Tropical Grasses and Legumes: Optimizing genetic diversity for multipurpose use

Annual Report 2006

IP-5 PROJECT

Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture

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Tropical Grasses and Legumes: Optimizing Genetic Diversity for Multipurpose Use (Project IP5)

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Cover photo: The most important asset of the Forage Project: Our people

Tropical Grasses and Legumes: Optimizing genetic diversity for multipurpose use
(Project IP5). 2007. Annual Report 2006. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. 230 p

Dr. Carlos E. Lascano

The Tropical Forages Project Staff dedicate this 2006 Annual Report to Dr. Carlos E. Lascano in recognition of his outstanding contribution to Tropical Forages Research. His research into the nutritive value and utilization of tropical forages has been exceptional, and his leadership of the tropical forages project superb. He has been very committed to the training of young scientists, has been dedicated to CIAT and strongly represented CIAT in international fora. He has collaborated admirably with national institutions and the private sector, and he has ensured that research results are passed on to farmers to improve their livelihoods.

Carlos began his undergraduate training in agronomy at the Escuela Agrícola Panamericana in Zamorano, Honduras. He continued his studies at the University of Arizona, Tucson, USA and received a BSc (1967) and a MSc in Animal Science (1970) from that university. After completing his MSc, he worked with SOLLA SA as Nutritionist and later in 1972 joined CIAT as a Coordinator of International training Courses in Tropical Livestock at the Farm Level and worked for 4 years before he left to USA for his graduate training. In 1979, he was awarded a PhD in Ruminant Nutrition from Texas A&M University, College Station, USA.

Carlos joined CIAT again as a Post Doctoral Fellow in the Tropical Pastures Program in the area of Animal Nutrition in late 1979 and in 1981 he was appointed as Senior Staff responsible for research on forage quality and utilization. His research activities are noteworthy for both their breadth and depth. He is a prolific author, having published more than 70 articles in international, peer reviewed journals, 25 book chapters, 70 conference papers, and 12 technical bulletins and co-edited eight books. These publications reflect Carlos' comprehensive knowledge of the development and utilization of multipurpose tropical forages, as well as animal nutrition and forage utilization.

At the Carimagua research station in the Llanos of Colombia, he carried out the long-term grazing experiments on the productivity of improved grass and grass-legume pastures for beef production. These studies provided strategic knowledge on the sustainability and productivity of these new systems. They also served as a valuable resource for studies, by his colleagues, of soil physical, chemical and biological processes.

Carlos established a dairy unit at the CIAT-Quilichao experiment station. This allowed for the quantitative measurement of forage quality, using short-term changes in milk production as an indicator of forage quality. Research on the contribution of legumes to increasing milk production revealed that some herbaceous, and most shrub, legumes did not give the expected response due to the presence of anti-quality factors. This research led to more strategic investigations of the nature of anti-quality factors and to management studies to improve intake and digestibility of forages.

Carlos became the Leader of the Tropical Forages project in 1996 and as an inspiring Leader for 10 years, he ensured that the focus of research was demand driven. This led to a focus on the identification of forage germplasm options for particular production niches, where there was real need, interest and ability of producers to adopt new technology. He has been outstanding both in his leadership and in fund raising for research on tropical forages. He provided direction and, through the use of interpersonal skills, motivated team members. He has a particular strength in enabling staff, whatever their skills and capabilities, to do their best. He obtained commitment from people at all levels, be they field workers, technicians or experienced scientists.

Carlos has always had a strong commitment to training of young scientists. This included undergraduate and graduate students, scientists from national organizations and his research support staff. He supervised 9 PhD, 19 MSc and 16 BSc students.

Carlos is a vital and inspiring intellectual force with outstanding communication skills. He is a bright, diligent and highly motivated individual. He is very open to new ideas and also has a creative imagination and an enthusiasm for exploring and gaining new knowledge. He has been a key team member in several collaborative projects at CIAT. He has developed strong research linkages with both public and private sector partners. He has actively contributed to institutional development (RIEPT, Tropileche-SLP) and to strengthening our ties with numerous national research programs.

Carlos is highly regarded for his professional expertise in the international community. An outstanding reputation led to his being invited to present plenary papers at several international conferences, as well as reading the Harry Stobbs Memorial Lecture in Australia in 1990. He has served on committees of international organizations such as the International Grassland Society and the Latin American Association for Animal Production and served as President of the latter association (1997-1999). Several countries in the tropics have sought his advice on research strategy in animal production.

In recognition of his exemplary contributions to global forage research, animal production in the tropics, and his distinguished professional career as an Animal Nutritionist, Dr. Lascano has received CIAT's Outstanding Senior Staff Award in 1996.

Carlos is a person of outstanding personal character. He is not only a very good scientist but also a good man. He is committed to helping people, and brings this commitment to every task. He is known for his frankness, in-depth analysis, and intellectual approach to life, and for his charming personality. He invariably seeks to nurture a spirit of harmony amongst his colleagues. He has a delightful sense of humor that is highly appreciated by his friends. He is wise enough to criticize himself. He is a person who puts all his energy and skills into whatever he does, with outstanding results.

The success of the Tropical Forages Project, through its varied partnerships and the tremendous dedication of its staff, has positively affected the lives of resource-poor farmers and consumers in the developing world. Carlos has been a great inspiration to his colleagues at CIAT and CIAT's partners. We will miss him greatly, but we will strive to live up to his exacting standards as we continue our work in developing superior tropical forage options for resource-poor farmers.

Carlos is a great family man, married to Mercita for 34 years. He is a father of two professionally successful children (Dr. Martin Lascano, MD and Claudia Patricia, Veterinary student) and a doting grandfather of Martina and Simón.

The Tropical Forages Team wants to sincerely thank Carlos for the unconditional support he has always offered and for making the Tropical Forages Project one of the most successful projects at CIAT in terms of both the quality of science and improvement of livelihoods for farmers.

We warmly wish him every success in his new endeavors.

Tropical Forages Team, April, 2007

Table of Contents

Tropical Grasses and Legumes:
Optimizing Genetic Diversity for Multipurpose Use

Project Description

Rationale: Livestock development is recognized as a key element for increasing the income of poor smallholders given the increased demand for animal products that is being experienced in developing countries. However, a high proportion of smallholder crop/livestock systems are located in areas with prolonged dry seasons and with land in different stages of degradation, which lead to an inadequate supply of high quality feed for livestock throughout the year. In addition, in many cases smallholders with livestock and limited land (i.e. South East Asia) do not have easy access to fodder and have to walk long distances to harvest forages. Thus development and expansion of high yielding and high quality forages, particularly at the livestock – crop interface needs to take place if small farmers are to benefit from increased market opportunities for livestock products.

To accomplish the objectives of the Forage Project, the research is organized around 4 major outputs: 1) Defined forage quality attributes, 2) Defined plant interactions with parasites (insects, bacteria, and fungi), 3) Defined mechanisms of plant adaptation to environmental constraints and 4) Delivered superior grasses and legumes to partners for further evaluation, adaptation and innovation in crop/livestock productions systems and eventual release and adoption by farmers.

Partnerships are formed with ARIs, universities and NARS to carry out strategic research on improving forage quality (output 1), on developing screening methods based on improved knowledge on mechanisms of adaptation of forage species to biotic (output 2) and abiotic stress factors (output 3) and on developing improved feeding systems using an innovative approaches (output 4).

The delivery of superior forage genotypes is accomplished through partnerships with NARS, NGO's, farmer groups and the private seed sector. To better target forage options to different environments and production systems (output 4) databases and decision support tools are being developed for use by researchers, development workers and for capacity building. Finally, as part of the delivery process we form partnerships with different groups to document on-farm performance of released grass and legume cultivars and quantify the impact of selected forages (output 4) in LAC and in Southeast Asia.

CGIAR System Priorities: Among the CGIAR Research Priorities (2005-2015), livestock is recognized as being crucial to improving the livelihoods of many poor rural and peri-urban farmers in tropical regions. It is recognized however, that for poor farmers to capitalize on evolving commodity markets, there is a need to improve the availability and access of improved feed resources in areas of low and high potential. This implies the challenge of developing forages capable of producing high quality biomass to feed ruminant animals in environments characterized by having pest and disease pressures, low fertility soils, long dry seasons or poorly drained soils. Development of forage-based feeding systems for monogastric animals to complement existing home grown feed resources and replace expensive commercial concentrates is also seen as an important research output to assure improved productivity and competitivity of pig, poultry and fish in smallholder systems.

To address the priorities of the CGIAR on livestock, the Tropical Forage Project of CIAT has had the global mandate of developing forage-based technologies suitable for extensive and intensive crop-livestock systems in contrasting environments. Selected forages are expected to perform well in infertile soils and to contribute to reduced seasonal variation in both feed quality and quantity and as a result reduce livestock mortality and increase productivity. In addition, grasses and legumes with broad adaptation to soils and climate in sub-humid and humid environments can contribute to better use of family labor (particularly women) and to recuperate degraded soil/pastures in pastoral and crop-livestock systems through the enhanced capacity of grasses with their deep root systems to improve physical structure of soils and of legumes to improve soil fertility through their contribution via biological N_2 fixation. Furthermore, improved forages contribute to soil improvement through improved soil organic matter quality thereby enhancing soil biological activity and belowground biodiversity. The benefits of legumes are captured by forming strong research linkages with the Research for Development Challenge (RDC) dealing with Agroecosystems Management and with TSBFI (Tropical Soil Biology and Fertility Institute) of CIAT.

Specific activities carried out by the Forage Project to contribute to the CGIAR Priorities are:

- Development of methodologies for screening forages for improved quality and for adaptation to major abiotic and biotic stress factors
- Characterization of the genetic diversity in legume collections from the Gene Bank of CIAT, other CG Centers and research institutions to select new alternatives with superior forage quality, yield and resistance to biotic and abiotic stress factors
- Breeding to develop superior grasses (*Brachiaria*) that combine quality attributes with adaptation to major abiotic and biotic stress factors
- Development of methods for evaluating forages in different production systems with farmer participation
- Development of Data Bases and Decision Support Tools to help target forages to different environments and production systems

Impact Pathways: To contribute to the improvement of livelihoods of poor rural livestock owners through high quality forages (output 1) adapted to major biotic (output 2) and abiotic (output 3) constraints, forage researchers rely on natural genetic diversity from core germplasm collections housed in the GRU of CIAT and other international and national centers. Artificial hybridization to create novel genetic combinations is used when major limitations in successful commercial cultivars have been identified and where evaluation of large germplasm collections has failed to identify the required character combinations (e.g. spittlebug resistance and acid soil tolerance in *Brachiaria*). Screening methods and selected genotypes with superior forage quality, with resistance to major pest and diseases and with adaptation to acid, low fertility soils, to poorly drained soils and to drought are the output targets to be used by different partners engaged in research and development activities. To improve the efficiency of partners to better target forages to diverse environments and production systems in defined target areas, the forage team develops methods on participatory evaluation of forages and decision support tools (output 4). Selected forage genotypes are evaluated by partners in different environments and production systems. The superior grass and legume genotypes are released by NARS and private seed companies and tested and adopted by farmers to intensify and diversify their production systems. Adoption of new forage varieties results in more income to livestock farmers through more efficient use of land and, labor, and more animal products for urban consumers.

International Public Goods: In the past there were a number of strong organizations in developed countries (i.e. Australia, USA) involved in development of forages for sub-tropical and tropical environments. However, currently there are only few suppliers of improved forages with an international mandate as is the case for CIAT, ILRI and ICARDA. The forage work carried out by the CGIAR centers is complementary. For example, forages developed at ICARDA are mostly for the arid and semi-arid regions, while forages being developed by ILRI are for areas with better soils and cooler environments while forages developed by CIAT are for lowlands to mid-altitudes. An additional important participant in Forage R&D is EMBRAPA in Brazil but with a national mandate.

The research outputs of CIAT's Tropical Forage Project are in line with the mandate of the CGIAR of producing international public goods (IPGs). The IPGs of the research outputs of the Forage Project can be grouped into the following categories:

1. Mechanisms/Processes (to assist in the development of screening methods)
    - Understanding how forages adapt to acid soils with high levels of Al
    - Understanding how forages adapt to soils with low levels of P
    - Understanding how grasses resist pests (spittlebug) and diseases (Rhizoctonia)

2. Screening and evaluation methods (to select improved genotypes)
    - Forage quality (i.e. crude protein and *in vitro* digestibility)
    - Biotic constraints (i.e. Spittlebug and Rhizoctonia)
    - Abiotic constraints (i.e. adaptation of grasses to low soil nutrient status and high Al; adaptation to drought and to poorly drained soil conditions)
    - Selection of forages by farmers using participatory methods

3. Superior grass and legume genotypes and cultivars (to contribute to increased livestock productivity)
    - Grasses and legumes selected from germplasm collections that have broad adaptation to environmental factors prevailing in target areas and with multiple uses in crop/livestock production systems.
    - Grasses with high forage quality and combined resistance to biotic and abiotic constraints

4. Databases and Tools (to better target forages)
    - Forage databases on adaptation of forage species to diverse edaphic and climatic conditions
    - Decision Support Tool with information on adaptation, uses and management of different forage species

Partners: Through partnerships with different organizations from developed and developing countries, the Forage Project conducts research to develop improved grasses and legumes as feed resources. In what follows we present some key partnerships and the nature of the work being done as it relates to the 4 outputs of the Forage Project shown in parenthesis.

1. Australia- CSIRO and QDPI; Germany- U of Hohenheim and ILRI: (output 4). Development of a tool- Selection of forages for the tropics (SoFT). Funds from ACIAR, DFID and BMZ.

2. Australia- CAMBIA: (output 3). Mechanisms of resistance of *Brachiaria* to Al. Funds from BMZ.

3. Brazil- EMBRAPA: (output 3). Development of a multidisciplinary network for research on acid soils involving different systems, crops and forages. Funds from Brazil to the CG.

4. Costa Rica – SIDE; Guatemala – ICTA and MAGA; Honduras- DICTA; Nicaragua- IDR, IICA and ILRI: (Output 4). Analysis of the Beef Chain in Central America. Funds from CFC.

5. Colombia- CORPOICA and Mexico- PAPALOTLA -Seed Company: (output 4). On- farm evaluation of selected *Brachiaria* hybrids. Funds from PAPALOTLA.

6. Germany- U of Hohenheim; Colombia -CORPOICA and U del Cauca: (outputs 1 and 3). Development of multipurpose forage legumes for smallholder crop-livestock systems in the hillsides of Latin America Funds from Volkswagen Foundation

7. Germany – U of Goettingen: (output 3). Genotypic variation in P acquisition and utilization in Arachis. Funds from the University.

8. Germany – U of Hannover: (output 3). Mechanisms of aluminum resistance and drought tolerance. Funds from BMZ.

9. Germany—U of Hohenheim; Nicaragua- INTA; Honduras- DICTA: (outputs 1 and 4). Demand-Driven Use of Forages in Fragile, Long Dry Season Environments of Central America to Improve Livelihoods of Smallholders. Funds from BMZ.

10. Germany- U of Hohenheim: (outputs 1and 3). Genetic diversity of multipurpose legumes (*Flemingia* and *Cratylia*). Funds from the University.

11. Guatemala- ICTA and PAPALOTLA- Seed Company: (output 4): On- farm evaluation of selected *Brachiaria* hybrids. Funds from PAPALOTLA.

12. Honduras- DICTA and PAPALOTLA -Seed Company: (output 4). On- farm evaluation of selected *Brachiaria* hybrids. Funds from PAPALOTLA.

13. Lao PDR- National Agriculture and Forestry Research Institute, Australia- Department of Primary Industry and Forestry (DPI & F), Queensland and Canada- Nutrition Prairie Swine Centre, Saskatoon (Output 1 and 4) – Forage legumes for supplementing village pigs in Lao PDR. Funded by ACIAR

14. Japan- JIRCAS: (output 3). Nitrification inhibition in *Brachiaria humidicola*. Funds from Ministry of Agriculture of Japan.

15. Japan- Hokkaido University: (Output 3). Mechanisms of plant adaptation to low P and high Al in contrasting forages. Funds from the University

16. Japan- Yamagata University: (Output 3). Mechanisms of Al resistance in *Brachiaria*. Funds from the University

17. Switzerland – ETHZ; and Colombia- CORPOICA, Universidad Nacional de Colombia- Bogotá: (output 1). The forage potential of tannineforus legumes. Funds from ZIL- SDC

18. Switzerland –ETHZ; and Colombia- CORPOICA: (output 3). Adaptation of *Brachiaria* grasses to low-P soils. Funds from ZIL- SDC

19. Switzerland- ETHZ; and INTA- Nicaragua: (output 1). Improved feeding systems for dairy cattle in tropical smallholder farms. Funds from ZIL-SDC

20. United States- U of Kentucky: (output 2). Endophytes in grasses- Alkaloid detection. USAID linkage fund

21. United States- U of Florida- (Output 2). Biochemical mechanisms of resistance of *Brachiaria* to spittlebug. USAID linkage fund

Project IP-5 Logframe (MTP 2006-2008)

	Outputs	Intended User	Outcome	Impact
OUTPUT 1	Grasses and legumes with high forage quality attributes developed	CIAT and NARS researchers, seed companies and farmers	New cultivars of *Brachiaria* and legumes with high quality are released and adopted by farmers in LAC, Asia and Africa	Increased production of livestock fed high quality grasses and legumes
Output Targets 2006	Selected at least 10 *Brachiaria* hybrids with high leaf digestibility (>60%) and protein (>10%) Defined the role of tannins and fiber quality in legumes on methane production	CIAT researchers CIAT, ARIS and NARS researchers	New genotypes incorporated into the Brachiaria breeding program to develop high quality cultivars Development of feeding systems that contribute to less methane emissions by ruminant animals	
Output Targets 2007	Defined effect of environment (soil fertility and rainfall) on quality of 5 selected shrub legumes	CIAT and NARS researchers	Environmental "niches" to grow shrub legumes with tannins in LAC and Africa better defined	
Output Targets 2008	Nutritional synergies of using mixtures of shrub legumes with and without tannins assessed with sheep and milking cows	NARS researcher and farmers	Farmers in LAC, Asia and Africa adopt the use of legume mixtures to maximize efficiency of use of forage- based supplements	
OUTPUT 2	Grasses and legumes with known reaction to pest and diseases and interactions with symbiont organisms developed	CIAT and NARS researchers, seed companies and farmers	New cultivars of *Brachiaria* and legumes with resistance to prevalent pests and diseases are released and adopted by farmers in LAC	Increased profitability and sustainability of livestock production through planting grasses and legumes resistant to major pests and diseases

Output Targets 2006	At least 10 *Brachiaria* hybrids with combined resistance to at least 3 species of spittlebug developed Screening method to assess resistance to *Rhizoctonia* foliar blight in *Brachiaria* streamlined in the breeding program	NARS researchers CIAT and NARS researchers	Selected *Brachiaria* hybrid with resistance to spittlebug tested in different regions in LAC Selected *Brachiaria* hybrids resistant to *Rhizoctonia* foliar blight tested in different regions in LAC and Asia	
Output Targets 2007	Alkaloid profile of the endophyte (*Acremonium*)/Brachiaria association elucidated	NARS and ARIS researchers	Defined if alkaloids present in endophyte- Brachiaria association are toxic to sheep	
Output Targets 2008	At least 20 tetraploid *Brachiaria* hybrids identified with *Rhizoctonia* foliar blight resistance as high as that of the commercial *B. decumbens* cv Basilisk	NARS researchers	*Brachiaria* hybrids with resistance to *Rhizoctonia* selected in multilocational trials in LAC and Asia	
OUTPUT 3	Grasses and legumes with adaptation to edaphic and climatic constraints developed	CIAT, ARIS and NARS researchers, seed companies and farmers	New cultivars of *Brachiaria* and legumes with adaptation to low fertility soils, drought and poorly drained soils released and adopted by farmers in LAC, Asia and Africa	Increased livestock/crop production and improved NRM through planting multipurpose forage species adapted to low fertility soils, drought and waterlogged soils

Output Targets 2006	Selected a genotype of *Brachiaria* that combines resistance to ate least two species of spittlebug with good adaptation to acid –low fertility soils Genetic variability for nitrification inhibition in the collection (40 accessions) of *Brachiaria humidicola* held by CIAT determined	NARS researchers and seed companies CIAT, ARIS and NARS researchers	A new *Brachiaria* hybrid is made available to NARS partners for field testing in LAC, Africa and Asia Selection for nitrification inhibition incorporated in the Brachiaria improvement programs in LAC	
Output Targets 2007	Screening method for selecting *Brachiaria* genotypes adapted to poorly drained soils developed	CIAT and NARS researchers	New genotypes incorporated into the Brachiaria breeding program to develop cultivars with adaptation to poor soil drainage	
Output Targets 2008	Tradeoff of using drought tolerant legumes as cover crops and dry season feed defined	NARS researcher and farmers	Farmers adopt legumes as green manure and as feed resource for the dry season in LAC and Africa	
OUTPUT 4	Superior and diverse grasses and legumes evaluated in different production systems are disseminated	NARS researchers, development programs and farmers	New cultivars of grasses and legumes with adaptation to biotic and abiotic stresses are adopted by farmers in LAC, Africa and Asia	Livelihoods of small livestock farmers improved through adoption of forages that result in more efficient use of family labor and higher income from crop and animal products

Output Targets 2006	Two forage seed delivery systems developed to pilot stage to test linking small seed producers to large company/export market opportunities A superior *Brachiaria* hybrid combining drought tolerance, resistance to spittlebug and adaptation to acid infertile soils released by a commercial seed company in LAC countries	Forage seed companies, development programs and farmers Forage Seed companies, development programs and farmers	Alliance with large seed companies reduces risk and increases income of small farmers engaged in seed multiplication Seed of a superior grass genotype available to small and large farmers in LAC, Asia and Africa	
Output Targets 2007	Elite accessions (5- 10) of shrub legumes (*Flemingia macrophylla* and *Desmodium velutinum*) and short term herbaceous (*Vigna unguiculata, Canavalia brasilensis, Lablab purpureus*) deployed in NARS forage evaluation programs	NARS researchers and development programs	Researchers in LAC, Asia and Africa incorporate into their forage evaluation programs new shrub legume alternatives	
Output Targets 2008	A superior *Brachiaria* hybrid with resistance to spittlebug and adaptation to acid soils and drought planted in over 50,000 ha	Small and large farmers	Farmers in LAC, Africa and Asia who adopt new pasture species increase milk and beef production	

Output Targets (MTP 200 6-2008)

Project	Outputs	Output Targets 2006	Category of Output Target	Achieved?
IP- 5	**Output 1** Grass and legume genotypes with high forage quality attributes are developed	Selected at least 10 *Brachiaria* hybrids with high leaf digestibility (>60%) and protein (>10%)	Materials	**Achieved** Ten hybrids with IVDMD > or = to 59% and CP > 14% identified. Need to refine logistic to streamline in the breeding program. Annual Reports 2004, 2005 and 2006
		Defined the role of tannins and fiber quality in legumes on methane production	Other kind of knowledge	**Achieved** 1. Annual Report 2006 2. One. Journal article (Animal and Feed Science and Technology. 13 (Suppl): 95-98) 3. Proceeding of Workshop (Publication CIAT 2006 No 352. 52p)
IP- 5	**Output 2** Grass and legume genotypes with known reaction to pests and diseases and interaction with symbiont organisms are developed	At least 10 *Brachiaria* hybrids with combined resistance to at least 3 species of spittlebug developed	Materials	**Exceeded** Annual Report 2006
		Screening method to assess resistance to *Rhizoctonia* foliar blight in *Brachiaria* streamlined in the breeding program	Practices	**Achieved** Annual Reports 2005 and 2006. Need to refine logistics to streamline in the breeding program.
IP- 5	**Output 3** Grass and legume genotypes with superior adaptation to edaphic and climatic constraints are developed	Selected a genotype of *Brachiaria* that combines resistance to at least two species of spittlebug with good adaptation to acid-low fertility soils	Materials	**Exceeded** 1. Annual Report 2005 and 2006 2. One Journal article (Crop Science. 46: 968-973, 2006)
		Genetic variability for nitrification inhibition in the collection (40 accessions) of *Brachiaria humidicola* held by CIAT determined	Other kind of knowledge	**Achieved** 1. Annual report 2004 and 2006 2. One Journal article (Plant and Soil, 2007 in press)

IP- 5	**Output 4** Superior and diverse grasses and legumes evaluated in different production systems are disseminated	Two forage seed delivery systems developed to pilot stage to test linking small seed producers to large company/export market opportunities	Capacity	**Achieved** A pilot smallholder forage seed enterprise is in place in Honduras. Small farmers in Nicaragua are multiplying seed from legumes. 1. Annual Report 2005, 2006 2. Legal constitution of the enterprise
		A superior *Brachiaria* hybrid combining drought tolerance, resistance to spittlebug and adaptation to acid infertile soils released by a commercial seed company in LAC countries	Materials	**Achieved** 1. Annual Reports 2005 and 2006 2. Technical Bulletin (Spanish, English and Portuguese) of cultivar released 3. Certification by Papalotla of seed produced and sold in different countries in 2005/2006

Research Highlights (2006)

Output 1: Grasses and legumes with high forage quality attributes developed

- **Legumes mixtures with and without tannins when fed to cows as supplements result in increased milk production**
  Previous studies had shown that supplementation with hay of *Calliandra calothyrsus* (Calliandra) did not increase milk production of dual purpose cows grazing low quality pastures during the dry season. This lack of response to supplementation with Calliandra has been associated to its high level of tannins, which results in low levels of ammonia production in the rumen and as a result bacterial protein synthesis is reduced. It has been hypothesized that mixing legumes with and without tannins could contribute to improve the effects of supplementation of legumes with tannins on milk production due to increased production of rumen ammonia and flow of total nitrogen to the lower digestive tract. To test this hypothesis, four supplements consisting either of the tanniniferous Calliandra or the tannin free high quality *Vigna unguiculata* (Cowpea) alone or in mixtures were offered to grazing cows. Replacing in the supplement 1/3 of Calliandra with cowpea resulted in a 22% increase in daily milk yield relative to the Calliandra alone supplement. Thus it can be concluded that legume based supplements based on high yielding shrub legumes with tannins can be significantly improved by mixing them with small proportions of a high quality legume without tannins.

Output 2: Grasses and legumes with known reaction to pest and diseases and interactions with symbiont organisms developed

- **Demonstrated possibility to overcome negative attributes of *Brachiaria humidicola* through breeding**
  The grass specie *Brachiaria humidicola* has a number of highly desirable attributes, notably its strongly stoloniferous growth, good resilience under grazing mismanagement, and tolerance to poorly drained soil conditions. However, available cultivars of the species have a number of defects such as poor nutritional quality, susceptibility to spittlebug, poor seed yield, and strong physiological seed dormancy. Two tetraploid accessions of *B. humidicola* with different reproductive modes (CIAT 26146, sexual; CIAT 26149, apomictic) were selected for carrying out experimental crosses. A number of microsatellite markers were assessed on the two parental genotypes. Informative markers – those present in the male (apomictic) parent and absent in the female (sexual) parent – were identified. Fourteen putative hybrid seedlings were obtained. Detection of the band present in the male parent and absent in the female in all 14 putative hybrids, confirmed that all hybrids were true hybrids. These results open the possibility of genetic improvement in *B. humidicola*, particularly if inheritance of reproductive mode (sexuality vs. apomixis) is found to be simply inherited.

Output 3: Grasses and legumes with adaptation to edaphic and climatic constraints developed

- **Selected apomicitic hybrids of *Brachiaria* with moderate level of tolerance to waterlogging based on green leaf biomass and leaf chlorophyll content and low proportion of dead leaves**
  In the tropics, *Brachiaria* pastures during the rainy season occasionally confront waterlogging conditions that severely limit pasture productivity and animal performance. In 2005, we developed a screening method to evaluate waterlogging tolerance in *Brachiaria* hybrids. Waterlogging for 21 days resulted in senescence and death of a great proportion of shoot biomass of the majority of hybrids and affected development of adventitious roots in some hybrids. In late 2005, we implemented the screening method for waterlogging tolerance and screened 48 BR04NO series of hybrids and identified three hybrids (BR04NO/3069, BR04NO/3207 and BR04NO/2774) that were superior in their tolerance to waterlogging based on greater values of green leaf biomass production, and leaf chlorophyll content and lower values of dead leaf biomass. These three plant attributes could serve as criteria for selection for waterlogging tolerance in *Brachiaria*.

Output 4: Superior and diverse grasses and legumes evaluated in different production systems are disseminated

- **Improved forages adopted by smallholders in SE Asia increased income and returns to labor and opened opportunities to link to markets**
  CIAT commenced forage research in Southeast Asia in 1992 with the introduction of a large range of forge accessions. In 2005, two major CIAT forage projects – the regional Livelihood and Livestock Systems Project and the bi-lateral Forages and Livestock Systems Project in Laos were completed. By this time, the long-term commitment of CIAT and its partners had led to significant livelihood benefits and adoption of planted forages by a large number of smallholder households in the region. These were documented in a survey and impact studies carried out in 2005. Planted forages significantly improved household income

and, most importantly, the returns to labor from livestock production. The initial benefit from planted forages was in labor savings from easy access to feed. Subsequently, improved growth of animals receiving planted forages emerged and farmers looked for ways of maximizing the opportunities provided by the new feed resources. Participatory approaches to technology development were an essential component of success. The key role of planted forages in enabling smallholder farmers to intensify their extensive livestock production system and become more market-oriented has been accepted by development agencies. Similarly, the participatory approaches developed for forage technology development and scaling out have attracted interest from development practitioners.

PROJECT OUTCOME: A superior *Brachiaria* hybrid combining drought tolerance, resistance to spittlebug and adaptation to acid infertile soils released by a commercial seed company in LAC countries

The output was first identified in the MTP 2005-2007. This outcome contributes to improved rural livelihoods through increased efficiency of livestock production and through the sale of seed, and vegetative propagules and fodder.

Brachiaria hybrid cultivar 'Mulato II' (CIAT 36087) is an apomictic selection from a hybrid population generated in 1995. It produces high yields of very high quality forage. It has antibiotic resistance to a range of Colombian and Brazilian spittlebug species as well as moderate tolerance of Rhizoctonia foliar blight. It has good drought tolerance and better acid soil adaptation than the common commercial cultivar Marandu. Seed yields, while low in comparison with conventional brachiariagrass cultivars, are at least double those of cv. Mulato. The cv. Mulato II was released by our private sector collaborator, Grupo Papalotla, in 2005. Seed sales in 2005 and 2006 total over 63.5 tons with another 700 kg distributed as "donations". Hence, seed sufficient to sow nearly 13,000 ha (assuming a sowing rate of 5 kg/ha) were delivered in the first two years of commercialization, generating over one million dollars in revenues. Seed sales projected for 2007 total over 400 tons, which will generate an estimated US$4.5 million in revenues and sow an additional 80,000 ha.

Livestock producers who benefit from cv. Mulato II range from large ranchers in Latin America to smallholders in Asia who grow Mulato II to produce high quality forage to feed work animals. Additional economic benefits, particularly to smallholders, are derived from artisanal seed production of cv. Mulato II in Bolivia and Thailand. In Thailand, Mulato II seed production by smallholders is far more profitable than any alternative crop (e.g., rice, sugarcane, maize, cassava, or two alternative forage seed crops).

The information on seed distribution in this document is based on seed sales data provided by the Papalotla Seed Company, experimental data on animal performance on cv. Mulato II (CIAT Forages Project Annual Reports 2004, 2005, 2006), direct observation of spontaneous smallholder adoption by vegetative propagation, and published economic analysis of crop production in Thailand (M.D. Hare: in proceedings of Forage Symposium: Forages- a pathway to prosperity of smallholder farmers, March 5-7, 2007. Faculty of Agriculture, Ubon Ratchathani University, Thailand (In press).

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

1.1 Selection of *Brachiaria* genotypes for high quality

Highlight

- In vitro dry matter digestibility (IVDMD) values for individual *Brachiaria* hybrids ranged from 48 to 63% over three sampling dates, while crude protein (CP) values ranged from 12 to 25%. Hence, we reconfirm the ample variation for these two important forage quality parameters for future genetic improvement

1.1.1 Screening of sexual and apomictic *Brachiaria* hybrids for digestibility and protein

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

Rationale

One of the outstanding attributes of the first two hybrid *Brachiaria* cultivars (Mulato and Mulato II) is their very high nutritive quality, which leads to outstanding animal productivity. Hoping to maintain or improve forage quality in future commercial hybrids, special attention is required to monitor quality attributes (crude protein and dry matter digestibility), particularly in our synthetic, tetraploid breeding population where genetic gain can be achieved by selection.

Materials and Methods

Vegetative propagules of 209 BR05 and RZ05 hybrids were delivered to the Forage Quality laboratory in April 2006. Individual plants were propagated vegetatively and transplanted to single-plant experimental units in a 3-replicate, space planted (1.00 x 0.75 m) field experiment on 07 June 2006. Plants were cut to a uniform height on 27 July. Individual plants were sampled three times, at 6-wk intervals, on 07 September, 19 October, and 30 November 2006. Urea was applied at a rate of 1 gm per plant following each sampling harvest. Approximately 23-25% of plants failed to establish or died and could not be sampled. On each of the three sampling dates, 300-gm samples of leaf tissue were taken, dried at 45°C for 48 hours, and ground to pass through a 1 mm screen. Dried, ground leaf tissue samples were analyzed in a NIRS System 6500 using software ISISCAN (IS-2250) version 2.71, Win ISI-III IS-1485.

Results and Discussion

Sampling date was by far the major source of variation. Genotypes differed at each sampling date and over sampling dates. Differences among genotypes were on the order of 15 percentage points for IVDMD and 12 percentage points for CP. Ten hybrids had IVDMD $\geq$ 59% with CP in excess of 14%.

Mean squares for genotypes were 2.9 times or 4.7 times larger than the mean squares for the genotype-sampling date interaction for IVDMD or CP, respectively. Correlations of genotype values between sampling dates were positive, but of only moderate magnitude: on the order of 0.3 to 0.4 for IVDMD, and on the order of 0.3 to 0.6 for CP. These results suggest that there is ample variability for genetic improvement in forage quality parameters in the *Brachiaria* breeding populations. While the digestibility and protein data reported were determined on sexual-by-apomictic hybrids, breeding progress will (or will not) be made in our synthetic tetraploid sexual breeding population. The logistics of obtaining reliable forage quality data on candidate sexual clones in an opportune manner needs to be worked out.

1.2 Forage quality of promising grasses and legumes

Highlights

- Feeding *Canavalia brasiliensis* to sheep as a supplement to a low quality grass resulted in a linear increase in dry matter intake as level of the legume offered increased

- Rumen degradability of tropical legume fibers is highly variable and at least as important to plant quality as secondary plant metabolite (tannin, saponin) content.

1.2.1 Feed value of *Canavalia brasiliensis* fed to sheep

Contributors: J. Carabalí, C. Lascano, P. Avila and M. Peters (CIAT)

Rationale

The evaluation of herbaceous legumes with farmer participation in hillsides of Central America resulted in the selection of *Canavalia brasiliensis* (Canavalia) given its high biomass production and dry season tolerance. On farm evaluation of Canavalia demonstrated that it was an excellent cover legume and that when sown at the end of the dry season with maize resulted in higher grain yield in the subsequent rainy season. Anecdotal evidence suggested that Canavalia was well accepted by grazing cows in the dry season. However, given the presence of a toxic amino acid (canavalin) in Canavalia species, known to affect monogastric animals, there was concern that cows consuming the legume could exhibit some form of toxicity leading to mortality.

To investigate the feed value of Canavalia as a supplement to low quality grasses and to define if animals consuming the legume showed toxicity problems a feeding trial with sheep was carried out. Results of the feeding trial are reported in this section.

Materials and Methods

In the Quilichao research station 10 African hair sheep grouped as light (5) and heavy (5) were assigned to 5 treatments in a replicated 5 X 5 Latin Square design.

The feeding trial had duration of 70 days, divided in 5 experimental periods (14 days/ period). Each period in turn was divided in 7 days for adjustment and 7 days for measurements. Animals were housed in metabolism crates and offered sun dried *Canavalia brasiliensis* and a low quality grass (*Brachiaria humidicola*) as a basal diet in the following arrangement of treatments:

T1: Canavalia 100%
T2: 25% Canavalia + 75% Grass
T3: 50% Canavalia + 50% Grass
T4: 75% Canavalia + 25% Grass
T5: 100% Grass

Each animal received a total of 80 g of DM /kg of BW $^{0.75}$ daily in two rations AM and PM. The legume was offered alone from 8 to 10 AM and from 1 to 3PM. After the end of the two hour period the legume not consumed was measured and animals were offered the grass basal diet. In addition all animals received water and a mineral mix ad lib. Forage consumed by each animal daily was calculated by difference of grass and legume offered and grass and legume refused.

Animals were fitted with fecal collection bags on day 6 of the adjustment period. Feces from each animal were weighed from day 7 to day 14 of the measurement period. A 10% aliquot was taken from each animal and stored in a freezer for subsequent freeze drying and chemical analysis.

Laboratory measurements in freeze dried feed offered and refused and in feces included DM, OM, NDF, ADF, IVDMD, CP (N x 6.25) and minerals (P, Ca, M, K and S).

Daily intake of total DM and of grass and legume were calculated by difference of forage offered and forage refused. Digestibility of DM was calculated as follows: DM Digestibility = DM consumed (g) – DM excreted in feces (g)/ DM consumed (g).

The data was subject to an analysis of variance with animal group (heavy and light), period, animal (group), Treatment (group) and Group x Treatment as sources of variation. Given that one animal died (not related to the diet received) the SAS GLM procedure was used.

Results and Discussion

As expected the CP content of Canavalia (17%) offered was higher than in the grass (7 %). The levels of P and Ca were also higher in Canavalia (0.26 and 1.9%) than in the grass (0.09 and 0.22%). The fiber content (NDF) was lower in Canavalia (59.7%) than in the grass (83%), which did not result in higher IVDMD in the legume (61 %) as compared to the grass offered (59%).

Total intake of DM was higher for the light (31.6 g/ BW $^{0.75}$) than for the heavy sheep group (31.6 g / BW $^{0.75}$). However more interesting was that DM intake increased linearly regardless of weight of the animals as the proportion of Canavalia increased in the forage offered (Table 1).

However, results showed that DM digestibility did not differ among treatments as shown in Table 1. These results are consistent with the in vitro digestibility values recorded for Canavalia and for the grass.

One important reason for running this feeding experiment was to define if the forage of *Canavalia brasiliensis* produced toxicity

Table 1. Intake and digestibility of *Canavalia brasiliensis* alone and as a supplement to a low quality grass.

Treatments	Intake (g DM/kg BW $^{0.75}$)	Digestibility (%)
Grass 100%	29.7 c	56.3
25% Canavalia + 75% Grass	31.1 c	57.5
50% Canavalia + 50% Grass	33.5 b	55.6
75% Canavalia + 25% Grass	35.4 a	56.3
Canavalia 100%	36.6 a	57.5

symptoms when feed to sheep alone or in mixture with a low quality grass. To define if Canavalia had any effect on liver function we measured two enzymes (GGT- Gamma Glutamil Transferase and ASAT- Aspartate amino transferase). Our results showed that GGT ranged from 13 to 22.0 with an average of 17 U/L (reference values 22 – 44 U/l).

In the case of ASAT the values recorded ranged from 38 to 97 with an average of 65 U/L (reference values 49 -123 U/L). Thus it would seem that short term intake of *Canavalia brasiliensis* did not result in any apparent liver damage. Through out the trial light or heavy sheep did not exhibit any strange behavior or toxicity symptom.

From the results of the feeding experiment we conclude that the *Canavalia brasiliensis* fed to sheep as a protein supplement resulted in a 5 to 19% increase in dry matter intake as the legume supplement increased from 25 to 75% of the forage offered. In addition, it would seem that forage of Canavalia does not produce toxic effects on sheep at least in short periods of time (70 + days).

1.2.2 In vitro degradability and methane production with purified fiber from legumes with and without tannins using the Rumen Simulation Technique (Rusitec)

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Rationale

Fiber content and degradability are key factors in determining forage quality. Ruminants in tropical regions derive a major portion of their energy from the fiber fraction of forages. The low rumen digestibility of tanniniferous legumes has been mostly associated with their high tannin contents. The present experiment was conducted to better define the role of fiber quality of two tanniniferous shrub legumes (*Calliandra calothyrsus* and *Flemingia macrophylla*) on ruminal fermentation of legumes with tannins.

Material and Methods

Plant material of *Calliandra calothyrsus* (CIAT 22310) and *Flemingia macrophylla* (CIAT 21083) was harvested manually eight weeks after a uniformization cut. The material was immediately stored at 20°C and subsequently freeze dried. For *Vigna unguiculata* (cowpea; CIAT 391) eight week old herbage (the whole plant, before flowering) was harvested and sun dried for three days. The tropical low quality grass *Brachiaria humidicola* (CIAT 6133; formerly *B. dictyoneura*) was cut after a growing period of 12 weeks and sun dried for two days. The dried plant material of all species was ground in a laboratory mill using a 5 mm screen.

The material harvested was analyzed for dry- and organic matter, nitrogen, fiber, lignin and ruminal degradability. The N associated to fiber (NDF-N) was measured. Fiber content of *B. humidicola*, *C. calothyrsus*, *F. macrophylla* and *V. unguiculata* was determined with the detergent method. In the case of fiber determination in Calliandra and Flemingia sodium sulfite was added to the detergent solution.

The fiber used in the in vitro experiment was obtained applying a slightly modified detergent method. The amount of plant material used to extract NDF was 5 g placed in 250 ml of detergent solution instead of 0.5 g in 100 ml solution. The detergent was removed by intensive washings with water and ethanol 70-95% in 20 to 25 washing steps, till the detergent could not be perceived not by smell nor flavor and the fiber did feel fluffy and not sticky anymore. The isolated fiber fraction was analyzed for the same parameters as described above for the entire plant material.

Employing the in vitro rumen simulation technique (RUSITEC) fiber extracted from the three legumes was evaluated for its rumen degradability during 2×10 days periods with two replications of each treatment per period (n=4). The first four days served for adaptation of microbes to the fermentation substrates and the following 6 days for data and sample collection. As a protein source for the fermentation media, 3 g of casein were added per day.

The daily dry matter supply to the fermenters was maintained constant at 10 g fiber + 3 g casein. Gas production and methane concentration were determined daily. Substrates and solid fermentation residues were analyzed for organic matter, N, neutral detergent fiber (NDF), and acid detergent fiber (ADF). Data were statistically analyzed with an ANOVA using the GLM procedure in SAS.

Results and Discussion

Chemical composition of the tested plants. The chemical analysis of the test plants showed large differences in fiber composition and N bound to fiber (Table 2). The grass Brachiaria had by far the highest NDF value of all tested plants. Of the legumes Flemingia had the highest NDF content (590 mg/g) followed by Vigna (530 mg/g) and Calliandra (358 mg/g). Lignin content was highest in Flemingia (154mg/g) followed by Calliandra (99 mg/g), Vigna (82 mg/g) and Brachiaria (56 mg/g).

Indigestible fiber was highest in Flemingia with 508 mg/g that corresponds to 86% of the total fiber content. In Calliandra the indigestible fiber content was 273 mg/g, corresponding to 76% of the total fiber content of the plant (Table 2). In Vigna and Brachiaria the indigestible fiber was only 170 and 192 mg/g (32% and 25% of total fiber), respectively. The hemicelluloses fraction was highest in Brachiaria followed by Vigna, Flemingia and Calliandra. Relatively to the total fiber content hemicellulose proportion in Flemingia was lower than in Calliandra, Brachiaria and Vigna (Table 2). With the fiber purification procedure used only 90-95% purity of NDF could be achieved. As we could not identify the impurities, the purified fiber was treated as homogeneous fraction.

The N bound to fiber in the plants was 59% in Brachiaria, 56% in Flemingia and 37 and 36% for Vigna and Calliandra (Table 2). Previous Rusitec trials had shown that the availability of this NDF-N for the rumen microorganisms was not sufficient to cover their needs. Thus casein was added to the medium since it is considered a good source of N for rumen microbes.

Table 2. Chemical composition of grass and legume species and their purified fibers (mg/g).

	Brachiaria		Vigna		Calliandra		Flemingia	
	plant	fiber	plant	fiber	plant	fiber	plant	Fiber
Nitrogen content	7.03	5.81	24.76	19.02	28.65	26.41	28.65	28.74
NDF-N in the plant	4.14	-	9.09	-	16.06	-	16.06	-
Not NDF-N in the plant	2.89	-	15.67	-	12.59	-	12.59	-
Neutral Detergent Fiber (NDF)	754	944	530	901	590	909	590	947
Acid Detergent Fiber (ADF)	386	468	309	507	406	547	406	636
Acid Detergent Lignin (ADL)	56	56	82	103	154	167	154	243
Hemicelluloses	368	476	221	394	184	362	184	311
Celluloses	330	412	227	404	252	380	252	393
Indigestible ADF (IADF)	192	203	170	327	508	409	508	584
Extractable condensed tannins	nd	nd	nd	nd	95	0	95	0
Bound condensed tannins	nd	nd	nd	nd	10	0	10	0.3
IVDMD	659	620	767	595	388	462	388	349

Fiber degradation and methane production. The in vitro dry matter degradation (DMD) and the degradation of organic matter (OMD) and neutral detergent fiber (NDF) differed ($P<0.05$) between all purified fibers tested. Vigna showed always the highest degradability followed by Brachiaria, Calliandra and Flemingia. Acid detergent fiber degradation was highest in Calliandra and Brachiaria and by far lowest ($P<0.05$) in Flemingia. Hemicelluloses degradation was highest ($P<0.05$) in Vigna, followed by Brachiaria, Flemingia and Calliandra. Celluloses degradation was lowest ($P<0.05$) in Flemingia and highest in Calliandra and Brachiaria.

In general, results in fiber degradation in vitro showed clear differences among legumes in fiber quality. The tanniniferous species had overall less digestible fibers than Vigna or Brachiaria. Legumes with tannins also showed particularly much lower digestibility of the hemicelluloses fraction.

Methane emission expressed as ml/d as well as methane emitted per unit of degraded organic matter differed ($P<0.05$) among species and was highest with Vigna, followed by Brachiaria, Calliandra and Flemingia (Table 3). Methane produced per unit of NDF did not differ between Brachiaria and Calliandra, and was highest with Vigna and lowest with Flemingia. These results indicate that the reduction in methane observed in other in vitro experiments and in an in vivo experiment reported in a different section when legumes with tannins were used to supplement low quality grasses was due not to the tannins per se affecting rumen methanogenic bacteria but rather to the very low degradability of the fiber of such legumes.

Table 3. Methane emission from the fermentation of purified fiber of different plant materials in an in vitro Rumen simulation system (RUSITEC) [ml/g].

	Brachiaria	Vigna	Calliandra	Flemingia
Methane [ml/d]	68b	120a	45c	13d
Methane/degraded OM [ml/g]	29.7b	38.2a	23.4c	11.8d
Methane/degraded NDF [ml/g]	25.2b	38.0a	20.1b	7.9c
Methane/degraded ADF [ml/g]	65b	119a	36c	19c
Methane/degraded Hemicelluloses [ml/g]	24.4b	81.1a	18.7b	6.5c
Methane/degraded Celluloses [ml/g]	74b	149a	51bc	30c

1.3 Utility of mixtures of legumes with and without tannins for ruminants

Highlights

- The cellulolytic bacteria *Ruminococcus flavefaciens* was not affected by tannins extracted from legumes which was not the case for *Fibrobacter succinogenes*.

- Mixing of legumes with and without tannins for silage production did not decrease nitrogen losses during storage but decreased ruminal degradability of protein which in turn could result in a more efficient utilization of nitrogen.

- Feeding tanniferous legumes to sheep as a supplement to a low quality grass decreased methane production as a result of reduced ruminal fiber degradation and not by adverse action on methanogenic microbes.

- Confirmed the high value of *V. unguiculata* (cowpea) as supplement for dual purpose cows grazing low quality pastures during the dry season as milk production increased with increasing proportion of the legume in the supplement.

- The feed value of a legume supplement based on *C. calothyrsus* high in tannins can be significantly improved by including a small proportion (e.g. 1/3) of *V. unguiculata* (cowpea) in the mixture.

1.3.1 Effect of tanniniferous forages Leucaena and Desmodium on rumen microbial populations

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Rationale

The composition of the rumen microflora can be affected by many factors one of them being the nature of the diet. A diet with high proportion of fiber tends to favor the growth of cellulolitic bacteria such as *Fibrobacter succinogenes* and *Ruminococus flavefaciens*. Thus it is possible that the inclusion in the diet of ruminants of tropical shrub legumes could favor the growth of cellulotic bacteria given their relative high fiber content. However, it is also possible that condensed tannins (CT) present in some shrub legumes could limit the availability of protein and energy needed for bacterial growth.

The present experiment was conducted to assess the effect of adding tannins extracted from *Leucaena leucocephala* and *Desmodium heterocarpum* (ovalifolium) on microbial ecology and in vitro fermentation of alfalfa.

Materials and Methods

A series of *in vitro* fermentations were carried out with rumen fluid and the addition of tannins (CT) extracted from *Leucaena leucocephala* **(**Leucaena) and *Desmodium heterocarpum* (Desmodium). Volatile fatty acid (VFA) production (mM), gas production (ml), and the relative abundance of the cellulolytic species *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* by qRT-PCR were measured.

The effects of purified CT were evaluated after 24 hours of in vitro fermentation. The fermentation took place in 50 ml bottles following standard protocols. Each bottle contained 100 mg of dried and ground alfalfa, 10 mg of CT from Leucaena or Desmodium and 10 ml of a buffer solution (20% of rumen liquor and 80% of growth medium).

Results and Discussion

The CT of Leucaena or Desmodium did not cause negative effects on the abundance of *Ruminococcus flavefaciens*. In contrast numbers of *Fibrobacter succinogenes* decreased (P < 0.05) after treatment with CT from Leucaena and Desmodium (Table 4).

Table 4. Effect of CT from tanniniferous forages on the abundance of cellulolytic rumen microbes *F. succinogenes* and *R. Flavefaciens* ($2^{-\Delta\Delta Ct}$).

Time (h)	Treatment	*Fibrobacter*	*Ruminococcus*
0	Control	1	1
	Leucaena	0.519 a	2.64 a
	Desmodium	0.298 b	2.61 a
12	Control	1	1
	Leucaena	0.595 a	4.67 a
	Desmodium	0.221 b	4.53 a
24	Control	1	1
	Leucaena	0.266 a	3.08 b
	Desmodium	0.054 b	32.76 a

Different letters show significant differences P< 0.05

It is possible that the presence of glycocalix that is associated to cell-wall topology and chemical composition of Gram + celluloytic rumen microbes (i.e. *Ruminococcus flavefaciens*) protect them from the negative effects of CT. The mechanisms by which CT of the two tanniniferous forages increased (P < 0.05) numbers of *Ruminococcus flavefaciens* remain unknown. Until now there is no evidence that rumen bacteria use tannins as a carbon source. Gas production was significantly (P<0.05) higher when CT from Leucaena were added as compared to CT from Desmodium.

Degradability of the substrate was correlated to gas production in presence of CT of both tanniniferous legumes. Other researchers also found a negative effect of CT from *D. ovalifolium* on IVDMD degradability. Total VFA production decreased in presence of tannins extracted from Desmodium, although a positive effect was found on propionate accumulation (Table 5).

Favoring propionate production by using tannins of some tropical forage would offer an optimal scenario in the energy balance of rumen function. As found by other researchers, the CT of Leucaena increased (P < 0.05) VFA production as compared to the control or to the treatment with Desmodium. In summary, our results indicate that the cellulolytic bacteria *Ruminococcus flavefaciens* was not affect by tannins extracted from legumes which was not the case for *Fibrobacter succinogenes*. These results are interesting since they suggest that different species from the rumen cellulolytic population would show different tolerance to tannins present in tropical forages.

Table 5. Effect of CT of *D. ovalifolium* and *L. leucocephala* on volatile fatty acids (VFA) production after 24h of fermentation.

		Volatile Fatty Acids (nM)					
Time (h)	Treatments	Acetate	Propionate	Butyrate	Isobutyrate	Total	Ratio
1	Control	16.08 c	6.91 a	3.38 a	0.33 a	26.70 b	2.33 c
	Leucaena	20.37 a	6.63 b	2.47 b	0.17 b	29.64 a	3.07 b
	Desmodium	17.29 b	4.61 c	1.98 c	0.21 b	24.09 c	3.76 a
12	Control	23.50 c	9.74 b	4.24 b	0.89 a	38.36 b	2.42 c
	Leucaena	26.06 b	6.84 c	4.38 b	0.34 c	37.62 c	3.81 a
	Desmodium	30.76 a	10.57 a	6.93 a	0.52 b	48.78 a	2.91 b
24	Control	39.57 a	13.11 a	6.41 b	1.07	60.17 a	3.02 c
	Leucaena	39.67 a	10.29 b	5.65 c	1.08	56.70 b	3.85 a
	Desmodium	33.88 b	10.4 b	7.40 a	1.09	52.78 c	3.26 b

Different letters show significant differences $P < 0.05$

1.3.2 Nutritional value of dried or ensiled mixtures of legumes with contrasting tannin contents

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Rationale

Forage conservation represents an interesting alternative for dry season supplementation of ruminant livestock in the tropics. The Tropical Grass and Legume Project of CIAT has developed alternative technologies such as the “bag silage” that are appropriate for livestock systems operated by smallholders. However, one important constraint of silage preparation is the loss of nitrogen during the fermentation process, particularly when legumes with high concentrations of soluble nitrogen are conserved.

Since tannins which are present in many tropical forage legumes protect proteins form microbial degradation, mixing legumes with and without tannins could contribute to reduce N losses during silage preparation and storage. The objective of the present investigation was to evaluate the ruminal fermentation characteristics and nutrient degradation of hay and silage of tropical forage legumes with (*Calliandra calothyrsus*, *Flemingia macrophylla*) or without (*Cratylia argentea*, *Vigna unguiculata*) tannins incubated either alone or in mixtures.

Materials and Methods

In Experiment 1, the shrub legume species *Calliandra calothyrsus* (CIAT 22310), *Flemingia macrophylla* (CIAT 17403) and *Cratylia argentea* (CIAT 18516 and 18668), and the herbaceous legume *Vigna unguiculata* (CIAT 1088/4, 288, 391, 9611 and 715) were cultivated at CIAT's research station Quilichao (Cauca, Colombia). All legumes were tested individually and the shrub legumes were additionally incubated in combination with *V. unguiculata* in a proportion of 1:2. Individual legumes and legume mixtures were evaluated as hay and as silage and all diets were incubated with or without the addition of polyethylene glycol (PEG) to inactivate tannins. This resulted in a total of 28 treatments (7 diets x 2 conservation methods x 2). The experiment was conducted using the gas-transducer technique.

In Experiment 2, the tanniniferous shrub legume *Calliandra calothyrsus* (CIAT 22310) and the herbaceous legume *V. unguiculata* (CIAT 1088/4, 288, 391, 9611 and 715) were tested either alone or in combination with each other in proportions of 1:2 and 2:1. Individual legumes and mixtures were evaluated as hay and as silage. This resulted in a total of 8 treatments (4 diets x 2 conservation methods). Hay was prepared by sun-drying for 3 days and silage was stored for 56 days. The experiment was conducted using a rumen simulation technique (RUSITEC).

Results and Discussion

In Experiment 1, accumulated gas production and rate of gas production were higher ($P<0.05$) with silage than with hay and were not affected ($P>0.05$) by the addition of PEG. Fermentation dynamics were clearly affected be the botanical composition of the diets. The highest ($P<0.05$) accumulated gas production and gas production rate were observed with *V. unguiculata* alone and with the mixture consisting *V. unguiculata* and *C. argentea*. The lowest values ($P<0.05$) were observed in the treatments with tanniniferous legumes alone. The treatments with *C. argentea* alone and the mixtures with *V. unguiculata* and tanniniferous legumes showed intermediate values.

In Experiment 2, chemical composition of hays and silages varied depending on the botanical composition and the conservation method. Compared to hay preparation, ensiling decreased the crude protein (CP) content in legumes without tannins (*V. unguiculata, C. argentea* and their mixtures with legumes with tannins). On the other hand, the fiber content increased with ensiling in the legumes with no tannins (*V. unguiculata* and *C. argentea*) and corresponding mixtures with legumes that had tannins. Apparent organic matter (OM) degradation was also affected by the botanical composition and the conservation method. The highest ($P<0.05$) OM degradability was observed in the treatments with *V. unguiculata* alone and in the mixtures with high proportion of this legume. The mixtures with low proportion of *V. unguiculata* showed intermediate values and the lowest OM degradability was found in the treatments with *C. calothyrsus* alone. Compared to sun drying, ensiling increased ($P<0.05$) the OM degradability in all forages except with *C. calothyrsus* alone. Apparent CP degradability was highest ($P<0.05$) with *V. unguiculata* alone and with the mixtures containing a high proportion of this legume. Ensiling increased ($P<0.05$) apparent CP degradability in *V. unguiculata* alone but had no effect ($P>0.05$) on protein degradation in the legume mixtures.

These results confirm that ensiling may result in losses of nitrogen in legumes with no tannins. However, these losses could not be minimized by mixing legumes with and without tannins. On the other hand these results indicate that ensiling compared to sun drying increases the apparent ruminal CP degradability in tannin-free legumes, which was not the case in tannin-rich legumes. It can be concluded that mixing of legumes with and without tannins for silage production does not decrease nitrogen losses during ensiling and storage of the forage but decreases the ruminal degradability of CP which in turn could result in a more efficient utilization of nitrogen.

1.3.3 Ruminal fermentation and duodenal protein flow in sheep supplemented with legume mixtures with contrasting tannin contents

Contributors: L.M. Monsalve (Universidad Nacional Palmira), P. Avila, G. Ramírez (CIAT), H.D. Hess ALP Posieux) and C.E. Lascano (CIAT)

Rationale

Nutrition of ruminant livestock in the tropics is mainly based on grasses. In general tropical grasses are of low to moderate digestibility, deficient in one or more essential nutrients (e.g. crude protein) and contain high amounts of fiber and low concentrations of soluble carbohydrates and starch. This in turn, results in low microbial activity in the rumen and may cause imbalances in digestive products and result in an inefficient use of metabolizable energy. Therefore alternative feeding strategies are needed which contribute to improve fermentation efficiency in animals fed diets based on grasses of low quality by assuring adequate ruminal ammonia levels, microbial protein synthesis and duodenal flow of undegraded feed protein. This may be achieved by supplementation with legumes which generally contain higher amounts of both rumen degradable and undegradable protein than tropical grasses. If the deficiency of fermentable nitrogen in the diet is eliminated, this may increase the activity of fibrolytic microorganisms resulting in an improved degradation of fibrous feeds.

Many of the legumes which could be used to supplement ruminant livestock in the tropics contain condensed tannins (CT). In higher concentrations, CT may have detrimental effects on animal production (e.g. suppressed intake and digestibility of nutrients). However, in lower concentration CT could have beneficial effects, such as reducing nitrogen losses during ruminal fermentation and increasing the flow of protein to the duodenum and the absorption of amino acids in the lower gut. The objective of the present investigation was to evaluate the effects of supplementing legume mixtures with different types and concentrations of CT on ruminal fermentation and the utilization of nitrogen in sheep fed a basal grass diet of low quality.

Materials and Methods

A feeding trial with sheep was conducted at CIAT's research station Quilichao (Cauca, Colombia). Then adult, castrated male sheep, fitted with rumial and duodenal canulae were divided into two groups with an average bodyweight of 26.1 and 34.6 kg, respectively. Animal were kept in metabolic crates and assigned to five treatments according to a repeated 5x5 Latin square design.

Diets consisted of *Brachiaria humidicola* (55% of dietary dry matter) and *Vigna unguiculata* either alone or in mixtures with the tanniniferous shrub legumes *Calliandra calothyrsus* and *Flemingia macrophylla*. The proportion of tanniniferous shrub legumes in the legume mixtures was either 1/3 or 2/3. This resulted in a total of 5 treatments. Animals were offered daily 45 g of forage dry matter per kg of metabolic bodyweight ($BW^{0.75}$). Animals were offered fresh water three times per day and had free access to a mineralized salt mixture. The experimental periods consisted of 18 days each, 10 days adaptation to the experimental diets and 8 days of data and sample collection. Bodyweight was determined at the beginning and the end of each experimental period after a fasting period of 17 hours.

During days 1 to 6 of each collection period, feed refusals, and urine and feces excreted were recorded daily and samples of feeds offered and refused and of urine and feces were taken. Duodenal digesta was sampled on days 6 and 7 and on day 8 ruminal fluid was sampled every 6 hours. Indigestible acid detergent fiber (IADF) was used as internal marker to estimate duodenal dry matter and nutrient flow. Forage offered, refusals, feces and the solid phase of duodenal digesta were analyzed for organic matter (OM), neutral detergent fiber (NDF), acid detergent fiber (ADF), FAID and total nitrogen (crude protein (CP) = N x 6.25). Microbial nitrogen in duodenal digesta was estimated using purines as marker. Ruminal fluid and duodenal digesta were further analyzed for ammonia concentration and feeds offered for total condensed tannin concentration.

Results and Discussion

The grass used in this investigation presented a low CP content (64 g/kg) and high contents of NDF and ADF (810 and 425 g/kg, respectively). The tanniniferous shrub legumes presented a high CP content which was similar to that found in *V. unguiculata*. Condensed tannin content was almost three times higher in *C. calothyrsus* than in *F. macrophylla*.

One factor which probably affected the animal response variables was the fact that the proportions of tanniniferous legumes consumed were clearly below the proportions in the diets offered. As a result of this, the proportions of tanniniferous legumes in the diets consumed were 10 and 15%, respectively and not 15 and 30% as intended. Furthermore there was a relatively high variability among individual animals.

In general, OM intake tended to decrease with increasing proportion of tanniniferous legumes in the diet, and was clearly lower ($P<0.001$) with diets containing *F. macrophylla* or *C. calothyrsus* than with the diet free of tannins. No differences in OM intake were observed between the two tanniniferous legumes. Apparent OM and ADF digestibilities were lower ($P<0.05$) with diets containing high proportions of tanniniferous legumes than in the diet free of tannins. Nitrogen intake and rumen ammonia concentrations and duodenal flow of total nitrogen were also decreased ($P<0.05$) by the inclusion of tanniniferous legumes. The proportion of undegraded feed protein reaching the duodenum was not increased by the inclusion of tanniniferous legumes in the diet.

The reasons for these unexpected results are unknown but could be related to the relatively high variability in legume consumption among individual animals.

The intake of small proportions of tanniniferous legumes in mixtures with a legume free of tannins decreased OM and CP intake and suppressed fiber digestibility in sheep fed a basal grass diet of low quality. On the other hand, consumption of tanniniferous legumes did not affect N utilization in the way as it was expected. Although the inclusion of higher proportions of tanniniferous legumes decreased ruminal ammonia concentration, duodenal flow of total nitrogen was also decreased when compared to the diet free of tannins.

It can be concluded that the inclusion of small proportions of tanniniferous legumes in mixtures with legumes free of tannins did no have any positive effects on digestion and utilization of nitrogen.

1.3.4 Effects of feeding legume mixtures on energy and nitrogen utilization by sheep

Contributors: T. Tiemann (ETH Zurich), Patricia Avila (CIAT), H.R. Wettstein (ETH Zurich), M. Kreuzer (ETH Zurich), H.D. Hess (ALP Posieux) and C.E. Lascano (CIAT)

Rationale

Tropical fodder legumes are often characterized by high contents of condensed tannins (CT). The effect of CT on protein digestion is well-known but few data are available on their effects on energy utilization and methane production. To investigate effects of CT in legumes on energy and protein utilization and on methane production, an in vivo feeding trial in metabolic cages and respiratory chambers was conducted. This study formed part of a larger project on the use of tanniniferous shrub legumes as potential forage supplements in smallholder systems.

Material and Methods

The study carried out in the ETH animal facilities in Zurich, Switzerland focused on two promising tanniniferous tropical shrub legumes: *Calliandra calothyrsus* and *Flemingia macrophylla*. Six diets were tested for their effect on energy turnover and methane release in a respiratory chamber experiment in a 6 x 6 Latin square design with six lambs of the Swiss White Mountain breed (n=6) (Table 6). Dry matter offered daily was 60 g/kg of metabolic bodyweight ($BW^{0.75}$). Five of the diets consisted of mixtures of grass (*Brachiaria brizantha*) and legume (55% grass and 45% legume foliage), with a grass-only diet serving as control. The legume supplements consisted either of sun-dried CT-free Vigna unguiculata alone or of mixtures of *V. unguiculata* with air-dried leaves of *C. calothyrsus* or *F. macrophylla* in ratios of 2:1 and 1:2.

Animals were adapted to the experimental diets for 2 weeks. In the third week we measured intake, and fecal and urine excretion. In addition, samples of rumen fluid and blood were taken. For 2 consecutive days, the gaseous exchange of the animals was measured using dual open-circuit respiratory chambers.

Subsequently diets and samples of feces and urine were analyzed for nitrogen, carbon and energy content, as well as fiber composition. Blood was analyzed for nitrogen (BUN) and in the rumen fluid bacteria, protozoa, ammonia and volatile fatty acids (by HPLC) were measured. Data were subjected to analysis of variance with SAS considering diet, animal and experimental periods as sources of variation.

Results and Discussion

It is often stated that legumes with high tannin content are not palatable and therefore avoided by herbivores. However in this trial the intake of the tanniniferous legumes was high (>85% of the amounts offered) despite relatively high total CT content in Calliandra (182 g kg^{-1}) and Flemingia (219 g kg^{-1}). The differences in intake due to plant species were much smaller than differences due to animals. Also some animals became used

Table 6. Diets utilized in the feeding trial with sheep.

Diet	Grass	High quality legume	Tanniniferous legume
1	*Brachiaria brizantha* 100%		
2	*Brachiaria brizantha* 55%	*Vigna unguiculata* 45%	
3	*Brachiaria brizantha* a 55%	*Vigna unguiculata* 30%	*Calliandra calothyrsus* 15%
4	*Brachiaria brizantha* 55%	*Vigna unguiculata* 15%	*Calliandra calothyrsus* 30%
5	*Brachiaria brizantha* 55%	*Vigna unguiculata* 30%	*Flemingia macrophylla* 15%
6	*Brachiaria brizantha* 55%	*Vigna unguiculata* 15%	*Flemingia macrophylla* 30%

to the tanniniferous legumes and showed intake rates of 100% while others did not. The intake of the basal grass diet exceeded 90% of the forage offered throughout the experiment.

Rumen ammonia concentration was low in the treatment with Brachiaria only (3.9 mmol/l) and with the high proportion of Calliandra (3.8 mmol/l), which in both cases is below the minimum requirement of ruminants. The ammonia level with the Brachiaria-Vigna only diet was high (6.5 mmol/l) compared to the other treatments. Plasma urea nitrogen (PUN) was also lowest for the grass alone and high Calliandra diets, followed by the two Flemingia treatments and the diet with the low Calliandra level.

The apparent digestibility of OM was low in diets with high proportions of tanniniferous legumes and increased when their proportion was reduced. The apparently digested N in relation to the $BW^{0.75}$ was for all diets (except for the high Calliandra diet) higher than for the pure Brachiaria diet. Apparent N digestibility was lowest for the high Calliandra and for the diets with high Flemingia and low Calliandra. However, the amount of protein retained did not differ ($P > 0.05$) between diets.

Fecal N relative to N intake was elevated as the proportion of tanniniferous species increased in the diets. On the other hand N loss through urine in relation to total N loss was higher when tanniniferous legumes were include at low levels. These results suggest that N of tanniniferous legumes is mainly lost via feces as tannins protect protein from degradation in the rumen. Results also showed that the expected increase in CP absorption due to tannins in the legumes fed did not take place in a significant degree. Total N loss and N retention did not differ between diets.

Apparent digestibility of NDF showed a tendency ($P<0.1$) to be lower with higher proportions of tanniniferous legumes. Apparent digestibility of ADF was reduced in all diets containing Calliandra or Flemingia.

Apparent energy digestibility and energy metabolizability were lower in the diets with tanniniferous legumes as compared to the diet with the high quality legume and the pure Brachiaria diet. The heat energy/kg $BW^{0.75}$ was lower ($P<0.01$) for the high tannin diets than for the high quality diet. The metabolizable energy was utilized mainly for fat tissue deposition, whereas the high tannin diets also showed lower fat gain than the high Vigna diet.

Volatile fatty acids did not differ between treatments. In contrast, methane production/ $BW^{0.75}$ was reduced by the inclusion of tanniniferous legumes. This reduction was 8.4% and 25.7% for the diets containing low and high proportions of Calliandra, respectively and 9.1% and 21.8% for the diets containing low and high Flemingia, respectively. When related to intake of gross energy this effect was significant only for the diets with high proportions of legumes with tannins. There was no effect of treatments on methane production when expressed per unit of digested energy, digested NDF or digested OM.

In general, our results demonstrate that a high level of Calliandra calothyrsus in the diet resulted in lower metabolic protein and energy supply as compared to the high-quality legume diet. It was also evident that *Flemingia macrophylla* supplementation was less detrimental than that of *Calliandra calothyrsus* in metabolic protein supply, but not in energy supply. However, a high proportion of *Flemingia macrophylla* in the diet had a similar adverse effect on protein supply as the low *Calliandra calothyrsus* diet. Regarding energy supply both legume species had similar effects.

The suppressing effect of tanniniferous legumes on methane seems to have been mainly mediated by reductions in ruminal fiber and organic matter degradation and less by a direct adverse action against the methanogenic microbes. Finally, our results suggest that the CT-free Vigna could be partially replaced by *Flemingia macrophylla* for improving low-quality tropical grass-only diets in the tropics if no other option is available.

1.3.5 Milk production of dual purpose cows supplemented with legume mixtures with and without tannins

Contributors: L. Bernal (Universidad Nacional Palmira), P. Avila, C. Ramírez (CIAT), H.D. Hess (ALP Posieux) and C.E. Lascano (CIAT)

Rationale

Milk production of dual purpose cows in livestock systems operated by smallholders in the tropics is heavily affected by the low availability and quality of the traditional forage resources during the dry season. One alternative to minimize the decrease in milk production during the dry season is to supplement livestock with legume foliage. These strategies allow smallholders to maintain livestock productivity throughout the year and reduce the dependence on purchased supplements during the dry season.

Previous studies had shown that supplementation with hay of *Calliandra calothyrsus* (Calliandra) did not increase milk production of dual purpose cows grazing low quality pastures during the dry season. This lack of response to supplementation with Calliandra has been associated to its high level of tannins, which results in low levels of ammonia production in the rumen and as a result bacterial protein synthesis is reduced. It has been hypothesized that mixing legumes with and without tannins could contribute to maximize the effects of legume supplementation on milk production due to increased production of rumen ammonia and flow of total nitrogen to the lower digestive tract.

A feeding trial with lactating dual purpose cows was conducted to test the effect of mixtures of legumes with and without tannins as supplements for milking cows grazing a low quality pasture.

Materials and Methods

The feeding trial was performed at CIAT's research station Quilichao (Cauca, Colombia). Eight Holstein x Cebu crossbred cow were assigned to 4 supplementation treatments using a repeated 4 x 4 Latin square design. The 4 supplements consisted either of the tanniniferous *Calliandra calothyrsus* (CIAT 22310) or the tannin free *Vigna unguiculata* (CIAT 1088/4, 288, 391, 9611 and 715) alone or of mixtures of these 2 legumes in proportions of 1:2 or 2:1. Legume foliage was harvested and sun dried for 3 days prior to the experiment.

The supplements were offered at a level of 1% of the cows bodyweight in two meals per day (at 05:00 and 13:00) during milking. To improve palatability of the legume foliages, the individual portions of the supplements were mixed with 100 g of molasses and 50 g of a mineralized salt for dairy cattle. The remaining time of the day, cows were grazing a pasture based on *Paspalum notatum* with an average dry matter availability of 983 kg/ha. The pasture area of 3 ha was divided into 2 paddocks of 1.5 ha each which were grazed alternately with 7 days of grazing and 7 days of rest period. The average stocking rate during the experiment was 2.6 animal units per hectare.

The experimental periods were of 14 days each, with 7 days of adaptation to the respective supplements and 7 days of measurement of daily milk production. Additionally samples of milk were taken for the determination of the contents of fat, total solids and urea nitrogen.

Results and Discussion

Despite the addition of molasses and salt to the legume foliage, consumption of the supplement was affected by its composition (Table 7). The highest ($P<0.05$) intake was observed when the supplement consisted of *V. unguiculata* (cowpea) alone or of the mixture containing 2/3 of this legume. With the supplement containing only 1/3 of cowpea, intake was intermediate and the lowest (P<0.05) supplement intake was observed with Calliandra alone. These differences in the consumption of the supplements were also reflected in daily milk production.

The lowest (P<0.05) amount of fat corrected milk was produced in the treatment with Calliandra alone. When legume mixtures were supplemented, milk production was intermediate and when cowpea was supplemented alone, milk production was higher (P<0.05) than in any other treatment. Milk contents of fat and total solids were not affected (P>0.05) by supplementation. However, milk urea nitrogen was higher (P<0.05) with cowpea than with Calliandra, which is probably due to the higher content of readily fermentable crude protein in *V. unguiculata*.

These results confirm the high potential of cowpea as supplement for dual purpose cows grazing low quality pastures during the dry season and they indicate that milk production increases with increasing proportion of cowpea in the legume supplement.

It can be concluded that a legume supplement based on Calliandra can be significantly improved by including a small proportion (e.g. 1/3) of cowpea in the mixture. It is worth mentioning that milk urea nitrogen levels were very low throughout the experiment, indicating that rumen degradable crude protein was a limiting nutrient even in the treatment with Cowpea alone. Therefore it is not surprising that the mixtures of Calliandra and cowpea did not result in a higher milk production than cowpea alone, because a positive effect of such legume mixtures can only be expected when degradable crude protein is not limiting ruminal fermentation.

Table 7. Amount of legume foliage consumed, milk production and milk urea nitrogen concentration of cows supplemented with contrasting legume mixtures.

	Botanical composition of supplement (% of dry matter)			
C. calothyrsus	100	67	33	0
V. unguiculata	0	33	67	100
DM offered (kg/d)	4.2	4.4	4.3	4.3
DM consumed				
kg/d	1.2c	2.6b	3.5a	3.8a
g/kg BW	2.8c	6.0b	8.2a	8.7a
Milk production (kg/d)	3.6c	4.4b	4.7b	5.3a
Milk urea N (mg/dL)	3.7b	3.1b	4.5a	6.3a

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 resistance to biotic and abiotic constraints

Highlights

- Open-pollinated progenies of 95 selected BR05 hybrids were assessed to infer reproductive mode of the parent hybrids. Of the 95 progenies, 23 were found to be fully apomictic as well as having desirable agronomic characteristics (including seed set).

- Presence/absence of SCAR marker N-14 was in good agreement with visual assessment of reproductive mode based on uniformity of OP progeny.

2.1.1 Establishment of a hybrid population as spaced plants in two field trials (Quilichao & Matazul)

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

Rationale

In each breeding cycle, several thousand new hybrids are evaluated in field trials to discard plants with obviously irrelevant phenotypes (low vigor, stemmy, diseased, deficiency symptoms). These field trials are established in two contrasting environments in Colombia. Following an initial cull on periodic visual assessments of clones at the two field sites, "pre-selected" hybrids are vegetatively propagated to CIAT-Palmira to produce propagules for further evaluations (entomology; pathology; plant nutrition; quality). Open pollinated seed is harvested (by enclosing inflorescences in mesh bags) from the ''pre-selected" plants in the CIAT-Quilichao trial to produce progenies for assessment of reproductive mode. "Pre-selects" are initially culled on seed fill (percent full spikelets, by weight) of the single-plant seed harvested.

Materials and Methods

Testcross seed was produced during 2005 at CIAT-Popayán, by exposing 565 sexual clones (series SX05NO) to pollen of *B. decumbens* cv. Basilisk in the field at CIAT-Popayán. Testcross seed was hand scarified and sown in sand in planting flats. Only 233 of the 565 sexual clones produced sufficient testcross seedlings for inclusion in the 2006 field trials.

Seedlings were individually transplanted to 10.16-cm diameter (4-in) plastic pots. Each seedling was then vegetatively propagated to produce two sets of 2250 individual genotypes in 233 testcross families. Seedlings were established in 5-plant family plots with from 1 to 4 replications depending on final family size. Four check genotypes (cvs. Basilisk, Marandu, Mulato, and Mulato II) were included in the trial, with three replications (of 5-plant plots) each. Entries (families and checks) were completely randomized among 462 5-plant plots.

Superior families and individuals within families were identified on periodic visual inspection of the two field trials. Open-pollinated seed was harvested on these "pre-selected" plants by enclosing inflorescences in mesh bags. Full caryopses were separated from empty spikelets by passing through a seed blower. Percent full seeds (weight: weight) was calculated for each individual "pre-selected" individual plant.

Results and Discussion

Initially, 353 individual testcross hybrids were identified as promising “pre-selections”. Based on poor seed fill, 189 of these (53.5%) were culled. The remaining 164 selections (from 77 testcross families) will be progeny tested (to identify apomicts) and evaluated for reaction to spittlebugs, rhizoctonia foliar blight, aluminum, and forage quality.

2.1.2 Reconfirming apomictic reproduction of selected hybrids and multiplication of seed for distribution

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

Rationale

Reproductive mode (apomixis vs. sexuality) of promising hybrids is assessed by progeny test. The progeny test serves the additional function of generating a first seed increase. Where any doubt remains regarding reproductive mode, or seed multiplied is insufficient for continued testing, a further seed increase/progeny test is conducted.

Materials and Methods

Seedlings of nine BR04 series hybrids were produced and a 45-plant plot of each hybrid established at CIAT-Popayán on 18 July 2006. Seed harvest and processing is still underway at the time of preparation of this report (09-Feb-07 14:05). Seedlings of 11 MX02 series hybrids were produced and these progenies were included in a progeny trial established at CIAT-Palmira on 14 July 2006. Four 5-plant plots were established for each hybrid.

Inflorescences were enclosed in mesh bags to recover mature seed. Total weight of crude seed was recorded. Full spikelets were recovered by passing crude seed through a seed blower. Seed fill was expressed as weight: weight of full: crude seed.

Results and Discussion

Progenies of all nine BX04 and 11 MX02 hybrids being assessed were uniform, confirming apomictic reproduction. Six of the 11 MX02 hybrids were culled on poor performance. The 14 remaining hybrids will be included in further evaluations leading eventually to possible cultivar release.

2.1.3 Reproductive mode of pre-selected Brachiaria hybrids at Quilichao and Matazul

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

Rationale

Only apomictic hybrids are of interest as possible commercial cultivars. In any hybrid population, approx. half of the individuals will turn out to be sexually reproducing. A progeny test is conducted to identify fully apomictic hybrids (based on uniformity among sibs). The progeny test serves the additional functions of reconfirming (or not) desirable agronomic characteristics of the promising hybrids and of generating the initial seed increase for further distribution and testing.

Materials and Methods

OP seed of 127 BR05 series “pre-selections” was hand scarified and germinated. Thirty-two progenies with fewer than five seedlings were culled, leaving 95 progenies. Seedlings were transplanted to 5-plant plots (one to four replications) on 14 July 2006. Entries (families) were completely randomized among available plots. Reproductive mode of each hybrid was inferred from the uniformity or variability of the siblings within families.

Seed of uniform families was recovered by enclosing inflorescences in mesh bags. Full spikelets were recovered from crude seed by passing through a seed blower. Percent full seeds were estimated on a weight: weight basis.

Results and Discussion

Approximately half of the BR05 hybrids were judged to be apomictic. Percentage full seed ranged from zero to 35%. Based on seed fill, the apomicts were culled to 23, where sufficient seed for further evaluation is available.

These 23 promising BR05 hybrids along with nine BR04 hybrids and seven MX02 hybrids will be submitted to further agronomic testing leading to possible commercial release of one or more of the 39 hybrids. Our partner, Semillas Papalotla, will assume major responsibility for these advanced evaluations.

2.1.4 Genetic control and molecular markers for spittlebug and reproductive mode in *Brachiaria*: Assess presence/absence of the "apomixes locus" marker (SCAR N-14) in hybrids

Contributors: J. W. Miles, J. Tohme and C. Quintero (CIAT)

Rationale

A reliable molecular marker of the "apomixis locus" would improve the efficiency of determination of reproductive mode by eliminating the necessity of field progeny testing of approx. half of candidate hybrids. These could be culled as sexuals at a very early stage in the evaluation process – perhaps as early as prior to initial agronomic evaluation – based on marker phenotype.

Materials and Methods

Leaf tissue samples of the 95 BR05 series hybrids with cv. Basilisk included in the 2006 progeny trial were taken and standard PCR procedures applied to assess the presence or absence of SCAR marker N-14.

Results and Discussion

Marker results were in essential agreement with progeny test results, within the inevitable uncertainty of the progeny test assessment of reproductive mode. I.e., hybrids classified as facultative apomicts might, in fact, be sexuals, and hybrids classed as sexual might be facultative apomict, and many hybrids simply cannot unequivocally be classified on the field progeny test.

Thirty-five of the 95 hybrids were classified as "apomictic". Of these, all were positive for presence of the marker. Twenty-six hybrids were classified as "sexual". Twenty-five of the 26 were negative for the marker, and one positive. Of 12 hybrids classified as "sexual or facultative apomict", all 12 lacked the marker, and, hence are probably real sexuals. A number of progenies could not be classified unequivocally, and N-14 data are uninformative (Table 8).

Table 8. Visual classification of open-pollinated progenies as to reproductive mode and corresponding presence/absence of SCAR marker, N-14 of 95 BR05 progenies assessed at CIAT-Palmira.

CLASSIFICATION	TOTAL	N-14 +	N-14 -
APOMICTIC, UNEQUIVOCAL	35	35	0
SEXUAL, UNEQUIVOCAL	26	1	25
SEXUAL OR FACULTATIVE APOMICT	12	0	12
APOMICTIC OR FACULTATIVE	3	2	1
QUESTIONABLE APOMICTIC	4	1	3
FACULTATIVE APOMICTIC	4	2	2
FACULTATIVE APOMICTIC OR APOMICTIC	1	1	0
FACULTATIVE APOMICTIC OR SEXUAL	2	1	1
QUESTIONABLE FACULTATIVE APOMICTIC	5	3	2
QUESTIONABLE SEXUAL	1	1	0
UNCLASSIFIABLE	2	1	1
TOTALS	95	48	47

2.2 Potential for improving *Brachiaria humidicola* through breeding

Highlights

- Fourteen *B. humidicola* hybrid seedlings produced
- A diagnostic microsatellite marker to identify putative *B. humidicola* hybrids was found
- Nineteen *B. humidicola* accessions reported to be tetraploids are available in the *Brachiaria* germplasm collection of CIAT's Genetic Resources Unit.
- Two field crossing blocks and a small-plot, 2-replicate observation trial were established to broaden genetic base of a proposed synthetic sexual breeding population in *B. humidicola.*

2.2.1 Evaluation of open-pollinated seed harvested from putatively sexual tetraploid accession *Brachiaria humidicola* exposed to pollen of putatively apomictic tetraploid accession

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

Rationale

B. humidicola, as a species, has a number of highly desirable attributes, notably its strongly stoloniferous growth, good resilience under grazing mismanagement, and tolerance to poorly drained soil conditions. Currently available cultivars of the species have a number of defects, notably poor nutritional quality, poor seed yield, and strong physiological seed dormancy.

Materials and Methods

Two tetraploid accessions of *B. humidicola* were selected. Their reproductive modes (CIAT 26146, sexual; CIAT 26149, apomictic) were obtained from cytological work done at Embrapa's Beef Cattle Center in Campo Grande, Mato Grosso do Sur.

Vegetative propagules of CIAT 26146 were established in 2005, as a series of single, spaced plants in a seed multiplication plot of CIAT 26149 at CIAT-Popayán and allowed to open pollinate. Seed was hand harvested on the plants of CIAT 26146. After several months to overcome dormancy, seeds were hand scarified and germinated.

A molecular marker allele present in the male parent (CIAT 26149) and absent in the female (26146), if detected in putative hybrids, would provide convincing evidence of their hybrid origin. Thus a number of microsatellite markers were assessed on the two parental genotypes. Informative markers – those present in the male (apomictic) parent and absent in the female (sexual) parent – were identified. Each of the 14 putative hybrid seedlings was assessed with one such informative microsatellite marker.

Results and Discussion

Fourteen putative hybrid seedlings were obtained. Detection of the band present in the male parent and absent in the female in all 14 of putative hybrids confirmed that all putative hybrids are in fact true hybrids. These results open the possibility of directed genetic improvement in *B. humidicola*, particularly if inheritance of reproductive mode (sexuality vs. apomixis) is found to be simply inherited.

2.2.2 Screen tetraploid *B. humidicola* germplasm accessions for reproductive mode

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

Rationale

Having demonstrated the feasibility of achieving genetic recombination between two reproductively characterized tetraploid *B. humidicola* accessions, it should be feasible to access all of the genetic diversity in CIAT's collection of *B. humidicola* germplasm at the tetraploid level. A first step to accessing this genetic diversity in a plant breeding program is to characterize reproductive mode in each of the tetraploid *B. humidicola* germplasm accessions. Existing information on reproductive mode of these accessions is incomplete and may be erroneous.

Materials and Methods

CIAT's collection of *B. humidicola* contains 19 accessions identified as tetraploid, based on work done at Embrapa's Beef Cattle Research Center and published in Penteado et al., 2000. Vegetative material of these 19 accessions was collected from the *Brachiaria* collection maintained at CIAT-Popayán by the Genetic Resources Unit. Three plantings have been established with these accessions (and 14 tetraploid hybrids): a space-planted crossing block established with vegetative transplants at CIAT-Popayán; a crossing block of pot-grown plants at CIAT-Palmira, and a small-plot (4-plant plots) 2-replicate observation trial at CIAT-Quilichao. OP seed will be harvested and individual genotypes – accessions and hybrids – will be progeny tested to assess their reproductive behavior.

Results and Discussion

Only one of the accessions (CIAT 16517) is flowering profusely at the time this report was prepared (13 February 2007), but this accession is mis-identified since it is not *B. humidicola*. Flowering is beginning on plants of other accessions at CIAT-Popayán, but not at CIAT-Palmira or at CIAT-Quilichao. No seed has been harvested to date (12-Feb-2007) from any of the three field plantings.

2.2.3 Recombination of tetraploid *B. humidicola* germplasm accessions to broaden genetic base of a proposed synthetic sexual breeding population

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

Rationale

To allow effective *B. humidicola* plant breeding, a broad-based, sexual breeding population needs to be synthesized from available tetraploid germplasm.

Materials and Methods

All available germplasm accessions reported as tetraploid, as well as 14 tetraploid hybrids are being allowed to open pollinate in field trials in order to: 1) assess reproductive mode by progeny testing all tetraploid genotypes, and 2) identify fully sexual genotypes in hybrid populations to recombine into a broad-based, synthetic sexual *B. humidicola* breeding population.

Results and Discussion

Several "crossing blocks" have been established. Seed produced in these crossing blocks, resulting from uncontrolled open pollination, will allow progeny testing of hybrids and accessions.

Superior individuals in the (segregating) progenies of sexual accessions will then have to be progeny tested to identify sexual hybrids. These hybrids will then form the "founder" parental set for the proposed tetraploid sexual *B. humidicola* breeding population.

2.2.4 Assessing reproductive mode of *Brachiaria humidicola* hybrids

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

Rationale

We know nothing of the inheritance of reproductive mode in *B. humidicola*. Progeny testing the 14 hybrids available to date will provide preliminary (but probably not conclusive) information on inheritance of apomixis in this species.

Materials and Methods

Open-pollinated seed from each of the 14 *B. humidicola* hybrids will be used to establish progenies. The reproductive mode of each hybrid will be inferred from the uniformity or variability of its progeny.

Results and Discussion

None of the *B. humidicola* hybrids is flowering yet in any of the field plantings.

2.3 Screening *Brachiaria* genotypes for spittlebug resistance

Highlights

- A high level of combined (multi-species) antibiotic resistance to spittlebug was identified in a number of hybrids, including nine apomictic hybrids, in spite of all BR05 hybrids being from crosses with the highly susceptible cv. Basilisk

- Very high levels of antibiotic resistance to *P. simulans*, *M. trifissa* and *Z. pubescens* were detected in elite apomictic hybrids (series BR04) previously selected for high resistance to *A. varia*, *A. reducta*, and *Z. carbonaria*.

- High levels of antibiosis resistance to six major spittlebug species were found in 6 apomictic hybrids of the series BR05. Three of these hybrids combine resistance to spittlebug with adaptation to acid soils

- Three apomictic hybrids of the MX02 series (selected in previous evaluations for resistance to *Prosapia simulans*, *A. varia*, *A. reducta, Z. carbonaria*, and *M. trifissa*) also showed resistance to *Z. pubescens*

- High levels of antibiotic resistance to three major Brazilian spittlebug species were detected in the commercial cv. Mulato II.

2.3.1 Continuous mass rearing of spittlebug species in Palmira and Macagual

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 both nymphs and adults of 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.

2.3.2 Identification of *Brachiaria* genotypes resistant to spittlebug

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

2.3.2.1 Greenhouse screening of *Brachiaria* accessions and hybrids for resistance to multiple spittlebug species

Rationale

Assessment of resistance to spittlebugs is an essential step in the process of breeding superior *Brachiaria* cultivars at CIAT. In 2006, intensive screening of selected hybrids was conducted under greenhouse and field conditions. A grand total of 1,150 available genotypes were evaluated. All six major spittlebug species were used.

Materials and Methods

Screenings for resistance in the greenhouse were conducted with different species of spittlebug (*Aeneolamia varia, A. reducta, Zulia carbonaria, Z. pubescens, Mahanarva trifissa* and *Prosapia simulans*). Test materials were usually compared with six 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 we reported on the levels of resistance to *A. varia*, *A. reducta,* and *Z. carbonaria* in 141 apomictic BR04 hybrids. Elite materials were screened in 2006 for resistance to *Z. pubescens, Mahanarva trifissa*, and *Prosapia simulans*. As usual, correlations between damage scores and percentage nymph survival were high and significant ($P < 0.01$): 0.889 for *P. simulans*, 0.939 for *M. trifissa*, and 0.917 for *Z. pubescens*. Very high levels of antibiotic resistance to all three spittlebug species were detected in these selected genotypes (Table 9).

Table 9. Percentage nymph survival in selected *Brachiaria* genotypes screened for resistance to three major spittlebug species. Means ± SEM of five replications per genotype.

Genotype	Spittlebug species		
	Prosapia simulans	*Mahanarva trifissa*	*Zulia pubescens*
BR04NO/1751	0	0	0
BR04NO/1819	0	0	0
BR04NO/1889	0	3.3 ± 3.3	0
BR04NO/1900	0	10.0 ± 6.7	6.7 ± 6.7
BR04NO/2007	6.7 ± 6.7	0	0
BR04NO/2109	0	0	0
BR04NO/2405	0	0	0
BR04NO/2455	0	0	0
BR04NO/2515	6.7 ± 6.7	10.0 ± 6.7	0
BR04NO/2557	0	0	0
BR04NO/2793	0	0	0
BR04NO/3119	33.4 ± 10.5	3.3 ± 3.3	3.3 ± 3.3
CIAT 6294[1]	10.0 ± 3.7	1. 7 ± 1.7	11.7 ± 7.9
CIAT 36062[1]	5.0 ± 2.5	0	3.3 ± 3.3
CIAT 36087[2]	0	0	0
SX01NO/0102[1]	0	0	0
BRX-44-02[3]	92.8 ± 4.9	80.0 ± 4.8	61.7 ± 7.9
CIAT 0606[3]	81.7 ± 6.8	85.0 ± 3.9	75.9 ± 4.0

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

Ten hybrids — BR05NO/0048, BR05NO/0267, BR05NO/0293, BR05NO/0537, BR05NO/0563, BR05NO/0760, BR05NO/0913, BR05NO/1402, BR05NO/1447, BR05NO/1520 — are highly resistant to six different spittlebug species (Table 10). Further, 6 of these 10 multiple species resistant hybrids — BR05NO/0334, BR05NO/0537, BR05NO/0563, BR05NO/0760, BR05NO/0913, and BR05NO/1520 — are apomicts, and hence candidates for cultivar release.

Three of the hybrids — BR05NO/0334, BR05NO/0537, and BR05NO/0563 — combine resistance to four spittlebug species and tolerance to aluminum with apomictic reproduction.

Table 10. Percentage nymphal survival in selected *Brachiaria* genotypes screened for resistance to six species of spittlebug.

Genotype	*Aeneolamia varia*	*Aeneolamia reducta*	*Zulia carbonaria*	*Zulia pubescens*	*Prosapia simulans*	*Mahanarva trifissa*
BR05NO/0048	0	3.3	3.3	0	13.3	0
BR05NO/0267	16.7	0	0	0	0	0
BR05NO/0293	0	3.3	0	0	11.7	0
BR05NO/0537	23.3	20.0	16.7	16.7	33.3	16.7
BR05NO/0563	13.3	10.0	6.7	0	6.7	6.7
BR05NO/0760	10.0	30.0	20.0	3.3	16.7	6.7
BR05NO/0913	3.3	3.3	13.3	0	0	0
BR05NO/1402	20.0	6.7	10.0	3.3	30.0	13.3
BR05NO/1447	0	13.3	3.3	0	23.3	6.7
BR05NO/1520	4.2	0	0	0	3.3	0
CIAT 6294[1]	36.6	76.7	30.0	26.7	46.7	0
CIAT 36062[2]	3.3	23.3	23.3	6.7	20.0	0
SX01NO/0102 01NO/0102[2]	6.7	0	0	0	23.3	0
CIAT 36087[3]	13.3	40.0	6.7	0	6.7	0
CIAT 0606[4]	93.3	93.3	93.3	90.0	76.7	66.7
BRX 44-02[5]	90.0	100.0	76.7	66.7	80.0	80.0

[1] Resistant commercial check (cv. Marandu).
[2] Resistant checks.
[3] Resistant commercial check (cv. Mulato II).
[4] Susceptible commercial check (cv. Basilisk).
[5] Susceptible check (Tetraploid *B. ruziziensis*).

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. In 2006 the genotypes were tested for resistance to *Z. pubescens*. Those with high levels of antibiotic resistance are shown in Table 11.

Work done in Brazil by Alejandro Pabón in cooperation with the Empresa de Pesquisa Agropecuaria de Minas Gerais (EPAMIG) revealed high levels of resistance to three major Brazilian spittlebug species in cv. Mulato II (Table 12). Mulato II was recently released in Brazil and elsewhere.

Table 11. Damage scores and percentage nymph survival in selected *Brachiaria* apomictic hybrids tested for resistance to *Zulia pubescens*. Means ± SEM of ten replications per genotype.

Genotype	Damage scores[1]	Percentage nymph survival
MX02NO/1905	1.0 ± 0	0
MX02NO/2273	1.3 ± 0.10	0
MX02NO/3056	1.3 ± 0.14	0
MX02NO/1388	1.4 ± 0.19	3.3 ± 2.22
MX02NO/3213	1.6 ± 0.18	3.3 ± 3.32
MX02NO/1769	1.7 ± 0.15	5.0 ± 2.55
MX02NO/1423	1.3 ± 0.16	5.6 ± 3.73
MX02NO/1561	1.3 ± 0.13	6.7 ± 4.43
MX02NO/3861	1.3 ± 0.13	8.3 ± 5.12
CIAT 6294[2]	1.6 ± 0.12	31.7 ± 6.77
CIAT 36062[2]	1.2 ± 0.11	0
SX01NO/0102[2]	1.0 ± 0.05	1.7 ± 1.66
CIAT 36087[3] (Mulato II)	1.3 ± 0.07	0
BRX-44-02[4]	4.8 ± 0.11	90.7 ± 2.78
CIAT 0606[4]	3.9 ± 0.16	81.6 ± 4.61

[1] On a 1 – 5 visual score scale (1, no visible damage; 5, plant dead).
[2] Resistant check.
[3] Resistant commercial check.
[4] Susceptible check.

Table 12. Levels of resistance to three Brazilian spittlebug species in CIAT 36087 (cv. Mulato II).

Genotype	*Notozulia entreriana*		*Deois flavopicta*		*Deois schah*	
	Damage scores[a]	Percentage survival	Damage scores[a]	Percentage survival	Damage scores	Percentage survival
CIAT 0606[b]	3.7a	76.0a	4.3a	84.7a	4.3a	80.9a
CIAT 6294[c]	1.9b	25.5b	2.4b	25.3b	2.0b	28.2b
CIAT 36087	1.6c	12.2c	1.8c	14.8c	1.7b	16.6b

[a] On a 1 – 5 visual score scale (1, no visible damage; 5, plant dead).
[b] Susceptible commercial check (cv. Basilisk).
[c] Resistant commercial check (cv. Marandu).
Means within a column followed by the same letter are not significantly different at the 5% level by LSD. Means o three trials, 10 repetitions per trial with *N. entreriana* and *D. flavopicta*; 20 repetitions with *D. schach*.

We continued with our studies on possible genotype x by insect species interactions. Pending data with two species from Mexico and final statistical analysis, our results suggest the occurrence of interesting genotype x spittlebug species interactions (Table 13), which may have important implications for breeding for resistance. This work (including field data from Caquetá, four spittlebug species) will be reported in full in 2007.

Table 13. Mechanisms of resistance to eight spittlebug species in selected *Brachiaria* genotypes. R, high antibiosis; I, intermediate antibiotic resistance; T, tolerance; S, susceptibility.

Genotype	*Aeneolamia varia*	*Aeneolamia reducta*	*Zulia carbonaria*	*Zulia pubescens*	*Prosapia simulans*	*Mahanarva trifissa*	*Notozulia entreriana*	*Deois schach*
CIAT 06294	I	T	T	R	R	R	R	R
CIAT 16106	S	T	R	S	T	S	R	R
CIAT 16307	R	R	I	R	R	R	-	-
CIAT 16310	-	-	-	R	R	R	-	-
CIAT 16493	I	I	S	T	T	R	S	S
CIAT 16827	I	T	T	I	R	R	R	R
CIAT 16829	R	T	T	I	I	R	R	R
CIAT 16830	R	I	I	T	I	R	R	R
CIAT 16835	I	T	T	I	I	R	R	R
CIAT 16843	I	S	S	S	S	I	R	R
CIAT 16844	I	S	I	S	S	S	R	R
CIAT 16867	S	S	T	S	S	S	T	T
CIAT 16886	S	S	T	T	S	S	T	T
CIAT 26110	T	T	T	R	R	R	T	R
CIAT 26288	S	S	S	S	S	I	S	S
CIAT 36061	S	S	S	R	I	R	T	T
CIAT 36062	R	R	R	R	R	R	R	R
CIAT 36087[a]	T	T	R	R	R	R	R	R
SXNO/0102[b]	R	R	R	R	R	R	-	-
CIAT 0606[c]	S	S	S	S	S	S	S	S
CIAT 0654[c]	S	S	S	S	S	S	-	-

[a] co. Mulato II.
[b] Resistant check.
[c] Susceptible check.

2.3.2.2 Field screening of *Brachiaria* accessions and hybrids for resistance to several 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, 12 major screening trials (three with *A. varia*, four with *Zulia carbonaria,* three 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 (BR04) and 18 CIAT accessions that had been previously evaluated in Palmira under greenhouse conditions.

Results and Discussion

Using tiller ratios (the ratio between tillers per plant at the end of the infestation process and tillers per plant at the beginning of the infestation process) as the main selection criterion, we found that most of the BR04 hybrids tested were susceptible to spittlebug. Those combining resistances to two or more species are listed in Table 14.

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			
BR04NO/1197	1.18	0.97	0.90	1.08
BR04NO/1950	1.06	0.81	1.15	0.99
BR04NO/2007	1.07	0.87	1.01	1.02
BR04NO/2069	1.27	0.89	1.10	0.82
BR04NO/2093	1.02	0.93	0.88	1.01
BR04NO/2405	1.00	0.96	1.07	0.92
BR04NO/2557	1.19	0.93	1.09	0.98
BR04NO/2833	1.28	1.13	1.03	0.88
BR04NO/2940	1.02	1.04	0.91	0.84
BR04NO/2983	1.09	1.04	0.94	0.97
BR04NO/3056	1.27	0.88	0.95	0.88
BR04NO/3077	1.02	0.79	1.07	0.84
BR04NO/3119	1.20	0.96	1.12	0.89
Mean selected hybrids	1.12a	0.94a	1.02a	0.93b
	Resistant checks			
CIAT 6294	1.06	1.00	1.06	1.09
CIAT 36062	1.09	1.00	1.00	1.10
Mean resistant checks	1.07a	1.00a	1.03a	1.09a
	Commercial check			
CIAT 36087 (Mulato II)	1.13a	1.00a	1.02a	1.00ab
	Susceptible checks			
CIAT 0606	0.49	0.36	0.58	0.48
BRX44-02	0.43	0.43	0.53	0.40
Mean susceptible checks	0.46b	0.39b	0.56b	0.44c

Means of 10 reps per genotype per species per trial; 2 trials in the case of *A. varia*, and *Z. pubescens,* 3 trials with *Z. carbonaria* and one 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.

2.4 Identify host mechanisms for spittlebug resistance in *Brachiaria*

Highlights

- A reliable mass screening technique to screen for resistance to adult feeding damage was developed.
- Adult survival was not affected by *Brachiaria* genotypes used in the experiments at the level of infestation used, which suggests that antibiosis does not play a role in resistance to adult feeding damage.

2.4.1 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 different 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 to adult damage as a possible component of resistance to spittlebug. In 2006 we successfully developed a reliable screening technique to evaluate *Brachiaria* genotypes for resistance to adult feeding damage.

Materials and Methods

Three spittlebug species were utilized: *A. varia, A. reducta*, and *Z. carbonaria*. Based on results obtained in 2005, twenty-day old plants were infested with four neonate adults per plant and the infestation was allowed to proceed until all adults died (usually, 8-10 days after infestation). Percentage adult survival was recorded on a daily basis. Damage scores in a 1-5 visual damage score scale were taken 10 days after infestation.

To measure chlorophyll loss as a result of adult feeding, we used a SPAD-502 chlorophyll meter 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. These methodologies were used to test for resistance six *Brachiaria* genotypes of well-known reaction to nymphal attack. Two evaluation units were compared: Muslin cages and plastic bottles.

Results and Discussion

At the level of infestation used in these experiments, adult survival was not affected by the genotypes. This means that antibiosis does not seem to play a role in resistance to adult feeding damage. Susceptible and tolerant reactions of genotypes were very similar using the muslin cage technique or the plastic bottle technique (Table 15). The latter has been adopted to conduct large-scale screenings of *Brachiaria* genotypes for resistance to adult feeding damage.

Table 15. Levels of resistance to adults of three spittlebug species in *Brachiaria* genotypes detected by means of two screening methodologies.

Genotype	Damage scores[a]		Percentage chlorophyll loss		FPLI (%) [b]	
	Muslin cages	Plastic bottles	Muslin cages	Plastic bottles	Muslin cages	Plastic bottles
Aeneolamia varia						
CIAT 0654	4.6a	4.3a	42.3a	41.5a	93.4	93.5
CIAT 0606	3.4b	3.6b	27.7b	26.1b	85.5	67.4
CIAT 6294	1.8d	1.9e	6.8d	15.7c	26.7	17.3
CIAT 36062	2.9c	2.7cd	12.2cd	22.8c	70.6	48.1
CIAT 36087	2.2d	2.2de	6.8d	20.9bc	42.1	47.3
SX01NO/0102	3.2bc	3.0c	17.1bc	25.1b	75.5	64.5
Zulia carbonaria						
CIAT 0654	5.0a	4.8a	65.7a	77.6a	100.0	96.4
CIAT 0606	3.9c	3.7b	37.9b	49.0b	78.0	72.3
CIAT 6294	2.6d	2.0d	12.0c	26.0d	49.5	50.5
CIAT 36062	4.3bc	2.9c	39.2b	39.4c	87.8	61.5
CIAT 36087	2.6d	2.3d	26.1b	30.8d	44.2	48.0
SX01NO/0102	4.7ab	3.1c	66.9a	40.3bc	96.1	75.4
Aenolamia reducta						
CIAT 0654	-	4.0a	-	35.3a	-	83.7
CIAT 0606	-	3.1b	-	26.0a	-	71.2
CIAT 6294	-	2.0c	-	6.5c	-	53.6
CIAT 36062	-	2.9b	-	27.3a	-	61.6
CIAT 36087	-	1.8c	-	12.7b	-	52.4
SX01NO/0102	-	2.9b	-	34.4a	-	67.1

[a] On a 1 – 5 visual scale (1, no visible damage; 5, severe damage, plant killed).
[b] Functional Plant Loss Index = $1 - \left\{\left[\frac{\text{Weight of infested plant}}{\text{Weight of uninfested plant}}\right] \times \left[1 - \frac{(\text{damage score})}{5}\right]\right\} \times 100$

2.5 Define interactions between host and pathogen in *Brachiaria*

Highlight

- A total of 10 Brachiaria hybrids (9 from the series RZ 05 and 1 from the series BR05) exhibited similar resistance (2.0-2.5) to *Rhizoctonia* foliar blight than the check (CIAT 16320)

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

Contributors: G.. Segura, W. Mera, X. Bonilla, J. Miles and S. Kelemu

Rationale:

Rhizoctonia foliar blight, caused by *Rhizoctonia solani* Kühn, is an important disease on a wide range of crops around the globe. 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).

R. solani is a basidiomycete fungus that does not produce any asexual spores (called conidia). In nature, the fungus reproduces mainly asexually and exists as vegetative mycelia and/or dense sclerotia. In the absence of a susceptible host, these sclerotia, that are irregular-shaped, brown to black structures, can survive in soil and on plant debris for several years.

The fungus can also survive as mycelia by colonizing soil organic matter as a saprophyte. When a susceptible host is available, sclerotia can germinate and produce hyphae that can infect host plants. The fungus is a very common soil-borne pathogen that primarily infects below ground plant parts in a great diversity 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. As symptoms progress, 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. A number of constitutive factors including cell wall calcium content, and cuticle thickness may contribute to resistance.

Other factors expressed after infection also play a role in resistance. These components of resistance may also be influenced by factors such as age and maturity of the plant as well as other external factors such as plant nutrition and environmental conditions (e.g. field vs controlled environmental growth conditions).

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. Measurement of resistance is based on quantification of disease symptoms or the growth and expansion of the pathogen on its host.

The objectives of this study are to: 1) artificially inoculate and induce uniform disease development in selected *Brachiaria* genotypes generated by CIAT's tropical forages project, 2) accurately measure resistance and identify resistant materials among these *Brachiaria* genotypes.

Materials and Methods

Plant materials: Two-hundred nine *Brachiaria* genotypes (127 with BR05 series and 82 with RZ 05 series) 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^{o}C and an annual rainfall of 3793 mm.

Field layout, artificial inoculations and disease evaluations: Twelve 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 2 m between blocks.

The entries were replicated 4 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, 25, 35, 45, 55 and 65 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 in susceptible genotypes 10-15 days after inoculations. There was a high degree of correlation in disease evaluation data among the various evaluation dates. The resistant control CIAT 16320 was consistently evaluated at scale less than 2. The disease evaluation data taken 65 days after inoculations represented well-developed disease symptoms that correlated well with data taken at various dates.

Based on the results we formed three groups:

Group 1 (highly resistant):
Ten genotypes, RZ05/3635, BR05/0262, RZ05/2721, RZ05/3551, RZ05/3634, RZ05/3738, RZ05/2738, RZ05/2919, RZ05/3394, and RZ05/3575 were evaluated at an average between 2.0 and 2.5.

Group 2 (moderately resistant):
Eighty-one others, R05/0048, BR05/0377, BR05/0555, BR05/0591, BR05/1482, CIAT 36087, BR05/0753, BR05/0760, BR05/0777, BR05/1359, RZ05/2699, RZ05/2816, RZ05/2842, RZ05/3021, RZ05/3362, RZ05/3397, RZ05/3405, BR05/0156, BR05/0537, RZ05/3063, BR05/0114, BR05/0115, BR05/0118, BR05/0379, BR05/0629, BR05/0714, BR05/0744, BR05/0913, BR05/0914, BR05/1455, RZ05/2682, RZ05/2764, RZ05/2942, RZ05/3173, BR05/0071, BR05/0092, BR05/0408, BR05/0549, BR05/0586, BR05/0701, BR05/1352, RZ05/3226, RZ05/3343, RZ05/3472, RZ05/3524, RZ05/3579, BR05/0731, BR05/0746, RZ05/3361, RZ05/3645, BR05/0303, RZ05/0462, RZ05/3158, RZ05/3541, RZ05/3576, RZ05/3434, BR05/0605, BR05/0707, BR05/1467, BR05/1717, RZ05/2838, BR05/0830, BR05/1433, BR05/1434, BR05/1469, BR05/1494, BR05/1865, BR05/1872, RZ05/2786, RZ05/3106, RZ05/3262, RZ05/3335, RZ05/3452, RZ05/3483, RZ05/3539, BR05/0150, BR05/1149, RZ05/2937, RZ05/3630, BR05/0475, BR05/0561 scored with an average rating scale of 2.6-2.9.

Group 3 (susceptible):
All remaining 118 materials, BR05/1830, BR05/0637, BR05/0642, BR05/0933, BR05/1440, BR05/1609, BR05/0406, BR05/0733, BR05/1449, BR05/0265, BR05/1401, RZ05/2938, RZ05/2985, BR05/1853, RZ05/3101, RZ05/3332, BR05/1879, RZ05/3371, RZ05/3528, RZ05/3608, RZ05/3333, RZ05/3590, BR05/1426, BR05/1462, BR05/1611, RZ05/2932, BR05/0508, BR05/0545, BR05/0671, BR05/0708, BR05/1460, RZ05/2801, RZ05/2847, RZ05/3107, RZ05/3355, RZ05/3574, BR05/0120, BR05/0284, BR05/0351, BR05/1574, BR05/1706, RZ05/3128, RZ05/3312, RZ05/3495, BR05/0117, BR05/0334, BR05/0609, BR05/0931, BR05/1464, RZ05/3244, RZ05/3466, BR05/0702, BR05/1302, RZ05/2802, RZ05/3485, RZ05/2992, BR05/0990, BR05/1520, BR05/1857, RZ05/3365, BR05/0354, BR05/0743, BR05/1173, BR05/1308, BR05/1402, BR05/1447, BR05/1623, RZ05/3589, BR05/1249, BR05/1586, BR05/0577, BR05/0627, BR05/0995, BR05/1344, BR05/1435, BR05/1444, BR05/1475, BR05/1738, RZ05/2831, RZ05/3311, BR05/0563, BR05/1361, BR05/1420, BR05/1479, BR05/1835, BR05/0244, BR05/0267, BR05/0891, BR05/1019, BR05/1429, BR05/1826, BR05/1493, BR05/1702, RZ05/3377, BR05/1376, BR05/0020, BR05/0159, BR05/0293, BR05/1040, BR05/1480, RZ05/3359, BR05/0209, BR05/1490, RZ05/2668, RZ05/2873, RZ05/3253, BR05/1331, BR05/1610, BR05/1883, RZ05/3398, CIAT 36061, RZ05/2641, RZ05/3378, RZ05/3391, RZ05/3367, RZ05/3629, BR05/1059, BR05/1647, RZ05/3585, RZ05/3616 scored between 3.0-5.0.

In Figure 1 we show a graphical representation of the results using data from representative genotypes from each of these groups.

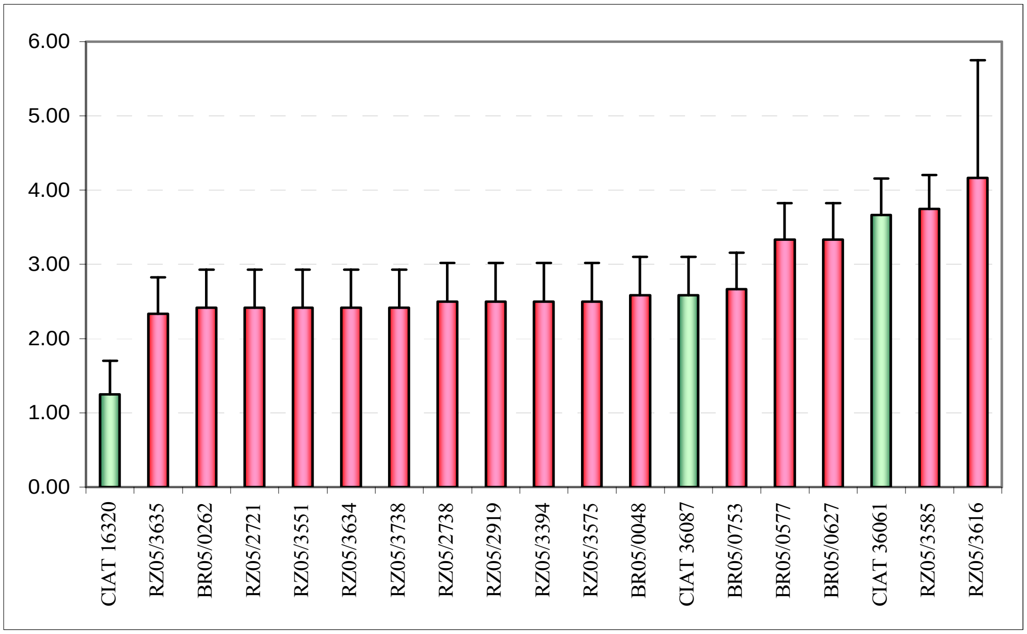

Figure 1. Ratings of *Brachiaria* genotypes for foliar blight disease reaction on a 1-5 scale 65 days after inoculations with sclerotia of *Rhizoctonia solani* under field conditions, Caquetá, Colombia. Bars indicate standard deviation.

2.6 Endophytes in tropical grasses

Highlights

- The transformation and expression of the GFP (green fluorescent protein)-encoding gene in an isolate of an endophytic plant growth promoting bacterium associated with species of *Brachiaria* is described. To the best of our knowledge, this is the first report on transformation of this endophytic bacterium. This is also the first report of a plant growth promoting endophytic bacterium associated with *Brachiaria* that contains nif-gene sequences.

- The introduction of three strains of endophytic bacteria, originally isolated from *Brachiaria* hybrid CIAT 36062, had a positive effect on plant growth and development in the recipient plant *B. brizantha* CIAT 6294. There was more tiller and root development in artificially inoculated plants than control plants. Results suggest a close and beneficial interaction between the introduced endophytic bacteria and *B. brizantha* CIAT 6294, resulting possibly in nitrogen fixation and enhancement of plant growth.

2.6.1 Bacterial endophytes in *Brachiaria*

Contributors: J. Abello, P. Fory and S. Kelemu (CIAT)

Rationale

Bacterial endophytes 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.

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.

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 Reports 2003, 2004]. 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.

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. This work describes the establishment of a transformation protocol and expression of the green fluorescent protein (GFP) gene in an isolate of a bacterial endophyte associated with species of *Brachiaria*. The purpose of this study is to evaluate the use of GFP in host-parasite interactions.

Material and Methods

Bacterial isolate and growth conditions*:* a bacterial isolate designated as CIAT 36062R2 (IP5 Annual Report 2005), isolated from roots of *Brachiaria* hybrid CIAT 36062, was marked for antibiotic resistance (rifampicin, rif^r). This isolate tested positive for *nif*H gene (the gene that encodes nitrogenase reductase) sequences (IP-5 Annual Report 2005; Kelemu *et al*., 2006, Phytopathology 96:S59) Bacterial cells were collected from a single colony and cultured on Luria agar medium containing rifampicin (LB; tryptone 10 g/l, NaCl 5g/l, yeast extract 5 g/l and agar 15 g/l; rifampicin 50 µg/ml) and incubated at 28 ^{o}C for 24 hours in darkness.

Plasmid: Plasmid pGT-Kan was kindly provided by Dr. Steve Lindow of the University of California, Berkeley. pGT-kan was constructed using plasmido pPROBE-GT (Miller *et al*., 2000, Molecular Plant-Microbe Interactions. 13: 1243-1250) as a base and it contains gfp under the promoter *nptII* and confers resistance to Kanamycin as well as gentamycin.

Transformation of the bacterial endophyte CIAT 36062R2: *E. coli* strain DH5á was electrotransformed with the plasmid pGT-kan for maintenance of the plasmid. CIAT 36062R2/rif^r was electrotransformed using a protocol described by Dulk-Ras and Hooykaas (1995, Methods Molecular Biology. 55: 63-72) with some modifications. To prepare competent bacterial cells, the cells were grown in LB medium at 28^{o}C with shaking at 250 rpm for 16 hours till a growth density of OD_{600}= 0.5. The cells were collected after centrifugation at 4,000 rpm, 4^{o}C for 15 minutes. The cells were rinsed three times with 20 ml solution of 10% glycerol and 1mM HEPES (pH: 7.0). They were then resuspended in 3 ml of 10% glycerol, 200 µl aliquots were made and stored at -80°C for subsequent use. Electroporation was conducted using a BIO-RAD® gene pulser at 12,5 Kv/cm, 200 Ω of resistance and 25 µF of capacity. Forty µl of competent cells were mixed with 100ng/µl of plasmid pGT-kan and electric pulse was applied to the mixture. The cells were then transferred to a 1 ml LB medium and incubated for 3 hours at 28°C. One hundred µl of this culture was plated on Luria agar plates containing 50 µg/ml rifampicin and 15 µg/ml of gentamycin for selection of transformants. Putative transformants appeared on the selection plates after 48 hours of incubation.

PCR analysis of bacterial transformants*:* Genomic DNA was isolated from putative transformants using a protocol described by Cheng *et al.* (2006, Biotechnology Letters. 28: 55-59.). Identification of GFPmut1 gene in transformants was conducted using specific primers T14GFP5´ (5´ATTCCCTAACTAATAA-TGATTAACTTTATAAGGAGGAAAAAC 3´) and T1GFP3´ (5´ GATGCCTGGA-ATTAATTCCTATTTGTATAGTTCATCC 3´) (Miller *et al*., 2000, Molecular Plant-Microbe Interactions. 13: 1243-1250). Amplifications were carried out in a Programmable Thermal Controller (MJ Research, Inc) programmed to 30 cycles comprised of I minute denaturation step at 95°C (3 minutes for the first cycle), followed by 2 min at 50°C, and primer extension for 3 minutes (10 minutes in the final cycle) at 72°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.

Plant inoculation: 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 these tillers were immersed in a beaker containing 200 ml of bacterial (transformant 36062R2/gfp) suspensions. 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 and soil in 3:1 proportion and maintained in the greenhouse under natural day light and at temperatures between 19 and 30°C. At 1, 2 3 and 5 months after inoculations, tissue samples were taken and examined under the microscope.

Test for stability of bacterial transformants: Transformant colonies were isolated and plated on Luria agar media without selection antibiotics and subsequently transferred for several cycles on media without selection pressure. These colonies were then examined for expression of GFP.

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. For observations of GFP expressions inside plant tissues, young roots and leaves were sectioned with diameters of approximately 0.5-1.5 mm.

Results and Discussion

Transformation of endophytic bacterium CIAT 36062R2/rif^r*:* Putative transformants appeared on selection plates after 48 hours of incubation. Colonies with a diameter of approximately 1-mm were isolated for analysis. Bacterial cells grown to an optical density (OD_{600}) = 1.0 were examined for green fluorescence. All cells examined demonstrated strong fluorescence indicating successful expression of gfp. Control colonies showed no fluorescence. 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.

PCR analysis of putative transformants*:* The putative bacterial transformants selected on the selection media were further examined using fluorescence microscope, and PCR analysis. DNA isolated from these transformants was examined for gfp sequences using PCR analysis. Transformants that contain gfp gene sequences produced an amplified DNA product of 750 bp-size, confirming successful transformation of endophytic bacterial cells with gfp. Negative controls produced no amplified product. The PCR method allowed us to quickly examine and further confirm putative transformants that have been selected on antibiotic selection media.

Test for stability of transformants: Selected bacterial transformants were cultured sequentially 15 times on media without selection antibiotics. Although stable in expression of gfp, the fluorescence intensity declined after the 9th transfer on media without the selection pressure for some of the transformants. This indicates that the gene of interest was not incorporated with the bacterial genome in some of these colonies that showed a decline in fluorescence intensity when maintained on media without antibiotic selection.

Microscopic examination: Microscopic examinations of selected bacterial transformants demonstrated strong expression of *gfp* as evidenced by the intense fluorescence emission at a range of wavelength (Photos 1and 2). The strongest emission was observed at a 355-425 nm range with Leica D filter. The emission intensity was somewhat lower when a filter Leica H3 was used with a 420-490 nm range.

Root and leaf tissues from *Brachiaria* plants inoculated with endophytic bacterial cells

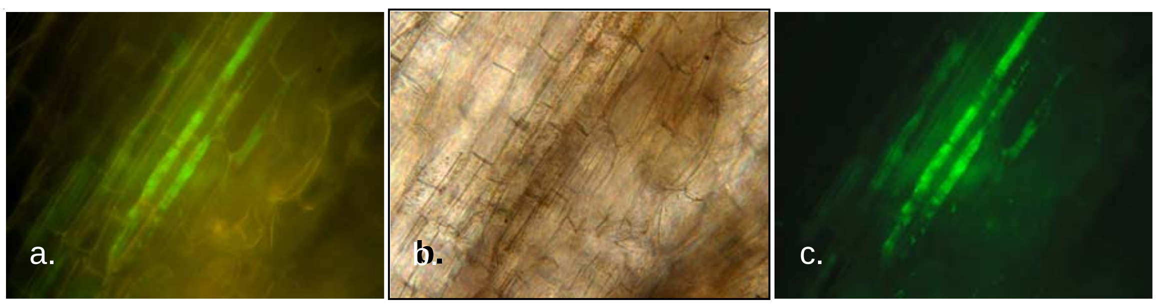

Photo 1. *Brachiaria* tissues from plants inoculated with bacterial endophyte transformed with green fluorescent protein gene *egfp*). a) fluorescence emission under UV light with Leica H3 filter, b) under normal lighting, c) fluorescence emission under UV light with Leica D filter.

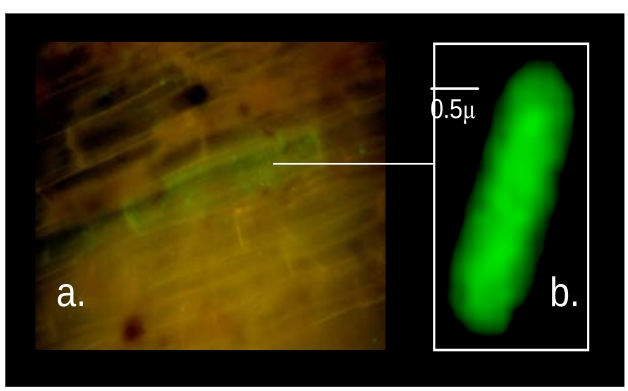

Photo 2. Endophytic bacterial cells transformed with a gfp gene. a) initial colonization of *Brachairia* roots inoculated with transformed bacterial cells one month after inoculation; b) a single bacterial cell (average length of 2,5 ìm). Photographed with Leica D filter.

transformed with gfp were examined under the microscope at 1, 2, 3 and 5 months after inoculations. Bacterial cells were localized in intercellular spaces. No fluorescent bacteria were observed in young leaves during the period of evaluations. It is possible that the transformed bacteria largely remained localized in the root zone within the period of the evaluations.

Although the transformation protocol functioned well for the endophytic bacteria, the recombination of the introduced gene to that of the bacterial genome was not evident, as the transformed bacteria lost their green fluorescence with time. Preliminary data showed that *Brachiaria* tissues taken from plants inoculated with GFP-transformed bacterial endophytes expressed fluorescence emission (Photo 1). This will allow us to study the endophyte-*Brachiaria* interaction, endophyte distribution within the plant tissue, and the correlation of endophytic bacterial colonization with *Brachiaria* plant growth and other related benefits.

Although various transformation systems have been developed and reported for many microbes, successful application of the technology is still not routine in many species. Furthermore, developing an efficient transformation system for a previously untransformed microbe can be a technical obstacle. This work describes the transformation and expression of the GFP (green fluorescent protein)-encoding gene in an isolate of an endophytic plant growth promoting bacterium associated with species of *Brachiaria*. To the best of our knowledge, this is the first report on transformation of this endophytic bacterium. This is also the first report of a plant growth promoting endophytic bacterium associated with *Brachiaria* that contains nif-gene sequences.

2.6.2 Endophytic plant growth promoting bacteria associated with *Brachiaria*

Contributors: P. Fory, X. Bonilla, 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.

In 2005 Annual Report, we demonstrated the effect of endophytic bacteria on the growth of *Brachiaria* hybrid CIAT 36061 (cv. Mulato). *Brachiaria* hybrid CIAT 36061 had 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. More tiller and root development were observed in artificially inoculated CIAT 36061 plants than plants containing only indigenous endophytic bacteria.

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 (IP-5 Annual Report 2005).

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 (IP-5 Annual Report 2005). The data presented indicate that a close and beneficial interaction existed between the introduced as well as indigenous endophytic bacteria and *Brachiaria* hybrid CIAT 36061, resulting possibly in nitrogen fixation and enhancement of plant growth.

In this study, we artificially introduced strains of endophytic bacteria into *Brachiaria brizantha* CIAT 6294 cultivar Marandu and examined the effect on plant growth.

Materials and Methods

Plant materials: Twelve *Brachiaria brizantha* CIAT 6294 (cv. Marandu) that are approximately one month old were used for inoculation. These plants were selected after examining with nested PCR, and showed no amplified products for sequences of *nif*H (the gene that encodes nitrogenase reductase) gene, indicating the absence of endophytic bacteria containing these sequences.

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: Twelve tillers of *Brachiaria brizantha* CIAT 6294 that are about a month old were prepared, their roots washed with sterile distilled water and made ready for inoculations. The roots of six 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 six 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 days after inoculations of *B. brizantha* CIAT 6294, the following measurements were taken in control and treated plants: 1) plant growth and development based on plant height, number of tillers, number of leaves and leaf area, 2) leaf chlorophyll content 3) leaf and stem nitrogen content, and 4) soluble protein content in leaves. 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 cm^2/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 samples was taken from each individual plant *Brachiaria brizantha* CIAT 6294, 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 6 plants (6 repetitions) 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

B. brizantha CIAT 6294 had no indigenous endophytic bacteria that have *nif*H gene sequences. We introduced three strains of bacteria, originally isolated from *Brachiaria* hybrid CIAT 36062, into CIAT 6294. In general, the introduction of these bacteria had a positive effect on plant growth and development in the recipient plant CIAT 6294. There was more tiller and root development in artificially inoculated CIAT 6294 plants than control plants.

Analysis of variance showed that the total biomass production (leaf, stem and root) collected from control *Brachiaria* CIAT 6294 plants was significantly ($P< 0.05$) less than that from inoculated ones (Figure 2). The data presented indicate that a close and beneficial interaction existed between the introduced bacteria and *B. brizantha* CIAT 6294, resulting possibly in nitrogen fixation and enhancement of plant growth. These results are consistent with the results reported in IP-5 Annual Report 2005 with *Brachiaria* hybrid CIAT 36061 (cv. Mulato).

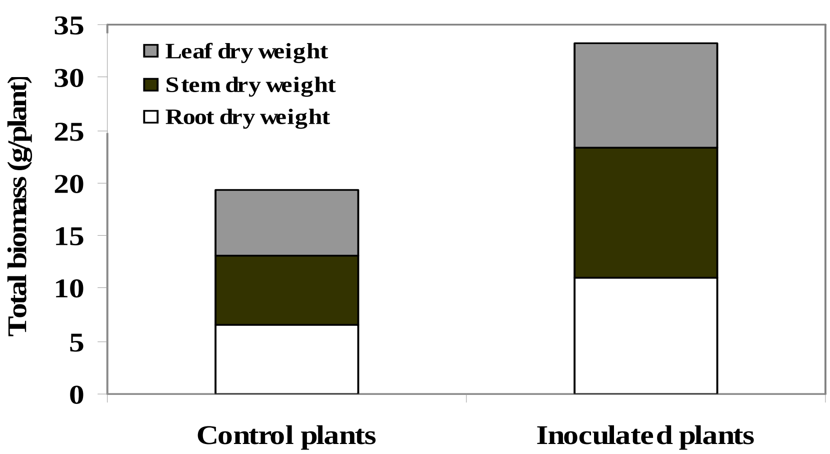

Figure 2. Total tissue biomass production in *Brachiaria brizantha* CIAT 6294 control plants, and inoculated with a mixture of 3 bacterial strains 01-36062-R2, 02-36062-H4, and 03-36062-V2 (originally isolated from *Brachiaria* CIAT 36062), 60 days after inoculations and maintained under greenhouse conditions with no nutrients. Values are average of 6 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, plant height, number of tillers, number of leaves, and leaf area than those control plants (Figure 3).

Analysis of variance showed that the chlorophyll content (SPAD units) collected from control *Brachiaria* CIAT 6294 plants (43.4 SPAD units) was significantly ($P< 0.05$) less than that from inoculated ones (50.34 SPAD units).

These data strongly suggest that endophytic bacteria have a direct beneficial effect on plant growth and development, and possibly on associated nitrogen fixation in *Brachiaria*. The possibility that this plant growth is through associated nitrogen fixation is further corroborated by the endophytic bacteria sequence data described in the report in section 2.6.3.

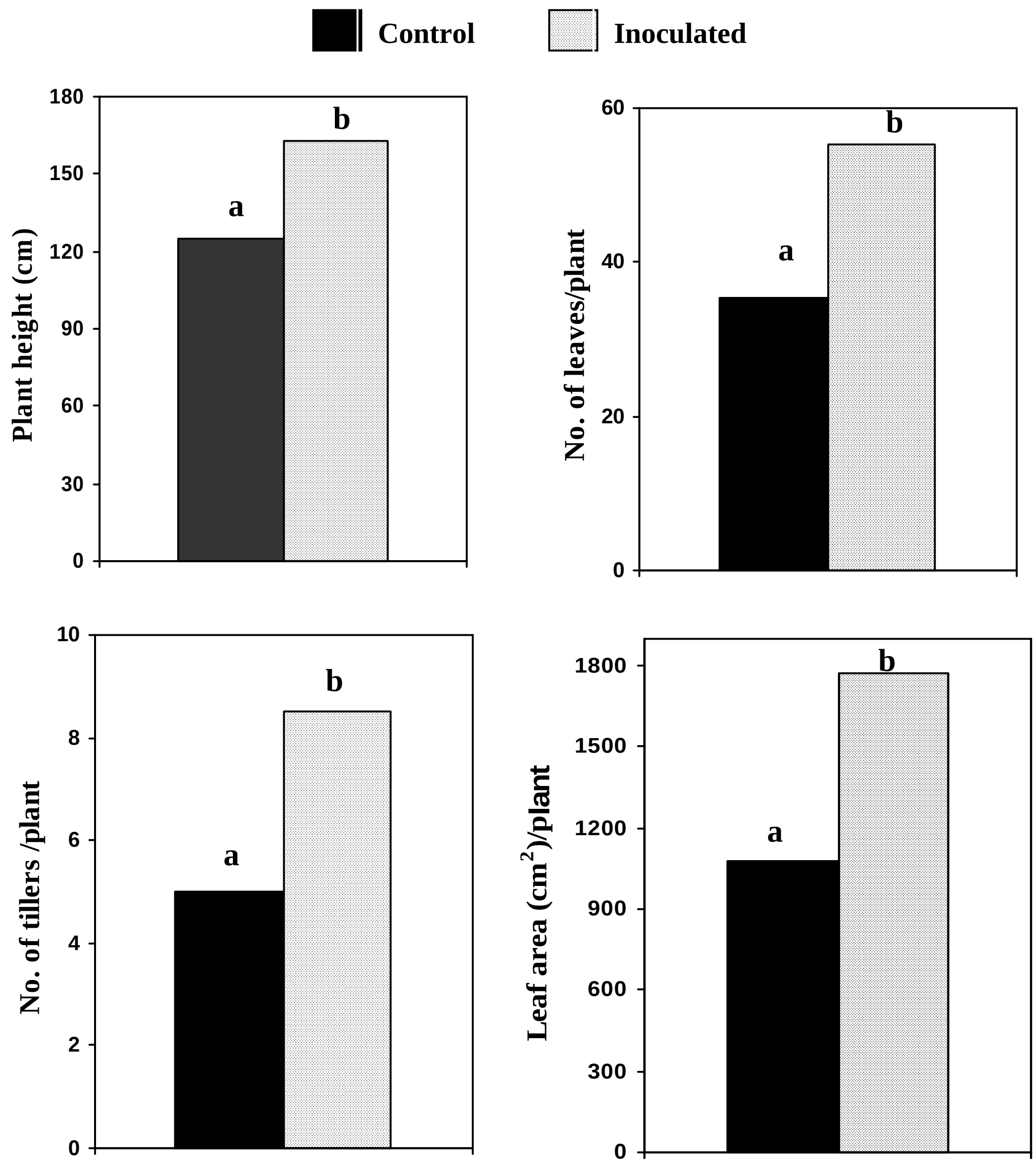

Figure 3. Effect of bacterial isolates (a mixture of 3 bacterial strains 01-36062-R2, 02-36062-H4, and 03-36062-V2 (originally isolated from *Brachiaria* CIAT 36062) on the growth of *Brachiaria brizantha* CIAT 6294 at 60 days after inoculations. Plants were grown under greenhouse conditions with no nutrients. Values are average of 6 plants per treatment.

2.6.3 Characterization and comparison of partial sequence of *nif*H gene in four strains of endophytic bacteria associated with *Brachiaria* genotypes

Contributors: P. Fory and S. Kelemu (CIAT)

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 nitrogen fixing organisms.

We have reported the isolation of three strains of bacteria from *Brachiaria* hybrid CIAT 36062 (BR97-1371) 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 previously (IP-5 Annual Report 2004) 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*). We reported (IP-5 Annual Report 2005) the cloning and sequencing of a 371 bp nested PCR amplified product (with *nif*H gene specific primers) isolated from an endophytic bacterium strain 01-36062-R2 associated with *Brachiaria* hybrid CIAT 36062. Using this sequence data, specific primers were designed and synthesized in order to develop a simple diagnostic tool that enables us to do one step PCR analysis and avoiding nested PCR methods, that will eventually allow us to detect, evaluate and select *Brachiaria* genotypes that harbor *nif*H gene containing endophytic bacteria (IP-5 Annual Report 2005). In this study, we continued to clone and analyze the sequences of *nif*H gene in other native strains of endophytic bacteria associated with *Brachiaria* genotypes.

Materials and Methods

Bacterial isolates: For cloning and sequence analysis, endophytic bacterial strains isolated from roots, leaves and stems of Brachiaria hybrids CIAT 36062 (designated 01-36062 -R2, 02-36062 –H4, 03-36062 –V2) and a strain isolated from Brachiaria hybrid CIAT 3061 were used for this study.

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 2 mg/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.

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 M 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.

Cloning and digestion 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 (size 320-322 bp) were ligated to the cloning vector PCR ® 2.1 (Invitrogen, Carlsbad, CA, USA) [Figure 4] 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)

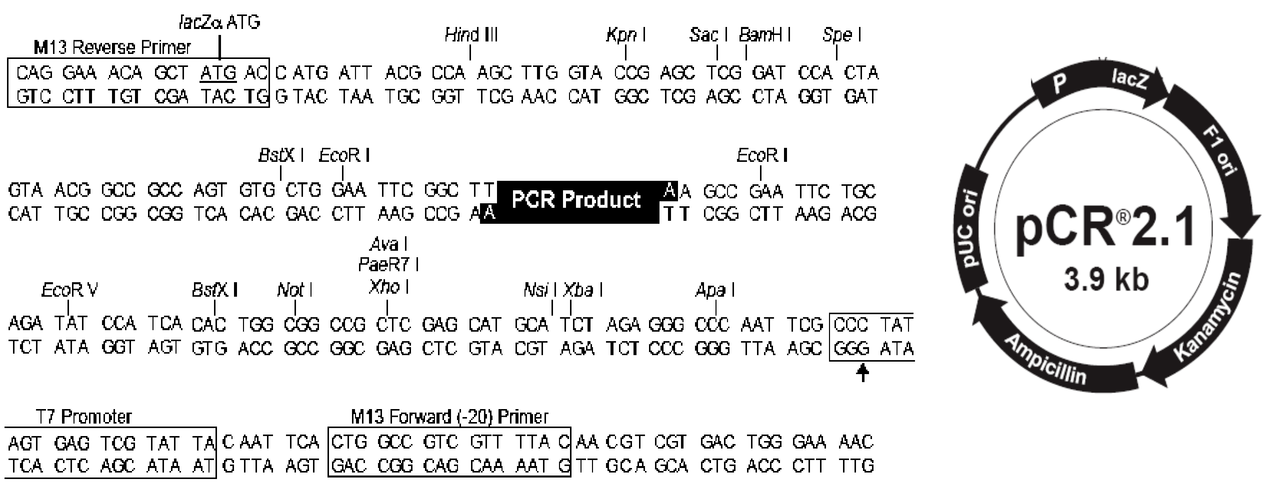

Figure 4. Cloning vector PCR ® 2.1. The vector has genes for resistance to the antibiotics ampicillin (Amp^r), kanamycin (Km^r), and *lac*Z.

Transformed colonies of *E. coli* DH5á were selected on Luria agar supplemented with n 1-mM of Isopropyl â-D-1-thiogalactopyranoside (IPTG) and 40-µg of X-Gal. 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, the plasmid DNA was digested to completion with the restriction enzyme *Eco*RI (Gibco/BRL). The digested products were separated by electrophoresis on a 1% agarose gel (Bio-Rad, NJ), stained with ethidium bromide and photographed under UV light. 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.

Phylogenetic anaysis: Phylogenetic analysis of the nucleotide sequences was conducted using *Neighbor-Joining* (NJ) method applying the parameters described in the program MEGA 3.1 (Kumar *et al*., 2004, Brief Bioinform 5:150-163). *Bootstrap resampling test* with 1.000 replications was applied.

Results and Discussion

Nucleotide similarity comparison: The sequences corresponding to *nif*H gene sequence were edited, cleaned and assembled using the program Secuencher v 3.0 (Sequencher 3.0 User Manual, 1995). The fragments that showed homology were aligned using the program Clustal version W (1.8). The sequences that correspond to strains designated as 01-36062-R2; 02-36062-H4 and 03-36062-V2, isolated from roots, leaves and stems, respectively, were identical to each other. The sequence analysis demonstrated the presence of *nif*H gene sequences in these sequenced clones, with a similarity of 89% in 283 bp with the *nif*H gene sequence of *Klebsiella pneumoniae* with a GenBank as AF303353.1. Further more the sequences had an 88% similarity with *Klebsiella* sp. Y83 (DQ821727.1) and *Enterobacter* spp. (Y79DQ821726.1) The clone from the endophytic bacterium isolated from *Brachiaria* hybrid CIAT 36061 had a 97% sequence similarity in 290 bp with three accessions registered in the GenBank, designated as DQ982313.1, DQ982300, and DQ982299.1. These sequences correspond to clones isolated from uncultured diazotrophes (nitrogen fixing organisms) isolated from roots and stems of maize plants. Nif genes that encode the nitrogenase complex and other enzymes involved in nitrogen fixation have consensus sequences identical in various nitrogen-fixing bacteria

Sequence comparison: The strains 01-36062-R2; 02-36062-H4, 03-36062-V2, and 36061 were compared with 16 nucleotide sequences that were selected with a maximum identity, Score and E-value, registered in the *GenBank* (Table 16). Furthermore, 18 nucleotide sequences of nitrogen-fixing organisms used in the studies by Franke *et al*., (1998, Lett. Appl. Microbiol. 26:12-16). Reiter *et al*., (2003, Can J. Microbiol. 49: 549-55). Bacteria with other characteristics were also included in these comparisons. Figure 5 clearly shows the sequences analyzed are phylogenetically grouped in three groups, A, B, and C, with high bootstrap values of 80, 82, 100 %, respectively. Group A contained 20 accessions, 8 of these belonging to protobacteria, one actinobacteria, one unidentified bacterium, 6 clones from uncultured organisms, and the four endophytic bacterial strains used in this study.

The three sequences from the endophytic bacteria isolated from *Brachiaria* CIAT 36062, designated 01-36062-R2; 02-36062-H4, and 03-36062-V2, are closely related to *Klebsiella, Enterobacter and Micrococcus*. Microorganisms in these three genera are known to be nitrogen-fixers. These results are in agreement with biochemical analysis (fatty acid analysis) of the isolate 01-36062-R2 conducted in earlier studies that showed a match with *Leclercia adecarboxylata*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*, at 0.879, 0.841, and 0.820 similarity index, respectively (IP-5 Annual Reports 2003/4). The sequence of the endophytic bacterial strain isolated from *Brachiaria* hybrid CIAT 36061 grouped 100% with three clones coded as DQ982313.1, DQ982300, and DQ982299.1, and 87 % with the rest in Group A (Figure 5).

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).

Group B consists of 15 accessions subdivided into three subgroups B-1, B-2, B-3. The sub-group B-1 contains 2 species of *Frankia* (a soil-inhabiting nitrogen-fixing bacterium) and one betaproto-bacteria. The subgroup B-2 consists of three accessions that belong to the genus *Azotobacter*,. Sub-group B-3 consists of 9 accessions. The group C consists of 2 species of the genus *Clostridium.* For a better understanding of the endophytic microbial diversity and identity associated with species of *Brachiaria*, it is important to extend the work to include more enophytic bacterial isolates from a number of *Brachiaria* hybrids. This work further complements the results reported in IP-5 Annual Report 2005 on the role of these bacterial endophytes in *Brachiaria* plant growth possibly through nitrogen-fixation.

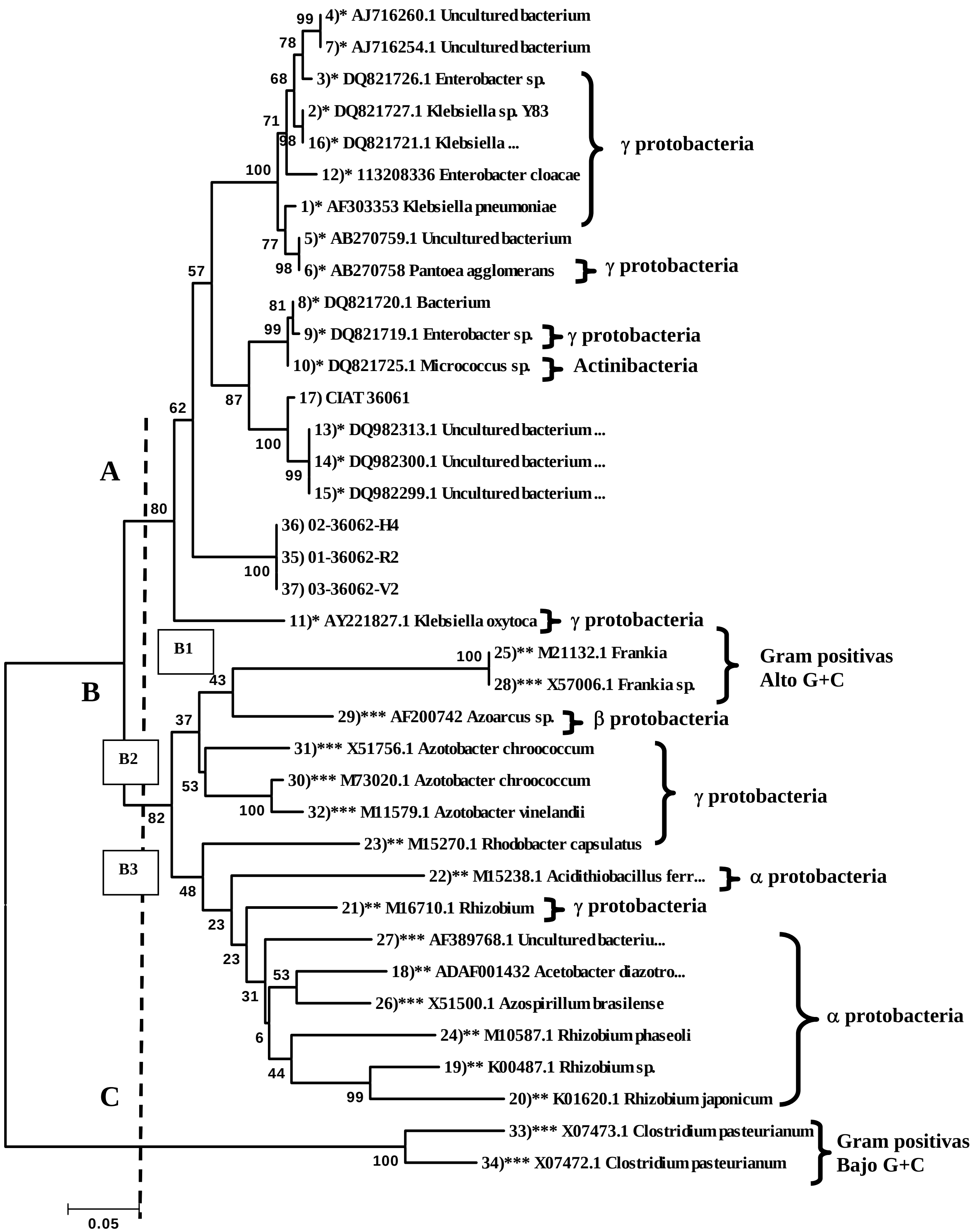

Figure 5. Phylogenetic tree generated for nucleotide sequences evaluated using Neighbor Joining analysis. The values represent 1,000 replications in bootstrap method.

Table 16. Nucleotide sequences and values generated using *GenBank* data for four endophytic bacterial isolates associated with Brachiaria hybrids in relation to other microbes.

No	Accession	Description	bp	Organism	Score	E value	Max Identity (%)
1	DQ982300.1*	uncultured bacterium	322	Bacteria; environmental samples	531	$5e^{-148}$	97
2	DQ982313*	uncultured bacterium	518	Bacteria; environmental samples	531	$5e^{-148}$	97
3	DQ982299.1*	uncultured bacterium	322	Bacteria; environmental samples	531	$5e^{-148}$	97
4	DQ821721*	*Klebsiella pneumoniae*	322	Bacteria; Proteobacteria; Gammaproteobacteria Enterobacteriales; Enterobacteriaceae; *Klebsiella*	385	$7e^{-104}$	90
5	AF303353	**Klebsiella pneumoniae**	518	Bacteria; Proteobacteria; Gammaproteobacteria Enterobacteriales; Enterobacteriaceae; *Klebsiella*	365	$7e^{-98}$	89
6	DQ821727	*Klebsiella* sp. Y83	322	Bacteria; Proteobacteria; Gammaproteobacteria Enterobacteriales; Enterobacteriaceae; *Klebsiella*	377	$1e^{-89}$	88
7	DQ821726	*Enterobacter* sp. Y79	322	Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Enterobacter	377	$1e^{-89}$	88
8	AJ716260	uncultured bacterium	360	Bacteria; environmental samples	325	$6e^{-86}$	87
9	AB270759.1	uncultured bacterium	360	Bacteria; environmental samples	321	$9e^{-85}$	88
10	AB270758	**Pantoea agglomerans**	360	Bacteria; Proteobacteria; Gammaproteobacteria Enterobacteriales; Enterobacteriaceae; Patoea	321	$9e^{-85}$	88
11	AJ716254	uncultured bacterium	360	Bacteria; environmental samples	317	$1e^{-83}$	87
12	DQ821720	Bacterium Y41	322	Bacterium Y41	313	$2e^{-82}$	87
13	DQ821719.1	*Enterobacter* sp. Y11	322	Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Enterobacter	305	$5e^{-80}$	87
14	DQ821725.1	*Micrococcus* sp. Y70	322	Bacteria; Actinobacteria;Actinobacteridae;Actinomycetales Micrococcineae; Micrococcaceae; Micrococcus.	297	$1e^{-77}$	87
15	AY221827.1	*Klebsiella oxytoca*	327	Bacteria; Proteobacteria; Gammaproteobacteria Enterobacteriales; Enterobacteriaceae; *Klebsiella*	293	$2e^{-76}$	86
16	AB270754	*Enterobacter cloacae*	359	Bacteria; Proteobacteria; Gammaproteobacteria;	281	$8e^{-73}$	86

Note: The first 4 accessions noted with * correspond to the endophytic bacterial strain isolated from *Brachiaria* hybrid CIAT 36061.

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

3.1 Genotypes of *Brachiaria* with adaptation to edaphic factors

Highlights

- Showed that the high level of Al resistance of signalgrass (*B. decumbens*) does not appear to be related to organic-acid secretion at root apices (a widespread Al-exclusion mechanism in plants). Instead, Al resistance appears to be a facet of a more generic resistance mechanism that prevents intoxication by inorganic cations, possibly as a result of the change in composition of the lipid bilayer of root cell plasma membranes.

- Showed that in signalgrass and also in ruzigrass grown under P deficiency decreases in internal plant P concentrations could be related to exudation of both oxalic acid and acid phosphatases. But the two grasses differed with regard to the magnitude of the internal leaf P concentration needed for the induction of these root-mediated rhizospheric mechanisms for P acquisition.

- Selected 9 hybrids (BR05NO/0406, BR05NO/0563, BR05NO/0334, BR05NO/0830, BR05NO/1173, BR05NO/0671, BR05NO/0120, BR05NO/0048, and BR05NO/0537) that were superior to *B. decumbens* parent in terms of aluminum resistance. Three of the 9 Al resistant hybrids (BR05NO/0334, BR05NO/0537, BR05NO/0563) also combined resistance to four spittlebug species and exhibited apomictic mode of reproduction.

- Showed that among the 15 *Brachiaria* hybrids tested under acid soil conditions in the Llanos of Colombia, BR02NO/0465 and BR02NO/1728 were more productive than the other BR02NO hybrids in terms of green forage (leaf + stem) yield after establishment with low initial fertilizer application and no maintenance fertilizer application. But neither of these hybrids was superior to cv. Mulato II.

3.1.1 Edaphic adaptation of *Brachiaria*

3.1.1.1 Advances in defining physiological mechanisms of aluminum resistance and developing screening methods to identify aluminum resistant genotypes

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Perennial brachiaria grasses (*Brachiaria* spp. Griseb.) are the most widely sown forage grasses in tropical America. CIAT and EMBRAPA are developing interspecific hybrids to combine traits of three parental species: acid-soil adaptation of signalgrass (*B. decumbens*) and spittlebug resistance of palisadegrass [*B. brizantha* (A. Rich) Staff], both tetraploid apomicts, and sexual reproduction of a tetraploidized, sexual biotype of ruzigrass (*B. ruziziensis* Germain &Evard), 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 selection and recombination. Edaphic adaptation is particularly difficult to select for because it is only manifest in the persistence of pastures over several growing seasons. The following is the summary of research accomplishments for the past few years towards the development of tools and knowledge that aid the genetic improvement acid soil tolerance in brachiariagrasses.

Aluminum toxicity and other acid-soil constraints

In the past, edaphic adaptation of brachiaria grasses was exclusively evaluated by quantifying forage yield and pasture persistence in field trials. These trials have resulted in the release of several well-adapted cultivars such as cv. Basilisk (signalgrass), Tully, and Llanero. Leaf area, biomass and N content as well as the partitioning of N and P to leaves were found to be useful predictors of adaptation and persistence on infertile, acid soils. Adapted genotypes typically had root and shoot attributes that facilitated acquisition and/or efficient use of key nutrients (N, P, and Ca) in a low-pH, high-Al soil. These attributes include: (i) maintenance of root growth at the expense of shoot growth; (ii) an extensive root system and association with arbuscular mycorrhizae; (iii) a highly branched root system with many apices facilitating Ca uptake (e.g., *B. ruziziensis*); (iv) the ability to acquire and utilize both NO_3^- and NH_4^+ (e.g., *B. humidicola*); and (v) the ability to acquire N through associative fixation (e.g., signalgrass). It was not clear whether there was genetic variation among brachiaria grass genotypes for Al resistance *per se*.

In an attempt to disentangle the various stress factors affecting growth of brachiaria grasses on acid soils, we designed a nutrient solution that simulated the ionic composition of soil solutions extracted from two Oxisols collected in the Colombian savannas. Relative growth of seedlings in this solution (compared to unstressed conditions) ranked the three parental genotypes of the *Brachiaria* breeding program the same way they had been ranked in field trials based on pasture persistence over several growing seasons. A comparison among several growth conditions suggested that Al sensitivity of less-adapted ruzigrass increased disproportionately under low nutrient supply. This conclusion was further verified by measuring root elongation of seedlings in solutions containing only Ca^{2+}, Al^{3+}, H^+ and Cl^- ions. These assays showed that well-adapted signalgrass could tolerate an approximately fivefold higher level of Al than poorly-adapted ruzigrass, even though the resistance level of ruzigrass was comparable to wheat, triticale and maize genotypes previously classified as Al-resistant.

Physiological mechanisms of Al resistance

Aluminum exclusion: The outstandingly high level of Al resistance identified in signalgrass has triggered a series of experiments to investigate its physiological foundations. The close relationship between Al accumulation in root apices and inhibition of root growth could suggest that exclusion mechanisms might contribute to Al resistance. However, secretion of organic acids and phosphate at root apices, a widespread mechanism of Al exclusion in plants, was clearly not the main Al exclusion/resistance mechanism in signalgrass. First, apices of signalgrass secreted quantities of organic acids only moderately larger than those of ruzigrass. Second, organic acid and phosphate efflux rates at signalgrass apices were 3.4 to 30fold lower than those of Al-resistant genotypes of buckwheat, maize and wheat, which are several-fold more sensitive to Al than signalgrass. These results suggest that hitherto uncharacterized mechanism(s) are responsible for exclusion of Al from root apices of signalgrass.

A separate line of evidence suggests that Al-resistance of signalgrass may only be a facet of a more generic resistance mechanism to inorganic cations. We found that the difference in Al resistance between signalgrass and ruzigrass coincided with a similar difference in resistance to a range of trivalent lanthanide cations and some divalent cations. It seems plausible to assume that resistance to a range of different toxicants can be more easily achieved through an exclusion mechanism than through an internal, detoxification mechanism; different toxicants are likely to have different intracellular targets and may thus require different internal modes of action.

It may be no coincidence that recent experiments at Yamagata University are pointing towards a possible role of root plasma membrane (PM) negativity and/or PM composition in Al resistance of signalgrass. When examining signalgrass, ruzigrass and 16 different cultivars belonging to eight other plant species, Al resistance was negatively correlated with the degree to which protoplasts isolated from root apices could be stained with methylene blue (MB), either externally or internally. (External staining was assumed to reflect differences in cell surface negativity and internal staining was interpreted as reflecting PM permeability.) Signalgrass was the most Al-resistant and least MB-stainable plant, and a short-term Al treatment seemed to permeabilize the PM of root apices of signalgrass less than those of ruzigrass. Fluorescence microscopy suggested that signalgrass apices contained elevated amounts of flavonoids. Because incorporation of the flavonoid catechin into artificial lipid bilayers decreased their Al permeability, we speculate that the PM of root apices of signalgrass contains flavonoids or other compounds that may modulate the physical characteristics of the lipid bilayer such that it becomes less permeable to Al.

Internal Al detoxification: Both Al-resistant signalgrass and less resistant ruzigrass accumulated high concentrations of Al in roots. Approximately two thirds of the total Al was complexed by soluble low-molecular-weight ligands, suggesting that it had been taken up into the symplasm. This conclusion was recently confirmed by a ^{27}Al NMR analysis of the *Brachiaria* hybrid cv. Mulato, which showed that Al in the root symplasm was present as a complex with ligand(s). Possible candidates for such ligands include citric acid, malic acid, *trans*-aconitic acid, oxalic acid and 1, 3-di-*O*-*trans*-feruloylquinic acid, a chlorogenic-acid analogue previously isolated from brachiariagrass roots. These ligands may constitute a sink for Al ions in mature roots because very little Al was translocated to shoots.

Root apices accumulated significantly larger amounts of citric, malic, *trans*-aconitic and oxalic acid than mature root sections, apparently by specifically retaining (rather than secreting) a greater proportion of the synthesized acids within the tissue. The accumulation occurred in dose-dependent manner as the Al content of apices increased under Al-toxic growth conditions. Although these data suggest a role of organic acids in internal detoxification of Al in brachiaria grass apices (the most Al-sensitive site), they do not fully account for the superior resistance level of signalgrass.

Interactions between Al toxicity and P deficiency: Aluminum toxicity and P deficiency tend to occur in parallel in infertile acid soils, largely because Al forms insoluble precipitates with phosphate. Chemical interactions between Al and P within plant tissues are commonly considered an important secondary effect of Al toxicity. In brachiaria grasses, Al had no effect on P concentrations in root apices of Al-resistant signalgrass but led to a severe decline in apices of ruzigrass, thus suggesting an Al-induced inhibition of acropetal P transport in roots.

Immobilization of P by Al within plant tissues could be prevented through compartmentalization of Al in vacuoles (roots, shoots) or inhibition of root-to-shoot translocation of Al (shoots; see previous section). Alternatively, a range of metabolic adjustments and accelerated P recycling could increase the efficiency with which P is taken up and/or used in Al-stressed plants. A recent study showed that, when grown on a low-P soil, the *Brachiaria* hybrid cv. Mulato had a higher P use efficiency (PUE: biomass produced per amount of P taken up) than wheat or rice. In contrast to wheat and rice, PUE of the *Brachiaria* hybrid increased under more severe P deficiency and soil acidity. The *Brachiaria* hybrid synthesized larger amounts of organic acids such as oxalate and fumarate in leaves. Phosphorus deficiency further increased organic acid accumulation, presumably as a consequence of a twofold increase in leaf phospho*enol*pyruvate carboxylase (PEPC) activity and a decreased the malate inhibition ratio of the enzyme. This PEPC stimulation was even more pronounced in *Brachiaria* roots, where it could play a role in the synthesis of organic acids exuded by roots to aid P acquisition from poorly soluble sources such as Al phosphate. Phosphorus deficiency also induced phosphohydrolases in the *Brachiaria* hybrid and enhanced the partitioning of photosynthate into amino acids and organic acids at the expense of carbohydrates. These metabolic adjustments appeared to generate a larger pool of inorganic P than in other species such as rice, which apparently stimulated P turnover and enabled the hybrid to use P more efficiently.

A solution-culture technique for simultaneously evaluating Al resistance and root vigor

The selection scheme of the *Brachiaria* breeding program at CIAT is based on the simultaneous assessment of a set of genotypes for a variety of traits including edaphic adaptation, insect and disease resistance, nutritional quality and seed production. All phenotypic assays, therefore, need to be based on vegetative propagules (rooted stem cuttings) rather than seedlings. We thus converted the seedling-based Al-resistance assay into a format that was suitable for the adventitious roots of stem cuttings, by increasing the concentration of Al (200 ìM $AlCl_3$, 200 ìM $CaCl_2$, pH 4.2) and simultaneously quantifying the intrinsic root vigor of each genotype in a solution containing only 200 ìM $CaCl_2$ (pH 4.2). The large differences in root vigor among the parental genotypes made it necessary to adjust total root length in the Al-containing solution for total root length in the absence of Al (or any other nutrients except Ca). Importantly, adjusted root-length values (RL_{ad}) ranked parental genotypes the same way they had been ranked in field trials and the seedling-based Al-resistance assay. RL_{ad} values differed quantitatively in a set of ruzigrass × signalgrass hybrids, consistent with multiple genes contributing to Al resistance of adventitious roots.

The concurrent assessment of root length in the Al-free solution revealed a large amount of genetic variation for root vigor in the absence of nutrients: the root system of the best (transgressive) segregant was more than eight times longer than that of poorly-adapted ruzigrass. Vigorous root growth should improve a plant’s nutrient-foraging ability (particularly for immobile nutrients such as P) and was previously identified as associated with pasture persistence. Root vigor, therefore, emerged as another selection target in the context of edaphic adaptation and was easily incorporated into the breeding program through our solution culture technique.

Implementation of a simplified version of this screening method, which allows simultaneous assessment of Al resistance and root vigor based on visual inspection, has facilitated breeding progress toward edaphic adaptation during the last five years. We have identified several well-adapted *Brachiaria* hybrids that had been pre-selected for insect (spittlebug) resistance. In 2002, we identified two sexual hybrids (SX01NO/3178) and SX01NO/7249) and one apomictic hybrid (BR99NO/4132) with Al resistance/root vigor better than that of the sexual parent (ruzigrass; BRUZ/44-02). In 2003, we identified two hybrids (BR02NO/1372 and BR02NO/1621) with better edaphic adaptation than that of most hybrids generated in the *Brachiaria* breeding program until then. In 2004, we evaluated a sexual population of 745 hybrids along with 14 reference genotypes. The improvement of Al resistance and root vigor of the sexual hybrids (SX03NO/0846, SX03NO/2367, SX03NO/0881) was very marked when compared with the sexual population of 2001. In 2005, we screened 139 apomictic/sexual hybrids and identified nine (BR04NO/1018, BR04NO/1552, BR04NO/1900, BR04NO/2110, BR04NO/2128, BR04NO/2166, BR04NO/2179, BR04NO/2201 and BR04NO/2681) that were superior to the well-adapted signalgrass parent, CIAT 606. Among these 9 hybrids, BR04NO/1018 also produced sufficient seed under field conditions. 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.

We use a multidisciplinary approach to cross-validate and integrate information from breeding, agronomy, physiology, soil science, plant nutrition and molecular genetics. Physiological and molecular studies of *Brachiaria* hybrids provide a path towards the identification of physiological mechanisms and genomic regions contributing to Al resistance, which in turn could lead to the isolation of genes contributing to Al resistance. The socioeconomic impact of improved acid soil adaptation of *Brachiaria* hybrids would be immense in terms of increased food production, more efficient use of purchased inputs, and improved integration of crop-livestock systems.

3.1.1.2 Adaptation of *Brachiaria* grasses to low-P soils: Role of exudation of P-mobilizing organic acids and enzymes in the rhizosphere on P acquisition

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Rationale

The elucidation of P efficiency mechanisms will be particularly helpful in the *Brachiaria* breeding program as it will contribute towards the design of appropriate selection criteria to permit the screening of genetic recombinants. Genotypic differences in P acquisition efficiency are related to adaptive changes in root morphology, biochemistry and physiology. In fact, the acquisition of fertilizer P on acid soils has been associated with an extensive root system in brachiaria grasses that permits exploration of a greater soil volume. Root development is remarkably sensitive to variations in the supply and distribution of P in the soils and roots respond in many ways to altering P availability in the rhizosphere. Root exudates affect P availability in the rhizosphere through various mechanisms that include (1) changing conditions in the soil solution (for example pH), thus promoting the dissolution of sparingly soluble P-containing minerals, (2) altering surface characteristics of soil particles, (3) competing with phosphate ions for adsorption sites, (4) complexing cations which are bound to phosphate, and (5) enzymatic hydrolysis of organic forms of P. The importance of each of these mechanisms will differ as rhizosphere conditions are highly variable, and depend on a wide range of factors, including soil type, plant species, soil microbial species and associated biological activities. It is widely accepted that organic acids cause a significant mobilization of P in the rhizosphere. However, there is little *in vivo* evidence to support this hypothesis apart from the observation that most plants experiencing P deficiency typically release more organic acids from their roots in comparison with P sufficient plants. In fact, organic acid anion exudation is considered to be a major mechanism of Al tolerance and P efficiency in certain plant species. The enhanced exudation of tri- and dicarboxylates, specifically citrate, malate and oxalate is considered to be a plant P deficiency response and these anions are also the major carboxylates released from Al-stressed plant roots.

For P to be available to plants, it should be present as orthophosphate in soil solution. Therefore, the exudation of acid phosphatases (APases) involved in the hydrolysis of organic P, is another important way for plants to enhance P availability in the rhizosphere, particularly as a large proportion of soil P (up to 80%) occurs in organic forms. The induction of APases is considered to be a universal response of vascular plants to phosphate starvation. Correlations between the intracellular and/or extracellular APase activity and cellular phosphate status have been shown to exist in plants.

The objective of this study as part of an output from a special project funded by ZIL-SDC, Switzerland was to investigate a possible link between the P nutritional status and physiological markers for P stress in *B. decumbens* (signalgrass - well adapted to P deficient acid soils) and *B. ruziziensis* (ruzigrass - less adapted to P deficient acid soils). In terms of root function, we examined the contribution of both organic acid exudation and acid phosphatase secretion during the development of P deficiency in plant tissue. In addition to these biochemical markers, we also evaluated changes in growth (biomass and root length production) as well as root biomass allocation which might be considered as morphological markers for P stress.

Materials and Methods

Growth of plants: Seedlings of *Brachiaria decumbens* cv. Basilisk (CIAT 606) and *Brachiaria ruziziensis* cv. Kennedy (CIAT 654) were pre-grown in minimally fertilized quartz sand in climate controlled growth chambers. Thereafter, selected seedlings were grown under the same controlled conditions, but under hydroponic system. The composition of the nutrient solution was based on previous literature reports for hydroponically-grown brachiariagrasses. In terms of phosphorus nutrition, we implemented the hydroxyapatite/dialysis pouch system which has been shown to permit the controlled release of phosphate from an apatite source as a function of pH. Previous experiments have shown the suitability of the system for the growth of the two *Brachiaria* species as biomass differences existed between species under different levels of available phosphate. The current experiment was performed under conditions were one gram of hydroxyapatite was added per dialysis bag in a total volume of 30 litres. The pH of the nutrient solutions was monitored to maintain a pH of 5.5.

Plant harvests: Three harvests were performed on days 7, 14 and 21. For each harvest, 10 bunches (3 plants/bunch) of each species were collected from 6 randomly assigned hydroponic tanks. Before the destructive harvests in which plants were separated into leaves, stems and roots, root exudates were collected for 6 h in exudation traps, using 0.1 mM $CaCl_2$ solution (containing 0.01% protease inhibitor cocktail; Sigma P2714) at pH 5.5. The exudation solution was analyzed for both organic acid content and acid phosphatase activity. At each harvest, the fresh mass was recorded for the leaves, stems and roots and the roots were analyzed for root length (RL) using WinRhizo root imaging software. Plant material was dried for 4 days at 45 °C, weighed and analyzed for the nutrient content. Data are expressed per plant bunch which is considered in the present study to represent one plant unit. Photo 3 outlines the different steps involved in the current protocol for the growth of plants and the collection of root exudates.

Phosphorus: For the determination of the P content in plant extracts, dried samples were incinerated at 550 °C followed by solubilization in 65% HNO_3 and analyzed using ICP-emission spectroscopy.

Organic acid analyses: Analysis of 100 µl of 5-fold concentrated exudation extracts was performed using a Dionex DX 500 ion chromatograph. For the detection of oxalic acid, an Ion Pac AS10 column in combination with a suppressor ASRS II with 50 mM NaOH as eluent, was used. The working standards included citric acid, malic acid, oxalic acid, trans-aconitic acid, fumaric acid, malonic acid, succinic acid, acetic acid, formic acid, tartratic acid, lactic acid, glycolic acid and galacturonic acid. Organic acids were positively identified by spiking samples with standards. Appreciable rates of dicarboxylate release were only found for oxalate. Citrate was also not detected. The rates of organic acid release are expressed per unit of root length, i.e. in meters.

Acid phosphatase activity: The activity of acid phosphomonoesterases was determined spectrophotometrically in root exudates using *p*-nitrophenyl phosphate as substrate and monitoring the release of *p*-nitrophenol from the substrate at 410 nm. Specifically, 400 µl of root exudate samples were added to 800 µl assay solution consisting of 25 mM MES buffer, 1 mM EDTA, 5 mM cysteine and 10 mM substrate at pH 5.5.

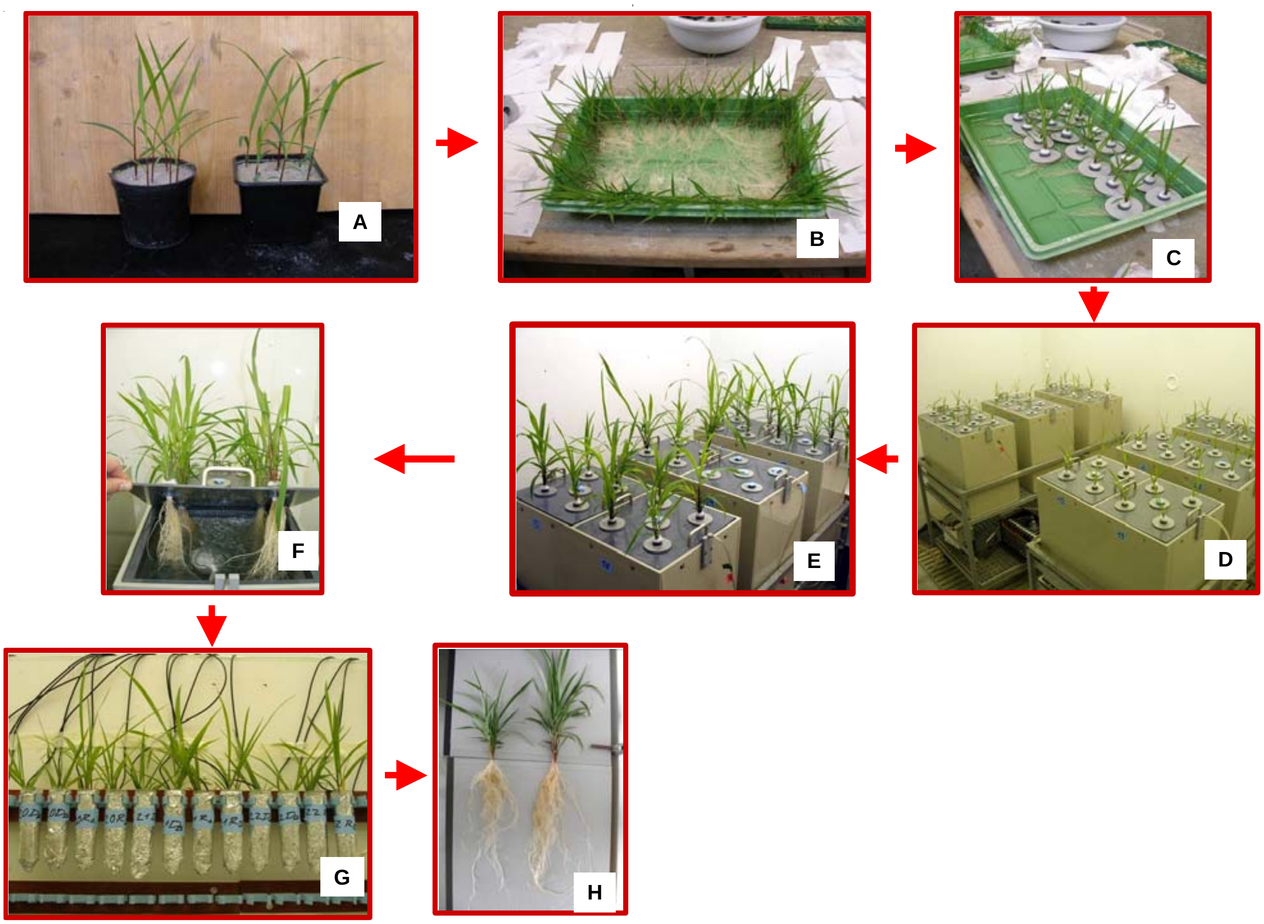


Photo 3. A scheme showing different steps involved for the growth of *Brachiaria* plants in hydroponic conditions and the collection of root exudates: **A.** Pre-grown seedlings (10-12 days old) in sand culture under minimal fertilization; **B.** Washed seedlings ready for selection and seedlings are kept in nutrient solution similar to that used in hydroponic experiment but without the addition of P; **C.** Preparation of uniform seedlings bunches (3 plants per bunch); **D.** Transfer of seedling bunches to hydroponic tanks and start of the experiment; **E.** *Brachiaria* grass seedlings grown for 7 days in nutrient solution in the presence of P; **F.** Hydroxyapatite-containing dialysis bag used for the constant release of phosphate; **G.** *Brachiaria* plants in exudation traps containing 0.1 mM $CaCl_2$ solution; and **H.** Visible differences in biomass and root length production (21 days) between signalgrass (on the left) and ruzigrass (on the right).

Samples were incubated for 40 minutes at 37^{o}C. The reactions were stopped by adding 600 µl of 0.5 M NaOH. Enzyme activity was calculated relative to standard solutions of *p*-nitrophenol. Enzyme activities are expressed in units where one unit (U) represents the release 1 µmol phosphate per minute. The activities of APase in root exudates were expressed per unit of root length. In our study, both organic acid and APase exudation rates are expressed for total root length as we found a strong linear correlation between root biomass and root length (data not shown).

Results and Discussion

Phosphorus relations during growth:
Comparison of both biomass production and root length production (Table 17) at each harvest time indicated highly significant genotypic differences. However, no differences in these two parameters existed between the species for the seedlings that were used in the study. Ruzigrass produced more biomass as well as more root length than signalgrass at each of the harvest times. In the first harvest, 40% more biomass was observed for ruzigrass while a 2-fold difference was found

in both the second and third harvest. The differences in root length were similar in the first two harvests (60% more root length) while in the third harvest a 2-fold difference was found. Comparison of the rates for both biomass and root length production also indicate differences between the species. Both rates were constant between harvest times in signalgrass and also no difference existed between the rates for both parameters. In ruzigrass, biomass production was higher than root length production between the first two harvests and also decreased towards the third harvest while an increase in root length production was found. Despite very low external availability of phosphate in the sand cultures used for the growth of the seedlings, the two species differed in the leaf P concentrations of the seedlings and the leaf P concentration in ruzigrass was 25% higher. This difference increased to more than 100% in the first harvest. However, between the second and third harvest, a decline of 50% in the leaf P concentration (Table 17) of ruzigrass was found followed by a further reduction of 50% between the last two harvests. The leaf P concentration was unchanged in signalgrass between the first 2 harvests, but a decrease of 50% was detected between the second and third harvest. Species differences existed only in the first harvest with a 2-fold higher level in ruzigrass.

The observed growth profiles could be anticipated as field studies have shown that although both *Brachiaria* species are P responsive, ruzigrass needs high P supply during early growth on a wide range of soils. A growth response at constant leaf P concentration was observed in signalgrass during the first two weeks while in ruzigrass, it appears that high initial P uptake efficiency resulted in high leaf P concentrations that might be the causal factor driving the higher levels of biomass production. Furthermore, the reduction in the growth rate in ruzigrass probably presented a penalty as a result of the earlier onset of decreasing leaf P concentrations and it is possible that in ruzigrass, crucial internal levels of P that are needed to maintain rapid growth, was reached. The reduced growth potential of ruzigrass might also be attributed to changes in the kinetics of P uptake. A higher leaf P concentration was found in ruzigrass only in the first harvest despite higher biomass production in all three harvests. Therefore, the tradeoff between factors governing P uptake supports an overriding role for limited external P availability to sustain high P uptake capacity in ruzigrass. Although biomass production was associated with growth dilution of P for both species towards the end of the growth period, it appears that in ruzigrass, the transition from P sufficiency to deficiency occurred at an earlier stage, already after the first harvest.

More support for the earlier development of P deficient *B. ruziziensis* plants is provided by the significant increase in the root mass fraction (RMF) (Table 17) between the last two harvests in ruzigrass. However, no significant genotypic differences were observed for the RMF, a morphological parameter that accounts for the fraction of biomass allocated to root biomass as a function of total biomass production. A role for the RMF as a morphological marker for P deficiency cannot be excluded in signalgrass as a differential increase tested significantly at a 90% level. Increases in the RMF are either because of allometric relationships or suggest that species allocate more biomass to root growth when P is limiting.

Synchronization between patterns for both oxalic acid exudation and acid phosphatase secretion and internal changes in the plant P status: Low levels of oxalate exudation (Table 17) were detected in the two species in the first harvest, especially in signalgrass. The rates of oxalate exudation increased in ruzigrass in the second harvest, reaching almost a six-fold higher level than in signalgrass. Between the last two harvests, the exudation of oxalic acid was also induced in signalgrass to a level slightly higher than was found in the second harvest in ruzigrass. In ruzigrass, the oxalic acid exudation profile is characterized by a maintenance response between the last two harvests. Temporal changes in APase activity (Table 17) showed a similar pattern for the two species, almost identical to the trend observed for oxalic

Table 17. Plant growth and root exudation characteristics of two Brachiaria grasses grown under P deficiency. Values indicate means ± standard deviation. RL = root length.

Plant characteristics	***B. decumbens* (signalgrass)**			***B. ruziziensis* (ruzigrass)**		
	Harvest 1 (after 7 days)	Harvest 2 (after 14 days)	Harvest 3 (after 21 days)	Harvest 1 (after 7 days)	Harvest 2 (after 14 days)	Harvest 3 (after 21 days)
Biomass (mg dry weight)	0.286 ± 0.026	0.680 ± 0.109	1.542 ± 0.431	0.406 ± 0.089	1.302 ± 0.304	2.971 ± 0.776
Root length (m)	10.81 ± 1.50	24.98 ± 6.01	51.10 ± 18.38	17.11 ± 3.02	41.45 ± 9.61	104.57 ± 41.98
Root mass fraction	0.271 ± 0.021	0.275 ± 0.024	0.296 ± 0.022	0.269 ± 0.027	0.267 ± 0.019	0.314 ± 0.030
Leaf P concentration (mg P/g dry weight)	2.110 ± 0.379	2.064 ± 0.469	1.185 ± 0.127	4.700 ± 1.071	2.108 ± 0.261	1.074 ± 0.214
Oxalate exudation rate (nmol/h/RL)	0.027 ± 0.042	0.141 ± 0.148	0.948 ± 0.532	0.312 ± 0.277	0.803 ± 0.665	0.824 ± 0.366
Acid phosphatase activity (units/RL)	5.128 ± 2.102	9.113 ± 2.156	28.183 ± 8.137	2.348 ± 1.031	22.816 ± 6.862	26.245 ± 9.339

acid exudation. Low rates of APase secretion was found in the first harvest which increased to a significantly higher level in ruzigrass (more than 2-fold) compared to signalgrass in the second harvest. Between the last two harvest times, the APase exudation rate in ruzigrass was maintained and similar to the trend for oxalic acid, extracellular APase activity could be detected in the third harvest in signalgrass at levels identical to that observed in ruzigrass in the second harvest.

Interestingly, the induction of both root exudate parameters appears to be linked to a decrease in internal plant P concentration. Furthermore, the manifestation of these two physiological markers for phosphate starvation stress in plants preceded the appearance of P limited growth in ruzigrass as both oxalic acid and APase exudation preceded the changes in biomass production and root growth. The differences in response specificity of physiological and morphological responses with the plant P status might also apply for signalgrass, but unfortunately, compelling evidence is lacking as growth was not affected. Species differed with regard to the exudation responses of oxalic acid and secretion response of APases as in ruzigrass, both responses required higher leaf P concentrations for induction.

Role of oxalate exudation in improving acid soil adaptation: Recently, more attention has been focused on the role of oxalate exudation in improving plant abiotic stress resistance, such as detoxification of aluminum and other heavy metals and overcoming P deficiency. Oxalate exudation in response to P deficiency has been reported to dominate in plant species that are considered to be extreme oxalate accumulators, e.g., sugar beet and spinach. However, recently a strong relationships between the exudation of oxalic acid and plant P deficiency was demonstrated in other plant species, e.g., in soybean and in lowland rice. Furthermore, several lines of evidence indicate that the exogenous application of oxalate to P deficient soils or rock phosphate increases P solubility and therefore P acquisition. Our results on brachiaria grasses are supportive of a role for oxalic acid in the P nutrition of both *Brachiaria* grass species.

Elevated rates of organic acid exudation were detected in response to Al stress in the two *Brachiaria* species, but these responses could not be linked to the external detoxification of Al. However, as Al toxicity and P deficiency co-exist in acid soils and are major constraints for *Brachiaria* pasture productivity in the tropics, a role for oxalic acid in Al resistance as a function of P deficiency cannot be excluded and needs further investigation. Thus it is likely that oxalic acid exudation might provide a dual ecological strategy for brachiaria grasses to alleviate the effects of soil P deficiency and Al toxicity.

Acid phosphatases and plant P acquisition: In general, enhanced activity of root phosphatases in response to P deficiency and higher levels of activity within the rhizosphere compared to bulk soil, are perceived as supportive evidence for the involvement of these enzymes in plant P nutrition. Recent studies using Arabidopsis mutants that were defective in APase secretion indicated that plant-derived rhizospheric APase activity is important for sustaining plant growth derived from substrates containing organic P.

Our results support these observations as increasing rates of APase exudation as a function of decreasing leaf P concentrations were found in both brachiaria grasses.

Functional synergism between oxalic acid and acid phosphatases in plant P acquisition: Our results are also suggestive of possible functional synergism between oxalic acid exudation and APases secretion in *Brachiaria* grasses for P acquisition in view of the proposed role for chelating organic anions in organic P release from organo-metallic P complexes in soils. Specifically, in cluster roots the acquisition of P was enhanced by the release of extracellular APases indicating that APases are likely an important adjunct to carboxylate exudation from cluster roots, because inorganic P, liberated by APases is more likely to be taken up in the presence of citrate, which can suppress re-adsorption and precipitation of inorganic P. Therefore, under these conditions, the efficiency of organic P mobilization could be greatly enhanced.

Conclusions

Results from the present study highlight the possibility that decreases in internal plant P concentrations could be related to exudation of both oxalic acid and acid phosphatases in signalgrass and also in ruzigrass. However, the two grasses differed with regard to the magnitude of the internal leaf P concentration needed for the induction of these root-mediated rhizospheric mechanisms for P acquisition.

Our results demonstrate a higher internal P requirement for growth in ruzigrass, a finding that is in agreement with published reports. In addition, the coordinated nature of the root exudation responses with plant growth and development indicates a regulatory function for internal P concentrations in leaf tissue to not only regulate physiological responses but also morphological responses of plant P deficiency.

It appears that the signalgrass can adjust its growth response to low P availability by regulating its P use while ruzigrass seem to use the available P more rapidly for its growth. Further research work is needed on differences in P utilization mechanisms between the two grasses.

3.1.1.3 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 five years, we have implemented hydroponic screening procedure to identify aluminum (Al)-resistant *Brachiaria* hybrids that were preselected for spittlebug resistance. In 2005, we evaluated the BR04NO series of 139 apomictic/sexual hybrids of *Brachiaria* and identified 9 hybrids (BR04NO/1018, BR04NO/1552, BR04NO/1900, BR04NO/2110, BR04NO/2128, BR04NO/2166, BR04NO/2179, BR04NO/2201 and BR04NO/2681) that were superior to the *B. decumbens* parent in terms of Al resistance.

This year, we evaluated 103 clones of the BR05NO series, 60 clones of the RZ05NO series, and 88 clones of the SX05NO series together with 3 parents and 8 checks for their level of Al resistance.

Materials and Methods

One hundred twenty-seven BR05 clones (obtained by crossing 34 highly spittlebug-resistant sexual clones with *B. decumbens* cv. Basilisk), were pre-selected on performance in two field trials during 2005. This population should segregate approx. 1:1 for reproductive mode.

The RZ05 plants are a recombined sexual population derived from crosses with the *Rhizoctonia*-resistant *B. brizantha* accession CIAT 16320. The SX05 plants are the most recent generation of our mainstream tetraploid sexual breeding population, which was originally synthesized in 1993. The population comprises germplasm from three *Brachiaria* species: *B. brizantha*, *B. decumbens*, and *B. ruziziensis*. This most recent generation has very high, multiple-species resistance to spittlebugs.

Among the clones selected by the *Brachiaria* breeding project, 103 BR05 clones, 60 RZ05 clones, and 88 SX05 clones together with 8 checks and 3 parents (*B. decumbens* CIAT 606, *B. brizantha* CIAT 6294 and *B. ruziziensis* 44-02) were included for evaluation of Al resistance. The clones that did not root in nutrient solution were excluded from further evaluation. Five incomplete sets (separate experiments) of BR05 plants were evaluated with and without Al in solution. Five incomplete sets of RZ05 plants were evaluated with and without Al in solution. The sets were incomplete because some of the hybrids did not root well in each experiment.

Only two incomplete sets of SX05 plants were evaluated in the presence of Al in solution. 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 nine 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, root systems were harvested. 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 5 years (see IP- 5 AR), Al resistant hybrids combine greater values of total root length per plant with lower values of mean root diameter relative to the mean values of the population when exposed to 21 days with toxic level of Al in solution.

Among the 103 hybrids (apomictic/sexual) of the BR05 population evaluated, nine hybrids (BR05NO/0406, BR05NO/0563, BR05NO/0334, BR05NO/0830, BR05NO/1173, BR05NO/0671, BR05NO/0120, BR05NO/0048, and BR05NO/0537) were superior to the *B. decumbens* parent in terms of root length with Al in solution (Table 18).

The relationship 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 6 to 9. Among these nine hybrids, BR05NO/0406 was markedly superior to *B. decumbens* in terms of fine root production as revealed by total root length and 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 26 hybrids (BR05NO/0406, BR05NO/0563, BR05NO/0334, BR05NO/0830, BR05NO/0120, BR05NO/0048, BR05NO/0537, BR05NO/0549, BR05NO/0707, BR05NO/0351, BR05NO/0117, BR05NO/1019, BR05NO/1467, BR05NO/0577, BR05NO/0760, BR05NO/0267, BR05NO/0746, BR05NO/1435, BR05NO/0150, BR05NO/0377, BR05NO/0159, BR05NO/0265, BR05NO/0605, BR05NO/0990, BR05NO/0115 and BR05NO/0586) compared with *B. decumbens*.

Table 18. Root length and mean root diameter of 103 hybrids of the BR05 population of *Brachiaria* hybrids evaluated with (200 µM Al) and without Al (0 µM Al) in solution in comparison with 3 parents and 8 checks.

Genotypes	Root length (cm plant^{-1})		Root diameter (mm)		Genotypes	Root length (cm plant^{-1})		Root diameter (mm)	
	0 µM Al	200 µM Al	0 µM Al	200 µM Al		0 µM Al	200 µM Al	0 µM Al	200 µM Al
Hybrids					***Hybrids***				
BR05/0406	547	360	0.294	0.346	BR05/1434	110	79	0.490	0.366
BR05/0563	504	272	0.259	0.351	BR05/1623	147	79	0.327	0.424
BR05/0334	670	263	0.305	0.378	BR05/0753	188	78	0.296	0.365
BR05/0830	440	259	0.287	0.345	BR05/0354	329	77	0.302	0.524
BR05/1173	346	240	0.290	0.346	BR05/1449	200	76	0.341	0.474
BR05/0671	292	237	0.293	0.344	BR05/1344	211	73	0.285	0.418
BR05/0120	451	221	0.309	0.365	BR05/1520	157	70	0.335	0.461
BR05/0048	688	217	0.289	0.382	BR05/0561	170	70	0.296	0.369
BR05/0537	495	212	0.268	0.362	BR05/1059	178	69	0.314	0.371
BR05/1040	371	206	0.272	0.360	BR05/0629	312	69	0.270	0.338
BR05/0549	502	205	0.297	0.400	BR05/1574	151	68	0.329	0.481
BR05/0707	546	205	0.238	0.304	BR05/1879	259	68	0.312	0.389
BR05/0351	529	199	0.286	0.379	BR05/1433	207	67	0.307	0.451
BR05/0156	262	194	0.372	0.416	BR05/1586	200	66	0.281	0.392
BR05/0545	308	184	0.267	0.356	BR05/1361	347	66	0.264	0.456
BR05/0117	489	181	0.278	0.342	BR05/0244	287	65	0.341	0.461
BR05/0555	330	179	0.299	0.361	BR05/1865	274	64	0.294	0.441
BR05/1019	415	178	0.290	0.382	BR05/1830	173	63	0.322	0.397
BR05/0293	377	171	0.383	0.486	BR05/1302	172	63	0.354	0.451
BR05/1467	395	169	0.306	0.406	BR05/0071	243	62	0.302	0.382
BR05/0577	447	168	0.254	0.343	BR05/1479	190	62	0.277	0.399
BR05/1702	298	165	0.321	0.400	BR05/0475	177	60	0.332	0.388
BR05/0760	561	163	0.274	0.390	BR05/1611	160	58	0.329	0.452
BR05/0267	447	158	0.292	0.395	BR05/1444	216	56	0.373	0.486
BR05/0746	418	156	0.281	0.371	BR05/0262	156	55	0.358	0.456

Continues…

Table 18. Root length and mean root diameter of 103 hybrids of the BR05 population of *Brachiaria* hybrids evaluated with (200 µM Al) and without Al (0 µM Al) in solution in comparison with 3 parents and 8 checks.

Genotypes	Root length (cm plant^{-1})		Root diameter (mm)		Genotypes	Root length (cm plant^{-1})		Root diameter (mm)	
	0 µM Al	200 µM Al	0 µM Al	200 µM Al		0 µM Al	200 µM Al	0 µM Al	200 µM Al
BR05/1435	394	153	0.277	0.371	BR05/1610	130	55	0.337	0.468
BR05/0150	422	151	0.282	0.419	BR05/1493	203	54	0.260	0.374
BR05/0377	428	151	0.277	0.339	BR05/1447	185	53	0.287	0.436
BR05/0159	428	151	0.324	0.487	BR05/1494	120	52	0.381	0.541
BR05/0609	259	150	0.308	0.326	BR05/1872	123	51	0.342	0.410
BR05/0265	411	148	0.325	0.454	BR05/1401	292	50	0.357	0.583
BR05/0379	328	146	0.342	0.417	BR05/1462	185	50	0.264	0.417
BR05/0605	469	145	0.278	0.374	BR05/1426	122	47	0.370	0.563
BR05/1460	327	145	0.338	0.428	BR05/0118	82	45	0.446	0.470
BR05/0990	407	142	0.292	0.434	BR05/0708	166	45	0.275	0.425
BR05/0995	369	142	0.293	0.393	BR05/1717	158	39	0.322	0.530
BR05/0114	335	141	0.308	0.408	BR05/1738	177	39	0.376	0.637
BR05/0508	376	127	0.278	0.417	BR05/0913	117	36	0.417	0.630
BR05/1440	358	124	0.343	0.510	BR05/1883	119	35	0.291	0.400
BR05/0115	439	121	0.302	0.456	BR05/0933	64	34	0.480	0.533
BR05/0586	518	119	0.278	0.458	BR05/1352	162	31	0.341	0.467
BR05/1826	241	117	0.265	0.332	BR05/1420	135	27	0.393	0.584
BR05/1609	342	113	0.269	0.392	BR05/1376	113	26	0.330	0.460
BR05/0743	240	111	0.287	0.329	BR05/1490	155	17	0.290	0.430
BR05/1464	277	108	0.341	0.457	**Parents**				
BR05/1857	319	107	0.300	0.424	CIAT606	386	210	0.241	0.323
BR05/1149	263	102	0.269	0.372	CIAT 6294	200	80	0.302	0.474
BR05/1706	231	102	0.296	0.335	Br44-02	92	36	0.421	0.542
BR05/0701	173	99	0.277	0.372	**Checks**				
BR05/1853	371	95	0.273	0.357	CIAT 26110	82	21	0.506	0.743
BR05/1455	284	94	0.352	0.448	CIAT679	263	126	0.221	0.262
BR05/0209	283	93	0.336	0.520	CIAT 6133	173	116	0.258	0.285
BR05/1475	252	90	0.330	0.486	CIAT 36061	220	76	0.369	0.490
BR05/0462	319	88	0.356	0.498	CIAT 36087	103	38	0.567	0.619
BR05/1359	373	85	0.279	0.464	BR02/1372	421	172	0.274	0.356
BR05/1835	363	84	0.272	0.406	BR02/1485	312	66	0.348	0.569
BR05/1402	310	84	0.335	0.461	BR02/1752	365	105	0.341	0.480
BR05/0092	237	83	0.333	0.431	**Means**	**307**	**117**	**0.314**	**0.421**
BR05/1308	112	83	0.330	0.450	**LSD(P<0.05)**	**221**	**96**	**0.058**	**0.068**

Among these hybrids BR05NO/0048 was outstanding in terms of its root vigor in the absence of Al in solution. Among the checks, an apomictic hybrid BR02NO/1372 showed greater level of Al resistance and also root vigor based on total root length per plant.

Among the 60 hybrids (apomictic/sexual) of the RZ05 population evaluated, none of the hybrids was superior to the *B. decumbens* parent in terms of total root length with Al in solution (Table 19).

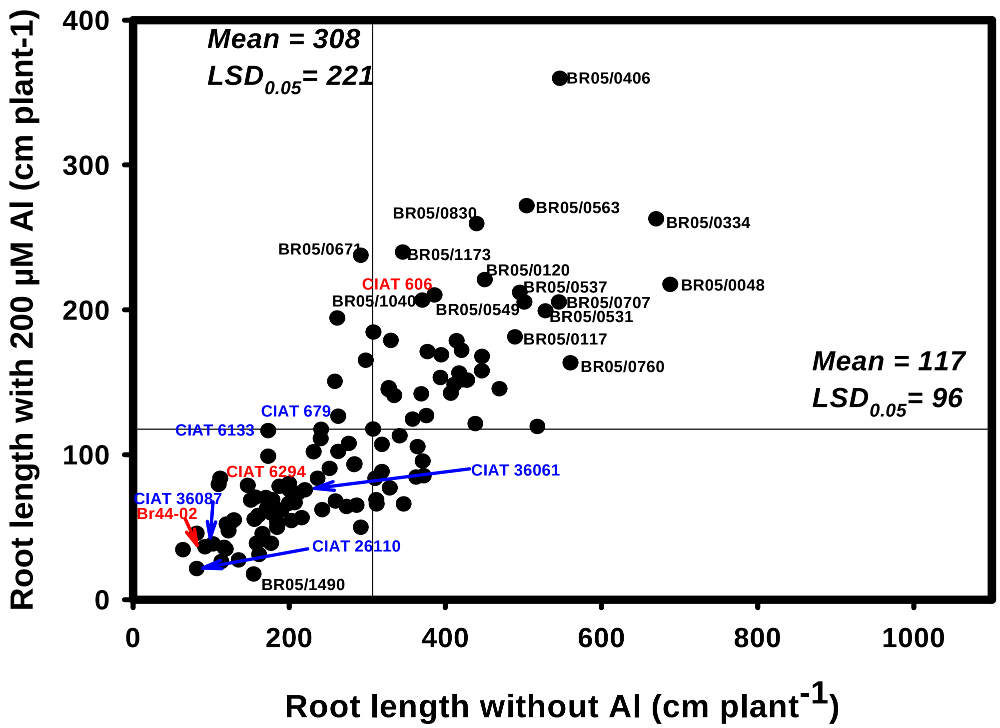


Figure 6. Relationship between total root length with Al and total root length without Al in solution of 103 hybrids, 3 parents and 8 checks of *Brachiaria*. Genotypes that developed greater root length under both conditions were identified in the upper, right hand quadrat.

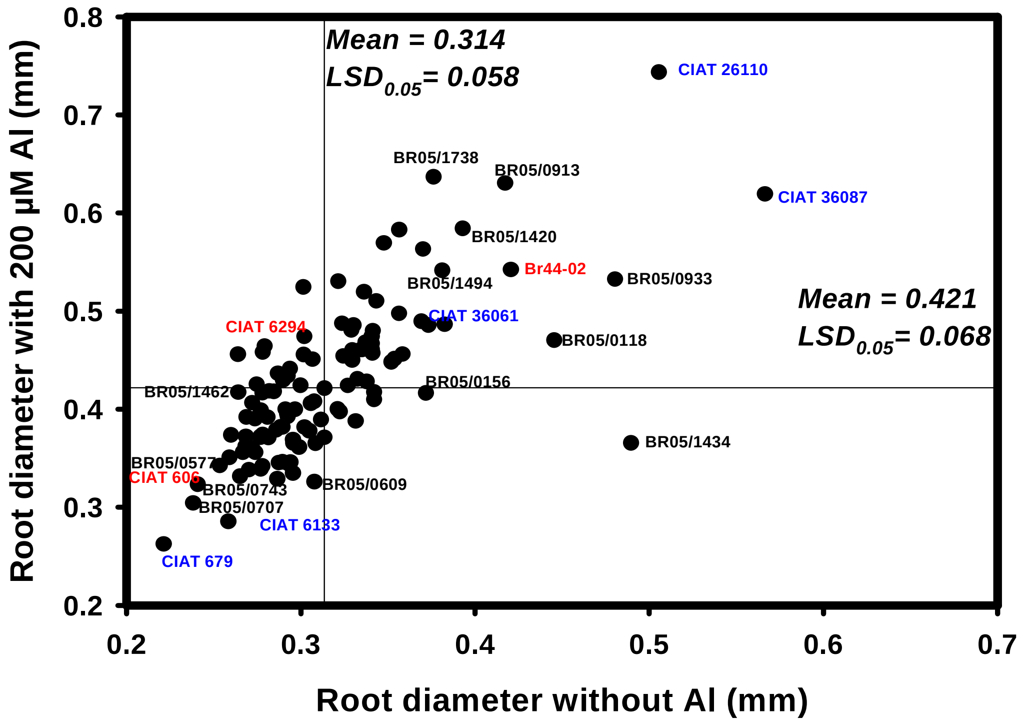


Figure 7. Relationship between mean root diameter with Al and mean root diameter without Al of 103 hybrids, 3 parents and 8 checks of *Brachiaria*. Genotypes that developed finer roots under both conditions were identified in the lower, left hand quadrat.

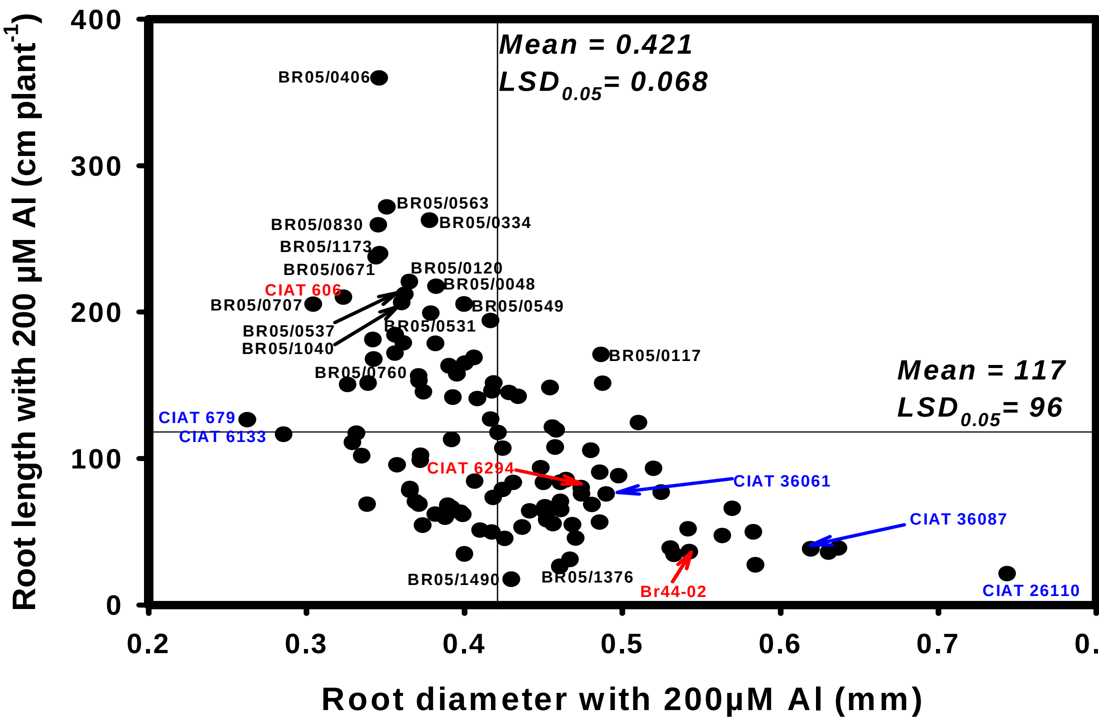


Figure 8. Relationship between total root length and mean root diameter of 103 hybrids, 3 parents and 8 checks of *Brachiaria* with presence of aluminum in solution. Genotypes that develop finer longer roots were identified in the upper, left hand quadrat.

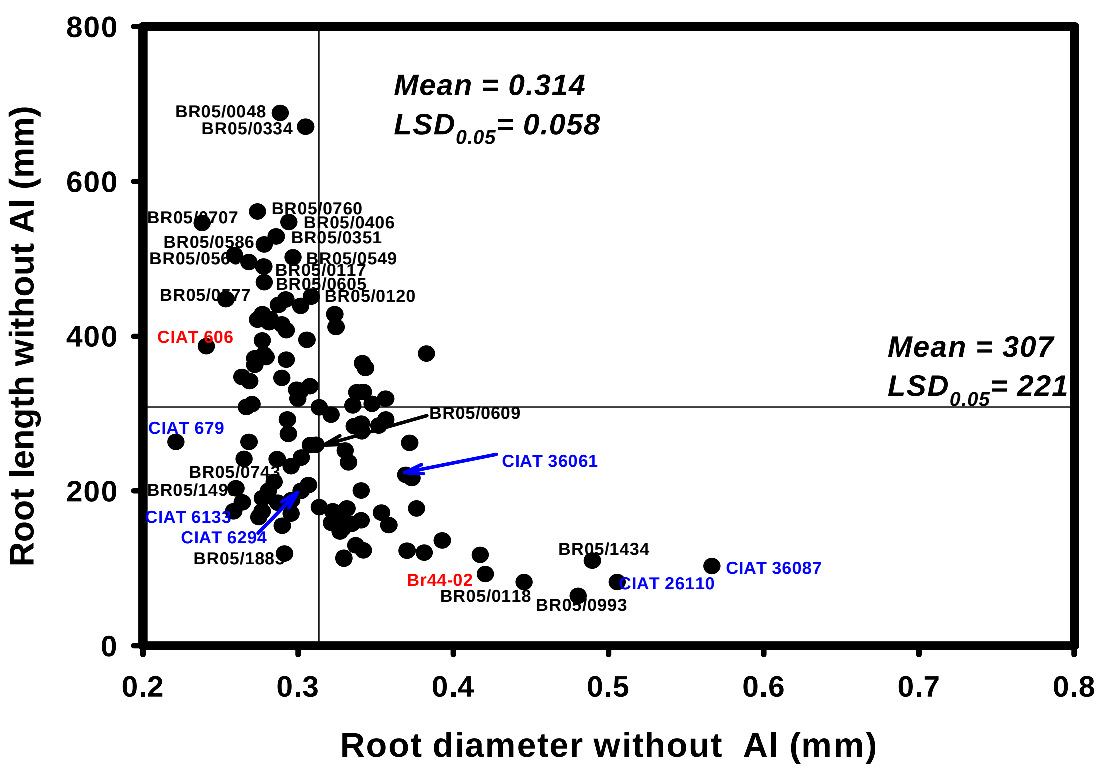


Figure 9. Relationship between total root length and mean root diameter of 103 hybrids, 3 parents and 8 checks of *Brachiaria* with absence of aluminum in solution. Genotypes that develop finer longer roots were identified in the upper, left hand quadrat.

Table 19. Root length and mean root diameter of RZ05 population of *Brachiaria* hybrids evaluated with (200 µM Al) and without Al (0 µM Al) in solution in comparison with 3 parents and 8 checks.

Genotypes	Root length (cm plant^{-1})		Root diameter (mm)		Genotypes	Root length (cm plant^{-1})		Root diameter (mm)	
	0 µM Al	200 µM Al	0 µM Al	200 µM Al		0 µM Al	200 µM Al	0 µM Al	200 µM Al
Hybrids					***Hybrids***				
RZ05/3244	318	157	0.377	0.439	RZ05/2801	128	60	0.435	0.536
RZ05/2932	317	132	0.363	0.470	RZ05/3635	224	60	0.371	0.477
RZ05/3107	260	128	0.301	0.366	RZ05/3738	182	60	0.412	0.542
RZ05/3158	394	122	0.300	0.376	RZ05/3361	199	60	0.314	0.412
RZ05/3332	350	118	0.336	0.442	RZ05/3063	328	59	0.354	0.466
RZ05/3362	372	117	0.360	0.391	RZ05/3101	143	50	0.426	0.736
RZ05/3333	197	106	0.455	0.496	RZ05/3398	141	47	0.447	0.608
RZ05/3021	358	106	0.318	0.394	RZ05/2682	158	46	0.363	0.566
RZ05/2985	259	105	0.375	0.411	RZ05/3359	223	43	0.404	0.648
RZ05/2919	305	103	0.339	0.447	RZ05/3541	91	37	0.488	0.557
RZ05/2937	249	103	0.295	0.388	RZ05/3616	113	36	0.428	0.627
RZ05/3226	285	101	0.364	0.363	RZ05/2838	65	35	0.588	0.550
RZ05/3378	396	96	0.303	0.471	RZ05/3589	58	32	0.554	0.640
RZ05/3394	266	95	0.360	0.513	RZ05/2938	148	31	0.366	0.487
RZ05/3630	276	93	0.394	0.531	RZ05/3579	106	30	0.379	0.554
RZ05/2873	316	93	0.356	0.470	RZ05/2802	116	29	0.575	0.661
RZ05/3106	231	93	0.355	0.490	RZ05/3434	92	28	0.377	0.499
RZ05/3365	221	91	0.370	0.497	RZ05/3576	109	27	0.387	0.609
RZ05/3629	265	91	0.381	0.551	RZ05/3483	86	23	0.485	0.708
RZ05/3466	230	88	0.332	0.490	RZ05/3634	73	21	0.588	0.699
RZ05/3262	269	86	0.375	0.350	RZ05/2842	54	19	0.523	0.655
RZ05/2992	250	84	0.263	0.377	RZ05/3485	56	17	0.499	0.497
RZ05/3173	241	84	0.306	0.374	**Parents**				
RZ05/3335	237	80	0.395	0.502	CIAT606	386	210	0.241	0.323
RZ05/2942	219	80	0.361	0.426	CIAT 6294	200	80	0.302	0.474
RZ05/2786	177	75	0.455	0.471	Br44-02	92	36	0.421	0.542
RZ05/3253	119	72	0.378	0.412	**Checks**				
RZ05/3391	183	68	0.298	0.411	CIAT 26110	82	21	0.506	0.743
RZ05/3312	322	68	0.365	0.569	CIAT679	263	126	0.221	0.262
RZ05/3371	167	67	0.428	0.500	CIAT 6133	173	116	0.258	0.285
RZ05/2847	127	67	0.441	0.501	CIAT 36061	220	76	0.369	0.490
RZ05/3585	229	66	0.366	0.450	CIAT 36087	103	38	0.567	0.619
RZ05/2738	167	66	0.340	0.476	BR02/1372	421	172	0.274	0.356
RZ05/3574	198	65	0.389	0.589	BR02/1485	312	66	0.348	0.569
RZ05/3311	193	64	0.376	0.550	BR02/1752	365	105	0.341	0.480
RZ05/3377	347	63	0.376	0.507					
RZ05/3452	216	62	0.316	0.444	**Means**	**226**	**83**	**0.372**	**0.479**
RZ05/2641	165	62	0.399	0.523	**LSD(P<0.05)**	**177**	**82**	**0.075**	**0.096**

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 10 to 13. Root vigor in terms of root length in the absence of Al in solution was similar or slightly superior for 2 hybrids (RZ05NO/3158 and RZ05NO/3378) compared with *B. decumbens*.

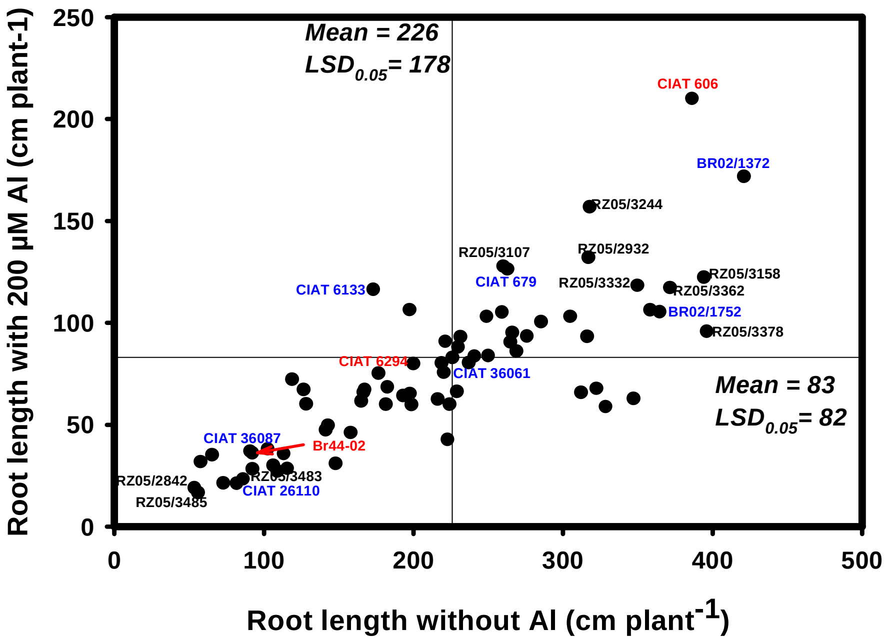


Figure 10. Relationship between total root length with Al and total root length without Al in solution of 60 hybrids, 3 parents and 8 check genotypes of *Brachiaria*. Genotypes that developed greater root length under both conditions were identified in the upper, right hand quadrat.

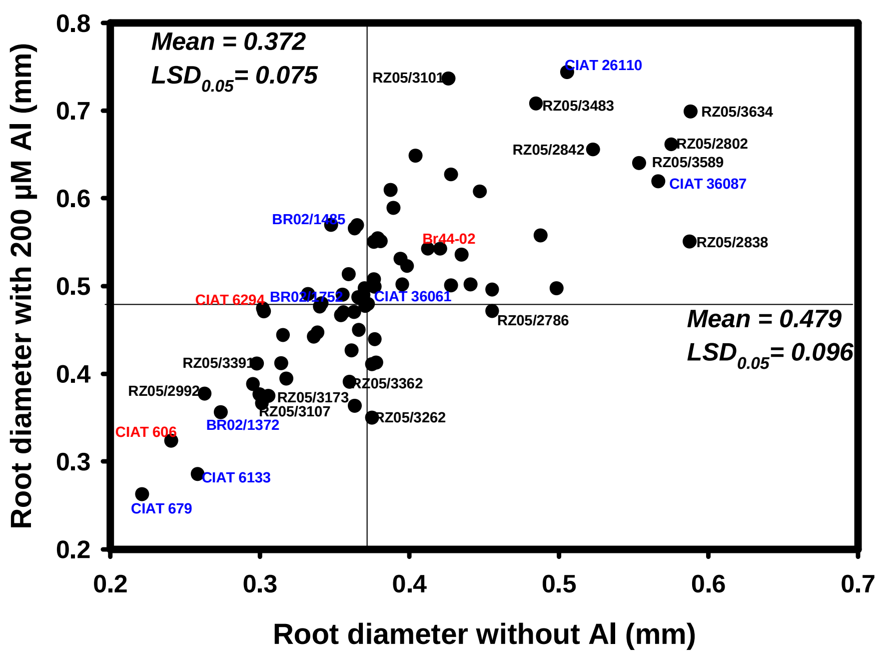


Figure 11. Relationship between mean root diameter with Al and mean root diameter without Al of 60 hybrids, 3 parents and 8 check genotypes of *Brachiaria*. Genotypes that developed finer roots under both conditions were identified in the lower left, hand quadrat.

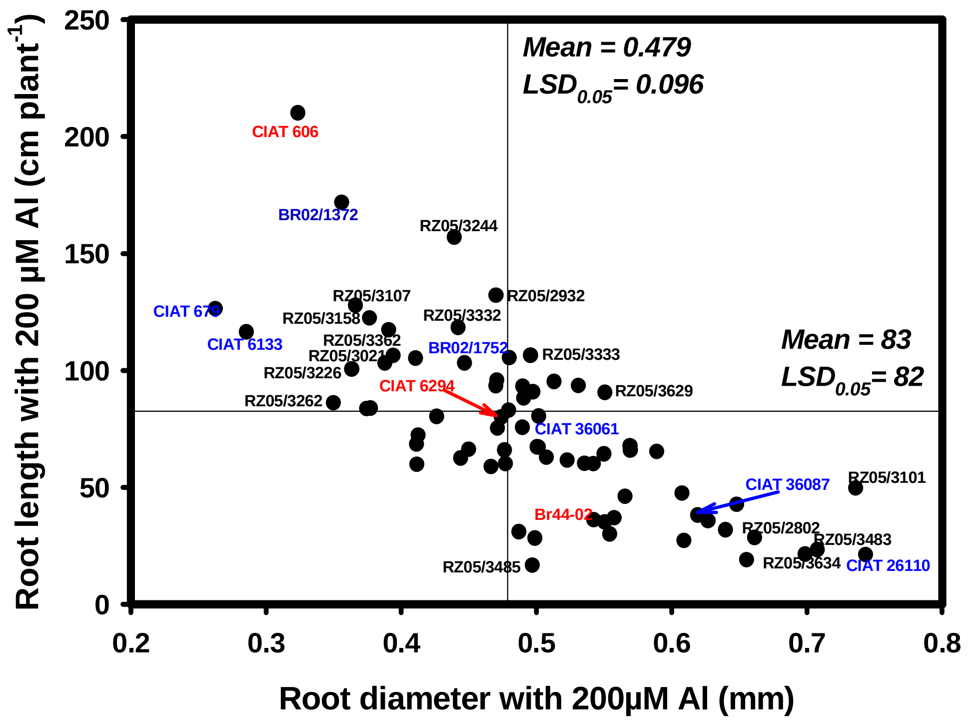


Figure 12. Relationship between total root length and mean root diameter of 60 hybrids, 3 parents and 8 check genotypes of *Brachiaria* with presence of aluminum in solution. Genotypes that develop finer longer roots were identified in the upper, left hand quadrat.

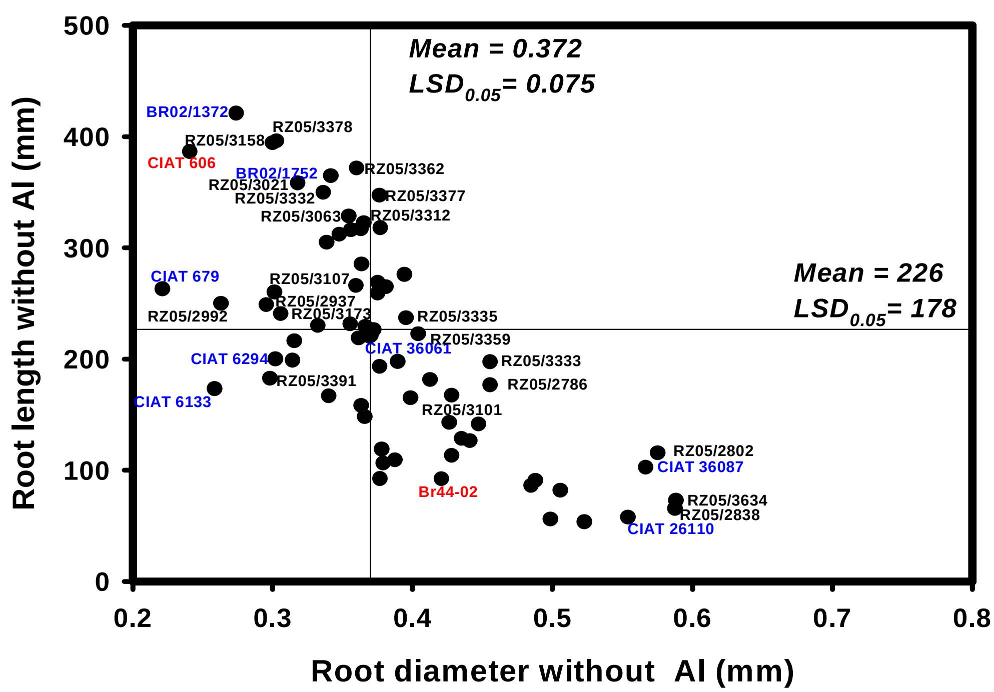


Figure 13. Relationship between total root length and mean root diameter of 60 hybrids, 3 parents and 8 check genotypes of *Brachiaria* with absence of aluminum in solution. Genotypes that develop finer longer roots were identified in the upper, left hand quadrat.

Among the 88 hybrids (sexual) of SX05 population evaluated, none was superior to the *B. decumbens* parent in terms of total root length with Al in solution (Figure 14). The relationship between root length with Al vs root diameter with Al showed that 2 sexual hybrids.

Results on spittlebug resistance of these hybrids are reported in section 2.2 of this report. Three of the 9 Al resistant hybrids (BR05NO/0334, BR05NO/0537, BR05NO/0563) also combined resistance to four spittlebug species and exhibited apomictic mode of reproduction. These hybrids will be further evaluated under field conditions for developing as potential cultivars.

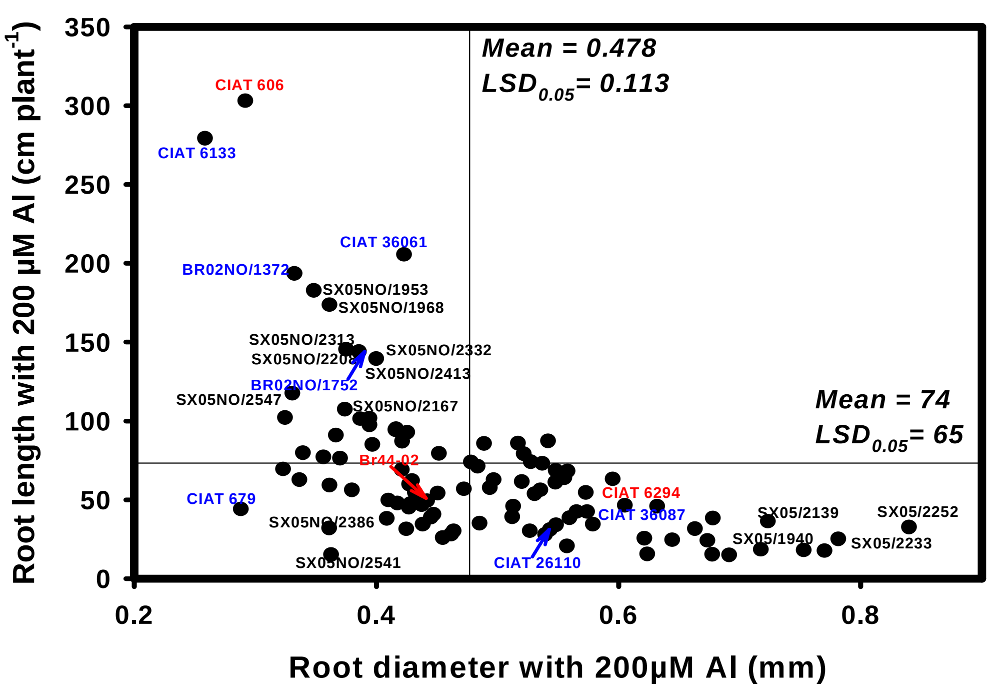


Figure 14. Relationship between total root length and mean root diameter of 88 SX05 hybrids, 3 parents and 8 check genotypes of *Brachiaria* with presence of aluminum in solution. Genotypes that develop finer longer roots with Al were identified in the upper, left hand quadrat.

Conclusions

We screened 103 apomictic/sexual hybrids of BR05 population of *Brachiaria* using hydroponic screening method and identified 9 hybrids (BR05NO/0406, BR05NO/0563, BR05NO/0334, BR05NO/0830, BR05NO/1173, BR05NO/0671, BR05NO/0120, BR05NO/0048, and BR05NO/0537) that were superior to *B. decumbens* parent in terms of aluminum resistance. Three of the 9 Al resistant hybrids (BR05NO/0334, BR05NO/0537, BR05NO/0563) also combined resistance to four spittlebug species and exhibited apomictic mode of reproduction. Results from this BR05NO population in Al resistance, as observed last year, 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.

3.1.1.4 Identification of candidate genes for Al resistance in *Brachiaria*

Contributors: M. E. Rodriguez,, A. F. Salcedo, M. E. Recio, A. L. Chavez, , J. Thome. M. Ishitani (SB-2 Project), and I. M. Rao (IP-5 Project)

Rationale

Previous studies have demonstrated that there is pronounced difference in aluminum (Al) resistance between *B. decumbens* (resistant) and *B. ruzisiensis* (susceptible). The objective of this work is to identify candidate genes responsible for high levels of Al resistance in *B. decumbens* using PCR-based technology.

Materials and Methods

Plant material: Seeds of *B. decumbens* and *B. ruziziensis* were germinated in 200 µM $CaCl_2$ (pH 4.2) for 4 - 5 days in the greenhouse. Similar seedlings with root length between 4 and 5 cm were subjected to Al treatment with continuously aerated solutions consisting of 200 µM $CaCl_2$ (pH 4.2) with and without 200 µM $AlCl_3$. Root tips (1cm length) of *B. decumbens* and *B. ruzisiensis* were collected at 0, 3, 6, 24 and 72 h after with or without Al treatment.

Candidate genes for Al resistance: 18 candidate genes have been isolated from a cDNA subtraction library between *B. decumbens* and *B. ruziziensis* and were used for this study.

cDNA synthesis: Total RNA was isolated from the root tips using Trizol® (Invitrogen, USA) according with manufacturer's protocol. Total RNA was treated with DNaseI (Invitrogen, USA) to remove genomic DNA. cDNA for PCR experiments was synthesized using SuperScript III reverse transcriptase (Invitrogen, USA). We used a co-amplification reverse transcription (Co-RT) strategy for priming cDNA, which combines oligo-(dT) with an 18S-RNA-specific primer in the initial reverse transcription reaction.

Gene expression analysis: Comparative expression analysis of each 18 genes at 0, 6 and 24 hours in *B. decumbens* and *B. ruziziensis* root tips was conducted by real time PCR using a gene-specific primer. Real-time PCR was carried out in MJ research Opticon II as follows: 20ìl reaction volume containing: 10ìl of Master Mix (2X) SYBR Green I kit (Stratagene, USA), 175 nM of each primer, and 5ìl of 1:10 diluted cDNA template. PCR conditions were 95 °C for 10 minutes, followed by 40 cycles of 95 °C for 10 seconds, 50-60 °C for 20 seconds and 72 °C for 30 seconds. The fluorescence reading was done at 72 °C and 83 °C and specificity of amplified products were confirmed by a melting curve from 65 °C to 95 °C.

Data analysis: Sequence analysis and homologous searches were performed with Vector NTI (Invitrogen, USA) and the BLAST algorithm of NCBI. We used qBase software v 1.3.5 (http://medgen.ugent.be/qbase) to analyze real time PCR data. The software employs a delta-Ct relative quantification model with PCR efficiency correction and multiple reference gene normalization. To estimate efficiency of PCR amplification of each gene, a standard curve was prepared using a serial dilution of cDNA or plasmid DNA carrying candidate genes. We used 18S-rRNA gene as reference gene for normalization.

Results and Discussion

Among the 18 genes we examined, six genes named *AlBdec3*, *AlBdec5*, *AlBdec8*, *AlBdec10*, *AlBdec15* and *AlBdec16* showed significant differential expressions in *B. decumbens* in response to Al toxicity compared with *B. ruziziensis*. *AlBdec3, 8, 10 and 16* showed a similar pattern of gene expression. At 0 h, the constitutive expression of each gene was observed. Upon exposure to Al toxicity, transcript level of these genes increased at 6 h.

Interestingly, *B. decumbens* maintained the same expression level for these genes at 24 h as found at 6 h. On the other hand, we observed significant reduction of the transcript level of these genes in *B. ruziziensis* at 24 h. Other two genes, *AlBdec5* and *15* had a similar type of expression pattern which indicated higher expression in *B. decumbens* at all time points we tested compared with *B. ruziziensis*. Figure 15 shows gene expression patterns of *AlBdec3* and *AlBdec15* as examples of the two expression patterns described above.

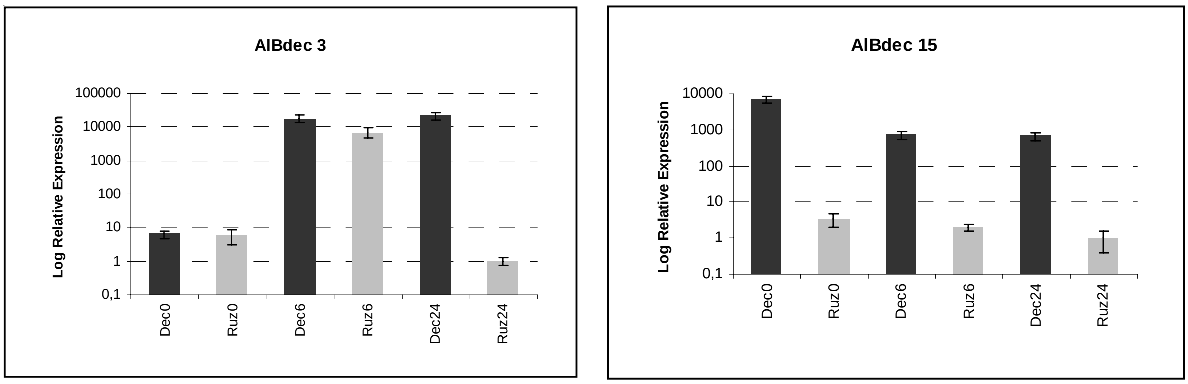


Figure 15. Transcript analysis of *AlBdec3* and *AlBdec15* at 0, 6 and 24 h of Al treatment. Black bar: *B. decumbes*; Grey bar: *B. ruziziensis*. The relative expression level was shown in a logarithmic scale.

Last year, we described 3 candidate genes that are presumably regulatory genes such as transcription factors and kinases based on BLAST analysis. However, these genes did not show any difference in transcript levels at the time points that we tested. Among the six genes confirmed by this study as differently expressed genes in *B. decumbens, AlBdec3*, *AlBdec5*, *AlBdec15* and *AlBdec16* did not show any significant homology or showed no homology to any genes in the NCBI database. This could be in part due to the short cDNA sequence of the gene with less than 300 bp. The other two genes, *AlBdec8* and *AlBdec10* showed a high homology to a putative cysteine proteases and a metal-dependent protein hydrolase family protein found in *O. sativa*, respectively (Table 20).

Once comparative analysis of the genes is completed we will obtain full length cDNA of selected genes by Rapid Amplification of cDNA Ends (RACE) technology to conduct functional

Table 20. Summary of Blastx search for six candidate genes in the NCBI database.

Name	Organism	E-value	Score (Bits)	Description
AlBdec03 (262bp)	No significant HIT			
AlBdec05 (356bp)	*Solanum tuberosum*	6.3	36.8	Low E-value, probably unknown gene: acidic ribosomal protein P1a-like
AlBdec08 (247bp)	*O. sativa*	3e-46	191	Transglutaminase-like enzymes, putative cysteine proteases
AlBdec10 (548bp)	*O. sativa*	3e-35	151	Metal-dependent protein hydrolase family protein
AlBdec15 (242bp)	No significant hit			
AlBdec16 (271bp)	No significant HIT			

analysis of genes by transgenic approach. Dr. Koyama at University of Gifu in Japan is collaborating with us to conduct the POC (proof of concept) work. An aluminum treatment experiment will be performed in 2007 for different genotypes of *Brachiaria* which show different degrees of Al resistance, including *B. decumbes (*Alresistant*), B. brizantha* cv. Marandú (intermediate level of Al resistance), hybrid Mulato 2, and *B. ruziziensis* (Al sensitive). Total RNA will be extracted from root tips and levels of expression for each candidate gene will be evaluated.

3.1.1.5 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

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 3 parents and 4 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. In 2005 evaluation, we showed that among the 15 *Brachiaria* hybrids tested, BR02NO/1794 and BR02NO/0465 were more productive than the other hybrids in terms of green forage (leaf + stem) yield in rainy season at 13 months after establishment with low initial fertilizer application and this adaptation seems to be closely associated with lower amounts of stem N content. In 2006, we tested the performance of these hybrids in the rainy season at 26 months after establishment under no maintenance fertilizer application.

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- Mulato and CIAT 36087- Mulato II). 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 and P) uptake were measured in the rainy season at 26 months after establishment (October 2006).

Results and Discussion

At 9 months after establishment CIAT 606, CIAT 36061, CIAT 36087, BR02NO/1452, BR02NO/ 1485, BR02NO/1720, BR02NO/1747, BR02NO/ 1752 and BR02NO/1794 were affected by bacterial infection and this affected their performance.

At 26 months after establishment, the dead shoot biomass was greater with 2 hybrids (BR02NO/ 0465 and BR02NO/1728 than with the other hybrids tested (Figure 16). Production of green

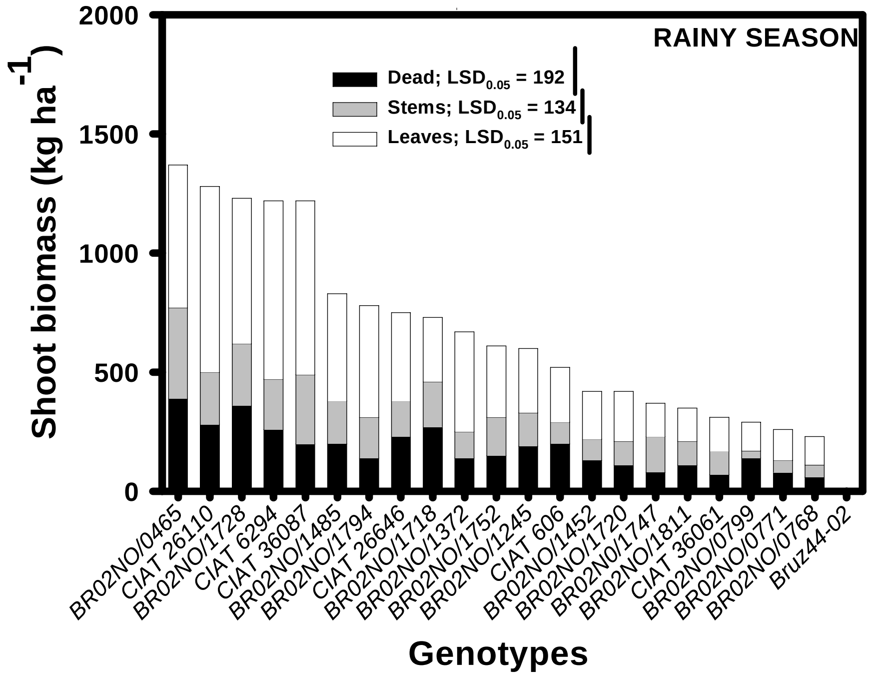


Figure 16. Genotypic variation in shoot biomass production (forage yield) of three parents (CIAT 606, 6294 and *B.ruziziensis* 44-02), four CIAT's accesions (26110, 26646, 36061 and 36087) and 15 genetic recombinants (BR02NO/0465; 0768; 0771; 0799; 1245; 1372; 1452; 1485; 1718; 1720; 1728; 1747; 1752; 1794 y 1811) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 26 months after establishment (October 2006). LSD values are at the 0.05 probability level. NS = not significant.

leaf biomass was greater with CIAT 26110, CIAT 36087 and CIAT 6294. The hybrid BR02NO/0465 was outstanding in the production of stem biomass and therefore also in total biomass production. As expected BRUZ 44-02 (sexual parent) did not persist under no maintenance fertilizer. Among the hybrids tested, BR02NO/0465 and BR02NO/1728 were more productive than the other BR02NO hybrids in terms of green forage (leaf + stem) yield. But these 2 hybrids were less productive than CIAT 36087 (cv. Mulato II). The hybrid Mulato II outperformed the checks and the hybrids. Results on shoot nutrient uptake also indicated that the hybrid BR02NO/0465 was superior to the other BR02NO hybrids (Figure 17).

Nitrogen uptake of this hybrid BR02NO/0465 was particularly outstanding among the BR02NO hybrids and was similar to CIAT 26110 (cv. Toledo) and CIAT 36087 (cv. Mulato II). Correlation coefficients between green forage yield and other plant attributes indicated that greater nutrient acquisition contributed to superior performance (Table 21). Significant negative correlation was observed between stem N and P content and live forage yield. Adaptation to infertile acid soil conditions seem to be closely associated with lower amounts of stem N and P content.

Conclusions

Table 21. Correlation coefficients (r) between green forage yield (t/ha) and other shoot traits of *Brachiaria* genotypes grown with low initial 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)	0.58***
Leaf biomass (t/ha)	0.96***
Stem biomass (t/ha)	0.85***
Leaf N content (%)	-0.03
Leaf P content (%)	0.10
Stem N content (%)	-0.31**
Stem P content (%)	-0.25**
Shoot N uptake (kg/ha)	0.95***
Shoot P uptake (kg/ha)	0.89***

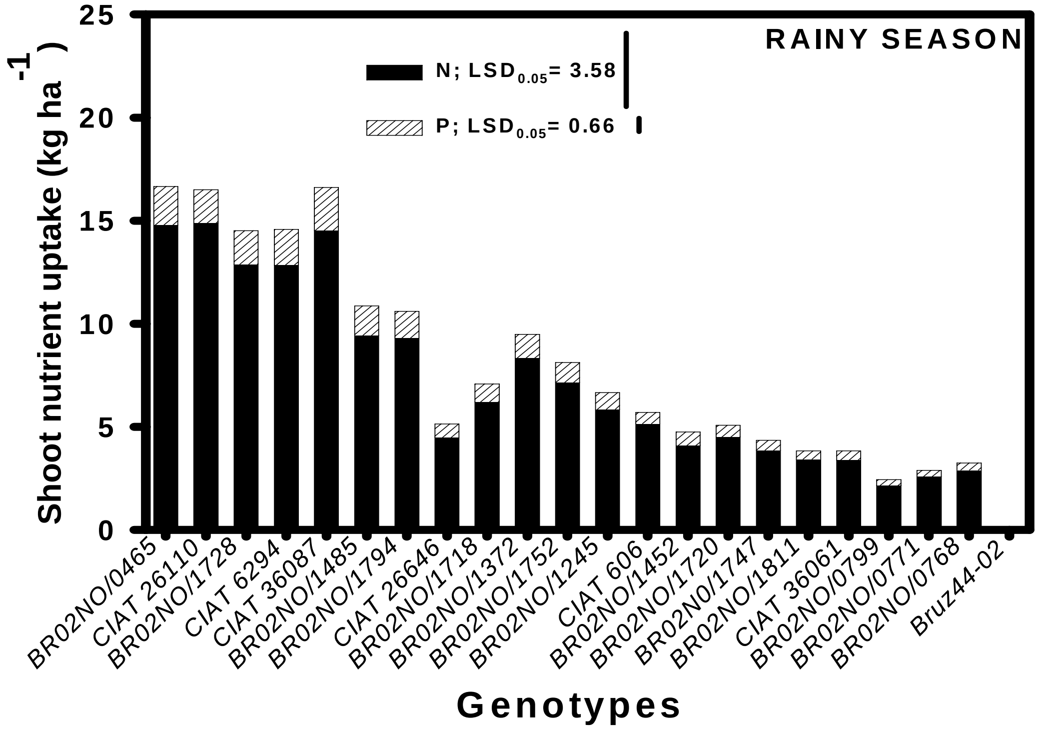


Figure 17. Genotypic variation in living shoot nutrient uptake (N, P, K, Ca and Mg) of three parents (CIAT 606, 6294 and *B.ruziziensis* 44-02), four CIAT's accesions (26110, 26646, 36061 and 36087) and 15 genetic recombinants (BR02NO/0465; 0768; 0771; 0799; 1245; 1372; 1452; 1485; 1718; 1720; 1728; 1747; 1752; 1794 y 1811) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 26 months after establishment (October 2006). LSD values are at the 0.05 probability level. NS = not significant.

Results from this field study indicated that among the 15 *Brachiaria* hybrids tested, BR02NO/0465 and BR02NO/1728 were more productive than the other BR02NO hybrids in terms of green forage (leaf + stem) yield in rainy season at 26 months after establishment with low initial fertilizer application and no maintenance fertilizer application. But neither of these hybrids was superior to cv. Mulato II. The superior adaptation of the two hybrids seems to be associated with lower amounts of stem N and P content.

3.2 Genotypes of *Brachiaria* with dry season tolerance

Highlights

- Among 6 genotypes tested for response to soil drying, two genotypes, *Brachiaria brizantha* CIAT 6294 cv. Marandú and *Brachiaria decumbens* CIAT 606, showed delay in stomatal closure compared to other genotypes as evident from lower values of $FTSW_c$ (critical value for the fraction of transpirable soil water at which transpiration is first reduced during a drying cycle) at which the NTR (normalized transpiration ratio) starts declining.

- Showed that among the 15 *Brachiaria* hybrids tested under acid soil conditions in the Llanos of Colombia, the hybrids BR02NO/1794 and BR02NO/1718 were more productive than the other BR02NO hybrids in terms of green forage (leaf + stem) yield in dry season at 19 months after establishment with low initial fertilizer application and no maintenance fertilizer application. But neither of these hybrids was superior to cv. Mulato II. The superior adaptation of the two hybrids seems to be associated with lower amounts of stem and leaf N and P contents and greater uptake of nutrients.

- Showed that the apomictic natural accession *B. brizantha* CIAT 6294 and the apomictic hybrid cultivar Mulato II (CIAT 36087) could produce greater amounts of live shoot biomass (forage) with maintenance fertilizer application. The productivity of these two genotypes was superior to *B. decumbens* CIAT 606 and this superior performance was associated with coarse root development.

3.2.1 Differences in regulation of water use, water use efficiency and growth of contrasting *Brachiaria* genotypes subjected to drought stress

Contributors: V. Hoyos, J. Polania and I.M. Rao (CIAT)

Rationale

Adaptation to drought involves complex multigenic components that interact holistically in plant systems and maintaining root growth plays a key role. Soil drying decreases shoot growth rate, plant height, and yield, but affects root growth less. Water loss may be reduced by leaf morphological structures or early stomatal closure in response to abscisic acid (ABA) transported in xylem from root to shoot and perceived at the guard cell apoplast. There is very limited knowledge on the physiological and biochemical bases of brachiariagrass' adaptation to drought.

Seasonal drought affects both quantity and quality of forage in tropical savanna environments. *Brachiaria* grasses differ in drought resistance; *B. brizantha* CIAT 6780 and *B. decumbens* CIAT 606 are relatively well adapted to drought stress. One physiological mechanism for improving drought resistance involves developing genotypes with high water use efficiency (WUE, the quantity of forage dry matter accumulated per unit of soil water transpired). Another physiological mechanism that also contributes to drought resistance is the decline in whole plant water use during soil water deficit or drought stress. During soil water deficit, plants undergo a transition between the water-replete phase where whole plant water use is not dependent on the soil water content and a second phase where water use is directly related to the availability of soil water. This transition is associated with a reduction in the average stomatal conductance and can occur at different soil water contents for different plant species or cultivars.

Our objective was to determine the differences in regulation of water use, water use efficiency and growth among the 4 cultivars and 2 hybrids of *Brachiaria* that were subjected to water deficit. This knowledge is needed to develop effective screening method(s) to evaluate drought resistance of *Brachiaria* hybrids generated by the *Brachiaria* breeding program at CIAT.

Materials and Methods

A greenhouse experiment was conducted to determine differences in regulation of water use, water use efficiency and shoot growth of 6 genotypes (*Brachiaria decumbens* CIAT 606 cv. Basilisk, *Brachiaria ruziziensis* 44-02, *Brachiaria brizantha* CIAT 6294 cv. Marandú, *Brachiaria brizantha* CIAT 26110 cv. Toledo, Mulato CIAT 36061 and Mulato II CIAT 36087) that were subjected to drought stress. Soil (3.5 kg) from Quilichao Experimental station with adequate supply of nutrients was used. The experimental design used was completely randomized arrangement with 2 levels of water supply (well watered and terminal drought) and six genotypes with 3 replications. One stolon was

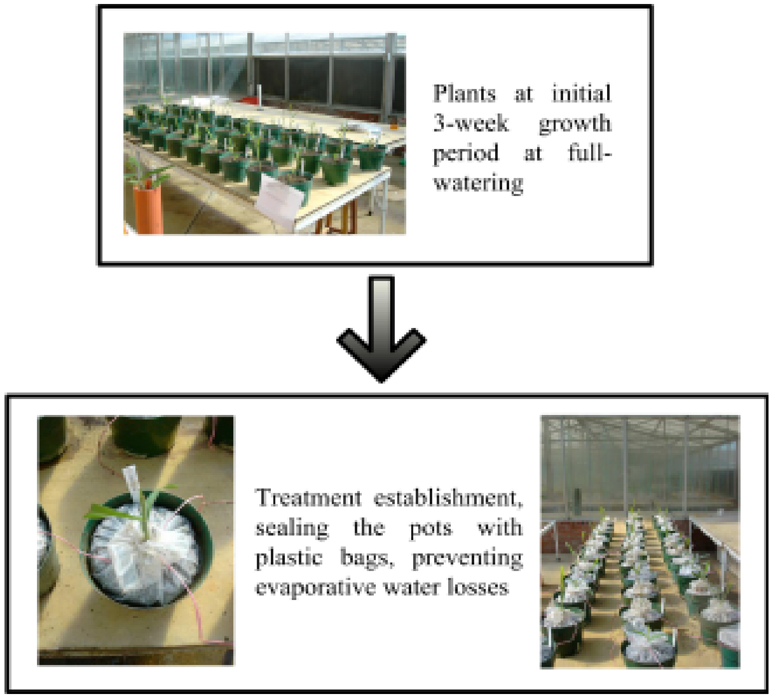


Photo 4. Methodology employed for determining FTSW response curves.

planted per pot and was well watered for 3 weeks. At the time of establishing the two treatments (100% field capacity (FC) and terminal drought), the pots were fully irrigated and allowed to drain until reaching a constant weight. The amount of water held at 100% FC is the maximum amount of water held in soil after free drainage. This weight was recorded and used to maintain 100% FC treatment. For inducing terminal drought stress, water supply was simply withheld. The surface of the pot was sealed with plastic in order to avoid evaporative water losses (Photo 4).

The weight of each pot was recorded every day in order to determine water loss due to transpiration based on weight difference between the days. At the end of the drying cycle, the transpiration data of the terminal drought pots was normalized by correcting the transpiration of the stressed plant against that of the control pots (100% FC) to obtain a transpiration ratio (TR), which helps to minimize the influence of large variations in transpiration across days. A second normalization was made using the TR of the same drought pot from the first days of the experiment to correct for any differences in plant size, this gives a normalized transpiration ratio (NTR), and the experiment was completed when this value reached ~0.1 for each pot, which was defined as the endpoint. This value in terms of experiment duration (days) is variable because of the differential response of the genotypes to applied stress. Nevertheless, the last pot was harvested at 51 days after planting (21 days after establishment and 30 days after stress induction). This was the experimental timeframe.

After reaching the endpoint, the plants were harvested and the total plant (shoot + root) dry weights were recorded. This weight was corrected with the weight of the plant before inducing drought by using an additional set of 3 plants per genotype to record fresh and oven dry weights. To obtain a value for soil moisture, a fraction of transpirable soil water (FTSW) was also calculated from:

$$\text{Daily FTSW} = \frac{\text{daily pot weight - final pot weight}}{\text{initial pot weight - final pot weight}} \quad (1)$$

The results from these two variables were fitted to the following equation to obtain a curve explaining the behavior of the plant during terminal drought stress:

$$NTR = \frac{1}{[1 + A \times \exp(B \times FTSW)]} \quad (2)$$

The results were analyzed using the GLM procedure and Tukey's HSD test from SAS v.9 for Windows.

Results and Discussion

Drought response curves: The daily values of normalized transpiration ratio (NTR) were the basis for expressing relative transpiration rate, and the values of (FTSW) expressed the relative soil water content. Results on NTR-FTSW curve for six genotypes of *Brachiaria* during soil drying or terminal drought are shown in Figure 18.

The level of adjustment (based on determination coefficients and 95% confidence intervals) of the transpiration values to the equation mentioned above and the inflection point that occurs in the resulting curve enables to determine the point at which transpiration began to decline for each genotype. This is represented as $FTSW_c$ (critical value for the fraction of transpirable soil water at which transpiration is first reduced during a drying cycle).

Two genotypes, *Brachiaria brizantha* CIAT 6294 cv. Marandú and *Brachiaria decumbens* CIAT 606, showed delay in stomatal closure compared to other genotypes during soil drying as evident from lower values of $FTSW_c$ at which the NTR starts declining.

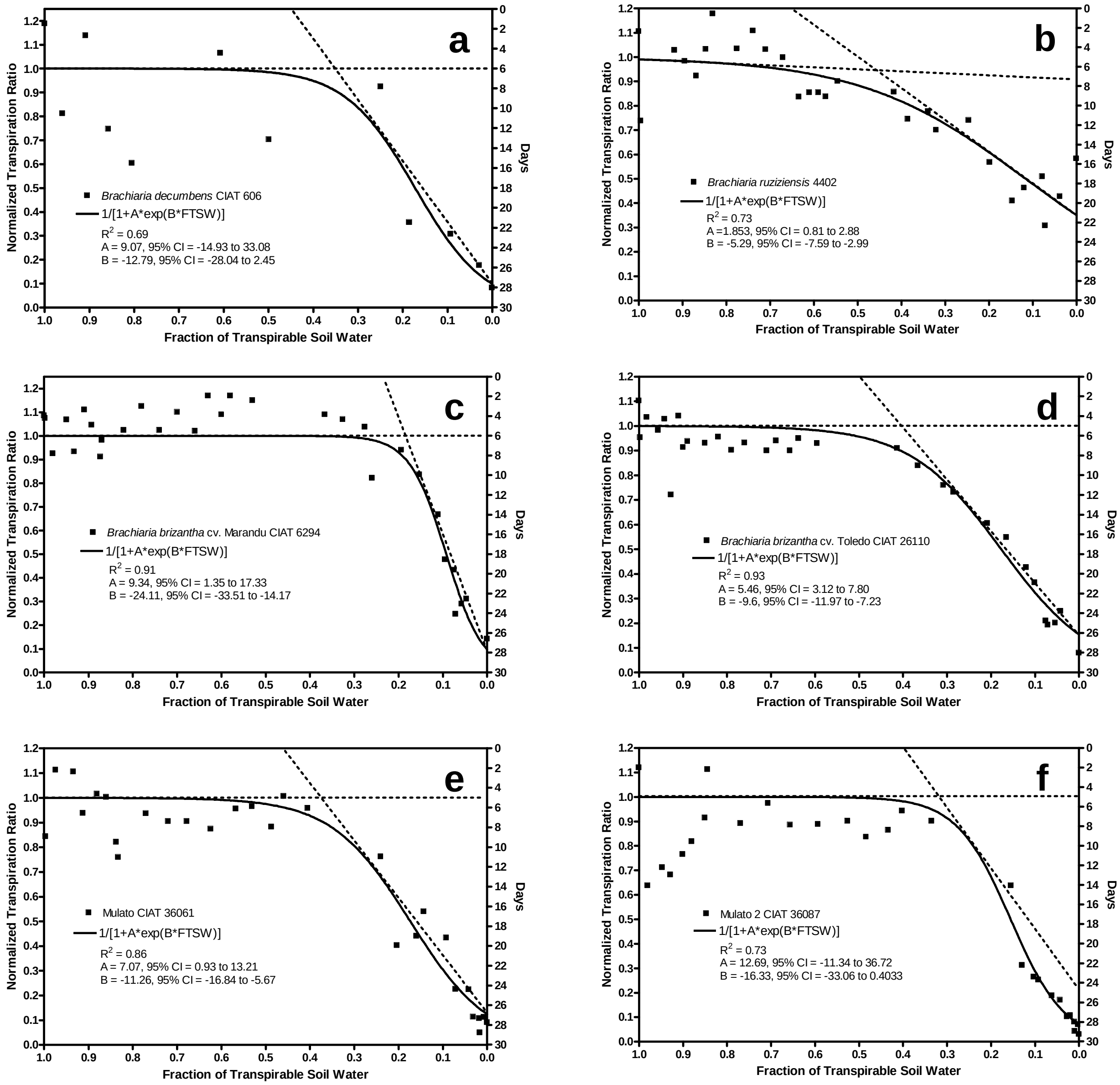
Cultivars Mulato and Toledo also showed delayed response compared with *B. ruziziensis* which showed stomatal sensitivity to soil drying. The response of cv. Toledo to soil drying seems to be somewhat different to the other genotypes, based on the time it took for cv. Toledo to achieve the endpoint (Table 22).

It seems to maintain higher rates of NTR during the drying cycle thereby allowing greater amount of dry matter accumulation during drought. We consider this as an important observation because cv. Toledo is found to be more adapted to seasonal drought in the Llanos of Colombia.

From this we can indicate that in terms of the expressed tolerance from the genotypes in this experiment, *B. brizantha* cv. Marandú was superior in the uptake of available water, but once the soil water has depleted, it seems to markedly decrease its transpiration (see Figures 18c and d). But cv. Toledo does not have such a water scavenging ability and therefore its water use regulation mechanisms allow it to maintain its functions for longer period (Table 22), enabling it with mechanisms to tolerate longer dry spells in the field.

Figure 19 shows the grouped response of the six genotypes in terms of the NTR vs FTSW relationship during soil drying. All six genotypes achieved in an average a complete closure of stomata, and their water uptake had been completely inhibited at 26.72 days. Dryness in the soil seems to be a signal translated as stomatal closure in the plant that starts with a mean FTSW value of 0.37.

In Figure 20 we illustrate that during soil drying, *Brachiaria decumbens* CIAT 606 was the most "conservative" genotype in the use of water by regulating the stomatal response and that the hybrids Mulato (CIAT 36061) and Mulato II (CIAT 36087) were the most demanding genotypes for water to maintain growth during soil drying conditions.



a. NTR – FTSW response curve for *Brachiaria decumbens* CIAT 606 cv. Basilisk
b. NTR – FTSW response curve for *Brachiaria ruziziensis* 44-02
c. NTR – FTSW response curve for *Brachiaria brizantha* CIAT 6294 cv. Marandú
d. NTR – FTSW response curve for *Brachiaria brizantha* CIAT 26110 cv. Toledo
e. NTR – FTSW response curve for cv. Mulato CIAT 36061
f. NTR – FTSW response curve for cv. Mulato II CIAT 36087

Figure 18. NTR (normalized transpiration ratio) – FTSW (fraction of transpirable soil water) response curves of 6 *Brachiaria* genotypes during the water deficit regime. Symbols represent mean daily values (n=3). The solid line represents the fit of the data to equation 2. The intersection of the dashed lines is the point at which stomata begin to close.

Table 22. Means for the $FTSW_C$ (critical values for the fraction of transpirable soil water at which transpiration is first reduced during a drying cycle), days to endpoint and FTSW value on endpoint for 6 genotypes during soil drying.

Genotype	$FTSW_c$	Days to endpoint (d)	FTSW on endpoint
B. brizantha CIAT 6294 cv. Marandú	0.194 A a[b]	27.00 B a	0.05 A a
B. decumbens CIAT 606 cv. Basilisk	0.357 B a	25.00 C a	0.10 A a
Mulato CIAT 36061	0.407 A ab	26.00 B a	0.03 A a
B. brizantha CIAT 26110 cv. Toledo	0.413 A ab	29.67 B a	0.03 A a
B. ruziziensis 44-02	0.417 B ab	26.00 C a	0.12 A a
Mulato II CIAT 36087	0.463 A a	26.67 B a	0.03 A a

[b]Means between columns/rows with the same capital/small letter are not significantly different at 0.05 level according to Tukey's HSD test.

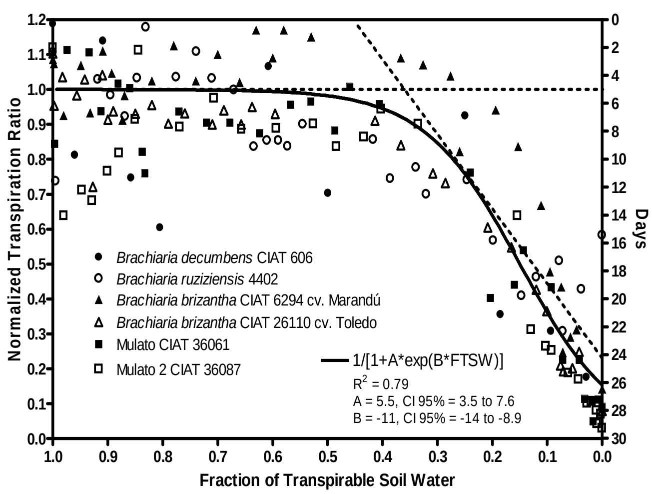


Figure 19. The relationship between the normalized transpiration ratio (NTR) and the daily values of the fraction of transpirable soil water (FTSW) for 6 genotypes of *Brachiaria* during terminal drought stress. The solid line represents the composite fit of all the data to equation 2. The intersection of the dashed lines is the point at which stomata begin to close.

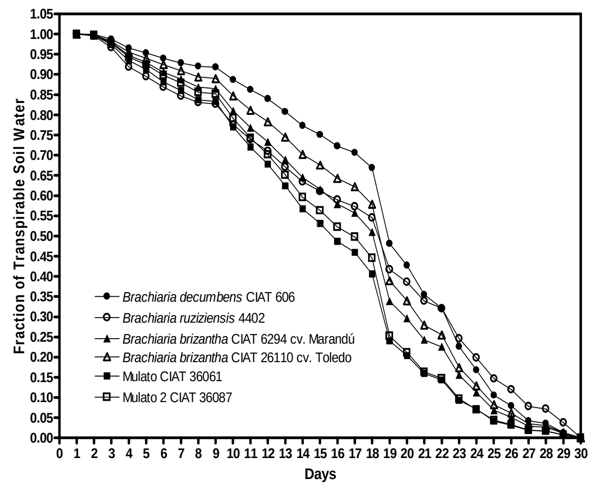


Figure 20. Differences in FTSW values over time for 6 genotypes of *Brachiaria* during soil drying.

The effect of drought on shoot dry matter accumulation and mean daily transpiration was more pronounced (inhibition of 62.26% for biomass and 67.11% for transpiration compared with well watered control) in the hybrid Mulato (CIAT 3606), which in well watered conditions is the genotype with the greatest dry matter yield, but it used very high amounts of water during soil drying as reflected by mean daily transpiration rate (Figures 21 and 22). The least inhibited genotype for both variables was *Brachiaria decumbens* CIAT 606, which showed greater level of drought resistance. In general terms, a highly significant correlation ($r = 0.93$; $P < 0.01$; $n = 36$) was found between mean daily transpiration and biomass production, and also a very highly significant difference ($P < 0.0001$) between genotypes according to analysis of variance for both variables.

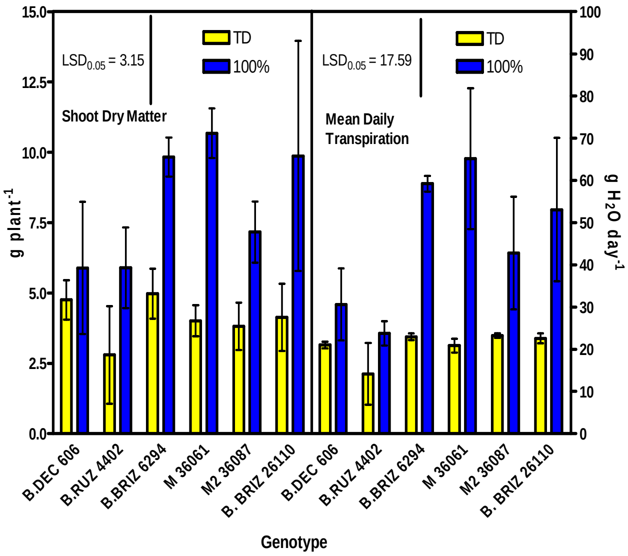


Figure 21. Differences in shoot dry matter and mean daily transpiration among 6 *Brachiaria* genotypes subjected to 100% FC (well watered) and terminal drought stress (TD).

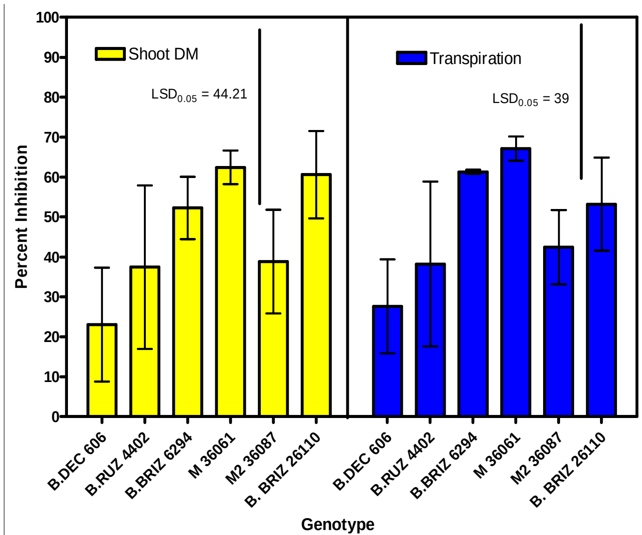


Figure 22. Effect of terminal drought stress on % inhibition of shoot dry matter accumulation and mean daily transpiration of 6 *Brachiaria* genotypes.

Conclusions

Among the 6 genotypes tested for response to soil drying, two genotypes, *Brachiaria brizantha* CIAT 6294 cv. Marandú and *Brachiaria decumbens* CIAT 606, showed delay in stomatal closure compared to other genotypes during soil drying as evident from lower values of $FTSW_c$ (critical value for the fraction of transpirable soil water at which transpiration is first reduced during a drying cycle) at which the NTR (normalized transpiration ratio) starts declining.

3.2.2 Dry season tolerance under field conditions of promising hybrids of *Brachiaria*

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. In 2005 evaluation at 9 months after establishment, we 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. In 2006, we tested the performance of these hybrids in the dry season at 19 months after establishment under no maintenance fertilizer application.

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 the end of the 3 month dry season at 19 months after establishment (March 2006).

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, BR02NO/1752 was relatively more affected. The dead shoot biomass was greater with 2 hybrids (BR02NO/1811 and BR02NO/ 1728) and a check (CIAT 26110) (Figure 23).

Two hybrids (BR02NO/1794 and BR02NO/1718) were outstanding among the BR02NO hybrids in producing the green forage (leaf + stem biomass) than the other hybrids. Mulato II (CIAT 36087)

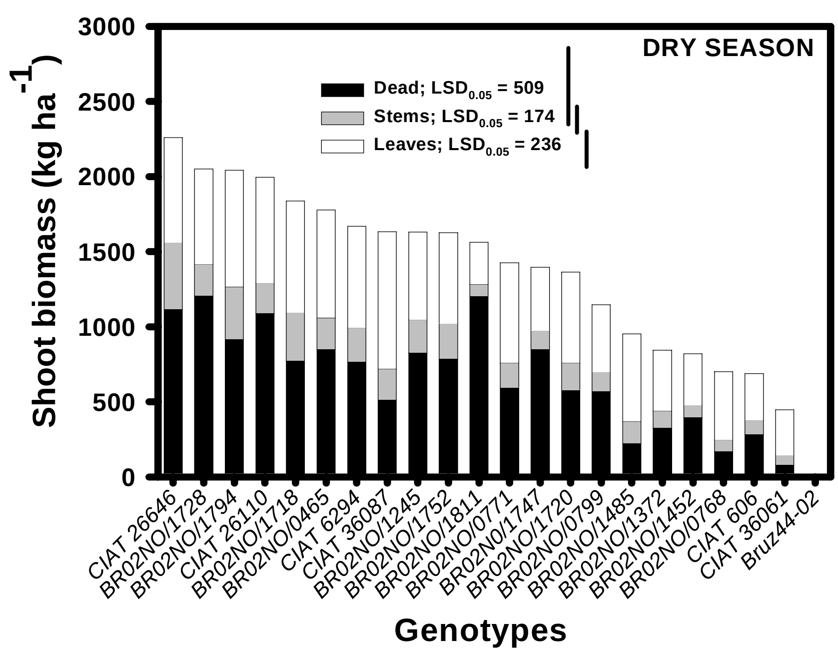


Figure 23. Genotypic variation in shoot biomass production (forage yield) of three parents (CIAT 606, 6294 and *B.ruziziensis* 44-02), four CIAT's accesions (26110, 26646, 36061 and 36087) and 15 genetic recombinants (BR02NO/0465; 0768; 0771; 0799; 1245; 1372; 1452; 1485; 1718; 1720; 1728; 1747; 1752; 1794 and 1811) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 19 months after establishment (March 2006). LSD values are at the 0.05 probability level. NS = not significant.

was outstanding in green leaf forage production as was observed last year. Among the BRO2NO hybrids tested BR02NO/1794 and BR02NO/1718 were outstanding in green leaf biomass production. As expected BRUZ 44-02 (sexual parent) did not persist under no maintenance fertilizer application. Results on shoot nutrient uptake also indicated that the hybrid BR02NO/1794 was

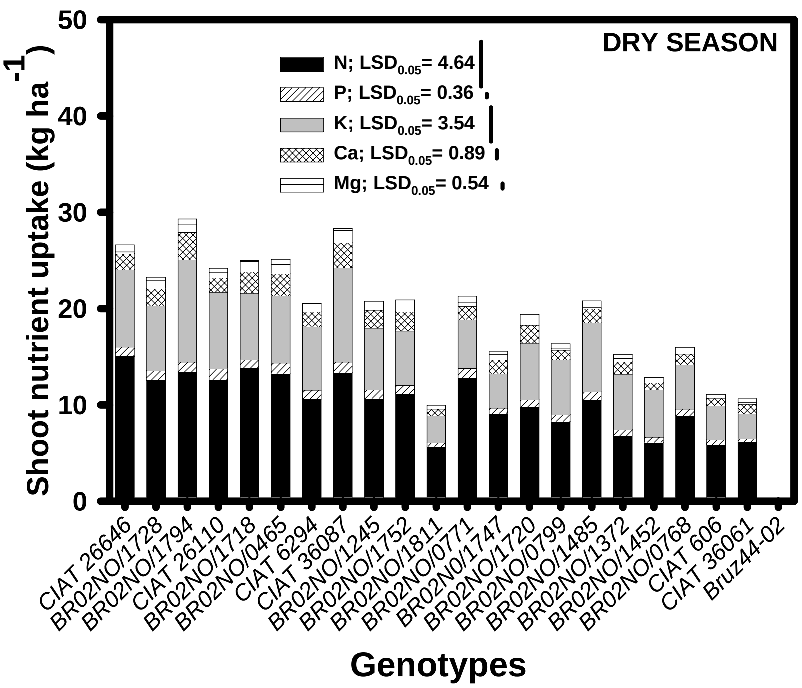


Figure 24. Genotypic variation in living shoot nutrient uptake (N, P, K, Ca and Mg) of three parents (CIAT 606, 6294 and *B.ruziziensis* 44-02), four CIAT's accesions (26110, 26646, 36061 and 36087) and 15 genetic recombinants (BR02NO/0465; 0768; 0771; 0799; 1245; 1372; 1452; 1485; 1718; 1720; 1728; 1747; 1752; 1794 and 1811) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 19 months after establishment (March 2006). LSD values are at the 0.05 probability level. NS = not significant.

superior to not only BR02NO hybrids but also the parents and the 4 checks (Figure 24). Mulato II was the only hybrid cultivar that was close in its nutrient uptake to this hybrid. Nitrogen uptake of the hybrid BR02NO/0771 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 23).

Significant negative correlation coefficients were observed between stem and leaf nutrient (N and P) contents and live forage yield. Adaptation to dry season under infertile acid soil conditions seem to be closely associated with lower amounts of nutrient contents in leaves and stems.

Table 23. Correlation coefficients (r) between green forage yield (t/ha) and other shoot traits of *Brachiaria* genotypes grown with low initial fertilizer application in a sandy loam oxisol in Matazul, Colombia.

Shoot traits	Low fertilizer
Total (live + dead) shoot biomass (t/ha)	0.86***
Dead shoot biomass (t/ha)	0.60***
Leaf biomass (t/ha)	0.96***
Stem biomass (t/ha)	0.89***
Leaf N content (%)	-0.45***
Leaf P content (%)	-0.34***
Stem N content (%)	-0.48***
Stem P content (%)	-0.49***
Shoot N uptake (kg/ha)	0.93***
Shoot P uptake (kg/ha)	0.84***
Shoot K uptake (kg/ha)	0.83***
Shoot Ca uptake (kg/ha)	0.86***
Shoot Mg uptake (kg/ha)	0.83***

Conclusions

Results from this field study indicated that among the 15 *Brachiaria* hybrids tested, BR02NO/1794 and BR02NO/1718 were more productive than the other BR02NO hybrids in terms of green forage (leaf + stem) yield in dry season at 19 months after establishment with low initial fertilizer application and no maintenance fertilizer application. But neither of these hybrids was superior to cv. Mulato II. The superior adaptation of the two hybrids seems to be associated with lower amounts of stem and leaf N and P contents and greater uptake of nutrients.

3.2.3 Root distribution and forage production of adapted *Brachiaria* grasses in acid soils of the savannas of Colombia

Contributors: J. Ricaurte, I. M. Rao, R.García, C.Plazas (CIAT) and J.C. Menjívar (National University, Palmira, Colombia)

Rationale

Brachiariagrasses are key components of agropastoral systems in acid soil savannas of Colombia. Previous research on shoot biomass production and shoot nutrient uptake of *Brachiaria* genotypes in the Llanos of Colombia showed differences in adaptation of *Brachiaria* species to these acid soils. It has been reported that *Brachiaria decumbens* cv. Basilisk, *Brachiaria brizantha* cv. Marandú, *Brachiaria brizantha* cv. Toledo and *Brachiaria* hybrid cv. Mulato 2 could persist and be productive over time with maintenance fertilizer in contrast to *Brachiaria ruziziensis* 44-02 which showed poor adaptation to acid soils after 1 year of establishment. The objective of the present study was to evaluate the differences in root distribution of well adaptated and poorly adapted brachiariagrasses and the impact of root development on forage production over time. We evaluated the impact of 6 *Brachiaria* genotypes and their root attributes (biomass production, length and specific root length) on forage production.

Materials and Methods

A field study was conducted at Matazul farm in the acid soil savannas of Colombia for 4 years. Six *Brachiaria* genotypes with variable adaptation to acid soils (two *Brachiaria* accessions *B.decumbens* CIAT 606 = cv Basilisk, *B.brizantha* CIAT 6294 = cv. Marandú, and four *Brachiaria* hybrids CIAT 36087 = cv. Mulato 2, BR98NO/1251, BR99NO/4015 and BR99NO/4132) were sown with two initial levels of fertilization ([kg ha^{-1}] low = 20 P, 20 K, 33 Ca, 14 Mg and 10 S; high = 80 N, 50 P, 100 K, 66 Ca, 28 Mg, 20 S, 2 Zn, 2 Cu, 0.1 B and 0.1 Mo and half of these amounts were applied for every 2 years as maintenance fertilization). Shoot biomass (forage) production was evaluated for 4 years (at the end of the dry and the rainy season of each year) while root distribution was measured at 33 (dry season) and 41 (rainy season) months after establishment.

Results and Discussion

Differences in production of live shoot biomass and total shoot biomass among the six *Brachiaria* grasses are shown in Figure 25. Significant differences were not observed in shoot biomass production among the 6 *Brachiaria* genotypes that were evaluated at 3 and 10 months after establishment. Mean value of live (forage) shoot biomass production was 5136 and 1979 kg ha^{-1} at 3 months after establishment for initial fertilizer applications of high and low, respectively. These values decreased to 860 and 601 kg ha^{-1} for high and low fertilizer application, respectively at 29 months after establishment. Later, the apomictic natural accession *B. brizantha* CIAT 6294 and the apomictic hybrid cultivar Mulato 2 (CIAT 36087) had significantly more live and total shoot biomass, followed by the apomictic natural accession of *B. decumbens* CIAT 606 and the hybrid BR99NO/4015. Two hybrids, BR99NO/4132 and BR98NO/1251, had markedly lower production of shoot biomass over time when compared to the other genotypes (Figure 25).

Total root length production during rainy season was twice that of the dry season (Table 24). Root

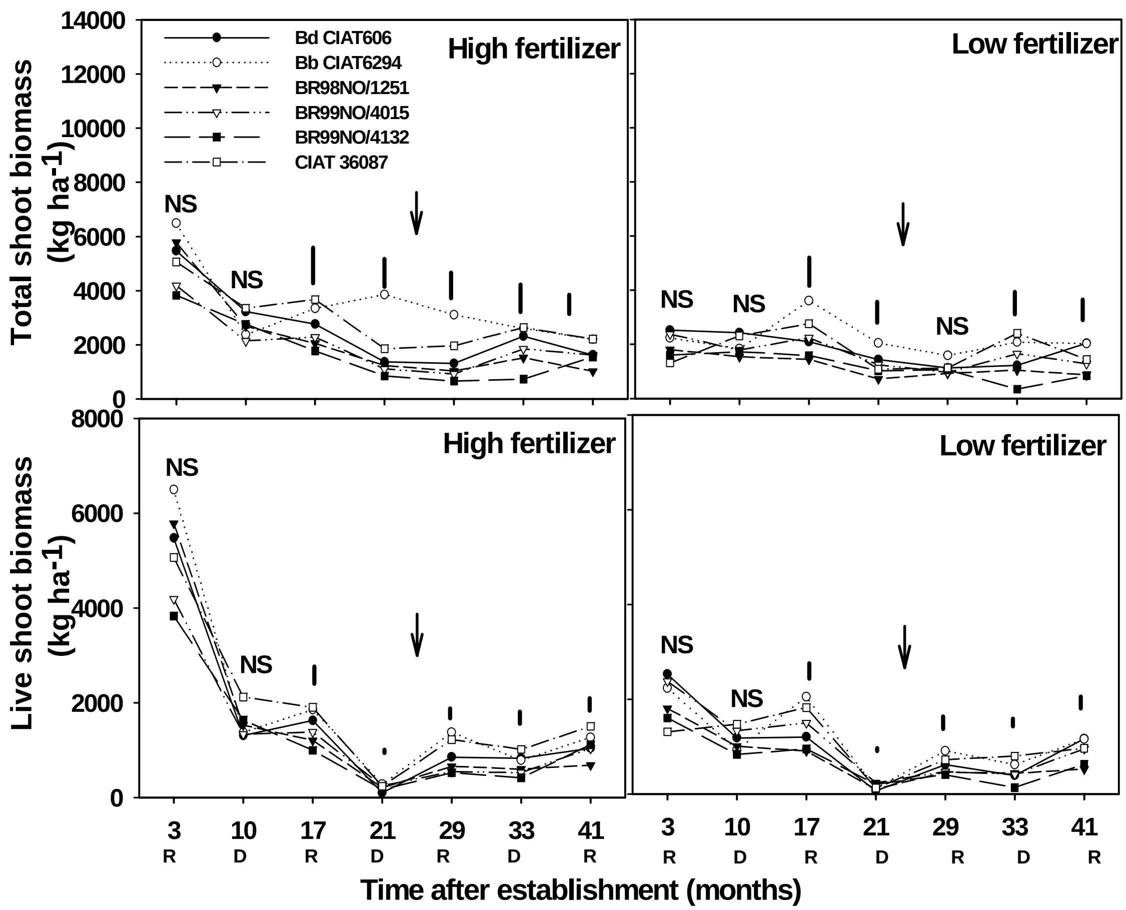


Figure 25. Total (live + dead) and live shoot biomass production (kg ha^{-1}) in 6 *Brachiaria* genotypes evaluated for 4 years. Measurements were made at the end of dry (D) and rainy (R) seasons in each year in an Oxisol of "Altillanura" in Puerto López, Meta (Colombia) with high (High F.) and low (Low F.) initial fertilizer application. The arrows indicate the time of application of maintenance fertilizer at half the levels of initial application. The bars or NS indicate values of $LSD_{p<0.05}$ or no significant differences between genotypes, respectively.

biomass was greater (more than 30%) and finer (25% more specific root length) in the rainy season than in the dry season (Table 24).

Differences among six *Brachiaria* genotypes in root biomass, root length and specific root length distribution across the soil profile at 33 (dry season) and 41 (rainy season) months after establishment are shown in Figure 26. Root biomass and root length decreased at deeper soil profile while the values of specific root length increased indicating the finer root development in deep soil layers. The very well acid soil adapted *B. decumbens* CIAT 606 showed finer root development (higher values of specific root length) in both dry and rainy seasons while the moderately adapted hybrids BR98NO/1251, BR99NO/4015 and BR99NO/4132 developed

Table 24. Total root biomass (kg ha^{-1}), total root length (km m^{-2}) and mean specific root length (m g^{-1}) (0 to 100 cm soil depth) of 6 *Brachiaria* genotypes evaluated at 33 (dry season - D) and 41 (rainy season - R) months after their establishment with high and low initial fertilizer application to an Oxisol of "Altillanura" in Puerto López, Meta (Colombia).

Genotypes	Root biomass (kg ha^{-1})		Root length (km m^{-2})		Specific root length (m g^{-1})		Soil bulk density (g cm^{-3})**	
	High Fert.	Low Fert.	High Fert.	Low Fert.	High Fert.	Low Fert.	High Fert.	Low Fert.
33 months (D)								
Bd CIAT 606	466	550	2.31	2.74	117	125	1.46	1.44
Bb CIAT 6294	1603	1364	4.07	3.38	44	49	1.43	1.42
BR98NO/1251	1391	1304	3.48	4.12	36	51	1.43	1.41
BR99NO/4015	1206	588	2.71	2.74	55	140	1.45	1.45
BR99NO/4132	609	599	3.30	3.46	64	79	1.42	1.42
CIAT 36087	1267	1149	3.28	3.01	51	49	1.43	1.42
Mean	1090	926	3.19	3.24	61	82	1.44	1.43
LSD(P<0.05)*	652	578	NS	NS	49	NS	NS	NS
41 months (R)								
Bd CIAT 606	816	884	4.127	5.734	132	137		
Bb CIAT 6294	1977	1635	5.006	6.298	42	74		
BR98NO/1251	1910	818	8.114	5.512	69	150		
BR99NO/4015	1161	1150	7.831	8.212	135	116		
BR99NO/4132	1313	987	7.942	7.892	103	100		
CIAT 36087	1572	1832	6.799	6.979	76	56		
Mean	1458	1218	6.637	6.771	93	106		
LSD(P<0.05)	786	482	3.071	NS	83	53		

* The least significant difference (LSD) values or not significant (NS) differences with 95% of probability.

** Mean soil bulk density at 0-40 cm depth.

finer root systems in the rainy season. Cultivar Mulato 2 (CIAT 36087) and CIAT 6294 developed thicker root systems both in rainy and dry seasons as revealed by lower values of specific root length.

The root biomass of the fine root genotype *B. decumbens* CIAT 606 was markedly lower than the values observed with the two thick root *Brachiaria* genotypes CIAT 6294 and CIAT 36087 (Table 24, Figure 26).

Soil from these two genotypes showed slightly lower values of bulk density that could result from turnover of thicker roots (Table 24). There is need to evaluate the impact of the adapted brachiariagrasses on soil quality parameters over time.

Conclusions

Results from this study indicate that the apomictic natural accession *B. brizantha* CIAT 6294 and the apomictic hybrid cultivar Mulato 2 (CIAT 36087) could produce greater amounts of live shoot biomass (forage) with maintenance fertilizer application. The productivity of these two genotypes was superior to *B. decumbens* CIAT 606 and this superior performance was associated with coarse root development. There is need to test the genotypic differences in root development and persistence with no maintenance fertilizer application. It is possible that fine root development that was observed with B. decumbens CIAT 6006 across the soil profile might contribute to its superior adaptation to infertile acid soils with no maintenance fertilizer application that is common in acid soil savannas.

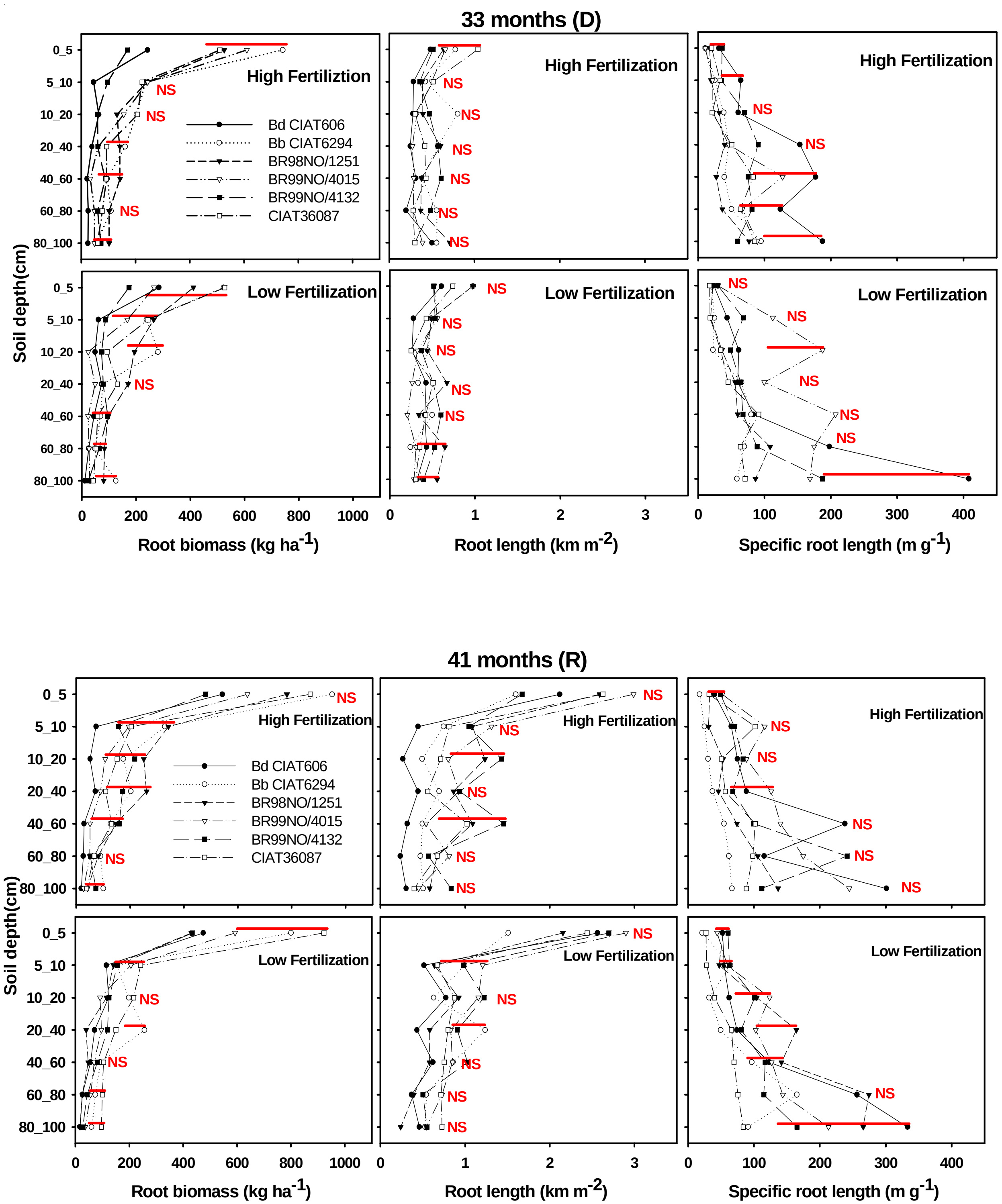


Figure 26. Root biomass (kg ha^{-1}), root length (km m^{-2}) and specific root length (m g^{-1}) distribution across soil profile of 6 *Brachiaria* genotypes evaluated at 33 (dry season - D) and 41 (rainy season - R) months after their establishment with high and low amounts of initial fertilizer application to an Oxisol of "Altillanura" in Puerto López, Meta (Colombia). Bars or NS by depth indicate least significant difference or not significant difference, respectively with 95% of probability.

3.3 Genotypes of Brachiaria with adaptation to poorly drained soils

Highlights

- Implemented a waterlogging screening method and evaluated 48 BR04NO series of hybrids and identified three hybrids (BR04NO/3069, BR04NO/3207 and BR04NO/2774) that were superior in their tolerance to waterlogging based on greater values of green leaf biomass production and leaf chlorophyll content and lower values of dead leaf biomass. These three plant attributes could serve as criteria for selection for waterlogging tolerance in *Brachiaria*

- Screened 109 hybrids of SX05NO series and found in general lower level of waterlogging tolerance in sexual hybrids than the apomictic hybrids and identified four sexual hybrids (SX05NO/1960; SX05NO/2156; SX05NO/2381 and SX05NO/1936) as superior in their level of tolerance to waterlogging based on their ability to maintain green leaf area and green leaf biomass production under stress. The level of waterlogging tolerance in these hybrids was markedly superior to the sexual parent *B.ruziziensis* 44-02.

3.3.1 Genotypic variation in waterlogging tolerance of *Brachiaria* genotypes

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

Rationale

In the tropics, *Brachiaria* pastures during the rainy season occasionally face waterlogging conditions that severely limit pasture productivity and animal performance. Waterlogging or flooding reduces the availability of soil oxygen to the plant. In 2005, we developed a screening method to evaluate waterlogging tolerance of 15 *Brachiaria* hybrids and 5 checks. Waterlogging for 21 days resulted in senescence and death of a great proportion of shoot biomass of majority of hybrids and affected development of adventitious roots in some hybrids. 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. 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. In late 2005, we screened 60 *Brachiaria* genotypes with an objective to quantify genotypic variation in waterlogging tolerance in *Brachiaria*.

Material and Methods

A pot experiment was conducted outside in the Forages patio area of CIAT Palmira between 23 November to 14 December 2005 to determine differences in tolerance to waterlogging among 60 *Brachiaria* genotypes (48 hybrids of BR04NO series; 3 parents - *B. decumbens* CIAT 606; *B. ruziziensis* 44-02; *B. brizantha* CIAT 6294; and 9 checks - *B. humidicola* CIAT 679; *B. humidicola* CIAT 6133; *B. brizantha* CIAT 26110; *Brachiaria* hybrid cv. Mulato CIAT 36061; *Brachiaria* hybrid cv. Mulato II CIAT 36087; BR02NO/1372, BR02NO/1485, BR02NO/1752, BR02NO/1245). Waterlogging treatment was imposed by applying excessive water to the pots (5 cm over soil surface) as shown in Photo 5.

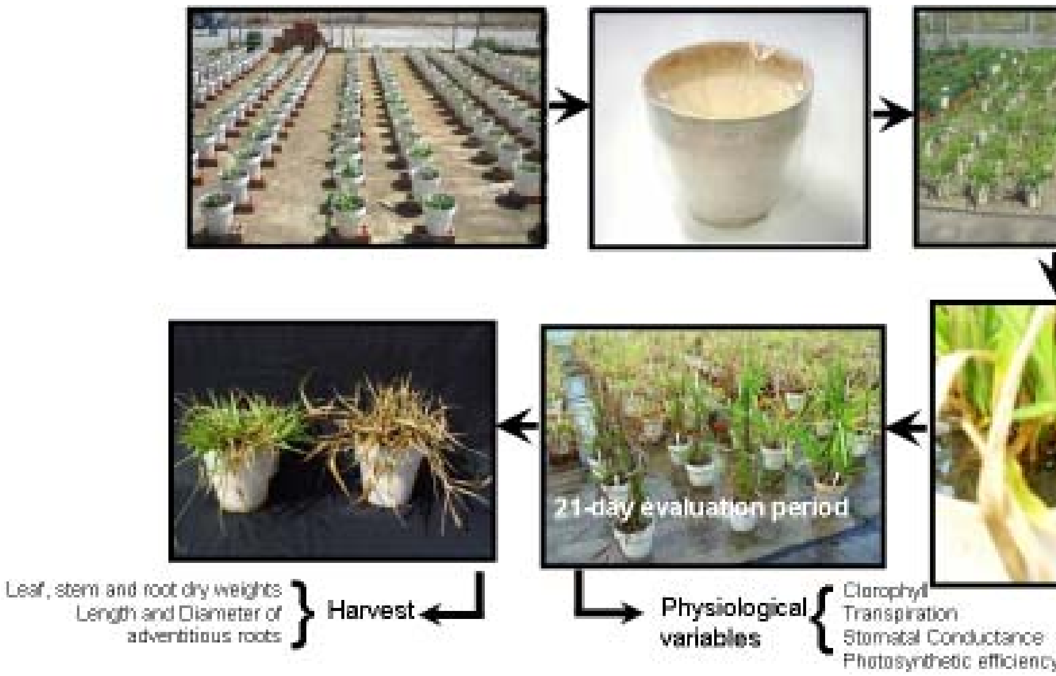


Photo 5. Different steps involved for inducing waterlogging treatment for 21 days and identification of waterlogging tolerant genotypes.

The trial was planted as randomized complete block with 3 replications. 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 amounts of fertilizer were supplied (kg ha^{-1}: 80 N, 50 P, 100 K, 66 Ca, 28 Mg, 20 S, 2 Zn, 2 Cu, 0.1 B and 0.1 Mo) to soil at the time of planting. Plants grew for 50 days under 100% field capacity of soil moisture.

Leaf chlorophyll content (in SPAD units) and leaf photosynthetic efficiency (Fv/Fm) were measured at weekly intervals for 3 weeks on a full expanded young leaf marked at the initiation of waterlogging treatment. After 21 days of waterlogging treatment, green leaf biomass (g pot^{-1}), total shoot and root biomass (g pot^{-1}), root volume (cm^3) and root length (m pot^{-1}) and mean root diameter (mm) of adventitious roots were measured.

Results and Discussion

The maximum temperature during the experimental period ranged from 26.1 to 30.6 °C while the minimum temperatures were 16 to 20.2 °C. The solar radiation was 10.6 to 21.4 MJ m^{-2} d^{-1}. At 7 days after of establishment of waterlogging stress, the majority of plants turned chlorotic. At 21 days after waterlogging treatment, several hybrids were dead. Significant genotypic variability in green leaf biomass was observed after 21 days of waterlogging treatment (Figure 27).

Two checks, *B. humidicola* CIAT 6133, BR02NO/1485, were outstanding in green leaf biomass production (Figure 26). Among the BR04NO series of hybrids tested, three hybrids BR04NO/3069, BR04NO/3207 and BR04NO/2774 were superior in their production of green leaf biomass than the others hybrids (Figure 27).

One of these three hybrids, BR04NO/3207 showed higher value of stomatal conductance and transpiration (data not shown) allowing green leaf growth during waterlogging stress (Figure 28). As expected, *B. humidicola* CIAT 679 and *B. humidicola* CIAT 6133 showed greater values of stomatal conductance and green leaf dry weight (Figure 27). But several hybrids also showed greater values of stomatal conductance while green leaf dry weight of those hybrids was lower.

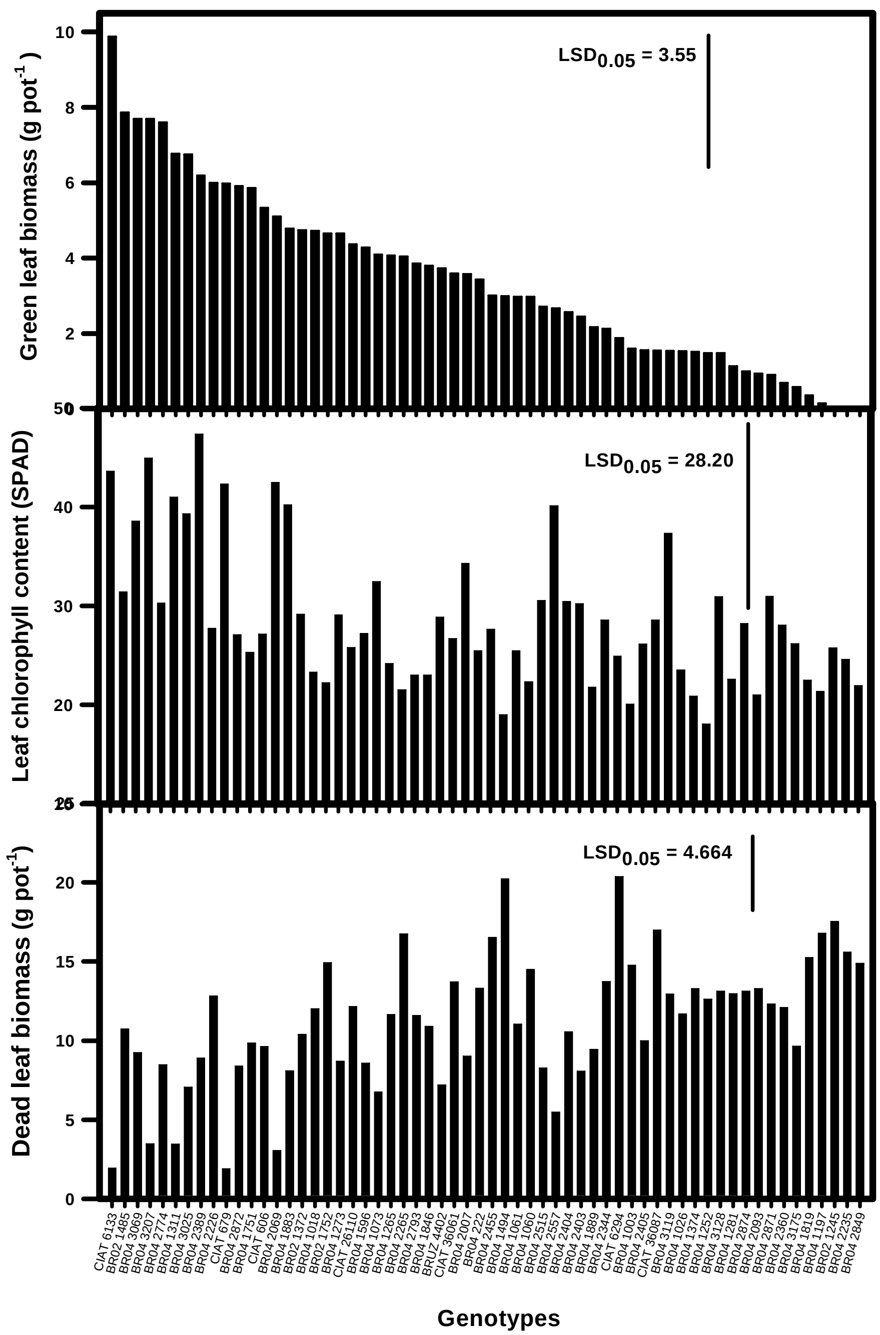


Figure 27. Influence of waterlogging on genotypic variation in green leaf biomass production, leaf chlorophyll content (SPAD) and dead leaf biomass (g pot^{-1}) of three parents (CIAT 606, 6294, *B.ruziziensis* 44-02), nine checks (CIAT 6133, 679, 26110, 36061 and 36087, BR02NO/1485, 1752, 1245, 1372) and 48 hybrids, of population BR04 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.

Leaf chlorophyll content measured as SPAD values also indicated that the genotypes that had greater amount of green leaf biomass also had higher values of SPAD, as expected (Figure 27). Genotypes that had greater amount of green leaf biomass and higher values of SPAD also showed less amount of dead leaf biomass (Figure 27).

These results indicated that greater values of green leaf biomass together with higher values of SPAD and lower values of dead leaf biomass could be good indicators of waterlogging tolerance.

Results on dry matter partitioning after waterlogging showed that cv. Mulato II (CIAT 36087) produced greater amount of root biomass but very small amount of green leaf biomass indicating that total root biomass production under waterlogging is not a good indicator of adaptation (Figure 28).

One of the checks, CIAT 679 which is very tolerant to waterlogging produced very small amount of root biomass. Among the three hybrids (BR04NO/3069, BR04NO/3207 and BR04NO/2774) that were superior in their

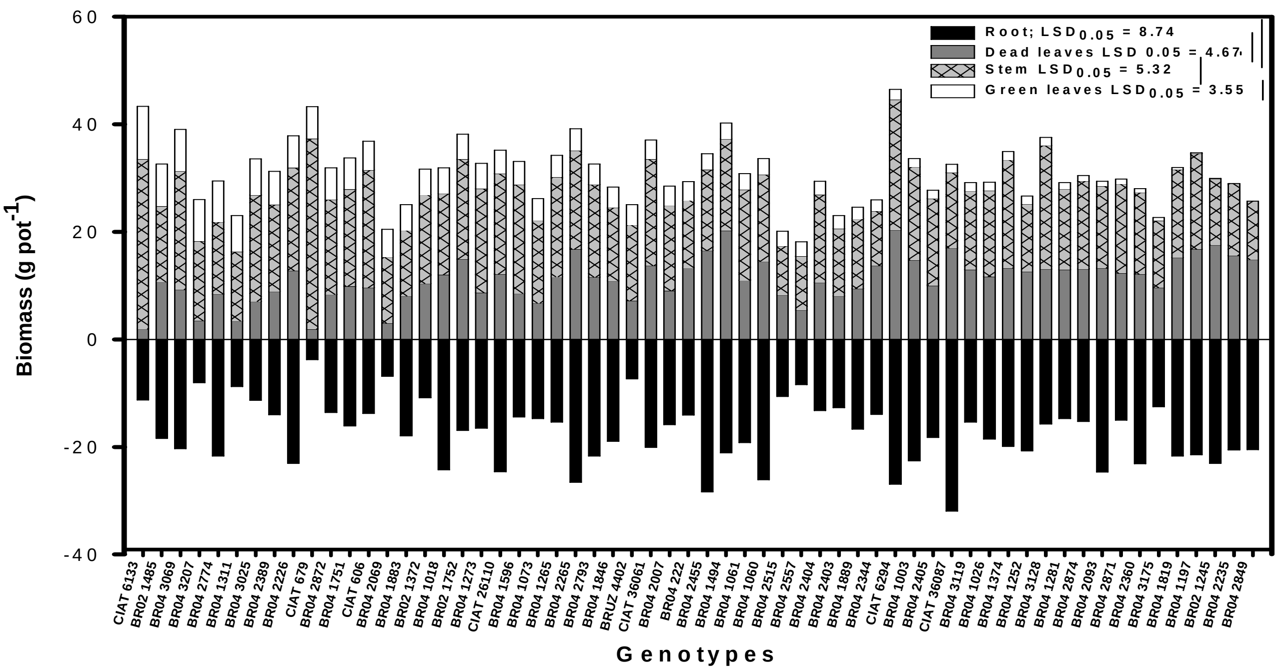


Figure 28. Influence of waterlogging on genotypic variation in biomass distribution among leaves, stems and root production of three parents (CIAT 606, 6294, *B.ruziziensis* 44-02), (CIAT 6133, 679, 26110, 36061 and 36087, BR02NO/1485, 1752, 1245, 1372) and 48 hybrids, of population BR04 of *Brachiaria*, 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.

production of green leaf biomass, BR04NO/3207 showed lower amount of root biomass. Results on adventitious root length showed marked genotypic variation but the genotypes that produced greater amounts of green leaf biomass showed less length of adventitious roots (Figure 29).

Genotypes that showed intermediate level of green leaf production produced greater length of adventitious roots. Results on mean root diameter of adventitious roots showed that some genotypes that produced greater leaf biomass also had greater values of mean root diameter of adventitious roots.

All the genotypes developed adventitious roots, except the hybrid BR04NO/2235 that also had no green leaf biomass production.

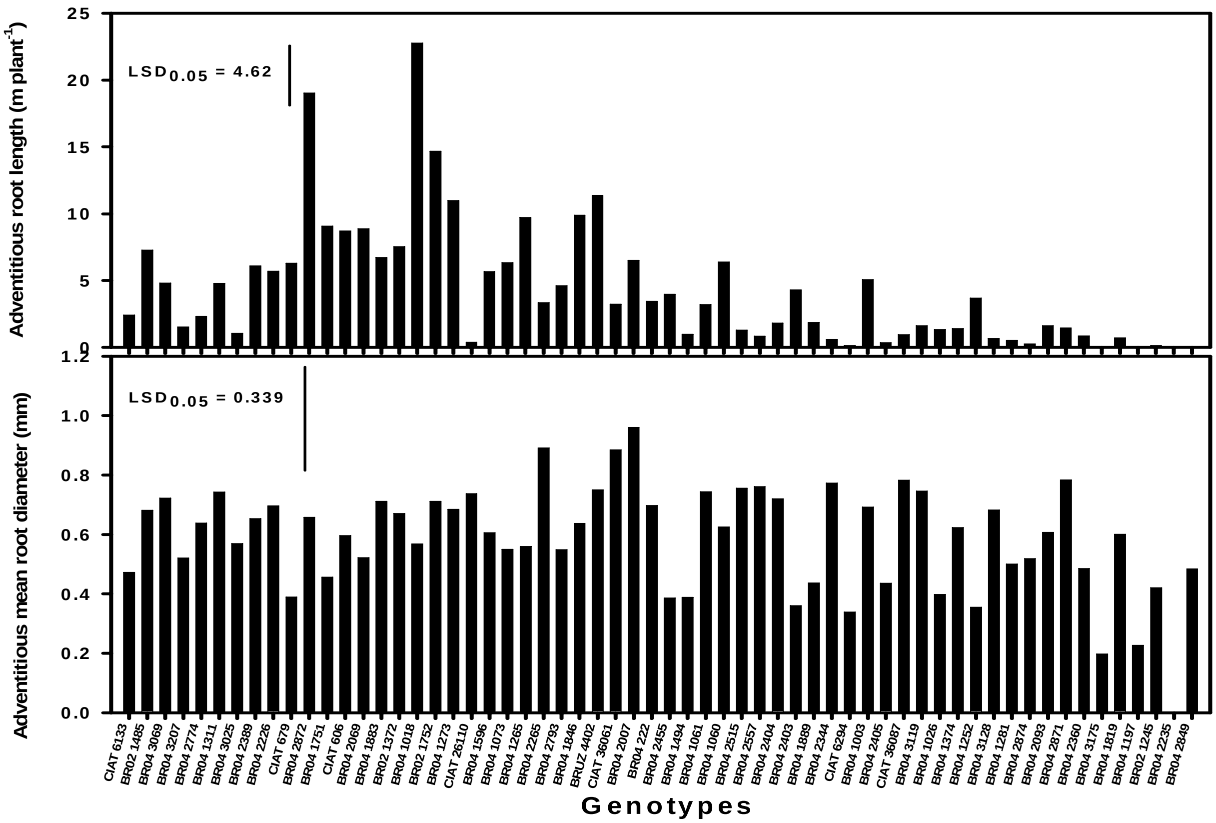


Figure 29. Influence of waterlogging on genotypic variation in adventitious root length production (m pot^{-1}) and mean root diameter of three parents (CIAT 606, 6294, *B.ruziziensis* 44-02), (CIAT 6133, 679, 26110, 36061 and 36087, BR02NO/1485, 1752, 1245, 1372) and 48 hybrids, of population BR04 of *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.

These results on adventitious root development during waterlogging indicated that this could be a strategy to overcome hypoxia but not all genotypes seem to use this as a mechanism. Correlation coefficients between green leaf biomass production and other plant attributes are shown in Table 25.

A higher value of positive correlation was found with leaf chlorophyll content (SPAD) while a higher value of negative correlation was observed with dead leaf biomass. Negative correlation was observed between green leaf weight and total root weight.

This indicates that the genotypes that adapt better to waterlogging conditions change the partitioning of biomass in favor of shoot growth at the expense of root growth.

Table 25. Correlation coefficients (r) between green leaf dry weight (g $plant^{-1}$) and other shoot traits of 60 *Brachiaria* genotypes grown under waterlogging in an oxisol from Santander de Quilichao, Colombia.

Plant traits	Waterlogging
Total chlorophyll content (SPAD)	0.649**
Dead leaf biomass (g $plant^{-1}$)	-0.576**
Leaf photosynthetic efficiency (Fv/Fm)	0.413**
Stomatal conductance (mmol $m^{-2}s^{-1}$)	0.392**
Transpiration (mmol $m^{-2}s^{-1}$)	0.392**
Stem biomass (g $plant^{-1}$)	0.028
Total root weight (g $plant^{-1}$)	-0.238**
Adventitious root dry weight (g $plant^{-1}$)	0.453**
Adventitious root length (m $plant^{-1}$)	0.417**
Adventitious mean root diameter (mm)	0.213**

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

Conclusions

We implemented the screening method for waterlogging tolerance and screened 48 BR04NO series of hybrids and identified three hybrids (BR04NO/3069, BR04NO/3207 and BR04NO/2774) that were superior in their tolerance to waterlogging based on greater values of green leaf biomass production and leaf chlorophyll content and lower values of dead leaf biomass. These three plant attributes could serve as criteria for selection for waterlogging tolerance in *Brachiaria*.

3.3.2 Genotypic variation in waterlogging tolerance of sexual hybrids of *Brachiaria*

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

Rationale

Evaluation of 48 BR04NO series of apomictic and sexual hybrids resulted in identification of three hybrids (BR04NO/3069, BR04NO/3207 and BR04NO/2774) that were superior in their tolerance to waterlogging. These hybrids showed greater values of green leaf biomass production and leaf chlorophyll content and lower values of dead leaf biomass after 21 days of waterlogging treatment. We suggested that these three plant attributes could serve as criteria for selection for waterlogging tolerance in *Brachiaria*. During 2006, we screened 121 *Brachiaria* genotypes including 109 sexual hybrids of SX05NO series with an objective to quantify the extent of genetic variability for waterlogging tolerance in *Brachiaria*.

Material and Methods

A pot experiment was conducted outside in the Forages patio area of CIAT Palmira between May 26 to June 20, 2006 to determine differences in tolerance to waterlogging among 121 *Brachiaria* genotypes (109 hybrids – SX05NO series; 3 parents - *B. decumbens* CIAT 606; *B. ruziziensis* 44-02; *B. brizantha* CIAT 6294; and 9 checks – *B. humidicola* CIAT 679; *B. humidicola* CIAT 6133; *B. brizantha* CIAT 26110; Brachiaria hybrid cv. Mulato CIAT 36061; Brachiaria hybrid cv. Mulato II CIAT 36087; BR02NO/1485; BR02NO/1372; BR02NO/1245; BR02NO/1752). The trial was planted as completely randomized block with 3 replications. Each experimental unit consisted of one pot filled with 3.5 kg of fertilized top's 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. Plants were grown for 50 days under field capacity conditions. Waterlogging treatment was imposed by an excessive water supply (5 cm over soil surface) for 24 days. After inducing waterlogging treatment, leaf chlorophyll content (SPAD), stomatal conductance (mmol $m^{-2}s^{-1}$), transpiration rate (mmol $m^{-2}s^{-1}$) were measured at weekly intervals on a full-expanded young leaf that was marked at the initiation of waterlogging treatment. At the end of the 24 days of treatment, green leaf area (cm^2 $plant^{-1}$), green leaf biomass (g $plant^{-1}$), total shoot and root biomass (g $plant^{-1}$), root volume (cm^3), root length (m $plant^{-1}$) and mean root diameter (mm) of adventitious roots were measured.

Results and Discussion

The maximum temperature during the experimental period ranged from 27 to 31.3 °C while the minimum temperature ranged from 17 to 20.4 °C. The solar radiation was 9.5 to 23.1 MJ m^{-2} d^{-1}. At 15 days after waterlogging treatment was imposed, some genotypes showed senescence and death of the leaves. A marked genotypic variation in waterlogging tolerance was observed among the sexual hybrids based on green leaf biomass, green leaf area, leaf chlorophyll content and dead leaf biomass (Table 26).

Table 26. Green leaf biomass, green leaf area , total chlorophyll content, dead leaf biomass and dead leaf biomass/green leaf biomass ratio of 121 *Brachiaria* genotypes after 24 days of waterlogging in an oxisol from Santander de Quilichao, Colombia. NN = no number.

Genotype	Green leaf biomass (g plant^{-1})	Green leaf area (cm^2 plant^{-1})	Total chlorophyll content (SPAD)	Dead leaf biomass (cm^2 plant^{-1})	Dead leaf biomass/ green leaf biomass ratio
Parents					
CIAT 606	3.89	331	21.4	6.11	1.6
Bruz 4402	2.38	167	21.8	11.83	5.0
CIAT 6294	8.79	976	31.0	6.29	0.7
Checks					
BR02NO/1245	5.86	626	22.0	4.06	0.7
BR02NO/1372	5.26	513	26.2	7.55	1.4
BR02NO/1485	2.63	206	5.9	17.76	6.7
BR02NO/1752	3.69	394	12.6	13.62	3.7
CIAT 26110	20.05	1483	37.1	5.17	0.3
CIAT 36061	3.31	362	10.8	10.06	3.0
CIAT 36087	4.53	477	18.4	13.33	2.9
CIAT 6133	10.55	1001	34.6	0.84	0.1
CIAT 679	6.03	574	28.6	2.58	0.4
Hybrids					
SX05NO/00NN	2.89	424	28.1	2.56	0.9
SX05NO/1894	0.99	87	9.0	12.50	12.7
SX05NO/1895	0.96	74	7.5	8.58	8.9
SX05NO/1901	2.77	148	16.3	7.69	2.8
SX05NO/1918	4.70	431	10.9	10.99	2.3
SX05NO/1931	3.81	333	24.2	7.40	1.9
SX05NO/1936	**4.93**	**519**	**14.2**	**7.14**	**1.4**
SX05NO/1940	3.93	439	7.4	11.81	3.0
SX05NO/1943	0.07	14	0.0	11.83	177.5
SX05NO/1953	1.00	106	0.2	13.47	13.5
SX05NO/1955	3.67	341	8.9	8.90	2.4
SX05NO/1960	**5.84**	**640**	**10.4**	**16.48**	**2.8**
SX05NO/1968	3.41	327	19.7	7.33	2.2
SX05NO/1981	3.13	379	19.0	3.97	1.3
SX05NO/1990	0.00	0	1.5	15.63	0.0
SX05NO/1993	0.84	73	2.4	13.75	16.4
SX05NO/2008	0.85	73	5.3	9.58	11.3
SX05NO/2011	2.48	322	18.3	9.01	3.6
SX05NO/2019	1.72	163	9.2	7.40	4.3
SX05NO/2026	1.35	100	1.3	15.97	11.8
SX05NO/2033	0.95	100	9.9	6.75	7.1
SX05NO/2061	3.30	355	23.9	6.90	2.1
SX05NO/2072	2.20	194	3.9	16.82	7.6
SX05NO/2074	0.46	41	2.8	10.25	22.5
SX05NO/2081	4.05	368	30.5	3.46	0.9
SX05NO/2107	2.63	230	7.7	9.91	3.8
SX05NO/2112	1.11	170	6.9	16.27	14.7
SX05NO/2120	2.40	216	24.8	7.47	3.1
SX05NO/2124	0.00	0	6.9	16.50	0.0
SX05NO/2130	0.34	31	12.1	8.23	24.0
SX05NO/2139	1.63	172	14.9	9.68	5.9
SX05NO/2143	0.14	19	0.6	16.74	119.5
SX05NO/2151	0.00	0	4.5	14.43	0.0
SX05NO/2155	0.65	75	4.8	11.31	17.3

Continues….

Table 26. Green leaf biomass, green leaf area , total chlorophyll content, dead leaf biomass and dead leaf biomass/green leaf biomass ratio of 121 *Brachiaria* genotypes after 24 days of waterlogging in an oxisol from Santander de Quilichao, Colombia. NN = no number.

Genotype	Green leaf biomass (g plant^{-1})	Green leaf area (cm^2 plant^{-1})	Total chlorophyll content (SPAD)	Dead leaf biomass (cm^2 plant^{-1})	Dead leaf biomass/ green leaf biomass ratio
SX05NO/2156	**5.20**	**529**	**22.7**	**3.36**	**0.6**
SX05NO/2158	2.49	325	17.8	7.12	2.9
SX05NO/2167	0.00	0	6.4	9.14	0.0
SX05NO/2177	2.02	174	2.9	7.82	3.9
SX05NO/2178	3.15	310	6.8	12.02	3.8
SX05NO/2198	2.72	368	8.1	12.31	4.5
SX05NO/2199	2.00	161	15.7	13.60	6.8
SX05NO/2202	3.06	381	1.2	10.00	3.3
SX05NO/2203	0.30	43	0.0	13.39	44.6
SX05NO/2208	0.19	25	6.4	6.87	36.2
SX05NO/2223	1.87	173	5.6	9.49	5.1
SX05NO/2225	3.39	214	23.3	4.97	1.5
SX05NO/2229	3.22	278	13.3	8.93	2.8
SX05NO/2233	1.22	133	6.6	9.44	7.7
SX05NO/2247	1.63	133	13.5	10.17	6.2
SX05NO/2252	0.00	0	6.2	8.10	0.0
SX05NO/2257	1.06	105	18.5	4.89	4.6
SX05NO/2265	0.92	101	20.1	9.85	10.7
SX05NO/2266	0.36	53	15.4	9.15	25.4
SX05NO/2270	0.00	0	1.8	8.84	0.0
SX05NO/2284	3.83	427	10.1	9.67	2.5
SX05NO/2293	2.00	178	7.4	10.77	5.4
SX05NO/2304	3.31	304	19.4	4.22	1.3
SX05NO/2313	1.09	166	0.3	7.93	7.3
SX05NO/2314	1.78	166	23.3	6.64	3.7
SX05NO/2319	0.16	21	10.1	11.33	70.8
SX05NO/2325	0.66	95	8.4	8.84	13.3
SX05NO/2332	2.50	377	5.4	8.70	3.5
SX05NO/2338	1.91	222	11.2	11.48	6.0
SX05NO/2340	2.06	198	1.8	10.60	5.2
SX05NO/2341	0.82	84	7.3	6.92	8.4
SX05NO/2353	0.55	73	7.8	11.30	20.7
SX05NO/2358	2.38	271	2.4	11.41	4.8
SX05NO/2362	1.34	142	12.2	10.02	7.5
SX05NO/2369	1.92	230	12.7	5.03	2.6
SX05NO/2375	2.36	329	31.3	2.01	0.9
SX05NO/2381	**5.13**	**494**	**27.4**	**4.58**	**0.9**
SX05NO/2382	1.84	185	25.4	3.33	1.8
SX05NO/2383	0.31	40	12.2	7.94	25.6
SX05NO/2386	1.02	106	11.4	8.95	8.8
SX05NO/2388	1.54	171	8.7	8.19	5.3
SX05NO/2389	0.67	135	5.8	11.33	16.9
SX05NO/2392	1.37	133	27.8	3.35	2.4
SX05NO/2393	0.65	63	6.5	12.08	18.5
SX05NO/2399	2.20	250	7.7	5.95	2.7
SX05NO/2403	0.00	0	5.1	9.95	0.0
SX05NO/2408	0.31	30	13.8	10.80	35.4
SX05NO/2413	3.25	369	16.5	11.20	3.5

Continues…

Table 26. Green leaf biomass, green leaf area , total chlorophyll content, dead leaf biomass and dead leaf biomass/green leaf biomass ratio of 121 *Brachiaria* genotypes after 24 days of waterlogging in an oxisol from Santander de Quilichao, Colombia. NN = no number.

Genotype	Green leaf biomass (g $plant^{-1}$)	Green leaf area (cm^2 $plant^{-1}$)	Total chlorophyll content (SPAD)	Dead leaf biomass (cm^2 $plant^{-1}$)	Dead leaf biomass/ green leaf biomass ratio
SX05NO/2414	2.61	280	15.6	9.02	3.5
SX05NO/2417	0.48	47	9.5	10.43	21.6
SX05NO/2423	1.36	210	30.6	1.94	1.4
SX05NO/2424	0.45	55	12.5	11.28	25.3
SX05NO/2425	1.01	94	11.9	7.06	7.0
SX05NO/2428	1.37	187	36.8	2.42	1.8
SX05NO/2450	0.00	0	2.4	12.35	0.0
SX05NO/2454	1.25	171	0.6	17.95	14.4
SX05NO/2473	1.81	165	36.4	3.64	2.0
SX05NO/2485	1.25	136	18.9	5.93	4.8
SX05NO/2494	0.95	113	15.4	7.63	8.0
SX05NO/2497	3.97	417	22.3	8.23	2.1
SX05NO/2502	2.54	381	12.1	9.95	3.9
SX05NO/2504	2.69	306	2.5	13.19	4.9
SX05NO/2507	2.02	73	10.2	13.90	6.9
SX05NO/2508	1.26	101	28.2	7.94	6.3
SX05NO/2511	2.38	295	31.2	5.35	2.3
SX05NO/2517	3.36	398	21.5	5.61	1.7
SX05NO/2525	4.04	381	33.2	2.99	0.7
SX05NO/2528	4.29	460	15.6	6.95	1.6
SX05NO/2530	3.24	200	26.5	5.30	1.6
SX05NO/2539	3.18	409	21.1	5.23	1.6
SX05NO/2541	2.20	243	22.7	3.88	1.8
SX05NO/2545	1.64	143	8.5	9.16	5.6
SX05NO/2547	3.68	335	20.5	7.31	2.0
SX05NO/2550	0.23	33	1.1	9.83	43.4
SX05NO/2558	2.34	343	11.7	8.96	3.8
Mean	**2.34**	**238**	**13.8**	**8.95**	**9.7**
LSD $_{0.05}$	2.19	283	11.95	4.02	

In general, the level of waterlogging tolerance in sexual hybrids appeared to be lower than the apomictic hybrids (see activity 3.3.1) and this could be due to high sensitivity of the sexual parent, Bruz 44-02 to waterlogging.

Four sexual hybrids (SX05NO/1960; SX05NO/2156; SX05NO/2381 and SX05NO/1936) were superior in their level of tolerance to waterlogging based on their ability to produce green leaf area and green leaf biomass production under stress. These hybrids also shoed higher values of leaf chlorophyll content and lower proportion of dead leaf biomass compared with green leaf biomass (Table 26).

The relationship between the green leaf biomass and dead leaf biomass indicated that the genotypes that combine greater values of green leaf biomass with lower values of dead leaf biomass could be considered as tolerant to waterlogging (Figure 30). Based on these criteria, *B. humidicola* CIAT 6133 and *B. brizantha* CIAT 26110 cv. Toledo were outstanding in their level of waterlogging tolerance.

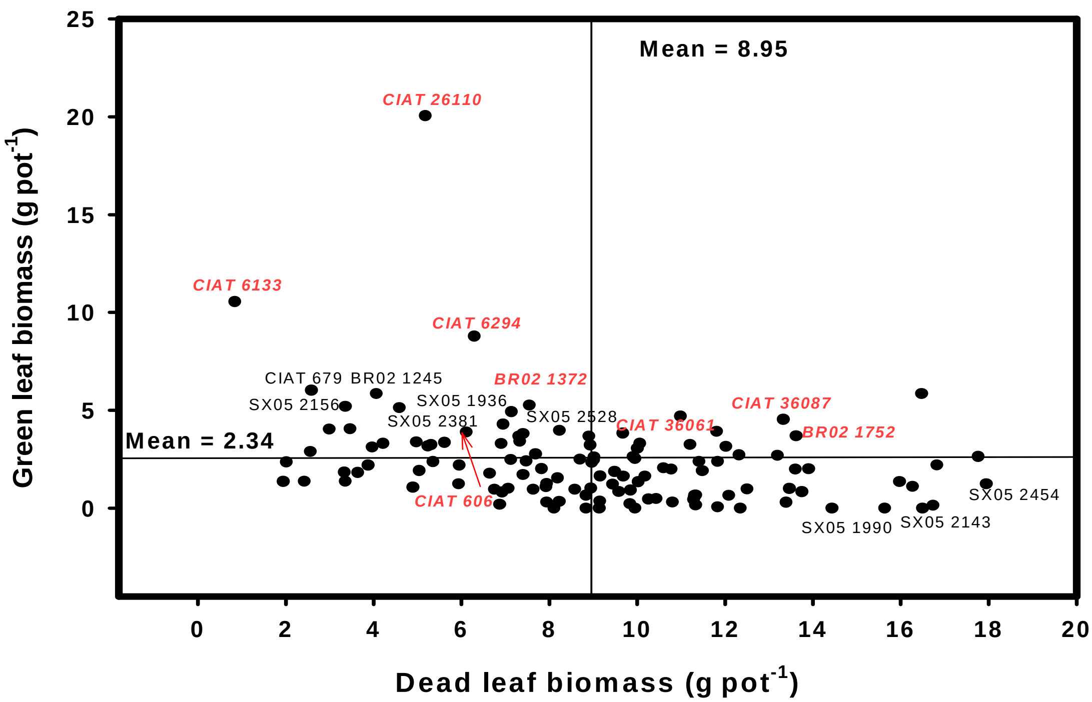


Figure 30. Relationship between green leaf biomass and dead leaf biomass of 109 sexual hybrids, 3 parents and 9 checks of *Brachiaria* after 24 days of waterlogging in an Oxisol from Santander de Quilichao, Colombia.

Table 27. Correlation coefficients (r) between green leaf biomass (g plant^{-1}) and other shoot traits of 121 *Brachiaria* genotypes under waterlogging in an oxisol from Santander de Quilichao, Colombia.

Plant traits	Waterlogging
Total chlorophyll content (SPAD)	0.537**
Stomatal conductance (mmol m^{-2}s^{-1})	0.366**
Transpiration (mmol m^{-2}s^{-1})	0.365**
Green leaf area (cm^2 plant^{-1})	0.944**
Stem biomass (g plant^{-1})	0.180**
Dead leaf biomass (g plant^{-1})	-0.355**

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

As expected, green leaf biomass showed very high positive correlation with green leaf area (Table 27).

Total chlorophyll content showed positive association and dead leaf biomass showed negative association with the green leaf biomass. Stomatal conductance and transpiration rate also showed positive association with green leaf biomass indicating that the higher values of stomatal conductance and transpiration contributed to improved growth of green leaves (Table 27).

Conclusions

We screened 109 hybrids of SX05NO series and found in general lower level of waterlogging tolerance in sexual hybrids than the apomictic hybrids and identified four sexual hybrids (SX05NO/1960; SX05NO/2156; SX05NO/2381 and SX05NO/1936) as superior in their level of tolerance to waterlogging based on their ability to maintain green leaf area and green leaf biomass production under stress. The level of waterlogging tolerance in these hybrids was markedly superior to the sexual parent Bruz 44-02. This pot study clearly showed that there is marked genotypic variation in waterlogging tolerance in *Brachiaria* and we could quantify the differences in waterlogging tolerance using the parameters of green leaf biomass, green leaf area, leaf chlorophyll content, dead leaf biomass and the proportion of dead leaf biomass to green leaf biomass.

3.4 Biological Nitrification Inhibition (BNI) in tropical grasses

Highlights

- Developed a highly sensitive bioassay using a recombinant luminescent *Nitrosomonas europaea*, that can detect and quantify the amount of the biological nitrification inhibition (BNI activity) produced by plants. A number of species including tropical and temperate pastures, cereals and legumes were tested for BNI activity in their root exudates and the results indicated that BNI activity varies widely among and within species, and that some degree of BNI capacity is likely a widespread phenomenon in many tropical pasture grasses. We suggest that the BNI capacity could either be managed and/or introduced into pastures/crops with limited expression of this phenomenon, via genetic improvement approaches that combine high productivity with the capacity to regulate soil nitrification process.

- Screening of 43 accessions of *Brachiaria humidicola* for specific and total biological nitrification inhibitory (BNI) activity resulted in quantifying genetic diversity in BNI and in identifying contrasting accessions with very high (CIAT 26573) and low BNI activity (CIAT 16880). Root biomass production showed negative association with specific BNI activity

- After four cycles of analyses for BNI activity in a field trial results indicate that nitrification rates were lower with the two accessions of *Brachiaria humidicola* than the accession of *Panicum maximum*. The soil incubation method used for this study to estimate nitrification rates seem to be highly sensitive to detect even small differences in nitrification rates among the grasses.

3.4.1 Biological nitrification inhibition (BNI) is a widespread phenomenon

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Nitrification, a microbial process, is a key component and integral part of the soil-nitrogen (N) cycle determining how N is absorbed, utilized or dispersed into the environment, all of which have large implications as to plant productivity and environmental quality. During nitrification, the relatively immobile NH_4^+ is converted to the highly mobile NO_3^-. This process strongly influences N utilization by plants, because the NO_3^- formed, is in many situations highly susceptible to loss from the root zone by leaching and/or denitrification. The loss of N from the root zone can also have large economic implications, valued at a US $ 15 billion annually fertilizer, along with environmental consequences such as nitrate pollution of ground water and eutrophication of surface waters. Management of nitrification by the application of chemicals is presently one of the few strategies available for improving N recovery, agronomic N-use efficiency (NUE) and limiting environmental pollution in some crops.

Suppression of soil nitrification has been observed to occur naturally in some ecosystems, termed biological nitrification inhibition (BNI) indicating that the inhibition originated from the plants in the ecosystem. The conservation of N and the resulting improved N status through BNI (this type of nitrification inhibition) is hypothesized as the driving force for the development of low-NO_3^- ecosystems. Some recent studies suggest that certain grass populations are able to influence nitrification in soil. Several researchers based on empirical studies also indicated that some tropical pasture grasses possibly inhibit nitrification. However, the concept remained largely unsupported because it was not feasible to conclusively demonstrate *in situ* inhibition of nitrification by chemicals released in the plant-soil system, due to lack of an appropriate methodology.

Recently, an assay using recombinant luminescent *Nitrosomonas europaea* has been developed at JIRCAS, Japan that can detect and quantify nitrification inhibitors released in the root zone. The present investigation was aimed at quantifying the inter- and intra-specific variability in BNI from various plant species, including pastures and field crops. This study focused primarily on BNI in tropical forage grasses, in particular *Brachiaria* species, considered to be well adapted to low-N environments of South American Savannas. A number of field crops were also included in the study to determine the likelihood of the widespread occurrence of BNI, as published information is not available in relation to the distribution of BNI ability.

The capacity to inhibit nitrification through the release of BNI activity from roots appears to be widespread among tropical pasture grasses such as *Brachiaria* spp. Among tropical pasture grasses, *B. humidicola* and *B. decumbens* have the highest BNI activity released from their roots. Among the tested cereal and legume crops sorghum, pearl millet, and peanut showed some degree of BNI capacity. Substantial genotypic variability for BNI was found in *B. humidicola* germplasm. Several high- and low-BNI genotypes were identified. Soils collected from high-BNI types showed little or no nitrification. This is in contrast to the soils from low-BNI types, which showed average nitrification (i.e. most of N in the soil converted to NO_3^-). The BNI activity from high-BNI type when added to soils showed a stable long-term inhibitory effect on nitrification. The more BNI activity added to the soil the greater was the inhibitory effect on soil nitrification. The BNI activity from high-BNI types had a stronger inhibitory effect on the HAO pathway than on the AMO pathway of *Nitrosomonas*, whereas BNI activity from standard cultivar (which is a medium-BNI type) had similar inhibitory effects on both enzymatic pathways.

Our results demonstrate that BNI may be widespread among plants with significant inter- and intra-specific variability. Thus, this genetically controlled BNI function could have the potential to be managed and/or introduced into pasture grasses/crops that do not exhibit the phenomenon *via* genetic improvement approaches that combine high productivity with the capacity to regulate soil nitrification.

3.4.2 Improvement of a bioluminescence assay using *Nitrosomonas europea* at CIAT to detect biological nitrification inhibition (BNI) activity from root exudates

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Rationale

Nitrification is a soil biological process where ammonium nitrogen (NH_4^+-N) is transformed into NO_2-N (by ammonia-oxidizing bacteria) and subsequently to NO_3-N (by nitrite-oxidizing bacteria). The NO_3 ion, unlike NH_4^+, is highly mobile in the soil, and thus is often leached from crop rooting zones by rain or irrigation. The nitrification process is performed by soil bacterial species including *Nitrobacter*. In this process, more than 70% of the fertilizer N used worldwide in agricultural systems is believed to be wasted due to nitrification-associated N losses. It is important to minimize the loses of N in crop-livestock systems by regulating the biological nitrification inhibition (BNI) process. Regulation of BNI in agricultural systems will improve N use efficiency.

Recently, JIRCAS, Japan has demonstrated that a tropical pasture grass *Brachiaria humidicola*, has the ability to inhibit nitrification using root exudates by a bioluminescence assay. The original assay was developed by Iizumi's group in Japan that used a recombinant *Nitrosomonas* strain which produced bioluminescence due to the expression of a *luxAB* gene. CIAT has been collaborating with JIRCAS on BNI. To extend CIAT's capacity for screening a large number of plant materials for BNI activity we adapted and modified the bioluminescence assay protocol developed at JIRCAS, Japan.

Materials and Methods

Bacterial strain and growth conditions: The recombinant *Nitrosomas europea* ATCC 19178 harbouring a gene cassette containing a *luxAB* gene, a gift from JIRCAS, Japan was grown aerobically at 30 °C in the dark in HEPES culture medium containing 4.0 g $(NH_4)_2SO_4$, 1.0 g KH_2PO_4, 11.93 g HEPES, 1.0 g $NAHCO_3$, 200mg $MgSO_4.7H_2O$, 10 mg $CaCl_2.2H_2O$ and 600 mg Fe (III) EDTA per liter of distilled water. The medium pH was adjusted to 7.5. The production rate of NO_2 during the bacteria growth period was monitored by a colorimetric method.

Bacterial cells for BNI assay: Bacterial cells from 500 ml of 4 day-old broth culture were separated by centrifugation at 10,000g, 4 °C for 10 min and re-suspended in 100 ml of P-medium, then kept in the dark for 30 min before the bioassay. An aliquot (5 µl) of test sample (root exudates or synthetic inhibitor) was mixed with 200 µl doubled distilled water. 250 µl of bacterial cells were added to this mixture and stirred and incubated at 15 °C for 30 min. After the incubation period, bioluminescence was measured. For each test sample three measurements were made.

Bioluminescence measurement: Bioluminescence was measured using a TECAN Genios luminometer multiplate reader. The luminescence reaction was started by mixing 100 µl of the sample as prepared above with 2.5 µl of 10% (v/v) n-decyl aldehyde dissolved in ethanol. The relative light unit (RLU) was used as a ¨full integration value¨ which means an average light output during 5 to 15 seconds after the initiation of the reaction.

Growth of B. humidicola and Panicum maximum in a hydroponic system to collect root exudates: Shoots of *B. humidicola* accessions (CIAT 679, 16888, 26159) and *Panicum maximum* (CIAT 16028) grown in soil were transferred to aerated nutrient solution consisting of 0.35mM $(NH_4)2SO_4$, 0.27mM K_2SO_4, 18µM H_3BO_3, 4.6µM $MnSO_4$, 1.5µM $ZnSO_4$ $7H_2O$, 1.5µM $CuSO_4$, 1µM Na_2MoO_4, 0.18mM Na_2HPO_4, 0.36mM $CaCl_2$, 0.46mM $MgSO_4$ $7H_2O$, 45µM Fe (III) EDTA. The shoots transferred to the nutrient solution were grown in 18 liter tanks on polyethylene blocks for two months. The pH of the solution was adjusted to 5.5. The nutrient solutions were replaced with fresh solution every four days. Root exudates were collected non-destructively as follows: 10-15 plants were briefly washed in 1mM NH_4Cl and the roots were placed in 500 ml of 1mM NH_4Cl solution where the root exudates were allowed to collect for 24 h.

Preparation of root exudates for bioassay: Root exudates were filtered through a filter paper to remove any debris such as root tissues and the pH of the filtered root exudates was adjusted to 7.8. The filtrate was centrifuged at 1,000g at 4 °C for 10 min. The supernatant was again filtered through 0.8 µm and 0.22 µm membranes to remove any contaminating organisms such as bacteria and then freeze-dried. The resultant pellet was dissolved in 10 ml methanol and further evaporated to dryness using a rotary-evaporator at 40 °C. The root exudates were extracted with 100-500 µl water and after centrifugation the supernatant (water-soluble compounds: WSC) was tested using bioluminescence assay. The remaining pellet was dissolved in 50-200 µl DMSO and centrifuged to collect the supernatant as water-insoluble compounds (WIC).

Results and Discussion

We have improved the bioassay by modifying several steps described in the bioassay that JIRCAS has developed for plants. The modified steps such as additional centrifugations, filtrations and extraction with different solvents allowed us to: 1) prevent biological contaminations from other sources in the process; and 2) test BNI activity in water-soluble and -insoluble fractions of root exudates. We standardized the method to calculate BNI activity as allylthiourea (AT) units using the synthesized inhibitor. Using water as control, various concentrations (0.01-0.22 µM) of AT were used to generate an AT standard curve for every set of experiments (Figure 31). The inhibitory effect of 0.2 µM AT in the bioassay medium was about 80% and was defined as one AT unit of BNI activity. Using this standard curve, the inhibitory effect of the test samples was converted and indicated as AT units.

JIRCAS previously found that grasses including *B. humidicola* and *P. maximum* have contrasting levels of BNI activity. Based on their observations, *B. humidicola* CIAT16888 had the highest BNI activity among the plant genotypes tested. As shown in Table 28, significant differences in BNI were found among *B. humidicola* accessions. We found that all tested materials have higher BNI activity in WSC fractions than that in WIC fractions. This strongly suggested that BNI compounds released from roots are water-soluble.

It was also found that *B. humidicola* CIAT16888 had the highest BNI activity (173 AT units g^{-1} DW) compared with other materials tested in this work as was reported previously from the collaborative research of JIRCAS-CIAT. We believe that our bioassay is now operational to test root exudates from any genotype of interest including crops such as rice germplasm (Table 28).

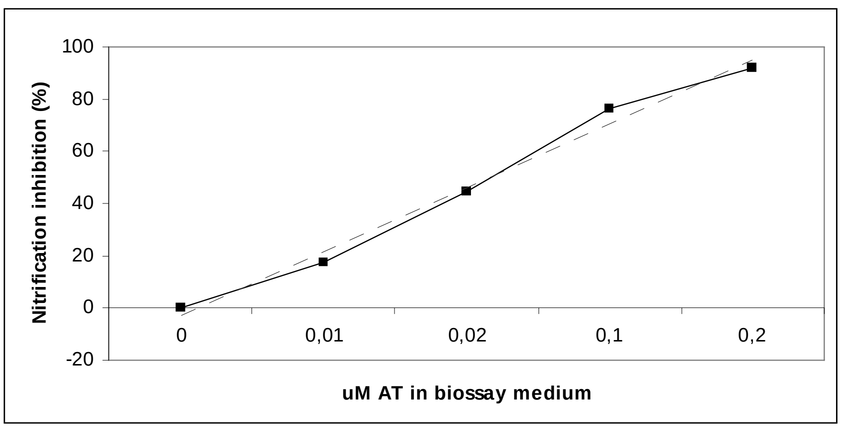

Figure 31. Transgenic *Nitrosomonas europea* response to synthetic nitrification inhibitor, allylthiourea (AT) in the bioassay medium.

Table 28. Biological Nitrification Inhibition (BNI) activity of root exudates of *B. humidicola* accessions. WSC = water soluble compounds; WIC = water insoluble compounds; SD = standard deviation.

Accession number	Specific BNI activity (AT units g^{-1} root DW)			
	WSC extract	SD	WIC extract	SD
CIAT 679	2.3	2.26	-0.7	0.28
CIAT 16888	173	33.6	-12.1	16.6
CIAT 26159	14.9	7.6	2.0	1.54

3.4.3 Screening for genetic variability in the ability to inhibit nitrification in accessions of *Brachiaria humidicola*

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Rationale

Ongoing collaborative research with JIRCAS, Japan, has shown that *B. humidicola* CIAT 679 (high BNI activity), *B. humidicola* CIAT 16888 (highest BNI activity) and Hybrid Mulato (intermediate NI activity) inhibits nitrification of ammonium and reduces the emission of nitrous oxide into the atmosphere. On the other hand, P.*maximum* CIAT 16028 does not have this property to inhibit nitrification. Given these findings with *B. humidícola* accessions and the fact that a range of inhibition of nitrification was observed among different tropical grasses, there is a need to determine the extent of genetic variation among the 69 accessions of *B. humidicola* that are part of CIAT germplasm bank. This information will be extremely useful to develop screening methods to select genetic recombinants of *Brachiaria* grasses that not only are resistant to major biotic and abiotic stress factors but also can protect the environment. Given the vast areas under B. *humidicola* in the tropics, reductions in net emissions of N_2O could also have important environmental implications.

The main objective of the study was to quantify differences among 43 accessions of B. *humidicola* in biological nitrification inhibition (BNI) activity from root exudates collected from plants grown under greenhouse conditions using infertile acid soil. Also we intend to test the relationship between nitrification inhibition and shoot and root production in terms of biomass.

Materials and Methods

A sandy loam Oxisol from the Llanos (Matazul) of Colombia was used to grow the plants (1 kg of soil/pot) under greenhouse conditions. A basal level of nutrients were applied before planting (kg/ha): 40 N, 50 P, 66 Ca, 100 K, 20 S and micronutrients at 2 Zn, 2 Cu, 0.1 B and 0.1 Mo. A total of 44 accessions were used (accessions P.*maximum* CIAT 16028 and B. *humidicola* CIAT 679, 682, 6705, 6738, 16180, 16181, 16182, 16183, 16350, 16867, 16870, 16871, 16873, 16874, 16877, 16878, 16879, 16880, 16883, 16884, 16887, 16888, 16889, 16890, 16891, 26145, 26151, 26152, 26155, 26160, 26181, 26312, 26411, 26413, 26415, 26416, 26425, 26427, 26430, 26438, 26570, 26573, 26638). A control without plants was also included. The experiment was arranged as a completely randomized block design with four replications. Each pot contained 6 plants to increase root biomass. After sowing, plants were allowed to grow for 15 weeks and were cut to 10 cm height to simulate grazing effects under field conditions.

NH_4- N (nitrogen source was ammonium sulfate) was added in four applications: 40 kg N/ha added to the soil when filling the pots, 40 kg N at times, 30, 60 and 90 days after planting. Four weeks later plants were harvested (at 16 weeks after sowing). At the end of the experiment, plants were carefully removed from soil minimizing mechanical damage to the roots. Soil adhered to the fine roots was removed and the roots were rinsed with deionized water. Once clean, the roots were fully submersed in 1mM NH_4Cl for 1 hour to trigger further BNI active compound exudation. Then the roots were immersed in 500 mL deionized water during 24 hours. The root extract was subsampled and around 100 mL were sent to JIRCAS in liquid form and inside a Styrofoam container with ice packs to maintain them refrigerated for testing the BNI activity level. Another 100 mL was stored in the cold room as a backup until the BNI was measured and then was discarded. The final concentrate was tested for its nitrification inhibitory activity using a specific bioassay developed at JIRCAS.

Harvested plants were separated into leaves, shoot and roots. Dry matter content and N status of leaves, shoot and root biomass was determined. At harvest time, soil samples were extracted with KCl and analyzed for nitrate and ammonium levels.

Results and Discussion

Results on dry matter partitioning among shoot and root biomass from the comparative evaluation of the 44 accessions are presented in Table 29. Significant differences were found in root biomass, stem biomass, leaf biomass and total biomass. The accessions CIAT 26425, 26438 and 26573 produced the highest values of total biomass while accessions CIAT 26427, 26312 and 16867 were lower than the rest of *B. humidicola* accessions. The accessions CIAT 26438 and CIAT 26425 produced the highest root biomass among the tested accessions. Values of root biomass of those accessions were more than eight fold greater than the value for the lowest in the group, the accession CIAT 26312.

Results from the bioassay indicated substantial level of genotypic differences in BNI (biological nitrification inhibition) activity in the root exudates of the accessions tested (Table 30). BNI activity is expressed as AT units; one AT unit is defined as the inhibitory activity caused by the addition of 0.22 µM of allylthiourea (AT) in the bioassay medium. Thus, the inhibitory activity of the test samples of root exudates is converted into AT units for the ease of expression in numerical form. The tested *B. humidicola* accessions presented a range in BNI activity between 44.8 AT units pot^{-1} and 207 AT units pot^{-1}, while the P.*maximum* 16028 exhibited lowest NI activity (0.55 AT units pot^{-1}). The highest values were observed with the accessions CIAT 26573 and CIAT 16887. However, lower values of AT units pot^{-1} were observed with the accessions CIAT 26312, CIAT 16890, CIAT 16884, CIAT 26413, CIAT 682, CIAT 16891 and CIAT 16880. No significant differences were found among the accessions CIAT 6705, CIAT 26430 and CIAT 16877 which showed intermediate level of BNI activity. The commercial cultivar, CIAT 679, which had been used in most of the previous work, presented similar values (66 AT units pot^{-1}) to the accessions CIAT 16883, CIAT 26155 and CIAT 16183.

Results on BNI activity indicate that wide genetic variability exists among accessions of *B. humidicola* in relation to the effectiveness of root exudates to inhibit nitrification in soils. This genetic variability for BNI activity could be exploited in a breeding program to select for genotypes with different levels of BNI activity. Accessions with superior BNI activity could be used as parents to regulate BNI activity in the genetic recombinants together with other desirable agronomic traits.

Correlation coefficients between plant (shoot and root) attributes and total or specific BNI activity indicated that root biomass is negatively associated with specific BNI activity ($r^2 = -0.26$) indicating that specific activity per unit root dry weight decreased with increase in root biomass. Total biomass (shoot + root) production also showed negative association with specific BNI activity ($r^2 = -0.17$). As expected specific BNI activity showed strong positive association ($r^2 = 0.6$) with total BNI activity.

The presence of substantially higher levels of BNI activity in the root exudates of two CIAT accessions (26573 and 16887) draws attention to the need to study these accessions in more detail to understand the BNI phenomenon. As a continuation of this work, we have assembled a set of 23 accessions of *B. humidicola* to conduct a study to test the genetic diversity in BNI in a single experiment under similar growing conditions and analyses of BNI. The *Brachiaria humidicola* breeding program at CIAT has generated a hybrid population and this population will be useful to analyze the tradeoffs of BNI in terms of the relationships among productivity, forage quality and BNI activity.

Table 29. Dry matter partitioning differences among 43 accessions of *B. humidicola* grown in pots under greenhouse conditions. Plants were harvested at four months after planting.

CIAT	Dry matter (g / pot)			
Accession Number	Root biomass	Shoot biomass	Root / Shoot	Total biomass
679	10.2 (3.26)	16.6 (2.36)	0.60 (0.13)	26.8 (5.50)
682	7.90 (2.25)	8.60 (1.05)	0.92 (0.24)	16.5 (2.83)
6705	4.73 (0.50)	9.32 (0.65)	0.51 (0.06)	14.0 (0.88)
6738	6.80 (0.97)	8.90 (0.76)	0.76 (0.05)	15.7 (1.71)
16180	7.30 (2.56)	6.94 (1.03)	1.11 (0.57)	14.3 (1.87)
16181	4.88 (1.40)	14.0 (2.04)	0.36 (0.13)	18.8 (2.07)
16182	5.33 (1.22)	9.51 (1.00)	0.57 (0.18)	14.8 (0.92)
16183	3.75 (2.70)	14.1 (4.60)	0.25 (0.13)	17.8 (6.99)
16350	3.96 (1.31)	9.99 (1.05)	0.40 (0.13)	13.9 (1.95)
16867	4.42 (0.29)	3.35 (0.47)	1.35 (0.23)	7.77 (0.46)
16870	7.42 (1.59)	6.60 (0.72)	1.12 (0.11)	14.0(2.30)
16871	7.43 (2.95)	12.9 (1.54)	0.60 (0.33)	20.3 (1.78)
16873	4.85 (0.35)	10.7 (1.36)	0.46 (0.08)	15.6 (1.17)
16874	6.52 (1.64)	7.68 (1.300	0.86 (0.24)	14.2 (2.44)
16877	8.77 (1.33)	6.55 (0.55)	1.34 (0.17)	15.3 (1.71)
16878	4.81 (0.59)	7.94 (1.51)	0.62 (0.11)	12.8 (1.89)
16879	6.85(0.88)	6.26 (1.04)	1.10 (0.11)	13.1 (1.85)
16880	6.10 (1.06)	5.77 (0.65)	1.05 (0.10)	11.9 (1.63)
16883	7.35 (1.00)	7.76 (0.70)	0.95 (0.10)	15.1 (1.52)
16884	9.36 (1.48)	9.33 (1.19)	1.00 (0.07)	18.7 (2.60)
16887	7.25 (0.34)	7.28 (0.50)	1.00 (0.05)	14.5 (0.79)
16888	7.31 (0.84)	5.90 (0.73)	1.26 (0.21)	13.2 (0.92)
16889	6.14 (1.22)	5.99 (1.58)	1.05 (0.23)	12.1 (2.48)
16890	5.75 (0.62)	6.12 (0.96)	0.95 (0.14)	11.9 (1.33)
16891	7.61 (0.47)	7.66 (1.15)	1.01 (0.18)	15.3(1.31)
26145	5.59 (1.05)	7.23 (0.73)	0.78 (0.19)	12.8 (1.14)
26151	7.18 (1.17)	10.1 (1.11)	0.71 (0.05)	17.3(2.26)
26152	5.22 (0.43)	8.04 (0.48)	0.65 (0.08)	13.3 (0.41)
26155	5.25 (1.01)	16.0 (2.78)	0.33 (0.03)	21.2 (3.70)
26160	5.40 (0.54)	9.06 (0.99)	0.60 (0.12)	14.5 (0.76)
26181	6.40 (4.25)	19.9 (4.86)	0.35 (0.23)	26.3 (5.43)
26312	1.65 (1.58)	8.25 (0.97)	0.19 (0.17)	9.90 (2.48)
26411	4.74 (0.72)	8.27 (2.04)	0.60 (0.16)	13.0 (2.50)
26413	5.21 (1.17)	10.1 (2.10)	0.53 (0.17)	15.4 (2.50)
26415	9.63 (2.27)	17.2 (2.58)	0.56 (0.07)	26.8 (4.69)
26416	6.21(0.91)	8.09 (0.39)	0.77 (0.14)	14.3 (0.64)
26425	13.3 (5.76)	18.6 (9.99)	0.91 (0.51)	31.9 (6.30)
26427	4.28 (0.64)	5.87 (0.94)	0.74 (0.17)	10.2 (1.16)
26430	6.36 (0.82)	4.66 (1.06)	1.46 (0.55)	11.0 (0.52)
26438	14.6 (1.73)	16.3 (0.85)	0.89 (0.08)	30.8 (2.46)
26570	7.87 (2.42)	9.27 (1.46)	0.84 (0.13)	17.1 (3.83)
26573	12.2 (4.25)	17.8 (4.58)	0.75 (0.38)	30.1 (3.41)
26638	3.75 (2.25)	9.15 (5.45)	0.47 (0.17)	12.9 (7.59)
Panicum maximum				
CIAT 16028	11.5 (4.26)	15.6 (2.02)	0.75 (0.28)	27.1 (4.75)
LSD	**2.69**	**3.44**	**0.30**	**4.13**

Table 30. Nitrification inhibitory activity (total BNI activity pot^{-1}) and specific activity (AT units g root dwt^{-1}) of the root exudates from 43 accessions of *B. humidicola* grown under glasshouse conditions. Plants were grown for four months before the collection of root exudates.

Accession CIAT No.	BNI activity (in AT units pot^{-1})	Specific BNI activity (in AT units g root dwt^{-1})	CIAT Accesion No.	BNI activity (in AT units pot^{-1})	Specific BNI activity (in AT units g root dwt^{-1})
679	66.4 (0.48)	6.83 (0.05)	16890	60.4 (17.6)	10.5 (2.66)
682	53.4 (16.0)	7.54 (3.85)	16891	60.0 (21.8)	6.74 (2.79)
6705	151 (26.1)	32.0 (4.59)	26145	95.8 (29.0)	17.5 (5.78)
6738	113 (25.7)	17.2 (5.23)	26151	124 (23.8)	17.9 (6.38)
16180	71.4 (10.1)	10.4 (2.55)	26152	141 (35.9)	27.1 (6.52)
16181	80.9 (0.57)	17.6 (0.12)	26155	67.9 (0.31)	13.0 (0.06)
16182	105 (30.9)	20.1 (5.19)	26160	70.9 (38.0)	12.7 (5.75)
16183	65.9 (0.27)	13.6 (0.06)	26181	93.5 (0.25)	20.6 (0.06)
16350	107 (38.2)	29.7 (13.6)	26312	62.4 (0.63)	21.4 (0.21)
16867	70.3 (6.5)	15.9 (1.16)	26411	118 (43.4)	24.6 (7.04)
16870	80.6 (19.9)	11.1 (3.10)	26413	56.3 (19.8)	11.0 (3.55)
16871	94.6 (0.26)	13.9 (0.04)	26415	93.3 (0.32)	9.26 (0.03)
16873	112 (66.0)	23.5 (14.4)	26416	128 (24.4)	20.5 (1.74)
16874	130 (84.1)	21.7 (15.0)	26425	71.6 (0.84)	6.42 (0.08)
16877	151 (50.5)	16.9 (4.65)	26427	93.6 (40.3)	24.2 (11.5)
16878	105 (33.3)	22.6 (9.89)	26430	151 (15.34)	24.1 (5.12)
16879	70.4 (9.51)	10.4 (1.46)	26438	93.5 (0.07)	6.53 (0.01)
16880	44.8 (14.1)	7.56 (2.90)	26570	168 (11.5)	22.6 (5.99)
16883	68.3 (26.8)	9.32 (3.51)	26573	207 (3.21)	24.3 (0.38)
16884	60.0 (31.2)	6.26 (2.88)	26638	93.0 (0.48)	19.9 (0.10)
16887	195 (66.0)	27.1 (10.0)	*P. maximum*	0.55 (0.02)	0.07 (0.00)
16888	174 (33.9)	24.2 (6.17)	**LSD**	**42.15**	**8.15**
16889	136 (45.8)	21.8 (4.38)			

Numbers in parenthesis indicate standard deviation. (LSD, $p<0.001$)

Conclusions

Screening of 43 accessions of *Brachiaria humidicola* for specific and total biological nitrification inhibitory (BNI) activity resulted in quantifying genetic diversity in BNI and in identifying contrasting accessions with very high (CIAT 26573) and low BNI activity (CIAT 16880). Root biomass production showed negative association with specific BNI activity.

3.4.4 Field validation of the phenomenon of biological nitrification inhibition from *Brachiaria humidicola*

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Rationale

A range of Biological Nitrification Inhibition (BNI) activity has been observed 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 BNI under field conditions and test the hypothesis that the BNI activity is a cumulative factor in soils under species that release the BNI activity from root exudates. Given the vast areas that are currently grown with tropical grasses, an understanding of the BNI process and the possibility of managing it to improve N use efficiency, reduce nitrate pollution of surface and ground waters 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 BNI activity were selected for the experiment and a soybean crop and a tropical grass (*P. maximum*) that lacks the BNI activity were selected as controls.

Materials and Methods

The field 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 ^{0}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* accessions 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 *Rizhobium* 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^{-1}): 48N, 24K, 8P, 0.2Zn, 0.2B. 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. Five samples were collected in each subplot and pooled to obtain a composite sample. Samples were carefully managed and only the soil adhered to the roots was removed and used for soils 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. Gas samples for measuring N_2O fluxes were collected monthly.

Results and Discussion

So far four soybean crops have been harvested (March and August, 2005; February and July, 2006). In this report we present the data collected during the fourth cropping season, nitrification rate, nitrate accumulation in the soil (Ion exchange resins), the net fluxes of N_2O and nitrate and ammonium levels in the top soil (0 – 10 cm) after fertilizer application.

In Figure 32 we show the differences in total shoot biomass harvested during the fourth crop cycle (March – July 2006). The results between treatments presented significant differences (LSD, p<0.001). Total shoot biomass yields of *P. maximum* and the *B. humidicola* accessions were similar but greater than that of the biomass from cv. Mulato and soybean. The decreased vigor of cv. Mulato was found to be due to reduced N availability in soil. Soybean had a total shoot biomass markedly lower than that of *B. humidicola* 679 and *B. humidicola* 16888. The growth of the *B. humidicola* accessions had been stimulated with the ammonium sulfate application as nitrogen source.

In Figure 33 we show total shoot biomass accumulated from the experimental plots. Total shoot yield of *P. maximum* and the cv. Mulato was significantly higher than that of soybean and the 2 accessions of *B. humidicola* (LSD, P<0.001). Total nitrogen uptake by different species showed significant differences (LSD, p<0.001) (Figure 34). Soybean and P.*maximum* accumulated considerably more nitrogen than cv. Mulato and the 2 accessions of *B. humidicola*. The *B. humidicola* plots removed less N than what is being added as fertilizer. The grain yield of soybean was similar to the first and second cropping seasons (1.4 Mg ha^{-1}) which was slightly lower than that of the commercial average in the region.

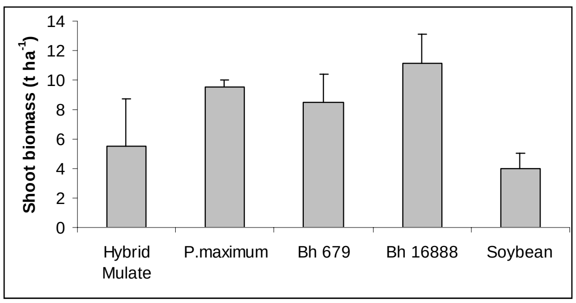

Figure 32. Shoot biomass production during the fourth cropping cycle (March -August 2005).

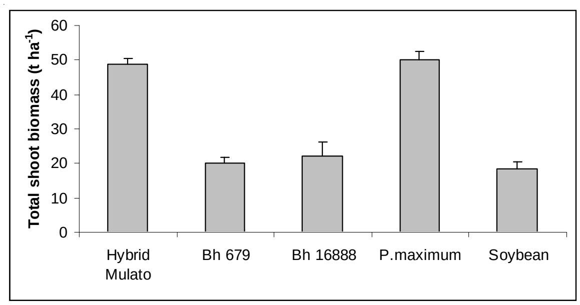

Figure 33. Total dry biomass production over the period of September 2004 and July 2006.

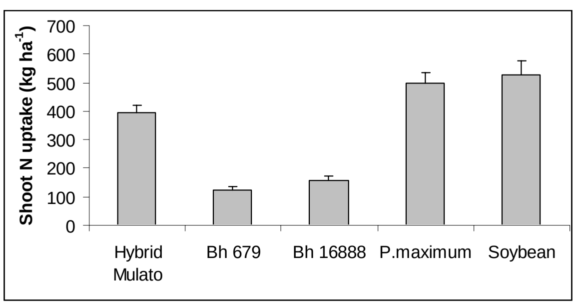

Figure 34. Total shoot nitrogen uptake for the period September 2004 and July 2006.

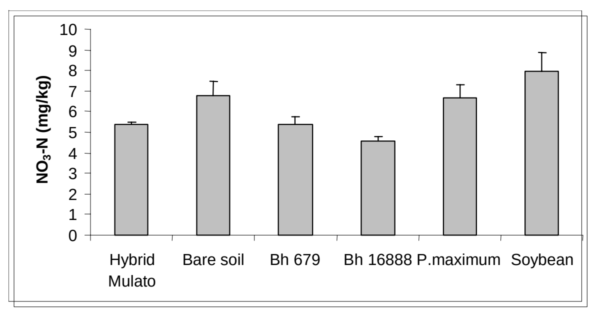

Figure 35. Nitrate levels in the top soil (0-10 cm) at harvest time of the fourth cropping cycle.

Soil ammonium and nitrate. Results on the nitrate levels in the top soil at harvest time showed significant differences (LSD, p<0.001) (Figure 35). The soybean plot showed higher levels of nitrate more likely as a result of lack of plant N uptake. The bare soil 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 two accessions of *B. humidicola* suggests a lower rate of nitrification with these two grasses or alternatively higher nitrogen losses.

The ratio of NH_4/NO_3 in soil over time showed that Bh 16888 had higher values after 20 days of fertilizer N application (Figure 36). Hybrid Mulato maintained the values over time compared with bare soil and *P. maximum*.

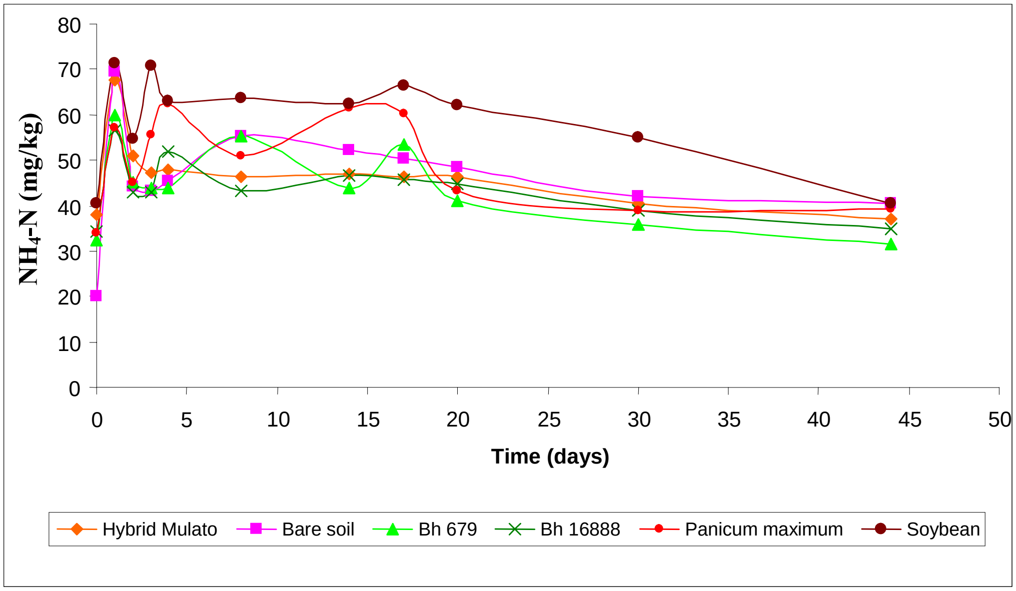

Figure 36. Ammonium to nitrate ratios in soil.

Nitrate accumulation in the soil (Ion exchange resins): Immediately after the second fertilizer application in June, 2006, Ion exchange resins (Western AG, Canada) were inserted vertically in the soil to a depth of 10 cm. Four anion exchange units were randomly distributed in each experimental subplot. Resins were collected 7, 14 and 29 days after fertilize, adhered soil carefully removed with a brush and resins extracted with 1M KCl. Analysis of nitrate was done on the resin elution solution. Results on the amounts of nitrate adhered to the nitrate resins showed significant differences (LSD, $p<0.001$) (Figure 37).

Seven days after fertilizer application, the soybean and bare soil plots showed higher levels of nitrate accumulated as a result of lack of plant N uptake. The P.*maximum* and Hybrid cv. Mulato also had high levels of nitrate accumulated in the ion exchange resins, while the B. *humidicola* accessions clearly showed lower nitrate concentrations. After fertilization and with the course of the days, the nitrate levels decreased gradually. Nevertheless, the accessions of B. *humidicola* showed lowest of NO_3-N at 7, 14 and 29 days after fertilizer.

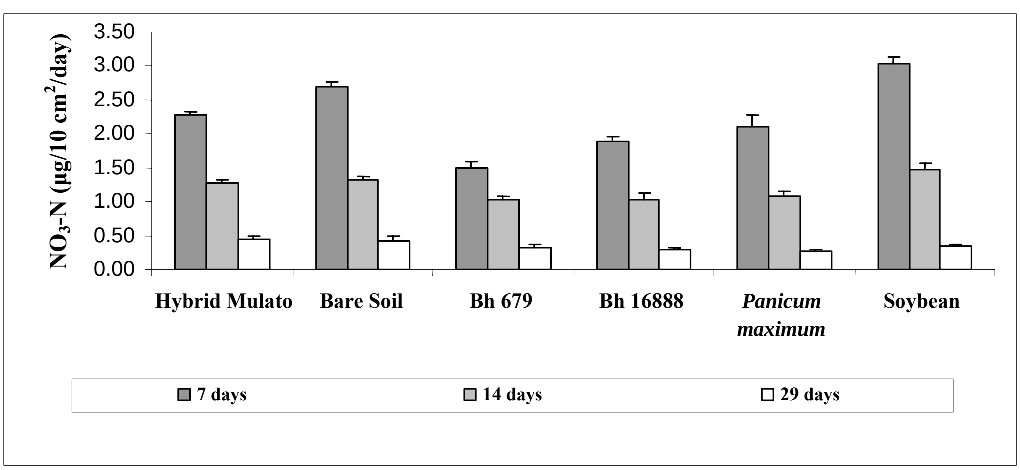

Figure 37. Nitrate retained in ion exchange resins over a 7, 14 and 29 days period after fertilizer application.

With the purpose of establishing the time at which the maximum levels of nitrate, nitrite and nitrous oxide occur in soil, the soil was fertilized and a continuous sampling was made during 44 days. In Figure 38 we show that a day after fertilizer application, the maximum concentration of nitrate was observed. The average levels of nitrate were increased from 6.12 mg/kg NO_3-N (before fertilizer application) to 12.90 mg/kg (1 day after fertilizer application). It is important to emphasize that the 2 accessions of *B. humidicola* exhibited lowest values of nitrate in the top soil.

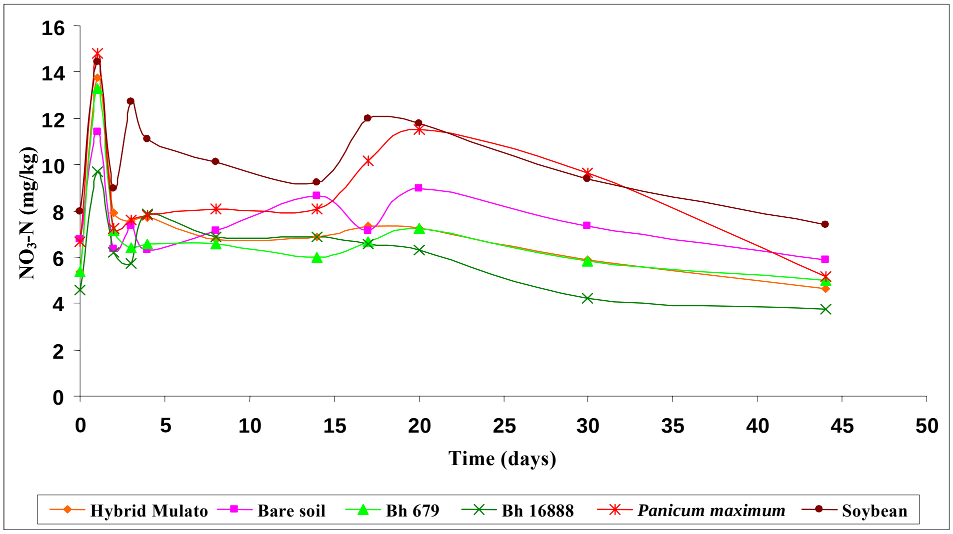

Figure 38. Nitrate levels in the top soil (0 – 10 cm) after fertilizer application.

Nitrous oxide emissions: Results on the behavior of the net fluxes of N_2O during 30 days after fertilizer application (March 31/2006 – May 4/2006) are shown in Figure 39. The highest concentrations were obtained at 1 and 3 days after fertilizer application while the lowest concentrations were observed at 20 days after fertilizer application. The 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* also showed 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.

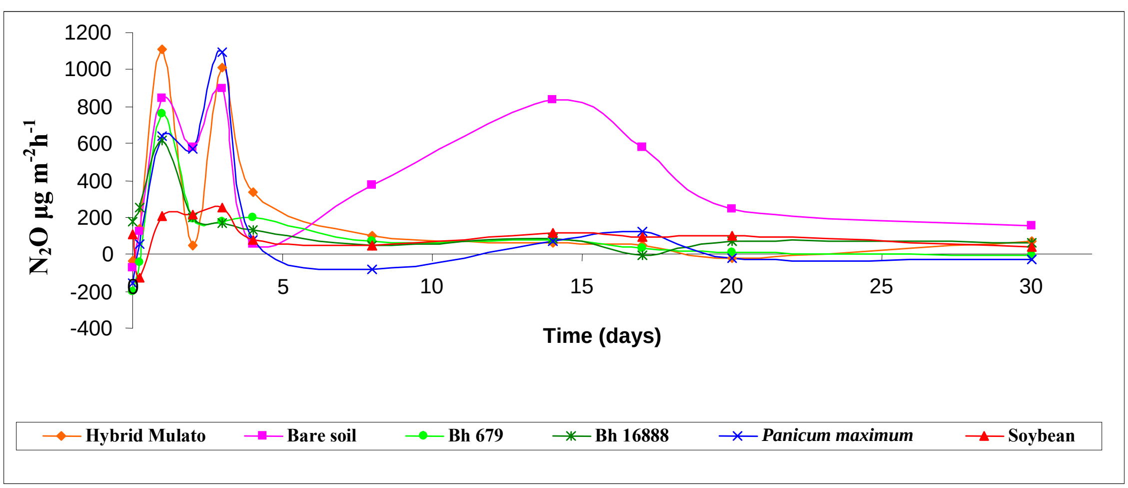

Figure 39. Net fluxes of N_2O during 30 days after fertilizer application.

Soil nitrification rates*:* Fresh rhizosphere soil was incubated to assess its mineralization rates during 30 days after fertilizer application. Soil samples were incubated with appropriate levels of ammonium and phosphate to favor nitrification. Chlorate was added to block the conversion of nitrite to nitrate and to measure rate of nitrite accumulation over time. Rate of nitrite accumulation was easier to measure than nitrate accumulation. Results presented in Figure 40 from the incubation test showed significant differences (LSD, $p<0.001$). Soybean showed stimulatory effect on nitrification while bare soil subplots presented the highest value of nitrification rate. P.*maximum* (No BNI activity) showed high levelof NO_2-N formation per day while cv. Mulato (intermediate BNI activity), *B. humidicola* 679 (high NI activity) and *B. humidicola* 16888 (highest BNI activity) showed lower rates of nitrification.

Nitrate to nitrite ratio values in soil showed that the values were markedly higher with the bare soil treatment and were lower with the two Bh accessions (Figure 41). These data also indicate greater inhibition nitrification by Bh accessions.

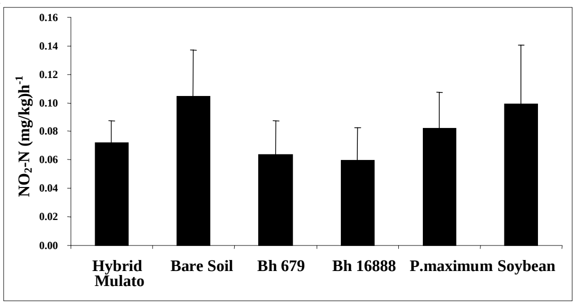

Figure 40. Average nitrification rates from incubated soils during 30 days after fertilizer application.

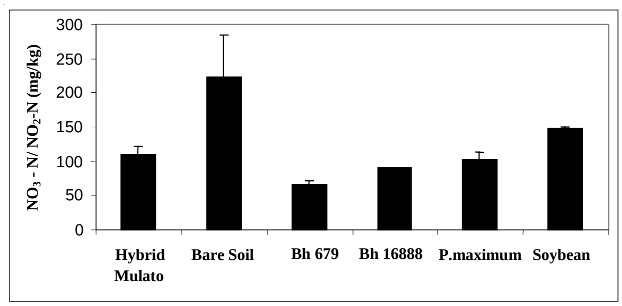

Figure 41. Nitrate to nitrite ratios in soil.

Conclusions

This field study after four cycles of analyses for BNI activity indicated that nitrification rates were lower with the two accessions of *Brachiaria humidicola* than the accession of *Panicum maximum*. The soil incubation method used for this study to estimate nitrification rates seem to be highly sensitive to detect even small differences in nitrification rates among the grasses. Further work is in progress to test the usefulness of this soil incubation method to quantify genotypic differences in BNI so that this method could be used as a screening method to quantify BNI among *Brachiaria* hybrids.

3.4.5 Soil microbial population analysis by Real-Time PCR to study Biological Nitrification Inhibition (BNI) activity of crops

Contributors: D. E. Moreta, A. Salcedo, M. Ishitani (SB-2 Project, CIAT); M. P. Hurtado, M. Rondón (PE-2 Project); I. M. Rao and C. Lascano (IP-5 Project, CIAT)

Rationale

In soil, nitrogen (N) available to plants in the form of nitrates (NO_3^-) does not bind to soil particles due to its negative charge. Therefore, this form of N is highly susceptible to leaching and thereby it is lost from the system. Nearly 70% of the N fertilizer applied to agricultural soils is lost due to the nitrification process (oxidation of the relatively immobile ammonium - NH_4^+ - into the highly mobile nitrate - NO_3^- -N). In the same way, the loss of N from soil to the atmosphere in the form of other compounds, cause a negative impact on the environment by contributing to worldwide global warming and the greenhouse effect. Moreover, the soil N that is lost by leaching and/or denitrification can be substantial, which promotes pollution of ground water.

For these reasons, it is imperative to identify natural compounds that inhibit the nitrification process, which is carried out by soil nitrifying microorganisms including *Archaea*. In collaboration with JIRCAS of Japan, CIAT found that *Brachiaria humidicola* has the ability to suppress the activity of specific nitrifying microorganisms (bacteria and *Archaea*) by releasing inhibitory compounds from its roots to the soil. The inhibitory effect was mainly demonstrated by a bioluminescence assay using root exudates which will give indirect evidence for the phenomenon. To demonstrate direct evidence of the BNI phenomenon in soil, we report here the implementation and standardization of a Real-Time PCR technique to monitor microbial populations in soil, which allows us to study direct effects of roots exudates of several crops on the soil microorganisms involved in the nitrification process.

Materials and Methods

Soil samples used in this report were collected from the field study at CIAT-HQ (see Activity 3.4.6). The field has been used for BNI field work for three years, since 2004, and the work was partially supported by JIRCAS. The crops used in the field study were: Soybean, *Panicum maximum,* Hybrid Mulato, *Brachiaria humidicola* 679, *Brachiaria humidicola* 16888 and bare soil (as a control). The soil sampling was done at the end of the 4th growing cycle of soybean and before and one day after nitrogen fertilization of the 5th growing cycle. The soil samples were taken from a depth of 10 cm from a 1 m x 1 m sub-plot for every treatment using a metal tube with one side open. The two soil samples were mixed and the soil DNA extraction was performed using a FastDNA SPIN kit for Soil (Q-BIOgene, USA) according to the manufacturer’s directions. Afterwards, the extracted DNA was quantified using the PicoGreen reagent and then was electrophoresed onto a 1% agarose gel to check its quality.

Four target genes (Bacteria and *Archea amoA* genes and 16S rRNA genes) were amplified using specific primer sets: *amoA*-1F/*amoA*-2R, *amoA*19F/*amoA*643R, BACT1369F/ PROK1541R, and Arch 20F/Arch 958R, respectively. These primers were demonstrated to be useful from published work. The *amoA* primers were designed to amplify t*amoA* genes, which encode a subunit of the ammonia-oxigenase enzyme that is presumably involved in the nitrification process in ammonia-oxidizing bacteria and *Archaea*. The other primers were used to amplify 16S rRNA gene, which was used as an internal control to track the population dynamics of the ammonia oxidizing bacteria and *Archaea* populations in soil.

Results and Discussion

To establish the PCR techniques for quantifing soil bacterial populations, we first generated standard curves to quantify the copy number of the target genes using gDNA from *Nitrosomonas europaea* for *amoA* gene and *E. coli* for 16S rRNA gene, and plasmid DNA with the specific DNA inserts for *Archaea* amoA and 16S rRNA genes (Figures 42 and 43). There were strong linear (R2 = 0.99) inverse relationships between Ct and the $\log_{10}$ number of amoA and 16s rRNA gene copies. This set a detection limit of gene copy numbers per DNA sample of interest in the reaction mixture.

In addition, the TaqMan technique was also carried out as a preliminary experiment to evaluate the viability of the technique to amplify the bacteria 16S rRNA gene using a specific probe and the primers mentioned earlier (data not shown). A universal Tm was used for all the primers to reduce variability among samples as much as possible, but the primer concentration was specific for each primer set. The TaqMan technique also proved to quantify accurately the

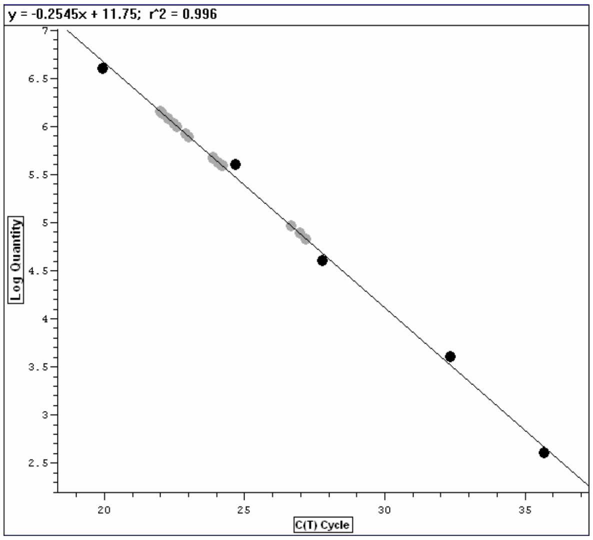

Figure 42. Standard curve generated from *E. coli* gDNA to quantify the copy number of the bacteria *amoA* gene.

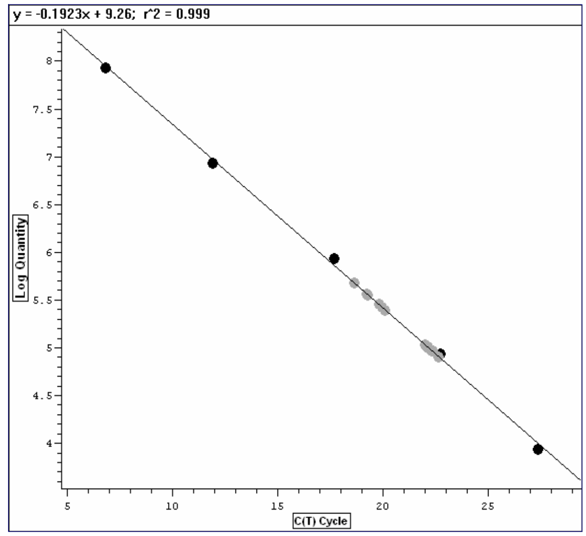

Figure 43. Standard curve generated from plasmid DNA to quantify the copy number of the *Archaea* 16S rRNA gene.

bacteria 16S rRNA (data not shown) and therefore can be used for further studies. Likewise, as we observed in Figure 44, the sharp fluorescence plot of the standard curve obtained from Real-Time PCR experiments will ensure an accurate quantification of the target genes. In addition, the fluorescence plot of the melting curve showed that specific PCR products are being obtained with the primers used, which gives more reliability to the experiment (Figure 45).

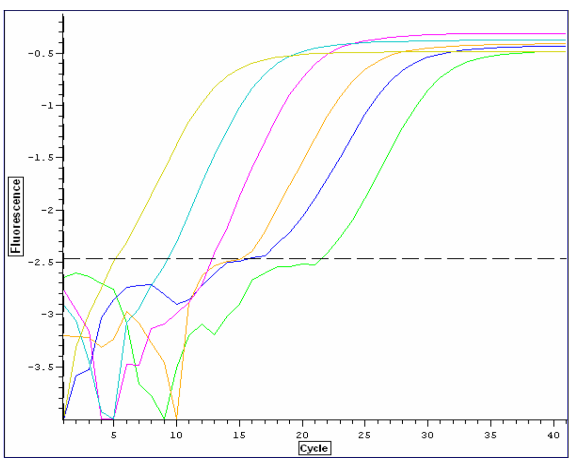

Figure 44. Fluorescence plot of PCR products of bacteria *amoA* gene using diluted *Nitrosomonas* genomic DNA

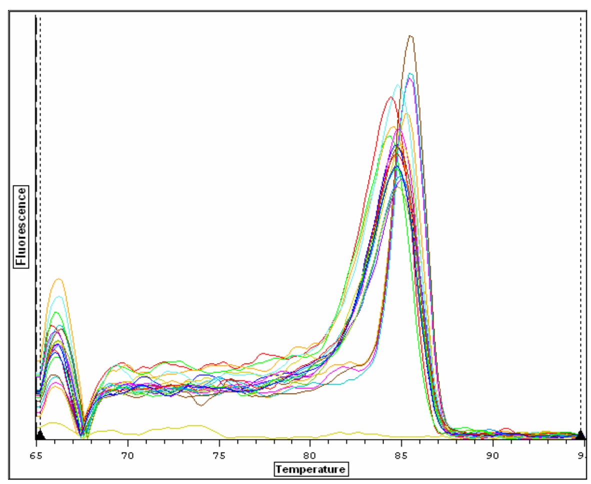

Figure 45. Melting curve of PCR products using a primer set for Archaea 16S rRNA gene
The DNA template used for this experiment was a diluted plasmid DNA containing Archaea 16S rRNA.

Our results suggest that the PCR technique developed is precise and reproducible for monitoring soil bacterial populations. For the soil samples, we have isolated good quality DNA from the soil samples that were described above.

Currently, we are implementing this technique along with a detailed statistical analysis to monitor effects of BNI in the field conditions on bacterial populations of ammonium oxidizing bacteria and *Archaea*.

3.5 Legumes (herbaceous and woody) with adaptation to acid soils drought and mild altitude environments

Highlight

- Selected accessions of *Leucaena diversifolia* CIAT 17248 and *L. trichandra* and 17249 combing high DM yields in both the wet and dry season, high CP content and an IVDMD of 70% or higher as materials of high potential for mid altitude sites with acid soils.

3.5.1 Evaluation of new collections of the multipurpose shrub legume *Flemingia* spp.

Contributors: M. Peters, L.H. Franco, R. Schultze-Kraft (University of Hohenheim), B. Hincapié, P. Avila and Statistician 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 well 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* and 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 6).

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, but some accessions did not reach cutting height during the establishment phase.

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 31). Vigor varied strongly among accessions and also among plants within accessions

After three years of evaluation 13% of the erect and 50% of the semi-erect accessions did not persist indicating low persistence due to low adaptation to the environment, to incidence of pests (e.g. stem borers) or to frequent cutting. The yield of the control accession CIAT 17403 was above the average of all accessions but lower then the highest yields accessions of the 2002 collection (CIAT 21083, 21090 y 21092) and the accessions CIAT 21116, 21091 y 21117 of this collection.

Photo 6. Collection of *Flemingia* spp., A) Habit erect, B) After standarization C) Habit Semierect.

Table 31. Vigor, height, diameter and branches of a collection (2004) of *Flemingia* spp in Quilichao

Accession No. CIAT	Vigor	Height	Diameter	Branches	Accession No. CIAT	Vigor	Height	Diameter	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
21098	3	79	66	8					
21103	3	66	86	8					
21106	3	53	85	8					
21099	3	58	72	8					
21102	3	60	86	7					
19458	3	55	90	7					
21097	3	45	73	6					
22064	3	46	60	6					
21105	3	55	78	5					
22092	2	48	57	5					
19625	1	25	73	5					
21107	2	39	54	5					
19626	1	31	44	4					
19627	1	24	41	4					
17402	1	49	40	3					
19623	1	25	49	3					
22084	2	72	46	3					
18046	1	27	20	2					
Range	1-5	24-112	20-125	2-14		2-5	59-141	39-143	3-17

In Tables 32 and 33 we summarize the performance of persisting accessions across seasons. In both the wet and dry season significant (P < 0.05) differences in DM yield were recorded for the erect as for the semi-erect accessions. Mean DM yields were higher for the erect accessions with 301 and 109 g/plant in the wet and dry season respectively, in contrast to 155 and 49 g/plant for the semi-erect accessions. No differences between seasons in the number regrowing points were found for the erect accessions whereas for the semi-erect accessions the number of regrowing points increased in the dry season.

Accessions CIAT 21033, 2116, 21090, 21092, 2109121117, 21492 and 20629 had the highest DM yields (350g/plant) in the wet season while in the dry season accessions CIAT 21083, 21090, 21092, 21091, 21117, 17403, 22058, 21115, 21089, 20629 y 19393 had the highest yields (>100g).

Table 32. Agronomic evaluation of a collection (2004) of *Flemingia* spp in Quilichao. Data of 4 evaluation cuts (two in the dry season and two in the wet season). Underlaid in grey: Accessions with digestibility >42 % and dry matter yield >300 g/plant in the wet season and digestibility >42 % and dry matter yield >150 g/plant in the dry season . (Erect).

Accession No. CIAT	Height	Diameter	Regrowing points	Mean DM yields	Height	Diameter	Regrowing points	Mean DM yields
	Wet				Dry			
	(cm)		(No.)	(g/pl)	(cm)		(No.)	(g/pl)
21083	141	121	81	488	109	111	93	225
21116	114	134	138	467	88	101	108	112
21090	119	145	106	460	113	127	115	261
21092	111	106	38	402	86	98	36	190
21091	107	88	33	376	90	86	37	199
21117	102	134	99	354	83	105	100	117
17403*	86	133	50	348	76	108	73	163
21115	101	116	98	338	91	105	95	136
22057	108	106	71	332	87	90	71	60
22058	123	92	22	322	114	94	23	170
22094	96	122	106	310	75	90	88	72
21118	86	118	80	291	65	86	74	47
21093	113	113	89	286	88	89	72	73
21113	115	120	86	275	86	96	91	75
21084	92	105	73	236	80	83	50	64
21089	96	86	21	227	73	79	24	106
22063	96	106	54	218	71	71	45	29
21094	99	108	75	194	90	93	68	86
22060	93	104	68	187	76	89	71	53
21111	91	102	60	178	77	85	63	68
21096	57	75	17	87	47	77	16	48
Mean	102	111	69	301	84	94	67	109
LSD(P<0.05)				191.2				91.7

The persistent accessions (42) were subjected to a cluster analysis (Figura 46), truncated at the 7 group level explaining 77% of the total variation. DM yield, diameter, digestibility in the dry season, regrowing points, vigor, and crude protein in the wet season were the parameters used for defining the clusters. The group including accessions CIAT 21083 and 21090 is characterized by the highest DM yields (225 and 261 g/plant), the highest value for diameter (1.1

Table 33. Agronomic evaluation of a collection (2004) of *Flemingia* spp in Quilichao. Data of 4 evaluation cuts (two in the dry season and two in the wet season). Underlaid in grey: Accessions with digestibility >35 % and dry matter yield >200 g/plant in the wet season and digestibility >35 % and dry matter yield >90 g/plant in the dry season. (Semi-Erect).

Accession No. CIAT	Height	Diameter	Regrowing points	Mean DM yields	Height	Diameter	Regrowing points	Mean DM yields
	Wet				Dry			
	(cm)		(No.)	(g/pl)	(cm)		(No.)	(g/pl)
21492	79	134	126	396	69	105	102	94
20629	86	119	129	390	74	88	93	105
17401	63	117	112	344	59	100	100	88
19392	83	130	109	344	69	96	89	77
19802	80	118	107	332	73	98	85	84
22043	64	149	181	317	46	103	121	28
19796	86	123	101	313	71	91	83	75
19803	79	118	105	311	67	91	82	82
21556	69	139	153	296	51	116	108	17
17406	75	114	112	282	112	89	78	84
21109	89	113	77	275	77	92	77	90
19393	69	112	110	265	60	92	95	101
17410	75	117	106	262	64	96	85	63
19455	66	110	77	250	59	89	68	59
7966	76	113	85	246	73	98	86	87
22047	70	124	140	231	48	115	118	29
20628	79	106	71	200	64	86	58	50
20627	67	107	110	186	56	80	72	40
19456	63	97	76	184	56	85	66	69
17453	57	100	81	174	49	73	60	26
22083	50	80	49	119	44	72	37	21
Mean	102	111	69	272	64	93	120	64
LSD(P< 0.05)				154.8				49.28

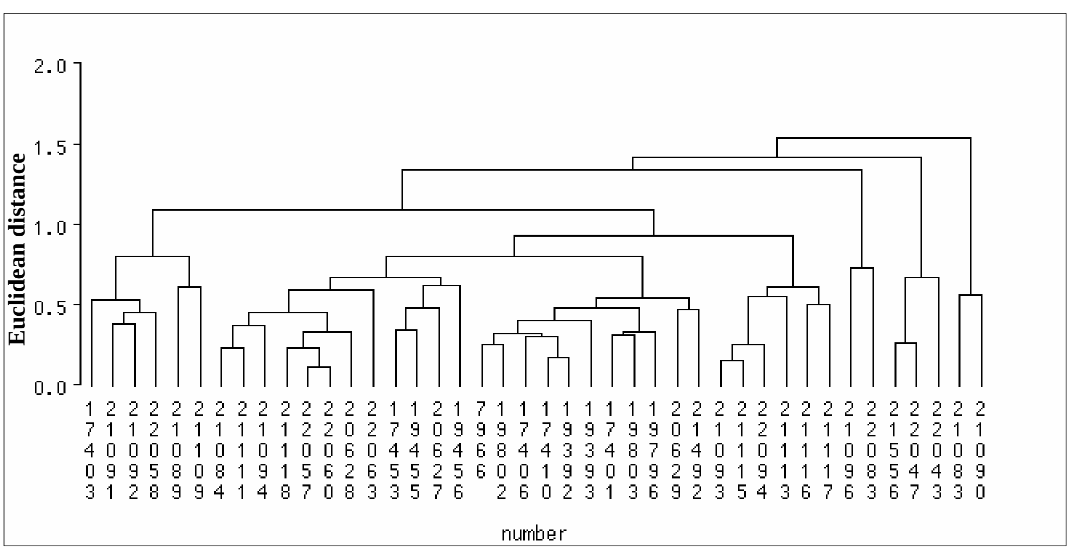

Figure 46. Dendrogram of a collection of *Flemingia* spp.

y 1.27 m), best vigor and a digestibility above 40%. The group of accessions CIAT 21093, 21113, 21115, 21116, 21117 and 22094 had the highest digestibility (46%) but low DM yields (88 g/plant). Forage quality in terms of IVMD and CP differed significantly (P < 0.01) among accessions in both seasons (Table 34). Although some accessions had a digestibility superior to the control CIAT 17403, average values were lower than those recorded in the 2002 collection of *Flemingia macrophylla*.

Table 34. Forage quality of accessions of *Flemingia* spp. evaluated in the wet and dry season in Quilichao, 2005-2006

Accession No. CIAT	Semi - Erect				Accession No. CIAT	Erect			
	IVDMD		C P			IVDMD		C P	
	Wet	dry	Wet	dry		Wet	dry	Wet	dry
	%					%			
21556	45	41	24	19	22094	47	47	23	19
22043	43	50	25	20	22060	46	40	21	18
22047	41	43	25	21	21118	45	42	21	18
21109	38	37	18	15	21093	45	46	22	18
21492	36	37	21	18	21116	45	47	22	19
19393	32	34	22	18	21090	44	46	22	19
20629	31	38	22	19	22057	44	40	21	18
17410	31	32	21	17	21083	43	43	22	20
19456	31	39	23	18	21115	43	46	22	21
19392	31	33	21	18	21111	40	46	22	19
19802	30	37	22	18	21094	40	46	20	19
19803	30	31	19	18	22063	40	41	21	19
20627	30	34	19	16	21117	40	44	22	19
19455	29	34	21	18	21084	38	48	21	18
17406	29	34	22	19	22058	38	35	18	15
17453	29	37	22	18	21096	38	40	18	15
20628	29	40	21	18	21113	36	46	24	21
22083	28	30	18	16	21092	36	37	20	18
7966	26	35	21	19	21089	32	37	17	17
19796	26	34	20	17	17403*	31	33	19	17
17401	25	34	19	18	21091	29	34	20	16
Mean	32	36	21	18		40	42	21	18
LSD (P< 0.05)	4.23	5.9	2.44	1.94		5.06	9.4	2.09	1.51

CIAT 17403 (IVDMD = 43.68 - 41.83; CP = 20.52 - 20.06; ADF = 20.36 - 24.09; T Sol= 4.32 - 9.62)

3.5.2 Field evaluation of a collection of the forage legumes *Leucaena diversifolia* and *Leucaena trichandra*

Contributors: M. Peters, L.H. Franco, R. Schultze-Kraft (University of Hohenheim), B. Hincapié, P. Avila, Katrin Zöfel (University of Hohenheim), Nelson Vivas (Universidad del Cauca) and Statistician G. Ramírez

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 collection (61 accessions) 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

The collection of *L. diversifolia* (50 accessions) and *L. trichandra* (11 accessions) was planted in jiffy pots in the green house. After eight weeks, seedlings were transplanted (June, 2005) to the CIAT station in Santander de Quilichao (Photo 7). 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, and 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, and glands), leaf area, and flowering and growth habit.

Results and Discussion

After two years of evaluation, some mortality of plants occurred mainly due to nematodes and leaf eaters, some accessions were strongly affected

Photo 7. Collection of L. *diversifolia* and *L. trichandra* planted at Quilichao, Cauca.

by *Camptomeris leucaenae*. The seasonal evaluation of Dry Matter yields showed significant (P<0.01) differences among seasons and accessions. DM yield in the dry season was only a 1/4 of the wet season yields (262g/plant vs. 64 g/plant). Accessions 46/87/15, 21242, ILRI-16507, K782, K787, 17271, ILRI-505, ILRI-15551, 45/87/05 and 46/87/12 yielded more than 330 g/plant in the wet season, while in the dry season only accessions 21242, ILRI-15551, 17248, K787, 17249 y K779 yielded more than 100g/plant (Table 35).

The control CIAT 17503 had DM yields below the mean of the collection in both seasons (262 and 64 g/plant, respectively). Average height and diameter were higher in the wet season; however, in the dry season more regrowing points were recorded. Flowering time varied among accessions as indicated by some early flowering accessions and some accessions which had not flowered under the conditions at Quilichao 18 months after planting.

Table 35. Agronomic evaluation of a collection (2005) of *Leucaena diversifolia* and *L. trichandra* in Quilichao. Data of 2 evaluation cuts (one in the dry season and one in the wet season (mean two cuts).

Accession No.	Height	Diameter	Regrowing points	Mean DM yields	Height	Diameter	Regrowing points	Mean DM yields
	Wet				Dry			
	(cm)		(No.)	(g/pl)	(cm)		(No.)	(g/pl)
46/87/15	175	107	49	423	77	107	63	64
21242	202	107	58	381	103	106	59	129
ILRI-16507	184	122	45	369	98	93	45	76
K782	175	110	51	368	76	102	59	85
K787	178	116	43	359	85	107	48	103
17271	188	147	59	352	103	110	61	92
ILRI-505	182	126	46	350	80	101	49	60
ILRI-15551	174	103	50	335	79	97	50	107
45/87/05	184	110	55	332	70	89	52	40
46/87/12	167	106	49	332	83	106	51	81
17248	200	122	62	329	140	93	54	107
45/87/09	168	106	37	329	73	108	55	67
22192	163	113	43	327	77	86	49	60
46/87/08	164	120	46	318	73	103	67	57
K780	176	110	39	316	83	108	43	91
46/87/02	179	103	44	305	89	94	50	60
17249	197	111	62	304	11	94	62	103
K779	174	104	41	303	88	104	52	102
46/87/14	165	116	35	300	76	102	49	63
17485	170	104	41	293	100	89	43	66
45/87/08	179	106	42	290	83	99	48	83
46/87/01	174	106	45	290	90	92	55	68
45/87/14	184	95	31	288	92	92	47	52
45/87/10	188	109	40	286	84	102	50	88
46/87/04	187	104	41	284	78	94	44	43
CPI-46568	169	115	46	280	86	90	41	69
K781	174	97	44	279	91	109	60	91
83/92	175	108	42	277	75	104	49	55
45/87/20	182	119	41	276	87	110	49	69
45/87/17	197	102	36	276	87	96	51	67
ILRI-523	163	104	42	274	109	103	43	94

Continues…..

Table 35. Agronomic evaluation of a collection (2005) of *Leucaena diversifolia* and *L. trichandra* in Quilichao. Data of 2 evaluation cuts (one in the dry season and one in the wet season (mean two cuts).

Accession No.	Height	Diameter	Regrowing points	Mean DM yields	Height	Diameter	Regrowing points	Mean DM yields
	Wet				Dry			
	(cm)		(No.)	(g/pl)	(cm)		(No.)	(g/pl)
46/87/09	175	96	30	261	87	80	36	52
4/91/17	198	85	33	256	112	79	34	43
45/87/18	182	109	32	255	82	99	67	48
4/91/04	190	108	45	254	95	93	50	31
K778	183	107	34	242	88	92	41	44
46/87/10	164	98	41	232	62	97	53	54
K784	144	96	34	229	75	98	47	72
45/87/01	194	106	29	228	89	88	45	60
17388	177	89	23	223	107	82	22	82
CPI-33820	154	100	46	223	74	108	46	63
17461	171	91	31	223	84	101	39	78
45/87/07	179	105	41	221	75	82	38	48
4/91/12	190	91	25	220	101	84	37	52
17264	135	113	37	219	69	89	43	47
46/87/06	174	92	25	219	76	82	37	40
ILRI-515	175	96	34	217	82	79	39	69
K793	166	94	24	209	75	92	43	44
46/87/07	190	110	42	207	81	85	36	41
17497	137	101	51	206	98	84	46	79
45/87/13	160	104	29	205	72	80	38	32
45/87/12	166	100	29	201	75	94	37	46
ILRI-14193	163	86	27	200	85	89	35	51
45/87/02	159	82	23	194	77	84	40	41
4/91/13	158	96	27	185	70	72	30	22
45/87/06	135	87	42	182	61	88	37	38
17268	171	87	25	178	117	91	39	95
17503*	163	83	35	175	106	73	21	60
45/87/11	140	103	38	172	70	84	33	28
82/92	153	93	28	149	71	82	39	39
17247	175	79	25	115	101	58	22	41
4/91/06	122	89	25	113	60	69	23	8
Mean	172	103	39	262	86	93	45	64
LSD(P<0.05)				207.96				77.46

* CIAT 17503 check

Significant (P< 0.01) differences among accessions were measured for IVDMD, but not for CP contents, in the wet season (Table 36).

Accessions 17247, 4/91/12, 17249, 17264, 17248, 4/91/13, 4/91/17, 4/91/06, 4/91/04, 17268 and CPI-33820 had higher digestibility and crude protein contents above 60 and 22%, respectively. Some of the high yielding accessions such as accessions 46/87/15, 21242, ILRI 15551 and ILRI-16507 had low digestibility < 53% while some of the materials with the highest digestibility 17247, 4/91/06 had low yields.

Based on the available results, accessions CIAT 17248 and 17249 appear to be of highest interest as they combine good DM yield and forage quality. Both originate from the state of Chiapas in Mexico, characterized by dry environments with a precipitation of 850 mm rain per year. In view the high forage digestibility in contrast to other *Leucaena diversifolia/L. trichandra* accessions it would be necssary to study the composition of the tannins. Accessions for higher altitudes need to be defined.

Table 36. Forage quality of accessions of *Leucaena diversifolia* and *L. trichandra* evaluated in the wet and dry season in Quilichao, 2005-2006.

Accession No.	IVDMD	C P	Accession No.	IVDMD	C P
	%			%	
17247	71	23	K781	51	22
4/91/12	70	23	ILRI-515	50	22
17249	70	25	45/87/08	50	21
17264	69	23	45/87/05	50	24
17248	69	23	CPI-46568	49	22
4/91/13	69	24	17485	49	24
4/91/17	68	23	45/87/07	49	21
4/91/06	68	24	ILRI-505	49	23
4/91/04	67	24	17497	49	22
17268	66	22	45/87/18	48	23
CPI-33820	63	22	17388	48	21
45/87/20	58	21	ILRI-15551	48	22
K787	57	22	45/87/14	48	23
46/87/08	56	23	K778	48	22
K779	56	20	45/87/01	47	23
K784	55	24	45/87/11	47	22
83/92	54	20	46/87/04	47	25
ILRI-523	53	22	45/87/10	47	24
K782	53	22	21242	47	22
45/87/06	53	22	45/87/12	47	22
17271	53	23	46/87/01	47	25
17503*	52	24	46/87/07	46	21
46/87/10	52	25	82/92	45	21
45/87/17	51	23	17461	45	24
ILRI-16507	51	25	45/87/13	45	23
K780	51	22	46/8714	45	23
46/87/09	51	22	46/87/06	44	23
22192	51	23	46/87/12	44	22
45/87/02	51	23	45/87/09	44	23
K793	51	21	46/87/02	43	22
ILRI-14193	51	22	46/87/15	41	22
Mean	53	23			
LSD(P<0.05)	7.161	4.782			

3.5.3 Field evaluation of a collection of the forage legumes *Dendrolobium* spp.

Contributors: M. Peters, L.H. Franco, R. Schultze-Kraft (University of Hohenheim), B. Hincapié, P. Avila and Statistician G. Ramírez

Rationale

As part of the effort to provide shrub legume alternatives with a wide range of adaptation and/ or suitable for defined niches in production system we are evaluating a collection of *Dendrolobium* spp. under acid soil conditions. As very little is known on *Dendrolobium* spp.

morphological studies are included in the research to define intra- and interspecific diversity.

Materials and Methods

A collection of 60 accessions of *Dendrolobium* spp was planted in jiffy pots in the green house (19 accessions of *D. lanceaolatum*, 33 of *D. triangulare*, 6 of *D.* sp and 2 of *D. umbellatum*). After eight weeks seedlings were transplanted (November, 2005) to the CIAT station in Santander de Quilichao (Photo 8).

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 1.5 m between rows. Fertilization was (kg/ha) P40, K50, Mg20, and S20. 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. Five months after planting, plant height, plan diameter and growth habit were recorded (Table 37).

The first cut in three replications was done on April, 2005, at a height of 0.5 m above the ground. In the fourth replication morphological evaluation was carried out seven month after transplanting.

Results and Discussion

Establishment of plants was completed five months after planting. The collection showed morphological differentiation, though most accessions had a low branching ability with 1 to 7 branches per plant. Most plants at this time showed low vigor but this may be due to late sowing and dry conditions during establishment. Height and diameter varied between 0.1 and 2.1 m and 1 and 1.8 m (Table 37), respectively. No mayor incidence of pest and disease were observed.

So far the collection has been evaluated only once in the dry and once in the wet season. The materials were divided in erect, semi-erect and prostrate accessions. Significant ($P<0.01$) differences in DM yield between accessions were observed for the erect materials; however, mean DM yields were low with 58 and 45 g/plant for the wet and dry season, respectively (Table 38).

Accessions CIAT 13710, 13259, 23412, 13262 and 33116 had DM yields above 100g/plant in the wet season, with CIAT 13710 also yielding more than 100g/plant in the dry season. The number of regrowing points doubled in the dry season; but this was not reflected in higher DM yield.

Photo 8 . Collection 2005 of *Dendrolobium* spp. in Quilichao.

Table 37. Vigor, height, diameter and branches of a collection (2006) of *Dendrolobium* spp. in Quilichao.

Accession CIAT No.	Ha*	Vigor	Height	Diameter	Branch	Accession CIAT No.	Ha	Vigor	Height	Diameter	Branch
		1-5	cm		No.			1-5	cm		No.
22004	P	3	56	89	3	23419	E	2	73	59	2
22005	P	3	42	128	4	23422	E	3	62	87	2
23733	P	4	63	136	3	23932	E	4	61	131	3
23933	P	3	45	93	2	23937	E	3	159	65	1
23935	P	4	35	165	4	33115	E	3	68	85	1
23936	P	3	38	118	3	33116	E	4	64	124	5
33515	P	3	36	78	3	33118	E	2	46	45	1
13258	E	3	88	59	2	33383	E	2	49	33	1
13259	E	2	67	70	3	33398	E	2	77	22	1
13260	E	3	77	43	1	33402	E	2	45	65	1
13261	E	3	96	54	1	33403	E	2	61	39	1
13262	E	3	73	78	2	33407	E	2	93	42	1
13528	E	2	87	67	2	33455	E	2	59	31	2
13529	E	3	53	98	5	33467	E	2	57	21	2
13546	E	2	60	49	2	33480	E	2	48	28	1
13705	E	3	83	34	2	35518	E	2	41	70	2
13706	E	2	43	52	2	23107	SE	2	46	39	2
13707	E	3	105	56	1	23239	SE	3	47	93	5
13710	E	4	79	119	2	23421	SE	2	37	88	2
13711	E	3	103	85	4	23734	SE	3	62	109	3
23104	E	2	50	52	2	23735	SE	4	75	135	5
23105	E	2	51	76	2	23934	SE	4	41	135	4
23106	E	2	63	51	2	33117	SE	2	53	87	3
23108	E	3	62	84	3	33119	SE	3	58	107	3
23412	E	3	75	93	1	33391	SE	3	49	95	3
23413	E	2	66	61	1	33508	SE	2	48	35	1
Mean								2.6	62.4	76.3	2.6
Range								1-5	0.1-2.1	6-1.77	1-7

***** Ha. Habit: E=Erect, SE=Semi-Erect, P=Prostrate

Similar to the erect materials, DM yield in the semi- erect group differed significantly (P<0.01) among accessions. Mean DM yields in the semi erect group were slightly higher than for the erect group, with 98 and 58 g/plant in the wet and dry season, respectively.

Highest wet season DM yields (> 100 g /plant) were measured for accessions CIAT 23239, 23735 and 23934, with only accessions CIAT 23239 reaching this yield level in the dry season. As observed with the erect accessions, regrowing points were higher in the dry than in the wet season (Table 39).

As observed in the erect and semi-erect accessions, the prostrate accessions also had more regrowing points in the dry than in the wet season (Table 40).

However, DM yields were very low and accessions did not persist under the cutting regime imposed in the experiment.

Dry season values for IVDMD and CP differed significantly (P<0.01) among accessions (Table 41).

Accessions CIAT 33518, 33383, 13259, 33119, 23239, 13268, 33508 and 23735 had a digestibility and crude protein above 50 % and 17 %, respectively.

Table 38. Agronomic evaluation of a collection (2006) of *Dendrolobium* spp. in Quilichao. Data of 2 evaluation cuts (one in the dry season and one in the wet season). (Erect materials).

Accession CIAT No.	Wet Height (cm)	Wet Diameter (cm)	Wet Regrowing points (No.)	Wet Mean DM yields (g/pl)	Dry Height (cm)	Dry Diameter (cm)	Dry Regrowing points (No.)	Dry Mean DM yields (g/pl)
13710	58	127	35	189	57	100	52	106
13259	77	116	18	146	58	81	34	75
23412	59	112	25	143	53	91	39	74
13262	60	108	15	134	52	87	39	87
33116	66	122	17	118	56	93	40	81
23413	56	107	11	96	48	103	32	78
23932	50	103	26	93	48	99	42	74
33115	48	117	14	85	46	88	35	39
13529	62	117	22	79	50	96	36	58
33402	49	94	11	73	44	84	26	55
23419	66	99	14	72	52	71	26	23
23108	65	98	13	72	55	104	29	54
23422	61	82	19	71	42	60	30	15
23105	55	93	17	70	42	71	26	20
23106	59	97	17	65	52	67	28	36
23104	50	88	11	52	38	59	21	14
13705	72	93	13	44	71	84	23	72
13546	70	87	12	43	76	96	22	43
13528	70	81	12	39	71	73	27	47
13711	64	72	10	35	79	95	24	69
13261	60	89	8	34	78	95	20	53
33518	46	85	6	32	50	63	7	23
13258	59	70	12	32	79	75	28	42
13707	66	121	7	31	73	106	23	50
33403	60	54	3	30	50	58	7	29
23937	76	76	10	28	90	100	23	84
13260	63	73	18	28	76	94	26	42
33407	52	56	2	27	53	58	6	24
33383	54	45	2	27	49	56	7	24
33118	53	59	2	24	40	71	16	18
33455	57	52	10	20	62	43	24	21
13706	52	75	4	17	61	67	20	16
33467	50	50	5	12	52	65	22	19
33398	70	64	4	10	79	73	13	19
33480	45	38	2	5	60	58	10	15
Mean	59	86	12	58	58	79	25	45
LSD(P<0.05)				41.58				35.67

Table 39. Agronomic evaluation of a collection (2006) of *Dendrolobium* spp. in Quilichao. Data of 2 evaluation cuts (one in the dry season and one in the wet season). (Semi-Erect materials).

Accession No. CIAT	Height	Diameter	Regrowing points	Mean DM yields	Height	Diameter	Regrowing points	Mean DM yields
	Wet				Dry			
	(cm)		(No.)	(g/pl)	(cm)		(No.)	(g/pl)
23239	57	127	27	242	48	99	52	116
23735	60	124	29	121	52	116	49	88
23934	50	123	28	119	48	115	49	94
23421	55	110	18	91	39	73	32	27
23734	58	122	19	89	52	83	41	48
33119	50	103	17	74	51	97	34	50
33117	56	81	13	55	46	72	32	37
23107	58	74	11	53	42	53	22	15
33391	44	76	20	47	48	88	40	62
35508	49	63	3	16	53	64	14	23
Mean	54	101	19	92	48	87	37	58
LSD(P<0.05)				88.8				42.25

Table 40. Agronomic evaluation of a collection (2006) of *Dendrolobium* spp. in Quilichao. Data of 2 evaluation cuts (one in the dry season and one in the wet season). (prostrate).

Accession No. CIAT	Height	Diameter	Regrowing points	Mean DM yields	Height	Diameter	Regrowing points	Mean DM yields
	Wet				Dry			
	(cm)		(No.)	(g/pl)	(cm)		(No.)	(g/pl)
23936	52	113	19	119	52	106	40	79
23935	47	125	27	108	43	121	42	81
22005	49	109	29	106	48	91	46	84
23933	53	112	18	98	47	104	37	75
23733	56	108	17	85	57	100	31	72
33515	40	90	15	53	43	83	37	27
22004	41	108	19	50	35	78	28	37
Mean	48	109	20	88	47	97	37	65
LSD(P<0.05)				37.93				39.84

Table 41. Forage quality of accessions of *Dendrolobium* spp. evaluated in the dry season in Quilichao, 2005-2006.

Accession No.	IVDMD	CP	Accession No.	IVDMD	CP
	%			%	
33518	59	19	23933	44	19
33383	53	17	33115	44	21
13259	52	23	23106	43	21
33119	52	19	23104	43	22
23239	52	21	23936	43	19
13262	51	22	23108	43	21
33508	51	18	23105	42	21
23735	50	20	33407	42	17
33402	49	20	33403	42	18
23734	48	20	23107	41	22
22004	48	20	13707	40	15
23932	48	19	13710	40	19
23934	47	20	23412	40	20
13706	47	17	23419	40	20
23733	47	19	13705	39	15
33117	47	21	13711	39	15
33118	45	17	33398	38	16
22005	45	20	13546	37	16
33391	45	20	33480	37	16
13529	45	17	23937	37	16
23935	44	19	13258	37	16
23421	44	21	13528	36	14
33116	44	19	13261	36	14
33515	44	19	33455	36	16
23413	44	21	13260	35	15
23422	44	22	33467	34	16
Mean				44	19
LSD(P<0.05)				7.07	2.8

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

4.1.1 Revised CIAT- ILRI strategy for forages/livestock R&D in Africa

Contributors: R. Roothaert (CIAT-ILRI), M. Peters (CIAT), C. Lascano (CIAT) and S. Tarawali (ILRI)

During 2005 and 2006 scientists from CIAT and ILRI discussed ways to collaborate in Africa in livestock/forage research based on the principles of comparative advantage and complementarity. One outcome of the discussions between the two centers was the preparation of strategy for R&D in Eastern and Southern Africa. The main elements of the strategy are outlined bellow. However, the strategy has not been implemented due financial limitations. Thus a priority in the near future is for CIAT and ILRI to appoint a Forage/Livestock scientist in Africa with the responsibility of creating partnerships and seeking funds to implement forage research.

The Problem

Most parts of sub-Saharan Africa are stricken by extreme poverty, and with limited resources for investment. HIV/AIDS is further impoverishing communities and is leading to increased labor scarcity. Livestock play an important role in the crop-livestock systems, which provide a livelihood for the majority of Sub-Saharan African (SSA) farmers. Specialized livestock production systems are mostly extensive in nature and concentrated in the arid and semi-arid zones. There is an evolution going on within the extensive livestock systems to integrate crop production. Productivity of livestock is generally low, and the potential positive contribution of livestock to food sufficiency, household income, asset building, equity, and integrated natural resources management has not been realized.

Problems related to stagnation of smallholder livestock production systems in SSA can be grouped as follows:

1. Poverty:
   a. Livestock systems are characterized by low inputs. Poverty is wide spread, and farmers in the crop-livestock systems prioritize their scarce investments to production of staples or high value crops.
2. Physical environment:
   a. Large parts of the continent have unfavorable conditions for fodder production such as low soil fertility, low annual rainfall and frequent droughts, and high prevalence of animal diseases.
3. Markets and economies:
   a. Low prices for animal products and lack of market orientation of livestock producers.
   b. Lack of infrastructure and policies that do not favor increase of livestock production.
   c. Poor integration of rural communities and private sector.
   d. Unstable political environments.
4. Research and information flows:
   a. Lack of access to information on improved livestock technologies.
   b. Livestock technologies don't bear relevance for end-users.
5. Cultural:
   a. Aim of livestock production for cultural reasons rather than for profit or maximizing productivity.
   b. Low levels of mechanization and peaks of labor demand.

The areas of common interest of research in Africa between CIAT and ILRI are tropical forages and livestock systems. In crop-livestock systems the introduction of high quality forages with adaptation to low fertility soils and to drought is seen as entry point not only to increase livestock productivity but also to reclaim degraded lands. In addition improved forages can result in more efficient use of scarce family labor for harvesting fodder and for feeding livestock. Required investments in smallholder forage systems are usually low, except for labor during the establishment phase, and for returning manure or slurry.

Forages have a high potential to improve livelihoods and environment in the following livestock systems in East and Southern Africa:

1. Intensive dairying. Smallholder stall fed systems with one or two improved cows integrated with crops. Sub-humid zones such as highlands of Kenya, Tanzania, Rwanda and Ethiopia. Although these farmers are resource poor smallholders, they are better off than others in their community who do not own dairy cattle. Some larger and wealthier ranches exist.
2. Semi-intensive dairying. Tethering and herding, sub-humid and semi-arid zones. e.g. cross-bred cattle in Kenya, Uganda, Tanzania. These households are poor to average in terms of wealth.
3. Semi-intensive and intensive small ruminant systems. These livestock systems are usually found across all wealth categories. e.g. Kenya, Uganda, Tanzania, Ethiopia.
4. Intensive beef ranches, e.g. Uganda, Malawi, South Africa. These are owned by wealthy farmers.

Forages can have a medium impact on livelihoods, but high impact on the environment in the following livestock systems:

1. Sedentary semi-intensive herds of cattle and small ruminants. Medium to large numbers of animals, mostly herding on unimproved common property pastures with strategic supplemental feeding. Animals are sold for slaughter during times of cash need or used for dowry payments and other ceremonies. Wealth status ranges from poor to rich, depending on numbers of animals owned. E.g. Uganda, Rwanda, Malawi.
2. Evolving pastoralist dual purpose and mixed animal species. Large herds of cattle, small ruminants and camels by trans-humant pastoralists. High value of property but rarely commercialized. Proportion of agro-pastoralists in this category, who are more market oriented, is growing. Agro-pastoralists have fewer animals and their wealth category is variable. e.g. Kenya, Uganda, Tanzania, Ethiopia.
3. Animal traction based highlands of Ethiopia. Primary purpose of cattle and equines: ploughing and transport. In addition, milk from local cows processed and sold by women. Poor households.
4. Non-ruminant systems, such as pigs, fish, apiculture. E.g. Malawi, Ethiopia, Kenya. Wealth ranges from poor to rich.

Although prices of livestock products are generally low in SSA compared to other parts of the world, livestock production can still be attractive compared to commercial crop production, for which prices are much lower. Grazing resources are becoming scarcer due to pressure on land for other uses by an ever-increasing human population. This has resulted in dwindling numbers of livestock in many places in SSA, which will drive up the price eventually. Alternative ways for increased prices for livestock products are in the export, which has a few yet hard to obtain openings.

The Strategy

Opportunities for forage/livestock based technologies in smallholder production systems in Eastern and Southern Africa will be identified and prioritized in areas of high and medium market potential, where high impacts of research can be expected in terms of adoption of improved forage technologies and poverty reduction. Except for the intensive beef ranches, all livestock systems described above are found among poor households. Poor households who do not own livestock, or which have low market potential for the livestock they own, will be targeted through production and sale of forage products which are consumed by the livestock systems in the medium to high market potential category. Forage as a marketable crop could be another arrow on a farmers' bow to kill poverty. Forage technologies to meet farmers' demands will be developed with participatory, market analysis and innovation systems. Partnerships with different public and private institutions will be strengthened to carry out joint research, training and dissemination activities. Public private linkages are a key mechanism for scaling. Thus linkages with private companies (i.e. Papalotla) for seed supply systems and NGO for marketing of dairy products will be assessed. South-south exchange will be promoted as a means of catalyzing dissemination of improved forage/livestock technologies and R&D methodologies. Close linkages will be observed with national partners, and existing networks will be building on such as the ASARECA - Animal Agriculture Network (AAARNET). The joint CIAT-ILRI work will enable national partners to find solutions rather than fix solutions by itself. Once a common research agenda between CIAT and ILRI has been agreed, discussions will be opened with EMBRAPA on a shared strategy for Africa.

Target research areas

Smallholder mixed crop-livestock and dairy systems linked to markets will be targeted, where improved forage systems can have a high chance of being adopted and contribute to reducing poverty. In addition, farming systems without livestock or with low livestock market potential will be targeted through fodder or seed production which feeds into other livestock systems.

The following criteria will be used to target the geographic areas and specific subjects of research:

1. High potential to extrapolate and scale out results of the research.
2. High potential to tap markets for livestock or their products.
3. High potential to reduce poverty.
4. Agreement with the partners operating in the area.
5. Close interaction with relevant staff from IRI and CIAT should be possible in the selected location(s).

Arusha, Tanzania will be an appropriate location to base the joint CIAT-ILRI staff. Smallholder integrated crop-livestock systems and intensive dairy systems are prevalent in this region. There are rural and national market opportunities for dairy products. Not far from Arusha, pastoral systems are evolving from extensive herding to mixed crop-livestock systems in large numbers. Innovations can be relatively easily scaled out and up in Tanzania and surrounding countries. Partners in Tanzania have been identified during the joint CIAT-ILRI reconnaissance study in 2001. Expertise exists within both institutions to improve the feed and soil systems through improved forage options, and a range of adapted forage germplasm is available. Arusha is centrally located in the region, with easy access to Nairobi (ILRI - HQ) and Kampala (CIAT Africa office).

Key activities of the joint research agenda

1. Selecting priority intervention areas

Areas with high concentration of smallholder intensive and semi-intensive dairying, intensive and semi-intensive small ruminant systems, sedentary semi-intensive herding, evolving pastoralist systems, animal traction based highlands, and non-ruminant systems will be mapped in East and southern Africa using secondary information where available, and through discussions with national partners. These maps will be overlaid with mixed crop-livestock systems (excluding the specialized livestock systems) with constraints of land and feed resources and at the same time market opportunities for livestock products, through discussions with partners. Initially, these systems will be mapped for Tanzania, Kenya, Uganda, Rwanda, Lake Kivu region, and Ethiopia. Later the same will be done for Malawi, Zimbabwe, Mozambique, Zambia, Burundi, Sudan and Madagascar. Targeted surveys and stakeholder meetings within the marked areas in the maps will be used to select sites for intervention. Additional criteria for targeting intervention areas are the maps of density of poor livestock keepers; Ethiopia, Eritrea, Uganda, Kenya, Tanzania, Rwanda, Burundi, Malawi and South Africa have high concentrations (2-20 persons per km^2) of poor livestock keepers in East and Southern Africa.

2. Identification of market opportunities

In order to get a good picture of market opportunities at micro level, one needs to have an understanding of the situation at meso and macro level, which will provide insight in the limitations of the opportunities. Data on production, consumption, export and import of milk and meat will be summarized initially for the priority countries Tanzania, Kenya, Uganda, Rwanda, Lake Kivu region, Ethiopia and later for other countries mentioned earlier. Existing marketing structures within countries will be summarized through discussions with local partners. Ex-ante market and impact studies will be carried out to assess the demand and price elastics of livestock products. Through combined surveys and stakeholder meetings mentioned at activity 1, market opportunities at micro-level will be assessed. A survey will be conducted in targeted areas on sales and demand of forage products, e.g. legume leaf meal, hay, or fresh forage.

3. Forage germplasm evaluation

Improved grasses and selected legume accessions with superior mechanisms to deal with abiotic stress factors prevalent in many parts of Africa such as soil acidity, low soil fertility and drought will be evaluated in multilocational researcher - led trials in terms of seasonal biomass production, forage quality and seed yield. A collection of forage species will be maintained in Africa to provide foundation seeds for national seed systems, private seed companies and R&D projects.

4. Development of forage and food-feed technologies with farmers

At selected sites, and through national partners, new forages and food-feed crops will be tested and evaluated with farmers. Principles of participatory and action research approaches and participatory monitoring and evaluation will be used to ensure appropriateness for and adoption by end-users. Processes of change will be evaluated in an innovation systems context; weak linkages among essential actor will be identified and strengthened, sustainable ways of information flows sought, and capacity strengthened to innovate. Territorial approaches for marketing related to dairy and crop-livestock systems will be tested and adapted.

5. Seed production and distribution systems

Work on forage seed supply systems is an important part of the joint CIAT-ILRI research agenda as a means to raise income and enable scaling. To identify suitable sites for seed production, tools such as Homologue, and CANASTA will be explored. With relatively little extra work, specific adaptation maps can be produced from ongoing CIAT and ILRI activities. Public-private linkages as well as complementary farmer - led efforts will be explored and developed. Linkages with private seed companies (i.e. Papalotla) will be strengthened. This work will also link with existing activities such as work of CIAT on seeds in Eastern Africa.

6. Reversing land degradation

Improved forages have the potential to improve soil nitrogen and organic matter, increase water holding capacity of the soil, and stabilize soil in erosion prone areas. These potentials of forages and food-feed crops will be evaluated and optimized in relation to dairy and crop-livestock systems with high market potential. The hypothesis that market opportunities improve farmer investments in natural resources through sustainable use of forages will be tested.

7. Information systems, capacity building, and enhanced learning

Rural communities need to have access to high quality and appropriate information about forages and food-feed crops in order to facilitate choices and decisions on what to grow. National partners have the prime responsibility to provide this information in at the places where it is needed. A system will need to be in place to facilitate knowledge management. The new package 'SoFT' provides a model for which forages can grow where. A-AARNET will be the appropriate channel to facilitate knowledge management and capacity building of national partners. Training on forage technologies, seed production, and agro-enterprise development will be targeted where necessary. A regional community of practice under the umbrella of A-AARNET will be established to exchange information and to enhance learning on research approaches which enhance uptake and impact of forage systems.

4.2 Partnerships in Asia to undertake evaluation and diffusion of new forage alternatives

Highlights

- A strategy for collaboration in forage/livestock R&D in Asia was agreed between CIAT and ILRI based on a common vision, comparative advantages and strengths of the two institutions

- Forage research from 1992 to 2006 has led to considerable adoption of forage technologies and has resulted in the development of new forage-based livestock systems that provide significant livelihood benefits to poor farmers

- Lessons learnt from developing and scaling out of improved forage technologies are providing useful guidance to development practitioners

- Forage legumes are emerging as a viable option for improving village pig production systems through improved protein nutrition

4.2.1 Revised CIAT- ILRI strategy for forages/livestock R&D in Asia

Collaborators: W. Stur (CIAT), R. Lefroy (CIAT), D. Grey (ILRI) and B. Thorpe (ILRI)

Scientists from CIAT and ILRI after several discussions have identified concrete opportunities for collaboration based on a common vision, comparative advantages and strengths of the two organizations. It is recognized that both institutions should continue their own activities in the area of livestock research for development and, as such, linkages between CIAT and ILRI will range from keeping each other informed of activities through the development and implementation of joint projects.

Past and current research areas

CIAT has implemented several regional and bi-lateral forage and livestock-related projects in upland areas of Southeast Asia, starting in 1992, with particular emphasis on

- identification of suitable forage varieties for upland areas in SE Asia,
- development of farmer participatory approaches to forage technology development,

- integration of forages into smallholder crop-livestock systems in upland areas
- developing approaches for scaling out identified forage systems
- formation of an active network of forage researchers and extension workers
- developing approaches for participatory livestock production to market constraints and opportunities
- linking livestock production with other aspects of farming systems (e.g. production and utilization of cassava, sweet potato, etc.)

As these projects developed, the emphasis of projects has broadened from forage evaluation to approaches of working effectively with farmers to integrate forages into crop-livestock systems, to scaling out successful examples, to identifying and overcoming marketing constraints. There is now a very active network of national partners who have been involved in these projects. Weaknesses are in the areas of animal nutrition and policy.

ILRI Initiated projects in the region in 1998, emphasizing:

- development of sustainable technologies for small holder crop-livestock systems
- analysis of existing policy and identification of policy options for smallholder production systems
- development of technologies and participatory approaches to small ruminant enterprises
- formation and support of an active network of livestock researchers and extension agencies

A common vision

Both CIAT and ILRI are working on ways of promoting increased market orientation of smallholder farmers in the region. This is based on the rapidly increasing demand for meat in Asia which makes livestock production an increasingly attractive farm enterprise. Innovative farmers are looking for ways of expanding livestock production to take advantage of this opportunity. This requires a more market-oriented production system but based on minimal cash inputs, at least during the transitional phase. Forages grown on farm are playing a key role as the enabling factor for intensification as they can provide the additional feed needed with minimal inputs. Access to markets and a good understanding of market demands for meat quality are also critical factors to enable farmers to benefit from improved livestock productivity.

Understanding enabling policies that promote increased smallholder livestock production are needed, particularly at local government level. The benefits of more intensive smallholder livestock production are significant at household level and can be widespread as the vast majority of livestock is in the hands of smallholders.

ILRI and CIAT have the complementary skills to work towards this vision of increasing the productivity and market orientation of smallholder farmers in Asia through better use of feed, livestock resources, marketing and policies through partnerships with the existing networks of national partners.

To achieve this vision the two centers should work together to develop:

1. A Knowledge Network for Livestock in South East Asia based on previous work and existing networks of national partners.
2. Technology, marketing and policy options that support the intensification of smallholder crop-livestock systems, to enable the transition from subsistence to market orientation.

Principles for collaboration and funding

- Try to seek funding/project opportunities that exploit the complementarity of the two institutions and should not attempt to do all livestock related work as joint activities
- CIAT has had a greater focus on SE Asia, with no activities in S Asia in the livestock area, while ILRI has worked in both areas, with a greater focus in S Asia. Within the obvious personnel constraints, the two centers should attempt to cross-fertilize these activities.

4.2.2 A survey of adoption of improved forages in Southeast Asia

Contributors: Werner Stür (CIAT), Phonepaseuth Phengsavanh (CIAT / NAFRI), Francisco Gabunada (CIAT/Leyte State University), Peter Horne (previously CIAT), Truong Tan Khanh (Tay Nguyen University), Viengsavanh Phimphachanhvongsod (NAFRI), John Connell (CIAT), and Federico Holmann (CIAT/ILRI).

Rationale

CIAT commenced forage research in Southeast Asia in 1992 with the introduction of a large range of forge accessions. In 2005, two major CIAT forage projects – the regional Livelihood and Livestock Systems Project (LLSP) and the bi-lateral Forages and Livestock Systems Project (FLSP) in Laos were completed (Table 42). By this time, the long-term commitment of CIAT and its partners had led to significant livelihood benefits and adoption of planted forages by a large number of smallholder households in the region. These were documented in a survey and impact studies and a summary of the results is provided in this section.

The survey commenced with assembling a list of households growing forages at pilot sites; from these lists up to 50 households were selected randomly for semi-structured interviews. More than 500 households were interviewed across all pilot sites. In addition, several well-targeted impact studies were conducted; these evaluated the impact of specific production systems such as cattle fattening, cow-calf production and herbivorous fish production.

Adoption of forages

Following a slow initial rate of uptake in the first few years, the adoption rate accelerated and almost 10,000 households had adopted planted forages at pilot sites by mid 2005 (Figure 47). Planted forages had also spread beyond project sites and the developed technologies were incorporated into development plans by local governments, NGOs and development projects. Adoption beyond project sites has been considerable (> 10,000 households) and is accelerating. Planted forages are becoming the 'normal practice' in many areas in the region.

The main forage species used were the grasses *Panicum maximum* 'Simuang', *Brachiaria humidicola* 'Tully' and 'Yanero', *Brachiaria* hybrid 'Mulato', *Brachiaria brizantha* 'Marandu', *Paspalum atratum* 'Terenos', *Setaria sphacelata* 'Lampung' and *Pennisetum* hybrid 'King grass' and the legume *Stylosanthes guianensis* 'Stylo184'. The reason farmers first grew grasses was that these have a much higher yield than legumes and quantity of feed (rather than quality) was the primary concern of farmers. The average area of planted forages on farms increased to about 2,500 m^2 with many farms having areas of 2,000 – 3,000 m^2 (Figure 48).

Farmers, almost exclusively, managed planted forages as cut-and-carry feed. Less than 5% of households at pilot sites reported that they occasionally graze their animals on planted forages. This is a significant departure from the perception commonly held in both the research and development community that forages should be used as grazed pastures. Farmers planted and managed forages like food crops, looking after each plant carefully. At several sites (e.g. Daklak, Vietnam), some households irrigated forages in the dry season. Another indicator of the intensity of forage production was the use of manure and fertilizer applied to forage areas. The vast majority of farmers (>90%) apply manure and/or fertilizer to their forages to ensure high productivity; only at sites with very extensive production systems (e.g. Malitbog, Philippines and Savannakhet, Lao PDR) was the use of manure for forages not yet adopted extensively.

Farmers use planted forages for many purposes (Figure 49). Almost all farmers used forages for cow-calf production with most using planted forages as a supplementary feed throughout the year or for providing feed when cows were kept

Table 42. CIAT forage research projects in Southeast Asia, 1992-2006.

Period	Project	Emphasis
1992–1994	'Forage Seeds Project', managed by CIAT and CSIRO (Australia) and funded by the Australian Government (AusAID). Working with national partners in Indonesia, Malaysia, Philippines and Thailand.	Introducing and screening of a broad range of forage germplasm (>500 accessions) for sub-humid environments.
1995–1999	'Forages for Smallholders Project' (FSP), managed by CIAT and CSIRO, and funded by the Australian Government (AusAID). Working with national partners in Indonesia, Lao PDR, Malaysia, Philippines, P.R. China, Thailand and Vietnam.	Developing appropriate forage technologies through regional and farmer participatory evaluation.
2000–2002	Phase-2 of the 'Forages for Smallholders Project', managed by CIAT and funded by the Asian Development Bank (ADB). Working with national partners in Indonesia, Lao PDR, Philippines, P.R. China, Thailand and Vietnam.	Participatory approaches to scaling out of forage technologies.
2003–2005	'Livelihood and Livestock Systems Project' (LLSP), managed by CIAT and funded by ADB. Working with national partners in Cambodia, Indonesia, Lao PDR, Philippines, P.R. China, Thailand and Vietnam.	Developing improved feeding systems (based on forages) to increase returns of livestock production and improve scaling out approaches.
2000–2005	'Forage and Livestock Systems Project' (FLSP), managed by CIAT and funded by the Australian Government (AusAID). The FLSP was a bi-lateral pilot development project in Lao PDR.	Participatory development and dissemination of forage technologies, including a large capacity building component.
2004-2005	Project Preparatory Technical Assistance (PPTA) to design a Participatory Livestock Development Project in Lao PDR, managed by CIAT in collaboration with ILRI and financed by the Asian Development Bank (ADB).	Working with ILRI to design a livestock development approach that would work in an ADB loan project in Laos. This integrated lessons learnt from past forage research in Southeast Asia.
2005–2007	Capacity Building for Smallholder Livestock Systems (CBSLSP), managed by CIAT and funded by the Asian Development Bank (ADB)	Using the approaches developed by the FSLP and LLSP, design an effective mentoring system that allows the rapid scaling out of forage and livestock innovations.
2006–2008	'Legumes for village pigs in Lao PDR' (L4PP), managed by CIAT and funded by the Australian Government (ACIAR).	Investigating the opportunities of using forage legumes as a protein source for pig production.
2007-2010	'Enhancing livelihoods of poor livestock keepers through increasing use of fodder', part of a SLP project operating in Ethiopia, Syria and Vietnam coordinated by ILRI; the Vietnam component is managed by CIAT; funded by IFAD.	Improve our understanding of the factors and processes that determine the success of fodder interventions in developing countries.

near the village for some weeks after giving birth. At some sites, farmers fed planted forages to draught cattle when they were used for ploughing or during period of flooding (e.g. Cambodia) when access to other feeds was difficult. Since 2002, a very exiting development has been the emergence of fattening systems for cattle. At first farmers in Daklak, Vietnam started to buy older thin cattle, to which they then fed planted forages for 2-3 months before selling them to traders for slaughter. This fattening/finishing of cattle before slaughter proofed to be a very profitable activity and many farmers, at other pilot sites where this idea was introduced, have

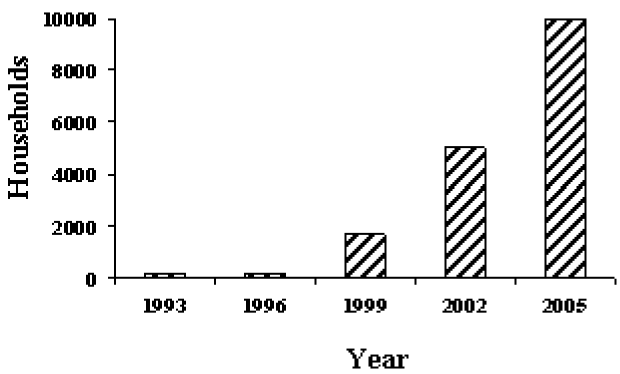

Figure 47. Farmers adopting planted forages at pilot sites (1993-2005).

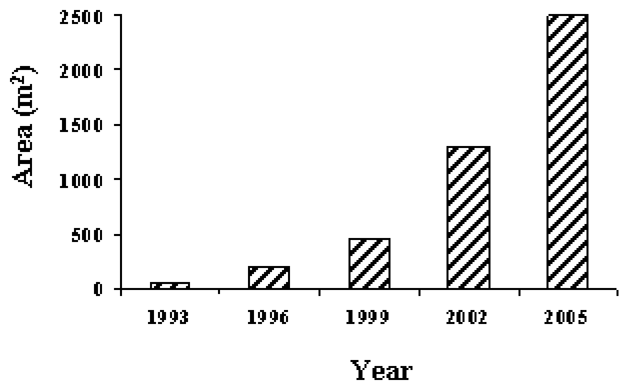

Figure 48. Average size of planted forages per household from 1993 to 2005.

also started to fatten cattle (Figure 49). In fattening systems, farmers used 100% planted forages rather than to use planted forages as a supplementary feed; this required approximately 800m^2 per animal. The main grasses used in these systems were *Panicum maximum* 'Simuang', *Pennisetum purpureum* 'Napier' and *Brachiaria* hybrid 'Mulato'. In these situations, farmers manage planted grasses very intensively with high rates of manure and fertilizer, and supplementary irrigation if available. Some farmers were using supplementary concentrate feed to achieve higher daily weight gains and there is an opportunity to introduce legumes as a source of cheap, farm-grown protein.

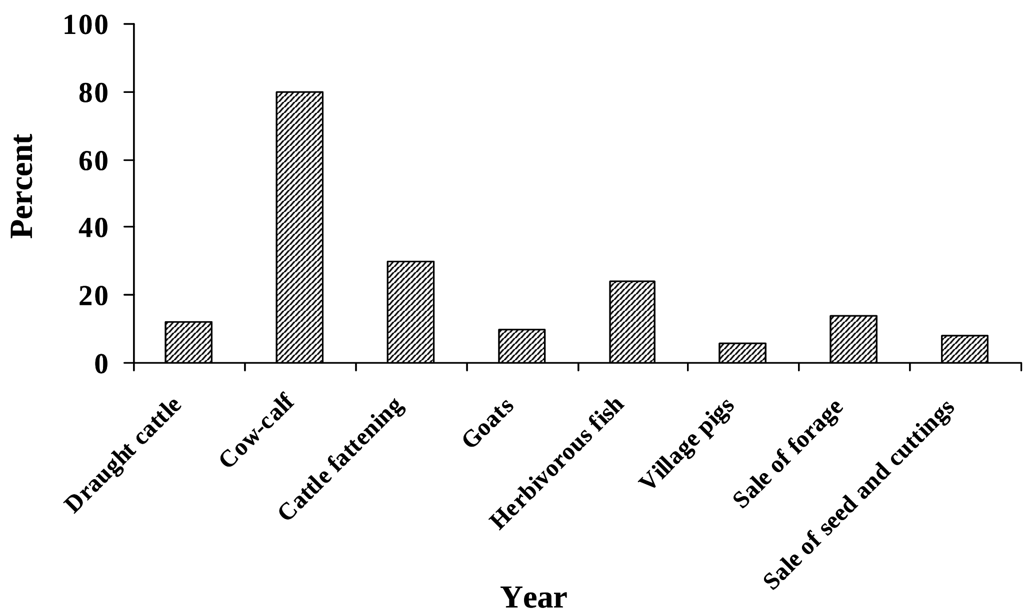

Figure 49. Use of forages in 2005.(total exceeds 100% as many farmers use forages for more than one purpose)

Several other unexpected forage uses developed. These were the feeding of planted grasses (mainly *P. maximum* ‘Simuang’) to herbivorous fish in Vietnam, feeding of the legume *Stylosanthes guianensis* ‘Stylo 184’ to village pigs in Lao PDR and the sale of fresh forage as feed to other farmers in Thailand and Vietnam (Figure 49).

Impacts

The main livelihood impacts of planted forages were considerable labor savings and higher income from increased sales of animals (from both improved animal productivity and the ability to raise more animals per household). These resulted in a significant increase in the return to labor from livestock production. The area of forage planted by farmers at almost all project sites was sufficiently large to experience not only labor saving but also substantial improvements in animal production (Table 43). An investment of 0.2 ha of planted forages is sufficient for fattening two cattle. At most sites, the area of planted forages was much larger than these minimum areas.

Several impact studies were conducted to document the impact of planted forages on the livelihood of households. Initially farmers grew forages in small areas on non-cropping land (e.g. road sides, between fields, on slopes not suitable for crops), however, households wanting to increase their forage area had to use areas that had previously been grown to crops. This has occurred at most sites with farmers converting their less productive cropping areas such as upper paddy fields to planted forage areas. This replacement of crops with planted forages reflects the higher returns from livestock production. Below are three examples.

Table 43. Minimum area of planted forages required for livelihood benefits.

Use of forages	Forage area
Saving labor (convenience)	300-500 m^2/farm
Fattening cattle or buffalo	800-1,000 m^2/animal
Cow-calf production	500-1,000 m^2/cow
Forages for herbivorous fish	500-700 m^2/pond
Legumes for pigs	100-250 m^2/pig

1. Cattle fattening: In Daklak, Vietnam, smallholder farmers started short-term fattening to finish cattle for sale to the slaughter house. Planted forages replaced less productive coffee plantations which had been planted when coffee prices were high. An impact study was conducted with 30 randomly selected households which compared cattle fattening with the previous use of the area where planted forages were now grown for cattle fattening. The average area of coffee replaced was 1,200 m^2. The mean daily liveweight gain of cattle in the fattening system was 669 g, based on planted grasses (mainly the grass *Panicum maximum* ‘Simuang’) and a small amount of concentrate feed (on average 2 kg/day). The net profit from fattening cattle was USD 511 per year compared with USD 90 for coffee from a 1,200 m^2 field, making cattle fattening a very attractive option.

2. Grass carp fish production: In Tuyen Quang, northern Vietnam, many households have fish ponds for producing grass carp. An impact study was conducted with 30 randomly selected households which compared fish production before and after adoption of planted forages. On average, farmers in the study had 2,400 m^2 of fish pond and had planted 540m^2 of forages (mainly *Panicum maximum* ‘Simuang’) to feed to their fish. One of the most important benefits of having planted forages was a saving of labor for feeding fish. The mean labor requirement for producing fish over one production cycle (8-10 months) was 648 hours before households had access to planted forages and 308 hours since planting forages, a very significant saving of scarce family labor. At the same time pond productivity increased from 75 kg to 122 kg of fish harvested per 100m^2 of pond, a 38% increase in productivity. Households also reported that they had been able to increase the area of fish ponds by almost 30% since using planted forages. The net income per fish pond increased from USD 84 to USD 283 and the return to labor increased from USD 0.25 to USD

1.28 per hour. The very significant benefits of using forage-based feeding systems, both in terms of net income and the much more attractive return on labor, explains the rapid uptake of this technology. The opportunities provided by planted forages – reduced labor and increased pond productivity – enabled many households to shift from raising some fish for home consumption to producing fish for the local market; a very profitable livelihood activity for households including those with very small land holdings as only small areas are required for ponds and forage plots.

3. Cow-calf production systems: A study, conducted in Ea Kar, Daklak, Vietnam, assessed the impact of adoption of planted forages on households practicing cow-calf production. The study used farmer group discussions and conducted 47 individual household interviews (27 household with planted forages and 20 households practicing traditional cow-calf production based on native feeds and extensive grazing). The main impacts of planted forages were larger herd size, a change in the management system from grazing to partial confinement (and providing cut-and-carry feed), a change from native cattle breeds to cross-bred animals, increased sales and higher returns to labor. The mean herd size was 6.9 animals for adopters and 4 animals for non-adopters (which was close to the average herd size of adopters before they had planted forages). Adopters were also able to raise crossbred (Red Sindhi x Native) cattle (77% for adopters and 27% for non-adopters), which have higher nutritional requirements but also a higher sale value than native cattle. The average income from the sale of cattle during the preceding year was USD 756 for adopter and USD 441 for non-adopters. Farmers who adopted forages were able to substantially reduce or eliminate altogether the large amount of labor needed for supervised grazing, with only a small additional amount of labor required for cutting grass. On average, adopters were spending less than half the amount of time looking after their cattle than the non-adopters (3.0 versus 6.8 hours/day) resulting in higher returns to labor. Returns to labor for adopters were USD 0.69 per hour, compared to USD 0.18 per hour for non-adopters.

Other very significant cash income generation opportunities were the sale of fresh forage to livestock producers and traders, particularly in Thailand and in northern Vietnam, and from feeding legumes to pigs (see next section, 4.2.2). At many sites, early adopters also obtained benefits from the sale of planting material and more recently from the sale of seed. In all cases, households used the additional income from sales of livestock to improve living conditions for the family, for educational expenses of children and to invest into their agricultural production.

Lessons learnt

Many important lessons for the successful development of planted forage systems and scaling out of forages for smallholder farmers emerged from this research. These can be grouped into those that are essential, and those that make technology development and scaling out easier or more difficult.

1) Essential components

- Livestock have to be important to the livelihood of farmers in the target area otherwise they will not be willing to invest the time and effort needed to evaluate and integrate planted forages.

- Farmers must have and recognize that they have a problem with feeding their animals. Traditional, communal feed resources are insufficient to support the production system and farmers are forced to invest more and more time in feeding their livestock. This must be recognized as a problem by farmers, and provides the entry point for working together.

- Employing a participatory approach to engage with farmers in developing and integrating forages into their farming system. Addressing the main problem (often labor shortage or lack of feed) ensures that farmers are willing to invest time and effort in evaluating the use of planted forages.

- Encouraging farmer learning, experimentation and innovation (Horne and Stür 2005); farmers will develop uses and ways of integrating and managing planted forages that are appropriate for their situation (e.g. forages for herbivorous fish, legumes for village pigs, using cut-and-carry for *Brachiaria humidicola*). This has resulted in high-impact systems that are compelling examples for others to adopt.

- Having suitable, well-adapted forage varieties that can deliver significant improvements to livestock production systems. There are many cases where ill-adapted species had been introduced previously without success, but widespread adoption occurred once a well-adapted variety was introduced (Tuhulele *et al.* 2007; Gabundada *et al.* 2007).

- Having long-term commitment. The forage technology development phase takes several years, as those involved have to evaluate, adapt and innovate with planted forages before these will provide significant livelihood benefits. Often, farmers realized that planted forages opened new opportunities and changed their livestock management and feeding system to take full advantage of the new feed resource. This process of learning and innovating takes time, however, the process can be quite fast when new sites are linked to more advanced sites where expertise in participatory forage technology development has already been developed. One example is Cambodia which benefited from experience from other countries and was able to develop fodder banks for feeding cattle during the flooding period within 2 years; a process that would have taken 3-5 years previously.

- Scaling out has to be based on compelling examples of a group of farmers receiving significant livelihood benefits from having adopted planted forages. These become learning sites for scaling out.

- Engaging key stakeholders such as development practitioners (extension service, animal health worker, NGOs and development projects) and service providers (such as traders and suppliers) is needed in scaling out successful forage technologies.

- Linking producers to markets. A better understanding of what markets demand and pay for different products generates interest and demand for improved feeding systems among farmers.

2) Factors that make it easier or more difficult to develop and scale out planted forages

- The degree of change of the production system required to integrate planted forages effectively. For example, the idea of planting forages on their own land and using this for cut-and-carry is relatively easy for farmers who already keep animals in pens and go out to cut natural feed from communal areas. The required system change is relatively small. On the other hand, farmers who manage their livestock in extensive systems (such as free-range grazing) have to make several significant changes to their management system to be able to take advantage of planted forages.

- The need for fencing increased the cost of planting forages. It is easier and cheaper to grow planted forages in areas where all animals are already constrained or penned, as no fences are required to protect the forage plots from grazing animals. In areas with unsupervised grazing, the need for a secure fencing adds significantly to the cost of utilizing planted forages and greater benefits are needed to offset these costs. There has been a trend for local government to prohibit free grazing, at least for part of the year, and to make animal owners responsible for damage to crops and planted forages. Such regulations help the adoption of planted forages.

- Ease of propagation; being able to propagate forages vegetatively promotes the spread of forages as farmers are not dependent on suppliers of seed. Dependence on seed requires the development of seed supply systems which provides an additional hurdle.

- Champions of particular forage technologies can accelerate the scaling out process. Without a project or a local champion, scaling out will still happen as long as the developed planted forage examples provides significant livelihood benefits but the rate of spread may be slow (Tuhulele *et al.* 2007).

- Population density and infrastructure also play a role in scaling out of forage technologies. Intensive farming systems with high population density are more conducive to the spread of good ideas and technologies from farmer-to-farmer than more extensive systems where there is less contact between farmers. For example, the rate of adoption was much slower in the extensive farming system (and poor road system) of Central Kalimantan compared with the fast uptake of planted forages for cattle fattening in more intensive farming systems in the Central Highlands of Vietnam.

Conclusions

Planting forages on their own land was the key factor that enabled smallholder farmers to improve livestock production. Planted forages significantly improved household income and, most importantly, the returns to labor from livestock production. The initial benefit from planted forages was, almost invariably, labor savings from easy access to feed. Subsequently, improved growth of animals receiving planted forages emerged and farmers look for ways of maximizing the opportunities provided by the new resource. This led to improved feeding and management systems, which provided significant benefits to farmers.

Participatory approaches to technology development were an essential component of success and produced several unexpected innovations such as forages for herbivorous fish production. Scaling out requires different methodology from participatory technology development and the involvement of a different set of stakeholders. This was most successful in cases where scaling out was based on high-impact, compelling examples which had been developed and adopted by a group of smallholder farmers.

The key role of planted forages in enabling smallholder farmers to intensify their extensive livestock production system and become more market-oriented has been accepted by development agencies in Laos. Similarly, the participatory approaches developed for forage technology development and scaling out have attracted interest from development practitioners. Both forage technologies and approaches for working with smallholder farmers have been integrated into large development project, ensuring that the results of our research have widespread impact.

Adoption of planted forage technologies is continuing to accelerate and the main challenges now are to (a) help farmers to continue to improve animal productivity to become more competitive, enable regular supply of animals and to link more effectively with markets to ensure maximum returns for higher quality animals, (b) address non-feed production constraints such as animal health, animal management, input supplies and marketing, and (c) address factors limiting scaling out such as supply of planting material of the most suitable forage varieties, and ensure access to useful information and training for new practitioners engaged in forage and livestock research and extension.

4.2.3 Legume supplementation of village pigs in Lao PDR

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Rationale

An unexpected outcome of the introduction of planted forages in Southeast Asia was the use of the forage legume *Stylosanthes guianensis* CIAT 184 (Stylo 184) as a supplementary feed for village pigs. Farmers found that pigs liked Stylo 184 and reported (i) significant labor savings as Stylo 184 could be used instead of naturally occurring green feeds which take a long time to collect, and (ii) improved growth rates and productivity of pigs.

Rearing pigs is a widespread smallholder livelihood activity in the northern mountainous regions of Lao PDR, contributing substantially to household incomes, especially among upland ethnic groups where rural poverty is endemic. Traditional feed resources for pigs (native tubers, banana stems and leafy vegetables from the forest) are, however, declining from overuse. Labor to collect pig feed and fuel to cook it, mainly provided by women, is also a major constraint. Consequently, village pigs are commonly underfed and chronically protein deficient, resulting in poor productivity.

A project was designed to investigate the potential of using forage legumes as a supplement for village pigs. ACIAR agreed to fund this research from January 2006 to December 2008. The objectives of the project are:

1. To collate and analyze baseline information of the existing pig production systems in upland Lao villages.
2. To determine the nutritional factors of legumes that are responsible for improved pig productivity, and evaluate best-bet legumes for their feeding value for pigs.
3. To scale-out the integration of Stylo 184 in smallholder pig feeding systems, using Stylo 184 as a model for investigating on-farm factors that influence adoption of forage legumes.
4. To develop guidelines for scaling out of improved pig feeding systems using forage legumes.

The project is managed by CIAT and the National Agriculture and Forestry Research Institute (NAFRI). The QDPI&F is providing support in the area of pig nutrition.

The project commenced in May 2006 and has completed two studies which are reported below. In 2006, other project activities (not yet completed) include nutritional analysis of commonly used feeds and potential forage legumes, a feeding experiment to document the potential growth rate and protein deposition of native pigs (information needed for evaluation of current feeding systems and to provide a basis for feed formulation), establishment of a community of practice and development alliance for scaling out of Stylo 184 with interested researchers, NGOs, local government extension services and development projects. Results of these activities will be reported in 2007.

A study of the impact of feeding Stylo to village pigs

This study was designed to quantify the impact of feeding Stylo 184 to pigs in smallholder pig production systems in Lao PDR. Feeding Stylo to pigs was a farmer innovation and reports from individual farmers indicated substantial benefits in terms of time saved for collecting green feeds and improved growth of pigs. The study was carried out in 11 villages in two districts in Luangphabang and Xiengkhuang provinces. The survey team consisted of CIAT staff, national partners from the National Agriculture and Forestry Research Institute (NAFRI) and staff of the Provincial Agriculture and Forestry Offices (PAFO) from Luangphabang and Xiengkhuang provinces and the District Agriculture and Forestry Offices (DAFO). Villages included in the study were selected on the basis of experience with using Stylo for pigs and those with longer experience of growing and using Stylo for pigs were selected preferentially as the study aimed to capture the experiences of farmers as well as impact on production and livelihood of producers. The only other selection criterion was to ensure that the main ethnic groups engaged in pig production were included in the study (Lao-loum, Hmong and Khmu).

Two study methods – Farmer focus group discussions and semi-structured interviews of randomly selected households - were used in the survey. Farmer focus meetings were organized in each village to gain a general understanding of pig management in each village, experiences of utilization of Stylo for feeding pigs and production and livelihood impacts. Household interviews were conducted immediately following each village meeting. A total of 30 households, including 7 women were interviewed. These represented the three main ethnic groups engaged in pig production in Laos: Hmong (4 households), Lao-loum (10 households) and Khmu (15 households).

The result of the study showed that there were two main impacts: (1) Improved growth rate of pigs, and (2) Time savings, as farmers (mostly women) no longer needed to collect naturally-occurring green feeds. Growth rates were estimated by asking respondents to estimate the initial and final sale weight of pigs kept in pens for fattening, and to recall the length of time taken from the start of the fattening period to sale of the animal. This information was used to calculate an average daily growth rate (ADG).

The mean age and weight of piglets at the start of fattening was 4 month when piglets weighed 14-15 kg, and sale weight was estimated at 65kg. Using Stylo as a supplementary feed reduced the length of the fattening period from 18 to less than 9 months (Table 44). This effect was consistent across villages and meant that Stylo supplementation increased average daily gain (ADG) from 107g per day in traditional feeding system to 207g per day for pigs supplemented with Stylo. Clearly, there may be factors other than Stylo supplementation that also played a role (e.g. better management, Stylo being fed in addition to other feeds rather than as a substitute for other feeds) but the consistency and magnitude of the response shows that Stylo has had a major impact on pig productivity. The average area of Stylo grown per household was 320 m^2.

The second impact was the saving of time and labor for collecting and cooking pig feed. If farmers had plenty of Stylo 184, the time needed to feed pigs could be reduced from more than 3 hours to 1.5 hours (Table 45). Farmers feeding only rice bran and Stylo were able to reduce the time needed for feeding to 40 minutes a day, as they no longer needed to cook feed. Even farmers with small areas of Stylo saved almost one hour per day.

Feedback from farmers showed that this time saving is regarded as highly significant as labor during the crop growing season is in short supply. Villages engaging in shifting cultivation require a huge amount of labor for weeding crops (estimated at 136 person-days per hectare). Freeing labor at this time of year is valued

Table 44. Productivity of growing pigs supplemented with traditional green feeds or Stylo.

	Traditional green feeds (no Stylo)	Supplemented with fresh Stylo	SE
Duration of production cycle, months	18.0	8.7	0.95
Initial Weight, kg	14.0	15.0	0.4
Final Weight, kg	65.3	65.1	3.2
Calculated ADG, g/day	107	207	12.2

Table 45. Time needed to feed village pigs before and after adoption of Stylo.

Items	Before		Now (with Stylo)			
	Time spent (min)	Who does the work?	Small Stylo area (not enough for feeding daily)	Large area of Stylo (enough to feed daily)		Who does the work?
				Mixed feed + Stylo	Rice bran + Stylo	
Collecting feed	125	W/M	55	0	0	W/M
Cook	50	W	50	50	0	W
Feeding	20	W	20	20	20	W
Collecting Stylo	-	-	20	20	20	M
Total	195		145	90	40	

tremendously. Farmers invested the 'freed' time in other farm activities including better management and health care of pigs. Reducing labor requirements was an excellent entry point for working with pig farmers in upland areas of Laos.

This study showed the potential of forage legumes to provide significant benefits in terms of improved growth and greatly improved the returns to labor by halving labor inputs into pig production. The improved growth response to Stylo 184 supplementation is likely to be related to improved protein supply and this will be investigated in controlled feeding experiments in 2007.

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Survey of village pig production systems in northern Laos

A survey of a broad range of smallholder pig systems was conducted to supplement the information collected in the impact study reported above. The impact study was carried out in villages where Stylo 184 had already been adopted and thus represented a small and biased sample. This survey covered a broad range of pig systems and included the three main ethnic groups engaged in pig production in Laos. The rationale for this decision was that different ethnic groups raise and manage pigs in different ways. The survey was conducted in 6 villages in three districts in Luangphabang and Xiengkhuang provinces from 13 March to 12 April 2006. The survey team consisted of L4PP staff, national partners from NAFRI, PAFO and DAFO.

Two study methods - village group discussions and semi-structured interviews of randomly selected households - were used in the survey. Village meetings were organized in each village to gain a general understanding of pig management in each village, the importance of pig production to farmers' livelihoods and to provide a list of pig-raising households in each village for random selection of households for interviewing. The team encouraged participation of women in the village discussions as the raising of small animals (such as pigs and poultry) is usually the responsibility of women. Household interviews were conducted immediately following each village meeting. A total of 30 households were interviewed representing the three main ethnic groups engaged in pig production in Laos: Hmong (13 households), Lao-loum (11 households) and Khmu (6 households). There were 12 women among the respondents.

The survey showed that there are three main pig production systems in the uplands of Laos: (1)

Free scavenging system; (2) Confining pigs in enclosures; and (3) Penning. The type of system employed was related to the purpose of raising pigs and ethnicity of the producer. The two main purposes of raising pigs were: (1) Piglet production and (2) Fattening pigs. Half of the respondents were engaged mainly in fattening while the other half was producing piglets (Table 46).

Table 46. Main purpose of pig production, stratified by ethnicity of producer.

Ethnicity of producer	Number of households	
	Piglet production	Fattening
Hmong	10	3
Khmu	4	2
Lao-loum	1	10

All Lao-loum producers, except one, were engaged in pig fattening, buying weaned piglets from piglet producers and fattening for sale. Most Hmong and two thirds of Khmu producers were producing piglets for sale. Just over 50% of producers were keeping pigs in pens (Table 47); these were mainly producers fattening pigs for sale while piglet producers mostly kept sows and piglets in enclosures or used a semi-scavenging system.

Table 47. Production systems, stratified by ethnicity of producer.

Ethnicity of producer	Number of households using		
	Semi-scavenging	Enclosure	Pens
Hmong	4	6	3
Khmu	1	2	3
Lao-loum	0	1	10

All respondents kept native pigs; these were either Moo Lao-soung (Hmong producers) or Moo lat (Khmu and Lao-loum producers). These breeds are well adapted to free range systems, where they can scavenge part of their feed. Local breeds are high-fat, swaybacked breeds, which produce more fat than meat. This has been important traditionally as pig fat has been the only oil/fat available for cooking in remote villages.

Farmers producing piglets mostly kept 1-2 sows (mean = 1.3) and, at the time of the survey on average had 5-6 piglets (mean = 5.5). Many piglet producers also fattened 1-2 pigs (mean = 1.4) which they had not able to sell or which they especially selected for fattening for special traditional ceremonies (such as New Year, weddings or religious celebrations). Farmers, who specialized in fattening pigs for sale, on average, produced 2-3 pigs per fattening cycle. Not every farmer keeps a boar. In most villages, there were only a few boars available for servicing sows and in some village no boars were available and the service had to come from another village.

The main feeds for pigs were planted crops such as maize and cassava (and to a lesser extent canna and sweet potato with leaves fed to pigs as green feed), crop by-products such as rice bran and broken rice, and green feed occurring naturally in local areas. Almost all producers reported that they fed rice bran and some green feed (fresh leaves) to their pigs (Table 48). In Lao-loum villages (lowland rice producers), producers fed mainly rice bran, sometimes mixed with broken rice or brewery waste (rice grain) and green feeds. Maize and cassava was used by most Hmong producers, while Khmu producers used maize and some cassava.

Feeds are not available year-round and cannot be stored safely for long periods. Therefore, feed is mostly poorly balanced in terms of energy and protein. Protein, in particular, appears to be

Table 48. Feed resources for pigs, stratified by ethnicity of producers

Feed resources	Number of respondents		
	Lao-loum	Hmong	Khmu
	(n = 11)	(n = 13)	(n = 6)
Rice bran	11	13	6
Broken rice	5	0	0
Maize	2	11	5
Cassava	2	12	2
Green feed	9	13	6

Table 49. Time spent collecting and preparing feed for pigs.

Activity	Wet season			Dry season		
	Mean	Median	Range	Mean	Median	Range
		(minutes)			(minutes)	
Collecting feed	113	105	30-120	126	120	30-180
Preparing and cooking	57	60	30-60	56	60	30-60

lacking in the diet for much of the year. Most farmers fed pigs twice a day, in the morning and late afternoon. All pigs received the same diet, and most farmers fed pigs were fed as a group with larger and dominant pigs being able to secure a larger amount of feed than smaller, more timid animals. Respondents reported that women spend up to 3 hours per day collecting natural green feeds and cooking feed for pigs (Table 49). This is a very time consuming activity for upland farmers, who need a lot of time for weeding and tending to upland crops.

The survey estimated growth rate of pigs in fattening systems by establishing the initial weight of pigs when they enter the fattening pen, the final weight at sale and the length of time taken for fattening. The mean length of the fattening cycle of pigs fattened for sale was 15.4 months which resulted in a calculated ADG of 111g (Table 50). Growth rate of pigs fattened by Hmong farmers for traditional feast was very similar with 110 g per day. The growth rate data collected in this survey are almost identical to those established in the Impact study reported in the previous section.

Sows produce, on average, 1.5 litters per year. Mean litter size was 7.4 piglets per litter, but only 3.9 piglets survived to weaning. The high mortality appeared to be related to poor hygiene (many dying from diarrhea caused by unspecified bacterial diseases) and poor nutrition of the sows during lactation. Squashing of piglets was not mentioned as a cause of mortality. Disease epidemics are a major concern of producers. All villages reported disease outbreaks (most likely Classical Swine Fewer) that killed 90% or more pigs in the village within the last few years. The results of this survey provide a baseline for subsequent impact assessment. They also showed that significant improvements in pig production can only be achieved by addressing the three main constraints of (i) poor feeds (both quality and quantity), (ii) high mortality of piglets, and (iii) outbreak of disease epidemics. In areas, where Stylo 184 has been introduced, farmers have started to improve not only feeding but also management (e.g. better housing, clean water supply) and health (vaccination and quarantine of pigs coming into the village); we hypothesize that is related to the improved growth rates and better returns to labor, which have made pig production a more attractive farm enterprise and thus worthy of investment. Forage legumes are emerging as a pivotal factor that enables and encourages farmers to develop more market-oriented pig production systems.

Table 50. Growth rates of pigs in fattening systems.

	Fattening pigs for sale (n = 16)			Fattening pigs for traditional feasts among Hmong producers (n = 13)		
	Mean	Median	Range	Mean	Median	Range
Length of fattening period (months)	15.4	16.5	7-24	21.3	24.0	8-24
Initial Weight (kg)	12.7	13.5	6-20	43.3	40.0	30-50
Final Weight (kg)	63	60	40-100	117	110	60-130
Average daily gain (g)	111	110	83-195	108	111	83-145

4.2.4 Future forage research in Southeast Asia

Contributors: Werner Stür (CIAT)

Forage research in Southeast Asia had several phases. The early phase (1992-1999) was characterized by forage accession introductions, nursery and regional evaluations and development of forage technologies with farmers using participatory approaches.

From 2000-2005, more applied research projects integrated forage technologies into a broad range of farming systems by working closely with farmers and these resulted in new forage-based livestock systems that provide significant benefits to farmers' livelihood. Improved livestock production based on forage technologies reduced labor inputs in livestock production and increased the income of poor households, resulting in significantly improved returns to labor.

These results 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. These projects also resulted in the identification of new research issues such as Stylo 184 for village pigs and forages for herbivorous fish production.

In 2007, a new research project in Vietnam will aim at (i) better understanding the factors and processes that influence the success of fodder innovations by analyzing forage adoption patterns in Vietnam and (ii) further develop forage-based livestock production systems using 'smart' feeding strategies designed to increase the returns from livestock production.

This 4-year project is part of a multi-country project (Syria, Ethiopia and Vietnam) managed by ILRI on behalf of the Systemwide Livestock Programme; the Vietnam component of this IFAD-funded project will be managed by CIAT. We will also pursue opportunities for funding for (i) research on feeding forages to herbivorous fish, (ii) research on forage-based cattle production systems for flood-prone areas in Cambodia, (iii) research on developing appropriate private sector supply systems for forage seed and planting material, and (iii) developing a knowledge management system for forage and livestock technologies in collaboration with ILRI to make innovations in livestock research more easily available to the development sector.

4.3 Partnerships in LAC to undertake evaluation and diffusion of new forage alternatives

Highlights

- Improved grasses were planted in 62 collaborating farms in 4 countries of Central America. Forage yield of the different cultivars has been variable across farms but consistently superior to the local pasture.

- Farmers in a pilot study in Northern Valle del Cauca, Colombia selected the following forage options to be sown in semi-commercial plots of 1 to 10 ha: *Brachiaria* hybrids cvv. Mulato, Mulato II, *B. brizantha* cv.*Toledo, B. dictyoneura*, *Cratylia argentea*, *Leucaena leucocephala*, *Vigna unguiculata* and *Lablab purpureus.*

4.3.1 Revised CIAT- ILRI strategy for forages/livestock R&D in Central America

Contributors: F. Holmann (CIAT), C. Lascano (CIAT), E. Perez (ILRI) and B. Perry (ILRI)

CIAT and ILRI have identified Central America as the focal region for their joint activities in Latin America because of the region's combination of large numbers of poor households dependent upon livestock and the potential for research-based improvements to their livelihoods. Thus scientists from the two centers developed a long-term R&D program that uses a demand-driven and production-to-consumption approach to improving rural livelihoods and increasing employment through livestock-based enterprises. Current and projected increases in the demand for livestock products both from within the region and to satisfy international markets are the basis for the program and the approach summarized bellow.

Outputs

Through policy options, institutional reforms and technological interventions the joint agenda should deliver improved:

1. input and output markets for smallholder livestock producers;
2. smallholder farm productivity;
3. value-added post-harvest;
4. management of natural resources,
5. rural livelihoods and increased employment.

Strategy

The program's emphasis will be to deliver outputs and outcomes that contribute significantly to poverty reduction and that are applicable regionally. To achieve significant impacts on poverty, the program should target resource-poor producers and their input and output market agents. Because of their importance to resource-poor producers in Central America, the program will focus on dual-purpose (meat and milk) cattle systems and with legume based feed resources for monogastric (swine and poultry) as means of linking small farmers to markets. The marketing of milk and value-added dairy products has a particularly important role to play in improving rural livelihoods based on these systems. Resource-poor families can take advantage of new market opportunities by raising pigs or chickens, activities that require small initial investments. Such production of monogastrics is a livelihood diversification strategy that can improve family nutrition, provide much needed cash income.

It is expected that opportunities for reducing poverty will arise mainly from identifying and responding to domestic, regional and international market demands rather than from providing a technology "push". Responses to these demands are expected to call upon CIAT's forage technologies (which may include supporting non-livestock keepers to supply processed forages) and its expertise in natural resource management, rural agro-enterprises and participatory methods, while ILRI will provide expertise in marketing, animal genetics, health and nutrition, policy analysis and livestock systems. The program's emphasis will be group activities to increase the bargaining power of the smallholder livestock producers and of their market agents along the production-to-consumption chain and to improve the responsiveness and effectiveness of services. A key international public good to be delivered by the program will be the process by which an integrated natural resource and production-to-consumption systems perspective is developed in a region with close links to a large neighboring market.

Implementation

In the short and medium term it is expected that the funds to finance the staff and operational costs for the implementation of the R&D program will come from special projects aimed at o strengthening the R&D program and to ensure the continuity of the joint agenda's implementation.

4.3.2 On farm evaluation in Central America of selected forage accessions and cultivars

Contributor: P. J. Argel (CIAT)

As part of the ILRI/CFC Project, the establishment of improved forage component was completed in 62 collaborating farms of Honduras (15 farms), Guatemala (9), Nicaragua (20) and Costa Rica (18). A total of 2,242 kg of experimental forage seed was delivered to collaborating institutions during the course of the project, and this represented 207 ha established with improved pastures in farms of participating countries.

Grasses established are dominated by improved cultivars of the genus *Brachiaria,* mainly the hybrids cvv. Mulato and Mulato II; also *B. brizantha* cv. Toledo is an important component in farms located in heavy soils exposed to prolonged dry seasons. Meanwhile, *Cratylia argentea* cv. Veraniega has been the forage shrub legume more widely planted.

On-farm dry matter yields (DM) of improved forages was measured in Costa Rica in collaborating farms during the reported period. Results showed that yields of the introduced grasses were very similar, with the exception of the naturalized grass *Hypharrenia rufa* (Jaragua) that produced lower yields and is probably the more common grass in all cattle farms of the subhumid tropics in Central America (Table 51). Within grass cultivar there is ample variation in forage yield due to different regrowth ages and soil and climatic conditions of the farms. However, it is clear that improved grasses have high DM yields that can contribute to higher stocking rates compared to the naturalized grass.

Table 51. Dry matter yields of improve and native grasses in collaborating farms of the ILRI/CFC Project in Costa Rica.

Species/Cultivar	kg DM/ha	Days of rest (No.)	Farms (No.)
B. brizantha cv. Marandu/	2746		
B. decumbens cv. Basilisk	(1532-3961)*	25	2
B. brizantha cv. Toledo	2368	21	1
B. hybrid cv. Mulato	2250	22	
	(1281-2948)	(20-25)	4
P. maximum cv. Tanzania	2223	25	1
Hyparrhenia rufa (Jaragua)	1100	18	1

*In brackets DM yield range

Monitoring productivity responses of improved forages in Guatemala, Honduras, Nicaragua, and Costa Rica (on-farm monitoring and evaluation during rainy season of improved pasture technologies) has been difficult to implement in the Project. Poor management practices and the difficulty to have permanent group of animals in the farms to monitor, has made it difficult to measure animal production. However, both farmers and technicians are aware that milking cows increase milk yields when grazing improved pastures. On farm monitoring of animal performance in improved pastures will be emphasized during 2007.

4.3.3 On-farm evaluation of forage options in Norte del Valle del Cauca, Colombia

Contributors: C.V. Durán (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; in 2005 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), in 2006 the municipalities of Buga, Tulúa and Zarzal were added. The initiative now reaches directly 300 farmers. Altitudes in the 8 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 a voting 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

In 2006, twenty-two technical visits were carried out, to follow up experiments including semi-commercial plots and participatory evaluations with the farmers of Roldanillo, Versalles, Sevilla, Bolívar and El Dovio (photo 9). So far the species considered to be the best adapted were the grasses *Brachiaria* hybrids cvv. Mulato, cv. Mulato II, *Brachiaria brizantha* cv. Toledo, the herbaceous legumes *Canavalia brasiliensis*, *Centrosema pubescens* and *Arachis pintoi,* the annual legumes *Vigna unguiculata* and *Lablab purpureus* and the shrubs *Cratylia argenteas*, *Leucaena leucocephala* and the local control *Tithonia diversifolia*. Adaptation of *Panicum maximum* and *Brachiaria humidicola* was also good although in some cases limited by contamination of the commercially obtained seed. *Desmodium velutinum* performed well in only one site, while *Clitoria ternatea* was not well adapted to the conditions of the study site.

For farmer selection the following parameters were important: Palatability, color, forage on offer, adaptation to low fertility soils, tolerance to drought, tolerance to pest and diseases, dry matter production, cover, rooting capacity, persistence and adaptation to variable altitude and soil fertility. Based on these criteria, farmers selected the following forage options to be sown in semi-commercial plots of 1 to 10 ha, and a total of 25 ha sown: *Brachiaria* hybrids cvv. Mulato, Mulato II, *B. brizantha* cv.*Toledo, B. humidicola*, *Cratylia argentea*, *Leucaena leucocephala*, *Vigna unguiculata* and *Lablab purpureus*. A strategy of shared expenses was employed, with the Project providing half of the seed, while the farmer bought the other half.

Among the forages , the associations (10 ha) of the grasses Toledo, Mulato and Mulato II with the leguminous shrubs *Cratylia argentea* and *Leucaena leucocephala* and the multipurpose legumes *Vigna unguiculata* and *Lablab purpureus* established best.

Several meetings with farmers to advance testing with semi-commercial plots were realized and the farmer cooperative COGANCEVALLE added to the group of partners. As well results were socialized to farmers and technicians including employees UMATAS (Government extension service) and COGANCEVALLE. Further faros have been selected for semi-commercial plots (40 ha), with 40 and 100 farmers involved directly and indirectly respectively. Training is another component of the initiative having trained so far more than 150 farmers and technicians in establishment, management, utilization and conservation of forages.

Photo 9. Farmer field visit to semi-commercial plots in Roldanillo, Norte del Valle.

4.4 Adaptation of forage conservation technologies by smallholders in hillsides livestock systems

Highlights

- Farmer-led experiments showed higher profitability of farm made legume products (i.e. cowpea hay and cowpea-based concentrates) compared to commercial concentrates for milk production

- Farmer-led experiments showed higher profitability for grass (*Brachiaria brizantha* cv Toledo) and sorghum silage compared to maize silage.

- The little bag silage technology was found suitable as a) tool in the introduction, promotion, and extension of silage technology and b) entry point for silage making in dual –purpose farms.

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 strategy in the co-development of forage conservation technologies followed the subsequent steps: 1. Site selection based on diagnosis; 2. Farmer trainings (in theory and practice); 3. On-farm evaluation of technology; 4. Multi-actor information exchange and scaling out; and 5. Monitor adaptation, adoption and diffusion processes.

Steps 1 and 2 were elaborated in detail in the Annual Report 2005. Here we report advances on step 3 (technology evaluation), in which the potentials of innovative forage technologies as dry season feed alternatives are assessed. In feeding experiments, their effect on livestock production is compared to prevalent dry season feed supplements such as maize silage and commercial concentrates.

In the Yoro area, as in many similar environments elsewhere in Honduras, there is a shift from meat oriented production with Brahman cattle to milk oriented production with an increasing share of dairy breeds. This implies a change from traditional low input, low output livestock farming systems to higher input, higher output farming systems. Animal nutrition in terms of feed quality and quantity is a key element in supporting this change, especially during the dry season. Improved forages, silage, hay and concentrates that are increasingly being used by farmers in the area can contribute to overcome seasonal feed constraints and maintain farm productivity. However, there is a lack of information how to use these technologies and resources more efficiently. The following constraints to efficient technology use have been identified:

1) Natural and introduced forage resources are often not managed adequately i.e. pasture and cut and carry grasses are generally used in an advanced stage of maturity, thus their potential, in terms of quality and quantity forage production, is under-exploited. During the dry season, overgrazing of pasture resources frequently occurs, leading to pasture degradation and reduced productivity.

2) Maize is the predominant forage for silage production in the Yoro area, however, the opportunity costs for maize silage increase as maize prices increase.

3) Farmers often supplement unreasonable high levels of concentrate in order to maintain body condition of animals and/or to increase milk production, which increases production costs significantly. Alternative native or introduced protein rich forage resources are little used.

Different collaborative on-farm trials were conducted to address these constraints and validate different conserved forage options under local socio-economic and biophysical conditions with the aim to increase livestock production and productivity to respond effectively to future market challenges.

The following forages were tested on-farm: *Vigna unguiculata* (cowpea) hay and grain concentrate, *Brachiaria brizantha* cv Toledo silage, Sorghum silage, and *Cratylia argentea* silage.

4.4.1 Effect of harvest time and drying procedures on quality and losses of hay of three cowpea (*Vigna unguiculata*) accessions

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Rationale

In systems of small and medium producers livestock productivity is limited by low quality and quantity of feed in critical periods such as long dry seasons. One alternative is the production of high quality legume hay at the end of the wet season. However quality, appearance and palatability of hay from legumes such as cowpea can be affected by cutting time, drying process and climate; losses in the process of hay making until utilization can be considerable depending on the process employed. Moreover, in contrast to temperate climate, where haymaking has a long tradition and normally relies on special machinery for cutting and drying, in smallholder systems in the tropics the lack of these direct manipulation of the hay crop is an alternative. The cowpea materials selected for this study have also been utilized in haymaking in Central America where quality problems have been reported and the possibly factors are considered for this study.

Material and Methods

Contrasting cowpea accessions, i.e. early, medium and late maturing types were used for the study. The materials selected, 9611, IT95K-52-34 y IT89KD-288, are characterized by a high forage and good grain production and have a wide adaptation to both acid and alkaline soils. The experiment was established in two contrasting environments, CIAT – Palmira (alkaline soils, lower rainfall) and CIAT – Quilichao (acid soils, higher rainfall), the cowpea sown at a density of 190.000 plants/ha).

In addition to the different cowpea accessions the following treatments were included: a) two cutting times, i.e. 6 and 8 weeks after sowing, 8 weeks corresponding to pre-flowering time; b) three drying methods i.e. field drying (SC), on cement and cut (SPP) and on cement without cutting (SPN), representing potential scenarios under farmer conditions. A split-plot design with three replications was utilized. The variables included forage quality analysis (IVDMD, CP, NDF and ADF), agronomic evaluation (DM yield, hay yield, leaf: stem ratio) and organoleptic observation (smell, color). For the assessment of color a scale from 1 to 7 was employed, where 1) represents green-reddish, 2) variable, 3) brown 4) light brown 5) 50/50 (green brown), 6) opaque green y 7) dark green.

Results and Discussion

Biomass yields were higher at the later cutting time (pre-flowering time) in both Palmira and Quilichao, with accession IT95K-52-34 having the highest yields. Comparing DM yield with hay yield, as expected the latter were lower due drying and handling losses; in Palmira water-logging and diseases let to yield reductions.

In Table 52 yield losses at the time of utilization as affected by treatments are shown. Whereas in Palmira the effects of drying method varied between accessions ($P > 0.05$), in Quilichao highest losses were recorded for field drying for all three accessions, though again not significant ($P < 0.05$). In general losses were below 10%, independent of drying method.

Pooling the three accessions drying method and time of cutting resulted in significant ($P<0.05$) differences in IVDMD, with treatment SPN (cement drying, not cut) and the earlier cut having the higher values in both locations, but no significant ($P > 0.05$) differences were found for CP (Table 53.). The low digestibility of the later cut in Quilichao could be accounted for by a longer drying time than in Palmira; moreover the dark green color of the hay indicates the presence of saprophytic fungi with sign of initial decomposition of tissue though the smell remained pleasant.

Table 52. Yield loss for haymaking as affected by drying method for three accessions of *Vigna unguiculata* (Caupi) in Palmira and Quilichao, 2006.

Site	Accession	Drying method*	Yield loss for haymaking %
Palmira	IT95K-52-34	SC	3
Palmira	IT95K-52-34	SPP	3
Palmira	IT95K-52-34	SPN	2
Palmira	9611	SC	3
Palmira	9611	SPP	5
Palmira	9611	SPN	2
Palmira	IT89KD-288	SC	5
Palmira	IT89KD-288	SPP	9
Palmira	IT89KD-288	SPN	3
Mean			4
			ns
Quilichao	IT95K-52-34	SC	8
Quilichao	IT95K-52-34	SPP	6
Quilichao	IT95K-52-34	SPN	5
Quilichao	9611	SC	9
Quilichao	9611	SPP	7
Quilichao	9611	SPN	5
Quilichao	IT89KD-288	SC	9
Quilichao	IT89KD-288	SPP	8
Quilichao	IT89KD-288	SPN	5
Mean			7
			ns

*SC: field drying; SPP: on cement and cut; SPN: on cement without cutting

Table 53. Yield loss for haymaking and hay quality of three accessions of *Vigna unguiculata* (Caupi) in Palmira and Quilichao, 2006.

Site	Drying method*	Yield loss for haymaking	IVDMD %	CP
Palmira	SC	3.8	73	20
Palmira	SPP	5.7	73	20
Palmira	SPN	2.4	75	20
Mean		4	73	20
LSD (P<0.05)		1.6	1.5	NS
Quilichao	SC	8.8	64	21
Quilichao	SPP	7	73	21
Quilichao	SPN	5	75	23
Mean		6.9	71	21
LSD (P<0.05)		1.7	1.4	NS

*SC: field drying; SPP: on cement and cut; SPN: on cement without cutting

Leaf:stem ratio in Quilichao was not affected by cutting time, while in Palmira the earlier cutting time resulted in a higher leaf:stem for two of the accessions, though not significant (P > 0.05) due to interaction between accession and cutting time (Table 54).

In the organoleptic assessment only samples from Quilichao showed the variable color characteristic, confirming the negative effects of alternate drying and wetting due to rain during haymaking. The green-reddish colour was found in only a few samples from Palmira, likely due to infection with *Oidium* sp., with the fungus turning reddish during drying. The dark, light green and opaque green, green/brown (50/50) and light brown colors considered desirable were found with hay from both locations, with the opaque green natural color of the plant characterized in the literature as indicator of a good hay being the most abundant.

The smell of all treatments, including the hays affected by disease and sub-optimal drying was pleasant, varying slightly between sweet and herbal. In contrast to Honduras no serious quality effects due to drying method, climate and cutting method were encountered. Of particular interest are the positive results from Quilichao where haymaking met wet conditions unusual for the season of the year.

Table 54. Yield loss for haymaking and leaf:stem ratio of has for three accessions of *Vigna unguiculata* (Caupi) as affected by cutting time (6 and 8 weeks after planting) in Palmira y Quilichao, 2006. The 8 weeks cut represents the preflowering stage

Site	Accession	Cutting time in weeks after planting	Yield loss of haymaking %	Leaf:stem ratio
Palmira	IT95K-52-34	8	3	1.2
Palmira	IT95K-52-34	6	2	1.5
Palmira	9611	8	2	1.5
Palmira	9611	6	4	2.2
Palmira	IT89KD-288	8	4	1.6
Palmira	IT89KD-288	6	7	2.3
Mean			4	1.7
(P<0.05)			NS	
Quilichao	IT95K-52-34	8	6	2.1
Quilichao	IT95K-52-34	6	7	2.4
Quilichao	9611	8	7	2.1
Quilichao	9611	6	7	1.9
Quilichao	IT89KD-288	8	7	2.1
Quilichao	IT89KD-288	6	7	2.1
Mean			7	2.1
(P<0.05)			NS	

4.4.2 Effect of feeding cowpea (*Vigna unguiculata*) hay and grain on milk yield

Contributors: C. Reiber, R. Schulze-Kraft, M. Peters, P. Lentes, V. Hoffmann, H. Cruz and C. Lascano

Supplementation of dairy cows with commercial concentrates is a practice widely used by farmers in the Yoro area, especially during the dry season. This practice elevates production costs per litre of milk and reduces farm productivity. Alternative protein sources, which can be produced on-farm, promise higher returns per litre of milk. Well adapted, i.e. drought tolerant, forage legumes such as cowpea (*Vigna unguiculata*) have been promoted among farmers. Cowpea is a highly palatable, digestible and nutritive feed source with a crude protein (CP) content of 14-21% in the foliage, and 18-28% CP in the grains. A number of studies have shown that the use of cowpea as fodder has a positive effect on ruminant performance. The first experiment reported here focuses on cowpea hay while the second experiment on cowpea grain. Both were tested as partial substitutes of commercial concentrate with milking cows.

4.4.2.1 Effect of feeding cowpea hay on milk production

Materials and Methods

The type of experiment that was carried on the farm was agreed with the farmer and this included the selection of the cows and the feed rations (collaborative-collegiate mode). The study was conducted in a farm near Victoria, Yoro, Honduras. The area is located at an altitude of 395 m.a.s.l and has a temperature ranging from 22 to 32 °C and an average annual rainfall of 1150 mm with a 6- month dry season.

Fresh forage production of cowpea (accession IITA 284/2) was approximately 18 tons/ha. An area of 3600 m² of cowpea was cut for hay in the early flowering stage (mid November), sun-dried or dried under a roof for one day each case. Drying under a roof was applied to minimize leaf losses normally occurring the field. About 1.2 tons of cowpea hay was harvested of which about 0.46 tons were used for the experiment.

The experiment was conducted in the dry season of 2006 (February to March). Eight crossbred cows (Holstein x Brown Swiss x Brahman) were selected. Criteria for the selection of the cows were a lactation period between three and five months, number of weaned calves, similar live weight and body condition. Based on these criteria cows were equally distributed in two groups and included in a Double Reversal Design. The experiment lasted 42 days divided in three periods of 14 days each.

The basic feed ration (BFR) was maintained during the whole experiment and was the same for all cows: BFR = 9.1 kg maize silage (DM 35.3%) + 13.6 kg sugar cane (DM 25%) + 6.8 kg maize straw (DM 70%).

The following treatments were applied:

1. Treatment A (“cowpea hay mix”): BFR + 3.64 kg concentrate + 2.73 kg cowpea hay

2. Treatment B (“concentrate mix”): BFR + 5.45 kg concentrate (control)

In treatment A 1.8 kg of commercial concentrate (PC 22%) was substituted by 2.73 kg of cowpea hay (PC 16.2%).

The feed was supplemented in two rations, one in the morning and one in the afternoon during milking. Cows did not graze during the experiment. Live weight was measured every seven days, in the beginning and in the end of each sub-period using a digital livestock balance. Measurements were always done at the same time of the day (in the morning after milking) to avoid live weight changes due to differences in the degree of rumen fill. Milk production for each cow was recorded twice daily.

For statistical analysis of milk production, data from the first seven days (adaptation phase) of each period were excluded and data from day 8 to day 14 were taken for each treatment and group and subsequently analyzed using non-parametric tests (Mann-Whitney Test).

Results

Feed intake: The cows consumed all the offered BFR ration (11.4 kg of DM/cow -Table 55). on average, daily DM intake was 3.5% of live weight.

Table 55. Total feed consumption in kg DM/cow/day

	BFR	Cowpea Hay	Concentrate	Total DM intake
Treatment A (cowpea hay mix)	11.4	2.3	3.2	16.9
Treatment B (concentrate mix)	11.4	0.0	4.8	16.2

5.45 kg commercial concentrate (88%) corresponds to 4.8 kg DM
3.64 kg commercial concentrate (88%) corresponds to 3.2 kg DM
2.73 kg cowpea hay (85%) corresponds to 2.32 kg DM

Milk production: Statistical analysis revealed no significant difference between treatments ($P > 0.05$). In the overall experiment, the "concentrate treatment" showed 0.64 kg higher milk production than the "cowpea hay" treatment (Table 56).

Table 56. Average milk production per treatment

	N	Mean (kg/cow)	Std. dev.	Range for 95% confidence interval for mean (kg/cow)
Cowpea hay mix	84	12.54	1.18	12.28 - 12.79
Concentr ate mix	84	13.18	1.41	12.88 - 13.49

Liveweight changes: The overall weight averages of both groups showed that cows lost weight when supplemented with the concentrate treatment (-0.59 kg/cow/day) whereas they gained weight during the cowpea hay treatment (0.19 kg/cow/day). The difference between the treatments, however, was not significant ($P > 0.05$).

Cost-benefit analysis: The cost of the feed ingredients, income and profitability were with a 95% confidence interval for mean milk production. Table 57 shows the costs for the feed components of the basic feed ration (BFR) totalled 0.31 $US/cow.

Table 57. Costs of ingredients in basic feed ration (BFR)

Feed	$UScent/ Kg FM	$UScent/ Kg DM	Kg FM/cow	$UScent/ cow
Maize silage (35.5% DM)	1.40	3.96	9.1	12.74
Sugar cane chopped (25% DM)	0.81	3.24	13.6	11.02
Maize straw (90% DM)	1.03	1.14	6.8	7.00
TOTAL				30.76

The total feed ration (TFR) costs were 1.43 and 1.78 $US/cow for Treatment A and Treatment B, respectively (Table 58).

The cost: benefit analysis showed that the net income and the profitability per unit of milk were significantly greater (probability of 100%) for the cowpea hay treatment than for the concentrate treatment (Table 59).

This means that the lower milk production with the cowpea hay treatment was more than compensated by the lower feed cost due to the use of the relatively cheaper cowpea hay compared to commercial concentrate.

Table 58. Costs for total feed ration per cow and treatment

	Basic feed ration	Concentrate ($US/cow)[1]	Cowpea hay ($US/cow)[2]	Total
Cowpea hay mix (A)	0.31	0.98	0.14	1.43
Concentrate mix (B)	0.31	1.47	0.00	1.78

[1] Concentrate cost: 0.27 $US/kg; [2] cost per kg of cowpea hay: 0.05 $US/kg

Table 59. Ranges (lower and upper bound of standard deviations) and mean values for income, costs and profitability of cowpea hay and concentrate rations

	Cowpea hay ration	Concentrate ration
Total (dry season) feed cost ($US/cow/day)	1.43	1.78
Dry season feed cost + labour cost[1] ($US/cow/day)	1.55	1.90
Milk production (kg/day)	12.28 - 12.79	12.88 - 13.49
Cost of milk ($US/kg)	0.126 - 0.121	0.147 - 0.141
Gross income from milk sale[2]/cow	3.56 - 3.71	3.74 - 3.91
Net income/cow/day	2.01 - 2.16	1.84 - 2.01
Net income/kg of milk	0.164 - 0.169	0.143 - 0.149
Net income/cost (profitability) per liter milk	130 - 140	97 - 106

[1] Milk price: 0.29 $US/liter (1 liter is considered as 1 kg)

Discussion

Milk production was higher with the all concentrate treatment. However, analysis of live weight changes indicated that, on average, cows gained weight during the cowpea treatment whereas they lost weight during the concentrate treatment. Although there is a significant difference in milk production in favour of the commercial concentrate-treatment (0.64 kg/cow/day), the cowpea hay treatment resulted in a significantly higher net income and a significantly improved cost-benefit ratio of 130-140% compared to 97-106% for the commercial concentrate treatment. This is due to the lower cost for cowpea hay compared to commercial concentrate.

4.4.3 Partial substitution of commercial concentrate with cowpea grain

Contributors: C. Reiber, R. Schulze-Kraft, M. Peters, P. Lentes, V. Hoffmann, H. Cruz and C. Lascano

Rationale

An innovative farmer participating in the project added cowpea grains and maize to a relatively low cost commercial concentrate (min 12% CP, 8.4 $US per 50 kg bag) and compared it to another more expensive higher quality commercial concentrate (min 20% CP, 12.1 $US per 50 kg bag). Cows accepted the concentrate with cowpea which resulted in higher milk production . In order to test the effect of cowpea as a concentrate substitute a more formal experiment was agreed with the farmer (collaborative-collegiate participation mode).

Material and Methods

The experiment was carried out in a farm near Sulaco, Yoro at an altitude of about 446 m.a.s.l. The temperature ranges from 22 to 32 °C with an average annual precipitation of about 1000 mm and a 6 months dry season. The experiment was conducted in the dry season 2006 (March to April). Six crossbred cows (Brahman x Brown Swiss) were selected. The cows had between 2 and 6 births and more than 2 months of lactation at the time of the experiment. Their body condition score was 2.75-3.00. The cows were distributed in two groups of three cows each. The experiment, employing Double Reversal Design lasted 30 days divided in three periods of 10 days each whereas in the first period, all cows passed through the control treatment. In following periods cows in the two groups switched between treatments:

Control: During the first period, the cows of the two groups were supplemented with the same concentrate that was already used by the farmer (control). During the second and third period, each group rotated thorough treatments 1 and 2.

Treatment 1 (T1): BFR + 1.4 kg commercial concentrate (maintenance, min 12% CP) + 1.4 kg ground maize + 1.4 kg ground cowpea grain

Treatment 2 (T2): BFR + 4.1 kg commercial concentrate (min 20% CP)

Where: Basic feed ration (BFR): 12.3 kg silage (mix of 85% sorghum, 7.5% maize and 7.5% cowpea foliage) + pasture; Control: BFR + 2.7 kg commercial concentrate (maintenance, min 12% CP) + 1.4 kg maize

For statistical analysis of milk production data from day 4 to day 10 were taken for each treatment and group and subsequently analyzed using non-parametric tests (Mann-Whitney Test).

Results

Feed quality and intake: The elaborated concentrate included 33% commercial concentrate (13.8% CP), 33% ground maize (10.3% CP) and 33% ground cowpea (26% CP), and had a CP content of 16.7%. The concentrate mixture used as control (66% commercial concentrate "maintenance" and 33% ground maize) had 12.6% CP. Both concentrates were accepted readily by the cows. The commercial concentrate "lechera nutricia" reached a CP content of 22.5%.

The dry matter consumed averaged 3.7 kg for the silage mixture, 3.6 kg for the concentrate and about 1.4 kg of pasture grass with a total of about 8.7 kg DM/cow/day (on average 2.5% of live weight).

Milk production: Milk production was highest for the control (8.47 kg/cow), followed by T2 (8.26 kg/cow) and T1 (7.68 kg/cow). The difference between T1 and T2 as well as the difference between the control and T2 were not significant ($P > 0.05$). However, the difference between the control and T1 was significant ($P < 0.05$) (Table 60).

Table 60. Average milk production (kg/cow) per treatment

	N	Mean	Std. deviation
Control	36	8.47	1.26
T1	42	7.68	1.14
T2	42	8.26	1.72

Cost-benefit analysis: In Table 61 we show the costs for the different feed ingredients. Beside pasture, silage (mainly sorghum) is the cheapest (0.012 $US) available forage source. The costs per kg of maize silage and cowpea grain depend mainly on the harvested amount and therefore have to be calculated for each case. The total feed costs per ration was highest for T2 (1.13 $US) and cheapest for T1 (0.75 $US).

Table 61. Feed costs (US$/kg and US$/cow).

	Silage	Concentrate (maintenance)	Concentrate ("lechera nutricia")	Cowpea Grain	Maize grain	Total
US$/kg	0.012	0.17	0.24	0.11	0.16	
Control (US$/cow)	0.15	0.46	0.0	0.0	0.22	0.83
T1 (US$/cow)	0.15	0.23	0.0	0.15	0.22	0.75
T2 (US$/cow)	0.15	0.0	0.98	0.0	0.0	1.13

Table 62. Mean values for costs, income and profitability for the treatments

	Total feed cost ($US/cow)	Milk production (kg)	Cost/kg of milk ($US)	Gross income/cow through sale of milk[1]	Net income/ cow	Net income/ kg of milk	Profitability (benefit/cost) per liter of milk
Control	0.83	8.47	0.098	2.46	1.63	0.190	196%
T1	0.75	7.68	0.098	2.23	1.48	0.191	200%
T2	1.13	8.26	0.14	2.40	1.27	0.147	112%

[1] Milk price: 0.29 $US/liter (1 liter is considered as 1 kg)

The cost-benefit analysis (Table 62) reveals that the net income and profitability per unit of milk was highest for the cowpea treatment (200%) and lowest for the commercial concentrate treatment (112%). This means that the lower milk production produced with the cowpea treatment (and the control) is more than compensated by its lower cost.

Discussion

The experiment confirms farmers' observation that there were no significant differences in the acceptance and in milk production between the farm-prepared concentrate including 33% cowpea grain and the more expensive commercial concentrate. Cost-benefit analysis revealed significant differences for net income (0.21 $US/cow/day) and profitability in favour of the cowpea mixture. Considering a farm with 10 cows and a supplementation period of four months, the farmer would save about 250 $US by substituting the expensive commercial concentrate with the cowpea-maize-mixture.

General discussion

Cowpea hay as well as cowpea based concentrate proved effective as partial substitute to commercial concentrate in terms of cost-benefit ratio. Further experiments are needed in order to investigate the effect of a total substitution of commercial concentrate with cowpea products. On-farm produced protein sources such as cowpea fodder and grain would have likely less price fluctuations and are less dependent on international markets than concentrates based on imported ingredients.

Regarding the current development of concentrate costs, it is expected that they become even more expensive, partly due to the increased demand of land and agricultural products for bio-fuel production. Therefore, it is important to offer farmers alternatives such as forage legumes to remain competitive. Both farmers, who conducted the cowpea-concentrate experiments, continued cultivating it for concentrate, increasing the area planted or subcontracting to other farmers. These farms are used as demonstration cases for farmer to farmer dissemination.

In Yorito, the farmer association "CREL" is forming a concentrate enterprise as result of institutional cooperation and as consequence of the continuously increasing costs of commercial concentrates. Moreover, poor non-livestock farmers, i.e. local CIAL (local agricultural research committee) groups are presently producing cowpea with the objective of establishing trade relations with CREL livestock farmers. Cowpea based concentrates need to compete with imported feed ingredients such as soybean, However, cowpea can be intercropped in maize or produced at the end of the dry season, filling a niche that could contribute to additional food, feed and/or income generation of small farmers.

4.4.4 Effect of substituting maize silage with *Brachiaria brizantha* cv. Toledo silage on milk production and productivity

Contributors: C. Reiber, R. Schulze-Kraft, M. Peters, P. Lentes, V. Hoffmann, H. Cruz and C. Lascano (CIAT)

Rationale

Maize is currently the most widely used forage source for silage in the area of Yoro. However, maize production for silage is considered by farmers to be expensive and could compete with maize production for food. During the last three years the use of alternative forages for silage such as sorghum, King Grass, sugarcane and recently Toledo has been increasing. Farmer-led on-farm experiments were conducted in order to evaluate the potential of sorghum and *Brachiaria brizantha* cv. Toledo silage compared to maize silage in milk production and productivity.

Brachiaria brizantha cv. Toledo was first introduced to the Yoro region in 1998 by DICTA and CIAT. Toledo was selected by farmers as one of the most promising pasture grasses for the region. Criteria of farmers were its adaptability, production and drought tolerance. Since 1999, the total area cultivated with Toledo has been increasing rapidly to about 400 ha in 2004. It is estimated that about 40-50% of the livestock keepers have adopted Toledo.

Until now, Toledo has been used under direct grazing. Nevertheless, in view of its high biomass production (up to 30 tons DM/ha/year), it could also be used as cut-and-carry grass for supplementation as fresh feed, or as conserved forage in form of hay or silage. This experiment compares Toledo silage with maize silage with respect to animal production and productivity.

Material and Methods

The farmer elaborated Toledo silage independently, and a formal experiment was planned with input from farmer and the researchers (collaborative mode). The experiment was carried out in a farm near Victoria, Yoro, at an altitude of 363 m.a.s.l. The temperature ranges from 22 to 32 °C with an average annual precipitation of 1150 mm and a 6-month dry season.

The feeding experiment was conducted in the dry season of 2006 (February to March). Eight crossbred cows (Simmental x Brahman, Brown Swiss x Brahman) were selected, with a lactation period of more than two months, two to three calves and a similar live weight and body condition. Cows were distributed in two groups of four cows each. The cows were fed on a time-restricted ration of maize silage and Toledo silage from 9 am to 2 pm. After that cows grazed on *Andropogon gayanus* and *Brachiaria* hybrid cv. Mulato pastures.

The following treatments were applied:

Treatment A: Maize silage + concentrate + pasture
Treatment B: Toledo silage + concentrate + pasture

Cows were assigned to a Double Reversal Design that lasted 42 days divided in three periods of 14 days each. Milk production for each cow was recorded daily in the morning. For statistical analysis of milk production, data from the first seven days (adaptation phase) of each period were excluded and data from day 8 to day 14 were taken for each treatment and group and subsequently analyzed using non-parametric tests (Mann-Whitney Test). Live weight was measured every seven days. This was done at the beginning and end of each sub-period using a digital livestock balance.

Results

Feed quality: The maize silage (31% DM) had a pH 4, which indicates that it fermented well. The good quality of the silage was confirmed by the pleasant sweet smell and its green colour

Table 63. Average milk production per group and treatment in kg/cow

		Group 1	Group 2	Average	Range for 95% confidence interval for mean (kg/cow)
Maize silage (A) (N=63)	Mean	5.50	5.65	5.55	5.32 – 5.78
	N	42	21	63	
	SD	0.88	0.99	0.91	
Toledo silage (B) (N=63)	Mean	5.02	5.64	5.43	5.18 – 5.68
	N	21	42	63	
	SD	0.83	1.02	0.99	
Difference (A) –(B)		0.48	0.01	0.12	

(organoleptic mark 1 = very good). Toledo silage had a lower DM content (25.6% DM), with a pH 5which indicated that fermentation was not optimal. However, organoleptic evaluations of Toledo silage including smell, texture, and colour indicated a good quality. Maize silage had a higher crude protein content than Toledo silage (6.44% vs. 5.04%). FDN content was 73.42% for Toledo silage and 70.61% for maize silage.

Feed intake: The cows consumed all the maize silage (31% DM) offered (13.6 kg of FM per day which equals 4.2 kg/cow/day of dry matter). During the first days of the adaptation phase, Toledo silage (25.6% DM) offer was varied to ensure maximum intake, which turned out to be 11.1 kg FM/cow/day or 2.8 kg DM per cow. Thus intake of Toledo silage was considerable lower than the maize silage DM intake. Average commercial concentrate (22% CP) supplementation was 2.6 kg/cow in both treatments.

Milk production: Results indicated no significant difference ($P > 0.05$) in milk production between Group 1 and Group 2. However, when the results of the groups were analyzed separately higher milk production due to the maize silage treatment was significant for Group 1 (0.48 kg/cow/day; $P < 0.05$) but not for Group 2 ($P > 0.05$; 0.01 kg/cow/day) (Table 63). Averaging the two groups there was a difference of 0.12 kg/cow in milk yield in favor of the maize silage, but this difference was not significant ($P > 0.05$).

Cost-benefit analysis: In Table 64 we show the feed costs for each treatment, which averaged 0.90 $US and 0.81 $US for treatment A and treatment B respectively. In Table 65 we present the range and mean values for income, costs and profitability of the two silage treatments.

The cost-benefit analysis indicates that net income and profitability were slightly higher for treatment B (Toledo silage) compared to treatment A (maize silage). This means that the lower milk production (and lower gross income from milk sale) was more than compensated by the lower cost of Toledo silage (Figure 50).

Table 64. Feed costs ($US)

	Silage Consumed (kg FM)	Silage cost/kg	Silage cost/cow	Concentrate[1] cost/cow	Total feed cost/cow
Treatment A (with Maize silage)	13.6	0.015	0.20	0.70	0.90
Treatment B (with Toledo silage)	11.1	0.01	0.11	0.70	0.81

[1] Concentrate cost: 0.27 $US/kg

Table 65. Ranges (lower and upper bound of standard deviations) and mean values for income, costs and profitability of maize and Toledo silage

	Treatment A	Treatment B
Total (dry season) feed cost ($US/cow/day)	0.90	0.81
Dry season feed cost + labour cost[1] ($US/cow/day)	1.018	0.928
Milk production (kg)/day	5.32 – 5.78 (5.55)[2]	5.18 – 5.68 (5.43)
Cost of milk ($US/kg)	0.191 – 0.176 (0.183)	0.179 – 0.163 (0.171)
Gross income from milk sale[3]/cow	1.54 -1.68 (1.61)	1.50 – 1.65 (1.57)
Net income/cow/day	0.52 – 0.66 (0.59)	0.57 – 0.72 (0.64)
Net income/kg of milk	0.098 – 0.114 (0.106)	0.110 – 0.127 (0.118)
Net income/cost (profitability) per litre milk (%)	51 – 65 (58)	61 – 78 (69)

[1]Labour (milker) cost: 0.118 $US/cow/day;
[2] Numbers in brackets are mean values; [3] Milk price: 0.29 $US/litre (1 litre is considered as 1 kg)

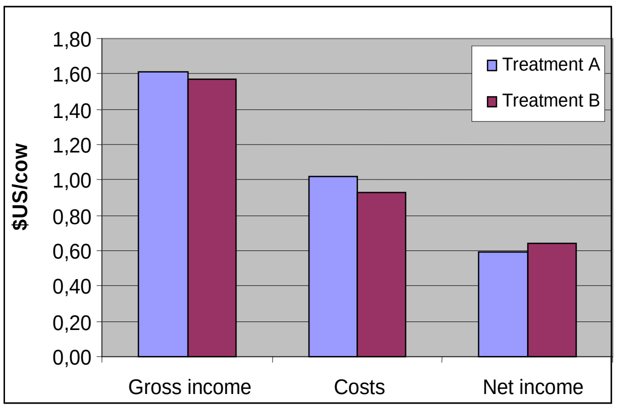

Figure 50. Gross income, costs and net income.

The mean difference of net income per kilogram of milk between treatments is 0.012 $US. For a farm producing 100 litres per day, this would mean about 1.2 US$ more net income per day. However, non-parametric tests didn't reveal significant differences between treatments for net income and profitability.

Discussion

Irrespective of the treatments, average milk production slightly and constantly decreased whereas average live weight increased during the experiment. Differences between maize silage and Toledo silage were not significant in terms of milk production and live weight. Moreover, net income and profitability did not show significant differences between treatments.

However, the trend of results shows that the slightly lower milk production with the Toledo silage treatment was more than compensated by lower treatment costs, which resulted in a slightly higher profitability compared to the maize silage treatment. The experiment indicates that Toledo silage is a feasible, valuable and economically attractive alternative to maize silage.

4.4.5 Effect of sorghum and maize silage on milk yield

Contributors: C. Reiber, R. Schulze-Kraft, M. Peters, P. Lentes, V. Hoffmann, H. Cruz and C. Lascano (CIAT)

Rationale

The majority of the farmers using maize for silage believe it to be superior to other forages. However, we have been observing an increasing use of sorghum silage in the last three years in Honduras. In a farmer-led experiment, forage sorghum and maize silage were supplemented in an alternated manner to milking cows. The farmer and the workers observed a higher milk production always when sorghum silage was fed the day before with a difference of 8-10 litres from 14 cows (0.6-0.7 litres/cow) for each day compared to maize silage. The farmer was encouraged to continue with his experiment under conditions of prolonged periods of maize and sorghum silage supplementation (collegiate-collaborative mode) in order to evaluate the production and productivity of maize and sorghum silage supplementation in milk production.

Materials and Methods

Maize and sorghum (Christiani, HF 802 hybrid) were cut for silage at maturity (milky to doughy). In September 2005, an area of 0.79 ha of maize was ensiled in a trench silo with 45 m^3 capacity which was opened after three months in December. An area of 1.4 ha of sorghum was ensiled in December in a heap silo with 48 m^3 capacity which was opened after two months in February 2006. Fresh probes were taken along in a cooler for laboratory analysis of pH and quality measurments. Fermentation quality of silages was additionally assessed by organoleptic characteristics of smell, colour and texture. Three Brahman-Brown-Swiss (37/63%) crosses in good body condition, with a lactation period of more than two months and more than 3 births were selected. Sorghum and maize silage were offered from 5 to 11.30 am and from 3 to 4.30 pm (time restricted supplementation). The following treatments were applied:

Treatment A: Sorghum silage + 2 kg concentrate (min 20% CP) + pasture (Toledo)

Treatment B: Maize silage + 2 kg concentrate (min 20% CP) + pasture (Toledo)

The feeding trial, lasted 30 days divided in three periods of 10 days, i.e. period 1: Sorghum silage (A); period 2: Maize silage and period 3: Sorghum silage (A). Milk production for each cow was recorded daily. For statistical analysis of milk production, data from the first three days (adaptation phase) of each period were excluded and data from day 4 to day 10 were taken for each treatment.

Results

Silage quality and intake: The pH values in combination with the DM contents indicate that both silages had fermented well and were an-aerobically stable (Table 66). This was confirmed through organoleptic evaluations of smell (pleasantly sweet, absence of butyric or strong acid smell), colour (green) and texture (intact), in which both silages resulted with grade “very good”. It is possible that the pH values of the silages increased during the period when the probes were transported to the laboratory and therefore it is expected that the real pH was lower in the silos.

Visible losses due to spoilage at the side walls were minimal (< 5%) in both silos. Crude protein contents were similar with 7.5% for sorghum silage and 7.8% for maize silage. Neutral detergent fibre (NDF) content was higher for sorghum silage (85.3%) than for maize silage (75%).

Intake of fresh maize silage (30.5% DM) averaged 18.2 kg FM , whereas intake of fresh sorghum silage (35% DM) averaged 22.8 kg. In addition to the higher FM intake of sorghum silage compared to maize silage, difference of total daily DM intake were even greater (8.0 vs. 5.6) due to the higher DM content of sorghum silage.

Milk production: The average milk production with sorghum silage was 11.33 litres/cow

Table 66. Silage quality

Material	DM (%)	pH	CP (%)	Ether Extract (%)	NDF (%)	Ash (%)
Sorghum silage	35	4.8	7.52	2.46	85.32	7.77
Maize silage	30.5	4.6	7.84	2.61	75.01	7.88

Table 67. Cost for maize and sorghum production

Item	Maize silage		Sorghum silage	
	Lps[1]/mz	$US/ha	Lps/mz	$US/ha
Ploughing with tractor	800	60.2	800	60.2
Oxen for seedbed preparation	500	37.6	500	37.6
Labour for sowing	200	15.0	200	15.0
Seed	40	3.0	409	30.8
Fertilizer (urea)	200	15.0	0	0
Fertilizer (NPK:18-46-0)	200	15.0	200	15.0
Labour application fertilizer	150	11.3	150	11.3
Plant protection measures	90	6.8	90	6.8
Labour for weeding	1000	75.2	1000	75.2
Total	3180	239.1	3349	251.8

[1]Lps = Lempiras (Honduran currency)

(N = 42; Std. Dev. = 1.82), which is 2.54 litres higher than with maize silage (8.79 litres/cow, N = 21; Std. Dev. = 1.25). The reason for the great difference in intake and milk production between the two silages could not be explained by quality parameters.

Silage costs: In Table 67 we show that sorghum production cost was slightly higher than maize due to higher seed cost.

In Table 68, silage production costs for maize and sorghum are listed. The cost of sorghum silage per kilogram was slightly higher compared to maize silage mainly due to a lower biomass production (17.1 tons/ha compared to 28.6 tons/ha), which is very low compared to results from elsewhere in Honduras, differences between maize and sorghum due to lower fertilization of sorghum (maize received an extra does of 130 kg/ha urea and less water availability for sorghum as it was planted after maize harvest at the end of the rainy season. Therefore, production costs can not directly be compared. In the calculation for sorghum silage cost, it is not considered that sorghum re-growth was harvested two times more. Silage from the second (and third cut) is cheaper since land preparation and establishment costs are not included in the calculation.

Cost-benefit analysis: The cost-benefit analysis was calculated with (a) sorghum silage costs of 0.024 $US/kg (only considering the first cut), and (b) the same costs for maize and sorghum silage (0.019 $US/kg). Case (b) serves as example how the higher palatability (or intake) of sorghum silage in this case influenced income and profitability. In Table 69 we show the feed costs for each treatment, with total feed costs for treatment A of 1.09 $US/cow, and 0.97 $US/cow, respectively, and 0.89 $US/cow for treatment B.

Table 68. Cost for maize and sorghum silage

	Maize silage		Sorghum silage	
	Lps/1.125 mz	$US/1.125 mz	Lps/2 mz	$US/2 mz
Production cost	3578	188.3	6698	352.5
Labour for ensiling process (harvest, transport, compaction)	2640	138.9	2080	109.5
Plastic	360	18.9	400	21.1
Gasoline for chopper	173	9.1	173	9.1
Chopper cost	1000	52.6	1000	52.6
Total	7751	407.8	10351	544.8

Table 69. Feed costs ($US)

	Silage intake (kg FM)	Silage cost/cow	Concentrate[1] cost/cow	Total feed cost/cow
Sorghum ration	22.8	(a) 0.55 (b) 0.43	0.54	1.09 (a) 0.97 (b)
Maize ration	18.2	0.35	0.54	0.89

[1] Concentrate cost: 0.27 $US/kg; sorghum silage costs: 0.024 (a) and 0.019 (b) $US/kg

In Table 70 we present the ranges and mean values for income, costs and profitability of the two silages. The cost-benefit analysis showed that net income and profitability were higher for treatment A (sorghum silage) compared to treatment B (maize silage), for both scenarios, (a) and (b). This means that the higher cost of sorghum silage (due to higher consumption) is more than compensated through higher milk production.

For (a): Non-parametric tests revealed significant differences between treatments for net income per cow but not for net income per litre and profitability. The mean difference of net income per cow between treatments is 0.54 $US. For a farm with 10 cows producing the same amount of milk over a 3 month period, this would mean about 486 $US more net income.

For (b): Non-parametric tests revealed significant differences between treatments for net income per cow, net income per litre and profitability. The mean difference of net income per cow between treatments is 0.66 $US. For a farm with 10 cows producing the same amount of milk over a 3-month period, this would mean about 594 $US more net income.

Conclusion

The results confirm farmers' experience that with sorghum silage milk production was higher compared to maize silage under prevalent farm conditions. Sorghum provides additional advantages over maize such as its ability to re-sprout reducing production costs and production delay and its higher drought tolerance allowing farmers to extend production into the dry season. As a consequence, maize is being substituted by sorghum for silage in the Yoro region.

Table 70. Ranges (lower and upper bound of standard deviations) and mean values for income, costs and profitability of treatments with maize silage and sorghum silage

	Treatment A (sorghum silage)	Treatment B (maize silage)
Total (dry season) feed cost ($US/cow/day)	1.09 (a) 0.97 (b)	0.89
Dry season feed cost + labour cost[1] ($US/cow/day)	1.19 (a) 1.07 (b)	0.99
Milk production (litres/day)	10.76 – 11.89 [2] (11.33)	8.22 – 9.35 (8.79)
Cost of milk ($US/litre)	(a)0.111 – 0.100 (0.105) (b) 0.099 -0.090 (0.094)	0.120 – 0.106 (0.113)
Gross Income from milk sale[3]/cow	3.12 – 3.45 (3.29)	2.38 – 2.71 (2.55)
Net income/cow/day	(a) 1.93 – 2,26 (2.10) (b) 2.05 – 2.38 (2.22)	1.39 – 1.72 (1.56)
Net income/litre of milk	(a) 0.179 – 0,190 (0,185) (b) 0.191- 0.200 (0.196)	0.169 – 0.184 (0.177)
Net income/cost (profitability) per litre milk (%)	(a) 161 – 190 (176) (b) 192 – 222 (207)	141 – 174 (157)

[1]Labour cost: 0.1 US$/cow; [2] Numbers in brackets are mean values; [3] Milk price: 0.29 $US/litre

4.4.6 Participatory experimentation with little bag silage technology (LBS)

Contributors: C. Reiber, M. Peters, R. Schultze-Kraft, P. Lentes, H. Cruz and C. Lascano (CIAT)

Rationale

Little bag silage (LBS) is seen as a promising technology for small-scale farmers. In Honduras, LBS can play an important role in creating awareness on the suitability of forage conservation for smallholder farmers and has been employed during farmer trainings and field days to train farmers in concepts and principles as an entry point for forage conservation technologies. The objective of the research is to evaluate the potentials and constraints of LBS for smallholders under Honduran conditions. Results presented in the Annual Report 2005 are complemented with this contribution.

Results

During a field day in Las Vegas/Victoria, seven LBS of about 40 kg each were elaborated. Wilted and chopped Toledo was ensiled using different additives. For demonstration purposes, one bag was made without additives, two bags with chopped sugarcane and two bags with citrus fruits (limón Persa) as additive. During a fair, two of the bags were presented to farmers. The smell of the silage with citrus fruits was reported to be extremely good and farmers were impressed about both, the bag silage technology and the feasibility of making good quality with citrus pulp added to Toledo silage. The good quality was confirmed by pH 3.8 and the ready acceptance by cows who had never been fed silage before. In Candelaria, problems with grass silages were reported and farmers complained about the bad smell. The main problem detected was the low amount of molasses added to the grasses and high moisture content in the silos, both due to the plant material itself and due to far too much water added to the molasses. A workshop was organized in which 21 silage bags of 30 kg each and different treatments were elaborated with farmers and subsequently stored in a closed room. The purpose of the workshop was to demonstrate farmers, a) that bag silage is a feasible alternative, and b) that the quality of Toledo silage can be improved by wilting and adequate addition of water soluble carbohydrates. After four months, another workshop was held in which the bags were opened for evaluation. In Table 71 we summarize the results.

Farmers' reported the best smell for the wilted silages with molasses and sugar cane followed by the moist silage with molasses. Additional pair-wise rankings revealed that wilted silage was preferred to moist silage and silage with additive was preferred to silage without additive. Nevertheless, losses due to fungi were greater in the wilted silages. This can be explained by the

Table 71. Toledo silage bags with different treatments

Treatment	Number of bags	pH	Smell (1-5)[1]	Farmers' ranking	Losses (%) Range and (average)
1. Moist (22% MS), without additive	3	4,4	2	6	0-10 (5)
2. Moist (22% MS) with molasses (6%)	4	4,5	4	3	0-7 (4)
3. Wilted (40% MS) without additive	2	6,0	3	5	0-100 (50)
4. Wilted (40% MS) with molasses (6%)	4	3,9	4	1	0-80 (32)
5. Wilted (40% MS) with sugar cane (20%)	4	4,7	4	1	0-15 (5)
6. Wilted (40% MS) with dissolved sugar blocks (6%)	4	4,2	3-4	4	10-100 (40)

[1] 1 = rotten, disgusting; 2 = bad; 3 = acceptable; 4 = good; 5 = very good

fact that air exclusion is more problematic with forages showing higher DM contents.

Constraints to adoption of LBS

Even though many farmers participated in events where the LBS technology was presented and/or personally elaborated LBS for testing, after two years, no adoption has yet occurred. Restrictions to success with LBS include availability of suitable and cheap plastic bags, high silage losses due to perforation of plastic bags caused by inappropriate handling and rodents (e.g. rats and mice), and lack of adequate storage facilities in many smallholder farms. Moreover silage adoption by smallholders is often restricted by access to a chopper as hand-chopping is cumbersome, time- and/or labour-intensive. Small-scale farmers with chopper rather adopted other silo types with higher capacities (i.e. heap and earth silos), either instantly or after having tried LBS.

Potential utility of the LBS Technology

In spite of no adoption, the LBS technology can be employed as a) a useful demonstration, experimentation and learning tool that can be used as adaptable prototype in farmer trainings and field days due to its rapid elaboration, b) to get farmers started in experimenting with silage technology at a low risk. LBS does not require additional manpower and can be elaborated in periods of low labor demand, can make use of small areas of cultivated high quality forages and other feed resources and can be ensiled solely or in combination. To be successful the choice of plastic material appears important We identified a bag having a high density, caliber 7, double layered, sealed at sides and rolled up as one piece, with high tensile strength material resistant to tearing as most suitable. The bags can be cut according to the needs, the two ends to be sealed through knotting. The plastic material is available in many remote areas and sold at a price of 57-81 US$ cent per meter depending on the location and the quantity purchased. Per silage bag of 30 kg, about 1.4 meters of plastic are necessary, which costs about 1 US$/bag. If intact, it can be used again reducing costs. The use of any form, e.g. a plastic barrel, in which the plastic bag is elaborated, revealed to be very useful, especially when using bags of higher capacity in order to improve compaction at the margins (Photo 10A), to avoid stretching and to prevent perforations of bags by coarse stems. The form is cut open at one side and clasped or held together by a rope or belt while filling and compacting the forage. It needs to be opened in order to be able to take out the bag of the barrel (Photo 10B). Perforation and tearing of bags during silage preparation, caused by e.g. plant parts, fingers or shoes, as well as during storage need to be sealed immediately with a high quality adhesive tape. Adequate storage, i.e. protection from animals (as well as sun-light), is key for the success with LBS. Possibilities to reduce risk of perforation caused by animals are natural or chemical pesticides, cats and burying of the bags in the ground.

Photo 10A. Compaction of LBS

Photo 10B: Opening of the barrel

4.4.7 Stimulating innovation among small farmers of low cost forage conservation technologies in Nicaragua

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Rationale

Feed shortage during the 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. Results indicate that feeding silage or hay to milking cows is profitable. In Las Segovias farmers produced hay and silage amounting to a total of almost 14 MT (Table 72). In San Dionisio (Matagalpa) only three farmers out of an initial nine produced hay or silage: sorghum silage from 0.15ha, Cratylia silage from 0.1 ha and Mulato hay from 0.7 ha. The produced material in Las Segovias was used for the feeding experiments outlined in Table 73.

Table 72. Hay and silage production in Las Segovias

Farmer	Locality	Cattle	Technology	Area (ha)	Prod. (kg)	Yield (MT/ha)	Remark.
Alejandro Rugama	La Trinidad	no	Cratylia silage	0.11	-		failed
			Vigna hay	0.14	164	1.1	
			Sorghum + Lablab	0.05	300	6.7	
José Angel Alaníz	La Trinidad	no	Mulato silage	0.18	680	3.9	failed
Martín Joya	La Trinidad	no	Mulato silage	0.20	-	-	failed
			Toledo hay	0.17	2681	15.5	
José Daniel Rodas	La Trinidad	no	Cratylia silage	0.06	273	4.5	
José Ines Rayo	La Trinidad	yes	Cratylia silage	0.20	-		failed
J. Cruz Garcia	La Trinidad	yes	Lablab hay	0.32	300	1.0	
Orlando Rodas	Condega	yes	Toledo hay	0.53	6720	12.7	
Rodolfo Valdivia	Estelí	yes	Cratylia silage	0.20	1140	5.7	
Wilfredo Castillo	Estelí	yes	Mulato silage	0.53	1360	1.4	

Table 73. Feeding experiments with dry season forages in Las Segovias

Farmer	Locality	Technology	Treatment
J. Cruz Garcia	La Trinidad	Lablab hay	T1: grass+sorghum T2: grass+sorghum+hay
Orlando Rodas	Condega	Toledo hay	T1: pasture T2: pasture + hay+ molasses
Rodolfo Valdivia	Estelí	Cratylia silage	T1: crop residues (70%) + sugarcane (30%) + molasses T2: crop residues (70%) + sugarcane (30%) + molasses + Cratylia silage
Wilfredo Castillo	Estelí	Mulato silage	T1: farmer’s practice + concentrates T2: farmer’s practice + Cratylia silage

One experiment showed that a daily ration of 4.5 kg of Cratylia silage substituting 3.6 kg of concentrates (basal ration: sorghum + *Pennisetum* spp. + molasses) did not have any negative effect on milk production, whereas the variable costs of the Cratylia supplement were only 5-10% of the concentrates.

4.4.8 Improved feeding systems for smallholder dairy cattle in Nicaragua with emphasis in the dry season

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Rationale

The objective of this project jointly carried out by ETH (Zürich), CIAT and INTA (Nicaraguan national agricultural research institute) is the participatory development of alternative and environmentally sound dry season feeding options in different agro-ecological zones in the hillsides of Latin America, which contribute to sustained milk production and improved milk quality during the dry season and reduce the dependence on purchased supplements.

Availability and quality of local and introduced forages:

To assess seasonal variations in dry matter production and feeding value of local and introduced forage species, in Las Segovias (El Tule) plots were established at two locations with eight grasses (four local and four introduced), seven legumes (two local, five introduced) and five (two local, three introduced) shrubs. Of the legumes, *Mucuna pruriens*, *Canavalia brasiliensis* and *Vigna unguiculata* performed best in terms of agronomic characteristics. In verification on-farm trials, both in El Tule and in San Dionisio (Matagalpa), differences in biomass production between the different grasses were considerable, *Brachiaria* hybrid 36061 “Mulato” showing a yield of 7.8 MT/ha, followed by *Brachiaria brizantha* “Toledo” (6.2 MT/ha), *Hyparrhenia rufa* (4.2 MT/ha) and *Brachiaria* hybrid 36087 “Mulato II” (4.0 MT/ha).

Improved forage management and supplementation strategies for the dry season

In 2006 dry season experiment cycles with introduced (i.e. *Brachiaria* hybrids, *Brachiara brizantha* “Toledo”) and local (i.e. *Hyparrhenia rufa*) pastures were conducted in El Tule and San Dionisio. In El Tule cows produced more milk when grazing introduced pastures (0.1-0.3 lts per cow per day), in San Dionisio there were no differences between introduced and local species. Fat content was also similar, but “Mulato” showed a considerably higher content in protein (Table 74).

Table 74. Pasture yields, milk production and milk quality, El Tule / San Dionisio, 2006

	El Tule		San Dionisio			
	Yield (MT/ha)	Milk production (lt/cow/day)	Yield (MT/ha)	Milk production (lt/cow/day)	Fat (%)	Protein (%)
Brachiara hybrid 36061 “Mulato”	7.8	2.9	4.4	3.9	4.7	4.0
Brachiara hybrid 36087 “Mulato II”	4.0	3.1				
Brachiaria brizantha 26110 “Toledo”	6.2	3.0	5.8	4.0	4.8	3.4
Hyparrhenia rufa (“Jaragua”)	4.2	2.8	1.4	4.0	4.6	3.4

These small differences in milk production are probably due to the limited genetic potential of the animals in the trials. Differences in live-weight gain and other production characteristics were not taken into account in the measurements.

4.5 Promotion of forage technologies to enhance competitiveness of livestock systems in LAC

Highlights

- Costa Rica's livestock and beef industry performs very unsatisfactorily and as a result farmers cannot recover the opportunity cost of the capital invested in the land, making this beef activity uncompetitive

- In dual purpose cattle systems in Olancho, Honduras, Central America purchased supplements (62% of the farms) is the most important cost driver in the dry season.

- Low and lowest performers operating dual purpose cattle farms use purchased feed supplements in the dry season to maintain body condition of the herd, while farms with a better feed base produce milk.

- Lowest performers operating dual cattle farms in Honduras combine resource constraints with deficiencies in management. Obstacles for the adoption of multi-purpose forage options are related to lack of cash and low return to investment on supplemental feeding to low genetic potential of cows for milk production.

- Early adoption by farmers in Yoro, Honduras of improved forage technologies to supplement milking cows in the dry season is most probable among top and medium performers given that they have financial reserves to test new technologies, and possess cows with better genetic potential to respond to high quality feed

- Incorporation of new forage/crop technologies in the current livestock systems in the Llanos of Colombia would constitute a powerful tool to stimulate regional agriculture, while improving the productive capacity of the soil.

- Forestry production in the Llanos of Colombia would be a good option to the extent that the region invests in adequate infrastructure for the management and processing of forest products.

4.5.1 Critical Issues to Promote Technical Change and Enhance the Efficiency and Competitiveness of the Beef Sector in Costa Rica

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This study aims to: (1) describe the economic agents of the meat chain in Costa Rica as well as its commercial and legal relationships; (2) identify the inter-relationships between links, technological levels, efficiency indicators, installed capacity (scale), and level of occupation; (3) characterize and estimate cost and price structures and the generation of value along the different links of the chain; (4) identify critical costs that can be modified through technological interventions, policies, or other actions; and (5) determine biological and economic risk factors throughout the chain. A methodology that identifies and determines the costs and benefits in each segment was developed to estimate the generation of value in monetary terms throughout the meat chain.

Costa Rica's meat sector has clearly suffered a downward trend since the mid-80s, with an annual decrease in production of 0.1% over the past 20 years despite the reduction of the herd inventory, which decreased from 2.3 million heads in 1985 to only 1.1 million in 2004. Government investment in the sector fell from 5% of the national budget in the early 1990s to only 1.5% at the beginning of this decade. Total farm credit of both public and private sectors has suffered a marked decline. In 1990 it represented 15% of total placements (4% in livestock production) and in 2002 these had fallen to only 5% (1.7% corresponded to livestock credit).

Productivity indicators reflect the poor dynamics of Costa Rica's livestock sector. The annual gross earning per unit area was estimated at US$44/hectare for cattle ranches, at $126/hectare for dual-purpose farms (including income from sale of milk), and at $135/hectare for farms where development and cattle fattening activities were carried out. This gross income is considered extremely low if the commercial value of land on beef farms is used as reference. This value ranges between US$1,000 and $2,000/hectare.

The aforementioned biological inefficiencies, combined with the high cost of land, hinder the recovery of the opportunity cost for capital invested in the land, making meat-related activities fairly uncompetitive. Because of its low productivity, the cattle-raising system pays family labor wages under the legal minimum.
Based on the assumption that the only cost in cash is that of farm labor, cattle ranches would be paying family labor a wage equivalent to 60% the legal minimum. Therefore it is imperative that the public and private sectors join efforts throughout the chain to increase the productivity and efficiency of this primary sector by facilitating the adoption of improved technologies.

Auctions yield a relatively good profit; however, when analyzed on a calendar day basis, they are not so attractive because of the low use of installed capacity (see Table 75).

A strategy that could prove useful to improve the efficiency of Costa Rica's auction system would be to integrate the different events in order to share fixed operational costs. Administrative and operational staff could rotate among existing auctions since their dates are different. This scheme would help reduce fixed costs and the commission charged, without affecting profits and improving efficiency in this link of the chain.

The industrial sector (municipal and industrial slaughterhouses) shows a low occupation of installed capacity, which results in high operational costs and very low labor efficiency. The estimates of total operational costs of slaughter range between US$32 and $66 per animal (Table 76).

If the estimated unit costs are compared with the rates collected for slaughtered beef (US$15-$23), municipal slaughterhouses would appear to work at a loss and industrial slaughterhouse with a very low margin of profit thanks to by-products (hide and viscera). The retail sector (butchers and supermarkets) present the best performance in terms of efficiency and profitability. The rate of profit, expressed as the fraction of the final price paid by the consumer that remains in hands of the butcher as retribution for his/her work, varied broadly—from 3% to 40%, with an average of 32% (Table 77). If these rates of profit are compared with those of other alternative retail businesses (approximately 8%), then this type of activity generates excellent margins of profit at very low risk. The value generated throughout the chain, as percentage of the final value of young bulls at retail price, is distributed as follows (Figure 51): rancher (19%), auctioneer (1%), fattener (34%), transporters (6%), slaughterhouse (7%), and retailers (33%). The

Table 75. Operational characteristics of auctions: type of animals bought or sold, operating costs, and income.

Indicator	Auction					Average
	1	2	3	4	5	
Year of establishment	1997	1993	1984	2001	1993	1994
Commission collected (%)	4	3.5	3.8	3.5	4.0	3.8
Installed capacity (# animals/day)	900	500	600	500	800	660
Average no. transactions per event (# heads)	500	390	300	290	750	446
Capacity used (%)	55	78	50	58	94	68
Weekly operation (# of days)	1	2	1	1	2	1.4
Real capacity used (%)	9.2	26	8.3	9.7	31.3	15.8
Categories of animals bought or sold at the auction (%)						
* Culled cows	10	60	35	6	30	28
* Weaned calves	25	15	15	20	15	18
* Weaned female calves	20	5	5	9	10	10
* Young bulls for finishing	30	5	10	25	20	18
* Heifers for slaughter	10	10	30	33	20	21
* Finished males	5	5	5	7	5	5
Most frequent distance from the auction to the farm (km)	25	40	30	60	50	41
Labor at the auction (# people)						
* Auction day	32	25	29	16	34	27
* Day without auction	9	9	6	4	12	8
Monthly operational costs[1] ($)						
* Labor	7,440	6,200	5,790	3,500	11,363	6,859
* Services	250	220	240	200	290	240
Gross monthly income[2] ($)	22,733	36,151	14,679	12,076	76,048	32,337
Net income per event ($)	3,474	3,433	1,997	1,934	7,437	3,655
Net income per animal bought or sold per event ($)	6.94	8.80	6.66	6.67	9.92	7.80
Net income per animal bought or sold per calendar day ($)	0.99	2.51	0.95	0.95	2.83	1.65

1 Estimate based on an average cost of US$550/permanent worker, including social benefit costs for days without auction and US$25/day for transitory workers on auction days.

2 Estimate based on the proportion of animals, according to category, that arrive at the auction, number of animals bought or sold per event, 2005 sale price, and commission collected by each auction.

distribution of the value generated along the meat chain is completely inequitable and is not consistent with the risk faced by the different actors forming the chain. The inequity observed in the distribution of the added value reflects a clearly dominant position in the market of several actors of the chain, which allows them to capture a very high fraction of the benefits. The generation of value along the chain ranges from US$0.28/animal per day for the rancher to US$46/animal per day for the butcher. The highest proportion of added value is concentrated at the final end of the chain. The butcher or supermarket obtains 164 times greater value from the same animal in the same time unit than the rancher but faces a lower risk because his/her raw materials, equipment, and infrastructure are usually covered by insurance policies.

Table 76. Some operational characteristics of municipal and industrial slaughterhouses in Costa Rica.

Variable	Municipal Slaughterhouses			Industrial Slaughterhouse
	1	**2**	**3**	
Volume slaughtered (heads/month)	45	150	650	7,635
Days of operation per month (#)	17	13	26	26
Capacity of daily slaughter (heads)	7	50	85	500
Capacity currently used (heads)	38	23	29	59
Initiation of operations (year)	1985	2002	1974	1964
Annual proportion of post-slaughter rejections (%animals)	<0.1	<0.1	<0.1	<0.1
Origin of cattle slaughtered (%)				
a. Small producer			4	NA
b. Medium producer		50	12	NA
c. Large producer			54	NA
d. Butcher's shops	100	50	30	NA
e. Supermarkets				NA
f. Others				NA
Agent of the chain that assumes the post-slaughter risks of confiscation	Cattle owner	Cattle owner	Cattle owner	Cattle owner
Availability of insurance policy (Yes, No)	Yes	Yes	Yes	Yes
Permanent employees (#)[1]	3	16	33	757
Productivity of labor (# of animals slaughtered per worker)	15	9.4	19.7	76.3
Operational costs ($/month)				
g. Labor	1,650	8,800	18,150	416,350
h. Electricity	140	1,070	2,525	64,080
Cost of slaughter ($/head))	39.80	65.8	31.8	62.9
Cost of maquila ($/head)	20	23	20	15

[1] Of the total no. of employees, about 100 work in slaughter-related activities.

The competitiveness of this meat chain is the sum of the efficiency and productivity of all the links that form it. A weak and rather poor demand for beef at the final link of the chain hinders the adoption of technology in the primary link of the chain, so it becomes a vicious cycle that generates low productivity and competitiveness. The low demand for beef implies reduced levels of slaughtering, which impedes the full use of the installed capacity of slaughterhouses and processing plants. This, in turn, hinders the generation of economies of scale and causes high unit costs that reduce the competitiveness of meat products in both domestic and foreign markets.

To promote technical change and enhance the efficiency and competitiveness of the value chain of Costa Rica's beef sector, we recommend the following: (a) learn from other chains, for example the poultry chain, by identifying actions that could improve the meat chain; (b) milk breeding cows when a milk market exists as a mechanism to increase family income because wages are currently below the legal minimum; (c) promote the creation of livestock funds as a mechanism to create social capital, reduce transaction costs, and improve chain productivity and profitability; (d) promote massive adoption of forage species with an emphasis on summer feeding to reduce weight losses of the national herd, improve farm profits, and promote modernization through the adoption of improved technologies; and (e) establish a standard systems for beef cuts based on quality and price, allowing the differentiation of offers for different consumer groups, among others.

Table 77. Monthly operational costs, breakeven point, and profits of urban and rural butcher's shops in Costa Rica (US$/kg).

Variable	Butcher's Shop							Average
	Urban neighbor-hood	Urban neighbor-hood	Urban neighbor-hood	Urban market place	Urban market place	Rural market place	Rural market place	
Workers (#)	22	13	3	24	5	2	4	10.4
Labor cost[1]	7150	12100	1650	13200	2750	1100	2200	5735
Energy cost	787	886	886	591	303	290	394	591
Lease of locale	3937	4000	3937	350	295	300	280	1871
Cost of insurance policy	157	158	160	158	157	150	140	154
Operational cost	12031	17144	6633	14299	3505	1840	3014	8351
Beef sales (kg/month)	6495	25980	3464	30310	8660	4243	3810	11852
Total sales of meat (kg/month)[a]	12990	43300	4619	43300	12371	7072	6350	18635
Breakeven point[2] (kg beef/month)	4500	4500	800	5000	1400	1200	1200	2800
Operational cost per kg meat sold ($/kg)[3]	0.93	0.40	1.44	0.33	0.28	0.26	0.47	0.45
Average cost of kg dressed carcass and viscera[4]	3.06	3.05	3.06	3.06	3.06	3.06	3.06	3.06
Average sale price of kg meat for breakeven point[5]	3.99	3.45	4.50	3.39	3.34	3.32	3.53	3.51
Average sale price to consumer[6] of dressed carcass plus viscera	4.63	4.63	4.63	4.63	4.63	4.63	4.63	4.63
Net earnings per kg meat sold ($/kg)	0.64	1.18	0.13	1.24	1.29	1.31	1.10	1.12
Net earnings per kg meat sold (%)	16.0	34.2	2.9	36.6	38.6	39.5	31.2	31.9

a. Includes all species.
1 Assuming an average cost per month of US$550 per worker, including social benefit costs.
2 No. kg beef that should be sold monthly to cover operational costs of butcher's shop.
3 Calculated by dividing total operational cost of butcher's shop by kg meat of all species sold monthly.
4 Calculated on the basis of the sale price of one 276-kg carcass at $611 by the slaughterhouse to the butcher's shop plus 16 kg viscera at $35 for a total of $646 divided by 211 kg salable meat (267 kg carcass multiplied by 78% salable meat minus 6% fluid loss). The survey did not ask for this value, but it was estimated on the basis of carcass sales of slaughterhouses.
5 Calculated on the basis of the sum of operational cost per kg beef sold plus average cost of purchasing kg meat from the slaughterhouse.
6 Estimate based on Table 4 for a young male bull and does not reflect the differences of prices that exist between butcher's shops; as a result, this is an approximate indicative.

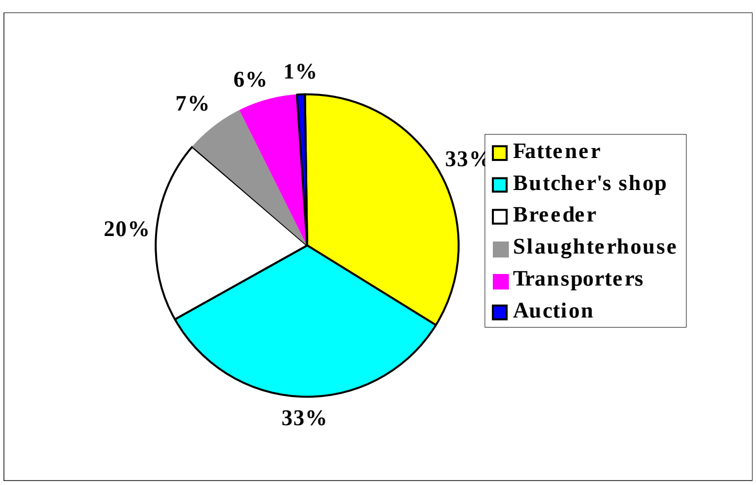

Figure 51. Value generated throughout the chain as percentage of the final value of a fat young bull at retailer price.

4.5.2 Management and farm characteristics that favor or impede efficient resource use in dual-purpose cattle systems in Central America

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Rationale

Since the 1950s, pasture area in Central America has more than tripled. This growth was accompanied by a diminution of forest land, since progress in livestock technology was slow and the widening pasture area was the dominant response to the incrementing demand for livestock products.
During the last 30 years, meat and milk consumption in developing countries has grown 3 times as fast as in developed countries. Projections to 2020 foresee an annual growth of meat demand by 3% and an increase in milk demand by 2.9 % for developing countries. However, between 2001 and 2003 the milk production in Honduras, a developing country, staggered 14% behind milk consumption. Thus the Honduran livestock sector has yet to take advantage of this comparably favorable market. In Honduras, there is a drastic contrast in poverty rates between urban areas (27.6 %) and rural areas (72.2 %). Moreover, the livestock sector is a major employer in rural areas; hence improvements in the livestock sector will likely have positive effects on livelihoods of the rural poor.

The low milk productivity of Central American cattle systems is related to various factors, including the low genetic potential of the commonly used dual-purpose cattle and, the low quality and quantity of feed resources, used in the prolonged dry season of commonly between 4 and 8 months in Honduras and Nicaragua. During the dry season of Central America milk production drops sharply, about 40 % lower than in the rainy season, when feed resources from green pasture are abundant.
The national and regional statistics for Honduras confirm the Central America-wide trends in milk production. The department of Olancho accounts for 20.14% of the national pasture land. In Olancho, cultivated and improved pastures make up 23.7 % of the agricultural land and natural pastures 36.9 %. The share of natural pastures in Olancho exceeds the country average by 14 %. Among the 18 departments, Olancho has the highest total number of milking cows in Honduras (167.107 heads), of which 45.4 % are milked in the dry season, while the share of milked cows rises to 63.2 % in the wet season. Total dry season milk production of Olancho is 41.48 % lower than in the wet season.

The data on poverty, land use, and milk production reveal that an intensification of the livestock sector through enhanced resource use would strongly contribute to sustain incomes of farmers. At the same time, the intensification of the livestock systems, in the sense of an increased production per unit, is as an opportunity to prevent land degradation by overgrazing. The experience of the past also indicates that the intensification of the livestock sector is needed to prevent further loss of forest areas. .

More intensive cattle management through an optimized use of forages can help reduce the degradation of natural resources and lead to a scale neutral increase of incomes from smallholders to large cattle farmers. One option for farm intensification is the use of tropical forage grasses and legumes, as cattle farmers largely depend on grazing of naturalized pastures and degraded improved pastures as a feed resource. Forages can help maintain the natural resource base and mitigate the farmers' dependency on external inputs. A key aspect of livestock system intensification is the correct application of forage technologies.
Socio-economic studies for the livestock farming systems of Olancho have concentrated on specific parts of the farming system, and have

not covered the representative zones of the province.

There are no studies available which analyzes the socio-economic situation of the typical farmers of Olancho using levels of success in milk production in the dry season. Such an analysis contributes to deriving more appropriate extension and development concepts for each category of farms. The objective of this paper is to explore which types of management and farm characteristics favor or impede efficient resource use. The paper develops pathways out of unproductive systems to more efficient income generation in milk and beef production enterprises from representative zones of Olancho.

The strategies farmers follow are analyzed first for their economic efficiency, followed by an assessment of the eco- and social- efficiency of the production. It is hypothesized that the low or inefficient productivity of farms in the dry season is related to an exaggerated use of purchased supplements. Farms which do not possess sufficient forage as a feed resource are caught by the "concentrate trap", when they feed commercial concentrates to animals of low genetic potential for milk production to maintain the herd in times of forage scarcity. In order to achieve this, the milk production systems and the underlying strategies of farms are analyzed and compared on their cost efficiency to derive appropriate solutions for intensification.

Materials and methods

Questionnaire Databank and Data Collection: The data used for this paper were collected by means of a comprehensive socio-economic questionnaire. This procedure was applied in order to take into account the diverse structures of farms and the different feeding strategies.. Prior to developing the questionnaire, a contextual analysis was carried out to define which areas of the farming systems had to be included in the data collection. The focus was laid on dry season problems and on the definition of farm and family income generating activities. Using the information gathered from the pre-survey activities, a standardized questionnaire and database structure were 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.

The population of the study consists of typical livestock farmers in Olancho and was split in two samples, each with distinct sampling procedures.

a. To assess the economic conditions of the representative livestock holder, the sampling plan covered a randomized sample of 69 farms distributed to five sub-study areas, which represent the most important agricultural zones of Olancho. These zones were chosen to represent as much area as possible of prolonged dry season in Olancho. Zones were selected consulting local experts and maps. Gradients between the study areas represent altitudinal and ecological change, as well as a distance gradient from the departmental capital. The largest distance between the two sampled farms is 91 km. The municipalities covered are Juticalpa and San Francisco de Becera in the valley of Guayape river (22 farms sampled), San Francisco de La Paz at the foot of the mountain (23 farms sampled), Gualaco on the top of the mountain (7 farms sampled), and San Esteban with two ranching zones descending the mountain (9 and 8 farms sampled).

b. A sample of 13 farms, referred to in the text as positive deviances, was selected using expert knowledge. This sample covers farms that use a higher level of technology, specifically forage technology. Positive deviances were defined by outstanding use of forage options, such as silage, hay, improved grasses, and legumes. Extra large farms were not accepted as positive deviances.

For the randomized sample the survey team consisted of two technicians from DICTA (Dirección de Ciencia y Tecnología Agropecuaria), the Honduran national agricultural research institute, and two CIAT staff members (a research assistant and the first author). The survey was conducted between July and

September 2005. The selection of the individual farms for sample a was done randomly in the field as follows. Without knowing the farms in the area, the team selected the route to follow for the day and interviewed every third household, provided that they had cattle. Targeted sampling for sample b was done employing expert knowledge from partner institutions, such as DICTA and UNA (Universidad Nacional de Agricultura, Catacamas). Unknown farms were identified by asking farmers about farms in the area that apply forage conservation or another forage technologies. The team for the assessment of the positive deviances consisted of the first author accompanied by a DICTA technician and sometimes by a collaborator from UNA. The data collection for the positive deviances took place between September and December 2005.

Once the entered data were verified, full cost accounting was used for the generation of results from the survey. Before interpreting the results of the analysis, data were checked for outliers on the family income in US$ per year when stratified according to herd size classes. Extreme values were found for 6 cases. These cases were not considered in the representation of average values thus the number of farms shrinks 76.

Assessment of costs and Income calculations: The gross income from milk per farm per year was calculated, by summing up the liters of milk produced in the dry season and the wet season and multiplied by the respective milk price without deducting any cost.
To obtain net income, all production costs were deduced from the gross income. Production costs include all purchased inputs and farm inputs, costs for renting machinery and services, e.g., hiring of external labor force, and contracting a team of oxen.

The production costs also include the opportunity cost of the family labor force used on the farm. The degree to which family members participated in farm work differed from case to case and was valued with the wage paid for external labor force assessed for each farm. Working on other farms with this salary would be the alternative for the farmers.

Production costs for the dry season were calculated in detail using the following variables: feeding cost of purchased supplements, production cost of forage grown for the dry season, cost of silage and hay, cost of cut and carry forage production, the salaries for workers, the opportunity cost of family labor used for dry season milk production (in Honduras most farms milk by hand), cost of veterinary services and medicines.
For the wet season, costs include feeding of purchased supplements, cut and carry forage production, weeding cost of pastures fertilization of pastures, the salaries for workers, the opportunity cost of family labor, veterinary services and medicines.

Farm income includes the income from livestock and the income from annual and permanent crops. As farms usually use a part of their products for home consumption farm income includes products sold (cash income) and the opportunity cost of products consumed (kind income).

The extent to which a farmer falls into the concentrate trap, as defined above, was assessed by the performance of the herd in milk production during the dry and wet seasons and this was set in relation to the expenses for commercial concentrate.

Grouping of farms: The grouping of farms applied uses gross margins to measure economic success. Performance groups can be formed in various ways e.g., based on superficial areas or units of production. For a comprehensive comparative analysis of dry season milk production in Olancho, data were grouped into four classes, using percentile intervals of the monthly dry season net milk income per cow in milk. Farms that did not benefit from milk production in the dry season were considered in a separate group.

The performance classification is as follows:
1. Lowest performers: farms that did not make money out of milk in the dry season and those losing money
2. Low performers: between 0 and 50 % percentile,
3. Medium performers: between 50 and 80 % percentile,
4. Top performers >80 % percentile

For the grouping of the performance classifications all farms surveyed in both samples were joined, combining the positive deviance farms with the typical farms. This was done to see if the dry season milk production strategies of the positive deviances bring higher returns per cow than in the rest of the sample.

Methods applied for the comparative analysis between groups of farms include descriptive statistics (mean averages, standard deviations, frequencies etc), stem and leaf tests, and linear regression models and non-parametric tests (Mann-Whitney test).

Results

Characterization of production and income status: When farms are grouped according to the net income per cow per month of dry season, the income differences between these groups are highly significant (P-value <0.01) (Table 78). Since the sample covers various climatic zones, it was checked if the performance in milk production was due to these climatic differences, which were assessed by farmer information on the length of the dry season. The shortest dry season is on the mountain top (2.7 moths), while the other zones have between 4.6 and 5.5 months of dry season. Although the difference is high, statistical tests proved that success category was not determined by climatic conditions. Other parameters, which one could expect to differ between performance groups, e.g., the age of the farm owner or his years of experience in milk production, were tested for differences between groups, but were shown to be similar, and thus these parameters do not differentiate the groups.

Income from milk in the performance groups: The comparison of net and gross income per cow per month for the dry and wet seasons is shown in Figure 52 and Table 78. Irrespective of the season, on average the lowest performers do not recuperate their expenses. The gross income is eaten up by production costs even in the wet season. While some farms lose only a little, others lose a lot. The error bars representing the standard deviation in the figures show that there are farms, which lose even in the wet season.

Table 78. Income per cow of performance groups in the dry and wet seasons, Olancho.

		Lowest N = 24	Low N = 13	Medium N = 23	Top N = 16
		A	B	C	D
Net income per cow per month dry season	Mean	-15.63	5.62	14.29	32.49
	Std. Deviation	22.90	3.09	3.15	13.20
	Significance	B,C,D[a]	C,D[a]	D[a]	
Net income per cow per month wet season	Mean	-1.83	18.41	18.16	27.90
	Std. Deviation	24.26	7.64	9.45	14.59
	Significance	B,C,D[a]	D[c]	D[b]	

[a] the hypothesis of non-significant differences between groups could be rejected with a probability of >= 99 % according to Mann-Whitney U test.
[b] the hypothesis of non significant differences between groups could be rejected with a probability of >= 95 % according to Mann-Whitney U test.
[c] the hypothesis of non significant differences between groups could be rejected with a probability of >= 90 % according to Mann-Whitney U test.

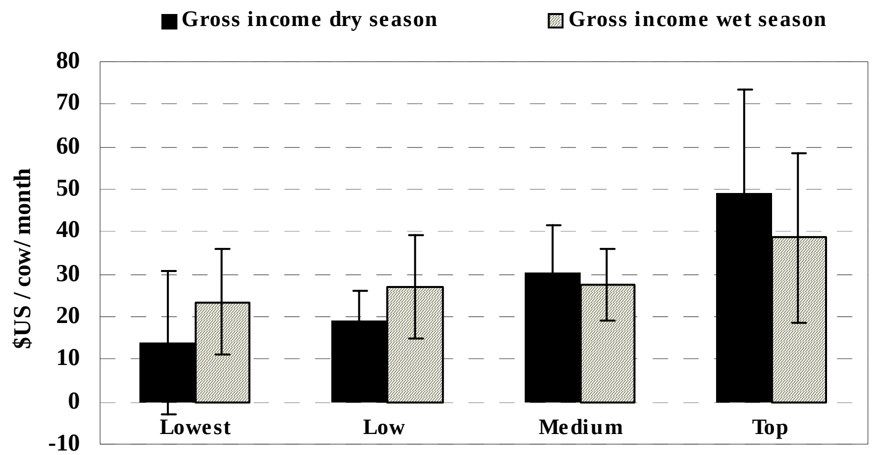

Figure 52. Comparison of wet and dry season gross income from milk of success groups, Olancho, 2005.

The dry season gross income per cow of the low performers is about 73 % of their wet season gross income. But when the production costs are deducted, wet season income is more than 3 times higher.

When the gross income from milk in the dry and wet season (Figure 52) is compared, medium and top performers show higher income per cow in the dry season. On gross income level, the lower production in the dry season (Table 85) is compensated through the higher milk prices (Table 84). Transferring this to net income (Table 78), the medium performers do not manage to benefit from the higher milk price, because their net income from milk in the dry season is lower than in the wet season. Only the top performers are able to increase their benefit in the dry season.

Gross milk income and value per cow: Throughout the sampled farms, the value of cows in milk was higher than the one of dry cows i.e. cows currently not in lactation (Table 2). The value per cow in milk serves as an indicator for the genetic potential for milk production. This relation was confirmed by farmers' experience: As a rule of thumb, farmers add 1,000 Lempira (52 $US) to the basic value of the cow for each liter of milk produced per day.

As shown in Table 79, positive deviances show the highest commercial value per milking cow. The differences between positive deviances, small and large farms were confirmed by non-parametric tests (p-value < .05), and to a lesser extent for medium size farms (p-value 0.08). Although the average price per cow in milk among extra large farms is considerably lower than the average of the positive deviances, the differences were not significant. This is due to heterogeneity among extra large farms.

To illustrate this, the yearly gross income per cow was set into a linear regression model, as

Table 79. Average farm assets in $US according to herd size classes: Per livestock head and totals, Olancho.

	Small		Medium		Large		Extra large		Positive deviances		Total
Value per head	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	Mean
Cows in milk	25	520	17	569.66	11	468.9	11	584 .69	12	780.7	581.6
Dry cows	19	385.04	15	429.82	11	373.21	11	476.08	12	567.98	450.4

Note: small: 1- 19, medium: 20 – 49 large, 50-99: extra large: > 100 dependent variable, and is described as: the number of cows in milk in the dry season and the value per milking cow (in $US). Model quality estimators (Table 80) confirm a good quality for the model in general and for the individual variables. The regression explained 81.2 % of the variation of the dependent variable. Values of the t-test for the coefficients of the individual variables were good and show high levels of significance. But the standard error of the estimation is high.

Model description:
Yearly Gross income from milk =
a + b * X1 + c * X2

a =	Constant
b =	Coefficient for X1
c =	Coefficient for X2
X1 =	Value per cow in $US
X2 =	Number of cows in milk in the dry season

There are differences in the value per cow and as the regression shows, these differences reflect in the genetic potential for milk production of the herds (Tables 80 and 81). As one would expect from the regression model, lowest performers have the cheapest cows and top performers the most expensive ones. The herds of the low and medium performers are comparable concerning the price per animal. With cows of the same price, medium performers are significantly more cost efficient in milk production than low performers. The lower cost efficiency in milk production of low performers has something to do with the management of the herds.

Table 80. Regression model for the whole sample $R^2 = 0.821$.

	Unstandardized Coefficients		T	Sig.
	B	Std. Error		
(Constant)	-3281.892	974.034	-3.369	.001
X1	6.922	1.499	4.619	.000
X2	292.807	19.485	15.027	.000

Representation of herd sizes in performance groups: To see how herd size plays on performance, farms were divided into herd size classes and plotted in respect to their performance group membership (Figure 53). The positive deviances were kept separate, irrespective of their herd size. The bulk of the lowest performers are small farms, but it is not automatic that small farms lose. They are present in each success category.

Medium size farms are more or less equally represented from lowest to medium performers. Medium size farms and positive deviances are equally represented among top performers. Together, they make up 70 % of the top performers. Large and extra large farms typically perform medium and low. About 23 % of the low performers are extra large farms. Those farms identified as positive deviances in Olancho appear among low medium and top performers.

Table 81. Value per milking cows in performance groups.

		Lowest N = 24	Low N = 13	Medium N = 23	Top N = 16
		A	B	C	D
Value per milking cow $US	Mean	482.46	562.75	565.22	723.68
	Std. Deviation	117.86	249.78	253.52	314.91
	Significance	C[c],D[a]			

[a] the hypothesis of non-significant differences between groups could be rejected with a probability of >= 99 % according to Mann-Whitney U test.
[b] the hypothesis of non significant differences between groups could be rejected with a probability of >= 95 % according to Mann-Whitney U test.
[c] the hypothesis of non significant differences between groups could be rejected with a probability of >= 90 % according to Mann-Whitney U test.

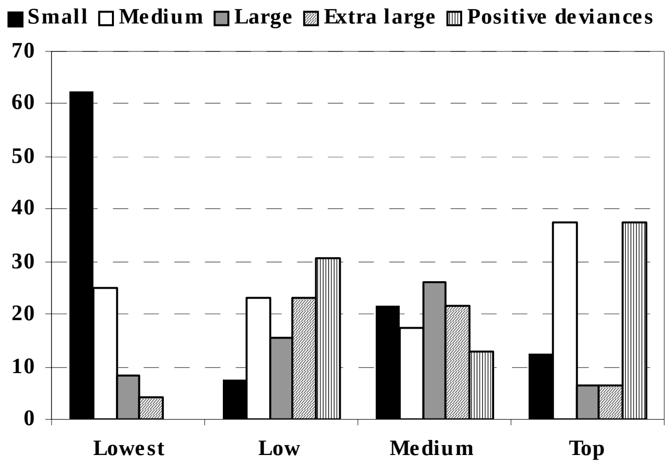

Figure 53. Performance group breakdown into cattle herd sizes, Olancho.
Note: small: 1- 19, medium: 20 – 49 large, 50-99: extra large: > 100

In the dry, lowest performers have proportionately fewer cows in milk than the other groups. This can be explained by a high presence of small farms (62.5%) among the lowest performers (Figure 53 and Table 82). Lowest performers have a lower share of improved pastures than the other groups and thus use a high percentage of naturalized pastures, which lose nutritive quality faster in the dry season. Apart from the observation that low performers have a slightly higher share of cows in milk in the dry season than the other groups from low to top, the differences are not marked. Only the lowest performers show a clear decline of cows in milk in the dry season, while the others maintain nearly the same shares of cows in milk throughout the year.

The efficiencies per unit of land are summarized in Table 83. The comparison of the yearly livestock income per ha of pastures and other forages

Table 82. Production conditions of farms in performance groups.

		Lowest N = 24	Low N = 13	Mediu m N = 23	Top N = 16
		A	B	C	D
Improved pastures % of area	Mean	46.79	62.74	74.70	61.77
	Std. Deviation	42.10	37.68	28.20	33.98
	Significance	C[b]			
Cows in milk dry season	Mean	35.74	64.97	60.43	61.50
	Std. Deviation	37.56	19.28	21.30	14.61
	Significance	B, C, D[a]			
Cows in milk wet season	Mean	61.24	58.62	63.92	62.4
	Std. Deviation	24.94	9.82	21.09	23.89

[a] the hypothesis of non-significant differences between groups could be rejected with a probability of >= 99 % according to Mann-Whitney U test.
[b] the hypothesis of non significant differences between groups could be rejected with a probability of >= 95 % according to Mann-Whitney U test.
[c] the hypothesis of non significant differences between groups could be rejected with a probability of >= 90 % according to Mann-Whitney U test.

Table 83. Land use efficiency per farm in US$ \ha\ year, Olancho.

		Lowest N = 24	Low N = 13	Medium N = 23	Top N = 16
		A	B	C	D
Livestock income per ha of pasture and other forages	Mean	-59.61	163.34	173.33	215.28
	Std. Deviation	377.31	130.28	101.74	191.96
	Significance	B,C,D[a]			
Farm income per ha of arable land crops and livestock	Mean	53.32	145.92	186.39	219.30
	Std. Deviation	144.90	104.92	135.75	170.06
	Significance	B,C[a]			

[a] the hypothesis of non-significant differences between groups could be rejected with a probability of >= 99 % according to Mann-Whitney U test.
[b] the hypothesis of non significant differences between groups could be rejected with a probability of >= 95 % according to Mann-Whitney U test.
[c] the hypothesis of non significant differences between groups could be rejected with a probability of >= 90 % according to Mann-Whitney U test.

between the performance groups includes the complete cattle production, which consists of beef, milk and sales of young stock. Even when all these products are considered, the lowest performers do not recuperate their losses from the dry season during the rest of the year. A tendency of a more efficient use per unit of land is noticeable among those farms who win, but differences are comparatively small and the within group variation is high.

The performance per unit of land does not show to be as distinctive as the performance per cow in milk in the dry season. Only when all farm products are considered (all annual and perennial crops, milk, beef and young stock sales), the lowest performers make a small benefit in total farm income per unit of land. This means that the lowest performers are better in crop production than in livestock production. Low performers are better in livestock production per ha than in crops, while overall income per area of land rises slightly between medium and top performers.

Milk prices and milk market in performance groups: In Olancho, the majority of the farmers sell to intermediates that collect the milk on the farm and resell it to artesian milk processing plants, where it is processed into the popular fresh cheese. In 1999, there were an estimated 600 artesian milk processing plants in Honduras. The difference of the dry and wet season price for milk is between .05 and .086 US$ per liter (Table 84). The higher milk price in the dry season is a measure of the dual increase in demand and scarcity of fresh milk during this period. The milk shortage that occurs during the dry season leads to a market potential for additional milk to be produced.

Table 84. Dry and wet season milk price in $US per liter, Olancho.

		Lowest N = 24	Low N = 13	Medium N = 23	Top N = 16
		A	B	C	D
Dry season milk price	Mean	0.2614	0.2506	0.2570	0.2776
	Std. Deviation	0.0248	0.0349	0.0274	0.0263
	Significance		D**	D**	
Wet season milk price	Mean	0.1919	0.2000	0.1805	0.1911
	Std. Deviation	0.0380	0.0374	0.0294	0.0468

The highest dry season and wet season amplitude of milk price per liter was found among the top performers. Their dry-season milk price is significantly higher than the one of low and medium performers. Some farms with higher production volumes sell to milk collection centers at a stable price. The dry season milk price of lowest performers was also higher than the one of the low and medium performers categories, but this difference was not significant. Yet, the trend of a higher product price among lowest performers can be attributed to a low volume of milk production, which is often sold locally as fresh milk in the village, especially among small farmers. Opportunities to sell milk were available for all farms sampled. For 95 % of the farms, it was always possible to sell the milk. Those with stagnation of sales in some periods during the wet season (typically around Christmas and Easter) processed their milk to curd cheese, which sells easily in the villages.

Milk production costs in different performance groups: During the wet season, when pasture feed is abundant, only 18.9% of the farms purchased feed supplements. For the low and medium performers, this results in a comparatively low milk production cost and higher income per cow. Lowest performers still produce at considerably higher costs. The supplement use changes in the dry season, when 57.9 % of all farms use purchased supplements and the production cost per liter of milk rises.

The production cost per liter of milk in the dry season differs significantly between all success groups (Table 85). In the case of the lowest performers, the production cost per liter is the highest and exceeds the milk price per liter. The smallest margin of profit of 0.0812 $US per liter produced is found among the low performers, which is less than half the profit than the top performers (0.1918 $US/liter). The margin of the Medium performers is 0.1329 $US per liter. Top performers have the lowest production costs and highest selling prices. With the exception of top performers, who spend more on supplements, there are no big differences in the dry season expenses of purchased supplements per animal between the performance groups (Figure 54). In all groups, average milk yield per cow drops in the dry season. Dry season milk yield per cow of lowest and low performers is considerably lower than the one of medium and top performers (Table 85). The expenses for forage production rise from the lowest to the medium performers, while the expenses for supplements fall. Lowest performers spend very little on forage production and a lot on supplements, while producing little milk. The spending of the top performers is concentrated on supplements. Their expenses for forages are comparable to those of the low performers. Top performers have the highest milk production per cow throughout the year. The structure of the expenses and the management of dry season feeding are made up of different components within the performance groups. In Table 86, dry season practices are summarized and are presented as frequencies among the performance groups.

Lowest and low performers show the highest dependency on purchased supplements, while pure farm feed is more widespread between medium and top performers. Although roughly 26% of the medium performers do nothing specific for dry season feeding, their situation is different. Their relative success is a result of favorable conditions available on these farms that provide more feed in the dry season, e.g., improved grasses and high quantities of crop residues. Mixing farm and purchased feed is a common practice among those groups who make profit from milk production in the dry season (Table 87). For a deeper interpretation of the production parameters and production conditions the following sections treat the performance groups separately, referring to Figures 54 and 55 and to Tables 85 and 86. To complete the picture of dry season milk production, farms in the performance groups were summarized to characteristic subgroups according to similar strategies.

Milk production of the lowest performers: About 17 % of the lowest performers do not use any purchased feed or farm supplements. Their usual dry season feed consists of pasture. This

Table 85. Dry and wet season milk production and costs in US$ of different performance groups, Olancho.

		Lowest N = 24	Low N = 13	Medium N = 23	Top N = 16
		A	B	C	D
Production cost per liter dry season	Mean	0.4941	0.1694	0.1241	0.0858
	Std. Deviation	0.3427	0.0601	0.0495	0.0381
	Significance	B,C,D[a]	C[b],D[a]	D[a]	
Production cost per liter wet season	Mean	0.2171	0.0586	0.0581	0.0561
	Std. Deviation	0.2124	0.0412	0.0347	0.0394
	Significance	B,C,D[a]			
Monthly supplementation cost per cow dry season	Mean	4.81	4.67	3.50	7.33
	Std. Deviation	9.26	4.61	5.95	11.08
	Significance	B[c]			
Monthly forage production cost per cow dry season	Mean	0.28	1.78	2.42	1.84
	Std. Deviation	1.39	3.92	4.00	2.46
	Significance	B,C,D[a]			
Dry season daily milk yield per cow liters	Mean	1.77	2.52	3.98	5.88
	Std. Deviation	2.10	0.80	1.42	2.87
	Significance	C[b],D[a]	C,D[a]	D[a]	
Wet season daily milk yield per cow liters	Mean	4.12	4.48	5.10	6.80
	Std. Deviation	2.08	1.93	1.52	3.35
	Significance	C[b], D[a]	D[b]	D[b]	

Note: All income figures are net income
Monetary units $US
[a] the hypothesis of non-significant differences between groups could be rejected with a probability of >= 99 % according to Mann-Whitney U test.
[b] the hypothesis of non significant differences between groups could be rejected with a probability of >= 95 % according to Mann-Whitney U test.
[c] the hypothesis of non significant differences between groups could be rejected with a probability of >= 90 % according to Mann-Whitney U test.

results in a low production of milk, because lowest performers have the smallest share of improved pastures and those who rely on pasture alone do not possess extraordinary favorable conditions, e.g., low groundwater surfaces on

Table 86. Frequencies of dry season feed sources in performance groups.

	Lowest	Low	Medium	Top
Nothing specific	17.39	7.69	26.09	12.50
Pure purchased feed	26.09	23.08	0.00	12.50
Pure farm feed	43.48	23.08	30.43	31.25
Mixture of purchased and farm feed	13.04	46.15	43.48	43.75

Table 87. Average number of dry season feed sources per farm in performance groups.

	Lowest	Low	Medium	Top
Total feed	1.22	2.23	2.39	2.81
Purchased feed	0.48	0.92	0.70	0.81
Low quality farm feed	0.52	0.62	0.74	0.88
High quality farm feed	0.22	0.69	0.96	1.13

Note:
Purchased feed: Commercial concentrate and molasses.
Low quality farm feed: dry pasture if fertilized, Maize and Bean straw.
High quality farm feed: Cut and carry grasses, Fresh maize and Sorghum, Hay, Silage, Legumes.

pastures. For the most part, lowest performers possess low quality natural pasture (Table 86). A phenomenon, which is introduced here as the "concentrate trap", describes those farmers who spend on purchased supplements without recuperating these expenses in milk production. About 39.1% of the lowest performers fall into this category. Supplementing concentrate, which is the most expensive form of supplementation, as the sole supplement is common practice for nearly 17.4 % of the lowest performers. Another 8.6% rely only on feeding concentrate and molasses. The rest of the lowest performers who fall into the supplement trap (13.1%) use farm feed of low quality combined with the purchased supplements. Only 17.3 % of the lowest performers dispose of cut and carry forages, a forage of better quality, but in limited quantity. The lowest performers with pure farm feed (43.4 %) mostly rely on maize stover of low quality. Only 4.3 % had cut and carry forages. With this poor feed base, 42 % of the lowest performers do not milk their cows in the dry season. The most important reason for not milking in this time is scarcity of feed for the herd. Cows are put to grazing on dry pastures, where they cannot get enough feed to produce. In some cases herd sizes are small and the milking period of the cows falls into the wet season, a good strategy for those farms with insufficient farm feed resources.

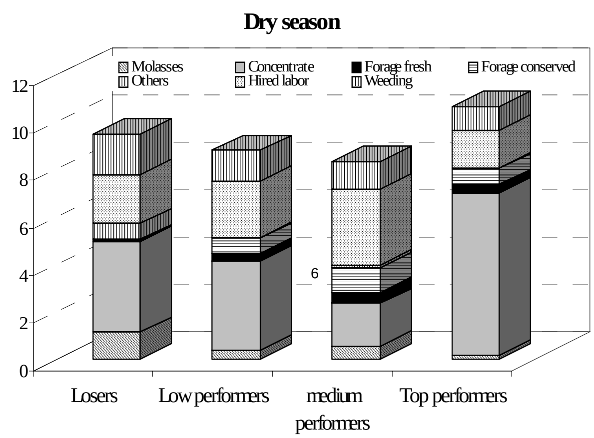

Figure 54. Monthly expenses for milk production per cow in milk in $US, dry season.

For 90 % of the lowest performers who did not milk in the dry season, it is characteristic that they do not spend on dry season feeding. Weight losses are characteristic for such a practice. Approximately 58 %, or 14 farms, of the lowest performers produce milk in the dry season. With the exception of one case, which has a comparatively high dry season milk production, lowest performers had an average milk yield of 2.6 liters per cow per day. This corresponds to the low price per milking cow, which indicated a low genetic potential for milk production. Dry season feeding of lowest performers who milk:

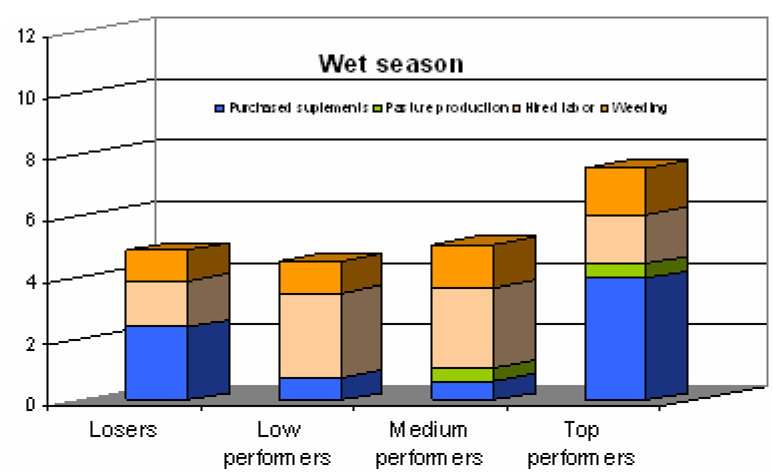

Figure 55. Monthly expenses for milk production per cow in milk in $US, wet season.

1. Limited quantity of farm supplements without use of purchased supplements, (one case).
2. No dry season supplementation was found in 4 cases. This results in a low production, which is not sufficient to recuperate the costs of animal husbandry (labor and vaccinations).
3. Seven farms purely relied on purchased supplements and dry pastures. Concentrates are most frequently used.
4. In two cases, farm and purchased supplements were combined. Quantities of farm feed were insufficient and expenses for purchased inputs turned out too high to recuperate costs.

With the lowest dry season milk yield per cow the lowest performers spend more on purchased supplements than low and medium performers. The high production cost per liter of milk and the low return per cow can be explained by a combination of traps in which these farmers repeatedly fall, i.e., the comparatively high use of purchased supplements, the low use and availability of farm-produced forages, and the low genetic potential of the cows. Even in the rainy season lowest performers have the highest costs per liter of milk. On average lowest performers used more purchased supplements and spent much more on purchased supplements than the low and medium performers. In the wet season 16.6 % of the lowest performers still fall into the concentrate trap. Family labor force is an under utilized resource among lowest performers. Although their herd sizes are small, the expenses for hired labor force per cow are comparable, to those of the top performers. The number of animals in milk and the total volume of production are low, family labor can only be accounted for those animals the family works or is supposed to work for. In the dry season, when few animals are in milk, the average opportunity cost for family labor is 20.52 $US per cow per month, while the rest of the costs sum up to 9.47 $US per cow. Average gross income per cow is only 13.96 $US per month. The family labor force is under exploited.

Milk production of the low performers: All 13 low performers produced at least some milk during the dry season. The monthly dry season net income from milk per cow is about 5.62 $US, and thus less than half of what medium performers generate. Gross income from dry season milk production among low performers is only about 5 $US per cow higher than that of the lowest performers. Moreover, low performers spend less on animal nutrition, and labor per cow is used more efficiently than among lowest performers, because low performers have more cows in milk.

Dry season feeding strategies among the low performers:

1. Two cases of the low input low output system, in which 1 case did neither use purchased nor produced forage and the other case only used crop residues (maize stover).
2. Three cases worked with purchased supplements exclusively.
3. Two cases worked with conserved forage. These farms had only recently adopted silage and the forage ensiled was not sufficient for the whole dry season.
4. Four farms work with a mixture of farm feed of low and high quality, among which low quality farm feed is more available, plus purchased inputs.
5. The remaining 2 cases combine farm grown forages with purchased inputs. One case has a better farm forage base than the other.

The average dry season milk yield of the low performers is 2.5 liters per cow per day, a very low milk yield to produce efficiently. Lowest performers milk 1.77 liters per cow per day. Such production levels can be reached without any purchased supplement, just by farm-produced forage, if made available. The wet season milk yield and the price per milking cow of the low and medium performers are comparable. Thus the genetic potential to produce more in the dry season is available among the low performers as well. In the dry season, all low performers who use purchased supplements fall into the supplement trap (69.2%) because they could do

better without it by changing their feed production strategy. The concentrate trap catches 46 % of the medium performers, but they do not fall as deeply as the lowest performers.
In the wet season low performers and medium performers spend a similar amount on purchased feed, but do not apply fertilizers to their improved pastures. Wet season net income per cow is nearly equal between low and medium performers.

Milk production of the medium performers: All medium performers produce milk during the dry season. Their average milk yield per cow in the dry season is lower than the one of the top performers, but greater than that of the low performers and the lowest performers. In the cases of top and medium performers, the gross income from milk in the dry season exceeds the one of the rainy season. For the medium performers, this difference is small, but the trend to exploit the herd more intensively in periods of high prices is noticeable.

Dry season feeding strategies of medium performers:

- Nine cases did not use purchased or produced forage. These farms have more favorable site conditions than others,. such as dry season pastures made up of improved pastures, or pastures near rivers, which stay green during the dry season and some crop residues.
- One case had high quality farm feed, such as conserved forage.
- Four cases feed conserved forage and purchased supplements.
- The remaining nine (9) cases apply strategies, including the use of fresh Maize and dry Sorghum forage and cut and carry forage, which is usually complemented with commercial concentrate.

Differences in management let the production cost per liter of milk fall below the one of the low and lowest performers. There is not a single case among medium performers that relied on purchased inputs alone. Medium performers show the highest percentage of improved pastures (Table 82).

Medium performers are marked by a comparatively low use of purchased supplements and by the use of a variety of farm produced dry season feed. Costs of production for farm-produced forages are higher than in the other groups. In the dry season feed of medium performers forages are used in various ways, including supplementation of fresh and conserved forages. This makes the cost for hired labor per production unit rise.

Milk production of the top performers: The situation among the top performers is twofold and needs to be interpreted separately. Some top performers generate a high income per cow without purchasing supplements, while others have huge expenses for purchased supplements. Among the top performers, the average monthly dry season income from milk per cow (Table 78) is $32.49, which is even higher than in the rainy season (27.90 $). On average, this can be explained by the higher milk price in the dry season. Typically, the milk production per cow drops is a little in the dry season. There are also farmers whose milk production rises in the dry season. They state that this is the result of a better care and alimentation during the dry season. Thus dry season feed is of better quality than rainy season feed, which usually consists of pasture and less concentrate. The top performers are made up of farms from all sizes with a high share of medium size farms and positive deviances (37.5 % for both).

The variety of dry season feeding strategies is the highest among top performers:

1. There are 2 cases, which do not use feed supplements or produce forage on the farm. These farms have favorable site and climatic conditions.
2. Three more farms of the top performers have favorable climatic conditions, but they supplement with molasses during the dry months.

3. Frequent strategies among top performers include feeding of cut and carry grasses, cut forage Maize and Sorghum in sufficient quantities. Grazing of crop residues, accompanied with the previously mentioned feed items, enables them to produce milk comparatively cheap. Concentrate is always a part of the diet.
4. Six of the top performers belong to the positive deviances, which use between 3 and 6 sources of feed in adequate quantities. These feeds include conserved forage (hay and silage), cut and carry grasses, Maize and Sorghum for forage, crop residues, and the purchased inputs in considerable quantities.

Milk production of the positive deviances among performance groups: Knowing that the positive deviances apply forage technologies in a more advanced way than the rest of the farms lets the expectation that they all should belong to the top performers group rise. The sample of positive deviances consists of normal farms that have adopted some forage technology. The economic valuation of these farms does not necessarily demonstrate what can be achieved with an appropriate use of forage technology. For such questions, controlled field trials or farmer field experiments would be more appropriate. The socio/economic assessment of positive deviances shows how these farmers work.

The positive deviances were selected to assess examples of technology adopters. Technologies included the more advanced technologies of livestock production systems and of forage production. Positive deviances were defined by outstanding use of forage options, such as silage, hay, improved grasses, and legumes. The expectation that the application of forage technology makes all those farms perform better could not be met because more factors have a great influence on the economic success of dairy production and those factors, such as e.g. the quality of the milking cows and to which costs forages are produced could not be eliminated during sampling.

Comparatively few farms could be identified that qualified as positive deviances. Thus we had to sample each farm and were not able to choose only farms that had applied the same forage technology.

The positive deviances that are top performers use over 4 times more money for purchased supplements than the medium performers (Figure 56). They seem to use purchased supplements in an exaggerated amount. The success of farms in milk production depends greatly on the volume of milk produced per production unit. Gross income from milk in the dry season depends on the value of the cow and the number of cows in milk in the dry season (Table 80).

The success or failure among positive deviances is explainable through the value, and thus the quality, of the milking cows (Table 88). The top performers have the most expensive cows. About 69 % of the cows from these farms are crossbreeds, with more than 50% being European breeds. These crossbreeds are able to transform concentrate into milk in a profitable way. Low performers among positive deviances feed good forage to inefficient dairy cows, which is obviously not as profitable as with good dairy cows.

General Discussion

The reduction of the number of cows in milk during the dry season is a common strategy which can be clearly observed among the lowest performers. Insufficient availability of farm fed lets the feeding costs rise and the income decline.

Considering their scarce feed sources, low performers do not respond by reducing the number of cows in milk. They have a slightly higher proportion of cows in milk during the dry season than the others. But their benefit is small because their production per cow is significantly lower than among the other winning categories, and they spend more on purchased inputs than the medium performers. Without changing the feed base, better timing of insemination to get cows to calving during the period of feed

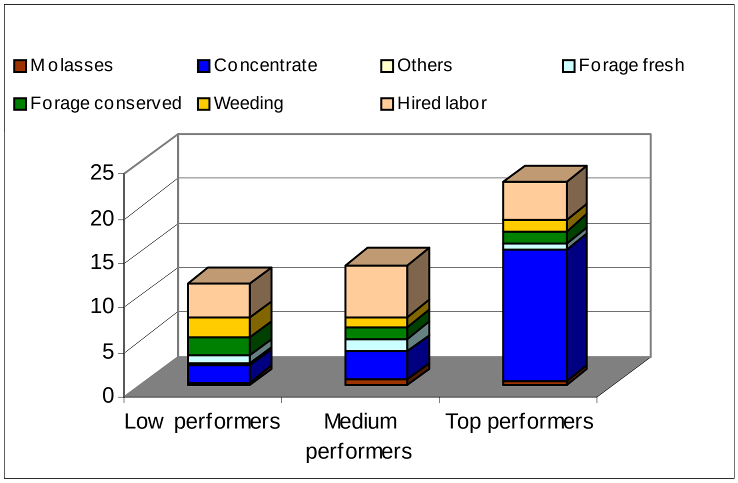

Figure 56. Monthly expenses of positive deviances in performance groups

Table 88. Average Value and breed composition of dairy herds among positive deviances in performance groups.

Category	Average value per dairy cow $US		Cattle cross breeds percentage of the dairy herds	
Low performers	Mean Std. Deviation	513.16 187.93	75 % Brahman 25 % Pardo	62.50
			50 % Brahman 50 % Pardo	25.00
			25 % Brahman 75 % Pardo	12.50
Medium performers	Mean Std. Deviation	789.47 157.89	75 % Brahman 25% Holstein	26.67
			50 % Brahman 50 % Holstein	16.67
			50 % Brahman 50 % Pardo	56.67
Top performers	Mean Std. Deviation	956.14 395.85	75 % Brahman 25 % Holstein	30.83
			50 % Brahman 50 % Holstein	39.17
			50 % Jersey 50 % Holstein	1.67
			75% Holstein 25 % Brahman	28.33

abundance, would make the use of their actual feed resources more profitable.

The decision of top performers to milk cows with an adequate provision of feed is a strategy that pays off well because efforts are concentrated on animals of good quality, which produce more per unit of feed.

Although fed in various intensities, purchased supplement are shown to be the most important cost driver in the dry season and are applied on 62 % of the farms.

Low and lowest performers do not benefit adequately from their herd. They are in a situation in which they use purchased feed supplements in order to keep the herd alive/ in a good condition, while other farms with a better feed base are producing milk. This situation of using purchased concentrate to replace farm feed or to maintain the herd can be described as the bottom of the concentrate trap. Expenses are high and few returns are generated, if any. Ideally, concentrate should be fed as an additional protein source to enrich the diet, and not as a substitute for forages.

With the exception of the top performers, as the expenses and variety of farm feed rise, the expenses for purchased supplements decline. The rising income per cow and per unit of land is a logical consequence of the more adequate use of farm resources for production and the purchase of inputs.

Lowest performers combine the disadvantages of resource-poor farms with deficiencies in management. Obstacles for the adoption of multi-purpose forage options are related to cash scarcity and the experience that the cows do not bring much. The lowest performers gain this experience, because the genetic potential for milk production of their cows is low and because their feed base is insufficient. The gross production of milk in the dry season is low and continuous cash flow is not available in these farms. Milk is thus "purchased", paid for with supplements. They are at the bottom end of the concentrate trap.

Their situation is not likely to change without intervention. Possibilities for policy and development interventions in these systems should aim at a consequent improvement of the forage feed base for the dry and the wet season and in the improvement of the genetic potential for milk production of the herds. The objective of such a development strategy should be to facilitate a continuous cash flow through year-round milk production, which is based on as much as possible farm feed. Recommendations for the development of losers include the subsidized promotion of well-adapted improved grasses (e.g. *B. brizantha cv* Toledo) and their conservation. Cut and carry grasses could also be promoted where appropriate land is available. Low quality farm feed in the form of maize stover is the most accessible dry season low cost feed for losers. This low quality feed resource can be improved by using *Lablab purpureus* as an intercrop species with maize to provide better legume/ stover feed and at the same time increase maize yield, as experiences from Olancho have demonstrated. Parallel to these forage based measures which aim at creating a good feed base, farms of poor communities should be enabled to improve the genetic potential of the milking cows by provision of adequate (milk cross breed) bulls to farmer groups. Technical assistance in pasture management and rearing of heifers would complement the package.

In the case of the low performers the low returns can be attributed to, inadequate feeding in the dry season with high share of purchased supplements and a low availability and use of high quality farm feed, such as fresh forage and conserved forage. The genetic potential for higher milk production in the dry season is generally available on the farms of low performers. Lack of forage and excessive supplement use causes them to fall behind the medium performers. Although to a smaller degree than the losers, these farms also fall into the concentrate trap because they substitute forage with concentrate to fill the cows stomach.

Possibilities for intervention lie in the improvement of the farm feed base by promoting a more adequate pasture management (rotation pasture and fertilization including organic and chemical fertilizers), the restoration of degraded unproductive pasture land and the improvement of maize stovers with legume intercrops. As long as the improvement of the feed base is in process and unreached, low performers should follow the example of medium performers, which keep the share of milking cows a little lower in the dry season, and thus corresponding to their feed availability. This could mitigate the effects of the concentrate trap.

The variety of feeding strategies rises for the dry season among medium performers because there is more farm feed available. Medium performers did not show any dominance of a specific herd size. The important message of this distribution is that milk production can be profitable irrespective of the size of the herd. What inhibits better performance among the medium performers is that they lack sufficient quantities of cheap forage and of high quality supplements. Limitations For higher volumes of dry season milk production and possibilities for the reduction of costs are related to the dry season forage shortage, which makes farmers reduce the number of cows in milk.

Most dry season feeding strategies are used among top performers, but expenses for purchased supplements are clearly higher than in the other groups. Top performers spend on average 7.33 $US per cow on purchased supplements. This is about the double of the medium performers. But they use the purchased input for production and not to fill the cows, as it is the case on many farms that perform low and medium. The genetic potential of their cows is considerably better than in the rest of the farms. Top performers should rethink their dry season feed strategy and make efficient use of high quality forages in conserved form or in the form of self or locally made concentrates.

Although top performers do not fall into the concentrate trap, they base their milk production on high levels of commercial concentrate. This dependency could possibly change by a sound use of legumes and enhanced use of conserved forage. Nevertheless, there is room for more intensive use of farm resources among top performers and among medium performers. The high use of purchased supplements (top performers) shows, that there is a need to improve the dry season feed base with conserved forage.

Milk production in Olancho is highly dependent on commercial concentrates. Although legumes provide high quality protein feed, they are rarely used. Adoption of such technologies is most probable among top and medium performers because they have some financial reserves, which could be used to test new technologies. Moreover, top performers possess good cows, which have the potential to respond to high quality feed. Once technology has sufficiently spread between top and medium performers, the message that it is possible to work more profitably with forages will probably reach and convince low and lowest performers and is likely to take over to low performers.

4.5.3 Diversified production systems for the Colombian Llanos: An economical assessment

Collaborators: Libardo Rivas (CIAT), Federico Holmann (CIAT-ILRI), and James García (CIAT)

Colombia's Ministry of Agriculture and Rural Development (MADR, its Spanish acronym) has launched an all-inclusive "Rebirth of Colombia's Upper Orinoquia" mega-project proposal. The main objective of this project is to generate environmental services associated with carbon (C) fixation or sequestration in an attempt to mitigate the effects of progressive global warming. The project will involve the large-scale planting of plant species that fix C in their aerial parts (foliage) and roots as a commercial product for the international market.

The proposal contemplates the establishment of 6.3 million hectares of trees species over a 20-year period. Upon termination of the project, the region's population is expected to have reached 5 million inhabitants, with 1.5 million jobs generated. The total cost of the agricultural component of the megaproject is estimated at US$15,000 million, in addition to the investments required in physical and social infrastructure and in public utilities. This initiative is encompassed within the national policy on productive forest development, which considers Forestry Incentive Certificates, known as CIFs, as one of the main incentive tools for the sector. The policy aims to stimulate the use and offer of forest products in Colombia, while generating environmental benefits such as erosion control, conservation of water sources, C fixation, reduced felling, and less pressure on natural forests. Using an economic, social and environmental approach, the present study evaluated, within the aforementioned global framework, new farming models that include various livestock, agricultural, and forest components for the production of food crops, raw materials for industrial use, and environmental products, such as C sequestration.

This *ex ante* evaluation aims to generate pertinent information as a way of supporting decision-making about investment in the public and private sectors, allowing a sustainable and competitive development scheme to be implemented in the region, with a high economic, social, and environmental impact. The techniques, based on linear programming, are used to address the major economic problem of efficient allocation of limited resources among multiple alternative uses. The theoretical model proposed is as follows: maximize $Z = CX$, subject to: $AX \le b$; $X_1, X_2 ...... X_n \ge 0$; Z being the target function, which in the present case is defined as the total net profit resulting from the implementation of several production options at the farm level. The C row vector corresponds to the coefficients of net profit per unit of product generated and the X column vector includes the latter.

The modified model used in the study extends the evaluation period to 19 years and is limited to the analysis of livestock, agricultural, and forest alternatives and carbon sequestration by different types of plant coverage.

The following variables are included: (1) decision options—also known as activities—whose level is directly controlled by the producer and are part of the production plan of any given farm and may include pasture-crop rotations, sale and purchase of products and inputs, access to credit, and use of cash over time and (2) internal (endogenous) and restrictive variables that include all those variables resulting from the internal operation of the model as well as economic, technical, and environmental restrictions. Production activities feasible of being carried out in Colombia's Orinoquia region include: (1) different alternative uses of land resources and how their efficient use can generate social, economic, and environmental benefits; (2) potential land use in livestock production, agricultural crops, and activities involving reforestation or natural forests; (3) alternatives that generate commercial products such as meat, milk, and wood, and environmental services such as Ca sequestration by pastures and forest coverage; (4) economic, social, and environmental benefits derived from land use occurring at the farm, region, and national levels.

Two types of livestock systems were evaluated. The first, dual-purpose systems, involve the production of both beef and milk on the same farm, with emphasis on milk production. The productive capacity of cows is improved by incorporating genes of dairy breeds and by offering a better quality diet to the bovine herd. The second, the cow-calf operation is a basic livestock production phase aimed at the raising of cattle for livestock farms specialized in cattle fattening.

The forage on offer for livestock comes from the following alternatives (Table 89): (1) pastures of native savanna alone, improved *Brachiaria*, and *B. decumbens*-legumes in association, and (2) pastures resulting from three different crop rotations. Rotation 1 starts with a period of 7 years of native savanna, followed by a 4-year cycle of semiannual crops rice-soybean and maize-soybeans in rotation, and ends with a pasture of *B. decumbens-D. ovalifolium* in association that remains productive over an 8-year period. Rotations 2 and 3 are similar, and begin and end with crop cycles of 6 years each, including an improved pasture in the intermediate phase, which, in the case of Rotation 2, is improved *Brachiaria* and, in Rotation 3, an improved pasture of *B. decumbens-D. ovalifolium*. The pastures alone were evaluated over the entire 19-year period, including renewals in years 8 and 15. The forest component is represented by the planting of Caribbean pine, which produces wood as well as environmental services in the form of Ca sequestration. To improve the soil's physical and chemical conditions, an improved grass was initially planted that remains productive for 4 years, before the pine plantation was established.

Several sequential scenarios were constructed to simulate the gradual incorporation of new technological components into existing livestock systems (Table 90). The livestock production model used, whether cattle-raising or dual-purpose, is based on the extensive use of pastures alone. During the following phase, the model adds a component of pasture-crop rotation in a process oriented to gradually improve soils by building arable layers.

Table 89. Farm model with dual-purpose livestock production system: livestock production, with availability of adapted crop germplasm, soil improvement practices, forest options, and payment for environmental services (sale of carbon) at different levels of working capital.

Initial availability of capital ($x 10^3$US$)		Land use (ha)				Cows at final evaluation (no.)	Net generation of employment (no. workdays)[a]	Value target objective ($x10^3$ US$)
Total ($x 10^3$ US$)	Per ha (US$)	Caribbean pine	Crop-pasture rotation R1	Crop-pasture rotation R2	Brachiaria *cv. Toledo*			
300	600	414.1	37.8	0.6	47.5	162	709	7851
200	400	287.0	117.7	47.6	47.7	312	873	6850
100	200	160.0	190.4	101.9	47.7	443	1062	5840
50	100	96.6	226.7	129.1	47.6	509	1156	5334
25	50	48.0	274.9	127.0	50.1	601	1147	5080
10	20	15.3	310.0	122.6	52.1	668	1129	4927
5	10	4.4	321.7	121.1	52.8	691	1123	4876

a. Annual average. Total area available: 500 ha.

The incorporation of trees and the offer of environmental services in the form of Ca sequestration represent the following stage in the process of transforming production systems. Finally, to evaluate the impact of the economic policy on production systems and land use, scenarios are built in which the production systems are supported by promotional policies such as the CIFs or prepayment of environmental services, rather than by including new technological components. The model considers a 500-hectare farm that operates with costs considered average for the region and with a working capital that can vary, alternatively, between US$5000 and US$300,000. Results showed the following possibilities for the region, among others:

(1) The incorporation of new technological components into traditional livestock production systems of the Llanos of Colombia results in a significant growth of net farm income, employment, production, and productivity. As can be expected, the use of improved pastures in the traditional livestock production system is conditioned by the availability of funds. The appearance of pasture-crop rotations excludes native savannas from the optimal solution and improved of cash flow facilitates the expansion of improved pastures on farms with lower availability of funds.

(2) The level of technology development in cattle-raising systems was lower as compared with dual-purpose systems. Therefore the economic effect of introducing

Table 90. Structure of the net present value of income according to degree of intensification of livestock systems in the Colombian *altillanura*[a].

Activities and products	Production system							
	Cattle-raising				Dual-purpose			
	Based on native savanna		Intensified[b]		Based on native savanna		Intensified[b]	
	PVGI[c] ($x10^3$ US$)	% total	PVGI[c] ($x10^3$ US$)	% total	PVGI ($x10^3$ US$)	% total	PVGI ($x10^3$ US$)	% total
Livestock								
Beef	174.4	78.7	143.0	9.2	221.2	44.2	1262.1	41.7
Milk	—	—			246.4	49.3	1405.4	46.4
Crops	—	—	457.0	29.3	—	—	237.4	7.8
Forest								
Wood	—	—	932.0	59.8	—	—	101.8	3.5
Sale of C			18.0	1.2	—	—	19.0	0.6
Sale of workdays	47.3	21.3	8.0	0.5	32.9	6.5	1.2	0.0
Total gross income	221.7	100.0	1558.0	100.0	500.5	100.0	3026.9	100.0

a. Working capital: US$10,000.
b. Includes improved pastures, pasture-crop rotations, forest species, and sale of C.
c. PVGI: present value of gross income, for example 5%.

improved technologies was greater in the former.

(3) The establishment of forest plantations for sale of wood and C sequestration has greater possibilities of being adopted in cattle-raising systems. The simulation exercise showed that trees would enter this system at all levels of available capital, being a promising alternative to cattle-raising systems, which are usually relegated to isolated areas far from the markets. The above can occur if the government invests in road infrastructure and transportation, in tandem with the development of complementary services, especially those related to the processing, management, and marketing of forest products.

(4) Technical advances significantly improve net farm income—the target objective of the model—but especially on farms with less available capital. For example, the implementation of pasture-crop rotations in cattle-raising systems increased net farm income by 1.8 with high capital availability and by 6 in farms facing greater financial restrictions.

(5) The intensification of production systems increases the capacity to generate employment, which has an important impact on the achievement of the social goals of equity and reduction of poverty.

(6) Forest development policies, for example the CIFs, have greater impact on production units with more working capital because it allows them to expand their area under forest. When capital is below US$25,000, the impact is null.

(7) Current prices of carbon are low on the international market and, according to several experts, will remain stagnant through 2012.

On-farm research projects and ex ante economic studies have demonstrated the feasibility of new technological options. However, the adoption of new technologies in the region does not yet have the necessary dynamics to trigger a significant impact on production, productivity, employment, and prices.

Agriculture is risky in the Llanos because it faces numerous restrictions (technical, economical, physical, social infrastructure) as documented in surveys conducted by CIAT in 2004 and 2005. Many of the producers interviewed perceive the enforcement of NAFTA as threatening, increasing the risks faced by the region's crop production. This bilateral agreement, however, also opens significant opportunities for the production of beef, milk and dairy products, forest and fruit trees—all activities that can adjust to the resources found in the Llanos. In a scenario where the price of grains falls more than 10%, the net income contributed by the crops to the rotations would be negative. Despite this, Rotation 1 (native savanna–crops–improved grass) would continue to be profitable because of its solid livestock component.

The research conducted on current crops to improve their yields and the search for new options of adapted, high-yielding crops to establish rotations with grasses emerge as alternatives to face the economic risks posed by prices and NAFTA challenges. In a growth environment supported by policies on investment in physical and social infrastructure, official credit programs, and compensatory measures for sectors adversely affected by the bilateral treaty, grain production is expected to continue to be economically viable in the Colombian Llanos. To materialize the advantages of participating in a broad, high-value market and to take maximum advantage of the Llanos natural resource pool, the country must consider comprehensive development programs that, in addition to promoting the on-farm application of new technology alternatives, apply appropriate policies to overcome the constraints hindering technical advances.

4.6 Forages for Monogastrics

Highlights

- Monogastric animal production is an integral component of many smallholder farms. Many farm families produce mostly chickens and pigs but other monogastric animals are common including duck, guinea pigs, rabbits and fish. Input and output opportunities restrict the earnings capacity of many farmers.

- Larger scale monogatric farmers face expensive feed costs. With cost of imported grain prices increasing, domestic production of grain and legumes may become more attractive. In addition, these alternative feed sources can be better adapted to specific production niches.

- Monogastric production is an important strategy to diversify household risk for many smallholder farmers. Occasional and/or informal sales of meat, eggs, and animals provide an additional income source, especially for women. A challenge for research and development is to foster enhanced market access and feed inputs according to local context.

4.6.1 Opportunities and constraints of smallholder farmer feed production for monogastric animals in Colombia, Honduras and Nicaragua

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Rationale

Demand for animal products is growing in developing countries. Pork and poultry rather than beef dominate consumption changes. These monogastric animals accounted for 76% of the increase in demand in developing countries between 1983 and 1997. Smallholder farmers, however, may not benefit from this opportunity. Trade liberalization and superior market access provide competitive advantages to larger-scale commercial operations.

A scarcity of high-quality feed restricts animal production and marketing of both small and large scale operations. A reliance on imported ingredients exposes concentrate companies to volatile prices and unreliable supplies. Difficulties in obtaining low-cost, high-quality feed ingredients may worsen; particularly since grain prices are anticipated to increase. Smallholder swine and poultry producers, without access to alternative feeds, may become even less competitive.

The aim of these diagnostic studies is to characterize areas with a high potential for smallholder production of monogastric animals and define opportunities and constraints. Objectives included poverty reduction and counteracting environmental degradation in specific areas in Honduras, Nicaragua and Colombia.

Materials and Methods

Three areas were selected for the diagnostic studies, Cauca, Colombia, Olancho, Honduras and Chinandega and Léon, Nicaragua. A quick diagnostic survey approach was employed to rapidly obtain in-depth knowledge of constraints and opportunities in specific social, economic, and natural environments. The objective was to understand smallholder monogastric production systems and identify the opportunities and constraints to substitute purchased (and imported) feeds with forage-based protein feed.

The following topics and questions were addressed:

General, monogastric systems
- General farm data such as area, crop and livestock enterprises;
- Livestock holdings and composition;
- Enterprise (crop and livestock) management and reasons for their selection;
- Livestock feeding systems (including on-farm production, purchases, pasture management);
- Use of outputs (i.e., household consumption, local market and/or commercial sales) and underlying reasons for end-use;
- Changes and trends in livestock composition and underlying reasons;
- Problems, difficulties, constraints, and opportunities;
- Animal health.

Markets
- Other on-farm commercial enterprises (besides monogastrics);
- How animals are sold, market competition;
- Seasonal demand and price variation;
- Price differentials according to product quality;
- Enterprise profit margins.

Feeds
- Feed use, composition, production and purchase;
- Feed sufficiency, critical times of the year;
- Strategies used to alleviate feed problems;
- Feed quality and quantities;
- Concentrate and supplement use.

Organization
- Participation in groups or associations;
- Technical support, sources and quality;
- Capital and credit availability.

Results

A) Cauca, Colombia.

The team visited small farms near the town of Pescador and Popayan. In Pescador (1200-1500 masl), most farmers had secure rights to land, as is the case throughout the Cauca region. Farmers with monogastrics generally relied on coffee and/or cattle as primary commercial activities. Approximately 70% of respondents raised chickens for household consumption that were fed household waste, some concentrates and, in some cases, farm-produced forages. Production of monogastrics was mostly a family activity, involving women, men and children. In most cases, women were responsible for the daily management of poultry; care of swine was more diverse.

Nearly 25% of farmers raising monogastric animals produced broilers, approximately 20% raised swine and 20% rabbits. A few raised guinea pigs (Table 91). Farmers producing poultry commercially had better access to technical support and to capital, the latter either through credit or earnings from coffee production.

Monogastric production was made possible by links with other farm activities. Earnings from coffee harvests were often invested in fattening poultry. A similar situation occurred with swine, Capital to obtain piglets came from coffee sales or credit provided by farmers' associations. For both commercial poultry and swine production, investments of returns from coffee into livestock production are a means to diversity production and to ensure cash flows over a greater part of the year. Nevertheless, investments were a greater priority than monogastric production. In some cases, farmers refrained from monogastric production due to limited available labor and fear of damage to the coffee crop by free roaming animals.

A lack of technical support and capital limited commercial production. Constraints to poultry production included animal diseases, insecure markets, price fluctuations, and resulting low profit margins. Lack of water, unfavorable prior experience and theft were also mentioned. Many farmers preferred either poultry or swine production. Lower market risk and higher profit margins were claimed for swine and guinea pigs, although the latter had a very limited market. The high cost of concentrates restricted profit margins.

In areas around the city of Popayan (1500-1700 masl), commercial production of monogastrics were dominant. As with farms near Pescador, coffee based systems provided capital for animals and feeds. Such an arrangement also limited monogastric production in peak coffee management and harvest times. Most farmers concentrated on animal fattening rather than egg production, thereby enhancing the flexibility for labor inputs. Fifty-six percent of respondents only fed their poultry purchased concentrates; while 40% mixed purchased and on-farm produced feeds. While nearly half of swine producers combined purchased feed with farm-produced feed, only 30% of poultry producers did the same. Less than 5% of producers completely relied on feed produced on-farm: these cases were mostly guinea pig producers.

Expansion of on-farm animal production in coffee based systems will depend on labor and capital availability. There was limited knowledge regarding on-farm feed production. A few farmers recognized the value of (forage based) on-farm feeds in reducing costs of concentrates and increasing product quality (e.g., texture and taste of meat and of egg yolk color). Forages

Table 91. Predominant monogastric systems and research priorities in northwestern Nicaragua.

System	Strategy	Research Needs
1. Small-scale swine fattening for 0 to 6 months or to 6 to 12 months of relatively poor farmers. Feed produced on farm and purchased	A	a) Introduction and testing of: i. Improved sorghum and (QPM) maize ii. Protein-rich germplasm (legumes--annuals and perennials) b) Participatory on-farm systems trials with attention paid to gender and to land and labor. c) Work with organized groups or with groups facilitated by project d) Improvement of animal breeds e) Facilitate relations with projects or programs providing credit, animal loans and/or training (e.g., on animal and human health relations).
2. Fattening of 20-100 pigs reared in pens; feed purchased and produced on farm	A	
3. Pigs produced using residues from peanut production	-	No research planned due to unique and inequitable system
4. Swine fattening of 20 to 50 unpenned pigs, peanut residues not available	A	
5. 20 to 50 pigs, with animals penned during crop production period	B	Some farmers interested in producing their own feed concentrates. Research to include model A with additional attention to production and formulation of feed concentrates including co-development of complementary feed ingredients. Organizational and business development for feed utilization and sale of feeds and livestock products.
6. Small and medium 7. scale poultry production with and without confinement, for hh consumption and sale	A	Particular attention to be given to protein nutrition for more eggs and better weight gain
8. Other monogastric animals solely for hh consumption	-	No research due to few or no incentives to improve production

produced for monogastrics included *Axonopus*, *Pennisetum* hybr., *Saccharum officinarum*, *Manihot esculenta*, *Bohemia nivea*, *Tripsacum laxum*, *Arachis pintoi*, *Alocacia macorrhiza* and *Trichantherea gigantea*.

The tendency to combine purchased and on-farm produced feeds was greater in swine compared to poultry production. Profit margins for swine appeared to be relatively healthy; while poultry production operated on smaller margins. Guinea pig production margins were high but restricted to a very limited niche market. Rabbit production in the Cauca region appeared to be the least commercially attractive.

Conclusions

In the majority of cases, coffee and monogastric production co-existed, with coffee providing necessary capital and labor but also competing during certain periods. Impediments to on-farm feed production include:

- Competition for land by coffee and cattle (although recently renovated coffee parcels may be suited to feed production);
- Labor peaks in coffee production limit availability of labor for on-farm production of feed;
- Credit for purchase of concentrates is readily available;
- Technical support for monogastric production is often provided by feed companies
- Technical information on producing alternative feeds is not readily available.
- Many farms are managed by caretakers who have little if any incentive to increase their labor inputs for on-farm feed production.

Factors favoring on-farm feed production include:

- Forages that do not compete with coffee in terms of cash flow and labor;
- High demand for high-quality feed concentrates;
- Expensive concentrates exacerbate household cash flow problems;
- On-farm produced feeds can impart product qualities favored by consumers;
- Variable quality of purchased concentrates recognized by farmers but is not specified by manufacturers;
- Farmers have experience cultivating mixes of crops and could easily add forages;

Additional analysis is needed regarding the costs of on-farm feed production vs. purchased feeds and concentrates vs. local artesanal production of concentrates (using either purchased or on-farm produced ingredients). Forage-based feed would likely be used in combination with purchased concentrates.

Forage-based protein feeds appear particularly attractive given widespread lack of sufficient protein in livestock diets. Opportunities for tropical forage-based protein feeds appear to be greatest in areas of 1400-1600 masl, to diversify production in areas suboptimal for coffee production. Potential for artesanal production of raw materials for sale appears greatest at lower altitudes, where many tropical forages have their highest productivity. Further work would need to focus on owner-managers of farms. Quicker success is likely with farmers who are (1) already organized or who could easily be organized and (2) already engaged in commercial production of monogastrics. Willingness and feasibility of commercial production needs to be explored further.

Specific areas of further research include:

- Cost-benefit and household cash flow analyses of forage-based on-farm feed production;
- Feeding strategies including trade-offs amongst feed cost, quality, and quantity;
- Effectiveness of forage-based protein feeds in the diets of different monogastric animals;
- Locally available forage and feed materials and local practices;
- On-farm trials with the participation of farmer groups;
- Value chains of both monogastric feeds and products;
- A focus on swine and poultry, and possibly guinea pigs, is appropriate. Rabbits and fish production appeared to be less attractive in the Cauca region.

B) Léon, Sauce and Chinandega el Viejo Nicaragua

The diagnostic team worked in areas around Chinandega and Léon. Areas visited differed by agroecological conditions, wealth levels, access to market and importance of cash crops.

Léon. Most of the area around the town of *Léon* is characterized by poverty and small-scale livestock systems. Animals were for household consumption and some sales in local markets. Mean farm size was slightly above 10 mz. Main crops were maize and sorghum cultivated on an average area of 1-2 mz and used for household and animal consumption. Mango and plantain was a cash crop in limited areas. Farmers raised multiple types of livestock, with a few having a few cattle; and most having a few chickens and fewer pigs. Approximately half of the respondents considered mongastrics as a commercial activity. Broilers and eggs were consumed and sold; while pigs were raised mostly for sale. Sheep, turkeys, geese and other animals were found and raised for home consumption. Smallholders had a mean of 9 pigs and approximately 25 chickens, though differences among individuals were large.

Maize and sorghum were the basic feeds of monogastric animals, sometimes combined with residues of plantain, mango, cassava and squash. Where available, farmers used jicaro fruit. Concentrates or purchased feed were used for very young animals, during feed shortages, final fattening, and largely as a supplement rather than a complete feed ration. While concentrates for very young animals appeared to be formulated, concentrate composition for other animal stages was unclear. In some cases, farmers mixed feeds themselves using available ingredients such as maize and sorghum.

Approximately 2/3 of farmers purchased supplements such as concentrates, mixed feeds (i.e. composites of maize and sorghum), semolina (maize meal), or maize and sorghum itself. Only 1/3 of farmers bought commercial concentrates, some of which may be locally fabricated. Use of peanut residues was limited but of importance where encountered.

Expansion of swine production was limited by lack of capital and/or lack of access to water in some cases. For both chicken and swine, theft appeared to be a problem. Other risks were animal diseases and instability of rainfall.

Sauce. Farm size and level of monogastric production in the area around Sauce were similar to those around Léon. Production of monogastrics was a diversification strategy with various interactions among different types of animals and between animals and crop production. Farmers fed milk whey to pigs; and in rare cases, chicken manure was used as a protein supplement to swine. Good crop harvests allowed animal feeding to be based almost completely on feed produced on-farm, while poor harvests led to a need to purchase at least some feeds. Animal production also had monetary interactions. While chickens were used more for home consumption and some cash flow, swine were a ‘bank account’ for investment or emergencies. Pig sales served to purchase crop production inputs; while crop harvests allowed purchase of the next set of piglets. Some farmers aspire purchase cattle from the earnings realized from pig production.

Animal feeding was largely based on farm inputs and cheaper feeds such as semolina. Concentrates—although recognized as being of higher quality—were considered too costly for continual feeding.

Limitations to the expansion of swine (and to some extent chicken) production included the danger of crop damage by roaming animals. Construction and use of pens, however, was unattractive to farmers taking advantage of open access field crop residues in the off-season.

Although the production of different types of animals was generally viewed as favorable, a cultural preference for cattle was common. Nevertheless, cattle are not easily attained since they require a much higher initial investment. A major benefit of cattle is that they are considered more secure (e.g., less prone to diseases than either swine or poultry).

Animal production has gender implications. Cattle raising was more a men’s activity; while poultry and swine at smaller scale (i.e. < 10 pigs and < 50 chickens) were mostly managed by women.

In some cases, off-farm work may prevent the raising of any animals. Feed availability was limited by crop yields and a lack of access to larger areas of land. Some farmers stated that local breeds of swine have low liveweight increases. Others recognized the robustness of such animals and saw liveweight gains because of feed quality. High mortality of young animals was a problem.

Almost all farmers had at least a few monogastrics. Swine and chicken production fulfilled different functions. Poultry provided a somewhat continuous household cash flow and food for home consumption. Small-scale poultry sales were often seasonal. Prices paid for poultry did not suffer price drops but increased seasonally and from year-to-year. Higher returns were realized during the Christmas period, for which some farmers planned accordingly. While demand/prices paid for cattle products rose 10-20%, swine increased20-40% over the last few years, making pig production relatively attractive. Farmers having to sell pigs in the face of personal emergencies possibly received lower prices from buyers.

Farmers employed different strategies for swine production. Most commonly, poor farmers with pigs kept one or two animals fed largely with farm-produced feed. Some farmers sold chickens to help pay for pig feed with pigs sold at 6 months. Others fattened pigs for up to 12 months to obtain higher weights and prices. A very small number of producers fattened up to 100 animals in pens. Other monogastrics, including ducks, geese, guinea fowl, sheep, or goats were raised by a few farmers. Crop outputs and earnings from crop sales supported animal production; animal sales helped to finance crop inputs; and crop residues supported pig production.

Chinandega el Viejo. Notably different in the Chinandega area was that pig production took advantage of crop residues from large-scale peanut production. Peanuts became an important cash crop when a processing plant opened in 2001. Rice is another major crop in the area and may have also inadvertently ended up supporting pig production. Farmers browse their pigs on previously harvested fields, some paying rent and others not. Crop residues are often sold at low prices. Peanut residues comprise the bulk of feed for farmers fattening pigs in the dry season (when there are no crops in the fields). The system based on peanut residues is highly attractive to fortunate locals who obtain high-quality feed at very low cost. One group of less-advantaged farmers was organizing itself to produce their own feed mixes and was interested in feed alternatives.

Conclusions

- Nearly all households raised monogastric animals.
- Many poor smallholders worked off-farm.
- Feed, mostly maize and sorghum, was usually produced on-farm and was supplemented with purchased feed (mixtures of maize and sorghum, maize and rice meals) and concentrates.
- In some cases lack of land and labor limited on-farm feed production.
- High costs prevented the feeding of animals solely concentrates.
- A lack of protein from purchased and farm-produced feeds limited animal productivity.
- An exceptional case of a highly productive crop residue / animal feed system was based on use of underpriced peanut residues.
- Various interactions were common between crop and livestock production (crops and crop incomes supporting animal production and vice-versa) and among animal classes (the use of chicken manure or whey in pig production).
- Farmers maintained animal and crop mixtures as a risk reduction strategy.
- Demand for pigs was high and increasing.
- Price premiums were not paid for higher animal product quality.
- Monogastric production was more risky than cattle production due to diseases.
- With some exceptions, farmer organizational levels were low.
- In some cases, farmers were interested in producing their own feed concentrates and in forage alternatives.
- Further investigation is needed to assess the relation between human and animal health (e.g. trichinosis).

Table 91 provides a summary of monogastric systems encountered and the appropriate research and development strategy for each of the systems. Research and development strategy A addressed the needs of resource-poor smallholder farmers, while strategy B improves the competitiveness of medium-scale farmers.

B) Olancho, Honduras

The diagnostic survey contrasted two focal groups: farmers with monogastrics for home consumption and farmers producing a higher volume for commercialization.

Small scale production. Thirty-eight small farms around Catacamas (La Pita, El Pescador) at about 400 masl were visited. Families rely on livestock, annual crops, off-farm employment and remittances. Although many farmers mentioned land availability as a problem, land rights were generally secure. About 73% of the group had poultry, mostly layers and some broilers. Chickens were kept unpenned around the house and in the gardens. About 43% had chickens for exclusively for subsistence, while 57% also sold some of their poultry products. No farmers exclusively produced eggs for sale. All fed their chickens maize, with 46% producing the maize on-farm. Ten percent purchased concentrates, and 25% purchased rice derivates like rice bran (3%) and broken rice (21%).

Approximately 60% of small-scale producers raised pigs. More than half had both chicken and pigs. Reasons for having pigs and chickens differed with 65% producing pigs for sale, 18% for consumption, and 13% for both purposes. With small-scale production, pigs were sold to passing buyers or were slaughtered at home and sold by section. Passing buyers fixed prices based on estimated live weight. Pigs were fed whey (78%) from milk processing plants. Whether farmers bought (52%) or received whey at no cost (26%) depended on relations with plant owners. Rice bran was purchased by 48%. Use of concentrates was moderate (43%). Concentrates were purchased, with the exception of one farmer who mixed his own. Pigs were also fed kitchen scraps, sweet potato, plantains and sugar cane.

Reasons for not having monogastrics or not having more monogastrics fell into four groups: (a) *social*— the smell of pigs, lack of space, and accidents with cars, (b) *experience*—animal diseases and associated knowledge, and (c) *economic*—lack of financial resources for concentrates and pens , and (d) *market bias*– purchases of chicks for broiler production in small quantities was not possible without futures obligation to buy concentrates.

Commercial production. Medium to large farms around Catacamas and Juticalpa were visited. The Universidad Nacional de Agricultura (UNA) in Catacamas has a donor-supported breeding program that sells breeding pigs and provides limited technical assistance in hygiene, breeding and feeding. Many of the farmers interviewed were in contact with the university and some had adopted recommended technologies: pen construction, concentrate mixes and improved pig breed. A similar program in Comayagua financed by Chinese donors is selling breeding pigs over a larger area.

Commercial pig producers typically had various income sources, and land and financial resources. Pig, poultry, and fish producers with limited access to land specialized in one product. Pig farmers with sufficient land usually had cattle as their main source of income.

Approximately 92% of commercial pig producers used concentrates. Nearly 40% purchased from four major producers and from stores in Catacamas and Juticalpa. Concentrate purchased from producers was delivered, paid for directly, and accompanied by technical assistance that also promoted the product. Concentrate prices were reportedly stable. Although packaging did not in all cases indicate the percentage of proteins, farmers were generally satisfied with quality and quality consistency.

Over half of farmers mixed their own concentrates. In 38% of cases, all raw materials were purchased, while 17% used purchased and farm-produced products like maize, sorghum, soya and semolina. Soy production was said to be impossible because machinery required for seeding and harvesting unavailability. This situation may favor alternative protein sources and smallholder production. An advantage of purchasing raw material was that its processed form of meal. Prices for raw material were not stable, with soya fluctuating 12% and maize 18%. Farmers were aware of the changes but were not able to store raw materials due to financial constraints.

A total of 44% used specific concentrate mixtures matched to growth stages. Concentrate firms offered five different products. Farmers mixing their own concentrates elaborated up to four different mixes. Table 92 compares prices of purchased and farm-mixed concentrates using purchased inputs.

Most farmers sold slaughtered pigs to middlemen. Other market channels were meat-processing plants in Catacamas and Tegucigalpa, the local market, and consumers. Prices paid were US $ 0.63/lb liveweight and US$ 0.79-0.95/ lb for meat. Farmers claimed that a higher quality did not increase price. The local market for pigs is saturated; and many producers ceased production due to market constraints.

Poultry was not as widespread as pig production and was differentiated between layer and broiler production: i.e., farms producing chickens specialized in one product. Broiler producers tended to purchase rather than mix concentrates; while farms with layers tended to mix their concentrate. Of all poultry producers, 70% used different concentrates for specific fattening steps or age periods. Sources of commercial concentrate were the same as for pigs. Prices for concentrates did not vary. Farmers who bought concentrate reported that changes from one brand to another changed production efficiencies. Farmers who mixed their own concentrate reported that sometimes protein content would be insufficient (Table 93).

Supply of chicks differed among farms and depended on the quantity purchased at a time. Large producers are able to order chicks from large firms without conditions. Medium size producers bought chicks from stores in Catacamas with the condition to use specific concentrates. One producer self prepared concentrates from purchased raw materials to avoid this dependency and purchases chicks delivered from El Salvador.

Eggs are marketed in the towns of Juticalpa, Catacamas, and San Francisco de la Paz). Some farmers sell to middlemen, others to small local stores. Eggs sold to the market and to resellers are paid for in cash. Food stores pay with a delay of seven days. Three large national producers regulate egg and broiler prices. A box of 320 eggs is worth US $27. Large broiler producers had more market channels than small ones. The most important channels for eggs were supermarkets, food stores and sales consumers or restaurants. Higher quality did not earn higher prices.

Chicken meat is worth US $0.73-0.89/ lb. A premium was paid for delivery to stores, restaurants and consumers. Some farmers provided delivery, built good relations with

Table 92. Purchased and self-mixed concentrates.

	Purchased ready n = 2		Farm mixed n = 1	
Concentrate	US$/100 lb	% Protein	USD/100 lb	% Protein
Pre starter	28.15			
Starter 1	19.78	22	12.63	22
Starter 2	15.26	15	11.57	18
Development	12.10	16	10.79	14
Final	11.44			
Pregnancy		13	10.26	13

Table 93. Sources of feed for monogastrics, Olancho, 2006

	Pigs		Layers		Broilers		Fish	
	Observations	% of total	Observations	% of total	Observations	% of total	Observations	% of total
Bought concentrate	8	33	2	40	4	80	8	89
Bought concentrate plus feed from farm	1	4		0		0		0
Feed from farm	2	8		0		0		0

costumers, and provided flexible short-term credits (7-20 days).

Prices rose around Christmas due to high demand. Small and medium producers were not able to increase production to take advantage of the high price because the large producers do not sell chicks to them in this period. Large producers said that they produced more broilers for the high price periods.

Fish production depended on availability of permanent water sources and was found on both specialized and diversified farms. All but one farmer purchased commercial concentrate for fish production. The one farmer mixed concentrates using purchased raw material. Different feeds for different fish growing stages are commercially available and were used by 2 out of 9 farmers. People typically did not mix their own fish concentrates because it was not easy to produce a product that floats on water. Only one farmer fed fish with cut-and-carry grass as a supplement to concentrates.

Fingerlings were purchased and transported from places like Campamento about 100 km away or from Comayagua about 300 km away.

Marketing of fish was not seen as problematic; there is not much production in the area, and fried tilapia is a typical dish. Market channels included direct sales to consumers (US $1.47/lb), restaurants (US $1.26/lb) and local markets. Some producers have restaurants where they sell fried fish for US $3.40-4.20. Fish producers respond to high price/demand periods like Easter week or the time around Christmas. They either stop selling fish before the high price period or produce more fish for the period.

Monogastrics producers mentioned the following reasons for not producing concentrates:

- Lack of machinery for mixing
- Less expensive to purchase concentrate (a large scale producer with 560 pigs)
- Obligation to purchase concentrates (medium broiler producers)
- Credit availability with concentrate purchase (laying hens)
- Not possible to produce floating concentrate for fish
- Lack of knowledge of concentrate composition
- Monogastrics are often an activity of secondary priority

Producers who mixed their own concentrate mentioned reasons for not producing raw material:

- High opportunity cost of land
- Lack of interest and knowledge
- Lack of machinery for crop production
- High production cost of maize and sorghum
- Specialization on egg production
- Scarcity of land
- Insufficient time or financial means for diversification

The most frequently mentioned market problems were:

- Not possible to sell large quantities at once (pigs and broilers)
- Quality does not fetch a premium
- Difficult to respond to price fluctuations
- The end consumer prefers to purchase known brands
- Transport problems to reach the national market

Conclusions

- Cost of producing raw materials on-farm should be further analyzed and compared with imported soy prices.
- Lack of knowledge of alternative high value protein sources may limit monogastric production.
- Monopoly power of concentrate companies can be challenged with alternative sources of chicks and feed.
- For all monogastrics products, quality is not awarded a price premium. Marketing initiatives would require consumer awareness.
- Although many medium-scale pig producers do not have land available for raw material production, opportunities exist for the production of forage-based protein feeds for others who already mix their own concentrates and who have sufficient land.
- Locally available materials and local practices need further diagnosis and analysis.
- Value chains of both monogastric feeds and products need further analysis and development.

General conclusions

Farming contexts differ greatly within and amongst countries of Latin America. Monogastric animal production is an integral component of many smallholder farms. Many farm families produce mostly chickens and pigs but other monogastric animals are common per region including duck, guinea pigs, rabbits and fish. Input and output opportunities restrict the earnings capacity of many farmers.

Larger scale monogatric farmers face expensive feed costs. With imported grain prices increasing, domestic production of grain and legumes may become more attractive. In addition, these alternative feed sources can be better adapted to specific production niches.

As ample feed for animals constrains production, markets are rarely formal or fair for smallholder producers. In Honduras, chick purchases require the purchasing of feed in the future. Enhanced farmer organization can help overcome policy and institutional barriers.

Monogastric production is an important strategy to diversify household risk for many smallholder farmers. Occasional and/or informal sales of meat, eggs, and animals provide an additional income source, especially for women. A challenge for research and development is to foster enhanced market access and feed inputs according to local context.

4.7 Promotion of artisanal seed multiplication and scaling of forage technologies in Central America

Highlights

- In Honduras the pilot farmer led seed enterprise EMPRASEFOR (Empresa de Producción artesanal de Semilla Forrajera) was regognized as an enterprise in early January, giving access to formal seed markets.

- In Nicaragua, various farmer group are initiating the production of legume seeds such as *Lablab purpureus*, *Cratylia argentea*, *Canavalia brasiliensis* and *Vigna unguiculata.*

4.7.1 Promotion of farmer led forage seed multiplication enterprises in Honduras

Collaborators: P. Lentes, H. Cruz, M. Posas (SERTEDESO), M. Peters, C. Lascano and C. Burgos (DICTA)

Rationale

The adoption of forage technologies in intimately related to the availability of good quality seed at reasonable prices. Therefore, taking into account the current 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.

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 locations.In 2001, production of loosely organized farmers in Honduras began with 286 kg of seed of *Brachiaria brizantha* cv. Toledo. During 2002 the 13 members now organized in EMPRASEFOR produced a total of 720 kg of Toledo on 10.4 ha, doubling the cultivated area of the year before. In 2003, production volume and area rose to nearly 1.5 tons, produced on 18.5 ha, 8 ha more than in 2002. For 2004, the production target of 1.6 tons was over shot by 300 kg, meanwhile increasing the area by only 2 ha. As the production volume of 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 (Table 94).

The limited local market was the entry point for linking the seed producers to a large company and export market opportunities. Thus, in April 2005, CIAT facilitated the contact of EMPRASEFOR with the Mexican seed producer and distributor PAPALOTLA, a partner of CIAT for several years. Once the core points of the alliance were agreed upon, PAPALOTLA ensured to buy the entire harvest of EMPRASEFOR, with defined minimum quality parameters to be met. To meet Honduran legal requirements EMPRASEFOR registered as certified seed producer with SENSASA, the Government body for Agricultural Health and is formally recognized as an enterprise since January 2006.

The group extended the area of cultivation to a total of 37.4 ha, 71 % more than in 2004. PAPALOTLA provided a credit and organized the delivery of fertilizers and agrochemicals for production through their local reseller.

Table 94. Development of seed production, EMPRASEFOR.

Year	area (ha)	Production (kg)	Production / ha	Increased production area (%)
2001	5.25	286	54.5	
2002	10.4	720	68.8	99.3
2003	18.5	1465	79.0	77.3
2004	21.8	1915	87.5	17.9
2005	37.4	954	25.4	71.2
2006	7	345	49.3	

Farmers prepared themselves to meet a production target of 4 tons of seed and during flowering, the Toledo plots were in perfect condition. Between October and December 2005, several hurricanes hit Central America. Due to the unfavorable weather conditions, the Toledo seed harvest of EMPRASEFOR was much below the expectations in quantity and quality, a severe backstroke for the recently founded enterprise. Total production was about 954 kg and thus only 23.85 % of what was expected. Since volume and quality were not sufficient for the export by PAPALOTLA, the partners agreed to continue selling the seed locally at a price of 10 $ per kg. Farmers have gained the experience that high volume production, is necessary to compete on the international seed market. However, the secure market offered by the alliance with the PAPALOTLA was paid for with a lower sale price per kg (6.50 $US) of seed.

For small farmers, like the members EMPRASEFOR, the investment and the associated individual risk of an expansion of seed production were comparatively high and met adverse environmental conditions. As a consequence of the negative experience in 2005 the area of seed production shrank to 7 ha in the following year. Farmers changed their priorities of land use, by using the established plots of Toledo as pasture and for the production of conserved forage. Farmers were refocused for the 2006 season and, despite the losses incurred during 2005 honored the credit provided by PAPALOTLA. As long as the local market for seed is good enough to sell for around 10 $US per kg, farmers meanwhile opted for a lower individual risk of production. Under the present context it appears to be more secure for them to use the area under Toledo liberated through the decline of seed production for milk and beef production, capitalizing on higher productivity of the improved pastures.

Another effort of Toledo seed production has been undertaken by CIAT´s national partner DICTA. Activities were related to training of individual farmers in Olancho, who just started to produce seed. In 2006, 6 farmers and 2 DICTA field technicians were trained in seed production. On nine farms in Olancho approximately 700 kg of seed were harvested. The area under seed production was 15.75 ha. This seed was produced for individual use of the producers and for local sales. Similar efforts are underway with the production of *Vigna unguiculata* (cowpea) seed.

The support of farmer led seed supply system producing forages in the process of adoption is supported by production of basic seed of pipeline materials for further experimentation, testing and technology adaptation, so that this seed is available in the country in time for semi-commercial evaluation and scaling.

4.7.2 Promotion of farmer led forage seed multiplication enterprises in Nicaragua

Contributors: A. Schmidt, M. Mena (INTA), R. van der Hoek, C. Davies and M. Peters (CIAT)

In Nicaragua farmer led seed production commenced later than in Honduras and hence the process of enterprise formation is still on going. However in San Dinisio in the Matagalpa department three farmers produced resp. 38, 38 and 9 kg seed of *Cratylia argentea* while seven farmers are producing seed of *Canavalia brasiliensis*. One of the farmers has extended his area to 0.3 ha and is likely to produce more than 200 kg this year. The other farmers all planted 0.15 ha and the total estimated production for this year is 700-800 kg (Table 95).

Table 95. Seed production of *Canavalia brasiliensis.*

Community	Farmer	Area (ha)	Projected production (kg)	Observations
Susuli Central	Matilde Zamora	0.15	130	
El Chile	Agustín Escoto	0.15	25	Drought
Susuli	Juan Hernandez	0.3	200	
San Ramón	Clark Davies	0.15	140	
San Cayetano	Yuri Lopez	0.15	0	Drought
El Zarzal	Salome Zeledon	0.15	120	
Los Limones	Migdonio Campos	0.15	140	
Total		1.2	755	

In 2006 in Las Segovias, three farmers produced a total of 650 kg seed of *Vigna unguiculata*. This was an important increase compared to 2005, during which 135 kg of *Vigna unguiculata* and 45 kg of *Lablab purpureus* was produced. Apart from this, one farmer is expected to produce a considerable quantity (50-100 kg) of seed of *Cratylia argentea*.

As a result of the collaboration between CIAT (providing seed and technical support) and the national agricultural research instute in Nicaragua (INTA), the latter institution produced 400 kg seed of Cratylia argentea in a plot of 0.4 ha in the valley of Sébaco, Matagalpa department. In the South Pacific zone a similar plot was established, and some individual farmers also started to produce Cratylia seed.

Cratylia seed was released for use by farmers in Nicaragua (registered as INTA Cratylia). INTA plans to enhance seed production by establishing 5 plots of 2 ha each in its five intervention zones (Las Segovias, Centro-Norte, Centro-Sur, Pacifico-Norte, Pacifico-Sur).

4.8 Forage seeds: Multiplication and delivery of experimental and basic seed

Highlights

- The Seed Unit at Atenas Costa Rica continued to produce, procure and deliver under request experimental and basic seed of promising forage germplasm. This year 505 kg of seed were delivered in response to 52 requests from 11 countries; the bulk of the seed was formed by *C. argentea* (124.3 kg) and *Brachiaria* spp. (314.1 kg).

- A total of over 800 kg of seed was produced in the Seed Unit of Palmira during 2006. This total included seed of 44 different accessions representing 17 grass and legume species. Six hundred seventy kg of seed were dispatched. This included 329 seed samples of 16 genera. Seed was shipped to 13 different countries.

4.8.1 Multiplication and delivery of selected grasses and legumes in the Seed Unit of Atenas

Contributors: Guillermo Pérez and Pedro J. Argel (CIAT)

Seed multiplication activities of promising forage germplasm continued during 2006 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 2005 through August 2006 a total of 245 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* (95 kg), *Brachiaria* spp. (11kg), *Brachiaria* hybrids cv. Mulato and cv. Mulato II (12 kg), *Arachis pintoi* (46 kg), *Leucaena* spp. (5 kg), *Stylosanthes guianensis* AFT 3308 (26.7 kg), *Vigna* spp. (21.2 kg), *Panicum maximum* (6 kg), *Paspalum* spp. (5 kg) and 18 kg of other forage species.

During the period August 2005-November 2006 a total of 504.9 kg of experimental and basic seed was delivered by the Seed Unit of Atenas (Costa Rica).

Table 96 shows that 52 seed requests were received from 11 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 (95.5 kg) and Panama (82.5 kg), followed by Nicaragua.

A total of 314 kg of basic seed of promising *Brachiaria* specie, particularly of cvv. Mulato and Mulato II, were also delivered this year.

Future of the Atenas Seed Unit

In December 2006 the CIAT´s Tropical Forage Project ended activities in Costa Rica and the office that supported the activities for the last 19 years was closed. However, given the importance of the Seed Unit in producing and delivering experimental and basic seed of promising forage germplasm for the region an agreement that is its final stages of negotiation allows national institutions of Costa Rica to continue the activities of the Seed Unit for the coming years.

The main supporters of the seed unit in the agreement are Corporación de Fomento Ganadero (CORFOGA), that will cover salaries and related expenses of one technician and two field labor; the Escuela Centro Americana de Ganadería (ECAG) and Cámara de Ganaderos del Sur (CEGUS) will cover office and field expenses.

On the other hand, CIAT will continue to supply promising forage germplasm to the seed unit based on demand. An international expert on forage seed will be contracted by CORFOGA to supervise the technical aspects of the seed unit.

It was agreed by the representatives of the institutions that will run the seed unit that the

Table 96. Countries, number of requests and amount of experimental/basic forage seed delivered by the Seed Unit of Atenas (Costa Rica) during the period August 2005-November 2006.

Country	No. of Requests	Forage species (kg)				Total
		Brachiaria spp.	*A. pintoi*	*C. argentea*	Other species	
Costa Rica	30	172.80	0.50	63.50	14.40	251.20
Dominican Republic	1				4.00	4.00
Guatemala	2	52.00		29.00	14.50	95.50
México	1		0.25	0.25	0.54	1.03
Honduras	2			2.00		2.00
Alemania	1	0.10	0.10	0.10	1.00	1.30
Nicaragua	5	31.00	4.00	15.00	8.00	58.00
Uruguay	1	5.00				5.00
Venezuela	3	1.20	0.30	0.90	1.00	3.40
Antillas	1			1.00		1.00
Panama	5	52.00	8.00	12.50	10.00	82.50
Total	**52**	**314.10**	**13.15**	**124.25**	**53.44**	**504.93**

Table 97. Activities programmed for the Seed Unit of Atenas with promising forage germplasm likely to become new forage cultivars during the next 6 years.

Year/Species	2007	2008	2009	2010	2011	2012
Panicum maximum CIAT 16051	Seed multiplication	Seed multiplication On-farm validation	Seed multiplication On-farm validation	Basic seed production	Cultivar release	
Panicum maximum CIAT 6799	Seed multiplication	Seed multiplication On-farm validation	Seed multiplication On-farm validation	Basic seed production	Cultivar release	
Brachiaria brizantha CIAT 26124	Seed multiplication	Seed multiplication On-farm validation	Seed multiplication On-farm validation	Basic seed production	Cultivar release	
Vigna radiata	Seed multiplication On-farm validation	Seed multiplication On-farm validation	Basic seed production	Cultivar release		
Cratylia argentea CIAT 22386	Seed multiplication	Seed multiplication	Seed multiplication On-farm validation	Seed multiplication On-farm validation	Basic seed production	Cultivar release
Cratylia argentea (Yacapani)	Seed multiplication	Seed multiplication	Seed multiplication On-farm validation	Seed multiplication On-farm validation	Basic seed production	Cultivar release
Stylosanthes guianensis (Multilínea)	Seed multiplication On-farm validation	Seed multiplication On-farm validation	Basic seed production	Cultivar release		

immediate activities on seed multiplication would concentrate on forge species listed in Table 97. The expectations are to multiply seed of selected accessions for on-farm demonstrations and eventual cultivar release. These forage germplasm are under advanced stage of evaluation and have seed available is a key for supporting research and promoting them at the farm level.

4.8.2 Multiplication and delivery of selected grasses and legumes in the Seed Unit of Palmira

Contributors: J.W. Miles (CIAT) A. Betancourt (CIAT) E. Pizarro (PAPALOTLA)

Diffusion of new forage genetic technology is generally through the vehicle of seeds. For novel plants, no commercial seed supply exists. In order to meet experimental (including participatory research) needs, CIAT maintains a small seed multiplication capacity at headquarters in Palmira. Seeds are multiplied in field plots established at CIAT-Popayán, CIAT-Quilichao, and CIAT-Palmira. A total of over 800 kg of seed was produced during 2006 (Table 98).

Table 98. CIAT Forage Seed Unit. Seed produced during 2006.

Species	Number of accessions	Weight (kg)
Brachiaria brizantha	7	33.50
Brachiaria humidicola	2	0.35
Brachiaria lachnantha	1	0.40
Brachiaria ruzizienzis	1	4.50
Brachiaria sp.	1	10.00
Cajanus cajan	3	66.00
Cannavalia brasiliensis	1	32.00
Centrosema macrocarpon	1	10.00
Centrosema molle	1	19.00
Cratylia argentea	2	322.70
Desmodium heterocarpon	2	16.80
Desmodium velutinum	7	23.36
Flemingia macrophylla	1	1.00
Lablab purpureus	4	62.50
leucaena diversifolia	1	0.40
Leucaena leucocephala	1	40.00
Vigna unguiculata	8	171.00
Total	44	813.51

This total included seed of 44 different accessions representing 17 grass and legume species. Six hundred seventy kg of seed were distributed. This included 329 seed samples of 16 genera (Table 99). Seed was shipped to 13 different countries (Table 100).

Table 99. CIAT Forage Seed Unit. Dispatches 2006, by genus.

Genus	Number of samples	Weight (kg)
Arachis	11	1.12
Brachiaria	91	67.46
Cajanus	3	0.30
Calliandra	4	0.08
Cannavalia	19	7.06
Centrosema	11	5.33
Clitoria	4	5.10
Cratylia	94	310.52
Desmodium	12	3.65
Flemingia	6	0.51
Lablab	12	5.86
leucaena	17	24.92
Mucuna	3	1.12
Pueraria	3	0.19
Stylosanthes	6	11.48
Vigna	33	225.38
Total	329	670.07

Table 100. CIAT Forage Seed Unit. Dispatches 2006, by recipient country.

Country	Number of samples	Weight (kg)
Colombia	247	628.82
Costa Rica	1	3.00
Germany	3	0.45
Guatemala	1	10.00
Honduras	8	6.35
Japan	21	3.37
Malawi	8	1.06
Nicaragua	21	7.66
Ruanda	8	1.06
Switzerland	1	0.50
Vietnam	6	0.60
Zimbabwe	3	7.00
Mauritius	1	0.20
Total	**329**	**670.07**

4.9 Tools to target forages

Highlights

- SoFT has been well accepted by the international community as a useful tool compiling information on tropical forages.

- More than 1000 CD copies of SoFT have been distributed and almost 100.000 visits have been recorded to the web site mostly from Research and Development institutions working in tropical and subtropical and environments. Another not foreseen group of users include educational institutions not only in tropical countries and the private seed sector.

4.9.1 Expert systems for targeting forages and extension materials for promoting adoption of 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

The product itself has been described in previous annual reports. Here we will report on dissemination of the product and future needs.

In 2006 we recorded almost 100.000 visits to the www.tropicalforages.info web site and more than 1000 CD copies of the tool have been distributed. There continues to be a large number of visits from Australia, while we have an increase in visits from the CG, commercial institutions and networks. We also experience more

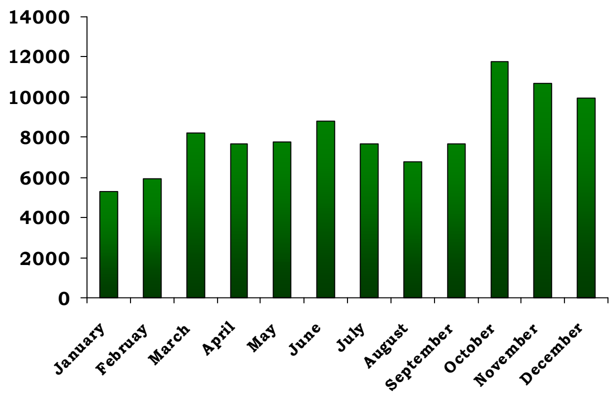

Figure 57. Number of visits to the forage web page in 2006: 98157

frequentation from LAC countries in particular Brazil, Mexico, Colombia, Argentina and European countries, the latter likely related to teaching. A number of Asian countries are regularly accessing the tool while use of the web site from African countries is comparatively low (though still recording a few thousand visits) (Figure 57).

Outlook

There is a need to include more recent information in the facts sheets and in some cases add new species. It is also necessary to translate the tool into other languages (i.e. Spanish and French, various Asian languages) to encourage its use and application in Latin America and the Caribbean, francophone Africa and Asia, respectively.

It is anticipated that the number of visits from Sub-Saharan Africa will increase over time as access to Internet will improve. Meanwhile it remains necessary to produce CD versions of the tool in particular in locations with limited internet access.

4.10 Facilitate Communication through journals, workshops and the Internet

Highlights

- There has been a sharp increase in the use of the forage web page with close to 340,000 downloads in 2006.
- Pasturas Tropicales is increasingly accessed on line with about 130.000 downloads in 2006.
- A CIAT-ETH-CORPOICA-U. Nacional collaborative workshop on forage potential of tanniniferous legumes was held in Bogotá, with funding by ZIL-SDC.

4.10.1 Diffusion of research results: Pasturas Tropicales

Contributors: C. Lascano (CIAT) and A. Ramirez (Independent Publisher)

After 22 years (1985-2007) the Journal Pasturas Tropicales will no longer be published due to financial limitations of the Forage Project in CIAT. The last number will come out in March 2007. During its existence a total of 85 numbers were published distributed in 28 volumes which contained 610 scientific articles and research notes on tropical forages. Most of the papers published in Pasturas Tropicales were from researchers working in R&D institutions in LAC.

At the beginning Pasturas Tropicales was the vehicle for publishing results obtained by researchers from CIAT and by researchers from different institution participating in the CIAT- led international forage network (Red Internacional de Evaluación de Pastos Tropicales —RIEPT). However, it later evolved into a journal in great demand by researchers from many institutions in LAC to publish their work and by a wide range of subscribers. The Journal was particularly popular in University Libraries.

One of the most important contributions of Pasturas Tropicales was to stimulate forage researchers from different institutions in LAC to publish their work. The fact that the Journal had a process for reviewing papers submitted assured contributors that only relevant, high quality and original work would be published with an international distribution.

Finally, as members of the Editorial Board of Pasturas Tropicales we want to express our appreciation to all those institutions and researchers that in one way or another contributed to the success of Pasturas Tropicales as a specialized publication on tropical forages.

4.10.2 Training courses on utilization of improved forages in Central America

Contributor: P. J. Argel (CIAT)

As part of the ILRI/CFC project this year we carried out a number of training courses on utilization and management of improved forages, which were directed to mainly farmers.

In Costa Rica a group of 25 farmers from Panama members of the livestock association called ANAGAN attended a training course between October 31st and November 4. The training course included a field trip to visit a cattle auction in San Isidro and improved pastures in one of the projects small cattle farms that has a fattening system based on *B. brizantha* cv. Marandú supplemented with chicken manure and minerals. In each cycle the farmer fattens 10 steers with a mean animal live weight gain of 600 to 800 g per day.

The trip continued with visits to a milk processing plant called Dos Pinos and the slaughtering plant called Montecillos, and finished at the facilities of the Escuela Centramericana de Ganadería (ECAG), a livestock training school, were CIAT's Tropical Forage Project have experimental plots for evaluation and seed multiplication of selected forage germplasm. It is worth to mention that Panama is not part of the ILRI/CFC Project, but farmer associations in that country have shown considerable interest in the advances and on the results of the Project.

As part of the diffusion and training activities we organized the 1st of December a workshop directed to farmers and technicians of Santa Cruz cattle association. Twenty five participants assisted and the topics dealt with cattle phytosanitary aspects and pasture management practices. The group responded positively and showed high interest in the presentations. Technical documents were handed out to the group.

4.10.3 Workshop on Tannins in Ruminant Nutrition

Contributors: C. Lascano (CIAT) and D. Hess (ALP Posieux)

A workshop was carried out as part of a collaborative CIAT- ETH-CORPOICA-U. Nacional- Bogotá Project entitled "The forage potential of tanniniferous legumes: Search for sustainable ways to cope with nutritional limitations in smallholder systems" funded by ZIL- SDC. A total of 16 papers were presented in the two day workshop held in December 2006 in the National University in Bogotá with the participation of 63 persons.

The overall aim of the collaborative project was to develop efficient feeding systems based on tanniniferous shrub legumes in order to improve livestock productivity in smallholder systems in the tropics. To accomplish this objective a number in vitro and in vivo experiments were carried out by mostly graduate students from the UNAL- Palmira and UNAL- Bogotá to test the utility of mixtures of legumes with and without tannins as protein supplements to low quality grasses.

One of the most important conclusion that came out of the presentation and discussions of results during the workshop was that the feed value of high yielding shrub legumes with tannins could be significantly improved by mixing them with small quantities of high quality legumes with no tannins. Additional results showed that reduction in methane observed when feeding legumes with tannins was related to their low fiber quality rather than to a direct effect of tannins on methanogenic bacteria.

4.10.4 Dissemination and facilitation of communication through the forage web site

Contributors: Simone Staiger, M. Peters, C. Lascano and B.Hincapié (CIAT)

The web site of CIAT's Tropical Forages Project, is the result of teamwork between all Project members, under the general web site coordination of the Communications Unit and with the support of both the Systems and the Information and Documentation units. In view of the target users the web site is available both in English and Spanish. In 2007 the web site was redesigned to comply with CIAT new standard aiming to be more functional for the variety of users including universities, research institutes, collaborators, donors, and the scientific community in general (Photo 12).

Figure 58 shows the number of visits to the web site in the period from March 2006 to February 2007. 9242 and 21617 visits were recorded for the English and Spanish version, respectively, a substantial increase to the previous year.

Downloads: A total of 338927 documents have been downloaded form the forage web site in the period between March 2006 and February 2007 (Table 101). Of particular interest to users were downloads from the journal Pasturas Tropicales, with in average more than 10000 downloads per month (Figure 59).

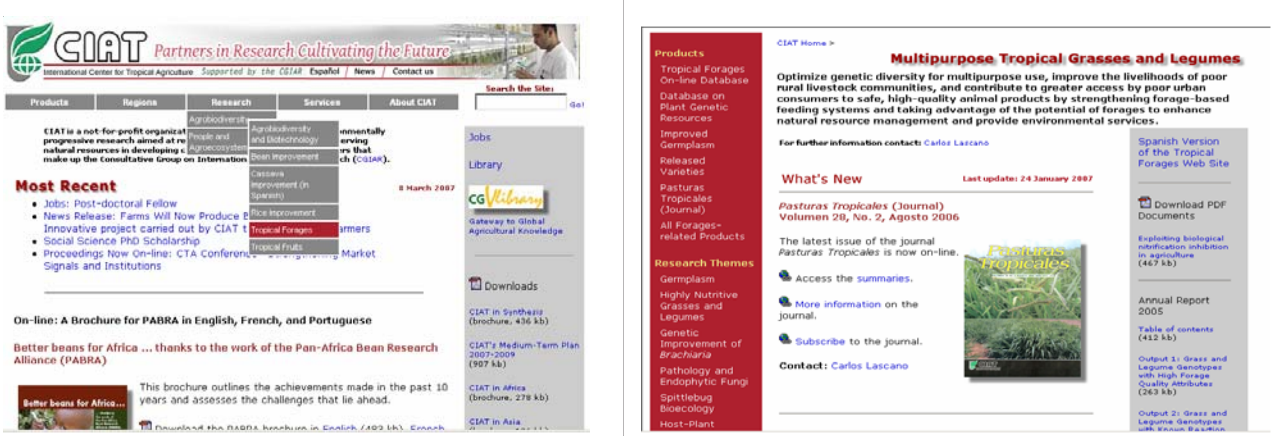

Photo 12. The site, accessible under the URL http://www.ciat.cgiar.org/forrajes/index.htm

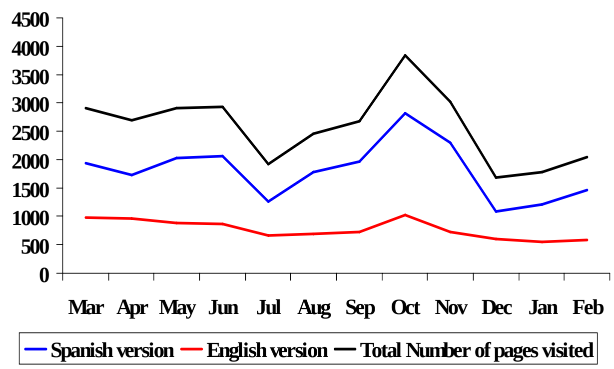

Figure 58. Number of visits to the forage web page

Table 101. Downloads from the forage web site in 2006

Publication	Downloads
Pasturas Tropicales (Indices and summary)	129840
Annual Report 2005	34852
Brachiaria brizantha cv. Toledo (Pasto Toledo)	26292
Cratylia argentea cv. Veranera	18906
Evaluación Pasturas	15359
Producción Artesanal de Semillas de Pasto Toledo	13263
Desmodium heterocarpon cv. Maquenque	10421
Producción Artesanal de Semillas de *Cratylia argentea*	4467
Brachiaria hibrido cv. Mulato	1033
Others	84494
Total	338927

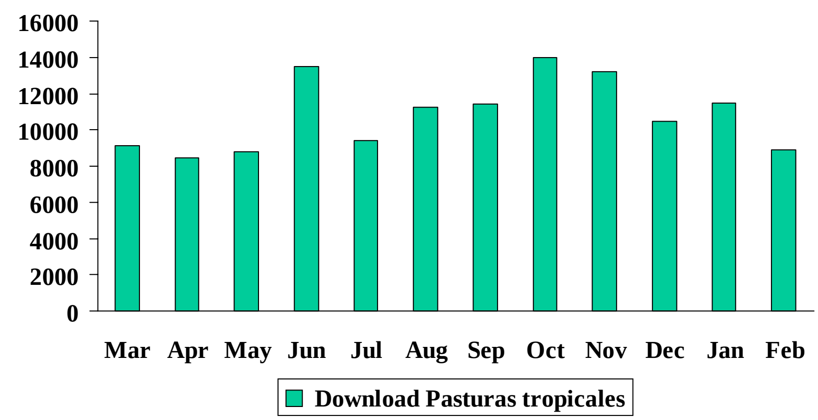

Figure 59. Downloads in the period March 2006 and February 2007 for the journal of Pasturas Tropicales.

Annex

List of publications 2006

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Andersson, M. S.; Peters, M.; Schultze-Kraft, R., Gallego, G.; Duque, M. C. 2006. Molecular characterization of a collection of the tropical multipurpose shrub legume *Flemingia macrophylla*. Agroforest Systems 68(3): 231-245

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Wenzl, P.; Chaves, A.; Buitrago, M.; Patino, G.; Miles, J.; Rao, I. 2006. Development and validation of a hydroponic screening method to identify acid soil adapted genotypes of the tropical forage grass Brachiaria. Paper presented at the 18th World Congress of Soil Science, Philadelphia, USA. July 9-15, 2006 (Oral and poster paper).

Zöfel, K.; Schultze-Kraft, R.; Peters, M. 2006. Field Characterisation of a Collection of the Forage Tree Legumes *Leucaena Diversifolia* and *L. tyrichandra* – an Ongoing Project in Colombia. Tropentag 2006, Prosperity and Poverty in a Globalized World – Challenges for Agricultural Research October 11 - 13, 2006, University of Bonn, Bonn, Germany, p. 351.

Awards to staff in the Project

Segenet Kelemu. **Friendship Award 2006** “Most outstanding award for contribution to the economic and social development of the people’s Republic of China by the State Administration of Foreign Experts Affairs, authorized by the State Council of the People's Republic of China. Beijing, China, September 27, 2006.

Capacity Building (Training)

Internship

Name	Status	University	Title
Schoonhoven, Diane	Completed	Wageningen University, The Netherlands	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
Carabalí Jenny	On-going	Universidad Nacional, Palmira, Colombia	Efectos de la suplementación con *Cannavalia brasiliensis* en la utilización por ovinos de un heno de gramínea de baja calidad
Cuéllar Ortega Diana	On-going	Universidad del Cauca, Colombia	Evaluación agromorfológica de la colección mundial de *Desmodium velutinum*
Hoyos Valerio	On-going	Universidad de Caldas, Colombia	Physiological screening of *Brachiaria* spp. genotypes for their tolerance to drought stress and aluminum toxicity in greenhouse conditions
Vallejo Camila	On-going	Universidad de Caldas, Colombia	Efecto de tiempo de cosecha y secado en la calidad, presentación y ocurrencia de pérdidas del heno de tres accesiones de caupí (*Vigna unguiculata*)
Zúñiga Edier	On-going	Universidad Nacional, Palmira, Colombia	Correlation between nymph survival and tolerance to adult feeding damage as components of resistance to spittlebug in *Brachiaria* spp.

MS Thesis

Name	Status	University	Title
Atzmanstorfer Karl	On-going	University of Salzburg, Austria	GIS-Based Analyses of Cowpea Adaptation to Colombian Hillside Environments
Castro Ulises	Completed	Colegio de Postgraduados de Chapingo, Chapingo, Mexico	Mechanisms of resistance to *Aeneolamia albofasciata* and *Prosapia simulans* en *Brachiaria* spp.

Name	Status	University	Title
Bernal Laila	On-going	Universidad Nacional, Colombia	Efecto de taninos en las leguminosas *Vigna unguiculata* y *Calliandra callotyrsus* evaluados a nivel *in vitro* e *in vivo* en bovinos de leche
Bystricky Maria	2006	University of Hohenheim	Floral Biology of *Cratylia Argentea*
López Machado 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
Pabón Alejandro	Completed	Universidad de Viçosa, Brazil	Mechanisms of resistance to *Deois incompleta* and *Notozulia entreriana* en *Brachiaria* spp.
Ricaurte José Jaumer	On-going	Universidad Nacional de Colombia, Palmira, Colombia	Impact of aluminium tolerant *Brachiaria* genotypes on soil quality characteristics of an oxisol of the altillanura of the Meta Department of Colombia
Sanabria Adriana	On-going	Universidad Nacional, Bogotá, Colombia	Genetic diversity among casual agents of anthracnosis in tropical fruits

PhD Thesis

Name	Status	University	Title
Sanchez Elsa	On-going	University of Hohenheim, Germany	From subsistence to market oriented livestock smallholder development in Nicaragua and Honduras
Castañeda Nelson	Completed	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	Completed	Universidad Nacional de Colombia, Bogotá, Colombia	Integration of maize with forages to recuperate degraded pastures in the Llanos of Colombia
Tiemann Tassilo	Completed	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 systems

List of Donors

Asian Development Bank (ADB)
Capacity Building for Smallholder Livestock Systems (Lao PDR)
2006-2008

Australian Center for International Agricultural Research (ACIAR)
Forage legumes for supplementing village pigs in Lao PDR
2006-2008

Common Fund for Commodities (CFC)
Enhancing beef productivity, quality,
safety, and trade in Central America
(Guatemala, Nicaragua, Honduras)
2003-2006

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

Semillas Papalotla, S.A. de C.V. 2001-2010
Brachiaria Improvement Program

Switzerland – SDC- ZIL
- Adaptation of Brachiaria grasses to low P soils
2003-2006
- The forage potential of tannaniferous
legumes: The search for sustainable
ways to cope with nutritional limitations
on smallholder systems (with ETHZ)
2004-2006

BMZ-GTZ
"Fighting drought and aluminum toxicity"
(Euros 1, 100, 000 for 3 years) (PI)
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