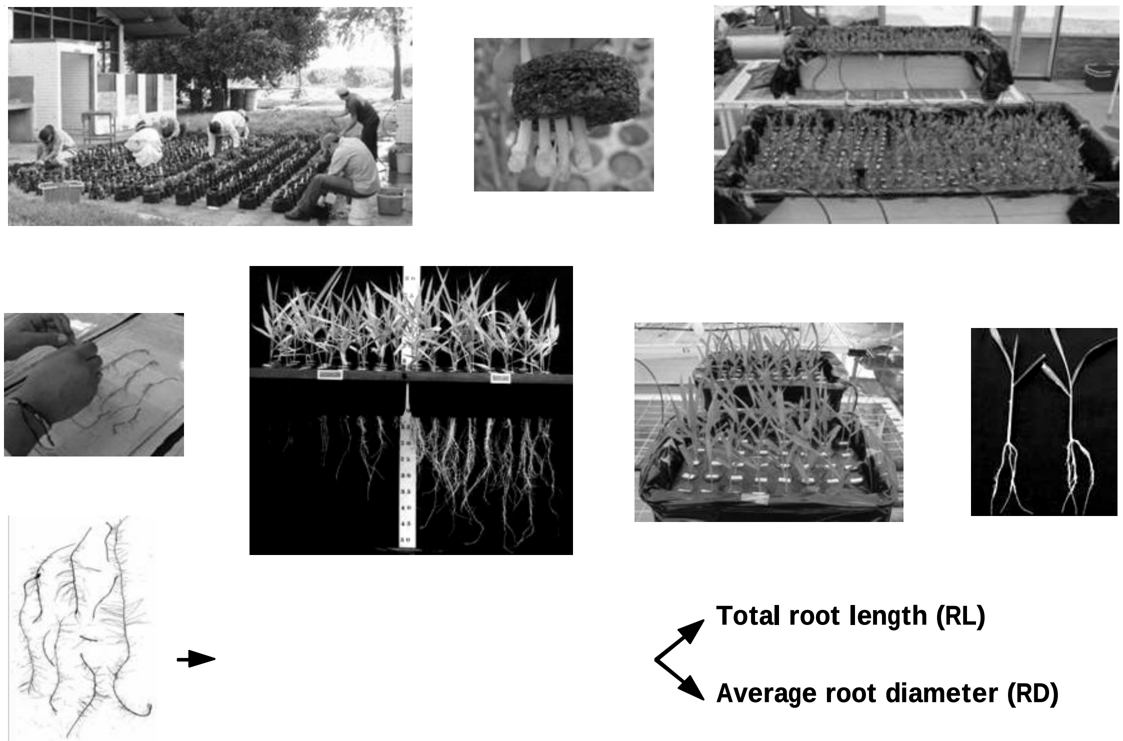

Photo 17. Procedure to identify acid soil-adapted *Brachiaria* genotypes. Plants were propagated in a mixture soil and sand (3:1). Vegetative propagules (stem cuttings), excised from these plants, were floated at the surface of a low-ionic-strength nutrient solution to produce adventitious roots. After nine days, pairs of rooted stem cuttings were selected for homogeneity. One propagule of each pair was transferred to solution 1 (200 ìM $CaCl_2$, pH 4.20), the other to solution 2 (200 ìM $CaCl_2$, 200 ìM $AlCl_3$ pH 4.20). Twenty-one days after transfer, roots were separated from stems, stained and scanned on a flatbed scanner to determine total root length (RL) and average root diameter (RD). Genotypes with vigorous root growth were identified based on RL in solution 1. Aluminum-resistant genotypes were identified based on RL in solution 2 after removing the variance component that was due to differences in root vigor.

During the last experiment, a small number of root apices were excised from adventitious roots of stem cuttings from *B. ruziziensis*, *B. decumbens* and two hybrids with contrasting levels of Al resistance (all grown in solution 2 for 12 days). The apices were stained with hematoxylin. Zones of Al-induced damage were visualized by fixing apices in a 1:1 mixture of 3.7 % phormol (pH 7.4) and glutaraldehyde, and cutting 70-µm thick longitudinal sections.

The pooled RL and RD data from the ten experiments designed to test the refined screening procedure were log-transformed nd adjusted, by linear regression, for harvest mean and the dry weight of stem cuttings. This procedure was designed to remove the variance components caused by differences among replicate experiments in growth conditions as well as differences in the amount of carbohydrates and nutrients supplied by stem cuttings to roots.

Aluminum resistance was quantified after regressing the adjusted logarithms of the RL (or RD) values from the Al treatment (solution 2) on those from the basal treatment (solution 1) to remove the variance component reflecting the inherent differences in root vigor among the hybrids. The residual values after regression were expected to be a more informative measure of true Al resistance than the original values from the Al treatment if root vigor and Al resistance were not correlated (Zeegers et al., 2004). Lack of correlation between the two traits was independently confirmed by comparing the genotype means for the adjusted logarithm of RL in solution 1 (root vigor) against the genotype means for an alternative Al-resistance index (the log-transformed ratio of RL in solution 2 to RL in solution 1): the two sets of hybrid means were indeed virtually uncorrelated ($r^2 = 0.02$).

Results and Discussion

The commercial *Brachiaria* grass cultivars are widely propagated by stem cuttings, a feature that enables breeders to generate genetically identical clonal propagules for evaluation of phenotypic characters. When the basal node of stem cuttings was incubated in a low-ionic-strength nutrient solution for nine days, all tested *Brachiaria* genotypes typically produced two to four adventitious roots (Photo 17). We initially used the three parental species (*B. ruziziensis*, *B. decumbens*, *B. brizantha*) to establish the effectiveness of the hydroponic solutions designed to simulate stress factors of the acid-soil syndrome. Solution 1 contained a low concentration of Ca^{2+} to protect root plasma membranes, but lacked other nutrients (200 ìM $CaCl_2$, pH 4.20). Solution 2 was identical to solution 1, but also contained 200 ìM $AlCl_3$. Root growth in solution 1 should reflect the plants' ability to produce an extensive root system that explores a large volume of soil for nutrient uptake. A comparison of root growth between the two solutions should provide a measure of Al resistance.

Stem cuttings of all three parental genotypes continued to produce leaves during the duration of the experiment. Leaves of *B. ruziziensis*, but not the other two parents, tended to become slightly chlorotic towards the end of the experiment. Roots of *B. decumbens* and *B. brizantha* continued to elongate in solution 1 for the entire period of evaluation (three weeks). Those of *B. ruziziensis*, by contrast, ceased to elongate after approximately one week and were considerably shorter (Figure 27, left panel). Presence of Al in solution 2 strongly inhibited root elongation of *B. brizantha*, but had only little effect on roots of *B. decumbens*. Root growth of *B. ruziziensis* in this solution was negligible (Figure 27, right panel).

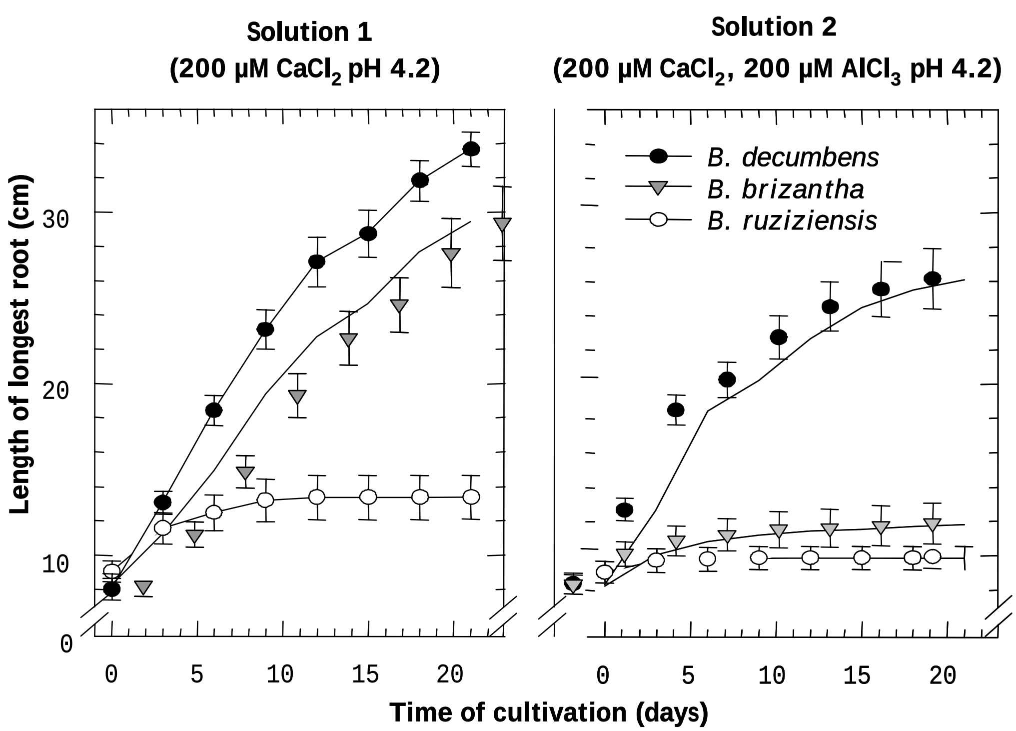

Figure 27. Initial test of the two treatments (without and with Al) using the three parental genotypes of the *Brachiaria* breeding program. The length of the longest root was recorded for up to 21 days of growth in the basal treatment (left panel) and the Al treatment (right panel).

The growth of the three *Brachiaria* genotypes in the two solutions coincides with well-established differences in adaptation to infertile, acid soils: *B. decumbens* is well-adapted, adaptation of *B. brizantha* is intermediate, and *B. ruziziensis* performs poorly. The results of this experiment suggest that vigorous root development under nutrient deprivation and a high level of Al resistance may both contribute to the excellent edaphic adaptation of *B. decumbens*.

We next evaluated, in a series of replicate experiments, the 21-d growth performance of a broader range of genotypes. We selected 38 *B. ruziziensis* × *B. decumbens* F1 hybrids (full siblings), which were expected to segregate for edaphic adaptation because of the heterozygosity of apomictically reproducing *B. decumbens*. In this set of experiments we characterized root growth by measuring total root length (RL) and average root diameter (RD).

Total root length in solution 1 ranged from 0.7 to 6 m. The latter value is quite remarkable considering the biomass of stem cuttings (mean dry weight: 0.42 g) and the fact that the growth medium contained only 200 ìM $CaCl_2$. Not surprisingly, roots of *Brachiaria* grasses in neotropical savannas represent a carbon sink of world-wide significance. The RL values showed a considerable degree of positive transgressive segregation, perhaps as a result of hybrid vigor (Figure 28, left panel). The striking difference in RL between *B. ruziziensis* (and one of the hybrids) and the other genotypes probably was an artifact of the particular subset of hybrids used for this experiment, because a recent screening of a larger group of *B. ruziziensis* × *B. decumbens* hybrids has identified hybrids that are intermediate between those two groups of genotypes. The performance of individual genotypes was consistent across harvests, as can be deduced from the mean standard error (0.034 log units) compared to the range of RL values measured for the group of 38 hybrids (0.933 log units) (Figure 28, left panel). This result confirmed the suitability of solution 1 for quantifying differences in root vigor among a set of full sibs. Root vigor as measured by this method probably comprises different physiological components expressed in mature plants, such as the frequency of initiation of adventitious roots, the tendency of nutrient-deprived plants to allocate carbon to roots rather than shoots, and the efficiency with which nutrient reserves in stem cuttings are remobilized to sustain root growth. Any of these components is likely to influence a plant's adaptation to infertile and acid soils.

Aluminum resistance was less straightforward to measure because the inherent differences in root vigor had to be taken into account to accurately quantify the effect of Al toxicity on root development for different genotypes. A similar problem has been encountered previously with rye seedlings, but was much more pronounced in the case of vegetative *Brachiaria* propagules. We used a residual-variance approach to compute a root vigor-adjusted Al-resistance index (see *Materials and Methods*). The right panel in Figure 28 displays the distribution of this index for the 38 *B. ruziziensis* × *B. decumbens* hybrids. The two genotypes with poor root vigor (*B. ruziziensis* and one hybrid; see left panel) showed quite different levels of Al-resistance (0.09 vs. 0.34 log units; right panel). The Al-resistance index, therefore, quantifies Al resistance even for genotypes with low root vigor, for which the greater contribution of the root length present at the initiation of treatments may introduce a bias toward higher resistance levels.

Aluminum resistance among the hybrids seemed to vary quantitatively. In agreement with the results from a seedling-based root elongation assay, Al resistance of *B. decumbens* was significantly superior to Al resistance of *B. ruziziensis*. In contrast to root vigor, the two parents were close to the two extremes of the Al-resistance distribution (Figure 28, right panel). Though based on a limited number of segregants, this segregation pattern would be consistent with multiple genes contributing to Al resistance, a similar situation as in species such as rice and probably maize. The absence of positive

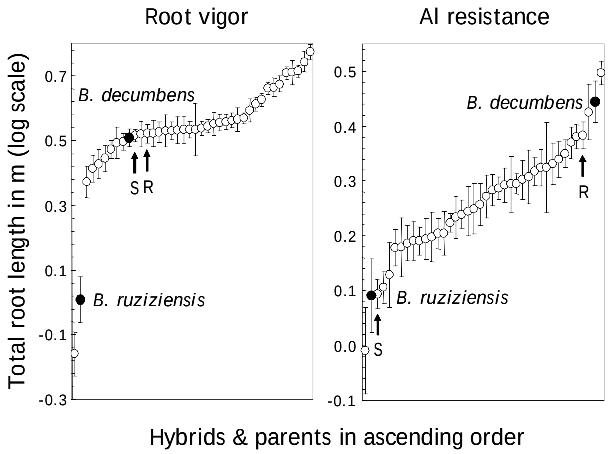

Figure 28. Segregation of root vigor (left panel) and Al resistance (right panel) in a group of 38 *B. ruziziensis* × *B. decumbens* F1 hybrids. Root vigor is the total root length (RL) in solution 1 (200 ìM $CaCl_2$, pH 4.20), adjusted for the effects of stem-cutting biomass and harvest. Aluminum resistance is the stem-cutting-biomass and harvest-adjusted RL in solution 2 (200 ìM $CaCl_2$, 200 ìM $AlCl_3$ pH 4.20), after removing the variance component caused by differences in root vigor. The two parents are highlighted by black symbols. The two hybrids with contrasting levels of Al resistance that were used for the hematoxylin-staining test (Photo 18) are designated as "R" for Al-resistant and "S" for Al-sensitive.

transgressive segregation suggests that *B. decumbens* contains most of the alleles that contribute importantly to Al resistance.

Aluminum toxicity not only inhibits root elongation, but also induces lateral swelling of roots. Aluminum-sensitive genotypes, therefore, should not only be characterized by a decrease in RL, but also an increase in RD. We found that the RL-based Al-resistance index was indeed negatively correlated with a similar index based on RD ($r^2 = -0.75$). We also validated our method of quantifying Al resistance against the well-established hematoxylin-staining method, using the two parents and two hybrids that were close to the extremes of the range of Al-resistance levels (see arrows in Figure 28). In agreement with our classification, root apices of Al-sensitive genotypes stained strongly; while those from Al-resistant genotypes remained clear (Photo 18).

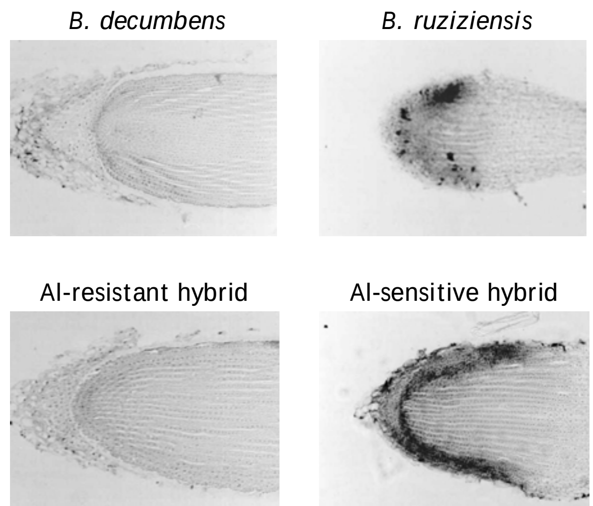

Photo 18. Hematoxylin-staining patterns for root apices of genotypes identified by the screen as Al-resistant (left side) and Al-sensitive (right side). The two *B. ruziziensis* × *B. decumbens* hybrids used for this test are also highlighted in Figure 28.

From the combined results of this study we conclude that our screening procedure quantifies component traits expressed in mature plants which are likely to influence the adaptation of *Brachiaria* genotypes to infertile, acid soils (root vigor, Al resistance). This conclusion was further verified by testing *Brachiaria* hybrid "Mulato", a recently released cultivar. The glasshouse screen revealed good root growth and intermediate Al resistance, consistent with its good vigor and responsiveness to applied nutrients on acid soils.

We characterized root growth and root-system morphology in detail to test the effectiveness of the two experimental treatments (solution1 and 2) in revealing genetic differences in edaphic adaptation. Preliminary data from a larger hybrid population suggest that this approach is also useful for identifying QTLs contributing to acid-soil adaptation. In order to maximize the number of segregants that can be screened, the procedure may be simplified. Plants could be cultivated in solution 1 (basal treatment), but transferred to solution 2 (Al treatment) a day or two before harvest, followed by hematoxylin-staining of root apices. This would enable breeders to separately assess root vigor (size of root system) and Al resistance (absence of staining), exclusively by visual inspection. Alternatively, plants could be cultivated in solution 2 only, thus simultaneously selecting for both component traits (Photo 18, right panel).

The latter approach may exclude potentially useful segregants for component traits. However, this approach has significantly increased the efficiency of the *Brachiaria* breeding program at CIAT by enabling breeders to quickly discard a large number of non-adapted genotypes. In a more recent breeding cycle, 745 sexual segregants were screened for both edaphic adaptation and spittlebug resistance in the course of six months, and the best 5 % that combine both traits were used for further genetic recombination and improvement. Several hybrid-derived sexual genotypes that are markedly superior to the original sexual tetraploid *B. ruziziensis* have been identified using this procedure.

Root vigor of mature plants does not appear to be a widely-used selection criterion in breeding programs targeting edaphic adaptation. Root vigor of seedlings has received some breeding attention yet is unlikely to bear much relevance for edaphic adaptation of *Brachiaria*, which is manifest in the persistence of pastures over several growing seasons. Stem cuttings from mature plants are probably a more suitable material to assess long-term edaphic adaptation because the dramatic differences in root vigor between *B. ruziziensis* and *B. decumbens* were not expressed at the seedling stage.

Aluminum resistance is usually assessed in seedling-based assays, either by quantifying root elongation or apical callose concentrations, or by staining root apices with hematoxylin. Although seedling-based assays have been successfully applied to *Brachiaria* grasses, poor germination of *Brachiaria* seeds at the surface of nutrient solutions and the poor viability of hydroponically-grown seedlings upon transplantation to soil limit their applicability in a breeding program. The Al-resistance screen based on stem cuttings circumvents the transplantation problem and enables the concurrent assessment of root vigor of mature plants as a second component trait contributing to edaphic adaptation. Vegetative propagation also permits simultaneous assessment of a single genotype (clone) for other traits such as insect or disease resistance, nutritional quality and seed production.

In summary, we developed and validated a hydroponic screening procedure that uses vegetative propagules (stem cuttings) of *Brachiaria h*ybrids to rapidly evaluate root vigor and aluminum resistance, two key components of edaphic adaptation. A simplified version of this procedure is being used to select for aluminum resistant apomictic and sexual hybrids of *Brachiaria*. Adaptation to infertile, acid soils is almost certainly a trait under complex genetic control. Ultimately, well-adapted genotypes will be confirmed only when plants are subjected to the stress syndrome *in situ*. However, the *Brachiaria* breeding progress achieved during the last four breeding cycles, indicates that the screening procedure outlined enables breeders to recover some key genetic components of acid-soil adaptation.

3.1.3 Mapping of QTLs associated with aluminum resistance in *Brachiaria* species

Contributors: C. Rosero, G. Gallego, J. Vargas, C. Quintero, M.C. Duque, M.E. Buitrago, P. Wenzl, I.M. Rao, J.W. Miles and J. Tohme (CIAT)

Rationale

Acid soils have been estimated to occur on about 40% of the arable land (3.95 billions of ha). Plant growth on these soils is constrained mainly by aluminum (Al) toxicity and deficiencies of nutrients such as phosphorus (P), nitrogen (N), and calcium (Ca). There is considerable variation within and between plant species in their ability to resist Al, and this variation within some species has allowed breeders to develop genotypes that are able to grow on acid soils. Within the *Brachiaria* genus, Al resistance of signalgrass (*B. decumbens* Stapf cv Basilisk), a widely sown tropical forage grass, is outstanding compared with the closely related ruzigrass (*B. ruziziensis* Germain and Evrard cv Common). Our main objective was to identify specific genes that are related with high level of Al resistance in signalgrass. Our research approach was to combine traditional genetic improvement tools with those that are available on structural and functional genomics. For this purpose, a molecular genetic map has been developed, using a F1 population (previously charaterized under Al stress conditions) formed by 180 individuals from *Brachiaria ruziziensis* x *Brachiaria decumbens* cross. This molecular map was built by means of 169 AFLPs and SSR markers that were distributed in 19 linkage groups. The map was the base for QTLs analysis procedures, establishing a relation between phenotypic data with molecular data.

Materials and Methods

An F1 hybrid population (263 plants) from the interspecífic cross between *B. decumbens (*CIAT 606, Al resistant) and *B. ruziziensis* (BRUZ 44-02, susceptible), was used for this study. SSRs primers developed from *B. decumbens* and AFLPs primer combinations (EcoRI/MseI), previously tested in the parents and determined as polymorphic were evaluated in the population. Additionally, SSRs primers from rice were evaluated and few SCARs markers that were developed from AFLPs alleles were tested in this population. Polymorphic markers of each parental were subset, and a X^2 analysis was made (global α=0.05%) for each group tor test the goodness of fit to 1:1 and 3:1 or 5:1 ratios, for single dosage markers (SDM) and double dosage markers (DDM). A linkage map was constructed (only with SDMs) with the Mac computer program MapMaker/EXP v3.0b (LOD min=12.0 and r= 0.3), calculating the distances (in cM) by Kosambi's mapping function. Five quantitative variables (root length, root diameter, abundance of root tips, root length/shoot biomass and specific root length) that are indicative of Al resistance were employed for QTLs analysis. The aluminum effect (eal) over each genotype of the population was calculated by doing a LS mean over each data variable. Genotypes without replications were excluded of the posterior phase of the analysis, as well as the data with total root length of less than 90 cm, because of its great response variability to Al stress conditions. QTLs analysis was preformatted in WinQTLCartographer v2.5 program (9). Three kind of analysis was made: Single point analysis (SPA), Interval mapping (IM) and Compositive interval mapping (CIM). The threshold to report a QTLs (LOD) was calculated by permutations methodology with 1 000 permutations ($\propto$= 0.05).

Results and Discussion

From 73 SSRs primers sets of *Brachiaria*, only 36 were tested in the population due to their polymorphism, these primers amplified 66 alleles for Al resistant parent CIAT 606 and 33 for Al sensitive parent BRUZ 44-02. Because of amplification problems, we were not able to evaluate 27 SSRs. Five primers were monomorphic, and the other five were not yet standarizathed. Three SSRs primers from rice chromosome one, have been evaluated in the population producing five alleles for the parent 606, and it was also possible to score two SCAR markers (ScC6b2 and ScC6b2).

Twelve AFLP's primer combinations were evaluated for their high polymorphism, scoring 454 markers allelic loci due to their complex banding pattern. Of these, 229 bands were shared, the rest were polymorphic, but from these ones almost 3/4 are bands from the Al resistant parent 606 (179). This amount of molecular information sharing shows a strong relationship between the genomes of the two contrasting parents, 606 and 44-02.

A low percentage from AFLP's polymorphic alleles of each parent (14.5% for 606 and 15.2%

to 44-02) were not evaluated, because of lack of segregation in the population. This in fact depends on the marker dosage, being one of the biggest limitations of using polyploid species such as *Brachiaria*. The SSRs are more effective markers for SDMs detection. This separation is very important when working with polyploids species because the other configurations used could endanger the map precision, affecting the distance as well as the position of markers.

A linkage map with 19 linkage groups was constructed for the tetraploid species *B. decumbens*, the map has 180 SDMs from which 114 are AFLPs, 64 SSRs and 2 SCAR markers, covering 1362.9 cM (Figure 29). The 19 linkage groups obtained are closer to the haploid number of this specie (18 chromosomes). Perhaps, the small size of *B. decumbens* chromosomes explain the fact that with only 180 markers we achieve a linkage group number closer to the search, and point out to a considerable genome coverage in the present linkage map.

Five phenotypic and quantitative variables including root length (*rl*), root diameter (*rd*), number of root tips (*t*), root length to shoot dry weight (*rlsdw*) and specific root length (*srl*) that are indicative of Al resistance (*eal_rl, eal_rd, eal_t, eal_rlsdw, eal_srl*) were employed for QTLs analysis. These pheotypic variables showed transgresive segregation that could be due to polyploid condition thereby contributing to heterosis or hybrid vigor.

With the three QTLs analysis methods (SPA, IM and CIM) it was possible to establish putative associations between moleculars markers, finding six putative QTLs that are associated with Al resistance. Three were localizated in LG5, the others in LG3, LG4 and LG15. The associations found by the mapping analysis are compiled in the Table 32 (see also Figure 29).

The small percentage of variation explained for each QTL, could be due to complexity that involves the aluminium resistance trait, and may be the six putative QTLs found point out some genes of small efect that could interact and produce the observed phenotype. However, more saturation level and/or fine mapping is hended to make further progress. Finally, it is also necessary to confirm the putative QTLs identified so far. This is because the phenotipic evaluation was made only under hydroponic conditions.

Further Work is in Progress to; (i) saturate the linkage map of *B. decumbens* (CIAT 606) with SSRs of genetically related species and with some *Brachiairia* SSRs primers that are not yet standarizathed, and (ii) conduct QTLs analysis for Al resistance with additional phenotypic data.

Table 32. Mapping analysis results. LG, position of the first marker, and the flanking markers associated with aluminum-resistance variable are indicated. The most closer markers with correlation are indicated in bold and with underline.

Method	Variable	LG	Flanking Markers	QTL Position	Markers Position	LOD	Aditive	R2
IM	eal_rd	15	GM_24 - **C5b10**	36,68	21,7 - **36,6**	3,014	0,052	0,053
IM	eal_slr	3	**rGM_72a** - rRM_46b	112,16	**88,3** - 113,5	3,008	0,110	0,058
IM	eal_slr	5	**GM_11a** - rGM_11b	104,22	**104,1** - 110,4	2,297	-0,089	0,041
CIM	eal_lr	5	**GM_12a** - GM_11a	103,45	**103,3** - 104,1	3,260	-0,072	0,052
CIM	eal_lr	5	**GM_11a** - rGM_11b	108,22	**104,1** - 110,4	3,098	-0,072	0,053
CIM	eal_rd	5	**GM_11a** - rGM_11b	108,22	**104,1** - 110,4	2,993	0,051	0,050
CIM	eal_rd	15	GM_24 - **C5b10**	35,83	21,7 - **36,6**	3,317	0,052	0,054
CIM	eal_lrsdw	4	**C1b12** - rC10b8	52,34	**52,3** - 63,3	3,024	-0,081	0,050
CIM	eal_slr	3	**rGM_72a** - rRM_46b	112,16	**88,3** - 113,5	4,111	0,120	0,068
CIM	eal_slr	5	**rRM_151** - rRM_466a	0,01	**0,0** - 14,1	2,801	-0,091	0,043
CIM	eal_slr	5	**GM_11a** - rGM_11b	104,22	**104,1** - 110,4	3,010	-0,094	0,045

LG Linkage group, IM Interval mapping, CIM Compost interval mapping.

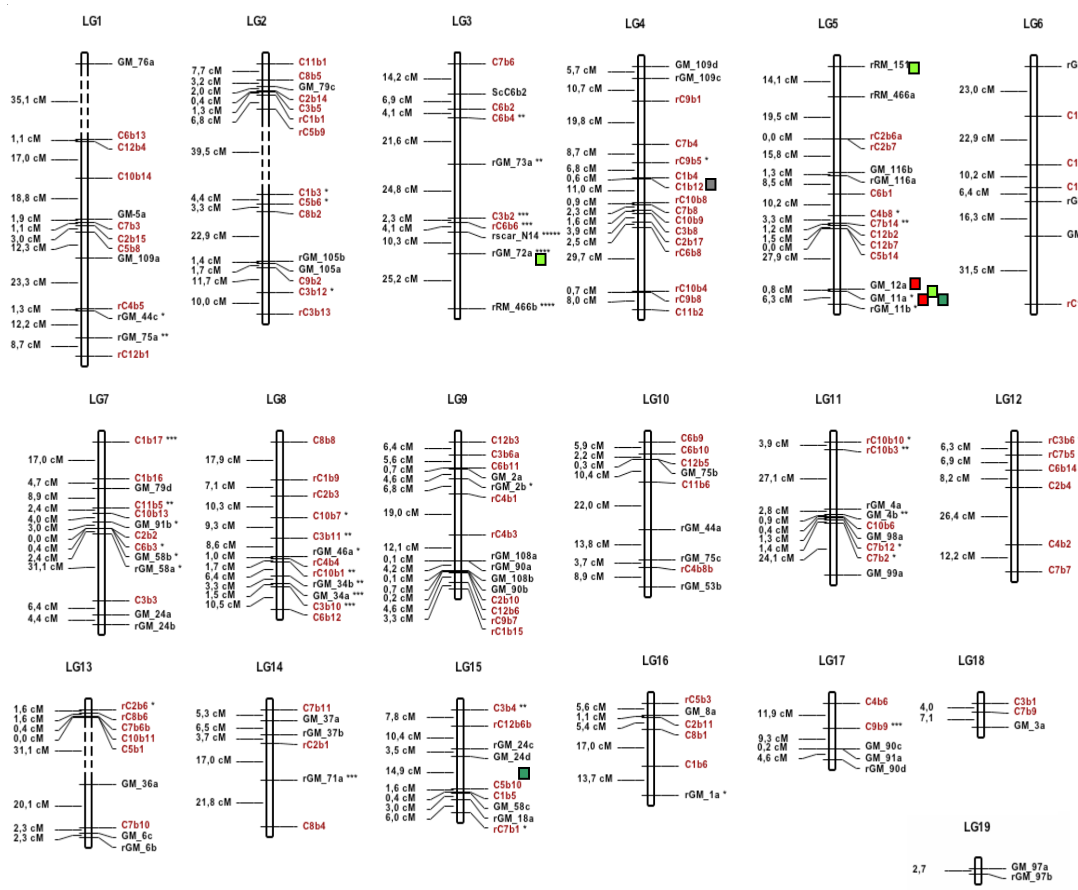

Figure 29. Genetic Linkage Map of *B. decumbens*, with localization of QTLs for Al resistance. SDMs of AFLPs, SSRs, and SCARs were used in this map. Distances between markers more than 30 cM, are shown with broken line. The boxes show the most closer marker associated to the region with the trait of interest: (■) eal_rl; (■); eal_rd, (■); eal_rlsdw and (■) eal_srl.

3.1.4 Isolation and characterization of candidate genes for Al tolerance in *Brachiaria*

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Rationale

Previous results demonstrated that there is pronounced difference in aluminium (Al) resistance between *B. decumbens* (resistant) and *B. ruzisiensis* (susceptible). The objective of this work is to identify candidate genes responsible for high level of Al resistance in *B. decumbens* using PCR-based technology. Identification of these candidate genes would enhance our understanding of molecular mechanisms of Al resistance, thereby contributing to genetic improvement of both forage and field crops for Al resistance.

Materials and Methods

Seeds of *B. decumbens* and *B. ruziziensis* were germinated in 200 M $CaCl_2$ (pH 4.2) for 4 - 5 days in the greenhouse. Homogeneous seedlings with root lengths between 4 and 5 cm were transferred to continuously aerated solutions, of 200 µM CaCl2 (pH 4.2) with and without $AlCl_3$. Seedlings were left to grow in the greenhouse. Root length was measured at 0 h, 3 h, 6 h, 24 h, and 72 h after Al treatment. Root tips (1 cm length) from *B. decumbens* and *B. ruzisiensis* were collected at 0 h, 3 h, 6 h, 24 h, and 72 h after Al treatment. Total RNA was isolated from the root tissue using Trizol® reagent by following manufacture's protocol (Invitrogen, USA). The

RNA samples from each time point were pooled to isolate poly(A) RNA using PolyATtract® mRNA Isolation Systems (Promega, USA). mRNAs (300 ηg each) were used for cDNAs synthesis using SMART cDNA synthesis kit (Takara-Clontech, USA). Differential expressed genes between *B. decumbens* and *B. ruziziensis* were selected by PCR-Select cDNA subtraction kit (Takara-Clontech, USA). The cDNA fragments obtained from a forward subtractive library (cDNAs from *B. decumbens* and *B. ruziziensis* as tester and driver, respectively) were directly cloned into a T/A cloning vector pGEMT-easy (Promega, USA) in Escherichia coli DH5á.

In order to obtain candidates for differentially expressed genes in *B. decumbens* the subtracted library was screened using a PCR-Select Differential Screening Kit (Takara-Clontech, USA). Individual colonies that showed the presence of inserted DNA were picked, and grown in a 384 microplate with LB-ampicillin freezing solution, (yeast extract 5 g, tryptone 10 g. NaCl 10 g, $K_2HPO_4.3H_2O$ 63 g, KH_2PO_4 18 g, $MgSO_4$ 0.4 3g sodium citrate 4.4 g, $(NH_4)_2SO_4$ 9 g and 349 ml of glycerol autoclaved until 1L). The colony array was blotted onto nylon membranes (Amersham, USA) resting on LB-agar and then cultured overnight at 37 °C. Cloned DNA then was denatured, neutralized and affixed to the membranes by using UV (120 mJ).

Probes for hybridization were derived from the forward- and reverse-subtractive libraries as described in the PCR-Select Differential Screening Kit (Takara- Clontech, USA). Radio labeled probes with (á-^{32}P)-dATP were purified by spin filtration (Takara-Clontech, Chroma-spin 400 columns). Hybridization was carried out by using a Hibridizer HB-1(Techne). Membranes were pre-hybridized for 6 hours at 70 °C in a solution of 1% BSA, 1 mM EDTA, 7% SDS, and 0.25 M sodium phosphate. Denatured probes were added and were hybridized to the cloned DNA's overnight at 70 °C. After hybridization, membranes were washed two times for 20 minutes in a low-stringency washing buffer [2× standard saline citrate (SSC)/0.5% SDS] and one time for 20 minutes in a high-stringency washing buffer (0.2× SSC/0.5% SDS). Membranes were exposed to film (Kodak MXG-1) for 16-72 hours. Positive clones were sequenced and homology analysis was undertaken using the BLAST-X algorithm of GeneBank NCBI, (http://www.ncbi.nlm.nih.gov/) and Gramene (www.gramene.org) databases.

Results and Discussion

We tested root growth of two *Brachiaria* genotypes, *B. decumbens* and *B. ruziziensis* under Al stress conditions used in this work to understand phenotypic responses to Al toxicity of the genotypes. As shown in Figure 30, root growth of both genotypes was not significantly different under control conditions (-Al). Under Al treatment (+Al) conditions root growth was inhibited for both genotypes. However, the Al resistant genotype, *B. decumbens* had less inhibition of root elongation compared with the Al sensitive genotype, *B. ruziziensis*. The small difference in root elongation with Al treatment between the two genotypes appeared at 24 hours after treatment. Since the root phenotype was present at the measured time point, gene expression related to that phenotype must occur prior to the expression of the phenotype.

To identify genes involved for Al resistance in roots, differentially expressed genes were isolated using a Clontech PCR-Select cDNA subtraction kit followed by PCR-Select Differential Screening Kit. Photo 19 shows clones derived from this process. 35 clones were identified as differentially expressed genes and were sequenced. 20 of the 35 were found to have sequence homology in the GenBank and Gramene protein databases as shown in Figure 31 and Table 33.

Among 35 clones, 8 clones of differentially expressed genes had the same sequence which

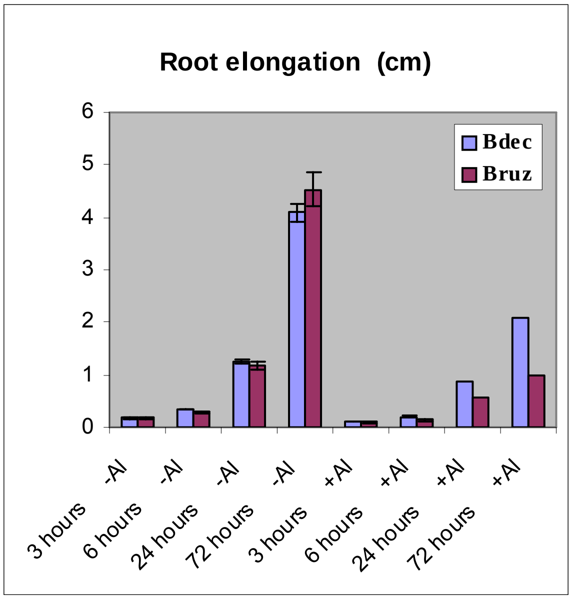

Figure 30. Comparison of root elongation of *B. decumbens* and *B. ruziziensis* exposed to 0 µM (-Al) aluminum (Control treatment), and 200 µM (+Al) aluminum treatment.

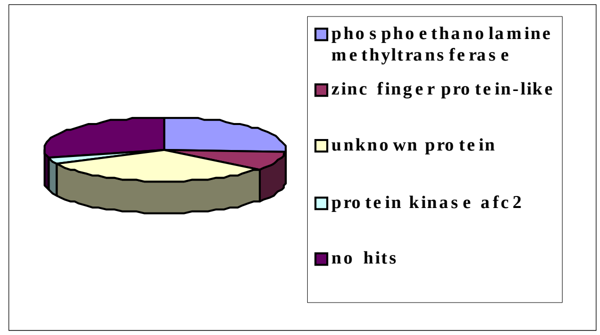

Figure 31. Functional categories found in candidate clones of differentially expressed genes as a response to high level of Al supply to rotos of *B. decumbens*.

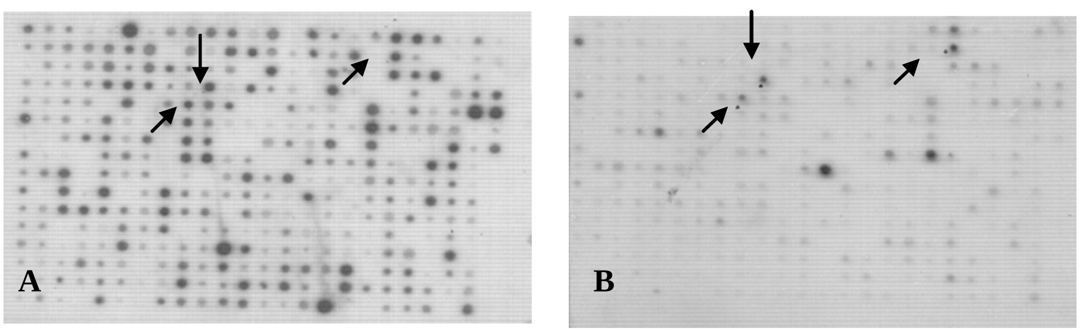

Photo 19. Array of 376 clones shown after hybridization of probes derived from a subtracted population of *B. decumbens* under Al toxicity (forward subtractive library). **A**. Subtracted library **B**. Control non-subtracted library. Dark spots in the autoradiography indicate clones that were expressed in high levels (arrows), or low abundance transcripts enriched during subtraction, which are candidates for differentially expressed genes.

Table 33. List of some candidates of differentially expressed genes as a response to Al toxicity in *B. decumbens* obtained after screening for differential expression. The top Blastx hit match is shown. Size refers to the number of nucleotide base pairs in the sequence. ^{AA} means poly A signals that were found in the cDNA clones.

Clone category	Size (bp)	Blastx similarity	bits	E value
Contig ^{AA}	835	Phosphoethanolamine methyltransferase	162	2e-39
Contig	635	Protein kinase afc2	275	1e-72
Contig ^{AA}	426	Zinc finger protein-like	79.0	5e-14
contig	603	Unknown protein	219	5e-56
singleton	247	hypothetical protein LOC_Os12g3865	87.0	2e-16

had high sequence similarity with phosphoethanolamine methyltransferase (Table 33). This enzyme catalyzes sequential methylations to form phosphocholine, a key precursor in the synthesis of phosphatidyl-choline and glycine betaine in plants. High levels of salinity and hyperosmotic stress is known to induce rapid increases in phosphatidylcholine in *Arabidopsis*.

Three clones showed sequence similarity to a zinc finger-like protein from rice. This protein contains a domain that is related to the zinc finger (FYVE type) family of proteins. It functions in membrane recruitment of cytosolic proteins by binding to phosphatidylinositol 3-phosphate (PI3P), which is found mainly on endosomes. It was suggested that these proteins regulate multiple functions in cells. It is known that Al is capable of affecting all isoforms of phosphatidylinositol specifically in roots. Other 2 clones showed similarity to protein kinase afc2 from rice (*Oryza sativa*).

10 independent clones matched against unknown sequences or expressed proteins found in the Genbank database. 9 clones did not match significantly with any existing sequences. Thus, all ten appear to be unique sequences at the cDNA level. 18 clones contained a poly (A) signal, suggesting that part of the sequence consists of UTR 3´region.

Candidate genes listed above will be analyzed for their expressions at different time points using Real Time PCR technology to confirm differential expression under Al toxicity. To evaluate function of the candidate genes we plan to isolate the full-length cDNA to evaluate the phenotype under Al toxicity *in planta* with altered expression of the gene of interest. The sequences of genes obtained will be used for gene mapping in *Brachiaria* and will also aid in the identification of molecular markers and QTL´s associated with Al resistance in *Brachiaria*.

3.1.5 Screening of *Brachiaria* hybrids for resistance to aluminum

Contributors: I. M. Rao, J. W. Miles, R. Garcia and J. Ricaurte (CIAT)

Rationale

For the last four years, we have implemented screening procedure to identify Al-resistant *Brachiaria* hybrids that were preselected for spittlebug resistance. In 2002, we identified 2 sexual hybrids (SX 01NO3178 and SX01NO7249) and one apomictic hybrid (BR99NO/4132) with greater level of Al resistance than that of the sexual parent, BRUZ/44-02. In 2003, we identified 2 hybrids (BR02NO1372 and BR02NO1621) with greater level of Al resistance than that of the most hybrids generated from the *Brachiaria* breeding program. In 2004, we evaluated Al resistance of a sexual population of 745 hybrids along with 14 checks. The increase in Al resistance of the sexual hybrids (SX03NO/0846, SX03NO/2367, SX03NO/0881) has been very marked compared with the sexual population of 2001. This year, we evaluated the BR04NO series of hybrids generated by the breeding team in 2004.

Materials and Methods

The BR04NO series of hybrids belong to eight families that were generated by crossing two spittlebug-resistant sexual clones identified in 2002 (SX01NO/0101 and SX01NO/0233) by four apomictic clones – three accessions of *B. brizantha* (CIAT 6387, CIAT 16121, and CIAT 16212) and *B. decumbens* cv. Basilisk (CIAT 606). An original population of 2,167 hybrids was propagated and transplanted to two field trials (CIAT-Quilichao and Matazul). Periodic visual rating during the 2004 season resulted in culling the original population down to 340 "pre-selected" clones. These were further culled, to 139 clones, on the basis of yield of seed harvested on single plants at CIAT-Quilichao and also through an initial screening with 200 µM Al treatment to eliminate the Al sensitive hybrids. These clones together with 10 checks including 3 parents (*B. decumbens* CIAT 606, *B. brizantha* CIAT 6294 and *B. ruziziensis* 44-02) were included for evaluation of Al resistance.

Four cycles of plants were evaluated with and without Al. Mean values from all the experiments are reported. Stem cuttings of hybrids and checks were rooted in a low ionic strength nutrient solution in the glasshouse for 9 days. Equal numbers of stem cuttings with about 5 cm long roots were transferred into a solution containing 200 µM $CaCl_2$ pH 4.2 (reference treatment) and a solution containing 200 µM $CaCl_2$ and 200 µM $AlCl_3$ pH 4.2 (Al treatment).

The solutions were changed every second day to minimize pH drifts. At harvest on day 21 after transfer, the dry weight of stems was measured. Roots were stained and scanned on a flatbed scanner. Image analysis software (WinRHIZO) was used to determine root length and average root diameter.

Results and Discussion

As reported for the past 4 years, higher values of total root length per plant and lower values of mean root diameter after exposure to 21 days with or without toxic level of Al in solution indicate superior of Al resistance. Among the 139 apomictic/sexual hybrids evaluated, 9 hybrids (BR04NO1018, 1552, 1900, 2110, 2128, 2166, 2179, 2201 and 2681) were superior to *B. decumbens* parent in terms of toot length with Al in solution (Table 34).

Relationships between root length with Al vs root length without Al, root diameter with Al vs root diameter without Al, root length with Al vs root diameter with Al and root length without Al vs root diameter without Al are shown in Figures 32 to 35. Among these 9 hybrids, BR04NO2681 was superior to *B. decumbens* in terms of fine root production as revealed by the lower value of mean root diameter in the presence of Al.

Root vigor in terms of root length in the absence of Al in solution was greater for 18 hybrids (BR04NO1018, 1265, 1273, 1552, 1592, 1629, 1751, 1819, 1900, 2110, 2128, 2166, 2179, 2201, 2414, 2458, 2969 and 3058) compared with *B. decumbens*. Among these hybrids BR04NO2201 was outstanding in terms of its root vigor in the absence of Al in solution. Among the checks, an apomictic hybrid BR02NO1372 showed greater level of Al resistance and also root vigor based on total root length per plant.

Results on spittlebug resistance of these hybrids are reported in section 2.2 of this report. The promising hybrids that combine spittlebug resistance with Al resistance and other desirable attributes will be field tested for mode of reproduction and seed yield so that the potential cultivars can be selected for further field evaluation.

In summary, we screened 139 apomictic/sexual hybrids of *Brachiaria* using a hydroponic screening method and identified 9 hybrids (BR04NO1018, 1552, 1900, 2110, 2128, 2166, 2179, 2201 and 2681) that were superior to *B. decumbens* parent in terms of aluminum resistance.

Results from this BR04NO population on Al resistance clearly indicated that the level of Al resistance is improving for each breeding cycle illustrating the genetic gain from a very efficient recurrent selection program.

Table 34. Root length and mean root diameter of *Brachiaria* sexual hybrids evaluated with (200 µM Al) and without Al (0 µM Al) in solution in comparison with their parents and other checks.

	Root length ($m\ plant^{-1}$)		Root diameter (mm)			Root length ($m\ plant^{-1}$)		Root diameter (mm)	
Genotypes	0 µM Al	200 µM Al	0 µM Al	200 µM Al	Genotypes	0 µM Al	200 µM Al	0 µM Al	200 µM Al
Hybrids					***Hybrids***				
BR04NO/1003	300	76	0.294	0.440	BR04NO/2344	104	37	0.487	0.508
BR04NO/1018	542	222	0.272	0.341	BR04NO/2346	99	31	0.353	0.492
BR04NO/1021	311	133	0.305	0.385	BR04NO/2360	342	115	0.353	0.478
BR04NO/1026	181	76	0.324	0.380	BR04NO/2389	130	58	0.281	0.399
BR04NO/1058	228	73	0.285	0.424	BR04NO/2396	55	38	0.491	0.557
BR04NO/1060	322	166	0.319	0.426	BR04NO/2403	286	67	0.333	0.438
BR04NO/1061	263	124	0.303	0.379	BR04NO/2404	213	53	0.346	0.515
BR04NO/1073	138	79	0.279	0.364	BR04NO/2405	212	71	0.390	0.476
BR04NO/1081	313	63	0.273	0.406	BR04NO/2414	574	152	0.274	0.370
BR04NO/1097	393	86	0.248	0.384	BR04NO/2429	266	40	0.334	0.497
BR04NO/1113	176	96	0.330	0.407	BR04NO/2455	190	68	0.346	0.409
BR04NO/1141	268	82	0.337	0.498	BR04NO/2457	62	55	0.384	0.469
BR04NO/1197	245	56	0.315	0.518	BR04NO/2458	495	167	0.294	0.358
BR04NO/1214	95	31	0.383	0.536	BR04NO/2515	113	52	0.347	0.447
BR04NO/1219	227	67	0.329	0.499	BR04NO/2518	175	37	0.329	0.491
BR04NO/1252	286	73	0.356	0.494	BR04NO/2532	143	59	0.321	0.455
BR04NO/1265	502	116	0.263	0.374	BR04NO/2557	134	29	0.407	0.626
BR04NO/1272	385	120	0.206	0.286	BR04NO/2577	123	31	0.401	0.521
BR04NO/1273	478	169	0.296	0.402	BR04NO/2670	171	49	0.421	0.532
BR04NO/1281	371	91	0.370	0.472	BR04NO/2681	481	315	0.263	0.296
BR04NO/1296	139	36	0.425	0.501	BR04NO/2774	203	66	0.403	0.509
BR04NO/1309	172	95	0.274	0.399	BR04NO/2793	405	150	0.267	0.364
BR04NO/1311	248	60	0.389	0.603	BR04NO/2833	182	127	0.345	0.426
BR04NO/1349	276	95	0.329	0.472	BR04NO/2841	92	38	0.400	0.522
BR04NO/1358	148	54	0.402	0.462	BR04NO/2849	177	67	0.356	0.469
BR04NO/1360	275	59	0.356	0.489	BR04NO/2854	301	96	0.305	0.442
BR04NO/1374	396	140	0.314	0.361	BR04NO/2863	184	39	0.316	0.408
BR04NO/1377	238	63	0.360	0.496	BR04NO/2871	329	132	0.353	0.393
BR04NO/1428	338	146	0.269	0.350	BR04NO/2872	379	109	0.303	0.450
BR04NO/1494	330	54	0.280	0.510	BR04NO/2874	43	37	0.408	0.486
BR04NO/1503	178	58	0.283	0.383	BR04NO/2938	89	35	0.416	0.470
BR04NO/1519	172	60	0.372	0.457	BR04NO/2940	127	50	0.302	0.352
BR04NO/1552	601	195	0.294	0.382	BR04NO/2954	199	58	0.295	0.442
BR04NO/1570	308	130	0.333	0.455	BR04NO/2969	441	106	0.297	0.410
BR04NO/1592	483	147	0.307	0.409	BR04NO/2983	128	77	0.359	0.369
BR04NO/1601	342	103	0.294	0.381	BR04NO/2987	27	18	0.417	0.438
BR04NO/1629	454	130	0.254	0.348	BR04NO/3051	217	97	0.340	0.420
BR04NO/1633	275	28	0.336	0.530	BR04NO/3056	126	52	0.344	0.473
BR04NO/1697	381	111	0.303	0.440	BR04NO/3058	429	122	0.304	0.410
BR04NO/1751	454	172	0.283	0.366	BR04NO/3066	57	29	0.448	0.505
BR04NO/1754	382	99	0.310	0.416	BR04NO/3068	119	72	0.402	0.369
BR04NO/1796	221	104	0.314	0.449	BR04NO/3069	239	146	0.321	0.409
BR04NO/1819	444	131	0.292	0.349	BR04NO/3119	192	63	0.315	0.495
BR04NO/1846	338	128	0.284	0.348	BR04NO/3128	213	65	0.348	0.401
BR04NO/1889	263	133	0.294	0.397	BR04NO/3130	32	21	0.494	0.562
BR04NO/1900	553	217	0.279	0.386	BR04NO/3134	97	29	0.354	0.455

Continues……..

Continued…..

Genotypes	Root length (m plant^{-1}) 0 µM Al	200 µM Al	Root diameter (mm) 0 µM Al	200 µM Al
Hybrids				
BR04NO/1941	225	102	0.306	0.441
BR04NO/2007	167	90	0.318	0.422
BR04NO/2093	305	184	0.313	0.389
BR04NO/2109	372	144	0.298	0.383
BR04NO/2110	599	252	0.275	0.371
BR04NO/2118	393	137	0.308	0.441
BR04NO/2128	504	224	0.276	0.366
BR04NO/2156	292	73	0.291	0.441
BR04NO/2163	376	101	0.250	0.422
BR04NO/2166	499	214	0.260	0.346
BR04NO/2179	494	190	0.267	0.368
BR04NO/2201	712	246	0.235	0.344
BR04NO/222	89	44	0.343	0.591
BR04NO/2226	393	71	0.295	0.534
BR04NO/2235	201	58	0.291	0.387
BR04NO/2265	331	57	0.328	0.497
BR04NO/2275	65	25	0.522	0.495
BR04NO/2285	233	72	0.306	0.381
BR04NO/2290	142	60	0.385	0.471
BR04NO/2338	266	109	0.317	0.379
Hybrids				
BR04NO/3175	170	64	0.351	0.369
BR04NO/3207	140	53	0.270	0.400
BR04NO/3214	128	41	0.350	0.490
BR04NO/3221	120	27	0.305	0.442
Parents				
B.decumbens CIAT606	403	178	0.238	0.309
Bbrizantha CIAT6294	261	72	0.326	0.491
B.ruziziensis 44-02	125	34	0.314	0.471
Checks				
Toledo	96	37	0.421	0.643
Mulato	454	149	0.332	0.432
B.humidicola CIAT6133	497	140	0.211	0.277
B.humidicola CIAT679	259	141	0.206	0.249
FM9503-S046-024 (CIAT 36087-Mulato II)	153	44	0.462	0.671
BR02NO/1372	763	245	0.258	0.314
BR02NO/1752	484	146	0.329	0.394
Mean	293	103	0.324	0.428
LSD (P<0.05)	161	73	0.056	0.076

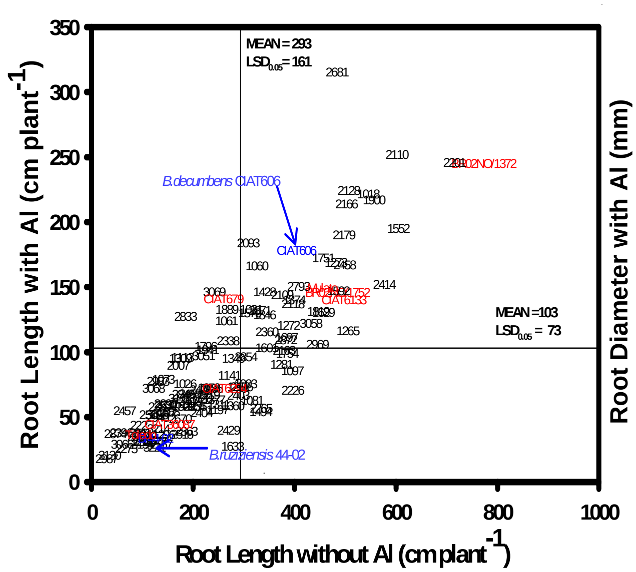

Figure 32. Relationship between total root length with Al and total root length without Al in solution of 149 genotypes of *Brachiaria*. Gentoypes that developed greater root length under both conditions were identified in the upper box of the right hand side.

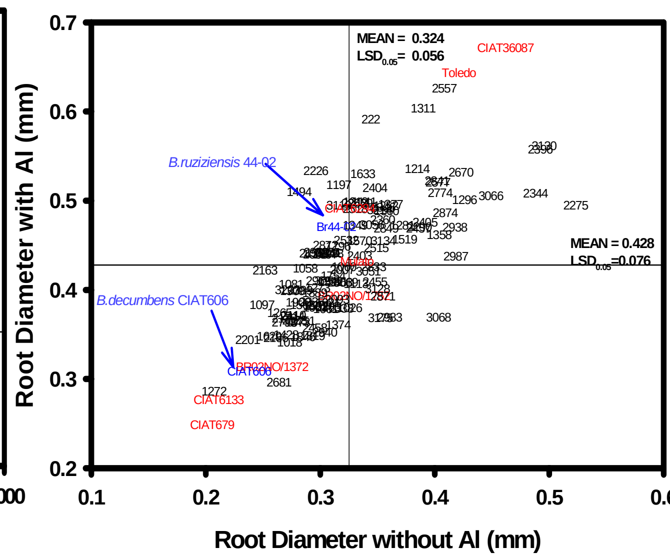

Figure 33. Relationship between mean root diameter with Al and mean root diameter without Al of 149 genotypes of *Brachiaria*. Gentoypes that developed finer roots under both conditions were identified in the lower box of the left hand side.

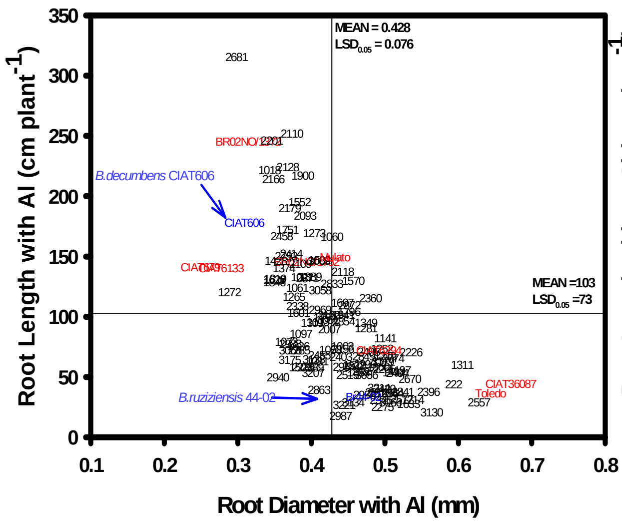

Figure 34. Relationship between total root length and mean root diameter of 149 genotypes of *Brachiaria* with presence of aluminum in solution. Gentoypes that develop finer root system were identified in the upper box of the left hand side.

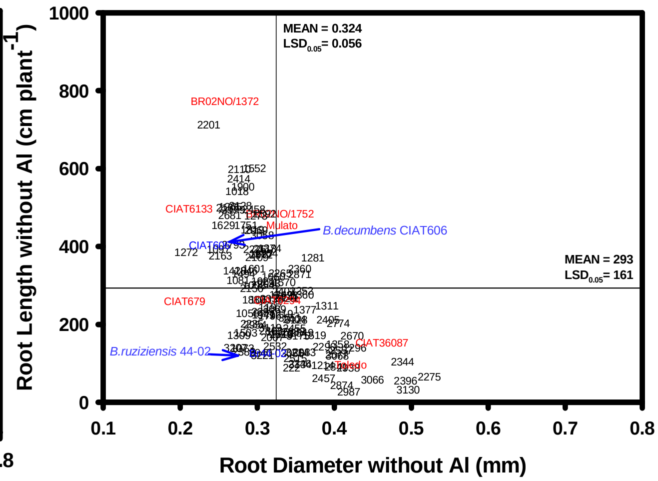

Figure 35. Relationship between total root length and mean root diameter of 149 genotypes of *Brachiaria* with absence of aluminum in solution. Gentoypes that develop finer root system were identified in the upper box of the left hand side.

3.1.6 Field evaluation of promising hybrids of *Brachiaria* in the Llanos of Colombia

Contributors: I. M. Rao, J. Miles, C. Plazas and J. Ricaurte (CIAT)

Rationale

Evaluation of a large number of *Brachiaria* hybrids for their resistance to spittlebug and adaptation to infertile acid soils resulted in identification of a few promising *Brachiaria* hybrids. We selected 4 of these hybrids for further field-testing in comparison with their 2 parents. The main objective was to evaluate growth and persistence with low nutrient supply in soil at Matazul farm of the altillanura (Llanos of Colombia).

At 30 months after establishment, live forage yield with low fertilizer application was greater with one spittlebug resistant hybrid, FM9503-S046-024 and one parent (CIAT 6294). Results on performance of 4 hybrids are reported this year.

Materials and Methods

A field trial was established at Matazul farm on 31 May of 2001. The trial included 4 *Brachiaria* hybrids (BR98NO/1251; BR99NO/4015; BR99NO/4132; FM9503-S046-024) along with 2 parents (*B. decumbens* CIAT 606 and *B. brizantha* CIAT 6294). The trial was planted as a randomized block in split-plot arrangement with two levels of initial fertilizer application (low: kg/ha of 20P, 20K, 33Ca, 14 Mg, 10S; and high: 80N, 50P, 100K, 66Ca, 28Mg, 20S and micronutrients) as main plots and genotypes as sub-plots with 3 replications. Maintenance fertilizer (half the levels of initial applications) was applied at 2 years after establishment. The plot size was 5 x 2 m. A number of plant attributes including forage yield, dry matter distribution, nutrient (N, P, K, Ca and Mg) uptake, leaf and stem total nonstructural carbohydrate (TNC) content and leaf and stem ash (mineral) content were measured at 42 months after establishment (November 2004).

Results and Discussion

After 42 months of establishment, the dead shoot biomass with low fertilizer application was greater with 2 parents (CIAT 606 and CIAT 6294) than with the 4 hybrids (Figure 36). One spittlebug resistant genetic recombinant, FM9503-S046-024 (*Brachiaria* hybrid cv. Mulato II) was superior in production of green leaf biomass with relatively less stem biomass in comparison with the parents under both low and high fertilizer application. With high initial fertilizer application the hybrid Mulato II and the parent 6294 were outstanding in green forage yield (Figure 36). As observed in the previous years, among the 4 hybrids tested, Mulato II was also outstanding in its adaptation to both low and high initial fertilizer application. It is important to note that Mulato II had greater amount of dead biomass and stem biomass under low fertilizer application (Figure 36).

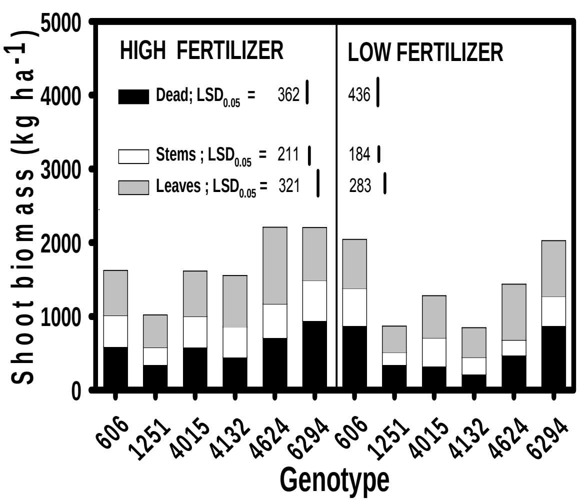

Figure 36. Genotypic variation as influenced by fertilizer application in shoot biomass production (forage yield) of two parents (CIAT 606, 6294) and four genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 42 months after establishment (November 22 2004). LSD values are at the 0.05 probability level. NS = not significant.

As observed for the past 2 years, results on shoot nutrient uptake indicated that the hybrid, cv. Mulato II was superior to CIAT 606 under both low and high fertilizer application (Figure 37). Nutrient acquisition by Mulato II was also greater than the rest of the hybrids with high initial fertilization. But with low fertilizer application, hybrid 4015 was superior to the other 3 hybrids in total nutrient acquisition. These results are consistent with the results reported for the past 2 years from the same experiment.

Correlation coefficients between green forage yield and other plant attributes indicated that greater nutrient acquisition with low initial fertilizer application contributed to superior performance (Table 35). These

Table 35. Correlation coefficients (r) between green forage yield (t/ha) and other shoot traits of *Brachiaria* genotypes grown with low or high initial fertilizer application in a sandy loam oxisol in Matazul, Colombia. Plant attributes were measured at 42 months after establishment (November 2004).

Shoot traits	Low fertilizer	High fertilizer
Total (live + dead) shoot biomass (t/ha)	0.91***	0.29
Dead shoot biomass (t/ha)	0.67***	-0.28
Leaf biomass (t/ha)	0.92***	0.92***
Stem biomass (t/ha)	0.84***	0.75***
Leaf N content (%)	-0.46*	-0.47*
Leaf P content (%)	0.00	0.07
Leaf TNC content (mg g^{-1})	0.19	-0.18
Leaf ash content (%)	-0.09	-0.09
Stem N content (%)	-0.74***	-0.31
Stem P content (%)	-0.03	-0.37
Stem TNC content (mg g^{-1})	-0.19	0.24
Stem ash content (%)	0.02	0.44*
Shoot N uptake (kg/ha)	0.93***	0.93***
Shoot P uptake (kg/ha)	0.87***	0.90***
Shoot K uptake (kg/ha)	0.58**	0.83***
Shoot Ca uptake (kg/ha)	0.83***	0.88***
Shoot Mg uptake (kg/ha)	0.84***	0.92***

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively

results are consistent with the results obtained for the past 2 years. Significant negative correlation was observed between leaf N content and live forage yield under both low and high fertilizer application. Adaptation to low fertilizer application seem to be closely associated with lower amounts of stem N content. However, no significant correlations were found between live forage yield and leaf and stem TNC or ash contents.

Results from this field study confirmed the previous findings of the superior adaptation of the hybrid *Brachiaria* cv. Mulato II, recently released.

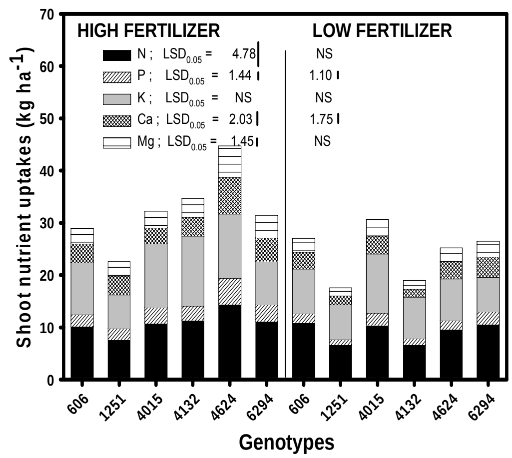

Figure 37. Genotypic variation as influenced by fertilizer application in nutrient uptake (N, P, K, Ca and Mg) of two parents (CIAT 606, 6294) and four genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 42 months after establishment (November 2004). LSD values are at the 0.05 probability level. NS = not significant.

3.1.7 Field evaluation for edaphic adaptation of promising hybrids of *Brachiaria* in the Llanos of Colombia

Contributors: I. M. Rao, J. Miles, C. Plazas and J. Ricaurte (CIAT)

Rationale

Field evaluation of a number *Brachiaria* hybrids under infertile acid soil conditions with low or high amounts of initial application of fertilizer and application of maintenance fertilizer with half of the amounts of initial applications at 2 year intervals improved the persistence of several hybrids and not allowed to distinguish differences between moderately adapted cultivars (cv. Marandu) and the promising hybrids. Therefore a field experiment was established in 2004 with 15 hybrids along with the parents and checks with low amounts of initial application of fertilizer and no maintenance fertilizer application to select hybrids that persist and produce green forage under acid infertile soil conditions.

Materials and Methods

A field trial was established at Matazul farm on 29 June of 2004. The trial included 15 *Brachiaria* hybrids (BR02NO/0465; BR02NO/0768; BR02NO/ 0771; BR02NO/0799; BR02NO/1245; BR02NO/1372; BR02NO/1452; BR02NO/1485; BR02NO/1718; BR02NO/1720; BR02NO/1728; BR02NO/1747; BR02NO/1752; BR02NO/1794 and BR02NO/1811), three parents (*B. decumbens* CIAT 606 and *B. brizantha* CIAT 6294 and *B.ruziziensis* 44-02) and four CIAT checks (*B.brizantha* CIAT 26110, *B.brizantha* CIAT 26646, *Brachiaria* hybrids CIAT 36061 and CIAT 36087). The trial was planted as a randomized block with 4 replications. One low level of initial fertilizer application was applied (kg/ha of 20P, 20K, 33Ca, 14 Mg, 10S) at the time of establishment. Maintenance fertilizer was not scheduled for application in order to identify genotypes that are bettter adapted to infertile acid soil conditions. The plot size was 5 m x 2.5 m. A number of plant attributes including forage yield, dry matter distribution and nutrient (N, P, K, Ca and Mg) uptake were measured at 13 months after establishment (August 2005).

Results and Discussion

At 13 months after establishment, the dead shoot biomass was greater with a check (cv. Mulato or CIAT 36087) and a parent (CIAT 606) than with the hybrids tested (Figure 38). Production of green leaf biomass with greater with CIAT 26110 and CIAT 36087. The hybrid BR02NO/1811 was outstanding in the production of stem biomass. As expected BRUZ 44-02 (sexual parent) was least productive of all the genotypes tested. Among the hybrids tested, BR02NO/1794 and BR02NO/0465 were more productive than the other hybrids in terms of green forage (leaf + stem) yield. Results on shoot nutrient uptake also indicated that the hybrid BR02NO/1794 was superior to other hybrids (Figure 39). Nitrogen uptake of the hybrid BR02NO/1752 was particularly outstanding among the hybrids.

Correlation coefficients between green forage yield and other plant attributes indicated that greater nutrient acquisition contributed to superior performance (Table 36). Significant negative correlation was observed between stem N content and live forage yield. Adaptation to infertile acid soil conditions seem to be closely associated with lower amounts of stem N content.

Table 36. Correlation coefficients (r) between green forage yield (t/ha) and other shoot traits of *Brachiaria* genotypes grown with low intial fertilizer application in a sandy loam oxisol in Matazul, Colombia.

Shoot traits	Low fertilizer
Total (live + dead) shoot biomass (t/ha)	0.94***
Dead shoot biomass (t/ha)	0.32**
Leaf biomass (t/ha)	0.82***
Stem biomass (t/ha)	0.84***
Leaf N content (%)	-0.12
Leaf P content (%)	0.13
Stem N content (%)	-0.43***
Stem P content (%)	-0.26*
Shoot N uptake (kg/ha)	0.92***
Shoot P uptake (kg/ha)	0.91***
Shoot K uptake (kg/ha)	0.78***
Shoot Ca uptake (kg/ha)	0.80***
Shoot Mg uptake (kg/ha)	0.89***

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

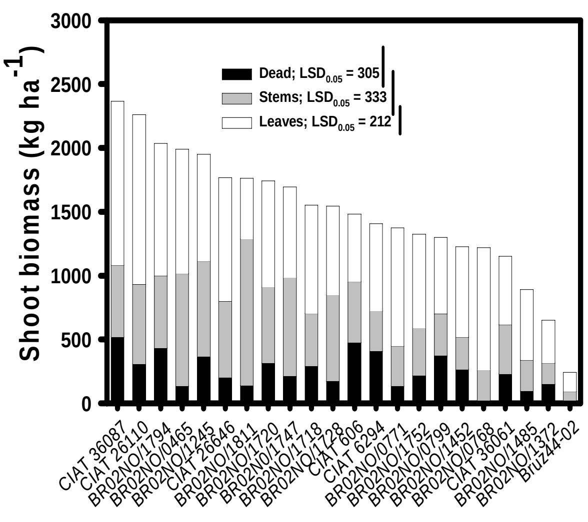

Figure 38. Genotypic variation in shoot biomass production (forage yield) of three parents (CIAT 606, 6294 and *B.ruziziensis* 44-02), four CIAT's accesions (26110, 26646, 36061 and 36087) and 15 genetic recombinants (BR02NO/0465; 0768; 0771; 0799; 1245; 1372; 1452; 1485; 1718; 1720; 1728; 1747; 1752; 1794 y 1811) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 13 months after establishment (August 2005). LSD values are at the 0.05 probability level. NS = not significant.

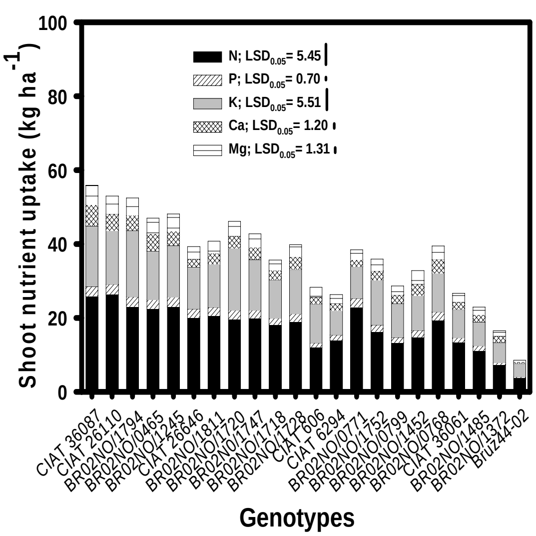

Figure 39. Genotypic variation in living shoot nutrient uptake (N, P, K, Ca and Mg) of three parents (CIAT 606, 6294 and *B.ruziziensis* 44-02), four CIAT's accesions (26110, 26646, 36061 and 36087) and 15 genetic recombinants (BR02NO/0465; 0768; 0771; 0799; 1245; 1372; 1452; 1485; 1718; 1720; 1728; 1747; 1752; 1794 y 1811) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 13 months after establishment (August 2005). LSD values are at the 0.05 probability level. NS = not significant.

3.2 Genotypes of *Arachis* with adaptation to edaphic factors

Highlights

- Showed that *Arachis pintoi* CIAT 18744 is very efficient in using phosphorus and this was not related to mycorrhizal association, acid phosphatase activity and root exudation.

- Found that *Arachis pintoi* CIAT 22172 has lower internal and external phosphorus requirements than the commercial cultivar (CIAT 17434).

3.2.1 Difference in P use efficiency in contrasting genotypes of *Arachis pintoi*

Contributors: N. Claassen, N. Castañeda-Ortiz (University of Goettingen, Germany), G. Neumann (University of Hohenheim, Germany) and I. M. Rao (CIAT)

Rationale

Large areas of acid soils in the tropics are of low P availability not because of low total P content but because P is strongly bound to Fe and Al or to Ca and therefore the P concentration in soil solution (C_{Li}) is very low, mostly below 1 µM. At this low P concentration transport to and uptake into roots is restricted. *Arachis pintoi* (Ap) is known to grow well on highly weathered low P soils of the Amazonia and the objective of this research was to investigate possible mechanisms of P efficiency, mainly whether arbuscular mycorrhizal (AM) infection, organic acid root exudates or acid phosphatase (Apase) activity could explain the P efficiency of *A. pintoi*. All parameter were measured on the same plants used to determine their P efficiency. Two genotypes, one P efficient and another P inefficient were used to better be able to pinpoint the reason for differences in P efficiency. Plant age is another factor affecting plant P efficiency, older plants being often more P efficient than younger ones. Therefore plants of different age were tested for their P efficiency.

Materials and Methods

The soil used was a fossil Oxisol from Germany taken from a pit, pH ($CaCl_2$) 5.2, 50% clay, P-CAL (calcium acetate lactate at pH 4.1 extraction) 0.4 mg kg^{-1} and soil solution (obtained by miscible displacement) P concentration (C_{Li}) of 0.12 µM. This C_{Li} is below C_{Lmin} of 0.2 µM known for *Arachis hypogea*. Two genotypes of forage groundnut, Ap CIAT 17434 considered as genotype P-inefficient (G-I) and Ap CIAT 18744 considered as genotype P-efficient (G-E) were used. Plants were grown in split-root pots, which consisted of a soil and a sand compartment. Phosphorus was only supplied in the soil compartment with two treatments of –P (0 mg P kg^{-1} soil) and +P [1000 mg P kg^{-1} soil as $Ca(H_2PO_4)_2$]. Other nutrients were supplied in sufficient amounts. The sand compartment was watered with a modified Hoagland nutrient solution without P. Seedlings were transplanted into the split-root pots (50% root in sand and 50% root in soil) and harvested after 30, 60 and 100 days of growth. At each harvest shoot dry weight, shoot and root P concentration, root fresh and dry weight as well as root length were determined. The P influx (mol cm^{-1} s^{-1}) between two harvests was calculated assuming a linear root growth. Root exudates were obtained by two procedures. The wall of the soil compartment possessed windows that were opened and filter paper discs were placed for two hours on roots growing at the exposed soil surface. The filter paper was extracted with water and organic acids (OA) analyzed by HPLC. Root exudates were also obtained by circulating water for two hours through the sand compartment. The solution was freeze dried and later redissolved for OA analysis by HPLC. APase activity was determined by suspending root segments form the soil compartment obtained at the windows in pnitrophenol phosphate and measuring the yellow colour development.

Results and Discussion

At ample P supply (data not shown) both genotypes had a similar, statistically not significant, yield of about 17 g plant^{-1}. But at limiting P supply (Figure 40a) the P efficient genotype (G-E) outyielded the P inefficient genotype (G-I) by a factor of about four. This superiority of G-E was due to a higher P uptake (Figure 40b). The superiority of G-E over G-I was already observed at younger age but the effect was much less pronounced, i.e. it was 50% at 30 days and 100% at 60 days. This shows that G-E became more P efficient as age progressed.

The higher P uptake of G-E was not because of more roots per unit of shoot (Figure 41a) but because of a higher P influx (Figure 41b). The increased P influx was most pronounced in the older plants of 60 to 100 days of age. Similar age effect was observed for maize by Bhadoria et al. (2001) who also found that older maize plants were more P efficient because their influx increased by a factor of around five with respect to younger plants.

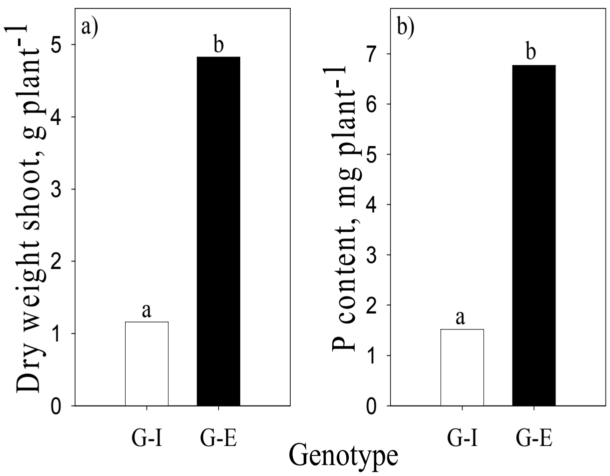

Figure 40. Yield and P content in shoot at P-0. Different letters are statistically different (Tukey-test, p<0.05).

To look for an explanation for different P influx AM infection, APase activity and organic acid exudation were investigated.

AM infection started only after 60 days and at 100 days the efficient genotype (G-E) showed a higher AM colonization than the inefficient one (G-I). But since differences in P efficiency already were evident before 60 days, AM could not be, at least not the only explanation for differences in P efficiency.

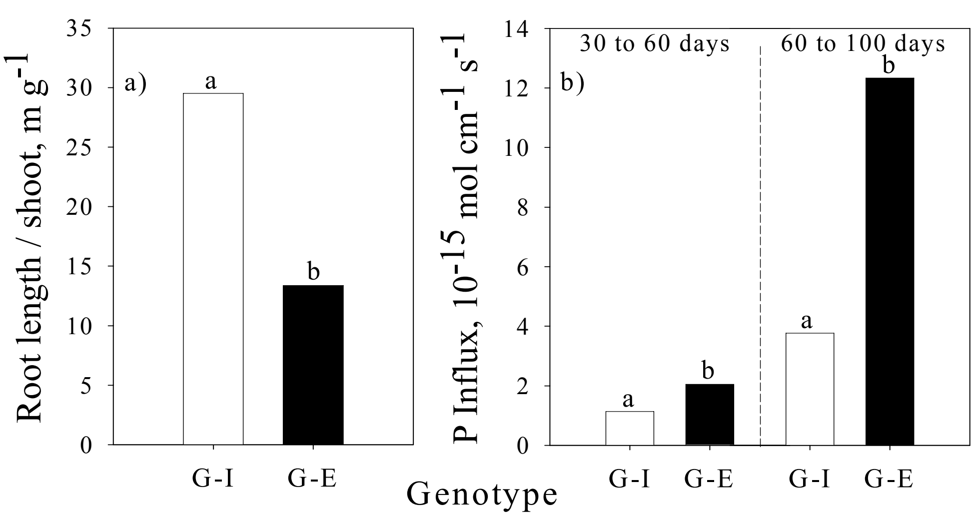

Figure 41. Root length-shoot ratio and P influx at P-0. Different letters are statistically different (Tukey-test, p<0.05).

Results shown in Figure 42 shows that the efficient genotype (G-E) had the same or lower APase activity indicating that this property was not responsible for its higher P influx nor for the increased P influx with age. The other factor investigated was the exudation of organic acids (OA). The roots that grew in soil did not show any OA exudation, and the portion of roots grown in sand exuded only measurable amounts of lactic and acetic acid while traces were only found for citric, malonic, malic, formic, fumaric, c-aconitic and t-aconitic acid. Oxalic, tartaric and succinic acid were not detected.

Results on P concentration in soil solution (C_{Li}) during the growth period and its relationship with the OA exudation rate by the two genotypes showed an increase in C_{Li} with time even though P was absorbed by the plants, i.e. the P mobilizing effect of the root exudates was stronger than the P depletion due to P uptake. P efficient genotype (G-E) showed a much stronger increase of C_{Li} than the G-I. This is in agreement with its much higher P influx shown in Figure 41. But the increased C_{Li} was not related to the exudation of OA, on the contrary higher exudation rates were related to lower value of C_{Li}.

In summary, the results of this research have shown that *A. pintoi* CIAT 18744 is very P efficient and is being able to thrive on soils with P soil solution concentrations that are below the C_{Lmin} value for other plants. The mechanism by which this high P efficiency was achieved was not because of AM infection, nor due to APase activity nor due to organic acid exudation. Further research investigating other mechanisms of P mobilization by *Arachis pintoi* is needed.

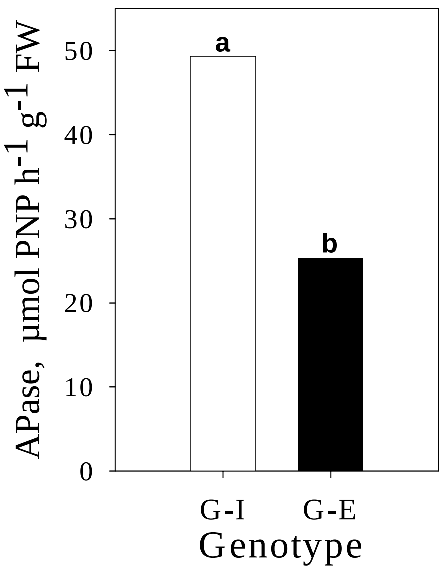

Figure 42. Acid phosphatase activity on the root surface at P-0. Different letters are statistically different (Tukey-test, $p<0.05$).

3.2.2 Phosphorus requirements of *Arachis pintoi* genotypes

Contributors: I. M. Rao, M. Rodríguez, E. Díaz, J. Ricaurte and R. García (CIAT)

Rationale

Previous field and greenhouse studies indicated genotypic differences in *Arachis pintoi* for phosphorus (P) acquisition. These genotypic differences in P acquisition among the accessions CIAT 17434, CIAT 18744 and CIAT 22172 were related to an increase of P in soil solution concentration with CIAT 18744 i.e. to P mobilization in the rhizosphere.

These results indicated that the rapid establishment as well as the sustained yield of CIAT 18744 and 22172 was due to their great ability to acquire P from P-deficient soil per unit root length. However, there was no significant difference among the genotypes in their ability to utilize acquired P. A greenhouse study was conducted with an objective to determine genotypic differences in P requirements of selected acessions compared to commercial cultivars. We tested the following three hypotheses: (i) internal P requirements of selected CIAT accessions are lower than those of the commercial cultivar (CIAT 17434); (ii) selected CIAT accessions are capable of acquiring greater amounts of P from lower level of P supply; and (iii) partitioning of P to leaves, particularly at low P supply, is grater with the selected CIAT accessions compared to commercial cultivars.

Materials and Methods

A greenhouse study was conducted using a sandy loam oxisol from Matazul farm in the Llanos of Colombia. The trial comprises 8 CIAT accesions of *Arachis pintoi* (17434, 18744, 18747, 18748, 18751, 22159, 22160 and 22172). A pot, containing 4 kg of soil/pot fertilized with basal nutrient (kg/ha) 80 N, 100 K, 66 Ca, 28.5 Mg, 20 S and micronutrients at 2 Zn, 2 Cu, 0.1 B and 0.1 Mo, sown with 2 plantlets germinated in quartz sand was the experimental unit. Six levels of P (0, 10, 20, 40, 80 and 160) were used. At the time of harvest (83 days of growth), plants were separated into the youngest fully expanded leaf (YEL), the remainder of the whole leaves (WL), the whole stems (WS) and roots (WR). The following measurements were made: leaf area, leaf biomass, stem biomass, root biomass, root length, specific root length, inorganic P in YEL, total P in YEL and WL, total P in leaf and stem biomass, leaf to stem ratio, shoot P uptake and leaf P partitioning index.

Genotypic differences in P requirements were determined by the calculation of 90% of maximum shoot biomass production using the equation $Y = a / \{1 + [e^{-(x - x0) / b)}]\}$. Y = shoot biomass production (g pot^{-1}); x = P supplied (kg ha^{-1}); e = 2.72; a, b were the coefficients of the equation.

Results and Discussion

Increase in P supply had markedly improved shoot biomass production of all the genotypes tested indicating that P supply in soil is a major limitation to forage yield (Figure 43).

Table 37. P requirements (external and internal) of eight genotypes of of *Arachis pintoi* grown in pots with acid soil from Matazul, Llanos of Colombia.

Accession number	External P requirement (kg ha^{-1})	Internal P requirement (mg g^{-1} dry weight)
CIAT 17434	53	1.23
CIAT 18744	55	1.25
CIAT 18747	122	1.42
CIAT 18748	75	0.84
CIAT 18751	85	1.34
CIAT 22159	110	2.00
CIAT 22160	76	1.25
CIAT 22172	41	0.80

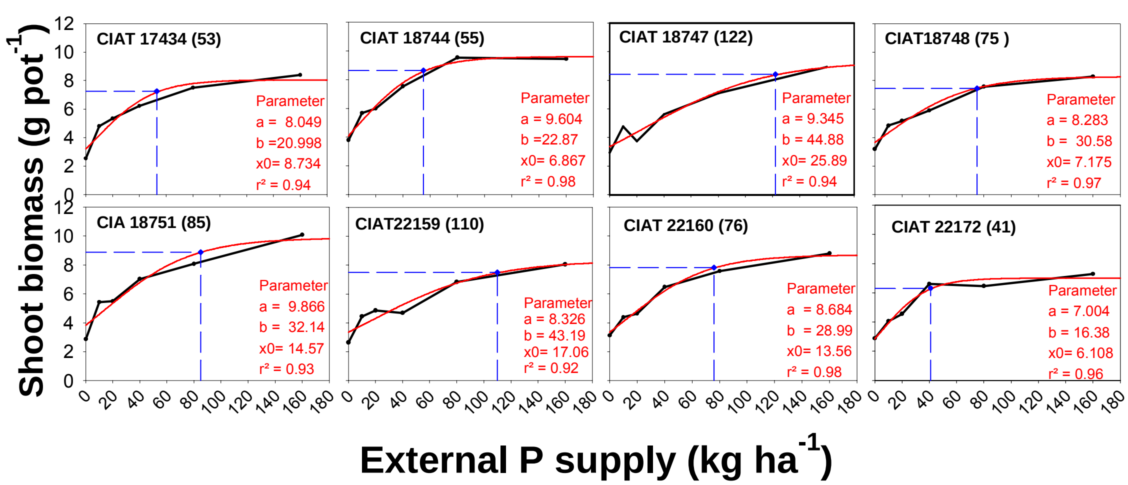

Figure 43. Shoot biomass production response to external P supply to soil of eight genotypes of *Arachis pintoi* grown in pots. Critical values for external P requirement are indicated in brackets. These were calculated with the parameters of the equation shown at right side of each plot.

The external P requirement of the accession CIAT 22172 was markedly lower (41 kg ha^{-1}) than that of the other genotypes (Figure 43, Table 37), particularly CIAT 18747 and CIAT 22159 (122 and 110 kg ha^{-1}, respectively). Internal P requirement of CIAT 22172 and CIAT 18748 were also markedly lower than that of the other genotypes (Figure 44, Table 37). The predicted P response of all genotypes was very closely related (r^2 values greater than 0.91) to the observed response (Figure 43). Internal P requirements based on P content in youngest expanded leaf were markedly lower for the accessions 18748 and 22172 (Figure 44).

Results from this study indicated that *Arachis pintoi* CIAT 22172 has lower internal and external phosphorus requirements than the traditional cultivars (CIAT 17434 and 22160).

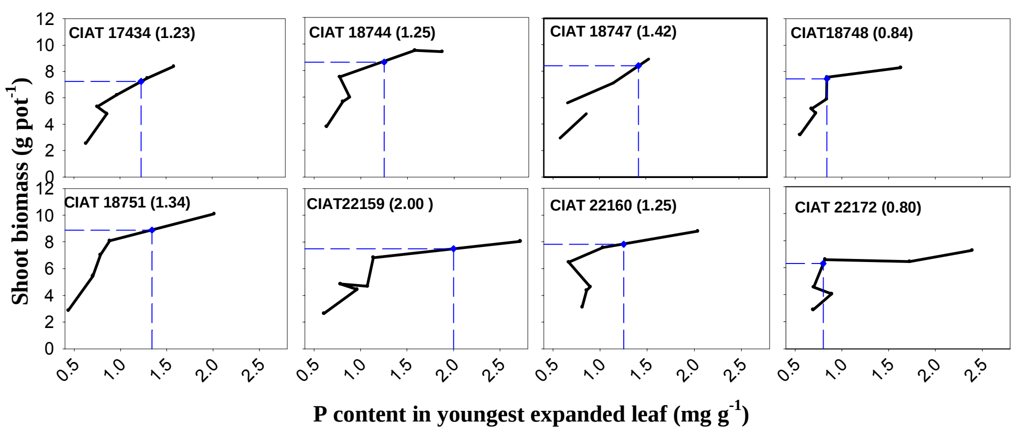

Figure 44. Shoot biomass production response to internal P content of the youngest expanded leaf of eight genotypes of *Arachis pintoi* grown in pots. Critical values for internal P requirement are indicated in brackets.

3.3 Genotypes of *Brachiaria* with dry season tolerance

Highlights

- Showed genetic variability for drought adaptation among *Brachiaria* genotypes grown in acid soil in pots.
- Developed transparent plastic tube screening system to evaluate the impact of drought on genotypic differences of *Brachiaria* genotypes in root growth and distribution in soil.
- Showed that the *Brachiaria* hybrid, cv. Mulato II) performed well into the third year after establishment in the Llanos and its superior performance at 30 months after establishment was associated with its ability to acquire greater amounts of nutrients from low fertility soil.
- Showed that among the 15 *Brachiaria* hybrids tested, BR02NO/1811 was more productive than the other hybrids in terms of green forage (leaf + stem) yield in the dry season and this adaptation seems to be closely associated with lower amounts of stem P content.

3.3.1 Genotypic variation in drought tolerance of *Brachiaria* under greenhouse conditions using small pots

Contributors: J. Rincón, F. Feijoo and I. M. Rao (CIAT)

Rationale

Identification of shoot and root attributes that are associated with superior drought adaptation will help to develop rapid and reliable screening methods. These methods are needed for the on-going *Brachiaria* genetic enhancement efforts to develop *Brachiaria* hybrids that combine drought adaptation with other desirable attributes. A greenhouse study was conducted to characterize shoot and root responses of 22 *Brachiaria* genotypes when subjected to two levels of water supply to pots.

Materials and Methods

A greenhouse study was conducted using a mix of sandy loam Oxisol from Matazul in the Llanos of Colombia with sand (2:1 w/w). The trial comprises 22 genotypes, including four parents (*B. decumbens* CIAT 606, *B. brizantha* CIAT 6294, *B. ruziziensis* 44-02 and CIAT 26646), three checks (cv Mulato CIAT 36061, cv Mulato 2 CIAT 36087 and cv Toledo CIAT 26110) and fifteen apomitic hybrids (BR02NO/ 0465, 0768, 0771, 0799, 1245, 1372, 1452, 1485, 1718, 1720, 1728, 1747, 1752, 1794, 1811) that were preselected for their resistance to spittlebug. Plants were grown in pots (8 inches diameter). The trial was planting as a randomized block in split-plot arrangement with two levels of water supply: 100% field capacity (well-watered) and 40% field capacity (drought stress) as main plots and genotypes as sub-plots.

Soil was fertilized with adequate level of nutrients (kg/ha of 80 N, 50 P, 100 K, 66 Ca, 28 Mg, 20 S and micronutrients). Treatments of water stress were imposed after three weeks of initial growth of plants established with stem cuttings with one plant per pot. Water stress was maintained by weighing each pot every two days and applying water to the soil at the top of the pot. By adding water to pots we simulated conditions similar to that of intermittent drought stress. After 2 months of growth (61 days after transferred into pot), shoot biomass distribution, root biomass, leaf area, photosynthetic efficiency, total chlorophyll content (SPAD), stomatal conductance, transpiration rate, and leaf and stem nutrient composition, ash content and TNC (total nonstructural carbohydrates) contents were determined.

Results and Discussion

Dry matter distribution with drought treatment (40% field capacity-FC) showed significant genotypic variation among checks and the hybrids of *Brachiaria* (Figure 45). Total

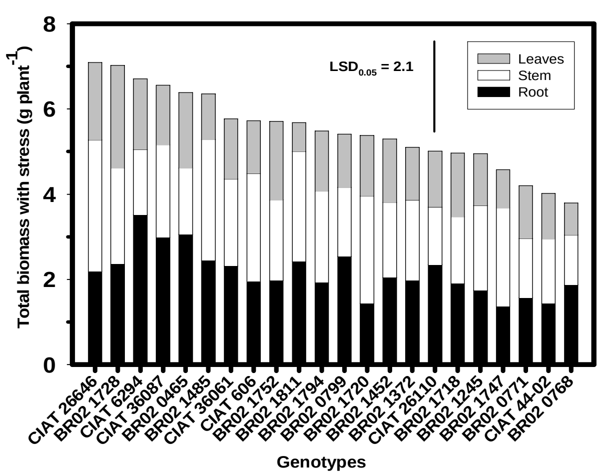

Figure 45. Influence of drought stress on genotypic variation in dry matter distribution among leaves, stems and roots of four parents (*B. decumbens* CIAT 606, *B. brizantha* CIAT 6294, *B. ruziziensis* 44-02 and CIAT 26646), three checks (cv Mulato CIAT 36061, cv Mulato II CIAT 36087 and cv Toledo CIAT 26110) and fifteen apomitic hybrids of BR02NO population of *Brachiaria* grown in pots. LSD values are at the 0.05 probability level.

biomass production under stress conditions was greater for CIAT 26646 (cv. La Libertad) and a hybrid 1728. This hybrid had greater proportion of biomass partitioned to leaves and roots compared with stems. One of the parents BRUZ 44-02 (sexual) was among the least productive genotypes under drought stress. Root biomass production was greater with another apomictic parent, CIAT 6294 (cv. Marandu). Root biomass production of the two cultivars, Mulato (CIAT 36061) and Mulato II (CIAT 36087) was also higher than several genotypes tested. These 2 cultivars also showed greater levels of leaf chlorophyll content and leaf N content (Table 38). Results on the influence of drought stress on a number of other plant attributes are shown in Table 38. Transpiration rate under drought stress was greater for the sexual parent BRUZ 44-02 and markedly lower for the apomictic parent CIAT 6294. Leaf and Stem TNC contents were markedly greater with Mulato II under drought stress indicating its ability to maintain greater forage quality under drought stress conditions.

In summary, this pot study showed genetic variability for intermittent drought adaptation in acid soil conditions. Based on this study, further studies were conducted to look at the effect of drought stress on root growth and distribution in transparent plastic cylinders.

Table 38. Influence of drought stress on leaf chlorophyll content, transpiration rate, leaf N content and leaf and stem TNC content of four parents (*B. decumbens* CIAT 606, *B. brizantha* CIAT 6294, *B. ruziziensis* 44-02 and CIAT 26646), three checks (cv Mulato CIAT 36061, cv Mulato 2 CIAT 36087 and cv Toledo CIAT 26110) and fifteen apomitic hybrids of BR02NO population of *Brachiaria* grown in pots.

Genotype	Leaf Chlorophyll Content (SPAD)		Transpiration Rate (mmol m^{-2} s^{-1})		Leaf N Content (%)		Leaf TNC Content (mg g^{-1})		Stem TNC Content (mg g^{-1})	
	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control
CIAT 26646	47.6	43.9	1.562	2.159	2.179	0.958	157.8	138.9	111.0	135.1
BR02 1728	52.6	48.4	1.558	1.928	2.029	0.942	159.5	129.7	179.4	96.6
CIAT 6294	51.1	47.2	1.399	1.917	2.187	0.900	161.6	133.2	209.8	126.4
CIAT 36087	55.4	52.6	1.461	1.813	2.361	1.098	202.8	144.7	227.4	111.8
BR02 0465	51.3	49.0	1.579	1.904	2.263	1.059	151.1	105.9	172.5	93.8
BR02 1485	51.2	49.6	1.655	2.221	2.160	0.995	192.4	185.4	107.1	127.5
CIAT 36061	56.2	48.7	1.550	2.031	2.284	0.927	159.8	114.1	188.0	137.5
CIAT 606	53.8	45.8	1.452	2.032	2.339	0.976	148.9	159.4	149.5	136.3
BR02 1752	56.6	46.4	1.610	2.174	1.895	0.945	142.5	126.0	222.7	123.9
BR02 1811	47.1	46.7	1.603	2.392	2.145	0.972	187.6	152.5	146.9	84.8
BR02 1794	54.9	50.4	1.800	1.994	2.169	0.948	204.0	137.5	203.2	162.5
BR02 0799	47.9	44.4	1.504	1.950	2.292	0.930	139.6	101.1	179.6	82.4
BR02 1720	50.7	49.9	1.537	1.934	2.577	1.037	208.5	132.0	185.1	117.7
BR02 1452	52.1	48.9	1.527	2.109	2.156	0.994	143.0	120.4	175.8	139.3
BR02 1372	53.4	46.4	1.580	2.137	2.823	0.934	166.0	132.8	240.1	113.6
CIAT 26110	45.8	42.9	1.547	2.044	2.290	1.062	143.2	165.1	160.4	83.1
BR02 1718	53.2	51.7	1.447	1.911	2.341	0.914	166.8	112.7	169.1	111.2
BR02 1245	53.1	49.6	1.461	1.963	2.327	0.875	215.5	156.5	181.2	101.7
BR02 1747	53.0	46.7	1.506	1.946	2.480	0.877	180.3	143.9	129.3	108.5
BR02 0771	43.0	43.5	1.783	2.185	2.405	1.041	168.4	138.6	152.0	141.0
CIAT 44-02	47.2	46.6	1.729	1.982	2.087	1.117	165.9	165.3	194.2	123.3
BR02 0768	47.0	47.8	1.494	2.192	2.118	1.060	152.2	138.4	166.7	111.5
Mean	**51.1**	**47.6**	**1.56**	**2.042**	**2.269**	**0.980**	**169.0**	**137.9**	**175.1**	**116.8**

3.3.2 Genotypic variation in drought tolerance of *Brachiaria* under green-house conditions using transparent plastic cylinders

Contributors: J. Rincón, F. Feijoo, and I. M. Rao (CIAT)

Rationale

Adaptive attributes to drought at root system level are difficult to dissect under field condition due to labor intensive nature of the work. Therefore we developed greenhouse methodology to study root development and distribution using transparent plastic tubes filled with soil. We tested the usefulness of this methodolgy by characterizing shoot and root responses of 16 *Brachiaria* genotypes when subjected to two levels water supply.

Materials and Methods

A greenhouse study was conducted using a mix of sandy loam Oxisol (from Matazul of the Llanos of Colombia) with sand (2:1 w/w). The cylinder contained 3 kg of soil, with bulk density of 1.45 g/cc. The trial comprised 16 genotypes, including four parents (*B. decumbens* CIAT 606, *B. brizantha* CIAT 6294, *B. ruziziensis* 44-02 and CIAT 26646), three checks (cv Mulato CIAT - 36061, cv Mulato II - CIAT 36087 and cv Toledo - CIAT 26110) and nine apomitic hybrids (BR02/0768, 0771, 1372, 1452, 1720, 1728, 1747, 1752, 1794) that were preselected for their resistance to spittlebug. Plants were grown in small transparent plastic cylinders (50 cm long and 7.5 cm diameter) covered with PVC tubes. The trial was planted as a randomized block in split-plot arrangement with two levels of water supply: 100% field capacity (well-watered) and 50% field capacity (drought stress) as main plots and genotypes as sub-plots.

Soil was fertilized with adequate level of nutrients (kg/ha: 80 N, 50 P, 100 K, 66 Ca, 28 Mg, 20 S and micronutrients). Treatments of water stress were imposed after two weeks of initial growth of plants established with stem cuttings with 1 plant per cylinder. Water stress was maintained by weighing each cylinder every two days and applying water to the soil at the top of the cylinder. By adding water to pots we simulated conditions similar to that of intermittent drought stress. By adding water to cylinders we simulated conditions similar to intermittent drought stress. After 45 days of growth, total dry matter distribution, root length distribution in different soil depths, leaf area were determined.

Results and Discussion

Significant genotypic variation was observed among checks and the hybrids of *Brachiaria* in terms of dry matter distribution under drought treatment (50% field capacity-FC) (Figure 46). Total biomass production under stress conditions was greater for cv. Mulato (CIAT 36061) and a hybrid 1728 due to increased leaf biomass production. Two parents (CIAT 6294 and BRUZ 44-02) produced greater proportion of root biomass under drought conditions. Total root

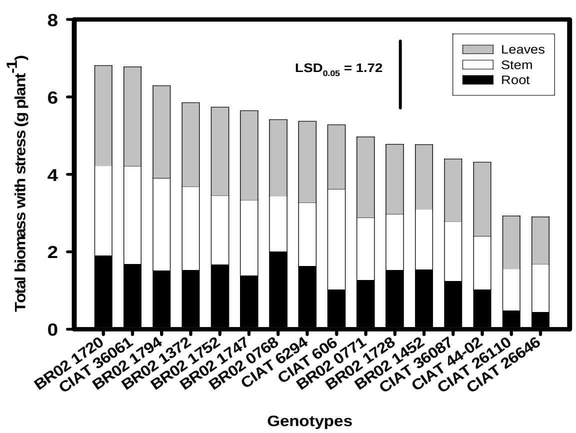

Figure 46. Influence of drought stress on genotypic variation in dry matter distribution among leaves, stems and roots of four parents (*B. decumbens* CIAT 606, *B. brizantha* CIAT 6294, *B. ruziziensis* 44-02 and CIAT 26646), three checks (cv Mulato CIAT - 36061, cv Mulato II - CIAT 36087 and cv Toledo - CIAT 26110) and nine apomitic hybrids of BR02 NO population of *Brachiaria* grown in plastic cylinders. LSD values are at the 0.05 probability level.

Table 39. Influence of drought stress on root and shoot attributes of four parents (*B. decumbens* CIAT 606, *B. brizantha* CIAT 6294, *B. ruziziensis* 44-02 and CIAT 26646), three checks (cv Mulato CIAT - 36061, cv Mulato II - CIAT 36087 and cv Toledo - CIAT 26110) and nine apomitic hybrids of BR02 NO population of *Brachiaria* grown in plastic cylinders.

Genotype	Specific leaf area (cm^2 g^{-1})		Mean root diameter (mm)		Specific root length (m $plant^{-1}$)		Root /shoot ratio	
	Stress	Control	Stress	Control	Stress	Control	Stress	Control
BR02 1720	114	173	0.370	0.393	119.68	134.67	0.390	0.284
BR02 1372	103	181	0.325	0.338	112.47	136.45	0.356	0.238
BR02 0768	145	202	0.347	0.361	104.95	73.73	0.592	0.231
BR02 1752	106	181	0.351	0.344	103.45	204.99	0.413	0.345
BR02 1728	152	183	0.351	0.307	99.08	210.86	0.472	0.492
BR02 1747	108	165	0.336	0.321	92.84	191.24	0.328	0.331
BR02 1794	108	190	0.365	0.371	90.60	198.39	0.319	0.348
CIAT 36061	114	180	0.365	0.403	90.13	195.26	0.332	0.389
CIAT 6294	127	175	0.350	0.313	87.24	112.38	0.439	0.428
BR02 0771	114	180	0.330	0.393	78.96	69.61	0.348	0.425
CIAT 606	156	231	0.351	0.356	70.09	71.36	0.243	0.168
CIAT 44-02	127	169	0.402	0.370	64.93	101.66	0.315	0.217
BR02 1452	168	188	0.381	0.379	63.56	54.41	0.477	0.146
CIAT 26110	145	143	0.352	0.258	41.79	78.38	0.198	0.147
CIAT 26646	125	158	0.320	0.339	41.61	36.35	0.181	0.351
CIAT 36087	138	195	0.394	0.446	41.39	74.31	0.398	0.468
Mean	**128**	**181**	**0.356**	**0.356**	**81.42**	**121.50**	**0.363**	**0.313**

length across the soil depth under drought stress was markedly greater with a number of hybrids compared with parents and the checks (Figures 47 and 49). Two hybrids 1720 and 1372 were particularly outstanding in their ability to develop fine root system.

Among these 2 hybrids, 1372 was also found he very resistant to aluminum toxicity in the hydroponic screening system. Results on genotypic variation in specific root length (root length per unit root weight), which is a measure of fine root development also showed that two checks (CIAT 26646 and CIAT 26110) and the hybrid 1372 were outstanding (Figures 48 and 50). Specific root length values were markedly lower for cv. Mulato II (CIAT 36087).

Results on the effect of drought on other root and shoot attributes are shown in Table 39. Mean root diameter values under drought stress indicated that the hybrid 1372 was outstanding in developing thin and long roots (Table 39).

Among the genotypes tested, the hybrid 1452 showed greater values of specific leaf area (leaf area per unit leaf weight) while the check cv. Toledo (CIAT 26110) was outstanding in combining greater values of specific leaf area and specific root length under drought stress (Table 39; Figure 48).

Based on this study, further studies will be conducted to characterize the effect of terminal drought stress (simulated by withholding water supply) on genotypic differences in root growth and distribution in transparent plastic cylinders.

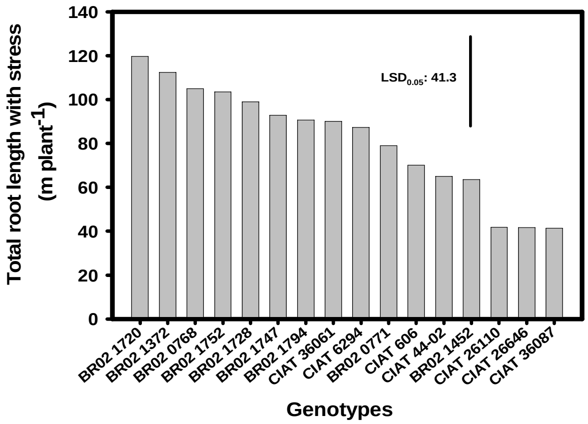

Figure 47. Influence of drought stress on genotypic variation in total root length per plant of four parents (*B. decumbens* CIAT 606, *B. brizantha* CIAT 6294, *B. ruziziensis* 44-02 and CIAT 26646), three checks (cv Mulato CIAT - 36061, cv Mulato II - CIAT 36087 and cv Toledo - CIAT 26110) and nine apomitic hybrids of BR02 NO population of *Brachiaria* grown in plastic cylinders. LSD values are at the 0.05 probability level.

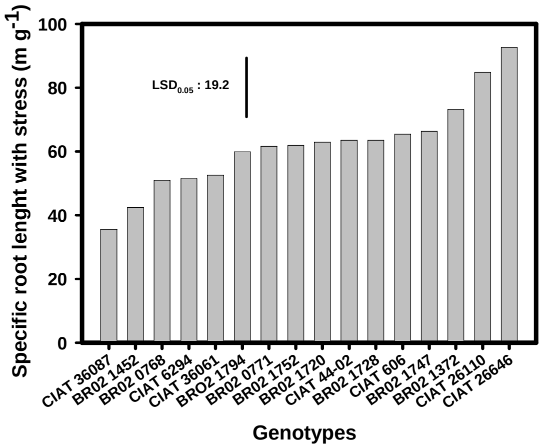

Figure 48. Influence of drought stress on genotypic variation in specific root length of four parents (*B. decumbens* CIAT 606, *B. brizantha* CIAT 6294, *B. ruziziensis* 44-02 and CIAT 26646), three checks (cv Mulato CIAT - 36061, cv Mulato II - CIAT 36087 and cv Toledo - CIAT 26110) and nine apomitic hybrids of BR02 NO population of *Brachiaria* grown in plastic cylinders. LSD values are at the 0.05 probability level.

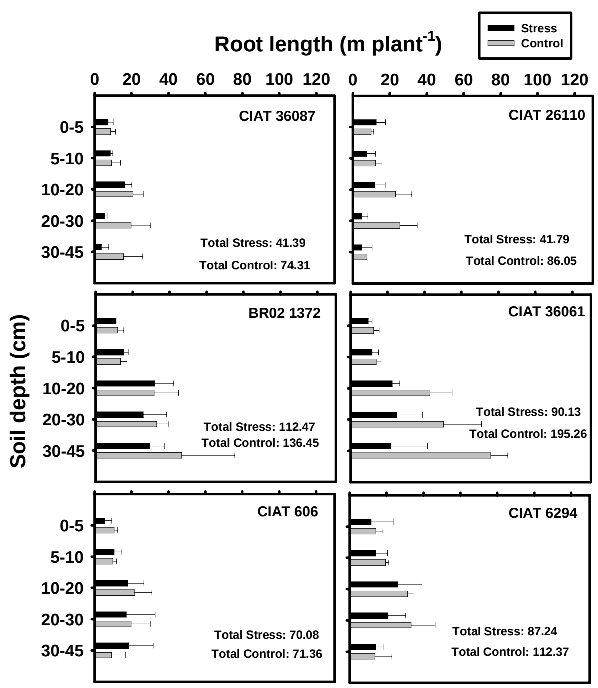

Figure 49. Influence of two levels of water supply (100% and 50% of field capacity) on root length distribution across soil depth in two parents (*B. decumbens* CIAT 606 and *B. brizantha* CIAT 6294), three checks (cv Mulato CIAT - 36061, cv Mulato II - CIAT 36087 and cv Toledo - CIAT 26110) and one apomyctic hybrid (BR02 NO 1372) of *Brachiaria* grown in plastic cilindres.

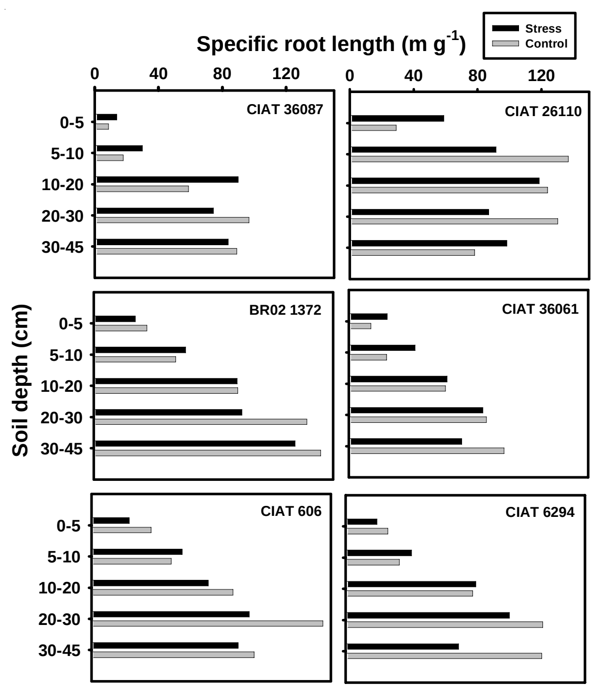

Figure 50. Influence of two levels of water supply (100% and 50% of field capacity) on specific root length distribution across soil depth in two parents (*B. decumbens* CIAT 606 and *B. brizantha* CIAT 6294), three checks (cv Mulato CIAT - 36061, cv Mulato II - CIAT 36087 and cv Toledo - CIAT 26110) and one apomitic hybrid (BR02 NO 1372) of *Brachiaria* grown in plastic cylinders.

3.3.3 Dry season tolerance of promising hybrids of *Brachiaria* in the Llanos of Colombia

Contributors: I. M. Rao, J. Miles, C. Plazas and J. Ricaurte (CIAT)

Rationale

Quantity and quality of dry season feed is a major limitation to livestock productivity in subhumid regions of tropical America. Evaluation of a large number of *Brachiaria* hybrids for their resistance to spittlebug and adaptation to infertile acid soils resulted in identification of a few promising *Brachiaria* hybrids. A field study is in progress at Matazul Farm in the Llanos of Colombia. We selected 4 of these hybrids for further field-testing in comparison with their parents. The main objective was to evaluate genotypic differences in dry season (4 months of moderate drought stress) tolerance of most promising genetic recombinants of *Brachiaria*. Results from this field study for the past 3 years indicated that the superior performance of the germplasm accession CIAT 26110 and the *Brachiaria* hybrid, FM9503-S046-024 (Mulato 2), which maintained greater proportion of green leaves during moderate dry season in the llanos of Colombia, was associated with greater acquisition of nutrients under water deficit conditions. This year, we report results from the dry season performance into the fourth year after establishment.

Field evaluation of a number *Brachiaria* hybrids under infertile acid soil conditions with low or high amounts of initial application of fertilizer and application of maintenance fertilizer with half of the amounts of initial applications at 2 year intervals improved the persistence of several hybrids and not allowed to distinguish differences between moderately adapted cultivars (cv. Marandu) and the promising hybrids. Therefore a field experiment was established in 2004 with 15 hybrids along with the parents and checks with low amounts of initial application of fertilizer and no maintenance fertilizer application to select hybrids that persist and produce green forage under acid infertile soil conditions.

Materials and Methods

A field trial was established at Matazul farm on 31 May of 2001. The trial included 4 *Brachiaria* hybrids (BR98NO/1251; BR99NO/4015; BR99NO/4132; FM9503-S046-024) along with 2 parents (*B. decumbens* CIAT 606 and *B. brizantha* CIAT 6294). The trial was planted as a randomized block in split-plot arrangement with two levels of initial fertilizer application (low: kg/ha of 20P, 20K, 33Ca, 14 Mg, 10S; and high: 80N, 50P, 100K, 66Ca, 28Mg, 20S and micronutrients) as main plots and genotypes as sub-plots with 3 replications. Maintenance fertilizer (half the levels of initial applications) was applied at 2 years after establishment. The plot size was 5 x 2 m. A number of plant attributes including forage yield, dry matter distribution, nutrient (N, P, K, Ca and Mg) uptake, leaf and stem total nonstructural carbohydrate (TNC) content and leaf and stem ash (mineral) content were measured at 46 months after establishment (April 2005).

Results and Discussion

At 46 months after establishment, live forage yield with low fertilizer application was greater with one spittlebug resistant genetic recombinant, FM9503-S046-024 (Mulato II) and one parent (CIAT 6294) (Table 40). With high initial fertilizer application also these two genotypes were outstanding in live forage yield (Figure 51). Among the 4 hybrids tested, Mulato II was outstanding in its adaptation to low initial fertilizer application. It is important to note that both Mulato 2 and CIAT 6294 had greater amount of dead biomass and stem biomass under low fertilizer application (Figure 51). Hybrid 4015 also performed well with low fertilizer application.

As observed last year, results on shoot nutrient uptake indicated that Mulato 2 was superior to CIAT 606 under low fertilizer application (Figure 52). Nutrient acquisition by Mulato 2 was also greater than the rest of the hybrids with high initial fertilization. These results are consistent with the results reported last year from the same experiment.

Correlation coefficients between live forage yield and other plant attributes indicated that greater nutrient acquisition with low initial fertilizer application contributed to superior performance (Table 40). These results are consistent with the results obtained for the past 2 years. Significant negative correlation was observed between leaf N content and live forage yield under low fertilizer application. Adaptation to low fertilizer application seem to be closely associated with lower amounts of both leaf and stem N content. However, no significant correlations were found between live forage yield and leaf and stem TNC or ash contents. Results from this field study confirmed the previous findings of the superior drought adaptation of *Brachiaria* hybrid Mulato II under infertile acid soil conditions.

Table 40. Correlation coefficients (r) between green forage yield (t/ha) and other shoot traits of *Brachiaria* genotypes grown with low or high intial fertilizer application in a sandy loam oxisol in Matazul, Colombia.

Shoot traits	Low fertilizer	High fertilizer
Total (live + dead) shoot biomass (t/ha)	0.84**	0.87***
Dead shoot biomass (t/ha)	0.50*	0.64**
Leaf biomass (t/ha)	0.97***	0.96***
Stem biomass (t/ha)	0.63*	0.84***
Leaf N content (%)	-0.40*	-0.35
Leaf P content (%)	-0.29	-0.03
Leaf TNC content (mg g-1)	-0.08	-0.12
Leaf ash content (%)	00.07	0.05
Stem N content (%)	-0.53**	-0.51*
Stem P content (%)	-0.38	-0.39
Stem TNC content (mg g-1)	-0.05	0.30
Stem ash content (%)	-0.01	0.07
Shoot N uptake (kg/ha)	0.82***	0.93***
Shoot P uptake (kg/ha)	0.82***	0.88***
Shoot K uptake (kg/ha)	0.78***	0.90***
Shoot Ca uptake (kg/ha)	0.61**	0.86***
Shoot Mg uptake (kg/ha)	0.61**	0.88***

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

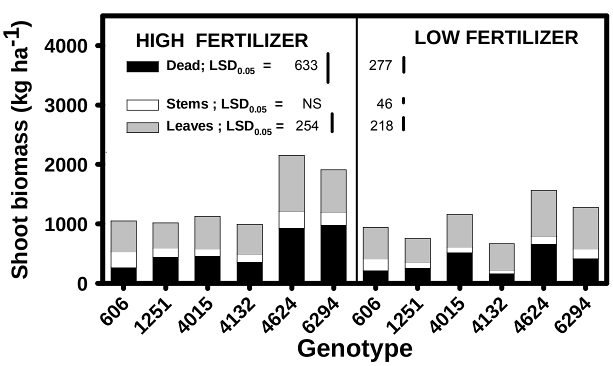

Figure 51. Genotypic variation as influenced by fertilizer application in shoot biomass production (forage yield) of two parents (CIAT 606, 6294) and four genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 46 months after establishment (April 2005). LSD values are at the 0.05 probability level. NS = not significant.

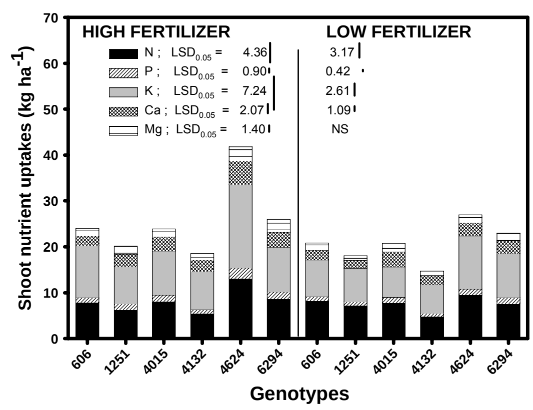

Figure 52. Genotypic variation as influenced by fertilizer application in nutrient uptake (N, P, K, Ca and Mg) of two parents (CIAT 606, 6294) and four genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 46 months after establishment (April 2005). LSD values are at the 0.05 probability level. NS = not significant.

3.3.4 Field screening for drought tolerance of promising hybrids of *Brachiaria* in the Llanos of Colombia

Contributors: I. M. Rao, J. Miles, C. Plazas and J. Ricaurte (CIAT)

Rationale

Field evaluation of a number *Brachiaria* hybrids under infertile acid soil conditions with low or high amounts of initial application of fertilizer and application of maintenance fertilizer with half of the amounts of initial applications at 2 year intervals improved the persistence of several hybrids and not allowed to distinguish differences between moderately adapted cultivars (cv. Marandu) and the promising hybrids in dry season tolerance.

Therefore a field experiment was established in 2004 with 15 hybrids along with the parents and checks with low amounts of initial application of fertilizer and no maintenance fertilizer application to select hybrids that persist and produce green forage under drought and acid infertile soil conditions.

Materials and Methods

A field trial was established at Matazul farm on 29 June of 2004. The trial included 15 *Brachiaria* hybrids (BR02NO/0465; BR02NO/0768; BR02NO/ 0771; BR02NO/0799; BR02NO/1245; BR02NO/1372; BR02NO/1452; BR02NO/1485; BR02NO/1718; BR02NO/1720; BR02NO/1728; BR02NO/1747; BR02NO/1752; BR02NO/1794 and BR02NO/1811), three parents (*B. decumbens* CIAT 606 and *B. brizantha* CIAT 6294 and *B.ruziziensis* 44-02) and four CIAT checks (*B.brizantha* CIAT 26110, *B.brizantha* CIAT 26646, *Brachiaria* hybrids CIAT 36061 and CIAT 36087).

The trial was planted as a randomized block with 4 replications. One low level of initial fertilizer application was applied (kg/ha of 20P, 20K, 33Ca, 14 Mg, 10S) at the time of establishment. Maintenance fertilizer was not scheduled for application in order to identify genotypes that are bettter adapted to infertile acid soil conditions. The plot size was 5 m x 2.5 m. A number of plant attributes including forage yield, dry matter distribution and nutrient (N, P, K, Ca and Mg) uptake were measured at 9 months after establishment and 3 months of dry season (April 2005).

Results and Discussion

At 9 months after establishment CIAT 606, CIAT 36061, CIAT 36087, BRO2NO/1452 , BRO2NO/1485, BRO2NO/1720, BRO2NO/1747, BRO2NO/1752 and BRO2NO/1794 were affected by bacterial infection. Among the hybrids, BRO2NO/1752 was relatively more affected. The dead shoot biomass was greater with a hybrid (BR02NO/1811) and a check (CIAT 26110) (Figure 53).

This hybrid was also outstanding among the hybrids in producing the green forage (leaf + stem biomass) than the other hybrids. Mulato 2 (CIAT 36087) was outstanding in green leaf forage production. Among the hybrids tested BR02NO/0768 was outstanding in green leaf biomass production.

As expected BRUZ 44-02 (sexual parent) was least productive of all the genotypes tested. Results on shoot nutrient uptake also indicated that the hybrid BR02NO/1794 was superior to other hybrids (Figure 54).

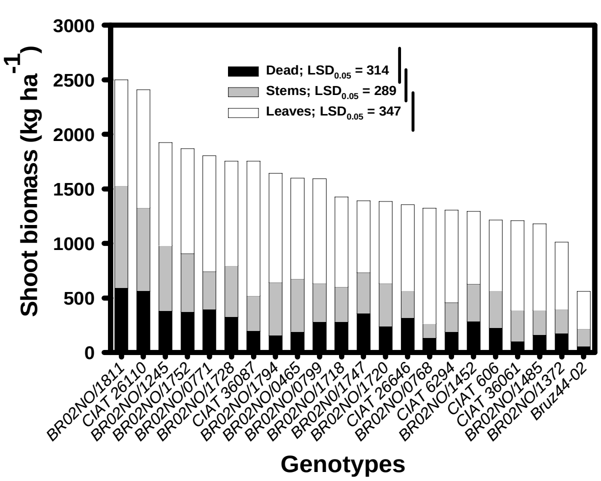

Figure 53. Genotypic variation in shoot biomass production (forage yield) of three parents (CIAT 606, 6294 and *B.ruziziensis* 44-02), four CIAT's accesions (26110, 26646, 36061 and 36087) and 15 genetic recombinants (BR02NO/ 0465; 0768; 0771; 0799; 1245; 1372; 1452; 1485; 1718; 1720; 1728; 1747; 1752; 1794 and 1811) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 9 months after establishment (April 2005). LSD values are at the 0.05 probability level. NS = not significant.

Table 41. Correlation coefficients (r) between green forage yield (t/ha) and other shoot traits of *Brachiaria* genotypes grown with low intial fertilizer application in a sandy loam oxisol in Matazul, Colombia.

Shoot traits	Low fertilizer
Total (live + dead) shoot biomass (t/ha)	0.97***
Dead shoot biomass (t/ha)	0.74***
Leaf biomass (t/ha)	0.90***
Stem biomass (t/ha)	0.88***
Leaf N content (%)	-0.13
Leaf P content (%)	-0.08
Stem N content (%)	-0.18
Stem P content (%)	-0.43***
Shoot N uptake (kg/ha)	0.85***
Shoot P uptake (kg/ha)	0.88***
Shoot K uptake (kg/ha)	0.88***
Shoot Ca uptake (kg/ha)	0.76***
Shoot Mg uptake (kg/ha)	0.79***

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

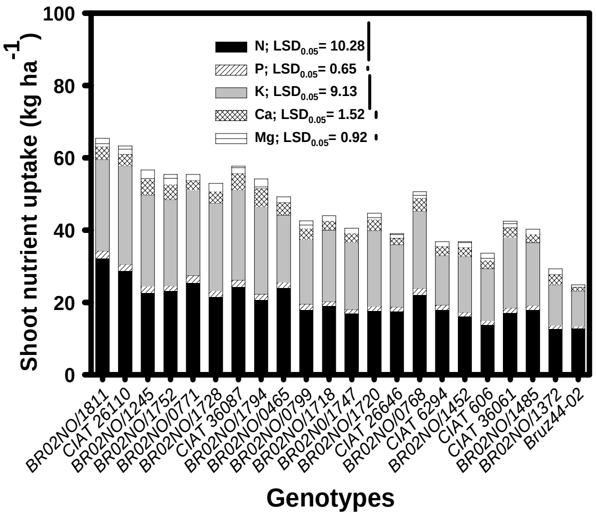

Figure 54. Genotypic variation in living shoot nutrient uptake (N, P, K, Ca and Mg) of three parents (CIAT 606, 6294 and *B.ruziziensis* 44-02), four CIAT's accesions (26110, 26646, 36061 and 36087) and 15 genetic recombinants (BR02NO/0465; 0768; 0771; 0799; 1245; 1372; 1452; 1485; 1718; 1720; 1728; 1747; 1752; 1794 and 1811) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 9 months after establishment (April 2005). LSD values are at the 0.05 probability level. NS = not significant.

Nitrogen uptake of the hybrid BR02NO/1811 was particularly outstanding among the hybrids. Correlation coefficients between green forage yield and other plant attributes indicated that greater nutrient acquisition contributed to superior performance (Table 41).

Significant negative correlation was observed between stem P content and live forage yield. Adaptation to dry season under infertile acid soil conditions seem to be closely associated with lower amounts of stem P content.

3.4 Grasses with adaptation to poorly drained soils

Highlight

- Made progress towards the development of a screening method to evaluate waterlogging tolerance and showed that the *Brachiaria* hybrid BR02NO1245 was particularly outstanding among the hybrids in its level of waterlogging tolerance due to its ability to maintain green leaf area and to develop adventitious roots.

3.4.1 Genotypic variation in waterlogging tolerance of *Brachiaria*

Contributors: I.M.Rao, J. Ricaurte, R. García and J.W. Miles (CIAT)

Rationale

Episodic waterlogging of the soil or deeper submergence (referred to collectively as flooding when a distinction is not necessary) occur when water enters soil faster than it can drain away under gravity. In the tropics, *Brachiaria* pastures during the rainy season occassionally face waterlogging conditions that severely limit pasture productivity and animal performance. Waterlogging causes major changes in the physical and chemical properties in the rhizosphere. Gaseous diffusion rate in waterlogged soil is about 100 times lower than air, and respiration of plant roots, soil micro-flora and fauna leads rapid exhastion of soil oxygen, and thereby causes hypoxia/anoxia condition. During waterlogging of the soil, anoxia will be preceded by a period of partial oxygen shortage (hypoxia). This will last for as long as it takes for dissolved oxygen in the floodwater to be consumed by roots and other aerobic soil organisms. This hypoxic interlude can act as a training period by improving the ability of the roots to survive subsequent anoxia by inducing biochemical (hypoxically-induced proteins) or anatomical (aerenchyma formation) acclimation. High temperatures tend to exacerbate the negative effects of waterlogging.

There is need to develop rapid and reliable screening methods to evaluate waterlogging tolerance of *Brachiaria* hybrids because the recently released Brachiaria hybrid (Mulato) is found to be relatively sensitive to waterlogging. Brazilian researchers evaluated waterlogging tolerance of different Brachiaria grasses and found that *B. brizantha* is intolerant, *B. decumbens* is moderately tolerant and *B. humidicola* is tolerant. They also evaluated 5 different accessions of *B. brizantha* and found genotypic differences in waterlogging tolerance based on relative growth rate, leaf elongation rate, adventitious root biomass, net photosynthetic rate and stomatal conductance. Our objective was to evaluate the recently developed *Brachiaria* hybrids for their waterlogging tolerance so that we can identify sources of resistance for genetic improvement and for genetic recombination of desirable attributes (e.g., biotic and abiotic stress adaptation, forage quality, seed production).

Material and Methods

A pot experiment was conducted outside in the patio area of CIAT Palmira between July 6 to 28 to determine differences in tolerance to waterlogging among 23 Brachiaria genotypes (15 hybrids - BR02NO/ 0465, 0768, 0771, 0799, 1245, 1372, 1452, 1485, 1718, 1720, 1728, 1747, 1752, 1794 and 1811; 3 parents - *B. decumbens* CIAT 606; *B. ruziziensis* 44-02; *B. brizantha* CIAT 6294; and 5 checks - *B.humidicola* CIAT 679; *B.dictyoneura* (*humidicola*) CIAT 6133; *B. brizantha* CIAT 26110; *Brachiaria* hybrid cv Mulato = CIAT 36061; *Brachiariia* hybrid FM9503-5046-024 = CIAT 36087).

Two treatments of water supply were imposed: control at field capacity (adequate water supply) and excessive water supply (5 cm over soil surface). The trial was planted as a randomized block in split-plot arrangement with 3 replications: water treatment as main plots and genotypes as sub-plots. Each experimental unit consisted of one pot filled with 3.5 kg of fertilized top soil (0-20 cm) from Santander de Quilichao's Oxisol and sown with two vegetative propagules (stem cuttings).

An adequate fertilizer was supplied (kg ha^{-1}: 80N, 50P, 100K, 66Ca, 28Mg, 20S, 2Zn, 2Cu, 0.1B and 0.1Mo) to soil at the time of planting; plantlets grew for 50 days under field capacity conditions and then 3 pots per genotype were waterlogged with 5 cm water column during 21 days. The other 3 pots per genotype were kept under field capacity conditions (control). Leaf Chlorophyll content (in SPAD units) and leaf photosynthetic efficiency (Fv/Fm) were measured at weekly intervals on a full expanded young leaf marked at waterlogging time 0. At the end of the 21 days treatment, gren leaf area (cm^2 pot^{-1}), green leaf biomass (g pot^{-1}), total shoot and root biomass (g pot^{-1}), root volume (cm^3) and root length (cm pot^{-1}) and mean root diameter (mm) of adventitious roots were measured.

Results and Discussion

The maximum temperature during the experimental period ranged from 27.6 to 34.5 °C while the minimum temperatures were 17.6 to 20.8 °C. The solar radiation was 14.0 to 22.2 MJ m^{-2} d^{-1}. Waterlogging for 21 days resulted in senescence and death of a great proportion of shoot biomass of majority of hybrids and development of adventetious roots in some hybrids (Photo 20).

The hybrid BR02NO 1245 was particularly outstanding in its ability to maintain green leaf area and to develop adventitious roots. Results on living leaf area and living leaf biomass production per pot indicated that one of the 5 checks, *B. humidicola* CIAT 6133, was outstanding in its level of tolerance to waterlogging (Figure 55).

Two other checks, *B. humidicola* CIAT 679 and *B. brizantha* CIAT 26110 also showed greater level of waterlogging tolerance in terms of green leaf area and green leaf biomass. Among the 15 hybrids tested BR02NO1245 was markedly superior in waterlogging tolerance than the other hybrids (Figure 56).

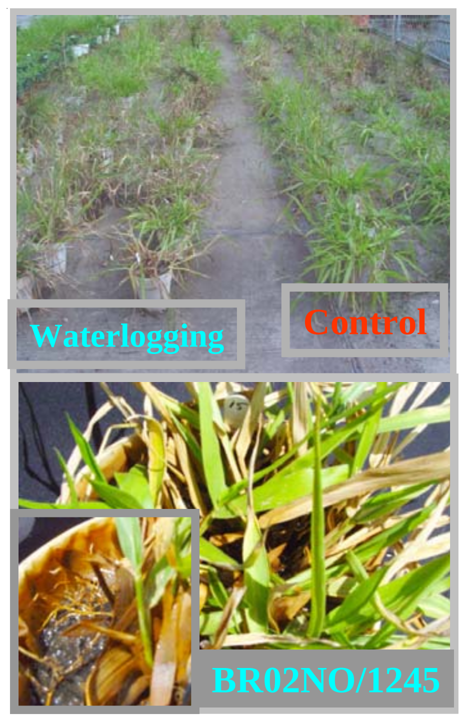

Photo 20. Influence of waterlogging on plant growth at harvest time (21 days of treatment) and a good hybrid of BR02NO population that showed good level of waterlogging tolerance due to development of adventitious roots (inset).

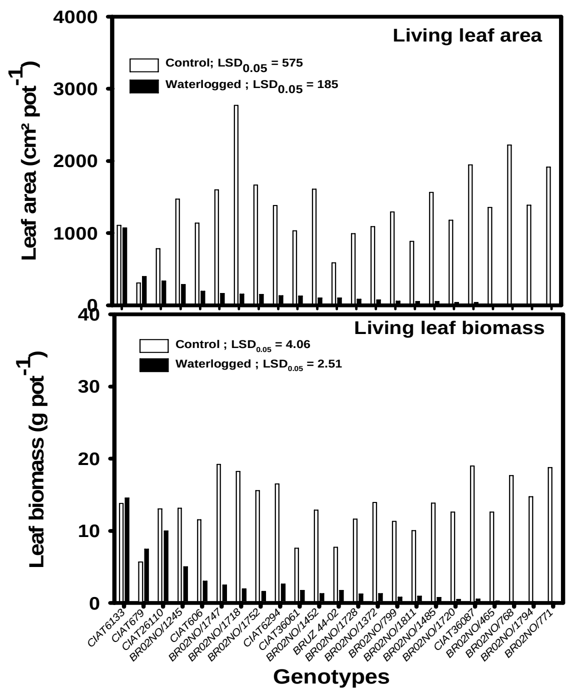

Figure 55. Influence of waterlogging on genotypic variation in gren leaf area and green leaf biomass production of three parents (CIAT 606, 6294, *B.ruziziensis* 44-02), 5 checks (CIAT 6133, 679, 26110, 36061 and 36087) and 15 hybrids of *Brachiaria* selected from BR02NO population of *Brachiaria* grown in an oxisol from Santander de Quilichao, Colombia. Plant attributes were measured at 21 days after waterlogging. LSD values are at the 0.05 probability level.

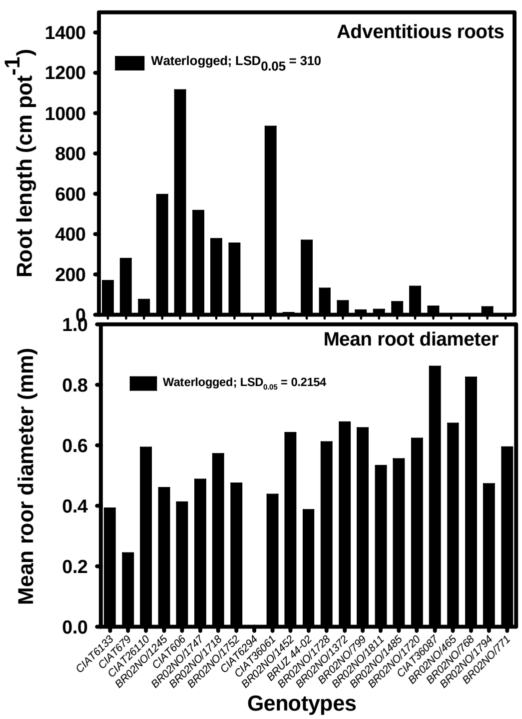

Figure 56. Influence of waterlogging on genotypic variation in adventitious root length production (cm pot^{-1}) and mean root diameter of three parents (CIAT 606, 6294, *B.ruziziensis* 44-02), 5 *Brachiaria* checks (CIAT 6133, 679, 26110, 36061 and 36087) and 15 hybrids of *Brachiaria* selected from BR02NO population grown in an oxisol from Santander de Quilichao, Colombia. Plant attributes were measured at 21 days after waterlo. LSD values are at the 0.05 probability level.

3.5 Nitrification inhibition in tropical grasses

Highlight

- Found that the Nitrification Inhibition activity of sexual accessions of *B. humidicola* was similar to the commercial apomictic cultivar.

3.5.1 Evaluation of *B. humidicola* accessions for nitrification inhibition ability

Contributors: M. Rondón, I.M. Rao, C.E. Lascano, J. Miles, J.A. Ramírez, M.P. Hurtado, J. Ricaurte (CIAT); G.V. Subbarao, T. Ishikawa, K. Nakahara, and O. Ito (JIRCAS, Japan)

Rationale

We have reported earlier that *B. humidicola* CIAT 679 has the ability to inhibit nitrification by releasing inhibitory activity from roots (NI activity). This is based on evaluation of germplasm accessions that have apomictic mode of reproduction. This makes it extremely difficult to use this ability in a breeding program to transfer the ability to inhibit nitrification to other *Brachiaria* grasses that lack such ability unless we find genetic variability for this trait among sexual accessions of *B. humidicola*.

Materials and Methods

During this year, we evaluated 11 accessions of *B. humidicola*, that are believed to have the sexual mode of reproduction, along with the standard cultivar of CIAT 679 and *Panicum maximum*. Sexuals of *B. humidicola* can be used in a *Brachiaria* breeding program as they can be hybridized with other *Brachiaria* species.

Plants were grown in a sandy loam Oxisol from the Llanos (Matazul) of Colombia (1 kg of soil/pot) under greenhouse conditions. The details of growing conditions and culture details were similar to that reported in the Tropical Grasses and Legumes annual report of 2004. After 120 days of growth, plants were removed from soil, and root exudates were collected and NI activity was extracted as described earlier (see annual report of 2004) and quantified using the modified bioassay which was developed at JIRCAS (see annual report of 2004 for details on the bioassay methodology).

Results and Discussion

Total NI activity released from roots of four plants during a 24 h varied from 62.4 to 207.2 AT NI among the sexual accessions of *B. humidicola*. The standard cultivar of CIAT 679 released about 66.4 AT NI. The NI activity released from *P. maximum* is only about 0.55 AT NI during 24 h period, thus confirming our earlier observations that this tropical grass lacked such NI ability.

Our results indicated that most of the sexual accessions of *B. humidicola* have similar NI ability to that of standard cultivar CIAT 679, and that only one sexual accession has nearly three times NI activity released compared to that of the standard cultivar. Thus, the NI ability of *B. humidicola* CIAT 679 is not confined to the accessions that have apomictic mode of reproductive behavior, but exists also in the accessions that have sexual mode of reproductive behavior. Our results indicate that some of these sexual accessions can be used as a source of NI trait for the *Brachiaria* breeding program to regulate NI activity to improve N use efficiency in pasture systems.

3.5.2 Field validation of the phenomenon of nitrification inhibition from *Brachiaria humidicola*

Contributors: M. Rondón, I.M. Rao and C.E. Lascano, Maria del Pilar Hurtado (CIAT); G.V. Subbarao, T. Ishikawa and O. Ito (JIRCAS, Japan)

Rationale

A range of Nitrification Inhibition (NI) activity has been measured for diverse accessions of *B. humidicola* and other tropical grasses under glasshouse conditions, as part of collaborative research between JIRCAS and CIAT. As a continuation of these research efforts, a long term field experiment was planned to validate the phenomenon of NI under field conditions and to test the hypothesis that the NI activity is a cumulative factor in soils under species that release the NI activity from root exudates. Given the vast areas currently grown in the tropics on tropical grasses, an understanding of the NI process and the possibility of managing it to improve N use efficiency, reduce nitrate pollution of surface and groundwaters as well as reduce net impact on global warming through reduced emissions of nitrous oxide, could have potentially global implications. Various tropical grasses showing a varying degree of NI activity were selected for the experiment and a soybean crop and a grass (*P. maximum*) that lacks the NI activity were selected as controls.

Materials and Methods

The experiment was initiated in September 2004 at CIAT-HQ at Palmira, Colombia on a fertile clayey Vertisol (pH 6.9), and with an annual rainfall of 1000 mm and mean temperature of 25 C. Two accessions of *B. humidicola* were used: the commercial reference material CIAT 679, which has been used for most of our previous studies, and the high NI activity *B. humidicola* accession CIAT 16888. The *Brachiaria* Hybrid cv. Mulato was included for having moderate NI activity and *Panicum maximum* var. common was used as a negative non-inhibiting control. Soybean (var. ICAP34) is also used as a negative control due to its known effect on promoting nitrification. A plot without plants is used as an absolute control.

Treatments were placed in plots of 10 m x 10 m with three replications and distributed in a completely randomized block design. Soybean was planted from seeds and the grasses were propagated from cuttings. Soybean was inoculated with the Ryzhobium strain CIAT 13232 to favor biological nitrogen fixation. Irrigation was provided to the field as required and two applications of broadcast fertilization were made at 30 and 60 days after planting on each plot, except within two 1 m^2 subplots demarcated in each plot, where the same levels of fertilizer were applied in solution to favor a more homogeneous distribution of the applied nutrients within the soil. Each application consisted of an equivalent dose of (kg/ha): 48N, 24K, 8P, 0.2 Zn, 0.2 B. The nitrogen source was ammonium sulfate. Weed control was done using Glyphosate in the bare soil plots and in the soybean plots before planting. During the soybean growing cycle manual weeding was done in such plots.

At harvest, soybean plants including roots were removed from the field when they had reached full maturity and the grain was already dry. The plants were separated into roots, shoots and grain, and a representative subsample taken for measuring dry matter content and N analysis. Plants of *P. maximum* were cut at approximately 20 cm height twice during the crop cycle. From each cut a representative subsample collected for dry weight and N analysis. The *Brachiaria* Hybrid cv. Mulato was cut at 20 cm height while the *B. humidicola* accessions were cut at 10 cm height. Similar procedure used for cv. Mulato was used for *P. maximum*. At harvest time, soil was carefully collected in the rizhopane of all species with an auger from the top 10 cm of the soil within each subplot.

Four samples were collected in each sub plot and pooled to obtain a composite sample. Samples were carefully managed and only the soil adhered to the roots was removed and used for soil analysis. Once the rhizosphere soil was collected, it was allowed to air dry and then was finely ground to <0-1 mm mesh. Soil was analyzed for nitrate and ammonium content using KCl extracts and colorimetric determination. Fresh rhizoplane soil was used for microbial counting of nitrifier organisms. Gas samples for measuring N_2O fluxes were collected monthly. Once a year, soil incubation studies were conducted using rhizosphere soil, to monitor nitrogen dynamics and fluxes of N_2O.

So far two soybean crops have been harvested (February and August, 2005). In this report we present the data collected during the second cropping season and the accumulated fluxes of N_2O over one year.

Results and Discussion

Biomass production. In Figure 57 we present the biomass harvested during the second crop cycle (April- August, 2005). Total yield of *P. maximum* and the Brachiaria hybrid Mulato were similar and clearly higher than the biomass from other species. Soybean had a total biomass slightly lower that the *B. humidicola*. Due to better plant coverage, biomass production of all the grasses but more particularly of the *B. humidicola* accessions was higher than during the initial cropping season.

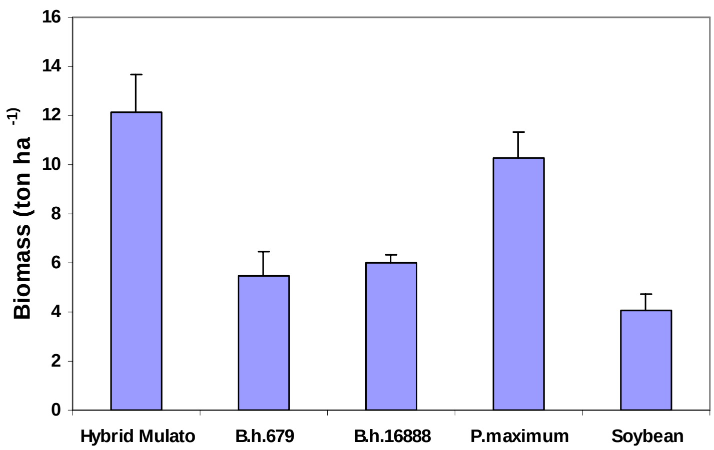

Figure 57. Total biomass harvested during the second cropping cycle (April- August 2005).

Total N uptake by plants (in the harvested components) followed a similar trend of biomass accumulation. It is evident that both *P. maximum* and the Hybrid Mulato are extracting more N than what is being added as fertilizer, and consequently a net N mixing from the soils is occurring. Soybean is balanced regarding N application/uptake while the *B. humidicola* plots are removing less N than what is being added as fertilizer. The yield of soybean was similar in the two cropping seasons (1.6 grain/ha) which is slightly lower that the commercial average in the region.

Soil Nitrate. In Figure 58 we show the nitrate levels in the top soil at harvest time. As expected the bare soil plots showed higher levels of nitrate more likely as a result of lack of plant N uptake. The soybean plots as well as the plots under the Brachiaria hybrid Mulato and *P. maximum* also had high levels of soil nitrate, while the *B humidicola* accessions clearly showed lower nitrate concentrations. The lower N uptake by 2 accessions of *B. humidicola* suggest a lower rate of nitrification with these two grasses or alternatively higher nitrogen losses.

Nitrous oxide emissions. In Figure 59, we show the accumulated fluxes of Nitrous Oxide (N_2O) over the period of September 2004 – August 2005. Annual emissions of N_2O were significantly lower in plots with *B. humidicola* and *P. maximum* than in the other plots. Fluxes were highest in the bare soil plots. These results support the view that *B. humidicola* is effectively inhibiting the nitrification process. However, *P. maximum* is also resulting in lower net emissions of N_2O but this may be attributable to the much higher nitrogen uptake by the plants which may limit the total amounts of N available for nitrification, assuming that the grass is able to take up N from the soil in ammonium form.

Soil Nitrification rates. Fresh rhizosphere soil was incubated to assess their mineralization rates using the procedure of Belser and Mays(1980) in which soil samples are incubated with appropriate levels of ammonium and phosphate to favor nitrification. Chlorate is added to block the conversion of nitrite to nitrate and rates of nitrite accumulation (which are easier to measure than nitrate accumulation) are registered over time. In Figure 60 we show the results from the incubation test. The trend was similar than that with the N_2O fluxes. Both *B. humidicola* and *P. maximum* showed significantly lower nitrification rates than bare soil and soybean.

No clear trend was observed for nitrate and ammonium levels in soil extracts. However, the significantly lower levels of inorganic soil under *B humidicola* suggest however that this species is likely favoring the flow of applied N into organic pools in the soil (Microbial N and Soil organic matter-N). Another indirect indication of this comes from the relatively low levels of nitrate estimated to be leached. This may need to be investigated in more detail in subsequent crop cycles. The incubation method to estimate nitrification rates used for this study is highly sensitive to detect even small differences in nitrification rates. In the next crop cycle, attempts will be made to monitor more frequently the nitrification rate of the plots during the crop season. The total amount of N lost to the atmosphere as N_2O in the bare soil plots corresponds to approximately 1.6% of the applied fertilizer-N. This figure falls within the range reported in the literature for tropical soils. With *B. humidicola* net N_2O were emission 12-20% less than the bare soil plots. This highlights the potential of these grasses in contributing to mitigation of climate change due to greenhouse effects.

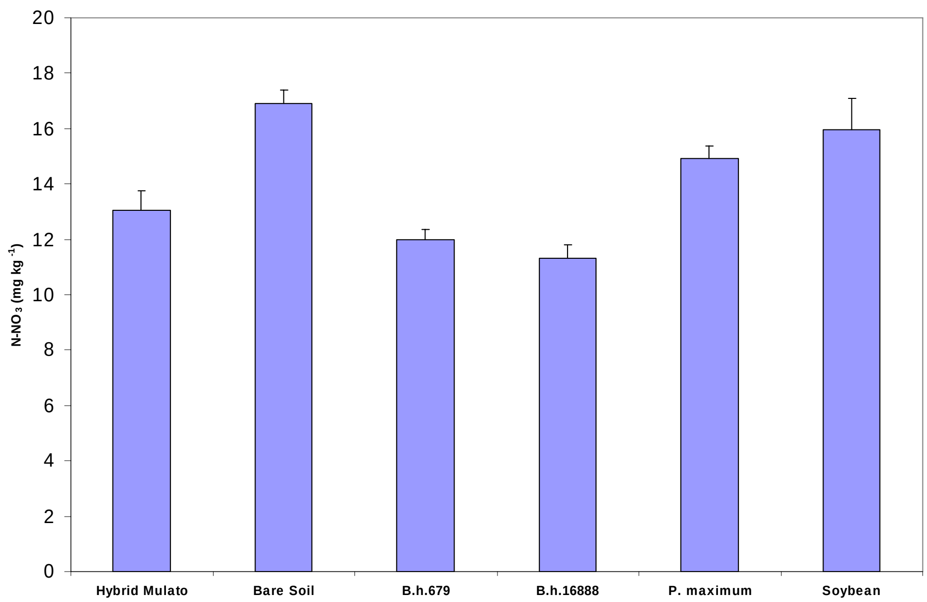

Figure 58. Nitrate levels in the top soil (0-10 cm) at harvest time.

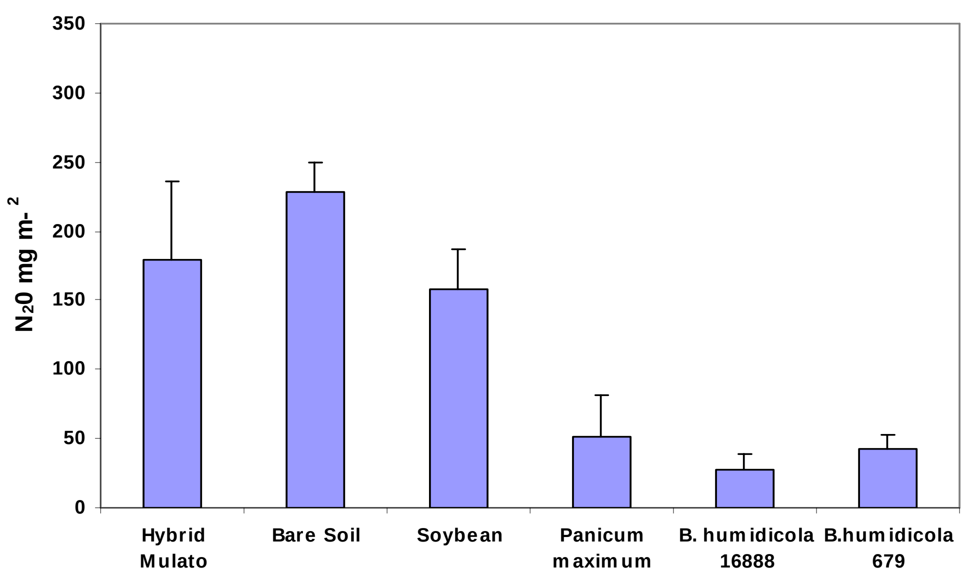

Figure 59. Accumulated fluxes of N_2O over one year period (September 2004 – August 2005). Two cropping cycles were included.

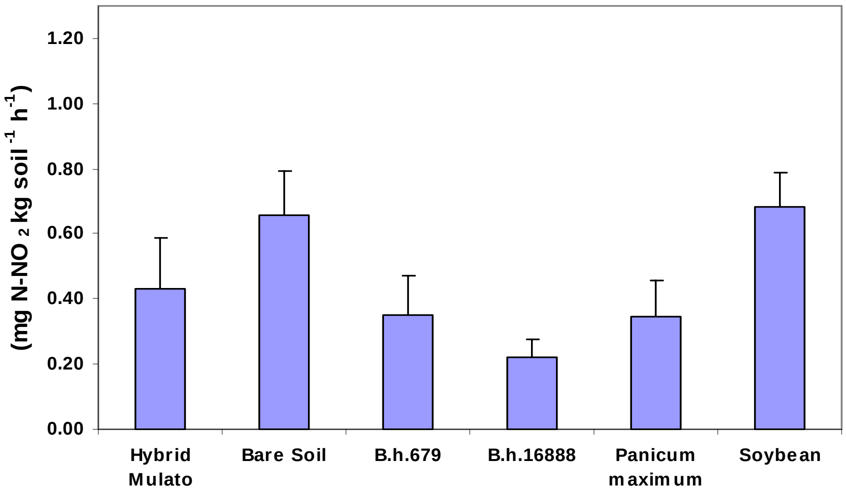

Figure 60. Nitrification rates from incubated soils.

3.6 Legumes with adaptation to acid soils, drought, and mild altitude environments

Highlights

- Identified accessions of *Leucaena diversifolia* and *L. trichandra* for further testing in mid altitude locations with acid soils.
- Selected accessions of *Desmodium velutinum*, (erect and semi-erect accessions) due to high productivity in the wet and dry season. All accessions selected had high digestibility and protein and were free of tannins.
- In Costa Rica plants originated from seed of *Cratylia argentea* showed very high variability in vigor and development and this masked the effect of application of fertilizer during establishment.

3.6.1 Evaluation of a collection of the multipurpose shrub legume *Flemingia* spp.

Contributors: M. Peters, L.H. Franco, (CIAT), R. Schultze-Kraft (U. of Hohenheim), B. Hincapié, P. Avila, and G. Ramírez (CIAT)

Rationale

The work of CIAT on shrub legumes focuses on development of materials to be utilized as a feed supplement during extended dry seasons. Tropical shrub legumes of high quality for better soils are readily available, but germplasm with similar characteristics adapted to acid, infertile soils is scarce. On the other hand, shrub legumes so far selected for acid soils do not perform well in mid altitudes (1200-2000 m.a.s.l.). For example, *C. argentea* is well adapted to acid soils but normally does not perform in altitudes above 1200 m.a.s.l.. One alternative for sites with acid soils and mid altitudes is *Flemingia macrophylla*. However its use has been limited due to poor quality and palatability. The research reported in this section had two main objectives:

1) Define the diversity available within the genus *Flemingia*.
2) Identify new, forage genotypes with superior agronomic traits and forage quality parameters.

Material and Methods

A collection of 63 accessions of *Flemingia* spp., originating in Asia was established in 2004 at CIAT's research station in Santander de Quilichao together with 3 control accessions (CIAT 21083, 21090 and 21092) which had been evaluated previously (Photo 21).

Passport information of all accessions was presented in the annual report 2004. Accessions were planted in jiffy pots in the green house and inoculated with adequate *rhizobium* strains; transplanting into the field was done after 8 weeks. Six months after transplanting plants were well established with good vigor and low incidence of pests and diseases. A standardization cut to 40 cm above soil was done, however some accessions did not reach cutting height during the establishment phase.

Photo 21. Collection of *Flemingia* spp. in Quilichao.

Results and Discussion

Accessions were grouped according to growth habit: (a) e = erect (24 accessions) and (b) se = semi-erect (42 accessions). For the semi-erect accessions the number of branches varied between 2 and 14, while for the erect accessions 3 to 17 branches per plant were counted (Table 42). Vigor varied strongly between accessions and also between plants within accessions.

During the first year, some accessions did not persist indicating low adaptation to the environment or to cutting. In Tables 43 and 44 we show the performance of accessions in the wet and dry season. Differences in DM yield between the erect accessions were not significant (P>0.05), with an average yield of 287 and 54 g/plant in the wet and dry season, respectively, indicating the low adaptation of these accessions to drought. Eventhough some accessions had higher branching in the dry season, this did not result in higher DM yields. Highest DM yields were recorded with the erect accessions CIAT 21083, 21092 and 21090 selected from the 2002 *Flemingia* collection (Table 43).

DM yield of the control accession CIAT 17403 was on average lower in the wet season but higher in dry season when compared with the mean over all accessions. The DM yields of the semi-erect accessions differed significantly (P< 0.05) in both the wet and dry season, but yields were lower than for the erect accessions. DM yields ranged between 1 and 354 g/plant in the wet season and between 1 and 115 g/plant in the dry season, indicating the low adaptation of these accessions to drought. Moreover, accessions were susceptible to pests (mainly stem borers), resulting in yield reduction and death of plants. It has to be noted that the stem borer incidence may be site specific as similar attacks were observed with other shrub species. The accessions CIAT 21556, 19803, 21109, 19392, 17406, 21492, 19796, 20629 and 19802 were the highest yielding (200/plant) accessions in the wet season. In the dry season highest yields (over 60 g/plant) were obtained with accessions CIAT 20629, 7966, 19802, 19455, 19393 and 17401. In terms of forage quality for wet season regrowth (8 weeks) significant differences (P< 0.05) between accessions were found for CP, NDF, ADF and soluble and insoluble tannin content; however IVDMD did not vary between accessions (Table 45). To what extent the observed differences in forage quality in *Flemingia* spp. accessions affect animal production needs to be assessed through animal trials using milking cows.

Table 42. Vigor, height, diameter and branching of a collection (2004) of *Flemingia spp* in Quilichao.

Accession No. CIAT	Vigor	Height	Diameter	Number of branches	Accession No. CIAT	Vigor	Height	Diameter	Number of branches
	1-5	Cm		No.		1-5	Cm		No.
	Semi – Erect					Erect			
21556	4	63	125	14	21093	5	141	143	17
19392	5	101	122	13	22060	4	95	96	16
19803	5	95	114	13	21117	5	101	129	15
17406	4	101	121	13	21112	5	121	134	15
17401	3	44	57	12	21113	4	106	108	14
21492	4	78	104	12	21116	5	124	137	14
19802	4	99	110	12	17403*	5	90	137	13
20629	4	90	110	12	21115	5	123	136	13
22047	3	55	107	12	22094	4	88	127	13
17408	5	97	109	11	21094	4	117	123	13
17410	4	94	107	11	21090	4	102	104	12
20628	5	112	121	11	22057	4	98	108	12
21104	3	58	78	11	21118	4	79	115	12
19393	4	85	109	11	22063	5	108	125	11
7966	5	85	105	9	21111	5	121	111	10
17453	4	90	112	9	22169	4	107	101	10
19455	4	61	117	9	21084	4	105	101	9
22083	3	60	100	9	21109	4	104	91	7
19796	4	86	108	9	21091	4	95	61	6
21100	2	43	67	9	21083	4	112	93	6
20627	3	69	100	9	22058	5	119	67	5
21101	3	62	90	9	21096	3	77	55	4
22043	4	48	129	9	21089	3	72	46	3
19456	4	59	100	8	21092	2	59	39	3
21105	3	55	78	5	21098	3	79	66	8
22092	2	48	57	5	21103	3	66	86	8
19625	1	25	73	5	21106	3	53	85	8
21107	2	39	54	5	21099	3	58	72	8
19626	1	31	44	4	21102	3	60	86	7
19627	1	24	41	4	19458	3	55	90	7
17402	1	49	40	3	21097	3	45	73	6
19623	1	25	49	3	22064	3	46	60	6
22084	2	72	46	3	18046	1	27	20	2
Range	1-5	24-112	41-129	3-14		1-5	27-141	20-1432	2-17

* Control

Table 43. Agronomic evaluation of erect accessions (collection of 2004) of *Flemingia* spp. in Quilichao. Data of 2 evaluation cuts (one in the dry season and one in the wet season).

Accession No. CIAT	Height	Diameter	Number of branches	Mean DM yields	Height	Diameter	Number of branches	Mean DM yields
	Wet				Dry			
	(cm)		(No.)	(g/pl)	(cm)		(No.)	(g/pl)
21116	115	127	97	466	81	98	146	105
21091	107	88	29	387	75	72	35	99
22058	135	99	21	364	101	95	24	105
21083	149	123	43	363	72	88	90	73
21092	107	99	29	354	72	85	35	101
22094	102	127	77	349	73	76	64	43
21090	110	128	65	346	81	99	125	92
22057	109	99	50	338	74	72	59	25
21115	100	111	68	321	81	96	110	108
21093	117	121	73	306	74	82	84	33
21117	98	128	61	298	70	96	108	42
21084	97	114	63	290	60	70	39	20
21118	94	125	70	282	56	81	72	20
22063	101	110	46	280	59	63	53	18
17403*	86	139	35	261	62	96	78	66
21112	101	110	65	257	70	79	76	39
21111	104	111	57	249	67	78	65	28
21113	116	115	70	249	74	91	99	71
22060	100	115	57	228	60	70	71	25
21094	100	108	59	210	63	78	88	43
21089	100	92	19	206	59	70	22	48
22169	79	70	30	119	57	47	30	16
21096	66	82	18	86	46	77	19	33
Mean	103	110	52	287	69	81	69	54
LSD (P< 0.05)				NS				NS

* Control

Table 44. Agronomic evaluation of semi-erect accessions (collection of 2004) of *Flemingia* spp. in Quilichao. Data of 2 evaluation cuts (one in the dry season and one in the wet season).

Accession No. CIAT	Wet				Dry			
	Height	Diameter	Number of branches	Mean DM yields	Height	Diameter	Number of branches	Mean DM yields
	(cm)		(No.)	(g/pl)	(cm)		(No.)	(g/pl)
21556	74	150	109	354	44	110	135	12
19803	81	116	64	299	58	81	86	39
21109	94	122	58	278	63	75	61	35
19392	82	131	65	276	60	81	83	35
17406	72	115	76	274	63	85	83	57
21492	75	127	72	274	60	94	102	57
19796	88	120	67	273	65	86	86	56
20629	81	108	73	260	68	78	107	115
19802	79	113	68	253	69	90	89	77
22043	64	145	114	248	39	105	148	17
22047	69	126	107	247	46	108	155	33
20628	78	115	68	227	58	80	59	23
7966	79	107	67	215	74	91	101	83
19455	62	107	48	208	53	77	83	73
19393	63	102	58	202	53	77	92	64
17401	58	98	78	195	51	83	103	61
17410	70	122	74	193	56	86	87	26
20627	72	113	81	172	48	76	83	26
17408	67	100	80	168	49	63	52	25
19458	64	95	60	158	54	79	85	52
17453	61	106	58	157	49	74	82	30
19456	63	103	58	120	45	73	68	44
21102	46	80	37	97	35	62	56	26
22083	47	78	35	97	41	67	48	24
21101	53	84	56	90	44	73	101	41
21098	64	67	41	89	51	41	22	10
21105	48	70	27	85	41	45	28	14
21099	47	74	39	80	42	61	56	42
21104	50	73	45	77	43	50	32	10
21097	39	63	22	59	30	46	17	10
21103	43	75	36	57	36	49	24	11
21106	41	76	39	53	34	58	37	19
17402	41	46	27	34	32	34	18	6
22092	45	58	18	21	36	36	24	8
21107	33	42	19	21	27	41	20	6
22064	36	51	18	19	28	32	12	4
22084	49	45	13	18	39	39	22	14
19625	32	40	19	15	22	40	19	1
21100	36	46	37	14	33	45	38	14
19627	26	43	26	10				0
19626	29	35	16	6	21	30	10	0
19623	22	34	14	5	30	30	12	0
18046	22	25	7	1	15	16	4	0
Mean	57	88	52	143	47	68	66	33
LSD(P< 0.05)				194				79

Table 45. Forage quality of selected accessions of *Flemingia* spp. evaluated in the wet season in Quilichao, 2005.

Accession No. CIAT	IVDMD	C P	FDN	FDA	TANSOL	TANINSO
	------------------------------	------------------------------	%	------------------------------	------------------------------	
22094	47	23	33.4	18.8	6.8	1.2
22060	46	21	36.9	21.7	7.1	1.0
21118	45	21	36.1	20.2	8.1	1.5
21556	45	24	40.0	22.2	7.6	1.2
21093	45	22	34.8	19.5	7.1	1.3
21116	45	22	35.8	19.7	8.2	1.4
21090	44	22	34.5	20.7	4.5	1.2
22057	44	21	32.1	19.1	8.5	1.2
21083	43	22	35.8	22.3	4.5	1.6
22043	43	25	44.6	20.4	7.8	1.6
21112	43	23	34.9	18.1	7.0	1.7
21115	43	22	36.3	19.8	7.6	1.7
22047	41	25	41.8	21.7	7.6	1.3
21111	40	22	40.2	22.4	6.9	1.7
21094	40	20	42.1	20.9	8.8	1.6
Mean	44	22	37.3	20.5	7.3	1.4
LSD(P< 0.05)	NS	2.4	4.0	2.6	1.9	0.7

Control CIAT 17403 (IVDMD = 43.68 - 31.23; CP = 20.52 - 19.0; ADF = 20.36 - 24.09; T Sol= 4.32 - 9.62)

3.6.2 Field evaluation of a collection of the forage legumes *Leucaena diversifolia* and *Leucaena trichandra*

Contributors: K. Zöfel (University of Hohenheim, CIAT), R. Schultze-Kraft (University of Hohenheim), M. Peters, L. H. Franco, B. Hincapié, and G. Ramirez (CIAT)

Rationale

Previous research had shown *Leucaena diversifolia* and *Leucaena trichandra* are well adapted to acid and infertile soils and mid altitudes (up to 2000 m.a.s.l.), in contrast to the widely used *Leucaena leucocephala* that does not perform well under these conditions. However, the evaluation of *L. diversifolia* and *L. trichandra* up to now has been restricted to only few accessions. A comprehensive collection of the two species, was put together in order to screen for genetic diversity in agronomic traits and nutritive value. Moreover, the study aims to find morphological differences both between the two species as well as among accessions.

Materials and Methods

A collection of 61 accessions of *L. diversifolia* and *L. trichandra* was planted in jiffy pots in the green house (50 accessions of *L. diversifolia* and 11 of *L. trichandra*). After eight weeks seedlings were transplanted (June, 2005) to the CIAT station in Santander de Quilichao. A randomized block with four replications was employed; three replications were used for agronomic and forage quality evaluation, and one for morphological characterization. Each replication consisted of five plants per accession. The plants were spaced 1 m, with 1.5m between rows. Fertilization was (kg/ha) P40, K50, Mg20, and S20. *Rhizobium* bacteria were applied in the field as a mixture of water and bacteria, given to each plant directly into the soil. During the first eight weeks after transplanting plants were irrigated when necessary to safeguard the establishment of the trial. Equally pesticides were applied when pests appeared to threaten the survival of the plants.

Agronomic evaluation included regular assessment every four weeks of plant vigor and symptoms of nutritional deficiencies, pests and diseases. Three months after planting, plant height, diameter and growth habit were recorded. The first cut in three replications was done on October, 2005, at a height of 0.5m above the ground. In the fourth replication morphological evaluation was carried out four month after transplanting, and comprised the assessment of leaf morphology (form, color, pubescence, glands), leaf area, flowering and growth habit.

Results and Discussion

All accessions showed good vigor under field conditions in Quilichao. Partly critical were the first eight weeks after planting, as planting was done at the beginning of the dry season, and as a result adverse conditions for plant establishment prevailed. Several plants died and had to be replanted.

Three months after transplanting to the field, plant height and diameter ranged from 0.2 to 1.7 m and 0.1 to 1.8 m, respectively. Four months after planting plants reached 2.3 m and 2.4 m for height and diameter, respectively. Growth habit showed great variability. Erect to prostrate forms were found. Angles between main bole and branches ranged from up to 90° to around 20°. Also the number of branches below 0.5 m (height of standardization cut) fluctuated greatly between accessions, indicating differing regrowth capacity.

The morphological data confirm the large genetic variability within the two species as has been reported previously in the literature. Features like coloring of pinnules' border, pubescence, length and width of leaves and length and width of pinnules varied greatly within one species. Main morphological differences between *L. diversifolia* and *L. trichandra* are length and width of the pinnules (4.0 to 8.0/0.8 to 1.4 mm and 5.2-12.6/1.6-3.2 mm, respectively), number of pinnae per leaf and number of pinnules per pinnae, both being distinctly lower for *L. trichandra* (Photo 22).

Several accessions, for which information to which species they belong was not available in the pertinent databases, could not be assigned to one of the two species based on their morphological characteristics.

Photo 22. Top: *L. diversifolia,* bottom: *L. trichandra*

3.6.3 Diversity in the shrub legume *Desmodium velutinum*

Contributors: R. Schultze-Kraft (University of Hohenheim), F. Parra (Corpoica), N.Vivas (Universidad Nacional de Colombia, Palmira), M. Peters, L.H. Franco, B. Hincapié, and G. Ramírez (CIAT)

Rationale

Tropical shrub legumes of high forage quality are available for medium to high-fertility soils (e.g. *Leucaena leucocephala*), which is not the case for germplasm with adaptation to acid, low-fertility soils in drought-prone environments. Species such as *Desmodium velutinum* may offer an option in such environments. There are very few studies on *D. velutinum* and those published mostly concentrate on one or two accessions. Thus a study was initiated to explore the diversity of *D. velutinum* in terms of morphology, yield and quality parameters. A core collection based on agronomic and morphological parameters, origin information, using GIS tools, and characterization with molecular markers, will be assembled for more detailed regional evaluation.

Materials and Methods

A total of 137 accessions of *Desmodium velutinum*, mostly originating from Asia and to a lesser extent from Africa, were planted at Quilichao (Photo 23). Plants were sown in jiffy pots and transplanted 6 weeks later into the field in single-row plots, with 4 replications. Dry matter yield, drought tolerance and forage quality are the main parameters being measured.

Results and Discussion

Five months after transplanting plants were well established, having good vigor and free of pests and diseases. Accessions were classified into three groups according to their growth habit: (a) e-erect (54 accessions), (b) se-semi-erect (66) and (c) r-prostrate (17 accessions).

In Tables 46, 47 and 48 we summarize DM yields of promising materials, averaged over four cuts in the wet and three cuts in the dry periods. Each growth type was analyzed separately as these are likely to occupy different "niches". Significant differences ($P < 0.01$) between semi-erect accessions were encountered in both the dry and wet seasons. For the erect types differences ($P<0.05$) were found only in the wet season and for the prostrate accessions no differences ($P>0.05$) due to season were detected. For the group classified as erect, DM yields were slightly higher in the wet than in the dry season, with accessions CIAT 33443, 13953, 33352, 33118, 23116, 23985 and 23994 producing more than 200g DM/plant after 8 week regrowth in the wet season and more than 100g DM/plant in the dry season. Branching after cutting was not affected by season, with the exception of accession CIAT 33443 which had a lower number of branches in the dry season (Table 46).

Photo 23. *Desmodium velutinum* at Quilichao Left: Cut in dry season, Right: Evaluation with the contributors.

Table 46. Agronomic evaluation of selected accessions of *Desmodium velutinum* in Quilichao. Data of four evaluation cuts (three in the dry season and four in the wet season). Growth habit: e = erect.

Accession No. CIAT	Height	Diameter	Branches	Mean DM yields	Height	Diameter	Branches	Mean DM yields
	Dry				Wet			
	(cm)		(No.)	(g/pl)		(cm)	(No.)	(g/pl)
33443	90	162	69	159	77	184	102	297
13953	86	136	69	153	85	160	78	327
33352	85	136	68	143	79	146	82	223
33138	108	118	73	135	93	126	64	228
23136	97	138	60	133	93	144	57	209
23985	83	139	80	126	98	158	82	269
23994	102	108	59	123	73	155	72	225
23079	93	111	61	108	85	130	56	185
D81995	80	120	63	108	67	143	52	189
33247	88	120	74	104	77	155	72	221
23081	83	117	81	95	78	144	88	220
23086	81	124	80	91	70	142	75	189
23988	78	139	69	91	74	153	60	158
23989	96	102	47	85	88	128	44	169
14314	90	106	51	85	76	118	54	150
13947	92	99	58	84	89	124	54	167
23133	88	102	62	84	97	124	50	132
D3456	77	125	58	78	69	142	61	149
33250	86	107	58	78	71	120	67	127
13391	80	108	67	78	74	130	66	151
33254	79	115	53	72	71	144	65	133
23132	84	96	54	69	83	135	51	150
D2430	72	108	71	66	71	137	73	151
13948	74	99	51	66	76	132	48	142
Mean	86	118	64	101	80	141	66	190
LSD(P< 0.05)				NS				115

Among the semi-erect accessions, differences in DM yield between accessions were significant (P<0.05) in the dry and wet season (Table 47). Wet season yields of above 200 g/plant were obtained with CIAT 23981, 23983, 23996, 23982, 33396, 33463, 13218, 33428, 23928, 23276 y 23991; however only 3 of the accessions (CIAT 23996, 23981 and 23983) had dry season DM yields above 150 g DM/plant. The number of regrowing branches was higher than for the erect types (Table 47).

For the prostrate accessions no differences (P>0.05) in DM yield were measured and in general yields were lower than for the semi-erect and erect types (Table 48). Only accessions CIAT 33481 and 13212 had DM yields above 100g /plant in the dry season, and only accessions CIAT 33520 and 33471 had DM yields above 150 g/plant in the wet season. Branching was slightly higher in the dry than in the wet season (Table 48).

In plants belonging to the threee growth types we observed a yield decline overtime (Figure 61).

The yield reduction were in part due to reduced plant population as a result of stem borer. Plant survival varied between 20-100%, 20-93%, and 46-100 % for the erect, semi-erect and prostrate growth types, respectively. Forage quality varied significantly (P< 0.05) between the 20 most productive accessions (Table 49).

Differences were found for IVDMD and NDF in both the dry and wet season, and for CP only in

Table 47. Agronomic evaluation of selected accessions of *Desmodium velutinum* in Quilichao. Data of four evaluation cuts (three in the dry season and four in the wet season). Growth habit: se = semi erect.

Accession No. CIAT	Height	Diameter	Branches	Mean DM yields	Height	Diameter	Branches	Mean DM yields
	Dry				Wet			
	(cm)		(No.)	(g/pl)	(cm)		(No.)	(g/pl)
23996	75	153	85	158	65	172	66	246
23981	81	153	84	155	72	176	79	321
33003	87	154	76	154	76	160	84	195
33451	66	164	68	149	55	171	75	192
23983	74	160	63	149	64	191	84	289
33396	67	154	73	141	53	182	75	233
23276	65	148	80	136	54	182	77	210
23928	77	162	76	137	68	176	64	211
23920	67	131	84	135	58	169	77	195
13216	71	147	63	134	57	154	76	164
23977	76	158	74	125	66	171	62	199
33463	74	143	79	121	63	157	76	223
23980	78	150	75	120	66	166	74	160
33428	81	140	84	119	69	162	91	212
23991	64	153	71	115	55	175	69	204
23982	81	142	74	115	72	169	75	235
23993	60	150	75	114	51	159	57	144
23325	81	134	73	112	70	156	71	192
13218	69	139	72	110	62	159	73	220
13691	74	129	70	105	64	160	75	199
33459	75	142	70	99	71	161	74	172
13220	81	120	54	95	60	146	60	168
13204	62	124	76	94	46	132	71	116
23921	68	139	63	93	60	157	60	182
23973	58	129	60	86	48	153	63	160
23975	72	132	73	85	60	132	83	122
33242	75	120	63	84	68	137	57	144
13692	51	127	64	84	42	148	59	140
23974	61	126	69	83	50	146	68	130
23923	72	132	68	82	68	154	62	163
23922	66	130	70	79	57	140	80	122
23275	60	114	61	79	62	135	72	141
13417	68	130	52	78	67	159	43	112
Mean	71	140	71	113	61	160	71	186
LSD(P< 0.05)				48				94

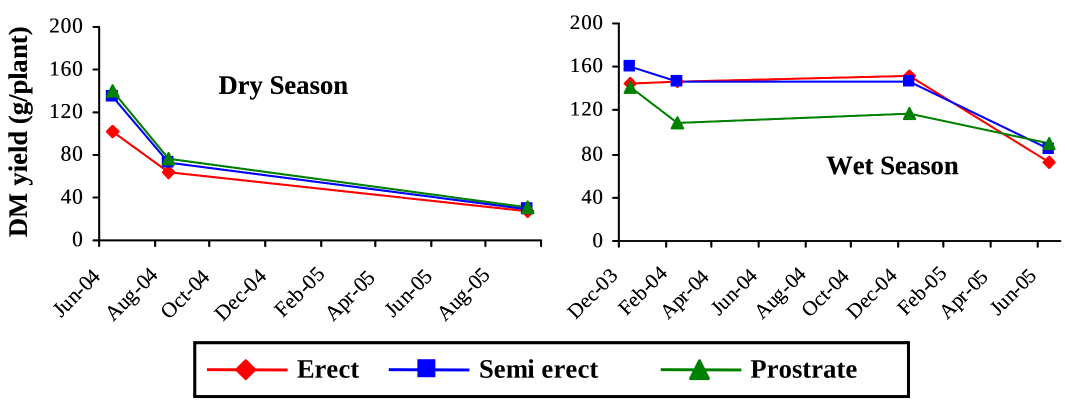

Figure 61. DM yield (g/plant) over time for different growth types of *Desmodium velutinum*, in Quilichao, Colombia

the wet season. Mean IVDMD across seasons were above a 65 % and CP above 19%. No measurable condensed tannins were detected using the Butanol-HCL assay (Table 49). From the results presented, it is evident that there is scope for selecting accessions of *D. velutinum* based on high dry matter yields in the wet and dry seasons. In addition, it is evident that under cutting the dry matter yields of *D. velutinum* decline over time.

Table 48. Agronomic evaluation of selected accessions of *Desmodium velutinum* in Quilichao. Data of four evaluation cuts (three in the dry season and four in the wet season). Growth habit: r = prostrate.

Accession No. CIAT	Height	Diameter	Regrowing points	Mean DM yields	Height	Diameter	Regrowing points	Mean DM yields
	Dry				Wet			
	(cm)		(No.)	(g/pl)	(cm)		(No.)	(g/pl)
33481	39	147	63	111	38	157	58	130
13212	46	138	63	103	37	143	57	116
33471	52	156	79	99	49	163	53	163
33520	47	142	63	98	34	174	57	190
13697	45	143	71	95	28	158	55	128
13213	49	135	67	89	43	139	65	136
13217	34	134	50	88	28	130	51	73
33484	49	144	59	86	38	160	58	104
Mean	45	142	64	96	37	153	57	130
LSD(P< 0.05)				NS				NS

Table 49. Forage quality of selected accessions of *Desmodium velutinum evaluated* in Quilichao, 2003-2004.*

Accession	IVDMD	CP	NDF	ADF	IVDMD	CP	NDF	ADF
	Wet				Dry			
	%							
23988	73.2	22.5	29.8	21.4	70.5	19.7	35.5	21.2
33451	70.6	22.7	30.8	21.6	69.8	19.1	38.4	24.1
23986	70.1	24.2	32.2	20.6	71.0	21.0	36.7	20.8
23994	69.7	22.0	32.8	23.9	66.6	20.7	38.4	25.7
23982	69.7	23.3	31.5	21.9	67.5	20.7	34.2	22.0
23981	69.2	21.7	34.4	23.8	68.9	21.1	35.2	22.6
23985	68.6	21.8	33.0	23.1	65.0	20.6	33.6	23.5
33463	68.3	23.4	36.0	25.8	64.6	20.4	39.6	26.5
13953	68.0	19.9	31.5	23.3	66.4	19.1	35.5	24.2
23921	67.9	21.9	33.8	24.0	63.5	17.7	41.7	28.5
23996	66.8	20.9	38.7	26.5	67.1	20.6	40.7	25.8
33443	66.8	20.5	37.1	28.2	63.9	18.9	39.4	27.3
23325	66.5	22.9	32.9	23.9	60.8	19.8	43.3	29.6
13218	66.1	20.9	36.3	25.1	63.2	20.2	39.2	26.8
23275	66.1	21.9	33.2	23.2	62.5	19.4	36.3	26.9
13691	65.8	21.6	35.4	25.2	60.3	18.9	44.0	29.2
23928	65.8	21.7	34.6	24.8	65.3	20.2	36.9	25.7
23081	64.9	20.9	36.5	26.6	61.6	20.1	43.3	26.6
23086	64.1	20.7	38.2	26.8	62.9	19.0	43.1	27.8
13952	63.8	19.3	35.2	25.6	61.4	18.4	41.1	27.2
Mean	67.6	21.8	34.2	24.3	65.2	19.8	38.8	25.6
LSD (P< 0.05)	4.2	3.5	5	3.7	7.9	4.1	5.2	4.7

3.6.4 Response of *Cratylia argentea* (Desvaux) O. Kuntze cv. Veranera to different establishment methods

Collaborators: J. Rosero, L.H. Franco, M. Peters, P. J. Argel, M. S. Sanchez, (Universidad Nacional - Palmira), B. Hincapié, and G. Ramírez (CIAT)

Rationale

Cratylia argentea is one of the tropical shrub legumes available that is suited to low fertility acid soils. The species is highly drought tolerant remaining productive over a 5 to 7 month dry season. Thus it offers the opportunity to smallholders in drought prone environments to maintain forage and consequently livestock productivity year round. Thus *Cratylia argentea* is progressively being adopted by farmers, particular those with small and medium size dual purpose cattle farms in Central America. However, as other shrub legumes the plant establishes slowly and farmers regularly complain that the period from establishment to utilization is too long. Very little research has been carried out to find better alternatives of establishment using either fertilizer or other soil amendments; thus it was justified to investigate methods for a better establishment of *Cratylia*.

3.6.4.1 Sites-Palmira and Quilichao, Colombia

Materials and Methods

The experiment established in 2004 aimed to assess the effects of method of planting (direct seeding, transplanting), fertilization with nitrogen and/or phosphorus and inoculation with *Rhizobium* on ease of establishment. In this section we report results from two sites, (Quilichao and Palmira) in Colombia. Results from Costa Rica are reported in the following section.

The following treatments were established using unscarified seed:

- Direct seeding or transplanting at 8 weeks;
- Inoculation-nitrogen fertilization: (a) Nitrogen (18 kg/ha), (b) Inoculation + nitrogen, (c) Inoculation alone.
- 4 levels of phosphorus fertilization: 0, 20, 40, (80 kg P/ha).

Fertilizers were applied 2 weeks after sowing/ transplanting. Evaluation was continued until plants reached 1 m in height, considered that plants have successfully established.

Results and Discussion

With direct seeding a high emergence percentage was observed in Quilichao, with 85 to 97 % of seed having germinated after 25 and 35 days, respectively; inoculation improved germination slightly (86 and 99 %) compared to non inoculated seed (84 y 96 %) (Table 50). In Palmira germination was relatively lower, with

Table 50. Survival rates of *Cratylia argentea* cv. Veranera plants in two sites, Quilichao and Palmira.

Treatment	Survival of plants (%)	
	Quilichao	Palmira
Method of sowing		
Direct	94	79
Transplanting	97	88
Inoculation-N application		
Control (0N – No inoculation	95	82
18 N	94	84
18 N + Inoculation	98	81
Inoculation	92	85
Phosphorus application		
0	96	84
20	96	82
40	98	80
80	95	86
Mean	96	83

92% of plants germinating 35 days after sowing. Interestingly, inoculation resulted in lower germination possibly due to the high clay content in Palmira, which under very wet conditions led to hard surfaces impeding germination and rooting and as a consequence making inoculation inefficient. In Quilichao, transplanting slightly improved plant survival, with 97% of seedlings surviving with transplanting compared to 94% with direct seeding. Inoculation or nitrogen application had no effect on establishment, but combining inoculation and nitrogen application lead to slightly better establishment (98% versus 94% of plants surviving). Phosphorus had no effect on establishment though applying 40 kg P lead to the highest survival rates with 98 %; higher P fertilization reduced survival (95 %). In Palmira, transplanting lead to an 88% survival rate, compared to 79% with direct seeding (Table 50). On the other hand, N fertilization or inoculation alone did not have any effect on survival, but phosphorus application up to 80 kg P slightly improved establishment. In Palmira Fe and Mg deficiencies were observed during establishment, indicating the need for micronutrient fertilization. Although emergence did not vary largely between treatments in Palmira and Quilichao, establishment (plant

Table 51. Days to establishment of *C. argentea* cv. Veranera under different methods of sowing, nitrogen application and inoculation and phosphorus application, in Quilichao and Palmira.

Method of sowing	N application (kg/ha) and inoculation	P application kg/ha	Quilichao	Palmira
			Days*	
Direct seeding	18N	0	157	195
		20	157	200
		40	148	200
		80	152	202
	18N+Inoculation	0	147	195
		20	151	209
		40	171	182
		80	152	188
	Control	0	171	204
		20	170	214
		40	165	181
		80	161	186
	18N	0	157	204
		20	163	209
		40	171	204
		80	125	195
Mean:			**157**	**199**
Transplanting	18N + Inoculation	0	139	188
		20	143	195
		40	123	195
		80	148	195
	Control	0	171	218
		20	147	202
		40	153	169
		80	157	216
	18N	0	151	195
		20	152	195
		40	128	186
		80	143	182
	Inoculation	0	144	195
		20	148	182
		40	167	204
		80	143	200
Mean:			**147**	**197**
LSD (P< 0.05):			**4.85**	**39 (ns)**

*Plants with 1 m height.

reach 1 m height) was faster in Quilichao with in average 157 days compared to 197 days in Palmira; at the same time survival of plants in Palmira was much lower than in Quilichao, with 50 and 90% of plants surviving, respectively (Table 50). In Quilichao the method of sowing had a significant ($P< 0.05$) effect on time for establishment (plant reach 1 m height), requiring 147 days with transplanting compared to 157 days with direct seeding (Table 51); however, survival rates were similar. Inoculation alone had no effect on establishment. Additionally a better response to combining N with inoculation was observed with transplanting than when seeding directly, i.e. 138 ± 16 days and 155 ± 24 days, respectively. Phosphorus had no effect on time to establishment.

No nutritional deficiencies were observed in any of the treatments in Quilichao (Photo 24), while in Palmira, a deficiency of micro nutrients in particular Fe was found (Photo 24), leading to yellowing of plants. Thus there may be a need for micronutrient fertilization in environments suboptimal for *Cratylia*.

Photo 24. Left: *C. argentea* at Quilichao, Right: *C. argentea* at Palmira

3.6.4.2 Site-Atenas, Costa Rica

Contributors: P J. Argel and G. Pérez (CIAT)

Materials and Methods

An experiment was established at ECAG facilities in Atenas to investigate factors associated with slow establishment of *C. argentea.* The site is located in a subhumid environment with a total annual rainfall of 1600 mm, and 5 to 6 months dry from December to May. The soils are Inceptisol of medium fertility with pH 5.0, low in organic matter and in both, P and Al content.

The experiment was planted using a split-plot design, replicated 3 times. Main plots were the control, inoculated seed (IS), 18 kg/ha of N and IS + 18 kg/ha of N. Subplots were P levels (0, 20, 40 and 80 kg/ha). CIAT 3564 was the rhizobium strain used to inoculate the seeds. Plots consisted of 10 individual plants spaced 0.5 m between plants and 1.5 m between plots. Plants were first planted in jiffy pots in a glasshouse and transplanted to the field 6 weeks later, followed by the application of treatments. Plant re-growth, number of leaves and plant vigor was measured 4 and 6 weeks after planting. Plant height has been measured every four weeks and will continue to the end of the experiment one year later. Finally DM yields of edible forage will be measured harvesting 8 central plants of each plot.

Results and Discussion

The cultivar Veraniega originated from a physical mixture of the accessions *C. argentea* CIAT 18516 and CIAT 18668. These accessions were chosen out of 11 different lines that had been evaluated across sites in the Latin tropics. The plant has a self pollinating (autogamy) reproductive system but also certain degree of allogamy (pollination of a flower with pollen from another flower).

Therefore the genetic purity of the original accessions is probably lost, since experimental and basic seed has been always harvested from non- spaced plants of the different accessions.

This is probably the reason why stands of cv. Veraniega vary considerably in plant vigor and plant height during establishment, making it difficult to measure plant responses to fertilizer application.

In this experiment we used *C. argentea* CIAT 22386, which was not part of the initial collection that gave origin to cv. Veraniega. This accession is late flowering and produces very vigorous plants; therefore we thought it was a better candidate to include in the fertilizer response trial.

The evaluations are still underway, but in Table 52 we show that after 5 months of growth there is no treatment effect on plant height, although there is a tendency for taller plants when N was applied either to inoculated or not inoculated seeds.

The interactions of main treatments with P levels were not significant ($P>0.05$), even though there is a tendency for taller plants with increasing rates of P application, except for the inoculated seed treatments where plant growth is reduced with P levels above 40 kg/ha .

Table 52. Response to phosphorus (P) of inoculated and non-inoculated seed *Cratylia argentea* CIAT 22386 with or without Nitrogen (N) at Atenas, Costa Rica. (Means of 5 months old plants).

Treatments	Plant height (cm)	Mean (cm)
Control		
0	157.3 b*	
20	155.6 b	
40	159.0 b	158.7
80	163.1 b	
Inoculated (I)		
0	163.1 b	
20	164.7 b	
40	180.3 ab	165.6
80	154.4 b	
Nitrogen (N)		
0	156.3 b	
20	177.7 ab	
40	168.9 ab	169.0
80	173.1 ab	
Inoculated + Nitrogen		
0	169.0 ab	
20	184.8 ab	
40	188.8 a	177.2
80	166.3 ab	

3.7 Herbaceous legumes with adaptation to acid soils and drought

Highlights

- Identified accessions of *Canavalia brasiliensis* that behaved as annuals and identified others that were persistent under cutting.
- Selected early and late flowering accessions of cowpea with relatively broad edaphic adaptation.

- The legume *Vigna umbellata* does not appear to be adapted to acid-low fertility soils.

- The legume *L. purpureus* cv. Highworth showed good adaptation to sub-humid conditions in Central America and together with accessions L-987, 21603 and four lines coming from CSIRO (CPIs) produced more than 3.3 t/ha of dry matter yields of high quality forage.

3.7.1 Evaluation of a core collection of *Canavalia brasiliensis* for multipurpose uses (Quilichao, 2004-2005)

Contributors: M. Peters, R. Schultze-Kraft (U. of Hohenheim), L.H. Franco, B. Hincapié, G. Ramírez (CIAT)

Rationale

Canavalia brasiliensis Mart. ex Benth. ("Brazilian jackbean") is a weakly perennial, herbaceous legume with a wide natural distribution in the New World (tropics and subtropics). In comparison with *C. ensiformis* ("jackbean"), research reports on *C. brasiliensis* are scattered and restricted to studies done in few sites in Latin America. The species develops a dense and extensive, deep-reaching root system and subsequently tolerates a 5 to 6 month dry period. Based on studies that generally were done with only one genotype, it is adapted to a wide range of soils, including very acid, low-fertility soils. Its main use so far has been as green manure, for fallow improvement and erosion control. Due to medium rate biomass decomposition, nutrient release of *C. brasiliensis* green manure has the potential to synchronize well with the nutrient demand of the succeeding crop. In Central America, the legume has been used experimentally to improve the value of crop residues utilized under grazing in the dry season. There is limited information on the nutritive value and toxicity to ruminants of the herbage of this species.

Materials and Methods

A total of 53 accessions of *Canavalia brasiliensis*, mostly from Latin America, was sown into jiffy pots and transplanted 4 weeks later to the field at CIAT's research station in Quilichao. Six plants per plot were sown, in a Randomized Complete Block Design with 4 replications. DM yield, drought tolerance and forage quality parameters were measured (Photo 25). The list and origin of germplasm evaluated was presented in the Annual Report 2004.

Results and Discussion

Plants established quickly, and incidence of pest and diseases was low, in particular when compared to a collection of *Canavalia* sp.

Photo 25. *C. brasiliensis* Top: Flower, Bottom: Regrowth after two weeks of cutting

Table 53. Vigor, soil cover (%) and dry matter yields of *Canavalia brasiliensis* during the establishment phase in Quilichao, 2004.

Accession	6 Weeks		12 Weeks		16 Weeks		
	Vigor	Soil cover	Vigor	Soil cover	Vigor	Soil cover	Mean dry matter yields
	1-5	%	1-5	%	1-5	%	Kg/ha
808	4	50	5	92	5	100	6320
17009	4	43	4	85	5	100	5333
8557	4	47	5	87	5	97	4693
17012	3	35	3	78	4	90	4560
21824	3	40	3	73	3	78	4440
20098	3	33	3	75	4	80	4440
18515	3	45	4	88	5	100	4333
20303	3	35	3	77	4	80	4333
17973	3	32	3	68	4	75	4293
7178	4	40	3	73	4	82	4200
20306	3	30	3	72	4	85	4187
7648	4	52	4	87	5	100	4107
7319	4	52	4	87	4	90	4067
20095	3	47	4	87	4	93	3907
7969	3	35	4	80	5	93	3880
22132	3	40	3	73	4	77	3840
7321	3	35	3	78	4	87	3787
7973	3	40	4	77	4	80	3760
18501	3	38	3	70	4	75	3720
17008	4	42	4	85	4	80	3680
8770	3	38	3	75	5	100	3680
19034	3	43	4	83	4	85	3653
17462	3	40	4	80	5	97	3627
905	3	43	3	63	3	67	3613
7647	3	37	4	77	4	87	3507
7971	3	30	3	72	4	82	3400
7175	2	27	3	63	3	72	3373
19029	4	43	4	83	4	83	3320
20518	3	42	4	83	4	80	3147
20096	3	37	4	85	4	83	3120
20516	3	35	3	73	4	83	3053
21825	3	33	3	73	4	82	3040
17010	3	35	3	70	4	80	2933
19035	3	35	3	72	3	63	2893
20514	4	52	3	83	3	80	2840
19361	4	43	3	78	4	88	2813
17011	3	40	3	80	3	85	2813
7972	3	32	4	80	4	90	2787
20513	4	50	4	75	3	82	2773
20301	3	27	3	78	3	83	2720
8768	3	32	3	63	3	72	2653
7970	3	43	4	87	4	90	2640
20295	3	35	3	77	3	77	2627
19359	3	37	3	73	3	73	2600
9146	3	37	3	75	3	80	2560
20304	2	28	3	63	3	75	2560
19632	3	27	3	65	3	67	2440
20296	2	23	3	60	2	67	2360
20090	2	22	3	70	3	70	2333
5033	3	38	3	70	3	80	2227
7174	3	32	3	67	3	70	2227
17828	3	28	4	68	3	63	2160
7894	3	28	2	55	3	72	1813
Mean	3.1	37	3.4	76	3.8	82	3400
Range	2-5	20-70	2-5	30-100	1-5	30-100	1000-8600
LSD P< 0.05							2855

planted at the same time in Quilichao. Twelve weeks alter transplanting the majority of accessions had soil covers above 70%, with accessions CIAT 808, 18515, 7319, 7648, 7970, 8557, 2009517008,17009 and 20096 showing the highest values (Table 53).

Total DM yields recorded16 weeks after transplanting varied significantly (P< 0.05) between accessions, with yields above 4 t/ha recorded for accessions CIAT 808, 17009, 8557, 17012, 20098, 21824, 18515, 20303,17973, 7178, 20306, 7648 and 7319. Accession CIAT 17009 is a material previously selected by farmers in Central America for use as green manure and to improve fallows and crop residues, due to its yield and drought tolerance. Despite a severe dry period materials remained green, with soil covers above 80%.

In view that many of the accessions had not completely covered the soil after 16 weeks a standardization cut to enhance sprouting was done in the early wet season. After 12 weeks of regrowth, soil cover and DM yields remained similar to the first cut; however some accessions such as CIAT 808, 17009 y 8557 had lower yields (around 1 t/ha DM less) indicating the more short term perennial characteristics of these particular accessions (Table 54.).

To assess persistence and regrowth ability under more dry conditions a cut was done at 15 cm, to evaluate biomass in the following dry season. Most plants persisted, but rooting and stolon formation was limited in the dry season. Thus no dry season evaluation was possible and DM yield was recorded in the following wet season after 24 weeks of regrowth (Table 54). On average DM yields were 37% lower than in the previous evaluation and significant differences (P< 0.05) in DM yield were observed between accessions. Accessions CIAT 808, 8768, 7175, 22132 and 17973 had reduced yields (55 to 66 %), while accessions CIAT 7971, 7972 and7321 had more stable yields with more than 3 t/ha DM; some materials such as CIAT 8557 and 905 increased DM yields by 10 to 25% from one year to another. The DM yield of the control accession CIAT 17009 was 39% lower than in the 1st year.

Other materials (CIAT 7319, 7114, 7894, 8770, 17973, 18501, 20514, 21825, 22132, 19034, 7178, 20518 y 20513) apparently have an annual behavior, a characteristic observed not only under cutting but also in adjacent uncut plots.

Forage quality was assessed by sampling a 16 week regrowth in the dry season and analyzing the samples for dry matter digestibility (IVDMD) and crude protein (CP). Mean IVDMD and CP values were 82% and 22%, respectively, and significant differences (P< 0.05) were observed among accessions. Accessions CIAT 7178, 20304 and 18515 had digestibilities above 84%, while accessions CIAT 5033 y 17828 were below 80%; CP contents ranged between 19% and 25%. The control accession CIAT 17009 had 82% and 22% of IVDMD and CP, respectively, which are values similar to the average of the collection (Table 55).

3.7.2 Evaluation of a core collection of *Canavalia* sp. for multipurpose uses (Quilichao, 2004-2005)

Contributors: M. Peters, L. H. Franco, R. Schultze-Kraft (University of Hohenheim), B. Hincapié, G. Ramírez, (CIAT)

Rationale

In view of the promising results obtained with *Canavalia. brasiliensis*, there was an interest to define the potential of other species of *Canavalia* for use mainly as green manure and for fallow improvement in low fertility, drought prone environments.

Table 54. Soil cover (%) and dry matter yields of *Canavalia brasiliensis* in Quilichao, 2004-2005.

Accession	Soil cover	Mean D M yields	Soil cover	Mean D M yields
	%	Kg/ha	%	Kg/ha
	Wet 2004 (12 Weeks)		Wet 2005 (24 Weeks)	
808	97	5493	83	2520
7175	78	5280	33	2160
17973	75	5280	40	1800
22132	73	5146	25	1880
8768	83	4733	50	2733
17008	97	4640	90	2480
17462	97	4627	90	3240
7648	95	4467	92	4067
7973	90	4387	57	2013
20303	92	4333	88	3373
18515	100	4240	83	2347
20306	83	4187	83	1973
17011	82	4160	80	2213
20295	80	4147	60	2160
17009*	82	4053	80	2493
7178	100	4053	48	1220
17012	93	4040	93	2507
19361	77	3840	45	2240
21824	75	3813	33	893
20095	93	3800	58	2147
7970	83	3707	57	2387
20304	90	3640	55	2720
20096	83	3627	53	2347
7319	92	3628	83	2960
20301	92	3533	68	2107
7971	73	3480	73	3787
7972	85	3440	60	3333
20514	80	3387	60	800
7174	77	3373	32	1000
19029	90	3333	43	1987
20098	73	3320	70	2893
20516	68	3237	33	2160
7969	97	3253	93	2827
8557	97	3187	92	3520
7321	73	3147	68	3360
18501	83	3120	33	1700
8770	70	3120	70	2460
19359	88	3093	55	1773
19034	77	3067	65	2680
17828	67	2813	43	1800
19035	60	2773	27	1467
7647	87	2760	83	2460
20090	82	2720	37	1187
5033	70	2707	47	1560
7894**	67	2653	.	.
9146	78	2613	50	843
21825	70	2600	20	520
20296	60	2587	7	1667
905	70	2493	83	3120
20513	77	2467	35	980
17010	87	2360	90	2773
20518	70	2067	20	1200
19632	67	1933	45	1120
Mean	82	3547	60	2254
LSD (P< 0.05)		3201		2805

***** Control accession, ****** Disappeared in 2nd year

Table 55. Forage quality of accessions of *Canavalia brasiliensis* sampled in the dry season, 16 week regrowth in Quilichao.

Accession No. CIAT	IVDMD	CP	Accession No. CIAT	IVDMD	CP
	%			%	
7178	85	21	20090	82	23
20304	85	21	9146	82	23
18515	84	20	7973	82	21
19029	84	22	7647	82	21
20296	84	25	20518	82	23
20514	84	20	17973	82	20
20306	84	22	8770	82	20
17011	84	23	19632	82	23
20303	83	23	7894	81	23
20516	83	22	7321	81	21
905	83	21	7969	81	25
20096	83	21	8768	81	21
17010	83	22	21825	81	20
7319	83	21	22132	81	22
20301	83	21	19359	81	25
20095	83	21	20295	81	23
7174	83	21	17012	81	23
7972	83	21	20513	81	20
7970	83	21	7971	81	24
808	83	23	19361	81	22
21824	82	21	7648	81	23
7175	82	19	17462	81	23
19034	82	22	19035	80	21
17009	82	23	18501	80	20
17008	82	23	5033	79	22
8557	82	22	17828	77	21
20098	82	21			
Mean				82	22
LSD (P<0.05)				4.5	5.0

Materials and Methods

A total of 47 accessions of *Canavalia* sp, originating from Latin America, China and Thailand were sown into jiffy pots and transplanted to the field in Santander de Quilichao. The design and variables are the same as described for *C. brasiliensis*. The list and origin of germplasm evaluated was presented in the Annual Report 2004.

Results and Discussion

Establishment of most accessions was slow, with soil covers below 53% when measured 12 weeks after transplanting. After 16 weeks of transplanting, only 11 materials had soil covers above 80% (Photo 26). Several materials appeared not to be well adapted to the acid soils in Quilichao and were severely affected by pests and diseases. Accessions with the best adaptation in the establishment phase were CIAT 21012, 21014, 19038, 21209, 7317, 7383, 8719, 21013, 21211 and 18587, all of which showed a good drought tolerance (Table 56).

Mean soil cover in the dry season was 60%; with a large variation between accessions (range: 5% - 98%). Accessions with soil covers above 80% were CIAT 19038, 21012, 7383, 7317, 21013, 21009, 21014, 8719 and 19033. Significant differences (P< 0.01) among accessions were also found for DM yield, with CIAT 7383, 8719, 20305, 20307 and 21209 yielding more than 3 t/ha DM (Table 56). However, many of the accessions tested showed signs of poor adaptation to dry season conditions and susceptibility to diseases and pests with yields below 1 t/ha DM. Mean soil cover and DM yield for the regrowth in the wet season were similar

Table 56. Soil cover (%) and dry matter yields of *Canavalia* sp in Quilichao, 2004-2005.

Accession	Soil cover %	Mean DM yields kg/ha	Soil cover %	Mean DM yields kg/ha	Soil cover %	Mean DM yields kg/ha
	Dry 2004 (16 weeks)		Wet 2004 (16 weeks)		Wet 2005 (24 weeks)	
7383	92	4100	95	2453	78	1893
8719	87	3660	92	2407	37	1253
20305	67	3347	63	2287	10	667
20307	63	3180	53	1100	20	413
21209	88	3040	95	2153	30	1867
18272	72	2820	73	1640	11	947
19038	98	2607	98	2707	68	2173
18271	73	2567	73	1820	25	1680
7317	92	2513	95	3233	32	1360
21014	88	2507	95	2387	93	2987
22031	70	2433	83	1587	20	1347
17451	70	2360	70	1407	25	1560
7318	40	2353	67	1460	17	520
17929	75	2327	63	1347	5	240
7322	63	2167	77	1773	17	800
21012	95	2087	98	1727	93	1827
18270	33	2013	53	1167	13	1603
8769	70	2000	77	1573	25	1680
18587	73	1833	67	2013	38	1540
20691	73	1820	82	2393	45	500
19031	65	1773	75	2340	20	1160
20803	75	1773	67	1947	5	93
20748	62	1573	70	1480	55	1140
21013	90	1480	92	1940	90	1800
19032	68	1460	53	1747	18	3100
8771	67	1453	47	1687	5	0
21211	75	1380	75	1600	33	660
19033	80	1347	77	2033	18	1040
20298	57	1173	60	1467	35	680
8185	47	1167	30	927	5	227
18580	67	1167	67	1540	17	627
21212	47	1147	50	930	4	480
19052	60	1060	70	1387	53	1853
19357	67	1040	60	1840	5	1120
20145	53	953	57	1227	35	640
20113	47	727	37	1180	5	340
21210	60	687	63	1127	7	640
20300	15	680	22	333	5	0
18268	38	540	53	1153	8	333
21487	47	507	47	1547	5	320
19356	43	507	65	1330	28	740
18263	22	373	17	213	6	53
18258	20	353	32	807	8	1360
18261	32	320	38	560	13	920
20297	10	207	25	187	20	453
20093	5	187	18	580	5	93
20299	5	0	20	240	35	480
Mean	60	1680	63	1657	28	1184
LSD (P< 0.01)		1829		1996		2176

Photo 26. Some of the best materials of *Canavalia* sp.

to dry season results; however, accessions CIAT 7383, 8719, 20305, 20307, 21209 and 18272 produced less dry matter than in the dry season. Highest yields in the wet season were obtained for accessions CIAT 7317, 19038, 7383, 8719, 20691 and 21014 (Table 56).

Utilizing the same methodology as for the C. *brasiliensis* collection, persistence under cutting was assessed by measuring regrowth and DM yield in the following dry season, after a cut to 15 cm above soil level. As for *C. brasiliensis*, a low capacity to form additional roots and stolons under drought conditions was observed. In addition, several accessions did not regrow after the wet season cut; hence DM evaluation was done in the following wet season after 24 weeks of regrowth (Table 56). Significant differences ($P< 0.01$) among accessions were observed, but mean productivity was lower than in the previous evaluation. Nevertheless a few accessions had high DM yields in the second year such CIAT 19032, 18270 and 19052, which increased yields from 1747 to 3100, 1167 to 1603 and 1387 to 1853 kg DM/ha, respectively. Accessions CIAT 19038, 17451, 21012, 8769 and 21013 maintained a stable productivity over time.

Only 32% of the accessions evaluated persisted for 2 years under cutting. The other accessions were annuals or had low environmental adaptation and a high susceptibility to pests and diseases. Persistent accessions with tolerance to drought and a high vigor include accessions CIAT 21012, 21013 and 21014.

Table 57. Forage quality of selected of accessions of *Canavalia* sp. sampled in the dry season in Quilichao.

Accession No. CIAT	IVDMD	CP	FND	FAD
	%			
21014	88	28	18	13
21013	88	25	19	13
21012	86	27	22	13
21209	86	24	22	13
19038	84	25	19	13
19033	84	25	25	15
18587	84	25	28	14
22031	84	27	24	11
18271	84	25	20	13
21211	83	27	22	12
18272	83	24	25	15
7317	81	26	21	13
8719	81	27	20	13
7383	80	27	21	13
17451	78	25	27	18
Mean	84	26	22	14
LSD ($P< 0.05$)	4	7	5	3

Forage quality of *Canavalia* sp. was high, based on samples obtained from plants showing high productivity and vigor. Significant ($P< 0.01$) differences were found for IVDMD, NDF and ADF, but not for CP; digestibility varied between 78 % and 88%, and CP content ranged between 18% and 28% (Table 57).

In general performance of the *Canavalia* spp collection was inferior to *C. brasiliensis*, with a higher susceptibility to pest and diseases. Selected accessions will be evaluated in 2006 larger plots with higher plant density.

3.7.3 Evaluation of a collection of *Vigna umbellata* for multipurpose uses (Palmira and Quilichao, 2005)

Contributors: M. Peters, L.H. Franco, B. Hincapié, A. Schmidt and G. Ramírez (CIAT)

In 2005 a small collection of 7 accessions of *Vigna umbellata* (rice bean) was evaluated in Palmira and Quilichao to assess the adaptation to variable soils (pH and fertility) and climate. Due to an intensive drought during establishment most materials did not develop well in Quilichao, with the exception of accession CIAT 522; however, in Palmira plants established well (Photo 27).

Significant differences ($P<0.05$) among accessions were observed for DM yield with CIAT4279, 24466 and 522 being the highest yielding accessions with yields above the mean of 3.5 t/ha DM as shown in Table 58. There was a high incidence of insect attacks; no flowering synchronization occurred, and some very late flowering materials were selected.

Table 58. Vigor, soil cover (%) and DM yield (kg/ha) of *Vigna unguiculata* in Palmira, 2005.

Accessions CIAT No.	Vigor	Cover (%)	DM (kg/ha)
4279	5	100	4900
24466	4.0	97	4400
522	4.3	100	3853
24360	4.6	100	3347
San Dionisio	3.6	87	3160
461	3	80	2527
24469	2.3	50	2400
Mean	3.8	88	3512
LSD ($P \leq 0.05$)			1792

Photo 27. *Vigna umbellata* at Palmira.

3.7.4 Evaluation of a core collection of *Vigna unguiculata* for grain production (Palmira and Quilichao)

Contributors: M. Peters, L. H. Franco, B. Hincapié, G. Ramírez (CIAT), and B.B. Singh (IITA, Nigeria)

Rationale

Cowpea (*Vigna unguiculata*) is utilized in the subhumid/semi-arid tropics of West Africa and India as a source of food and feed for livestock, but the utilization of cowpea in Latin America is so far limited. We visualize that cowpea could be an alternative crop for the second planting season in the central hillsides region of Nicaragua and Honduras where the legume could provide not only higher grain yields as compared to common beans, but could also allow for a third crop in November/December in order to provide hay as animal feed in the dry season or contribute to soil fertility enhancement for the following maize crop. Cowpea could also be used for hay, silage and feed meal production, which in turn could be an option for income generation by smallholder livestock and non-livestock owners. Adaptation to climatic and edaphic conditions, especially to water stress, are prerequisites for the successful development of cowpea as a forage options in traditional livestock maize-bean mixed systems in Central America. The evaluation of new selected sets of cowpea accessions from IITA continued in 2005, with the objective of identifying materials for specific uses such as grain materials for production of seed concentrate meals for feeding monogastrics.

Materials and Methods

A new set of 32 accessions of *Vigna unguiculata* obtained from Dr. B.B. Singh, cowpea breeder of IITA, was complemented with 5 local accessions from Honduras. The collection included 14 fast maturing and 11 late maturing materials, all with high drought tolerance. The collection was sown in Quilichao and Palmira to compare the effect of climate and soil on grain production. Emphasis is given to identifying accessions with broad adaptation, which is crucial for Central American Hillsides with highly variable soil and climatic conditions.

Results and Discussion

2005 collection

Cowpea grain yield was much higher in Palmira than Quilichao, with late maturing materials yielding 3 times more and the early materials yielding 4 times more. In both locations, the late materials had higher grain yields than early types (Tables 59 and 60). In Quilichao, the late accessions with more than 1 t/ha grain harvested included 9611, IT97K-1069-6, IT98K-503-1, IT99K-718-6 and IT98K-205-9; in Palmira grain yields of above 3 t/ha grain were obtained with accessions INTA-02, IT98K-205-15, IT99K-718-6, IT98K-128-3 and IT97K-1069-6 (Table 59). For the early types in Quilichao IT99K-316-2, IT00K-1217 and IT97K-568-18 yielded more than 1 t/ha grain while in Palmira more than 3 t/ha was achieved with accessions IT93K-625, IT97K-499-35, IT00K-1263, IT98K-1111-1 and IT99K-316-2 (Table 60).

In Palmira, some of the accessions including the control 9611, remained green after grain harvest, (Photo 28) which has also observed in other sites such as the Norte del Valle, but not in Quilichao; the edaphic and climatic effects on senescence of cowpea plants need further study, in particular as green material after grain harvest provides an added value as hay, silage or green manure.

Photo 28. Cowpea (*Vigna unguiculata*) accessions with high grain production at Quilichao and Palmira, remaining green after (photo 361-1) grain harvest.

Table 59. Biomass and grain yields of late maturing accessions of Cowpea (*Vigna unguiculata*) at Quilichao and Palmira, 2004-2005.

Accession	Quilichao		Palmira	
	Total (kg/ha)	Grain (kg/ha)	Total (kg/ha)	Grain (kg/ha)
9611 (control)	2047	1340	7007	2613
IT97K-1069-6	3348	1307	6207	3240
IT98K-503-1	2653	1240	4647	2840
IT99K-718-6	2620	1213	5227	3767
IT98K-205-9	2247	1093	5347	2840
IT98K-205-15	2460	987	8140	4100
INTA-03	3173	927	6687	2873
IT97K-556-4	2840	880	5287	2933
IT98K-128-3	2793	767	5367	3567
IT98K-491-4	2053	720	4287	2567
IT00K898-5	2100	680	3073	1593
INTA-02	5013	387	8033	4860
Mean	2779	962	5776	3149
LSD (P≤0.05)	1826	NS	2615	1643

Table 60. Biomass and grain yields of early maturing accessions of Cowpea (*Vigna unguiculata*) at Quilichao and Palmira, 2004-2005.

Accession	Quilichao		Palmira	
	Total (kg/ha)	Grain (kg/ha)	Total (kg/ha)	Grain (kg/ha)
IT99K-316-2	1933	1440	3800	3073
IT00K-1217	1540	1093	3193	2687
IT97K-568-18	1637	1090	3167	2987
IT98K-128-4	2130	930	3283	2960
IT97K-499-35	1787	880	5100	3333
IT00K-1150	1707	753	2240	2287
IT00K-901-5	2217	707	4393	3157
IT98K-1111-1	2220	687	2700	3320
IT98K-589-2	3100	560	5360	2007
IT99K-491-7	1260	533	3160	2347
IT93K-452-1	1813	460	2233	2340
IT93K-625	2880	453	5113	4880
IT00K-1263	3143	423	5310	3327
IT99K-529-1	2687	373	2933	2513
Mean	2147	742	3713	2944
LSD (P≤0.05)	1231	790	1821	1404

3.7.5 Evaluation of a core collection of *Lablab purpureus* for multipurpose use (Atenas, Costa Rica)

Contributors: P. J. Argel and G. Pérez (CIAT)

Rationale

In Central America the legume *Lablab purpureus* (syn. *Dolichos lablab* L.) has been experimentally used as green manure and to improve crop residues such as maize in sites with a prolonged dry season with promising results; however, the commercial use of the legume is limited basically because seed availability of existing lines is scarce and there is no diffusion process in place including on-farm demonstrations. Within the genus considerable variability exists that justifies characterization in order to identify more productive and better adapted lines.

Under this assumption, new accessions that came from ILRI/CSIRO are under evaluation in the subhumid environment of Atenas, Costa Rica.

Materials and Methods

Twelve accessions of *L. purpureus* were established for evaluation of adaptation in a randomized complete block design with three replicates at Atenas, Costa Rica. The accession I-6533 failed to establish well and consequently did not produce measurable biomass. Plot size was 2.0 m wide x 1.5 m long (3 m^2), and the planting distances were 0.25 m and 0.50 m between plants and rows respectively.

Plots were fertilized with 50 kg/ha of P_2O_5, 50 kg of K_2O, 20 kg of S and 20 kg of Mg. Plant emergence, plant vigor and cover were measured respectively 4 and 6 weeks after planting (see IP-5 Annual Report 2002). A first harvest was carried out at 16 weeks of age to measure dry matter yields, in vitro dry matter digestibility and crude protein of total plants.

Results and Discussion

Plant emergence was acceptable and there was no need for replanting. Differences in plant cover and vigor were also observed as reported before. Dry matter yields and forage quality varied among accessions as shown in Table 61.

The cultivar Highworth showed good adaptation to the site and together with accessions L-987, 21603 and four accessions coming from CSIRO (CPIs) produced more than 3.3 t/ha of dry matter yields of high quality. Digestibility and crude protein was high for all accessions, particularly for cv. Highworth, which confirms the excellent forage quality of this legume.

This cultivar is early flowering and has intermediate seed yields compared to other accessions (see IP-5 2004 Annual Report); however, the outstanding forage yield produced by this cultivar merits seed multiplication and on-farm validation and demonstration.

Table 61. Dry matter yields (DMY), In vitro dry matter digestibility (IVDMD) and Crude Protein (CP) of *Lablab purpureus* lines harvested at 16 weeks of age in Atenas, Costa Rica.

Accessions	DM Yield (kg/ha)	IVDMD (%)	CP (%)
cv. Highworth	5121.6 a*	70.3	13.8
CPI-106471	3807.5 ab	69.1	14.7
L-987	3783.8 ab	62.8	12.6
CPI-67639	3640.6 ab	63.3	14.1
CPI-52535	3320.8 ab	63.0	13.5
21603	3316.8 ab	66.2	11.5
CPI-34777	3310.0 ab	60.6	14.2
I-14442	2858.5 b	67.3	14.0
I-11632	2492.8 b	67.5	15.6
CQ-2975	2423.9 b	68.6	13.4
CPI-36903	1964.1 b	65.8	15.0

* $P<0.05$

3.7.6 Selection of *Vigna unguiculata* for seed multiplication and on-farm testing

Contributors: M. Peters, L. H. Franco, B. Hincapie, and G. Ramírez (CIAT)

In recent years, CIAT and its partners have evaluated a range of multipurpose accessions *Vigna unguiculata* (cowpea), mainly obtained from IITA, for inclusion in smallholder production systems, as fresh and conserved feed, for leaf and seed meals, and green manure.

It was thus timely to synthesize the information obtained so far and to identify superior accessions for variable soil conditions and different potential uses, with the aim of selecting accessions testing in diverse smallholder production systems.

The following approach was taken to select cowpea accessions for different niches:

1. Trials were analyzed separately using standard statistical procedures.

2. Mean biomass and grain yields were obtained for each material evaluated in Quilichao (AC - Acid Soil) and Palmira (AL - Alkaline Soil).

3. Using descriptive statistics a graphical analysis was elaborated classifying materials for grain and biomass (Figures 62 and 63).

4. The most promising materials for multiple uses were those above the mean in grain and biomass yield in both acid and alkaline soils (upper right quadrant of graph).

5. The most promising materials for grain yield were those above the mean (lower right quadrant of the graph).

6. The most promising materials for biomass were those with yields above the mean (upper left quadrant).

7. Visual observation was employed to classify materials as late or early maturing using the days to flowering as an indicator. At the same time the capacity of materials to regrow after cutting/harvesting of grain was assessed.

8. Results were then compared and validated with results from an earlier multilocational trial of a limited set of accessions.

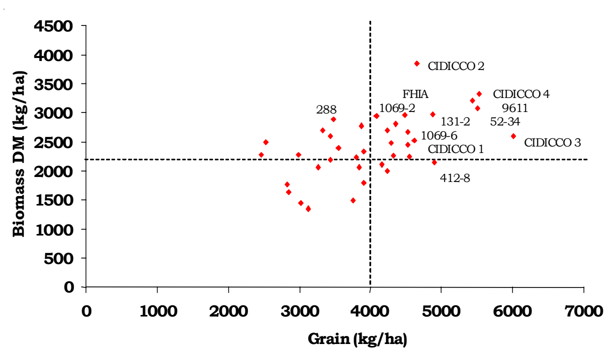

Figure 62. Clasification of materials for grain and biomass yield in Palmira

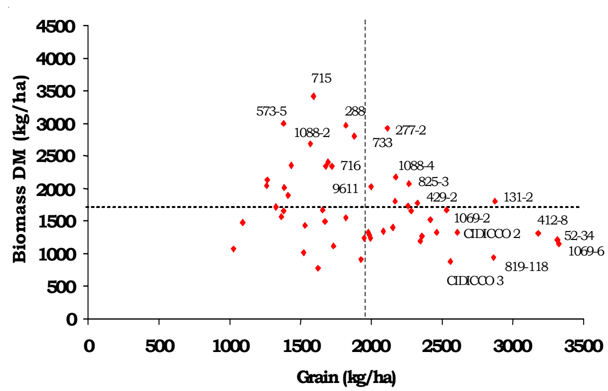

Figure 63. Classification of materials for grain and biomass yield in Quilichao

Table 62. Selection criteria for *Vigna unguiculata* materials

Biomass types				Grain types				Dual purpose types (biomass and grain)			
Material	Green regrowth*	M	Soil adaptation	Material	Green regrowth	M	Soil adaptation	Material	Green regrowth	M	Soil adaptation
IITA-277-2	Yes	P	AC	IITA-52-34	Yes	T	Both	DICTA-9611	Yes	T	Both
IITA-715	Yes	P	AC	CIDICCO 3	Yes	T	Both	IITA-131-2	No	T	Both
IITA-573-5	Yes	P	AC	IITA-1069-6	Yes	P	Both	CIDICCO 2	Yes	T	AL
IITA-288	Yes	P	AC	IITA-412-8	No	T	Both	CIDICCO 4	Yes	T	AL
IITA-1088-2	Yes	P	AC	IITA-316-2**	Yes	P	Both	IITA-52-34	Yes	T	AL
IITA-1069-2	Yes	T	AL	IITA-625**	No	T	AL	CIDICCO 3	Yes	T	AL
INTA 02*	Yes	T	Both	IITA-718-6**	Yes	T	AL	CIDICCO 1	Yes	T	AL
				IITA-205-15**	Yes	T	AL	IITA-1069-6	Yes	P	AL
				INTA 02**	Yes	T	AL				

M = time to maturity, AC = Acid soil, AL = Alkaline soil, T = late maturing, P = early maturing

* After harvesting grain
** Only based on first year evaluation (new collection)

In Table 62 selected *Vigna unguiculata* materials for feed use (biomass), grain use (seed meals) and dual purpose materials are presented. While materials selected for biomass production are sensitive to soil pH, materials for dual purpose use have a broader adaptation to soil pH.

Both early maturing and later maturing materials exist allowing the option of having alternatives based on labour peaks. Several of the materials selected have the ability to regrow after grain harvest allowing the harvesting of both grain and forage, however this ability is dependent on environment.

Output 4: Superior and diverse grasses and legumes delivered to NARS partners are evaluated and released to farmers

4.1 Partnerships in Africa to undertake evaluation and diffusion of new forage alternatives

Highlights

- For farmers in Uganda total forage yield was not a priority indicator to select new forage species. The value of new forages (improved Brachiaria cultivars) was mainly associated with dry season performance.

- The legume Stylo CIAT 184 was seen as an excellent option for dry season feeding, by farmers in Uganda but seed availability limits its use and diffusion. Development of farmer led forage seed systems linked to commercial components is of high priority.

4.1.1 Lessons learned from participatory evaluation of improved forages with farmer groups in Tororo, Uganda

Contributors: Ralph Roothaert (CIAT/ ILRI, Ethiopia); Grace Nalukwago, Paul Nyende (Africa 2000 Network, Uganda)

Rationale

Eighty percent of the Ugandan people depend on agriculture for their livelihood (Aliguma and Nyoro, 2004). Tororo district is one of the poorest areas in Uganda with more than 60 % of households falling below the absolute poverty line (Thornton et al, 2002). The farming system is characterised by annual crops, local cattle and goats. Although Tororo lies in a tsetse fly infested area, there is potential for improved dairy production for smallholders which can contribute to increased farm income, and better nutrition for children and sick household members. A few NGOs are introducing dairy production using improved breeds.

A participatory diagnosis conducted in 2003 by the NGO Africa 2000 Network (A2N) and CIAT revealed many problems related to productivity of the farming system:

- High incidence of pests and diseases in crops.
- Lack of seeds and knowledge of improved crops varieties.
- Feed shortage for improved dairy and goat breeds, and for cross breds, especially during the dry season which lasts from December to March.
- Cross border trade with Kenya causing labour shortage.

In addition to problems described, the soils are infertile and deficient in K, P and N. The district has a high population density, (more than 280 people per km^2). 82% of the land is under cultivation . As population increases, so does the demand for land.

Partnerships for research to improve soil management

In 1997, a consortium of district based R&D organisations was formed to improve soil fertility to overcome food insecurity and poverty. The consortium was called INSPIRE and consisted of the district government's department of production, Africa 2000 Network (A2N), Sasakawa Global 2000 (SG2000), Tororo District Farmers Association (TODIFA), Cash Farm, Plan International, DATIC, CARITAS, Appropriate Technology (AT) Uganda, and various national and international research institutes. A2N has been coordinating the activities. Among different soil fertility improvement strategies, research was carried out with farmers to evaluate the performance of several cover crops: *Crotalaria grahamania*, *C. pancilla*, mucuna, canavalia, sesbania and tephrosia. Although the evaluation criteria were mostly agronomic in nature, an additionally appreciated attribute of mucuna was its use as animal feed.

Objectives of the experimentation with forages

A2N works with 45 farmer groups in Tororo District, and some of these groups have received Friesian cattle through a dispersal scheme to start up dairy production. In 2003, CIAT and A2N selected two groups to start participatory evaluation of improved forage varieties. Some farmers of one group, Katamata, had already received a cow. In the other group, Umoja, selection had been made of farmers who would qualify to receive a cow. Although INSPIRE had tested forage crops for soil fertility improvement with farmers, most of the species used in these experiments were not that suitable for animal feed, due to palatability, digestibility or toxicity problems. On the other hand, farmers were already growing and feeding napier, calliandra, leucaena and sesbania to their livestock. Napier is a good forage in many aspects, but it needs high soil fertility and continuous rainfall throughout the year. Calliandra, leucaena and sesbania are especially useful to supply dietary protein for cattle, but their establishment and management is more intensive than other forage crops.

One objective of the new initiative was to evaluate alternative forage varieties which would provide high amounts of high quality feed for dairy cows during the critical dry season from December to March. The other objective was to study the research process and develop methods that optimise learning and impacts for forage systems with resource poor farmers in East Africa. Secondary objectives were to increase fodder availability for goats, and improve soil fertility.

Approach and methods

An action research approach was chosen to obtain the research outputs and to enhance learning and change among all parties involved. Several iterative cycles of planning-action–reflection–planning were envisaged over a period of two years. The first cycle started with reflection of past farmer experiences and collaborative participatory planning of on-farm forage evaluation experiments. The research was carried out with two farmer groups within a range of 10 km from Tororo town; Katamata and Umoja groups. Research was carried out simultaneously and separately with both groups. Both groups had mixed gender composition of members.

Results and Discussion

First cycle, Feb. – June 2004

Past experiences with livestock and forages were reflected with both groups. Seventeen women and 20 men farmers were part of the initial discussions. Ownership of local goats, local cattle, local pigs, and exotic cows were 59, 57, 24 and 11% respectively. Although none in Umoja group owned a dairy cow, 14 members were expecting to receive one soon. Farmers of both groups were familiar with the following fodder trees: Sesbania sesban, Calliandra calothyrsus and Leucaena leucocephala. Sesbania was said to be good for milk production; calliandra was said to be drought resistant, and leucaena was liked by goats and cattle. Experiences with other forages were also mentioned: Pennisetum purpureum grows well on deep soils, Lablab purpureus increases appetite of animals. Mucuna is dual purpose; it improves soil fertility, feeds cows and goats, and grows well during the rainy season.

Planning. Members of the groups expressed interest to experiment with new forage legumes and grasses that would provide high quality feed for their animals. Most members preferred to experiment on an individual basis rather than in a group; in previous group experiments the contribution of labour from group members had been skewed and unsatisfactory. Unfortunately there was not enough seed for experimentation on individual basis for everyone; a maximum of 5 replicates per forage species per group was possible. A compromise was reached in both groups: experimental plots would be established on 5 individual farms per group, and other members would choose the farm they would want to be associated with and contribute labour to. By contributing labour for an experimental plot, the member would have priority to use of fodder, seeds or planting materials produced from the plot. Consensus was reached for 5 men and 5 women to be allowed to take the lead and establish experimental plots on their farms. Each of them would become sub-group leader, and 4 additional members were allocated to each sub-group, making a total of 50 members directly involved in the experiment. A scientist described new forage varieties and explained their agronomic and nutritive attributes.

A plot layout was proposed by the scientist, and farmers suggested some modifications in terms of space. The agreed layout contained 4 grass species, 7 herbaceous legumes species and varieties, one tree legume, and a live hedge of the

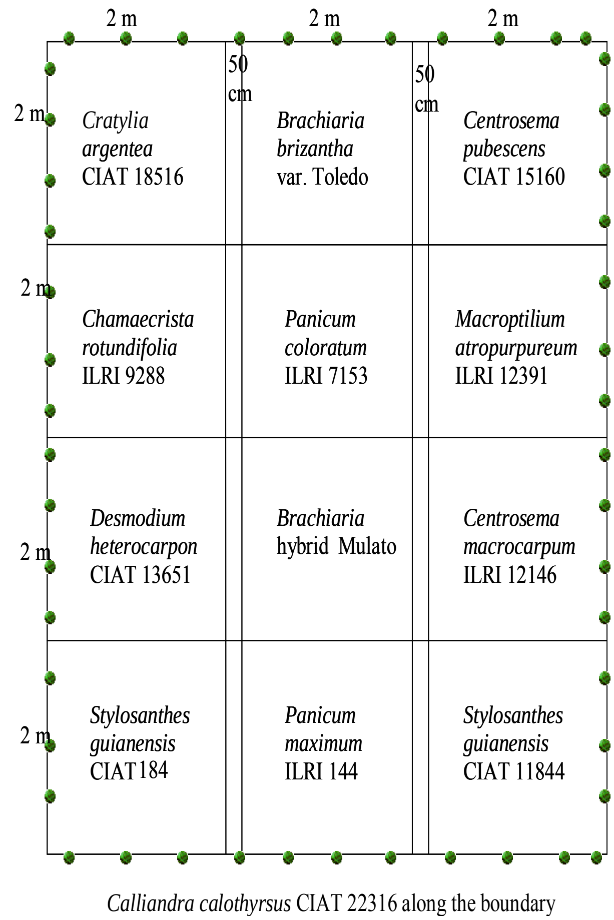

Figure 64. Experimental plot layout for farmer sub-groups in Tororo District.

Table 63. Description of forage types and spacing for planting in experimental plots.

Type	Botanical name	Accession number/ variety	Local name	Spacing
G	*Brachiaria* hybrid	Mulato 1	Mulato	Lines 40 cm
G	*Brachiaria brizantha*	Var. Toledo	Toledo	Lines 40 cm
G	*Panicum coloratum*	ILRI 7153	Makarakara	Lines 40 cm
G	*Panicum maximum*	ILRI 144	Odunyo	Lines 40 cm
HL	*Centrosema macrocarpum*	ILRI 12146	Centro 2	40 x 40 cm
HL	*Centrosema pubescens*	CIAT 15160	Centro 1	40 x 40 cm
HL	*Chamaecrista rotundifolia*	ILRI 9288	Chama	30 x 30 cm
HL	*Desmodium heterocarpon*	CIAT 13651	Desmodium	Lines 30 cm
HL	*Macroptilium atropurpureum*	ILRI 12391	Siratro	40 x 40 cm
HL	*Stylosanthes guianensis*	CIAT 184	Stylo 1	Lines 30 cm
HL	*Stylosanthes guianensis*	CIAT 11844	Stylo 2	Lines 30 cm
TL	*Calliandra calothyrsus*	CIAT 22316	Calliandra	1 m
TL	*Cratylia argentea*	CIAT 18516	Cratylia	1 x 1 m

G = grass, HL = herbaceous legume, TL = tree legume fodder tree calliandra surrounding each total plot (Figure 64; Table 63).

All forages were planted directly in the soil through using seeds which were scarified when appropriate. The location of the plots would be near roads, so that it would generate interest of passers-by.

Second cycle, June – Aug. 2004

Farmers suggested many parameters to be measured from the plots, and the methods of monitoring were discussed (Table 64). In addition, ease of management, wood production, and milk production were mentioned to be measured, but it was decided that assessment of those parameters would be more appropriate during a following season, since they would require a longer period after planting.

Group discussions were held in June 2004 on the first season's evaluation. Things that reportedly went well, in terms of forages performance and experimental process, were timely seed availability and planting, and growth of some species. The facilitators had been visiting the groups regularly.

The following factors were associated with problems encountered:

- Identification of germinating and emerging forages were difficult; farmers did not know what kind of plants to look for. This delayed weeding. On the other hand, some farmers recognised the patterns of lines or matrices in which the forages germinated, and had started weeding.
- There was some damage by stray animals feeding on the young plants.
- Some farmers found the plots too small; they would rather start feeding large quantities from the plots at once.
- Cratylia was attacked by unidentified pests.
- Several species germinated 4-5 days after planting, but the panicums and siratro did not germinate well, even after weeks.
- Although farmers had a good impression about germination variability, no quantitative records were taken. Equally, the other planned

Table 64. Agreed parameters to be measured and their methods for forages in the experimental plots.

Parameter	Method and time
Germination	Visual estimated percentage of emergence, one month after planting.
Growth and vigour	Visual observation and ranking of species and varieties, 2 months after planting.
Pest and disease resistance	Visual observation and description of symptoms and insects, 3 months after planting.
Plant height	Tallest plant per species or variety in cm, 3 months after planting.
Fodder biomass production	Harvesting demarcated sections of 1 x 1 m per species in each plot, 3 months after planting. Fresh fodder is weighed using simple scales.
Maturity	Time after planting of flowering and seed setting.
Drought resistance	Visual observation and ranking of species and varieties, one month after rains stopped.
Palatability	Separate for cattle and goats. Small heaps of different forages are placed on the ground. One animal at a time is allowed to nibble at the heaps. A few farmers observe the behaviour of the animal. The preference of forage is ranked for each individual animal. The test is repeated with fresh materials for 5 cattle and 5 goats. A relaxed environment is provided for the animals during observation.
Seed production	Seeds are harvested from plots whenever they are mature. They are stored in paper bags and weighed by facilitating staff.
Best general performance	Group discussion, ranking of species and recording reasons.

monitoring of parameters described in Table 64 proved ambitious; most was not done. The groups needed to prioritise on what o measure, also considering that they already had monitoring and evaluation plans associated with other activities. Forage experimentation needed to be integrated in these plans.
- One group experienced organisational problems, there was a dispute about leadership. This might have affected recording exercises.

Planning. The following actions were planned:

- Address the problem of stray animals damaging the experimental plots. Local leaders would be invited to a meeting. There would be an opportunity to agree on a by-law for restricting movements of stray animals in the location and enforcement of the by-law.

- Reduce number of parameters measured by farmers to the ones of greatest interest by the community: palatability, pests and diseases, and drought resistance. Fodder biomass production remained of great interest to scientists and it was agreed that facilitators would take data from the plots. A portion of 1 x 1 m of each variety or species in each plot was reserved for these measurements. The plots were maintained for another rainy season and the subsequent dry season. The facilitators would also measure seed production from the plots.

Third cycle, July – September 2004

Palatability tests had been conducted with local and dairy cattle and with local goats. Although repetitions of the tests with individual animals had been recommended and explained, in order to increase reliability of results, farmers observed all animals together during the same test. There was consensus about the ranking of palatability of the forages. Tests for grasses and legumes were done separately. For the local cattle, Mulato ranked highest (in terms of animal's first choise and quantity consumed), followed in rank by Toledo, *Panicum maximum*, and *P. coloratum*. Among the legumes, they only ate stylo. The dairy cattle ate all grasses and legumes without any noticeable preference.

Palatability of grasses by goats was observed as follows with decreasing ranks: *P. maximum*, Toledo, Mulato, *P. coloratum.* Palatability of legumes by goats was ranked as follows with decreasing ranks: *Desmodium heterocarpon*, *Centrosema pubescens*, *C. macrocarpum*, Chamaecrista, siratro, stylo 184, Cratylia and stylo 11844.

Vigour of forages was discussed in a meeting. In both groups, Mulato, Toledo, and stylo 184 were among the top three forages in terms of yield (Table 65). No disease or pest was recorded, except for mole rats infestation which affected the whole plot.

There was a discussion about the functioning of the sub-groups. Not all members contributed labour equally for weeding and data collection, which was the cause of some dissatisfaction. It was decided that members were free to leave the group if they didn't feel happy there, or join another group. Active sub-group members would benefit from harvested forage and seeds. It was acknowledged that not every sub-member had an interest in forage, which cleared the confusion.

Planning. Several farmers reported that the plots were too small to harvest substantial amounts of feed. Thus plans were made to expand the forages to other areas on the farms. Recommendations were made on which species would be suitable for intercropping, barriers, cut and carry from fodder banks, or grazing, and how they needed to be managed.

Fourth cycle, September 2004 – March 2005

During the rainy season, biomass was harvested from the species and varieties in every plot. Results are summarised in Table 65.

Meetings were held in December 2004 and Feb 2005. Rains had been bad in the past season, to the extent that it had not been possible to expand and plant forages in other places. One farmer

Table 65. Fresh biomass production tonnes/ha of forages planted in Tororo, 2 harvests.

Species or hybrid	Accession	Sept. 04	June 05	Total
Brachiaria hybrid	Mulato 1	36.6	19.6	56.2
Brachiaria brizantha	Var. Toledo	32.2	23.1	55.4
Panicum coloratum	ILRI 7153	6.0	5.3	11.3
Panicum maximum	ILRI 144	9.2	1.3	10.5
Centrosema macrocarpum	ILRI 12146	6.0	4.0	10.0
Centrosema pubescens	CIAT 15160	14.9	8.0	22.9
Chamaecrista rotundifolia	ILRI 9288	20.2	8.4	28.6
Desmodium heterocarpon	CIAT 13651	7.6	6.1	13.7
Macroptilium atropurpureum	ILRI 12391	9.7	2.0	11.7
Stylosanthes guianensis	CIAT 184	42.9	16.0	58.9
Stylosanthes guianensis	CIAT 11844	22.1	10.9	33.0
		$P < 0.001$	$P < 0.001$	

had experimented with intercropping of centro in an existing napier fodder bank. The dry season reminded everyone of the need of having forages which could tolerate drought. Severe forage shortage existed at Umoja group. A few forages from the experimental plots remained green.

There had been no systematic evaluation for drought tolerance. In March, a tour was conducted among all plots and pictures were taken of all sub-plots. After comparing the digital photos, drought resistance of the species and varieties was ranked. The following ranks were made:

1. Stylo (both accessions)
2. Mulato
3. Toledo
4. Chamaecrista
5. *Centrosema pubescens*
6. *Desmodium heterocarpon*
7. all others

When the first rains hit the ground at the end of March, farmers were surprised to see that the forages which were at a bad stage of drying up all regenerated fully and within a very short time.

With the help of facilitators, shorter and easier names were given to the forage species and varieties (Table 63). This greatly enhanced communication and comparison of forage performances.

Monitoring of the forage experiment had been integrated in the general PME plans. One important indicator for learning from the experiment was added to the plan: the number of visitors, their purpose, and their opinion about the forages shown.

Harvesting of seeds remained a problem. An opportunity arose during an interaction with scientists from a nearby institute, the Livestock Health Research Institute. The Director was interested in forage experimentation and some collaboration took off. One of the benefits for the groups in Tororo was that the institute would also try to produce seeds of the most preferred forages.

Planning. Farmers were expecting a better rainy season. Fourteen farmers were planning to expand 2 or 3 of the following species integrated on their farms: stylo 184, Toledo, Mulato, Calliandra, Chamaecrista, Desmodium, and Panicum. They were planning on using vegetative materials collected from the experimental plots to expand the new forage options. Some Desmodium seeds were still available from the original planting, and would be given to the groups. Seedlings of Calliandra would be bought from nurseries in Tororo.

In order to facilitate a second data collection on fodder biomass production, all plots would be slashed to remove dead and dry material. The weighing of this dry material was not considered relevant, because animals had been browsing on the plots during the drought, and weights of the materials left was considered to bear little relation to real biomass production during the dry season.

Fifth cycle, April – October 2005

Mole rats continued to cause severe damage in some plots. Two factors probably play a role: (1) the plants are perennial and provide food for the mole rats when the other crops have been cleared from the land after harvest, and (2) the plants have been selected for their palatability to livestock, with a high probability that they are also palatable to mole rats.

The artificial insemination (AI) service in Tororo District had collapsed more than 2 years ago. Farmers depend on an unreliable and expensive AI service from Mbale, some 50 km away. As a result, cows have remained without a calf and without milk for up to 2 years. Whether cows are being milked or not, they need to be sprayed weekly with acaricides to keep them free of ticks. Farmers had relaxed on the treatments due to money constraints and lack of income from sales of milk. As a result, most cows were affected by tick born diseases, especially East Coast Fever, and only one cow in the whole group has survived.

It was observed that the expansion of forages into the farms at the Katamata group had stagnated. We hypothesised that the problems with the lack of AI and the mortality of cows were the cause of the reduced enthusiasm for improved forages, and we decided to test this with the farmer group. The question was raised why forages were no longer adopted in the farms and a candid discussion revealed the reasons in order of importance:

1. Farmers had other priorities, commitments, or thought the planting of forages was too much work.
2. Although it had been iterated at the beginning that there would be no dispersal of animals implicated with the forage evaluation project, expectations on the contrary were hard to die out. When it became clear that no animal dispersal was taking place, enthusiasm for forages dropped.
3. Loss of animals.

At the Umoja group most cattle had survived, and expansion of forage in is progress. The group still struggles with lack of leadership, which is seriously affecting the social capital. It is difficult to bring members of the group together for a meeting.

One farmer has been able to produce and collect a small amount (about 30 g) of Chamaecrista seeds. Chamaecrista produces large amounts of seeds under Tororo farm conditions, but most is left on the ground. Compared to Stylo, it is relatively easy to harvest the dry pods of Chamaecrista and thresh the seeds.

In the second season of 2005, A2N has started to scale out forages to 11 other groups. Members were selected who had dairy cows or improved goats. Mulato, Toledo, Chamaecrista and Lablab are now grown in fodder banks on-farm. Lack of seeds has prevented the scaling out of Stylo. A new initiative is started to bring research partners in Uganda (NARO, CGIAR, CIRAD, Makarere University), development partners (MAAIF, NGOs, NAADS, Local Governments), farmer organisations, and private seed companies together to analyse the national forage seed system and explore new partnerships which will enhance the availability of priority forage species, varieties and hybrids.

Lessons learned

After two years of evaluation with farmer participation of new forage options the following lessons were learned:

1. The value of the new forages for farmers tested was related to availability of green forage during the dry season and not on total forage yield. For this reason, the Brachiaria cultivars tested in this project are a welcome addition to the napier grass that some farmers are already using. Napier normally stays green during the dry season, but stops growing and becomes stemmy. Brachiaria has the potential to continue growing during at least part of the dry season, and remains a high leaf:stem ratio. The dry matter production of the two Brachiarias obtained from this experiment was 55 – 56 tonnes fresh weight over a year, equivalent to 13.9 – 14.1 tonnes dry matter (DM) per year. This compares favourably to expected yield of unfertilised napier which is normally in the range of 2 – 10 t DM/yr under similar rainfall conditions (Mwangi et al., 1998; SoFT 2005).

2. Replacement of napier by Brachiaria cv. Mulato or Toledo is seen as a way to deal with a mycoplasma causing the 'stunt' syndrome affecting napier grass in East Africa. The diseases is transmitted by plant hoppers. Ninety-nine percent of all farmers' napier plots across the country are affected, and the disease causes an average of 60 % decline in forage yield (Kabirizi, pers. com).

3. Although grasses normally out-yield herbaceous legumes under similar conditions, biomass production of *Stylosanthes guianensis* CIAT 184 was comparable to the highest yielding grasses in this experiment, (i.e. the two Brachiarias). The advantages of Stylosanthes over all other grasses is that it has a higher crude protein content which remains stable during the dry season. Stylosanthes can be used as a protein supplement to balance protein deficient forages such as maize stover and grasses during the dry season.

4. If seeds were available of Stylo, dairy farmers in Tororo District would plant it. Stylo does produce seeds, but they mature unevenly, scatter on the ground, and are too small to pick up by hand. Technologies exist for seed collection from the ground, involving either plastic sheets or progressive sieving of particles, soil and seed. We recommend that some of these technologies are tested with farmers, and that links with commercial seed companies are made to add monetary incentives to seed production and processing. Adoption of Chamaecrista, Centrosema, Mulato and Toledo would also be enhanced through availability of seeds.

5. An alternative way of spreading Mulato and Toledo among farmers is through root splits. One tuft of grass can produce up to 50 splits. In Indonesia this method has been instrumental in spreading improved *Brachiaria spp.* to thousands of farmers. The Forages for Smallholders Project initially subsidised the production of root splits by farmers, but soon after the production and sale started running without subsidies (CIAT Forage AR2004). Projects might want to continue to facilitate through providing information to producers about where the demands for planting materials are, so that more smallholder farmers are able to increase their incomes through the business of forage planting material production.

6. Adoption of forages in East Africa is highly correlated to intensification processes and market success of livestock enterprises. In the case of smallholder dairy systems, many factors contribute to its market success, such as adequate AI service, veterinary service, input and output systems, and dairy management expertise. The Katamata experience shows that when one factor breaks down, in this case the AI service, the whole system breaks down. The lesson learned is that when improved forages are introduced into a smallholder dairy system, the whole dairy innovation system should be analysed, and an action plan made to strengthen its weak linkages.

If the introduction of improved forages is associated with a livestock dispersal program, the ones likely to adopt forages are the farmers who have received an animal. The dispersal program enhances adoption of forages among those who receive animal, but discourages others who don't benefit from the dispersal. This limits the scale of adoption of forages. In locations without a dispersal program, but where other factors stimulate intensification of livestock production, the scope for scaling out improved forages is less limited. Many examples of this have been described by the Forages for Smallholders Project (CIAT Forage AR2004).

4.2 Partnerships in Asia to undertake evaluation and diffusion of new forage alternatives

Highlights

- The Forages and Livestock Systems Project (FLSP) in Laos, funded by the Australian Agency for International Development (AusAID) was completed in June 2005. The project achieved its targets of developing and disseminating forage technologies to smallholder farmers, resulting in significant household impacts. More than 1300 farmers in 106 villages (covering 5 districts in 2 provinces) had adopted planted forages for livestock feeding. A total of 900 farmers were benefiting from significant impacts such as labor saving, improved animal production and increased household income. More than 150 farmers report they have been able to reduce or stop shifting cultivation as a direct result of intensifying their livestock production. More than 200 farmers report that intensifying their livestock production systems has allowed their children to attend school. The technologies deployed by this project and the approaches of working with farmers to achieve adoption has attracted considerable interest by large development projects, NGOs and the donor community in Laos, and have been incorporated into several project as a major component.

- The Southeast Asian Regional "Livelihood and Livestock Systems Project (LLSP)", funded by the Asian Development Bank, was completed in December 2005. More than 8,000 farmers have adopted forage technologies developed and introduced through this project and its predecessor, the Forages for Smallholders Project (FSP). Impact assessment studies were carried out late in 2005 to capture household impacts of forages on major production systems. These will summarized in a working document and published as individual papers in the scientific literature in 2006.

- A study of seed production of Mulato and Mulato II confirmed the higher seed yield of Mulato II, particularly at low altitudes where seed set of Mulato was particularly low. While seed set of Mulato increased with altitudes to about 20%, this was still much lower than seed set of Mulato II and Ruzi.

4.2.1 Forages and Livestock Systems Project (FLSP), Lao PDR

Contributors: Peter Horne (CIAT), Viengsavanh Phimphachanhvongsod (NAFRI), Viengxay Photakoun (NAFES) and John Connell (CIAT)

Rationale

In 2000, the Australian Agency for International Development (AusAID) approved a 5-year project, the Forage and Livestock Systems Project (FLSP), to capitalize on the promise of earlier forage research work conducted by CIAT and CSIRO in the Forages for Smallholders Project (FSP). The FSP identified a small suite of robust, broadly adapted forage varieties that had the potential to provide significant benefits in SE Asian smallholder livestock systems.

AusAID funded the FLSP so that CIAT could demonstrate this potential by working with farmers to integrate improved forage and livestock management strategies into smallholder upland farming systems in northern Lao PDR. In the process, CIAT was able to further develop participatory approaches to action research and learn more about the opportunities and constraints of these forage varieties in smallholder farming systems. The FLSP was completed in June 2005.

Livestock are found on most farms in the Lao PDR with 89% of all farm households raising one or more livestock types. The importance of livestock to households is very high typically providing both a livelihood safety net and the majority of cash income. Traditional livestock production systems in the north of the Lao PDR are, however, characteristically extensive, low-input, low-output and opportunistic. These systems neither assist farmers to move out of shifting cultivation nor help them overcome poverty. In most cases, the major constraint to livestock keepers becoming more market-oriented is livestock disease, but there is little farmers can do about this while their livestock systems are extensive. Developing feed resources near the village is an ideal way to help farmers raise their animals closer to home, enabling better management and health. The FLSP was developed to use forage varieties and other livestock technologies to help farmers make the jump from being livestock keepers to being livestock producers.

Results and Impacts

By the end of the fourth wet season (Nov 2004), the project was supporting 26 field staff in 5 districts working with >1,300 farmers in 106 villages. Most farmers were planting grasses for cattle and buffalo and about 950 farmers were planting *Stylosanthes guianensis* to feed their pigs. By this time, 900 farmers (65% of the farmers working with the project) were benefiting from significant impacts and 790 said they were achieving at least one significant livelihood impact. More than 150 farmers report they have been able to reduce or stop shifting cultivation as a direct result of intensifying their livestock production. More than 200 farmers report that intensifying their livestock production systems has allowed their children to attend school. More than 670 farmers said that labor savings have allowed them to start other livelihood activities. These impacts have been wealth, gender and ethnicity neutral.

For farmers in northern Lao PDR to develop these kinds of impacts required significant systems change. To bring about these kinds of changes is not a trivial matter and required the development of extension approaches that would help farmers make these systems changes of their own accord, driven by the demonstrated potential for significant livelihood impacts. A participatory extension process was developed and used as the vehicle for providing intensive, on-the-job, mentored learning. In addition, the project provided 68 more-formal training events, totaling >900 individual training experiences. The main focus of this training was on improving the technical, extension and planning skills of provincial and district staff.

In the 2005 wet season (starting just before the end of the project in June 2005), the project substantially increased the spread and significance of these impacts with the number of villages increasing to at least 119 and the total number of farmers using and benefiting from forages reaching more that 2,000 households. The work continues with funding from local government, albeit at a much lower level of support.

Lessons learned

Some important lessons about forages and livestock systems development have been learnt from this project:

1. Market-oriented livestock production systems are now a proven option for poverty alleviation and reduction of shifting cultivation in the uplands of the Lao PDR

2. Forages as ‘entry points’, providing quick solutions to simple immediate problems, are a powerful tool in extension, especially in complex upland systems. They build confidence among farmers and encourage them into further innovation.
3. The impact-yielding forage systems developed by farmers usually result not from resolving immediate problems but from changing their livestock systems to take advantage of new opportunities.
4. Few of the impact-yielding forage systems can be ‘photocopied’ from one place to another. New farmers will always need to adapt the systems to their own realities.
5. A managed feed resource is the key mechanism enabling livestock systems change in the uplands. Relatively small areas of forages managed in this way can give relatively large impacts.
6. There is little likelihood of “magic bullet” solutions to the problems of animal disease in smallholder livestock systems in the uplands of the Lao PDR. Integrated solutions involving better feeding and management combined with strategic use of veterinary medicines are likely to be far more effective, achievable and sustainable.
7. There are simple ways of helping district staff develop a vision for how research and extension processes can work and then acquire the technical skills and extension tools that allow them to put this vision into practice within the context of smallholder livestock systems.

Through the demonstration of the ability of forages to delivery significant impacts to smallholder livestock keepers, the project has had a significant influence on the goals and plans of the Lao government. The FLSP demonstrated that market oriented livestock production can be a practical alternative to shifting cultivation and provide a means for farmers to work their way out of poverty. One result is that this work will continue beyond the completion of the FLSP as the Lao government, with support from the Asian Development Bank (ADB), is planning to invest US$10 million in a new project to “improve the income and livelihood of about 20,000 farming families by introducing animal health and productivity enhancement technologies, improving marketing opportunities and the regulatory environment, and encouraging the development of private livestock service providers”. CIAT and the International Livestock Research Institute (ILRI) managed the design of this project for the ADB. It will build strongly on the experiences, technologies, methodologies and lessons learned in the FLSP. Other rural development projects and NGO’s have also adopted the technologies developed in the FLSP as part of their activities.

Recognizing that the field staff of FLSP have important skills for such scaling-out, the ADB has also to fund a project with CIAT to start in early 2006 which aims to approximately double the number of skilled field teams, using the FLSP field staff as mentors. The context of this project will be a seamless continuation of the development process of the FLSP in the same districts. In essence it will provide a bridge between the FLSP and the subsequent loan project to ensure that the momentum is not lost. It is a measure of the abilities of the field staff that they can confidently take on such a task.

As the FLSP moved from relatively simple technical issues to dealing with impacts from forages, new issues have arisen that have significant consequences for CIAT’s future forages research in Lao PDR and the wider region:

1. **systems-level technical issues**, including (i) nutrient decline in regularly cut forage plots and (ii) the need for farmers to reinvest profits in basic livestock inputs
2. **systems level technical opportunities**, including (i) new feed resources for small animals, (ii) ‘smart’ feeding using a range of new and traditional feed resources, (iii) better utilization of forage surpluses in the wet season for large animals, (iv) making a transition from cut & carry feeding to grazed forage plots to both reduce labor inputs and

improve nutrient cycling and (v) more rapid off-take of animals to optimize returns

3. **encouraging the development of livestock enterprises** to help livestock producers become better livestock producers

4.2.2 The Livelihood and Livestock Systems Project (LLSP) in Southeast Asia

Contributors: Werner Stür, Francisco Gabunada, Phonepaseuth Phengsavanh and John Connell (CIAT)

Rationale

The Asian Development Bank (ADB) funded 'Livelihood and Livestock Systems Project' (LLSP) started in January 2003 for a period of three years. The LLSP is a collaborative research for development project bringing together livestock researchers and extension workers in seven countries in Southeast Asia. The purpose of the project is to improve (i) sustainable livelihoods of smallholder farmers in the uplands through intensification of crop-livestock systems, using farmer participatory approaches to improve and deliver forage and feed technologies, and (ii) delivery mechanisms for the dissemination of these technologies.

The LLSP follows the Forages for Smallholders Project (FSP) which developed forage technologies with smallholder farmers and disseminated these to other farmers in target districts in partner countries in Southeast Asia. The activities of the new project are broader as it works with farmers to maximize the benefit from having planted forages through the development of improved livestock production systems (with emphasis on feeding), analysis of production and marketing constraints and opportunities, and the efficient dissemination of new technologies to new areas and farmers.

In each partner country, the project collaborates with a national research and/or development agency. Within countries, one or more provinces and districts are involved in the project with site coordinators (provincial or district) and several extension workers involved at project sites.

Results and Impacts

By September 2005, more than 7,000 smallholder farmers had adopted forage technologies and were growing forages on their farms. The mean area of planted with forages per farm was 2,500 m^2 with individual forage areas ranging from 300 m^2 to 7,000m^2 in some areas. This was utilized almost exclusively as cut-and-carry feed and fed to rabbits, goats, pigs, cattle and buffaloes. Farmers with small areas were using planted forages as a supplementary feed to communally available feed resources while those with larger areas tend to use planted forages as the main feed sources. The main forage type grown by farmers is grass. Legumes were grown mainly for special purposes such as *Stylosanthes guianensis* CIAT 184 as supplementary feed for pigs (either fresh or dried and chopped, and fed as leaf meal) or as cover crop in tree plantations. The main purpose of growing planted forages by smallholder farmers can be summarized as:

- Improved cow-calf systems
- Fattening (finishing for sale) of cattle
- Forage for fish production
- Legumes for supplementation of village pigs
- Sale of fresh forage for feed
- Use of forages for convenience only (ie. saving labor)

The main benefits of growing planted forages initially were time saving as feed was conveniently located near the house or animal pen. This meant that farmers could keep their animals closer to their house and many farmers then started to expand their forage area. They started to experience productivity increases or increased their herd size to take advantage of the additional feed available. At many sites, farmers are now fattening cattle and buffalo for 2-3 months before selling the animals. This added considerable value to the animals and farmers became more aware of market opportunities. This process of increased market-orientation of livestock production is continuing at project sites.

Associated benefits for early adopters accrued from sale of vegetative planting material and seed. All farmers have more manure available and this is another source of income for farmers at some sites where manure is highly valued. Impact studies of planted forages on the main production systems were conducted late in 2005 and the results will be summarized in a working document and published as journal articles in 2006. The results of the LLSP attracted interest by local and national governments, and donor-funded projects. Many of the technologies developed are being taken up by development projects.

4.2.3 Seed Production of new *Brachiaria* hybrids on the Bolovens Plateau, Lao PDR

Contributors: Madeleen Husselman (MSc. student, Wageningen Agricultural University), and Peter Horne (CIAT)

Rationale

Seed production of the *Brachiaria* hybrids (Mulato and Mulato II) in Thailand showed that seed yield of Mulato is lower than Mulato II, and this was attributed largely to low seed set in Mulato. As seed set is controlled partially by environmental factors (mainly temperature and moisture) an experiment was designed which compared seed production of these Brachiaria hybrids (and a control species – B. ruziziensis) at varying altitudes on the Bolovens Plateau, Lao PDR.

Methodology

The experiment was conducted at three sites, comprising different agro-climatic zones on the Bolovens Plateau (latitude: 15° N). At each site three varieties (Mulato, Mulato II and Ruzi) were planted in small plots of 3 m x 3 m, in a randomized block design with four replicates. Plots were 1 m apart and 3 m from outside borders. Plant spacing was 50 by 50 cm and only the inside nine plants were used for measurements. For practical reasons all the plants were sown in seed beds at Nong Hine on May 20th 2004 and transplanted to the other sites on June 23rd. During the first month of their establishment the plots were manually kept weed free. On the 17th August the plants were cut approximately 5 cm above the ground. The plots were fertilized with NPK (15-15-15) at a rate of 100kg N/ha, in split applications. Two weeks after transplanting they received a third of this amount and after the closing cut the remaining two thirds.

Seed was harvested by bundling seed heads and covering them with nylon bags, when the first seeds in a plot started to mature. The bags were emptied once a week until all the seed had been shed. The seed was dried in a shed for at least three days, then half a day in the sun. The seed was cleaned using a fan to separate empty from full spikelets. The cleaned seed was weighed and recorded per plot. Seed of each species at each site was bagged and analyzed for seed quality factors in Khon Kaen, Thailand.

Results and Discussion

Seed yield (pure live seed) of *B. ruziziensis* was higher than that of the two Mulato hybrids with higher seed yields at the two higher altitudes (Table 66). Mulato II produced its highest seed yield at the lowest altitude and its lowest seed yield at the highest altitude. This was contrary to expectations. Unfortunately, variability was high and the seed yield differences between the two Mulato hybrids were statistically not different. Nevertheless, seed yield of Mulato II was double

Table 66. Pure Live Seed Yield (kg/ha) of three Brachiaria varieties at three sites

Species/ Site	Nong Hine (1280 m asl)	Ban Itou (940 m asl)	Ban Houy Hee (200 m asl)
Mulato	191 bc	127 c	143 c
Mulato II	158 c	235 abc	307 abc
Ruzi	376 ab	420 a	209 bc

Values followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Table 67. Seed set (%) of Mulato, Mulato II and Ruzi at three sites

Species/ Site	Nong Hine (1280 m asl)	Ban Itou (940 m asl)	Ban Houy Hee (200 m asl)
Mulato	22 e	23 e	6 f
Mulato II	54 c	39 d	68 ab
Ruzi	76 a	60 bc	52 c

Values followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

the seed yield of Mulato at the lowest altitude and the differences were smaller at the highest altitude. Seed set of Mulato was lower than that of the other two varieties at all altitudes, and extremely low at the lowest altitude (Table 67). The 6% seed set of Mulato at low altitude is similar to results reported in Thailand. It also shows that seed set of Mulato improves with altitude but the maximum percentage seed set in this experiment was still only 23%. Seed set of Mulato II and Ruzi were much higher. Seed set of Ruzi improved with altitude while seed set of Mulato II was highest at the lowest altitude.

An important aspect of seed quality for farmers is germination percentage (both maximum germination percentage and speed to reach that maximum). Germination percentages of both Mulato hybrids at all sites were very low and significantly lower than for Ruzi (Table 68) due to dormancy of this variety. Seed viability of both hybrids was, however, good at all sites except for Mulato at the medium altitude site. This experiment confirmed the low seed set of Mulato. Seed set of this variety appears to improve with altitude and higher seed yields should be achievable at higher altitude locations. The experiment also confirmed the higher seed set of Mulato II at low altitude and its potential for higher seed yields than Mulato at low altitude sites. A significant issue for future research will be whether this high percentage dormancy in the Mulato hybrids is easily overcome simply with storage time or simple treatment.

Table 68. Germination (%)after 14 days and viability (%) of Mulato, Mulato II and Ruzi seed at three sites.

	Nong Hine (1280 m asl)	Ban Itou (940 m asl)	Ban Houy Hee (200 m asl)
Germination			
Mulato	10 c	13 c	12 c
Mulato II	10 c	11 c	40 b
Ruzi	90 a	90 a	94 a
Viability			
Mulato	81 b	69 c	80 b
Mulato II	90 a	81 b	92 a
Ruzi	90 a	90 a	94 a

Values followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

4.2.4 Strategy for future activities in Southeast Asia

Contributors: Peter Horne and Werner Stür (CIAT)

The FLSP and LLSP projects were in essence "Proof of Delivery" projects that built of earlier research by CIAT on forage technologies and participatory approaches ("Proof of Concept"). Both project proved to local and national governments and donor-funded projects that forages play a pivotal role in developing more market-oriented smallholder livestock production systems. They also showed that improved livestock production based on forage technologies can increase the income of poor households dramatically and help them to escape the poverty trap of having to spend more and more time in producing food for home consumption. The labor saving achieved by planting forages gives farmers the opportunity to work more effectively and quickly realize production gains. These "Proof of Concept" projects led to

(i) New ideas and opportunities for research that would never have arisen had we not delved into a Proof of Delivery mode. Examples are the feeding of forage legumes to village pigs, an innovation that emerged from farmer experimentation and has led to a new project funded by ACIAR which will commence in 2006. Another example is the feeding of forage grasses to fish which has enabled farmers to increase fish production and is highly profitable. Both of these uses of forages are now to researchers and we are pursuing ideas for projects in these areas.

(ii) Forage technologies are adapted to local conditions, delivering significant impacts to many farmers and being taken up by farmers' groups, projects and government and non-government organizations. An example of the uptake of research outcomes from the FLSP is the development of an ADB-funded investment project, the "Participatory Livestock Development" loan project in Laos. ADB asked CIAT and ILRI to design this project and this was completed in 2006. This would not have occurred from the usual research projects but required a "proof of delivery" project.

In 2006, we will build on the outcomes of the FLSP and LLSP, and pursue funding opportunities for research on feeding of legumes for pigs and feeding forages to fish. We will also develop new 'proof of delivery' projects to continue ways of achieving more market-oriented livestock smallholder production, and form alliances with development partners to scale out forage technologies in the region. Finally, we would like to pursue the development of a knowledge network on forages and livestock technologies to make innovations in livestock research available to the development sector.

4.3 Partnerships in LAC to undertake evaluation and diffusion of new forage alternatives

Highlights

- Species selected and criteria used by farmers in hillsides of Colombia to select grasses and legumes were similar to those used by farmers in hillsides of Central America.

- Showed that grain yields of cowpea in hillsides (marginal areas for coffee) were depedent on genotype and on altitude.

- *B. brizantha* cv. Toledo continues to show good adaptation in the llanos piedmont, where soils have high levels of moisture in the wet season.

- Results with *Brachiaria* hybrid cv. Mulato in Central America continue to show that its main benefit relative to other grasses is higher animal productivity per unit area.

- Improved forage species were introduced in 60 collaborative farms in Central America to monitor impact on beef and milk production.

4.3.1 On-farm evaluation of forage options in Norte del Valle del Cauca, Colombia

Contributors: C.V. Duran (Universidad Nacional de Palmira), Luz Mary Ocampo, Mario Carvajal (Secretaría de Agricultura del Valle), M. Valderrama (Instituto Técnico de Roldanillo, INTEP), farmers from the Grupo de Productores de la Ondina, J.I. Roa (IPRA), L.H. Franco and M. Peters

Rationale

The Norte del Valle of Colombia is an important livestock area. However forage options available to livestock holders are limited and hence restrict productivity of livestock operations. Through a participatory approach we aim to define and adapt forage technologies suitable to smallholder production systems to improve livelihoods of farmers.

Material and Methods

Forage technologies developed with farmers include germplasm options and forage conservation technologies. A participatory process is followed facilitating adaptation, innovation and adoption by farmers. The main collaborators in the process are the farmer group 'la Ondina', the Universidad Nacional de Palmira, the Instituto Técnico de Roldanillo (INTEP), the Secretaría de Agricultura y Pesca del Valle del Cauca. Initially, in 2004, the main beneficiaries of this work were a group of farmers (30) from the municipality of Roldanillo; currently the work has been expanded to 5 groups in 5 municipalities in the Norte del Valle del Cauca (Roldanillo, Bolívar, El Dovio, Versalles and Sevilla) reaching directly a total of 200 farmers. Altitudes in the 5 municipalities range from 1000 to 2000 m.a.s.l., representative of the variable environments in the region. From the onset, a participatory approach was employed, in order to understand farmers demands and livestock systems, with the aim to select and co-develop different forage alternatives suitable to the prevalent farming systems. In each of the 5 municipalities a participatory diagnosis was carried out to identify opportunities and constraints of livestock holders. The methodology employed used a group brainstorming approach, with farmers further stratifying and prioritizing opportunities and constraints through an individual votation process. Farmer cross visits and visits to on-station trials further supported the process through exposure to new technologies and sharing of experiences with technicians and farmers.

Eight experiments were established in five municipalities, representing different climatic (altitudes between 1000 and 2000 m) and edaphic niches. At each site 16 multipurpose forages were sown. These experiments were used for the participatory selection of forage technologies and lead to further on-farm testing. The innovation and adoption process is accompanied by training in pasture establishment and management as well as on the utilization of hay and silages. The training is supported by extension type publications.

Results and Discussion

Participatory cross visits. The cross visits were very effective to facilitate the interchange between farmers and farmers and technicians/ researchers and served also as an opportunity for farmers to familiarize themselves with forage technologies available (Photo 29). Farms with established pastures of *Brachiaria* hybrid

Photo 29. Participatory process of farmers at "Norte del Valle" and *Leucaena* with *Brachiaria* hybrid cv. Mulato

cv. Mulato in Zarzal and *Brachiaria brizantha* cv. Toledo in Pereira were visited as well as the Cenicafe farm ‘La Romelia’ in Chinchiná; these visits allowed to see many forage options under utilization by farmers as well as observing the persistence of some forage options as in Romelia associations of Leucaena and Arachis with *Brachiaria decumbens* and *Cynodon persist* for 23 years under hillside conditions. At the CIAT station in Quilichao multipurpose forage germplasm adapted to different climates and acid low fertility soils such as *Cratylia, Flemingia, Desmodium, Canavalia, Lablab* and *Vigna* (Caupi) and animal nutrition trials including work on hay and silages were visited by farmers.

Experiments. While grasses showed a wide range of adaptation establishing well across sites, performance of herbaceous legumes was more site specific. As with grasses, the shrub species were well adapted across sites. A participatory evaluation was carried out in each of the sites to assess farmer preferences and selection criteria and to observe adaptation of the different forage options to farm conditions. In Table 69 farmer preferences for the different forage options are presented. The most important criteria for the farmer selection of forages were:

- Palatability
- Color
- Forage on offer
- Dry season performance
- Tolerance to ants
- Cover (aggressiveness)
- Rooting capacity
- Persistence in pasture
- Adaptation to soil fertility gradients

The criteria selected are very similar to results obtained in evaluations with farmers in Central America, further confirming the validity of the participatory approach taken.

As a result farmers are now sowing larger areas (0.5 to 7.5 ha per farm) of selected materials e.g. *Brachiaria* hybrids cv. Mulato, Mulato II, *Brachiaria brizantha* cv. Toledo, *Cratylia argentea*, *Leucaena leucocephala*, *Vigna unguiculata* and *Lablab purpureus*, to be utilized for further on-farm testing, adaptation and expansion. In total it is estimated that by February 2006, 40 ha of improved forages will be sown in the Norte del Valle.

Table 69. Ranking of different forage technologies by farmers in the ‘Norte del Valle’, Colombia.

Ranking	Grasses	Shrub legumes	Herbaceous legumes
1	*Brachiaria brizantha* cv. Toledo	*Cratylia argentea* cv. Veranera	*Canavalia brasiliensis*
2	*B.* hybrid Mulato	*Leucaena diversifolia*	*Centrosema molle*
3	*B.* hybrid Mulato II	*Leucaena leucocephala*	*Arachis pintoi*
4	*Panicum maximum* Guinea	*Flemingia macrophylla*	*Desmodium heterocarpon cv. Maquenque*
5	*Brachiaria dictyoneura*	*Desmodium velutinum*	*Lablab purpureus*

4.3.2 Exploring opportunities for alternative forage crops in Hillsides: Spatial genotype x environment and economic analysis for Cowpeas in Hillsides of Cauca

Contributors: K. Atzmanstorfer, T. Blaschke (University of Salzburg), D. White, T. Oberthür, G. Escobar, L.H. Franco, M. Peters and G. Ramirez (CIAT)

Rationale

One of the most widely grown legumes in tropical and subtropical regions, is Cowpea, *Vigna unguiculata* (L.) Walp. This annual legume is of major importance to the livelihoods of millions of poor people, especially in West Africa and China. It is grown usually as a companion or relay crop with major cereals. Development of new disease and insect resistant varieties with shorter harvest cycles have contributed to its increased cultivation over the last decade. Apart from providing inexpensive and nutritious food (grain, pods), this annual multi-purpose legume, gives excellent forage (grain concentrate, leaves and haulm) and could be used for making hay and silage. Farmers can obtain additional agronomic and environmental benefits to their farmlands using cowpea as green manure. The plant improves soil quality by fixing soil nitrogen; it is drought and heat resistant, and prevents erosion given that it is a fast growing ground cover plant.

Forage seed and leaf meals obtained from cowpea may be a promising economic alternative for many smallholders in marginal coffee growing areas in the tropical hillsides at an altitude. Coffee cultivated in suboptimal altitudes normally cannot compete with coffee produced in higher areas in terms of quality, and therefore farmers rely on the highly volatile markets for volume coffees. Currently cowpea is not widely grown in most of Colombia or other tropical hillsides. Very scarce evidence exists of its use as a forage plant in Latin America and the Caribbean and less regarding its use as an input to commercial feed concentrates and its production potential. Moreover its performance in the hillside eco-region is not yet fully understood.

The objectives of this research effort are: to (a) examine effect of environmental conditions on cowpea production, (b) identify optimal growing areas and (c) estimate financial viability of cowpea as component of animal feed concentrates. We used a hillside site in southern Colombia as case study area, in which we carry out the following research sequence:

a) As a first step, a genotype by environment performance analysis is conducted, relating performance of cowpea lines to specific environmental characteristics such as topography, soil and climate. The data is generated in semi commercial field trials (2100 m^2 each – areas big enough to get economically valid and farm size agronomic data) located in contrasted landscape positions in the Cauca Province.

b) Secondly, GIS is used for (i) describing the environments in which the trials are conducted as well as for (ii) identifying areas in marginal coffee growing regions of Colombia with similar environmental conditions to those of the trial sites. This data is required to conduct an extrapolation as to how much area and therefore how much yield the animal feed industry can potentially count on. GIS-based prediction models for environmental niche identification such as CaNaSTA (Crop Niche Selection Tool for Tropical Agriculture) or Homologue are used for these analyses.

c) A complementary financial analysis will be carried out to determine yield-price-profit thresholds using the agronomic trial data. Results will enable farmers and farmers and the feed concentrate industry to determine the financial feasibility for commercial use of cowpea as an animal feed. For large-scale production, cowpea needs to be competitive with alternative feeds such as imported soybeans. Nevertheless, the production of cowpea may be attractive to farmers during the fallow period of coffee plots. Both financial benefits of cowpea and its effect on coffee systems will be estimated.

Materials and Methods

Three cowpea accessions (DICTA 9611, IITA 5234 and IITA 1088-4) were established at five different sites in the department of Cauca: Mondomo, Suarez, but complete data was only available for three sites - El Tablón, CIAN and Mr. Manuel Trujillo (Photo 30). The plot size was approximately 500 m^2 in average, simulating commercial cultivations and the planting distances were 10 cm between plants and 0.5 m between rows. The plots were fertilized with 50 kg/ha of P_2O_5, 50 kg of K_2O, 20 kg of S and 20 kg of Mg. Plant emergence, plant vigor, cover, and incident of pest and diseases and dry matter yields were measured to 8 weeks after planting. The harvest of grain was carried out at 13 weeks.

A

B

Photo 30. Grain yield production of cowpea in two sites: (A) CIAN and (B) El Tablón.

Results and Discussion

Due to a severe attack of insects and problems with one collaborating farmer, complete data was only available for three sites. The highest grain yields were achieved on the relatively low altitude trial sites of El Tablón (1138m) and CIAN (1109 m) with a mean grain yield over all accessions of 1388 kg/ha and 599 kg/ha, respectively (Photo 30). The trial site of Mr. Manuel Trujillo (1558 m), characterized by higher altitude, more rainfall, lower temperature and less fertile soils, showed distinctly less grain yield (481 kg/ha).

The best performing accession in terms of grain yield in the three sites analyzed was IITA 5234 (1047 kg/ha) followed by DICTA 9611 (865 kg/ha[1]), and IITA 1088-4 (557 kg/ha) (Figure 65). The highest grain yield was achieved by DICTA 9611 (1735 kg/ha) followed by IITA 5234 (1453 kg/ha) in the 'El Tablón' site.

For financial analysis, production costs were examined for each trial site. Assuming that cowpea is sold at the soybean meal market price of 0.19-0.23 US$/kilo (USDA-soybean meal price forecast for 2005/06), calculations showed that the maximum revenue would be around 217 and 261 US$$ per ha cultivated cowpea for the best performing trial site, El Tablón. (Calculated for the mean grain yield over all accessions). A second round of field trials is being performed at different sites with different grain-type accessions in order to gain more information on genotype x environment interactions as well as on economical figures. Work will be completed by March 2006.

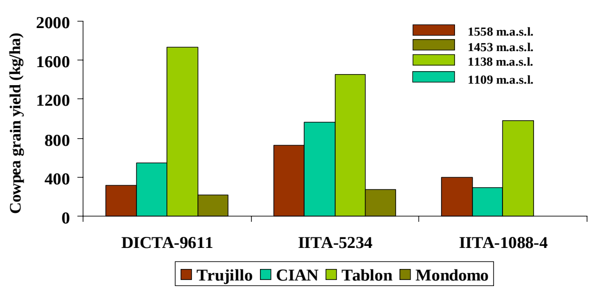

Figure 65. Cowpea grain yield of different accessions in contrasting sites in hillsides of Cauca, Colombia

4.3.3 On-farm evaluation of new forage options for pasture rehabilitation in the Llanos of Colombia

Contributors: C. Plazas, D. Vergara, J. W. Miles and C. Lascano (CIAT)

Rationale

Degradation of introduced pastures is one of the main constraints in livestock production systems of tropical America. This degradation results from poor pasture management and overgrazing. To address problems of pasture degradation in the llanos of Colombia in 1998- 1999 we introduced new grasses and legumes in degraded pastures in the well- drained savannas and in the piedmont. For 6-7 years we have been monitoring the reclaimed pastures in commercial farms and under the management of the farmers.

Results and Discussion

Legumes

Legumes introduction in farms of the Piedmont: In a farm (San Pedro) a pasture of *A. pintoi - B. humidicola* (10 ha) was established in an area that was degraded and invaded by *Homolepis aturensis*, a low quality grass. After 7 years, the legume content varies from 30 to 40% (Figure 66). The weed *H. aturensis* has practically disappeared while the content of *B. humidicola* has stabilized at 60-70% .

The pasture has been fertilized only once (2004) with P (250 kg /ha of rock phosphate) and Ca (250 kg/ha of lime). In spite of the low fertilization applied to the pasture, the carrying capacity has increased over time. Currently 40 steers with an average initial LW of 250 Kg are maintained in the pasture for 10-15 days with a rest period of 15 days.

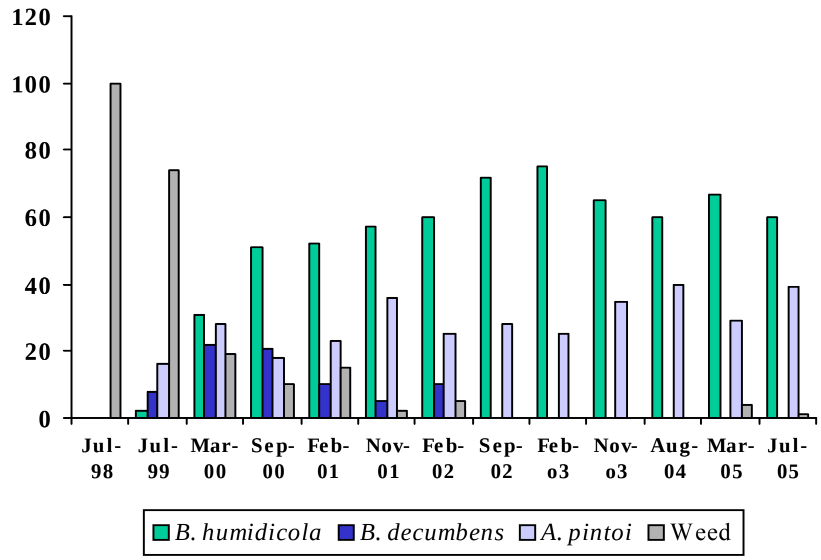

Figure 66. Botanical composition (%) in a pasture of *Brachiaria humidicola* associated with *Arachis pintoi* after 7 years of establishment at the farm San Pedro in Piedmont of the Llanos of Colombia.

Woody legumes under grazing in the piedmont: Between 2001 and 2003 we carried out a project to validate the utility of *Cratylia argentea* as feed resource for milking cows in smallholder systems in the piedmont. A total of 14 farms were selected to establish Cratylia for different uses (cut/carry, direct grazing and seed production) with farmer participation. We continued to monitor the stands of Cratylia under different management and to see to what extent farmers had made any innovations on the utilization of this legume. In 11 farms Cratylia is being used as a protein bank with direct and controlled (1 to 3 hours/day) grazing by cows in both the dry and wet seasons. In 2 farms the legume is being used to produce silage for dry season feeding and in only one farm the legume is used in a cut/carry system. In the initial conversations with farmers we had recommended that the legume be used in a cut/carry system. However, it is evident that farmers preferred to use direct grazing in order to save money and time. Labor is not always available and when it is available the cost is high.

We have been monitoring the performance of *Cratylia* under grazing and after 4-5 years it is evident that the productivity of the stands has declined from 2 tons of DM/ha to 500 kg of DM/ha. Plant mortality has also been high (30%) as opposed to almost no mortality in farms where the legume has been managed under cutting. Thus it would seem that Cratylia when managed under grazing should be replanted at least every 4-5 years.

Grasses

Performance of *B. brizantha* cv. Toledo in the piedemont and well-drained savannas: In 2000 at the San Pedro farm, located in llanos Piedmont, a pasture of 2,5 ha of *Brachiaria brizantha* cv. Toledo (CIAT 26110) was established in a degraded area. A group of 13-15 steers with average LW of 320 kg grazed the pasture this year with an occupation period of 8-10 days and 25- 30 days of rest. Forage availability in this pasture has varied between 3,5 and 6,3 t/ha of DM with a content of CP between 7,5% and 11% (Table 70).

Results from the farm trial indicate that *Brachiaria brizantha* cv. Toledo is a commercial cultivar that has excellent productive potential in the Piedmont. The most important attributes of *B. brizantha* cv Toledo as an alternative for the llanos piedmont continue to be high carrying capacity and adaptation to poorly drained soils.

Table 70. Forage availability (t/ha of DM) and CP content (%) of *Brachiaria brizantha* CIAT 26110 cv. Toledo under grazing at farms of Piedmont and Altillanura of the Llanos of Colombia.

Season	Piedmont [a]		Altillanura[b]	
	DM (t/ha)	CP (%)	DM (t/ha)	CP (%)
March/2000	-	-	1.3	3.5
September/2000	-	-	6.8	4.2
February/01	4.8	11	5.9	4
November/01	4.4	10	3.4	5
February/02	3.5	8	2.6	2.5
September/02	3.5	7	3.9	5
February/03	5.8	8	5.5	4
November/03	4	6	4.2	4
August/04	3.5	10	4.9	4
March/05	6.3		Burnt	
July/05	5.5		2.7	

a Farm San Pedro.
b Farm El Porvenir.

In 2000, at the farm El Porvenir in a well -drained savanna with acid infertile soils, a pasture (3, 5 ha) was established with *B. brizantha* cv. Toledo. Results after 5 years indicated that forage availability varied between 1, 3 and 6, 8 t/ha of DM, with relative low CP content (3 % and 5%) in the absence of N fertilization (Table 71). The pasture has been managed with 12-15 steers of 280 kg of average LW, with 8 days of occupation and between 35 to 40 days of rest. Productivity of Toledo in the well-drained savannas has not been as good as in the piedmont. However, the grass has persisted under an intensive grazing regime and with limited maintenance fertilization. In 5 years the pasture has only been fertilized once with 50 kg/ha of 15-15-15 and 50 kg/ha of KCl.

Performance of different accessions of *B. brizantha* in well-drained savannas. In 2000 at the farm El Porvenir, well drained savanna site, 5 ha of *B. brizantha* CIAT 26318 were established. In the following year, 3,5 ha of each of the accessions of *B. brizantha* CIAT 26990 and 26124 were also established in the same farm. Forage on offer in these pastures has varied from 3,4 to 5,2 t DM/ha in *B. brizantha* CIAT 26318 and from 2,3 to 5,1 t DM/ha of DM in *B. brizantha* CIAT 26990. Production of DM with *B. brizantha* CIAT 26124 has been low (between 2,0 and 0,4 t/ha of DM) due to its excellent palatability and heavy grazing system used (Table 71). The CP content of these accessions has been low in the dry season (3 to 4%) and average for a grass in the wet season (6 to 7%).

In general results indicate that after 5 years of evaluation of the different *Brachiaria brizantha* accessions it continues to be evident that each accession should be managed in a different manner. For example, *Brachiaria brizantha* CIAT 26318 (the prefered accession of the farmer) is being used finish steers with 8 days of occupation and 35 of rest, while *B. brizantha* CIAT 26990 is used with good results with cows with 5 days of occupation and 30 days of rest. The accession CIAT 26124 has practically

Table 71. Forage availability and crude protein (CP) content of accessions of *Brachiaria brizantha* at the farm El Porvenir in the Altillanura of the Llanos of Colombia.

Sampling Date	DM (t DM /ha)			CP (%)		
	CIAT 26318	CIAT 26993	CIAT 26124	CIAT 26318	CIAT 26993	CIAT 26124
March/2000	1.4	-	-	2.6	-	-
September/2000	4.7	-	-	4.2	-	-
February/01	4.8	4.2	2.0	3.7	7.2	7.4
November/01	4.3	4.5	3.8	5.2	6.5	5.2
February/02	3.4	2.4	2.5	3.7	2.8	3.3
September/02	5.2	5.0	2.4	3.7	4.3	6.1
February/03	4.7	4.7	2.9	3.0	3.9	3.3
November/03	4.7	4.8	3.1	3.9	4.1	5.9
August/04	5.0	4.3	2.0			
March/05	Burnt	Burnt	Burnt			
July/05	4.6	2.8				

disappeared due to high palatability and limited maintenance fertilization. In 5 years the three pastures has only received 42 kg of N, 8 kg of P and 33 kg of K per hectare.

Performance of *Brachiaria* Hybrid cv. Mulato. This hybrid with multiple positive attributes (i.e. rapid establishment, excellent forage quality and drought resistance) continues to be evaluated with milking cows in the piedmont and as a component in crop- pasture systems in the llanos of Colombia.

Piedmont of the Llanos: In a clay loam oxisol at the farm La Isla, Piedmont 7, 5 ha of Mulato were established in July 2001 in a plot of *B. decumbens* in advanced stage of degradation. At the beginning of the evaluation process 3, 2 steers /ha at the finishing stage were maintained in the pasture for one month. All animals received 1 kg daily of commercial concentrate as supplement and liveweight gains were in the order of 2 kg/d... In a second phase of utilization, milking cows were introduced in a rotational grazing system in five plots of 0, 75 ha and 3 days of occupation. With this system 12 milking cows (3, 2 cows/ha) were maintained during a complete cycle of 15 days. Cows in pastures of *B. decumbens* produced on average 5 liters of milk in the morning milking and 4 liters in the afternoon milking. In the Mulato pasture, the same cows produced daily 6.5 liters in morning milking and 5 liters in the afternoon milking, which represented 23 liters of additional milk per day.

One major problem of Mulato in La Isla farm has been plant mortalility due to poor soil drainage in parts of the pasture and as result Mulato has been replaced by *Homolepis aturensis*. However, measurements carried out after 4 years of establishment showed that the availability of Mulato in the well drained areas is 3 and 3.5 tons of DM/ha in the dry and wet seasons, respectively in spite of heavy grazing and no fertilization.

Well -drained savannas: Sowing commercial crops with grasses or crop-pasture rotations are alternatives to reduce establishment costs of pastures, to improve their productivity and quality due to high residual fertilizer and for sustainability of the crop phase over time. With the support of Papalotla, of Mexico in 2001 we established Mulato (15 ha) in association with maize. Grain yield after 138 days of sowing the maize was 3, 7 tons /ha, while *Brachiaria* hybrid cv. Mulato produced 4,2 tons of DM /ha. Due to N deficiency, in June 2004 fertilizer was applied (67 kg/ha of N and 38 kg/ha of K) and the forage on offer increased from 2,9 to 5,1 t DM/ha. However, soon after the fertilization Mulato exhibited nutrient deficiency indicating that it requires frequent fertilization when sown in low fertility soils. Currently in approximately 10 (plot 3) to 25% (plot 1) of the grazing area, the proportion of Mulato decreased due to poor drainage and plants were replaced with native vegetation. However, the pasture in well drained plots remains productive and as a result support 2.5 animals/ ha with a grazing frequency of 15-20 days and rest a period of 30-40 days.

4.3.4 On-farm evaluation of *Brachiaria* hybrid cv. Mulato

Contributors: A. Mendoza and C. Burgos (DICTA), H. (CIAT), B. Pinzón, E. Santamaría and J. Girón (IDIAP), and H. Cuadrado (CORPOICA)

Rationale

On-farm validation of new promising forage species complements the results reported from on-station research sites. This has more relevance if considering that farmers have their own preferences and management practices that influence productivity and performance of pastures, and in all cases they adapt the use of new forage options to their particular needs. Under this consideration the *Brachiaria* hybrid cv. Mulato has been monitored during the last three years in dual purpose cattle farms of Central and South American tropics, and the results continue to confirm that this grass offers an important alternative for dual purpose farmers compared with some traditional grasses, both for the high quality and high forage production reported that allows more stocking rate per unit area.

Materials and Methods

As mentioned in Project IP-5 2002 Annual Report, a protocol for on-farm validation/promotion of *Brachiaria* hybrid cv. Mulato was developed and proposed to national institutions of Panamá, Guatemala, Nicaragua, Costa Rica, Honduras and Colombia. Animal liveweights gains have also been measured on research stations in Panamá (IDIAP's research station in Gualaca), and in Colombia (Corpoica's research station in Cereté). In both sites a rotational grazing system was used (3/21 occupation/rest in Panamá and 2/22 occupation rest during the wet period and 3/33 during the dry season in Colombia). In Panamá the soils are acid of Inceptisol type and were fertilized annually with 80-30-20 kg/ha of N, $P_2 0_5$ and $K_2 0$ respectively, meanwhile the site in Colombia has alluvial soils and the paddocks were not fertilized during the 525 days that the experiment lasted.

Results and Discussion

On-farm monitoring: Honduras continues to be the country where *Brachiaria* hybrid cv. Mulato has been more closely monitored in dual purpose cattle farms (see IP-5 Annual Report 2004). Colleagues from the national institution (DICTA) have carried out important field work in this regards along the Atlantic coast, the north-west and the central part of the country. In Table 72 we show that from January to May 2005, covering practically the whole dry period in this country, the major effect of cv. Mulato was on higher stocking rates and not on individual milk production per cow, therefore contributing to more milk produced per unit area as was reported last year with a set of different farms. It is interesting to note that a large variation in

Table 72. Area planted, stocking rate and milk production per animal and per hectare of dual purpose cows grazing *Brachiaria* hybrid cv. Mulato and other grasses during January-May 2005 in different farms of Honduras. (Information supplied Conrado Burgos and Alejandro Mendoza of DICTA).

Farm/ Grasses	Area planted (ha)	Stocking rate (cows/ha)	Milk (kg/cow/day)	Total milk (kg/ha/day)
1. Mulato*	7.0	7.5	3.5	25.9
Swazi	11.2	3.7	3.2	14.7
2. Mulato	4.2	5.6	4.4	24.1
Swazi	11.2	2.1	3.6	7.8
3. Mulato	7.0	0.9	4.8	6.8
Swazi	14.0	1.3	4.9	3.4
4. Mulato	9.8	4.8	6.0	28.4
Swazi	14.0	3.3	5.9	19.5
5. Mulato	2.1	4.0	4.3	20.6
Basilisk	6.0	2.4	5.1	12.2

* Mulato (*Brachiaria* hybrid), Swazi (*Digitaria swazilandensis*), Basilisk (*B. decumbens*).

stocking rate exists between farms with the same type of pastures, an indication of the effect of site and the type of animal the farmers have. Both milk and beef on-farm monitoring continues in selected farms of Costa Rica, Nicaragua, Honduras and Guatemala.

Controlled grazing trial: The grazing trials ended during 2005 after 525 and 638 days in locations of Colombia and Panamá, respectively. In Table 73 we show the estimated stocking rates and the daily animal liveweight gains at both sites. In Cereté, Colombia cv. Mulato significantly out-yielded *B. decumbens* cv. Basilisk (P<0.05) in both stocking rate and total animal liveweight per ha per year.

The stocking rates for cv. Mulato were similar in Cereté and Gualaca; however animal liveweights were slightly higher at the latter site. At this locality the animals used were cross-bred young zebu steers, meanwhile that at Cereté the animals used were zebu steers and F1 cross-bred zebu x romo sinuano, a local race known by the adaptation to warm humid conditions.

Table 73. Stocking rates and animal livewights in grazing trials of cv. Mulato and *B. decumbens* (Basilisk) in Gualaca (Panamá) and Cereté (Colombia). (Information supplied by Bolívar Pinzón and Eliut Santamaría of IDIAP (Panamá) and Hugo Cuadrado of Corpoica (Colombia)).

Site	Pastures	Stocking rates (AU/ha)	Animal Liveweights (g/day)	(kg/ha/yr)
Gualaca	Mulato	3.4	544	879
Cereté	Mulato	3.5 a *	503 a	796 a
	Basilisk	2.0 b	532 a	580 b

* P<0.05

4.3.5 On-farm evaluation of different forage alternatives in Central América

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Rationale

Low animal productivity is the standard in beef and dual purpose production systems in Central América, despite that during the last decade there has been an increasing demand for improved pastures. The forage species are dominated by *Brachiaria* cultivars followed by *Panicum* species; few forage legumes are in the list. Both farmers and technicians are nevertheless aware that improved pasture technologies, including the use of legumes, are viable options that need more promotion and on-farm demonstration to increase adoption. Therefore, presently there are regional projects that develop forage alternatives with the participation of private companies, national institutions and farmer organizations; some results are presented.

Results and Discussions

Technological components based on improved grasses and legumes

The Tropical Forage Project of CIAT carried out activities in the ILRI/CFC Project ‘Enhancing Beef Productivity, Quality, Safety and Trade in Central America’. During 2005 the establishment of improved forage components in 60 selected farms of Honduras, Guatemala, Nicaragua and Costa Rica was completed. Monitoring of animal and pasture production, as well as persistence of introduced grasses and legumes was also initiated, mainly in Honduras. However, in all participating countries some pasture components needed replanting, and in some farms additional grass-legume plantings were contemplated. For these reasons more seed was distributed during the first semester of 2005, as shown in Table 74.

Table 74. Forage species procured and distributed to each of the collaborating country of the ILRI-CFC Project in Central America during the period January-June 2005.

Species/Cultivar	Countries				Total
	Guatemala	Honduras	Nicaragua	Costa Rica	
	(kg)				
Brachiaria brizantha cv. Marandú	8	-	15	-	23
B. brizantha cv. Toledo	-	-	125	-	125
B. decumbens cv. Basilisk	8	-	26	34	68
B. humidicola (ex-*B. dictyoneura*) cv. Llanero	-	-	-	5	5
B. hybrid cv. Mulato	50	-	22	218	290
B. hybrid cv. Mulato II	-	18	8.5	28	54.5
Paspalum atratum cv. Pojuca	-	-	3	-	3
Panicum maximum cv. Mombasa	-	-	-	11	11
Arachis pintoi cv. Porvenir	53	-	10	52	115
Cratylia argentea cv. Veraniega	9	-	6	-	15.5
Leucaena leucocephala CIAT 17263	3	-	3.5	-	6.5
Stylosanthes guianensis AFT 3308	3	3.5	-	9	15.5
Pueraria phaesoloides cv. Kudzú tropical	-	-	-	22	22
Total	**134**	**21.5**	**193.5**	**379**	**754.5**

A total of 754.5 kg of pasture seed has been procured and distributed during 2005. The grasses cultivars dominate the deliveries (579.5 kg), which is an indication of farmer preferences. Honduras was the country that received less forage seed (21.5 kg), and showed adequate pasture establishment in the collaborating farms during the 2004 planting season. Costa Rica received the largest amount of seed (379 kg), particularly for the high request of *Brachiaria* hybrid cv. Mulato (290 kg), which is an improved grass of high quality that farmers begin to appreciate as important forage component for their farms. This grass has also been planted successfully in other collaborating countries particularly Honduras, and the impact on animal production, particularly the increase in milk yields in dual purpose cattle is now well documented. (See activity 4.1.4). Another country where forage components were established successfully was Guatemala. The proper forage plantings is attributed to the fact that most of the small and medium size collaborating farmers combine both annual crops and cattle in their farms, therefore they use the same crop practices to establish forage components that without doubt leads to excellent forage establishment.

In Table 75 we show grass cultivars and area planted in 8 collaborating farms distributed along the localities of El Reposo, Coatepeque, Nueva Concepción and Cuyuta in Guatemala. A total of

Table 75. Area and forage components established in collaborating farms of the ILRI/CFC Project in Guatemala during 2004/2005.

Species/Cultivar	Area (ha) planted as: Pure grass	Mixed with *A. pintoi*
B. brizantha cv. Toledo	4.7	-
Brachiaria hybrid cv. Mulato	15.5	1.7
Brachiaria hybrid cv. Mulato II	3.8	1.4
B. brizantha cv. Marandú + *B. decumbens* cv. Basilisk	4.2	1.5
B. brizantha cv. Marandú	-	3.0
Cynodon nlemfuensis cv. Estrella	-	1.4
Digitaria eriantha cv. Pangola	-	0.4
Total	**28.2**	**9.4**

38.6 ha of new pastures are now ready to be monitored during the present growing season; although we received news that the hurricane Stand caused considerable damage in both pastures and crops in the sites in Guatemala.

Again, the grass cv. Mulato dominates the areas planted, followed by cv. Toledo and the mixture of the grasses cvv. Marandú and Basilisk. The perennial peanut *Arachis pintoi* cv. Porvenir was the only forage legume used in this case, but for the present planting season other legumes such as *Stylosanthes guianensis* and *Leucaena leucocephala* will be planted as well.

4.4 Adaptation of forage conservation technologies to smallholder systems

Highlights

- Demand for forage conservation technologies and reasons for low adoption by small farmers in a region of Honduras were identified.
- The benefit of silage on milk production was estimated to be greater for small scale farmers than for large scale farmers in Central America.
- In the study area in Honduras (Yoro) a sharp increase in silo use was found mainly due to farmer training and field days. The number of farms using silage increased 90% and silage volume increased by 120% in one year.
- Plastic bag silage offers a low cost opportunity for small scale farmers, but the non-availability and high cost of suitable bags in Honduras are seen as constraints for further uptake.
- Feeding silage or hay to milking cows seems profitable. However, farmers in Honduras fed more hay than silage, while in Costa Rica the reverse was true.

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4.4.1 Stimulating innovation among small farmers of forage conservation technologies

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Rationale

Feed shortage during the five to six months dry season in many areas of Central America severely limits livestock production and farm income. Alternative strategies to level milk and meat production include hay and silage preparation for the dry season. However, adoption of forage conservation methods by small-scale farmers so far has been low. Reasons include technologies not suitable to smallholder conditions that require high investments (e.g. machinery and/or large bunker silos) and lack of knowledge about appropriate low cost alternatives such as heap silo, earth silo, wrapped silage and little bag silage (LBS). The research carried out in Honduras aims to define criteria and pathways to enable small-scale farmers to adapt forage conservation technologies to local conditions through facilitation of innovation. Alternative forage-based products for ruminants will be validated as dry season feed and potential income-generating options.

The specific research objectives are:

1) To determine the effect, costs and benefit of hay and silage supplementation from different forage legumes (*Vigna unguiculata*, *Lablab purpureus* and *Cratylia argentea*) and improved grasses (*Brachiaria brizantha* cv. Toledo and *Brachiaria* hybrid cv. Mulato) on milk production and productivity.
2) To determine constraints for adoption, conservation technologies and to determine ways to overcome such constraints through innovation processes.

Target areas and farmer groups. Based on a diagnosis ("Forage Technologies to Alleviate Dry Season Feed Shortages - A Diagnosis of Honduras and Nicaragua") in 2004 carried out by CIAT and partners (see Annual Report 2004) with the collaboration of national and local organizations (DICTA, SERTEDESO and FHIPA, Fundación Hondureña de Investigación Participativa Agrícola), two main target areas in Honduras were selected (Yoro and El Paraíso) for the study on innovation of forage conservation for dry season feeding. Additionally, in Olancho and Intibuca two farmer groups were selected (Table 76). The areas are characterized by a long dry season (up to seven months), no or little use of conserved forages, grazing mainly on naturalized pasture (*Hyparrhenia rufa*), little use of improved pasture such as *Brachiaria* spp. and low milk production in the dry season.

The results presented in this report give an overview on the potential and constraints for adoption of forage conservation technologies in the target areas.

Table 76. Farmer groups in Honduras involved in forage conservation research.

Departments	Zones	Groups
Olancho	Catacamas	Group San Pedro de Catacamas
Yoro	Sulaco,	AGASUL
	Victoria,	EMPRASEFOR, CREL and individuals
	Yorito,	CREL
	Yoro	CREL
	La Savanna	Non-livestock farmer group
Danli	Alauca,	Independent farmers
	Jamastrán	Independent farmers
Intibuca	Jesús de Otoro	CIAL-group

Adaptation of forage conservation technologies

Two contrasting extension strategies (promotion of adoption and promotion of innovation) are compared with groups of small-scale farmers in the project areas. Each strategy represents a different approach to technology development and transfer, with differences evaluated in terms of adoption, benefit-costs of both the technology and the strategy of R&D and extension.

Promotion of adoption. In 2003 and 2004, DICTA (Dirección de Ciencia y Tecnología Agropecuaria), the main National Agricultural Research and Extension Institution, promoted little bag silage technology during field days in Choluteca, Olancho, El Paraíso and Comayagua where the theory of making silage was explained, little bag silage demonstrated and plastic bags distributed to farmers. Some farmers were visited again by DICTA to further promote the standarized technology.

Farmers at the beginning of 2006 participated in these meetings as well as farmers who received plastic bags will be visited in order to investigate the adoption processes as well as reasons for non-adoption of silage technologies.

Promotion of Innovation. Innovation, adaptation and diffusion processes are facilitated to different farmer groups through an interactive (including farmer to farmer) and experimental learning process involving selection, and modification of best-bet technology options. We focus on innovative farmers who adapt forage conservation technologies to their local conditions and then the innovation and adaptation processes are documented. The other group we focus is on non-livestock farmers who could have as an objective the marketing of dry season forage alternatives like legume hay, legume based concentrates and little bag silage.

Farmer training events. Improved forage grasses (*Brachiaria brizantha* cv. Toledo and *Brachiaria* hybrid cv. Mulato), herbaceous (*Vigna unguiculata*, and *Lablab purpureus*) and shrub (*Cratylia argentea*) legumes as well as their conservation in form of hay and silage (e.g. little bag silage were offered to farmers during farmer trainings in events).

A) Theoretical training. In June 2005, with the help of local farmers, DICTA and the NGO SERTEDESO (Servicio Técnico de Desarollo Sostenible), farmers were invited to participate in training courses on "efficient use of forages and their conservation". During the meetings, basic farm data (e.g. farm size, number of cattle and milk production in the dry and rainy season) of the participants were gathered using a structured interview. Furthermore, participatory methods were applied to obtain diagnostic data (e.g. problems in the dry and rainy season and feeding strategies in the dry season) which served as rising awareness of problems and introduction to the topic. The program of the first meetings was:

- Introduction and presentation of the project.
- Participatory diagnosis on farmers' interest, problems in the dry and rainy season and feeding strategy in the dry season.
- Theoretical part: Background of forage conservation (problems, definition, objectives, advantages and disadvantages), technical aspects of silage and hay making (optimal cutting time, important steps, forages to conserve, additives, characteristics of a good silage/hay, different silo types) and its use.
- Participatory part: Sourcing interest in hay and silage, market for hay and silage including price evaluation and interest in different kind of silos.
- Open discussion and questions.
- Distribution of information sheets and small quantities of seeds (e.g. *B. brizantha* cv. Toledo, *B.* hybrid cv. Mulato, *Vigna unguiculata*, *Lablab purpureus* and *Cratylia argentea*).

B) Field days (practical training). In September 2005, more training events directed to farmers were carried out in prototype farms. The training centered on a revision of the theoretical aspects of forage conservation (since there were some new participants) and a practical part in which little bag silage and hay was prepared. Different silage bags were evaluated afterwards.

In November and December 2005, at the beginning of the dry season, different grass and legume hays and silages (see above for the species) were elaborated in order to carry out on-farm experiments (researcher-led) with milking cows supplemented with materials during the dry season 2005/2006.

In 2004 and 2005, in the departments of Yoro (Yoro, Yorito, Sulaco and Victoria), El Paraíso (Alauca and Jamastran), Olancho (San Pedro de Catacamas) and Intibuca (Jesus de Otoro) several farmer trainings (theory and practice) and field days were conducted (Table 77).

Table 77. Training events related to forage conservation and participants in different regions of Honduras.

Region	Event	Participants
Yorito	Farmer training (theory)	22 CREL members + 1 non-CREL
Sulaco	Farmer training (theory)	23 participants (10 pupils, 7 AGASUL and 6 more individual farmers)
Victoria	Farmer training (theory)	14 participants (7 from EMPRASEFOR)
	Two field days, one about RTM* including silage use (DICTA) and one about LBS	RTM: 15 participants Silage: 23 participants from Victoria, Sulaco, Yoro.
Yoro	Field day (practice) LBS	14 participants
Alauca	1 Farmer training (theory)	14 participants
	2 Field days	About 20 participants
Jamastran	3 Farmer trainings (RTM+LBS)	RTM: 27
	Including theory and practice	LBS: 13
Catacamas	1 Farmer training (theory)	35 (12 students)
Jesús de Otoro	Farmer training (theory)	Group of 10 farmers

RTM= Ración Total Mezclada

In Yorito, except one person, the participants (23) were members of the milk affiliation "CREL" (Centro de Recolección y Enfriamiento de Leche). Five of the farmers already had a silo. In Sulaco, 23 persons participated of which 10 were interested young students invited by their teacher, seven farmers were from the AGASUL-group of which three farmers have already a silo, two from another nearby village (La Albardilla) and 3 more individual farmers. In Las Vegas near Victoria a farmer training event was carried out with 14 participants of whom 7 were members of the forage seed enterprise EMPRASEFOR (Empresa de Producción Artesanal de Semilla Forrajera). Two field days were carried out: (a) in Victoria in an innovators' farm where little bag silage (LBS) was elaborated and a heap silo was demonstrated and (b) in Yoro where LBS technology was demonstrated.

In Alauca, two farms were selected to introduce forage innovations. Beside several visits and field days in 2004 and 2005 with demonstration of LBS technology and hay making a farmer training event with 14 participants was carried out including theory on forage conservation. In collaboration with DICTA, 3 farmer training events were carried out in a prototype farm in Jamastran that used conserved forages. A total of 35 farmers participated in the event in San Pedro de Catacamas of which 12 were students from the college and 23 were farmers. Three farmers had already experience in silage making but ceased it due to failure. Another farmer training was held with the farmer group of Jesus de Otoro.

Characterization of farmer groups. Farmers participating in the training events on forage conservation were grouped in categories according to the number of livestock they own. As Figure 67 illustrates most of the participants can be grouped into the categories of small and medium.

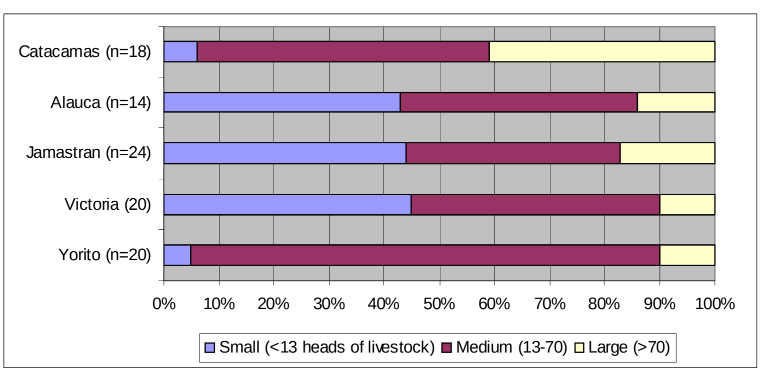

Figure 67. Share of small, medium and large scale farmers in different groups included in surveys in Honduras.

4.4.2 Survey with farmer groups to estimate current and potential use of forage conservation technologies

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In the section that follows we report the main results of a survey carried out with farmers (N=101) selected to participate directly or indirectly in the research. Detailed information on how the surveys were carried out is presented in Section 4.5 of this report.

Milk production in the dry and rainy season. Considering all farm size categories over all regions (101 farmers), on average milk production in the dry season drops about 0.7 liters/cow (from 4.92 in the rainy season to 4.25 liters) in the dry season. In Alauca, the difference in milk production between the dry and rainy season was highest (2.44 liters/cow) (Table 78). In general, milk yield difference between the rainy season and dry season seems to be higher in small (<13 heads) farms (1.12 liters/cow) than in medium and large scale farms (0.51 and 0.75 liters/cow respectively) (Table 79). This can be explained by the fact that farmers grouped in categories medium and large are more specialized in milk production and invest more capital concentrates and silos than small scale farmers in order to take advantage of the higher milk price paid in the dry season.

Problems faced by farmers with milking cows in the dry and rainy seasons. During the meetings farmers were asked about their most important problems in the rainy and dry seasons.

a) Problems in the rainy season

In the rainy season problems most mentioned were:

- Low price for milk with a minimum of 2 Lempira/litre (about 0.11 US$/litre) in the rainy season compared to about 5.5 Lempira/litre in the dry season (about 0.30 US$/litre).

- Lack of marketing possibilities for dairy products.

- Pests (flies and external bloodsucking parasites, ticks) and diseases (estomatitis) diarrhea of calves, fungal diseases in the hoof.

- Weeds in the grazing areas.

- Inundation and mud (that are breeding places for pests that transmit diseases) during the rainy season.

- Lack of financial resources, land and technical assistance and non-availability of improved pasture in their farms.

b) Problems in the dry season

In all regions the most severe problem in the dry season was feed shortage (in average 86% of all farmers) followed by water shortage (50%). Other problems mentioned were “little or less milk production” (14%), “diseases”, “bad management”, “high prices for concentrates”, loss of weight” and “cattle mortality”, “pests”, “lack of money”, “bad pasture quality” and “lack of knowledge on forage conservation”.

Feeding strategies used by farmers in the dry season. Farmers use a wide range of feeding alternatives to overcome dry season feed shortages of which the most common is the utilization of forage sugar cane (caña forrajera/filipina), followed by feeding crop residues like

Table 78. Milk production (liters/cow) in the rainy season (wer) and dry season (dry) of different farmer categories in different regions.

			Farm category			
Department	Region		Small (20)	Medium (64)	Large (17)	Average
El Paraiso	Jamastran (20)	Dry	4	5.29	4.82	4.75
		Wet	5.14	5.84	4.65	5.36
		Diff	1.14(7)	0.55(9)	-0.17(4)	0.61
	Alauca (10)	Dry	2.33	2.53	5.79	3.39
		Wet	2.66	4.02	10.47	5.83
		Diff	0.33(2)	1.49(6)	4.68(2)	2.44
Olancho	Becerra (19)	Dry	4.3	5.22	3.59	4.78
		Wet	4.53	5.35	5.01	5.19
		Diff	0.23(2)	0.13(13)	1.42(4)	0.41
	SF de la Paz (17)	Dry	4.39	4.16	4.5	4.28
		Wet	5.65	4.01	3.67	4.18
		Diff	1.26(3)	-0.15(11)	-0.83(3)	-0.02
Yoro	Yorito (20)	Dry	6.67	3.57	5.22	3.89
		Wet	10	4.59	5.18	4.92
		Diff	3.33(1)	1.02(17)	-0.04(2)	1.03
	Victoria (15)	Dry	4.11	4.02	4.41	4.11
		Wet	5.31	4.24	4.85	4.62
		Diff	1.2(5)	0.2(8)	0.44(2)	0.51
Average			**1.12**	**0.51**	**0.75**	**0.67**

Small (<13 heads of livestock); Medium (13-70 heads); Big (>70 heads); (x) = number of farmers; Ver = dry season, Inv = rainy season, Diff = difference

maize and sorghum straw and corn husk (tuza), concentrate, cut and carry grasses like "Camerun" or King grass (*Pennisetum purpureum*) and renting land for grazing. Other mentioned strategies are grazing improved grasses like *Brachiaria* hybrid cv. Mulato and *Brachiaria brizantha* cv Toledo, use of molasses, gallinaza (chicken manure) and urea.

Silage. Of all participants 14% already used silage (mainly of maize and/or sorghum), and 5% used hay. There are only two cases of silage marketing in the study area. One is in Sulaco

Table 79. Milk production in the dry and wet season.

	Dry	Wet	Diff
Small (20)	4.09	5.29	1.12
Medium (64)	4.21	4.71	0.51
Large (17)	4.59	5.44	0.75
Average	4.25	4.92	0.67

where a farmer sold maize silage (from a big silo) at a price of 25 Lempira (1.3 US$) in bags of 70 pounds (about 0.04 US$ per kg silage). In Yorito, SERTEDESO (a local NGO) sold silage in 2003: The silo had a dimension of about 8 x 4 x 2 m and a capacity of about 30 tons of silage. They sold the whole silo to a price of about 6,500 Lempira (about 350 US$) equivalent to about 0.01 US$ per kg fresh material. Other farmers stated a price of 0.05-0.08 US$/kg FM when asked to what price they would sell silage.

Straw and hay. In Alauca, farmers usually rent a piece of land with maize straw for a price of 115 US$ per ha or the maize straw can be bought at a price of 5 Lempira/10 pounds (0.06 US$/kg). A big bale of hay (600 pounds) costs 1,200 Lempira (0.24 US$/kg).

In Yorito, farmers stated a price of 2.7 US$ per head for 2 month rent of piece of land with maize straw.

In Jamastran sorghum straw is sold at a price of 1.33 US$ per 16 kg bale (0.08 US$/kg).

In Catacamas, farmers told us that the supply is higher than the demand for hay, and as a result people won't buy it. One bale of hay costs 11-15 Lempira/20 pounds (0.065-0.09 US$/kg).

In Victoria, a bale of Estrella (*Cynodon plectostachyus*) or Suazi (*Digitaria swazilandensis*, *D. didactyla*) hay (15 libras) costs 10 Lempira (0.08 US$) with a production cost of 0.04 US$/kg.

In Sulaco, farmers mentioned 0.07 US$/kg of hay, in Yorito about 0.1 US$/kg. Another dry season feeding option is "tuza" which is the dried husk of maize and is sold at a price of 0.04–0.06 US$ per kg. In Sulaco, a 32 kg bag of sugar cane costs 25 Lempira (0.04 US$/kg).

Another alternative is the pod of the legume tree "Espino" which costs 0.02 US$ in Sulaco and 0.03 US$ in Victoria. Other edible pods are from the tree "Cablote" ("tapa culo") with a price of 20 Lempira/100 pounds (0.02 US$ per kg) and Guanacaste.

Costs for different silo types and silage costs. In Table 80 we show initial investment costs, depreciated annual costs and the cost per kg of silage (DM) made in different silos.

Initial investment costs for different silos including plastic costs differ from up to 820 US$ for a brick silo with roof to about 50 US$ for an earth silo with the same capacity. The initial investment and annual costs for 800-1200 plastic bags that are necessary to ensile the same amount of silage (36 tons) would be very high. However, this value is very theoretical since the purpose of plastic bag is to ensile little amounts of high quality forage. Nevertheless, the cost per kg of maize silage does not differ much for all silos (0,04-0,05 US$) except for LBS which is 0.05-0.07 US$, since it requires a proportional higher amount of plastic bags per unit of silage compared to other silo types. However, for small-scale farmers who can not afford high initial investments and whose objective is to ensile little amounts, LBS is an economic alternative. Furthermore, LBS does not necessarily require extra labour costs since employees paid on day basis can additionally fill some bags every day. Other advantages of LBS are presented below.

Table 80. Initial investments, annual cost (depreciated) and silage cost/kg of different silo types (US$).

Type of silo	Initial investment (+ plastic)	Annual cost (+ plastic)	Cost/kg DM maize silage²
Bunker silo (30 years use)	500-800 (+20)	17.5-27 (+20)	0.04-0.05
Plastic bag silage[1] (2 years use)	0 (+ 168-400 for 800-1200 bags)	112-200	0.05-0.07
Earth silo (5-10 years)	30 (hole) (+20)	3-6 (+20)	0.04-0.05
Heap silo (with earth filled bags)	51 (324 bags) (+30)	15 (bags) (+30)	0.04-0.05
Heap silo (without bags)	0 (+ 65)	0 (+ 32.5)	0.04-0.05

Size of silo: 8*4*2 m (for ca. 36 t)

[1] Cost for bag: 0.14 $ (30kg/bolsa) - 0.5 $ (45 kg/bag)

² The silage cost is based on a maize production cost of 0.04 US$/kg DM

Priority of farmers for investment. Farmers were asked in what they would like to invest in order to improve there farm if they had financial resources available. The objective was to evaluate farmer priorities and detect differences between small, medium and large scale farmers in order to estimate the potential of forage conservation technologies.

Most farmers mentioned that priority on investments were‘: (a) improved pasture, (b) purchase of cows, (c) installations (stable, trough), (d) silo and (e) improve herd composition (breed) (Figure 68). The order of importance of the intended farm improvements for small, medium and large scale farmers are shown in Table 81.

Small scale farmers would like to increase the number of livestock (40%) and improve pasture (37%) before investing in barns (29%), silo (14%) and fences (12%). The priority of medium scale farmers’ is to improve the feed base (pasture, 56%) for their herd before investing in breed improvement (16%) followed by silo and irrigation (both 11%) and buying cows (10%).

Large scale (as well as medium scale) farmers’ priority is to improve pasture (32%) followed by breed improvement and irrigation system (both 23%), buying cows, milking machine and silo (all 18%). They intend to intensify their farm through high cost investments like irrigation system, milking machines, wells (14%) and machinery (9%).

In summary the sequence of most important investments to improve farms of small and large scale Honduran farmers are: 1. Improve pasture and increase number of livestock, 2. Construct/ improve barns, 3. Silo and improve breeds, 4. Fencing and 5. Irrigation.

This reveals that farmers are not only aware of silage technology but it is already an important component in the planning of their projected farm improvements.

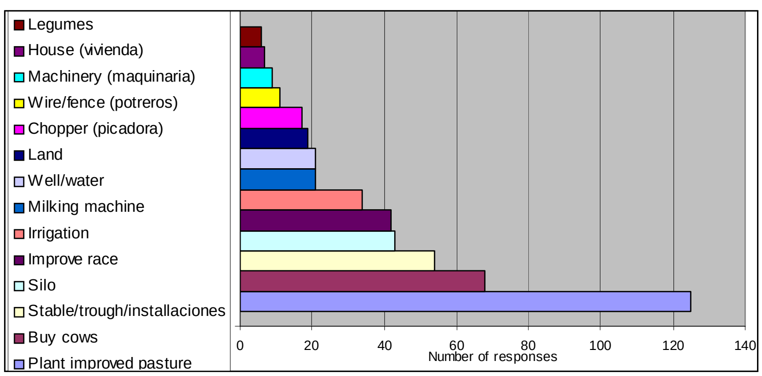

Figure 68. Preferences of farmers for farm improvement

Table 81. Order of importance of farm improvements for small, medium and large scale farmers:

	1. place	2. place	3. place	4. place	5. place
Small (n=35)	Buy cows (40%)	Pasture (37%)	Stable/trough (29%)	Silo (14%)	Wire/fence (12%)
Medium (n=61)	Pasture (56%)	Stable/trough (20%)	Improve herd composition (16%)	Silo (11%), Irrigation (11%)	Buy cows (10%)
Large (n=22)	Pasture (32%)	Improve herd composition (23%), Irrigation (23%)	Buy cows (18%), Milking machine (18%), Silo (18%)	Well (14%)	Machinery (9%)

Demand for forage conservation technologies by farmers. Farmers were asked about their motivation for participating in training events in order to define their interest and demand for forage conservation. In Yorito, 84% of the group mentioned to be interested in forage conservation, 77% in Sulaco, in Las Vegas all farmers (100%) and in Jamastran 85%. In Jesus de Otoro and Catacamas farmers' main interest was to learn about improved pasture species and their management whereas only 18% and 12% respectively voted for forage conservation.

A reason for farmers' preference in Jesus de Otoro and Catacamas might be that in these areas in comparison to the areas in Yoro improved forages like *Brachiarias* were not yet promoted and there is still little use of these. Thus it would seem that the first priority of farmers is to improve their pastures before investing in forage conservation technologies. Another question concerning interest of farmers in different forage conservation possibilities revealed that hay making, LBS as well as trench silo were the most favoured (Table 82).

The fact that the most severe problems in the dry season mentioned by farmers is feed shortage and their interest in forage conservation for the dry season, clearly illustrate the demand for feed alternatives like hay and silage.

Comparison of milk production in farms with and without silage. Milk production in the dry and rainy season of farms with silo was compared to milk production in farms without silo in order to estimate benefits of forage conservation in the form of silage (Table 83). In general, farms having silo compared to farms without silo not only produce

Table 82. Number of farmers interested in hay production and different silo types.

Item	Ala	Jam	Yor	Sul	Cat	LV	Total
Hay	n.d.	3	4	n.d.	n.d.	8	15
Little bag silage	6	2	0	5	2	4	19
Trench silo	1	0	4	13	0	3	21
Heap silo	3	1	0	3	2	0	9
Bunker silo	1	0	7	0	0	2	11

n.d. = no data

56% more milk in the dry season (difference of 2.13 liters/cow) but also 34% more in the rainy season (difference of 1.53 liters/cow). This milk yield difference between dry and rainy season is smaller for farms having silo (0.14 liters/cow) than for those without silo (0.74 liters/cow) and highest for small scale farmers without silo (1.56 liters/cow).

Level of milk production is not exclusively dependent on the season of the year or the use of silage. The higher milk yield in the rainy season of farmers having silo (34%) reflects improved and intensified livestock production systems characterized by the use of improved pasture, breeds more appropriate for milk production, use of concentrate, and/or better management (e.g. pasture rotation, milking frequency).

Assuming that small scale farmers without silo fed silage in the dry season and that the ratio of milk production in the dry and rainy season is equal to the ratio of dry and rainy season found in farms with silo, the potential milk yield is 4.41 liters/cow which is 1.12 liters (or 34%) more than presently being collected. Thus the effect of

Table 83. Milk production (liters/cow) of farms with silo compared to farms without silo.

With silo (n=49)				Without silo (n=139)				Diff2 - Diff1
	Dry season	Rainy season	Diff1		Dry season	Rainy season	Diff 2	
Small (2%)	5.71	6.25	0.54	Small (19%)	3.29	4.85	1.56	1.02
Medium (45%)	5.98	5.59	-0.39	Medium (59%)	3.96	4.39	0.43	0.82
Large (53%)	5.92	6.48	0.56	Large (22%)	3.87	4.73	0.86	0.30
Average	5.94	6.08	0.14	Average	3.81	4.55	0.74	0.60

Small (<13 heads of livestock); Medium (14-70 heads); Large (>70 heads)

Table 84. Estimated average milk yield increases if silo was utilized in farms without silo.

Farm size	% milk yield in dry season of milk yield in the rainy season (with silo)	% milk yield in dry season of milk yield in the rainy season (without silo)	Farms without silo: Potential yield in dry season if used silo (liters/cow)[a]	Farms without silo: Potential milk yield increase if used silo (liters/cow)[b]	% milk yield increase if used silo (estimated effect)
Small	91	68	4.41	1.12	34%
Medium	107	90	4.70	0.74	19%
Large	91	82	4.30	0.43	11%
Average	98	84	4.46	0.65	17%

a = (Liters rainy season without silo) • (% milk yield in dry season (with silo)
b = Potential yield dry season - actual yield dry season of farms without silo

using silage can be greater for small scale farms (1.12 liters or 34%), followed by medium scale and large scale farms (Table 84). These calculations do not take into account the variability of the data within categories and assumes that milk yield difference in the rainy season between silo and non-silo farms is mainly the effect of breed differences.

Cost-benefit of using silage. Assuming an increase of 1.5 liters/cow if cows were fed with silage, a price of 0.3 US$/liter, a silage cost of 0.015 US$/kg FM and an intake of silage of 20 kg/cow/day, the benefit of feeding silage can be calculated (the following only considers the benefit from milk production) using the following:

Milk price • milk yield increase – cost of silage/kg • silage intake = benefit.
Using this formula a farmer with 10 cows would have a benefit of 1.5 US$/day (0.3 US$/liter • 1.5 liters – 0.015 US$/kg • 20 kg/cow = 0.45 US$ – 0.3 US$ = 0.15 US$ per cow/day.

Calculation of break even point (income = costs) only considering milk production:
0.3 US$/litre • x liters = 0.3 US$. The break even point is 1 liter/cow which means in this case that a cow should increase milk production by more than 1 liter if supplemented with 20 kg of silage in order to produce a profit.

The benefit of feeding silage on milk production is highly dependent on the breed, silage quality and quantity. In order to evaluate the overall benefit of feeding silage, these factors and effects on reproduction and live-weight as well as effects on stocking rate have to be considered. More detailed economic assessments are under way.

Reasons for non-adoption of silage technology by small scale farmers. Out of 49 farmers with silo, only one (2%) was a small scale farmer.

Some reasons for the little use of silage by small scale farmers are the following:

- The investments required for (renting) machinery and/or for constructing a silo are high.
- Non availability of a forage chopper ("picadora").
- Lack of knowledge and/or experience on silage technology in general and/or alternative low cost silo technologies.
- Preference of investing in cows, improved pasture, land or barns before constructing a silo.
- Have breeds of low milk production potential and the milk produced is used for household consumption.
- Lack of infrastructure (e.g. roads, milk associations (CRELs).
- Unsatisfactory experiences leading to bad reputation of silage.
- Aversion to innovation and risk.
- Traditional extensive farming systems.
- Lack of planning and motivation.

Nevertheless, about 13 small scale farmers with 3 to 7 milking cows are presently beginning with silage preparation after its socialization in the field days.

Acceptance of plastic bag silage by farmers. Comments made by farmers reveal that LBS is an attractive technology for its low costs, easy handling, low labor requirement and efficient use of labor, reduced risk of losses due to rapid filling and small size, adaptability of bag size and marketing possibilities. Furthermore, LBS was mentioned to be an alternative to ensile high quality forages of smaller plots whose amount would not be sufficient to fill a large silo (Photo 31).

The main criteria used by farmers for selecting plastic bags were: (1) market availability, (2) cost, (3) resistance and (4) size (handling). After the evaluations, farmers voted for their preferred plastic bags. Small bags were preferred i.e. a green self made bag elaborated from a plastic in tube form and the double layer concentrate bag-plastic bag (calibers 2 and 3). For the big bags, farmers preferred the bag of the same raw plastic material as the self made small bag (caliber 6).

The evaluations revealed that farmers preferred plastic bags of at least 4 caliber and low elasticity (low density) or a concentrate bag in order to facilitate the compaction and reduce the risk of damages and consequent silage losses.

In Table 85 we summarize the main advantages and disadvantages as viewed by farmers of different plastic bags for making LBS.

(a) **(b)**

(c) **(d)** **(e)**

Photo 31. Different bag types elaborated and evaluated during field days. (a) Manual adaptation: Plastic tube is cut to appropriate size and sealed using a hot machete; (b) Evaluation of different silage bags; (c), (d), and (e) use of barrels, garbage cans or (used) particularly thick animal feed concentrate bags in order to facilitate filling, compaction and reduce risk of perforation of plastic bags

Table 85. Advantages and disadvantages of different bag types.

Bag type	Advantages	Disadvantages
Transparent bag (Calibre 3)	- More resistant than rubbish bag - Easy handling - More docile	- Not available in the market - fragile
Little rubbish bag (Calibre 2)	- Available in the market - Cheap - More docile than the bluish one	- Very fragile
Two-layer resistant concentrate bag – plastic bag	- Better than blue transparent bag - More resistant - Double protected - More practical for compaction,	- Higher costs but we can save the concentrate bags
Green small bag self-made (Calibre 6)	- Available in the market - Adaptable in size and form - Better for compaction and air removement - Easy handling	- Higher costs - More time requirement for elaboration of bag
Green big tube bag with barrel (Calibre 6)	- Available in the market - Resistant - The size can be adapted - Safer for compaction - Greater capacity - Less plastic requirement (less costs)	- More air in the sides and ex-tremes (sealing at two extremes) - Too big and heavy (need 2 persons to carry)
Big black rubbish bag (Calibre 4)	- Available in the market - Greater capacity - The bigger the cheaper per kg of silage	- More expensive - Less resistant than green bag - Need for 2-3 persons

4.4.3 Case study: Adoption of silo technologies in the area of Yoro, Honduras

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Rationale

In many parts of Honduras, silage preparation for dry season feeding is a strategy rarely employed by smallholder farmers. Nevertheless there are regions where some farmers have adopted the silo technology but its diffusion is slow. Reasons include high initial investments for the most common large bunker silo inaccessible to smallholder farmers, lack of know-how on silage production and lack of knowledge of appropriate low cost silo alternatives such as heap silo, earth silo and little bag silage (LBS).

In this section we present a successful case of adoption of forage conservation in the forma of silage in Yoro, Honduras and the conditions that favoured diffusion and adoption of silage technology.

Materials and Methods

In 2002, CIAT and its partners identified the need and demand for silo conservation technologies by farmers in the area of Yoro (a reference site for forage related R and D led by CIAT). Silo types such as heap and earth silos and especially little bag silage were offered during farmer trainings and field days in order to catalyze innovation, adoption and dissemination processes of forage conservation technologies with and by small-scale farmers. In this case study we analyze the most relevant factors of the diffusion process at the social system level. An informal expert interview and structured farmer interviews were conducted in order to document this process. Further research will be carried out on the diffusion and the individual adaptation and adoption process of forage conservation technologies influenced by farmer socio-economic conditions.

Results

Factors influencing the spread of silage innovations were among others, promotion of silage technology (farmer training and field days), extension and interaction among farmers, demand and market price of milk in the dry season, presence of milk associations, and key farmers with innovative ideas.

Farmer trainings and field days. CIAT and DICTA have been promoting forage conservation (maize and sorghum) in form of silage and its use since 2002 when a field day including theory and practice about silage making (earth silo) and a feeding demonstration was held in a prototype farm in Victoria. A total of 17 farmers from Victoria, Sulaco and Yorito participated in this event. Additionally, individual farmers visited this farm at the time when the innovator elaborated his silo. In 2003, there was only one silage field day event with about 20 farmers and 10 students from Yoro, Victoria and Sulaco. The following year, CIAT and DICTA carried out further field days in prototype farms addressing topics such as improved pasture, its use and conservation. In subsequent years (2004-2005) courses on forage conservation were carried out in the Yoro area (Yorito, Yoro, Sulaco and Victoria) with special focus on plastic bag silage. For more details on the events and their results see section 4.2.1 of this report.

Development of silage use in the Yoro area (regions of Yoro, Yorito, Sulaco and Victoria). In Figure 69 we illustrate the increase of farms using silos in the different regions around Yoro over time. The diffusion process began in Sulaco and Victoria in 2002, originating from 11 farms with silo of which four were "fincas prototipo", i.e. farms where new forage technologies are introduced, monitored, evaluated and scaled out. Since then, the adoption rate has followed a linear increase. In Yorito and Yoro this process started two years later with a sharp increase of the number of farms using silo.

The curve in Figure 70 represents the aggregated number of farms making silo in the Yoro area. There is not only an increasing number of silo farms (190% in the last year) and total silage production (220% in the last year), but also an

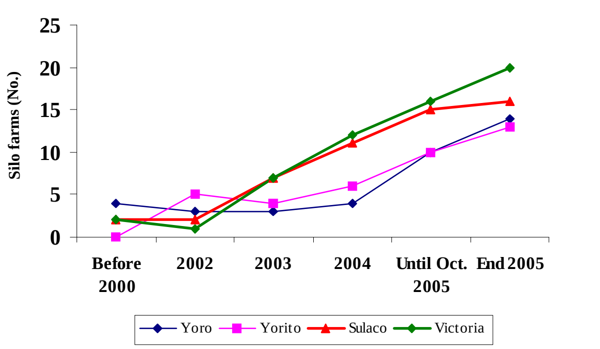

Figure 69. Number of silo farms in different regions of Yoro.

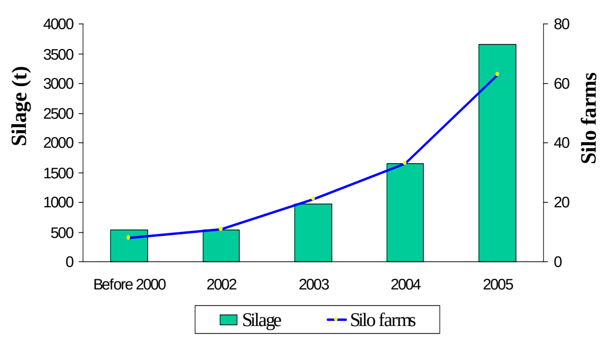

Figure 70 Start up phase of silo diffusion process in the Yoro area: number of silo farms and silage produced.

increase in silage production per farm over the last years (e.g. from 46 t in 2003 to 58 t per farm in 2005). Some innovators even tripled their silage production since 2003.

In 2003 and 2004, there were only medium and large scale farmers using silo. Currently, 13 small scale farmers in the region with 3 to 7 milking cows are starting to use mainly heap and earth silo as well as little bag silage. Since 2005, farmers also started to ensile improved forages such as *B. brizantha* (cv. Toledo) and *Brachiaria* hybrid (cv. Mulato) as well as the shrub legume *Cratylia argentea*.

Presently, there are 14 farmers in Yoro, 13 in Yorito, 16 in Sulaco and 20 in Victoria who integrated silage technology as part of their dry season feeding strategy. About 3.700 t from about 130 ha of mainly maize and sorghum are ensiled in the area as feed for more than 1000 heads of cattle over a 6 months dry period (assuming a consumption of 20 kg/head/day).

(a) Bunker silo **(b) Heap silo**

(c) Earth silo **(d) Little bag silos**

Photo 32. Different silo types: (a) Common silo type, and alternatives (b, c, and d).

Silo types. The most common silo type used in the area is a bunker silo constructed of bricks and/ or concrete with a size of 8 x 4 x 2 m (capacity of about 35 tons of ensiled forage) and an opening at the front side. In Yorito and Sulaco, some of these silos are with roof to protect the silage from water and direct sunlight (Photo 32). In 2003, this silo type was still used by 92% (24 out of 26) of the farmers compared to 50% in 2005. As illustrated in Figure 71, the use of alternative silo types such as little bag silage (LBS), earth and heap silos has increased in the last two years with a present share of 11%, 22% and 17%, respectively.

Silo farmers and CREL (Centro de Recolección y Enfriamiento de Leche)**.** In Yoro, Yorito and Victoria some livestock farmers (20, 25 and 21 respectively) are affiliated to milk associations (CRELs). Milk is stored and cooled in these centres and subsequently delivered to a milk processing enterprise (“SULA”).

By the end of 2001, farmers in these regions got together and started the process of organization, legalization and training. In Yoro, the CREL group has been delivering milk to SULA since June 2003, in Yorito and Victoria since February 2004. Farmers associated to CRELs can deliver every amount of milk at a fixed and steady price for both the dry and rainy season, which in 2005 was 0.32 $US (5.93 Lempiras) per liter for Yorito and Victoria and 0.34 $US per liter for Yoro.

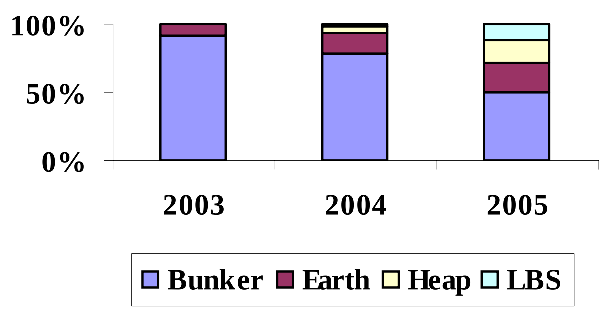

Figure 71. Percentage of different silo types over years

In Victoria and Yorito about 40% and in Yoro about 63% of the farmers in the CREL group have a silo. In total, about half of the farmers (47%) who are affiliated to a CREL group feed silage in the dry season 2005/2006 (first column in Figure 72). Considering all farmers in the Yoro area who have a silo, 53% are affiliated to a CREL (second column in Figure 72). In Victoria and Yoro, about 45% and in Yorito 67% of CREL farmers have a silo. In Sulaco, about 15 farmers are affiliated to a group called AGASUL (Asociación de Ganaderos y Agricultores de Sulaco, founded in the 80s). Milk is sold at the local market at a price of about 0.22 $US (4 Lempiras) per litre in the rainy and about 0.30 $US (5.50 Lempiras) in the dry season. About two thirds of the group have a silo.

Discussion

Promotion of Innovation. It is evident from the Yoro case study that activities like farmer training and field days have an accelerating effect on the diffusion of silage technology. Many farmers mention that there is a lack of know-how concerning silage making. Awareness creation on dry season forage scarcity, its consequence on animal productivity (e.g. milk production, liveweight gain and fertility), the transfer of silage technology as well as the demonstration on how to elaborate silage are very important for the adoption of the technology. Subsequently, farmers can adapt the silo type to their specific situation. The most frequently used bunker silo was first adapted by an innovative farmer (Oscar Nuñez) who participated in a field day where an earth silo was demonstrated. He wanted to construct it with concrete walls and an extended roof at one side to have a feeding place for his livestock. Although this silo type was not promoted specifically (since it requires high investment) farmers nearby adopted it.

In 2004 and 2005, alternative silo types like plastic bag silage, earth and heap silos were offered and this had an immediate effect on the number of farmers who got interested in making silage.

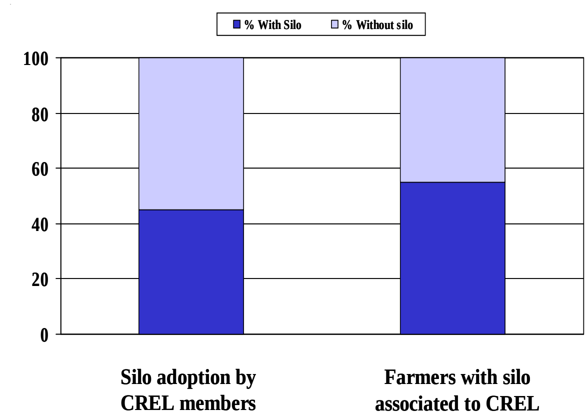

Figure 72. Relation of silo adoption to CREL affiliation in the Yoro area

Interaction and communication. Some argue that the speed of the diffusion process of a successful innovation of a technology is mainly determined by the extent of the interaction between the farmers who have already adopted and potential adopters. Livestock farmers in Honduras affiliated to milk associations like the CRELs as well as other farmers in these regions have permanent contact with each other and most of them exchange their experiences, especially those concerning improved production systems, almost every day.

Although there appears to be a relation between the existence of CREL (and the secured purchase of milk at a fixed and higher market price) and silage use, this may not have been the decisive factor as a similar diffusion process can be observed in Sulaco where no CREL exists. Furthermore, in Victoria and Yorito the CRELs deliver milk since February 2004 but the silo diffusion process in Victoria already started two years before. Another argument is that in the CREL group there are as many farmers using silo as there are without silo. Nevertheless, only considering Yorito and Yoro, there is a coincidence of the existence of CRELs and the diffusion of silage technology.

Key persons. According to some authors it is important for the speed of diffusion of a given technology whether the innovators are at the same time respected communicators. One of the pioneer innovators in the region whose farm is one of the prototype farms in Victoria is additionally working with CIAT and DICTA since 1996 in the forage area as technical and scientific assistant and extensionist. He is well known in the region and is regarded more as a farmer than a technician. He visits farms and farmers come to him in order to pick up information, advice and recommendations. He undoubtedly can be regarded as an innovator, key communicator and influential at the same time not only within the social system group and community but also at higher system levels connecting the regions and groups through communication and exchange. But this is not the only case. Other farmers like e.g. Oscar Nuñez, another "prototype" farmer, fulfil these conditions for the social system level group (CREL) and community (Yorito).

Projected adoption of silage technology. The curve shown in Figure 69 illustrates a slow initial adoption of silage technology but a subsequent steep increase indicating the beginning of a diffusion process. Considering that silo technology starts to be transferred from farmer to farmer and that an estimated 10-20% of the livestock farmers in the regions of Yorito, Sulaco, Victoria and Yoro will have a silo by the end of 2005, it can be hypothesized that silo diffusion will continue independently from any further interference. However, results need to be further validated since there are 30 farmers implementing silage technology for the first time in 2005/2006 and the outcome and acceptance first have to be confirmed, especially for the small scale farmers using alternative silo types.

The projected adoption of little bag silage technology for small scale as well as for medium scale farmers depends on the success with the technology in at least some cases. Success will be determined by: (1) the elaboration process (e.g. type and humidity of the forage used, type of bag, additives used, compaction, sealing) which will have consequences for the quality of the silage (e.g. smell, presence of moulds and acceptance by the animal), and (2) the storage management (e.g. in a closed room) in order to protect the bags from rodents, insects and sunlight. Successful cases with plastic bag silage could then be used for the further dissemination of the technology by a guided interaction between first adopters and those who have not yet adopted (farmer to farmer transfer of technology).

In general, based on comparison with the literature and the observed situation in the Yoro area, it can be concluded that:

- Farmers' participation in the meetings, their interest in forage conservation as well as the annual silo adoption rate demonstrate the demand for silo technology.

- Earth and heap silos as well as plastic bag silage seem to be appropriate for adoption by small scale farmers.

- Adoption, adaptation and innovation of alternative silo types by smallholders are likely to continue and expand in the next years.

- Silo technology requires precise instruction either by technicians or by experienced farmers in order to reduce the risk of failure and subsequent discouragement.

- Research and/or development projects can initiate and accelerate the diffusion process of silage technology by e.g. farmer trainings and field days with multi-actor exchanges.

- After a certain level of adoption the diffusion process is likely to continue independently from any outside interference.

- Farmer associations like CREL foster farmer exchanges and transfer of technology but are no prerequisite for the diffusion of forage conservation adoption or innovations.

- Key persons like innovators who have an influence on the decision making of other farmers can accelerate the innovation, adoption and diffusion process.

4.4.4 Case study: Innovation of the bag silage technology by farmers

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Case study-Ignacio Sanchez, Olancho: Earlier, the farmer fed maize silage produced in earth and heap silos, but he discontinued the use of silage and is now feeding Mulato hay. He argued that it is more comfortable, practical and cheaper to make hay (elaborated with machinery). A neighbour told us that this farmer was the silo pioneer in the region, but he discontinued its use it because his silo had fungi and some cows died feeding on it.

In 2004, he had 1000 bales of hay and with this he maintained his milk production and his cows. The cost to elaborate the hay is about 0.6US$ per bale and the market price is about 1.5US$/bale.

In 2003, in collaboration with DICTA Mr. Sánchez elaborated 25 bags of Cratylia silage of which two were ruined in the end and were not fed. The others had fungi on the top layer (2.5 kg) that was still fed after sun drying. He prepared most of the bags without molasses with one day of dehydration and preferred them to those with molasses ("helps to compact, works better"). The cost was estimated to 25-30 Lempiras/bag (1.2-1.6 US$).

Presently and motivated by CIAT and DICTA, he is elaborating plastic bag silage again (Photo 33) which will serve for a milk production experiment and additionally he wants to sell some bags.He is ensiling Cratylia and not corn or sorghum as most farmers. For him, the advantages of the Cratylia silage bags are the time flexibility in its elaboration and its feeding to calves and weak cows.

Case study-Rigoberto Nolasco, Juscarán: The owner of another prototype farm (DICTA) began feeding silage in the 80's. He filled a large bunker silo and later a heap silo every year. In 2004, he was told about the little bag silage technology and experienced a successful case in another region which convinced him. Presently and for the first time, he is elaborating big bag silage and invented the use of a barrel which eases the compaction and protects the plastic bag from perforation.

The silage consists of a mixture of various species of cut and carry grasses like *Pennisetum purpureum* cultivars Camerun and King Grass, *Panicum maximum* (Mombaza) and the legume *Cratylia argentea*. Salt and citrus fruits (orange and/or lemon) cut in half are added as preservatives in layers (Photo 34). Molasses which was said to be only available for a short period after the harvest of sugar cane is not added. Presently, 100 bags of about 100 kg each are elaborated and another 200 bags will follow.

Problems with the large silos he used to make were the long time requirement to fill (one week), high costs, high labour requirement in times of labor scarcity, effort, bad smell, silage drainage problems and the soiling of the silage caused by muddy water coming from the slope above. He mentioned that he does not have these problems

Photo 33. Silage bags of Cratylia in 2004

Photo 34. Silage of different species and mixture with fruits.

with the bags. Furthermore, he does not have extra costs for labor; the workers who are paid on day basis can additionally fill some bags. Another advantage of the bags mentioned is that once opened a bag, the silage is used immediately and because of that there are less losses due to prolonged exposure to air and the consequent spoilage than with a large silo. A disadvantage of the bag silage was the high cost of the bag (0.46 US$) and the higher cost per kg of silage compared to a big silo.

Case study-Antonio Polanco, Yoro. A farmer who had participated in a field day in Yoro in September 2005 prepared on his own initiative the first 15 plastic bag silage. These bags were small and he said that the forage ensiled (*Pennisetum purpureum*) had a high water content so that liquid leaked out of the bags. After the second field day, he prepared 20 larger bags of about 60 kg and dehydrated the material for some hours. He mentioned as an advantage that the dehydrated silage does not weigh that much. The silage was prepared without any additives except for one bag in which he added ground maize as an experiment (Photo 35). The plastic tube used is very resistant, adaptable in size and locally available to a price of 10 Lempira (about 0.5 US$) per yard. Compaction is done with a wooden stake and at the margins carefully by hand in order to prevent damage of the plastic. On each bag the date of production is noted down in order to control the smell and the acceptance by the cows after a specific time. The first feeding experience revealed acceptance of the silage by the cows. He noted that the effort and cost is only worth it if high quality forage is used.

The smell of the silage of an opened bag was slightly acetic. Thus, we suggested adding some molasses or other sugar-containing products in order to improve fermentation and agreed on an experiment of different additives: dissolved sugar, cut sugar cane, orange pulp and ground maize.

Constraints for the adoption of the bag silage technology

Initial results reveal the following constraints expressed by farmers for the use of bag silage:

1. Bags are susceptible to infestations by rodents, ants, worms, and insects that damage the bags and lead to growth of fungi and consequent losses of silage in quantity and quality (Photo 36).

Photo 35. Silage of different species and mixture with maize.

Photo 36. Worms and fungi in LBS

2. Plant stems can perforate the bags and the holes have to be sealed by an adhesive tape.
3. Little availability of adequate bags in the rural areas.
4. Suitable resistant plastic bags are expensive.
5. Compaction, sealing and transport have to be done with great care in order not to damage the bags.
6. Higher cost per unit of silage.
7. Proportional quality losses are usually greater than in large silos due to proportionally greater margin area per unit of silage and insufficient compaction at the sides.

Experience and innovation with hay

In November 2004, 1 kg of *Brachiaria* hybrid cv. Mulato was planted in a selected plot (about 875 m²). Two months later, the farmer transplanted Mulato for the first time, another 6 weeks later for the second time. The area was about 2400 m² (total area of 3275 m²) and was irrigated in 5 days interval during the dry season. The cost of transplanting was 700 Lempira (about 38 US$). The farmer was trained on hay making including technical aspects on cutting, drying (including turning) and elaboration of bunches (5-10 kg). In March 2005, the farmer elaborated the first hay from the area which was planted with the grass (Photo 37). He dehydrated the material in the sun for 2 days before feeding it to the cows and calves. We recommended cutting the grass at 10-15 cm since it was cut too low affecting its sprouting potential. In August, he reported that he had to fertilize the field twice since plants appeared yellowish. After two applications of about 240 kg of urea fertilizer, Mulato grew vigorously.

In the rainy season, the grass was cut at 4 week intervals and carried to an unused pig barn for final drying, since excessive rain did not allow proper sun-drying. The grass was spread on wooden sticks bend over the divisions of the pig stable. When the farmer considered the material as dry, he bound it in bunches and put it into the small storing room in the same pig stable building.

However, part of the stored hay was considered to be not dry enough in the centre of some bunches. Thus the grass had to be removed and

(a)

(b)

(c)

Photo 37. (a) Mulato established; (b) hay of Mulato (c) bunches of Mulato spread outside in the sun for one more day. It was recommended to cut early in the morning of days with less probability of rainfall and to cut only the amount that could be dried and managed in time. Furthermore, it was pointed out that although the material may appear dry, residual moisture may remain inside the thick stalks damaging the stored hay. It was demonstrated how to estimate the dry matter content by a wring probe.

A constraint to manual hay making of Mulato is that farmers do not like handling the material due to the pricking characteristic of the fine hairs which cause burning skin irritations. In addition in the rainy season 2005, it was very difficult to make hay since rainfall was abundant, and Mulato requires at least two days of sunshine for proper drying. In case of daily precipitation, silage seems to be the more appropriate alternative for conservation.

In summary, based on the case studies and interviews to farmers, the following points can be drawn:

- The main problem in the dry season stated by farmers is forage scarcity leading to low(er) milk production.
- Farmers are interested in and demand for forage conservation technologies.
- For small scale farmers, the estimated milk yield increase if supplemented with silage would be about 1.1-1.9 liters/cow/day (34-74%).
- In spite of constraints of bag silage technology, it is an attractive alternative for small as well as for medium scale farmers. It is adaptable to local conditions and its advantages (like for example the flexibility, low labour requirement and low investment cost) favour its adoption at a larger scale.
- Favouring conditions for the adoption of bag silage technology are, beside others, the availability of a chopper, improved forages and milk races, adequate promotion, the availability of appropriate plastic bags and the existence of innovators.

4.4.5 Economical benefits of feeding hay and silage during the dry season on commercial dual-purpose farms in Central America

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The objectives of this study were to (a) estimate and compare the increase in milk and beef production due to feeding hay and silage during the dry season, (b) estimate and compare the production costs of making silage and hay, and (c) estimate and compare the net benefits as a result of feeding silage and hay.

In Honduras thirteen farmers were personally interviewed: seven farmers who produced silage and six farmers who produced hay. All surveyed farms were located in Yoro and its surroundings, a region with a prolonged dry period. In Costa Rica nine farmers were interviewed: seven who produced silage and two who produced hay. Two farmers were selected in the Esparza-Puntarenas region and seven farmers in the Nicoya Peninsula, both regions on the Pacific Coast with a prolonged dry season.

General information on the silage and hay

In Tables 86 and 87 we present general information on farms using silage and hay in both countries. Farmers in Yoro had to deal with a longer dry season and therefore, the use of conserved feedstuffs was desired for a longer period. Hence, as can be seen in Table 86, farmers in this part of Honduras compared with farmers in Costa Rica, allocated more land to

Table 86. General information on silage.

	Honduras (n=7)	Costa Rica (n=7)
Used forage (% of farms)		
Silage made of corn	42.9	71.4
Silage made of (corn and) *Cratylia*	14.2	28.6
Silage made of corn and other forages	42.9	0
Used type of silo (% of farms)		
Little bag silage	0	14.3
Monton silage	14.3	85.7
Bunker silage	85.7	0
Amount of land allocated to produce silage (ha)	3.9	1.1
Yield (mt/ha)	23.9	16
Yield as DM (mt/ha)	8.4	5.6
Size of silo (m^3)	134.1	24.3*
Construction costs (US$/$m^3$)	19.08	0
Waste (%)	4.5	2.7
Duration of silage in store (months)	6	3
Years producers have been feeding silage	6	7
Producers in neighbourhood using silage (%)	16.3	6.3

*n = 6

make silage (i.e. 3.9 ha vs. 1.1 ha) and fed it for a longer period (i.e. 6 mo vs. 3 mo). Likewise, Table 87 shows that farmers in Honduras compared with farmers in Costa Rica, allocated more land to produce hay (i.e. 6.4 ha vs. 3.5 ha) and fed this for a longer period (i.e. 6 mo vs. 5 mo).

Results in Table 86 indicate that in Honduras the yield of silage was higher than in Costa Rica (i.e. 24 mt/ha vs. 16 mt/ha). This could be explained by the use of a combination of maize and sugarcane and/or sorghum by 43% of farmers in Honduras. A study executed in Honduras and Nicaragua revealed that sugarcane had a yield 80 mt/ha, while maize had 33 mt/ha. Sorghum had a yield of 45-50 mt/ha. Table 87 shows that in Costa Rica the yield of hay was higher than in Honduras (i.e. 10.5 mt/ha vs. 8.2 mt/ha). In Costa Rica and Honduras, on average, the resting period before harvesting the pasture was 90 and 37 days, respectively. The longer resting period in Costa Rica resulted in a higher yield.

The results of this study suggest that in Honduras the adoption rate of silage was higher than of hay, while in Costa Rica the adoption rate of hay was higher. In Honduras, the interviewed farmers mentioned that 16% of the farms in their vicinity utilised silage and 6% utilised hay, while this percentages in Costa Rica were 2% and 20%, respectively (Tables 86 and 87). As can be seen in Table 86, farmers in Honduras chose to construct an expensive and durable type of silo; 86% of farmers used bunker silo compared to 14% who used a monton silo. Contrarily, 86% of farmers in Costa Rica used a monton silo. On the other hand, farmers in Costa Rica constructed larger and more expensive stores to stockpile their hay than farmers in Honduras despite the fact that farmers in Costa Rica produced less of it.

Costs of hay and silage

In Tables 88 and 89 we present the costs of silage and hay. The costs of making silage were subdivided in labour costs (costs for clearing and preparing the land, applying fertilizer, planting, controlling weed and harvesting), machinery costs, and other costs (ie., herbicide, seed, fertilizer, additives, and plastic). The costs of making hay were subdivided in labour costs (although labour costs most of the time were included in the rental costs of machinery), machinery, and other costs (ie., fertilizer).

Table 87. General information on hay.

	Honduras (n=6)	Costa Rica (n=2)
Used forage (% of farms)		
Hay made of improved grass	66.7	100
Hay made of (improved grass and) corn	33.3	0
Amount of land allocated to produce hay (ha)	6.4	3.5
Yield (mt/ha)	8.2	10.5
Yield as DM (mt/ha)	7.2	9.2
Size of storing facility (m^3)	298*	480
Construction costs of storage facility (US$/$m^2$)	15.62	25.89
Resting period before harvesting hay (days)	37*	90
Duration of hay in store (months)	6	5
Price of hay (US$/mt)	46.55*	93.96
Years producer has been feeding hay	5	10
Producers in neighbourhood using hay (%)	2.0	20

*n = 5

Silage. In Table 88 we show the costs of making silage in Honduras and Cost Rica (i.e. $16/mt and $46/mt, respectively). The production costs of silage was significantly lower in Honduras than in Costa Rica, mainly explained by the lower labour cost. The higher labour costs in Costa Rica were due to more expensive labour (the salary of a worker was $8.47/day, compared with $2.57/day in Honduras) and an higher amount of labour per hectare (i.e. 32 man-days/ ha vs.18 man-days/ha in Honduras). In Costa Rica more labour was involved in land clearing (i.e. 20 man-days/ha vs. 2 man-days/ha in Honduras). This could be explained by the fact that three smallholders produced silage for the first time, and therefore had to clear the land thoroughly. In Costa Rica also more labour was allocated to planting (i.e. 7 man-days/ha vs. 2 in Honduras) because of the higher amounts of seed used. In addition, in Costa Rica more labour was engaged in harvesting, transporting and filling the silo, although the yield (mt/ha) was lower compared with Honduras (i.e. 16 mt/ha and 24 mt/ha, respectively, see Table 88).

Machinery costs were also lower in Honduras than in Costa Rica. This was because the rental costs of a tractor or cutter were lower and because of the use of oxen instead of tractors by about half of the farmers in Honduras.

Furthermore, silage-using farmers in Costa Rica used more expensive management practices than farmers in Honduras: (a) almost every interviewed farmer in Costa Rica used herbicides, while in Honduras just about half of farmers applied it, (b) farmers in Costa Rica applied more seed (24 kg/ha vs. 19 kg/ha) and (c) farmers in Costa Rica used higher amounts of fertilizers per hectare (386 kg/ha vs. 226 kg/ha in Honduras). In addition, the yield (mt/ha) of silage was lower in Costa Rica (Table 86). Therefore, the costs, which were independent of the quantity of silage (like costs of cleaning the land) were more expensive than in Honduras.

Table 88. Costs of silage.

	Honduras (n=7)	Costa Rica (n=7)
Costs of making silage		
Labour costs ($/mt)	6.32	23.57
Machinery costs ($/mt)	3.73	7.82
Other costs ($/mt)	6.43	14.24
Total costs ($/mt)	16.48	45.63
Total costs as DM ($/mt)	47.08	130.37
Costs of feeding silage ($/head/day)	0.21	0.33
Costs of feeding silage ($/farm/year)	511.56	565.19

All costs are expressed in US dollars.

Hay. In Table 89 we show the costs of making hay in Honduras and Costa Rica ($20/mt and 39/mt, respectively). The higher cost of producing hay in Costa Rica was due to the high machinery costs. The rent of machinery for baling (with labour costs included) was more expensive in this country. Two farmers in Honduras did not bale the hay, but stored it as a heap, which was a cheaper option than baling. Comparing the costs of silage and hay, it appeared that in the case of hay the machinery costs were much higher. This was because labour costs were (in almost all cases) included in the rental costs of machinery. On the other hand, other costs were lower for hay production. This could be explained by the use of improved pastures. Farmers didn't need to buy seed and didn't use herbicides, which were necessary for silage.

Comparing figures in Tables 88 and 89 revealed that in Honduras the cost of producing silage was higher than the cost of producing hay (i.e. $47/mt DM vs. $23/mt DM). The feeding costs were lower when hay was fed (i.e. $0.19/cow/day vs. $0.21/cow/day), although the amount fed was higher (i.e. 6.2 kg DM/cow/day vs. 4.1 kg DM/cow/day). In Costa Rica, the cost of producing hay was lower than the cost of producing silage (i.e. $44/mt DM vs. $130/mt DM). The feeding costs were lower when hay was fed (i.e. $0.19/cow/day vs. $0.33/cow/day), although the amount fed was higher (i.e. 4.4 kg DM/cow/day vs. 2.7 kg DM/cow/day). Thus, these data demonstrated that, both in Honduras and Costa Rica, feeding hay was cheaper than feeding silage.

Benefits from feeding hay and silage

Based on the increased milk production or the maintained body weight and the prices of milk or beef, the net income and net benefit due to feeding hay and silage were calculated. In Tables 90 and 91 these benefits are given. As described before, due to higher amounts of silage fed in Honduras, farmers obtained a higher increase in milk yield. Additionally, profits from milk were higher in Honduras due to higher milk prices (i.e. $0.29/lt vs. $0.24/lt in Costa Rica). Therefore, as shown in Table 90, the net income due to feeding silage was higher in Honduras than in Costa Rica (i.e. $0.93/cow/day vs. $0.52/cow/day). The same was true for hay (Table 91). Farmers in Honduras fed more hay and therefore, obtained a higher increase in milk yield. Consequently, the net income due to feeding hay was also higher in Honduras than in Costa Rica (i.e. $1.16/cow/day vs. $0.35/cow/day), although it has to be mentioned that the net income due to feeding hay

Table 89. Costs of hay.

	Honduras (n=6)	Costa Rica (n=2)
Costs of making hay		
Labour costs ($/mt)	13.67*	NA
Machinery costs ($/mt)	13.05	34.6
Other costs ($/mt)	5.01	4.37
Total costs ($/mt)	20.34	38.94
Total costs as DM ($/mt)	23.11	44.25
Costs of feeding hay ($/cow/day)	0.19**	0.19
Costs of feeding hay ($/calf/day)	0.03**	
Costs of feeding hay ($/farm/year); cows	447.69**	990.65
Costs of feeding hay ($/farm/year); calfs	61.99**	

All costs are expressed in US dollars.

*n = 1

**n = 3

NA = Not Available. Labour costs are included in the rental cost of machinery

Table 90. Benefits from feeding silage.

	Honduras (n=7)	Costa Rica (n=7)
Milk		
Price of milk in dry season ($/lt)	0.29	0.24
Income from milk without silage ($/cow/day)	1.83	0.36*
Income from milk with silage ($/cow/day)	2.76	0.88*
Net income due to feeding silage ($/cow/day)	0.93	0.52*
Net income due to feeding silage ($/farm/year)	3318.30	725.93*
Income:cost-ratio	6.1	1.6
Net benefit due to feeding silage ($/cow/day)	0.72	0.20*
Net benefit due to feeding silage ($/farm/year)	2806.74	512.03*
Beef		
Price of cow meat ($/kg)	1.14?	0.9??
Price of calf meat ($/kg)	1.2?	0.94??
Income loss due to not feeding silage ($/head/day)		0.68**
Net income due to feeding silage ($/head/day)		0.68**
Net income due to feeding silage ($/farm/year)		1485**
Benefit:cost-ratio		2
Net benefit due to feeding silage ($/head/day)		0.45**
Net benefit due to feeding silage ($/farm/year)		1052.07**

All costs are expressed in US dollars.
*n = 5
**n = 2

in Costa Rica was based on just one farmer, therefore, this number was may not be representative.

Farmers in Honduras fed more hay to their milking cows than silage. Therefore, farmers realised a higher increase in milk yield and, as Tables 90 and 91 show, a higher income due to feeding hay (i.e. $1.16/cow/day vs. $0.93/cow/day). In Costa Rica the silage-using farms achieved higher increases in milk yield than the hay-using farmer, which resulted in a higher income (i.e. $0.52/cow/day and $0.32/cow/day, respectively).

As shown in Table 90, the income-cost ratio of feeding silage [net income ($/cow/day) divided by total costs ($/cow/day)] of farms with milking cows was positive in both countries; 6.1 and 1.6 respectively. Also the net benefit due to feeding silage [net income ($/cow/day) minus costs ($/cow/day)] of farms with milking cows was positive in both countries; $0.72/cow/day and 0.20/cow/day, respectively. This indicated that feeding silage to milking cows was profitable in both countries. In Honduras, the lower production cost and the higher net income explained the higher income-cost ratio and net benefit on farms with milking cows compared with Costa Rica. In addition, due to the higher amount of milking cows fed during more months, the net annual benefit due to silage was much higher in Honduras than in Costa Rica ($2,807/farm/year and $512/farm/year, respectively). This same scenario was true for the case of hay in both countries (Table 91). Comparing Tables 90 and 91 revealed that in Honduras the income-cost ratio of hay was higher than of silage (i.e. 9.1 vs. 6.1). Also the net benefit due to feeding hay was higher (i.e. $0.97/cow/day vs. $0.72/cow/day). Therefore, the annual net benefit from feeding hay (i.e. $6,885/farm/year) was higher than from silage (i.e. $2,807/farm/year) and appeared therefore to be more profitable. Thus, the low adoption rate of the use of hay in Honduras, as described before (2% vs. 16% of silage, Table 86), seemed not logical.

Table 91. Benefits from feeding hay.

	Honduras (n=6)	Costa Rica (n=2)
Milk		
Price of milk in the dry season ($/lt)	0.29	0.24
Income from milk without hay ($/cow/day)	2.06**	0.75*
Income from milk with hay ($/cow/day)	3.22**	1.10*
Net income due to feeding hay ($/cow/day)	1.16**	0.35*
Net income due to feeding hay ($/farm/year)	7684.80**	945*
Income:cost-ratio	9.1**	1.9*
Net benefit due to feeding hay ($/cow/day)	0.97**	0.17*
Net benefit due to feeding hay ($/farm/year)	6884.90**	419.85*
Beef		
Price of cow meat ($/kg)	1.14?	0.9??
Price of calf meat ($/kg)	1.2?	0.94??
Income loss due to not feeding hay ($/head/day)	0.28**	0.45*
Net income due to feeding hay ($/head/day)	0.28**	0.45*
Net income due to feeding hay ($/farm/year)	1220.40**	3240*
Benefit:cost-ratio	16.59	2.2*
Net benefit due to feeding hay ($/head/day)	0.25**	0.24*
Net benefit due to feeding hay ($/farm/year)	1158.41**	1751.76*

All costs are expressed in US dollars.
*n = 1
**n = 3

A comparison within Costa Rica revealed that the income-cost ratio of hay was higher than of silage (i.e. 1.9 and 1.6, respectively), although the net benefit due to feeding silage was higher (i.e. $0.20/cow/day vs. $0.17/cow/day) due to the fact that the interviewed silage-using farms in Costa Rica were all low-milk yielding farms, while the hay-using farmer realised a higher milk production. Because silage-using farmers fed this feedstuff to more cows, their net benefit ($/farm/year) was higher than the net benefit ($/farm/year) of hay-using farmers ($512 and $420/farm/year, respectively). Again, it has to be mentioned that the net benefit due to feeding hay in Costa Rica was based on just one farmer; therefore, this number may not be representative.

In the case of beef production, in Honduras, the net income and net benefit due to feeding hay were $0.28/calf/day and $0.25/calf/day. The income-cost ratio was 16.6. In Costa Rica the net income and net benefit due to feeding silage were $0.68/cow/day and $0.38/cow/day and due to feeding hay were $0.45/cow/day and $0.24/cow/day. The income-cost ratio was 2 in the case of silage and 2.2 in the case of hay. These results indicate that feeding silage in Honduras and hay or silage in Costa Rica to young and mature non-milking animals was profitable.

Market potential

Hay. All interviewed farmers in Honduras and Costa Rica suspected that other producers were willing to buy the surplus of hay. Except for one farmer in Honduras, all farmers knew the selling price. On average, this was more than twice the price of producing it; $47/mt in Honduras and $94/mt in Costa Rica. This indicates that farmers were aware of the market value or their hay. In Costa Rica, actually, the two interviewed farmers sold hay to neighbours. In Honduras, the adoption rate of hay was low. However, the income-cost ratio and net benefit due to feeding hay was higher than silage, which possibly will bring along a increased demand, higher adoption rate and the development of a market. However, at this moment, just informal sales on very small scale occurred.

In Costa Rica, the adoption rate of hay, compared with silage, was high. Since many years, bales of hay mostly from rice straw or from "Transvala"grass (ie., *Digitaria decumbens*) had been marketed. In most cases, the demand, and not the quality and price, played an important role.

The ministry of Agriculture and Livestock of Costa Rica developed, in cooperation with agriculture organisations, a program to stimulate the production and sale of high quality hay. Possibly, due to this program, the hay market will expand in Costa Rica. Currently, the development of a national market for hay is also part of a project of the Instituto Nacional de Innovación y Transferencia de Tecnología Agropecuaria (INTA). The development of this market is based on auctions, where hay with different prices, which reflect different qualities, is auctioned and sold to the higher bidder. In April 2005, the Asociación de Productores Agroindustriales de Bagaces (APAIB) held the first auction in Costa Rica, where six farmers sold hay (between 150 and 250 bales per farmer). The hay was classified in three different qualities with prices ranging from $0.11/kg for the highest quality (i.e. 9% CP and 55% DIVMS) to $0.08/kg for the lowest quality (i.e. 4% CP and 35% DIVMS). The APAIB intends to have another auction in February 2006, with 5 different types of qualities and corresponding prices and with a higher amount of hay.

Silage. All farmers in Honduras and Costa Rica assumed they could sell an excess of silage to other producers. However, farmers didn't have a surplus and therefore didn't sell it. Most of the silage-producing farmers didn't know the selling price. It appeared that neither in Honduras nor in Costa Rica existed a market for silage. This was possibly due to the fact that it is cumbersome to transport, which makes it difficult to market. Introducing the little bag silage technology seems promisory as this constraint can be solved.

In Honduras the adoption rate of silage was higher than hay. However, the income-costs ratio and net benefits due to feeding hay were higher. This meant that the use of hay was more profitable. Therefore, the adoption rate and demand for hay may increase in the future at the expense of silage.

In Costa Rica, although the use of silage was profitable, its adoption rate was low. Opposite to the efforts which are made to develop a market for hay, no attempts are made to develop a market for silage.

4.5 Adoption of forages-based technologies in Central America

Highlights

- Documented that in positive deviance farms (successful adopters) included in a survey it is posible to maintain or even increase milk yield in the dry season with proper feeding strategies.

- To assist in the extrapolation of results the interrelation of spatial factors with pathways of decision-making among farmers is being analyzed along an altitudinal gradient in Olancho, Honduras.

- The adoption of cowpea in a reference site in Honduras has been low, mainly due to lack of seed and lack of market for the grain and fodder.

- The prototype EMPRASEFOR former-led seed company in Honduras expanded the seed plots by 70% as a result of an alliance with a large seed company who will purchase the seed at an agreed price.

- The farmer group EMPRASEFOR has joined its formal enterprise.
- Baseline data for farming systems analysis available for most tipical climatic zones of Olancho.

4.5.1 Potential for adoption of forage-based technologies in representative climatic zones of Honduras: Base line study

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Rationale

Farmers in Honduras located in areas with a prolonged dry season face severe animal nutrition problems. The length of the dry season, up to 8 months in the driest parts of the country, is roughly equivalent with the period of forage shortage in the typical Honduran farm. Thus, in many small and medium size farms, milk production ceases during most of the dry season. Livestock malnutrition increases risk of animal losses and diseases and decreases performance in meat production and in reproduction.

Despite widespread awareness among farmers concerning dry season forage shortage and low livestock productivity, few farmers have adopted multipurpose forage-based technologies. By adopting one or several options out of the available range such as drought tolerant germplasm or forage conservation technologies, farmers can overcome fodder shortages period and eliminate forage shortage in the long term in a sustainable way. Initial adoption may start on a small scale e.g. by dedicating a small investment to plant an improved grass and a shrub legume. The benefit gained from this can be used for a stepwise increase of the area of improved grasses. Once improved grasses are available, their conservation as hay or silage could be the next step to eliminate forage shortage.

The underlying reasons for the decision to adopt improved forages can be many: the socio-economic situation of the farms, and non-economic reasons of decision-making, such as traditions, different ways of seeing the problem and potential solutions. The assessment and comparative socio-economic analysis of bottlenecks and opportunities for adoption of improved forage will give an insight on principles and pathways to introduce feeding systems into smallholder farms.

In the analysis, a ‘typical farmer’ and the early or successful adopters (positive deviances from normality) are considered in two separate groups to enable researcher to potentially identify enabling conditions and actions required for the adoption of forage technologies.

Materials and Methods

The potential for adoption of multiple purpose forage-based technologies in representative climatic zones of Honduras is being assessed by means of a financial farming systems analysis. A comprehensive socio-economic data collection must take into account the diverse structures of farms and how livestock holders use wide a range of feeding strategies. A holistic farming systems approach is most suitable to assess decision making of farmers and to assess possibilities for adoption of multi purpose forage options.

In some farms, feeding strategies may include native pasture and crop-residue grazing, silage, hay, cut-and-carry forages, and purchased inputs. Other farms may only rely on native pasture. In addition, farmer’s decision making usually depends on more factors than livestock and livestock feeding alone, such as for example availability of resources like land, labor, cash and food security. To define which areas of the farming systems had to be included in the data collection, a contextual analysis was carried out in which we looked at dry season problems and areas of the farm are important for income generation.

In order to develop a feasible and cost-effective survey instrument, the contextual analysis used participatory methods in areas with a prolonged dry season of Yoro, Olancho and Danli where small and medium farms prevail. For socio-economic data collection, a standardized questionnaire and database structure was developed, adapting and extending existing material to the requirements of this survey. After the pre-testing phase, the questionnaire was improved with field results and the database was constructed. Sampling was done in two distinct stages following the objective of a comparative analysis between adopters and the typical conditions found in each of the zones. Two separately selected samples were chosen:

a) Through randomized data collection in several sub study areas, the typical socio-economic situation of the farmers in the area is assessed and
b) A separate sample is selected, using expert knowledge in order to find successful examples of adopters and reasons for adoption.

To assess the economic conditions of the representative livestock holder, the sampling was done in a randomized way. In several sub study areas, spread over Olancho, the farmers were selected randomly from the total population of farmers. After the elaboration and testing of the survey instrument, detailed sampling activities were started in Olancho in two blocks: a) Collection of representative data in 5 sub study areas representing altitudinal and ecological change, as well as a distance gradient from the province capital. b) assessment of positive deviances by means of targeted sampling.

Targeted sampling is done employing expert knowledge from partner institutions, like DICTA (Dirección de Ciencia y Tecnología Agropecuaria). Additional farms were identified asking farmers whom they know that was already using forage conservation or another forage technology in the area. This was in order to effectively search for farms that already have adopted one or several of the improved feeding strategies for milking cows.

The data were checked for their correctness and formula were developed for the calculation of parameters. Results were analyzed statistically, using non parametric tests.

Results and Discussion

Being a recently initiated activity (running since May 2005), many of the result presented indicate tendencies rather than final results.

In the frame of the contextual analysis information was collected informally (in the provinces of Yoro, El Paraiso and Olancho) to gain a basic understanding of the how farming systems work in different areas and which problems farmers perceive as most important. Much of this work was done in conjunction and complementary with other activities such as participatory assessment of problems and objectives with farmer groups, taking advantage of group trainings on dry season forage conservation techniques (hay and silage). An important objective of this investigation was to obtain detailed knowledge about dry season-problems on the farms, as perceived in the different regions by the livestock producers. In the view of farmers, the key problems in all zones is feed shortage, water shortage, drought and low milk production. More explicit results of this activity are presented in Section 4.2 of this report.

Semi-structured interviews with individual farmers revealed, that livestock production is one of many components in income generation. With the priority of family food security and risk management, small and medium size cattle farms rely on the production of basic grain (maize and beans) for home consumption and to cash income generation. Thus both, the livestock and cropping system components of the farming system needed to be included in the survey. Further, characteristics of the family and institutional aspects were parts of the structured survey. Farm decisions are made based on the whole situation of the family and thus each activity can be influence the allocation of resources in the farm. The same holds for the capability to invest in new forage options.

The survey in Olancho covered 66 randomly selected farms in 5 different zones of the province and was complemented with targeted sampling of 5 successful adopters of improved forage based technologies. There is a wide range of livestock herd sizes and production strategies found within the five zones. To facilitate the comparative analysis of livestock production, the farms were classified according to farm size. In this case, the approach of Fujisaka et. al (2005) was employed for the classification of farm sizes using the number of cattle heads for differentiation: small (1-12 head of cattle), medium (13-70), and large (>70).

To get an initial insight, the analysis focused on differences in milk production between the dry and the wet seasons and between farm sizes (Table 92). Out of 66 interviewed farmers, 12 do not produce milk in the dry season. This is distributed as follows: Of the medium producers, 8 farmers (22 %) did not produce milk in the dry season, of the small producers, 3 farmers (20 %) cease milk production and of the large producers only one case (6.6 %) did not produce milk in the dry season, respectively.

The reasons to stop milk production in the dry season as indicated by the farmers are the lack of pasture and conserved forage. Two farms did not produce milk in the wet season. Asked for the reason, they said there would not be enough forage available in pastures even during this time. One farm did not produce milk at all, although 5 milking cows were available. In this case, the milk was used to feed the calves.

Results on milk yield per cow in the dry and wet season between the different farm sizes indicate that there is no large difference in the intensity of milk production between small and the medium producers. Also the difference in milk yield per cow was found not to differ significantly between the small and large producers.

The hypothesis of farm size having no effect on milk yield is rejected (with a probability of >95 % according to Mann-Whitney test) when dry season milk yield of medium size and large size

Table 92. Daily milk production for the dry and wet season in conventional farms of different sizes, Olancho (2005).

Herd size		Livestock head	Dry season, Cows in milk	Wet season, Cows in milk	Dry season, milk per cow (l)	Wet Season, milk per cow (l)
Small 1-12 head 22.70%	Mean	7.1	1.9	2.6	3.6	5.3
Total n = 15	SD	2.4	1.4	1.1	2.3	3.1
Medium 13 - 70 head 54.50%	Mean	32.5	7.4	9.4	3.9	4.6
Total n= 36	SD	17.8	6.5	5.5	1.5	1.7
Large > 70 head 22.70%	Mean	182.5	37.4	37.5	2.8	4.7
Total n = 15	SD	102.8	22.1	20.5	1.4	1.9
Total 100%	Mean	60.8	13.0	14.4	3.6	4.8
Total n = 66	SD	83.5	17.6	16.9	1.7	2.2

farms is compared. Average milk production per cow in large size farms is 1.1 liters lower than in medium size farms, although it is nearly the same in the wet season. Mean wet season milk production of small producers is highest, but the high variability observed in the sample for this parameter did not allow to pick up statistical difference with the other the groups. These differences show that the importance of production per animal declines as the herd size increases.

In the dry season, the milk prices only differ (probability > 95 % according to Mann-Whitney test) between the small and the large farms. This is due to a higher milk price obtained by the small farmers, who often market their milk directly in the village without intermediaries. For the large producers, the comparatively lower milk price obtained in the dry season and the slightly higher milk price in the wet season reflects the smaller amplitude in milk prices for this group during the year. Some of them possess long-term contracts with the milk processing industry, which are usually available for members associations called CREL. The wet season milk price of the medium size farms is significantly lower than the price of the small farms (probability of >94%) and the price from large farms lower (probability of > 90 %) than that from the other two groups.

In Table 93 we present figures for gross revenue from milk as affected by farm size and season of the year. It should be noted that these figures do not represent net income, because they were calculated without consideration of production cost. Detailed income analysis is forthcoming. Nevertheless some comparisons are given here in order to identify tendencies.

Large and medium size farms showed differences in monthly revenue per cow between dry and wet seasons (probability of >96%). While the dry season revenue of the medium size farms was higher than in large farms, the situation is contrary during the wet season. Small farms have the highest mean revenue per cow in the wet season, but with a high variability within the group.

Table 93. Dry and wet season milk revenue and prices for conventional farms of different sizes, Olancho (2005).

Herd size		Dry season, monthly revenue/cow	Wet season, monthly revenue /cow	Dry season milk price	Wet season milk price	Improved pastures
		Lps	Lps	Lps/l	Lps/l	% of farm area
Small 1-12 head 22.70%	Mean	552.5	614.2	5.1	3.9	41.7
Total n = 15	SD	349.0	377.0	0.4	0.9	41.4
Medium 13 - 70 head 54.50%	Mean	584.6	443.2	4.9	3.4	55.7
Total N= 36	SD	229.2	205.4	0.4	0.6	37.0
Large > 70 head 22.70%	Mean	401.8	503.6	4.7	3.7	67.2
Total N = 15	SD	209.8	175.3	0.5	0.6	30.4
Total 100%	Mean	532.5	498.3	4.9	3.5	55.1
Total N = 66	SD	262.7	256.3	0.4	0.7	37.2

Note: Monetary units in Lempira (Lps); 1 USD is approximately 19 Lps.

On average, small and large farms experience income losses during the dry season, which was not observed on the average medium farms, but the variability in this group is high. Differences in the availability of improved pastures between the groups contribute to a high variability in the sample, although the use of improved pastures increases with farm size. For a complete analysis of pasture use, data on the areas of each variety were collected and will be analyzed further on.

The analysis of farms included in the positive deviances is based on a very small sample size, thus results in Tables 94 and 95 need to be interpreted with care. Of the five sampled farms, 2 are medium size and 3 are large. The number of milking cows differs only slightly between the seasons for the whole group, a feature that reflects better dry season forage availability. The share of improved pastures is high in this innovative group (82 %), as compared with the conventional farms (37 %).

In the conventional farms the dry season milk production is generally lower than in the wet season. The reduction in milk yield due to the dry season is 1.7, 0.7 and 1.9 l/day for small, medium and large farms respectively. In the non-conventional (positive deviance) farms, an average increase of milk production of 1.4 l could be found between dry and wet season.

The increase of milk production in the dry season relative to the well season in the positive deviances farms (Table 94) shows the potential impact dry season forage management can bring to farmers.

This difference is likely due to a better dry season forage management. So the first lesson learned from the positive deviances is that it is possible to maintain or even increase milk production in the dry season. This is potentially interesting from the financial point of view, because of the current differences in the milk price between seasons.

In the dry season, the monthly revenue per cow in the positive deviances farms is nearly 2.5 times greater than the one of conventional farmers. Differences were also observed with daily milk yield per cow, which is 2.6 times higher in the dry season and about 1.5 times higher in the rainy season than revenues obtained in the typical farm.

The decrease in income from milk was very variable among conventional farms. The small farms lose about 10 % of their revenue in the dry season (Table 93). Although production of the medium size farms drops by 0.7 l per cow in the dry season and the number of cows milked is lower in the dry season, their revenue rises by 31 % as compared to the wet season. The large farms experience the highest percent losses of revenue from milk in the dry season (20 %). A striking difference could be found for the positive deviances.

These farms prepare themselves for the long dry season and have about 80 % more revenue in the dry than in the wet season. The monthly revenue per cow in the dry season is 590 lps (about 31 US$) higher than in the rainy season in the case of the farms in the positive deviances group.

Table 94. Daily dry and wet season milk production in farms included in the positive deviances analysis, Olancho (2005).

Positive deviances N = 5	Livestock head	Dry season, milking cows	Wet season, milking cows	Dry season, milk per cow (l)	Wet Season, milk per cow (l)
Mean	99.6	24.8	25.6	8.5	7.1
Standard desviation	60.0	16.6	15.7	4.0	3.9

Table 95. Dry and wet season milk revenue and prices of positive deviances, Olancho (2005).

Positive deviances N = 5	Dry season, monthly revenue/cow	Wet season, monthly revenue / cow	Dry season milk price	Wet season milk price	Improved pastures
	Lps	Lps	Lps/l	Lps/l	% of farm area
Mean	1326.1	735.3	5.2	3.4	81.7
Standard desviation	655.2	421.8	0.3	0.4	26.1

Note: Monetary units in Lempira (Lps), 1 USD is approximately 19 Lps

4.5.2 Socio-economic factors associated with livestock/forage farming systems in Honduras: spatial variation

Collaborators: P. Lentes, M. Peters, D. White, T. Oberthur (CIAT), F. Holmann (CIAT-ILRI), and C. Burgos (DICTA)

Rationale

Within the on-going project led by CIAT entitled "Understanding and Catalyzing Learning-Selection Processes of Multi-Purpose Forage based Technologies in Central-America" in collaboration with DICTA (Dirección de Ciencia y Tecnología Agropecuaria), numerous socio-economic and non-economic factors change along spatial gradients. Such gradients may describe differences in farm types in two directions: (a) from the valley bottom to the top of the mountain, and (b) from areas with good infrastructure to remote areas. Another possibility of a gradient could be the description of gradual change of environmental characteristics between zones of farm types and land use.

Gradients generally can serve for the regionalization and scaling of socio-economic data that are usually collected for specific locations. The characteristics of farms and conditions for farming usually do not change abruptly but follow a continuum of change e.g. market distance or climatic conditions. Reasons of decision making in the farms and possibilities for adoption of multi purpose forage options are different between regions. This variation limits scaling of results from surveys that do not consider geographic factors in sampling. Since farming systems are to a great extent dependent on geographical site conditions, gradients are in this case used to establish relations between socio-economic conditions in the area and spatial factors.

The gradient or transect approach is used to consider the geographic characteristics of the study areas. Thus the plan is to scale results from other areas of investigation (socio-economics and learning selection). In doing so, social and spatial sciences provide means to detect similarities between regions. The knowledge gained in one region may then be transferred to a similar region. Using GIS as a tool to detect congruent features between regions, information can be extrapolated, constructing and using specific regionalization models. This enables a wider coverage of the information gained from socio-economic surveys, which have to be more or less punctual. Extrapolation of socio-economic factors to areas where no survey was done have the advantage of lower cost and workload. Regionalization models allow the estimation of future development on regional scale, considering both, spatial and socio-economic aspects.

Material and Methods

A focal area of our investigation in CA is to assess the interrelation of spatial factors with the pathways of decision-making processes among farmers from a geographical point of view. Along a transect line, principal zones are defined. Within these zones socio-economic interviews are conducted, using random sampling to cover the typical conditions of farmers for each zone. The exact location of each surveyed farm is measured using GPS. In order to scale this information of individual farms to the area of the village, interviews with key persons or village and communal level statistical data are required. GIS data are used to define the spatial parameters for the linkage of the empirical data with regionalization models.

From June to September 2005 socio-economic surveys were conducted, using a transect approach at the same time. In Olancho, data collection covered 66 farms from 5 different zones along an altitudinal gradient that was selected to cover as much as possible the ecological conditions prevailing in the region. The survey includes small, medium and large-scale animal holders. Amongst them there are adopters of new technologies as well as non-adopters. Economic parameters (e.g. income, gross margins and milk production parameters) were calculated in order to compare groups and to identify and understand driving forces and main determining variables for the adoption process.

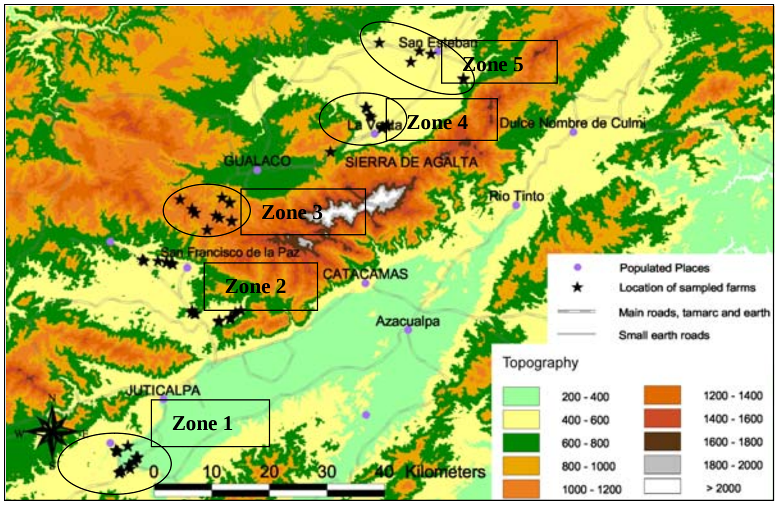
In the section 4.5.1 of this report the data were interpreted according to herd sizes. Information obtained from conventional farmers and those who already adopted multi purpose forage options was compared. In this section, the results are interpreted according to geographical zones. Field visits to the zones of interest were carried out to define interesting and representative areas for data collection and to define the course of the transect in the main study areas. In order to obtain a representative cut through Olancho, the characteristics of each major climatic zone were discussed with experts from the collaborating institution DICTA, using a map to localize the areas. From their experience, similar regions were defined and for each of this regions a parallel region was selected along the transect. Criteria for the definition of regions were the length of the dry season, the prevailing farm sizes and similar altitudinal and slope conditions. Some compromises were necessary in order to avoid dangerous areas and to keep work feasible, the area east of Catacamas, where the dry season is comparatively short, was excluded.

In Olancho, the transect line follows an altitudinal gradient, that begins in the valley around Juticalpa (zone 1). To leave the valley of Guayape river, a side road between Juticalpa and Catacamas was selected and interviews were conducted along the course of the road and following side roads. The transect takes course up the mountain through the municipality of San Francisco de la Paz (zone 2), that covers the intermediate area around 500 to 550 m.a.s.l. and further up to the municipality of Gualaco (zone 3), crossing a pass of about 1200 m.a.s.l.. The transect goes down to an intermediate zone (4) again. At about 94 km from the crossing at the main road from Juticalpa to Catacamas, the dirt road reaches San Esteban (zone 5) with its large-scale cattle farms again in a waste plain (Map 1).

Results and Discussion

In Table 96 we present the differences in the dry season length and the periods of forage shortage for the selected zones. Along with the altitudinal gradient goes a moisture gradient. Several factors more, including the adoption of dry season forage options and soil properties influence the length of the forage shortage period.

To assess the effect of feed shortages on livestock production, milk yields were estimated for each of the zones (Table 97). The number of animals per farm is higher in the two zones far away from the main road (probability > 95 % according to Mann-Whitney test). While the valley around Juticalpa (zone 1) and the intermediate area around San Francisco de la Paz (zone 2) have comparable farm sizes. Zone 3 shows the lowest number of animal heads. This is due to the more diverse structure of farms in the area on the mountain, where coffee is grown and generates additional cash income. In the zones 4 and 5, where the land price drops, as compared to zone 1, the farms are larger in area and possess more livestock.



Map 1. Zones of principal investigation, Olancho
Data sources: CIAT (1999): Atlas de Honduras- Datos Mitch, (Versión Beta – Enero 1999) , CGIAR - Consortium for Spatial Information (CGIAR-CSI) (2004)

Table 96. Farmer´s perception of dry season and forage shortage length in Transect zones, Olancho (2005).

Zone	Months of dry season	Months of forage shortage
1	5.5	3.5
2	5.3	3.9
3	2.7	1.5
4	4.6	3.1
5	5.0	3.7

On the top of the mountain the shorter dry season and the favorable conditions of forage availability result in a better milk yield per cow in the dry season. Zone 3 clearly exceeds milk yield as compared to the other areas (probabilities > 90 % for zones 1, 2 and 4). The intermediate type zone 4, where herd size is comparatively large shows the lowest milk production per cow in the rainy season (probability > 95 % for zones 1 and 3). The difference in milk prices also shows a spatial trend. In the dry season, the milk price is higher in zones 1, 2 (probability > 95 %) and 3

Table 97. Milk production and milk prices in the dry and wet season in the zones along Transect, Olancho (2005).

Region		Livestock head	Dry season, milk per cow	Wet season, milk per cow	Dry season milk price	Wet season milk price	Improved pastures
			l	l	Lps/l	Lps/l	% of farm area
Zone 1	Mean	42.57	3.52	5.01	4.98	3.39	46.44
	SD	57.55	1.69	2.20	0.29	0.46	37.39
Zone 2	Mean	50.21	3.54	4.72	5.06	3.50	38.73
	SD	71.50	1.11	1.63	0.38	0.82	34.57
Zone 3	Mean	30.33	4.98	5.99	4.94	3.78	63.32
	SD	28.43	1.85	3.06	0.58	0.94	36.59
Zone 4	Mean	93.00	3.06	3.42	4.83	3.36	88.31
	SD	104.94	1.96	1.05	0.50	0.54	20.44
Zone 5	Mean	162.50	3.01	4.71	4.38	4.17	78.15
	SD	144.52	2.02	2.39	0.33	0.08	23.99
Total	Mean	60.88	3.62	4.82	4.92	3.54	55.11
	SD	83.52	1.74	2.15	0.43	0.68	37.16

(probability > 90 %), as compared to zone 5. The wet season milk prices also differ between the same areas (probability > 95%). But in the wet season the milk price in zone 5 is higher. This can be explained by the presence of CRELS in zone 5. These associations offer long-term contracts with stable milk prices between the seasons, an advantage that is also felt in zone 5 and 4, where herd sizes are greater.

4.5.3 Learning selection processes on the use of different forage based options

Collaborators: P. Lentes, M. Peters, D. White, B. Douthwaite (CIAT), and C. Burgos (DICTA)

Rationale

As mentioned in the section 4.5.2 of this Report, learning selection, socio-economics and spatial conditions are interrelated. The research we are carrying out adopts the learning selection view of innovation (Douthwaithe 2003) in which "people engaging in an innovation process experiment and generate novelties (i.e., adaptations) through experimental learning. They make sense of these novelties by internal reflection and talking to colleagues, neighbors and friends. They then choose to continue to use the novelty, or not. At the same time they will influence others who are going through their own learning selection cycles. The net result is a continual process of novelty generation, selection and promulgation that improves the "fitness" or adoptability of the technology. As the adoptability improves more people adopt.

Four phases in the innovation process can be used in the context of multi purpose dry season forage options: (a) Development phase, (b) Start-up phase, (c) Adaptation phase and (d) Expansion phase. The combination of learning selection with socio-economics and thus the use of different methodologies makes it possible to assess the decision making of farmers from the economic point of view and simultaneously consider non-economic factors. This combination is believed to be of great value in the case of small scale and often mixed farming systems in zones with prolonged dry season forage shortage in Honduras. Usually small-scale farmers are constrained by low availability of resources and low levels of education and training for the utilization of multi purpose forage options.

Another main point of interest is the assessment of a learning selection processes to get to know farmers' pathways to get out of dry season forage shortage and to learn more about bottlenecks of adoption of forage-based technologies as a response to these constraints in Honduras.

The identification of bottlenecks and chances for adoption of multipurpose dry season forage, options for first time users and for advanced users has to be analyzed in order to define which difficulties can be eliminated. The bottlenecks of adoption differ between the replication cycles of learning. A range of typical bottlenecks of first time users can be defined for each technology option and by a process of adaptation. By adapting first time users farmers improve the technology and make it more viable for others.

Materials and Methods

Two approaches are being used to analyze the learning selection processes:

a) New farmers: initiation of contacts and introduction of specific best bet technologies (hay, silage, improved grasses, forage legumes) through training events.

b) Positive deviances farmers: group of farmers already applying multipurpose forage options are identified. These farms also serve for the assessment of technology spread from early adopters to more farms.

Among both groups of farmers, we will analyze how adoption takes place, which phases of adoption can be distinguished and how adaptation of technology works. Research questions like the following are to be answered.

Questions:

- What are the benefits of the technology from the point of view of the adopters?
- Which stages of the learning process can be distinguished in the regions covered by the study (Yoro, Olancho El Paraiso)?
- What are the characteristics of the adopters?
- Can there be principles derived from these characteristics?
- What were the circumstances under which the positive deviances adopted?

The following hypothesis will be tested during the course of the study:

- When farmers start adopting one of the multi purpose forage options, the chances are high that they continue adopting other options in the future or increase the use of the first option.
- A good entry point for the introduction of the whole package can be one specific forage option that is easy to start with.

Results and Discussion

During field training on forage conservation technologies, which served for the collection of socio-economic information, first contacts with farmers were established in Olancho. Farmers were instructed on how the information they provide was going to be used and that the possibility of training on e.g. hay, silage and improved grasses and legumes was available from CIAT and DICTA. This procedure served as a good starting point for the establishment of good working relations with farmers.

The intensive contact during the interviews created an interest and awareness among the farmers. Many of persons interviewed expressed their interest in training, they knew what all the questions were about. At the end of the interviews, an open discussion was usually conducted with the farmers, in which they asked questions back. At a later stage, these farmers were invited for training on hay and silage preparation. The introduction of more technology options is planned with these groups. Follow up visits with individual farmers and the facilitation of experience exchange in the form of group discussions will bring insight on adoption and adaptation processes. This is necessary to facilitate and enhance the application of technology and to get to know which obstacles farmers face and what they actually do with the introduced technologies. Since the socio-economic situation of the farmers trained was already assessed in base line surveys, it will be assessed for interrelations between adoption and socio-economic factors.

In Olancho, two training events were organized for focus groups defined by the sample of the socio-economic survey: (a) one for the group from the valley area around Juticalpa (27 participants) and (b) one for the group of farmers from the area of San Francisco de la Paz (25 participants) (Photo 38). Farmers were invited personally with a flyer that addressed the problem, objective, and the topics of the training. The possibility to invite more interested farmers was offered to each person. The training was conducted at a prototype farm, where improved grasses and forage legumes are cultivated.
In the group of the valley, some farmers already had experience with GOs and NGOs that offer training, while the second group did not have this experience. Many families in the valley receive remittances from their children that migrated to the US. This creates a dependency on money transfers, which may reduce motivation to change dry season feeding strategies. Young and middle age farmers were more interested in the training events. A possible explanation could be that older farmers are less motivated in trying out new options. In general, the group from San Francisco de la Paz was more interested and curious. This was already noticed during data collection.

(a) (b)

Photo 38. Farmer during events (a) hay production and (b) silage production using plastic bags.

For the training, a DICTA (Dirección de Ciencia y Tecnología Agropecuaria) prototype farmer was ready to host the groups for the two days. This farm was selected because it offered the possibility to include practical examples in forage conservation. The content of the training events carried out was designed to give the farmers an overview on several options that were identified as their points of interest during the survey when they were first contacted. These included:

1. Welcome and introduction of the objectives of the workshop
2. Presentation of results from farming systems survey in Olancho
3. Improved pastures as a forage crop, requirements on soils and climate, advantages and disadvantages
4. Forage legumes as sources of high quality feed (annuals and perennials)
5. Establishment, disease and pest management of forage grasses and legumes
6. Farmers experience with multipurpose forage options. (3 farmers that have a good dry season forage management reported their experiences)
7. Visit of Pasture plots (Brachiaria hybrid (Mulato), Mulatto associated with Arachis pintoi, *Cratylia argentea*
8. Presentation of the theory on hay and silage as alternative for dry season feeding.
9. Practical training hay drying (turning was done 2 times in the course of the day) and making of hay bunches.
10. Elaboration of little bag silage, using Craylia and Sugarcane
11. Discussion and evaluation of different forms and sizes of hay bunches
12. Discussion and evaluation of different sizes of little bag silage

The farmers gave good feedback on the presentations and they actively participated in discussions and the evaluations of hay and silage preparation. Participants also appreciated that the events had practical training and that the location offered the possibility to see much of the forage options mentioned in the training event. The involvement of positive deviances farmers (i.e. farmers having had successful experiences with improved forage technologies) in the training was also positive for the credibility and establishment of a trusty environment. Participants took advantage of the presence of these farmers who readily shared their experience.

At the end of the workshop, small samples of seed of *Cratylia argentea* (about 23 lb to 35 farmers for both workshops) were distributed to farmers who were interested to seed directly (seed value per farmer was about 2.50 US $). For the distribution of Vigna *unguiculata* the quantity of 10 lb was distributed to interested farmers. These farmers were ready to plant half a manzana (0,35 ha) with cowpea accessions IITA 9611 (8 farmers), IITA 284/2 (2 farmers) and CIDICCO 4 (2 farmers). With these farmers and also with others that expressed their intention to make hay or silage on their farms, follow up is scheduled. Once the technology is introduced, the farmers can be observed to monitor success, adaptation, spread of technology and the adoption process during the whole project period.

In summary, with the two farmers groups in Olancho, new forage-based technology was introduced and the next step is to observe and follow up what each participant is going to do with the knowledge gained in the training. For each case, it is now possible to survey the farmers individually or in groups on what they do and which constraints they have in their specific cases.

More training events will be organized in the future in other focal areas of the project to allow comparison between regions with different climatic and socio-economic conditions. Cases of more experienced farmers, which have already passed a few replication cycles will be used to observe the adaptation and innovation process. Another activity will be to monitor the dissemination of technology from farmer to farmer where prototype farmers will play an important role. The results achieved with these activities will be integrated into the spatial context and will be used in spatial analysis.

4.5.4 Case study: Adoption of cowpea in Hillsides of Honduras

Contributors: C. Reiber, S. Biller and R. Schultze-Kraft (U. of Hohenheim), M. Peters and P. Lentes (CIAT)

Rationale

Research on the opportunities and constraints of different cowpea accessions for integration into smallholder farming systems was carried out in the Yorito area during 2003. A total of 57 farmers had experimented with cowpea on small plots, mainly with the objective to use cowpea for human nutrition and soil improvement. The results revealed a high acceptance of cowpea by farmers who employed grain and biomass yield, drought tolerance, low disease infestation and taste as the main criteria of selection. It was concluded that cowpea had a high potential of being adopted at a larger scale. Since 2003, the farmers did not get any more seeds and assistance. A follow-up study was carried out in September 2005 to examine process related for non-adoption of cowpea, and farmers assessment on price and potential uses of cowpea.

Materials and Methods

Structured interviews with 18 farmers were performed in 10 different communities. As farmers in the communities who have experience with cowpea are in contact frequently and live close to each other, this sample was considered to be sufficient to investigate who had continued cultivating or still had seed of cowpea. A standardized questionnaire with both quantitative and qualitative questions was used. The study only considered farmers who planted cowpea in 2003.

Results

Cowpea adoption. Since 2003, the number of cowpea planters decreased from 57 to an estimated 13 in the second cropping season (“postrera”) of 2003 (Figure 72). In 2004, only four farmers grew cowpea. In 2005, there were 5 cowpea growers and a total of 6 farmers still had seeds.

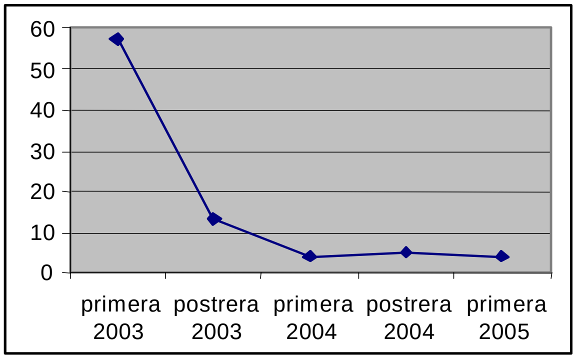


Figure 72. Number of cowpea planters in different seasons during a two year period.

Reasons for not growing cowpea. When farmers were asked for the reasons why they ceased planting cowpea, their main response was that they did not have any seeds left. The causes for the loss of seeds varied. On the one hand, seed production was limited due to climatic constraints (drought or too much rain), pest problems (rabbits and leaf cutter ants) and the use of plant biomass for green manure. On the other hand, seeds were damaged during storage due to pest attacks, were consumed, fed to chicken or distributed to others.

Process of innovation. The following briefly describes four cases of cowpea users as a result of promotion in 2002 and 2003.

1. A group of young people (CIAL-youth) was supported with additional cowpea grains in order to raise young pigs. The piglets had an age of 2 months and a weight of about 17 kg when cowpea supplementation started. The first week, 500 g of crude cowpea were mixed with 500 g of concentrate in order to adapt the piglets to the new feed. Then, cowpea was fed alone (2 kg per day and pig) for a period of 68 days before they were slaughtered. The pigs reached a final live-weight of 94 and 81 kg respectively which means that the average live-weight gain for this period was about 1 kg per pig/day.

2. During the survey in 2003, a farmer already used pre-cooked cowpea grain to feed fish (Tilapia) in his pond. Presently, he prepares fish concentrate composed of ground grains from legumes such as Cowpea, Mucuna, "Cablote" (*Guazuma ulmifolia* Lam.) and if available soybean grains as well as salt. He mentioned that he had given some cowpea seeds for grain production to another farmer nearby who was interested in his fish concentrate idea. This farmer, also having a fish pond, then copied his idea and is presently also elaborating his own fish concentrate.

3. A woman who was not included in the survey in 2003 produces cowpea to sell bundles of green pods in the streets of Yorito for 5 Lempira/ bundle.

4. A farmer planted 3 cowpea accessions in association with maize. Although he had problems due to drought and leaf-cutter ants he continued to produce cowpea for household consumption. Presently this farmer associates cowpea with sugar cane.

Interest and estimated market price of cowpea seeds. The farmers were asked if other people who were not reached by the 2003 promotion activities had requested seeds or information on cowpea from them. In total, there were 23 additional persons who asked 10 cowpea planters for seeds or information. Eight of the farmers gave seeds as a gift to 15 other farmers. Additionally, 3 farmers sold seeds at prices of 2.5, 3 and 5 Lempira/pound to 8 other farmers.

Farmers were asked if they would be willing to produce cowpea in case a market (demand) for cowpea existed and 16 out of the 18 farmers said that on this condition they would (of course) produce cowpea. In order to investigate the cowpea market potential, farmers were asked at what price they would sell the seed. The price range mentioned was in between 3-10 Lempira/ pound: 10 farmers stated a price of 3-5 Lempira/ pound and 4 farmers mentioned a price of 10 Lempira/pound which is double the price of common bean.

A total of 14 farmers (78%) mentioned that they would buy seeds if available at the local market of which 6 already tried to get some. The mostly stated price at which cowpea would be purchased was at 5 Lempira/pound (range from 3-10 Lempira).

Farmer experiences with cowpea. When farmers were asked about their experience of using cowpea to improve soil fertility, 83% replied that they had observed improvements after cowpea. Of this 50% indicated growth improvement of the succeeding crop after cowpea (in the same field). Other farmers (25%) observed a general improvement of the soil and 13% argued that subsequent crops were less susceptible to pests and diseases.

Farmers were also asked if they thought that cowpea could be used to improve the food situation in their villages. A total of 67% agreed and were then asked for their reasons. The "greater food variety", "high yields", "good taste" and "high quality" of the bean were statements of the farmers.

Other advantages mentioned:

- Cowpea can be used as a multiple purpose forage
- Vigorous and fast growth
- It can be harvested several times (undetermined maturation) leading to high grain production
- Good resistance against pests and diseases.

Promising cowpea accessions: Farmers gained experience with different accessions during the evaluation period in 2003 and two years later they were asked which of these accession(s) they preferred. FHIA, which is an early maturing accession and used for seed/grain production was mentioned 5 times. Verde Brazil, an accession with high biomass production (for soil improvement and forage) and DICTA 9611 (intermediate, erect type that adapts well to different biophysical conditions) were mentioned 4 times. In general, red seeded materials including the accessions FHIA, DICTA 9611 and CIDICCO 4 were preferred to white seeded accessions.

Farmers' selection criteria were based on the possible uses of the different accessions, in their production systems as well as their seed appearance, which is an important factor for marketing purposes.

Agronomic constraints: Based on measurements, observations and farmers' experience, cowpea did not tolerate high precipitations (especially the accession Verde Brazil) and altitudes above 1500 m.a.s.l. Furthermore, cowpea is highly vulnerable to pests such as leaf-cutter ants and rabbits that could cause partial or even complete crop failures if no pesticides are applied.

Uses of cowpea: If farmers could get seeds of cowpea, they would like to produce cowpea mainly for food purposes (13 times mentioned). Other uses mentioned were for example the sale of the grain if a market existed, for soil improvement as well as for feed purposes (for chicken and for fish).

Discussion

Some answers of farmers may have been biased as they may have answered some questions as they thought interviewer wanted to hear and in order not to lose possible fringe benefits. For example, when farmers were asked if they would produce cowpea in case of market demand, hardly anyone would say no although they might think differently. Regarding the low adoption rate and seed availability, one might assume that farmers' interest in cowpea is limited. However, the fact that seeds were lost due to unpredictable circumstances (e.g. rabbits, drought, and spoilage of stored seeds) and the non-availability of more seeds could also explain the low adoption.

Nevertheless, there are some farmers who still produce cowpea for different purposes (concentrate, green pods) and some already began to sell seeds to other interested farmers indicating its high potential demand in the region studied.

The existence of a local market for cowpea products seem to be a prerequisite for greater adoption since farmers additionally want to sell at least a part of their cowpea production. The use of cowpea as feed for ruminants as well as monogastrics could fill the niche for as alternative high quality fodder and protein grain for feeding monogastrics.

In summary, results from the survey indicate:

- Problems associated with non-adopotion are pests such as leaf-cutter ants and rabbits, low tolerance to high soil moisture content, altitudes above 1500 m.a.s.l. and no seed availability at the local market.
- Some farmers have learned how to use cowpea (food, feed and soil improvement) and are aware of its benefits.
- Farmers would produce cowpea in case of the existence of a local market.
- Most promising accessions are FHIA (for its early maturation and high seed production), Verde Brazil (for its high biomass production) and 9611 for its adaptability.

The way forward

Presently, and as part of the CIAT-project on forage alternatives for the dry season, cowpea hay and grains for the elaboration of concentrates are being produced in different cattle farms in the area in order to evaluate its effects on animal production. In addition, four farmers (of the former cowpea planters) without cattle were selected for cowpea forage production (hay). Trade linkages between cowpea and poultry pig producers and feed companies will be established in order to create the basis for possible production expansions.

4.5.5 Case study: Prototype forage seed enterprise of small farmers in Honduras

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The adoption of forage technologies is dependent on availability of good quality seed at reasonable prices. Therefore, taking into account the current forage seed market in Central America, the promotion of seed supply systems with a focus on farmer-led enterprises is one of our strategies for scaling up selected forage technologies. At the same time, seed production offers a means of income for small farmers. A farmer group in Honduras working under their own label – ‘EMPRASEFOR’ (Empresa de Producción Artesanal de Semilla Forrajera), formerly PRASEFOR, produces seed since 2001; this farmer-led seed enterprise was established with very limited financial support (i.e. less than US$ 2000), hence the approach could easily be replicated at other regions. As shown in Table 98 in 2001, seed production in Honduras began with 286 kg of seed, produced by 11 members of EMPRASEFOR. During 2002 the 13 members of EMPRASEFOR produced a total of 720 kg of *Brachiaria brizantha* cv. Toledo on 10.4 ha, doubling the cultivated area of the year before. In 2003, the production volume and area again rose to nearly 1.5 tons, produced on 18.5 ha. The 13 members of the group nearly reached 2 tons of

Table 98. Evaluation of seed production of *B. brizantha* cv. Toledo in EMPRASEFOR.

Year	Area	Production	Production	Increased production area
	ha	kg	ha	%
2001	5.25	286	54.5	
2002	10.40	720	68.8	99.3
2003	18.50	1465	79.0	77.3
2004	21.80	1915	87.5	17.9
2005	37.40	4000 *	106.8	71.2

*Estimation of what is expected.

seed in 2004. As the production volume of *B. brizantha* cv. Toledo seed rose, the group faced the problem of a limited local market and extended its sales area to more clients in the wider region during 2004 and 2005.

In April 2005, CIAT facilitated the contact of EMPRASEFOR with a Mexican seed company (PAPALOTA), which is a partner of CIAT in the development and distribution of Brachiaria hybrids. During a workshop on seed production and marketing in Yoro, an open discussion was held among EMPRASEFOR members (about 10 people attended), SERTEDESO (the NGO collaborating with EMPRASEFOR) and PAPALOTLA on the benefits of forming an EMPRASEFOR - PAPALOTLA alliance.

The objective of this alliance was to facilitate the expansion of seed production by PRASEFOR through an assured market offered by PAPALOTLA. The underlying principle of the alliance was that both PAPALOTLA and EMPRASEFOR should see economic benefits.

In a closed door meeting, PAPALOTLA and EMPRASEFOR discussed potential agreements, i.e. the sale of the seed produced to PAPALOTLA, to access a wider market for PRASEFOR utilizing the commercialization channels of PAPALOTLA and reducing the need for import expenses (quarantine, paper work for import, shipping cost etc.) for PAPALOTLA. The final agreement reached include:

a) minimum production target of 3 t/year of *B. brizantha* cv Toledo (in high demand) seed, with well defined quality requirements,
b) a price that could compete with the price of seed imported from Brazil and
c) access by PRASEFOR farmers of needed agricultural inputs such as fertilizers, herbicides at preferred rates through the PAPALOTLA distributor in Honduras.

After the workshop the group met several times to further define points of negotiation, concerning production volume, price per kg of seed and quality parameters (purity and germination). CIAT facilitated the negotiations and communication between the two parties. Once the core points of the alliance were agreed upon, PAPALOTLA agreed to buy the seed produced by EMPRASEFOR during 2005 with minimum quality parameters.

Quality will be assessed by the neutral government organization, Servicio Nacional de Sanidad Agropecuaria (SENASA). Minimum quality requirements are purity of 90% and germination > 65 %. The price of 6.50 US$ per kg was based on a purity of 95 % and germination of 70 %. For inferior quality still in the range of the minimum requirements, a formula to apply on the price per kg was also agreed.

The commercial seed company PAPALOTLA agreed to include the EMPRASEFOR label in the form of a sticker on the seed package sent to the market. To meet the production target, the group started to extend the area of cultivation to a total of about 37.4 ha, 71% more than in 2004. PAPALOTLA financed and organized the delivery of fertilizers and agrochemicals for production through their local reseller.

In October 2005, CIAT trained the farmers once on seed production of Toledo given emphasis on establishment, management and harvest of seed multiplication plots. It was found that post harvest processing was not done in an optimal way by EMPRASEFOR. In the process of purification, a better calibration of the processing equipment would probably lead to better results in terms of volume of pure seed. In order to be able to sell the seeds in accordance with Honduran laws, EMPRASEFOR had to be certified as seed producer by a government agency in Honduras (SENASA).

CIAT established the contact and started the registration process of the group. In the course of the certification process SENASA trained the farmers on legal issues of seed production and the correct use of agrochemicals. SENASA also had to examine the plots before the official registration document was handed out to

EMPRASEFOR.

One more precondition to start business with PAPALOTLA was that EMPRASEFOR had to legalize the enterprise based on laws of Honduras. This is currently in the process. In collaboration with SERTEDESO, CIAT established the contact with MIPIME (the government organization that issues the legal constitution of the company), helped the farmers to define the objectives of the company and to collect all documents necessary to obtain the legal status of the company.

A specialized lawyer was contracted to develop a constitution act for EMPRASEFOR. The official documents of the firm foundation was expected of the end of 2005.

4.6 Analysis of current and improved livestock/forage systems in LAC from an economical perspective

Highlights

- Investment on improved forages by farmers in Central America is economically profitable, but for the investment to be viable, producers need a line of credit of several years since they do not have the cash flow necessary for the financing of the necessary investments.

- Adoption of *Brachiaria* hybrid Mulato for direct grazing during the rainy season and *Cratylia argentea* for feeding during the dry season significantly improves the productivity of milk and beef as well as stocking rate, which allows the option of expanding the herd size.

- Costa Rica's beef industry performs very unsatisfactorily. At the farm level, beef production systems generate low gross incomes if we take into account that the commercial value of beef farmland ranges between $1,000/ha and $2,000/ha. As a result, the gross income cannot recover the opportunity cost of the capital invested in the land, making the beef industry uncompetitive.

- Intensification of beef and dual-cattle systems in the Llanos of Colombia would result in dramatic increases in rural employment.

- Forestry production would be a good option in the Llanos of Colombia to the extent that the region invest in reasonable means of transportation and adequate infrastructure for the management and processing of forest products.

4.6.1 Improved forages as drivers of economic growth in Central America

Contributors: Federico Holmann (CIAT-ILRI) and Libardo Rivas (CIAT)

This study aims at the evaluation of the economic viability of new forage options in different farm sizes and production systems in Central America, within the framework of the improvement of the competitiveness of the livestock sector. It is the goal to contribute relevant economic information, that helps to improve the design of economic and technological policies that accelerate the processes of innovation and technical change in the region. The economic return and the economic viability of investments in improved forages are analyzed, in order to evaluate its economic potential in different contexts of farm sizes, production systems and internal and external constraints to producers.

Data came from a random sample of 123 farms located in Costa Rica (30), Nicaragua (32), Honduras (35), and Guatemala (26). Producers were interviewed in order to obtain information regarding herd structure, land use, milk and beef production, and input use for animal feeding. This information allowed the estimation of technical and reproductive parameters as well as labor use, both family and hired. Based on this information it was possible to define 3 farm sizes, that represent groups of producers with different orientations and economic possibilities, thus: (a) the sector of subsistence where the objective function of the producer is household consumption and its main activity agriculture; (b) the small commercial livestock producer; and (c) the livestock producer of medium size.

A multi-period, linear programming model was used to analyze and evaluate the various scenarios and calculate the profitability indicators of the alternatives under evaluation. The model evaluates each of the 3 farm sizes and includes the following scenarios: (a) The producer adopts a *Brachiaria* hybrid in the areas within the farm which are presently planted with native pastures and also adopts the dry season feeding system based on the shrub legume *Cratylia argentea.* The increase in productivity and income is estimated based on the profits from the increase in animal response with respect to the baseline; and (b) the same scenario described in (a) but assuming alternatively that the farm changes the production system from dual purpose to specialized beef production.

The results obtained indicate that the investment in improved forage is economically profitable and that it represents a good option in order to increase the well-being of producers. However, in order for the investment in improved forages to be viable, producers need a line of credit of several years (i.e., 2-7, depending on the production system and country) since they do not have the cash flow necessary for the financing of the necessary investments. The adoption of the *Brachiaria* hybrid for its utilization in direct grazing during the rainy season and that of the shrub legume *Cratylia argentea* for feeding during the dry season significantly improved the productivity of milk and beef as well as the stocking rate, which generates the possibility of expanding the herd size. The number of cows can be increased between 2.1 and 3.5 times in the dual purpose system and between 2.6 and 6 times in the specialized beef production system. Milk production per hectare can be increased up to 2.3 to 3.5 times in the dual purpose system and beef production can be increased between 3.7 to 4.5 times, in the specialized beef production system. Table 99 contains this information taking as an example the dual purpose production

Table 99. Herd inventory, milk and beef production, employment generation, net income, number of years credit is needed, and time necessary to achieve maximum herd growth in small dual purpose farms in Central America.

	Guatemala		**Honduras**		**Nicaragua**		**Costa Rica**	
	Base line	Goal	Base line	Goal	Base line	Goal	Base line	Goal
Number of mature cows	14	30	14	30	14	30	14	26
Milk production (kg/farm/day)	31	66	31	66	31	66	31	56
Beef production (kg/farm/mo)	167	352	167	352	167	352	167	305
Area under improved forages (ha)								
* *Brachiaria* hybrid	0.0	6.9	0.0	6.9	0.0	6.9	0.0	10.2
* *Cratylia argentea*	0.0	6.1	0.0	6.1	0.0	6.1	0.0	2.8
Employment generation (# full-time persons/yr)	0.9	1.9	0.9	1.9	0.9	1.9	0.9	1.4
Net income ($/farm/mo)	126	378	94	336	70	305	90	271
Number of years to pay back credit	NA	4	NA	2	NA	2	NA	3
Years needed to achieve maximum herd growth	NA	9	NA	9	NA	9	NA	7

NA = Not apply

system and the small farm size. The investment in improved forages not only brings economic benefits for the producers, but also social, since the adoption of new technologies based on improved forages increases the generation of rural employment and food availability. In the dual purpose system it is possible to increase employment between 1.5 to 4 times and in the specialized beef system between 1.8 to 3 times.

The parameter of greatest impact in terms of net income is fertility. An increase of 10% in the annual calving rate improves net income between 12-19% in subsistence farms and small, and between 14-21% in medium size farms, depending on the country and on the prevailing production system. Table 100 contains this information for all farm sizes using the dual purpose production system. The second parameter in importance with regard to net increases in income is milk productivity in dual purpose farms or beef productivity in farms with specialized beef production. A 10% increase in the current productivity per cow results in 10-13% increase in net income in subsistence farms, 12-15% in small farms, and 12-19% in medium farms. Similar improvements in other parameters such as calf mortality results in a very low response in terms of net income/farm (i.e., < 3%).

The dual purpose system turned out to be a better economic option than the specialized beef production system. (Table 101). It can be observed that the potential for generating income is strongly associated with the economic orientation of the livestock activity. Indeed, subsistence farms with dual purpose systems have the capacity to generate from 28 to 35% more income that their homologous specialized in beef. In the small farms, that proportion ranges between 68 and 84% and in the medium farms the range of increase in income is between 107

Table 100. Sensibility of net income to a change in 10% improvement of herd fertility and beef and milk productivity, and a reduction of 10% in calf mortality and price of milk and beef for different farm sizes and production systems in Central America. 1/.

Parámetro	Guatemala	Honduras	Nicaragua	Costa Rica
	(% change in net income)			
Subsistence farm size				
Herd fertility (+10%)	+ 17.9	+ 18.3	+ 17.7	+ 19.4
Milk yield (+10%)	+ 12.9	+ 12.8	+ 12.5	+ 13.1
Beef yield (+10%)	+ 5.0	+ 5.2	+ 5.1	+ 4.4
Calf mortality (-10%)	+ 1.0	+ 1.1	+.1	+ 0.8
Milk price (-10%)	- 16.4	- 19.1	- 16.4	- 13.8
Beef price (-10%)	- 12.3	- 11.4	- 12.4	- 8.9
Small farm size				
Herd fertility (+10%)	+ 16.7	+ 16.9	+ 15.7	+ 19.1
Milk yield (+10%)	+ 13.0	+ 12.9	+ 12.3	+ 15.0
Beef yield (+10%)	+ 3.9	+ 4.0	+ 3.6	+ 4.6
Calf mortality (-10%)	+ 0.5	+ 0.5	+ 0.5	+ 0.8
Milk price (-10%)	- 14.2	- 14.3	- 13.6	- 16.3
Beef price (-10%)	- 9.7	- 10.4	- 9.6	- 10.7
Medium farm size				
Herd fertility (+10%)	+ 15.5	+ 15.1	+ 14.2	+ 19.8
Milk yield (+10%)	+ 13.0	+ 12.5	+ 11.8	+ 17.9
Beef yield (+10%)	+ 2.9	+ 3.0	+ 2.8	+ 3.9
Calf mortality (-10%)	+ 0.5	+ 0.5	+ 0.5	+ 0.5
Milk price (-10%)	- 14.3	- 13.6	- 12.8	- 19.9
Beef price (-10%)	- 8.9	- 9.2	- 8.1	- 12.3

1/This is a partial analysis in the sense that when a variable changes, the others remain constant.

Table 101. Net income by farm size and production system in Central America.

Country	Production system					
	Dual purpose			Specialized beef		
	Subsistence	Small	Medium	Subsistence	Small	Medium
	(US$/cow/yr)					
Guatemala	120	152	193	112	113	118
Honduras	105	135	178	94	98	104
Nicaragua	94	123	163	87	86	93
Costa Rica	122	127	129	105	112	118

and 145% compared to specialized beef. The reduced cost shows the income that is forgone because a forage technology which is not in the optimal solution is forced to enter. The shadow price represents the quantity of money that the producer would be willing to pay for an additional unit of a given limiting factor that has been exhausted. Eliminating a hectare of the *Brachiaria* hybrid in order to replace it with a hectare of the traditional grass *B. decumbens* would generate a loss per year between $57 and $115 dollars in subsistence farms. Furthermore, replacing a hectare of *Cratylia* with one of King grass would generate a loss per year between $136 and $195 dollars, depending on the country (Table 102**).**

Table 102. Reduced cost of traditional forage alternatives and shadow prices of land, protein, and digestible energy in different farm sizes using the dual purpose production system in Central America.

Variable	Guatemala	Honduras	Nicaragua	Costa Rica
	(net imcome per annual unit)[1]			
Subsistence farm size				
Reduced cost				
Brachiaria decumbens	- 115	- 108	- 104	- 57
King grass	- 195	- 161	- 136	- 192
Shadow price				
Land	131	127	124	47
Digestible protein dry season	1.7	1.4	1.2	1.5
Digestible energy dry season	0	0	0	0
Small farm size				
Reduced cost				
Brachiaria decumbens	- 163	- 151	- 142	- 98
King grass	- 223	- 186	- 159	- 229
Shadow price				
Land	205	194	185	102
Digestible protein dry season	2.0	1.7	1.4	1.9
Digestible energy dry season	0	0	0	0
Medium farm size				
Reduced cost				
Brachiaria decumbens	- 212	- 204	- 189	- 60
King grass	- 252	- 217	- 186	-200
Shadow price				
Land	282	212	258	51
Digestible protein dry season	2.3	2.0	1.7	1.6
Digestible energy dry season	0	0	0	0

[1] In the case of *Brachiaria*, King grass and land, the unit is represented by one hectare. In the case of protein, by a kilo, and in the case of energy, by a Mcal.

The shadow price of land in farms with subsistence size ranges from $47/year in Costa Rica to $131/year in Guatemala. The shadow price of the energy nutrient is zero, which means there is an energy surplus in the ration (ie., it is exceeding) and as a result, it is not limiting. On the other hand, the shadow price of the protein ranges from $1 to $1.7 per kilogram of digestible protein, which indicates that what is being lacking in the diet during the dry season is a little more protein in order to optimize the existing excess energy in the diet. This optimization can be made by either fertilizing the bank of *Cratylia* in order to increase the quantity of biomass, or supplementing during the dry season with a low-cost protein supplement such as chicken manure. The shadow price of capital in the dual purpose system ranges from $1.38 in subsistence farms to $2.14 in medium farms. In the specialized beef system this ranges from $1.11 in subsistence farms to $1.37 in medium farms (Table 103). This means that the investment in improved forages makes it possible to pay very high interest rates.

Table 103. Shadow price of capital available for investing in improved forage technologies in livestock production systems in Central America.

Production system	Guatemala	Honduras	Nicaragua	Costa Rica
	(US dollars willing to pay for each dollar invested in forages)			
Dual purpose				
Subsistence farm size	1.41	1.38	1.51	1.48
Small farm size	1.92	1.70	1.60	1.57
Medium farm size	2.13	1.78	1.89	2.14
Beef				
Subsistence farm size	1.12	1.11	1.11	1.12
Small farm size	1.21	1.18	1.14	1.19
Medium farm size	1.37	1.26	1.16	1.25

4.6.2 Beef chain analysis in Costa Rica: Identifying critical entry points to improve the efficiency of the sector

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Rationale

Costa Rica's livestock and beef industry generally performs very unsatisfactorily. The national herd has declined significantly, as has beef production. This evolution is closely associated with two forces: inefficiency in breeding and fattening, with revealing indices of an underexploited industry; and an industry that is inefficient and uncommitted to forging partnerships with the production sector to promote the production of better quality animals. Intermediaries abound, and their place in the system, together with established economic relationships, debilitates the producer's capacity to influence the distribution of the aggregate value he helps create. This translates to economic performances that do not correspond with the time and capital invested, or with the economic risks faced.

The study's overall objective is to characterize and analyze the beef chain in Costa Rica, and thus to identify and suggest lines of action that would optimize its operation and facilitate greater participation, particularly that of small-scale actors, in processes other than those of simple primary production. Such a strategy would help improve technological adoption, and the productivity and profitability of livestock production. The goal is to generate strategic information that helps public and private actors set priorities and implement lines of action that promote technological change and improve the competitiveness of the nation's livestock agribusiness. The specific objectives of the study were to:

1. Identify the articulations between links, technological levels, efficiency indicators, installed capacity (scale), and types of occupation
2. Describe the economic agents in the chain and their commercial and legal relationships
3. Characterize and estimate cost and price structures, and the generation of aggregate value in different links of the chain
4. Identify critical costs that can be modified through interventions, whether technological, policy, or other
5. Determine the biological and economic risks throughout the chain

To obtain primary, qualitative, and quantitative information (Table 104), selective samplings at different levels of the chain were taken, using structured and semi-structured surveys of different actors, including producers, intermediaries, traders, processing plants, and supermarkets.

Results and Discussion

Primary production

In Tables 105 and 106 we show the results of a survey conducted of 1074 livestock farms throughout the country during the first semester, 2005. The farms' principal activities were cow-calf, dual purpose, or fattening. The average herd size was 93 heads and of farm size 67 ha. Farms ranged from 82 heads and 55 ha in dual-purpose systems to 105 heads and 80 ha for fattening.

These production systems generate an annual animal extraction rate between 8% in dual-purpose systems and 24% in fattening systems, with a national average of 13%. In contrast, annual rate of cow replacement is very low, ranging from 5.1% in dual-purpose systems to 3.9% in fattening systems. This annual extraction rate is caused mainly by the low rate of annual calving, which ranges from 49% in fattening systems to 63% in dual-purpose systems, with a national average of barely 54%.

This low extraction rate generates an annual average of animals sold per farm of 12 heads, ranging from 7 in dual-purpose systems to 25 in fattening systems. However, the average weight of animals sold was 164 kg and 158 kg for male and female calves, respectively; 330 kg for young bulls (<2 years old); 252 kg and 440 kg for heifers and young bulls (2–3 y o), respectively; 335 kg for young cows; 582 kg for bulls; and 439 kg for cows.

This number of animals, multiplied by the average weight at sale for each category, generates very low annual sales that range from 2,260 kg in dual-purpose systems to 10,140 kg in fattening systems, with a national average of 3,980 kg. This means that annual productivity is about 60 kg beef/ha, ranging from 41 kg/ha in dual-purpose systems to 126 kg/ha in fattening systems.

In 2004, farm gate prices for beef (live weight) for steers and heifers slaughtered in abattoirs were US$1.11/kg and $0.95/kg, respectively. Auction prices for other categories were $0.82/kg and $1.02/kg for female and male calves, respectively; $0.88/kg for heifers; $0.96/kg for young bulls (<2 y o); $0.76/kg for culled cows; and $0.95/kg for bulls.

The annual sales in kilograms of beef, translated into gross income, varied from US$2,050 for the average dual-purpose farm to $10,090 for the average fattening farm, generating an annual gross income of $37/ha in the dual-purpose system to $125/ha in the fattening system. Such

Table 104. Lists of specific information to collect at different levels of the beef chain, Costa Rica.

Level in the chain					
Producer	Livestock trader or auctioneer	Slaughterhouse	Meat wholesaler	Processing plant	Supermarkets and markets
Farm size		Number of animals slaughtered	Volumes of meat and purchase and sale prices by type of meat	Volumes of meats processed	Type and volumes of meats and processed products Consumer prices
Area under pastures		Use of the capacity installed	Volumes of byproducts and purchase and sale prices	Classes of cuts and processed byproducts	Percentage of losses of meats and processed products Agents who assume them
Sales of livestock by category	Volume of purchases by category	Type of installations Technological level	Percentage of physical losses Agents who assume them Commercial arrangements with suppliers	Type of installations and technological level Use of capacity installed	Commercial arrangements with suppliers Insurance policies and risks covered
Age at sale and by category					
Average weight of animals sold by category	Travel expenses	Costs or fees for dressing		Type of products Prices at the processed meat plant	
Total inventory	Buying prices by category	Class of services Products and byproducts generated	Volumes and types of meats marketed	Percentage of losses of meats and processed products Agents who assume them	Purchase prices of meats on the carcass at the slaughterhouse Purchase prices of processed products
Farm gate price (US$/kg on the hoof) by category		Prices of slaughtered cattle Prices of byproducts	Purchase and sale prices of meats and byproducts	Commercial arrangements Insurance policies and risks covered	
Technical parameters: • birth rate (%) • mortality (%) • weight gains		Commercial arrangements Insurance policies and risks covered	Percentages of physical losses of meats and byproducts Agents who assume them	Sale conditions and agreements	
Technical and economic limitations		Percentage of losses of products and byproducts Agents who assume them	Identification of business risks as perceived by the wholesaler		
Sale conditions and agreements	Sale conditions and agreements		Sale conditions and agreements		

Table 105. Inventory of the average herd, annual animal sales, extraction rate, and average weight of animals sold during 2004, according to a 2005 survey of livestock producers who own breeding (602 farms) and dual-purpose (298 farms) production systems, Costa Rica.

	Production system											
	Breeding[a]						Dual purpose[a]					
Variable[b]	1 (n=139)	2 (n=203)	3 (n=175)	4 (n=57)	5 (n=28)	Average (n=602)	1 (n=70)	2 (n=114)	3 (n=74)	4 (n=30)	5 (n=8)	Average (n=296)
Inventory herd (no.)												
Cows	7.8	21.4	42.8	87.5	192.9	38.7	7.5	20.5	44.1	84.8	171.3	33.9
Young cows	2.1	6.6	12.7	29.4	47.0	11.4	1.5	6.1	12.3	20.5	51.3	9.2
Heifers	1.3	4.7	10.2	19.1	50.4	9.0	1.9	6.2	10.9	25.4	62.1	9.8
Female calves	2.8	7.7	15.8	27.9	87.7	14.6	3.0	7.9	17.2	23.2	71.4	12.3
Male calves	2.1	5.9	11.3	19.0	42.4	9.5	2.3	5.7	11.8	24.2	47.0	9.4
Young bulls (<2 y o)	0.6	2.8	5.8	9.1	15.2	4.5	0.1	1.4	4.2	9.0	1.8	2.9
Young bulls (2–3 y o)	1.4	0.9	3.7	5.3	27.5	3.3	1.4	0.7	2.6	11.1	7.0	2.3
Bulls	0.7	1.6	2.2	3.6	5.1	1.9	0.6	1.2	1.9	3.3	6.6	1.6
Total animals	**18.7**	**51.7**	**104.6**	**200.7**	**468.1**	**93.0**	**18.3**	**49.7**	**105.1**	**201.4**	**418.4**	**81.5**
Area (ha)	48.6	41.1	72.2	127.2	217.9	68.4	15.5	45.7	67.8	123.8	136.9	54.7
Annual calving (%)	53	52	48	53	53	51	67	64	62	54	34	63
Mortality (%)												
Calves	2	2	2	2	2	2	2	2	2	1	1	2
Adults	1	1	1	0	1	1	0	1	1	1	1	1
Annual sales (no.)												
Cows	0.5	0.7	2.6	5.1	17.9	2.5	0.7	1.6	2.8	4.3	4.3	2.0
Young cows	0.2	0.2	0.9	1.2	0.5	0.5	0.1	0.3	0.4	0	3.8	0.3
Heifers	0.1	0.3	0.7	1.9	4.1	0.7	0	0.5	0.5	0.6	0	0.4
Female calves	0.1	0.3	1.4	1.1	5.0	0.9	0.2	0.4	0.8	0.6	2.6	0.5
Male calves	0.5	1.3	4.4	2.9	10.3	2.6	0.4	1.4	3.5	2.3	3.5	1.8
Young bulls (<2 y o)	0.2	1.1	1.2	4.4	0	1.3	0.3	0.2	0.9	1.5	0	0.5
Young bulls (2–3 y o)	0.7	0.1	1.1	0.9	19.6	1.4	0.1	0	1.4	4.0	0	0.8
Bulls	0.2	0.1	0.5	0.3	0.6	0.3	0	0.1	0.4	0.5	12.5	0.5
Total animals	**2.5**	**4.1**	**12.8**	**17.8**	**58.0**	**10.2**	**1.8**	**4.5**	**10.7**	**13.8**	**26.7**	**6.8**
Extraction rate (%)	**13.4**	**7.9**	**12.2**	**8.9**	**12.4**	**11.0**	**9.8**	**9.1**	**10.2**	**6.9**	**6.4**	**8.3**
Replacement rate (%)	**3.9**	**3.7**	**5.1**	**6.4**	**8.8**	**4.7**	**5.1**	**5.1**	**6.3**	**2.3**	**3.1**	**5.1**
Weight at sale (kg)												
Cows	403	425	430	426	456	428	404	539	412	419	400	466
Young cows	301	325	354	400	405	336	338	326	250	N/A	350	320
Heifers	253	236	255	236	303	252	220	241	250	237	N/A	239
Female calves	108	157	172	175	191	164	85	159	129	168	150	136
Male calves	151	175	174	180	213	175	150	150	156	127	125	149
Young bulls (<2 y o)	293	353	328	307	N/A	330	205	269	282	250	N/A	263
Young bulls (2–3 y o)	277	542	460	580	491	470	470	385	467	378	N/A	433
Bulls	615	579	650	650	660	627	450	500	550	615	550	532
Meat sold (kg)	**768**	**1134**	**3620**	**4911**	**20225**	**3018**	**551**	**1428**	**3095**	**5097**	**10750**	**2261**
Income from meat sold (US$)[c]	**705**	**1054**	**3322**	**4370**	**19163**	**2789**	**493**	**1193**	**2870**	**4856**	**9881**	**2053**

a. Herd size: 1 = smaller than 30 heads per farm; 2 = 31–75 heads; 3 = 76–150 heads; 4 = 151–300 heads; 5 = more than 300 heads.
b. y o = years old
c. Prices in 2004: live steers slaughtered in abattoirs = $1.11/kg and females, $0.95/kg. Auction prices for other categories were $0.82/kg for female calves, $1.02/kg for male calves, $0.88/kg for heifers, $0.96/kg for young bulls (<2 y o), $0.76/kg for culled cows, and $0.95/kg for bulls.

Table 105. Inventory of the average herd, annual animal sales, extraction rate, and average weight of animals sold during 2004, according to a 2005 survey of livestock producers who own breeding (602 farms) and dual-purpose (298 farms) production systems, Costa Rica.

	Production system											
	Breeding[a]						Dual purpose[a]					
Variable[b]	1 (n=139)	2 (n=203)	3 (n=175)	4 (n=57)	5 (n=28)	Average (n=602)	1 (n=70)	2 (n=114)	3 (n=74)	4 (n=30)	5 (n=8)	Average (n=296)
Inventory herd (no.)												
Cows	7.8	21.4	42.8	87.5	192.9	38.7	7.5	20.5	44.1	84.8	171.3	33.9
Young cows	2.1	6.6	12.7	29.4	47.0	11.4	1.5	6.1	12.3	20.5	51.3	9.2
Heifers	1.3	4.7	10.2	19.1	50.4	9.0	1.9	6.2	10.9	25.4	62.1	9.8
Female calves	2.8	7.7	15.8	27.9	87.7	14.6	3.0	7.9	17.2	23.2	71.4	12.3
Male calves	2.1	5.9	11.3	19.0	42.4	9.5	2.3	5.7	11.8	24.2	47.0	9.4
Young bulls (<2 y o)	0.6	2.8	5.8	9.1	15.2	4.5	0.1	1.4	4.2	9.0	1.8	2.9
Young bulls (2–3 y o)	1.4	0.9	3.7	5.3	27.5	3.3	1.4	0.7	2.6	11.1	7.0	2.3
Bulls	0.7	1.6	2.2	3.6	5.1	1.9	0.6	1.2	1.9	3.3	6.6	1.6
Total animals	**18.7**	**51.7**	**104.6**	**200.7**	**468.1**	**93.0**	**18.3**	**49.7**	**105.1**	**201.4**	**418.4**	**81.5**
Area (ha)	48.6	41.1	72.2	127.2	217.9	68.4	15.5	45.7	67.8	123.8	136.9	54.7
Annual calving (%)	53	52	48	53	53	51	67	64	62	54	34	63
Mortality (%)												
Calves	2	2	2	2	2	2	2	2	2	1	1	2
Adults	1	1	1	0	1	1	0	1	1	1	1	1
Annual sales (no.)												
Cows	0.5	0.7	2.6	5.1	17.9	2.5	0.7	1.6	2.8	4.3	4.3	2.0
Young cows	0.2	0.2	0.9	1.2	0.5	0.5	0.1	0.3	0.4	0	3.8	0.3
Heifers	0.1	0.3	0.7	1.9	4.1	0.7	0	0.5	0.5	0.6	0	0.4
Female calves	0.1	0.3	1.4	1.1	5.0	0.9	0.2	0.4	0.8	0.6	2.6	0.5
Male calves	0.5	1.3	4.4	2.9	10.3	2.6	0.4	1.4	3.5	2.3	3.5	1.8
Young bulls (<2 y o)	0.2	1.1	1.2	4.4	0	1.3	0.3	0.2	0.9	1.5	0	0.5
Young bulls (2–3 y o)	0.7	0.1	1.1	0.9	19.6	1.4	0.1	0	1.4	4.0	0	0.8
Bulls	0.2	0.1	0.5	0.3	0.6	0.3	0	0.1	0.4	0.5	12.5	0.5
Total animals	**2.5**	**4.1**	**12.8**	**17.8**	**58.0**	**10.2**	**1.8**	**4.5**	**10.7**	**13.8**	**26.7**	**6.8**
Extraction rate (%)	**13.4**	**7.9**	**12.2**	**8.9**	**12.4**	**11.0**	**9.8**	**9.1**	**10.2**	**6.9**	**6.4**	**8.3**
Replacement rate (%)	**3.9**	**3.7**	**5.1**	**6.4**	**8.8**	**4.7**	**5.1**	**5.1**	**6.3**	**2.3**	**3.1**	**5.1**
Weight at sale (kg)												
Cows	403	425	430	426	456	428	404	539	412	419	400	466
Young cows	301	325	354	400	405	336	338	326	250	N/A	350	320
Heifers	253	236	255	236	303	252	220	241	250	237	N/A	239
Female calves	108	157	172	175	191	164	85	159	129	168	150	136
Male calves	151	175	174	180	213	175	150	150	156	127	125	149
Young bulls (<2 y o)	293	353	328	307	N/A	330	205	269	282	250	N/A	263
Young bulls (2–3 y o)	277	542	460	580	491	470	470	385	467	378	N/A	433
Bulls	615	579	650	650	660	627	450	500	550	615	550	532
Meat sold (kg)	**768**	**1134**	**3620**	**4911**	**20225**	**3018**	**551**	**1428**	**3095**	**5097**	**10750**	**2261**
Income from meat sold (US$)[c]	**705**	**1054**	**3322**	**4370**	**19163**	**2789**	**493**	**1193**	**2870**	**4856**	**9881**	**2053**

a. Herd size: 1 = smaller than 30 heads per farm; 2 = 31–75 heads; 3 = 76–150 heads; 4 = 151–300 heads; 5 = more than 300 heads.
b. y o = years old
c. Prices in 2004: live steers slaughtered in abattoirs = $1.11/kg and females, $0.95/kg. Auction prices for other categories were $0.82/kg for female calves, $1.02/kg for male calves, $0.88/kg for heifers, $0.96/kg for young bulls (<2 y o), $0.76/kg for culled cows, and $0.95/kg for bulls.

gross incomes are extremely low if we take into account that the commercial value of beef farmland ranges between $1,000/ha and $2,000/ha. As a result, the biological inefficiencies mentioned above, combined with high land costs, make it impossible to recover the opportunity cost of the capital invested in the land, making this beef activity uncompetitive.

Table 107 shows the use and cost of labor in the above-mentioned production systems, taking as value the cost of the minimum wage in the case of family labor. The value of labor represents the principal cost in these production systems, ranging from an average of 1.7 full-time people in the cow-calf operations at an annual cost of US$3,346 per farm to 2.2 people in the fattening system at $4,330 per farm.

If we compare these values with the annual gross income per beef sale in Tables 105 and 106, then this labor cost represents, on average, between 43% of sales in the fattening system (i.e., US$4,330 in Table 107 divided by $10,088 in Table 106) and 201% in the dual-purpose system (i.e., $4,133 in Table 107divided by $2,053 in Table 105). The latter is understandable as most sales come through selling milk, not beef. However, the labor cost for the cow-calf system represents, on average, 120% of beef sales. This is worrying, as it implies that this system is not profitable, with the recompense for family labor being lower than the minimum wage.

Risks of beef production

Figure 73 shows a steer's life cycle from conception to slaughter. For 1.3 years (i.e. 15.5 months), the producer does not obtain any profit, as the animal is not born. The mating period is about 6.5 months until the female becomes pregnant (i.e., 12 months × 0.54, which is the annual rate of calving). Gestation takes another 9 months.

Once the male calf is born (weighing about 35 kg and worth US$50 dollars), it starts growing through three commercial phases: (1) pre-weaning period, which lasts between 7 and 10 months, with a 5% risk of mortality, (2) development, which usually lasts another year, with a 2% risk of mortality, and (3) fattening, which takes a further year, with a 2% risk of mortality, plus a conservative 3% risk of theft for being a fat appetizing animal.

In addition, in beef production, the annual replacement of the beef-producing machinery, that is, the cow and bull, must be considered, thus adding an annual 2.4% cost for replacing the cow (i.e. the annual 4.7% is divided between the two as 50% of all births are male; Table 106), and an annual 0.1% cost for replacing the bull (assuming a ratio of 25 cows per bull). That is, to each young bull that reaches slaughter age, a 14% mortality rate must be added from birth to slaughter, 3% for rustling, and 2.5% for replacing his parents, totaling 19.5%. That is, 1.2 young bulls are needed for one to reach the slaughterhouse, or 4 out of 5 male calves born.

This risk, taken over 4.2 years (i.e., 50 months), is assumed totally by the producer. Other links of the chain, such as the slaughterhouse or supermarket, protect against risks by buying insurance policies that are then transferred to the consumer and/or producer.

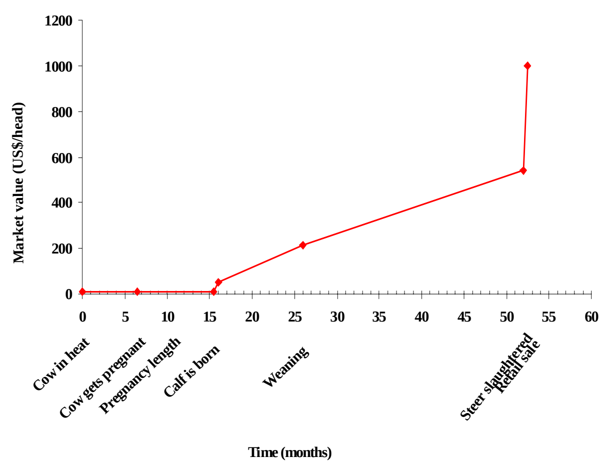

Figure 73. A young bull's life cycle from conception to slaughter, and its commercial value.

Table 107. Quantity and cost of contracted and family labor allotted to livestock activities on 1074 farms, Costa Rica, 2005.

Herd size[a]	Labor type		Dual purpose (n=296)		Breeding (n=602)		Fattening (n=176)		Total (n=1074)	
			Quantity	Cost	Quantity	Cost	Quantity	Cost	Quantity	Cost
1	Contracted		0	0	0	0	0	0	0	0
	Family		0.6	1180	0.4	787	0.7	1378	0.6	1180
		Total	0.6	1180	0.4	787	0.7	1378	0.6	1180
2	Contracted		0	0	0	0	0	0	0	0
	Family		1.1	2165	0.6	1180	1.1	2165	0.9	1771
		Total	1.1	2165	0.6	1180	1.1	2165	0.9	1771
3	Contracted		0	0	0	0	0.8	1574	0.3	590
	Family		1.6	3149	1.5	2952	0.8	1574	1.3	2558
		Total	1.6	3149	1.5	2952	1.6	3148	1.6	3148
4	Contracted		1.3	2558	1.9	3739	1.7	3346	1.6	3149
	Family		1.4	2755	1.1	2165	0.8	1574	1.1	2165
		Total	2.7	5313	3.0	5904	2.5	4920	2.7	5314
5	Contracted		4.1	8069	4.0	7872	6.0	11808	4.3	8462
	Family		1.1	2165	1.0	1968	1.0	1968	1.0	1968
		Total	5.2	10224	5.0	9840	7.0	13776	5.3	10430
Avge	**Contracted**		**1.0**	**1968**	**1.0**	**1968**	**1.2**	**2362**	**1.1**	**2165**
	Family		**1.1**	**2165**	**0.7**	**1378**	**1.0**	**1968**	**0.9**	**1771**
		Total	**2.1**	**4133**	**1.7**	**3346**	**2.2**	**4330**	**2.0**	**3936**

a. Herd size: 1 = less than 30 heads per farm; 2 = 31–75 heads; 3 = 76–150 heads; 4 = 151–300 heads; 5 = more than 300 heads.

Livestock marketing

Much of the livestock trade is carried out through the country's 19 auctioneering entities, which together carry out 24 events per week. The auctioneers act as intermediaries by receiving the animals from a producer and, once having sold them, pays the seller according to the price at which each animal was sold but discounting the corresponding percentage for the commission by sale. The commission varies from auction to auction, with most collecting between 3.8% and 4%.

The producer is paid 5 to 20 minutes after the animals have been auctioned, going to the cashier's window to collect the check, which is payable in cash. On closing the books, the difference between purchases and sales should equal the percentage of the collected commissions. The sum of commissions, minus the expenses generated in developing the auction, is the profit made by the auction owner. Every auction is obliged by law to contract a veterinary to verify the health of the animals admitted into the auction. The animals enter the installations 2 hours before the auction. The event lasts as long as the number of animals admitted. An auctioneer can negotiate 110 to 125 animals per hour, with the average number of animals traded per event being 450.

The trade of live cattle is governed by the balance between supply and demand. If the auction prejudices the seller, he will then seek other means of marketing his livestock. If the buyer finds prices are too high, then he will also seek better options elsewhere. To achieve the balance, auction owners must carry out the process transparently. Base prices should reflect current market value.

For small producers, auctions are a major outlet for selling cattle. An analysis carried out by Servicios Integrados para el Desarrollo Empresarial (SIDE) showed that, during 2003, auctions traded for 2,850 sellers. Only 0.2% of these (i.e., 6 people) were large producers, 1.8% were medium-scale producers (i.e., 45 sellers), and 98.2% were small producers[1].

For most cases, the producer markets his livestock once a year and that, in 63% of cases, he sells less than 10 animals. Another 17% market their livestock at auctions two times a year, and 9% three times. Only 5% of all producers marketing livestock sell more than 40 animals. As a result, small producers find this environment a transparent and safe option for marketing their livestock.

4.6.3 New diversified livestock/agriculture/forestry production systems: An economic evaluation in the Colombian Llanos

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The objective of this study is to evaluate from the economic, social, and environmental optics, new models of farm that include various livestock, agricultural, and forest components for the production of food and raw materials, and in addition, generation of environmental services such as carbon sequestration. To meet the objective a multiperiodic linear programming model was used to optimize different production systems and to evaluate environmental externalities. For this analysis, the progressive incorporation of new technological components into the current livestock production systems are simulated, constructing various sequential technological scenarios. The starting point is a livestock farm with an extensive production system based on native sabanas in monoculture. In the following phase, the model adds a component of pasture

[1] According to the classification of producers developed by the Consejo Nacional de Producción de Costa Rica. This established that producers who obtain a gross income of up to US$25,000 per year are small producers; from $25,000 to $70,000 as medium-scale producers, and more than $70,000 as large producers.

rotations and crops, in a process oriented to improve the soil conditions through the construction of arable layers. The incorporation of forest and the sale of environmental services, in the form of carbon sequestration represent the following stage in the path of transformation of the productive systems.

For the animal feeding component the forage sources considered are: 1) Pastures in monoculture: a) native savanna, b) improved *Brachiaria brizantha* (cv. Toledo) and c) a mixed pasture of grass and legume (*B. decumbens* + *D. ovalifolium*); and 2) Forage from the rotations of grasses and crops. It is assumed that the improved pastures in addition to providing forage also fix carbon into the soil.

The evaluation period is 19 years. The semiannual crops that enter in the rotation scenarios are rice, corn, and soybeans. Three pasture rotations are considered. Rotation 1 is composed of three segments. The initial segment is a native pasture that remains in production during 7 years, followed by a cycle of 4 years of semiannual crops: rice–soybeans and corn–soybeans. This rotation culminates in the seeding of a mixed pasture, of grass and legume, with a productive life of 8 years.

Rotations 2 and 3 are very similar. Both begin and end with crop cycles of 6 years each. They differ in that in rotation 2, in the intermediate segment an improved *Brachiaria* is established, remaining in production during 7 years. In rotation 3, in the same segment, an mixed grass-legume pasture is established.

The forest alternative is represented by planting Caribbean pine, a specie with a high degree of adaptation that produces wood sequesters carbon. Given that the plantation is to be established in native savanna pastures, and because the soil conditions are not appropriate, the strategy is to plant first an improved grass during 4 years, after which it is introduced the pine for the remaining 15 years.

It was decided to simulate a typical farm of 500 hectares which operates with regional cost averages and that has a working capital that has a range of US$ 5,000 to 300,000. For simplicity, the results presented in this report are for the dual purpose production system (DP).

Dual purpose extensive system. At all levels of starting capital, the DP is more attractive economically than the cow-calf (ie., specialized beef) system. When the initial working capital is US$ 300,000 the value of the objective function of the DP is almost 6 times higher than in the specialized beef system. If producers with limited working capital went from specialized beef to DP, their net income would grow 2.5 times.

The greatest economic incentive of the DP is reflected in better shadow prices of the land. The maximum price that a producer with a specialized beef system would be willing to pay per additional hectare would be US$ 135 on average. If the farm were dual purpose, the amount would be US$ 449/ha. The optimal strategy for farms with high starting capital would be to establish a very high proportion of its area with improved forages, 89% (Table 108). On the other hand, farms with low starting capital would appeal to native sabannas as the main source of livestock feeding. Under these conditions, the DP can only generate employment when the starting capital is above US$ 200,000.

Dual purpose system including rotations of grass–crops and conservationist practices of land use. The adoption of rotations between improved forages and crops in the DP system displaces the native sabannas. When high levels of operating capital are available, the improved grass (ie., *B. brizantha* cv Toledo) is highly competitive, occupying a fraction that ranges between 64 and 79% of the available farm area. The rest of the farm is allocated to rotation 2 (Table 109). As the availability of operating capital decreases, the area cultivated with rotation 1 increases. The association *B. decumbens* + *D. ovalifolium* does not appear in the optimal solution per se, but is part of rotation 1.

The introduction of grasses–crop rotations increases significantly the capacity of employment generation of the mixed crop-livestock systems.

Table 108. Dual purpose system Scale*: 500 ha* Extensive system, with no improved forages, no crops, and no forestry

Starting capital available		Land use Ha				Herd inventory (heads)	Net employment generation (# journals)	Value of objective function US$ 000
			Improved forages					
Total US$ 000	Per hectare US$	Native sabanna	Brachiaria Toledo	Association *B. decumbens* + *D. ovalifolium*	Total area utilized (ha)			
300	600	55.7	444.3	0.0	500.0	990	236	6815
200	400	200.9	299.1	0.0	500.0	693	28	4746
100	200	346.2	153.8	0.0	500.0	384	-180	2677
50	100	418.8	81.2	0.0	500.0	231	-283	1643
25	50	455.2	44.8	0.0	500.0	155	-336	1125
10	20	484.3	15.7	0.0	500.0	94	-377	778
5	10	494.0	6.0	0.0	500.0	73	-391	649

As the area under grasses in monoculture decreases, the net generation of employment tends to rise. In farms with the highest levels of starting capital, the introduction of rotations and of conservationist practices of land use increases net income by 13%. Likewise, in the specialized beef system the increase in net income is about 44%. In situations of low availability of starting capital the economic impact of technological adoption is substantially greater. The jump in productivity that implies the adoption of rotations of grasses–crops represents an increase of almost 7 times the net income (Tables 108 and 109).

Dual purpose system with grass–crop rotations, conservationist techniques of land use and forest activities. The addition of the option of forestry in the mixed crop-livestock system influences land use in situations where the availability of starting capital is over US 10,000. As the level of starting capital begins to increase above $10,000 the area under forestry plantations comes to occupy a high fraction of the total available land (81%, Table 112).

The presence of forestry plantations reduces the competitiveness, within the system, of the pastures in monoculture. The improved pastures, that at high levels of starting capital and in a scenario without the option of forestry, occupy 79% of available land, now reduce their participation to only 12% when the option of forestry is included. As in the case of the specialized beef system, the option of carbon sale, given its low current prices, would have limited importance as revenue-producing in dual purpose livestock farms (Table 110). Changes in the price of the carbon, in the range of US$ 5 to 100/mt, would have little incidence on land use changes (Table 111).

Due to the greatest initial productivity of the dual purpose system compared to specialized beef, the introduction of trees in the first one has a smaller economic incidence. The introduction of trees in DP would not lead to substantial changes in the value of the objective function, which means that the shadow prices of land would remain stable. An economic and social externality, resulting from the expansion of areas allocated to forestry, is the reduction of the capacity to generate employment since this activity is less intensive in labor compared to the crop-livestock options.

Forest incentive policies

The application of a forest incentive, equivalent to a CIF of 50%, would stimulate the forestry option among producers with high levels of starting capital, but with a poor effect on the weakest economic groups of producers (Table 113). However, the combination of a forest incentive such as the CIF and an agreement of prepayment of the carbon sequestered would have a great impact in terms of expansion of the forest areas within producers with availability of starting capital under US$ 200,000 (Table 114). Under

Table 109. Dual purpose system Scale: 500 ha. Dual purpose system with improved forages in rotation with crops and conservationist soil practices

Starting capital available		Land use Ha							Herd inventory (heads)	Net employment generation (# jornals)	Value of objective function US$ 000
			Rotations crop-grass			Improved grass					
Total US$ 000	Per hectare US$	Native sabanna	Rotation 1	Rotation 2	Rotation 3	*Brachiaria* cv. Toledo	Association *B. decumbens* + *D. ovalifolium*	Total area utilized			
300	600	0.0	0.0	106.5	0.0	393.5	0.0	500.0	871	780	7684
200	400	0.0	0.0	179.7	0.0	320.3	0.0	500.0	709	1099	6889
100	200	0.0	69.4	235.0	0.0	195.6	0.0	500.0	567	1400	5995
50	100	0.0	205.0	174.7	0.0	120.3	0.0	500.0	661	1253	5535
25	50	0.0	272.8	144.5	0.0	82.7	0.0	500.0	709	1180	5305
10	20	0.0	312.0	128.2	0.0	59.8	0.0	500.0	734	1142	5163
5	10	0.0	319.0	130.2	0.0	50.8	0.0	500.0	727	1157	5102

Rotation 1: Native sabanna – rice/corn ~ soybean –. *B. decumbens* + *D. Ovalifolium*. Rotation 2: = rice/corn ~ soybean - *Brachiaria* cv. Toledo - rice/corn ~ soybean
Rotation 3: rice/corn ~ soybean - *B. decumbens* +*D. Ovalifolium* – rice/corn ~ soybean

Table 110. Economic impact of the sale of carbon in a farm with a dual purpose production system of the Altillanura with different levels of starting capital

Level of starting capital US$ 000	Value of objective function US$ 000		Change in the value of the objective function due to sale of carbon	
	With sale of carbon	Without sale of carbon	Total (US$ 000)	%
300	7932	7788	144	1.8
200	6995	6899	96	1.4
100	6057	5995	62	1.0
50	5588	5535	53	1.0
25	5353	5305	48	0.9
10	5209	5163	46	0.9
5	5145	5102	43	0.8

Table 111. Impact of changes in the price of carbon on the profitability and land use in a dual purpose farm of the Colombian altillanura.

Carbon price US$/mt	Land use			Value of the objective function US$ 000
	Rotation grass-crops		Forestry	
	Rotation 1	Rotation 3		
5	6.3	281.8	211.9	3797
10	6.3	281.8	211.9	3861
20	6.3	281.8	211.9	3995
30	30.3	261.7	208.0	4130
40	30.3	261.7	208.0	4266
50	57.3	239.1	203.6	4402
60	57.3	239.1	203.6	4541
70	57.3	239.1	203.6	4680
80	83.2	217.5	199.3	4821
90	83.2	217.5	199.3	4962
100	83.2	217.5	199.3	5104

Starting capital: US$ 100,000

this scenario, producers with low starting capital (US$ 5,000) would incorporate the option of forest in their production systems by almost one third of the farm (173 ha), which would imply an increase of 9% in net income (Tables 113 and 114).

Impact of technological transformation of livestock systems in situations of low availability of working capital. One of the main problems to transform the predominant extensive livestock systems in the Altillanura, other than the limitations of road infrastructure, access to markets and technological offer, is the lack of working capital which on many occasions impedes the adoption and dissemination of new alternatives of production. To analyze this constraint, different technological scenarios were designed for a livestock farm with a limited starting capital of US$10,000. The transformation of the system with a low starting capital, from the primary stage where the farm depends almost exclusively on native savannas until reaching a phase of modernization and diversification, involves a dramatic growth of its net income, as observed in Table 115. The technological adoption not only makes it possible to utilize all the available family labor, but also generates additional employment.

With intensification through the incorporation of new technological components, the demand on labor increases. When the specialized beef system adopts strategies of conservationist land

Table 112. Dual purpose system. Scale: 500 hectares. Livestock, with improved forages rotation with crops, conservatist soil practices, forrestry options and payment for environmental services (sale of carbon.)

Availability of starting capital		Land use (ha)										
				Rotations grass-crops			Improved grasses					
Total US$ 000	Per ha US$	Forestry Caribbean Pine	Native savanna	Rot 1	Rot 2	Rot 3	*Brachiaria* cv. Toledo	Association *B. decumbens* + *D. ovalifolium*	Total area utilized (ha)	Herd inventory (heads)	Net employment generation (journals)	Value of objective function US$ 000
300	600	405.3	0.0	28.1	8.7	0.0	57.9	0.0	500.0	182	729	7932
200	400	275.8	0.0	139.1	37.5	0.0	47.6	0.0	500.0	374	837	6995
100	200	134.3	0.0	235.4	80.0	0.0	50.3	0.0	500.0	565	983	6057
50	100	63.6	0.0	283.6	101.3	0.0	51.5	0.0	500.0	661	1055	5588
25	50	28.3	0.0	307.7	111.9	0.0	52.1	0.0	500.0	708	1092	5353
10	20	0.0	0.0	312.0	128.2	0.0	59.8	0.0	500.0	734	1142	5209
5	10	0.0	0.0	319.0	130.2	0.0	50.8	0.0	500.0	727	1157	5145

Table 113. With the application of a scheme of forest incentives (CIF of 50%).

Availability of starting capital		Land use (ha)										
				Rotation grass-crops			Improved grasses					
Total US$ 000	Per ha US$	Forestry Caribbean Pine	Native savanna	Rot 1	Rot 2	Rot 3	*Brachiaria* cv. Toledo	Association *B. decumbens* + *D. ovalifolium*	Total area utilized (ha)	Herd inventory (heads)	Net employmen t generation (journals)	Value of Objetive Function US$ 000
300	600	453.7	0.0	0.0	0.0	0.0	46.3	0.0	500.0	102	709	8239
200	400	311.6	0.0	116.8	32.8	0.0	38.8	0.0	500.0	311	829	7209
100	200	151.8	0.0	224.6	77.7	0.0	45.9	0.0	500.0	534	979	6161
50	100	71.9	0.0	278.4	100.2	0.0	49.5	0.0	500.0	646	1054	5637
25	50	31.9	0.0	305.4	111.4	0.0	51.3	0.0	500.0	702	1091	5375
10	20	0.0	0.0	312.0	128.2	0.0	59.8	0.0	500.0	734	1142	5209
5	10	0.0	0.0	329.0	130.2	0.0	50.8	0.0	500.0	727	1157	5145

Table 114. Dual purpose system Scale: 500 ha. Anticipated payment of environmental services d application of a CIF of 50%.

Land use (ha)										
Forestry Caribbean Pine	Native savanna	Rotation grass - crops			Improved grasses		Total area utilized	Herd inventory (heads)	Net employment generation (journals)	Value of objective function US$ 000
		Rot 1	Rot 2	Rot 3	*Brachiaria* cv. Toledo	Association *B. decumbens* + *D. ovalifolium*				
203.9	0.0	0.0	0.0	0.0	296.1	0.0	500.0	656	492	8786
361.7	0.0	0.0	0.0	0.0	138.3	0.0	500.0	306	620	8443
376.9	0.0	53.6	34.2	0.0	35.3	0.0	500.0	182	837	7507
256.9	0.0	110.7	93.3	0.0	39.1	0.0	500.0	300	1040	6556
206.5	0.0	134.6	133.0	0.0	25.9	0.0	500.0	317	1189	6045
181.7	0.0	146.4	162.4	0.0	9.5	0.0	500.0	303	1306	5718
173.4	0.0	150.4	172.1	0.0	4.1	0.0	500.0	299	1345	5609

Table 115. Technological evolution of a cow-calf operation with low starting capital in the Colombian Altillanura.

Level of development of the production system	Land use (ha)							Value of the objective function		Land shadow price	
	Pastures in monocultive			Rotations grass - crops			Forestry	US$ 000	Index	US$/ha	Index
	Native savanna	Improved *Brachiaria*	Association Grass - legume	Rot 1	Rot 2	Rot 3					
1. Extensive livestock with native savanna	482.2	17.8	0.0	-	-	-	-	277	100.0	136	100.0
2. Livestock + grass – crop rotations	0.0	13.7	0.0	343.5	9.9	132.9	-	1560	563.1	2692	1979.4
3. Livestock + grass – crop rotations + forestry	0.0	0.0	0.0	0.0	0.0	359.8	140.2	2704	976.2	4680	3441.2
4. Livestock + grass - crop rotations + forestry + sale of environmental services (C)	0.0	0.0	0.0	0.0	0.0	359.8	140.2	2750	992.8	4763	3502.2
5. Alternative 4,with policy to incentivate forestry (CIF)	0.0	0.0	0.0	0.0	0.0	359.8	140.2	2847	1027.8	4936	3629.4
6. Alternative 5, with anticipated payment for environmental services	0.0	0.0	0.0	9.1	0.0	254.6	236.3	4215	1521.7	6631	4434.6

Table 116. Technological evolution of a dual purpose livestock system with low starting capital in the Colombian Altillanura.

Level of development of the production system	Land use (ha)							Value of the objective function		Land shadow price	
	Pastures in monocultive			Rotations Grass - crops							
	Native savanna	Improved *Brachiaria*	Association Grass - legume	Rot 1	Rot 2	Rot 3	Forestry	US$000	Index	US$/ha	Index
1. Extensive livestock with native savanna	484.3	15.7	0.0	-	-	-	-	778	100.0	546	100.0
2. Livestock + grass – crop rotations	0.0	59.8	0.0	312.0	128.2	0.0	-	5163	663.6	9736	1783.1
3. Livestock + grass – crop rotations + forestry	0.0	59.8	0.0	312.0	128.2	0.0	0.0	5163	663.6	9736	1783.1
4. Livestock + grass - crop rotations + forestry + sale of environmental services (C)	0.0	59.8	0.0	312.0	128.2	0.0	0.0	5209	669.5	9829	1800.2
5. Alternative 4,with policy to incentivate forestry (CIF)	0.0	59.8	0.0	312.0	128.2	0.0	0.0	5209	669.5	9829	1800.2
6. Alternative 5, with anticipated payment for environmental services	0.0	9.5	0.0	146.4	162.4	0.0	181.7	5718	735.0	10317	1889.6

use, crop rotations in order to construct arable layers, the increase in employment is estimated in 1,121 wages. If the farm had the dual purpose system, the increase in employment would exceed 1,500 wages/year. The forestry alternative to produce wood and sequester carbon, at this level of starting capital, is a more attractive optioin in the specialized beef farm. In the dual purpose farm, the forestry option only appears in the optimal solution when the combination of a forest incentive is accompanied by an anticipated payment of carbon sales (Tables 115 and 116).

Conclusions

The traditional livestock systems in the savannas of Llanos of Colombia based to a great extent in the utilization of native pastures as the main forage source, generates levels of productivity and profitability that makes their survival in a globalized world very difficult. The savannas provides a large geographical space with the opportunity of high productive potential, which should be used strategically to conserve its soil resources, classified by experts as very fragile from the physical and chemical standpoint. The simulations of several technological scenarios carried out in this study show that the incorporation of new components to the current livestock systems would constitute a powerful tool to stimulate regional agriculture, while improving the productive capacity of the soil.

In the evaluated scenarios, the level of starting capital determines to a high degree the capacity of the systems to incorporate new technological options. Forestry production would be a good option to the extent that the region invest in reasonable means of transportation and adequate infrastructure for the management and processing of forest products. The current prices in the international carbon market are low and this trend is expected to continue in the next 5–6 years. This circumstance determines that the sale of carbon sequestered by trees and pastures do not represent a significant income in the farm economy and has a low impact on land use.

4.7 Multiplication and delivery of experimental and basic forage seed

Highlights

- Over one ton of seed was produced by the Forage Seed Multiplication Unit at CIAT-Palmra during the 15-month reporting period (September 2004 and December 2005). Five hundred forty-three seed samples, totaling 1.2 t, were dispatched during the same period to eleven different countries.

- The Seed Unit at Atenas continues to produce, procure and deliver under request experimental and basic seed of promising forage germplasm. This year 491 kg of seed were delivered in response to 53 requests from 9 countries; the bulk of the seed was formed by *C. argentea* (96.6 kg), *Brachiaria* spp. (115 kg) and *A. pintoi* (184.4 kg).

4.7.1 Multiplication and delivery of selected grasses and legumes from the Seed Unit in CIAT-Palmira, Colombia

Contributors: A. Betancourt, J. Muñoz and J.W. Miles (CIAT)

Rationale

The delivery mechanism for our technology — improved germplasm — is generally in the form of seed. For many of the plants we are developing, no commercial seed supply exists. While we seek to encourage private initiative in supplying seed, we recognize that in the early stages of development a need for seed for experimental purposes and initial distribution can most reliably be met by internally generated supplies. The Project maintains a modest seed multiplication and processing capacity at headquarters to meet this demand.

Materials and Methods

Seed multiplication field plots are established and maintained at headquarters (CIAT-Palmira) and at substations at CIAT-Popayán and CIAT-Quilichao. Final seed processing and all aspects of seed distribution are handled at CIAT headquarters, where routine seed quality determinations are also conducted.

Results and Discussion

Just over one ton of seed was produced and processed by the Forage Seed Multiplication Unit at CIAT during the 15-month reporting period (September 2004 to December 2005) (Table 117). Project priorities, as reflected by volumes of seed produced of the different forage species, are similar to last year's. A significant proportion of the total (nearly 40%) was seed of *Cratylia argentea*. Significant quantities of *Lablab purpureus* (167.2 kg) and *Canavalia brasiliensis* (59.4 kg) were also produced. Smaller quantities of seed of 34 accessions of 13 additional species completed the total (Table 117).

Seed distribution was 20% in excess of production during the reporting period, relying on carryover stocks from 2004 (Table 118). A total of 543 individual samples were distributed to a diversity of end users in 11 different countries (Table 119).

Table 117. Seed of 63 accessions of 15 species produced and processed by the Forage Seed Multiplication Unit (CIAT-Palmira) between September 2004 and December 2005.

Genus	Species	Number of accessions	Harvest (kg)
Lablab	*purpureus*	13	167.2
Arachis	*pintoi*	1	3.5
Cratylia	*argentea*	9	385.5
Leucaena	*leucocephala*	6	155
Pueraria	*phaseoloides*	2	10
Canavalia	*ensiformis*	3	22.2
Canavalia	*brasiliensis*	7	59.4
Brachiaria	sp.	2	8.5
Brachiaria	*brizantha*	7	101
Brachiaria	*decumbens*	1	50
Brachiaria	*lachnantha*	1	16
Calliandra	*calothyrsus*	5	13.5
Centrosema	*acutifolium*	2	2
Stylosanthes	sp.	1	0.4
Stylosanhtes	*guianensis*	3	37.6
	Totals	63	1031.8

Table 118. Volumes of seed of 24 forage genera distributed by the Forage Seed Multiplication Unit (CIAT-Palmira) between September 2004 and December 2005.

Genus	Kilograms	Genus	Kilograms
Andropogon	4.4	*Hyparrhenia*	0.8
Arachis	2.1	*Lablab*	29.1
Brachiaria	223.0	*Leucaena*	33.8
Cajanus	9.4	*Melinis*	5.2
Calliandra	1.0	*Mucuna*	19.3
Canavalia	24.8	*Panicum*	17.9
Centrosema	17.3	*Paspalum*	3.5
Clitoria	1.0	*Pennisetum*	0.01
Cratylia	738.6	*Pueraria*	1.3
Desmodium	27.3	*Stylosanthes*	7.1
Dioclea	1.5	*Zornia*	7.4
Flemingia	31.8	Total	1207.6

Table 119. Forage seed samples dispatched by the Forage Seed Multiplication Unit (CIAT-Palmira) to eleven countries between September 2004 and December 2005.

Country	Number of Samples
Germany	6
Bolivia	2
Colombia	502
Costa Rica	1
Ecuador	1
United States	1
Honduras	7
Kenya	5
Nicaragua	15
Thailand	1
Trinidad and Tobago	2
Total	543

4.7.2 Multiplication and delivery of selected grasses and legumes from the Seed Unit of CIAT-Atenas, Costa Rica

Contributors: Guillermo Pérez and Pedro J. Argel (CIAT)

Seed multiplication activities of promising forage germplasm continued during 2005 at the Atenas Seed Unit (Costa Rica) in collaboration with the Escuela Centroamericana de Ganadería (ECAG). The seed either produced or procured is destined to support advanced evaluations and promotions of forage germplasm both by CIAT's projects and regional research/development institutions.

From September 2004 through August 2005 a total of 392.6 kg of experimental and basic seed was either produced at Atenas or procured from associated collaborators. The bulk of the seed was formed by *Cratylia argentea* (105.5 kg), *Brachiaria* spp. (5.3 kg), *Brachiaria* hybrids cv. Mulato and cv. Mulato II (211.9 kg), *Arachis pintoi* (19.9 kg), *Leucaena* spp. (5.8 kg), *Centrosema* spp. (0.3 kg), *Stylosanthes guianensis* AFT 3308 (20.7 kg), *Vigna* spp. (6.5 kg) and *Panicum maximum* (1.3 kg) and *Paspalum* spp. (3.40 kg). Also 12.0 kg of other forage species.

During the period September 2004-August 2005 a total of 490.6 kg of experimental and basic seed was delivered by the Seed Unit of Atenas (Costa Rica).

Table 120 shows that 53 seed requests were received from 9 countries, where most of the requests came from Costa Rica, the host country of the forage project. However, a significant amount of experimental seed was delivery to Guatemala (154.0 kg) and to Honduras (100.7 kg), both countries involved in forage projects with the participation of CIAT.

A high amount of basic and experimental seed of the promising forage legume *Arachis pintoi* (184.4 kg) was delivered, and of *Brachiaria* species, particularly of cv. Mulato, the new hybrid of this genus that is being promoted regionally with the assistance of the private sector.

Table 120. Countries, number of requests and amount of experimental/basic forage seed delivered by the Seed Unit of Atenas (Costa Rica) during the period August 2004-August 2005.

Country	No. of Requests	Forage species (kg)				Total
		Brachiaria spp.	*A. pintoi*	*C. argentea*	Other species	
Colombia	1			0.8		0.8
Costa Rica	35	67.5		41.1	74.5	183.1
Guatemala	6		105.0	43.0	6.0	154.0
Haití	1			1.0		1.0
Honduras	4	18.0	78.0	2.2	2.5	100.7
Kenya	1	2.0				2.0
Nicaragua	3	26.0	1.4	8.5	11.6	47.5
Perú	1	1.0				1.0
Venezuela	1	0.5				0.5
Total	53	115.0	184.4	96.6	94.6	490.6

4.8 Facilitate communication through journals, workshops and the Internet

Highlights

- An expert system to target forages (SoFT) was completed in 2005 and launched at the International Grassland Congress in 2005. Since the time of release of SoFT, 700 to 800 CD copies have been distributed.

- A total of 178 technicians from National Institutions in four Central America countries were trained on pasture establishment, forage seed quality and forage conservation for dry season feeding.

- An international workshop on adaptation of forages/crops to acid soils was organized by EMBRAPA and CIAT. Progress on improving acid soil adaptation of forages/crops was presented and future areas for collaborative research were identified.

- Three issues of Pasturas Tropicales were published in 2005, with contributions from forage researchers from Brazil, Colombia, Costa Rica, and Argentina.

- The newly launched SoFT (Selection of Forages for the Tropics) included in the Forage web page has had a large frequency (11,000-15,000/month) of visit.

4.8.1 Expert systems for targeting forages: Selection of forages for the tropics (SoFT)

Contributors: B.C. Pengelly (CSIRO), B.G. Cook (QDPI), I. J. Partridge (QDPI), D.A. Eagles (CSIRO), M. Peters (CIAT), J. Hanson (ILRI), S. D. Brown (CSIRO), J. L. Donnelly (CSIRO), B. F. Mullen (CSIRO) , R. Schultze-Kraft (University of Hohenheim), A. Franco and R. O'Brien (CIAT)

Rationale

Forage research over the last 50 years has identified many tropical grasses and legumes that have a role in farming systems in developed and developing countries. Information on the adaptation and use of these species has resided in peer-reviewed literature, research reports with limited distribution and, often most importantly, in the memories of forage agronomists with decades of experience of working with a wide range of forages in diverse farming systems. Selecting the right species and germplasm for particular environments and farming systems is a complex task and there is often poor access to information. This has frequently resulted in researchers not being able to learn from past experience, and there has always been a risk that repeating the mistakes of the past will result in lost opportunities and poor use of resources. Moreover, researchers and advisors in contact with communities have usually had poor access to up-to-date information on tropical forages, often resulting in suboptimal suggestions to farmers; a situation further aggravated by the decline in the overall number of forage experts over the last 20 years. In this context the main objectives for development of SoFT were:

- To develop a knowledge system for the identification of forages suitable for specified niches within smallholder farming systems in the tropics and subtropics.
- To promote the system within the "communities" who are using tropical forages.
- To develop a strategy for maintenance and updating the knowledge system.

Results and Discussion

This project attempted to access the best available information that would allow the adaptation and potential use of 180 tropical forage species and their elite cultivars or accessions to be defined and integrated in a single user-friendly database. The database, which includes a simple-to-use tool to assist in the selection of the best-bet species, is now freely available on the Internet (www.tropicalforages.info) and on CD (Photo 39).

The database has five main features:

i) information in fact sheets on the adaptation, uses and management of forage species, cultivars and elite accessions
ii) a selection tool built on LUCID™ that enables easy identification of best-bet species
iii) a bibliography of more than 6,000 references and abstracts on forage diversity, management and use which will enable users with poor library facilities to access summaries of some of the key literature
iv) global maps of climate adaptation for each species
v) a collection of photographs and images of species to help in their identification and use

The database selection tool is an expert system based on the experiences of forage specialists who have worked for many years in tropical and subtropical regions of Africa, lower latitude USA, Central and South America, South and South-east Asia and Australia. Selections are made on the basis of 19 criteria (Table 121). The project brought the teams of experts together in workshops in Africa, Asia, South America, Central America, Europe and Australia over a two year period and had input from other forage specialists during the database development.

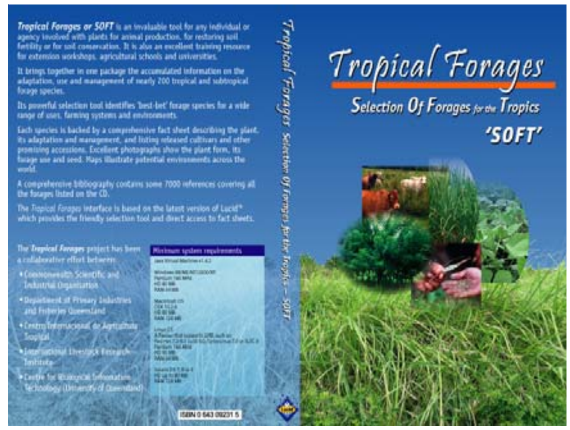

Photo 39. Screenshot Tropical Forages Database "SoFT".

The principal outcome has been summarised information on tropical forage adaptation and use from expert knowledge, available literature and experiential sources made available in a readily accessible and consistent format. With availability on DVD and the Internet, the database allows researchers and advisors to select those forages most suitable for local conditions. Although the database was initially designed for use in developing countries, its content includes species adapted to farming systems in developed countries and is equally applicable in these regions. The database covers a wide range of forage uses and allows users to select among many different farming systems ranging from permanent to short term pastures, with applications in agroforestry, inter-row cropping, cut-and-carry, hedgerows, green manures and ground covers.

The database has also been recognised as a valuable teaching tool for colleges and universities with feedback from many university

Table 121. Selection criteria available in the SoFT database for selecting the most suitable forages for environments and uses.

Climate/farming system attributes	Soil environment attributes	Plant attributes
Latitude x altitude	Soil pH	Plant family (legume or grass)
Rainfall (average annual)	Level of available soil Al/Mn	Life cycle
Length of dry season	Level of soil salinity	Growth form
Inundation	Soil drainage	Stem habit
Intended forage use	Soil texture	Cool season growth
Grazing pressure	Soil fertility	Frost tolerance (foliage damage)
Shade environment		

staff from a wide range of countries attesting that the database and selection tool will have a major role in improving the way tropical forage science is taught.

The database was promoted during its development via the regional forage workshops attended by most practicing forage agronomists from different regions. Since then promotion has been through the partner organisations, particularly through the International centers (CIAT and ILRI), and in the delivery of a paper at the XXth International Grassland Congress in Ireland in June 2005. In addition to this paper, the project team conducted demonstrations at the Congress and over 100 tropical forage agronomists were able to test the selection tool and trawl the 180 fact sheets. The database is on the internet and each of the partner organisations is taking every opportunity to promote both database and selection tool. This is particularly so with the international institutes who have strong roles in their regions. Since the release in June 2005, there has been a steady increase in the number of visits to the Internet site, from 249 visits in June, to 360 visits in July, 755 in August, 2953 in September and 4810 in October. While larger number of these have been from Australia and the US, the latter with a large participation from Educational Institutions, there have been significant and increasing visits by users in Colombia, from CG centers Mexico, Peru, Brazil. Argentina, France, The Netherlands, Nepal, Guatemala, Switzerland, Thailand, Germany and Kenya.

Future actions

It was agreed at the commencement of the project that the database would reside on the CIAT Internet server and that CIAT would be responsible for its maintenance and its updating. This plan has now been put into place. However it has become apparent even after only a few months that new information needs to be added to the fact sheets. It will be also necessary to translate the database into other languages (i.e. Spanish and French) to encourage its use and application in Latin America and the Caribbean and francophone Africa, respectively.

4.8.2 Training courses in Central America

Contributors: P. J. Argel, G. Pérez, P. Lentes (CIAT), C. Reiber (U. of Hohenheim), H. Cruz (CIAT-DICTA)

During the period October 2004 through September 2005, six training courses on topics related with forage technology were held in Nicaragua, Honduras, Guatemala and Costa Rica, within the frame of the Project Enhancing Beef Productivity, Quality, Safety and Trade in Central America financed by CFC and coordinated by ILRI.

In Table 122 we show that 178 technicians from different national institutions participated in the workshops on topics related with pasture establishment and measurement, forage seed quality, and forage conservation practices. A considerable number of farmers participated as well, particularly in Guatemala and Nicaragua where silage of forages in small plastic bags is being promoted as viable practice of forage conservation for small farmers. Many of the technicians trained are responsible for on-farm forage monitoring in collaborating farms of the ILRI/CFC Project, thus facilitating the documentation of the impact on farm productivity due to the establishment of improved pastures.

During 2005 training courses and field days to demonstrate to farmers how to make hay and silage were also carried out in Honduras as part of BMZ, Germany Special Project. The events carried out are summarized in Table 123.

Table 122. Training courses carried out in participating countries of the project Enhancing Beef Productivity, Quality, Safety and Trade in Central America during the period October 2004 to September 2005.

Country	Date	Participants (No.)	Themes	Participating Institutions/Farmers
Nicaragua	28-29 Oct. 2004	55	Pasture establishment Forage seed quality	IDR*, INTA, UNA, MAG/FOR, Duwest
Honduras	9-11 Nov. 2004	12	Pasture establishment Forage seed quality	DICTA, Duwest
Guatemala	18 March 2005	16	Pasture establishment Forage seed quality	ICTA, U. de San Carlos, MAGA, FECAGUATE
Costa Rica	1 July 2005	15	Pasture measurements	CORFOGA, CGUS, Dos Pinos, Coopemontecillos, ECAG
Nicaragua	21 Sept. 2005	15	Forage conservation	INTA
	22 Sept. 2005	12	Forage conservation	INTA/ Small Farmers,
	23 Sept. 2005	25	Forage conservation	IDR
Guatemala	28 Sept. 2005	28	Forage conversation	MAGA, Small Farmers

* IDR, Instituto de Desarrollo Rural; UNA, Universidad Nacional; INTA, Instituto Nicaraguense de Tecnología Agropecuaria (Nicaragua) and Instituto Nacional de Innovación y Transferencia de Tecnología Agropecuaria (Costa Rica) respectively; MAG/FOR, Ministerio de Agricultura, Ganadería y Forestal; DICTA, Dirección de Ciencia y Tecnología Agropecuaria; ICTA, Instituto de Ciencia y Tecnología Agrícolas; MAGA, Ministerio de Agricultura y Ganadería; FECAGUATE, Federación Guatemalteca de Ganaderos; CORFOGA, Corporación de Fomento Ganadero; CGUS, Cámara de Ganaderos Unidos del Sur; ECAG, Escuela Centroamericana de Ganadería.

Table 123. Training courses carried out in Honduras as part of BMZ, Germany Special Project during the period October 2004 to December 2005.

Event	Dates	Location	Participants		
			M	F	Total
Pasture establishment Pasture seed quality IDR*, INTA, UNA, MAG/FOR, Duwest	28-29/10/2004	Nicaragua			55
Pasture establishment Pasture seed quality DICTA, Duwest	9-11/11/2004	Honduras			12
Pasture establishment Pasture seed quality ICTA, U. de San Carlos, MAGA, FECAGUATE	18/03/2005	Guatemala			16
Pasture measurements CORFOGA, CGUS, Dos Pinos, Coopemontecillos, ECAG	1/07/2005	Costa Rica			15
Pasture conservation INTA INTA/ Small Farmers, IDR	21/09/2005 22/09/2005 23/09/2005	Nicaragua			15 12 25
MAGA, Small Farmers	28/09/2005	Guatemala			28
Training course on pasture seed harvesting and seed quality with emphasis on Toledo grass designed to Victoria small farmers associated in Prasefor (a pasture seed cooperative)	24/04 to 05/05 2005	Yoro, Honduras			
Management and conservation (hay and silage) of forages, theoretical part with participative evaluations	5/21/2005	Sulaco, Yoro, Honduras	12	1 (10 pupils)	23
Management and conservation (hay and silage) of forages, theoretical part with participative evaluations	6/7/2005	Yorito, Yoro, Honduras	20	4	24
Establishment and use of legumes for concentrate production	6/8/2005	Salitre, Yoro, Honduras	4	8	12
Characteristics and management (establishment) of Cowpea, *Lablab purpureus* and *B. brizantha* cv Toledo, hay and concentrate production and its use	6/8/2005	La Savanna, Yoro, Honduras	3	6	9

Continues……

Continued……

Table 123. Training courses carried out in Honduras as part of BMZ, Germany Special Project during the period October 2004 to December 2005.

Event	Dates	Location	Participants		
			M	F	Total
Management and conservation (hay and silage) of forages, theoretical part with participative evaluations	6/9/2005	Las Vegas/Victoria, Yoro, Honduras	13	1	14
Management and conservation (hay and silage) of forages, theoretical part with participative evaluations	6/14/2005	Alauca, El Paraiso, Honduras	13	1	14
Management and conservation (hay and silage) of forages, theoretical part with participative evaluations	6/15/2005	Jamastrán, El Paraiso, Honduras	15	3	18
Management and conservation (hay and silage) of forages, theoretical part with participative evaluations	6/16/2005	Jesus de Otoro, Intibuca, Honduras	6	1	7
Management and conservation (hay and silage) of forages, theoretical part with participative evaluations	6/17/2005	Catacamas, Olancho, Honduras	21	5 (12 pupils)	38
Forage conservation with practical training in little bag silage production	9/13/2005	Yoro, Yoro, Honduras	22	0	22
Silage making with special focus on little bag silage, practice	9/22/2005	Alauca, El Paraiso, Honduras	20	4	24
Silage making with special focus on little bag silage, practice	9/23/2005	Jamastrán, El Paraiso, Honduras	45	5	50
Silage making with special focus on little bag silage, practice	9/24/2005	Victoria, Yoro, Honduras	26	3	29
Forage conservation with practical training in hay and little bag silage, the characteristics of improved forages	9/27/2005	Las Tres Ceibas Olancho, Honduras	22	3	25
Forage conservation with practical training in hay and little bag silage, the characteristics of improved forages	9/26/2005	Las Tres Ceibas Olancho, Honduras	19	8	27
Forage conservation with practical training in silage making (LBS and other silos), the characteristics of improved forages	11/29/2005	El Rodeo, El Paraiso, Honduras	7	2	9
Use of legumes for hay and concentrate preparation presented to 2 non-cattle farmer groups	12/9/2005	Yorito, Yoro, Honduras	2	10	12

4.8.3 International workshop on advances in improving acid soil adaptation of tropical crops and forages (Organizers: EMBRAPA, CIAT and IRD)

Contributor: I. M. Rao (CIAT)

An international workshop on "Advances in improving acid soil adaptation of tropical crops and forages, and management of acid soils" took place in Brasilia, Brazil from 18 to 21 October 2005. This workshop was the result of interaction of a group of EMBRAPA forage researchers that visited CIAT last year to strengthen collaboration between CIAT and EMBRAPA. The event was organized by EMBRAPA (Drs. Ronaldo Andrade and Leide Andrade of CPAC), CIAT (Carlos Lascano and Idupulapati Rao) and IRD (Dr. Thierry Becker outposted staff at CPAC) with funds from Brazilian Government to the CGIAR that are administered via CIAT. The workshop had 30 participants from several centers of EMBRAPA, CIAT, Cornell University, IRD and other partners of EMBRAPA.

The main objectives of the workshop were: (i) To review the progress in improving acid soil adaptation of major food and feed crops and management of acid soils; (ii) To identify future research needs; and (iii) To develop collaborative research program (short and medium term) among EMBRAPA-CIAT and other partners to improve acid soil adaptation of tropical crops and forages and management of acid soil. The program for the Workshop included 1.5 days of invited presentations, 0.5 day of a session on future research needs, 1 day of field visit to CPAC labs and field experiments and a farm in the Cerrados, and 0.5 day of conclusions and final recommendations.

Highlights of the Workshop

- Professor Leon Kochian from USDA-ARS and the Cornell University, USA made the keynote presentation on physiological and molecular mechanisms of aluminum (Al) resistance in crops. Last year, the first gene for Al r ALMT1) was identified and cloned from wheat by a group of Japanese and Australian researchers. Attempts are being made by Cornell and EMBRAPA to clone the Al resistance gene (ALT_{SB}) from sorghum. It is clear that the mechanisms of Al resistance are more complex in rice and maize than in wheat and sorghum.

- CNPMS-EMBRAPA presented their work on breeding, physiology and molecular genetics of maize and sorghum. Researchers at this center have been very successful in releasing a number of acid soil adapted maize and sorghum cultivars for the past few years. The biotechnology group of CNPMS is working closely with the breeders and physiologists in identification and characterization of Al-induced genes in the root apex of grass species (maize, sorghum, rice, wheat, barley, oat and *Brachiaria*).

- CNPGC-EMBRAPA presented the results of team-work on agropastoral production in no-tillage systems in the Cerrados of Brazil. Great progress has been made in understanding vegetation and soil dynamics in a long-term experiment (12 years-old) on agropastoral systems. At present, there are about 6 million hectares that are under no-till systems in the Cerrados. One important observation made at this workshop is the dramatic increase in adoption of *Brachiaria brizantha* cv. Marandu in the Cerrados for the past 15 years due to its productivity potential and spittlebug resistance. This is an indication of need for spittlebug resistant and productive brachiaria grasses for acid soils.

- CIAT- TSBF – LAC presented the work with different partners (University of Hannover, Germany; Diversity Arrays Technology P/L, Australia; Hokkaido University, Japan; Yamagata University, Japan) on screening for Al resistance in common bean and *Brachiaria* and on gene identification for high level of Al resistance in signalgrass (*B. decumbens*).

- CIAT-TSBF-LAC- CORPOICA team working on acid soil management emphasized the contrasting differences in soil bulk density between the Llanos of Colombia and the Cerrados of Brazil (bulk density values are much higher in the Llanos and restrict root development). Results were also presented to demonstrate the need to build-up an arable layer to improve soil quality to facilitate no-till systems in the Llanos using the data from the long-term experiment of CULTICORE and the satellite experiments from Matazul in the Llanos of Colombia.

Conclusions and Recommendations

- Establish a network to develop research on acid soils involving Embrapa Centers (wheat, soybean, maize and sorghum, Cerrados, beef cattle, milk cattle, cotton, rice and beans, western crop-livestock, CENARGEN), International institutions (CIAT, IRD, CIMMYT) and universities (Cornell, UnB, UFRGS, UFLA, UFMG);
- Prioritize multidisciplinary approach including production systems with different crops and

forages;

- Identify prospective funding opportunities;
- Establish a network on phenotyping and genotyping for aluminum resistance and phosphorus efficiency;
- Develop a program for capacity building (e.g., short-term, long-term) in topics related to plant adaptation to acid soils and management of acid soils;
- Provide opportunities for special training in bioinformatics tools;
- Create databases on instrumentation, germplasm characterization and technical capabilities of EMBRAPA and partners; and
- Develop a plan for germplasm exchange and intellectual property rights and credit sharing among the partners.

4.8.4 Diffusion of research results: Pasturas Tropicales

Contributor: A. Ramírez (Independent Publisher) and C. Lascano (CIAT)

In 2005, three issues corresponding to Volume 27 of Pasturas Tropicales were published. The contributions came from researchers in institutions from Brazil (21), Colombia (4), Costa Rica (1) and Argentina (1) (Table 124). As in previous years, a large number of contributions coming from Brazilian institutions were observed. This is a reflection of the importance given to R&D in this country as compared to other countries in the region. In addition, it was interesting to note that publications in 2005 covered research topics not previously received from contributors, such as: agroforestry systems, nutritional value of fodder shrubs, use of organic

Table 124. Subjects and number of published contributions in Tropical Pastures during 2005.

Subject	27(1)	27(2)	27(3)	Institution*	Country
Adaptation of forages	1	1	1		Brazil
Rehabilitation pastures			1	UFRPE, ESALQ, Univ. de Hohenheim-Embrapa	Brazil
Inoculation methods	—	—	1	UFRRJ	Brazil
Seed production	1	—		EPAMIG-Brazil	
Agroforestry	1	1	1	Univ. de Brazilia	Brazil
				Embrapa-Agrobiología	
				EARTH	Costa Rica
Phytopathology	—	1	—	Embrapa-CNPGC	Brazil
Entomology	—	1	—	CIAT	Colombia
New cultivars	—	1	—	CIAT	Colombia
Nutritious quality-shrubs	1	—	—	Embrapa-CPAC	Brazil
Fertilization	—	—	1	UFLA	Brazil
				UFFRJ	
Organic fertilization	1	—	1	Embrapa-Agrobiología	Brazil
Green manure	1	1	1	Embrapa-Agrobiología	Brazil
Establishment	—	1	1	UFRPE	Brazil
				INTA-Univ. de Tucumán	Argentina
Animal production	1	2	—	Corpoica, CIAT	Colombia
				Embrapa-Amazonia	Brazil
Impact of germplasm adoption	—	—	—	CIAT	Colombia
Simulation model/growing	—	2	1	ESALQ	Brazil
Total	**7**	**11**	**9**		

* UFRPE = Universidade Federal Rural de Pernambuco, ESALQ = Escola Superior de Agricultura Luiz de Queiros, UFRRJ = Universidade Federal Rural de Rio Janeiro, EARTH = Escuela de Agricultura de la Región de Trópico Húmedo, CNPGC = Centro Nacional de Pesquisa de Gado de Corte, CIAT = Centro Internacional de Agricultura Tropical, CPAC = Centro de Pesquisa Agropecuária dos Cerrados, Corpoica = Corporación Colombiana de Investigación Agropecuaria.

and green manures, and simulation models. As in previous years, we received contributions related to quality, animal production, and fertilization of fodder species were received and published (Table 124). Finally, the magazine is being edited and produced completely by Tropical Forages Project and this has contributed to its timely distribution.

4.8.5 Update on the Forage Web Site

Contributors: S. Staiger, B. Hincapie, A. Franco and M. Peters (CIAT)

The Tropical Forages Web site is the result of team work between all project members, under the general coordination of the Communications Unit and Support of both the Systems and the Information and Documentation Unit. The website has allowed us disseminate our research results extensively and promptly communicate important news. The site is accessible under the URL http//:www.ciat.cgiar.org/forrajes/index.htm.

In 2005 about 80.000 pages were visited (i.e. 6.625 pages per month) with a high frequency of visits on the Spanish version of the web page. The highest number of downloads were recorded for manuals on *Brachiaria brizantha* cv. Toledo and *Cratylia argentea* cv. Veranera and the Pasturas Tropicales journal. Noteworthy is also the high number of downloads of manuals on seed production of cv. Toledo indicating the high probability of on-farm seed multiplication of this species.

An additional 53000 pages (4.484/month) were visited on the Tropileche website, with a very high number of about 74,000 downloads (6131/month)

In July 2005 the Selection of forages for the tropics (SoFT) web site was launched in collaboration with CSIRO, QDPI, ILRI and the University of Hohenheim the web site can be accessed under URL http//:www.tropicalforages.info (see section 4.8.1). After a slow start the site has been very well accepted by users around the world, with between 11000 and 15000 pages visited per month from September to October. The site is most frequented by users from Australia, the US including educational institutions, Mexico, Colombia and Brazil.

Annex

Journal Articles in Refereed Journals (Published, In press and Submitted)

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Awards to Staff in the Project

Outstanding Research Publication Award (ORPA): Cardona, César; Fory, Paola; Sotelo, Guillermo; Pabón, Alejandro; Díaz, Giovanna, and Miles, John. " Antibiosis and tolerance to five species of spittlebug (Homoptera: Cercopidae) in *Brachiaria spp*: Implications for breeding for resistance". Apr. 2004. Journal of Economic Entomology 97(2): 635-645.

Outstanding Support Staff Contribution Award (OSSCA): Guillermo Sotelo, Research Assistant, Tropical Forages Entomology

Francisco Luis Gallego Award: Sotelo Paola; Cardona César; Guillermo Sotelo, and Montoya James. "Resistencia de *Brachiaria spp* al salivazo: Efectos subletales de cultivares resistentes sobre los adultos de *Aeneolamia varia* (S. (Homptera:Cercopidae))"

Thesis list

Internship

Name	Status	University	Title
Schoonhoven, Diane	Completed	Wageningen University, Holland	Estimation and comparison of benefits due to feeding silage and hay during the dry season on commercial dual-purpose cattle production systems in Honduras and Costa Rica
Zöfel, Katrin	Completed	University of Hohenheim, Germany	Morphological and phenological characterization and analysis of origin information of a collection of *Leucaena diversifolia*

BS Thesis

Name	Status	University	Title
Abello Javier F.	Completed	Universidad Nacional, Bogotá, Colombia	*Brachiaria* endophytes as gene delivery system
Betancourth Martha	On-going	Universidad Nacional, Palmira, Colombia	Evaluación de la producción de leche con Brachiaria (Toledo y dos híbridos Mulato y Mulato 2) en suelos ácidos
Miller María Fernanda	Completed	Universidad del Valle, Cali, Colombia	Resistencia de *Brachiaria* spp. al salivazo*:* Efectos subletales de cultivares resistentes sobre los adultos de *Zulia carbonaria* (Lallemand) (Homoptera: Cercopidae)
Real Posada Franklin Rigoberto, Rayo Carazo Omar Antonio Ramírez Edwin José López Suárez Cheyla Matilde Romero Duarte Juan Adán Luna García Álvaro José	On-going	Universidad Nacional Agraria (UNA), Managua, Nicaragua	Survey on dry season feed resources in three different livestock regions of Nicaragua
Rincón Lozano Joisse Dayana	Completed	Universidad Nacional de Colombia, Palmira, Colombia	Evaluación del efecto de la sequía en genotipos de *Brachiaria* bajo condiciones de invernadero
Rosero Jaime	Completed	Universidad Nacional, Palmira	Ensayo Multilocacional de Sistemas de Establecimiento de *Cratylia argentea* cv. Veranera
Schöber Johanna	On-going		Literature review of *Pueraria phaseoloides* (in progress)

MS students

Name	Status	University	Title
Atzmanstorfer Karl	On-going	University of Salzburg, Austria	2006. Semi commercial production of cowpea in Cauca and Valle
Castro Ulises	On-going	Colegio de Postgraduados de Chapingo, Chapingo, Mexico	Mechanisms of resistance to *Aeneolamia albofasciata* and *Prosapia simulans* en *Brachiaria* spp.
Cortés Cortés Javier E.	On-going	Universidad Nacional de Colombia, Bogotá, Colombia	Efecto de los taninos de leguminosas tropicales sobre la degradación in vitro de la proteína con fluido ruminal y pepsina
Husselman Madeleen	On-going	Wageningen Agricultural University	Evaluation of potential production of seed from the hybrid Brachiaria "Mulato" with small plot and on-farm trials on the Bolovens Plateau in southern Lao
Hernández Chaves Moisés	Completed	University of Costa Rica	Evaluación de la selectividad de herbicidas y el control de malezas durante la fase de establecimiento de los pastos *Panicum maximum, Brachiaria brizantha* y *B. decumbens*
López Francisco	On-going	Universidad del Valle	Caracterización de la tolerancia al daño causado por adultos como componente de resistencia a *Aeneolamia varia* (F.) en genotipos de *Brachiaria* spp
Monsalve Castro Lina Maria	On-going	Universidad Nacional de Colombia, Palmira, Colombia	Efectos sobre la fermentación ruminal, el flujo de proteína duodenal y la absorción de nitrógeno en ovinos alimentados con leguminosas con y sin taninos
Nieto B. Juan C.	Completed	Universidad de Costa Rica, Costa Rica	Caracterización nutricional de material fresco y ensilado de Maní forrajero (*Arachis pintoi*) cultivado en asocio con Maíz (*Zea mays*) a tres densidades de siembra
Pabón Alejandro	On-going	Universidad de Viçosa, Brazil	Mechanisms of resistance to *Deois incompleta* and *Notozulia entreriana* en *Brachiaria* spp.
Payan Arlen	On-going	Centro Agronómico de Investigación y Enseñanza (CATIE), Costa Rica	Efecto de *Cratylia argentea* sobre la producción animal en la cuenca de Jucuapa, Matagalpa, Nicaragua
Ricaurte José Jaumer	On-going	Universidad Nacional de Colombia, Palmira, Colombia	Impact of aluminum tolerant *Brachiaria* genotypes on soil quality characteristics of an oxisol of the altillanura of the Meta Department of Colombia
Schulz Tatjana	Completed	University of Hohenheim,, Germany	Evaluation of *Brachiaria brizantha* cv. Toledo and *Brachiaria* hybrids Mulato and Mulato II conserved as hay
Vivas Nelson	Completed	Universidad Nacional de Colombia, Palmira, Colombia	Evaluación agronómica de 144 accesiones de *Desmodium velutinum* como alternativa forrajera para las zonas de ladera del norte del departamento de Cauca

PhD students

Name	Status	University	Title
Andersson Meike Stephanie	Completed	University of Hohenheim, Germany	Genetic diversity and core collection approaches in the multipurpose shrub legumes *Flemingia macrophylla* and *Cratylia argentea*
Bartl Karin	On-going	Swiss Federal Institute of Technology (ETH), Zurich, Switzerland	Effects of improved feeding systems for dairy cattle in tropical smallholder farms on milk production and quality at high altitudes
Castañeda Nelson	On-going	University of Goettingen, Germany	Genotypic variation in P acquisition and utilization in *Arachis pintoi*
Hernández Luis Alfredo	On-going	University of Hohenheim, Germany	A participatory procedure applied to selection and development of forages with farmers
Louw-Gaume Annabé	On-going	ETH, Zurich, Switzerland	Adaptation of *Brachiaria* grasses to low P soils
Mejia Sergio	On-going	Universidad Nacional de Colombia, Palmira, Colombia	Identification of candidate genes responsible for adaptation of tropical forage grass, *Brachiaria* to low phosphorus soils
Reiber Christoph	On-going	University of Hohenheim, Germany	Encouraging adoption of research-based offerings with contrasting extension approaches
Rincon Alvaro	On-going	Universidad Nacional de Colombia, Bogotá, Colombia	Integration of maize with forages to recuperate degraded pastures in the Llanos of Colombia
Tiemann Tassilo	On-going	Swiss Federal Institute of Technology (ETH), Zurich, Switzerland	The forage potential of tanniniferous legumes: Search for sustainable ways to cope with nutritional limitations in smallholder livestock
Van der Hoek Rein	On-going	University of Hohenheim, Germany	Participatory research methods for forage- based technologies in Central-American hillsides.

List of Donors

Asian Development Bank
Livelihood and Livestock Systems
Project
2003-2005

Australia – ACIAR
Development of a knowledge system for the selection of forages for farming systems in the tropics (co-financed with BMZ-DFID), 2002-2005

Australia – AusAID
Forages and Livestock Systems
Project
2000-2005

Austria - KEF Commission for Development Studies at the Austrian Academy of Sciences
Development of low input systems such as organic farming by optimizing the use of legumes in a dry region of Nicaragua to strengthen soil fertility, yield, human nutrition and farm income. Use of legumes in low input systems (ULLIS), 2005-2007

Colombia - SAG Valle del Cauca
Development of agricultural production in the Valle de Cauca (University; Universidad Nacional, Government), 2004-2006

Common Fund for Commodities (CFC)
2003-2006
Enhancing beef productivity, quality, safety, and trade in Central America (Guatemala, Nicaragua, Honduras)

FUNICA - Nicaragua 2003-2004
Validación de sistemas de cultivos con introducción de leguminosas como abonos verdes y coberturas sobre la sostenibilidad de sistemas de producción tradicionales en una microcuenca, San Dionisio, Nicaragua

Germany- BMZ
- Development of a knowledge system for the selection of forages for farming systems in the tropics (cofinanced with ACIAR-DFID), 2002-2005
- Demand-Driven Use of Forages in Fragile, Long Dry Season Environments of Central America to Improve Livelihoods of Smallholders, 2004-2007
- PostDoc proposal Understanding and Catalyzing Learning Selection processes, 2005-2008

Germany - Volkswagen Foundation
- Research and development of multipurpose forage legumes for smallholders croplivestock systems in the hillsides of Latin America (with the U. of Hohenheim and CORPOICA, 2003-2006

Japan – The Ministry of Foreign Affairs
– The tropical Forage Project (core funds)

Semillas Papalotla, S.A. de C.V. 2001-2007
Brachiaria Improvement Program

Systemwide Livestock Program, 2005-2006
Jump-Starting Smallholder Farmer Participation in Public-Private Partnerships: Adapting Legume Forages for On-Farm Monogastric Production and the Feed Industry**,** Seed Grant

Switzerland – SDC- ZIL
- Adaptation of Brachiaria to low P (with ETHZ), 2003-2005
- The forage potential of tanniniferous legumes: The search for sustainable ways to cope with nutritional limitations on smallholder systems (with ETHZ), 2004-2006

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