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

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

Highlights

- Collaborative research conducted with Hokkaido University in Japan generated the first evidence based on ^{27}Al NMR analysis that organic acids within root tissue help detoxify aluminum in non-accumulator species such as in the *Brachiaria* hybrid cv Mulato.

- Showed that the high level of aluminum resistance in signal grass (*Brachiaria decumbens*) is part of a generic resistance mechanism that is effective against trivalent cations in general.

- Showed that hematoxylin staining could be employed as a quick selection criterion to discard Al-sensitive genotypes in the *Brachiaria* breeding program, because most of the Al accumulates in the external layer of root meristems and should be readily stainable with hematoxylin in intact root apices.

- Constructed a genetic linkage map for aluminum resistance in *Brachiaria*, evaluated 50% of the polymorphic SSRs and AFLPs in the F1 cross of *B. decumbens* x *B. ruziziensis* and found preliminary associations between markers SSRs and AFLPs and three phenotype root traits of aluminum resistance.

- Using microarray technology, the 3'-UTR sequences of candidate genes associated with aluminum (Al) resistance in *Brachiaria decumbens* were identified by comparing expression levels between genotypes and treatments.

- Using screening procedure to evaluate aluminum resistance, 3 sexual hybrids (SX03NO/0846, SX03NO/2367, SX03NO/0881) were identified with greater level of Al resistance than that of the sexual parent. One of the apomictic hybrids (BR02NO1372) was outstanding in its level of Al resistance and this hybrid is also resistant to spittlebug

- Showed that the *Brachiaria* hybrid, FM9503-S046-024 (Mulato 2) performed well into the third year after establishment in the Llanos and its superior performance at 30 months after establishment was associated with its ability to acquire greater amounts of nutrients, particularly Ca and Mg from low fertility soil

- Results from a 4-year field study in the Piedmont showed that the *Arachis pintoi* accessions CIAT 18744, 18751 and 22159 were superior to the commercial cultivar (CIAT 17434) in terms of persistence with low amounts of initial fertilizer application.

- Collaborative research conducted in Goettingen, Germany, under controlled environmental conditions in a growth chamber, showed that the *Arachis pintoi* accessions CIAT 18744 was more efficient acquiring P from less available P-pools in a low-P oxisol than the commercial cultivar, CIAT 17434. This high P efficiency and the increase of P uptake were found to be due to a high P influx. The activity of acid phosphatase on root surface and exudation of organic acids (lactic and acetic) did not contribute to this increase in P influx.

3.1.1 Edaphic adaptation of *Brachiaria*

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Previous research 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 to evaluate Al resistance 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 Investigating 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*.

A) Aluminum resistance coincides with differential resistance to trivalent lanthanide cations in *Brachiaria*

Contributors: **P. Wenzl (CAMBIA, Australia), A.L. Chaves, M.E. Recio, J. Tohme and I.M. Rao (CIAT)**

Rationale

Signalgrass (*Brachiaria decumbens*) has evolved a highly effective Al-resistance mechanism that does not appear to rely on chelation of Al ions with organic-acid anions. Electrical charges at the external surface of root cells generate an electrostatic potential that modulates cell-surface ion activities and hence ion uptake and intoxication. We hypothesized that the superior Al resistance of signalgrass compared to closely related ruzigrass (*B. ruziziensis*) could be due to a less negative surface potential at root cells that are critical to root growth and elongation. We tested this hypothesis by investigating whether Al resistance of signalgrass was associated with superior resistance to other cations toxicants and greater susceptibility to anionic toxicants.

Materials and Methods

Seeds were germinated in continuously aerated 200 μM $CaCl_2$ (pH 4.20) for 3-4 days. Homogeneous seedlings were selected and their root lengths were recorded. The seedlings were then transferred to continuously aerated treatments solutions in the greenhouse (toxicant + 200 μM $CaCl_2$, pH 4.20; see left panel below). After three days root length were measured again. Each experiment comprised six toxicant levels plus the toxicant-free basal solution. Three independent experiments were performed for each toxicant. The concentration of a toxicant that inhibited relative root elongation (RRE) by 50 % (C_{50}) was determined for each of the two species after fitting a Weibull function to the pooled data from the three replicate experiments by using the Marquardt-Levenberg algorithm (right panel above). The SE of C_{50} was computed based on error propagation rules. The C_{50} values of the two species for a particular toxicant were considered to be different if their 95 % confidence intervals did not overlap.

Results and Discussion

The superior Al resistance of signalgrass compared to ruzigrass was associated with greater resistance to all the trivalent lanthanide cations tested (Figure 11). If a lower root cell surface negativity was the cause for the greater lanthanide resistance of signalgrass, signalgrass should be more resistant to other cationic toxicants and more sensitive to anionic toxicants. The two species, however, were equally sensitive to the majority of divalent and monovalent cations (Figure 10b) and most anions (Figure 10c). Apart from lanthanides and Cd^{2+}, signalgrass was more resistant than ruzigrass only for those inorganic toxicants that are in redox equilibrium with a trivalent cationic form: $Fe^{2+} \leftrightarrow Fe^{3+}$, $Co^{2+} \leftrightarrow Co^{3+}$, $Cr_2O_7^{2''} \leftrightarrow Cr^{3+}$. An organic trivalent cation (spermidine^{3+}), by contrast, was equally toxic to both species.

These results suggest that Al resistance in signalgrass is part of a more generic resistance mechanism that is effective against trivalent cations in general, a finding that confirms the unique physiological basis of Al resistance in signalgrass.

The pattern of resistance to cationic and anionic toxicants, however, is not consistent with the idea that a less negative root cell surface potential confers resistance to cationic toxicants as a result of electrostatic interactions, that is, solely based on the charge but not the structural properties of a toxicant. The cross-resistance of signalgrass to Al and other, mostly trivalent inorganic cations may instead be based on interspecific differences in critical cellular sites to which trivalent cations such as Al^{3+} and lanthanides bind. More work is required to elucidate the nature of these sites and to develop biochemically-based strategies to isolate the underlying genes. It may be possible to use lanthanide cations as proxies for Al to circumvent some of the problems and ambiguities caused by the difficulties to predict Al speciation.

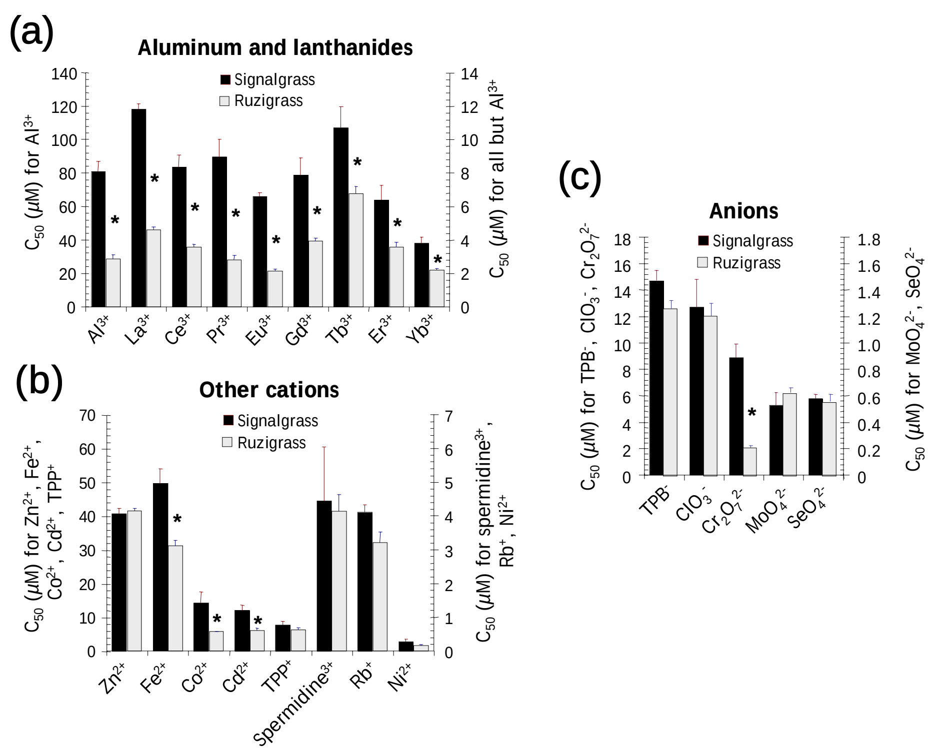

Figure 11. Concentrations of cationic and anionic toxicants required for inhibiting root elongation of signalgrass and ruzigrass by 50 % (C_{50}).

B) Accumulation of callose and aluminum in root tips of *Brachiaria* spp.

Contributors: A. Arango, P. Wenzl, I.M. Rao, and J. Tohme (CIAT)

Rationale

The effects of aluminum (Al) toxicity on callose accumulation were evaluated in root tips of *Brachiaria* populations previously evaluated for physiological and genetics response (IP-5 Annual report 2002, 2003). *Brachiaria decumbens* (Al resistant), *B. ruziziensis* (Al sensitive) and two contrasting *B. ruziziensis* x *B. decumbens* hybrids were evaluated after 3, 12 and 21 days of Al treatment (200 µM $AlCl_3$).

Materials and Methods

Rooted stem cuttings of *B. decumbens*, *B. ruziziensis* and two contrasting hybrids were cultivated as described previously (IP-5 Annual Report, 2003). Root apices (5 mm), collected after different times of exposure to Al, were fixed during 48 hours in 2.5 % glutaraldehyde to detect callose with aniline blue, or in a 1:1 mixture of 3.7 % phormol (pH 7.4) and 2.5 % glutaraldehyde to detect Al accumulation with hematoxylin.

Samples were cut (7 µm) and processed and aluminum was visualized by staining with 0.1% (w/v) hematoxylin and 0.01% (w/v) KIO_3 for 20 min. Callose was visualized by staining with 0.1% (w/v) aniline blue and 1M glycine NaOH (pH 9.5).

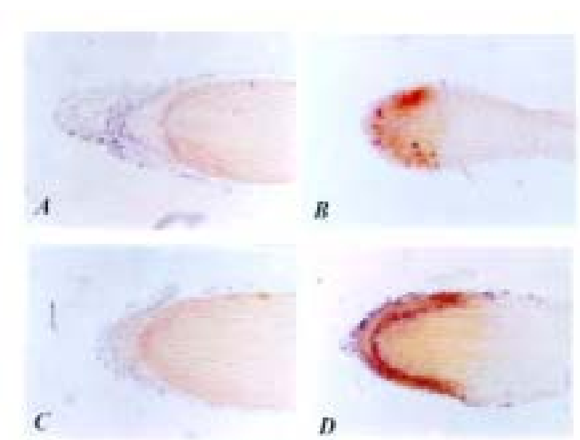

Figure 12. Hematoxylin staining of *Brachiaria*.root apices. **A**. Al-tolerant parent (*B. decumbens*); **B**. Al-sensitive parent (*B. ruziziensis*); **C**. Al-tolerant hybrid; **D**. Al-sensitive hybrid (12 days of Al treatment).

Results and Discussion

Differential hematoxylin staining was observed between sensitive and tolerant genotypes. Root apices of *B. decumbens* (Figure 12A) and an Al-resistant hybrid (Figure 12C) did not accumulate much Al. By contrast, root apices of *B. ruziziensis* (Figure 12B) and an Al-sensitive hybrid (Figure 12D) accumulated Al in the outer layer of root meristems. Aniline-blue staining of histological sections revealed a higher content of callose for Al-sensitive genotypes.

The pattern of callose accumulation was only partially correlated with that of Al (visualized by hematoxylin). Al-tolerant *B. decumbens* accumulated callose exclusively in the root cap and at the surface of the root meristem. Al-sensitive *B. ruziziensis* accumulated a large amount of callose in cortical and vascular tissues, an area where little Al was detected in the hematoxylin stain (Figure 13).

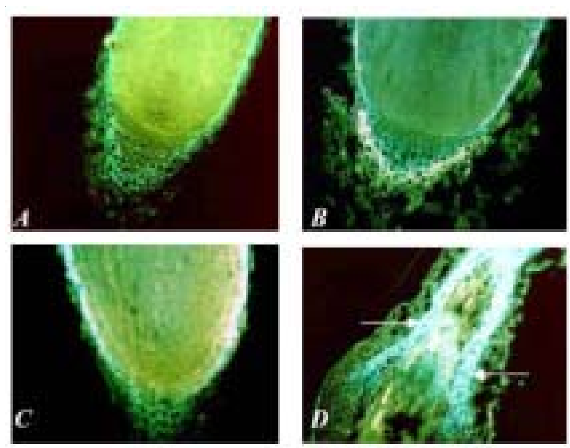

Figure 13. Callose detection by aniline-blue staining in histological sections of *Brachiaria* root apices. **A, B**. *B. decumbens* (without and with Al). **C, D**. *B. ruziziensis* (without and with Al). Arrows designate callose deposition (3 days of Al treatment).

Hematoxylin staining could be employed as a quick selection criterion to discard Al-sensitive geotypes in the Brachiaria breeding program, because most of the Al accumulates in the external layer of root meristems and should be readily stainable with hematoxylin in intact apices.

C) Identification of molecular markers and QTLs associated with gene(s) conferring aluminum resistance in a *Brachiaria ruziziensis* × *Brachiaria decumbens* cross (F1)

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Rationale

Acid soils have been estimated to occur on about 40% of the arable land (3.95 billions of ha). Plant growth on these soils is constrained mainly by aluminum (Al) toxicity and deficiencies of nutrients such as phosphorus (P), nitrogen (N), and calcium (Ca). There is considerable variation within and between plant species in their ability to resist Al, and this variation within some species has allowed breeders to develop genotypes that are able to grow on acid soils. Within the *Brachiaria* genus, Al resistance of signal grass (*B. decumbens* Stapf cv Basilisk), a widely sown tropical forage grass, is outstanding compared with the closely related ruzigrass (*B. ruziziensis* Germain and Evrard cv Common). The main objective of this work is to identify microsatellites, AFLPs and QTLs associated with the gene(s) conferring aluminum resistance in a *Brachiaria ruziziensis* × *Brachiaria decumbens* cross.

Materials and Methods

An F_1 hybrid population of 263 individuals (*B. ruziziensis* x *B. decumbens*) was used for this study. Young leaves were cut and placed in paper bags in an incubator previously set at 45-50°C. Samples were allowed to dry for at least 20h, or until the leaves were dry enough that they break easily. Samples were stored at –80°C until grinding. Dried leaf tissue was grinded with stainless steel spheres with vigorous shaking. Genomic DNA was extracted using a CTAB-Chloroform protocol with some modifications for small amounts of tissue. DNA was quantified on a DyNA Quant 200ä Flourometer (Hoefer Scientific Instruments) and diluted at 4ng/ul for SSRs amplification and 25ng/ul for AFLPs amplification. Methods for the isolation of microsatellites, and the methodology for PCR amplification and evaluation of polymorphism, were as described previously (SB-2 Annual Report, 2000; 2001) with some modifications. The AFLP Analysis System I kit, and AFLP Analysis System II Small Genome, from Invitrogenâ were used for AFLP amplification, following the instructions, with some modifications. Silver staining (Promega Inc., USA), was used to visualize allele segregation of the markers on 6% denaturing polyacrylamide gels with 5M Urea and 0.5X TBE.

Results and discussion

Microsatellites: 73 SSRs were evaluates in the parental genotypes of which 40 were found to be polymorphic. When run in the progeny, three sets of primers did not amplify in 30% of the progeny, so they were discarded together with three more monomorphic microsatellites. Ninety-seven polymorphic alleles were scored in the population, out of which 63 were found with the *B. decumbens*, Al-tolerant, 606 genotype, while 34 carried the *B. ruziziensis*, Al-sensitive, 44-2 genotype (Figure 14).

AFLPs: 64 combinations of primers were assayed with the two parental genotypes. Among them, 12 having high number of polymorphic bands, were chosen. To date 3 combinations (E-ACC/M-CAC; E-ACT/M-CTA and E-ACG/M-CAG) were run in the progeny and yielded 63 polymorphic bands distributed as follows: 50 were found in the Al-tolerant parental and 13 in the Al-sensitive genotype. (Figure 15).

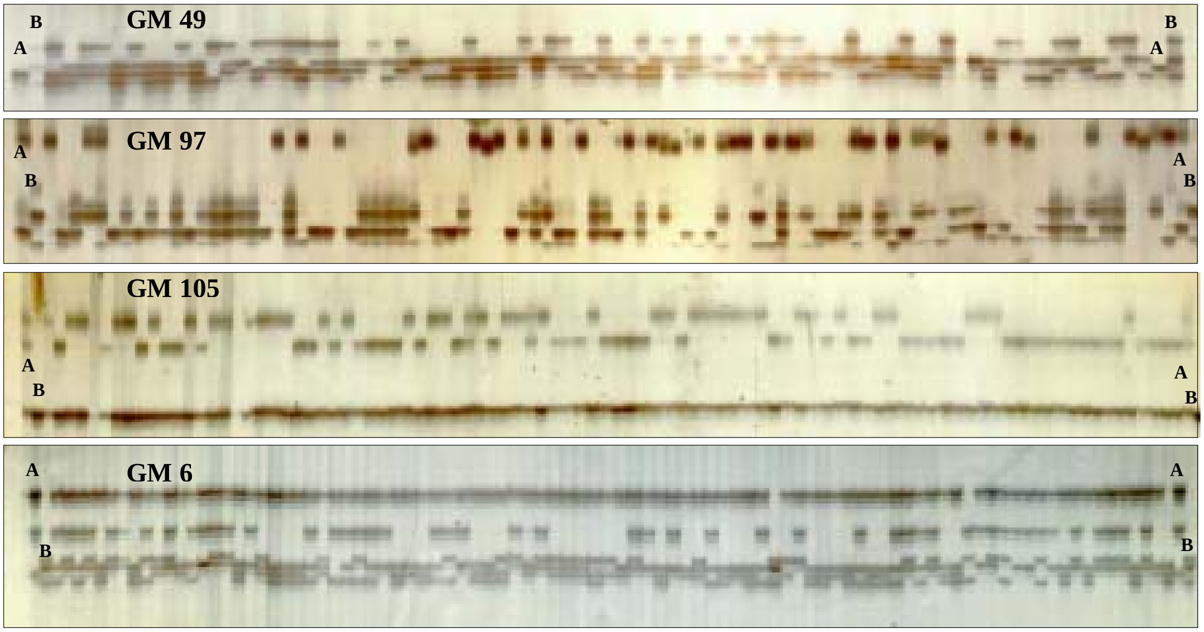

Figure 14. Segregation of four SSR alleles in some individuals of the F_1 *B. ruziziensis* x *B. decumbens* progeny. **A**, Parental genotype 606 (Al-resistant) and **B**, parental genotype 44-2 (Al-sensitive).

Linkage analysis: Segregation of markers in a 1:1 ratio, as single dose restriction fragments (SDRF), will be determined by a Chi-square test. The data matrixes for presence or absence of bands were analyzed with MAPMAKER v3.0b for PC, and MAPMAKER v2 for Macintosh. Using 56 SSR and 50 AFLPs molecular markers, we constructed a putative linkage map with 18 linkage groups. These linkage groups span 445.3 cM, and the average marker density is one per every 6.1 cM. The position of 78 markers is shown in Figure 15, on the framework molecular genetic map of *Brachiaria* (LOD = 25 and tetha (θ) = 25). Map distances are shown in Kosambi map units.

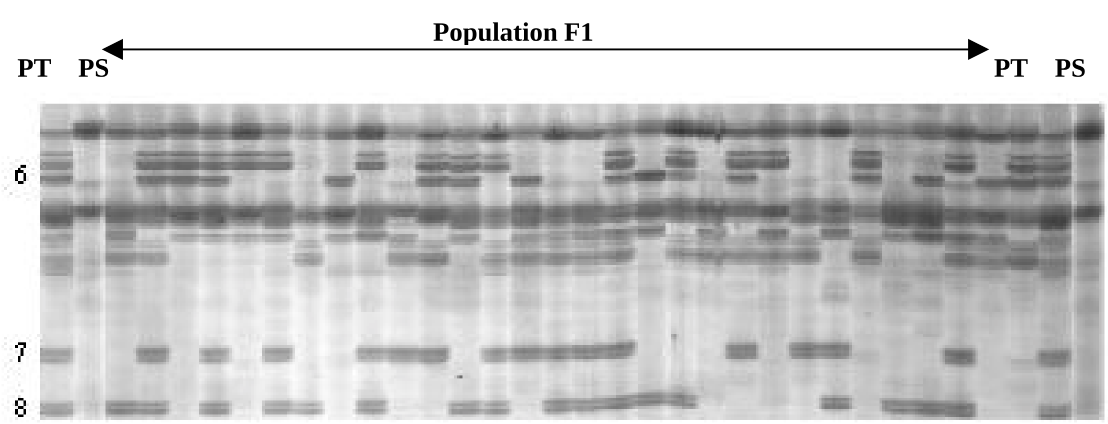

Figure 15. Segregation of AFLPs bands in individuals of the F_1 *B. ruziziensis* x *B. decumbens* progeny. **PT**, Parental genotype 606 (Al-resistant) and **PS**, parental genotype 44-2 (Al-sensitive).

Association between Molecular Markers and Al resistance: To find association with molecular markers, a preliminary analysis of 106 markers at the 10% level was done using SAS. Putative associations were found between 78 SSRs and AFLPs markers and the phenotypic characterization under greenhouse conditions. The three phenotypic variables for Al resistance (root length, abundance of root tips, mean root diameter) were analyzed for association with molecular markers. We found 13 molecular markers with R^2 between 0.0124-0.0267 that explain the variance for these traits; molecular markers associated with phenotypic characterization are in blue (Table 15 and Figure 16).

Further work is in progress: (i) to saturate linkage map of *B. decumbens* CIAT 606 parent with SSR and AFLPS; (ii) to analyze data for mapping for Al resistance; (iii) to conduct QTLs analysis for Al resistance; and (iv) to design of SCARs for marker assisted selection.

Table 15. Association analysis between molecular markers and phenotipic variable for Al resistance in a *Brachiaria decumbens* x *Brachiaria ruziziens*es hybrid population.

Marker	R^2	Linkage Group	Phenotipic Variable
GM 44d2	0.0267	B	Rl[a]
GM 44d2	0.0218	B	Tips[b]
C1b4	0.0181	R	Tips
GM 109c	0.0177	N	Rd[c]
C1b17	0.166	D	Rd
C2b17	0.0161	R	Tips
C2b15	0.016	I	Rd
GM 109d	0.0156	N	Rd
GM 79c	0.0156	A	Rl
GM 58a	0.0151	D	Rd
GM 44d1	0.0149	B	Tips
C2b12	0.0145	B	Rd
C1b12	0.014	R	Tips
GM 79d	0.013	D	Rd
GM 44d2	0.0124	B	Rd

a Root Length
b Abundance of root Tips
c Root Diameter

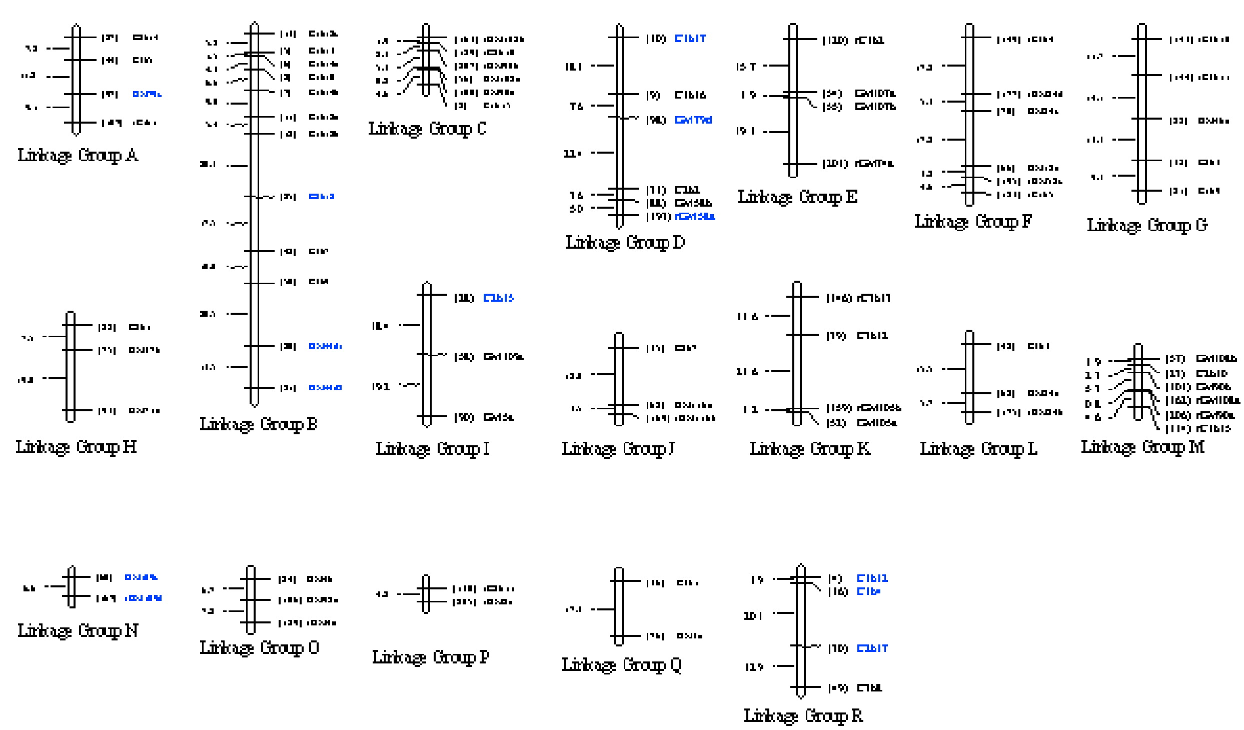

Figure 16. Preliminary *Brachiaria* linkage map of a *B. ruziziensis* × *B. decumbens* F1. Markers in blue indicate putative association with Al resistance.

D) Identification of candidate genes associated with aluminum resistance in *Brachiaria decumbens*.

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Rationale

The 3'-UTR sequences of candidate genes associated with aluminum (Al) resistance were identified by comparing expression levels between genotypes and treatments. Subtractive libraries were prepared from root apices of contrasting genotypes grown in the presence and absence of Al: *Brachiaria decumbens* (Al-resistant parent), three Al-resistant *B. ruzizie*nsis x *B. decumbens* hybrids, *B. ruziziensis* (Al-sensitive parent), and seven Al-sensitive hybrids (Annual Report 2003). Microarray technology was then used to catalogue clones derived from genes that were differentially expressed between samples.

Materials and Methods

Subtractive libraries of 3'UTR fragments were prepared with the differential subtraction chain (DSC) method (IP-5 Annual Report, 2003). Inserts were amplified and arrayed in duplicate on glass slides. Pairs of contrasting RNA populations (control, target) were hybridized to microarrays (Table 16). Two pairs of dye-swap hybridizations were performed per combination of control and target.

Microarray sample pools (MSPs) were synthesized from 2.4 to 240 ng of cDNA to cover a 100-fold range of signal intensities. They were arrayed together with other controls, such as negative controls (spotting buffer, polylinker of the vector used for library preparation, unrelated genes such as insulin, Sp1 β-cell, HPH) and positive controls (GADPH of *Brachiaria*, α-tubulin, Spy genes). A total of 768 controls were spotted onto the array. The logarithms of the crude ratios between the two channels were first normalized by using the lowess algorithm and then analyzed with the Significance Analysis of Microarrays (SAM) software.

Differentially expressed clones were amplified, purified (Qiagen kit) and sequenced (ABI BigDye terminator kit). The sequences obtained were compared against those in the UTR database at http://bighost.area.ba.cn.it/BIG/UTRHome.

Results and Discussion

The microarray hybridizations identified a total of 35 3'-UTR fragments of candidate genes that were expressed differentially in the four comparisons between target and control pools (Figure 17, Table 16).

Seven clones contained the post-transcriptional control sequence 15LOX-Dice (15-Lypoxigenase Differentiation Control Element), seven clones contained the ribosomal regulatory element IRES (Internal Ribosome Entry Site), and one clone was homologous to the 3'-UTR of a *Arabidopsis thaliana* gene coding for the germination protein

Table 16. Populations evaluated in conditions of Al toxicity by Microarrays.

Combination	Control	Treatment		Target	Treatment
1	Al-sensitive parent + hybrids	$AlCl_3$ (200 σM)	*vs*	Al-resistant parent + hybrids	$AlCl_3$ (200 σM)
2	Al-resistant parent + hybrids	Al (0 σM)	*vs*	Al-resistant parent + hybrids	$AlCl_3$ (200 σM)
3	*B. ruziziensis*	$AlCl_3$ (200 σM)	*vs*	*B. decumbens*	$AlCl_3$ (200 σM)
4	*B. decumbens*	Al (0 σM)	*vs*	*B. decumbens*	$AlCl_3$ (200 σM)

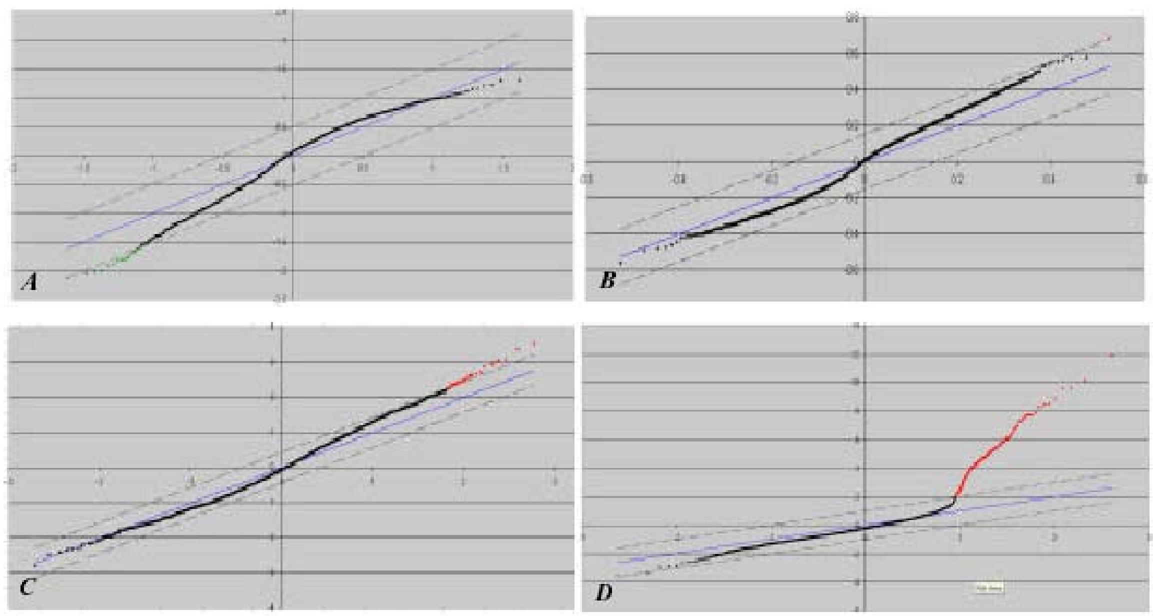

Figure 17. Differentially expressed genes associated with Al resistance (red points). **A**. Al-sensitive parent + hybrids in Al treatment *vs* Al-resistant parent + hybrids in Al treatment; **B**. Al-resistant parent + hybrids in control treatment *vs* Al-resistant parent + hybrids in Al treatment; **C**. Al-sensitive parent (*B. ruziziensis*) in Al treatment *vs.* Al-resistant parent (*B. decumbens*) in Al treatment; **D**. Al-resistant parent (*B. decumbens*) in control treatment *vs.* Al-resistant parent (*B. decumbens*) in Al treatment.

GLP2 (EMBL: BT002170). Five clones had no match in the data base. The 3'-UTR clones of differentially expressed genes identified in these experiments will be used as probes to isolate the corresponding full-length genes in a cDNA library previously prepared from root apices of the Al-resistant parent (*B. decumbens*).

E) Internal mechanisms of plant adaptation to aluminum toxicity and phosphorus starvation in three tropical forages

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Rationale

Soil acidity inhibits plant growth, principally because of toxicity from excess aluminum (Al) and lack of nutrients, especially phosphorus (P). Plant mechanisms for tolerating toxic levels of Al are usually grouped into two: external mechanisms to prevent Al invading root cells, or internal mechanisms that provide tolerance to excess Al. One significant external mechanism is for roots to exude organic acids into the rhizosphere, where they then make stable complexes with Al. Internal mechanisms are often found in Al-accumulator species, which prevent Al toxicity within the plant by creating Al complexes with organic acids or silicon.

Phosphorus is a major nutrient that plays a role in forming phospholipids, nucleic acids, nucleosides, coenzymes, and phosphate esters in plants. Under P starvation, adapted plants produce more fine roots or root hairs and/or increase root mass to acquire more P from soils. The roots release chelating compounds (e.g. organic acids) to mobilize and use insoluble phosphate compounds (e.g. those with Al, Fe, and Ca). Roots also release enzymes (e.g. acid phosphatase or APase) to use organic P. In addition, under P deficiency, adapted plants have the strategy of efficiently using P in cells. Phosphohydrolases may function as a P-recycling mechanism in plants. Inorganic P, liberated by APase or ribonuclease (RNase) in old tissues are retranslocated to young tissues. Induction of APase and RNase in plants under P deficiency has been intensively studied. Another strategy—mycorrhizal symbiosis—is also important for many plant species.

Brachiaria species are adapted to low-fertility acid soils in the tropics, tolerating both excess Al and P starvation very well. However, their mechanisms of high level of adaptation have yet to be defined. In *B. decumbens*, for example, Al-exclusion mechanisms, such as exudation of organic acids and rhizosphere alkalinization, are not involved in its high level of resistance to Al. Many other tropical forage grasses and legumes also grow well in acid soils, adapting to excess Al and P starvation stresses by using mechanisms that are still unclear. We therefore studied the mechanisms of adaptation to Al toxicity and P starvation in three tropical forages: two grasses (a *Brachiaria* hybrid and *Andropogon gayanus*) and one legume (*Arachis pintoi*).

Materials and Methods

For both experiments, we used seeds from three tropical forages: the grass *Brachiaria* hybrid (*B. ruziziensis* Ger. & Ev. clone 44-06 × *B. brizantha* (A. Rich.) Stapf CIAT 36061, also known as cv. Mulato); the grass *Andropogon gayanus* Kunth (CIAT 621); and the legume *Arachis pintoi* Krap. & Greg. (CIAT 17434). The seeds were sterilized with sodium hypochlorite for 10 min, washed with deionized water, and sown on a moderately moist perlite-vermiculite mixture (1:1, v/v) in a greenhouse at Hokkaido University, Sapporo, Japan. Uniform seedlings (shoot height = 10 cm) were transplanted to containers containing a standard nutrient solution with 0.06 mM P at pH 4.0, and grown for one week to adapt to hydroponic conditions.

Experiment 1: Tolerance of Al stress and P starvation: After precultivation, seedlings were transferred to 36-litre containers containing treatment solutions, which comprised: the standard nutrient solution with 0.06 mM P (control); the same solution but no P (-P, i.e. 0 mM P); and the same solution but with Al added (+Al, i.e. 0.37 mM Al and 0.06 mM P). Al and P were added as $Al_2(SO_4)_3$ and NaH_2PO_4, respectively. At the end of 10 days of treatment, the roots of seedlings from each treatment were first washed with tap water, then with deionized water, and transferred to polyethylene bottles containing 0.1 mM $CaCl_2$ (pH 4.0) for collecting root exudates. After being left overnight from 17:00 to 8:00 (i.e. 15 h) in a greenhouse, the exudates were collected, the roots washed with deionized water, and the seedlings cut to separate roots from shoots. The fresh weight of each set of organs was determined. Half of the fresh samples were dried in a forced-air oven at 80°C for 72 h, then weighed and digested with H_2SO_4-H_2O_2 for mineral analysis. The other half of the samples was used to determine organic acids. Acid phosphatase activity in root exudates was determined. One unit of acid phosphatase activity was defined as the amount of enzyme that hydrolyzed 1 µmol of *p*NPP per minute. Soluble organic acids in fresh samples were determined and the concentration of organic acids was measured by capillary electrophoresis. The levels of Al, K, Ca, and Mg in leaves and roots were determined. Concentrations in leaves and roots were determined by the semi-micro Kjeldahl method for N and the vanado-molybdate yellow method for P.

Experiment 2: ^{27}Al NMR study: *Brachiaria* seedlings were prepared as described above, and transferred to 36-litre containers carrying the standard nutrient solution, but with 2.8 mM Al at pH 3.7 added, and left to grow for 1 month. The much higher Al concentration was used to ensure clear peaks in the ^{27}Al NMR spectrum. Even so, the *Brachiaria* seedlings grew well (data not shown). After treatment, roots were removed from the seedlings and washed, first with tap water, then with deionized water. The roots were grouped into three: Fraction (a), roots given the water washings only, and used to determine total amounts of Al and organic acids; Fraction (b), roots were also washed with 0.1 N HCl for 5 min to remove apoplastic, soluble or loosely bound, components; and Fraction (c), roots were also washed with 0.1 N HCl for 5 min, frozen at -50°C for 1 h to rupture cell membranes, thawed, and washed again with 0.1 N HCl for 5 min to remove symplastic, soluble or loosely bound components. Al and organic acid concentrations in each fraction were determined as described for Experiment 1. Each fraction of *Brachiaria* roots was placed in a 10-mm-diameter NMR tube. $AlCl_3$ (0.1 M) solution was used as an external reference to calibrate the chemical shift (0 ppm). ^{27}Al NMR spectra were recorded, using a Bruker MSL400 spectrometer at 104.262 MHz. The spectra were obtained by using a frequency range of 62.5 kHz, a pulse width of 12 µs, a delay time of 0.16 ms, a cycle time of 0.5 s, and 4000 scans.

Results and Discussion

Experiment 1: Tolerance of Al stress and P starvation. The effect of each treatment on plant growth was expressed as dry matter accumulation in each treatment relative to that of the control treatment (Figure 18). The +Al treatment (+0.37 mM Al) did not affect growth in any of the species used in the study. The -P treatment did not inhibit growth in *Brachiaria* and *A. pintoi*, but did in *A. gayanus*. Relative growth rate (RGR) in the control treatment was much higher in the two grasses than in the legume (Figure 18). The root-to-shoot ratio in *Brachiaria* and *A. pintoi* increased remarkably with -P treatment, whereas it remained unchanged in *A. gayanus* (Figure 18). The treatments hardly affected acid phosphatase activity in root exudates of seedlings, except in *A. gayanus* where it increased with +Al treatment (Table 17).

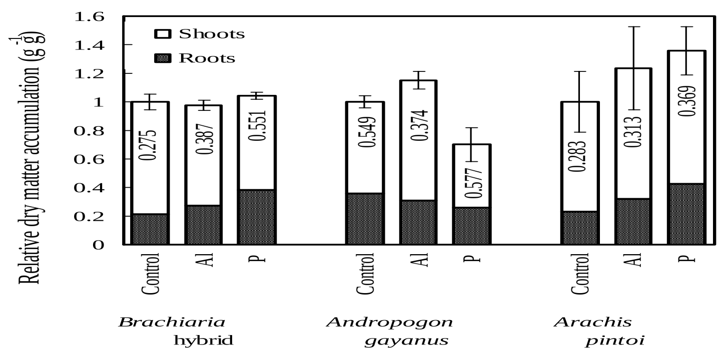

Figure 18. Effects of P starvation and Al toxicity on plant growth in Experiment 1. Plant growth was expressed as the relative dry matter accumulation (i.e. [dry weight after treatment – initial dry weight in each treatment]/[dry weight after treatment – initial dry weight in control treatment]). Bar values indicate root-to-shoot ratios, and the range for each bar indicates the ± SE value. Relative growth rate (dry weight after treatment – initial dry weight)/initial dry weight) in control treatment was 10.4, 8.5, and 1.1 g g^{-1}, in a *Brachiaria* hybrid, *Andropogon gayanus*, and *Arachis pintoi*, respectively.

Table 17. Acid phosphatase activity in root exudates of forage seedlings grown under three treatments

	Acid phosphatase (mU g^{-1} f. wt 15 h^{-1})		
Species	Control	+Al	-P
Brachiaria hybrid	170.9 à 72.2	100.7 à 42.1	202.9 à 24.9
Andropogon gayanus	182.3 à 3.2	213.6 à 24.5	166.6 à 21.4
Arachis pintoi	76.1 à 2.9	76.6 à 7.0	54.6 à 1.0

Mineral concentrations in leaves and roots, and total organic acid concentrations in roots are shown in Table 18. Nitrogen concentrations were hardly affected by the treatments. Phosphorus concentrations in leaves and roots of all species declined drastically in the -P treatment, especially in the *Brachiaria* hybrid and *A. gayanus*. Phosphorus concentrations tended to decrease as K concentrations decreased. Aluminum concentrations in *Brachiaria* leaves with the +Al treatment were less than 100 mg kg^{-1}, whereas in *A. gayanus* and *A. pintoi*, they were about 600 and 340 mg kg^{-1}, respectively. Organic acid concentrations in roots increased under the +Al treatment in the *Brachiaria* hybrid and *A. gayanus*.

Experiment 2: ^{27}Al NMR study. Aluminum, oxalate, malate, and citrate concentrations as determined by the ^{27}Al NMR spectrum are shown for each fraction in Figure 19. Aluminum concentration in fraction (b) (roots after removing apoplastic soluble components) did not differ significantly from that in fraction (a) (intact

Table 18. Concentrations of N, P, K, Ca, Mg (mg g^{-1}d. wt), Al (mg kg^{-1}d. wt) and total organic acid (μmol g^{-1}f. wt) in organs of *Brachiaria* hybrid, *Andropogon gayanus* and *Arachis pintoi.* Values are the means of three replicates±SE. SEs were not shown in organic acid concentrations because of two replicates. ND = not determined.

Species		Leaf						Root					
		Control		+Al		- P		Control		+ Al		- P	
Brachiaria	N	47.3	±1.7	48.0	±0.6	55.8	±0.8	27.0	±0.9	27.6	±1.3	25.4	±1.0
hybrid	P	8.8	±0.7	14.3	±0.3	0.8	±0.0	5.6	±0.2	8.8	±0.5	1.1	±0.0
	K	24.9	±0.5	25.3	±0.8	9.7	±0.0	33.7	±0.4	33.1	±2.2	16.1	±3.5
	Ca	4.5	±0.6	2.8	à0.3	2.3	±0.2	1.3	±0.1	1.3	±0.0	0.7	±0.1
	Mg	3.7	±0.3	3.7	±0.3	3.3	±0.1	3.5	±0.1	4.5	±0.3	2.3	±0.1
	Al	49	±6	75	±11	56	±7	357	±40	3285	±76	687	±24
	Organic acid	ND		ND		ND		0.79		4.71		0.23	
Andropogon	N	21.7	±0.2	27.0	±0.3	20.5	±0.2	18.7	±1.3	20.6	±0.2	26.0	±0.7
gayanus	P	6.5	±0.0	5.5	±0.2	0.6	±0.0	10.6	±0.9	7.0	±0.0	0.2	±0.3
	K	18.8	±0.2	20.7	±0.5	14.6	±0.7	24.9	±1.7	28.8	±0.2	10.8	±1.4
	Ca	6.5	±0.0	2.9	±0.0	4.4	±0.1	2.1	±0.1	2.0	±0.0	1.7	±0.1
	Mg	3.7	±0.1	3.0	±0.0	3.1	±0.0	3.5	±0.2	4.5	±0.0	2.8	±0.1
	Al	39	±3	594	±22	23	±4	219	±15	5822	±4	117	±139
	Organic acid	ND		ND		ND		3.44		10.70		3.71	
Arachis	N	49.5	±1.8	51.4	±1.4	56.0	±1.3	31.4	±0.5	35.1	±0.4	37.2	±1.0
pintoi	P	17.1	±0.5	10.1	±0.7	2.4	±0.1	31.1	±0.5	22.9	±1.3	2.3	±0.1
	K	27.5	±0.1	25.0	±1.6	22.6	±0.6	30.2	±0.7	28.8	±1.6	33.5	±1.8
	Ca	15.6	±0.2	19.5	±1.5	13.8	±0.8	5.1	±0.0	3.0	±0.6	3.5	±0.1
	Mg	8.8	±0.0	8.8	±1.6	6.5	±0.3	15.3	±0.4	5.0	±0.3	3.5	±0.2
	Al	83	±6	339	±15	58	±4	605	±1	4402	±143	356	±6
	Organic acid	ND		ND		ND		0.47		0.53		1.19	

roots). The differences in organic acid concentrations between fractions A and B were also small. ^{27}Al NMR spectra obtained from fractions A and B were very similar, and showed several peaks downfield and a small peak at 0 ppm. In contrast, in fraction (c) (roots after removing apoplastic soluble components, followed by removing symplastic soluble components), Al concentration was much lower than in fractions A and B. The ^{27}Al NMR spectrum was also different, with the resonance peaks downfield decreasing and a peak at 0 ppm becoming higher and sharper. Of the three organic acids, malate decreased drastically in fraction (c) (Figure 19).

All three tropical forages used in the present study were highly tolerant of Al and no growth reduction was observed with the +Al treatment (+0.37 mM Al) (Figure 18). Concentrations of Ca and Mg, the uptake of which appeared inhibited by Al, decreased in the +Al treatment (Table 18). However, concentrations of other major nutrients were not affected by Al application (Table 18), suggesting that Al did not affect root function for these nutrients.

To prevent or evade Al toxicity, plants growing in acid soils have developed mechanisms to exclude Al from roots or to tolerate high Al concentrations in tissues. Although organic acid exudation from roots is a major mechanism for excluding Al from roots, this mechanism was not evident in *Brachiaria* species. Previous research showed that Al application increases organic acid concentrations in roots of *Brachiaria* species. In our study, organic acid concentrations increased with Al application, but not with P starvation in *Brachiaria* and *A. gayanus* (Table 18).

We speculated before that the increase in organic acids in roots suggested that they play a role in the internal detoxification of roots from Al by acting as ligands for Al. In many Al-accumulator species, leaves with high concentrations of Al are detoxified by organic ligands, such as Al-oxalate, Al-catechin, and Al-citrate. The same mechanisms are considered possible in roots.

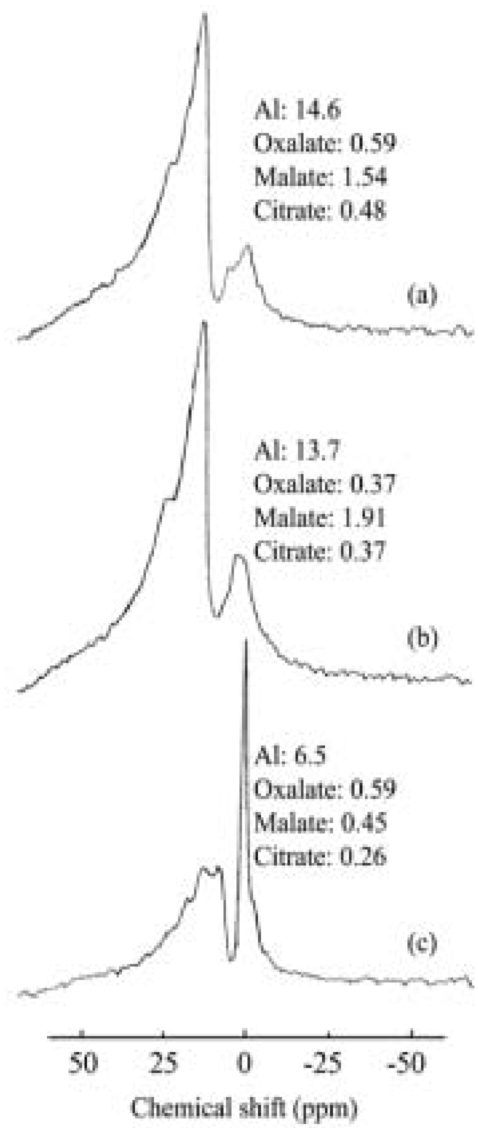

Figure 19. ^{27}Al NMR spectra and concentrations of Al (mg g^{-1} d. wt) and organic acids (µmol g^{-1} f. wt) in three root fractions of a *Brachiaria* hybrid. Fraction (a) = intact roots; fraction (b) = roots after removing soluble or loosely bound apoplastic components; fraction (c) = roots after removing soluble or loosely bound apoplastic and symplastic components. $AlCl_3$ (0.1 mM) was used as an external reference to calibrate the chemical shift (0 ppm).

An important result of our study was to demonstrate that Al-ligand complexes occur inside the root cells of a *Brachiaria* hybrid, an Al non-accumulator genotype. We applied simple fractionation of Al and ^{27}Al NMR to roots of the *Brachiaria* hybrid to evaluate the occurrence of Al-ligand complexes in root tissues. The ^{27}Al

NMR spectrum obtained from intact roots (fraction a) showed several peaks downfield at 10-20 ppm, suggesting that most of the soluble Al in roots makes octahedral complexes, presumably with organic acids. Both the ^{27}Al NMR spectrum and Al concentrations in fraction (b), in which soluble or loosely bound apoplastic Al was removed, were almost the same as those of intact roots (fraction a), indicating that root apoplast is not a primary site for Al accumulation in *Brachiaria* hybrid (Figure 19). In contrast, in fraction (c), the peaks downfield in the ^{27}Al NMR spectrum, Al concentrations, and malate concentrations all drastically decreased after removing symplastic components (Figure 19), indicating that most of the chelated Al occurs in root symplast, and that malate may participate in formation of Al complexes.

In Al-accumulator species, Al that penetrates the plasma membrane of root cells is immediately transferred to shoots. In *Brachiaria*, however, Al accumulated only in roots and Al concentrations in shoots were kept very low (Table 18). In shoots of buckwheat, an Al-accumulator species, Al-ligand complexes are mostly isolated in vacuoles. Al in the *Brachiaria* hybrid may also compartmentalize in vacuoles and, thus, not be translocated from roots to shoots.

Of the three species tested in this study, the *Brachiaria* hybrid and *A. pintoi* were extremely tolerant of low P stress (Figure 18). The *Brachiaria* hybrid grows well under P starvation, despite very low P concentrations in its leaves (Table 18). This suggests that *Brachiaria* can reuse P more efficiently. In *A. gayanus*, growth under the -P treatment was inferior to that of the control treatment, probably because of a higher relative growth rate (RGR). Based on values of P-use efficiency (the reciprocal of P concentration in plant), both *A. gayanus* and the *Brachiaria* hybrid appear to be tolerant of P starvation (Table 18). Higher P-use efficiency was observed before in *Brachiaria humidicola (dictyneura)* than in forage legumes, including *A. pintoi*, in acid Oxisols.

Although APase activity in root exudates, which contributes to the use of organic P in the rhizosphere, was hardly affected by P deficiency in any of the species (Table 17), it was higher than that of other forage species. While the ability to use organic P in the rhizosphere did not affect plant growth in the solution culture experiment, high APase activity in root exudates may significantly affect P acquisition in soils. In addition, the higher root-to-shoot ratio under P starvation in *Brachiaria* hybrid and *A. pintoi* would increase P uptake in soils (Figure 18).

This study showed that the *Brachiaria* hybrid, *Andropogon gayanus*, and *Arachis pintoi* adapt well to acid soils with low P and excess Al. The results indicate that the *Brachiaria* hybrid makes chelating complexes of Al (possibly with organic acids) inside root cells (symplast) to detoxify the cells. Until now, Al detoxification with organic acids in plant tissue has been observed mainly in Al-accumulator species.

This study provides direct evidence for the first time that organic acids inside root tissues help detoxify Al in non-accumulator species of Al. In Al non-accumulator species, Al is assumed to be detoxified outside the root cells, in the rhizosphere and/or root apoplast, by exuded organic acids. However, the *Brachiaria* hybrid, an Al non-accumulator, accumulates large amounts of Al in the root symplast (Figure 19), probably in the vacuoles. Thus, in some cases, Al accumulation and detoxification in roots involve the same mechanism in Al-accumulators and non-accumulators. Differences in characteristics of Al accumulation in shoots between Al-accumulators and non-accumulators may be caused by the existence of ligands for controlling translocation from roots to shoots.

Conclusions

We tested how Al toxicity and P starvation affect growth, concentrations of minerals and organic acids, and acid phosphatase activity in root exudates of *Brachiaria* hybrid, *Andropogon gayanus* and *Arachis pintoi*. The two tropical grasses tolerated high levels of Al toxicity and P starvation, with the *Brachiaria* hybrid maintaining very low levels of Al concentration in shoots. ^{27}Al NMR analysis revealed that, in *Brachiaria* hybrid, Al makes complexes with some ligands in root symplast. The three forages are probably adapted to P starvation through high P-use efficiency. These experiments provide the first evidence we know of that organic acids within root tissue help detoxify Al in non-accumulator species such as the *Brachiaria* hybrid.

3.1.1.2 Screening of *Brachiaria* hybrids for resistance to aluminum

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

Rationale

For the last three years, we have implemented screening procedure to identify Al-resistant *Brachiaria* hybrids that were preselected for spittlebug resistance. In 2002, 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. Last year, 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. 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 a sexual population of 745 hybrids along with 14 checks. The increase in Al resistance of the sexual hybrids has been very marked compared with the sexual population of 2001.

Materials and Methods

A total of 745 sexual hybrids generated from 2003 population together with 14 checks including 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 sexual hybrids were screened for spittlebug resistance (C. Cardona, personal communication; also see Output 2 and Activity 2.2 of this report). All the hybrids that rooted well were screened initially using 200 µM Al treatment to eliminate the Al sensitive hybrids. From this initial screening, a total of 124 sexual hybrids were selected to test under both treatments of with Al (6 experiments) and without Al (3 experiments) in solution. Out of these 124 sexual hybrids, data were obtained for 86 sexuals for both treatments. Mean values from all the experiments (ranging from 3 to 20 observations) are reported. Stem cuttings of hybrids and checks were rooted in a low ionic strength nutrient solution in the glasshouse for 9 days. Equal numbers of stem cuttings with about 5 cm long roots were transferred into a solution containing 200 µM $CaCl_2$ pH 4.2 (reference treatment) and a solution containing 200 µM $CaCl_2$ and 200 µM $AlCl_3$ pH 4.2 (Al treatment). The solutions were changed every second day to minimize pH drifts. At harvest on day 21 after transfer, the dry weight of stems was measured. Roots were stained and scanned on a flatbed scanner. Image analysis software (WinRHIZO) was used to determine root length and average root diameter.

Results and Discussion

As reported for the past 3 years, higher values of total root length per plant and lower values of mean root diameter after exposure to 21 days with or without toxic level of Al in solution indicate that the parent *B. decumbens* CIAT 606 is outstanding in its level of Al resistance (Figures 20 and 21).

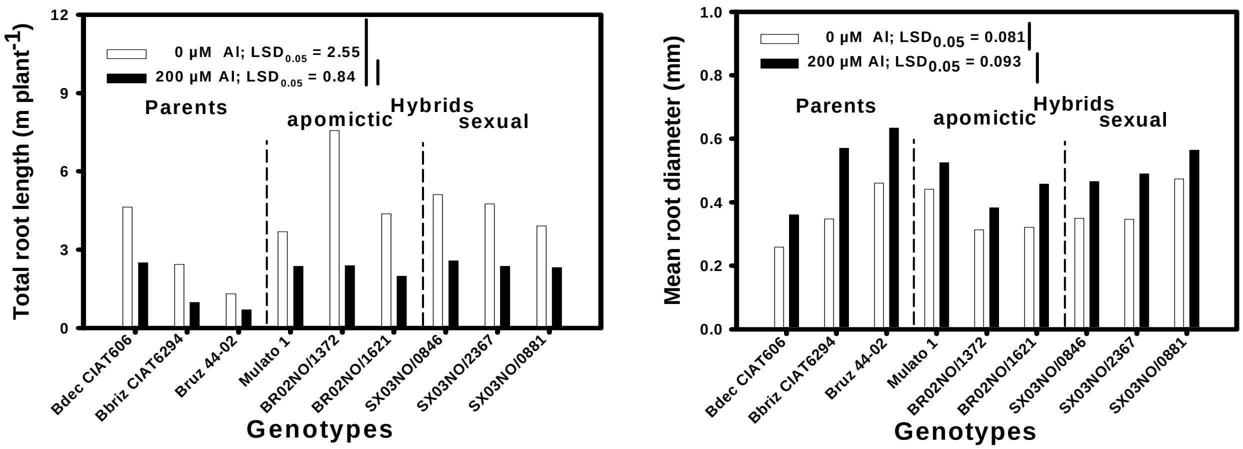

Figure 20. Al resistant apomictic and sexual hybrids of *Brachiaria* based on total root length and mean root diameter. Total root length and mean root diameter were measured after exposure to 0 or 200 ìM $AlCl_3$ with 200 ìM $CaCl_2$ (pH 4.2) for 21 days.

Among the 745 sexual hybrids and checks tested, 3 sexual hybrids (SX03NO/0846, SX03NO/2367, SX03NO/0881) and 3 apomictic hybrids (Mulato, BR02NO1372 and BR02NO1621) showed greater level of Al resistance based on total root length per plant (Figure 20; Table 19). Among these promising hybrids, BR02NO1372 showed greater fine root development than CIAT 606 in the absence of Al in solution (Figure 20). Total root length of the three sexual hybrids, both in the presence or absence of Al, was markedly superior to the sexual parent, *B. ruziziensis* (Figure 20). Among the hybrids and the checks tested, *B. humidicola* (*dictyoneura*) CIAT 6133 showed the lowest values of mean root diameter in the presence or absence of Al in solution (Table 19; Figure 22). This could be a desirable attribute for persistence under infertile acid soil conditions.

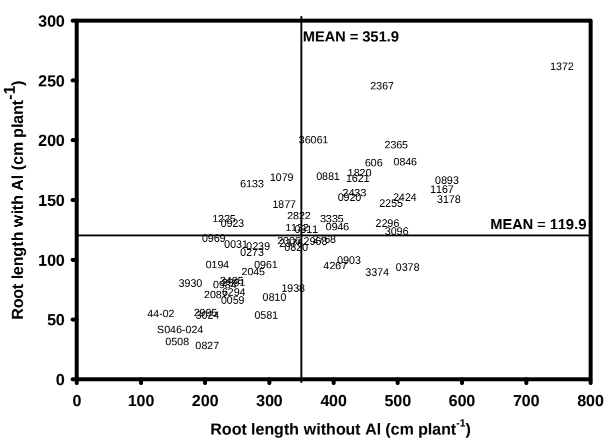

Figure 21. Relationship between total root length and mean root diameter of 78 genotypes of *Brachiaria* with presence or absence of aluminum in solution. Gentoypes that develop finer root system were identified in the upper box of the left hand side.

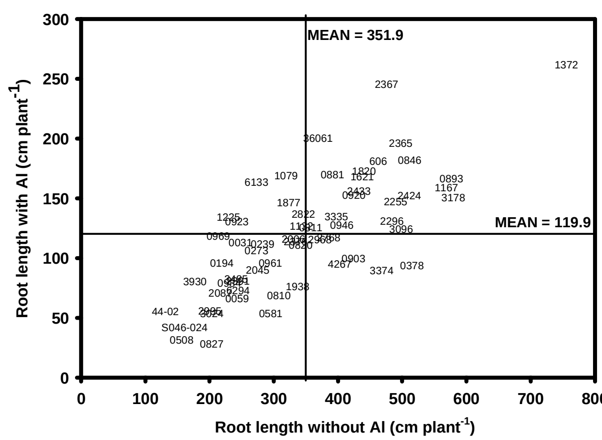

Figure 22. Relationship between total root length with Al and total root length without Al of 78 genotypes of *Brachiaria* with presence or absence of aluminum in solution. Genotypes that develop greater root length were identified in the upper box of the right hand side.

Table 19. Root length and mean root diameter of *Brachiaria* sexual hybrids evaluated with (200 µM Al) and without Al (0 µM Al) in solution in comparison with their parents and other checks.

	Root length ($m\ plant^{-1}$)		Root diameter (mm)	
Genotypes	0 µM Al	200 µM Al	0 µM Al	200 µM Al
Sexual hybrids				
SX03NO/0846	5.115	2.575	0.349	0.465
SX03NO/2367	4.756	2.365	0.346	0.490
SX03NO/0881	3.912	2.314	0.474	0.564
SX03NO/2433	4.323	2.170	0.314	0.448
SX03NO/2365	4.976	2.170	0.332	0.456
SX03NO/1167	5.685	2.069	0.349	0.505
SX03NO/1079	3.192	1.992	0.351	0.479
SX03NO/0311	3.573	1.969	0.350	0.483
SX03NO/2968	3.718	1.966	0.397	0.578
SX03NO/2424	5.109	1.855	0.389	0.521
SX03NO/0893	5.765	1.804	0.373	0.555
SX03NO/2255	4.896	1.797	0.390	0.584
SX03NO/1820	4.404	1.734	0.341	0.419
SX03NO/0273	2.731	1.726	0.394	0.502
SX03NO/0903	4.241	1.703	0.473	0.457
SX03NO/2296	4.839	1.634	0.317	0.532
SX03NO/1132	3.429	1.634	0.378	0.494
SX03NO/0961	2.946	1.593	0.386	0.554
SX03NO/2995	2.005	1.578	0.426	0.636
SX03NO/3096	4.983	1.535	0.320	0.475
SX03NO/2391	2.441	1.509	0.454	0.580
SX03NO/2006	3.305	1.486	0.375	0.532
SX03NO/2768	3.855	1.480	0.404	0.562
SX03NO/0946	4.060	1.454	0.405	0.549
SX03NO/0059	2.428	1.450	0.357	0.528
SX03NO/2045	2.749	1.449	0.360	0.539
SX03NO/0378	5.153	1.440	0.396	0.702
SX03NO/0920	4.249	1.400	0.357	0.554
SX03NO/3930	1.770	1.399	0.555	0.724
SX03NO/0810	3.079	1.398	0.371	0.610
SX03NO/0923	2.426	1.356	0.436	0.579
SX03NO/3335	3.973	1.338	0.426	0.625
Sexual hybrids				
SX03NO/2087	2.168	1.337	0.505	0.658
SX03NO/0969	2.135	1.294	0.399	0.561
SX03NO/0194	2.191	1.292	0.420	0.555
SX03NO/1225	2.294	1.292	0.430	0.476
SX03NO/2376	3.337	1.286	0.397	0.590
SX03NO/3374	4.679	1.263	0.367	0.609
SX03NO/1877	3.231	1.249	0.480	0.692
SX03NO/0031	2.481	1.237	0.463	0.535
SX03NO/0239	2.825	1.185	0.443	0.664
SX03NO/2822	3.459	1.181	0.393	0.576
SX03NO/4267	4.026	1.180	0.404	0.543
SX03NO/0820	3.418	1.108	0.393	0.516
SX03NO/0508	1.565	1.096	0.454	0.550
SX03NO/1938	3.371	1.065	0.417	0.649
SX03NO/3485	2.415	1.025	0.478	0.698
SX03NO/3024	2.034	1.009	0.400	0.575
SX03NO/0581	2.953	0.977	0.390	0.636
SX03NO/0984	2.308	0.944	0.419	0.548
SX03NO/0827	2.033	0.764	0.446	0.656
Parents				
B.decumbens CIAT606	4.625	2.496	0.259	0.360
B. brizantha CIAT6294	2.444	0.979	0.348	0.570
B.ruziziensis 44-02	1.310	0.705	0.461	0.634
Checks				
BR02NO/1372	7.557	2.395	0.313	0.383
Mulato	3.688	2.361	0.441	0.525
BR02NO/1621	4.377	1.980	0.321	0.458
B.humidicola (*dictyoneura*) CIAT6133	2.732	1.922	0.221	0.287
SX01NO/3178	5.794	1.341	0.394	0.653
FM9503-S046-024 (CIAT 36087-Mulato 2)	1.609	0.934	0.512	0.765
Means	3.519	1.537	0.395	0.553
LSD (P<0.05)	2.548	1.077	0.081	0.093

attribute for persistence under infertile acid soil conditions.

Relationship between root length and mean root diameter with Al in solution showed that several sexual hybrids were superior to the sexual parent, *B.ruziziensis* 44-02 (Figure 21). Exposure to Al decreased the mean value of total root length of the 78 genotypes from 352 to 120 cm $plant^{-1}$ (Figure 22). Relationship between total root length without Al and with Al in solution showed that several apomictic and sexual hybrids were superior to the Al resistant parent, *B. decumbens* CIAT 606 in the absence of Al in solution (Figure 22). The greater root vigor of these hybrids could

The sexual hybrids SX03NO/846 and SX03NO/0881 were found to be spittlebug resistant while SX03NO/0311 was resistant to Rhizoctonia foliar blight. Two sexual hybrids that were found to be spittlebug resistant, SX03/2483 and SX03/1820, were also found to be moderately resistant to Al stress. Another spittlebug resistant sexual hybrid, SX03/2694 was more sensitive to Al than the sexual parent, *B. ruziziensis* 44-02. The promising sexual hybrids that combine spittlebug resistance with Al resistance and other desirable attributes are being used in recurrent selection to generate superior hybrids of *Brachiaria*.

3.1.1.3 Field evaluation of most promising hybrids of *Brachiaria* in the Llanos of Colombia

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

Rationale

Evaluation of a large number of *Brachiaria* hybrids for their resistance to spittlebug and adaptation to infertile acid soils resulted in identification of a few promising *Brachiaria* hybrids. We selected 4 of these hybrids for further field-testing in comparison with their 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, nutrient (N, P, K, Ca and Mg) uptake, leaf and stem total nonstructural carbohydrate (TNC) content and leaf and stem ash (mineral) content were measured at 30 months after establishment (November 2003).

Results and Discussion

After 30 months of establishment, forage yield with low fertilizer application was greater with one spittlebug resistant genetic recombinant, FM 9503-S046-024 (CIAT 36087—Mulato 2) and one parent (CIAT 6294) (Table 20). With high initial fertilizer application also these two genotypes were outstanding in live forage yield (Figure 23). Among the 4 hybrids tested, Mulato 2 was outstanding in its adaptation to low initial fertilizer application. It is important to note that CIAT 6294 had greater amount of dead biomass and stem biomass under low fertilizer application (Figure 23).

Table 20. Correlation coefficients (r) between green forage yield (t/ha) and other shoot traits of *Brachiaria* genotypes grown with low or high initial fertilizer application in a sandy loam oxisol in Matazul, Colombia.

Shoot traits	Low fertilizer	High fertilizer
Total (live + dead) shoot biomass (t/ha)	0.84***	0.91***
Dead shoot biomass (t/ha)	0.54	0.76***
Leaf biomass (t/ha)	0.94***	0.94***
Stem biomass (t/ha)	0.69***	0.68***
Leaf N content (%)	-0.24	-0.35
Leaf P content (%)	0.21	0.06
Leaf TNC content (mg g^{-1})	-0.25	-0.31
Leaf ash content (%)	0.05	0.01
Stem N content (%)	-0.45	0.79***
Stem P content (%)	-0.43	-0.31
Stem TNC content (mg g^{-1})	0.45	0.12
Stem ash content (%)	-0.12	0.05
Shoot N uptake (kg/ha)	0.93***	0.85***
Shoot P uptake (kg/ha)	0.91***	0.93***
Shoot K uptake (kg/ha)	0.76***	0.78***
Shoot Ca uptake (kg/ha)	0.85***	0.81***
Shoot Mg uptake (kg/ha)	0.84***	0.89***

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

As observed last year, results on shoot nutrient uptake, particularly Ca and Mg, indicated that the hybrid, Mulato 2 was superior to CIAT 606 under low fertilizer application (Figure 24). Nutrient acquisition by Mulato 2 was also greater than the rest of the hybrids with high initial fertilization.

These results are consistent with the results reported last year from the same experiment. Correlation coefficients between live forage yield and other plant attributes indicated that greater nutrient acquisition with low initial fertilizer application contributed to superior performance (Table 20). No significant correlations were found between live forage yield and leaf and stem TNC or ash contents.

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

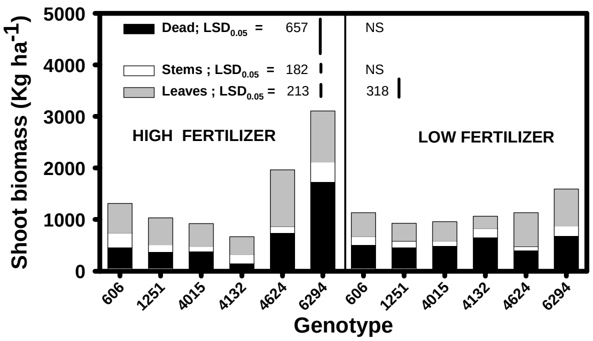

Figure 23. Genotypic variation as influenced by fertilizer application in shoot biomass production (forage yield) of two parents (CIAT 606, 6294) and four genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 30 months after establishment (November 2003). LSD values are at the 0.05 probability level. NS = not significant.

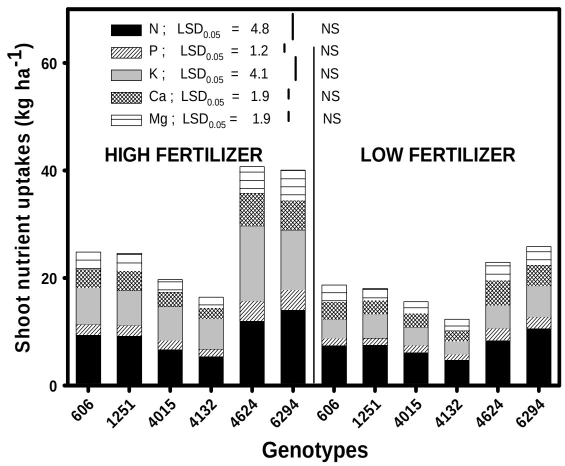

Figure 24. Genotypic variation as influenced by fertilizer application in nutrient uptake (N, P, K, Ca and Mg) of two parents (CIAT 606, 6294) and four genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 30 months after establishment (November 2003). LSD values are at the 0.05 probability level. NS = not significant.

3.1.2 Participatory evaluation of *Brachiaria* accesions/hybrids in comparison with commercial cultivars in Nicaragua

Contributors: A. Schmidt, C. Davis, M. Peters, J. Miles and I. M. Rao (CIAT)

Rationale

As part of the BMZ-GTZ project on developing aluminum resistant *Brachiaria* hybrids, in 2002 we initiated field studies in Nicaragua for evaluation of new hybrids of *Brachiaria* along with commercial *Brachiaria* cultivars with farmer participation. The opinion of farmers is very important in the process of identifying and selecting promising forage germplasm, because their selection criteria are not necessarily the same as those of researchers. 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 uses in crop-livestock systems.

Materials and Methods

The experiment site was chosen in Ubú Norte (12° 58’ 44" N, 84° 54’ 23" E, 261 masl) in the Region Autónoma del Atlántico Sur (RAAS) where acid soils are predominant. Soil characteristics and rainfall distribution from January to December are presented in Table 21 and Figure 24, respectively. 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” (no CIAT No.) 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 (5x4m) were sown early October 2002 upon the beginning of the second rainy season.

Table 21. Soil characteristics of experimental site Ubú Norte.

SOM (%)	pH	P-Bray (ppm)	K (meq)	Ca (meq)	Mg (meq)	Na (meq)
6.61	5.66	3.22	0.55	5.50	2.42	0.09

The establishment of grasses in the plots 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 (Photo 14). Two fertilization treatments (high/low) were applied upon plot establishment.

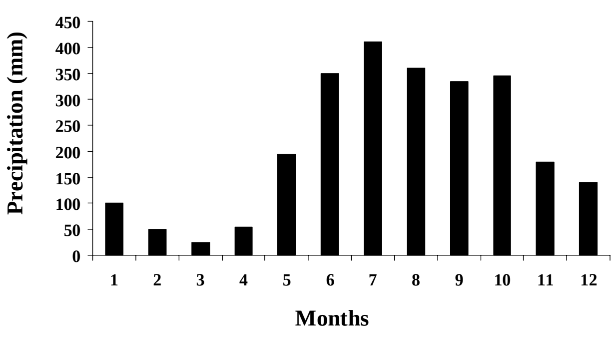

Figure 25. Rainfall distribution at the experimental site of Ubú Norte, Nicaragua.

Fertilization levels were adjusted to soil analysis results. Agronomic and participatory evaluations with farmers were conducted in August 2003 (max. rainfall.), March 2004 (min. rainfall) and July 2004 (max. precip) in each case 6 weeks after standardization cut (Figure 25).

Photo 14. *Brachiaria* accessions and hybrids tested on acid soils in Ubú Norte, Nicaragua.

Results and Discussion

Evaluation 2003: Results from agronomic evaluations showed no significant fertilizer effect on plant height, soil cover and dry matter yield (Table 22). Significant differences were found among accessions/ hybrids, but no fertilizer x accession/hybrid interactions were detected.

Data obtained in 2003 showed *Brachiaria brizantha* cv. Toledo (CIAT 26110) and *Brachiaria* hybrid cv. Mulato (CIAT 36061) as the top ranking accessions with regard to dry matter yield, followed by *B. brizantha* CIAT 26124 and *B. brizantha* cv. Marandú (CIAT 6780). The lowest yields were obtained with *B. humidicola* CIAT 679 and *B. humidicola (dictyoneura)* CIAT 6133 due to their slow 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). Plant height was greater with *B. brizantha* cv. Toledo (CIAT 26110) and *B. brizantha* cv. Marandú (CIAT 6780). *B. humidicola* CIAT 679 did not recover well from the standardization cut and was discarded from the experiment in 2004.

Table 22. Plant height, soil cover and DM yield of 14 *Brachiaria* accessions and hybrids in Ubú Norte, Nicaragua, 2003-2004.

Fertility Levels/ Accessions	2003						2004		
	Max			Min			Max		
	Plant height (cm)	Soil Cover (%)	DM yield (g/m^2)	Plant height (cm)	Soil Cover (%)	DM yield (g/m^2)	Plant height (cm)	Soil Cover (%)	DM yield (g/m^2)
Fert. Level									
- High *	88	64	802	56	73	310	76	75	338
- Low	82	60	747	54	69	283	71	67	303
No. CIAT									
606	90	76	578	56	73	244	73	82	329
654	71	48	538	39	52	239	55	37	188
679	32	17	-	-	-	-	-	-	-
6133	55	30	339	37	46	234	55	65	347
6780	126	89	1000	40	78	171	65	91	244
16322	98	88	646	72	84	252	94	88	348
26110	108	65	1682	72	70	375	76	64	363
26124	99	89	1239	64	67	264	75	66	320
26318	90	34	854	62	47	359	61	17	342
26646	75	48	505	65	70	305	76	76	275
26990	87	60	695	64	53	357	73	48	277
36061	99	93	1546	52	74	305	69	81	308
36062	70	32	440	52	76	322	54	49	253
“Mixe”	89	90	775	56	81	331	77	86	355
Mean Acc.	85	61	834	56	67	289	69	65	304
LSD (0.05)	31	30	645	13	24	190	21	30	263

(*Differences at fertilization levels were not significant at $p \leq 0.05$)

Results obtained during the period of low rainfall showed no fertilizer effect on agronomic parameters. Best performing accessions were *B. brizantha* cv. Toledo (CIAT 26110), *B. brizantha* CIAT 26318, *B. brizantha* CIAT 26990, and *B. brizantha* “Mixe”. In early 2004, Rhizoctonia foliar blight detected in *Brachiaria* hybrid cv. Mulato, had a negative effect on dry matter production. Plots of *B. brizantha* CIAT 16322 developed very well in the period of low rainfall and outperformed other accessions in plant height and soil cover, indicating good adaptation to prevailing environmental conditions. Dry matter yield, however, was below average because of the very fine leaf structure of this accession.

Evaluation 2004: Due to severe reduction in rainfall during the first semester of 2004 in Nicaragua, dry matter yields were significantly lower than in 2003. The highest yields were recorded for *B. brizantha* cv. Toledo (CIAT 26110), *B. brizantha* “Mixe”, *B. brizantha* CIAT 16322, and *B. brizantha* CIAT 26318. *Brachiaria* hybrid cv. Mulato showed good soil cover and above average dry matter yield. *B. brizantha* cv. Marandú (CIAT 6780), widely planted in the area of Ubú Norte, established well and showed good adaptation, soil cover and height, especially in the first year, but in subsequent evaluations dry matter yield was below average. Apart from the Rhizoctonia foliar blight attacks no significant or limiting pest and disease incidents were recorded throughout the experiment.

Participatory evaluations with farmers: Prior to agronomic data collection, farmer groups evaluated the plots in accordance with their own criteria. Their preference rankings are summarized in Table 23.

The main criteria applied by farmers throughout the experiment were: plant height, soil cover, foliage production, and ease of establishment, leaf size and color. While the high ranking of

Table 23. Preference ranking of *Brachiaria* accessions and hybrids at Ubú Norte, Nicaragua (2003-2004) (Max = Maximun rainfall; Min = Minimum rainfall).

Max 2003	Min 2003	Max 2004
B. brizantha cv Marandú	*B. brizantha* cv. Toledo	*B. brizantha* cv. Toledo
B. brizantha cv. Toledo	*B. brizantha* cv Marandú	*B. hybrid* cv. Mulato
B. hybrid cv. Mulato	*B. brizantha* "Mixe"	*B. brizantha* cv Marandú
B. brizantha CIAT 26990	*B. hybrid* cv. Mulato	*B. brizantha* "Mixe"
B. brizantha CIAT 26124	*B. brizantha* CIAT 16322	*B. brizantha* CIAT 16322
B. brizantha CIAT 16322	*B. brizantha* CIAT 26646	*B. brizantha* CIAT 26318

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 with bright green leaves. The fact that both materials were sold on the seed market could have influenced the ranking. Accessions such as *B. brizantha* CIAT 26990, 26124, 16322 were often classified as less productive because of their leaf size. Most other materials were rated low due to low soil cover or plant height.

As mentioned earlier, farmer rankings could have been influenced to some extent by the active presence of a livestock project in the area promoting *B. brizantha* cv. Toledo and *B. hybrid* cv. Mulato. The main difficulty during the participatory evaluation was the large number of accessions to be ranked. Some farmers had difficulties to keep track of all plots. In the future, smaller number of materials should be presented to farmers in order to avoid confusion, especially with farmers who do not have experiences with ranking of forage germplasm.

Although the chosen experimental site represented an acid soil region of Nicaragua, soil pH and aluminum contents were not limiting factors for the tested accessions/hybrids. Since real drought conditions did not prevail throughout the experiment, hybrids such as cv. Mulato could not express their full potential, but did show susceptibility to Rhizoctonia foliar blight. Nevertheless farmers seem to appreciate the Mulato hybrid, since seed is increasingly available to them. *Brachiaria brizantha* accession CIAT 16322 and *Brachiaria brizantha* "Mixe" are high-potential materials due to their excellent adaptation and growth. Their small leafs might be a decisive factor for their adoption by farmers.

3.1.3 Edaphic adaptation of *Arachis pintoi*

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

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

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 Piedmont 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 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 Piedmont in May, 2001 as monoculture. 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 42 months after establishment (December 2004) 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

At 42 months after establishment of the trial, the response in terms of shoot biomass production with fertilizer application was greater with CIAT 22160 (Figure 26). Overall the performance of CIAT 18744 and CIAT 18751 and CIAT 22159 was better than the other accessions. These results are consistent with the observations made last year. Shoot TNC content was markedly greater in CIAT 18751 under both low and high initial fertilizer application (Tables 24 and 25).

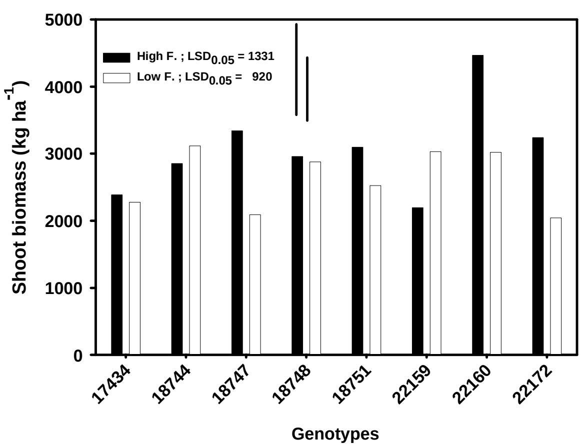

Figure 26. Genotypic variation in forage yield of 8 accessions of *Arachis pintoi* at 42 months after establishment (December, 2003) in forage yield (kg/ha) as influenced by initial level of fertilizer application to a clay loam oxisol at La Libertad, Piedmont.

Shoot ash (mineral) content was higher in CIAT 22172 under both low and high initial fertilizer application (Tables 24 and 25).

The superior performance of CIAT 18744 was associated with greater TNC (Total Non-structural Carbohydrates) and lower ash content in the shoot biomass under low initial fertilization indicating greater nutrient use efficiency for producing green forage. Measurements of nutrient uptake indicated that CIAT 22160 was outstanding with high initial fertilizer application

Table 24. Shoot biomass, cover, vigor and shoot total nonstructural carbohydrates (TNC) content and shoot ash content in 8 *Arachis pintoi* accessions evaluated with high initial fertilizer application to low fertility acid soils at La Libertad (December 2003).

Accession	Shoot biomass (kg ha^{-1})	Cover (%)	Vigor	Shoot TNC (mg g^{-1})	Shoot ash (%)
17434	2384	98	3.0	79.3	8.073
18744	2853	100	4.3	79.3	7.867
18747	3339	100	4.3	56.0	8.367
18748	2959	100	4.0	81.3	7.693
18751	3095	100	4.0	102.1	7.527
22159	2193	100	4.0	63.1	7.967
22160	4464	100	4.3	73.2	7.600
22172	3240	98	4.0	67.1	8.400
Mean	3066	99.5	4.0	75.2	7.937
LSD(P>0.05)	1331	NS	0.66	NS	NS

Table 25. Shoot biomass, cover, vigor and total nonstructural carbohydrates and ash content in 8 *Arachis pintoi* accessions evaluated with low initial fertilizer application to low fertilizer on low fertility acid soils at La Libertad (December 2003).

Accession	Shoot biomass (kg ha^{-1})	Cover (%)	Vigor	Shoot TNC (mg g^{-1})	Shoot ash (%)
17434	2276	100	3.3	90.7	8.033
18744	3114	100	4.0	135.9	7.240
18747	2088	98	3.7	64.2	7.673
18748	2878	97	3.3	84.3	7.927
18751	2525	100	4.0	125.1	7.040
22159	3028	100	4.0	69.7	7.560
22160	3021	100	4.3	100.8	7.373
22172	2043	97	3.0	65.1	8.187
Mean	2622	99	3.7	92.0	7.629
LSD (P>0.05)	920	NS	0.71	NS	0.611

while CIAT 22159 was superior with low initial fertilizer application (Tables 26 and 27).

Correlation coefficients between green forage yield and other shoot attributes indicated that the superior performance with low initial fertilizer application was associated with lower level of Ca content in the shoot tissue (Table 28).

Results from this field study indicated that after 4 years, the *Arachis pintoi* accessions CIAT 18744, 18751 and 22159 are superior to the commercial cultivar (CIAT 17434) in terms of persistence with low amounts of initial fertilizer application.

Table 26. Shoot N, P, K, Ca and Mg uptake of 8 *Arachis pintoi* accessions evaluated with high initial fertilizer application to low fertility acid soils at La Libertad (December 2003).

Accession	Shoot nutrient uptake (kg ha^{-1})				
	N	P	K	Ca	Mg
17434	65.5	7.5	32.5	51.0	23.3
18744	76.6	8.5	35.8	55.3	27.9
18747	88.6	10.1	46.9	84.8	34.2
18748	75.2	7.9	34.2	68.3	28.5
18751	80.0	8.0	31.0	70.7	28.0
22159	63.2	7.9	29.4	47.8	21.4
22160	113.6	12.0	59.6	90.2	43.9
22172	90.6	10.0	47.0	75.5	31.5
Mean	81.7	9.0	39.5	67.9	29.8
LSD(P>0.05)	28.8	3.1	23.5	NS	13.6

Table 27. Shoot N, P, K, Ca and Mg uptake of 8 *Arachis pintoi* accessions evaluated with low initial fertilizer application on low fertility acid soils at La Libertad (December 2003).

Accession	Shoot nutrient uptake (kg ha^{-1})				
	N	P	K	Ca	Mg
17434	60.0	7.3	26.7	52.6	23.9
18744	79.8	9.7	31.5	55.8	36.8
18747	61.4	7.1	20.2	48.3	19.8
18748	78.0	8.4	19.9	70.0	32.1
18751	64.3	8.2	27.5	47.2	21.8
22159	91.1	11.7	35.6	61.7	29.9
22160	75.2	9.0	21.2	62.3	35.2
22172	56.1	5.4	15.7	55.5	23.1
Mean	70.7	8.3	24.8	56.7	27.8
LSD (P>0.05)	24.7	3.0	14.0	NS	12.4

Table 28. Correlation coefficients (r) between green forage yield (t/ha) and other shoot traits of *Arachis pintoi* genotypes grown with low or high initial fertilizer application in a low fertility Oxisol in La Libertad, Colombia.

Shoot traits	Low fertilizer	High fertilizer
Soil cover (%)	0.20	-0.05
Vigor (visual scale)	0.44*	0.55**
Shoot N content (%)	-0.13	-0.49**
Shoot P content (%)	-0.06	-0.58**
Shoot K content (%)	0.11	0.28
Shoot Ca content (%)	-0.45*	0.04
Shoot Mg content (%)	0.13	-0.17
Shoot TNC content (mg g^{-1})	0.05	-0.01
Shoot ash content (%)	-0.29	-0.02

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

3.1.3.2 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

Several studies conducted in Colombia as well as in Goettingen confirmed that *Arachis pintoi* genotypes showed a different growth potential when grown in Ultisols or Osixols with extremely low P availability. A growth chamber study conducted last year in Goettingen indicated significant genotypic difference in P acquisition among the accessions CIAT 17434, CIAT 18744 and CIAT 22172. Therefore this P acquisition was related to an increase of P soil solution concentration with CIAT 18744 i.e. to P mobilization in the rhizosphere. These results indicated that the rapid establishment as well as the sustained yield of CIAT 18744 and 22172 was due to their great ability to acquire P from P-deficient soil per unit root length. There was no significant difference among the genotypes in their ability to utilize acquired P. This year, we continued our efforts to determine the physiological basis of differences in P influx between the 2 accessions, CIAT 17434 and 18744.

Material and Methods

Plant cultivation - Germinated seedlings in sand of *Arachis pintoi* CIAT 17434 and 18744 were pre-cultured in –P nutrient solution for 5 days and then the taproot was excised to develop lateral roots for additional 15 days till the lateral roots had reached about 5 cm long. The 20-day-old seedlings were transplanted to pots divided in three compartments (Figure 27) and placed on the dividing walls and their roots split so that 50% grew in soil and 50% in sand.

Each one of the outer compartments was filled with 2 kg of air dry clay loam fossil 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) from Lich in the Vogelsberg area (Hessen – Germany). The middle compartment was filled with 3 kg air dry sand (size 3-5 mm) in which P were removed using 5% HCl solution and then washing the sand till getting the same pH of distilled water. The sand compartment was watered three times per day with nutrient solution without P. Its composition (M) was: $Ca(NO_3)_2$ 5.0×10^{-3}, KCl 5.0×10^{-4}, K_2SO_4 3.5×10^{-3}, $MgSO_4$ 2.5×10^{-3}, H_3BO_3 5.0×10^{-5}, $MnSO_4$ 5.0×10^{-6}, $ZnSO_4$ 2.5×10^{-6}, $CuSO_4$ 1.0×10^{-6}, $(NH_4)_{6Mo7O24}$ $5.0\times10_{-8}$, and FeEDTA 1.0×10^{-4}. This compartment was allowed to percolate water through a hole in the bottom which were later leached the root exudates. Two levels of P supply (0, and 1000 mg kg^{-1}) as $Ca(H_2PO_4)_{2.H2O}$ were given only in the soil compartments. Basal nutrients were applied (mg kg^{-1}) every 30 days to each soil compartment: 50 K as K_2SO_4, 40 Mg as $MgSO_4$, 0.2 B as H_3BO_3, 0.1 Mo as $(NH_4)_{6Mo7O24}$ and 100 N as $Ca(NO_3)_2$. The soil surface in each soil compartment was covered with a layer of quartz sand (1 to 2 cm) to avoid the formation of a superficial crust due to the watering. Two weeks before transplanting, water was added to get moisture content of 25% w/w. One pot for each P treatment was left unplanted to measure soil moisture evaporation losses and as control of P dynamic under unplanted conditions. The pots were watered daily and water was added to

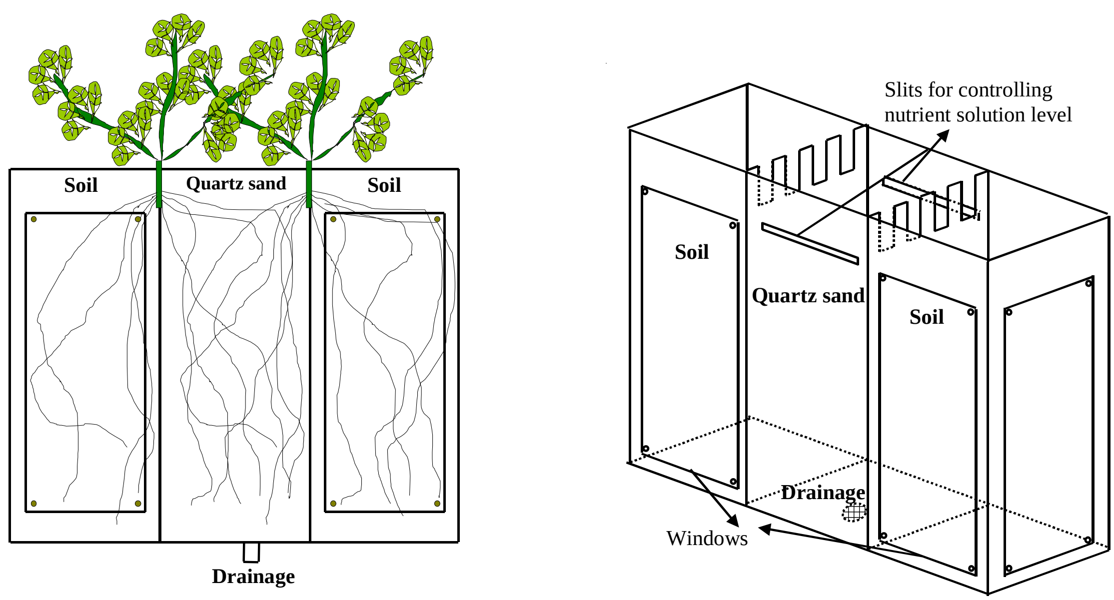

Figure 27. Schematic representation of plant growing technique with split root system.

maintain the soil with 60% of its water holding capacity by weight. The plants were grown 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.

There were four replicates for each treatment. The pots were completely randomized and re-positioned weekly to minimize any effect of uneven environmental factors.

Collection of root exudates using a percolation system - Prior to collection of root exudates, the sand compartment was thoroughly washed with distilled water until removing nutrient ions, specially NO_3, which affects accurate determination of organic acids by HPLC. The sand compartment drainage's was closed and then filled with bi-distilled water for 1 hour. After that the leached root exudates were collected to avoid O_2 stress (deficiency) in the roots and immediately the sand compartment was again filled with the collected root exudates for one hour. Finally, leaving the drainage open, the collected root exudates were once more added to the sand by a pressure bottle in order to leach all as possible root exudates from the sand. The collected root exudates were lyophilized to concentrate them and after that the dry root exudates were diluted in 1 mL bi-distilled water and put into 1.5 mL Eppendorf reaction vials. Thereafter, they were subjected to shaking for three-times (30 seconds each) for extraction of carboxylates. The aliquot was centrifuged at 2000g for 10 min. The supernatant was collected by a micropipette and stored at –20ºC for analysis of organic acid anions by HPLC.

Analysis of carboxylates - The organic acid anions in root exudate samples were analyzed by reversed phase HPLC in the ion suppression mode. Separation was conducted on a 250 × 4 mm reversed phase column (LiChrospher 100 RP-18, 5 µm particle size) equipped with a 4 × 4 mm LiChrospher 100 RP-18 guard column (Merck, Darmstadt, Germany). Sample solutions (100 µL) were injected onto the column, and 18 mM KH_2PO_4 adjusted to pH 2.2 with H_3PO_4 was used for isocratic elution, with a flow rate of 0.25 mL min-1 at 24°C and UV detection at 210 nm. Identification of organic acids was performed by comparing retention times and absorption spectra with those of known standards.

Determination of root surface APase activity - Excised segments (2-3 root tips at 1.5-2 cm) of root tips were harvested in the soil compartments and transferred to 1.5 mL Eppendorf reaction vials. The root segments were washed 2 times with distilled water for 5 min to remove contents of wounded cells, followed by adding 0.5 mL water, 0.4-mL Na-Ac buffer and 0.1 mL pNPP substrate. After a reaction time of 10 min at 25-30°C, an aliquot of 0.8 mL of the reaction medium was taken out and mixed with 0.4 mL of 0.5 M NaOH to terminate the reaction. The absorption of reaction solution was measured at 405 nm. The fresh weight of excised segments of root tips was recorded after determination of APase activity.

Quantitative determination of the pH values at the root surface with the antimony electrode In the soil compartments, the pH values at the root surface of young and old root were measured potentiometrically with an antimony electrode.

Plant and soil harvests - The plants were harvested as separate roots and shoots at 30, 60 and 100 days after transplanting (DAT) into split-root pots. The dry weight was determined by drying the shoots and roots in an oven at 65% for 1 day and then at 105°C till constant weight. After grinding, the plant material was used for determination of nutrient composition. To determine P concentration in plant tissue, shoot and root 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 (the amount P taken up per unit of root and time) were determined. Soil solution pH value was measured directly. Soil pH was determined in 0.01 M $CaCl_2$ in soil to solution ratio of 1:2.5. Data were subjected to an analysis of variance using the SAS computer program. Least-significant differences were calculated by an tuckey-test. A probability level of 0.05 was considered statistically significant.

Results and Discussion

At high P supply (P-1000) the shoot yield (Figure 28) and the shoot P concentration of both genotypes was similar. i.e. the growth potential of both genotypes is the same. But under low P availability, the shoot dry matter yield and shoot P concentration were different between the genotypes. Since under sufficient P condition the genotypes show the same growth, the difference at P-0 is because they have different P efficiency (Figure 29). At 30 DAT, the two genotypes had different shoot P contents (Figure 30) under low P availability and at 100 DAT the P content in the shoot of CIAT 18744 is 5 times higher than that of CIAT 17434. This shows, that the high shoot dry matter yield by CIAT 18744 was due to its high P uptake.

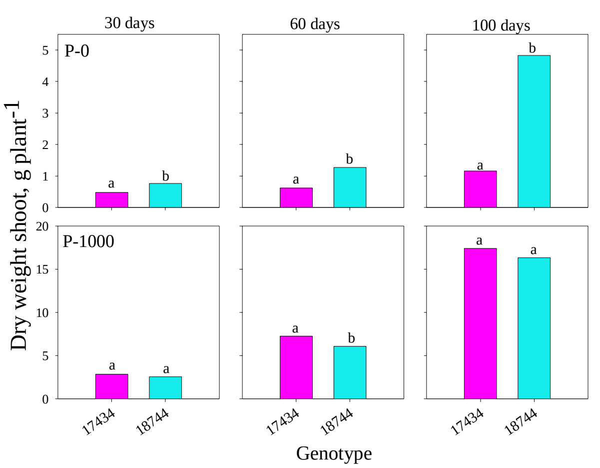

Figure 28. Influence of phosphorus supply (P-0 and P-1000) on genotypic differences in shoot biomass (forage) production of 2 accessions of *Arachis pintoi* grown for 30, 60 and 100 days in a clay loam oxisol in a growth chamber. Means are different (P<0.05) if letters above bars are different within P supply level at a given age. DAP = days after planting.

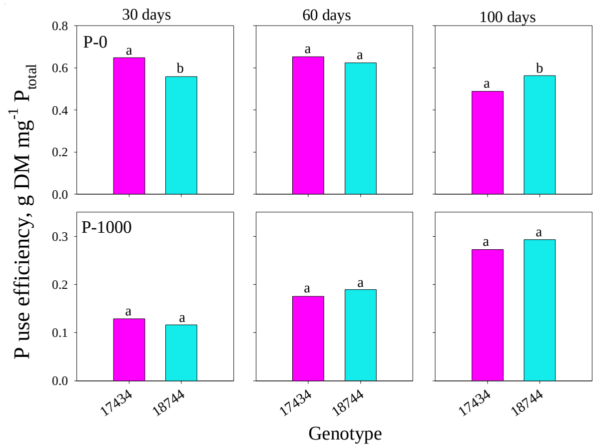

Figure 29. Influence of phosphorus supply (P-0 and P-1000) on genotypic differences in P use efficiency of 2 accessions of *Arachis pintoi* grown for 30, 60 and 100 days in a clay loam oxisol in a growth chamber. Means are different (P<0.05) if letters above bars are different within P supply level at a given age.

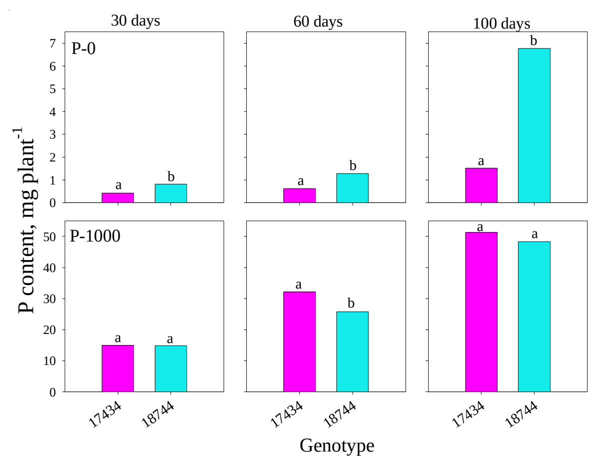

Figure 30. Influence of phosphorus supply (P-0, P-50 and P-1000) on genotypic differences in shoot P content of 2 accessions of *Arachis pintoi* grown for 30, 60 and 100 days in a clay loam oxisol in a growth chamber. Means are different (P<0.05) if letters above bars are different within P supply level at a given age.

Differences in P uptake by a plant could be due to differences in the size of the root system and/ or differences in P influx i.e. the amount taken up per unit of root and time. As a measure of the size of the root system, we used the root length-shoot biomass ratio (Figure 31). CIAT 18744 showed the lower ratio at each harvest. Thus the high shoot P content, of the efficient genotype, was not associated with more roots but to a greater P influx. The P influx (ability of plant P uptake per unit length of the root per unit time) was determined for the period between 30 and 60 days and 60 and 100 days. The P influx by the CIAT 18744 was markedly greater than that of CIAT 17434 (ca. 2 times and 3 times for the first and second harvest, respectively).

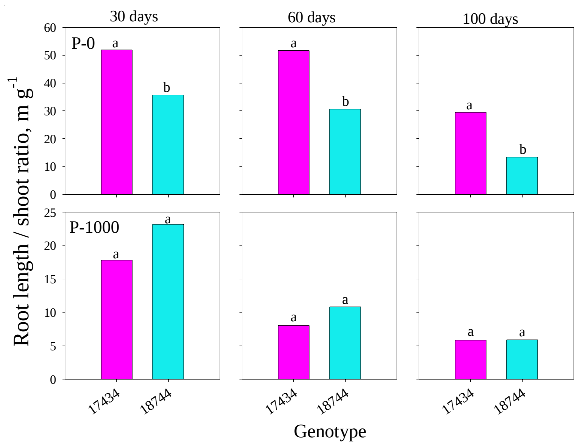

Figure 31. Influence of phosphorus supply (P-0 and P-1000) on genotypic differences in root length/shoot biomass ratio of 2 accessions of *Arachis pintoi* grown for 30, 60 and 100 days in a clay loam oxisol in a growth chamber. Means are different (P<0.05) if letters above bars are different within P supply level at a given age.

With age, the P influx increases 4 times higher for CIAT 17434 and 6 times higher for CIAT 18744 (Figure 32). One possible reason for high P influx could be due to increase in P absorbing surface area per cm root that was infected by hyphae of native arbuscular-mycorrhizae (AM). Only at 100 DAT and -under low P availability, CIAT 18744 had a higher colonization of native AM than CIAT 17434 (Table 29), which may be the reason for the higher P influx of CIAT 18744, during the second growth period. However, the infection found after 30 and 60 DAT cannot explain the P influx differences between the genotypes, at 30 DAT.

To explore further possible reasons for differences in P influx between the two accessions, we investigated the acid phosphatase (APase) acitivity on the root surface and exudation of organic acids from roots. At each growth stage, CIAT 18744 had a lower APase activity at the root surface than the CIAT 17434 (Figure 33).

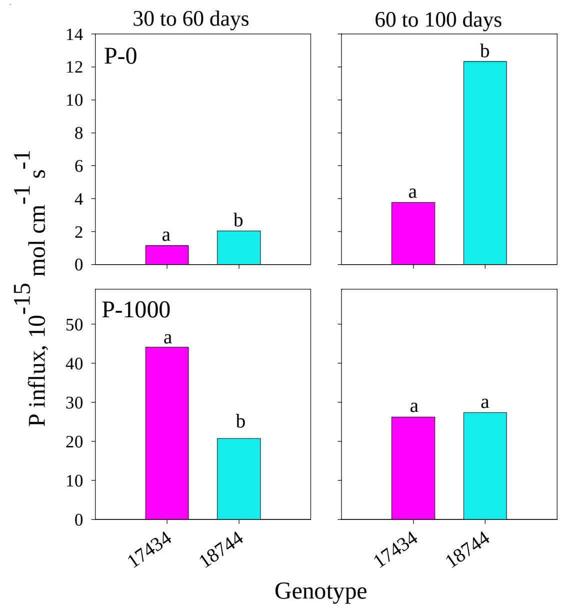

Figure 32. Influence of phosphorus supply (P-0 and P-1000) on genotypic differences in P influx of 2 accessions of *Arachis pintoi* grown for 30, 60 and 100 days in a clay loam oxisol in a growth chamber. Means are different (P<0.05) if letters above bars are different within P supply level at a given age.

Table 29. Influence of P supply on colonization of roots by mycorrhizae.

P-level	Genotype	Colonization %		
		30 d	60 d	100 d
P-0	17434	< 1	< 5	25 a
	18744	< 1	< 5	56 b
P-1000	17434	< 1	< 1	5 a
	18744	< 1	< 1	12 a

Thus the APase activity may not have contributed to either the high P influx of the efficient genotype or the increase of P influx with age. The P absorbing surface area and the hydrolysis of organic P cannot explain the higher P influx by CIAT 18744.

Another possibility could be that the plants mobilise P, i.e. increase P concentration in soil solution. P concentration in soil solution of unplanted and planted soil was also determined (Figure 34).

After 30 days of planting, the P concentrations for all were approximately 0.1 µM, which is

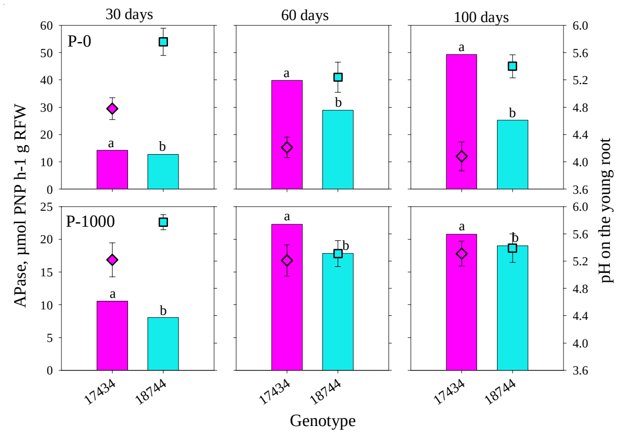

Figure 33. Influence of phosphorus supply (P-0 and P-1000) on genotypic differences on APase activity at the root surface and pH on the young root of 2 accessions of *Arachis pintoi* grown for 30, 60 and 100 days in a clay loam oxisol in a growth chamber. Means are different (P<0.05) if letters above bars are different within P supply level at a given age.

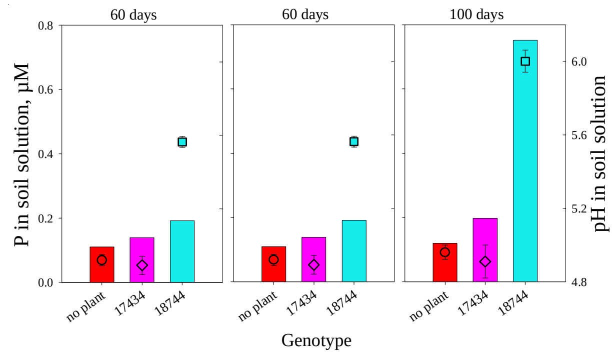

Figure 34. Influence of 3 accessions of *Arachis pintoi* and 1 accession of *Arachis hypogea* on P concentration in soil solution and pH in soil solution when grown with no phosphorus supply (P-0) for 30, 60 and 100 days in a clay loam oxisol in a growth chamber. Means are different (P<0.05) if letters above bars are different within P supply level at a given age.

below CLmin found for *Arachis hypogea*. At 60 DAT, especially at 100 DAT, the P concentration of the planted soil increased markedly and the efficient genotype showed the highest P concentration. It was 6 times higher than that of the unplanted soil

P mobilization has very often been related to organic acids exuded by roots. Twelve different organic acids were analyzed from the collected root exudates in which only lactic acid and acetic acid were detected in significant amounts with the HPLC. Organic acids, like citric acid or malic acid, that are often shown to be associated with P mobilization, were only found in trace amounts (Figure 35).

The observed organic acids were related to the P soil solution concentration. The highest exudation rate was associated with the lowest P concentration in soil solution with CIAT 17434, and the increase of the P concentration with age was associated with a decrease of organic acid exudation. Thus the organic acid exudation by the genotypes could not explain the increase of P concentration in soil solution. Moreover, monocarboxylic acids such as lactic or acetic acid have not been associated with P mobilization.

Further research work is necessary to identify the physiological mechanisms responsible for the high P uptake observed with the *Arachis pintoi* CIAT 18744.

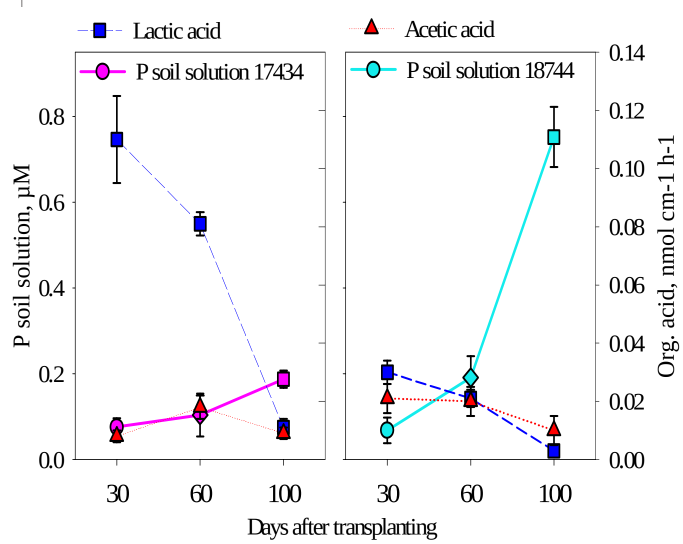

Figure 35. Influence of 3 accessions of *Arachis pintoi* and 1 accession of *Arachis hypogea* on P concentration in soil solution and organic acid exudation from roots when grown with no phosphorus supply (P-0) for 30, 60 and 100 days in a clay loam oxisol in a growth chamber. Means are different (P<0.05) if letters above bars are different within P supply level at a given age.

3.2 Genotypes of *Brachiaria* with dry season tolerance

Highlights

- Found that total nonstructural carbohydrate (TNC) content in stems of *Brachiaria* genotypes increased with increasing drought stress in large plastic soil cylinders under greenhouse conditions.

- Found that *Brachiaria* hybrid Mulato 2 is superior to cv. Mulato in terms of dry season tolerance under low fertility acid soil conditions in the Llanos of Colombia. This was associated with a greater proportion of fine roots in the top 5 cm of the soil profile.

3.2.1 Genotypic differences in root distribution and drought tolerance of 2 hybrids and 3 parents

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

Rationale

Identification of shoot and root attributes that are associated with superior drought adaptation will help to develop rapid and reliable screening methods. These methods are needed to develop *Brachiaria* hybrids that combine drought adaptation with other desirable attributes. Field studies conducted for the past few years in the Llanos of Colombia indicated that one of the *Brachiaria* hybrids, CIAT 36087 (FM9503-S046-024 or Mulato 2) is superior to its parents and other hybrids. This hybrid maintained greater proportion of green leaves during dry season under field conditions. A greenhouse study was conducted to characterize shoot and root responses of this hybrid in comparison to its parents and another hybrid, cv. Mulato when subjected to moderate and severe drought stress conditions.

Materials and Methods

A greenhouse study was conducted using a sandy loam oxisol from Matazul farm in the Llanos of Colombia. The trial comprises 5 entries, including three parents (*B. decumbens* CIAT 606, *B. brizantha* CIAT 6780, *B. ruziziensis* 44-02) and two hybrids (cv. Mulato and CIAT 36087). Plants were grown in large plastic cylinders (100 cm long and 15 cm diameter) covered with PVC tubes (Photo 15).

The trial was planted as a randomized block in split-plot arrangement with three levels of water supply: 100% field capacity (well-watered), 60% field capacity (moderate drought stress) and 30% field capacity (severe drought stress) as main plots and genotypes as sub-plots. Soil was fertilized with adequate level of nutrients (kg/ha of 80 N, 50 P, 100 K, 66 Ca, 28 Mg, 20 S and micronutrients).

Treatments of water stress were imposed after three weeks of initial growth of plants established with seed. Water stress was maintained by weighing each cylinder every week and applying water to the soil at the top of the cylider. After 2 months of stress treatment (at 85 days after germination), shoot biomass distribution, root

Photo 15. A large plastic cylinder showing root distribution across soil depth.

biomass and root length distribution in different soil depths, and leaf and stem nutrient composition, ash content and TNC (total nonstructural carbohydrates) contents were determined.

Results and Discussion

Reducing the water supply to 30% of field capacity markedly decreased the leaf, stem and root biomass of the three parents and the two hybrids (Figure 36). Leaf biomass of CIAT 606 was lower than the other genotypes tested at all three levels of water supply. Differences between the two hybrids (CIAT 36061 and CIAT 36087) in leaf biomass were not significant within each level of water supply.

Results on root length distribution showed that the hybrid Mulato 2 had a greater proportion of fine roots in the top 5 cm of soil profile at all 3 levels of water supply (Figure 37). Higher values of root length observed for 50 to 100 cm soil depth in some genotypes at 60% and 100% of field capacity indicates the growth of roots on the surface of the plastic tube and reaching to the bottom of the cylinder. We also noted problems of compaction in some cylinders. Therefore we conducted some additional studies to overcome these problems and found that use of 2:1 of soil and sand in smaller plastic tubes (50 cm long and 5 cm diameter) could overcome some of the problems encountered with large cylinders. Use of small cylinders will also facilitate evaluation of a larger number of genotypes.

Results on the determination of N, P, ash (mineral) and TNC contents in leaves and stem tissue indicated that water stress could markedly increase stem TNC content (Figure 38). Severe water stress also increased stem N, P and ash contents. But leaf N, P, ash and TNC contents were not markedy influenced by water stress conditions.

Work is in progress to evaluate the usefulness of fine root production in top soil, root penetration in subsoil and % increase of stem TNC as indicators of drought tolerance and green leaf production in *Brachiaria*.

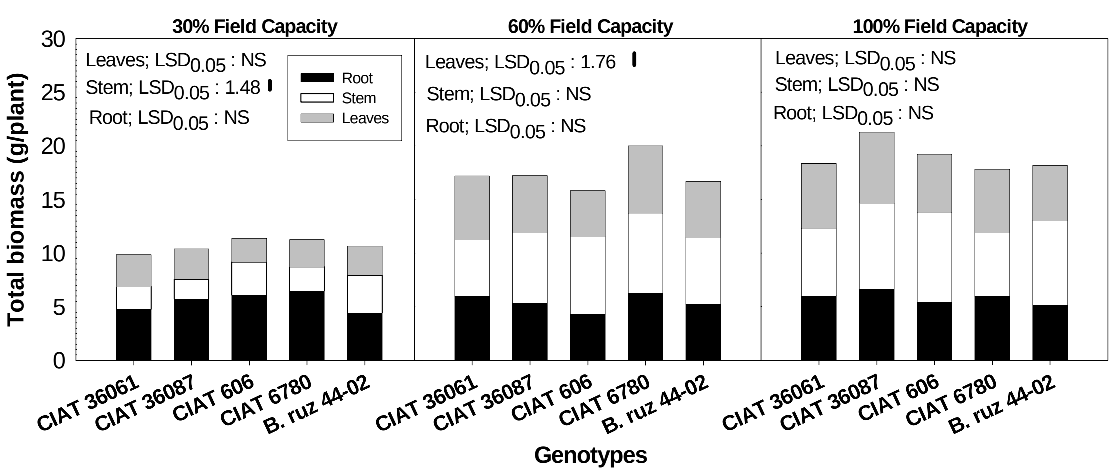

Figure 36. Influence of three levels of water supply (100%, 60% and 30% of field capacity) on dry matter distribution among leaves, stem and roots of five *Brachiaria* genotypes.

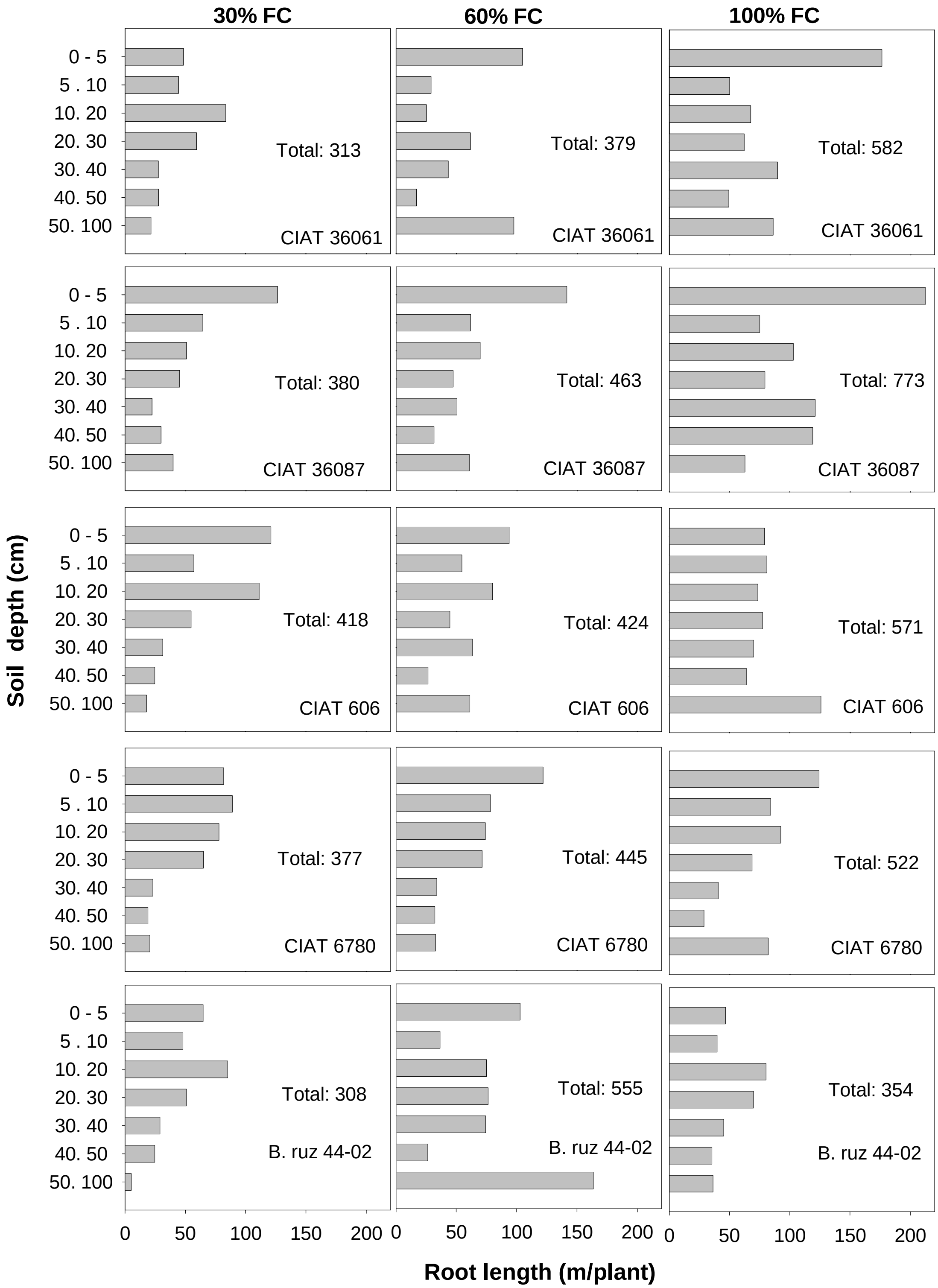

Figure 37. Influence of three levels of water supply (100%, 60% and 30% of field capacity) on root length distribution across soil depth in five *Brachiaria* genotypes.

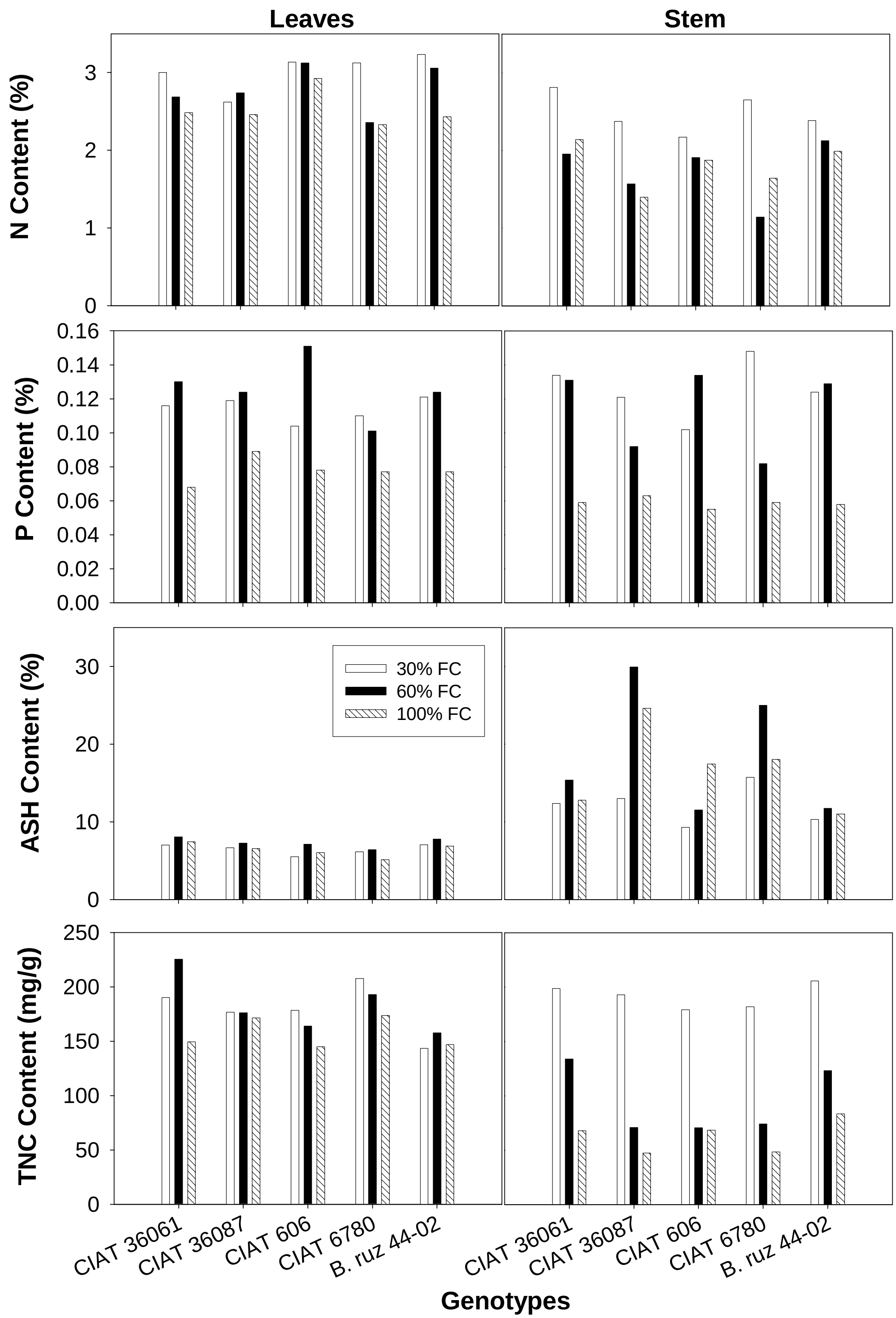

Figure 38. Influence of three levels of water supply (100%, 60% and 30% of field capacity) on leaf and stem N, P, ash and TNC contents of five Brachiaria genotypes.

3.2.2 Genotypic variation in dry season tolerance in *Brachiaria* accessions and hybrids 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 Mulato 2 (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 fifth 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) in six entries (2 hybrids, 3 parents and 1 accession) were measured at the end of the dry season (56 months after establishment; March 10, 2004). Maintenance fertlizer (half the levels of initial application) was applied at the beginning of the wet seasons of 2001 and 2003.

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 39; Table 30). At 56 months after establishment (4 months after dry season – March 10, 2004), live forage yield with low

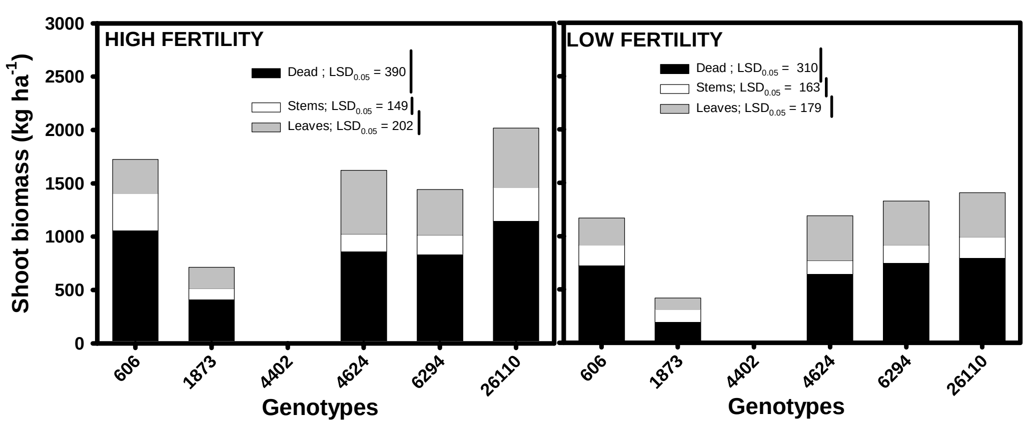

Figure 39. Genotypic variation as influenced by fertilizer application in dry matter distribution among green leaves, stems and dead biomass of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 56 months after establishment (at the end of the dry season – March 2004). LSD values are at the 0.05 probability level.

fertilizer application ranged from 0 to 609 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 3 parents, CIAT 6294 was outstanding in green live forage and dead biomass production with low fertilizer application. A spittlebug resistant genetic recombinant, Mulato 2 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 values of leaf to stem ratio of the genetic recombinant, FM9503-S046-024, were markedly superior to other genotypes under low and high levels of initial fertilizer application (Table 31).

Table 30. 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 56 months after establishment (at the end of the dry season – March 10 2004). LSD values are at the 0.05 probability level.

Genotype	Live shoot biomass ($kg\ ha^{-1}$)		Leaf to stem ratio (%)		Total forage yield ($kg\ ha^{-1}$)	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
Recombinants:						
FM9201-1873	223	301	0.9	2.0	420	714
FM9503-5046-024	542	760	3.4	3.7	1190	1622
Parents:						
CIAT 606	447	665	1.3	0.9	1169	1723
CIAT 6294	582	605	2.4	2.4	1329	1442
BRUZ/44-02	0	0	.	.	0	0
Accessions:						
CIAT 26110	609	872	2.1	1.8	1408	2019
Mean	401	534			919	1253
LSD (P>0.05)	327	318			582	667

Table 31. 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 56 months after establishment (at the end of the dry season – March 10 2004). LSD values are at the 0.05 probability level.

Genotype	Leaf N content (%)		Stem N content (%)		Shoot N uptake ($kg\ ha^{-1}$)	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
Recombinants:						
FM9201-1873	1.05	1.11	0.72	0.89	4.04	3.12
FM9503-5046-024	0.99	0.86	0.67	0.48	4.94	5.94
Parents:						
CIAT 606	1.28	1.34	0.62	0.63	4.45	6.57
CIAT 6294	1.24	0.94	0.72	0.58	6.13	4.99
BRUZ/44-02	.	.	.	.	.	.
Accessions:						
CIAT 26110	1.21	0.97	0.61	0.49	6.12	6.61
Mean	1.15	1.04	0.67	0.61	5.14	5.45
LSD (P>0.05)	0.26	0.17	NS	0.35	NS	2.90

Results on leaf N content indicated significant differences among genetic recombinants, parents and accessions with both levels of fertilizer application (Table 31). Shoot N uptake with both low and high fertilizer application was markedly greater for the hybrid Mulato 2 than the cv. Mulato (Table 31; Figure 39). Leaf and stem N content and shoot N uptake values indicated that the genetic recombinant Mulato 2 could use N more efficiently to produce green forage in the dry season (Table 31).

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

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

Rationale

Evaluation of a large number of *Brachiaria* hybrids for their resistance to spittlebug and adaptation to infertile acid soils resulted in identification of a few promising *Brachiaria* hybrids. We selected 4 of these hybrids for further field-testing in comparison with their parents. The main objective was to evaluate growth and persistence in dry season 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; Mulato 2) 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, nutrient (N, P, K, Ca and Mg) uptake, leaf and stem total nonstructural carbohydrate (TNC) content and leaf and stem ash (mineral) content were measured at 34 months after establishment (March 2004).

Results and Discussion

At 34 months after establishment, live forage yield with low fertilizer application was greater with one spittlebug resistant genetic recombinant Mulato 2 and one parent (CIAT 6294) (Table 32). With high initial fertilizer application also these two genotypes were outstanding in live forage yield (Figure 40).

Table 32. 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)	0.81***	0.87***
Dead shoot biomass (t/ha)	0.66**	0.70***
Leaf biomass (t/ha)	0.94***	0.89***
Stem biomass (t/ha)	0.77***	0.83***
Leaf N content (%)	-0.63**	-0.71***
Leaf P content (%)	0.05	-0.07
Leaf TNC content (mg g-1)	-0.08	-0.22
Leaf ash content (%)	0.15	-0.11
Stem N content (%)	-0.37	-0.58**
Stem P content (%)	-0.61	-0.19
Stem TNC content (mg g-1)	0.21	-0.2
Stem ash content (%)	0.40	0.53
Shoot N uptake (kg/ha)	0.95***	0.88***
Shoot P uptake (kg/ha)	0.93***	0.89***
Shoot K uptake (kg/ha)	0.61**	0.91***
Shoot Ca uptake (kg/ha)	0.86***	0.85***
Shoot Mg uptake (kg/ha)	0.86***	0.89***

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

Among the 4 hybrids tested, 4624 was outstanding in its adaptation to low initial fertilizer application. It is important to note that both the hybrid 4624 and CIAT 6294 had greater amount of dead biomass and stem biomass under low fertilizer application (Figure 40).

As observed last year, results on shoot nutrient uptake, particularly Ca and Mg, indicated that the hybrid, 4624 was superior to CIAT 606 under low fertilizer application (Figure 41). Nutrient acquisition by the hybrid 4624 was also greater than the rest of the hybrids with high initial fertilization. These results are consistent with the results reported last year from the same experiment. Correlation coefficients between live forage yield and other plant attributes indicated that greater nutrient acquisition with low initial fertilizer application contributed to superior performance (Table 32). No significant correlations were found between live forage yield and leaf and stem TNC or ash contents.

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

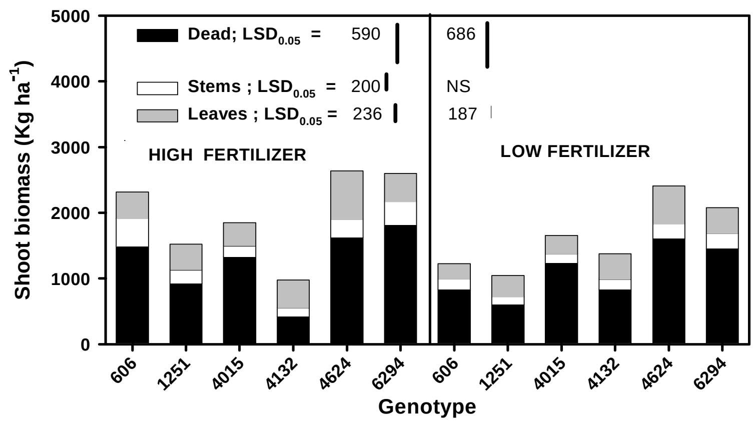

Figure 40. Genotypic variation as influenced by fertilizer application in shoot biomass production (forage yield) of two parents (CIAT 606, 6294) and four genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 30 months after establishment (November 2003). LSD values are at the 0.05 probability level. NS = not significant.

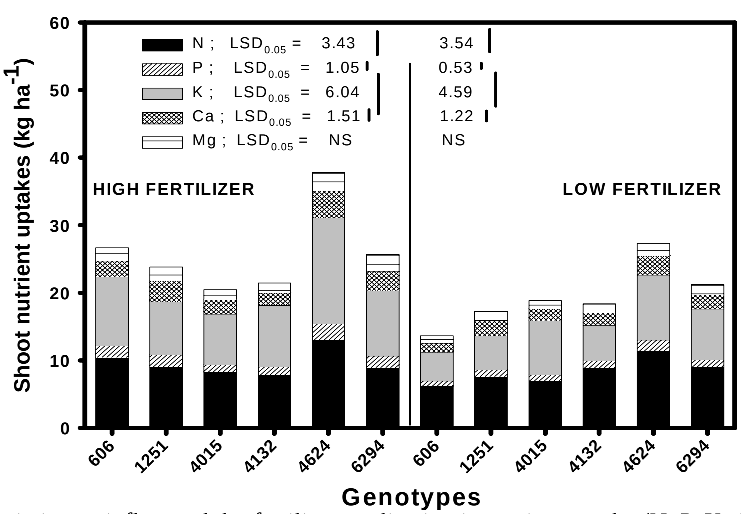

Figure 41. Genotypic variation as influenced by fertilizer application in nutrient uptake (N, P, K, Ca and Mg) of two parents (CIAT 606, 6294) and four genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 30 months after establishment (November 2003). LSD values are at the 0.05 probability level. NS = not significant.

3.3 Grasses with adaptation to poorly drained soils

Highlights

- Plant survival and yield of *Brachiaria* hybrids and accessions were affected by waterlogged conditions. However, *Brachiaria* hybrid CIAT 36087 (Mulato 2) showed less plant mortality than other hybrids under these conditions.

- *Paspalum atratum* cv. Pojuca showed no plant mortality and increased yield under waterlogged soil condition.

3.3.1 Field evaluation of *Brachiaria* and *Paspalum* genotypes in poorly and well drained sites in Costa Rica

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

Rationale

Poorly drained sites are frequently found in many areas of the low land tropics were cattle is an important economic activity. However, improved forage options are limited for permanent or periodically waterlogged conditions, and as a result native or naturalized grasses of medium to poor feeding qualities predominate in these areas. For this reason field tests are necessary to characterize the adaptation of promising forage germplasm to poorly drained soil conditions.

Material and Methods

As described in the IP-5 Annual Report 2003, seedlings of the *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 36087, CIAT 4015 and CIAT 36062, were transplanted for evaluation in a site with variable slope, sufficient to create three different moisture conditions. The soil is a heavy clay (45-55% clay) with the following characteristics: pH 5.6, 0.4 meq/100 ml of Al content, high content of Ca (26.9), Mg (10.4) and medium content of K (0.44). Phosphorous (4 ppm) and Zinc (2.5 ppm) contents are low, whereas Mn (27.5), Cu (16.3) and Fe (39.7) contents are medium.

Twelve plants were established in each strata as described in 2003, and in September of this year three dikes were built along the lower part of the plots to create variable gradients of soil humidity: (a) waterlogged, (b) moderately waterlogged and, (c) well drained condition. Plant mortality, vigor and plant yield were measured during the wet period of 2004 along the three humidity strata that were created.

Results and Discussion

The dikes built created the expected waterlogged conditions. At the lower part of the plots a permanent water table of 5 to 10 cm depth remained and covered partially the grass plants during the evaluation period. At the middle of the plots, moderately waterlogged conditions were also created, and the water table remained around 20 cm. The well drained conditions had a water table that ranged from 30 to 50 cm.

Plant vigor and plant mortality of all *Brachiaria* species were affected during the evaluation period by the soil moisture conditions created as shown in Table 33. With the exception of *P. atratum* cv. Pojuca, the *Brachiaria* species lost vigor as the soil humidity increased, indicating poor adaptation to waterlogged soils. At the waterlogged site, plant mortality was relatively high (3 plants out of 12 plants) with *Brachiaria*

Table 33. Plant vigor and plant mortality of grass species established along a humidity gradient formed by (1) well drained conditions, (2) moderately drained, and (3) poorly drained (waterlogged) conditions in a heavy soil of Atenas, Costa Rica.

Species	CIAT No.	Vigor*			Plant mortality (No.)		
		(1)	(2)	(3)	(1)	(2)	(3)
Brachiaria hybrid (cv. Mulato)	36061	3.8	4.0	2.0	0	0	3
B. hybrid (Mulato 2)	36087	4.2	4.3	3.5	0	0	1
B. hybrid	36062	4.8	2.8	2.3	0	1	2
B. hybrid	4015	3.2	3.3	1.8	0	0	3
B. brizantha	26124	4.3	2.8	1.7	0	0	3
B. brizantha	26318	2.5	2.2	1.5	0	0	2
B. brizantha	26990	2.5	2.0	1.2	1	2	2
B. brizantha	(Mixe)	3.7	3.2	1.8	0	0	3
Paspalum atratum cv. Pojuca	26986	3.5	4.0	5.0	0	0	0

* Vigor rated: 1.0 = poor vigor; 5.0 = highly vigorous plant.

hybrids cv. Mulato and CIAT 4015, for *B.brizantha* CIAT 26124 and Mixe, and moderately (2 plants dead out of 12 plants) for *Brachiaria* hybrid CIAT 36062, *B. brizantha* CIAT 26318 and CIAT 26990. The *Brachiaria* hybrid CIAT 36087 (Mulato 2) had only 1 dead plant at the flooded site, while *P. atratum* cv. Pojuca did not show any sign of plant mortality under these conditions, indicating the good adaptation of this species to waterlogged soils.

As the soil moisture increased, there was a tendency for reduced plant yields in all *Brachiaria* species. In contrast with *P. atratum* cv. Pojuca there was higher yields under water-logged conditions (Table 34).

It was interesting to observe that the yields recorded for the *Brachiaria* hybrid cv. Mulato and other *Brachiaria* species, were similar at the three humidity treatments, indicating that for the degree of plant mortality recorded, the survival plants compensated in growth for the lost ones, with the exception of *B.* hybrid CIAT 36087 (Mulato 2) and Mixe that produced significant more plant yield at well drained sites.

Table 34. Dry matter yields (DMY) of grass species established along a humidity gradient formed by (1) well drained conditions, (2) moderately drained, and (3) poorly drained (waterlogged) conditions in a heavy soil of Atenas, Costa Rica (means of 4 cuts of 5 weeks re-growths during the wet period 2004).

Species	CIAT No.	DMY (g/plant) Well drained	Moderately drained	Poorly drained	Probability
Brachiaria hybrid (cv. Mulato)	36061	86.7 a*	76.1 a	72.5 a	ns
Brachiaria hybrid (Mulato 2)	36087	99.4 a	75.0 b	75.2 b	p=0.02
Brachiaria hybrid	36062	71.3 a	59.3 a	56.7 a	ns
Brachiaria hybrid	4015	55.4 a	51.7 a	43.8 a	ns
B. brizantha	26124	67.7 a	62.3 a	61.2 a	ns
B. brizantha	26318	56.3 a	48.8 a	44.6 a	ns
B. brizantha	26990	37.0 a	30.6 a	30.0 a	ns
B. brizantha	(Mixe)	64.8 a	57.3 ab	47.2 b	p=0.10
Paspalum atratum cv. Pojuca	26986	101.5 a	104.3 ab	237.4 c	p=0.0001

*****Within the same line means followed by the same letter are not statistically significant

3.4 Nitrification inhibition in tropical grasses

Highlights

- Root exudates from *B. humidicola* are effective, persistent and stable at inhibiting nitrification up to at least 75 days.
- Presence of NH_4-N stimulates the synthesis and release of NI activity in exudates produced by the roots of *B. humidicola*.
- Genetic variability in capacity to inhibit nitrification was found among accessions of *B. humidicola* held by CIAT, which opens up the possibility for breeding for this trait

3.4.1 Bioassay – Further improvements and refinements to the methodology: Expression of NI activity in AT (equivalent to allylthiourea inhibition) units

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

Rationale

We have further improved the bioassay methodology and developed ways to express inhibitory effect (on nitrification from root exudates) in equivalent standard inhibitor, allylthiourea (AT) units. The transgenic *Nitrosomonas* -responds linearly to the AT concentration in the bioassay medium (Figure 42). Using this relationship, the inhibitory effect from root exudates (that is determined using bioassay) is expressed in AT units, which can be subjected to statistical analysis. One AT unit of NI is defined as the inhibitory activity caused by the presence of 0.22 µM of AT in the bioassay medium. These improvements in the bioassay methodology will make it now possible to characterize the nitrification inhibition phenomenon in root exudates of plants. Also, the bioassay methodology will make it possible for the evaluation and comparative analysis of crop and forage germplasm accessions and breeding lines for the NI (nitrification inhibitory) activity in root exudates.

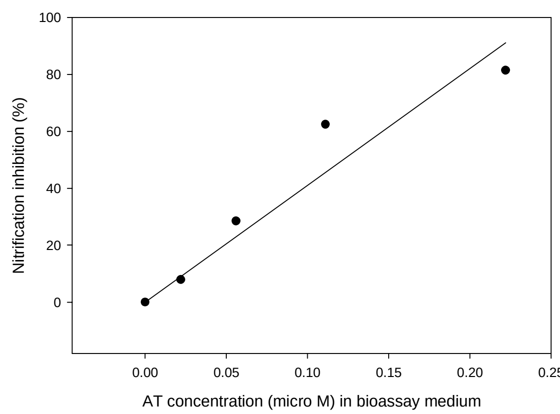

Figure 42. Transgenic *Nitrosomonas europaea* response to synthetic nitrification inhibitor, allylthiourea in the bioassay medium.

3.4.2 Stability, persistence and effectiveness of *Brachiaria humidicola* root exudates in inhibiting nitrification in soil

Contributors: G.V. Subbarao, H. Wong, T. Ishikawa and O. Ito (JIRCAS, Japan); M. Rondon and I.M. Rao (CIAT)

Rationale

This year, we have improved further the protocols in processing and testing of root exudates to determine the inhibitory effect on nitrification in soil (IP-5 Annual Report, 2003). We have tested the stability, persistence and effectiveness of the inhibitory effect from root exudates of *B. humidicola* on nitrification in soil. NI activity of 10 AT units g^{-1} soil (Soil from Tsukuba, Japan) was added to the soil with 182 ppm of N as $(NH_4)_2SO_4$ and incubated at 20 °C and 95% RH. The experiment was replicated three times. Sequential sampling was done at 25 d intervals and the incubation was continued for 100 days. NI activity of 10 AT units g^{-1} soil was very effective in inhibiting nitrate formation in soil (about 70% inhibition) and remained effective in inhibiting nitrification (about 50%) until 75 days. A substantial portion of the inhibitory effect from NI activity was lost between 75 and 100 period of incubation in soil.

The synthetic nitrification inhibitor, ©Nitrapyrin did not inhibit nitrification effectively (only about 20% inhibition on nitrate formation) at 4.5 ppm under these conditions and lost its effectiveness after 30 days of incubation (Figure 43).

Our results demonstrate that root exudates from *B.humidicola* are effective, persistent and stable in inhibiting nitrification in soil (up to 75 days at least). Our results indicate that two *B. humidicola* plants of 60 to 70 d old can release up to 100 AT units of NI activity (in 24 h period) under optimum conditions.

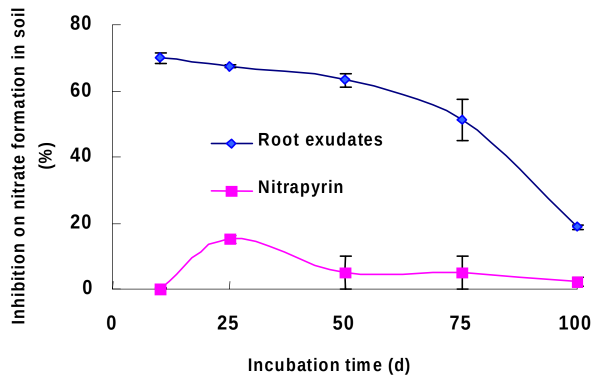

Figure 43. Inhibitory effect from root exudates (10 AT units NI activity g-1 soil) and nitrapyrin (4.5 ppm) on nitrate formation in soil during 100 d incubation period (Note: In control, nearly 90% of the added NH_4-N was nitrified by 75 days).

Our results also indicate that the NI activity release rates mentioned above can be maintained for long periods of time (we have tested up to 15 days and that the release rates were maintained).

This is the first time that we have demonstrated the effectiveness, stability and persistence of root exudates (from *B. humidicola*) inhibitory effect on nitrification in soil.

3.4.3 Influence of NH_4-N on expression/regulation and release of NI activity in root exudates of *B. humidicola*

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

Rationale

We have tested the hypothesis that nitrogen forms (NH_4-N *vs* NO_3-N) can influence the release of NI activity from roots in *B.humidicola*. Plants of *B. humidicola* were grown hydroponically with two sources of nitrogen – 1 mM N as $(NH_4)_2SO_4$ or KNO_3 for 70 days. The experiment was replicated three times. Root exudates were collected by keeping intact plant roots in distilled water, 1 mM NH_4Cl or 1 mM KNO_3 for 24 h. NI activity of root exudates was determined with the NI bioassay. Root exudates of NH_4-N grown plants showed NI activity, whereas NI activity was completely absent in the root exudates of NO_3-N grown plants (data not shown for NO_3-N grown plants as there was no NI activity detected in root exudates).

Presence of NH_4-N in the root exudates collection solutions further stimulated the release of NI activity in NH_4-N grown plants (Figure 44).

The NI activity released in the presence of NH_4-N was several-fold higher than in the absence of NH_4-N (i.e. when root exudates are collected using distilled water).

Our results support the hypothesis that presence of NH_4-N stimulates the synthesis and release of NI activity from root exudates (data not presented on the root tissue NI levels). The release of NI activity from roots appears to be a highly regulated phenomenon and NH_4-N in the rhizosphere is certainly one of the important regulating factors for the release of NI activity. Also, regulatory role of NH_4-N in the rhizosphere for the release of NI activity from roots further indicates the functional significance of NI activity in protecting NH_4-N in soil from nitrification.

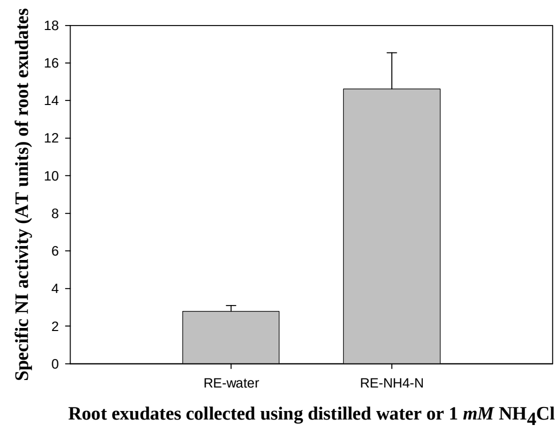

Figure 44. Influence of NH4-N in the root exudates collection medium on the release of NI activity into root exudates from *B. humidicola* roots (Specific NI activity = NI activity g^{-1} root dry weight).

3.4.4 Screening for genetic variability in the ability to inhibit nitrification in accessions of *B. humidicola*

Contributors: M. Rondón, I.M. Rao, C.E. Lascano, J.A. Ramírez, M.P. Hurtado, J. Ricaurte (CIAT); G.V. Subbarao, T. Ishikawa, K. Nakahara, and O. Ito (JIRCAS, Japan)

Rationale

Ongoing collaborative research with JIRCAS, Japan, has shown that *B. humidicola* CIAT 679 inhibits nitrification of ammonium and reduces the emission of nitrous oxide into the atmosphere. Given these findings with the commercial cultivar of *B. humidicola* CIAT 679, and the fact that a range of inhibition of nitrification was observed among different tropical grasses, there is a need to determine the extent of genetic variation among the 69 accessions of *B. humidicola* that are part of CIAT germplasm bank. This information will be extremely useful to develop screening methods to select genetic recombinants of *Brachiaria* grasses that not only are resistant to major biotic and abiotic stress factors but also can protect the environment. Given the vast areas under *B. humidicola* in the tropics, reductions in net emissions of N_2O could also have important environmental implications.

The main objective was to quantify differences among 10 accessions of *B. humidicola* regarding the nitrification inhibition activity of root exudates collected from plants grown under greenhouse conditions using infertile acid soil. Also we intend to test the relationship between nitrification inhibition and root production in terms of biomass and length.

Materials and Methods

A sandy loam Oxisol from the Llanos (Matazul) of Colombia was used to grow the plants (4 kg of soil/pot) under greenhouse conditions. 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 ten accessions were used (accessions CIAT 679, 6133, 6369, 6707, 16866, 16867, 16886, 16888, 26149, 26159). A control without plants was also included. The experiment was arranged as a completely randomized block design with four replications. Each pot contained four plants. After sowing, plants were allowed to grow for 15 weeks and were cut to 10 cm height to simulate grazing effects under field conditions. Plant tissue was dried and saved.

Plants were allowed to re-grow during 5 weeks more to promote a well developed root system and then ammonium sulfate was applied in solution at a rate of 38.5 mg N-NH_4/kg soil (equivalent to 100 kg N-NH_4 per hectare). Five weeks later plants were harvested (at 25 weeks after sowing). At the end of the experiment, plants were carefully removed from soil minimizing mechanical damage to the roots. Soil adhered to the fine toots was removed and the roots were rinsed with deionized water. Once clean, the roots were fully immersed in 1 liter of deionized water and were allowed to produce root exudates during 24 hours. Collected root exudates were kept in the refrigerator and were reduced in volume to approximately 100 ml using a freeze drier.

Harvested plants were separated into shoot and roots. Root length was measured using a root length scanner. Dry matter content and N status of both shoot and root biomass was determined. At harvest time, soil samples were extracted with KCL and analyzed for nitrate and ammonium levels. The concentrated root exudates were further concentrated using a rotovapor using protocols that were developed for this purpose. The final concentrate was tested for its nitrification inhibitory activity using a specific bioassay developed at JIRCAS.

Results and Discussion

Results on dry matter partitioning among shoot and root biomass from the comparative evaluation of the ten accessions are presented in Table 35. No significant differences were found in total biomass production among most of the CIAT accessions except for the accessions of 16866 and 16867, which were lower than the rest of the accessions. However, significant differences among accessions were found in root biomass production. The commercial cultivar, CIAT 679, which has been used in most of the previous work, seems to have root biomass around the average value for the group tested. The accession 6707 produced the highest root biomass among the tested accessions. Values of root biomass of this accession were more than two fold greater than the value for the lowest in the group, the accession 26149.

Table 35. Dry matter partitioning differences among ten accessions of *B. humidicola* grown in pots under greenhouse conditions. Plants were harvested at six months after planting.

CIAT Accession Number	Dry matter (g/pot)		
	Root biomass	Shoot biomass	Total biomass
CIAT 679	4.29 (1.19) a	14.76 (3.76) d	19.05 (3.68) f
CIAT 6133	4.14 (1.65) a	15.06 (1.90) d	19.20 (3.49) f
CIAT 6369	4.77 (1.58) b	14.35 (1.59) d	19.12 (2.52) f
CIAT 6707	4.92 (0.72) a	17.84 (2.75) d	22.75 (2.61) f
CIAT 16866	3.52 (0.89) a	13.45 (0.96) e	16.97 (0.95) g
CIAT 16867	3.50 (0.38) a	14.70 (1.65) e	18.20 (1.56) g
CIAT 16886	4.48 (1.09) b	15.53 (4.56) d	20.01 (5.12) f
CIAT 16888	3.26 (0.72) a	16.97 (1.40) d	20.22 (1.17) f
CIAT 26149	2.39 (0.30) c	17.31 (3.20) d	19.70 (3.09) f
CIAT 26159	2.96 (1.43) c	16.15 (2.09) d	19.10 (2.20) f

Numbers in parenthesis indicate standard deviation. In a given column, data followed by the same letter indicate non- significant differences (LSD, $p<0.05$).

Results from the bioassay indicated substantial level of NI (nitrification inhibitory) activity in the root exudates of most of the accessions tested (Table 36). However a range in NI activity was found among the tested accessions. Accessions could be grouped in 3 classes in relation to their specific NI activity. Group 1 with the accession CIAT 16867 showed no NI effects, behaving

Table 36. Nitrification inhibitory activity (total NI activity pot^{-1} and specific activity .g root dry $weight^{-1}$) of the root exudates from ten accessions of *B. humidicola* grown under glasshouse conditions. Plants were grown for six months before the collection of root exudates.

CIAT Accession Number	NI activity (in AT units pot^{-1})	Specific NI activity (in AT units g root dwt^{-1})
CIAT 679	68.84 (24.1) cd	17.48 (8.4) c
CIAT 6133	51.58 (16.9) cd	12.24 (2.83) c
CIAT 6369	86.94 (14.3) c	20.72 (4.2) c
CIAT 6707	69.68 (5.5) cd	14.86 (1.2) c
CIAT 16866	41.48 (6.9) d	11.26 (2.9) c
CIAT 16867	-48.55 (18.1) e*	-13.42 (3.35) d
CIAT 16886	128.05 (15.3) ab	27.95 (5.8) bc
CIAT 16888	160.95 (6.08) a	53.76 (17.45) a
CIAT 26149	33.5 (39.8) d	15.22 (18.15) c
CIAT 26159	126.17 (19.9) b	46.33 (19.0) ab

Note: Numbers in parenthesis indicate standard deviation. In a given column, data followed by the same letter indicate non-significant differences (LSD, $p<0.05$). NI activity is expressed as AT units; One AT unit is defined as the inhibitory activity caused by the addition of 0.22 µM of allylthiourea (AT) in the bioassay medium. Thus, the inhibitory activity of the test samples of root exudates is converted into AT units for the ease of expression in numerical form.

*Negative activity indicates that nitrification was stimulated by the root exudates.

similarly to other grasses such as *Panicum maximum,* which also lack the NI activity. Group 2 that included accessions CIAT 6133, 6707, 16866, 26149, 6369, and 6707 showed similar levels of NI that was observed with the commercial cultivar CIAT 679. Group 3 that included the accessions 16886, 16888, and 26159 showed significantly higher levels of NI than the accession 679. The accession 16888 was outstanding in its NI activity with a value of more than three times to that of the value of CIAT 679.

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

The presence of substantially higher levels of NI activity in the root exudates of the two CIAT accessions (16888 and 26159) draws attention to the need to study these accessions in more detail. The immediate task is to continue the screening of other accessions of *B. humidicola* from the gene bank and to initiate screening of other commercially important grasses and crops for their ability to inhibit nitrification. As a continuation of this work, this year we have initiated the screening of another 11 accessions of *B. humidicola* including all materials that are classified as putatively sexual. An additional experiment will be conducted to obtain and test NI activity of root exudates from maize, rice, sorghum, soybean, cowpea and common bean. Results from this study will be reported next year. Further research work is needed 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.

3.4.5 Field validation of the phenomenon of nitrification inhibition from *Brachiaria humidicola*

Contributors: M. Rondón, I.M. Rao, C.E. Lascano, M. P. Hurtado, J. Ricaurte (CIAT); G.V. Subbarao, T. Ishikawa and O. Ito (JIRCAS, Japan)

Rationale

Research conducted at JIRCAS and CIAT for the past three years using *B. humidicola* has shown that root exudates from this tropical grass have the capability to inhibit/suppress the nitrifying populations in the soil. Factors such as presence of NH_4-N in the soil seem to have a stimulating effect on the expression of nitrification inhibition (NI) activity in the root exudates of *B. humidicola*. Differences have been found among accessions of *B. humidicola* with regard to their NI activity. Also, our recent studies involving soils incubated with root exudates of *B. humidicola* and soybean have shown that root exudates from *B. humidicola* have suppressed the N_2O emissions and inhibited the nitrification process, while those of soybean seem to stimulate the nitrification process in soils. Soybean (usually in rotation with maize) is becoming increasingly important as a crop not only in Latin America but also in many tropical and temperate regions. Other grasses such as *Panicum maximum* lack the NI activity, while the *Brachiaria* hybrid cv. Mulato was found to have a moderate level of NI activity. The use of this hybrid is expanding rapidly in Latin America due to its high productivity and forage quality.

All these above studies were conducted either using hydroponic systems or soil in pots under greenhouse conditions to test and verify the concept of the biological phenomenon of nitrification inhibition. There is a clear need to validate some of these findings under field conditions. This year a collaborative (CIAT-JIRCAS) long-term experiment was initiated to validate the phenomenon of NI under field conditions and to monitor whether the NI activity is a cumulative process in the soil

Given the vast areas currently grown in the tropics on tropical grasses, an understanding of the NI process and the possibility of managing it to improve fertilizer N use efficiency, reduce nitrate pollution of surface and ground waters as well as reduce net impact on the atmosphere through reduced emissions of nitrous oxide, could have potential global implications for sustainable agricultural development and environmental protection.

Materials and Methods

The field experiment was established on 31 August 2004 at CIAT-Palmira on a Mollisol (Typic Pellustert) as a randomized complete block (RCB) design with six treatments and 3 replications. Annual rainfall at this site is about 1000 mm with a mean temperature of 25 ^{o}C. Soil is fertile with a pH of 6.9. Two accessions of *B. humidicola* were included: the reference material (CIAT 679) that has been used for most of our previous studies, and the high NI activity germplasm accession (CIAT 16888). The Hybrid Mulato was included as a moderate NI and *Panicum maximum* var. common was included as a negative non-inhibiting control. A crop rotation (maize-soybean) was included to assess under field condition the recent finding that Soybean lacks NI ability (indeed accelerate nitrification), while maize shows some degree of inhibitory capability. As first crop of the rotation we used maize variety (ICA V109). A plot without plants where emerging weeds are removed manually is used as an absolute control.

Plot size for each treatment was 10m x 10m. Irrigation will be provided if necessary. Maize was planted from seeds and the tropical forage grasses were propagated from vegetative cuttings. Fertilizer will be applied (broadcast) for every crop cycle, consisting of (kg/ha) 96 N (as urea), 48 K, 16 P, 0.4 Zn, 0.4 B and 8 S. The fertilizer is split into two equal applications: one at 20 days after sowing of each crop (either maize or soybean) and the other at flowering time at approximately 60 days after sowing.

A number of soil and plant parameters will be measured at every four months. These include nitrate and ammonium availability in the soil, dynamics of nitrifier organisms in soil, plant nitrogen uptake and nitrous oxide (N_2O) emissions. The NI activity of soil water extracts will be measured using the bioassay. Soils samples will be periodically collected and sent to JIRCAS to assess changes in inhibitory compounds in the soil. Gas samples for measuring N_2O fluxes will be collected every month. Once a year, soil incubation studies will be conducted using rhizosphere soil, to monitor nitrogen dynamics and fluxes of N_2O. Currently plants are growing well and the initial sampling is expected in January 2005. Results from this field study will be reported next year.

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

Highlights

- Selected accessions of *Desmodium velutinum* with superior productivity, forage quality and that have persisted over 2 years under cutting.

- Selected accessions of *Canavalia brasiliensis* with superior productivity and drought tolerance for seed multiplication.

3.5.1 Evaluation of new collections of the multipurpose shrub legumes *Flemingia macrophylla, Flemingia* spp. and *Cratylia argentea*

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

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*, *Flemingia* spp. and *C. argentea* with two 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 and (b) germplasm origin information.

Material and Methods

In the Annual Report of 2003 we presented results on the evaluations of large collections of both *Flemingia macrophylla* and *Cratylia argentea*. To complement this work, we acquired additional germplasm of *C. argentea* and *F. macrophylla*. In view of the interest of *Flemingia* as a genus; we are also evaluating a new collection of different *Flemingia* species.

In 2002, 8 accessions of *Cratylia argentea* - CIAT 22377, 22395, 22388, 22389, 22395, 22401, 22402, 22403 and Yacapani previously evaluated in Costa Rica were sown at CIATs research station in Santander de Quilichao (Photo 16). In contrast to other accessions of *C. argentea*, Yacapani is of prostrate growth habit. Accessions CIAT 18516 and 18668 were included as standards.

Photo 16. *Cratylia argentea* at Quilichao, 2002.

Plants were sown in the greenhouse in jiffys and transplanted to the field 6 weeks later. We employed the same evaluation methodology as for the larger collections of *C. argentea* and *F. macrophylla* described in the Annual Report 2003 (for more details on the methodology please refer to the Annual Report 2003).

Results and Discussion

Agronomic evaluation: Three cuts were carried out in each the dry and wet season and results on dry matter yield are presented for *Cratylia argentea* and *Flemingia macrophylla* in Tables 37 and 39.

***Cratylia argentea* (2002):** Except for the distinct growth habit of Yacapani, no morphological differences between accessions were observed. Though Yacapani has normally a prostrate growth habit, erect plants were also encountered; it is not clear if this variation is to be attributed to contamination of the seed, outcrossing or diversity within the accession. Additional plants of the accession were sown to study the growth habit of this particular accession in more detail. No disease and pest problems were observed.

In general, yields were much higher than previously obtained in Costa Rica for the same accessions. DM yields of the accessions CIAT 18516, 22377, 22395, 22389, 22403 and 18668 were higher than reported for the larger collection in the Annual Report 2003, most likely as a result of a more favorable growing conditions in 2003/2004. No significant (P>0.05) yield differences among accessions were recorded in either the wet or dry season. Mean dry season yields were superior to those recorded in the wet season, and better regrowth was also found under dry conditions.

Highest DM yields were obtained for CIAT 18516, which had a higher yield and regrowth ability than accession CIAT 18668. The mixture of these accessions was released in Costa Rica as cv. Veraniega and in Colombia as cv Veranera. Lowest DM yields in both seasons were recorded for accession CIAT 22401 (Table 37). In line with results obtained for DM yield, in-vitro dry matter digestibility (IVDMD) was higher in the dry than in the wet period. The accessions 18516 and 18668 (mixture of these two accessions formed cv. Veranera) had lower IVDMD and CP as compared to accessions CIAT 22402, 22389, 22041 and 22388. However, with the exception of CIAT 22389, accessions of higher quality had relatively low DM yields (Table 38).

***Flemingia macrophylla* (2002):** In contrast to the large diversity in growth types encountered in the larger collection of *F. macrophylla* (see

Table 37. Agronomic evaluation of a collection (2002) of *Cratylia argentea* in Quilichao. Data of six evaluation cuts (three in the dry season and three in the wet season). Underlaid in grey: Accessions CIAT 18516/18668.

Accession No. CIAT	Height	Diameter	Regrowing points	Mean DM yields	Height	Diameter	Regrowing points	Mean DM yields
	Wet				Dry			
	(cm)		(No.)	(g/pl)	(cm)		(No.)	(g/pl)
18516	169	149	28	580	151	126	31	683
22395	142	137	20	436	145	123	25	542
22389	152	121	17	389	156	111	22	515
22377	145	110	18	370	144	111	21	437
22403	148	124	18	351	144	113	23	437
18668	140	120	19	325	136	106	23	425
22388	146	118	14	321	150	101	18	411
22402	144	106	14	310	150	87	17	378
Yacapani	135	131	17	284	122	111	27	400
22401	121	107	17	219	129	93	17	247
Mean	144	122	18	351	143	108	22	439
LSD (P<0.05)				246.9				344.6

Table 38. Forage quality of accessions of *Cratylia argentea* evaluated in Quilichao, 2004. Grey underlaid: Accessions CIAT 18516/18668.

Accession No. CIAT	Wet IVDMD	Wet C P	Wet ADF	Dry IVDMD	Dry C P	Dry ADF
			(%)			
22402	60.2	20.2	24.1	70.6	22.8	22.8
22389	65.0	20.1	22.4	70.6	22.1	20.7
22401	60.1	21.7	24.9	68.4	22.4	24.9
22388	63.2	21.8	23.5	67.6	22.0	22.1
22395	60.6	21.1	25.1	65.7	23.5	25.1
22403	57.6	20.5	25.9	65.5	20.6	27.8
18668	58.6	19.3	25.2	65.1	20.8	25.4
18516	60.6	20.1	23.3	65.0	23.2	22.3
22377	55.9	21.2	27.0	64.3	22.8	26.4
Yacapani	56.8	21.5	23.5	64.1	22.0	22.7
Mean	59.8	20.8	24.5	66.7	22.2	24.1
LSD (P< 0.05)	6.9	2.8	3.7	3.7	2.1	2.9

Annual Report 2003), materials evaluated this year were more homogenous and all of erect growth habit (Photo 17).

Differences in yield between the wet and dry seasons were not significant. However, significant (P<0.05) differences in DM yield were found among accessions when averaged across seasons. The highest DM yields and the best regrowth were recorded for accessions CIAT 659 and 906 (Table 39).

Photo 17. Collection 2002 of *Flemingia macrophylla* in Quilichao.

Table 39. Agronomic evaluation of a collection (2002) of *Flemingia macrophylla* in Quilichao. Data of six evaluation cuts (tree in the dry season and tree in the wet season). Grey underlaid: Accessions with digestibility >44% and dry matter yield >250 g/plant.

Accession Number	Wet Height (cm)	Wet Diameter (cm)	Wet Regrowing points (No.)	Wet Mean DM yields (g/pl)	Dry Height (cm)	Dry Diameter (cm)	Dry Regrowing points (No.)	Dry Mean DM yields (g/pl)
659	127	117	37	416	119	116	46	419
906	134	108	30	409	134	102	40	409
870	126	107	37	378	124	115	45	350
591	135	114	34	374	129	102	38	315
914	134	105	31	370	137	114	40	395
816	119	108	31	347	119	109	41	304
595	133	108	19	335	128	101	26	300
615	123	107	28	328	104	97	34	280
780	128	108	30	321	130	110	35	298
857	126	99	25	314	118	97	32	324
632	124	97	29	310	120	101	39	273
753	109	93	25	297	109	94	31	246
601	126	108	26	297	134	108	34	353
629	110	94	25	291	105	101	42	274
821	110	104	33	274	112	99	31	266
804	117	103	25	274	119	101	30	313
576	130	96	20	267	129	93	25	303
542	122	96	18	206	123	80	22	186
Mean	124	104	28	323	122	102	35	312
LSD(P< 0.05)				166.62				180.62

In terms of quality parameters significant (P<0.01) differences among accessions were measured for IVDMD, ADF and soluble tannin content (Table 40). While some accessions had a higher digestibility than CIAT 17403 (control), values were lower than for the accessions with highest quality selected from the larger collection of Flemingia macrophylla previously evaluated (see Annual report 2003).

Table 40. Forage quality of accessions of *Flemingia macrophylla* evaluated in Quilichao, 2004.*

Accession Number	Wet				Dry			
	IVDMD	C P	ADF	Tan Sol	IVDMD	C P	ADF	Tan Sol
				(%)				
870	45.4	21.7	23.0	4.8	44.5	20.4	21.4	1.6
816	41.3	21.1	23.4	7.4	41.1	21.1	21.6	5.5
601	41.4	20.6	22.1	3.3	40.1	20.4	21.8	5.9
780	44.6	21.5	22.2	6.0	40.1	21.4	22.2	3.9
595	41.6	21.7	21.4	2.4	39.3	20.6	22.0	6.2
857	44.8	20.0	22.3	6.5	38.6	19.8	22.7	3.4
804	37.0	22.0	22.3	6.9	38.4	21.2	23.3	6.2
542	40.4	21.3	22.8	5.3	38.2	20.6	22.7	5.3
914	38.5	19.0	23.5	5.8	37.8	19.8	24.4	1.8
576	40.1	20.9	20.5	5.9	36.9	19.9	21.3	7.4
906	42.7	20.4	22.3	5.0	36.8	20.5	23.0	2.4
591	39.6	21.7	20.9	2.1	36.7	21.1	20.9	8.0
659	38.2	20.6	20.5	7.6	35.7	20.6	21.2	4.1
753	37.8	21.9	23.8	6.8	35.4	20.4	23.6	6.5
615	39.3	21.1	24.6	3.9	35.4	20.0	24.6	8.1
821	38.0	20.1	21.7	4.7	34.8	20.3	21.1	3.7
629	32.6	20.2	23.4	3.0	32.9	19.3	20.3	8.0
632	37.1	20.1	23.2	4.7	30.9	18.7	21.4	7.3
Mean	40.1	20.9	22.3	5.1	37.4	20.3	22.2	5.3
LSD(P< 0.05)	5.8	2.8	2.3	3.9	7.0	2.5	1.93	2.7

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

3.5.2 Genetic diversity in the multipurpose shrub legume *Desmodium velutinum*

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Rationale

For acid, low-fertility soils in drought-prone environments there are few options in terms of shrub legumes. 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 the ones available concentrates on one or two accessions. However, the available information indicates that this legume produces forage of high quality and has the potential to adapt to drought and (acid) low-fertility soils.

We are currently exploring the genetic diversity in a collection of D. velutinum held by CIAT in terms of morphology, yield and quality parameters. From this work we expect to derive a core collection based on agronomic and morphological parameters, origin information, (using GIS tools), and characterization with molecular markers, will be identified for more detailed regional evaluation.

Materials and Methods

A total of 137 accessions of *Desmodium velutinum*, mostly originating from Asia and to a lesser extent from Africa, were planted at Quilichao (Photo 18). Plants were sown in jiffy pots and transplanted 6 weeks later into the field in single-row plots, with 4 replications. Dry matter yield, drought tolerance and forage quality are the main parameters being measured.

Photo 18. Regrowth at 8 weeks of *Desmodium velutinum* at Quilichao

Results and Discussion

Five months after transplanting plants were well established, had a good vigor and were free of pests and diseases. Accessions were classified into three groups according to their growth habit: e = erect (54 accessions), se = semi-erect (66), r = prostrate (17 accessions).

DM yields, averaged over two cuts each in the wet and dry periods are presented in Tables 41, 42 and 43 for the 3 groups of *D. velutinum* that were formed based on growth habit. Each growth type was analyzed separately as these are likely to occupy different niches. Significant (P< 0.01) differences among accessions were recorded in each group.

In general, results indicate that for the group classified as erect, DM yields were slightly higher in the wet than in the dry season, with accessions CIAT 33443, 13953, 23985, 23994 and 33352 producing more than 200g DM/plant for an 8 week regrowth, in both seasons (Table 41). A higher number of regrowing points was recorded in the dry season, indicating that *D. velutinum* does not only survive dry periods but that it remains productive (Table 41).

Among the semi-erect groups DM yield differences among accessions were significant (P< 0.05), with slightly higher yields in the wet than the dry season. In this group only 2 accessions, CIAT 13218 and 23983 had DM yields above 200 g DM/plant (Table 42).

As observed in the erect group, there were more regrowing points in the dry than in the wet season. Quality of the semi-erect types was similar to the erect types, with a range of 59 to 76% and 17 to 25 % IVDMD and CP, respectively. A larger number of accessions in this group had digestibilities above 70%, with accessions CIAT 23992, 23923, 23922, 33387, 23986, 23995 and 23975 having values above 73% though at a low yield level.

In the prostrate group significant (P<0.01) yield differences among accessions were measured (Table 43). However yields were lower than for the other groups, with only CIAT 13212 having DM yields above 200g/plant (Table 43). As digestibilities were also relatively low, this group is probably of the least agronomic interest.

Forage quality parameters of some promising *Desmodiun velutinun* accessions in the Erect Group are presented in Table 44. The IVDMD and CP content ranged between 59% and 75%, and 19% and 26%, respectively.

Accessions CIAT 23988, 23079, 23272, 33138 and 13948 had digestibilities above 70%. Highest stability for digestibility and yield across seasons was measured for accessions CIAT 33443, 23985, 33352, 23994 and 33138 (Table 44).
The level of CP was high in all accessions evaluated.

Table 41. Erect Group: Agronomic evaluation of a collection of *Desmodium velutinum* in Quilichao. Data of four evaluation cuts (two in the dry season and two in the wet season). Grey underlaid: Accessions with digestibility > 67 % and dry matter yield >200 g/plant.

Accession No. CIAT	Height	Diameter	Regrowing points	Mean DM yields	Height	Diameter	Regrowing points	Mean DM yields
	Wet				Dry			
	(cm)		(No.)	(g/pl)	(cm)		(No.)	(g/pl)
33443	102	178	54	300	85	163	73	237
23985	109	151	41	230	105	143	76	215
33352	97	143	51	195	88	132	78	207
13953	99	152	61	301	88	140	60	204
23994	92	152	65	201	84	144	64	204
33138	108	134	48	185	106	107	69	194
23081	105	142	68	244	84	130	74	177
23086	90	143	62	184	78	136	72	170
33247	89	153	51	177	83	131	74	170
23136	112	143	43	151	103	123	52	158
23079	104	127	45	172	95	117	57	155
23989	112	134	37	165	101	115	46	153
D2430	80	133	53	145	81	129	65	141
23083	93	124	53	136	85	118	78	140
23132	98	131	38	129	90	107	56	134
23988	95	157	50	168	80	138	71	133
D3456	93	138	33	145	80	134	71	126
13947	108	119	42	159	93	112	59	125
13391	93	124	48	131	80	111	62	120
23133	118	131	35	148	98	108	47	116
13948	90	120	35	119	85	115	56	113
D81995	85	141	44	185	73	119	57	105
13222	90	119	37	132	78	120	56	105
23929	92	122	40	113	79	112	53	103
33250	94	125	43	121	81	101	62	103
23135	102	129	34	109	82	100	56	101
23930	77	135	42	92	73	133	56	100
14314	102	121	42	138	83	96	52	98
33254	85	134	36	117	74	112	53	93
23084	82	112	42	85	79	110	61	92
23158	89	135	39	91	84	132	53	89
23667	94	122	36	86	87	103	42	81
23272	80	128	48	82	78	124	53	79
23987	93	125	48	100	81	112	65	77
13945	105	102	29	102	94	97	47	74
23320	82	125	45	75	86	113	48	69
23669	86	103	41	67	83	101	49	69
23157	103	114	37	102	94	100	42	68
13954	88	121	34	104	70	111	46	68
23160	85	126	36	84	78	119	48	68
23324	85	130	43	70	85	120	50	65
D6	75	132	33	95	71	87	33	64
23322	77	132	43	64	78	119	62	64
23274	76	124	48	91	71	111	52	59
23326	89	123	46	66	80	107	48	55
23248	74	133	48	81	67	107	63	53
13221	95	119	32	57	84	106	32	52
23134	107	103	25	71	85	75	30	49
23321	79	112	33	41	79	114	46	46
23319	63	103	37	51	55	95	46	37
33255	74	83	17	32	70	68	23	33
D7NAPRI	67	110	24	77	56	86	24	30
23323	68	100	26	27	73	104	34	20
23082	71	118	34	44	67	92	42	19
Mean	91	128	42	123	82	114	55	105
LSD(P< 0.05)				112.099				124.81

Table 42. Semi-Erect Group: Agronomic evaluation of a collection of *Desmodium velutinum* in Quilichao. Data of four evaluation cuts (two in the dry season and two in the wet season). Grey underlaid: Accessions with digestibility >66 % and dry matter yield >190 g/plant.

Accession No. CIAT	Height	Diameter	Regrowing point	Mean DM yields	Height	Diameter	Regrowing points	Mean DM yields
	Wet				Dry			
	(cm)		(No.)	(g/pl)	(cm)		(No.)	(g/pl)
13218	78	163	61	218	73	148	61	225
23983	81	179	37	223	71	169	76	200
23982	90	166	51	225	79	148	72	198
23981	86	173	59	281	77	155	69	194
23276	70	171	51	224	60	172	81	194
33463	79	164	63	241	74	149	73	193
33003	98	162	53	209	87	152	79	177
33396	70	179	52	207	62	146	65	177
23928	87	173	55	217	76	159	70	171
23996	90	173	51	325	72	153	71	169
33459	88	154	59	172	88	144	65	168
33451	69	177	41	230	64	167	70	164
23920	69	144	52	210	63	151	78	161
33428	82	155	48	184	79	151	81	159
13216	75	162	32	185	73	150	81	159
23991	68	166	46	181	62	155	71	155
23923	82	156	54	176	79	140	55	154
23977	82	169	45	195	77	156	68	151
23325	86	160	61	228	74	135	62	146
13691	84	165	54	227	73	139	68	146
23980	88	174	59	197	74	147	73	142
23922	76	150	45	133	66	138	69	129
13220	100	148	41	176	69	123	58	127
23279	96	164	59	181	97	160	63	123
33242	84	142	41	123	73	126	60	122
23993	68	163	51	154	55	151	68	121
13219	67	161	51	141	59	144	48	115
23973	56	154	41	153	59	144	62	112
23927	86	136	42	122	80	129	60	111
23275	73	138	48	176	64	120	61	110
23921	76	159	56	224	67	148	52	106
13417	78	150	38	116	74	142	48	105
13952	78	134	51	141	75	129	37	105
33356	79	146	47	125	65	139	63	103
23975	86	145	52	179	67	117	74	103
13692	53	158	54	154	56	130	55	102
23986	77	153	62	160	63	129	73	99
13227	78	152	44	121	66	133	63	98
33249	71	145	44	115	58	111	52	90
23979	87	162	53	145	78	128	67	88
23278	92	161	53	116	83	142	56	87
23080	69	149	40	122	68	137	55	86
23668	82	131	38	87	65	123	58	84
13526	69	149	34	126	66	133	46	83
23974	63	143	50	142	54	124	61	82
23995	68	151	46	96	62	133	64	79
13676	94	146	51	95	77	128	62	76
23282	73	125	47	76	72	119	59	72
33401	77	142	46	112	68	115	52	72
13204	64	148	52	179	56	118	74	68
33464	80	130	40	100	71	116	50	67
23271	80	141	48	80	70	117	48	66
23277	90	135	44	91	75	111	58	64
23280	59	95	26	48	48	87	35	59
23992	75	140	45	104	61	110	59	58
33387	71	149	52	89	58	116	53	57
23327	74	129	41	88	70	117	59	56
23976	75	143	46	92	52	120	48	54
13690	73	137	54	125	58	120	69	49
23915	61	113	50	71	54	97	53	44
23273	74	113	37	59	53	88	46	41
23926	61	111	49	70	56	113	60	41
23925	69	111	41	69	53	106	45	37
13207	64	107	33	46	56	94	44	29
23924	49	83	33	30	47	84	44	19
23281	51	76	29	14	47	73	35	15
Mean	76	147	47	147	67	131	61	109
LSD(P<0.05)				113.1				110.6

Table 43. Prostrate Group: Agronomic evaluation of a collection of *Desmodium velutinum* in Quilichao. Data of four evaluation cuts (two in the dry season and two in the wet season).

Accession No. CIAT	Height	Diameter	Regrowing points	Mean DM yields	Height	Diameter	Regrowing points	Mean DM yields
	Wet				Dry			
	(cm)		(No.)	(g/pl)	(cm)		(No.)	(g/pl)
33520	44	171	41	179	42	154	59	143
13694	50	162	49	124	56	156	68	132
33481	42	174	45	158	45	154	59	121
13213	51	152	45	142	58	134	61	118
13215	53	167	50	148	48	151	68	94
33484	48	161	42	119	47	152	62	92
13693	41	160	51	127	38	142	58	92
13697	36	173	52	199	52	150	64	92
13212	52	163	54	206	45	141	55	91
33471	61	169	56	164	57	151	59	91
23990	56	168	43	126	50	158	54	82
13214	28	152	48	110	32	141	58	77
13695	46	162	50	145	37	151	53	75
13687	38	147	37	115	41	132	49	74
13688	53	139	42	81	34	132	52	70
13211	44	155	39	82	42	129	51	68
13217	37	147	48	171	38	144	45	58
Mean	46	160	47	141	45	145	57	92
LSD(P<0.05)				92.3				64.5

Table 44. Forage quality of selected accessions (erect group) of *Desmodium velutinum* evaluated in Quilichao, 2003-2004.

Accession	IVDMD	CP	NDF	ADF
			%	
23988	73.2	22.5	29.8	21.4
33451	70.6	22.7	30.8	21.6
23986	70.1	24.2	32.2	20.6
23994	69.7	22.0	32.8	23.9
23982	69.7	23.3	31.5	21.9
23981	69.2	21.7	34.4	23.8
23985	68.6	21.8	33.0	23.1
33463	68.3	23.4	36.0	25.8
13953	68.0	19.9	31.5	23.3
23921	67.9	21.9	33.8	24.0
23996	66.8	20.9	38.7	26.5
33443	66.8	20.5	37.1	28.2
23325	66.5	22.9	32.9	23.9
13218	66.1	20.9	36.3	25.1
23275	66.1	21.9	33.2	23.2
13691	65.8	21.6	35.4	25.2
23928	65.8	21.7	34.6	24.8
23081	64.9	20.9	36.5	26.6
23086	64.1	20.7	38.2	26.8
13952	63.8	19.3	35.2	25.6
Mean	67.8	21.6	34.2	24.3

3.5.3 Evaluation of a core collection of *Canavalia brasiliensis* for multipurpose uses in Santander de Quilichao, Colombia, 2004

Contributors: M. Peters, R. Schultze-Kraft (University of Hohenheim), Luis H. Franco, B. Hincapié, and G. Ramírez (CIAT)

Rationale

Canavalia brasiliensis Mart. ex Benth. ("Brazilian jackbean") is a weakly perennial, prostrate to twining herbaceous legume with a wide natural distribution in the New World tropics and subtropics. In comparison with *C. ensiformis* ("jackbean"), research reports on *C. brasiliensis* are scattered and restricted to studies done in Latin America. The species develops a dense and extensive, deep-reaching root system and subsequently tolerates a 5 month dry period. Based on studies that generally were done with only one genotype, it is adapted to a wide range of soils, including very acid, low-fertility soils. Its main use is as green manure, for fallow improvement and erosion control. Due to medium biomass decomposition, nutrient release of *C. brasiliensis* green manure has the potential to synchronize well with the nutrient demand of the succeeding crop and leads to high N recovery rates.

In Central America, the legume is being used to improve the value of stubble grazing in the dry season. Antinutritive substances such as toxic amino acids (e.g., canavanin), lectins (e.g., concanavalin Br) and trypsin inhibitors have been reported in seeds of *C. brasiliensis*. However, there is little information on the nutritive value of the herbage of this species for ruminants.

Materials and Methods

A total of 53 accessions of *Canavalia brasiliensis*, mostly from Latin America, were sown into jiffy pots and transplanted 4 weeks later to the field at CIAT's research station in Santander de Quilichao. A total of 6 plants per plot were sown, in a Randomized Complete Block Design with 4 replications. DM yield, drought tolerance and forage quality parameters are measured (Photo 19).

Photo 19. *Canavalia brasiliensis* sown at Quilichao, 2004

Results and Discussion

Plants established quickly, and incidence of pest and diseases was low, in particular when compared to a collection of *Canavalia* sp. planted at the same time in Quilichao. 12 weeks alter transplanting the majority of accessions had soil covers above 70%, with accessions CIAT 808, 18515, 7319, 7648, 7970, 8557, 2009517008,17009 and 20096 showing the highest values (Table 45).

Forage yields 16 weeks after transplanting varied significantly (P< 0.01) among accessions, with yields above 4 t DM /ha recorded for accessions CIAT 808, 17009, 8557, 17012, 20098, 21824, 18515, 20303,17973, 7178, 20306, 7648 and 7319.

Table 45. Vigor, soil cover (%) and dry matter yields of *Canavalia brasiliensis* in Quilichao, 2004.

Accession	6 Weeks		12 Weeks		16 Weeks		
	Vigor	Soil cover	Vigor	Soil cover	Vigor	Soil cover	Mean dry matter yields
	1-5	%	1-5	%	1-5	%	kg/ha
808	4	50	5	92	5	100	6320
17009	4	43	4	85	5	100	5333
8557	4	47	5	87	5	97	4693
17012	3	35	3	78	4	90	4560
21824	3	40	3	73	3	78	4440
20098	3	33	3	75	4	80	4440
18515	3	45	4	88	5	100	4333
20303	3	35	3	77	4	80	4333
17973	3	32	3	68	4	75	4293
7178	4	40	3	73	4	82	4200
20306	3	30	3	72	4	85	4187
7648	4	52	4	87	5	100	4107
7319	4	52	4	87	4	90	4067
20095	3	47	4	87	4	93	3907
7969	3	35	4	80	5	93	3880
22132	3	40	3	73	4	77	3840
7321	3	35	3	78	4	87	3787
7973	3	40	4	77	4	80	3760
18501	3	38	3	70	4	75	3720
17008	4	42	4	85	4	80	3680
8770	3	38	3	75	5	100	3680
19034	3	43	4	83	4	85	3653
17462	3	40	4	80	5	97	3627
905	3	43	3	63	3	67	3613
7647	3	37	4	77	4	87	3507
7971	3	30	3	72	4	82	3400
7175	2	27	3	63	3	72	3373
19029	4	43	4	83	4	83	3320
20518	3	42	4	83	4	80	3147
20096	3	37	4	85	4	83	3120
20516	3	35	3	73	4	83	3053
21825	3	33	3	73	4	82	3040
17010	3	35	3	70	4	80	2933
19035	3	35	3	72	3	63	2893
20514	4	52	3	83	3	80	2840
19361	4	43	3	78	4	88	2813
17011	3	40	3	80	3	85	2813
7972	3	32	4	80	4	90	2787
20513	4	50	4	75	3	82	2773
20301	3	27	3	78	3	83	2720
8768	3	32	3	63	3	72	2653
7970	3	43	4	87	4	90	2640
20295	3	35	3	77	3	77	2627
19359	3	37	3	73	3	73	2600
9146	3	37	3	75	3	80	2560
20304	2	28	3	63	3	75	2560
19632	3	27	3	65	3	67	2440
20296	2	23	3	60	2	67	2360
20090	2	22	3	70	3	70	2333
5033	3	38	3	70	3	80	2227
7174	3	32	3	67	3	70	2227
17828	3	28	4	68	3	63	2160
7894	3	28	2	55	3	72	1813
Mean	3.1	37	3.4	76	3.8	82	3400
Range	2-5	20-70	2-5	30-100	1-5	30-100	1000-8600
LSD (P< 0.05)							2855.21

Accession CIAT 17009 is a line previously selected by farmers in Central America for use as green manure and to improve fallows and crop residues, due to its high yield and drought tolerance. Despite a severe dry period all accessions remained healthy, with soil covers above 80%; in a few materials some leaf loss under drought conditions was observed.

3.5.4 Evaluation of a core collection of *Canavalia* sp. for multipurpose uses in Santander de Quilichao, Colombia, 2004

Contributors: M. Peters, Luis H. Franco, R. Schultze-Kraft (University of Hohenheim), B. Hincapié, G. Ramírez, (CIAT)

Rationale

In view of the promising results obtained with *Canavalia brasiliensis*, there is an interest to define the potential of other species of *Canavalia* for use mainly as green manure and for fallow improvement in low fertility, drought prone environments.

Materials and Methods

A total of 47 accessions of *Canavalia* sp, originating from Latin America, China and Thailand were sown into jiffy pots and transplanted to the field in Santander de Quilichao (Photo 20).

The design and variables are the same as described for *C. brasiliensis*. Establishment of most accessions was slow, with soil covers below 53% after 12 weeks of transplanting. On the other hand, 16 weeks after transplanting, only 11 materials had soil covers above 80%. Several materials appear not to be well adapted to the acid soils in Quilichao and were severely affected by pests and diseases.

Accessions with the best adaptation during establishment phase were CIAT 21012, 21014, 19038, 21209, 7317, 7383, 8719, 21013, 21211 and 18587, all of which are showing good drought tolerance (Table 46).

Photo 20. *Canavalia* sp. sown at Quilichao, 2004.

Table 46. Vigor and soil cover (%) of *Canavalia* sp. in Quilichao, 2004.

Number Accession	6 Weeks		12 Weeks		16 Weeks	
	Vigor	Soil cover	Vigor	Soil cover	Vigor	Soil cover
	1-5	%	1-5	%	1-5	%
19038	5	55	5	90	4	100
21209	5	52	5	80	4	97
7317	5	50	4	83	4	93
21012	5	42	4	60	5	92
21014	4	40	4	57	5	90
8719	5	57	5	83	4	87
18271	5	53	4	73	3	87
7383	4	40	4	67	4	87
7318	5	58	4	80	3	83
21013	4	37	4	63	4	83
21211	4	33	4	63	4	82
7322	5	58	4	72	3	78
19033	5	45	4	70	3	78
20803	4	42	4	63	2	75
19031	4	42	4	60	3	73
20307	4	40	4	73	3	73
18587	4	33	4	50	4	73
18272	5	52	4	73	3	72
20145	4	37	3	47	3	72
18580	3	32	3	47	3	72
22031	4	35	3	50	3	70
20691	4	32	3	50	2	70
20748	3	28	4	57	2	70
8769	4	40	4	57	2	68
19032	4	37	4	63	3	68
20305	4	32	3	50	2	68
20298	4	42	4	70	3	67
19357	4	35	4	67	2	67
18270	4	45	4	60	3	65
21210	4	45	4	57	3	65
17929	4	45	4	70	2	63
17451	4	38	4	57	2	63
8771	3	30	3	43	2	63
21212	4	32	3	40	2	60
8185	4	45	3	43	2	57
20113	4	40	3	47	2	57
21487	3	28	3	47	2	57
19052	2	17	3	33	3	57
18268	2	11	2	20	2	37
19356	2	13	2	20	2	33
18258	2	18	2	13	2	28
18261	2	8	2	13	1	28
18263	2	8	2	13	1	25
20300	4	37	3	40	1	23
20093	3	32	2	20	1	10
20297	2	7	2	10	1	10
20299	2	6	2	10	1	5
Mean	3.7	36	3.5	53	2.6	64
Range	1-5	1-70	1-5	10-95	1-5	5-100

3.6 Annual legumes for multipurpose use in different agroecosystems and production systems

Highlights

- Selected an accession (IT95K-52-34) of cowpea with superior grain yield as compared to local checks in acid infertile and fertile soils.

- Showed that cowpea as green manure can substitute the N applied (80 kg) to maize by farmers in hillside of Nicaragua.

- Selected an accession (CPI-67639-early flowering) of *Lablab* in hillsides of Nicaragua based on rapid establishment, high cover and high seed yield.

3.6.1 Evaluation of new Vigna *unguiculata* accessions in Quilichao and Palmira, Colombia

Contributors: M. Peters, Luis H. Franco, B. Hincapié, G. Ramírez, (CIAT), R. Schultze-Kraft (University of Hohenheim) and B.B. Singh (IITA, Nigeria)

Rationale

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

Good adaptation to climatic and edaphic conditions, especially to water stress, are prerequisites for a successful development of cowpea as an option for the traditional maize-bean cropping systems in Central America. It remains to be seen if cultural traditions allow for the inclusion of cowpeas in the daily menu of people in Central America.

Materials and Methods

A new collection of cowpea obtained from IITA was sown in Santander de Quilichao and Palmira in 2004 in order to select accession with both high forage and grain yields and good adaptation to contrasting soils. (Photo 21). Our previous selection criteria for cowpea had been mainly forage yield in alkaline and acid soils. The same evaluation methodology as presented in previous annual reports was utilized. The main variables measured are forage production and quality, grain yield and effect as green manure on a subsequent maize crop. A particular emphasis is given to material adapted to a wide range of soils.

Results and Discussion

The collection established was highly diverse in terms of flowering response, with very early to very late accessions present. The differential flowering pattern will be taken into account for the planning of subsequent trials. In Palmira it was necessary to replant one replication because of negative effects of waterlogging and consumption of seed by birds.

Soil cover was more rapid in Palmira than in Quilichao, with a mean of 80% and 64% covered respectively 10 weeks after planting. Pest and diseases were present in both sites but did not limit the development and productivity of plants. Grain was harvested 12

Photo 21. Cowpea (*Vigna unguiculata*) at Quilichao.

weeks after planting when pods where dry. Mean yields in Quilichao were above 2 t/ha and significant ($P \leq 0.05$) differences among accessions were measured.

The accessions IT97K-1069-6, IT95K-52-34 and IT98K-412-8, had yields above 3 t/ha. Mean grain yields in Palmira were double (4 t/ha) than those obtained in Quilichao. Differences among accessions were significant ($P \leq 0.01$) and the highest grains yields with more than 5 t/ha were achieved with local checks (CIDICCO3, CIDICCO4), and with the new accession, CIAT 9611, IT95K-52-34 (Table 47).

In terms of forage quality, significant ($P \leq 0.01$) differences among accessions were measured for IVDMD, P and K, but not for CP. In vitro dry matter digestibility was above 83% while CP contents ranged between 19.7% and 24.2 %, confirming the high quality of the forage from cowpea (Table 48).

Table 47. Vigor, soil cover (%) and yield (kg/ha) of *Vigna unguiculata* grain in Quilichao and Palmira, 2004.

Accession	Quilichao (acid infertile soils)			Palmira (fertile soils)		
	Vigor	Cover (%)	Grain (kg/ha)	Vigor	Cover (%)	Grain (kg/ha)
	1 - 5	10 weeks	12 weeks	1 - 5	10 weeks	12 weeks
IT97K-1069-6	5	85	3327	4	87	4360
IT95K-52-34	5	92	3313	5	100	5513
IT98K-412-8	4	72	3180	4	83	4900
IT98K-131-2	4	67	2873	3	77	4873
IT97K-819-118	4	68	2867	3	73	2460
IT98K-406-2	4	72	2607	3	77	3847
CIDICCO 3 (local check)	5	82	2560	5	100	6013
IT97K-1069-2	4	73	2533	4	90	4087
IT97K-818-35	4	72	2460	3	77	3020
CIDICCO 2 (local check)	5	85	2420	5	93	4653
IT99K-7-14	3	63	2360	3	63	3267
IT99K-409-8	4	77	2347	4	87	3440
IT99K-429-2	3	53	2327	3	67	3120
IT99K-1060	2	47	2280	3	62	3760
IT97K-825-3	4	75	2267	3	67	2853
IT98K-476-8	4	73	2260	3	73	3553
CIAT 9611 (local check)	4	60	2167	5	93	5433
IT98K-391-2	4	72	2153	5	93	4553
IT98D-1399	3	53	2087	3	60	3440
IT98K-412-13	4	67	1993	3	73	4533
IT96D-610	4	65	1987	4	83	4327
CIDICCO 1 (local check)	4	75	1980	5	93	4613
IT98K-428-3	4	70	1953	4	77	3333
CIDICCO 4 (local check)	5	70	1927	5	100	5533
IT97K-570-18	4	77	1820	5	93	5533
IT98K-506-1	3	55	1733	3	77	3913
IT97K-356-1	3	20	1673	3	70	3913
IT98K-205-8	3	53	1660	3	67	3800
IT97K-499-38	4	60	1627	4	78	2980
IT98K-390-2	4	63	1533	5	100	4233
IT99K-7-21-2-2	2	37	1520	3	67	2833
IT89KD-288[1]	2	43	1413	3	77	3480
IT97K-494-3	2	47	1380	3	70	2527
IT99K-216-24-2	2	53	1367	3	53	4153
IT99K-1122	2	50	1327	4	90	4233
IT97K-461-4	3	50	1260	5	87	4293
FHIA (local check)	2	43	1093	4	83	4487
IT98K-311-8-2	4	80	1027	4	90	3880
Mean	3.8	64	2070	3.8	80	4020
MSD ($P \leq 0.05$)			1810.2			3353.4

[1] Accession selected from the first core collection from IITA evaluated in Palmira and Quilichao.

Table 48. Quality of the forage in accessions of *Vigna unguiculata* grown in Quilichao, 2004.

Number Accession	Forage			
	IVDMD	Protein	P	K
		%		
IT98K-131-2	89.8	21	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
CIAT 9611 (local check)	88.8	20.2	0.16	1.44
FHIA (local check)	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 (local check)	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 (local check)	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 (local check)	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 (local check)	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 ($P \leq 0.01$)	5.74 ($P \leq 0.07$)	0.08 ($P \leq 0.01$)	0.71 ($P \leq 0.01$)

3.6.2 Evaluation of *Vigna unguiculata* germplasm in Nicaragua

Collaborators: A. Schmidt, C. Davies, E. López, M. Peters, L.H. Franco, and G. Ramirez (CIAT)

Rationale

We visualize that cowpea could be an alternative crop for a) the first planting season ("primera") as a soil improving starter crop for maize, and b) for the second planting season ("postrera") in low fertility soils in hillsides of Nicaragua and Honduras. This legume could allow for a third crop in November/December the maize/bean systems in order to provide grain, hay for animal 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.

Materials and Methods

During 2002 a small core collection of *Vigna unguiculata* from IITA comprising 14 accessions, which were selected in Quilichao and Palmira for good adaptation to soils (acid and alkaline) was introduced into Nicaragua. In October 2002 the core collection, complemented with 5 Central American accessions, was planted out in small plots at the SOL seco site in San Dionisio, Matagalpa, Nicaragua (Lat N 12° 45' 05.8", Long. W 85° 53' 16.5", Alt. 537 masl, rainfall 990 mm/a, mean temp. 26°C). Standard evaluation procedures were applied as in previous years. At flowering 50% of each plot was cut and biomass kept on plot surface as mulch; from the remaining 50% grain was harvested in December 2002. Crop residues were equally kept on plot surface for the rest of the dry season. The respective results were reported in AR 2003, p.116). Upon the outset of the rainy season 2003 a maize crop was established in each plot. Total dry matter and grain production were recorded in November 2003 in order to detect possible residual fertility effects from the preceding *Vigna unguiculata* accessions. Additional plots were established where 45, 80, 140, 200 kg/ha N, respectively, were applied. A non-fertilized (N 0) treatment was also included.

Early 2003, due to increasing interest in *Vigna unguiculata* by farmers in San Dionisio (who demanded the re-establishment of our collection for further participatory evaluations and for seed increase for on-farm evaluation) the accessions were re-established in 6m x 6m plots without replicates. After 2 field events with participatory evaluations (n=21 persons) plots were harvested and seed distributed to 35 farmers.

Results and Discussion

In Table 49 maize plant height, DM and grain yields are presented. No significant differences (P>0.05) were detected among accessions or between treatments (mulch vs. grain harvested). Mean plant height, DM and grain yield are in-line with farmer maize crops in San Dionisio. Farmers, depending on their economic possibilities, apply up to 80 kg/ha N to their maize crops. Results of our experiment showed that a cowpea crop can easily replace the application of these amounts of nitrogen, even as a preceding dry season crop. This corroborates our findings from on-farm experiments, reported in AR 2003 (p. 145), where we argued that with a legume crop planted at the end of the rainy season, traditional nitrogen fertilizer applications for the following maize crop can be substituted in the dry hillsides of Nicaragua and Honduras.

Our results this year with cowpea indicate that farmers can not only reduce production cost of maize, but also have an additional legume grain harvest for human consumption or animal feeding. Since no significant differences between plots with mulched or grain-harvested cowpeas were found, farmers can choose their cowpea accession based on their utilization preference.

In Table 50 we present the results of participatory evaluations of the cowpea collection in Nicaragua. Selection criteria employed by farmers were: grain yield, leafiness and leaf size, plant vigor, pod size, and plant height. Of the accessions selected, the local accessions Rojo, INTA and Negro are leafy types for animal feeding, while the introduced IT90K-284/2 accession is a dual purpose type as shown before (see AR 2003, p. 117). This accession is widely adapted across different environments in Colombia and Central America.

Seed production began in the first planting season 2004 with the production of 50 kg of accession Rojo and IT90K-284/2. In the second planting season seed production efforts will be increased. Evaluation of new accessions, both local and introduced materials, will continue throughout

2004-2005. With regard to soil fertility effects resulting from cowpea as a green manure, we plan to intensify our collaborative work with the TSBF/Soils Group in Central America (see also 4.1.7 in this report).

Table 49. Plant height, DM and grain yields of maize planted after *Vigna unguiculata* (cowpea) at San Dionisio, Nicaragua, 2003.

Accession	Maize yields - Cowpea mulch			Maize yields - Cowpea grain harvested		
	Plant height (cm)	DM total (kg/ha)	Grain yield (kg/ha)	Plant height (cm)	DM total (kg/ha)	Grain yield (kg/ha)
IT95K-1088/2	194	7999	3385	198	6512	3255
IT95K-1088/4	191	8279	3149	184	6408	2041
IT90K-277/2	198	8278	3284	189	6873	2785
IT90K-284/2	188	5763	2844	172	4967	2213
IT89KD-288	202	10206	3528	190	8930	4209
IT89KD-391	200	6858	2699	201	8448	4048
IT93K-503/1	181	6408	3224	176	7910	3448
IT93K-573/5	191	6185	2792	199	7611	3006
IT93K-637/1	194	7029	3050	209	9095	4516
IT86D-715	204	7937	3416	191	5643	3008
IT86D-716	198	6772	2015	200	7534	3294
IT86D-719	201	6537	4014	192	7735	3422
IT6D-733	187	5607	2824	197	8367	3804
IT96D-740	197	6188	2683	193	7628	3094
Café	212	8163	4082	208	8399	3671
INTA	199	6955	2873	196	9115	3253
Negro	209	8129	3845	185	8124	3905
Rojo	190	8667	3432	210	8688	3770
SF Libre	201	6461	2777	189	7015	2886
Mean Acc.	197	7285	3153	194	7632	3349
LSD ($P \leq 0.05$)	46.9	6873.9	3044.9	42.1	4619.7	2349
Nitrogen treatments						
N 0	180	4324	2239			
N 45	179	5104	2621			
N 80	177	6668	3085			
N 140	164	7169	4060			
N 200	184	7612	4368			

Table 50. Farmers ranking of *Vigna unguiculata* accessions and their potential uses expressed by farmers in San Dionisio, Nicaragua, 2003.

Framers ranking		Potential use expressed by farmers	
Good	Regular	Animal feed	Grain production
Rojo	IT86D-715	Rojo	Rojo
Café	IT86D-719	INTA	IT90K-284/2
IT96D-740	IT93K-637/1	Negro	
IT89KD-391	IT6D-733		
INTA	IT95K-1088/2		
Negro	IT93K-573/5		
IT90K-284/2			

3.6.3 Evaluation of *Lablab purpureus* germplasm in Nicaragua

Contributors: A. Schmidt, C. Davies, E. Lopez, M. Peters, L.H. Franco, G. Ramirez (CIAT)

Rationale

A major problem facing livestock producers in Central America is inadequate animal nutrition during the dry season when pastures, sorghum and maize stover are limiting in quality. Problems such as sickness and weight loss due to a poor nutrition are frequent. One way for improving the utilisation of such crop residues is by adequate supplementation with leguminous forages of high quality.

The legume *Lablab purpureus* is recognized not only as drought resistant, but also for its adaptability to a wide range of environmental conditions. Though the legume is widely known in Central America under a number of names (e.g. dolichos, caballero) and has the capability of being an outstanding resource for crop-livestock systems in this region, (e.g. the legume can be used as cover crop, grazed in a pasture setting or as a companion crop to maize, cut as hay, or mixed with corn silage), it is not being used to its full potential. So far only two commercial lines have been available to farmers in Nicaragua, but seed availability remains a major limitation. Thus to select for more productive and better-adapted germplasm for the dry hillside regions of Nicaragua, a *Lablab purpureus* core collection from ILRI/CSIRO is currently under evaluation.

Material and Methods

During 2003 a core collection from ILRI/CSIRO comprising 12 accessions was introduced into Nicaragua. In October 2003, accessions were planted out in 2m x 2.5 m small plots in three replicates in a randomized complete block design at the SOL seco site in San Dionisio, Matagalpa, Nicaragua (Lat N 12° 45' 05.8", Long. W 85° 53' 16.5", Alt. 537 masl, rainfall 990 mm/a, mean temperature 26°C). After an initial evaluation of plant emergence, accessions were evaluated in a two-weeks interval for plant height, soil cover, plant vigour, flowering patterns, incidence of pests and diseases, and seed production. No fertilizer was applied throughout the experiment. Dry matter yields prior to flowering were not recorded due to the small initial amount of seed introduced. Priority was given to genotype characterization and seed increase.

Result and Discussion

Accessions of the core collection established well at the experimental site with an average of 79% of all seeds emerging (Table 51). Plants reached an average height of 49 cm and covered 12 weeks after planting on average 74% of the plots. Plants showed good vigour and no incidence of pests or diseases throughout the experiment, with the exception of accession CPI-36903.

Accession CPI-67639 (early flowering) performed outstanding in this experiment in terms of establishment, soil cover and seed yield. It remains to be seen if this seed high yield is correlated with low biomass production. Biomass production and on soil fertility effects on subsequent maize crops will be obtained on larger plots in the *postrera* 2004 and primera season 2005. Enough seeds was harvested in the present experiment to include an additional experimental site in 2004-2005, which will be under the responsibility of our national partner INTA.

We conclude from this first experiment with *Lablab purpureus* that the accessions evaluated adapted well to the dry conditions of the central region of Nicaragua where small farmer crop-livestock systems are predominant. Our results showed variability within the collection with regard to seed yield and flowering patterns. Nevertheless, biomass production data and the results from participatory evaluation by farmers will determine which accession will be multiplied for forage production, soil improvement or grain production. The selected *Lablab* accessions will be an additional annual legume alternative to farmers in the drier regions of Central America.

Table 51. Plant emergence, plant height, soil cover, plant vigour, flowering behaviour and seed yield of a *Lablab purpureus* core collection at San Dionisio, Nicaragua, 2003-2004

Accessions	CIAT No.	Plant Emergence (%)	Plant height (cm)	Soil cover (%)	Plant Vigour (1-5)	Flowering (Early/Late)	Seed Yield (g/m2)
CPI-67639	17197	100	41	97	4	E	127
CPI-34777	22598	67	59	62	4	L	113
21603	21603	77	44	70	5	L	92
I-14442	22768	88	50	87	5	L	83
CQ-2975	22735	90	47	83	5	E	82
L-987	22660	97	60	91	5	E	74
CPI-52535	22604	63	42	63	3	L	70
cv. Highworth	22660	85	56	83	4	E	70
I-11632	22764	90	50	72	4	L	43
I-6533	22770	85	51	85	5	L	37
CPI-36903	22653	33	32	30	3	L	34
CPI-106471	22663	77	56	62	4	E	28
Mean		79	49	74	4.3		71
LSD ($P \leq 0.05$)		78.8	43.5	72.4			81.9

3.6.4 Effect of *Lablab purpureus* accessions as a green manure in Quilichao and Palmira

Contributors: M. Peters, L. H. Franco, B. Hincapié, and G. Ramírez (CIAT)

Rationale

Lablab purpureus is a free seeding, fast growing, short-term perennial legume, with widespread use through the tropics as a fodder plant. In Africa the use of Lablab for human consumption is also common. The origin of the Lablab germplasm currently utilized is mainly Eastern/Southern Africa and Asia. In addition, it is well documented that *Lablab purpureus* is best adapted to areas with rainfall regimes of 750–2000 mm/year. This species grows in a variety of soils, but the ideal pH for growing Lablab is reported to be between 5.0 and 7.5.

In order to evaluate the potential of Lablab in tropical America, we obtained a collection available at ILRI/CSIRO. 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 the evaluation of the Lablab collection in acid and neutral soils to define niches for this species as green manure and fodder (especially for hay and silage or deferred feed), with emphasis on Central America where soils are highly variable in pH.

Materials and Methods

A multilocational trial to evaluate of *Lablab purpureus* selected from previous work in Colombia was initiated in contrasting sites – soil, climate and altitude – in Colombia (6 sites), Costa Rica and Nicaragua. In this section we report results on effects of Lablab on a succeeding maize crop in Quilichao (acid low fertility soils) and Palmira (alkaline high fertility soils) (Photo 22).

Results and Discussion

Due to the short rainfall cycles in the bimodal rainfall system prevalent in Palmira and Quilichao, it is not possible to plant a crop directly after lablab as a green manure. Hence an alternative strategy was employed, with the lablab green manure being incorporated at the end of the dry season, followed by the crop sown at the beginning of the next wet season. Maize DM and grain yields after incorporating lablab as green manure are presented in Table 52.

Maize yields in Palmira were higher than in

Quilichao. In Palmira there was no positive effect neither of the green manure or N-fertilization, probably due to the inherent high fertility of soils. In contrast, in Quilichao, maize yields without fertilization were only 55% of the yields obtained with 120 kg/ha N. Following incorporation of lablab accessions CIAT 22663 and 21663, maize grain yields were similar to those recorded with the 3 levels of N applied.

Table 52. Effect of *Lablab purpureus* as green manure on biomass and grain yields of a succeeding maize crop (Palmira and Quilichao, 2004).

Accessions CIAT No.	Palmira (fertile soil)		Quilichao (acid infertile soil)	
	Yield (kg/ha)			
	DM Plant	Grain Maize	DM Plant	Grain Maize
22653	5648	6014	4548	4336
22768	3366	5319	4665	4554
22766	4411	5282	4381	4231
22762	4026	5162	4047	3268
22660	4385	4627	4242	3983
17197	4156	4870	4375	4080
22770	3591	4796	4175	3886
22652	5171	4657	3963	3886
N0	5824	4596	3240	2689
22598	4513	4545	5010	4426
22764	3738	4471	4431	3963
N120	5574	4439	4320	4826
21603	4012	4360	4821	4682
22663	5152	4179	5016	4826
22604	3897	4128	4336	4587
N80	4124	3929	4136	4292
N160	4694	3850	3674	4470
22735	2863	3627	4387	3607
N40	4138	3077	4119	3958
Fallow			3064	2906
Mean	4398	4558	4266	4087
LSD ($P \leq 0.05$)		3401		3134

(A)

(B)

Photo 22. (A) *Lablab purpureus* as green manure and **(B)** Maize after Lablab in Palmira.

3.6.5 Forage and green manure potential of a collection of *Mucuna* spp.

Contributors: M. Peters, L. H. Franco, B. Hincapié, and G. Ramírez (CIAT)

Rationale

Mucuna is a legume utilized by farmers in Central America (particularly in the humid tropics) as a green manure and rarely as forage. We were interested in determining the variability among Mucuna accessions when used as green manure.

Materials and Methods

Eight accessions of *Mucuna* sp., obtained from CIEPCA, and previously evaluated for L-Dopa content (see Annual report 2002) were planted in Quilichao (see Photo 23) to be used as a green manure for a succeeding maize crop. A Randomized Complete Block Design with 3 replications was employed.

Results and Discussion

The establishment of Mucuna was rapid, with the exception of *Mucuna* sp cv. Rayada-61. Mean soil cover was 90% 12 weeks after sowing. At 16 weeks soil cover declined to 81% due to some leaf loss. However, soil cover of *Mucuna*. sp cv. Preta-82 and *M. pruriens* CIAT 9349 remained stable with 100% soil cover even under drought conditions.

Biomass yields were above 5.8 t/ha both at 12 and 16 weeks, with *M. pruriens* cv. Jaspeada-106, *M. pruriens* CIAT 9349. These accessions had significantly (Pd≤0.01) higher DM yields after 16 weeks, of growth that other accessions (Table 53).

However, highest maize grain yields (6.1 t/ha) were achieved after incorporation of *M. pruriens* cv. Utilis-109. Interestingly the lower biomass yielding accession (cv. Rayada-61) had a better effect as green manure as compared to the higher biomass accession (cv. Jaspeada –106), probably related to immobilization of nutrients in the soil.

Digestibilities of Mucuna accessions under evaluation ranged between 52% and 62%, and digestibility values were below the values obtained for lablab included as a control. The CP contents ranged between17% and 21% (Table 54).

Table 53. Soil cover (%) and biomass yield (kg/ha) of *Mucuna* sp and maize grain yield in Quilichao, 2004.

Accession	Biomass Mucuna				Maize	
	Soil cover (%)		DM yield (kg/ha)		DM yield (kg/ha)	
	12 Weeks	16 Weeks	12 Weeks	16 Weeks	Plant	Grain
M. pruriens cv. Cochinchinensis	100	85	7987	5720	11768	5773
M. pruriens cv. Jaspeada-106	100	80	6940	10033	11206	5099
M. sp cv. Ghana-4	70	32	6433	3020	9413	3991
M. sp cv. Rayada-61	53	17	6213	1687	12291	5728
M. pruriens CIAT 9349	100	100	5427	6293	11723	5160
M. pruriens cv. IRZ-99	100	85	5347	7867	11367	5422
L. purpureus CIAT I-14442 (control))	77	68	5053	5507	11846	5544
M. pruriens cv. Utilis-109	100	97	4420	6580	12748	6140
M. sp cv. Preta-82	100	100	4400	6007	10449	4787
Fallow				5720	8676	4259
Mean	90	81	5802	5857	11234	5222
LSD (P≤0.05)			3295.41	4788.27	4657.72	2355.44

Photo 23. *Mucuna* sp. at Quilichao

Table 54. Forage quality of a collection of *Mucuna* sp evaluated in Quilichao, 2003-2004.

Accession	PC %	DIVMS %
L. purpureus (control)	18.1	72.7
M. pruriens cv. Cochinchinensis	20.2	61.6
M. sp cv. Ghana-4	15.1	60.4
M. pruriens cv. IRZ-99	20.8	58.1
M. pruriens cv. Jaspeada-106	16.9	57.9
M. pruriens CIAT 9349	21.8	57.5
M. sp cv. Preta-82	20.0	57.2
M. pruriens cv. Utilis-109	21.4	55.3
M. sp cv. Rayada-61	17.0	51.9
Mean	19.0	59.2
LSD (P≤0.05)	6.1	6.6

3.6.6 Evaluation of legumes as covers for plantations in the Llanos of Colombia

Contributors: C. Plazas, M. Peters, L.H. Franco, B. Hincapie (CIAT) and Oil Palm and Rubber Growers of the Colombian Llanos

Rationale

In plantations of the Llanos of Colombia there is a need to find sustainable ways to reduce weed infestation, to maintain and improve soil fertility, to control erosion and increase soil fauna biomass. There is currently a trend to promote plantation systems in the Llanos. In the rubber plantation the target group for this promotion are small to medium size farmers who want to diversify there farming operations. In the oil palm plantations plots of up to 5 ha are rented out to landless farmers to manage the oil palms for the oil palm industry.

In 1999 a range of legume species (*Arachis pintoi, Desmodium heterocarpon* subsp. *ovalifolium* and *Pueraria phaseoloides)* were sown under shade and no-shade conditions in the Meta department of Colombia.

Materials and Methods

In plots of 80 m^2 we established legumes covers in a commercial rubber (young and old) and oil palm plantations in the savannas and Piedmont areas of the Llanos. The following legumes were sown in a Randomized Block Design with three replications: *Arachis pintoi*: 17434, 18744, 18748, 22159, 22160 (seed rate 10 kg/ha); *Desmodium heterocarpon* subsp. *ovalifolium* (*D. ovalifolium)*: 350, 13105, 13110, 13651, 23762 (0.5 kg/ha); *Pueraria phaseoloides*: 8042, 9900 (3 kg/ha). Additionally a mixture of *Arachis pintoi* CIAT 18744 and *Desmodium ovalifolium* CIAT 13651 was sown.

These plots have been monitored through visual observation in regular intervals, to assess long-term persistence of legumes sown as plantation covers.

Results and Discussion

The legume covers were evaluated at the beginning of the wet season of 2004. Now 5 years after planting, several accessions sown under high shade conditions in established plantations disappeared. However, legume covers established in young plantations with moderate shade continue to be vigorous and cover the soil well. Several *A. pintoi* accessions have covers above 70%, while *D. heterocarpon* cv. Maquenque (CIAT 13651) covered 67% of the soil and *D. heterocarpon* (CIAT 23672) covered 80% of the soil. The control *P. phaseoloides* almost disappeared under low shade (Table 55).

Results confirm the utility of *D. heterocarpon* subsp. *ovalifolium* and *A. pintoi* as plantation covers, provided moderate light in available. These legume when used as covers should be established in the early phase of the plantation and/or utilized to cover more open spaces between rows of trees, where weeds are a major problem.

Table 55. Soil cover of different forage legumes under rubber 5 years after sowing, under high shade and low shade conditions, Llanos of Colombia.

Accession	High shade		Low shade	
CIAT No.	Soil cover (%)	Vigor	Soil cover (%)	Vigor
A. pintoi 17434	17	3	73	4
18744	5	3	73	4
18748	12	3	60	3
22159	5	2	72	4
22160	10	2	73	3
D. heterocarpon 350	0	0	57	3
13105	0	0	52	3
13110	2	1	52	3
13651 (cv. Maquenque)	2	1	67	3
23762	0	0	80	3
P. phaseoloides 8042 (control)	0	0	12	2
9900	2	1	2	1
Asoc. *A. pintoi – D. heterocarpon*	5	2	45	3