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

3.1 Genotypes of *Brachiaria*, other grasses and *Arachis* with adaptation to edaphic factors

Highlights

- Tolerance to low phosphorus (P) in the *Brachiaria* hybrid cv. Mulato involves two major strategies: (1) increasing the ability to use P efficiently by inducing phosphohydrolases (APase and RNase) in shoots with P deficiency; and (2) enhancing sugar catabolism and subsequent synthesis of amino acids and organic acids in leaves under P deficiency.
- Phenotypic characterization of *Brachiaria decumbens* x *Brachiaria ruziziensis* population showed that genotypes more severely affected by Al toxicity have smaller root systems (shorter root length) that are made up of thicker roots (greater root diameter) and are more heavily branched (more root tips per unit dry weight).
- Two hybrids (BR02NO1372 and BR02NO1621) were identified with greater level of Al resistance than other hybrids generated in the *Brachiaria* breeding program.
- Showed that the *Brachiaria* hybrid, FM9503-S046-024 performed well into the fourth year after establishment in the Llanos and its superior performance at 40 months after establishment was associated with its ability to acquire greater amounts of nutrients, particularly Ca and Mg from low fertility soil.
- Accesions of *Arachis pintoi* CIAT 18744 and 22172 were superior to the commercial cultivar, CIAT 17434 in acquiring phosphorus (P) from from less available P-pools in a low-P oxisol, under growth chamber conditions. The superior performance of CIAT 18744 and 22172 was related to greater P-acquisition efficiency rather than greater P-use efficiency
- Three accessions of *Arachis pintoi* (CIAT 18744, 18751 and 22159) were superior under field conditions than the commercial cultivar (CIAT 17434) in terms of persistence with low fertilizer application
- High farmer preferences for commercial *Brachiaria brizantha* cvv. Toledo, Marandú and La Libertad
- Recorded high and similar DM yields for both *Brachiaria* hybrid FM 9503-S046-024 and *Brachiaria* hybrid cv. Mulato

3.1.1 Edaphic adaptation of *Brachiaria*

Contributors: I. M. Rao, P. Wenzl, J. W. Miles, J. Tohme, M. Ishitani, J. Ricaurte, R. García, A. L. Chaves, M. E. Recio, M.E. Buitrago, A. Arango, D. F. Cortes, G. Gallego, E. Gaitán, and C. Plazas (CIAT), M. Nanamori, T. Shinano, T. Yamamura, M. Osaki (Hokkaido University, Japan)

Studies on mechanisms of adaptation of *Brachiaria* species to acid soil stress factors indicated that Brachiaria decumbens cv. Basilisk is highly resistant to toxic levels of Al and low supply of P. Based on this knowledge, rapid and reliable screening procedure was developed to improve the efficiency of the on-going *Brachiaria* breeding program. The use of improved screening methods and identification of QTLs and candidate genes responsible for Al resistance and adaptation to low P supply will contribute toward development of superior genotypes that combine several desirable traits to improve pasture productivity on acid, infertile soils and to combat pasture degradation.

3.1.1.1 Physiological and genetic aspects of aluminum resistance in *Brachiaria*

As part of the restricted core project funded by BMZ-GTZ of Germany, we continued our efforts to investigate physiological and genetic aspects of aluminum resistance in *Brachiaria*.

3.1.1.1.1 Surface charge density of root apices as a potential factor contributing to aluminum resistance of *Brachiaria decumbens*

Contributors: A.L. Chaves, M.E. Recio, P. Wenzl, J. Tohme and I.M. Rao

Rationale

There is a pronounced difference in aluminum (Al) resistance between *B. decumbens* and *B. ruziziensis* (Wenzl et al., 2001). Previous results demonstrated that this difference is not restricted to Al^{3+} ions but is also observed for trivalent lanthanide ions (see IP-5 Annual Report, 2002). We hypothesized that a less negative (or even positive) surface charge of root apices of *B. decumbens* compared to those of *B. ruziziensis* could be the underlying physiological mechanism.

Materials and Methods

Seeds of *B. decumbens* and *B. ruziziensis* were germinated in 200 µM $CaCl_2$ (pH 4.2) for 4 – 5 days. Homogeneous seedlings, with root lengths between 2 and 3 cm, were transferred to continuously aerated solutions containing 200 µM $CaCl_2$ (pH 4.2) and various concentrations of different cationic or anionic toxicants. The seedlings were left to grow in the glasshouse for 3 days. At harvest, root lengths were measured and root elongation was calculated by subtracting the root length at transfer. Relative root elongation (RRE) values were computed by referencing root elongation values against root elongation in the toxicant-free reference treatment.

Results and Discussion

Figure 28 displays RRE values of the two species exposed to various concentrations of a variety of cationic and anionic toxicants. Data points below the diagonal indicate that *B. decumbens* is more resistant than *B. ruziziensis*; data points above the diagonal indicate greater susceptibility of *B. decumbens* compared to *B. ruziziensis*. The left panel clearly shows that *B. decumbens* is more resistant to all the trivalent cations tested. The latter would be consistent with root apices of *B. decumbens* having fewer negative surface charges than those of *B. ruziziensis*. This is because a lower negative surface charge density would entail lower concentrations of cationic toxicants in the vicinity of root surfaces.

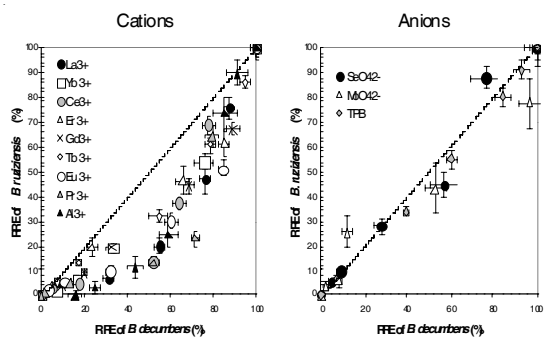

Figure 28. Comparison of relative root elongation (RRE) of *B. decumbens* and *B. ruziziensis* exposed to cationic (left panel) or anionic toxicants (right panel). Cationic toxicants included lanthanum (La^{3+}), ytterbium (Yb^{3+}), cerium (Ce^{3+}), erbium (Er^{3+}), gadolinium (Gd^{3+}), terbium (Tb^{3+}), europium (Eu^{3+}), praseodymium (Pr^{3+}), and aluminum (Al^{3+}). Anionic toxicants included selenate (SeO_4^{2-}), molybdate (MoO_4^{2-}) and tetraphenylborate (TPB). All values are means ± SE of 36 seedlings measured in three independent experiments.

A lower negative surface charge density, however, should also make *B. decumbens* more sensitive to anionic toxicants than *B. ruziziensis*. The right panel of Figure 1, however, does not appear to confirm this prediction because the

data points do not cluster above the diagonal. Results from a greater variety of anionic toxicants are required; yet these data do not appear to be consistent with the idea that Al resistance of *B. decumbens* is due to reduced electrostatic attraction of Al ions to the surface of root apices. Instead they appear to point to a mechanism that may be based on binding to cellular ligand(s). Work is in progress to further characterize this generic cation-resistance mechanism.

3.1.1.1.2 Physiological components of acid soil adaptation in a population of *Brachiaria ruziziensis* × *Brachiaria decumbens* hybrids

Contributors: M.E. Buitrago, M.E. Recio, A.L. Chaves, P. Wenzl, J. Tohme, J.W. Miles and I.M. Rao

Rationale

Last year we confirmed that a *B. ruziziensis* × *B. decumbens* hybrid population segregates for two physiological components that are important for growth in infertile acid soil: root growth vigor and Al resistance. We therefore continued to evaluate most of the 274 hybrids. The cumulative data from ten harvests are now ready to be combined with molecular marker data to identify QTLs underlying these traits.

Materials and Methods

Stem cuttings of hybrids and parents were rooted in a low ionic strength nutrient solution in the glasshouse during 9 days. Equal numbers of stem cuttings 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 $AlCl_3$ pH 4.2 (Al treatment). The solutions were changed every second day to minimize pH drifts. At harvest on day 21 after transfer, the dry weight of stems was measured. Roots were stained and scanned on a flatbed scanner. Image analysis software (WinRHIZO) was used to determine root length, average root diameter and number of root apices. The root growth data from ten harvests were log-transformed because such growth data tend to be log-normally distributed (Causton and Venus, 1981). They were then adjusted for harvest mean (based on the differences between harvest means and overall mean) and the dry weight of the stem cuttings (using linear regression).

Results and Discussion

The B. ruziziensis × *B. decumbens* hybrids showed a broad range of root growth vigor. A considerable number of individuals were superior to well-adapted *B. decumbens*, perhaps as a result of heterosis induced by the interspecific cross (see large number of data points to the right of *B. decumbens* in Figure 29). By contrast there were only few individuals whose roots elongated as poorly as those of *B. ruziziensis*.

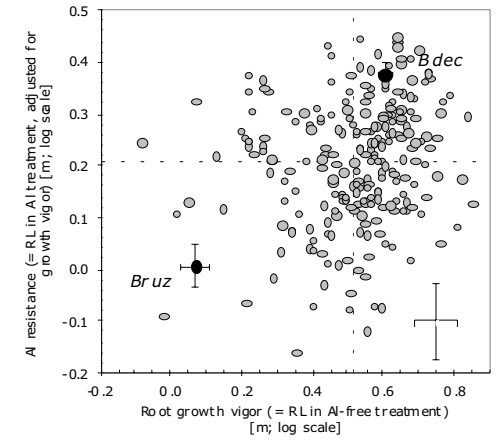

Figure 29. Segregation of root growth vigor (x axis) and Al resistance (y axis), both measured based on root elongation (RL = root length) in a *B. ruziziensis* x *B. decumbens* hybrid population. Al resistance was quantified by adjusting RL of plants in the Al treatment for their RL when grown without Al (see text for further details). Data points representing parents are highlighted with large black symbols. Error bars in the lower right corner denote the average SE of the hybrid population.

Because root growth vigor varies among hybrids, the inhibitory effect of Al on root length (RL) is not easy to measure, and RL in the Al treatment has to be properly referenced. We tested two approaches. First, we calculated relative root length values by comparing – for each pair of stem cuttings distributed among the two treatments – RL in the Al treatment vs. RL in the treatment without Al (the logarithm of the ratio was used for this purpose). The resulting "Al resistance index" was not correlated with root growth vigor. We therefore also tested adjusting RL values in the Al treatment for RL values in the Al-free treatment using linear regression.

The two approaches were compared using the degree of correlation among three parameters describing different effects of Al toxicity as a criterion. These were: (i) inhibition of root elongation (see above), (ii) lateral swelling of roots (resulting in a greater root diameter, RD), and production of a greater number or short laterals (resulting in a higher specific root tip number, sRT = number of root tips per unit dry weight). The RD and sRT parameters had been computed based on the two approaches described for RL. The three parameters were more tightly correlated in the second approach, thus suggesting superior data quality. The latter was also corroborated by the fact that the two parents were closer to the two extremes of the distribution. We therefore used the linear regression approach to present the data in Figure 29.

Figure 30 displays the relationship among the three different Al-toxicity parameters used above. Genotypes more severely affected by Al toxicity have smaller root systems (shorter RL)

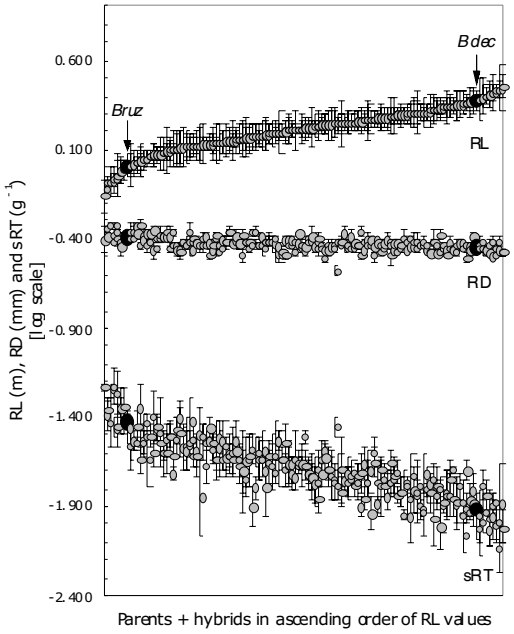

Figure 30. Comparison of three parameters that reflect Al resistance: root length (RL), average root diameter (RD), and number of root tips per gram of root dry weight (sRT = specific number of root apices). The parameters were measured with rooted stem cuttings in an Al-toxic solution. They were adjusted for harvest mean, the effect of the dry weight of stem cuttings used, and root growth vigor measured with another set of stem cuttings in an Al-free reference treatment (see x axis in Figure 1). Data points representing parents are highlighted with large black symbols.

that are made up of thicker roots (greater RD) and are more heavily branched (more root tips per unit dry weight, sRT). It may be possible to increase the robustness of detecting Al-resistant genotypes by computing the principal component of these three parameters to create "a composite Al resistance index" that captures the various Al-induced changes in root growth and architecture in a single parameter.

3.1.1.1.3 Identification of candidates genes associated with aluminum resistance in *Brachiaria*

Contributors: A. Arango, D. F. Cortes, G. Gallego, P. Wenzl, I.M. Rao, M. Ishitani and J. Tohme

Rationale

Last year we reported on the identification of individuals of a *Brachiaria ruziziensis* x *Brachiaria decumbens* hybrid population with contrasting degrees of Al resistance. This

suggested the feasibility of isolating candidate Al-resistance genes from root apices based on a comparison of gene expression patterns between an Al-resistant and an Al-sensitive bulk of hybrids. This year we pursued this approach.

Materials and Methods

Rooted stem cuttings of *B. decumbens*, *B. ruziziensis* and a group of hybrids with contrasting Al resistance levels were cultivated as described in "Evaluating traits associated with acid soil adaptation in a population of *Brachiaria ruziziensis* × *Brachiaria decumbens* hybrids" (see activity 3.1.1.1.2). Root apices collected at various harvests were pooled to create 6 samples for RNA extraction: two Al-resistant bulks of hybrids (grown with +/-Al), an Al-sensitive bulk of hybrids (grown with Al), two *B. decumbens* samples (grown with +/-Al) and a *B. ruziziensis* sample (grown with Al). Total RNA was isolated, mRNA was captured with magnetic beads, and ds-cDNA was synthesized using anchored oligo(dT) primer/adapters. To minimize cross-hybridization among genes belonging to the same family, 3'-UTRs were amplified by suppressive PCR after simultaneous digestion of cDNAs with MseI and MspI followed by adapter ligation. The resulting amplicons were then mixed in various combinations and subjected to three rounds of differential subtraction chain (DSC) (Luo et al., 1999).

Results and Discussion

Figure 31 displays the 3'-UTR amplicons obtained from the six mRNA samples. Control experiments confirmed that only 3'-UTR fragments and no internal gene fragments had been amplified (i.e. suppressive PCR was successful). In addition, sequencing of random 3'-UTRs fragments identified homologies to 3'-regions of known genes, including one coding for a root-specific protein. As a result of the double digestion with two 4-bp cutters, the fragments are small. This should have minimized cross hybridization among different genes during the subsequent subtractive hybridization steps.

The 3'-UTR fragments remaining after three rounds of subtractive hybridization were separated on a polyacrylamide gel. Distinct bands were visible in some combinations of tester and driver amplicons (Figure 32). This appears to suggest that the DSC procedure may have been successful.

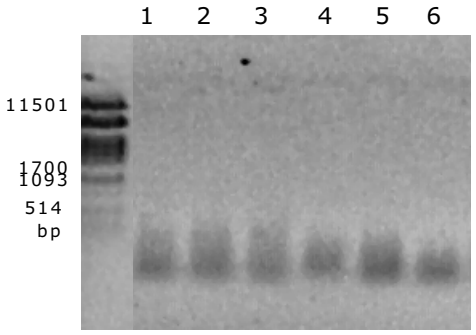

Figure 31. 3'UTR amplicons obtained from six cDNA samples: **1**, Al-resistant F_1 bulk grown at 200 µM Al^{3+}; **2**, Al-resistant F_1 bulk grown without Al^{3+}; **3**, Al-sensitive F_1 bulk grown at 200 µM Al^{3+}; **4**, *B. decumbens* grown at 200 µM Al^{3+}; **5**, *B. decumbens* grown without Al^{3+}; **6**, *B. ruziziensis* grown at 200 µM Al^{3+}.

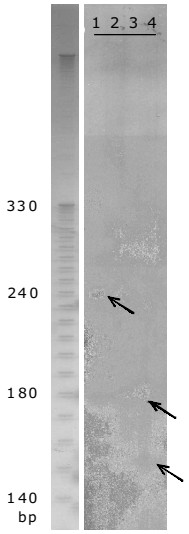

Figure 32. 3-UTR fragments remaining after three rounds of DSC. The numbers refer to the four combinations of tester and driver amplicons listed in Table 19.

Bands will be excised from the gel, cloned and sequenced. In addition, random fragments from the second round of subtraction will be cloned and sequenced. Depending on their redundancy they will be used to fabricate cDNA microarrays for screening for differentially expressed genes.

Table 19. Combinations of tester and driver cDNA samples used for subtractive hybridization.

Combination	Tester		Driver	
	Genotypes	Treatment	Genotypes	Treatment
1	Al-resistant F_1 bulk	200 µM Al^{3+}	Al-sensitive F_1 bulk	200 µM Al^{3+}
2	Al-resistant F_1 bulk	200 µM Al^{3+}	Al-resistant F_1 bulk	no Al^{3+}
3	*B. decumbens*	200 µM Al^{3+}	*B. ruziziensis*	200 µM Al^{3+}
4	*B. decumbens*	200 µM Al^{3+}	*B. decumbens*	no Al^{3+}

3.1.1.1.4 Isolating genes from root apices of *Brachiaria decumbens* that enhance Al resistance of yeast

Contributors: P. Wenzl, E. Gaitán, I.M. Rao and J. Tohme

Rationale

Some genes that when transformed into plants increase their resistance to Al, were originally identified based on their ability to enhance Al resistance of yeast. We used this approach to identify candidate Al-resistance genes in root apices of *B. decumbens* grown in the presence of Al.

Materials and Methods

Total RNA was extracted from root apices of rooted *B. decumbens* stem cuttings grown in a solution containing 200 µM $CaCl_2$, 200 µM $AlCl_3$ (pH 4.2). After capture of mRNA with magnetic beads, ds-cDNA was synthesized, ligated to adapters, size-fractionated and PCR-amplified. PCR products from the > 2 kb and < 2 kb fractions were ligated separately to linearized pYES2 plasmid and transformed into *E. coli*. The libraries obtained were amplified on plates. Plasmids extracted were mixed at a 1:1 ratio and re-transformed into yeast. Transformants were plated on a medium containing enough Al to arrest growth of yeast cells transformed with empty plasmid. Plasmids from the most quickly growing colonies were isolated, re-transformed into *E. coli*, and extracted for further characterization.

Results and Discussion

Approximately 100 yeast colonies were obtained from two million cfu plated on the Al medium (= 0.005 %). The plasmids isolated from 48 well-growing colonies were digested with a mixture of restriction enzymes to identify clones that had been isolated more than once, thus minimizing the chance of selecting false positives for further analysis. This fingerprinting experiment identified nine clones that had been isolated at least twice from different colonies. Work is underway to sequence and characterize inserts from these clones.

3.1.1.2 Low phosphorus tolerance mechanisms in *Brachiaria*: Phosphorus recycling and photosynthate partitioning in the *Brachiaria* hybrid

Contributors: M. Nanamori, T. Shinano, T. Yamamura, M. Osaki (Hokkaido University, Japan) and I. M. Rao (CIAT)

Rationale

The ongoing *Brachiaria* breeding program at the Centro Internacional de Agricultura Tropical (CIAT), conducted in collaboration with the Empresa Brasileira de Pesquisa Agropecuaria

(EMBRAPA), has generated several promising genetic recombinants (genetically recombined apomictic or sexual hybrids). Field evaluation of these hybrids identified one apomictic hybrid, FM9201/1873 (from *Brachiaria ruziziensis* clone 44-06 and *Brachiaria brizantha* cv. Marandú), as productive when grown on P-deficient, low-fertility, acid soils in both wet and dry seasons. This hybrid, the world's first commercial *Brachiaria* hybrid, was recently released in Mexico as cv. Mulato, and is being adopted by farmers in the tropics. Before this study, we had previously evaluated the *Brachiaria* hybrid's tolerance of low P supply, and found that it had very high P-use efficiency (g of biomass produced per mg of P uptake). Our current study aims to determine the hybrid's mechanisms for tolerating low P supply, that is, the physiological basis of its high P-use efficiency. We quantified the effects of P deficiency on phosphohydrolases and carbon metabolism in its leaves, and compared them against the values for the rice (*Oryza sativa* L.) cultivar Kitaake, which is also relatively tolerant of low P and low pH conditions.

Materials and Methods

Brachiaria hybrid cv. Mulato and rice (*Oryza sativa* L. cv. Kitaake) were grown hydroponically under greenhouse conditions (43°3' N, 141°2' E, altitude 17 m; maximum temperature 32°C; minimum temperature 16°C; average photoperiod during experiment = 14.8 h light and 9.2 h darkness; maximum photon flux density = 1550 μmol m^{-2} s^{-1}). Seedlings were pre-cultured in a 56-L vessel, containing a nutrient solution. After 1 week in pre-culture, 5 plants were transplanted to 56-L vessels containing nutrient solutions, each with one of three levels of P concentration (0 μM, 6 μM, and 32 μM) for 2 weeks. The experimental design was a randomized complete block with 3 replicates. *Brachiaria* hybrid and rice were cultured in different containers. Phosphorus concentration was adjusted every day, as was the pH (5.2 ± 0.1). Phosphorus depletion in each treatment was monitored daily to make sure that transient P depletion is not a major problem during plant growth. The P levels selected represent P deprived, low P and adequate P conditions for plant growth. The nutrient solution was completely renewed once a week. Three plants were pooled for one replicate, and each experiment conducted with three replicates (obtained from three different containers). Half of the collected plants were dried in an oven at 105°C for 3 days and weighed. The other half was frozen in liquid nitrogen and stored at -80°C until analysis of inorganic phosphate (P_i) and enzyme activities. Dried samples were digested with sulfuric acid and hydrogen peroxide to determine total N and P.

We used the Schmidt-Thannhauer-Schneider method with slight modifications to fractionate P compounds in plants. Phosphorus concentration in each fraction was measured by the molybdate-blue method. APase, RNase, PEPC, PEPP and PK activities were measured according to published methods. APase activities were assayed in sodium acetate buffer (pH 5.6), using *p*-nitrophenylphosphate as substrate. One unit of APase activity was defined as the activity that liberated 1 μmol of *p*-nitrophenylphosphate per minute. RNase activities were assayed in sodium acetate buffer (pH 5.6), using 250 μg of yeast RNA as the substrate. One unit of RNase activity was defined as the activity that liberated the amount of soluble nucleotide corresponding to one unit of nucleotide per minute. One unit is the amount of nucleotide that has an A_{260} of 1.0 in a volume of 1.0 mL. Values are the means ± SE of three replicates.

A lyophilized leaf sample (about 100 mg) was used to extract organic acids, after agitating with 5 mL of 0.01 N HCl for 1 h. After centrifuging at 3000 *g* for 10 min, the supernatant was collected and then filtered, using a 45-μm membrane filter. The organic acids were then analyzed, using capillary electrophoresis. A lyophilized leaf sample (about 50 mg) was incubated with 80% (v/v) ethanol at 40°C for 17.5 h to extract glucose, fructose, and sucrose. Carbohydrate contents were measured with an F-kit purchased from Roche Diagnostics Corporation.

For the experiment on ^{14}C partitioning analysis, we used plants grown under either the 0 µM P or the 32 µM P treatment for 2 weeks after pre-culture. $^{14}CO_2$ was generated by adding 30% PCA into $NaH^{14}CO_2$ (18.5 kBq). $^{14}CO_2$ was fed to the plants for 5 min in a vinyl package under natural lighting (photon flux density was immediately in liquid nitrogen. The sample was lyophilized and stored at -20°C until analysis for different fractions. ^{14}C assimilated in shoots was fractionated into amino acids, organic acids, phosphate esters, sugars, and residue, using the ion-exchange columns. The residual fraction is considered to consist of protein, starch, and constituents of cell walls such as cellulose, hemicellulose, and lignin. All of the fractions were concentrated, using a rotary-evaporator at 40ˆC, then making up their volumes to 10 mL by water. From each sample, 1 mL was mixed with toluene scintillator (DPO 4 g, POPOP 200 mg, and nonion 300 mL, made up to 1 liter with toluene) and placed in darkness overnight. Radioactivity was measured, using a liquid scintillation counter.

Results and Discussion

Plant growth and nutrient status: Results on changes in total P concentrations in the nutrient solution in each container within 24 h showed that the 3 levels of P treatment 0 µM P, 6 µM P and 32 µM P provided conditions of complete P deprivation, low P supply and adequate P supply, respectively, during the treatment period of 2 weeks. Maximum amount of P depletion of up to 85% to 100% was observed at 2 weeks after treatment with 6 µM P while it was only up to 40% with 32 µM P treatment.

Although plant growth decreased with P deficiency, the difference between the treatments 6 µM P and 32 µM P was not significant (Table 20). The total dry weight (DW) of the *Brachiaria* hybrid decreased by 33% in the 6 µM P treatment under P deficiency, and lower P supply had greater effect on leaf and stem growth than on root growth. Rice also decreased its growth with P deficiency, but its growth in the 0 µM P treatment was greater than that of the *Brachiaria* hybrid. This was because rice seed is larger and contains more P, and therefore contributed to a higher accumulation of P in rice seedlings before they were subjected to low-P treatments.

However, P-use efficiency (the inverse of P concentration) was greater in the *Brachiaria* hybrid (1.85 g DW per 1 mg P) than in rice (1.61 g DW per 1 mg P) (Table 21). Thus, in the 0 µM P treatment, the *Brachiaria* hybrid showed greater tolerance of low concentrations of tissue P, compared with rice. In contrast, P-use efficiency in the 32 µM P treatment was greater

Table 20. Dry weight (DW) among plant parts of a Brachiaria hybrid and rice grown at three different levels of phosphorus (P) supply.

Species	Treatment (µM P)	DW (g per plant)			
		Leaf	Stem	Root	Total plant
Brachiaria hybrid	0	0.425 [a]	0.248 [a]	0.438 [a]	1.11 [a]
	6	0.707 [b]	0.395 [b]	0.539 [a]	1.64 [ab]
	32	0.799 [b]	0.439 [b]	0.449 [a]	1.69 [b]
Rice	0	0.400 [a]	0.583 [a]	0.365 [a]	1.35 [a]
	6	0.487 [b]	0.645 [a]	0.403 [a]	1.53 [a]
	32	0.542 [b]	0.700 [a]	0.363 [a]	1.61 [a]

Means followed by the same letter within the column are not significantly different, according to Duncan's multiple range test ($P \leq 0.05$).

Table 21. Nitrogen and P contents in different plant parts of a *Brachiaria* hybrid and rice grown at three different levels of P supply.

Nutrient	Species	Treatment (µM P)	Concentration (mg per g DW)				P-use efficiency (g DW per mg P)
			Leaf	Stem	Root	Total plant	
Nitrogen	*Brachiaria* hybrid	0	44.7[ab]	43.3[b]	32.0[ab]	39.6[a]	
		6	42.0[a]	37.3[a]	29.2[a]	38.6[a]	
		32	45.2[b]	38.3[a]	33.1[b]	40.2[a]	
	Rice	0	33.4[a]	15.1[a]	18.0[a]	21.3[a]	
		6	44.9[b]	24.6[b]	23.9[b]	30.8[b]	
		32	50.5[c]	30.7[b]	29.6[c]	37.1[c]	
Phosphorus	*Brachiaria* hybrid	0	0.44[a]	0.62[a]	0.58[a]	0.54[a]	1.85[a]
		6	1.69[b]	1.94[b]	1.78[b]	1.78[b]	0.56[b]
		32	11.0[c]	6.92[c]	8.35[c]	9.26[c]	0.11[c]
	Rice	0	0.74[a]	0.57[a]	0.56[a]	0.62[a]	1.61[a]
		6	2.03[b]	1.83[b]	1.78[b]	1.88[b]	0.53[b]
		32	6.29[c]	7.00[c]	5.40[c]	6.40[c]	0.16[c]

Means followed by the same letter within the column are not significantly different, according to Duncan's multiple range test ($P \leq 0.05$).

in rice than in the *Brachiaria* hybrid. Apparently, the mechanisms of P-use efficiency differ between the two test crops. Although P-use efficiency was different, dry weight in the 32 µM P treatment was similar for the two test crops, indicating that both crop plants would serve as good models for understanding how different plant mechanisms function in relation to P nutrition. Nitrogen concentration was stable in the *Brachiaria* hybrid, regardless of P treatment, whereas it decreased markedly in rice with P deficiency (Table 20). Phosphorus concentration decreased markedly in both test crops with P deficiency (Table 21), which was lower at 0 µM P treatment, especially in leaves of the *Brachiaria* hybrid. Inorganic P (P_i) concentration, expressed on the basis of dry weight also decreased markedly with P deficiency in both test crops (Table 21), with P_i concentration being higher in leaves than in roots. P_i concentration in stems was higher in rice than in the *Brachiaria* hybrid, although when P was supplied at 6 µM P or 32 µM P, P-use efficiency was similar for both the *Brachiaria* hybrid and rice.

Fractions of phosphorus compounds: Acid-soluble P_i accounted for a large part of total P in both species under P-sufficient conditions (Figure 33). But the ratio of acid-soluble P_i to total P decreased drastically with P deficiency in plant parts (leaf, stem, and root) and total biomass, especially in rice (Table 22). In the 0 µM P treatment, the ratio of acid-soluble P_i accounted for 47% and 17% in the Brachiaria hybrid and rice, respectively. However, in both test crops, the RNA:P ratio increased with P deficiency as acid-soluble P_i decreased.

Phosphohydrolase activity: We measured APase and RNase activities as P-recycling enzymes to discover whether they have any role in increasing the efficiency of acquired P in the plant. APase and RNase activities were both induced by P deficiency. The *Brachiaria* hybrid had higher APase activity, especially in shoots, than did rice. Specific activity of APase in leaves was 5.2 to 7.7 times higher in the *Brachiaria* hybrid (Figure 34A) than in rice (Figure 34B) and, in stems, 2.7 to 3.5 times higher. APase activity in roots was noticeably induced in both test crops when they were grown without P. RNase activity in leaves and stem was also higher in the *Brachiaria* hybrid than in rice (Figures 34C and 34D), while being slightly lower in roots. RNase activities in *Brachiaria* hybrid leaves were 4.4 to 5.8 times higher than in rice leaves and 1.7 to 5.4 times higher in stems.

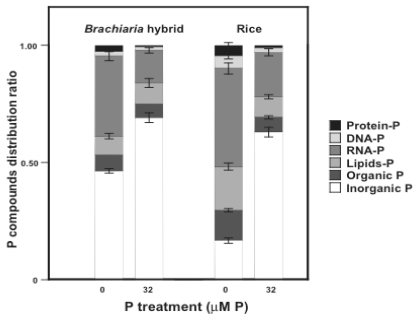

Figure 33. Distribution of P compounds in the *Brachiaria* hybrid and rice under three levels of P supply. Plants, 7 to 8 weeks old, were grown in P-deficit nutrient solution for 2 weeks. Phosphorus in plants was fractionated into the following six fractions: acid-soluble inorganic P, acid-soluble organic P, lipids-P, RNA-P, DNA-P, and residual P. Most of the residual-P fraction is considered to consist of protein P. Values are the means ± SE of three replicates.

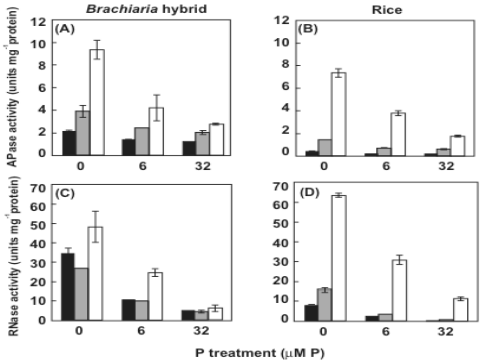

Figure 34. APase (A, B) and RNase (C, D) activities of a *Brachiaria* hybrid (A, C) and rice (B, D). Plants were grown at three different levels of P for 2 weeks. APase and RNase activities were measured in leaves (black bars), stem (hatched bars), and roots (white bars).

Organic acid concentration in leaves: Total organic acid concentration was higher in the *Brachiaria* hybrid than in rice (Figure 35). With P deficiency, organic acid concentration dropped by 37% in the Brachiaria hybrid and by 55% in rice. Results on organic acid composition showed thatoxalate and fumarate were the main organic acids in the *Brachiaria* hybrid, whereas oxalate concentration was high in rice only in the 32 μM P treatment. In rice, the marked decrease in oxalate was accompanied by increased malate and citrate concentrations in leaves.

Carbohydrates in leaves: The concentrations of glucose, fructose, and sucrose dropped with P deficiency (Figures 36A, 36B, 36C, respectively). These decreases were more prominent in the *Brachiaria* hybrid. Starch (Figure 36D), glucose, and fructose concentrations were higher in the *Brachiaria* hybrid than in rice, with sucrose concentration being higher in rice (Figure 36C). The level of starch decreased in the *Brachiaria* hybrid with P deficiency while it increased in rice.

Table 22. Contents of P_i and P_i:total P ratio in different plant parts of a Brachiaria hybrid and rice grown at three levels of P supply.

P_i parameter	Species	Treatment (μM P)	Leaf	Stem	Root	Total plant
P_i concentration (mg per g DW)	*Brachiaria* hybrid	0	0.13 [a]	0.08 [a]	0.06 [a]	0.09[a]
		6	0.73 [a]	0.22 [a]	0.30 [b]	0.47 [a]
		32	7.43 [b]	3.48 [b]	4.63 [c]	5.66[b]
	Rice	0	0.13 [a]	0.08 [a]	0.05 [a]	0.08[a]
		6	0.57 [b]	0.24 [a]	0.31 [a]	0.36[b]
		32	3.61 [c]	4.05 [b]	3.04 [b]	3.67[c]
P_i:total P ratio (%)	*Brachiaria* hybrid	0	29.6 [a]	14.1 [a]	11.3 [a]	17.5[a]
		6	43.5 [b]	11.1 [a]	16.7 [a]	26.2[b]
		32	67.1 [c]	50.2 [b]	56.0 [b]	61.1[c]
	Rice	0	17.0 [a]	13.7 [a]	8.5 [a]	13.6[a]
		6	27.8 [b]	13.3 [a]	17.3 [a]	19.1[b]
		32	57.4 [c]	57.8 [b]	56.2 [b]	57.4[c]

Means followed by the same letter within the column are not significantly different, according to Duncan's multiple range test ($P \leq 0.05$).

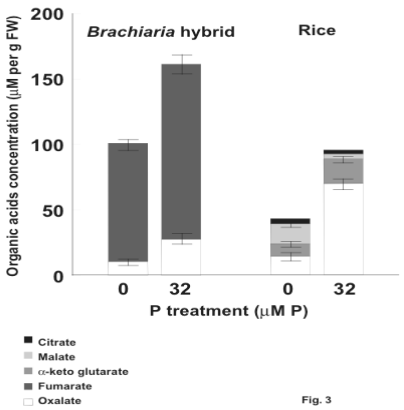

Figure 35. Organic acid concentration in *Brachiaria* hybrid and rice leaves. Plants were grown with or without P for 2 weeks. Organic acid content was measured, using capillary electrophoresis. We identified each peak of an organic acid by comparing the peaks with standards. Contents were determined by comparing the peak area of the sample with that of the corresponding standard. Values are the means ± SE of three replicates.

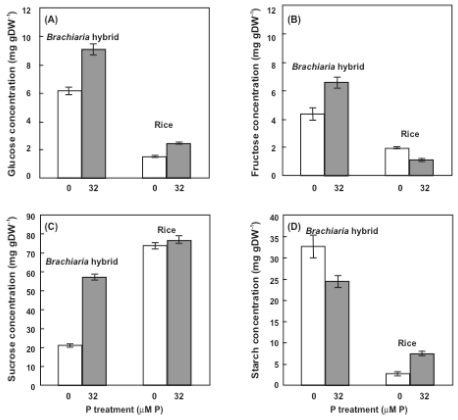

Figure 36. Carbohydrate concentration in *Brachiaria* hybrid and rice leaves: (A) glucose, (B) fructose, (C) sucrose, and (D) starch concentrations. Plants used for the assays were grown with P (white bars) or without P (hatched bars) for 2 weeks. Carbohydrate concentration Values are the means ± SE of three replicates.

Enzyme activities catalyzing the PEP-consuming reaction: The three enzymes that catalyze the PEP-consuming reaction in glycolysis are PEPC, PEPP, and PK. Phosphoenolpyruvate carboxylase (PEPC) activity was greater in the *Brachiaria* hybrid than in rice (Table 23). In rice, it increased by a factor of 2.4 with P deficiency, whereas it did not change in the *Brachiaria* hybrid. In both test crops, phospho*enol*pyruvate phosphatase (PEPP) activity was induced by P deficiency: 5.6 times in the *Brachiaria* hybrid and 6.0 times in rice, although in leaves, it was higher in the *Brachiaria* hybrid than in rice. Pyruvate kinase (PK) activity in rice was 1.6 times higher in the 0 μM P than in the 32 μM P treatment. Although PK activity in the *Brachiaria* hybrid was also higher in the 0 μM P treatment, it was not significant.

^{14}C partitioning: In both test crops, photosynthetically fixed carbon pools were mainly distributed to sugars, and the ^{14}C distribution ratio to sugars increased with P deficiency (Figure 37). The effect of P deficiency on ^{14}C partitioning to sugars was larger in rice than in the *Brachiaria* hybrid. The distribution ratio to sugars was 68% in rice and 50% in the *Brachiaria* hybid in the 0 μM P treatment. The ^{14}C distribution ratio to the amino acid and organic acid pools was greater in the *Brachiaria* hybrid than in rice, and slightly increased with P deficiency in the *Brachiaria* hybrid. The ^{14}C distribution ratio to phosphate esters markedly decreased with P deficiency in both test crops. The ^{14}C distribution ratio to the residue fraction, which is supposed to contain protein, starch, and nonstructural carbohydrate pools, increased with P deficiency in both test crops.

Table 23. Influence of P deprivation on enzyme activities that are invo metabolism in leaves of *Brachiaria* hybrid and rice.

Species	Treatment	Activity (μmole min^{-1} mg	
	(μM P)	PEPC	PEPP
Brachiaria hybrid	0	4.39[a]	0.218[a]
	32	3.79[a]	0.039[b]
Rice	0	0.04[a]	0.060[a]
	32	0.09[b]	0.010[b]

Plants used for the assays were grown with or without phosphorus for 2 effect of phosphorus concentration in the medium was determined sepa *Brachiaria* hybrid and rice by t-test ($P \leq 0.05$).

Results from this study show that tolerance of low P in the *Brachiaria* hybrid and rice involved marked differences in P recycling and carbon metabolism. For the *Brachiaria* hybrid, low-P tolerance involved two major strategies: (1) increasing the ability to use P efficiently by inducing APase and RNase in shoots with P deficiency; and (2) enhancing sugar catabolism and subsequent synthesis of amino acids and organic acids in leaves under P deficiency. For rice, strategies for low-P tolerance differed by involving (1) decreased carbon flow to amino acids and organic acids, and decreased N concentration; and (2) improved partitioning of photosynthates to sucrose, combined with restricted sugar catabolism.

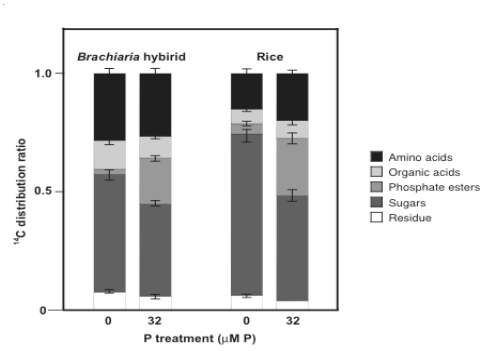

Figure 37. Distribution of photosynthetically assimilated ^{14}C in the *Brachiaria* hybrid and rice. $^{14}CO_2$ (18.5 kBq) was fed to P-deficient or P-sufficient plants under natural lighting. After $^{14}CO_2$ was fed for 5 min, only shoots were harvested and used for assays. ^{14}C assimilated in shoots was fractionated into amino acids, organic acids, phosphate esters, sugars, and residue, using the ion-exchange columns. The residual fraction is considered to consist of protein, starch, and constituents of cell walls such as cellulose, hemicellulose, and lignin. Values are the means ± SE of three replicates.

3.1.1.3 Screening of *Brachiaria* hybrids for resistance to aluminum

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

Rationale

For the last two years, we have implemented screening procedure to identify Al-resistant *Brachiaria* hybrids that were preselected for spittlebug resistance.Last year, we have identified 2 sexual hybrids (SX 01NO3178 and SX01NO7249) and one apomictic hybrid (BR99NO/4132) with greater level of Al resistance than that of the sexual parent, BRUZ/44-02. With the partial support of BMZ-GTZ of Germany and Papalotla (seed company) of Mexico to the *Brachiaria* improvement project, this year we evaluated Al resistance of the most promising *Brachiaria* hybrids that are resistant/tolerant to spittlebug.

Materials and Methods

A total of 79 genotypes (including 54 new hybrids and 24 checks that included previous selections together with 3 parents (*B. decumbens* CIAT 606, *B. brizantha* CIAT 6294 and *B. ruziziensis* 44-02)) were included for evaluation of Al resistance. All the new hybrids were screened for spittlebug resistance (C. Cardona, personal communication; also see Output 2 and Activity 2.2 of this report). Stem-cuttings were rooted in a low ionic strength nutrient solution containing $CaCl_2$ (200μM), selected for uniformity and transferred to a solution containing 200 μ*M* $CaCl_2$ (pH 4.2) and exposed to 2 levels of $AlCl_3$ (0 and 200μM). The solution was replaced every third day, and total root length and root biomass were measured after 21 days of Al treatment (Photo 13). Root architecture was measured using WINRHIZO software program. Results reported are mean values from 3 experiments.

Results and Discussion

As reported for the past 2 years, results on total root length per plant after exposure to 21 days with or without toxic level of Al in solution indicate that the parent *B. decumbens* CIAT 606 is outstanding in its level of Al resistance (Figure 38).

Photo 13. Different steps involved in screening for aluminum resistance: (a) growing of individual genotypes in soil; (b) cultivation of vegetative propagules in nutrient solution for inducing rooting; (c) generation of vegetative propagules with 4 to 5 cm long roots for evaluation; (d) treatment with or without aluminum in nutrient solution for 3 weeks; and (e) quantitative evaluation of root traits at the end of 3 weeks of treatment (Photos are from the MS thesis work of Adriana Arango).

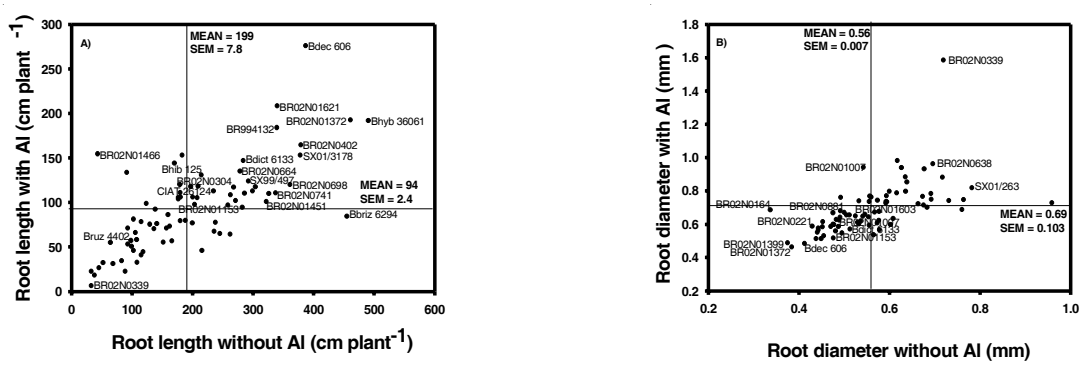

Figure 38. Identification of Al resistant hybrids of *Brachiaria* based on (A) total root length and (B) mean root diameter. Total root length and mean root diameter were measured after exposure to 0 or 200 ˜M $AlCl_3$ with 200 ˜M $CaCl_2$ (pH 4.2) for 21 days. Genotypes with (A) higher values of root length were identified in the upper box of the left hand side and (B) lower values of mean root diameter (fine roots) with no or high Al in solution were identified in the lower box of the left hand side. SEM = standard error of the mean.

Among the 54 new hybrids tested, 2 hybrids (BR02NO1372 and BR02NO1621) showed greater level of Al resistance based on total root length per plant (Figure 39). Among these two hybrids, BR02NO1372 showed greater fine root development than CIAT 606 in the absence of Al in solution (Figure 39). Another hybrid, BR02NO402 also showed moderate level of Al resistance based on root length value with the presence of Al. Exposure to Al decreased the mean value of total root length of the 79 genotypes from 199 to 94 cm $plant^{-1}$. Among the checks tested, 2 sexual hybrids (SX99/7075 and SX01/1378) that were selected before for Al resistance also showed moderate level of Al resistance. Among the apomictic hybrid checks cv. Mulato (CIAT36061) and BR99/4132 showed greater level of Al resistance.

Results on mean root diameter showed that the hybrid BR02NO1372 had the lowest values under both with and without Al treatments (Figure 39). This hybrid was superior to CIAT 606 in fine root development. One of the hybrids, BR02NO339

showed very high values of mean root diameter of about 1.6 mm (coarse root system) with Al in solution.

The relationship between total root length and mean root diameter in the presence of Al in solution showed that 2 hybrids (BR02NO1621 and BR02NO1372) were outstanding in developing fine root system (Figure 39). The same relationship without Al in nutrient solution indicated that the hybrid BR02NO1372 is superior to CIAT 606 in terms of total toot length The mean values of root diameter decreased from 0.69 to 0.56 mm with the exposure to Al in nutrient solution. of mean root diameter).

Results on spittlebug resistance of these hybrids are reported in section 2.2 of this report. The hybrid BR02NO1372 was selected as one of the spittlebug resistant hybrids. Two other hybrids (BR02NO402; BR02NO794) that showed moderate level of Al resistance were also found to be moderate in resistance to spittlebugs. This set of hybrids are being evaluated under field conditions to determine the mode of reproduction (sexual or apomictic) using progeny test. The hybrids that combine desirable attributes with apomictic mode of reproduction will be candidates for further field evaluation as potential cultivars for release. The promising sexuals could be used in recurrent selection to generate superior hybrids of *Brachiaria*.

We have identified 2 hybrids ((BR02NO1372 and BR02NO1621) with greater level of Al resistance than that of the most hybrids generated from the *Brachiaria* breeding program.

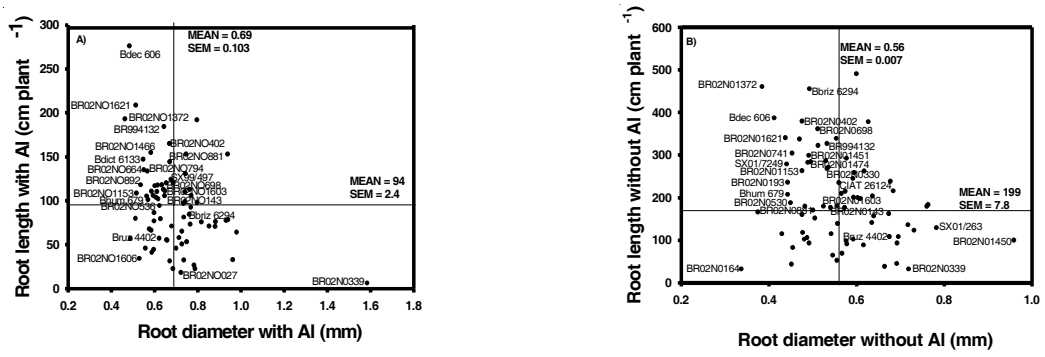

Figure 39. Relationship between total root length and mean root diameter of 78 genotypes of *Brachiaria* with (A) presence or (B) absence of aluminum in solution. Gentoypes that develop finer root system were identified in the upper box of the left hand side.

3.1.1.4 Identification of plant attributes in *Brachiaria* associated with persistence under low nutrient supply

Contributors: I. M. Rao, J. W. Miles, C. Plazas, J. Ricaurte and R. Garcia

Rationale

A field study is completed this year at Matazul Farm in the Llanos of Colombia. The main objective was to identify genetic recombinants of *Brachiaria* with tolerance to low nutrient supply and evaluate plant attributes that contribute to superior adaptation. Results obtained from this field study at 28 months after establishment indicated that the *Brachiaria* hybrid, FM9503-S046-024 was not only rapid in its establishment but also performed well into the third year after establishment. Its superior performance was associated with its ability to acquire greater amounts of nutrients, particularly Ca and Mg from low fertility soil. This year we report the

results on the performance of genetic recombinants in terms of their growth and persistence under low fertility acid soil conditions at 40 months after establishment.

Materials and Methods

A field trial was established on a sandy loam oxisol at Matazul farm in the Llanos of Colombia in July, 1999. The trial comprises 12 entries, including six natural accessions (four parents) and six genetic recombinants of *Brachiaria*. The trial was planted as a randomized block in split-plot arrangement with two levels of initial fertilizer application (low: kg/ha of 20P, 20K, 33Ca, 14 Mg, 10S; and high: 80N, 50P, 100K, 66Ca, 28Mg, 20S and micronutrients) as main plots and genotypes as sub-plots. Live and dead forage yield, shoot nutrient composition, shoot nutrient uptake and leaf and stem TNC (total nonstructural carbohydrates) were measured at the end of the wet season (November 19, 2002), i.e., at 40 months after establishment. Maintenance fertlizer (half the levels of initial application) was applied in July 2001.

Results and Discussion

As expected, application of high amounts of maintenance fertilizer at 2 years after establishment improved forage yield of most of the genotypes compared with low fertilizer application (Figure 40). At 40 months after establishment, live forage yield with low fertilizer application ranged from 0 to 2004 kg/ha and the high values of forage yield were observed with three germplasm accessions (CIAT 26110, 26318 and 6294) and one spittlebug resistant genetic recombinant, FM9503-S046-024 (Table 24). This spittlebug resistant genetic recombinant not only showed rapid establishment but also maintained greater level of forage production over time for the past 3 years with low initial fertilizer application. It also was very responsive to higher fertilizer application as revealed by live shoot biomass and total forage yield. As expected, the performance of one of the parents, BRUZ/44-02 was very poor compared with other parents and genetic recombinants. These results are similar to those observed at 28 months after establishment (IP-5 Annual Report, 2002). The values of leaf to stem ratio were markedly superior with the genetic recombinants than the parents and other germplasm accessions with both levels of fertilizer application (Figure 40). This is an important attribute for improving animal production through *Brachiaria* breeding activities.

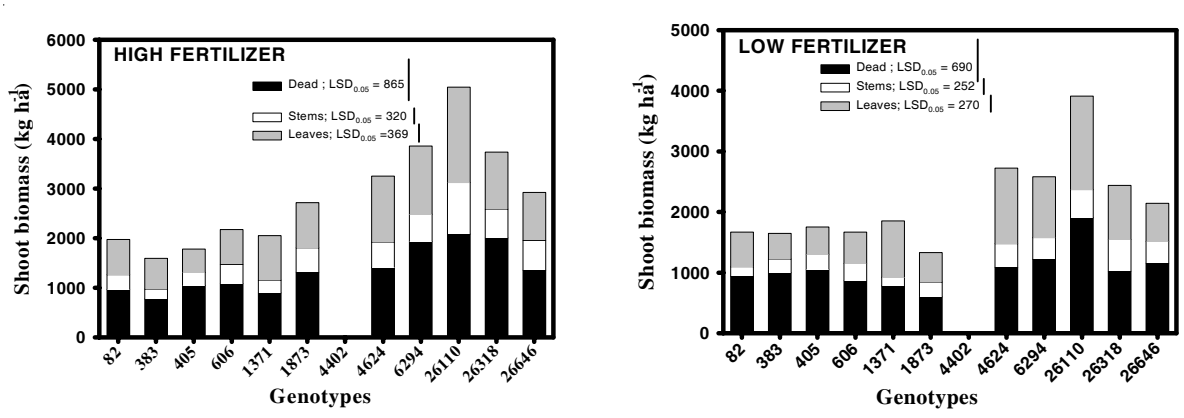

Figure 40. Genotypic variation as influenced by fertilizer application in dry matter distribution among leaves, stems and dead biomass of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 40 months after establishment (November 2002) LSD values are at the 0.05 probability level.

Among the genetic recombinants, FM9503-S046-024 was outstanding in production of green leaf biomass with both low and high fertilizer application (Figure 40). One of the germplasm accessions, CIAT 26110 was outstanding in its leaf biomass production and its production was almost 2 times greater than that of the mean value of 12 genotypes tested. These results are consistent with the observations made in previous years.

Shoot N uptake with low fertilizer application was greater for one accession (CIAT 26110), one parent (CIAT 6294) and two genetic recombinants (FM9503-S046-024, FM9301-1371) (Figure 41). These two genetic recombinants were also outstanding in their ability to acquire greater amounts of P, K, Ca and Mg from low fertilizer application when compared with other genetic recombinants (Figure 41). The ability of these two hybrids and CIAT 26110 to acquire Ca from soil with low fertilizer application treatment was particularly outstanding compared with other hybrids and germplasm accessions. This ability to acquire greater amounts of Ca from acid soil (Table 24) could not only contribute to its superior persistence on acid infertile soils but also could contribute to greater forage quality and animal production. Among the parents, *B. Brizantha*, CIAT 6294 was superior in nutrient acquisition from low and high fertilizer application (Figure 41). Shoot nutrient uptake by CIAT 26110 was outstanding with both low and high fertilizer application (Figure 41).

Production of live forage yield was associated with significantly lower contents of nutrients (P, Ca and Mg) in stem tissue of *Brachiaria* genotypes, particularly under low fertilizer application, indicating the importance of efficient mobilization of nutrients from stems to leaves and efficient utilization of nutrients for the production of green forage (Table 24). This could be an important physiological mechanism for superior performance with low fertilizer application. Leaf ash (mineral) and stem ash contents were also negatively associated with green forage yield indicating lower mineral status and greater nutrient use efficiency under low fertilizer treatment.

Results from this field study indicated that the *Brachiaria* hybrid, FM9503-S046-024 was not only rapid in its establishment but also performed well into the fourth year after establishment. Its superior performance at 40 months after establishment was associated with its ability to acquire greater amounts of nutrients, particularly Ca and Mg from low fertility soil.

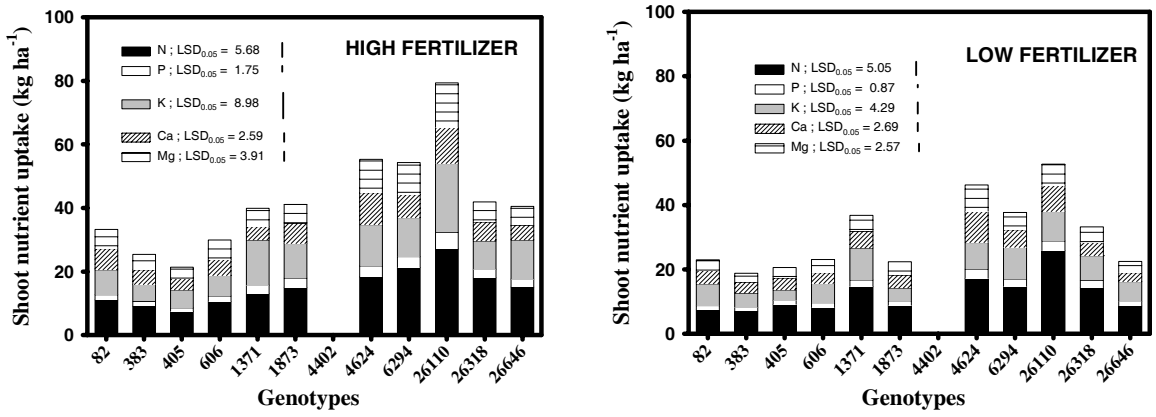

Figure 41. Genotypic variation as influenced by fertilizer application in nutrient (N, P, K, Ca, Mg) uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 40 months after establishment (November 2002) LSD values are at the 0.05 probability level.

Table 24. Correlation coefficients (r) between green forage yield (t/ha) and other shoot traits of *Brachiaria* genotypes grown with low or high intial fertilizer application in a sandy loam oxisol in Matazul, Colombia.

Shoot traits	Low fertilizer	High fertilizer
Total (live + dead) shoot biomass (t/ha)	0.89***	0.94***
Dead shoot biomass (t/ha)	0.61***	0.76***
Leaf biomass (t/ha)	0.95***	0.97***
Stem biomass (t/ha)	0.76***	0.92***
Leaf N content (%)	0.19	-0.20
Leaf P content (%)	-0.29*	0.03
Leaf K content (%)	-0.30*	-0.10
Leaf Ca content (%)	-0.22	-0.33*
Leaf Mg content (%)	-0.37**	-0.08
Leaf TNC content (mg g^{-1})	-0.39**	0.02
Leaf ash content (%)	-0.20	-0.15
Stem N content (%)	-0.22	-0.49**
Stem P content (%)	-0.37**	-0.06
Stem K content (%)	-0.06	0.04
Stem Ca content (%)	-0.36*	-0.57***
Stem Mg content (%)	-0.39**	-0.32*
Stem TNC content (mg g^{-1})	-0.05	-0.35*
Stem ash content (%)	-0.22	-0.01

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

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

Rationale

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

Materials and Methods

A field trial was established at Matazul farm on 31 May of 2001. The trial included 4 *Brachiaria* hybrids (BR98NO/1251; BR99NO/4015; BR99NO/4132; FM9503-S046-024) along with 2 parents (*B. decumbens* CIAT 606 and *B. brizantha* CIAT 6294). The trial was planted as a randomized block in split-plot arrangement with two levels of initial fertilizer application (low: kg/ha of 20P, 20K, 33Ca, 14 Mg, 10S; and high: 80N, 50P, 100K, 66Ca, 28Mg, 20S and micronutrients) as main plots and genotypes as sub-plots with 3 replications. The plot size was 5 x 2 m. A number of plant attributes including forage yield, dry matter distribution and nutrient uptake were measured at 18 months after establishment (November 2002).

Results and Discussion

At 18 months after establishment, live forage yield with low fertilizer application ranged from52 to 2049 kg/ha and the high values of forage yield were observed with one spittlebug resistant genetic recombinant, FM9503-S046-024 and one parent (CIAT 6294). With high initial fertilizer application also these two genotypes were outstanding in shoot biomass production (Figure

42). Among the 4 hybrids tested, 4624 was outstanding in its adaptation to low intial fertilizer application. It is important to note that CIAT 6294 had greater amount of dead biomass and stem biomass under low fertilizer application (Figure 42).

Results on shoot nutrient uptake indicated that the two hybrids, 4624 and 4015 were superior to CIAT 606 under low fertilizer application (Figure 43). Nutrient acquisition by the hybrid 4624 was also greater than the rest of the test materials with high initial fertilization. These results are consistent with previous field study conducted on the same farm.

The performance of the 4 hybrids in comparison with two parents with maintenance fertilizer application will be monitored for the next 2 years in terms of forage yield and nutrient acquisition.

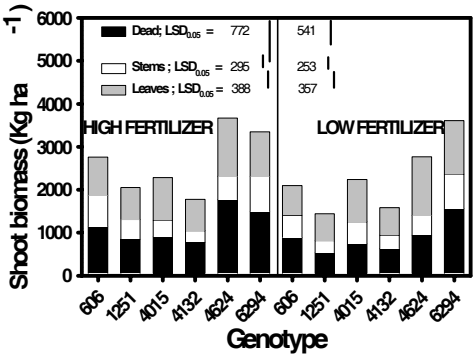

Figure 42. Genotypic variation as influenced by fertilizer application in shoot biomass production (forage yield) of two parents (CIAT 606, 6294) and four genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 18 months after establishment (November 2002). LSD values are at the 0.05 probability level. NS = not significant.

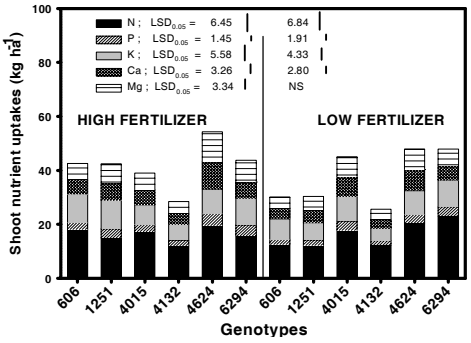

Figure 43. Genotypic variation as influenced by fertilizer application in nutrient uptake (N, P, K, Ca and Mg) of two parents (CIAT 606, 6294) and four genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 18 months after establishment (November 2002). LSD values are at the 0.05 probability level. NS = not significant.

3.1.2 Field trials in Costa Rica, Nicaragua and Honduras for participatory evaluation of *Brachiaria* hybrids in comparison with commercial cultivars

Contributors: Beatriz Sandoval and Marco Lobo (INTA-MAG), Pedro J. Argel, Guillermo Pérez, A. Schmidt, H. Cruz, L.A. Hernandez, L.H. Franco, M. Peters, J. Miles and I. M. Rao (CIAT)

Farmers' participation is very important in the process of identifying and selecting promising forage germplasm, because this group bases their selection criteria on particular plant attributes not necessarily observed by the researchers. Thus, in the selection process, farmers' participation complements additional experimental information generated on the same plots by technicians in charge of the experiments.

Participatory evaluation of *Brachiaria* hybrids and accessions is being carried out in hillsides of Costa Rica, Honduras and Nicaragua.

Rationale

As part of the BMZ-GTZ project on developing aluminum resistant *Brachiaria* hybrids, last year we initiated field studies in Costa Rica, Nicaragua and Honduras for evaluation of new hybrids of *Brachiaria* along with commercial *Brachiaria* cultivars in participation with farmers. Farmers' participation is very important in the process of identifying and selecting promising forage germplasm, because this group bases their selection criteria on particular plant attributes not necessarily observed by the researchers. Thus, in the selection process, farmers' participation complements additional experimental information generated on the same plots by researchers in charge of the experiments. Thus the main objective of participatory evaluation was to expose the promising hybrids to farmers and generate information on farmer selection criteria. This information is highly useful to *Brachiaria* improvement program to incorporate farmer perspectives on *Brachiaria* ideotypes for multiple use in crop-livestock systems.

Materials and Methods

Costa Rica - A field trial was established on 30 August 2002 in the area of Puriscal (Cantón Mora) in a farm called El Rodeo. National program (INTA) staff responsible for managing the trial are Ing.Beatriz Sandoval and Marco Lobo from MAG of Costa Rica. The design is a complete randomized block with 3 replications. The plot size was 2 m long x 4 m wide. The texture of the soil is loamy with the following chemical characteristics: pH 4.6, Al (0.8 ppm), Ca (6.2 cmol/kg), Mg (1.76 cmol/kg), K (0.46 cmol/kg), Zn (0.6 mg/kg), Cu (5.0 mg/kg), Fe (52 mg/kg); SOM (5.7%). The *Brachiaria* species planted had the following CIAT numbers: 606, 26110, 26990, 26318, 26646, 6133, 36061, 6789, 36062, 26124 and Mixe (no CIAT number). Among the 11 genotypes planted, CIAT 36061 and CIAT 36062 are apomictic hybrids. During 2003 we continued the participatory evaluation of promising lines and hybrids of *Brachiaria* at the locality of El Rodeo (Cantón Mora, Costa Rica). A number of plant attributes including forage yield, dry matter distribution and nutrient uptake are being monitored.

Nicaragua - The experimental site was chosen in Ubú Norte (12° 58' 44" N, 84° 54' 23" E, 261 masl) in the Region Autonoma del Atlantico Sur (RAAS) where acid soils are predominant. Soil characteristics are: SOM 6.61%, pH 5.66, P(BrayII) 3.22, K (meq) 0.55, Ca (meq) 5.5, Mg (meq) 2.42. A total of 14 *Brachiaria* accessions and hybrids (CIAT No. 606, 654, 679, 6133, 6780, 16322, 26110, 26124, 26318, 26646, 26990, 36061, 36062, and "Mixe") were sown in three replicates in a split-plot design with fertility levels as main plots and genotypes as subplots. Site preparation was initiated in September and plots (5 x 4m) were sown early October 2002 upon the beginning of the second rainy season. Plot establishment was heavily affected by unusual high precipitation, leaving Ubú with 1/3 more precipitation as the average of the last 20 years and causing floods in the area. Most of the plots established did not germinate and thus plots were replanted in May 2003. Two fertilization treatments were applied upon plot establishment. Fertilization levels were adjusted to soil analysis results. The first agronomic and participatory evaluation was conducted in August 2003 (7 weeks after standardization cut).

Honduras - The trial planted in the Yorito reference site in Honduras (neutral to slightly acidic soils, 6 months dry season) used the same experimental design as reported for the Costa Rica trial. Data for the participatory evaluation was analysed using correspondence factorial analysis (CAF; see section 4.1.9 of this report).

Results and Discussion

All *Brachiaria* lines established well, however differences existed in plant height 109 days after planting within and between *Brachiaria* species. Table 25 shows that *B. brizantha* cv. Marandú (CIAT 6780), cv. Toledo (CIAT 26110), one line of this species called Mixe, as well as *Brachiaria* hybrid cv. Mulato (CIAT 36061) and

Table 25. Plant height of *Brachiaria* lines established for participatory evaluation at El Rodeo farm (Puriscal, Costa Rica) 109 days after planting.

Species	Plant height (cm)
B. brizantha (Mixe)	67.8 a*
B. brizantha cv. Marandú (CIAT 6780)	66.7 a
B. hybrid cv. Mulato (CIAT 36061)	60.6 a
B. brizantha cv. Toledo (CIAT 26110)	56.7 a
B. decumbens cv. Basilisk (CIAT 606)	55.5 a
B. brizantha CIAT 26124	51.1 ab
B. brizantha CIAT 26990	49.4 ab
B. brizantha cv. La Libertad (CIAT 26646)	44.2 abc
B. hybrid CIAT 36062	30.5 bc
B. humidicola cv. Llanero (CIAT 6133)	25.3 c
B. brizantha CIAT 26318	24.5 c

*p<0.001 (Duncan's Multiple Range Test)

B. decumbens cv. Basilisk (CIAT 606), among others, had well developed plants and more vigorous establishment than other lines under test. However, *B. humidicola* (synonymous: *B. dictyoneura*) cv. Llanero is a highly stoloniferous species that covers very well the soil and establishes well although produces shorter plants.

Participatory evaluation: Two groups of farmers formed by 8 and 12 participants and coming from the localities of La Guácima and El Rodeo, were selected to participate in separate sessions for participatory evaluation of the trial, as illustrated in Photo 14. One session was carried out in February (dry period) and another in May (beginning of the wet season).

Photo 14. Participatory evaluation of Brachiaria species and hybrids established in El Rodeo, Costa Rica

The variables plant cover, leafiness, leaf texture and color, were chosen to measure degree of preference and probabilities of adoption of the *Brachiaria* germplasm under test. The data were pooled and statistically analyzed following a logistics regression model suggested by L. A. Hernández (CIAT, 2000).

A summary of the results is presented in Table 26. Farmers showed special preference for the commercial materials *B. brizantha* cv. Toledo, Diamantes 1 (Marandú) and La Libertad, particularly referred to plant cover and abundance of leaves. Cultivar Mulato had a medium acceptance for the same variables but was rated high for leaf texture and color. Similar preference was showed by *B.* hybrid CIAT 36062, although in this case plant cover was rated low. Other entries had low to medium acceptance for the variables measured, including the commercial line *B. decumbens* cv. Basilisk.

An additional participatory evaluation will be conducted next October, and the experiment will continue under evaluation for another growing season.

Participatory evaluation in Nicaragua

The first agronomic evaluation (Table 27) showed no significant fertilizer effect on plant height, soil cover and dry matter yield. This was mainly due to greater availability of exchangeable cations. There were significant differences among accessions/ hybrids. However, no accession/

Table 26. Farmer preferences of ten species and hybrids of *Brachiaria* established for evaluation at the locality of El Rodeo, Costa Rica (Information supplied by Beatriz Sandoval and Marco Lobo from INTA).

Species	Plant cover	Abundance of foliage	Foliage texture and color
B. decumbens cv. Basilisk (CIAT 606)	medium*	low	medium
B. brizantha cv. Diamantes 1 (CIAT 6780)	high	high	medium
B. brizantha cv. La Libertad (CIAT 26646)	high	high	medium
B. hybrid CIAT 36062	low	medium	high
B. hybrid cv. Mulato (CIAT 36061)	medium	medium	high
B. brizantha (Mixe)	low	low	medium
B. brizantha cv. Toledo (CIAT 26110)	high	high	high
B. humidicola cv. Brunca (CIAT 6133)	low	high	low
B. brizantha CIAT 26124	low	high	medium
B. brizantha CIAT 26990	medium	medium	low

* The groups high, medium and low presented significant differences between them ($p<0.05$, Chi-square Test).

Table 27. Plant height, soil cover and DM yield of 14 *Brachiaria* accessions and hybrids in Ubú Norte, Nicaragua

Parameter	Plant height (cm)		Soil cover (%)		DM yield (g/m^2)	
Fert. Level						
- High	88	ns *	64	ns	802	ns
- Low	82		60		747	
No. CIAT						
606	90	bcd	76	abc	578	cd
654	71	cde	48	de	538	cde
679	32	e	17	e	-	-
6133	55	ef	30	ef	339	de
6780	126	a	89	ab	1000	bc
16322	98	bcd	88	ab	646	cd
26110	108	ab	65	bcd	1682	a
26124	99	bc	89	ab	1239	ab
26318	90	bcd	34	ef	854	bcd
26646	75	cde	48	de	505	cde
26990	87	bcd	60	cd	695	bcd
36061	99	bc	93	a	1546	a
36062	70	de	32	ef	440	cde
"Mixe"	89	bcd	90	ab	775	bcd

(* $P \leq 0.05$; Duncan's Multiple Range Test; ns = not significant)

hybrid interactions with fertilizer levels were detected. *Brachiaria brizantha* cv. Toledo (CIAT 26110) and *Brachiaria* hybrid cv. Mulato (CIAT 36061) produced the largest amount of dry matter yield, followed by *B. brizantha* (CIAT 26124) and *B. brizantha* cv. Marandú (CIAT 6780). The lowest yields were obtained from *B. humidicola* (CIAT 679) and *B. dictyneura* (CIAT 6133) due to their slow and insufficient establishment. Best soil cover was observed in plots with *B.* hybrid cv. Mulato (CIAT 36061), *B. brizantha* "Mixe", *B. brizantha* (CIAT 26124), and *B. brizantha* cv. Marandú (CIAT 6780). The tallest plants were produced by *B. brizantha* cv. Toledo (CIAT 26110) and *B. brizantha* cv. Marandú (CIAT 6780).

Prior to agronomic data collection, a farmer group (7 pers.) evaluated the plots in accordance with their own criteria. Their preference ranking resulted as follows: *B. brizantha* cv. Marandú (CIAT 6780), *B. brizantha* cv. Toledo (CIAT 26110), *Brachiaria* hybrid cv. Mulato (CIAT 36061), *B. brizantha* (CIAT 26990), *B. brizantha* (CIAT 26124), *B. brizantha* (CIAT 16322), *B. decumbens* (CIAT 606), *B. brizantha* "Mixe", *B. ruzizensis* (CIAT 654), *B. brizantha* (CIAT 26318), *B. brizantha* cv. La Libertad (CIAT 26646), *B*. hybrid (CIAT 36062), *B. dictyoneura* (CIAT 6133), and *B. humidicola* (CIAT 679). The applied criteria were plant height, soil cover, foliage production, leave size and color. While the high ranking of *B. brizantha* cv. Marandú (CIAT 6780) was somewhat expected, the cultivar is known in the area for years and well-adapted to the prevailing conditions, *B. brizantha* cv. Toledo (CIAT 26110), *Brachiaria* hybrid cv. Mulato (CIAT 36061) were preferred because of their abundant foliage and green leaves. The fact that this year both materials were heavily sold on the seed market could have influenced the ranking. Accessions such as *B. brizantha* CIAT 26990, 26124, 16322 were classified as less productive because of their leaf size. All other materials were rated low due to low soil cover or plant height.

Through additional application of fertilizer it may be possible for detecting accession/hybrid interactions with fertility levels. Agronomic data and farmers' ranking presents a similar first picture of the materials established in Ubú Norte. Further evaluations will generate better conclusions.

Participatory evaluation in Honduras

Vigor, flowering time, competitiveness, earliness, robustness, growth, foliage abundance, rooting capacity, color and drought tolerance were the criteria employed by farmers in assessing forage grasses. The global analysis for wet season showed that the first 4 Dims (row coordinates factors) explained 86.9 % of the variation, a high percentage in analyzing participatory work. Growth, vigor and time of flowering were the most important criteria defined in Dim 1. Cover and Competitiveness were the most important criteria in Dim 2. Color and earliness were the most important criteria in Dim 3, while robustness was the most important criteria defined in Dim4.

The global analysis for the dry season showed that the first 3 Dims explained 87 % of the variation. Abundance of foliage, drought tolerance and robustness were defined in Dim 1, while rooting capacity, softness, and leafiness were the most important parameters in Dim2. Color and cover were the most important parameters in Dim3. Hence, for farmers it is important that independent of season, forages have good establishment and growth and compete effectively with weeds. In the dry season leaf color as an indicator of drought tolerance, i.e. staying green and retaining leaves becomes an important criterion for farmer selection.

In the dry season *Brachiaria* hybrid cv. Mulato (CIAT 36061) and *B. brizantha* cv Toledo (26110) were the forage options most preferred by farmers; *B. brizantha* CIAT 6780 and *Brachiaria* hybrid CIAT 36062 had an intermediate preference. In the wet season *B. brizantha* cv Toledo, *B. brizantha* CIAT 6780 and *Brachiaria* hybrid CIAT 36062 were the most preferred materials; *Brachiaria* hybrid cv. Mulato obtained an intermediate classification. Over seasons *Brachiaria brizantha* cv. Toledo, *B. brizantha* CIAT 6780, *Brachiaria* hybrid cv Mulato (CIAT 36061), and *Brachiaria* hybrid (CIAT 36062) were the grasses best responding to farmers' criteria.

Preferences were based on farmer evaluation of materials under cutting in small plots. For a final assessment, an evaluation including the effect of the grazing animal on the grasses may be required. Preliminary results from this multilocational participatory evaluation of *Brachiaria* genotypes indicate that farmers' criteria for identifying the promising genotypes include rapid establishment, vigor, competetiveness, leaf color and texture, and green leaf production in the dry season. Further evaluations are planned for evaluating persistence.

3.1.3 Edaphic adaptation of *Arachis pintoi*

3.1.3.1 Genotypic variation in *Arachis pintoi* for tolerance to low P supply

Contributors: N. Castañeda, N. Claassen (University of Goettingen, Germany) and I. M. Rao (CIAT)

Rationale

Field studies conducted in Caqueta, Colombia and greenhouse studies conducted at CIAT-Palmira indicated significant genotypic variation in P acquisition and utilization in *A. pintoi*. In order to identify P-efficient *Arachis pintoi* genotypes and to define specific mechanisms contributing to P efficiency, a Ph. D. theis work is in progress at the University of Goettingen in collaboration with CIAT. Two growth chamber studies were conducted and results from the first study to determine genotypic differences among ten accessions of *A. pintoi* in P-acquisition and utilization from low P soil were reported last year. Results from this study indicated that the commercial cultivar (CIAT 17434) is relatively less adapted to low P supply in soil. This study also confirmed the previous results obtained under field conditions that the accession CIAT 22159 is better adapted to very low P supply in oxisols. Results from the second study that included 3 contrasting accessions and aimed at identification of specific physiological mechanisms of P efficiency are reported below.

Material and Methods

A growth chamber trial was conducted at Institut for agricultural chemistry – Goettingen, Germany. An Oxisol (clay 50%, organic carbon 0.35%, pH_{CaCl2} 5.1, pH_{H2O} 5.2, P-CAL 0.4 mg /100 g soil and P-Bray II 1.4 mg/100 g soil, Fe/Al-P 788 mg kg^{-1} and Ca-P 330 mg kg^{-1}; in soil solution pH 4.9 and 0.1 µM P L^{-1}) was used for evaluating the P efficiency of 3 accessions of *A. pintoi* (CIAT 17434, 18747, 22172) in a pot experiment. A low P-adapted commercial peanut, *A. hypogea* cv. AK-12/24, from India was used as a crop control. Treatments were arranged in a complete randomised block design with 3 replications. Three levels of P supply were used and three harvests (30, 60 and 90 days after planting) were conducted in order to evaluate the influence of the plant growth on the P-influx. Among the 3 *A. pintoi* accessions tested, CIAT 17434 is a commercial forage cultivar in Latin America.

Plastic pots of 4 L capacity were filled with 2.7 kg air-dry soil at a bulk density of 1.2 g cm^{-3}. Three levels of P supply (0, 50 and 1000 mg kg^{-1}) as $Ca(H_2PO_4)_2.H_2O$ were given. Basal nutrients were applied (mg kg^{-1}) to each pot:, 50 K as K_2SO_4, 40 Mg as $MgSO_4$, 0.2 B as H_3BO_3, 0.1 Mo as $(NH_4)_6Mo_7O_{24}$ and 100 N as $Ca(NO_3)_2$ was applied every 30 days. Two weeks before sowing, water was added to get a moisture content of 25% w/w. Seeds were sown directly in the pots. One pot for each P treatment was kept unplanted to measure soil moisture evaporation losses from the pots. Plants were thinned at the earliest to maintain 4 plants in each pot. The soil surface in each pot was covered with a layer of quartz sand (1 to 2 cm) to avoid the formation of a superficial crust due to the watering. The pots were watered daily and water was added to maintain the soil with 60% of its water holding capacity. Pots were kept in a growth chamber, maintained at 25°C, with a photon flux density of 300 µmol m^{-2} s^{-1} and 80% relative humidity during 16 h day and at 20 °C and 70% relative humidity during 8 h night.

At harvest, quartz sand was removed form the soil surface and shoots were cut above the soil surface. The dry weight of shoot was recorded. Roots were carefully separated by washing the soil on a sieve with 200 µm mesh width. Roots were cleaned of any foreign material, surface moisture was removed by centrifuging and root fresh weight was determined. Subsamples of around 0.5 g were preserved in 20% ethanol for root length measurements using line intersection method. To determine P concentration in plant tissue, plant samples were wet digested in HNO_3

and P was determined with Molybdate-Vanadium method. Shoot P uptake, shoot growth rate, P acquisition efficiency (mg of P uptake in shoot biomass per unit root length), P use efficiency (g of forage production per g of total P acquisition), and P-Influx were determined. Data were subjected to an analysis of variance using the SAS computer program. Least-significant differences were calculated by an F-test. A probability level of 0.05 was considered statistically significant.

Results and Discussion

With no external supply of P (P-0) to an infertile oxisol, the commercial cultivar (CIAT 17434) was least productive over time (30, 60 and 90 days after planting) in terms of shoot biomass compared with the other 2 accessions (22172 and 18474) and commercial cultivar of peanut, *A. hypogea* (Figure 44). Among the 4 genotypes tested, CIAT 18744 was outstanding in shoot biomass production with different levels of P supply. This observation is consistent with field observations made at Carimagua (Oxisol) and Caqueta (Ultisol) where the commercial cultivar (CIAT 17434) was slow in establishment. Response to increase in P supply in terms of shoot growth was also markedly greater with *A. pintoi* accessions than A. hypogea. This was mainly due to belowground pod development and growth in peanut over time (Table 28). This greater development of pods in peanut had contributed to greater P uptake into the pods particularly with high P supply. Results on shoot growth rate over time showed that the accession CIAT 18744 was outstanding with P-0 treatment in exhibiting 100% of the growth rate of P-1000 between 60 and 90 days of growth (Figure 45).

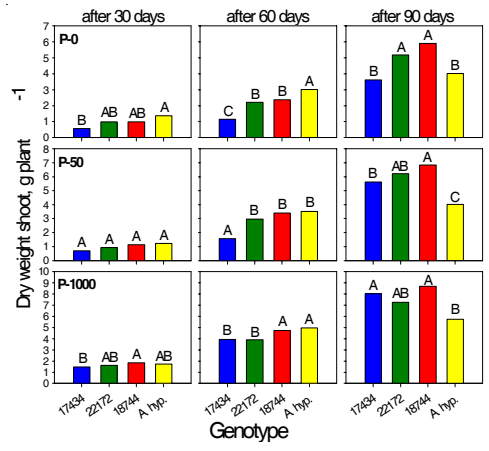

Figure 44. Influence of phosphorus supply (P-0, P-50 and P-1000) on genotypic variation in shoot biomass (forage) production of 3 accessions of *Arachis pintoi* and 1 accession of *Arachis hypogea* grown for 30, 60 and 90 days in a clay loam oxisol in a growth chamber. Means are different (P<0.05) if letters above bars are different within P supply level at a given age.

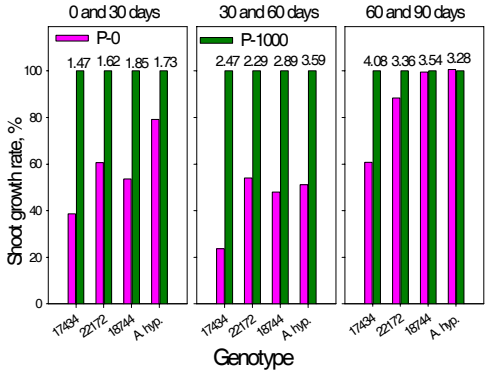

Figure 45. Genotypic variation as influenced by phosphorus supply (P-0 and P-1000) in shoot growth rate of 3 accessions of *Arachis pintoi* and 1 accession of *Arachis hypogea* grown for 30, 60 and 90 days in a clay loam oxisol in a growth chamber. Absolute values of plant growth rate (grams of dry weight per plant per month) with P-1000 are shown at the top of the bars for each genotype.

Differences in P use efficiency (g of shoot biomass produced per mg of P uptake in the plant) were not consistent among the 3 accessions of *A. Pintoi* indicating that internal P utilization efficiency is not that different among the accessions during establishment (Figure 46). But shoot P uptake was significantly greater with CIAT 18744 and 22172 than the commercial cultivar 17434 (Figure 47). This greater acquisition of P by these two accessions contributed to their superior performance under P-0 treatment. The shoot P uptake of peanut variety was markedly lower due to greater pod development (Table 28). The superior acquisition of P by CIAT 18744 and 22172 was not related to their root system development as revealed by the relationship of root length to shoot biomass ratio (Figure 48). These results on root length to shoot biomass ratio indicate that the greater

Table 28. Production of pods and their contribution to P uptake.

Genotype	Dry weight of pods (g plant^{-1})			Pod P uptake (mg plant^{-1})		
	P-0	P-50	P-1000	P-0	P-50	P-1000
60 DAP						
CIAT 17434	-	-	-	-	-	-
CIAT 18744	-	-	-	-	-	-
CIAT 22172	-	-	-	-	-	-
A.h AK-12/24	0.57	2.1	1.36	1.34	6.67	5.38
90 DAP						
CIAT 17434	0.05	-	-	0.1	-	-
CIAT 18744	-	-	0.05	-	-	0.1
CIAT 22172	-	-	-	-	-	-
A.h AK-12/24	10.37	11.03	11.31	22.57	33.67	47.48

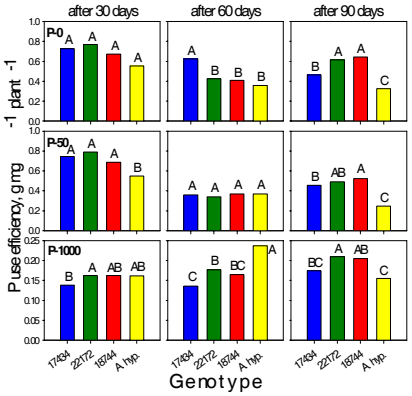

Figure 46. Influence of phosphorus supply (P-0, P-50 and P-1000) on genotypic variation in P use efficiency of 3 accessions of *Arachis pintoi* and 1 accession of *Arachis hypogea* grown for 30, 60 and 90 days in a clay loam oxisol in a growth chamber. Means are different (P<0.05) if letters above bars are different within P supply level at a given age.

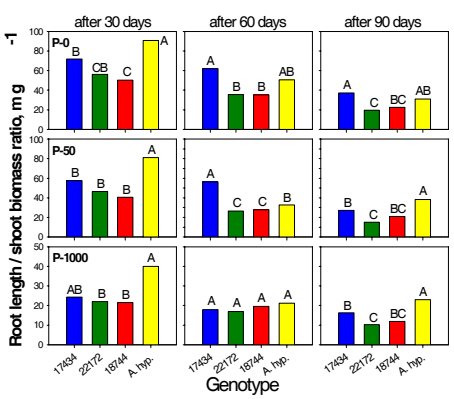

Figure 48. Influence of phosphorus supply (P-0, P-50 and P-1000) on genotypic variation in root length/shoot biomass ratio of 3 accessions of *Arachis pintoi* and 1 accession of *Arachis hypogea* grown for 30, 60 and 90 days in a clay loam oxisol in a growth chamber. Means are different (P<0.05) if letters above bars are different within P supply level at a given age.

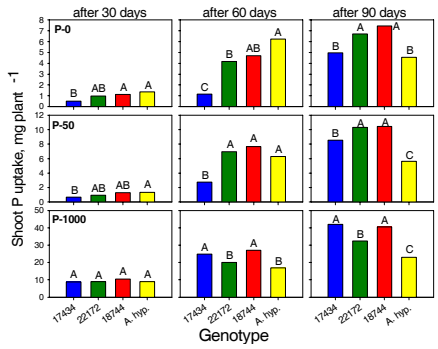

Figure 47 . Influence of phosphorus supply (P-0, P-50 and P-1000) on genotypic variation in shoot P uptake of 3 accessions of *Arachis pintoi* and 1 accession of *Arachis hypogea* grown for 30, 60 and 90 days in a clay loam oxisol in a growth chamber. Means are different (P<0.05) if letters above bars are different within P supply level at a given age.

ability to acquire P from P-0 treatment by these 2 accessions could be related to biochemical changes induced in the rhizosphere. For example exudation of organic acids or enzymes that can mobilize less available P forms in the rhizosphere.

Measurements of P influx at different times after planting showed that there was significantly greater P influx in CIAT 18744 and 22172 than CIAT 17434 between 30 and 60 days of growth with P-0 and P-50 treatments (Figure 49). Measurements of P concentration in soil solution showed that CIAT 18744 was particularly outstanding in increasing P level in soil solution with P-0 treatment. Further research work is in progress to evaluate the role of root exudation in this increase in P concentration in soil solution.

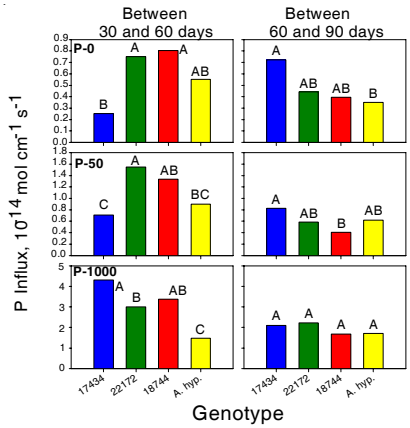

Figure 49. Influence of phosphorus supply (P-0, P-50 and P-1000) on genotypic variation in P influx of 3 accessions of *Arachis pintoi* and 1 accession of *Arachis hypogea* grown for 30, 60 and 90 days in a clay loam oxisol in a growth chamber. Means are different (P<0.05) if letters above bars are different within P supply level at a given age.

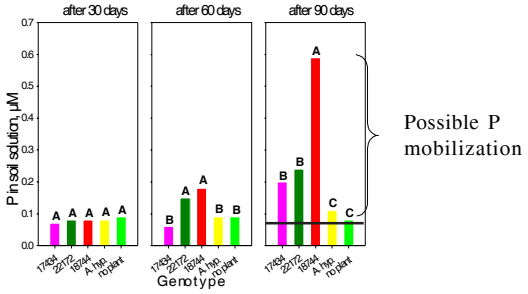

Figure 50. Influence of 3 accessions of *Arachis pintoi* and 1 accession of *Arachis hypogea* on P concentration in soil solution when grown with no phosphorus supply (P-0) for 30, 60 and 90 days in a clay loam oxisol in a growth chamber. Means are different (P<0.05) if letters above bars are different within P supply level at a given age. DAP = days after planting.

This study conducted under controlled environmental conditions in a growth chamber indicated that the rapid establishment and greater P efficiency of CIAT 18744 and 22172 are associated with their greater ability to acquire P from P-deficient soil but not due to their ability to utilize acquired P. (Figure 50).

3.1.3.2 Field evaluation of promising accessions of *Arachis pintoi* in the Llanos of Colombia

Contributors: I. M. Rao, M. Peters, C. Plazas and J. Ricaurte

Rationale

Based on field studies conducted in Caqueta, Colombia and the data collected from multilocational evaluation, we have assembled a set of 8 genotypes for further testing at two sites (Piedmont and Altillanura) in the Llanos of Colombia. The site in Piedmont is close to La Libertad (CORPOICA Experimental Station) and the soils in this region are relatively more fertile than in the Altillanura. The site in Altillanura is at Matazul farm where the soils are relatively infertile (sandy loam). The main objective of this work was to identify plant attributes related to superior adaptation of the most promising accessions for the llanos of Colombia.

Materials and Methods

A field trial was established in May, 2001. The trial was planted in Piedmont as monoculture. We expect multiple use for this legume in the Piedmont area (e.g., cover legume in plantations). The trial included 8 accessions of *Arachis pintoi* (CIAT 17434; 18744; 18747; 18748; 18751; 22159; 22160 and 22172). The trial was planted as randomized block in split-plot arrangement with two levels of initial fertilizer application (low: kg/ha of 20P, 20K, 33Ca, 14 Mg, 10S; and high: 80N, 50P, 100K, 66Ca, 28Mg, 20S and micronutrients) as main plots and genotypes as

sub-plots with 3 replications. Genotypic differences in agronomic performance were determined at 28 months after establishment (September 2003) at low and high initial fertilizer application. Maintenance fertilizer at half the level of initial applications was applied at 26 months after establishment (August 2003).

Results and Discussion

A visual assessment of vigor and soil cover was carried out at 27 months after establishment showed that under both low and high initial fertilization, CIAT 18751 showed greater vigor and soil cover.

At 28 months after establishment of the trial, the response in terms of shoot biomass production with fertilizer application was greater with the commercial check, CIAT 17434 and CIAT 22160 (Figure 51). Overall the performance of CIAT 18744 and CIAT 18751 and CIAT 22159 was better than the other accessions. A number of plant attributes including nutrient uptake are being monitored to evaluate persistence. This field study indicated that the *Arachis pintoi* accessions CIAT 18744, 18751 and 22159 are superior to the commercial cultivar (CIAT 17434) in terms of persistence into third year after establishment with low amounts of initial fertilizer application.

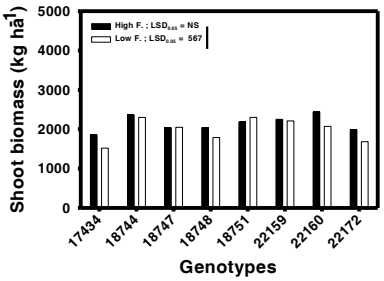

Figure 51. Genotypic variation in forage yield of 8 accessions of *Arachis pintoi* at 28 months after establishment (September 24, 2003) in forage yield (kg/ha) as influenced by initial level of fertilizer application to a clay loam oxisol at La Libertad, Piedmont.

3.2 Identication of genotypes of grasses and legumes with dry season tolerance

Highlights

- Superior performance of one germplasm accession (CIAT 26110) and one hybrid (FM9503-S046-024) of *Brachiaria* which maintained greater proportion of green leaves during dry season in the Llanos of Colombia, is associated with greater acquisition of nutrients under water deficit conditions.

3.2.1 Field evaluation of *Brachiaria* hybrids for drought tolerance in a subhumid environment of Costa Rica

Contributors: Pedro J. Argel and Guillermo Pérez (CIAT)

Rationale

Commercial lines of the genus *Brachiaria* are widely used in the tropics as forage plants. However given the diversity of ecosystems and systems of production a broad field still exists for *Brachiaria* lines of high forage quality and better adaptation to drought, waterlogged sites and poor soils. More recently new hybrids of *Brachiaria* are available from a breeding program carried out at CIAT headquarters, thus it is worth to investigate the climate and soil adaptation as well as the potential yield of this germplasm as compared with traditional lines of *Brachiaria.*

Material and Methods

Six *Brachiaria* hybrids were direct planted using acid scarified seeds in a randomized block design with four replicates at Atenas, Costa Rica. Plot size was 2 m wide x 3 m long (6 m^2) and the planting distances were respectively 0.50 m between plants and rows; plants were thinned to two per site two weeks after seedling emergence and plant height and plant cover measured 16 weeks after planting. Dry matter yield was measured by cutting 9 central plants at 10 cm height after 5 and 8 weeks re-growth respectively during the wet and dry periods; a total of 6 evaluation cuts during the wet period and 3 cuts during the dry period have been carried out up to date. The experiment will finalize at the end of the next dry period for a total of two years of evaluation. The site is located in a subhumid environment with a total annual rainfall of 1600 mm, and 5 to 6 months dry from December to May. The soils are Inceptisol of medium fertility with pH 5.0 and low P and low aluminum content.

Results and Discussion

All *Brachiaria* hybrids established well and no re-planting of the plots was necessary. The accession *Brachiaria* hybrid CIAT 36061 (cv. Mulato) presented high seedling vigor and two weeks after planting all experimental plots of this line had more developed plants than the other hybrids. It has been also observed that following evaluation cuts this hybrid produces rapid re-growths, comparable only to those observed in *Brachiaria* hybrid CIAT 46024.

Plant growth and DM yields: Four months after establishment cv. Mulato had significantly taller plants ($p<0.05$) than other *Brachiaria* hybrids under evaluation (Table 29). However at this date, plant cover was not hundred percent for any hybrid and it was very similar between hybrids with the exception of CIAT 4015 that had significantly less soil cover. None of these hybrids is truly stoloniferous, thus it cannot be expected that under a cutting regime as it was the case in this experiment, a complete soil cover will be observed.

Total DM yields have been very similar between cv. Mulato and the *Brachiaria* hybrids BR99NO/4015, C BR99NO/4132 and FM9503-SO46-O24 (Table 30). This tendency has been observed for both the wet and dry season yields ($p<0.1$). However, the hybrid CIAT FM9503-SO46-O24 had a tendency to produce more yield during the dry season, which is a desirable characteristic of this promising line. Less DM yield have been recorded for the hybrids BR98NO/1251 and CIAT 36062, which seem to be poorly adapted to the Atenas conditions.

Table 29. Plant height and plant cover of *Brachiaria* hybrids 4 months after establishment at Atenas, Costa Rica

Hybrid	Plant height (cm)	Plant cover (%)
CIAT 36061 (cv. Mulato)	73.5 a*	85.3 a*
BR99NO/4132	35.4 b	80.9 ab
FM9503-SO46-O24	44.9 b	74.7 ab
CIAT36062	36.9 b	73.3 ab
BR98NO/1251	40.3 b	68.0 ab
BR99NO/4015	42.7 b	57.3 b

Although total DM yields were not statistically different between the commercial *Brachiaria* hybrid cv. Mulato ($p<0.1$) and the highly promising new hybrid CIAT 46024 (Table30), there was a clear tendency of the latter to produce more yield at each evaluation cut along

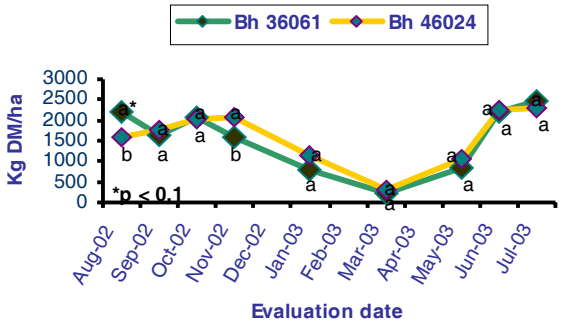

Figure 52. Dry matter (DM) yields under cutting of *Brachiaria* hybrid CIAT 46024 and cv. Mulato (CIAT 36061) from August 2002 through July 2003 in Atenas, Costa Rica.

Table 30. Dry matter yields during the wet and dry periods of *Brachiaria* hybrids established in Atenas, Costa Rica (mean of 6 cuts carried out every 5 weeks during the wet period and mean of 3 cuts carried out every 8 weeks during the dry season)

Hybrid	Mean dry matter yields (kg/ha)		
	Wet	Dry	Total
CIAT 36061 (cv. Mulato)	2030.9 a*	634.2 ab	1570.5 a
FM 9503-S046-024	2000.2 a	832.6 a	1611.9 a
BR99NO/4132	1890.1 ab	588.2 ab	1456.8 ab
BR99NO/4015	1530.8 abc	612.6 ab	1230.9 ab
BR98NO/1251	1360.3 bc	438.8 b	1054.7 b
CIAT 36062	1290.8 c	486.1 ab	1027.8 b

* p<0.1 (Tukey's Studentized Range Test)

the reported evaluation period, as shown in Figure 52. At the first evaluation cut in August 2002, cv. Mulato produced significantly more yield than CIAT 46024, but during subsequent cuts the latter consistently produced more DM yields even during the dry period from December 2002 to May 2003. These yields, although statistically similar, may however have some importance under forage utilization, given that grazing animals would have more forage available in paddocks planted with the hybrid CIAT 46024 in sites with prolonged dry season like Atenas.

3.2.2 Genotypic variation in dry season tolerance in *Brachiaria* in the Llanos of Colombia

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

Rationale

A major limitation to livestock productivity in subhumid regions of tropical America is quantity and quality of dry season feed. A field study is completed this year at Matazul Farm in the Llanos of Colombia. The main objective was to evaluate genotypic differences in dry season (4 months of moderate drought stress) tolerance of most promising genetic recombinants of *Brachiaria*. Results from this field study for the past 2 years indicated that the superior performance of the germplasm accession CIAT 26110 and the *Brachiaria* hybrid, FM9503-S046-024, which maintained greater proportion of green leaves during moderate dry season in the llanos of Colombia, was associated with greater acquisition of nutrients under water deficit conditions. This year, we report results from the dry season performance into fourth year after establishment.

Materials and Methods

A field trial was established on a sandy loam oxisol at Matazul farm in the Llanos of Colombia in July, 1999. The trial comprises 12 entries, including six natural accessions (four parents) and six genetic recombinants of *Brachiaria*. Among the germplasm accessions, CIAT 26110 was identified from previous work in Atenas, Costa Rica as an outstanding genotype for tolerance to long dry season (up to 6 months). The trial was planted as a randomized block in split-plot arrangement with two levels of initial fertilizer application (low: kg/ha of 20 P, 20 K, 33 Ca, 14 Mg, 10 S; and high: 80 N, 50 P, 100 K, 66 Ca, 28 Mg, 20 S and micronutrients) as main plots and genotypes as sub-plots. Live and dead forage yield, shoot nutrient composition, shoot nutrient uptake and leaf and stem TNC (total nonstructural carbohydrates) were measured at the end of the dry season (44 months after establishment; March 3, 2003). Maintenance fertlizer (half the levels of initial application) was applied at the beginning of the wet season of 2001 (July, 2001).

Results and Discussion

Because of the application of maintenance fertilizer, forage yields with high fertilizer treatment were greater than those with low fertilizer treatment (Figure 53). At 44 months after establishment (4 months after dry season – March 3, 2003), live forage yield with low fertilizer application ranged from 0 to 329 kg/ha and the highest value of forage yield was observed with a germplasm accession CIAT 26110. This accession was released in Costa Rica as cultivar Toledo and is known for its dry season tolerance. Among the 4 parents, CIAT 6294 was outstanding in live forage and dead biomass production with low fertilizer application. A spittlebug resistant genetic recombinant, FM9503-S046-024 was superior among the genetic recombinants in terms of greater live shoot biomass, both with low and high fertilizer application. As expected, the performance of one of the parents, BRUZ/44-02 was very poor compared with other parents and genetic recombinants as it produced almost no live forage after dry season. The leaf to stem ratio values of one of the genetic recombinants (BR97NO-0082) were markedly superior to other genotypes under low and high levels of initial fertilizer application (Table 31).

The superior performance of the accession CIAT 26110 with low fertilizer application was mainly attributed to its ability to produce green leaf biomass during dry season (Figure 53). Results on leaf and stem N content indicated significant differences among genetic recombinants, parents and accessions with both levels of fertilizer application (Table 32). But shoot N uptake with low fertilizer application was markedly greater for the hybrid, FM9503-5046-024 (Table 32; Figure 54). With high fertilizer application, the hybrid FM9503-5046-024 and CIAT 26110 were outstanding in shoot N uptake. Shoot uptake of P, K, Ca and Mg was also greater with the hybrid FM9503-5046-024 and CIAT 26110 (Tables 33 and 34; Figure 54). Among the parents, CIAT 6294 was superior in P, K, Ca and Mg acquisition from both low and high fertilizer application.

Correlation analysis between green leaf biomass produced in the dry season and other shoot attributes indicated that superior performance with low and high fertilizer application was associated with greater stem biomass indicating the importance of stem reserves for production of green leaf biomass (Table 35). No significant negative association was observed between green leaf biomass and level of nutrients in green leaves. But significant negative association was observed between green leaf biomass production and stem P

Table 31. Genotypic variation as influenced by fertilizer application in live shoot biomass, leaf to stem ratio and total forage yield of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 44 months after establishment (at the end of the dry season – March 2003). LSD values are at the 0.05 probability level.

Genotype	Live shoot biomass		Leaf to stem ratio		Total forage yield	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	(kg/ha)-					
Recombinants:						
BR97NO-0082	121	145	1.5	2.3	544	848
BR97NO-0383	130	227	1.5	0.7	969	1040
BR97NO-0405	116	181	1.1	0.5	1734	1759
FM9201-1873	136	238	1.1	1.1	460	1181
FM9301-1371	180	226	0.9	1.2	612	938
FM9503-5046-024	269	386	0.9	0.8	1323	1814
Parents:						
CIAT 606	100	108	1.4	0.7	870	758
CIAT 6294	326	288	0.6	0.6	2145	2093
BRUZ/44-02	0	0	.	.	0	0
CIAT 26646	88	198	0.8	1.0	1115	2397
Accessions:						
CIAT 26110	203	454	1.4	0.4	2536	3474
CIAT 26318	185	314	1.6	0.3	2498	2865
Mean	160	231			1309	1615
LSD *(P=0.05)*	156	171			1146	808

Table 32. Genotypic variation as influenced by fertilizer application in leaf N content, stem N content and shoot N uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 44 months after establishment (at the end of the dry season - March 2003). LSD values are at the 0.05 probability level.

Genotype	Leaf N content		Stem N content		Shoot N uptake	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	---------- (%) ---------		---------- (%) ----------		---------- (kg/ha) --------	
Recombinants:						
BR97NO-0082	2.02	2.21	0.84	0.91	1.75	2.64
BR97NO-0383	2.32	1.94	0.87	0.59	2.15	2.49
BR97NO-0405	1.98	2	1.03	0.63	1.79	1.89
FM9201-1873	1.78	2.02	0.97	0.55	1.91	3.06
FM9301-1371	2.42	2.16	1.15	0.98	3.07	3.63
FM9503-5046-024	2.26	2.02	0.85	0.5	3.63	4.77
Parents:						
CIAT 606	2.1	2.16	0.72	0.65	1.51	1.46
CIAT 6294	2.1	1.97	0.66	0.54	3.94	3.03
BRUZ/44-02	-	-	-	-	-	-
CIAT 26646	1.53	1.64	0.69	0.44	0.78	2.05
Accessions:						
CIAT 26110	2.32	2.14	0.68	0.51	3.18	4.36
CIAT 26318	2.18	2.27	0.45	0.4	2.80	2.65
Mean	2.08	2.05	0.78	0.61	2.41	2.98
LSD *(P=0.05)*	0.47	0.33	0.44	0.26	1.99	1.97

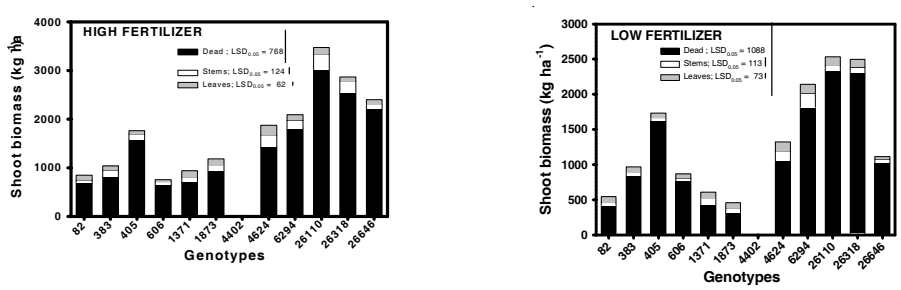

Figure 53. Genotypic variation as influenced by fertilizer application in dry matter distribution among green leaves, stems and dead biomass of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 44 months after establishment (at the end of the dry season – March 2003). LSD values are at the 0.05 probability level.

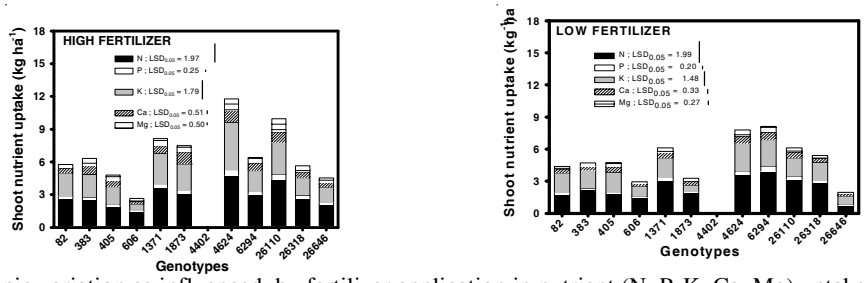

Figure 54. Genotypic variation as influenced by fertilizer application in nutrient (N, P, K, Ca, Mg) uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 44 months after establishment (at the end of the dry season – March 2003). LSD values are at the 0.05 probability level.

and Ca content. The usefulness of this trait for evaluating dry season tolerance needs further research work. Stem ash (mineral) content was also negatively associated with green leaf biomass. Results from this field study indicated that the superior performance of one germplasm accession (CIAT 26110) and one genetic recombinant (FM9503-S046-024) which maintained greater proportion of green leaves during dry season in the Llanos of Colombia, was associated with greater acquisition of nutrients under water deficit conditions.

Table 33. Genotypic variation as influenced by fertilizer application in leaf P content, stem P content and shoot P uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 44 months after establishment (at the end of the dry season - March 2003). LSD values are at the 0.05 probability level.

Genotype	Leaf P content		Stem P content		Shoot P uptake	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	---------- (%) ---------		---------- (%) ----------		------- (kg/ha) -------	
Recombinants:						
BR97NO-0082	0.19	0.18	0.13	0.09	0.20	0.22
BR97NO-0383	0.18	0.17	0.09	0.08	0.19	0.26
BR97NO-0405	0.30	0.24	0.10	0.10	0.24	0.26
FM9201-1873	0.14	0.18	0.08	0.07	0.15	0.33
FM9301-1371	0.21	0.20	0.11	0.11	0.28	0.36
FM9503-5046-024	0.19	0.20	0.08	0.06	0.34	0.50
Parents:						
CIAT 606	0.15	0.17	0.08	0.05	0.12	0.10
CIAT 6294	0.24	0.20	0.09	0.05	0.47	0.31
BRUZ/44-02	-	-	-	-	-	-
CIAT 26646	0.18	0.17	0.09	0.06	0.12	0.23
Accessions:						
CIAT 26110	0.23	0.23	0.07	0.06	0.32	0.53
CIAT 26318	0.20	0.20	0.06	0.06	0.27	0.29
Mean	0.20	0.19	0.09	0.07	0.25	0.31
LSD *(P=0.05)*	0.07	0.05	0.03	0.04	0.20	0.24

Table 34. Genotypic variation as influenced by fertilizer application in shoot K uptake, shoot Ca uptake and shoot Mg uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 44 months after establishment (at the end of the dry season - March 2003). LSD values are at the 0.05 probability level.

Genotype	Shoot K uptake		Shoot Ca uptake		Shoot Mg uptake	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
			(kg/ha)			
Recombinants:						
BR97NO-0082	1.82	2.14	0.35	0.41	0.25	0.34
BR97NO-0383	1.67	2.10	0.39	0.82	0.30	0.63
BR97NO-0405	1.81	1.59	0.52	0.54	0.39	0.50
FM9201-1873	0.59	2.41	0.36	1.11	0.24	0.57
FM9301-1371	1.85	2.79	0.49	0.69	0.43	0.64
FM9503-5046-024	2.56	4.34	0.70	1.08	0.55	1.08
Parents:						
CIAT 606	0.93	0.58	0.21	0.24	0.16	0.24
CIAT 6294	2.50	1.85	0.69	0.68	0.54	0.50
BRUZ/44-02	-	-	-	-	-	-
CIAT 26646	0.74	1.42	0.20	0.41	0.14	0.40
Accessions:						
CIAT 26110	1.7	2.88	0.52	0.98	0.42	1.18
CIAT 26318	1.71	1.60	0.35	0.53	0.28	0.53
Mean	1.63	2.16	0.44	0.67	0.34	0.60
LSD *(P=0.05)*	1.48	1.79	0.33	0.51	0.27	0.50

Table 35. Correlation coefficients (r) between green leaf biomass (t/ha) and other shoot traits of *Brachiaria* genotypes grown with low or high fertilizer application in a sandy loam oxisol in Matazul, Colombia.

Shoot traits	Low fertilizer	High fertilizer
Live forage yield (t/ha)	0.85***	0.77***
Total forage yield (live + dead) (t/ha)	0.57***	0.66***
Dead biomass (t/ha)	0.48***	0.57***
Stem biomass (t/ha)	0.93***	0.94***
Leaf TNC content (mg g^{-1})	0.13	-0.08
Leaf ash content (%)	-0.04	0.04
Stem N content (%)	-0.32	0.08
Stem P content (%)	-0.41*	0.04
Stem K content (%)	-0.27	0.21
Stem Mg content (%)	-0.25	0.11
Stem TNC content (mg g^{-1})	-0.13	0.23
Stem ash content (%)	-0.24	0.14

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

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

Contributors: I. M. Rao, J. Miles, C. Plazas, J. Ricaurte and R. Gracía

Rationale

Previous research on evaluation for dry season tolerance in *Brachiaria* grasses indicated that the superior performance of the *Brachiaria* hybrid, FM9503-S046-024 which maintained greater proportion of green leaves during moderate dry season in the Llanos of Colombia, was associated with lower levels of K and N content in green leaves. The main objective of this field study was to evaluate dry season tolerance of the more recent hybrids of *Brachiaria* in comparison with their parents when grown with low nutrient supply in soil at Matazul farm of the altillanura.

Materials and Methods

A field trial was established at Matazul farm on 31 May of 2001. The trial included 4 *Brachiaria* hybrids (BR98NO/1251; BR99NO/4015; BR99NO/4132; FM9503-S046-024) along with 2 parents (*B. decumbens* CIAT 606 and *B. brizantha* CIAT 6294). The trial was planted as a randomized block in split-plot arrangement with two levels of initial fertilizer application (low: kg/ha of 20P, 20K, 33Ca, 14 Mg, 10S; and high: 80N, 50P, 100K, 66Ca, 28Mg, 20S and micronutrients) as main plots and genotypes as sub-plots with 3 replications. The plot size was 5 x 2 m. A number of plant attributes including forage yield, dry matter distribution and nutrient uptake were measured at the end of dry season (march 2003; 4 months of drought stress), i.e., at 22 months after establishment of the trial. The trial was managed with strong and frequent mob grazing at 2 months interval.

Results and Discussion

At 22 months after establishment (4 months after dry season), live forage yield with low fertilizer application ranged from 65 to 235 kg/ha and the highest value of forage yield was observed with the hybrid 4624 (Figure 55). Differences in dry matter distribution among the hybrids and parents indicated that the parent CIAT 6294 was superior to other genotypes in terms of shoot biomass

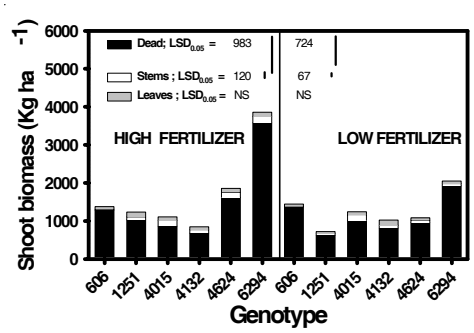

Figure 55. Genotypic variation as influenced by fertilizer application in dry matter distribution among green leaves, stems and dead biomass of two parents (606, 6294) and 4 genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 22 months after establishment (at the end of the dry season – March 2003). LSD values are at the 0.05 probability level.

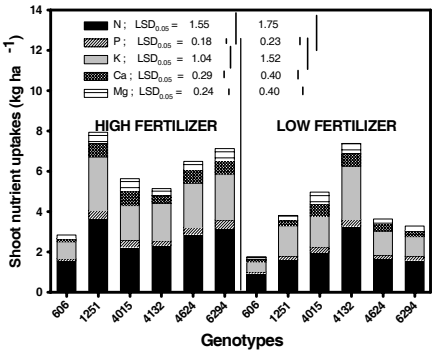

Figure 56. Genotypic variation as influenced by fertilizer application in nutrient (N, P, K, Ca, Mg) uptake of two parents (606, 6294) and 4 genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 22 months after establishment (at the end of the dry season – March 2003). LSD values are at the 0.05 probability level. NS = not significant.

production with both low and high initial fertilizer application. This parent produced greater dead biomass under both low and high initial fertilizer application. The dry matter distribution pattern of the some hybrids such as 4015 was in contrast with that of the parent CIAT 6294. These hybrids produced lower amounts of dead biomass and a greater proportion of aboveground biomass was in green leaves. Another hybrid, 4132 had markedly lower stem biomass compared with green leaf biomass.

The hybrid 4132 was also outstanding in its ability to acquire nutrients, particularly from low application of fertilizer (Figure 56). Results on nutrient uptake also showed that two other hybrids 1251 and 4624 were also superior in their ability to acquire nutrients. The results obtained on green leaf and stem N and P contents also indicated the superior nutritional quality of the hybrid 1251 under both high and low initial fertilizer application (Figure 57). The dry season performance of the 4 hybrids will be monitored for the next 2 years in comparison with the two parents in terms of forage yield and nutrient acquisition.

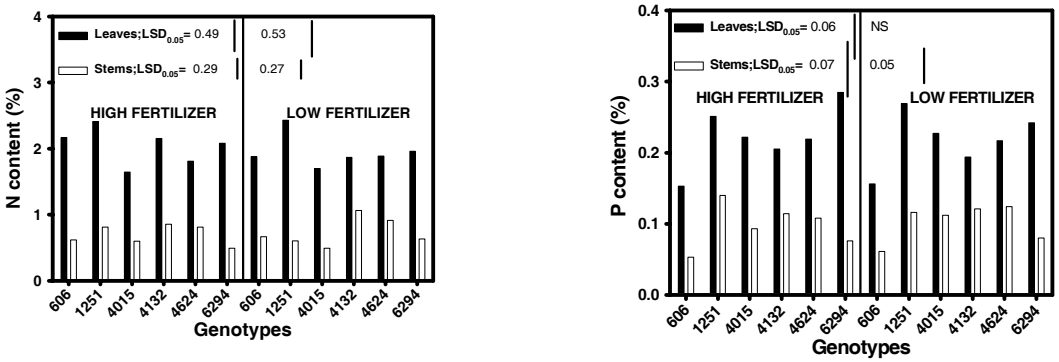

Figure 57. Genotypic variation as influenced by fertilizer application in nitrogen (N) and phosphorus (P) content of leaves and stems of two parents (606, 6294) and 4 genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 22 months after establishment (at the end of the dry season – March 2003). LSD values are at the 0.05 probability level.

3.3 Shrub legumes with adaptation to acid soils and drought

Highlights

- *Accessions of* Cratylia argentea *with superior productivity relative to* C. argentea *cv Veraniega were identified*
- Accessions of *Flemingia macrophylla* with digestibility and productivity superior to Control CIAT 17403 were identified
- RAPD molecular markers for *Cratylia argentea* and *Flemingia macrophylla* were identified

3.3.1 Genetic diversity in the multipurpose shrub legumes *Flemingia macrophylla* and *Cratylia argentea*

Contributors: M.S. Andersson (University of Hohenheim, CIAT), R. Schultze-Kraft (University of Hohenheim), M. Peters, J. Tohme, L.H. Franco, P. Ávila, G. Gallego, Belisario Hincapié, G. Ramirez, C.E. Lascano (CIAT)

Little variation in nutritive value among *Cratylia* accessions was observed. CIAT 18674, 22375, 22406, 22408 and 22409 had higher dry matter yields in the dry and wet seasons than CIAT cv Veranera (CIAT 18516/18668). These accessions, collected in the states of Goias and Mato Grosso in Brazil, were selected for seed multiplication given that they had DM production higher than 3 t/ha per cut, good seed production capacity and equal or superior digestibility and crude protein content than Veranera.

Three semierect accessions CIAT 18437 from Indonesia, and 21083 and 21090 from Thailand were superior in comparison to CIAT 17403 (from Thailand). They had digestibility values >48% and dry matter yields >2 t/ha. More detailed analysis of two subsets showed that accessions with higher feed quality in terms of digestibility have lower fiber and condensed tannin contents than low-quality accessions.

For *Cratylia argentea* analysis of origin, agronomic morphological and molecular marker information did not identify correlations between the clusters obtained in the different approaches. In the case of *Flemingia macrophylla*, clustering obtained by molecular marker information correlated well with morphological information and grouped accessions according to their different growth types.

Rationale

The work of CIAT on shrub legumes emphasizes the development of materials to be utilized as 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. *Flemingia macrophylla* and *Cratylia argentea* have shown promising results in such environments and hence work on these genera is part of the overall germplasm development strategy of the CIAT Forages team.

C. argentea is increasingly adopted and utilized, particularly in the seasonally dry hillsides of Central America, and more recently, the Llanos Orientales de Colombia. However, most research and development is based on only few accessions and hence activities to acquire and test novel germplasm of *C. argentea* are of high priority.

F. macrophylla also is a highly promising shrub legume with excellent adaptation to infertile soils. In contrast to *C. argentea*, whose adaptation is limited to an altitude below 1200 masl, *F. macrophylla* can successfully be grown up to altitudes of 2000 masl. However, the potential utilization of *F. macrophylla* is so far limited by the poor quality and acceptability of the few evaluated accessions.

The project aims to investigate the genetic diversity within collections of *F. macrophylla* and *C. argentea* with three main objectives:

1) To identify new, superior forage genotypes based on conventional germplasm characterization/evaluation procedures (morphological and agronomic traits, forage quality parameters, including IVDMD and tannin contents)
2) To optimize the use and management, including conservation, of the collections. For this, different approaches to identify core collections for each species were tested and compared based on: (a) genetic diversity assessment by agronomic characterization/ evaluation; (b) germplasm origin information; and (c) molecular markers (RAPDs).
3) To assist future germplasm collections on methodology, geographical focus and genetic erosion hazards.

Material and Methods

Agronomic characterization and evaluation*:* Space-planted, single-row plots in RCB design with three replications were established in Quilichao in March 1999 (*Cratylia argentea*, 39 accessions) and March 2000 (*Flemingia macrophylla*, 73 accessions). Additionally two replications were sown for seed production and morphological observations.

The following parameters were measured in the trials: vigor, height and diameter, regrowth, incidence of diseases, pests and mineral deficiencies, and dry matter yield during wet and dry seasons. For the analysis of nutritive value, crude protein content and *in vitro* dry matter digestibility (IVDMD) of the entire collections were analyzed. For the morphological evaluation, qualitative and quantitative parameters were measured, such as days to first flower, days to first seed, flower color, flowers per inflorescence, flowering intensity, pod pubescence, seeds per pod, seed color, leaf area, peduncle length, etc.

For *F. macrophylla*, a more detailed analysis of nutritive value was conducted of a representative subset (25 accessions), which included high, intermediate and low nutritive value accessions. The groups were selected based on crude protein content and IVDMD. The chemical analysis comprised fiber (NDF, ADF, N-ADF), extractable and bound condensed tannin (ECT, BCT) content and astringency (protein binding capacity). Monomer composition of the extractable condensed tannin fraction (procyanidin:prodelphinidin:proelargonidin ratio = C:D:P) was determined with high-performance liquid chromatography (HPLC). In the latter case, results were extremely variable both between laboratory replicates and among field repetitions.

In order to give at least an idea of the monomer composition of extractable condensed tannins in *F. macrophylla* accessions, results from only five accessions, which were consistent between duplicates and among repetitions, are reported. Additionally, another subset of 10 accessions (9 high-quality accessions (18437, 18438, 21083, 21090, 21092, 21241, 21580, 22082, 22327) and CIAT 17403) was sampled 4, 6 and 8 weeks after cutting, to investigate the effect of age on digestibility as well as on protein, fiber and condensed tannin content and astringency.

Based on data referring to the morphological, agronomic and feed quality variation of all accessions a core collection will be created, using multivariate statistic tools (Principal Component Analysis and Cluster Analysis).

Analysis of available origin information: Based on ecogeographical information on origin of accessions, a core collection was created, hypothesizing that geographic distance and environmental differences are related to genetic diversity. The analysis was conducted with FloraMap™, a GIS tool developed by CIAT, which allows the production of climate probability models using Principal Component Analysis (PCA) and Cluster Analysis.

Genetic analysis by molecular markers (RAPDs): Efforts made in genetic analysis showed that common manual DNA extraction

methods did not work well with *F. macrophylla* and *C. argentea*. A modified protocol, which was used to extract DNA showed promising initial results. However, frequent degradation, contamination and partial digestion of DNA occurred, due to secondary plant compounds, probably polyphenols. In preliminary trials with a commercial extraction kit instead, the DNA purity was higher but partial digestion continued to be a severe problem. Various studies with amplified fragment length polymorphism markers (AFLPs), the method of choice, did not succeed and finally studies using this methodology could not be completed. Instead, random amplified polymorphic DNA (RAPD) markers, which do not require enzymatic digestion, were successfully employed.

A total of 47 RAPD 10-mer primers (Operon Technologies, Alameda, CA, USA) were screened as single primers for the amplification of RAPD sequences. Primers with highest levels of polymorphisms were repeated to test for reproducibility and those that produced polymorphic, distinct and reproducible bands were chosen for RAPD analysis. Multiple Correspondence Analysis (MCA) was performed on a matrix created based on the presence (1) or absence (0) of amplified bands. Subsequently, cluster analysis was performed on the coordinates obtained by MCA. Dendrograms were generated using UPGMA method. Nei's coefficient was used as estimator of similarity between accessions in order to generate between- and within-group similarity tables. Diversity was estimated using Nei's H and G_{ST} estimators.

Data analysis and synthesis: Individual and combined data analyses of all generated information was carried out using multivariate statistics. We have applied principle component analysis in all data sets (agronomic, morphological, geographical and molecular). In addition, cluster analysis was performed and the resulting clusters were compared to identify similarities.

Results and Discussion

Agronomic characterization and evaluation*:* Results from evaluations per season carried out for *Cratylia argentea* and *Flemingia macrophylla* indicated considerable phenotypic and agronomic variation in the collections studied. Data for *C. argentea* and *F. macrophylla* have been presented already in previous reports. For *C. argentea,* IVDMD varied between 59 and 69% and crude protein content between 18 and 24%. Mean dry matter production was 2.2 (range 0.8 to 5.2) t/ha and 1.93 (range 0.6 to 3.3) t/ha in the wet and dry season, respectively. Dry season yields were relatively high and confirm the good adaptation of *C. argentea* to dry conditions. There was a pronounced effect of season on some agronomic and quality traits. DM production was higher in the rainy season than in the dry season whereas ADF was higher in the dry than in the wet season. A season x genotype interaction was detected for IVDMD. The cluster analysis dendrogram (Ward's Method) was truncated at the 6-group level. The detailed agronomic characteristics of each group are listed in Table 36. Group 4 was the agronomically most promising cluster. It contained three accessions with the highest DM production (3.2 t/ha in the rainy and 2.4 t/ha in the dry season), CP content, regrowth and plant diameter values. The highest dry matter yields (2.4 to 3.8 t/ha) were recorded in accessions 18674, 22375, 22406, 22408 and 22409 from groups 4 and 6. Productivity of these accessions was higher than yields of the cultivar released in Costa Rica (cv. Veraniega) and Colombia (cv. Veranera) - an accession mixture of CIAT 18516/18668 (yield 1.9 and 3 t/ha). In addition to the higher yield, these accessions also had equal or superior digestibility values (65 to 69%) and crude protein content (20 to 24%) in comparison to CIAT 18516/18668 (IVDMD 64 to 67%, CP 21 to 24%). Based on high forage yield and good seed production potential we selected CIAT 18674, 22375, 22406, 22408 and 22409 for seed multiplication and regional testing (Table 37).

Table 36. Identification of *Cratylia argentea* accessions of agronomic interest. * *rainy/dry season value*

Group 1 (average/low yields, low regrowth, high dry season digestibility, high CP, high ADF):* CIAT 22382, 22390, 22392, 22393, 22394, 22396, 22399, 22411
Group 2 (high/low yields, low regrowth, high dry season digestibility, average CP, high ADF): CIAT 18675, 22380, 22383, 22384, 22386, 22387, 22391
Group 3 (average/low yields, low regrowth, average digestibility, lower CP than group 1, high ADF): CIAT 18672, 22376, 22378, 22381
Group 4 (very high/average yields, good regrowth, high digestibility, high CP, low ADF, low NDF): CIAT 22374, 22375, 22406
Group 5 (high/average yields, good regrowth, average digestibility, average CP, high ADF): CIAT 18676, 18957, 22373, 22400, 22410, 22412
Group 6 (very high/average yields, good regrowth, high digestibility, high CP, low AFD, higher NDF than group 4): CIAT 18516, 18667, 18668, 18671, 18674, 22379, 22404, 22407, 22408, 22409

Table 37. Selected promising *Cratylia argentea* accessions and the two control accessions CIAT 18516/18668. Data of two evaluation cuts with 8 weeks of regrowth per season. IVDMD = *in vitro* dry matter digestibility, CP = crude protein.

Accession number	DM production (t/ha) [a]		IVDMD[b] (%)		CP[c] (%)		Seed production[d] (g/plant)
	Rainy	Dry	Rainy	Dry	Rainy	Dry	
18674	3.82	2.44	65	65	20	23	153
22375	3.12	2.41	65	66	21	23	255
22406	3.54	2.59	64	66	21	22	152
22408	3.25	2.45	69	67	21	22	153
22409	3.11	2.62	66	68	22	24	97
18516 (Control)	3.06	2.04	64	67	21	24	18
18668 (Control)	2.39	1.91	64	65	21	23	110

[a] Plant density 10 000 plants/ha
[b] Two-stage technique (Tilley & Terry 1963)
[c] Kjeldahl nitrogen x 6.25 (AOAC 2003)
[d] 15 months after sowing

In *F. macrophylla,* accessions evaluated differed in IVDMD, DM production, ECT, tannin extractability (ECT/total CT) and astringency (protein binding capacity) whereas CP and BCT showed only minor variability. IVDMD varied from 28 to 58% and crude protein content from 13 to 25%. Mean dry matter production was 2.08 t/ha in the wet and 1.18 t/ha in the dry season. The chemical composition of 25 *F. macrophylla* accessions with contrasting digestibility varied greatly among accessions and in response to harvest season (Tables 38 and 39).

Total condensed tannin content ranged from 1.5 to 16.7% in the rainy season and from 1.8 to 22.4% in the dry season. Astringency ranged from 1.7 to 6.8 (PBE) in the rainy season and from 2.4 to 7.9 in the dry season. The acetone-extractable CT among accessions ranged from 0 to 19.4%, whereas the content of acetone-bound CT ranged from 1.3 to 3.3%. The ECT represented 0% of total condensed tannins in CIAT 21090 but 95% in CIAT 20616. Positive correlations were found between ECT and astringency ($r_{rainy} = 0.712$, $r_{dry} = 0.721$, $P < 0.01$). IVDMD was negatively correlated with ECT ($r_{rainy} = - 0.694$, $r_{dry} = - 0.576$, $P < 0.01$) and astringency ($r_{rainy} = - 0.632$, $r_{dry} = 0.548$, $P < 0.01$).

The monomer composition of the extractable CT fraction in 5 accessions *F. macrophylla* was

Table 38. *In vitro* digestibility, fiber and crude protein content of a representative subset of *F. macrophylla*. Data of one evaluation cut in the wet season and one in the dry season. n = no. of accessions evaluated, IVDMD = *in vitro* dry matter digestibility, CP = crude protein, NDF and ADF = neutral and acid detergent fiber, N-ADF = nitrogen bound to acid detergent fiber.

Forage quality[a]	IVDMD[b] (%)		CP[c] (%)		NDF[d] (%)		ADF[d] (%)		N-ADF[d] (%)	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
High (n=6)	52.8	48.0	22.4	21.6	33.0	33.8	20.3	23.7	11.1	11.2
Medium (n=12)	46.4	43.7	21.3	20.5	34.6	36.1	23.7	24.9	12.0	11.2
Low (n=7)	42.4	40.1	20.5	20.2	34.6	35.9	23.1	24.4	11.5	12.0
Minimum	39.9	36.8	17.0	17.6	29.5	31.2	17.0	21.5	9.1	6.6
Maximum	56.2	51.3	24.4	23.6	39.3	39.8	27.6	29.2	15.4	16.9
Mean	46.8	43.7	20.9	20.7	34.2	35.6	22.7	24.5	11.7	11.5

[a] high: average IVDMD ≥48%. intermediate: ≥43-47%, low: <43%
[b] Two-stage technique (Tilley & Terry 1963)
[c] Kjeldahl nitrogen x 6.25 (AOAC 2003)
[d] van Soest et *al.* 1991, Robbins et *al.* 1987

Table 39. Condensed tannin content and composition in 25 *F. macrophylla* accessions with contrasting digestibility. Data of one evaluation cut in the wet season and one in the dry season. ECT = acetone-extractable condensed tannins, BCT = acetone-bound condensed tannins, PBE = protein-binding entities, ND = not detectable. n.a. = not available.

CIAT No.		**ECT[a] (%)**		**BCT[a] (%)**		**Extractability (ECT/TotalCT %)**		**Astringency[b] (PBE)**	
		Wet	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**
18437	High Quality	4.2	7.6	2.33	2.65	64.54	74.05	4.21	4.57
18438		0.2	1.6	1.76	2.02	12.00	44.81	3.33	3.78
20065		7.7	9.7	1.30	2.24	85.47	81.26	4.84	n.a.
21083		0.1	0.0	1.36	1.81	7.48	0.00	1.65	2.39
21087		7.2	6.6	1.57	1.98	82.04	76.95	4.40	6.44
21090		ND	ND	1.57	1.99	0.00	0.00	2.03	2.53
17403	Intermediate Quality	4.3	9.6	2.12	2.43	67.08	79.83	4.52	4.99
20622		12.3	13.5	3.27	1.20	79.05	91.83	4.60	5.85
20744		11.5	13.2	1.91	1.75	85.77	88.29	4.39	5.79
20975		13.5	14.5	2.59	2.01	83.90	87.80	6.77	6.33
20976		13.4	16.7	1.85	1.33	87.90	92.62	6.09	5.33
21092		6.6	3.8	1.53	2.21	81.11	63.35	2.81	3.55
21249		9.0	10.7	2.18	2.59	80.55	80.51	4.44	5.70
21529		9.3	10.3	2.90	3.32	76.19	75.64	4.88	5.15
21982		8.0	12.5	1.87	2.90	81.13	81.16	6.03	7.70
21992		7.8	12.0	2.19	2.37	78.17	83.51	5.12	7.91
22082		0.3	0.3	1.86	1.96	11.85	11.31	2.73	2.88
J 001		7.1	15.8	2.55	1.73	73.63	90.15	4.11	5.58
17407	Low Quality	7.3	11.5	2.13	2.25	77.41	83.58	5.46	5.33
19457		8.5	11.6	2.76	2.65	75.51	81.46	5.80	6.74
20616		5.9	15.7	2.00	0.84	74.59	94.92	4.71	5.97
20621		14.2	17.1	2.54	0.96	84.78	94.67	5.42	5.88
21241		10.0	8.8	2.16	2.11	82.22	80.62	3.81	6.13
21580		9.4	5.7	1.79	2.07	84.06	73.22	5.49	5.07
21990		12.4	19.4	2.30	2.98	84.38	86.67	6.37	6.95
High		3.9	5.1	1.6	2.1	41.9	46.2	3.4	3.9
Intermediate		8.6	11.1	2.2	2.2	73.9	77.2	4.7	5.6
Low		9.7	12.8	2.2	2.0	80.4	85.0	5.3	6.0
Mean		7.9	10.3	2.1	2.1	68.0	71.9	4.6	5.4

[a] Butanol-HCl (Terrill et *al.* 1992, Barahona et *al.* 2003)
[b] Radial diffusion assay (Hagerman 1987, Lareo et *al.* 1990)

quite variable due to accession but not due to season of the year (Figure A). In four accessions (CIAT 20621, 20744, 20975 and 20976) prodelphinidin made up more than half of the proanthocyanidins (range from 49 to 79%). The second most important constituent was propelargonidin, which ranged from 16 to 38%. Procyanidin was only present in small proportions (0 to maximum 16%). It was interesting to observe that in CIAT 21092 propelargonidin represented 82% of total proanthocyanidins in the rainy season and 95% in the dry season. Procyanidin was absent and prodelphinidin was less than 20%.

The five accessions for which we have reliable data on monomer composition of ECT are not representative of the entire *Flemingia* collection in terms of forage quality. However, four of them had very high ECT concentrations (13-17%) whereas CIAT 21092 presented relatively low ECT levels (7 and 4% in the rainy and dry season, respectively). The latter had an exceptionally high propelargonidin proportion but totally lacked cyanidin, which could indicate a relationship between monomer composition and forage quality.

Analysis of a subset of 10 high-quality accessions (including control) showed that forage quality varied over time. Patterns were different in the rainy and dry season for both the averaged values of the 10 accessions and for individual accessions (Figure 59). Correlations found in this analysis confirmed the negative correlations between IVDMD and ECT and IVDMD and astringency. Season had a large effect on IVDMD, DM production, plant height and diameter (higher in the rainy than in the dry season) and ADF, NDF, ECT and astringency (slightly higher in the dry season than in the rainy season). Extractability (percentage ECT of total CT) was relatively stable between harvest seasons (differences <10%). Only in six accessions (CIAT 17403, 18438, 20616, 20622, 21092 and J 001) differences up to 33% between rainy and dry season were found. No genotype x season interactions were detected for DM production and regrowth.

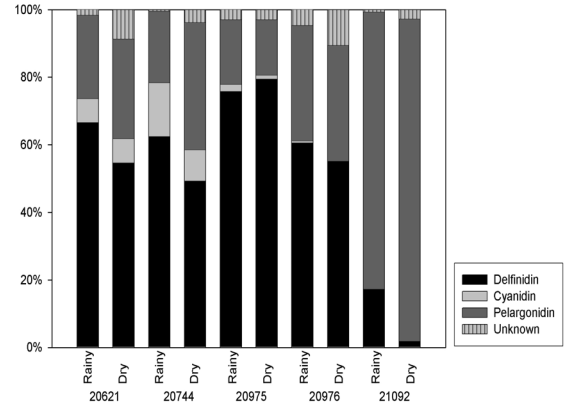

Figure 58. Monomer composition (procyanidin: prodelphinidin: propelargonidin ratios (C: D: P)) of the ECT fraction of five *F. macrophylla* accessions in rainy and dry season.

The accessions with the highest average *in vitro* dry matter digestibility were CIAT 18437, 18438, 21083, 21090, 21092 and 21241. The most productive accessions were CIAT 7184, 21090, 21241, 21248, 21249, 21519, 21529, 21580 and CPI 104890 with a total DM production >3.5 t/ha in the rainy and >2 t/ha in the dry season. Among the materials superior to CIAT 17403 (digestibility 36%, DM production 1.5 t/ha in the dry season) CIAT accessions 18437, 21083 and 21090 were identified for further testing as promising materials for dry season supplementation because they combined high digestibility with high productivity and low extractable condensed tannin content. These accessions had digestibility values > 48% and dry matter yields > 2 t/ha. Extractable condensed tannin content was 4.2 and 7.5% in the rainy and dry season for CIAT 18437 and nil in CIAT 21083 and 21090. However, their low seed production in the site where they were evaluated can limit their value.

The dendrogram (Ward's Method) was truncated at the 7-group level, explaining 72% of variation. The detailed agronomic characteristics of each group are listed in Table 40. Group 4 was the one that had the most promising accessions from an agronomic point of view. It contained eight accessions (7 semierect, 1 'tobacco') with the highest digestibility values of the collection (51% in

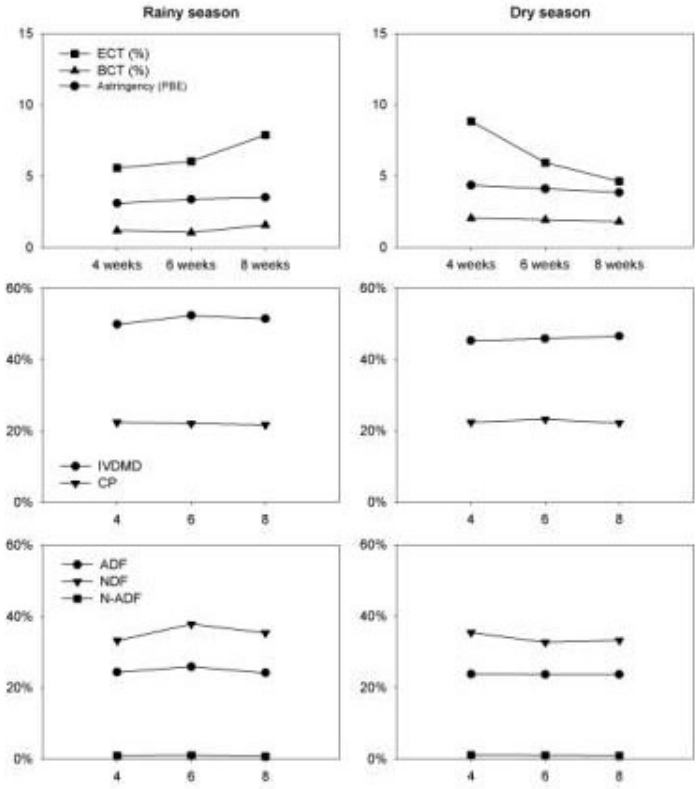

Figure 59. Variability in IVDMD, CP, fiber and tannin content of 10 *Flemingia macrophylla* accessions after 4, 6 and 8 weeks of regrowth in the rainy and dry season. ECT, BCT = acetone-extractable and bound condensed tannin, PBE = protein binding entities, IVDMD = *in vitro* dry matter digestibility, CP = crude protein, NDF and ADF = neutral and acid detergent fiber, N-ADF = nitrogen bound to ADF

the rainy and 47% in the dry season) and high DM production (2.6 t/ha in the rainy and 1.2 t/ha in the dry season). The three selected promising accessions CIAT 18437, 21083 and 21090 were contained in this cluster.

Genetic analysis by molecular markers (RAPDs): Out of 47 random primers tested, 9 were chosen that produced 171 RAPD bands ranging from 4 to 18 polymorphic bands per primer. Eight primers were selected for *Flemingia macrophylla* (D01, D04, D15, I07, J04, J06, J07, J12), and six for *Cratylia argentea* (D15, G12, I07, J06, J07, J12) (Table 41). Clustering of 47 *Cratylia argentea* and 1 *C. mollis* (outgroup) accessions resulted in 5 groups (Figure 60), plus two genetically very distinct materials: "yacapani" (the only prostrate *C. argentea* accession) and *C. mollis* (data not shown here). Group 1 included 28 accessions and group 2 twelve. Group 3 comprised accessions 884 and CIAT 18668 and 22389. Group 4 was conformed of CIAT 22386 and 22387. Group 5 contained only CIAT 18674, one of the two agronomically most promising accessions. No correlation was found between the clustering according to RAPD polymorphisms and agronomic, morphological or geographical characteristics. Analysis of genetic diversity within accessions revealed high variability. Nei and Li similarity between groups often was as high or higher than within groups (Table 42). This could indicate either seed contamination of accessions and/or outcrossing during multiplication in the field. Research on reproduction of *C. argentea* is urgently required to determine the rate and impact of outcrossing in this species.

Clustering of 111 *Flemingia macrophylla* and 2 *F. paniculata* (outgroup) accessions resulted in six groups (Figure 61), distinguishing well among the different morphotypes of this species, which have been described in the morphological evaluation (Photo 15, see also CIAT Annual Report 2002).

Group 1 included the two *F. paniculata* accessions. Group 2 was conformed by 55 of the 111 *F. macrophylla* accessions, which - with the exception of CIAT 20065 (prostrate) - belongederect growth type. Group 3 was composed of 23 semierect-1 and one "tobacco"-type accession and group 4 comprised 3 semierect-2 accessions. Group 5 included 8 prostrate, 14 semierect-1, 4 "tobacco" and 2 erect accessions and group 6 contained one semierect-2 and one semierect-1 accession.

No correlation was found between the clustering based on RAPD polymorphisms and agronomic or geographical characteristics. On the other hand, RAPD analysis proved to b useful for the identification/distinction of the different *F. macrophylla* morphotypes. It is suggested that the employment of the more powerful AFLP markers would detect higher polymorphisms within the morphotypes of this species.

Photo 15 . Four *F. macrophylla* morphotypes: 1=erect, 2=semi-erect 2, 3=prostrate, 4='tobacco'

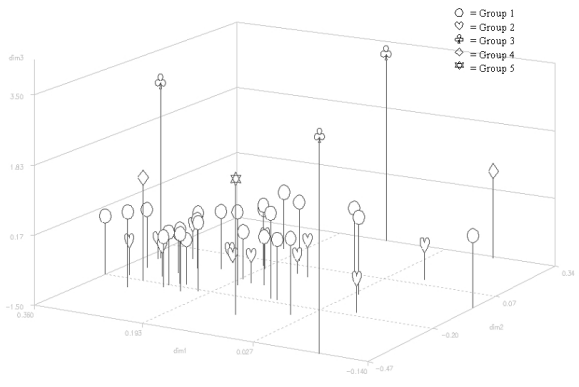

Figure 60. Tridimensional representation of five groups (without outgroup and prostrate accession "yacapani") resulting from clustering (UPGMA) of 47 *Cratylia argentea* and 1 *C. mollis* accessions according to molecular marker information (RAPDs).

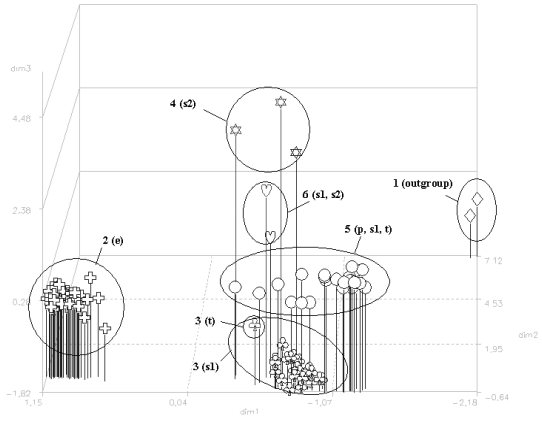

Figure 61. Tridimensional representation of the groups resulting from clustering (UPGMA) of 111 *Flemingia macrophylla* and 2 *F. paniculata* accessions according to molecular marker information (RAPDs). e = erect, s1 and s2 = semi-erect 1 and 2, p = prostrate, t = "tobacco" morphotype.

Table 40. Identification of *Flemingia macrophylla* accessions of agronomic interest. * *rainy/dry season value*

Group 1 (average/low digestibility, low yields, low regrowth, average CP, low vigor, low plant height):* CIAT 20973, 20977, 21080, 21086, 21994, 22053, 22087, 22090
Group 2 (average/low digestibility, average/low yields, high regrowth, average CP, low vigor, low plant height): CIAT 20979, 21079, 21990, 21993, 22285
Group 3 (high/low digestibility, low yields, average regrowth, high/average CP, low vigor, low plant height): CIAT 18048, 20972, 20976, 20978, 20980, 20982, 21982, 21991, 21992, 21995, 21996, 22327
Group 4 (high digestibility, high/average yields, good regrowth, high/average CP, high vigor, average plant height): CIAT 18437, 18438, 20975, 21083, 21087, 21090, 21092, 22082
Group 5 (average digestibility, high yields, average regrowth, high CP, high vigor, high plant height): CIAT 801, 7184, 20622, 20625, 20626, 20631, 20744, 21241, 21248, 21249, 21519, 21529, 21580, C104890, I15146, J001
Group 6 (like group 5, but lower yields, lower vigor and lower plant height): CIAT 19453, 19454, 19797, 19798, 19799, 20065
Group 7 (low digestibility, high/average yields, average regrowth, high CP, high vigor, average plant height): CIAT 17400, 17403, 17404, 17405, 17407, 17409, 17411, 17412, 17413, 18440, 19457, 19800, 19801, 19824, 20616, 20617, 20618, 20621, 20624

Table 41. Oligonucleotide primers employed in RAPD analysis, their sequence, number of bands obtained and percentage of polymorphic bands per species (% PBS).

Primer code	Sequence (5' to 3')	Number of bands		PBS (%)
		Polymorphic	Monomorphic	
Flemingia macrophylla				
D 01	ACCGCGAAGG	7	1	
D 04	TCTGGTGAGG	5	0	
D 15	CATCCGTGCT	18	1	
I 07	CAGCGACAAG	4	0	
J 04	CCGAACACGG	8	1	
J 06	TCGTTCCGCA	14	2	
J 07	CCTCTCGACA	13	0	
J 12	GTCCCGTGGT	6	2	
Total		***75***	***7***	***91.5***
Cratylia argentea				
D 15	CATCCGTGCT	10	4	
G 12	CAGCTCACGA	17	1	
I 07	CAGCGACAAG	17	4	
J 06	TCGTTCCGCA	13	1	
J 07	CCTCTCGACA	8	3	
J 12	GTCCCGTGGT	9	2	
Total		***74***	***15***	***83.1***

Table 42. Nei similarity within and between groups resulting from clustering (UPGMA) of 47 *Cratylia argentea* and 1 *C. mollis* accessions according to molecular marker information (RAPDs)

Group	**N**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Total**
1	28	0.825	0.814	0.759	0.774	0.769	0.487	0.413	
2	12		0.839	0.721	0.764	0.720	0.515	0.404	
3	3			0.757	0.748	0.754	0.457	0.388	
4	2				0.717	0.757	0.479	0.400	
5	1					1.000	0.426	0.433	
6	1						1.000	0.444	
7	1							1.000	
Total	**48**								**0.776**

3.3.2 Evaluation of a core collection of *Desmodium velutinum*

Contributors: M. Peters, L.H. Franco, B. Hincapié (CIAT), F. Parra (Corpoica), N.Vivas (Universidad Nacional de Colombia, Palmira) and R. Schultze-Kraft (University of Hohenheim)

Tropical shrub legumes of high forage quality are available for medium to high-fertility soils (e.g. *Leucaena leucocephala*), which is not the case for germplasm with adaptation to acid, low-fertility soils in drought-prone environments. Species such as *Desmodium velutinum* may offer an option in such environments (where they would complement *Cratylia argentea*). There are very few studies on *D. velutinum* and these mostly concentrate on one or two accessions. However, the available information indicates that this legume that produces forage of high quality has the potential to adapt to drought and (acid) low-fertility soils.

The proposed research attempts to explore the genetic diversity of *D. velutinum* in terms of morphology, yield and quality parameters through the evaluation of the world collection (138 accessions) held in CIAT, which come from a wide range of environments mainly in Asia and to a lesser extent in Africa. A core collection based on agronomic and morphological parameters and origin information, using GIS tools, will be identified for more detailed regional evaluation.

A total of 138 accessions of *Desmodium velutinum*, mostly originating from Asia, were planted at Quilichao. Plants were transplanted from jiffy pots into single-row plots, with 4 replications, in a randomized block design. Dry matter yield, drought tolerance and forage quality are the main parameters being measured. Currently the trial is in the establishment phase (Photo 16). Next year we will report results for a wet and for a dry period.

Photo 16. Plots of *Desmodium velutinum* established at Quilichao

3.4 Grasses with adaptation to poorly drained soils

Highlight

• Established a field trial to define adaptation of selected grasses to poorly drained conditions.

3.4.1 Field evaluation of *Brachiaria* and *Paspalum* genotypes in poorly and well drained sites in Costa Rica (Atenas)

Contributors: Pedro J. Argel and Guillermo Pérez (CIAT)

Rationale

The adaptation and response of forage plants to different climates and soils, as well as pests and diseases is variable. It is well known that some genotypes growth well in poor acid soils, other need medium to high fertility soils, and other do not perform well in heavy poorly drained soils. The expression of genetic combination of characters is so wide and variable that it is impossible to anticipate or predict the adaptation and potential production of a forage plant. For this reason field tests are necessary to characterize promising forage germplasm, particularly those related with tolerance to drought and poorly drained soil conditions.

Material and Methods

Seedlings of the forage species *Paspalum atratum* cv. Pojuca (CIAT 26986), *B. brizantha* CIAT 26124, CIAT 26318, CIAT 26990, a line of this species called Mixe, and the *Brachiaria* hybrids CIAT 36061 (cv. Mulato), CIAT 46024, CIAT 4015 and CIAT 36062, were transplanted for evaluation in a site with variable slope. The soil is a heavy clay with the following characteristics: pH 5.6 (medium), 0.4 meq/100 ml of Al content (medium), high content of Ca (26.9), high of Mg (10.4) and medium of K (0.44). Phosphorous content is very low (4 ppm), low in Zinc (2.5 ppm) and medium respectively in Mn (27.5), Cu (16.3) and Fe (39.7). Linear plots of 6 m long were used in a split plot design with 4 replicates. Main plot was formed by the forage species and subplots formed by different gradients of soil humidity, namely poorly drained, moderately drained and well drained area. Forage seedlings grown in jiffy pots of 45 days of age were established the 10th of July at a distance of 0.50 m between plants along a row perpendicular to the slope line for a total of 12 plants per plot.

By mid September a dike will be built at the lower part of the plot to create variable gradients of soil humidity. It is expected to have a waterlogged condition, a moderately drained area and a well-drained area. Mean plant height will be measured at the commencement of the waterlogged conditions, and plant mortality, vigor and growth will be measured every two weeks for a period of 16 weeks following the created soil moisture conditions. Forage DM yield will be determined for each species at the end of the observations at the different soil moisture conditions.

Results and Discussion

All *Brachiaria* lines and cv. Pojuca established well. Growth two months after planting shows vigorous plants along the slope line of the plots and none pests or diseases have been present. A measure of plant height immediately before the construction of the dike shows considerable variation in plant development: cv. Mulato (81.7 cm), *B. brizantha* (Mixe, 75.0 cm), cv. Pojuca (72.2 cm), *Brachiaria* hybrid CIAT 46024 (71.7 cm), *Brachiaria* hybrid CIAT 36062 (71.7 cm), *B. brizantha* CIAT 26124 (68.9 cm), CIAT 26318 (63.9 cm), CIAT 26990 (58.3 cm) and *Brachiaria* hybrid CIAT 4015 (48.3 cm).
This experiment will continue under evaluation during the present wet season and results will be report next year.

3.5 Legumes for multipurpose use in different agroecosystems and production systems

Highlights

- Accessions of *Vigna unguiculata* (cowpea) with good performance across sites in Central America, Haiti and Colombia were identified.
- Selected out of 17 accessions of *Vigna unguiculata* received from IITA, 5 accessions for acid soils (IT86D-715, IT89KD-288, IT6D-733, IT89KD-391 and IT95K-1088/4) and 5 for neutral soils IT95K-1088/4, IT86D-719, IT95K-1088/2, IT93K-637/1and IT96D-740); some promising local accessions identified for further testing.
- Three accessions of *Lablab purpureus* (CPI 34777, CPI 106471 and CPI 52535) were selected for high yield on acid and neutral soils.

3.5.1 Evaluation of annual legumes for drought tolerance in Central America

Contributors: Axel Schmidt (IP-5), Steve Hughes (SARDI, Australia), Clark Davies (IP-5), Michael Peters (IP-5), Luis Alfredo Hernández (SN-3)

Rationale

Farmers' adaptive capacity to cope with increasing drought incidents in Central America depends to a large extent on the availability of drought tolerant crops and forages. Limited by 6-7 months of dry season (November to May/June; 750-1100 mm/year), smallholders in the hillsides of Nicaragua are looking for crop varieties and forage species which safeguard their harvests and permit to feed animals during the dry season. Multipurpose legumes adapted to these dry conditions can play a significant role in enhancing livestock feed during the dry season and, if integrated in cropping systems, they may also improve soil fertility through nitrogen fixation and suppress weeds when used as cover crops (mulch).

Species requiring smaller amounts of water for considerable biomass production (feed, cover, green manure) might even offer the possibility to extent the growing season beyond the two traditional growing periods "*primera*" from May/June to September and "*postrera*" from September to November.

To match farmers' demand for such plant materials, it is necessary to screen available germplasm for adaptation to local conditions and for plant characteristics which allow for the integrations of these selected species into the traditional maize-beans cropping system. In order to improve adoption such germplasm evaluation and selection should be preferably based on farmers' criteria and in collaboration with them.

Material and methods

In collaboration with the South Australian Research and Development Institute (SARDI) and its Australian Medicago Genetic Resource Center, a set of 30 annual legume species (Mediterranean region – see Table 43) were selected for an adaptation experiment at the SOL SECO site (the Spanish acronym for Supermarket of technologies for hillsides – dry) in San Dionisio, Matagalpa, Nicaragua. The site is known by local farmers as the driest place in San Dionisio and is shared by other CIAT projects and national collaborators for the screening of different crops for their drought tolerance The selection criteria were: low water requirement, fast growth and soil cover, annual growth cycle, ease of establishment, late flowering and no seed shattering.
The experiment was reestablished in October 2002 since the first establishment failed due to the unusually early beginning of the dry season in October 2001. The experiment was designed as

randomized blocks with 3 replicates. Seeds were pre-germinated and seedlings transplanted in the 4.5 m x 1.5 m plots with an overall planting distance of 0.5 m between plants. The experiment protocol included periodical evaluations (every two weeks) of field emergence, plant height, ground cover, drought tolerance, flowering patterns, biomass production, seed production, incidents of pests and diseases, and for farmer selected species forage quality parameters.

Results and Discussion

Although rainfall conditions favored plant establishment this time, plant development was slow and most of the accessions died within three weeks after transplanting. Only *Hedysarum coronarium* (SARDI 32506) and *Hedysarum flexuosum* (SARDI 35361) showed some adaptation potential, but died during the first dry weeks. Since no accession showed promising signs of adaptation during the two years under characterization and evaluation, the collection will be dropped form the work plan.

Since the issue of drought tolerant multipurpose legume germplasm remains important, work will focus in 2004 on the evaluation of CIAT's collection of *Vigna umbellata* (rice bean), a plant species which raised high expectation this year among farmers during a small experiment at the SOL-Wibuse site in San Dionisio. While CIAT's *Vigna umbellata* collection (8 accessions) is diverse in its origin (Central America, Colombia, Africa, Southeast Asia), the collection was never evaluated under field conditions for drought tolerance and multiple purposes.

Table 43: Available germplasm passport data of annual legume species established at SOL SECO site in San Dionisio, Nicaragua.

SARDI Acc. No.	Genus	Species	Alt.	Lat.	Long.	Precip. (mm/a)	Origin
367	Trifolium	purpureum	60	33° 54' N	35° 28' E	890	Lebanon
687	Trifolium	hirtum	300	32° 33' S	118° 13' E		Australia
1011	Trifolium	vesiculosum					Italy
3400	Medicago	arabica					USA
4715	Trifolium	clypeatum		36° N	27° E		Greece
5036	Tetragonolobus	purpureus		45° 30' N	73° 36' W		Canada
5045	Trigonella	balansae					Sweden
6125	Trifolium	apertum	50	45° 02' N	39° 00' E		Russia
8687	Lotus	peregrinus	140	32° 42' N	35° 12' E		Israel
12701	Lotus	edulis	700	36° 30' N	03° 45' E	650	Algeria
12953	Lotus	maroccanus	91	34° 03' N	06° 33' W	425	Morocco
14935	Trifolium	spumosum	400	37° N	30° E		Turkey
17357	Trifolium	diffusum					USA
22575	Hymenocarpos	circinnatus	150	39° 41' N	19° 45' E	1100	Greece
24228	Hedysarum	carnosum	200	35° 50' N	09° 12' E	300	Tunisia
24578	Trifolium	isthmocarpum	250	34° 05' N	05° 00' W	750–1000	Morocco
32202	Trigonella	***Calliceras***					Canada
32233	Medicago	scutellata					Australia
32506	Hedysarum	coronarium					Morocco
32511	Tetragonolobus	palaestinus					Spain
33621	Trifolium	alexandrinum					Australia
33816	Vicia	sativa					Australia
33842	Lotus	ornithopodioides					Czechoslovakia
33884	Trifolium	incarnatum					Unknown
34647	Medicago	polymorpha					Turkey
35361	Hedysarum	flexuosum		35° 48' N	05° 45' W	750-1000	Morocco
35644	Trifolium	salmoneum	120	35° 07' N	35° 38' E	400	Israel
36323	Medicago	polymorpha					Australia
36400	Trifolium	squarrosum					Unknown
36441	Trifolium	michelianum					Bulgaria

3.5.2 Evaluation of a core collection of *Vigna unguiculata* for multipurpose uses in Colombia, Nicaragua, Honduras, and Costa Rica.

Contributors: M. Peters, L. H. Franco, A. Schmidt, H. Cruz Flores, P. Argel, P. Avila, B. Hincapié, G. Ramírez, (CIAT) and B.B. Singh (IITA, Nigeria)

Rationale

Cowpea (*Vigna unguiculata*) is utilized in the subhumid/semi-arid tropics of West Africa and India as a source of food and feed for livestock, but the utilization of cowpea in Latin America is so far limited. We visualize that cowpea could be an alternative crop for the second planting season in low fertility soils in the central hillsides region of Nicaragua and Honduras where the legume could provide not only higher grain yields as compared to common beans, but could also allow for a third crop in November/December in order to provide grain, hay for feeding in the dry season or contribute to soil fertility enhancement for the following maize crop. Cowpea could also be used for hay, silage and feed meal, which in turn could be an option for income generation by smallholder livestock and non-livestock owners. Adaptation to climatic and edaphic conditions, especially to water stress, are prerequisites for a successful development of a cowpea option for the traditional maize-bean cropping systems in Central America.

3.5.2.1 Evaluation of new cowpea accessions in Quilichao and Palmira, Colombia

In 2003, a new collection comprising 32 breeding lines obtained from IITA (B.B. Singh), complemented with 5 accessions locally used in Honduras and Nicaragua were evaluated in Quilichao and Palmira. The objective was to compare the effect of climate and soil on performance and to identify accessions with broad adaptation, which is key for Central American hillsides with highly variable soil and climatic conditions (Photo 17). Standard agronomic evaluation methods were employed as in previous years.

This year we report results from Quilichao only as the trial in Palmira is still in the establishment phase. In Quilichao (acid soils) no incidence of pests and diseases was reported. In table 44 results on vigor, soil cover and dry matter yield are presented.

Mean dry matter yields (1416 kg/ha) were lower than recorded with previous collections in Quilichao (mean yield 2229 kg/ha), possibly as a result of favorable climatic conditions during growth period. However, yield differences ($P \leq 0.05$) between accessions were measured at harvest in the pre-flowering stage (8 weeks after planting). Accessions IT97K-825-3 IT97K-461-4, IT89KD-288, IT98K-131-2 and 9611 had the highest yields with equal or more than 1800 kg/ha DM. After sampling, cowpea biomass was incorporated in the soil to measure the effect on a succeeding maize crop.

Photo 17. Plots of Cowpea (*Vigna unguiculata*) at Quilichao

Highly significant (P≤0.01) differences between accessions were measured for *in-vitro* dry matter digestibility (IVDMD), phosphorus (P) and potassium (K) content, but not for protein content (CP). In general results confirm cowpea as a very high quality material with dry matter digestibilities above 83% and protein contents ranging between 20 and 24 %. Several accessions had digestibilities above 88%, among these the high yielding IT98K-131-2, IT97K-825-3 and 9611 (Table 45).

Table 44 . Vigor, soil cover (%) and dry matter yield (kg/ha) of *Vigna unguiculata* in Quilichao, 2003

Accessions	Vigor	Soil Cover (%)	DM kg/ha
	1 – 5	8 weeks	8 weeks
IT97K-825-3	4.3	95	2067
IT97K-461-4	3.0	73	2040
IT89KD-288	4.0	90	1893
IT98K-131-2	4.3	92	1800
9611	5.0	100	1800
IT99K-429-2	4.0	87	1780
IT98K-476-8	4.0	88	1727
IT99K-1122	4.0	93	1720
IT97K-1069-2	4.3	93	1673
IT98K-205-8	3.3	80	1667
IT99K-1060	3.0	78	1660
IT97K-494-3	3.3	78	1653
IT99K-216-24-2	4.0	87	1567
IT97K-570-18	4.6	95	1553
Cidicco 2	4.6	97	1520
IT97K-356-1	3.6	87	1487
FHIA	3.3	72	1480
IT98K-390-2	4.6	97	1433
IT98K-391-2	4.0	90	1400
IT98D-1399	3.3	82	1340
Cidicco 1	4.6	93	1327
YT98K-406-2	4.0	87	1320
IT97K-818-35	4.0	90	1320
IT98K-412-8	3.3	77	1307
IT96D-610	3.3	82	1287
IT99K-7-14	2.6	68	1267
IT98K-428-3	3.6	87	1240
IT98K-412-13	4.0	92	1233
IT95K-52-34	4.6	95	1200
IT99K-409-8	3.6	77	1193
IT97K-1069-6	3.6	83	1147
IT98K-506-1	2.6	68	1120
IT98K-311-8-2	4.3	93	1067
IT99K-7-21-2-2	3.0	70	1020
IT97K-819-118	2.6	65	940
Cidicco 4	4.3	85	913
Cidicco 3	4.0	87	873
IT97K-499-38	2.5	65	780
Mean			1416
MSD (P≤0.05)			976.23

Table 45. Fodder quality of accessions of *Vigna unguiculata* grown in Quilichao, 2003.

Accessions	Forage			
	IVDMD	Protein	P	K
		%		
IT98K-131-2	89.8	21.0	0.16	1.46
IT97K-825-3	89.2	20.6	0.16	1.85
IT96D-610	88.8	19.8	0.13	1.21
9611	88.8	20.2	0.16	1.44
FHIA	88.6	19.4	0.13	1.57
IT98K-311-8-2	88.4	19.4	0.15	1.44
IT98K-476-8	88.4	19.9	0.12	1.77
IT99K-7-14	88.3	20.5	0.14	1.29
IT89KD-288	87.9	20.4	0.12	1.45
IT99K-216-24-2	87.8	21.5	0.16	1.51
IT98K-205-8	87.5	20.4	0.14	1.44
IT95K-52-34	87.5	19.7	0.14	1.44
IT97K-819-118	86.8	20.8	0.16	1.64
IT97K-570-18	86.5	20.0	0.13	1.49
Cidico 2	86.8	20.7	0.14	1.59
IT97K-356-1	86.7	20.3	0.15	1.54
IT97K-818-35	86.7	20.9	0.15	1.44
IT97K-499-38	86.6	22.2	0.15	1.78
IT99K-429-2	86.6	20.3	0.14	1.43
IT99K-1122	86.5	23.1	0.20	1.95
IT98K-506-1	86.4	21.1	0.16	1.69
IT97K-1069-2	86.3	20.9	0.17	1.37
IT98K-412-13	86.2	22.1	0.16	1.77
IT97K-1069-6	85.7	23.5	0.18	1.73
IT97K-461-4	85.5	22.4	0.16	1.69
IT97K-494-3	85.3	22.7	0.17	1.86
Cidico 4	85.1	22.1	0.15	1.80
IT98K-412-8	85.1	22.6	0.16	1.93
IT98K-428-3	85.1	22.5	0.16	1.5
IT98D-1399	85.0	20.5	0.13	1.77
IT99K-7-21-2-2	84.7	22.6	0.16	1.95
Cidico 1	84.7	21.1	0.14	1.77
IT98K-390-2	84.6	21.7	0.15	1.69
IT98K-391-2	84.6	20.6	0.10	1.78
Cidico 3	84.5	23.6	0.14	1.81
IT99K-1060	83.7	21.6	0.15	1.5
IT99K-409-8	83.7	22.5	0.15	1.7
YT98K-406-2	83.0	24.2	0.21	1.9
Mean	86.4	21.3	0.15	1.64
LSD	4.79	5.74	0.08	0.71
	($P \leq 0.01$)	n.s.	($P \leq 0.01$)	($P \leq 0.01$)

3.5.2.2 Evaluation of cowpea in Honduras

In 2002, an experiment was established in the SOL Yorito site (neutral pH) to evaluate a core collection of cowpea. *Lablab purpureus* DICTA was included for comparison. In the 2002 Forages Annual Report dry matter yields of the cowpea accessions were reported, hence here the green manure effects on a succeeding maize crop are presented.

Highest maize grain yields were obtained following accessions IT93K-637/1, IT86D-719, 9611, IT6D-733, IT93K-573/5 and IT90K-284/2. Maize grain yields in these treatments were above 2 t/ha and higher than obtained with N-fertilization. Maize grain yields following incorporation of the accession IT93K-637/1 were almost double as compared to the non-fertilized treatment (N 0). The green manure effect of the different accessions cannot be explained by dry matter production of cowpea materials (Table 46).

3.5.2.3 Evaluation of cowpea in Nicaragua

In 2002, a collection of cowpea (*V. unguiculata*) was established in the Sol Seco site in San Dionisio (for evaluation of drought tolerance). Standard agronomic evaluation methods were employed, to evaluate accessions planted in a Randomize Complete Block Design with 3 replications and to determine effects of.

Highly significant differences (Pd≤0.01) were recorded both for biomass as well as grain yields (table 47). The local cowpea type 'Café', IT90K-284/2, type 'INTA', type 'SF libre' and 'Negro' provided the highest dry matter yields with up to 4.7 t/ha while highest cowpea grain yields were measured for local types 'Negro', 'Café' and 'Rojo'.

From the results it appears that there are local materials available in Nicaragua with a production superior to most introduced lines under the dry conditions at the SOL Seco site. The performance of these lines across environments would need to be studied. Green manure effects on a succeeding maize crop are currently being investigated.

3.5.2.4 Genotype x Environment interactions

An adaptability index to assess the response of a set of 14 cowpea accessions across different climates and acid to alkaline soils in Honduras, Nicaragua, Costa Rica and Colombia was calculated. Accessions best adapted across environments but responding to improved environmental conditions were IT90K-284/2, IT89KD-391, IT95K-1088/4, IT95K-1088/2, IT86D-716, IT93K-637/1 (Figure 62). In view of their wide adaptation across environments these accessions will be multiplied and selected for further comparison with local varieties and new collections for use as fodder. For utilization as green manure, based on data from only 2 sites, accession IT90K-284/2 is the most promising material; however, data from other 2 sites in Nicaragua and Colombia will be key to confirm the superiority of this accession.

Table 46. Dry matter yields of *Vigna unguiculata* (cowpea) genotypes before incorporating into the soil and maize grain yields after incorporation of cowpea (Yorito, Honduras. 2002 – 2003)

Treatment	Cowpea	Maize	
	Biomass	Grain yield	DM Total
		Kg/ha	
IT93K-637/1	8397	2575	4905
IT86D-719	7422	2329	4571
9611	11440	2250	4401
IT6D-733	5349	2230	4179
IT93K-573/5	6866	2083	3837
IT90K-284/2	12132	2071	4179
N80*		2016	3571
IT93K-503/1	4484	2000	3798
IT86D-716	8134	1825	3742
Lablab purpureus DICTA	7368	1802	3310
N40		1790	3139
IT90K-277/2	6370	1742	3579
IT89KD-288	8086	1639	3718
IT96D-740	3229	1635	3238
IT89KD-391	10158	1619	3583
FHIA	3663	1607	3318
IT86D-715	5110	1603	3250
CIDICCO3	8718	1599	3528
CIDICCO1	5010	1591	3810
CIDICCO2	10724	1556	3206
IT95K-1088/2	7992	1500	3089
IT95K-1088/4	9208	1373	3071
N0		1357	2710
Mean	7545	1822	3649
LSD ($P \leq 0.05$)	4243	1030	1281.6

Table 47. Dry matter and grain yields of *Vigna unguiculata* (cowpea) genotypes before soil incorporation in San Dionisio (Sol Seco), Nicaragua 2002.

Treatment	Cowpea	
	Biomass DM kg/ha	Grain yield Kg/ha
Café	4730	2268
IT90K-284/2	3063	1243
INTA	2780	1180
SF Libre	2627	1060
Negro	2463	2630
Rojo	1920	2123
IT93K-503/1	1763	1290
IT86D-716	1737	1300
IT95K-1088/4	1727	1103
IT93K-637/1	1613	1053
IT86D-715	1467	747
IT95K-1088/2	1140	923
IT89KD-391	1057	980
IT86D-719	1000	1077
IT93K-573/5	927	500
IT96D-740	900	730
IT6D-733	563	797
IT89KD-288	377	533
IT90K-277/2	263	117
Mean	1690	1138
LSD ($P \leq 0.01$)	2268.6	1689.7

*Maize grain yields in N120, N160 and N200 treatments were similar to the N80 treat

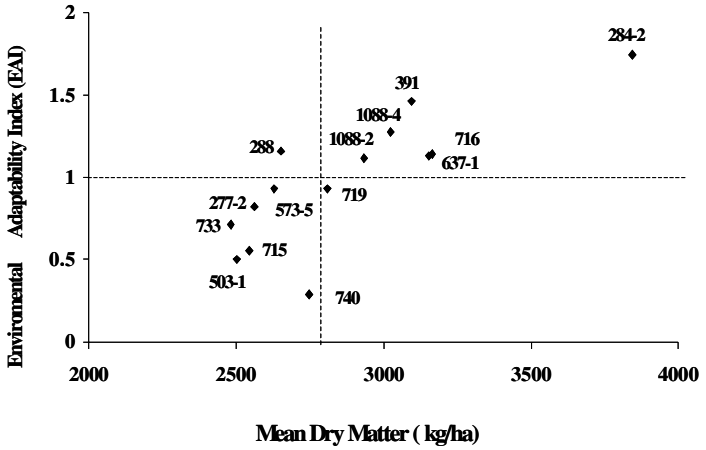

Figure 62. Adaptability Index for Dry matter production of a collection of *Vigna unguiculata* evaluated in contrasting environments in Honduras, Nicaragua, Costa Rica and Colombia

3.5.3 Evaluation of *Lablab purpureus* for multipurpose uses

Contributors: M. Peters, L. H. Franco, B. Hincapié, A. Schmidt, H. Cruz Flores, P. Argel and G. Ramírez (CIAT)

Lablab purpureus (syn. *Dolichos lablab* L.) is a legume with high potential as green manure and fodder in the tropics. Some commercial lines have been available to farmers for some time, but considerable variability exists within this genus that merits characterization with the aim to identify more productive and better-adapted lines. Under this consideration, new lines from a core collection from ILRI/CSIRO are under evaluation in the subhumid environment of Colombia and Central America.

Our main objective with the collection is to select accessions with broad adaptation to different soils and climate conditions in tropical America. However, of immediate interest is to evaluate the Lablab collection in acid and neutral soils to define niches of Lablab for green manure and fodder (especially for hay or deferred feed), with emphasis on Central America where soils are highly variable in pH.

3.5.3.1 Evaluation of collection *Lablab* in contrasting sites

Quilichao – Acid Soil: Most accessions had soil covers above 70% 12 weeks after establishment, showing strong ability to compete with weeds. At the 16 week sampling period, soil cover of some accessions was lower than at 12 weeks, due to defoliation triggered by flowering in early flowering material and attack by leaf eating insects at the beginning of the dry period.

No interactions between accession and harvest date were statistically detected ($P<0.05$); however, it was evident that for late flowering material dry matter yields increased over time while for some early flowering materials yields were reduced at the 16 week compared to the 12 week sampling. This has implications for the utilization of Lablab materials as green manure as different combinations of time available for green manure and Lablab material can address different temporal niches in the farming system.

Highest dry matter yields were recorded for accessions I-14442, CPI-106471, CPI-52535, CPI-52535, CPI-52535 ,I-11615, cv. Highworth and CPI-36903 with more that 3 t/ha 16 weeks after planting (Table 48).

Table 48. DM yield (kg/ha), vigor and soil cover (%) of *Lablab purpureus* accessions in Quilichao, 2003

Accessions	Soil cover (%)		DM yield (kg/ha)	
	12 Weeks	16 Weeks	12 Weeks	16 Weeks
I-14442	92	97	3153	4600
CPI-106471	92	83	2573	4467
CPI-52535	88	63	3720	4127
21603	97	97	2693	3367
I-11615	47	35	1693	3233
Cv. Highworth	88	80	2340	3160
CPI-36903	77	57	2360	3047
CPI-34777	92	65	3273	2650
I-11632	70	45	2413	2587
I-6533	73	77	1793	2513
CQ-2975	83	90	2327	2360
CPI-67639	77	73	1860	1973
L-987	87	78	2200	1950
I-14437	75	77	1560	1760
CPI-35894	50	20	1800	1560
Mean	79	69	2384	2918
LSD ($P \leq 0.05$)			2670.12	3060.16

Palmira-Neutral soils: As in Quilichao leaf eating insects attacked Lablab accessions at the beginning of the dry period. Dry matter yields, soil cover and vigor of Lablab accessions was better in Palmira than in Quilichao, confirming earlier results that *Lablab purpureus* responds to better soil conditions and higher pH. Average soil covers were above 90% both at the 12 and 16 week sampling, with mean dry matter yields above 4 t/ha and 7 t/ha 12 and 16 weeks alter planting, respectively (Table 49).

Costa Rica: Twelve lines of *Lablab purpureus* were established for evaluation of adaptation in a randomized complete block design with three replicates at Atenas, Costa Rica. Plot size was 2 m wide x 1.5 m long (3 m^2), and the planting distances were 0.25 m and 0.50 m between plants and rows respectively. Plots were fertilized with 50 kg/ha of P_2O_5, 50 kg of K_2O, 20 kg of S and 20 kg of Mg. Plant emergence and plant vigor and cover were measured respectively 4 and 6 weeks after planting. The experiment will continue under evaluation to measure DM yield and forage quality.

The experiment was initially planed for a plot size of 8 m^2 (3 m wide x 2.5 m long), but we had to adjust the size of the plots to the availability of seed quantities. Plant emergence was acceptable and there was no need for replanting, thus over 87% plant emergence was observed 4 weeks after planting as Table 50 shows. Differences in plant cover and vigor have been also observed up to date, and cv. Highworth and the lines CPI-67639 and L-987 have shown good growth and plant development. Less vigor and have shown the lines CPI-106471, 21603, CQ-2975 and CPI-34777, while other lines are intermediate in plant development.

Some variability in plant vigor has been observed within identical lines, but we are not sure if this is due to seed quality or caused by particular soil conditions. So far, low incidence of leaf eater insects, as well as foliar diseases have been observed.

Table 49. DM yield (kg/ha), vigor and soil cover (%) of *Lablab purpureus* accessions in Palmira, 2003

Accessions	Soil cover (%)		DM yield (kg/ha)	
	12 Weeks	16 Weeks	12 Weeks	16 Weeks
CPI 106471	75	90	6713	8567
CPI 52535	100	97	5500	8053
CPI 36903	97	83	4120	7633
I-11632	98	93	4213	7573
I-14437	100	95	4788	7487
I-6533	98	98	4427	7453
I-14442	100	98	4247	7340
CPI 34777	98	83	5853	7140
I-11615	90	80	4613	7060
CPI 67639	100	98	5580	6547
Highworth	100	95	4493	6527
CQ-2975	98	100	3813	6480
CIAT 21603	88	90	3420	6253
L-987	100	100	4920	6073
CPI-35894	100	65	3860	5273
Mean	96	91	4704	7031
LSD (P≤0.05)			2304.09	3073.92

Table 50. Plant emergence, plant vigor and cover of *Lablab purpureus* lines established in Atenas, Costa Rica

Accessions	Plant Emergence (%)	Plant Cover(%)	Plant Vigor (%)*
21603	95.8	25.0	2.3
CPI-106471	90.3	35.0	2.3
CPI-34777	87.5	38.3	2.8
CPI-36903	93.1	45.0	3.0
CPI-52535	100.0	43.3	3.2
CPI-67639	100.0	61.7	4.3
CQ-2975	98.6	22.5	2.3
Highworth	100.0	62.5	4.3
I-11632	87.5	42.5	3.0
I-14442	97.2	55.0	3.7
I-6533	90.3	55.0	3.8
L-987	88.9	57.5	4.3

* Visual rating (1 poor vigor, 5 highly vigorous plant)

3.5.3.2 Effect of lablab accessions as a green manure

In the 2002 Forages Annual Report data on biomass production of *Lablab purpureus* in Quilichao and Palmira were reported. In this report we summarize the effect of different accessions of Lablab on a succeeding maize crop on neutral to alkaline soils in Palmira.

Significant differences (P≤0.05) between accessions were measured for total maize biomass yield but not for maize grain yield.

Accessions CPI 34777, CPI 106471, CIAT 21603, CPI 52535, cv. Highworth and I-11615 resulted in maize grain yields above 4 t/ha, substantially higher than the maximum maize grain yield of 2.5 t/ha recorded with N160 - fertilization. Maize yields following lablab did not appear to be directly related to biomass production of lablab (Table 51).

Table 51. Dry matter yield (kg/ha), vigor and soil cover (%) of *Lablab purpureus* accessions and grain and dry matter yield of a following maize in Palmira, 2002-2003.

Treatment	*Lablab purpureus*			Maize	
	Vigor	Soil cover (%)	DM yield (kg/ha)	Grain	Total
	1 a 5	12 Weeks		(kg/ha)	
CPI 34777	5	100	5293	7902	22400
CPI 52535	5	100	4733	6116	20215
CPI 52544	4	97	3840	5789	19884
CPI 100602	4	97	3513	5773	19858
I-11630	5	100	4353	5706	15353
I-14411	5	100	3460	5525	17076
CPI 29398	4	97	4167	5261	17417
CPI 36903	4	97	3093	5240	17778
CQ-2975	5	100	4187	5157	17264
CPI 96924	3	63	3160	5101	14424
I-14437	4	100	4413	4916	19437
I-11615	3	87	4480	4897	16513
I-11613	4	90	3220	4823	18327
CPI 35894	3	90	3713	4740	16240
CPI 99985	3	93	3387	4704	14414
I-14442	4	100	3067	4580	18751
cv. Highworth	5	97	4620	4556	16323
I-6533	4	97	4033	4467	17458
I-14441	5	96	4040	4406	19951
CPI 106471	5	100	5053	4244	20201
CPI 106548	4	87	3200	4091	17343
CPI 76998	4	100	3613	3753	17097
CPI 67639	4	93	3673	3609	14952
CIAT 21603	5	100	4853	3387	15758
Endurance	3	80	2653	3267	12844
CPI 81626	3	93	4213	3257	13594
cv. Rongai	4	100	4113	2761	13219
L-987	5	100	4227	2745	12948
NI60				2474	8961
cv. Koala	3	80	3067	2106	16534
N80				2002	7756
N120				1747	8460
N0				1300	8597
N40				1105	6589
Mean		94	3919	4185	15813
LSD ($P \leq 0.05$)			1505	NS	11537

In view of the heterogeneity of the variances between the different treatments for yields of a succeeding maize crop, a smaller group of 19 treatments (14 accessions and including all 5 fertilizer control treatments) with relatively high homogeneity of variances within treatment was selected. With this smaller group highly significant ($P \leq 0.01$) differences in maize grain and total biomass yield were detected. *Lablab purpureus* CPI 34777, CPI 52535, I-11630 and I-14441 as green manure resulted in significantly ($P \leq 0.01$) higher maize grain yield than with N-fertilization (Table 52).

Table 52. Dry matter yield (kg/ha), vigor and soil cover (%) of *Lablab purpureus* accessions and grain and dry matter yield of a following maize in Palmira, 2002-2003.

Treatment	*Lablab purpureus*			Maize	
	Vigor	Soil cover (%)	DM yield (kg/ha)	Grain	Total
	1 a 5	12 Weeks		(kg/ha)	
CPI 34777	5	100	5293	7902	22400
CPI 52535	5	100	4733	6116	20215
CPI 52544	4	97	3840	5789	19884
CPI 100602	4	97	3513	5773	19858
I-11630	5	100	4353	5706	15353
I-14411	5	100	3460	5525	17076
CPI 29398	4	97	4167	5261	17417
CPI 36903	4	97	3093	5240	17778
CQ-2975	5	100	4187	5157	17264
CPI 96924	3	63	3160	5101	14424
I-14437	4	100	4413	4916	19437
I-11615	3	87	4480	4897	16513
I-11613	4	90	3220	4823	18327
CPI 35894	3	90	3713	4740	16240
CPI 99985	3	93	3387	4704	14414
I-14442	4	100	3067	4580	18751
cv. Highworth	5	97	4620	4556	16323
I-6533	4	97	4033	4467	17458
I-14441	5	96	4040	4406	19951
CPI 106471	5	100	5053	4244	20201
CPI 106548	4	87	3200	4091	17343
CPI 76998	4	100	3613	3753	17097
CPI 67639	4	93	3673	3609	14952
CIAT 21603	5	100	4853	3387	15758
Endurance	3	80	2653	3267	12844
CPI 81626	3	93	4213	3257	13594
cv. Rongai	4	100	4113	2761	13219
L-987	5	100	4227	2745	12948
NI60				2474	8961
cv. Koala	3	80	3067	2106	16534
N80				2002	7756
N120				1747	8460
N0				1300	8597
N40				1105	6589
Mean		94	3919	4185	15813
LSD ($P \leq 0.05$)			1505	NS	11537

Among the lablab accessions CPI 34777 and CPI 52535 are the most promising materials, producing high biomass yields and having a high green manure effect on succeeding crops. CPI The accession 34777 has rapid growth and needs to be utilized quickly while CPI 52535 can occupy niches with longer time spans for harvesting the forage and grain. Evethough yields of Lablab are generally higher on soils with a pH above 5.0, these two accessions were among the highest yielding in the Lablab collection on both acid and alkaline soils. Both accessions are included in a G X E experiment to assess performance over a wider range of environments (see below).

3.6 Nitrification inhibition in tropical grasses

Highlights

- Found substantial differences in total and specific NI (nitrification inhibition) activity among tropical grasses.
- Developed and tested a simple protocol to quantify the impact of addition of root exudates from plants on nitrification inhibition of incubated soils.

Last year, we showed the feasibility of using a bioassay (with recombinant Nitrosomonas europaea) that detects nitrification inhibitory activity in plant (such as root exudates or tissue extracts) or soil samples (such as soil water extracts). Using this bioassay we have shown that root exudates from B. humidicola inhibit nitrification. The inhibitory activity of the root exudates increased with the plant age (mostly because of the increase in root mass) and showed a sigmoid pattern; subsequently, NI activity in root exudates declined as the plants reach the maturity stage.

This year we report our ongoing research activities on methodology development, and comparative evaluation of other tropical grasses for the ability to inhibit nitrification. The other ongoing research activities in this collaborative project include: isolation of the active compound responsible for NI activity in B. humidicola, mechanisms underlying the inhibition of nitrification in root exudates, and factors that regulate the expression of NI activity. Results from these activities will be reported next year.

3.6.1 Bioassay – Further improvements and refinements in the methodology

Contributors: G.V. Subbarao, K. Nakahara, T. Ishikawa, K. Okada and O. Ito (JIRCAS, Japan)

The bioassay methodology was adopted from Izumi et al. (1998), which was initially developed to detect nitrification inhibitors in the municipal-waste water treatment plants. This methodology has gone through improvements to get reliable and stable measurements in detecting inhibitory effect on nitrification from root exudates, tissue extracts and soil-water extracts. Of the various factors, one of the most important is the incubation temperature of the bacterial culture with the test compound (i.e. root exudates) for the bioassay measurements. The bioassay appears to be at its best in detecting the inhibitory activity at 15^{O}C; the bioassay's ability to detect inhibitory effect from a known inhibitor (allylthiourea) decreased with an increase in incubation temperature. Also, the room temperature where the luminometer is located should be maintained close to 20 to 22^{O}C to obtain stable measurements.

The Nitrosomonas culture age of 6 to 7 days is found to be the optimum stage for the bioassay measurements; beyond this, the response to known inhibitor (Allyl thiourea) decreased with the culture age. These modifications are now part of the bioassay methodology (for the other details on the methodology please see CIAT, 2002 - IP-5 Annual Report) to evaluate nitrification inhibitory activity of the plant samples and will improve our ability to generate reliable and stable measurements for this research project.

3.6.2 Root exudates – Development of sample processing and preparation protocols for the determination of nitrification inhibitory activity using a bioassay

Contributors: G.V. Subbarao, K. Nakahara, T. Ishikawa, K. Okada and O. Ito (JIRCAS, Japan)

In most cases root exudates when collected from the plant roots (by keeping intact plant roots in deionised/distilled water for 24 hours) needs to be condensed several fold (about 50 to 100) before they can be used for the determination of NI activity. We have noticed several times that the contamination of chloride (either from water that is used for collecting root exudates or from the soil-water extracts) interferes with the inhibitory activity measurements. To avoid chloride contamination problems in the root exudates and soil-water extracts, we have developed a sample preparation protocol where the root exudates sample is evaporated to dryness using a rotari-evaporator at 45^{O}C, and then extracted with methanol; the methanol extract is further evaporated to dryness and then re-dissolved in dimethyl sulfoxide. Using this sample preparation protocol, we could eliminate completely the problems of chloride interference, as chloride does not dissolve in methanol.

Using several root exudates samples from B. humidicola, we have shown that the NI activity from the root exudates or plant tissue extracts can be recovered into the methanol extract. The remaining portion of the root exudates did not show inhibitory activity (data not shown). This sample preparation protocol has now become a standard procedure for processing the root exudates samples for the determination of NI activity.

3.6.3 Comparative evaluation of six tropical grass species for the ability to inhibit nitrification from acid soil

Contributors: M. Rondon, I.M. Rao and C.E. Lascano (CIAT); G.V. Subbarao, K. Nakahara, T. Ishikawa, K. Okada and O. Ito (JIRCAS, Japan)

Rationale

Collaborative research with JIRCAS colleagues has shown that *B. humidicola* CIAT 679 inhibits nitrification of ammonium and reduces the emission of nitrous oxide into the atmosphere (IP-5 2001 Annual Report). Given these findings with one genotype of *B. humidicola*, there is a need to determine the extent of genetic variation among tropical grasses in their ability to inhibit nitrification and reduce emissions of N_2O. 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 constraints but also can protect the environment. Given the vast areas under *B. humidicola* in the tropics, reductions in net emissions of N_2O could have important environmental implications. The main objective was to quantify differences among several tropical grasses to inhibit nitrification and associated reductions in N_2O emission under greenhouse conditions using infertile acid soil. Also we intend to correlate nitrification inhibition with root biomass and length, and to monitor nitrate and ammonium levels in the soil after addition of ammonium –N as fertilizer.

Materials and Methods

A sandy loam oxisol from the Llanos (Matazul) of Colombia was used to grow the plants (4 kg of soil/pot). A basal level of nutrients were applied before planting (kg/ha): 40 N, 50 P, 100 K, 66 Ca, 28.5 Mg, 20 S and micronutrients at 2 Zn, 2 Cu, 0.1 B and 0.1 Mo. A total of 6 different tropical grasses were used as test plants at two levels of ammonium sulfate application (0 and 100 kg/ha). The grasses

included: *B. humidicola* cv. *Humidicola*; *B. decumbens* cv. *Basilisk*; *B. dictyoneura* cv. *Llanero*; *B.* hybrid cv. *Mulato*; *B. brizantha* cv *Marandu*; *P. maximum* cv. *Common*. Two control treatments were included: soil without plants that had no application of ammonium sulfate or had application of ammonium sulfate. The experiment was arranged as a completely randomized block design with 4 replications. Plants were allowed to grow for 7 weeks and were cut to 10 cm to stimulate regrowth for 3 weeks and were cut again at 10 cm height to allow regrowth and to simulate grazing effects under field conditions. After another week (at 11 weeks) of regrowth, ammonium sulfate was applied in solution at a rate equivalent to 100 kg $N\text{-}NH_4$ per hectare. To favor a greater development of plant roots in the pots, which could increase the NI effects, the plants were allowed to grow for 13 weeks more (at 24 weeks) before making a second application of ammonium sulphate at the same dose. Plants grew for 4 weeks more before final harvest (at 28 weeks). Thus the total length of the experiment was 7 months. At the end of the experiment, plants were harvested and separated into shoot and roots. Root length was measured using a root length scanner. Dry matter content and N status of both shoot and roots was determined.

Results and Discussion.

Results from the comparative evaluation of six tropical grasses (that are predominantly grown in South America) indicate that substantial levels of NI (Nitrification inhibitory) activity is present in the root exudates of other Brachiaria grasses in addition to *B. humidicola* (Table 53). However, among Brachiaria grasses tested in this study, NI activity is substantially lower in *B. brizantha* cv. Marandú. It is interesting to note that a complete absence (below the detectable limit to our bioassay) of NI activity from the root exudates of *P. maximum*.

The total NI activity (i.e. NI activity from four plants pot^{-1}) of *B. humidicola*, *B. decumbens*, B. *dictyoneura* and *Brachiaria* hybrid cv. Mulato were similar based on our bioassay estimations (Table 53). Nevertheless, the NI activity of the root exudates needs to be confirmed further using soil incubation studies.

The presence of substantial levels of NI activity in the root exudates of *Brachiaria* hybrid cv. Mulato has immediate practical implications as this hybrid has a huge potential for being planted in large areas of South America. Also, the implications of such high levels of nitrification inhibition (if confirmed further from the soil incubation studies and field studies) in *Brachiaria* hybrid cv. Mulato opens the possibility to screen for this trait in the Brachiaria improvement program. The lack of nitrification inhibition ability in *P. maximum* is an interesting issue as this is the levels of N inputs.

Large differences in specific NI activity among the tropical grasses were found in this study, with *B. humidicola* exhibiting the highest level of inhibition (Table 54). Furthermore, the total and specific NI activities were substantially lower in the treatment without ammonium in all the Brachiaria grasses tested. Similar trends (data not shown) were found when the specific activity was calculated using total root length instead of root dry weight that is shown in Table 54.

The immediate task is to characterize and quantify this phenomenon further in grasses that have contrasting abilities in nitrification inhibition (e.g., *B. humidicola* vs. *Panicum maximum*) both under glasshouse and field conditions. This would help us understand the potential impact of this relatively new phenomenon in nitrogen dynamics in pasture systems. We also need to determine the relative importance of total NI activity *vs*. specific NI activity in influencing the nitrification process (i.e. inhibition) in a soil environment. Whether the presence of ammonium nitrogen (or any form of nitrogen) near the root zone of the soil acts as a triggering factor for the release of inhibitory compounds or if the plant nitrogen status regulates the NI activity in the root exudates, are some the other issues that need to be resolved through further research work.

Table 53. Nitrification inhibitory activity (total NI activity pot^{-1}) of the root exudates from 6 tropical grasses grown under two levels of N fertilization. Plants were grown for seven months before being used for collecting root exudates.

Pasture species	N Fertilizer Treatment	NI activity (in AT units pot^{-1})	SD
B. humidicola	With ammonium supply	81.95	7.35
B. decumbens	With ammonium supply	77.39	5.33
B. dictyoneura	With ammonium supply	70.54	11.30
B. hybrid Mulato	With ammonium supply	84.52	11.20
B. brizantha	With ammonium supply	49.62	13.33
P. maximum	With ammonium supply	-9.43*	8.80
B. humidicola	Without ammonium supply	13.04	6.46
B. decumbens	Without ammonium supply	21.43	1.28
B. dictyoneura	Without ammonium supply	19.22	1.90
B. hybrid cv. Mulato	Without ammonium supply	23.19	1.45
B. brizantha	Without ammonium supply	23.91	0.36
P. maximum	Without ammonium supply	1.81	2.39

Note: Ammonium was supplied as ammonium sulfate at 100 kg ha^{-1} rate. NI activity is expressed as AT units; One AT unit is defined as the inhibitory activity caused by the addition of 0.44 µM of allylthiourea 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.

*Negative activity - nitrification was stimulated by the root exudates. SD = standard deviation.

Table 54. Specific NI (Nitrification inhibitory) activity (NI activity g^{-1} root dry wt) of the root exudates from 6 tropical grasses grown under two levels of N fertilization.

Pasture species	N Fertilizer Treatment	Specific NI activity	SD
B. humidicola	With ammonium supply	17.30	0.34
B. decumbens	With ammonium supply	12.31	0.27
B. dictyoneura	With ammonium supply	5.93	0.68
B. hybrid *Mulato*	With ammonium supply	10.16	2.26
B. brizantha	With ammonium supply	8.35	2.20
P. maximum	With ammonium supply	-1.67	1.34
B. humidicola	Without ammonium supply	2.87	1.17
B. decumbens	Without ammonium supply	7.22	2.19
B. dictyoneura	Without ammonium supply	2.90	0.31
B. hybrid Mulato	Without ammonium supply	6.46	0.45
B. brizantha	Without ammonium supply	6.75	0.40
P. maximum	Without ammonium supply	0.69	0.75

3.6.4 Development of an incubation protocol to assess nitrification inhibition by addition of root exudates to soils.

Contributors: M. Rondon, I.M. Rao and C. E. Lascano (CIAT); G.V. Subbarao, K. Nakahara, T. Ishikawa, K. Okada and O. Ito (JIRCAS, Japan)

Rationale

According to the results presented in the preceding section, root exudates from various tropical grasses have differential ability to reduce nitrification as demonstrated by the bioassay. As indicated before, the next sequential step is to assess the effect of application of root exudates directly to soil in relation with Nitrification Inhibition. Given that the process of collecting and concentrating root exudates is time and labor intensive, and that it is difficult to generate large amounts of root exudates, it is desirable to develop a methodology to apply root exudates to small amounts of soil in conditions that allow a proper monitoring of inorganic nitrogen in the soil as well as reliable monitoring of fluxes of nitrous oxide. This later process is especially difficult to follow, given the inherent variability in gas fluxes from soil even under controlled incubation conditions.

The purpose of a recent scientific internship of Marco Rondon at JIRCAS laboratories was to develop and test a simple incubation methodology to quantify the effect of application of root exudates to soils on nitrate and ammonium levels and on fluxes of nitrous oxide. As nitrous oxide fluxes are highly dependent on the moisture content of the soil, the method needs to maintain moisture content at levels of 50-60% of water filled pore space (WFPS), which is considered optimum for nitrification in most soils (Del Grosse et al., 2002) After trying various alternatives, a suitable method was developed and tested. The final protocol is described here, and some results using it are presented.

Description of the method

Plastic syringes (20 ml) are used both as incubation containers and as gas collection chambers for monitoring gas fluxes. Ten g of air-dried soil are loaded inside the syringe on top of an inert fine nylon (100 µm mesh), which allows gas exchange but prevents soil to move out of the syringe. On top of the soil surface, another 2 discs of nylon mesh are placed to hold the soil in place and serve as barriers to reduce moisture evaporation from the soil surface. The nylon barrier also serves to isolate the soil from the syringe piston during the gas collection process. Once the soil and nylon discs are placed inside the syringe, the syringe is tapped several times until the soil redistributes itself into a minimum volume. Water, fertilizer solution or the root exudates to be tested are then added to the soil to reach 60% WFPS. Water is sprinkled slowly and uniformly on top of the nylon mesh with a fine syringe needle. The liquid progressively moistens the soil by capillary movement and after about one hour, the soil is well mixed with the liquid without a need for further disturbances. This procedure allows a very effective control of the moisture content in the syringes, which otherwise, is a very time consuming and error causing procedure. Temperature is maintained stable during the incubation time by placing the syringes in an incubation chamber. During the incubation time, the soil can freely exchange gases with the surrounding air. The combination of controlled temperature and moisture content permits reliable and reproducible monitoring of gas fluxes.

At the time of collecting gas samples, syringes are removed from the incubation chamber. A Teflon valve is attached to the lower end of the syringe. The syringe piston is inserted into the barrel and the piston is depressed fully (without compressing the soil) to force the air inside the soil pore space to move outside the syringe. The soil air is replenished with fresh air by moving backwards the syringe piston. This procedure is

repeated four times to warrant a complete exchange of the original air inside the soil pores. Once this is done, the gas sample corresponding to time zero could be collected. Then, the piston is raised at a predetermined height inside the syringe, to provide a preset soil to air volume ratio. The needle valve is closed creating a tight sealed chamber. The chamber is allowed to exchange gases during a determined gas collection period, typically one hour. After this time, the air inside the chamber could be directly analyzed by gas chromatography or transferred into pre-evacuated glass vials to be analyzed at a later time. Once the sample is collected, the syringe piston is removed to allow the soil to exchange again gases freely with the surrounding air. The procedure permits an easy way to adjust the air to soil volume ratio, so very low fluxes of gases could be detected. The procedure was tested repeatedly and good reproducibility in gas measurements was obtained.

3.6.5. Effect of application of root exudates on inorganic nitrogen and fluxes of nitrous oxides from incubated soils.

Contributors: M. Rondon, I.M. Rao and C. E. Lascano (CIAT); G.V. Subbarao, K. Nakahara, T. Ishikawa, K. Okada and O. Ito (JIRCAS, Japan)

Materials and methods

The procedure described in activity 3.8.4 was used to study the effect of application of root exudates on fluxes of N_2O from incubated soils. Root exudates were obtained from intact plants grown in solution media, which were transferred from the nutrient media into de-ionized water. Plants were allowed to exudate during 24 hours and the resulting root exudates were concentrated using a rotovapor at 45C.

Experimental treatments included:

1. Blank (W): Application of water
2. Control (AS): application of aqueous solution of ammonium sulfate at a rate of 91 ug N-NH_4/ g soil
3. Root exudates from *B. humidicola* at low concentration (BL). Exudates were concentrated from 30 liters into 900 ml with a rotovapor.
4. Root exudates from *B. humidicola* at high concentration (BH). Exudates were concentrated from 30 liters into 200 ml with a rotovapor.
5. Root Exudates from soybean at low concentration (SNB). Exudates were concentrated from 30 liters into 900 ml.

Treatments with root exudates received the same dose of ammonium sulfate as the Control AS. The ammonium was dissolved in the root exudates prior to adding the solution to soils.

A fertile Andisoil from Tsukuba was used. Soil was collected from the top 20 cm in the field, air dried and then sieved with a 2 mm mesh. Soil was well mixed and 10 g sub samples were loaded into plastic syringes. The corresponding treatment liquid (Water, ammonium sulphate, root exudates + AS) was then applied to the syringes. 3.6 ml of aqueous solution was used in each syringe, to raise the soil moisture content to 60%WFPS. Once wetted, the syringes were kept at 21C in an incubation chamber for 24 days. These syringes were subsequently used to monitor fluxes of N_2O over time, according to the procedure described above (activity 3.8.4). Gas samples were collected at 5, 9, 14, 19 and 24 days after ammonium sulphate application. 8ml of the gas sample were transferred into a 4ml preevacuated glass vial. Analysis of N_2O was performed typically within 2 days after collection using a Shimadzu 14B GC equipped with an ECD detector, and a stainless steel column packed with Porapaq Q80 mesh.

Gas fluxes were calculated by linear regression of chamber headspace concentration vs. time. The results are presented as arithmetic means of three replicates.

For monitoring of nitrate and ammonium levels, parallel samples of soil were incubated. Due to limited availability of root exudates, only 2 grams of air-dried soil was used for monitoring of inorganic N. The vials received the same treatments as the syringes and incubation was started at the same date. KCl extracts were made of the incubated soil in the vials at the same dates of gas collection. 20 ml of 2M KCl were used to extract the 2 g of soil in each vial. Shaking time was 1 hour. The KCl extract was filtered immediately after shaking using Whatman 5C filter paper that was prewashed with KCL. Extracts were maintained refrigerated until the time of analysis. Analysis was done colorimetrically using an Autoanalyzer.

Results and Discussion

As expected, (Figure 63), addition of water or aqueous solutions to air dried soil resulted in a temporary increase in fluxes of N_2O. The addition of root exudates from soybean strongly promoted the fluxes of the gas from the soil during the initial week of incubation compared to the Control treatment. Recent research (G. V. Subbarao, personal communication) has shown that root exudates from soybean also promote nitrification in the bioassay medium. This may be the result of addition of readily available carbon sources in the root exudates. The large peak of N_2O could be caused by rapid denitrification. Reports from the literature (Azam et al., 2002), indicate that when nitrogen is added to the soil, in addition to nitrogen sources, microbial respiratory activity is greatly enhanced and this may result in a rapid depletion of oxygen in the soil micropores which favors the formation of anaerobic microsites where denitrification may take place. After one week, the initial peak of nitrous oxide decays and soils that received root exudates from B. *humidicola* at high concentration, show subsequently lower emissions of nitrous oxide.

In Figure 64, accumulated net fluxes of nitrous oxide for a period of 24 days are presented. Exudates from soybean also result in appreciable higher net accumulated fluxes of N_2O during the incubation period. Whether these fluxes are resulting from enhanced nitrification or denitrification needs to be clarified in further studies. No appreciable reduction in fluxes of the gas appear to occur as a consequence of the addition of root exudates from *B. humidicola* at low concentration, suggesting that the added dose of the active compound was probably not high enough to interact with all the population of nitrifying bacteria in the soil. In contrast to this, the addition of exudates of *B. humidicola* at high concentration resulted in a net decrease of around 45% of total emissions of N_2O relative to the control treatment.

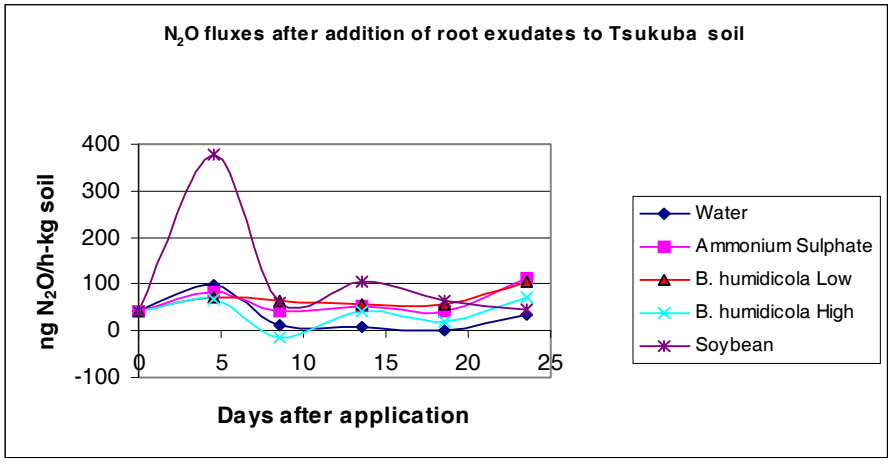

Figure 63 Fluxes of nitrous oxide following addition of root exudates from different species to incubated Andisol.

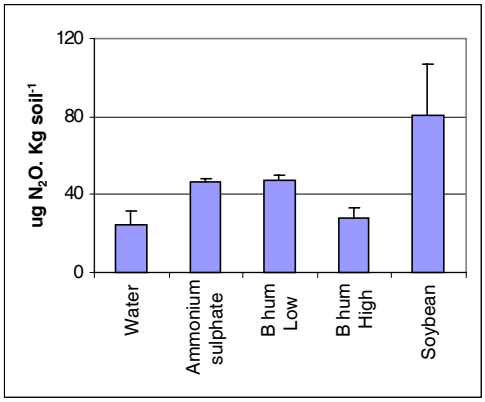

Figure 64 Accumulated fluxes of nitrous oxide over a 24-day incubation period.

Similar trends were observed in levels of ammonium in the soils, (Figure 65). Ammonium levels were consistently higher in the *B. humidicola* treatment indicating reduced nitrification rate, while consistently lower levels were found in the soybean exudates treatment confirming promoted nitrification.

The nitrification inhibition seems to be more effective between 5 and 9 days as indicated by the slope of the graphs in Figure 65. After two weeks of incubation, the curves show similar slopes suggesting that the residual effect of the root exudates could be of around two weeks. This is shorter residual time than the 3 weeks

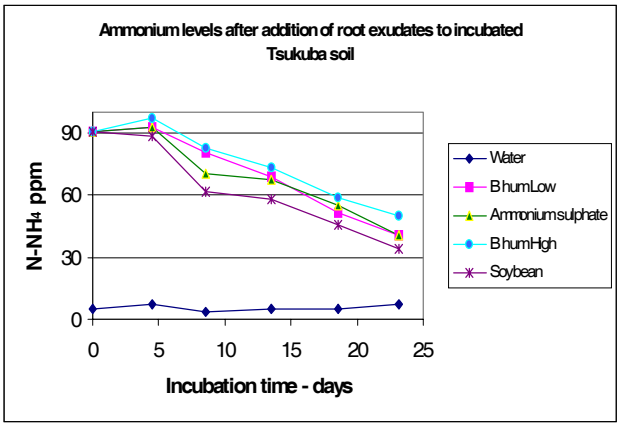

Figure 65. Ammonium evolution on incubated soils after receiving root exudates.

suggested by previous studies in pots (Ishikawa et al, in press), and could be an indication of low additions of the active compound with the root exudates as compared with concentrations generated by the root system in pot experiments or in the field.

Results from this study suggest that there is a minimum threshold concentration of root exudates (active compound) required to effectively reduce nitrification in soils. Once the active compounds in root exudates that are responsible for NI activity are identified, it would be possible to conduct more controlled experiments to add known amounts of the active compound to the soils.

The procedures developed and tested here, have proven indeed to be easy, and reliable for monitoring fluxes of nitrous oxide and appropriate to follow the levels of inorganic nitrogen in the soil. These methods could be applied to test the effect of additions of root exudates from different plant species to soils on Nitrification Inhibition and associated fluxes of N_2O and would contribute to rapid advances in this field of research.

The method is being currently used to assess the effect of application of various root exudates into soils of contrasting chemical and physical characteristics including oxisols from Colombian savannas and inceptisols from Andean hillsides. Results will be reported next year.