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

Activity 3.1 Genotypes of *Brachiaria, Panicum,* and *Arachis* with adaptation to edaphic and climatic factors

Highlights

- Found that two sexual *Brachiaria* hybrids (SX 2349 and SX 497) had greater level of Al resistance than sexual parent (BRUZ/44-02).
- Showed that the superior performance of the *Brachiaria* hybrid (FM9503-S046-024) after establishment was associated with its ability to acquire greater amounts of nutrients from low fertility soil.
- Showed that *B. humidicola* suppresses nitrification and nitrous oxide emission by inhibiting the activity of ammonium oxidizing bacteria in the soil.
- Using phosphorus isotope exchange kinetics technique, found that *Brachiaria decumbens* CIAT 606 acquires P from less available P forms in an oxisol.
- Field evaluation over 3 years showed that *Arachis pintoi* CIAT 22159 may be better than the commercial cultivar (CIAT 17434) in terms of persistence with no P fertilizer input.

Progress towards achieving milestones

- **New *Brachiaria* sexual hybrids with Al resistance identified**
  We were successful in identifying 2 sexual hybrids (SX 2349 and SX 497) with greater level of Al resistance than that of the sexual parent, BRUZ/44-02. These sexual hybrids are currently being used in the on-going breeding program to combine spittlebug resistance with high level of Al resistance.

- ***Brachiaria* hybrids with superior performance under low soil fertility identified**
  We selected a *Brachiaria* hybrid, (FM9503-S046-024) for its excellent adaptation to low fertility soils, which was associated with its ability to acquire greater amounts of nutrients from the soil as compared to other hybrids. Using phosphorus isotope exchange kinetics technique in collaboration with scientists from ETH, Zurich Switzerland, we showed that *Brachiaria decumbens* CIAT 606 acquires P from less available P forms from low P oxisol. Further research work is needed to evaluate the ability of most promising *Brachiaria* hybrids for this unique ability to acquire less available P forms from low fertility oxisols.

- **Ability to suppress nitrification and emission of nitrous oxide by *Brachiaria humidicola* quantified**
  Collaborative research with JIRCAS scientists from Japan showed that *B. humidicola* suppresses nitrification and nitrous oxide emission by inhibiting the activity of ammonium oxidizing bacteria in the soil. Further research work is in progress to determine genotypic variation in this ability of *B. humidicola*.

- **Field experiment with promising accessions of *Arachis pintoi* established in the Llanos**
  We identified *Arachis pintoi* CIAT 22159 as a promising accession for targeting to infertile acid soils. This accession along with a few other accessions (CIAT 18744, 18748, 22160) and commercial cultivar (CIAT 17434) are being evaluated in relatively better soils of the Llanos of Colombia (Piedmont region) for their suitability as cover or forage legumes.

Low supply of nutrients and aluminum (Al) toxicity are major limitations to tropical forage production on acid soils. Previous research indicated that tropical grasses and legumes that are adapted to infertile acid soils have root and shoot attributes that are linked to strategies to acquire nutrients in a low pH and high Al environment. Identification of those key plant attributes is fundamental to develop more efficient screening procedures for germplasm evaluation and/or improvement.

3.1.1 Development of improved tetraploid, sexual *Brachiaria* hybrid breeding population for resistance to edaphic factors and general environmental adaptation

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

The first criterion for selection in the tetraploid, sexual *Brachiaria* breeding population is performance in field trials on acid, Al-toxic soils at CIAT-Quilichao and the Matazul farm in Puerto López (Llanos Orientales). Over 4,300 progeny plants were established during 2001 in duplicate, space-planted field trials. Plants are being culled on visual assessment of vigor and freedom of obvious deficiency symptoms as well as other attributes. A manageable set of fewer than 1000 clones will be identified by 01 December 2001 and propagated for in vitro assessment of Al tolerance (and other attributes) during first semester of 2002, to identify a small set of parental sexual clones to be intercrossed in isolation to produce the subsequent cycle population.

3.1.2 Studies on mechanisms of acid soil adaptation in *Brachiaria* cultivars and development of screening methods

3.1.2.1 Identification of Al-resistant *Brachiaria* hybrids

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

Rationale

Last year, we implemented screening procedure to identify Al-resistant *Brachiaria* hybrids that were preselected for spittlebug resistance. As part of the restricted core project funded by BMZ-GTZ of Germany, this year we used this screening method to identify most promising sexual *Brachiaria* hybrids that combine Al resistance with spittlebug resistance.

Materials and Methods

A total of 46 genotypes including 3 parents (*B. decumbens* CIAT 606, *B. brizantha* CIAT 6294 and *B. ruziziensis* 44-02) were selected for evaluation of Al resistance. Among the 43 hybrids selected for screening, 41 were sexuals and two hybrids, CIAT 36061 and CIAT 36062 were apomicts. All the hybrids except CIAT 36061 were highly resistant to spittlebug infestation (C. Cardona, personal communication). Stem-cuttings were rooted in a $CaCl_2$ (200µM) solution, selected for uniformity and transferred to a solution containing 200µ*M* $CaCl_2$ (pH 4.2) and exposed to 2 levels of $AlCl_3$ (0 and 200µM). The solution was replaced every third day, and total root length and root biomass were measured after 21 days of Al treatment.

Results and Discussion

As observed before, results on total root length indicate that the parent *B. decumbens* CIAT 606 is outstanding in its level of Al resistance (Figure 29). Among the 43 hybrids tested, 2 apomictic hybrids (CIAT 36061, CIAT 36062) and 2 sexual hybrids (SX 2349 and SX 497) showed moderate level of Al

resistance (Figure 29). The level of Al resistance of these two sexuals was markedly superior to that of the sexual parent, BRUZ/44-02. Among the hybrids, CIAT 36061 showed greater level of Al resistance than that of the other hybrids. The relationship between root length with no Al and root length with high Al showed that the extent of total root length of the apomictic *Brachiaria* hybrid cv. Mulato (CIAT 36061) was similar to that of the outstanding parent *B. decumbens* CIAT 606 with no Al in solution (Figure 30). But in the presence of high level of Al in solution this hybrid did not perform as well as CIAT 606 but markedly superior to the rest of the hybrids. The hybrids that were identified in the upper box of the right hand side (Figure 30) are being utilized in the on-going breeding program.

The root apex (root cap, meristem, and elongation zone) accumulates more Al and attracts greater physical damage than the mature root tissues. Scanning electron microscopic observations revealed differences in Al toxicity effects on root tips of two parents and one hybrid (Photo 13). The extent of deformation caused by Al toxicity was minimum with *B. decumbens* and was marked with *B. ruziziensis*. The hybrid, CIAT 36061 showed relatively less deformation of root tips compared with *B. ruziziensis*.

Conclusions

We have identified 2 sexual hybrids (SX 2349 and SX 497) with greater level of Al resistance than that of the sexual parent, BRUZ/44-02. We are also in the process of evaluating a hybrid population of *B. decumbens* x *B. ruziziensis.* This will enable us to develop molecular markers for Al resistance in *Brachiaria*.

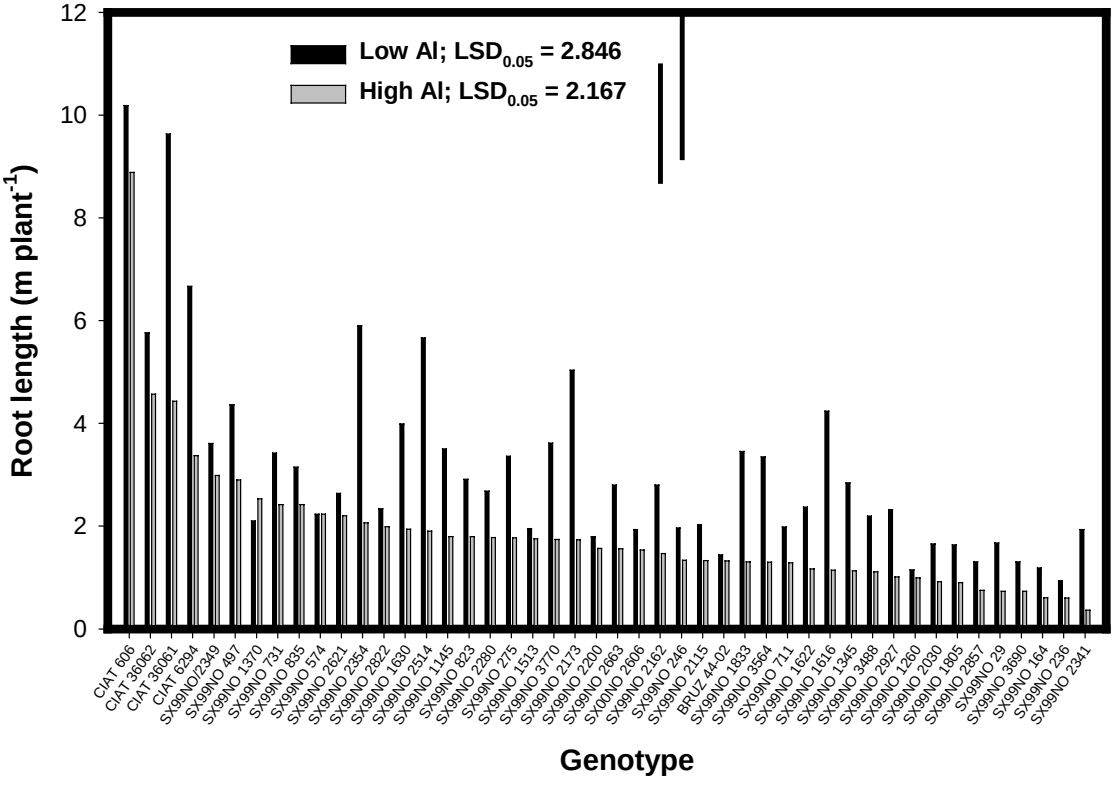

Figure 29. Screening for Al resistance among 46 genotypes of *Brachiaria*. Total root length was measured after exposure to 0 or 200 µM $AlCl_3$ with 200 µM $CaCl_2$ (pH 4.2) for 21 days.

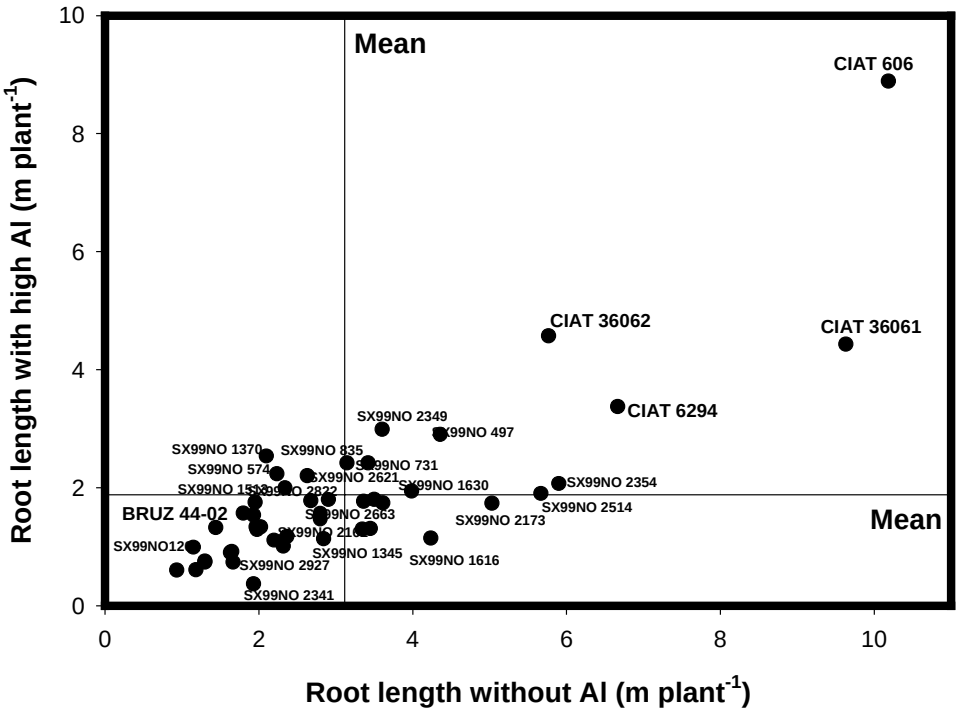

Figure 30. Identification of Al resistant *Brachiaria* hybrids. Hybrids that were superior in root length with no or high Al in solution were identified in the upper box of the right hand side. Total root length was measured after exposure to 0 or 200 µM $AlCl_3$ with 200 µM $CaCl_2$ (pH 4.2) for 21 days.

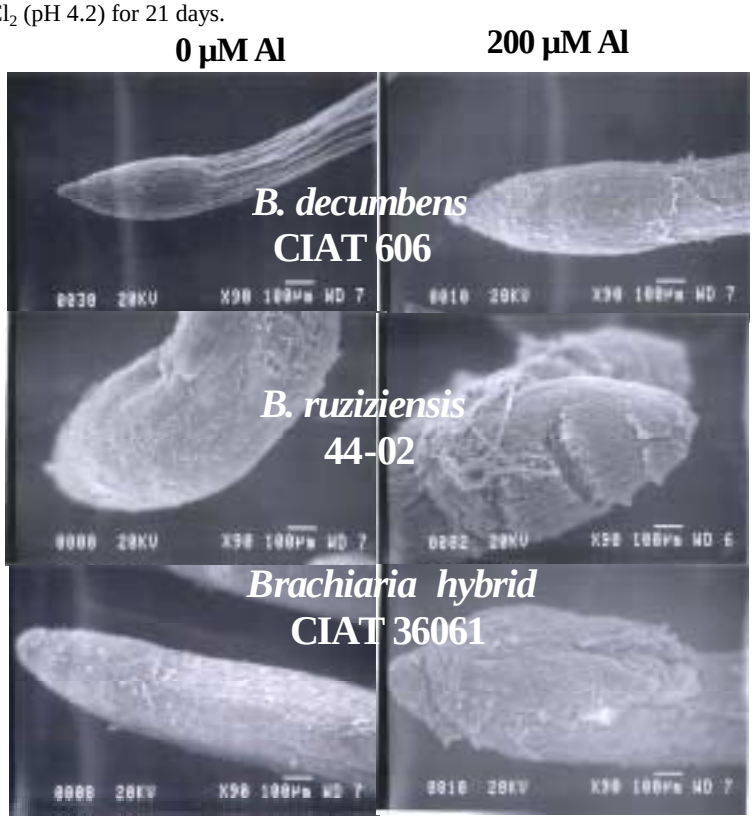

Photo 13. Scanning electron micrographs of root tips of two parents (*B. decumbens* and *B. ruziziensis*) and one hybrid (CIAT 36061) of *Brachiaria* exposed to either 0 or 200 µM of $AlCl_3$ for 21 days.

3.1.2.2 Identification of genetic recombinants of *Brachiaria* with tolerance to low nutrient supply

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

Rationale

A field study was conducted at Matazul Farm in the Llanos of Colombia. The main objective was to identify genetic recombinants of *Brachiaria* with tolerance to low nutrient supply and evaluate plant attributes that contribute to superior adaptation.

Materials and Methods

A field trial was established on a sandy loam oxisol at Matazul farm in the Llanos of Colombia in July, 1999. The trial comprises 12 entries, including six natural accessions (four parents) and six genetic recombinants of *Brachiaria*. The trial was planted as a randomized block in split-plot arrangement with two levels of initial fertilizer application (low: kg/ha of 20P, 20K, 33Ca, 14 Mg, 10S; and high: 80N, 50P, 100K, 66Ca, 28Mg, 20S and micronutrients) as main plots and genotypes as sub-plots. Live and dead forage yield, shoot nutrient composition, and shoot nutrient uptake were measured at the end of the wet season (October 2000).

Results and Discussion

Initial application of high amounts of fertilizer did not improve forage yield of most of the genotypes compared with low fertilizer application (Table 59). This indicates that the initial application of high amounts of N and K fertilizer at the time of establishment had very little residual effects into the second year. At 15 months after establishment, live forage yield with low fertilizer application ranged from 0.27 to 2.39 t/ha and the high values of forage yield were observed with two germplasm accessions (CIAT 26110 and CIAT 26318) and one spittlebug resistant genetic recombinant, FM9503-S046-024 (Table 59). As expected, the performance of one of the parents, BRUZ/44-02 was very poor compared with other parents and genetic recombinants.

One of the genetic recombinants, cv. Mulato (CIAT 36061), had more dead biomass with both levels of fertilizer application (Table 59). The gretest amount of total forage yield was obtained with one of the germplasm accessions, CIAT 26318 with both levels of fertilizer application. Two recombinants, cv. Mulato (CIAT 36061) and FM9503-S046-024 showed greater amount of dead biomass similar to one of the parents, CIAT 6294 with both levels of fertilizer application. These two recombinants were also superior in production of greater amount of green leaf and stem biomass (Table 60). Results on leaf and stem N content indicated that BRUZ/44-02 had greater amount of N per unit dry weight but its ability to acquire N (shoot N uptake) was lowest compared with other parents and genetic recombinants (Table 61). Shoot N uptake with low fertilizer application was greater for two accessions (CIAT 26110 and 26318), one parent (CIAT 6294) and one genetic recombinant (FM9503-S046-024). This genetic recombinant was also outstanding in its ability to acquire greater amounts of P, K, Ca and Mg from low fertilizer application when compared with parents, accessions and other genetic recombinants (Tables 62 and 63). Among the parents, *B. brizantha* CIAT 6294 was superior in P, K, Ca and Mg acquisition from low fertilizer application. It is important to note that live forage yield was associated with lower contents of not only essential nutrients but also Al in stems with both low and high fertilizer application. (Table 64). Live forage yield with low fertilizer application showed a significant negative relationship (-0.45**) with stem N content. This observation indicates that genotypes that are efficient in uitilization of N for the production of green forage is an important mechanism for superior performance with low fertilizer application.

Table 59. Genotypic variation as influenced by fertilizer application in live shoot biomass, dead shoot biomass 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 15 months after establishment (October 2000) LSD values are at the 0.05 probability level.

Genotype	Live shoot biomass		Dead shoot biomass		Total forage yield	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	(kg/ha)					
Recombinants:						
BR97NO-0082	793	1125	385	427	1178	1552
BR97NO-0383	934	1376	375	516	1308	1892
BR97NO-0405	1230	1061	518	537	1748	1598
cv. Mulato (CIAT 36061)	1419	1824	1378	1650	2797	3474
CIAT 36062	1145	1355	415	761	1560	2116
FM9503-5046-024	2082	1712	1429	814	3511	2527
Parents:						
CIAT 606	907	1204	361	215	1267	1419
CIAT 6294	2022	2429	1030	1580	3052	4010
BRUZ/44-02	274	268	244	212	518	480
CIAT 26646	1194	1854	865	709	2060	2563
Accessions:						
CIAT 26110	2390	2231	1364	777	3755	3007
CIAT 26318	2379	2568	1618	1287	3996	3856
Mean	1397	1584	832	791	2229	2374
LSD *(P=0.05)*	800	812	1138	1195	1559	1745

Table 60. Genotypic variation as influenced by fertilizer application in leaf biomass, stem biomass and leaf to stem ratio of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 15 months after establishment (October 2000). LSD values are at the 0.05 probability level.

Genotype	Leaf biomass		Stem biomass	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	(kg/ha)			
Recombinants:				
BR97NO-0082	578	741	215	384
BR97NO-0383	476	485	457	890
BR97NO-0405	629	523	601	538
cv. Mulato (CIAT 36061)	785	1014	634	810
CIAT 36062	808	1013	337	342
FM9503-5046-024	1356	1036	726	676
Parents:				
CIAT 606	571	779	335	425
CIAT 6294	1287	1184	735	1245
BRUZ/44-02	125	172	149	96
CIAT 26646	833	1185	361	668
Accessions:				
CIAT 26110	1371	1486	1019	744
CIAT 26318	1430	1412	948	1156
Mean	854	919	543	136
LSD *(P=0.05)*	440	428	415	522

Table 61. 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 15 months after establishment (October 2000). LSD values are at the 0.05 probability level.

Genotype	Leaf N content		Stem N content		Shoot N uptake	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	(%)		(%)		(kg/ha)	
Recombinants:						
BR97NO-0082	0.970	0.920	0.83	0.61	7.32	9.35
BR97NO-0383	1.050	0.960	0.91	0.62	8.75	9.61
BR97NO-0405	0.990	0.990	0.88	0.63	11.15	8.14
cv. Mulato (CIAT 36061)	0.740	0.830	0.86	0.56	10.88	13.06
CIAT 36062	0.880	0.910	0.65	0.53	9.08	11.06
FM9503-5046-024	0.980	0.900	0.90	0.54	19.86	12.53
Parents:						
CIAT 606	0.850	0.840	0.80	0.47	7.37	8.58
CIAT 6294	0.890	0.750	0.67	0.65	16.50	16.54
BRUZ/44-02	1.290	1.210	1.08	0.80	2.98	3.49
CIAT 26646	0.860	0.770	0.69	0.56	9.50	12.96
Accessions:						
CIAT 26110	0.900	0.720	0.40	0.51	16.41	13.75
CIAT 26318	0.770	0.700	0.65	0.48	16.99	14.98
Mean	0.930	0.870	0.76	0.58	11.40	11.33
LSD *(P=0.05)*	0.188	0.164	0.32	0.22	6.81	4.74

NS = not significant.

Table 62. Genotypic variation as influenced by fertilizer application in leaf P content, stem P content and shoot P uptake of genetic recombinants, parents and other germplasm accessions of Brachiaria grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 15 months after establishment (October 2000). LSD values are at the 0.05 probability level.

Genotype	Leaf P content		Stem P content		Shoot P uptake	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	(%)		(%)		(kg/ha)	
Recombinants:						
BR97NO-0082	0.154	0.141	0.127	0.075	1.11	1.34
BR97NO-0383	0.149	0.160	0.088	0.072	1.08	1.30
BR97NO-0405	0.146	0.166	0.119	0.079	1.55	1.31
cv. Mulato (CIAT 36061)	0.153	0.149	0.118	0.068	2.04	1.98
CIAT 36062	0.169	0.190	0.110	0.074	1.73	2.20
FM9503-5046-024	0.179	0.155	0.118	0.076	3.12	2.07
Parents:						
CIAT 606	0.162	0.147	0.180	0.106	1.49	1.57
CIAT 6294	0.153	0.151	0.112	0.086	2.75	2.84
BRUZ/44-02	0.193	0.166	0.110	0.087	0.42	0.45
CIAT 26646	0.142	0.129	0.117	0.067	1.56	2.00
Accessions:						
CIAT 26110	0.126	0.132	0.060	0.098	2.33	2.42
CIAT 26318	0.126	0.135	0.101	0.075	2.69	2.64
Mean	0.154	0.151	0.113	0.080	1.82	1.87
LSD (P=0.05)	0.049	0.041	0.050	NS	1.06	0.78

NS = not significant.

Table 63. Genotypic variation as influenced by fertilizer application in shoot K uptake, shoot Ca uptake and shoot Mg uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 15 months after establishment (October 2000). LSD values are at the 0.05 probability level.

Genotype	Shoot K uptake		Shoot Ca uptake		Shoot Mg uptake	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	(kg/ha)					
Recombinants:						
BR97NO-0082	9.67	11.87	3.23	5.40	3.19	5.51
BR97NO-0383	11.97	9.49	2.79	4.64	3.06	4.71
BR97NO-0405	15.59	11.25	3.72	3.45	4.00	4.02
cv. Mulato (CIAT 36061)	15.27	21.76	4.96	6.08	5.93	6.41
CIAT 36062	16.59	13.73	4.69	6.31	6.13	8.45
FM9503-5046-024	27.52	18.32	9.48	7.07	10.27	9.07
Parents:						
CIAT 606	13.34	15.13	4.00	4.34	4.96	6.23
CIAT 6294	24.98	21.09	7.04	7.01	8.65	10.66
BRUZ/44-02	3.07	3.12	0.98	1.39	1.07	1.60
CIAT 26646	14.94	18.14	3.42	5.12	4.72	8.64
Accessions:						
CIAT 26110	23.11	19.92	6.40	7.06	7.35	11.24
CIAT 26318	28.61	18.65	6.65	7.10	9.25	12.06
Mean	17.05	15.46	4.78	5.50	5.71	7.51
LSD *(P=0.05)*	11.46	5.80	2.90	2.89	3.13	4.11

Table 64. 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)	0.88***	0.89***
Dead shoot biomass (t/ha)	0.56***	0.59***
Leaf biomass (t/ha)	0.96***	0.88***
Stem biomass (t/ha)	0.93***	0.87***
Leaf N content (%)	-0.31*	-0.56***
Leaf P content (%)	-0.31*	-0.27
Leaf K content (%)	-0.14	-0.31*
Leaf Ca content (%)	-0.16	-0.44**
Leaf Mg content (%)	-0.10	-0.02
Leaf Al content (%)	-0.21	-0.37*
Stem N content (%)	-0.45**	-0.43**
Stem P content (%)	-0.34*	-0.33*
Stem K content (%)	-0.13	-0.17
Stem Ca content (%)	-0.33*	-0.46**
Stem Mg content (%)	-0.28	-0.30*
Stem Al content (%)	-0.30*	-0.23

Conclusions

Results from this field study indicated that the superior performance of the *Brachiaria* hybrid, FM9503-S046-024 at 15 months after establishment was associated with its ability to acquire greater amounts of nutrients from low fertility soil.

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

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

Rationale

Based on the data collected from greenhouse and field screening of a large number of *Brachiaria* hybrids, we selected 4 hybrids for further field testing to evaluate 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 this year. The trial included 4 *Brachiaria* hybrids (1251; 4015; 4132; 4624) 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.

Results

A number of plant attributes including forage yield, dry matter distribution and nutrient uptake are being monitored.

3.1.2.4 Screening accessions of *Brachiaria humidicola* for suppression of nitrification and nitrous oxide emission from soil

Contributors: T. Ishikawa (JIRCAS, JAPAN), and I.M. Rao (CIAT)

Rationale

Ammonium-N is transformed into nitrite-N and nitrate-N by soil microorganisms (Figure 31), a process known as nitrification. Nitrification leads to substantial losses of applied fertilizer N through nitrous oxide (N_2O) emission and runoff/leaching losses of nitrate from agricultural production systems. This is often associated with nitrate pollution of ground water and aquatic bodies. Nearly 50 to 70% of the applied fertilizer N is lost because of nitrification, causing enormous environmental pollution problems and also inefficiency in N utilization. Nitrous oxide, one of the greenhouse gasses, is emitted from the soil because of nitrification. Preliminary estimations indicate that N_2O emissions from the fertilizer N range from 7.3% of applied N for field crops such as maize to 12.0% of applied N for grasslands. By controlling nitrification in soils, it will be possible in future to reduce N fertilizer inputs into agricultural production systems and also minimize nitrate pollution in aquatic systems and ground water.

We found that a tropical grass, *Brachiaria humidicola* that is widely adapted to lowland agroecosystems (savannas) of humid and subhumid tropics, particularly in South America has the ability to suppress nitrification in soil and emission of N_2O to the atmosphere.

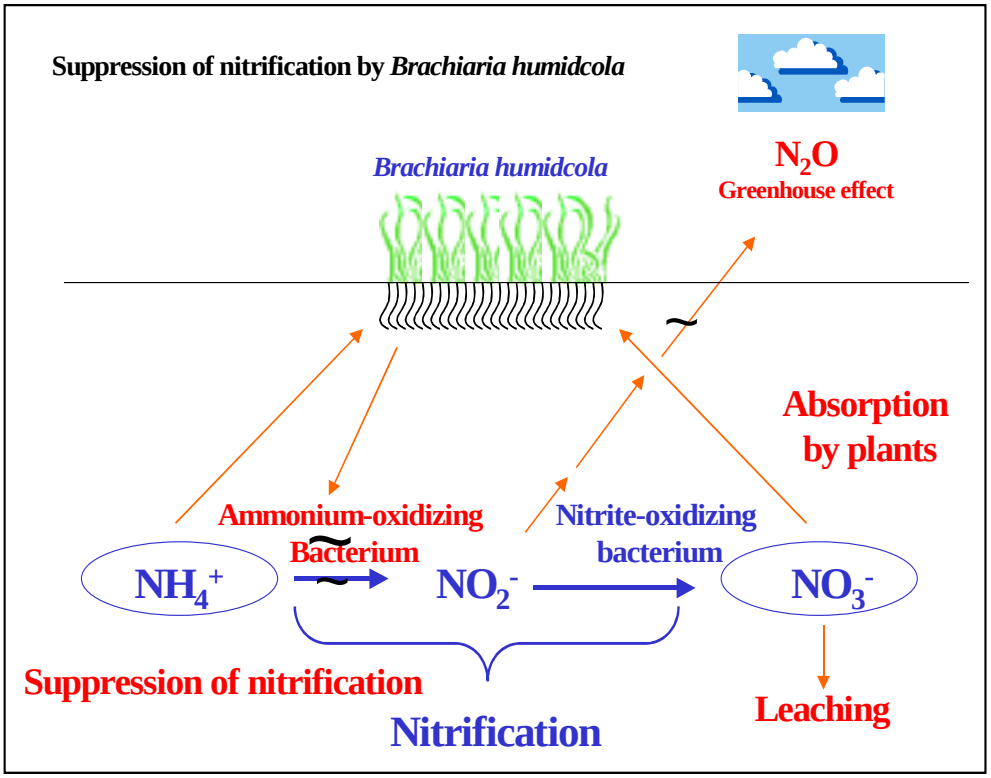

Figure 31. Mechanism of nitrification and nitrification suppression by *Brachiaria humidicola*

Materials and Methods

Three tropical grasses, *Brachiaria decumbens*, *Brachiaria humidicola* and *Melinis minutiflora* were grown in Wagner pots filled with Typic Hapludands [pH (H_2O) 6.0 and C/N ratio 11.68)]. Plants were grown in growth chamber with day/night temperature regimes of 30 ºC and 28 ºC, respectively with a 14 h photoperiod. Six weeks after sowing (WAS), 1.422 g of ammonium-nitrogen was supplied as ammonium sulfate. At 8 weeks after sowing, plants were separated from the soil. Root exudates were collected from these plants by keeping the plants in distilled water (1L pot^{-1}) for 24 h, plants were then harvested and dried for dry weight measurements. Soils were sampled for chemical analysis and for the initiation of nitrification study that followed. The effect of root exudates on the multiplication of ammonium oxidizing bacteria (AOB) was measured by MPN (most probable number) method.

Nitrification Study: Nitrification experiment was initiated to test the residual effect of the three grasses on soil nitrification during a 24 day period. Ammonium-N was applied (1.422 g NH_4 SO_4) to each of the sampled soil (2.3 kg pot^{-1}) and incubated for 24 days. Soil water content was maintained at 50% water holding capacity during this period (i.e. 24 day period after the harvest of plants at 8 WAS). Soil extract was made and its effect on multiplication of AOB was measured by MPN method. Soil samples were collected at various intervals and analyzed for NO_3 and NH_4 forms of nitrogen. Nitrous oxide emission from sampled soil was monitored by collecting the air samples at periodic intervals and N_2O levels were analyzed by gas chromatography.

Results and Discussion

Ammonia oxidizing bacteria (AOB) were nearly 10 times higher in soils where *B. decumbens* and *Melinis minutiflora* were grown at 8 WAS compared to soils where *B. humidocola* was grown (10,000 *vs* 1000 g^{-1} dry soil). Residual effect of *B. humidicola* on suppression of AOB lasted for about 12 days after the plants were harvested, but subsequently the AOB began to increase and reached levels similar to *B. decumbans* and *M. minutiflora* treatments at 24 days after plants were harvested (results not shown). For nitrite-oxidizing bacteria, however there were no significant differences among *B. decumbans*, *B. humidicola* and *M. minutiflora* treatments (results not shown).

Results on ammonium-N in soils were presented as the percentage of initial amount of ammonium-N applied (Figure 32). Nearly 50% of the ammonium-N was lost by 12 days after the initiation of nitrification treatment in soils where *B. decumbens* and *M. minutiflora* were grown (Figure 2). However, in soils where *B. humidicola* was grown, there was no significant change in ammonium-N levels up to 12 days but subsequently declined.

By 24 days after the nitrification study was initiated, NH_4-N in soils of *B. humidicola* treatment was similar to that of *B. decumbens* and *M. minutiflora*. Most of the applied ammonium-N was converted into nitrate-N or was lost as N_2O in all the three treatments by 24 days after the plants were harvested. Thus, the residual effect of *B. humidicola* on AOB has lasted only for about 12 days after the plants were harvested.

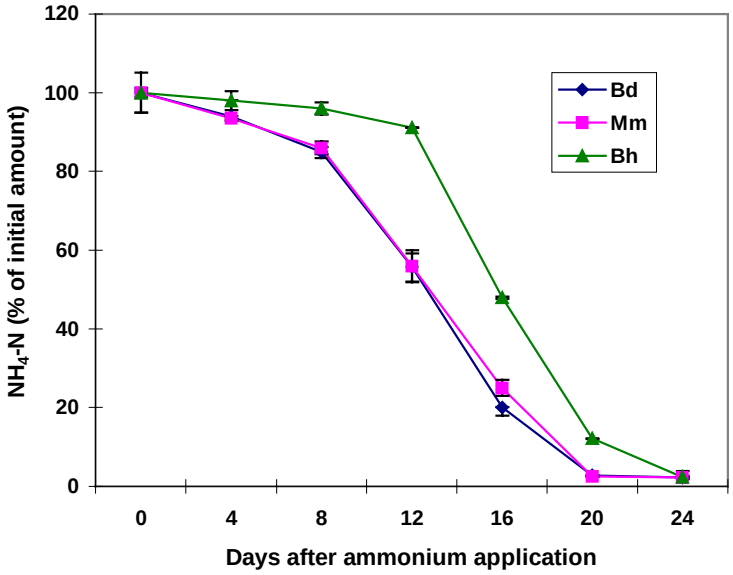

Figure 32. Percent of intial amount of ammonium-N in soil in relation to days after ammonium application

Nitrous oxide emission during the nitrification study was substantially higher for *B. decumbans* and *M. minutiflora* (31.0 and 29.3 µg-N m^{-2} hr^{-1}) compared to *B. humidicola* (5.0 µg-N m^{-2} hr^{-1}) treatment (Figure 33). For *B. decumbans*, and *M. minutiflora* treatments, N_2O emission reached the highest levels between 8 and 12 days after the initiation of nitrification study, which is similar to control pots (i.e. no plants). Soil

extracts and root exudates of *B. humidicola* treatment suppressed AOB, whereas no such effect was observed for *B. decumbans* or *M. minutiflora* treatments (results not shown).

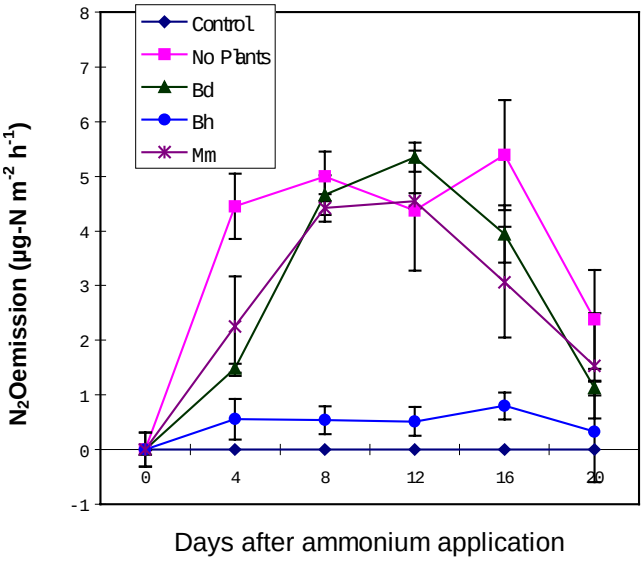

Figure 33. Nitrous oxide emission from soil in relation to days after application of ammonium. Control pots received no ammonium application while pots with no plants received ammonium application.

Conclusions

Our results strongly support the notion that *B. humidicola* has the ability to suppress nitrification by inhibiting the biological activity of ammonium oxidizing bacteria (AOB) in the soil. This was demonstrated by substantial decrease in AOB populations in soils where *B. humidicola* was grown. Also, nitrous oxide emissions, which is an indicator of the AOB biological activity was very low for *B. humidicola* treatment. Our results support the hypothesis that *B. humidicola* suppress nitrification and N_2O emission by inhibiting the activity of AOB in the soil. This may be achieved by secreting organic compounds from the roots that have the inhibitory effect on these AOB bacteria. Also, we have demonstrated that the residual effect of this tropical grass on nitrification of the soil will be about 24 days.

The Genetic Resources Unit of CIAT has a germplasm collection of about 62 accessions of *Brachiaria humidicola*. We plan to evaluate these germplasm accessions of *B. humidicola* in order to identify genotypic differences in their ability to suppress nitrification. This work is expected to contribute toward identification of germplasm accessions with greater ability to inhibit nitrification process in soil. The selected accessions could then be used in breeding programs for developing genetic stocks that combine greater forage production potential with high levels of nitrification inhibition capacity.

Currently we are also working on identification of the organic compound/s that are responsible for this unique property of nitrification inhibition in the root exudates of *Brachiaria humidicola*. Also, efforts are underway to understand the mechanisms of nitrification inhibition in these root exudates of *B. humidicola.*

3.1.3 Differences in phosphorus acquisition from less available phosphorus forms in an oxisol as determined by isotope exchange kinetics

Contributors: S. Buehler, A. Oberson, E. Frossard (ETH, Zurich, Switzerland), D. Friesen (IFDC-CIMMYT, Nairobi, Kenya) and I. M. Rao (CIAT)

Rationale

The ability to grow on low P soils differs widely between plant species and even among varieties. These differences have been attributed to several strategies for optimizing P uptake or P use efficiency. The strategies for P uptake improvement include root system morphology, root hair density, symbiosis with mycorrhizal fungi and modification of the rhizosphere by root exudates or phosphatases to access P forms of low availability. Previous research showed that *Arachis pintoi* accesses sparingly soluble P sources by high response to fertilization with Al-phosphate, Ca-phosphate or organic phosphate (phytic acid). *Brachiaria* species are reported to be well adapted to low P acid soils with the variety *Brachiaria decumbens* CIAT 606 being planted on over 40 million ha of low P acid soils in Latin America.

Adaptation of *Brachiaria* species to low-P soils is mainly attributed to the extensive fine root system, mycorrhizal association, and lower internal requirement of P for plant growth together with enhanced secretion of phytase under low P conditions (e.g., *Brachiaria decumbens*). Infertility of acid tropical soils is caused by multiple stress factors, among others Al-toxicity. *Brachiaria decumbens* also tolerates high Al-concentrations in the soil solution, possibly due to intracellular complexation of Al-ions with organic acids. Plants with special P uptake mechanisms may contribute to more efficient soil P use or could increase the recovery of applied fertilizer if they take up P that is normally not available to other plants. This might reduce the need of high P fertilizer inputs, although on the long-term the use of germplasm with special P uptake mechanisms would lead to soil P mining. A strategy to contribute to agricultural sustainability would be to minimize the P fertilizer requirement needed to produce an economic return through the use of more efficient crop and forage germplasm.

Differences between plants in the accessed P forms can be detected by growing plants on soils labeled with radioactive P isotopes and comparison of their isotopic composition. The **L** value is derived from the isotopic composition of the P taken up by the plant and expresses the total amount of soil P, which is potentially available to the plant. This amount can be compared to the E value of the same soil, which is based on the isotopic composition, measured or extrapolated, in the solution of a labeled soil suspension after a defined time of isotopic exchange. It was shown for a large range of soils, L values determined with common bentgrass (*Agrostis capillaris*) and E values calculated for the same time of isotopic exchange are not significantly different, and therefore *Agrostis capillaries* takes up only isotopically exchangeable P. In contrast, significant differences between E values and L values on the same soil for the same time of isotopic exchange are interpreted by plant P uptake from non-exchangeable P pools. Different L values of different plants on the same soil indicate that these plants do access P pools with different isotopic exchangeability. Higher L values were attributed to special P uptake mechanisms, especially exudation of organic acids, as citric acid in the case of lupine or piscidic, malonic or oxalic acid in the case of pigeon pea.

Seed P is an important factor affecting the isotopic composition of the P taken up by the plant and with this L values, especially under P-limitation and with little plant P uptake. Adding carrier-P, i.e. a simultaneous ^{31}P addition with the radioactive label, could increase plant growth and P uptake. It is assumed that L values are independent of the amount of carrier added to the soil. Besides the effect of enhancing plant growth, the application of carrier with the ^{33}P label is recommended to avoid the fixation of the label.

As part of a Ph.D. thesis study funded by a special project from SDC, Switzerland, two key forage species (*Arachis pintoi* and *Brachiaria decumbens*) were compared with three low-P adapted crop cultivars (maize, bean and upland rice) in terms of their ability for P uptake from sparingly available P pools based on their L values. A soil was chosen with very low available P (determined with Bray II) in order to guarantee P limitation for plant growth. The L value of *Agrostis capillaris* was determined as reference assuming that this plant does not access any non isotopically exchangeable P. E values were determined in a batch experiment without carrier application to determine the isotopically exchangeable soil P. L values were determined in two experiments, one without and one with a P carrier application of 10 mg P kg^{-1} soil. This amount was chosen as, with the application of the same amount to a similar soil, *Bachiaria* species and *Arachis pintoi* increased biomass production and P uptake but remained P limited.

Materials and Methods

The soil chosen (well-drained oxisol) for this study was cultivated as improved grass legume pasture starting with rice in 1993, with under sown pasture. The procedure to determine E values is based on the measurement of the specific activity ($^{33}PO_4$/ $^{31}PO_4$) of phosphate ions in the soil solution after an addition of carrier free $^{33}PO_4$ in a soil-solution system at steady state. The isotopically exchangeable P (E_t) was calculated assuming that, at any given exchange time, the specific activity of phosphate in solution is equal to the specific activity of the exchangeable phosphate on the solid phase:

$$\frac{r_t}{10 * C_p} = \frac{R}{E_t} \qquad \text{[Eq. 1]}$$

or:

$$E_t = R \times \frac{10 C_p}{r_t} \qquad \text{[Eq. 2]}$$

and r_t/R is extrapolated as:

$$\frac{r_t}{R} = \frac{r_1}{R}\left[t + \frac{r_1}{R}^{(\frac{1}{n})} \right]^{-n} + \frac{r_\infty}{R} \qquad \text{[Eq. 3]}$$

where R is the introduced radioactivity in MBq $ml^{-1}$and r_t is the radioactivity remaining in the solution after t minutes. The other parameters can be determined experimentally: n is a parameter calculated as the slope of the linear regression between $\ln(r_t/R)$ and $\ln(t)$ for $t \leq 100$ minutes of the measured values, r_1/R is the interception of the regression when t=1, and r_∞/R is calculated as:

$$\frac{r_\infty}{R} = 10 * \frac{C_p}{P_i} \qquad \text{[Eq. 4]}$$

Where P_i is total inorganic P and the ratio r_∞/R represents the radioactivity remaining in the soil solution at infinite time.

L value determination. The experimental conditions of the two pot experiments carried out to determine L values are summarized in Table 2. The cultivars used were *Brachiaria decumbens* (CIAT 606), *Arachis*

pintoi (CIAT 18744) and rice (*Oryza sativa* var Savanna-6) in experiment one. Additionally we used beans (*Phaseolus vulgaris* AFR 475) and maize (*Zea mays* NST 90201(s) co-422-2-3-1-7-2-1), an inbred line derived from a triple hybrid developed by the Thai Department of Agriculture, selected as tolerant to low-P conditions. In both cases common bentgrass (*Agrostis capillaris*) was grown as control plant without adaptation to low P conditions. The soil was labeled by adding the quantities of $^{32}PO_4$ or $^{33}PO_4$ ions in 10 mL water to portions of 1.5 kg incubated soil and were thoroughly mixed to ascertain an even distribution of the isotope (details given in Table 65).

Table 65. Soil (0-15 cm) properties determined on air-dried samples (except bulk density).

Total P (mg kg^{-1})	242
Total P_i (mg kg^{-1})	86.4
Resin P (mg kg^{-1})	1.5
Bray-II P (mg kg^{-1})	3.1
pH (in H_2O)	4.3
Total C (g kg^{-1})	23.7
Total N (g kg^{-1})	1.6
Aluminum-saturation (%)	68
Bulk density (g cm^{-3})	1.3

Agrostis was grown from 100 mg of seeds (corresponding to about 800 seeds) in both experiments, which were sown directly into each pot. All other plants were pregerminated on filter paper before planting into the pot at numbers indicated in Table 66. The pots with beans and *Arachis pintoi* were inoculated with a suspension of the *Rhizobium* strains CIAT 899 and CIAT 3101, respectively. During the experiment soil moisture was controlled by weighing and kept at 50 % of the water holding capacity.

Table 66. Experimental conditions in pot experiment 1 and 2 for determination of L values.

	Experiment 1	Experiment 2
Plant species, quantities of soil and plants per pot	*Arachis pintoi*, 2 kg soil, 2 plants *Brachiaria decumbens*, 2 kg soil, 2 plants Rice, 2 kg soil, 2 plants	*Arachis pintoi*, 0.9 kg soil, 1 plant *Brachiaria decumbens*, 0.9 kg soil, 2 plants Rice, 0.9 kg soil, 2 plants Beans, 0.9 kg soil, 1 plant Maize, 3.4 kg soil, 1 plant
	Agrostis capillaris, 500 g, 100 mg seeds	*Agrostis capillaris*, 400 g soil, 100 mg seeds
Labeling	$^{32}PO_4$, 5.2 MBq kg^{-1} soil	$^{33}PO_4$, 3.7 MBq kg^{-1} soil
Carrier	none	10.26 mg P as KH_2PO_4 kg^{-1} soil, applied with labeling solution
Replicates	4	5
Location	greenhouse, CIAT, Colombia	Biotron, ETH, Switzerland
Experimental conditions	Maximum light intensity ~ 1100 µ mol m^{-2} s^{-1}	16 h daylight, light intensity ~ 300 µ mol m^{-2} s^{-1}
Temperature	38/20 °C (max/min d/n, over whole growth period)	24/20 °C (constant)
Humidity	90/40 % (max/min)	65 % (constant)
Duration of plant growth:	2 months	11 weeks

After two months or eleven weeks, respectively, plant shoots were harvested and dry matter was weighed after 48 h drying at 80° C. About 200 mg of a homogenized sample of the whole shoot biomass in the first or half of the total shoot in the second experiment, cut in pieces <2mm, was calcinated at 550° for 4 hours. Plant P content (p) was determined after solubilization of the ash in 1-5 mL of 11.3 *M* HCl. The same method was used for the determination of the seed P content, measuring ball-milled samples of 100 mg (*Agrostis capillaris*), two seeds (*Arachis pintoi*, rice, beans and maize) or five seeds (*Brachiaria decumbens*), with five replicates each.

The plant ^{33}P (r) content was measured by scintillation counting of diluted (to avoid quench effect) samples using a liquid scintillation analyzer (Packard 2500 TR) and Packard Ultima Gold scintillation liquid. The measured radioactivity was decay corrected back to the day of soil labeling. The L values, expressed as mg P kg^{-1} soil, were calculated with the P-concentrations and activities measured in the total shoot.

Experiment 1:
Without carrier:

$$L = \frac{R * p}{r} \qquad \text{[Eq.5]}$$

Experiment 2
With carrier:

$$L = Q\left(\frac{R * p}{Q * r} - 1\right) \qquad \text{[Eq.6]}$$

The source of P taken up by the plant in the experiment with carrier addition can be calculated as:

$$P_{\text{soil}} = p - P_{\text{carrier}} \qquad \text{[Eq.7]}$$

$$P_{\text{carrier}} = \frac{Q * r}{R} \qquad \text{[Eq.8]}$$

where R is the quantity of $^{33}PO_4$ or $^{32}PO_4$ used to label exchangeable soil P (MBq kg^{-1} soil) and Q the quantity of carrier added (mg P kg^{-1} soil), r is the quantity of $^{33}PO_4$ or $^{32}PO_4$ (MBq kg^{-1} soil) and p is the quantity of $^{31}PO_4$ (mg kg^{-1} soil) in the plant shoots. $P_{carrier}$ and P_{soil} are the total amount of P derived from the carrier solution or from soil respectively. However, the P content of the seed is a third P source and uptake from this source could not be distinguished from the P taken up from soil. Therefore, P_{soil} is actually the sum of the P taken up from soil and from the seed, and the specific activity of the P taken up from soil is diluted. This results in an overestimation of the L value in both experiments. To increase the accuracy of the L value, the following correction was applied:

$$L_{\text{th}} = L\frac{p}{\left(p + P_{seeds}\right)} \qquad \text{[Eq.9]}$$

where L_{th} is the corrected value, L the value calculated with Eq. 5 or 6 and P_{seeds} the P content of the sown seeds per pot. Another possibility to correct for the seed P influence is to subtract the total seed P content from plant P uptake for the calculation of the L value: This correction assumes that 100 % of seed P was taken up by the plant and allocated to the shoot. Therefore, it corrects for the highest possible influence of seed P.

$$L = \frac{R\,(p - P_{seed})}{r} \qquad \text{[Eq. 10]}$$

Acid phosphatase activity determination: Three bulk soil samples were taken at random after harvesting plants in all pots, air dried and roots were removed carefully by sieving soil at 2 mm. Acid phosphatase activity at pH 6.5 of soil samples derived from the planted pots and soil incubated without plants at the same conditions was measured using 1 g air-dried soil.

Statistical Analysis: The effect of plants in the pot experiment and the effect of the experimental conditions on parameters of isotopic exchange in the E value determination were tested by analysis of variance (ANOVA). If the F-test was significant ($P<0.05$), the means were compared using Tukey's multiple range test.

Results and Discussion

L values determined without carrier and correction for seed P influence: The biomass production of all plants in the first experiment was very low and the total P uptake was hardly higher than the P content of the seeds (Tables 68 and 69). Correction for the contribution of seed-P according to Eq. 9 resulted in a marked reduction of the uncorrected L values (Table 68). It is, however, doubtful whether this correction, which was established for L value determination with *Agrostis capillaris* and *Lolium perenne* as model plants on sand culture, is also valid for other test plants and for all soil types.

The correction with Eq. 10 was only applicable in the case of *Brachiaria decumbens* as for all other test plants $P_{seed}>p$. Consequently, the corrected L values may rather give an impression of the order of magnitude of the seed P influence than represent exact values. Most studies comparing L values of different plants on low P soils, may have underestimated the problem of seed P influence. As the P reserves in the seed are in most cases relatively high in comparison to the P taken up from soil, the L value can not be calculated without correction for seed P uptake.

Due to the uncertain influence of seed-P, the interpretation of the L values remains limited. Additionally, the L value of *Agrostis capillaris* could not be used as reference as the ratio of plant P uptake to seed P was very low, too. However, in the case of *Brachiaria decumbens* with the smallest influence from seed P (Table 68 and 69), the corrected L_{th}-value remains much higher (131 mg kg^{-1} with Eq.9 or 127 mg kg^{-1} with Eq.10, respectively) than the extrapolated E_{8weeks}-value of 64 mg kg^{-1} determined for the same soil (Table 67). As Eq. 10, with the subtraction of total seed P from P export in the plant shoot, corrects for the highest theoretically possible influence of seed P, the L value of *Brachiaria decumbens* indicates that P additional to the isotopically exchangeable P was taken up. However, it should be mentioned that the extrapolation of E values on such very low P soils is difficult and the precision of the calculated E_{8weeks} is therefore limited. On the other hand, the L value of *Brachiaria decumbens* is also higher than the total soil P_i extracted with the sequential P fractionation (Table 65). This fact reinforces the assumption that organic P or very recalcitrant P forms contributed to the P uptake of *Brachiaria decumbens*.

Table 67. Parameters of isotopic exchange of the used soil.

r_1/R†1	0.03
c_p ‡	0.003 mg l^{-1}
n¶	0.43
E_1 #	1.1 mg kg^{-1}
E_{8weeks}#	64 mg kg^{-1}

† ratio of radioactivity remaining in soil solution to radioactivity added at time 0 after 1minute of isotopic exchange
‡ P concentration in the soil solution measured at soil:water ratio 1:10
¶ Parameter of isotopic exchange describing the decrease of radioactivity in the soil solution
#Quantity of P exchangeable within 1 minute or within 8 weeks (calculated with Eq. 3)

Table 68. Biomass production, P uptake and L values of the compared plants in experiments 1 and 2.

Plant Material	Shoot dry weight		P uptake		Shoot P concentration		L†		L_{th}‡	
	(g per pot)		(mg per pot)		µg g^{-1} dry weight		(mg P kg^{-1} soil)			
	Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2
A. pintoi	1.6a	2.4b	0.9a	2.1b	561ab	877b	185a	4.0	46b	3.1
B. decumbens	0.3bc	1.9bc	0.22b	1.1b	729a	581c	153ab	0.9	131a/ 127§	0.9
Rice	0.6b	2.3b	0.25b	1.1b	417b	478cd	125b	1.4	39b	1.1
Maize	-	6.3a	-	3.9a	-	622c	-	4.7	-	3.8
Beans	-	1.0c	-	1.5b	-	1350a	-	1.7	-	1.1
Agrostis capillaris	0.2c	1.0c	0.14b	0.4b	697ab	392d	128ab	3.3	6.7b	1.6
ANOVA	***	***	***	***	*	***	*	n.s.¶	***	n.s.

*,*** Significant at the 0.05 or 0.001 probability level, respectively. Values within columns followed by the same letter do not differ significantly (P=0.05) according to Tukey's test.
†L value without seed-P correction
‡L value with the seed -P correction, Eq. 9
§ second value: corrected with seed P correction, Eq. 10
¶ not significant

Table 69. Average seed weight and seed P content of the used varieties

Plant material	Weight per seed	Total P in sown seeds
	(mg)	(µg)
Arachis pintoi	158	1200/ 2 seeds
Brachiaria decumbens	4.6	26.7/ 2 seeds
Rice	44	282/ 2 seeds
Maize	306	900/ 1 seed
Beans	174	540/ 1 seed
Agrostis capillaris	0.125	480/100 mg seeds

The adaptation of *Brachiaria* species to low P soils is mainly attributed to soil exploration by an abundant fine root system and mycorrhizal association. In addition, it was shown in a pot experiment with different

added P sources, that *Brachiaria dictyoneura* cv. Llanero can acquire P from less available inorganic (aluminum phosphate, as $AlPO_4$) and organic (phytic acid) forms. Acid phosphatase activity in roots of *Brachiaria dictyoneura* was increased with decreasing soil P supply, and *Brachiaria decumbens* grown under low P condition in nutrient solution was shown to secrete the highest amount of phytase in comparison to 15 other plant species.

In our study acid phosphatase activity measured in the pot soil samples, and in turn the potential to mineralize available phosphomonoesters, was only significantly increased ($p<0.001$) for *Arachis pintoi* in the first experiment and was increased significantly ($p<0.001$) for all plants but *Agrostis capillaris* in comparison to the control soil without plant in the second experiment (Table 70). However, as the measurements were not restricted to rhizosphere soil, local effects in that zone would not have been detected.

Table 70. Phosphatase activity in soil samples derived from pots after plant harvest.

Plant species	Phosphatase activity	
	Exp. 1	Exp. 2
	μg nitrophenol g^{-1} h^{-1}	
Arachis pintoi	426a	322a
Brachiaria decumbens	285b	342a
Rice	295b	295ab
Maize	-	332a
Beans	-	286b
Agrostis capillaris	236b	242c
Control	219b	225c
ANOVA	***	***

***Significant at the 0.001 probability level, values within a column followed by the same letter do not differ significantly (P=0.05) according to Tukey's test.

The influence of carrier application: As the correction for seed P influence was difficult, the L value determination without carrier application was unsatisfying for the tested plants, with exception of *Brachiaria decumbens*. To overcome the difficulties of small total P uptake and biomass production, the second experiment was carried out with the application of KH_2PO_4 (10.3 mg P kg^{-1} soil) as a carrier with the labeling solution and the duration of plant growth was extended from two month to eleven weeks and smaller pots were used to reach higher biomass production and a higher soil exploration by the roots. The application of a P carrier resulted in much smaller L values (mean of all plants 2.7 mg P kg^{-1} soil) than without carrier (mean 148 mg kg^{-1}) and there were no significant differences between plants.

One possible explanation of the difference found between L values determined with or without carrier application is that an application of 10 mg P kg^{-1} to a soil with a very low P concentration in the solution (in this case approximately 3 μg l^{-1}) could have a high impact on the processes in this system. Instead of isotopic exchange, a net diffusion process may dominate and sizes of pools are changed. High influences of carrier application on E values, especially for high P sorbing soils, were found before and were explained by the influence of carrier P on the process of isotopic exchange as well as by the fact of $^{32}PO_4$ fixation.

Additionally to the carrier application also the different experimental conditions, especially the smaller pot size, might have influenced the L-value. However, a smaller soil volume and therefore higher root exploration and biomass production per kg soil should, if at all, lead to an increase of L values by higher

root activity and P mobilization and not to a decrease.

In our study, the nearly identical values of the specific activities of the plants and the applied carrier indicate that the carrier P was the main source for the P taken up by the plant. Separation of the P sources using Eq. 7 and 8 shows that on average 81% of P taken up by the plant derived from the carrier (Table 71). Of the 19 % of the total plant P uptake derived from another source a part is actually seed P. Therefore it can be assumed that almost no soil P was taken up and that the application of carrier is not valid for the determination of L values on low-P highly P sorbing soils.

Table 71. Amount of P derived from applied carrier and percentage of P derived from other sources in Experiment 2 (calculated with Eq. 7 + 8).

Plant species	Total P uptake in plant shoot	P derived from carrier	P derived from other sources (soil and seed)
	(mg per pot)		(%)
Arachis pintoi	2.1b	1.5b	27a
Brachiaria decumbens	1.1b	1.0b	9b
Rice	1.1b	0.9b	16b
Maize	3.9a	2.6a	26a
Beans	1.5b	1.0b	13b
Agrostis capillaris	0.4b	0.3C	24a
(average)			19
ANOVA	***	***	***

*** Significant at the 0.001 probability level, values within columns followed by the same letter do not differ significantly (*P*=0.05) according to Tukey's test.

Conclusions

A higher L value than E value for *Brachiaria decumbens* suggested P uptake from less available P forms from a low P soil. For all other plants, the contribution of the seed P to plant P uptake did not allow the calculation of exact L values. Therefore, drawing conclusions about the access of different P pools by different plants was not possible. L values determined with or without carrier P differed widely and suggested that a carrier application is not recommendable for using soils with very low P supply.

Results from this study indicate that it is possible to use L value determination as a screening method to identify the most promising *Brachiaria* hybrids with adaptation to low P supply in soil. Further research work is needed to identify specific physiological and biochemical mechanisms contributing to the ability of *B. decumbens* to acquire P from less aavailable forms from low P oxisol.

3.1.4 Studies on genotypic variation in *Arachis pintoi* for tolerance to low phosphorus supply

Contributors: N. Castañeda, N. Claassen (University of Goettingen, Germany); A. Betancourt, R. Garcia, J. Ricaurte and I. M. Rao (CIAT)

Rationale

For the last two years, we reported the progress from the field and greenhouse studies that were aimed to determine genotypic differences among ten accessions of *Arachis pintoi* in P-acquisition and utilization from low P soil. This year we report the progress made on persistence of these ten accessions.

Materials and Methods

A field study is in progress at "La Rueda" ranch, Montañita, Caquetá (latitude 1º 25' N, longitude 75º 27' W and 180 m.a.s.l). Plant growth was monitored since June 1998. The mean rainfall, temperature and relative humidity were 3500 mm/year, 25ºC and 75% respectively. The experiment was laid down in a split plot RCBD with three P levels [native P (NP), phosphate rock (PR), triple super phosphate (TSP)] as main plots and ten genotypes [CIAT 17434 (commercial), 18744, 18748, 22159, 18745, 18751, 22160, 18747, 22155, 22172] as subplots. The experiment was replicated three times. Application (kg P ha^{-1}) of PR and TSP was at 50 and 20, respectively. Plants were harvested at 32 months after establishment.

Results and Discussion

Last year, we reported that CIAT 22159 was outstanding in terms of persistence during second year as determined by leaf area index, shoot biomass and shoot P and Ca uptake. But this accession was relatively slow in establishment.

At 32 months after establishment and after a short dry spell, we evaluated the persistence of the same 10 accessions with 3 different sources of P applied at the time of establishment. Initial application of PR and TSP at establishment had no residual effect on the performance of 10 accessions. (Table 72). Live forage yield was greater for CIAT 22159 and CIAT 18744 with no P application treatment. This was not due to better leaf production but due to a large biomass of stolons (Table 73). With no P application treatment, CIAT 22159 which was reported to be better for persistance in association with aggressive grass, *B. dictyoneura* cv. Llanero at Carimagua was also superior in its ability to produce greater leaf area development and therefore leaf biomass production. Dead forage yields were greater with this accession with no P application.

Table 72. Influence of P fertilizer source on genotypic differences in leaf area index, live forage biomass and dead forage biomass of *Arachis pintoi* grown a low P soil at Montañita, Caquetá. Measurements were made at 20 months after planting.

Plant	P source	Accession of *A. pintoi*										$LSD_{0.05}$
Attributes		17434	18744	18745	18747	18748	18751	22155	22159	22160	22172	
Leaf area	NP	1.34	0.78	0.58	0.68	0.99	0.65	0.76	1.29	0.71	0.71	0.57
index	RP	0.89	0.75	0.72	0.44	1.10	0.81	0.69	0.78	0.63	0.77	0.48
(m^2/m^2)	TSP	1.19	0.78	0.76	0.56	1.06	0.93	0.58	0.71	0.87	0.93	0.49
Live forage	NP	2.22	3.08	1.39	2.32	1.93	2.15	3.07	3.54	1.74	2.84	1.14
yield	RP	2.31	2.38	1.60	1.86	2.83	2.33	2.19	3.39	2.15	2.40	0.91
(t/ha)	TSP	2.04	3.14	1.41	2.18	2.35	2.12	1.41	2.44	1.79	2.84	1.14
Dead forage	NP	1.03	1.55	0.96	1.05	1.11	1.52	1.71	1.80	1.22	0.99	0.65
yield	RP	1.39	1.92	1.16	1.23	1.40	1.34	0.84	1.22	1.12	0.76	0.64
(t/ha)	TSP	1.47	1.23	1.60	1.31	1.18	1.19	0.55	1.16	2.24	1.12	0.77

NP= Native phosphorus (0 kg P/ha); RP= Rock phosphate (50 kg P/ha); TSP= Triple super phosphate (20 kg P/ha).

The differences among the 10 genotypes in terms of leaf N and P contents were small when compared with leaf biomass production (Table 73). The content of N and P in green leaves was greater with the accession CIAT 18751.

Nutrient uptake, particularly P and N by green leaves during the dry spell was not significantly different among 10 acessions for no P treatment. Among the ten accessions, CIAT 17434, the commercial cultivar was outstanding in its ability to acquire Ca particularly from no P treatment (Table 74).

Table 73. Influence of P fertilizer source on genotypic differences in leaf biomass, leaf N content and leaf P content of *Arachis pintoi* grown a low P soil at Montañita, Caquetá. Measurements were made at 20 months after planting.

Plant	P source	Accession of *A. pintoi*										$LSD_{0.05}$
Attributes		17434	18744	18745	18747	18748	18751	22155	22159	22160	22172	
Leaf	NP	629	501	327	417	578	406	498	541	423	423	235
biomass	RP	518	431	388	258	666	576	417	477	389	438	267
(kg/ha)	TSP	562	505	409	329	568	548	361	431	522	564	NS
Leaf N	NP	24.9	32.6	27.8	32.8	26.8	32.6	30.1	29.5	30.5	28.5	NS
content	RP	30.2	36.2	36.4	38.1	35.1	34.9	29.5	31.5	33.9	30.9	4.4
(g/kg)	TSP	32.5	34.8	40.0	35.0	32.0	36.3	30.0	33.2	33.6	28.3	6.9
Leaf P	NP	2.1	2.2	2.5	2.4	2.1	2.4	2.4	2.1	2.1	2.0	NS
content	RP	2.1	2.7	2.8	2.8	2.4	2.6	1.9	2.3	2.3	2.2	0.4
(g/kg)	TSP	2.4	2.5	2.7	2.8	2.1	2.7	2.0	2.3	2.7	2.0	NS

NP= Native phosphorus (0 kg P/ha); RP= Rock phosphate (50 kg P/ha); TSP= Triple super phosphate (20 kg P/ha).

Table 74. Influence of P fertilizer source on genotypic differences in P, N and Ca uptake by green leaves of *Arachis pintoi* grown a low P soil at Montañita, Caquetá. Measurements were made at 20 months after planting.

Plant	P source	Accession of *A. pintoi*										$LSD_{0.05}$
Attribute		17434	18744	18745	18747	18748	18751	22155	22159	22160	22172	
	NP	1.30	1.10	0.84	0.98	1.22	1.02	1.05	1.16	0.90	0.85	NS
P uptake	RP	1.11	1.15	1.06	0.69	1.57	1.47	0.82	1.09	0.86	0.95	0.59
(kg/ha)	TSP	1.37	1.24	1.08	0.92	1.19	1.47	0.66	1.00	1.37	1.13	0.60
	NP	15.6	16.58	9.23	13.99	15.32	13.65	15.66	15.99	12.92	12.12	NS
N uptake	RP	15.77	15.50	13.98	9.72	23.37	20.10	12.52	15.11	12.50	13.47	9.2
(kg/ha)	TSP	18.77	17.54	15.98	11.77	18.43	19.85	10.63	14.39	17.09	15.97	NS
	NP	8.25	4.17	2.55	3.50	3.95	3.73	3.67	5.33	3.74	5.00	3.73
Ca uptake	RP	7.07	4.05	4.07	2.92	5.34	4.66	3.70	6.51	2.91	5.31	3.32
(kg/ha)	TSP	5.72	3.23	4.34	3.17	4.32	3.71	3.53	3.87	3.71	5.83	NS

NP= Native phosphorus (0 kg P/ha); RP= Rock phosphate (50 kg P/ha); TSP= Triple super phosphate (20 kg P/ha).

Conclusions

It appears from this field study that CIAT 22159 may be a better accession in terms of persistence with no P fertilizer input.

3.1.4.1 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

Last year, we reported the progress from the field studies carried out at Caqueta that were aimed to determine genotypic differences among ten accessions of *Arachis pintoi* in persistence with low P supply in soil. Based on these data and the data collected from multilocational evaluation, we have assembled a set of 8 genotypes for further testing at two sites (Piedmont and Altillanura) in the Llanos of Colombia. The site in Piedmont is close to La Libertad (CORPOICA Experimental Station) and the soils in this region are relatively more fertile than in the Altillanura. The site in Altillanura is at Matazul farm where the soils are relatively infertile (sandy loam).

Materials and Methods

Two field studies were established during May this year. The trial in Piedmont was planted as monoculture while the trial in Altillanura was planted in association with a grass. This is based on the expected end use of the legume. We expect multiple use for this legume in the Piedmont area (e.g., cover legume in plantations). The trial in the Piedmont included 8 accessions of *Arachis pintoi* (CIAT 17434; 18744; 18747; 18748; 18751; 22159; 22160 and 22172). The trial in the Altillanura included 4 accessions (CIAT 17434; CIAT 18744; CIAT 18748 and CIAT 22159) planted in association with *Brachiaria decumbens*. Both trials were 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.

Results

At 140 days after establishment of the trial in Piedmont, CIAT 18751 was found to be outstanding in its ability to establish rapidly. Two accessions (CIAT 17434 and 18751) were responsive to high level of fertilization while one of those two accessions (CIAT 18751) together with CIAT 18747 were among better performers with low fertilizer application as determined by soil cover.

A number of plant attributes including forage yield, dry matter distribution and nutrient uptake are being monitored for both trials.

Activity 3.2 Genotypes of grasses and legumes with dry season tolerance

Highlights

- Showed that the superior performance of the *Brachiaria* hybrid, FM9503-S046-024 which maintained greater proportion of green leaves during moderate dry season in the llanos of Colombia, was associated with lower levels of K and N content in green leaves.
- Field screening of 16 genotypes of Brachiaria and 13 accessions of *Arachis pintoi* with long dry season in Costa Rica resulted in identification of *Brachiaria* hybrid CIAT 36061 as not only adapted to drought but also superior in maintaining greater level of nitrogen (crude protein) in green leaves.

Progress towards achieving milestones

- ***Brachiaria* accessions and hybrids with superior tolerance to drought relative to commercial cultivars identified.**
  One hybrid of *Brachiaria* (FM9503-S046-024) was identified as promising material for areas with moderate drought stress in the acid soil regions. Recently released *Brachiaria* hybrid cv. Mulato (CIAT 36061) was identified as not only adapted to long dry season stress but also superior in its nutritional (protein) quality of green leaf forage to animals.

- ***Arachis* accessions with superior tolerance to drought identified.**
  Field testing of 13 accessions of *A. pintoi* did not result in identification of specific shoot attributes that are related to superior drought adaptation. Further research work is needed to evaluate shoot and root attributes under controlled conditions in the glasshouse.

- **Advanced in the development of an improved screening method to evaluate drought tolerance in *Brachiaria*.**
  Field screening of *Brachiaria* accessions at Atenas, Costa Rica were not very successful in identifying specific shoot attributes that contribute to superior adaptation to drought. Further research work is needed to evaluate shoot and root attributes under controlled conditions in the glasshouse to develop improved screening methods to evaluate drought adaptation of *Brachiaria* accessions and hybrids.

3.2.1 Determination of the genotypic variation in dry season tolerance in *Brachiaria* accessions and genetic recombinants in the Llanos of Colombia

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

Rationale

Quantity and quality of dry season feed is a major limitation to livestock productivity in subhumid regions of tropical America. A field study is in progress 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*. Last year, we showed that the superior performance of the *Brachiaria* hybrid, FM9503-S046-024, which maintained greater proportion of green leaves during dry season during the first year of establishment, was associated with lower levels of K and N content in green leaves. This year, we continued our efforts to monitor the dry season performance into second 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, *B. brizantha* (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 20P, 20K, 33Ca, 14 Mg, 10S; and high: 80N, 50P, 100K, 66Ca, 28Mg, 20S and micronutrients) as main plots and genotypes as sub-plots. Live and dead forage yield, shoot nutrient composition, and shoot nutrient uptake were measured at the end of the dry season (20 months after establishment; March 2001).

Results and Discussion

Initial application of high amounts of fertilizer (at the time of establishment) did not improve forage yield of most of the genotypes compared with low fertilizer application (Table 1). This indicates very little residual effects of initial application into the second year. At 20 months after establishment (4 months after dry sseason), live forage yield with low fertilizer application ranged from 0.14 to 1.48 t/ha and the highest value of forage yield was observed with one spittlebug resistant genetic recombinant, FM9503-S046-024 (Table 75).

Table 75. Genotypic variation as influenced by fertilizer application in live shoot biomass, dead shoot biomass 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 20 months after establishment (at the end of the dry season - March 2001). LSD values are at the 0.05 probability level.

Genotype	Live shoot biomass		Dead shoot biomass		Total forage yield	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	(kg/ha)					
Recombinants:						
BR97NO-0082	475	407	204	286	679	693
BR97NO-0383	502	449	223	310	725	759
BR97NO-0405	478	690	287	380	765	1070
cv. Mulato (CIAT 36061)	985	702	611	377	1596	1079
CIAT 36062	440	609	639	476	809	1085
FM9503-5046-024	1485	1106	626	510	2111	1616
Parents:						
CIAT 606	548	541	560	590	1108	1131
CIAT 6294	785	727	385	382	1170	1109
BRUZ/44-02	141	196	85	64	226	260
CIAT 26646	835	1077	792	1115	1627	2192
Accessions:						
CIAT 26110	1008	1266	407	732	1415	1998
CIAT 26318	1074	806	754	702	1828	1508
Mean	730	715	442	494	1172	1208
LSD *(P=0.05)*	552	452	379	438	821	786

As expected, the performance of one of the parents, BRUZ/44-02 was very poor compared with other parents and genetic recombinants. Among the four parents, CIAT 26646 performed better but it had greater amounts of dead biomass than the other test materials.The superior performance of the hybrid, FM9503-S046-024 was mainly attributed to its ability to produce green leaf biomass during dry season (Table 76). But this hybrid produced less stem biomass than another hybrid, FM 9201-1873. Among the parents, CIAT 26646 showed greater leaf and stem biomass (Table 76).

Results on leaf and stem N content indicated that BRUZ/44-02 had greater amount of N per unit leaf dry weight but its ability to acquire N (shoot N uptake) with low fertilizer application was lowest compared with other parents and genetic recombinants (Table 77).

Shoot N uptake with low fertilizer application was greater for two accessions (CIAT 26110 and 26318), one parent (CIAT 26646) and one genetic recombinant (FM9503-S046-024). This genetic recombinant was also outstanding in its ability to acquire greater amounts of P, K, Ca and Mg from low fertilizer

application when compared with parents, accessions and other genetic recombinants (Tables 4 and 5). Among the parents, CIAT 26646 and CIAT 6294 were superior in P, K, Ca and Mg acquisition from low fertilizer application.

Table 76. Genotypic variation as influenced by fertilizer application in leaf biomass, stem biomass and leaf to stem ratio of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 20 months after establishment (at the end of the dry season - March 2001). LSD values are at the 0.05 probability level.

Genotype	Leaf biomass		Stem biomass	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	Kg/ha			
Recombinants:				
BR97NO-0082	436	383	39	24
BR97NO-0383	431	381	71	68
BR97NO-0405	389	561	89	129
cv. Mulato (CIAT 36061)	493	605	492	97
CIAT 36062	402	563	38	46
FM9503-5046-024	1320	1044	165	62
Parents:				
CIAT 606	366	375	182	166
CIAT 6294	672	648	113	79
BRUZ/44-02	115	176	26	20
CIAT 26646	595	648	240	429
Accessions:				
CIAT 26110	827	985	181	281
CIAT 26318	640	581	434	225
Mean	557	579	173	136
LSD *(P=0.05)*	400	342	308	154

Table 77. 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 20 months after establishment (at the end of the dry season - March 2001). LSD values are at the 0.05 probability level.

Genotype	Leaf N content		Stem N content		Shoot N uptake	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	%		%		(kg/ha)	
Recombinants						
BR97NO-0082	1.360	1.140	ND	ND	ND	ND
BR97NO-0383	1.230	1.190	ND	ND	ND	ND
BR97NO-0405	0.910	0.970	1.11	0.83	3.72	6.31
cv. Mulato (CIAT 36061)	1.260	1.040	0.48	1.03	6.39	6.85
CIAT 36062	1.200	1.120	ND	ND	ND	ND
FM9503-5046-024	1.070	0.910	0.99	0.59	15.35	10.01
Parents:						
CIAT 606	1.360	1.180	0.84	0.91	6.21	5.10
CIAT 6294	0.990	0.900	1.05	0.67	6.99	6.06
BRUZ/44-02	2.240	1.900	ND	ND	ND	ND
CIAT 26646	1.060	0.960	0.68	0.67	8.05	8.72
Accessions:						
CIAT 26110	1.040	0.870	0.90	0.84	10.12	10.53
CIAT 26318	0.970	1.110	0.65	0.81	9.08	7.41
Mean	1.160	1.070	0.8	0.8	7.21	6.78
LSD *(P=0.05)*	0.451	0.481	NS	0.36	6.11	4.58

ND = not determined due to small size of the sample; NS = not significant.

Table 78. Genotypic variation as influenced by fertilizer application in leaf P content, stem P content and shoot P uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 20 months after establishment (at the end of the dry season.

Genotype	Leaf P content		Stem P content		Shoot P uptake	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	%		%		(kg/ha)	
Recombinants						
BR97NO-0082	0.116	0.126	ND	ND	ND	ND
BR97NO-0383	0.088	0.108	ND	ND	ND	ND
BR97NO-0405	0.101	0.116	0.124	0.130	0.42	0.78
cv. Mulato (CIAT 36061)	0.108	0.109	0.090	0.103	0.62	0.71
CIAT 36062	0.132	0.163	ND	0.152	ND	0.97
FM9503-5046-024	0.116	0.119	0.136	0.184	1.75	1.33
Parents:						
CIAT 606	0.118	0.137	0.117	0.172	0.63	0.76
CIAT 6294	0.113	0.109	0.165	0.099	0.87	0.74
BRUZ/44-02	0.092	0.143	ND	ND	ND	ND
CIAT 26646	0.104	0.127	0.084	0.106	0.82	1.24
Accessions:						
CIAT 26110	0.106	0.106	0.128	0.165	1.06	1.47
CIAT 26318	0.095	0.114	0.088	0.107	0.97	0.89
Mean	0.108	0.123	0.112	0.130	0.76	0.85
LSD *(P=0.05)*	NS	0.039	0.040	0.053	0.68	0.63

ND = not determined due to small size of the sample; NS = not significant.

Table 79. Genotypic variation as influenced by fertilizer application in shoot K uptake, shoot Ca uptake and shoot Mg uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 20 months after establishment (at the end of the dry season - March 2001). LSD values are at the 0.05 probability level.

Genotype	Shoot K uptake		Shoot Ca uptake		Shoot Mg uptake	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	(kg/ha)					
Recombinants:						
BR97NO-0082	7.62	7.24	0.90	1.23	0.83	1.15
BR97NO-0383	7.59	6.17	1.01	0.95	1.17	0.94
BR97NO-0405	7.00	11.86	1.08	1.93	1.08	1.99
cv. Mulato (CIAT 36061)	6.94	9.38	1.26	1.59	1.41	1.81
CIAT 36062	7.96	9.35	0.91	1.53	0.87	2.09
FM9503-5046-024	23.58	13.32	3.70	3.55	3.69	3.63
Parents:						
CIAT 606	9.55	8.16	1.01	1.04	1.30	1.62
CIAT 6294	10.48	9.53	1.47	1.49	1.75	1.82
BRUZ/44-02	2.49	4.20	0.30	0.75	0.31	0.85
CIAT 26646	9.50	13.95	1.28	1.84	1.62	2.88
Accessions:						
CIAT 26110	14.10	15.54	1.73	2.51	1.94	3.60
CIAT 26318	12.05	8.95	1.67	1.40	2.10	2.12
Mean	10.40	9.92	1.43	1.67	1.59	2.07
LSD *(P=0.05)*	8.25	6.14	1.70	1.22	1.73	1.60

Correlation analysis between green leaf biomass produced in the dry season and other shoot attributes indicated that superior performance with low fertilizer application was associated with lower level of N in green leaves (Table 80).

Significant negative association was also observed between green leaf biomass and lower level of K and N in green leaves with high fertilizer application. This observation indicates that genotypes that are efficient in uitilization of N for the production of green forage is an important mechanism for superior performance with low fertilizer application in the dry season.

Conclusions

Results from this field study indicated that the superior performance of the *Brachiaria* hybrid, FM9503-S046-024 which maintained greater proportion of green leaves during moderate dry season in the llanos of Colombia, was associated with lower levels of K and N content in green leaves.

Table 80. Correlation coefficients (r) between green leaf biomass (t/ha) and other shoot traits of *Brachiaria* genotypes grown with low or high fertilizer application in a sandy loam oxisol in Matazul.

Shoot traits	Low fertilizer	High fertilizer
Live forage yield (t/ha)	0.87***	0.72***
Total forage yield (t/ha)	0.81***	0.81***
Dead biomass (t/ha)	0.54***	0.51***
Stem biomass (t/ha)	0.20	0.45**
Leaf N content (%)	-0.33*	-0.45**
Leaf P content (%)	0.05	-0.10
Leaf K content (%)	-0.15	-0.54***
Leaf Ca content (%)	-0.16	0.06
Stem N content (%)	0.20	-0.11

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

3.2.2 Determination of the genotypic variation in dry season tolerance in *Brachiaria* and *Arachis* in Costa Rica

Contributors: I.M.Rao, P. J. Argel, J. Ricaurte and R. García (CIAT)

Rationale

Field studies were continued at Atenas, Costa Rica. The main objective was to evaluate genotypic differences in dry season (6 months) tolerance among 16 accessions of *Brachiaria* species and 13 accessions of *Arachis* species. We continued our work to test further the hypothesis that tolerance to dry season is greater in genotypes that accumulate greater amounts of total nonstructural carbohydrates (TNC) combined with less amounts of minerals (ash content) per unit dry weight of leaves and stems. The use of shoot attributes such as ash content, Ca content and nonstructural carbohydrate levels as selection criteria for dry season tolerance in *Brachiaria* is being tested further using green leaves developed during dry season compared to dry leaf and stem tissue.

Materials and Methods

Trial 1 included 16 genotypes (15 accessions and 1 hybrid) of *Brachiaria* species and trial 2 included 13 accessions of *Arachis* species selected from agronomic evaluation of the germplasm. Atenas site provided excellent field conditions to evaluate the impact of long dry season (5 months) while keeping nutrient supply in soil adequate for growth. Forage yield, nutrient composition, and nonstructural carbohydrates and ash content in green leaves, dry leaves and stem tissue were measured.

Results and Discussion

Trial 1 - Forage yield among *Brachiaria* species during dry season ranged from 2988 to 9988 kg/ha and the greatest forage yield was observed with *B. brizantha* CIAT 26646 (Table 81). The superior performance of this accession at Atenas site is consistent with its outstanding performance at Matazul site in the Llanos of Colombia (see above Activity 3.2.1). *B. brizantha* CIAT 26110, which maintained greater proportion of green leaves during dry season (visual observation) maintained greater amountn of N (crude protein) and TNC in green leaves while its ash (mineral) content was markedly lower than most accessions. This observation indicates that this accession combines drought adaptation with greater nutritional value of the green forage. One of the Brachiaria hybrids tested (CIAT 36061) was particularly outstanding in its N status of the green leaves. It apperas that this hybrid also combined adaptation to drought with greater nutritional value of the green forage. Among the 16 genotypes *B. brizantha* CIAT 667 was outstanding in maintaining greater amounts of nonstructural carbohydrates in green leaves.

Table 81. Genotypic variation in forage yield, green leaf nutrient composition, ash content and total nonstructural carbohydrates (TNC) of 16 genotypes of *Brachiaria* species grown during dry season at Atenas, Costa Rica.

Genotype (CIAT number)	Forage yield (kg/ha)	Green leaf composition							
		C	N	P	K	Ca	Mg	Ash	TNC
		(%)							(mg/kg)
B. brizantha (26646)	9988	25.3	0.45	0.074	1.345	0.546	0.648	9.0	156
B. brizantha (16305)	8828	27.8	0.67	0.057	1.468	0.418	0.513	8.3	141
B. brizantha (16322)	8132	26.5	0.56	0.050	1.150	0.566	0.721	11.4	174
B. brizantha (16319)	8068	27.0	0.39	0.038	1.379	0.337	0.517	6.8	180
B. brizantha (26110)	7028	27.1	0.71	0.049	1.472	0.409	0.295	7.5	178
cv. Mulato (CIAT 36061)	6692	28.0	0.81	0.062	1.334	0.701	0.755	11.4	161
B. brizantha (16300)	6640	26.9	0.47	0.045	1.139	0.495	0.623	7.1	158
B. brizantha (16467)	6628	27.2	0.57	0.076	1.860	0.606	0.519	9.6	146
B. brizantha (667)	6492	25.5	0.69	0.079	1.586	0.506	0.437	8.4	263
B. brizantha (16168)	5548	26.9	0.49	0.038	1.445	0.656	0.622	10.3	93
B. brizantha (16549)	5372	26.5	0.50	0.050	1.151	0.569	0.525	7.8	164
B. brizantha (16289)	5308	27.1	0.51	0.050	1.494	0.564	0.477	9.7	199
B. briznahta (16488)	5080	26.4	0.50	0.069	1.161	0.564	0.587	10.8	121
B. brizantha (16135)	4548	26.4	0.73	0.077	1.054	0.651	0.828	8.8	154
B. decumbens (16497)	3492	26.2	0.68	0.063	1.205	0.546	0.587	7.7	125
B. brizantha (6387)	2988	25.7	0.76	0.101	1.126	0.881	0.742	10.7	184
Mean	6302	26.7	0.59	0.061	1.336	0.563	0.587	9.1	162

Results on composition of dry leaves in terms of nutrients and TNC indicated that *B. brizantha* CIAT 667 is also outstanding in maintaining greater levels of TNC and nutrients (Table 82). Among the 16 genotypes tested. *B. brizantha* CIAT 16300 showed the lowest amount of ash content of both green leaves and dry leaves indicating that this genotype had greater nutrient use efficiency to produce forage

during dry season. Genotypic variation in nutrient and TNC composition of stems indicated that the hybrid CIAT 36061 is outstanding in maintaining greater composition of N and TNC than other genotypes. These results indicate that the hybrid is not only productive during dry season but also nutritive to the animals (Table 82).

Table 82. Genotypic variation in dry leaf nutrient composition, ash content and total nonstructural carbohydrates (TNC) of 16 genotypes of *Brachiaria* species grown during dry season at Atenas, Costa Rica.

Genotype (CIAT number)	Dry leaf composition							
	C	N	P	K	Ca	Mg	Ash	TNC
				(%)				(mg/g)
B. brizantha (26646)	25.4	0.32	0.073	0.873	0.537	0.629	9.5	140
B. brizantha (16305)	27.0	0.42	0.028	0.769	0.265	0.302	8.9	129
B. brizantha (16322)	26.5	0.36	0.048	0.868	0.561	0.565	11.0	134
B. brizantha (16319)	26.5	0.18	0.027	1.099	0.379	0.677	8.1	137
B. brizantha (26110)	26.5	0.51	0.041	1.211	0.460	0.361	8.9	154
cv. Mulato (CIAT 36061)	26.7	0.41	0.041	0.630	0.624	0.609	11.8	152
B. brizantha (16300)	28.6	0.33	0.054	0.780	0.568	0.631	7.7	247
B. brizantha (16467)	26.9	0.23	0.059	1.146	0.479	0.438	9.7	159
B. brizantha (667)	26.4	0.34	0.055	0.972	0.545	0.468	10.6	258
B. brizantha (16168)	28.7	0.43	0.044	1.098	0.547	0.533	10.1	201
B. brizantha (16549)	25.2	0.35	0.036	0.809	0.570	0.548	8.9	146
B. brizantha (16289)	27.9	0.48	0.054	1.027	0.479	0.398	9.0	139
B. brizanhta (16488)	27.0	0.38	0.065	1.012	0.435	0.540	10.9	143
B. brizantha (16135)	26.8	0.68	0.096	1.025	0.514	0.717	10.3	134
B. decumbens (16497)	26.5	0.58	0.074	0.870	0.573	0.560	8.6	90
B. brizantha (6387)	28.1	0.70	0.095	0.823	0.699	0.533	10.6	151
Mean	26.9	0.42	0.056	0.938	0.515	0.532	9.7	157

Trial 2- Although forage yield data were not available, among the 13 accessions of *Arachis pintoi* tested, three accessions, CIAT 17434, 22159 and 22161 maintained greater concentration of N in green leaf tissue (Table 83). One of the accessions, *A. pintoi* CIAT 22160, which was selected as dry season tolerant accession from field evaluation in cerrados of Brazil showed level of K in green leaf tissue. It also showed greater levels of Ca, Mg and TNC in green leaf tissue.

Results on genotypic variation in dry leaf nutrient composition, ash content and TNC content showed that CIAT 22160 had lower levels of TNC indicating that it may have greater ability to mobilize photosynthates (TNC) from older dry leaves to young green leaves (Table 84). This may be an important mechanism in the shoots in addition to its better rooting ability to avoid drought stress.

Further research is needed to test this accession compared with the commercial check, CIAT 17434 under glasshouse conditions to compare root and shoot attributes with drought stress.

Conclusions

Results from the above 2 trials did not provide any clear evidence that using green leaf nutrient status, ash content or TNC one good indicator of drought adaptation of *Brachiaria* and *Arachis* gentoypes.

Table 83. Genotypic variation in stem nutrient composition, ash content and total nonstructural carbohydrates (TNC) of 16 genotypes of *Brachiaria* species grown during dry season at Atenas, Costa Rica.

Genotype (CIAT number)	Stem composition							
	C	N	P	K	Ca	Mg	Ash	TNC
	(%)							(mg/g)
B. brizantha (26646)	27.3	0.11	0.040	0.374	0.169	0.334	3.9	144
B. brizantha (16305)	24.9	0.19	0.047	0.752	0.117	0.233	4.8	165
B. brizantha (16322)	26.1	0.19	0.042	0.582	0.148	0.202	5.8	116
B. brizantha (16319)	26.6	0.21	0.040	0.721	0.144	0.362	4.4	174
B. brizantha (26110)	25.4	0.30	0.046	0.723	0.151	0.157	4.4	99
cv. Mulato (CIAT 36061)	26.4	0.50	0.039	0.528	0.208	0.292	4.9	267
B. brizantha (16300)	27.4	0.25	0.050	0.561	0.164	0.374	4.6	153
B. brizantha (16467)	27.5	0.13	0.044	0.973	0.161	0.276	5.0	142
B. brizantha (667)	26.4	0.14	0.056	0.999	0.170	0.208	6.5	153
B. brizantha (16168)	27.6	0.07	0.025	0.513	0.110	0.179	4.4	152
B. brizantha (16549)	27.2	0.19	0.035	0.417	0.185	0.218	3.6	152
B. brizantha (16289)	24.8	0.11	0.024	0.863	0.141	0.193	5.6	150
B. brizanhta (16488)	26.3	0.11	0.042	0.600	0.151	0.320	6.4	133
B. brizantha (16135)	26.7	0.19	0.044	0.618	0.133	0.341	5.6	95
B. decumbens (16497)	26.0	0.26	0.057	0.626	0.145	0.200	4.7	116
B. brizantha (6387)	26.5	0.20	0.071	0.608	0.149	0.186	5.6	214
Mean	26.4	0.20	0.044	0.654	0.153	0.255	5.0	152

Table 84. Genotypic variation in green leaf nutrient composition, ash content and total non-structural carbohydrates (TNC) of 13 accessions of A*rachis pintoi* grown during dry season at Atenas, Costa Rica.

Genotype (CIAT number)	Green leaf composition							
	C	N	P	K	Ca	Mg	Ash	TNC
	(%)							(mg/kg)
A.pintoi (17434)	28.8	2.67	0.119	0.959	2.470	0.861	8.9	104
A.pintoi (18744)	26.2	2.62	0.103	0.785	2.423	0.981	9.1	69
A. pintoi (22148)	25.4	2.21	0.069	0.834	2.363	0.534	8.3	139
A. pintoi (22149)	26.3	2.48	0.091	0.929	2.371	0.501	8.8	127
A. pintoi (22150)	26.3	2.16	0.071	1.017	2.307	0.579	8.7	143
A. pintoi (22151)	26.3	2.31	0.076	0.571	2.590	0.710	8.9	64
A. pintoi (22155)	26.0	2.26	0.076	1.284	2.280	0.502	8.8	138
A. pintoi (22156)	-	-	-	-	-	-	-	-
A. pintoi (22157)	26.5	2.62	0.105	1.175	2.193	0.612	9.0	122
A. pintoi (22158)	26.7	2.47	0.086	0.956	2.403	0.765	9.5	143
A. pintoi (22159)	26.6	2.67	0.094	0.811	2.398	0.857	9.4	73
A. pintoi (22160)	27.2	2.35	0.077	0.490	2.511	1.004	9.5	117
A. pintoi (22161)	29.9	2.67	0.112	0.952	2.030	0.933	8.5	95
Mean	26.9	2.46	0.090	0.897	2.362	0.680	9.0	111

Table 85. Genotypic variation in dry leaf nutrient composition, ash content and total nonstructural carbohydrates (TNC) of 13 accessions of *Arachis pintoi* grown during dry season at Atenas, Costa Rica.

Genotype	Dry leaf composition							TNC
(CIAT number)	C	N	P	K	Ca	Mg	Ash	(mg/g)
A. pintoi (17434)	26.2	2.03	0.089	0.681	2.487	0.861	9.2	63
A. pintoi (18744)	28.6	2.15	0.092	0.654	2.886	0.981	10.3	42
A. pintoi (22148)	26.7	1.87	0.060	0.607	2.304	0.534	8.3	90
A. pintoi (22149)	26.3	2.21	0.081	0.544	3.096	0.501	8.2	80
A. pintoi (22150)	27.3	1.79	0.057	0.793	2.337	0.579	9.0	64
A. pintoi (22151)	26.7	1.59	0.045	0.377	3.017	0.710	10.3	61
A. pintoi (22155)	27.1	1.82	0.056	0.968	2.508	0.502	9.2	91
A. pintoi (22156)	26.7	1.90	0.055	0.789	2.524	-	9.1	53
A. pintoi (22157)	24.8	1.97	0.076	0.747	2.760	0.612	9.4	109
A. pintoi (22158)	27.1	2.11	0.088	0.685	2.798	0.765	9.8	82
A. pintoi (22159)	27.3	2.01	0.066	0.585	2.527	0.857	9.5	36
A. pintoi (22160)	28.5	1.99	0.069	0.355	2.598	1.004	9.3	52
A. pintoi (22161)	26.0	2.05	0.074	0.970	2.341	0.933	9.0	134
Mean	26.9	1.96	0.070	0.673	2.629	0.737	9.3	73

Activity 3.3 Shrub legumes with adaptation to drought and cool temperatures

Highlights

- Found significant differences within and between a collection of *Cratylia argentea* accessions and *Leucaena* spp. in quality attributes.
- Accesssions of *Cratylia argentea* with superior performance than *C. argentea* cv. Veraniega identified
- Developed map of potential distribution of *Flemingia macrophylla* in tropical Asia and found genetic variability for forage quality parameters in this legume species.

Progress towards achieving milestones

- **List of new accessions of *Cratylia argentea* and *Leucaena* species with known forage value**
  Our results show considerable variability in growth habit, DM yields, and quality parameters in accessions of *C. argentea*, which open the opportunity for the selection of new cultivars in the near future. Accessions CIAT 18674, 22375, 22406, 22408 and 22409 had higher dry matter yields than CIAT 18516/18668 (cv. Veraniega) in dry and wet seasons. The new accessions have also showed dry tolerance and good re-growth during prolonged dry seasons, which is one of the outstanding characteristics that makes *C. argentea* a valuable forage for dual purpose cattle farms.

  The legume *Leucaena leucocephala* is very well known for its high forage value. However, a great diversity exists within this genus that has not been fully characterized. For instance, our results showed that *L. macrophylla* susp. *nelsonii* OFI 47/85, species commonly found along the coasts of Oxaca and Guerrero in Mexico, showed high CP content (28.3%), and acceptable IVDMD (62.2%), indicating that it deserves to be evaluated with animals in futures studies.

- **List of *Flemingia macrophylla* accessions characterized for yield and quality**
  Results indicate that several accessions have superior dry matter yield and better digestibility than the control CIAT 17403. The most promising accession (CIAT 21090) will be multiplied for further

testing. Studies to better understand the difference in digestibility among *Flemingia* accessions were initiated and these studies will be complemented with palatability trials.

3.3.1 Characterization of a core collection of *Cratylia argentea* and *Leucaena* sp. in a subhumid environment of Costa Rica

Contributors: Diego Bolaños Crespo, Mayra Montiel L., Miguel Vallejo Solís y Carlos Jiménez Crespo (U. de Costa Rica); Guillermo Pérez y P. J. Argel (CIAT)

Rationale

One of the limitations of *Leucaena leucocephala* is its limited adaptation to acid soils and to pest such as psyllid. On the other hand, *C. argentea*, a shrub native to the South American tropics, has shown good adaptation to acid soils, and excellent tolerance to prolonged dry periods. Thus, we were interested in characterizing core collections of *C. argentea* and *Leucaena* for yield and quality attributes in a site characterized by having a long dry season and acid soils of medium fertility.

Materials and Methods

Leaves and young stems (edible forage) were harvested from 18 lines of *Leucaena* spp. and 30 lines of *Cratylia argentea* planted in Atenas, Costa Rica, that had been under cutting evaluation for 2 years. The site is located in a subhumid environment with a total annual rainfall of 1600 mm, and 5 to 6 months dry from December to May. The soils are Inceptisol of medium fertility with pH 5.0, and low P and low aluminum content. The samples were dried in an air forced oven set at 60 °C for 72 hours, and then ground to less than 5-mm particles. Dry matter (DM) and protein content (CP) were determined using standard AOAC procedures. Neutral-detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to Van Soest and Roberston (1979), while invitro dry matter digestibility (IVDMD) was determined as described by Tilley and Terry (1963).

Results and Discussion

Results on quality of *C. argentea* and *Leucaena* spp. are presented in Table 86 and Table 87, respectively. The mean CP was higher in *Leucaena* sp. (mean of 23.3%) compared to *Cratylia* (mean of 17.7%). *L. macrophylla* subsp. *nelsonii* OFI 47/85 had the highest value of CP (28.3%), while *L. pulverulenta* OFI 83/87 had the lowest (17.2%).

In general, variation of CP content within *Cratylia argentea* accessions was less than in the *Leucaena* species, which indicates more genetic variability within the latter group. On the other hand, *C. argentea* CIAT 22382 had the highest CP content (19.4%), and *C. argentea* CIAT 22389 the lowest CP value (15.1%).

The NDF contents for both *Cratylia* and *Leucaena* accessions are within the range reported in the literature for tropical tree legumes. The NDF fraction varied from 43-80% for the *Leucaena* species, and from 62-77% for *C. argentea*. Considerable interspecific variation in NDF content existed within the species of *Leucaena*, evaluated with *L. macrophylla* subsp. *nelsonii* OFI 47/85 showing the highest content of NDF (80%) while *L. diversifolia* subsp. *diversifolia* OFI 83/92 the lowest (43%). There was little variability of NDF values within the *Cratylia* group, indicating a good degree of genetic uniformity for the 30 accessions evaluated.

Table 86. Quality components of *Cratylia argentea* accesions established in the subhumid enviroment of Atenas, Costa Rica.

CIAT No.	DM (%)	CP (%)	NDF (%)	ADF (%)	IVDMD* (%)
22374	34.2	15.8	68.6	52.7	54.0
22386	30.8	16.8	69.9	50.2	60.9
22379	31.6	16.6	69.5	50.9	50.5
22382	33.0	19.4	67.3	46.4	64.9
22381	31.0	18.8	67.7	51.5	56.5
22375	32.8	15.7	66.5	49.9	54.1
22389	34.0	15.1	68.7	48.6	55.7
(BRA 000621)	42.1	18.2	71.1	57.0	-
22391	33.3	16.6	61.6	44.9	51.3
(BRA 000876)	31.6	19.3	62.3	45.3	61.6
22393	31.9	18.4	63.6	44.2	65.0
22378	32.3	18.5	68.1	46.4	58.8
(Yacapani)	32.9	18.6	64.4	43.2	55.8
22380	34.3	17.5	68.9	50.6	53.1
22385	37.2	17.1	65.4	45.6	54.8
22383	32.2	16.2	69.7	47.8	53.7
22384	34.5	16.1	65.8	48.2	57.2
22395	33.8	19.0	67.7	48.4	55.1
22373	32.1	18.6	67.6	52.2	57.1
22390	32.2	18.7	66.6	47.7	50.3
22392	31.2	19.0	64.2	44.8	57.3
22387	32.0	17.5	66.4	49.7	-
22394	34.2	18.4	67.9	48.7	60.3
(BRA 000884)	32.8	19.3	65.9	42.8	62.5
22388	31.2	18.2	68.4	48.4	58.9
(BRA 000604)	37.3	17.8	65.3	46.5	55.4
(BRA 000841)	34.5	18.4	65.6	47.1	55.9
22396	32.5	19.0	64.6	47.1	47.8
22377	33.8	17.9	67.3	54.4	48.1
22376	32.0	17.3	76.7	48.6	57.4
Mean	33.2	17.7	69.1	48.1	55.9
Sd	2.3	1.2	2.9	3.2	4.4

*Quality components of eatable forage (leaves and young stems) 8 weeks old

The mean level of ADF was slightly higher in *Cratylia* than in the *Leucaena* accessions, but within the range expected for tropical legumes. On the other hand, DM digestibility was higher, and more variable in *Leucaena* (range from 50-84%), compared to *Cratylia* (range from 48-65%).

Forage quality parameters of *C. argentea* cv. Veraniega (CIAT 18516/18668) at 90 days of re-growth, are within the range observed in the core collection of *C. argentea* accessions evaluated. On the other hand, *L. leucocephala* subsp. g*labrata* cv. Taramba en Australia (OFI 34/92), showed high CP (33%) and IVDMD (65%), indicating a high potential feed value; however, this line is susceptible to the psyllid, which may limit its commercial use in sites with high incidence of the insect.

Table 87. Quality components of *Leucaena* species established in the subhumid environment of Atenas, Costa Rica.

Species	ID No. (OFI)	DM (%)	CP (%)	NDF (%)	ADF (%)	IVDMD* (%)
L. trichanda	53/88	29.2	24.9	50.6	46.6	51.8
L collinsii	52/88	29.5	26.6	45.1	35.5	84.0
L. leucocephala subsp. *glabrata*	34/92	32.6	23.8	65.7	40.3	64.6
L. pallida	14.96	30.6	22.9	70.6	53.5	53.4
L. hybrid	1/95	31.8	21.3	63.3	42.3	-
L. macrophylla subsp. *nelsonii*	47/85	43.4	28.3	80.2	45.4	62.2
L. leucocephala CIAT	17263	31.4	24.3	46.9	41.6	71.5
Leucaena hybrid	52/87	32.3	25.0	52.3	41.3	-
L. salvadorensis	17/86	35.9	22.4	49.7	38.5	69.9
L. lanceolata	43/85	33.8	24.2	46.4	41.1	73.3
L. diversifolia subsp. *diversifolia*	83/92	30.1	26.1	43.4	38.0	55.7
L. pallida	79/92	31.4	24.4	55.2	49.6	50.1
L. esculenta subsp. *esculenta*	47/87	33.6	20.7	68.0	40.1	60.6
L. pulverulenta	83/87	38.0	17.2	62.7	47.4	73.1
L. collinsii subsp. *zacapana*	56/88	30.5	21.7	52.3	46.3	70.9
L. lempirana	6/91	31.3	24.5	47.6	36.0	80.7
L. shannonii subsp. *magnifica*	19/84	31.2	23.5	51.0	39.9	75.7
L. trichodes	61/88	32.3	22.7	55.9	45.2	66.6
Mean		32.4	23.3	54.3	42.2	64.9
Sd		3.4	2.2	10.2	4.8	10.2

*Quality components of eatable forage (leaves and young stems) 8 weeks old

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

Contributors: M. Andersson (University of Hohenheim), M. Peters, J. Tohme (CIAT), R. Schultze-Kraft (University of Hohenheim), and L.H. Franco (CIAT)

CIAT projects: SB-2

Rationale

Work on shrub legumes in CIAT, emphasizes the development of species to be utilized as feed supplement during extended dry periods. Tropical shrub legumes of high quality for better soils are readily available, but germplasm with similar characteristics adapted to acid, infertile soils is scarce. Shrub legume species, such as *Flemingia macrophylla* and *Cratylia argentea* are well adapted to low fertility soils and to prolonged drought, respectively. Thus, work on these genera is of high priority in CIAT's Forage Project.

In order to define the extent of genetic diversity within ex-situ collections of *F. macrophylla* and *C. argentea* we initiated a project with three main objectives. (a) 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), and (b) to optimize the use and management, including conservation, of the collections. To accomplish these objectives, different approaches are being used with a core collection: (a) genetic diversity assessment by a germplasm origin information; and (b) molecular markers (AFLPs). This information should also be useful to define future collection needs in terms of geographical focus.

Materials and Methods

Agronomic characterization and evaluation: Spaced-plants of *Cratylia argentea* (39 accessions) and *Flemingia macrophylla* (73 accessions) were established in Quilichao in March 1999 (Photo 13) and March 2000 (Photo 14), respectively. Additionally two replications were sown for morphological characterization and for seed production. The following parameters are being measured: vigor, height and diameter, regrowth, seasonal dry matter yield during incidence of diseases, pests and mineral deficiencies.

Photo 13. *Cratylia argentea* at Quilichao

Photo 14. *Flemingia macrophylla* accession at Quilichao

For the morphological evaluation, qualitative and quantitative parameters are recorded, such as days to first flower, days to first seed, flower color, flowers per inflorescence, flowering intensity, pod pubescence, seeds per pod, seed color, branching capacity, leaf length and width, peduncle length, etc. To assess nutritive value, we are measuring crude protein (CP) and in vitro dry-matter digestibility (IVDMD) in leaf samples of the two collections.

For *F. macrophylla*, a more detailed chemical analysis will be conducted on a representative subset which will include accessions with high intermediate and low CP and IVDMD. Other chemical analysis in selected accessions of *F. macrophylla* will include NDF, ADF, condensed tannins calcium, and phosphorus.

Analysis of available origin information: Based on geographical information on the site of origin of accessions, a core collection will be created under the assumption, that geographic distances and environmental differences are related to genetic diversity. The analysis will be conducted with FloraMap^{TM}, a GIS tool developed by CIAT

Genetic analysis by molecular markers (AFLPs): Genetic variability will also be assessed through AFLP molecular markers. Based on the results of molecular markers group of accessions will be formed, using multivariate statistic tools.

Data analysis and synthesis: Individual and combined analyses of all data generated will be performed, including the use of GIS tools and multivariate statistics. In the analysis of each of the different approaches (agronomic characterization, origin information, molecular marker analysis), Principal Component Analysis and Cluster Analysis will be utilized to assist in the formation of core collections. Correlation between the different approaches and clusters obtained will also be determined.

These results are expected to help in deciding which of the three methods or which combination is most appropriate (time and cost efficiency) to create a core collections. For example, if an agronomic evaluation of the entire collection is not feasible because of time constraints, a core collection may be created using origin information and/or molecular markers. In addition, based on similarity of molecular marker and GIS analysis, we hope to provide information that will be useful for defining future collections on areas with particularly high diversity. Accession duplicates in the world collections will also be identified.

Results and Discussion

Based on the origin of existing germplasm accessions (73), the potential natural distribution of *F. macrophylla* extends throughout vast areas of tropical Asia (Photo 15). This map, however, is not to be considered as a 'prediction' of the probable natural distribution of *F. macrophylla* but merely as an indication of conditions based on climate and latitude/longitude matching those of the germplasm collection sites.

Agronomic characterization and evaluation: Results from one evaluation in the dry season and one in the rainy season show that there is considerable phenotypic and agronomic variation in the collection of *Cratylia argentea* evaluated (Table 88) and *Flemingia macrophylla* (Table 89). In the case of *C. argentea* mean dry matter production was 45 g/plant in the wet and 60 g/plant in the dry season, whereas IVDMD varied between 61 and 67% and CP content between 18 and 21%.

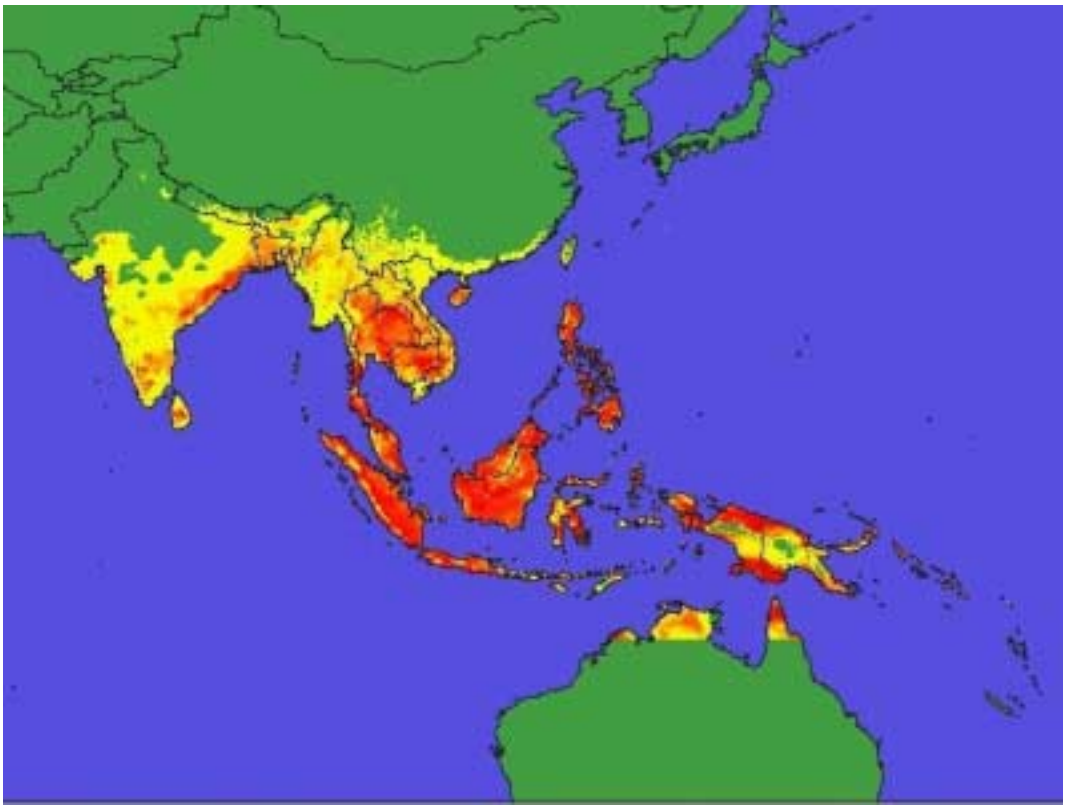

Photo 15. FLORAMAP analysis of potential natural distribution of *Flemingia macrophylla* in tropical Asia, based on passport data of the world germplasm collection maintained at CIAT.

Principal component analysis performed with the agronomic data of 39 accessions of *C. argentea* revealed high correlations between total dry matter production, diameter, regrowth points and vigour. Cluster analysis resulted in 9 groups and 5 of the clusters contained only one accession, among them three of the most productive accessions (CIAT 18674, 22406 and 22408).

Based to these initial results, we have pre-selected *C. argentea* accessions CIAT 18674, 22375, 22406, 22408 and 22409 given that productivity of these accessions is higher than the genotypes released in Costa Rica (an accession mix of CIAT 18516/18668) as cv. Veraniega.

In the case of *F. macrophylla* the average dry matter production was 60 g/plant in the wet and 42 g/plant in the dry season. The most productive accessions were CPI 104890, CIAT 21090, 21241, 21529 and 21580 with a total dry matter production >100 g/plant. We also found high variability in IVDMD (31 to 51%) and CP (16 to 24%), which is an interesting results since one limitation of *F. macrophylla* is low feed value.

Principal component analysis performed with the agronomic data of 73 accessions of *F. macrophylla* revealed high correlations between total dry matter production, plant height and diameter and vigour (>70%). Cluster analysis (UPGMA) resulted in 7 clusters and 2 of the clusters contained only one accession, among them one of the most productive accessions (CIAT 21090). Based on these results we selected the *F. macrophylla* accession CIAT 21090 (semi-erect type, high forage yield and quality for seed multiplication and evaluation with animals.

Table 88. Agronomic evaluation of a collection of *Cratylia argentea* in Quilichao. Preliminary data of four cuts (two in the dry season and two in the wet season).

Treatment No. CIAT	Height (cm)	Diameter (cm)	Regrowing points (No.)	Mean dry matter yields (g/pl)			IVDMD (%)	Crude protein (%)
				Wet	Dry	Mean		
18516	112	105	19	55	78	66	65.0	20.7
18667	112	101	18	45	68	56	64.6	20.4
18668	106	110	17	48	68	58	65.2	19.9
18671	111	106	20	54	55	54	64.3	18.3
18672	96	83	13	34	39	37	62.1	20.1
18674	118	122	23	91	109	100	63.9	20.0
18675	112	97	15	47	63	55	63.3	19.0
18676	105	93	14	46	50	48	61.2	19.7
18957	111	102	16	50	76	63	62.5	20.1
22373	109	93	15	38	57	48	64.4	20.2
22374	116	102	17	55	71	63	66.4	19.6
22375	125	98	16	59	76	68	67.0	21.2
22376	95	70	11	23	36	29	64.1	19.6
22378	103	81	12	34	39	36	61.7	19.8
22379	111	89	16	47	65	56	63.5	19.6
22380	107	90	11	31	43	37	61.3	20.4
22381	105	85	11	34	46	40	64.0	19.1
22382	110	92	12	41	62	52	64.2	20.4
22383	99	90	13	34	43	39	62.6	18.6
22384	113	91	9	43	47	45	64.5	18.9
22386	111	86	12	39	47	43	64.7	18.6
22387	111	90	12	41	57	49	62.5	19.1
22390	99	92	13	45	47	46	64.8	18.5
22391	108	96	15	44	62	53	63.4	18.9
22392	114	83	13	33	53	43	63.2	21.0
22393	110	92	17	41	58	49	63.5	20.6
22394	112	88	13	33	46	40	64.0	20.5
22396	101	79	10	30	43	36	63.8	21.3
22399	102	86	13	35	42	39	66.1	19.8
22400	119	104	16	52	74	63	61.7	20.7
22404	110	97	13	42	68	55	67.0	20.9
22405	111	96	16	41	61	51	62.9	19.9
22406	113	112	20	63	86	74	62.6	21.0
22407	111	99	16	46	59	53	65.3	20.8
22408	120	109	18	69	88	79	67.2	20.1
22409	113	115	17	57	81	69	66.5	21.2
22410	116	96	14	42	60	51	64.3	19.8
22411	103	88	14	37	58	47	64.5	20.2
22412	116	90	11	42	63	52	64.9	18.7
Mean	110	95	15	45	60	52	64.1	19.9
Range	95-125	70-122	9-23	23-91	36-109	29-100	61-67	18-21

Table 89. Agronomic evaluation of a collection of *Flemingia macrophylla* in Quilichao. Preliminary data of two cuts (one in each season). Growth habit: =erect, s=semierect, p=prostrate.

Treatment No. CIAT	Height (cm)	Diameter (cm)	Regrowing Points (No)	Mean dry matter yields (g/pl)			IVDMD (%)	Crude Protein (%)
				Wet	Dry	Mean		
J 001 (e)	125	85	30	102	58	80	40.1	22.3
801 (e)	125	90	29	103	62	82	36.3	22.9
7184 (e)	124	95	34	101	82	92	34.0	21.4
C 10489 (e)	108	99	34	121	79	100	33.6	22.7
I 15146 (e)	98	70	24	103	58	80	39.9	22.9
17400(s)	63	98	33	55	52	53	33.2	21.5
17403 (s)	67	96	32	68	57	62	35.8	22.2
17404 (s)	58	79	32	46	45	45	32.9	22.5
17405 (s)	65	94	36	71	67	69	36.1	21.9
17407 (s)	78	106	39	87	62	74	32.8	21.9
17409 (s)	56	109	35	87	66	77	33.0	20.2
17411 (s)	55	86	33	56	54	55	35.5	22.4
17412 (s)	73	96	39	61	63	62	38.6	20.2
17413 (s)	58	93	35	51	39	45	35.2	20.1
18048 (s)	32	43	19	12	8	10	42.8	20.4
18437 (s)	54	101	37	57	55	56	47.8	22.5
18438 (s)	58	71	31	36	22	29	51.5	23.5
18440 (s)	59	87	38	65	44	55	33.4	21.4
19453 (e)	105	78	20	65	33	49	36.0	21.6
19454 (e)	115	82	24	73	52	63	39.1	19.7
19457 (e)	116	85	25	52	64	58	33.1	21.3
19797 (s)	57	90	22	58	46	52	38.5	21.0
19798 (s)	55	95	27	61	55	58	38.3	20.9
19799 (s)	50	69	19	28	39	33	37.3	21.7
19800 (s)	65	85	29	34	48	41	32.0	20.7
19801 (s)	82	91	40	68	57	63	35.7	21.7
19824 (e)	62	93	35	54	61	58	35.6	21.3
20065 (p)	15	21	4	0	1	1	32.1	18.9
20616 (s)	67	108	34	86	54	70	32.1	22.0
20617 (s)	72	92	27	51	44	48	30.6	20.1
20618 (s)	74	95	31	57	60	58	33.2	21.6
20621 (e)	84	88	32	58	54	56	31.6	21.6
20622 (e)	146	88	30	105	76	91	42.8	22.9
20624 (s)	74	122	39	101	91	96	34.5	19.8
20625 (e)	128	86	26	105	69	87	42.5	22.8
20626 (e)	115	92	28	88	70	79	39.5	22.3
20631 (e)	121	90	25	97	75	86	41.5	20.9
20744 (e)	125	87	27	102	65	84	42.9	23.1
20972 (p)	24	56	31	12	14	13	39.6	23.4
20973 (p)	24	45	17	4	10	7	34.2	19.6
20975 (s)	52	83	45	44	24	34	45.3	20.3
20976 (s)	45	57	27	17	11	14	40.8	20.0
20977 (s)	33	35	9	5	4	4	46.1	18.5
20978 (s)	52	56	24	21	11	16	46.6	22.1

Continues…..

Table 89. Agronomic evaluation of a collection of *Flemingia macrophylla* in Quilichao. Preliminary data of two cuts (one in each season). Growth habit: =erect, s=semierect, p=prostrate.

Treatment No. CIAT	Height (cm)	Diameter (cm)	Regrowing Points (No)	Mean dry matter yields (g/pl)			IVDMD (%)	Crude Protein (%)
				Wet	Dry	Mean		
20979 (s)	48	76	38	27	25	26	38.9	21.2
20980 (s)	43	55	26	27	18	22	41.8	21.0
20982 (s)	49	61	28	26	23	25	41.0	19.9
21079 (s)	47	78	44	51	25	38	37.9	20.2
21080 (s)	41	58	13	32	11	21	39.2	15.5
21083 (e)	93	79	36	71	43	57	45.8	21.4
21086 (s)	27	29	4	NA	3	3	-	-
21087 (s)	64	66	46	47	32	39	42.3	20.4
21090 (s)	88	106	48	135	66	100	50.0	21.3
21092 (s)	72	81	23	57	39	48	49.2	18.0
21241 (e)	133	93	27	134	66	100	36.2	20.2
21248 (e)	127	92	30	106	77	91	33.5	23.6
21249 (e)	129	104	34	167	85	126	40.9	22.0
21519 (e)	127	101	28	109	67	88	39.5	22.3
21529 (e)	132	102	31	145	71	108	42.0	23.1
21580 (e)	131	101	32	184	86	135	39.1	19.8
21982 (p)	19	62	38	26	11	19	42.1	20.9
21990 (p)	35	66	43	27	19	23	31.9	19.1
21991 (p)	29	52	24	13	10	11	37.5	22.6
21992 (p)	29	50	24	12	9	11	48.5	20.2
21993 (s)	42	77	45	34	24	29	43.9	19.9
21994 (p)	24	42	9	8	7	8	34.5	16.4
21995 (p)	29	50	26	11	8	9	40.8	19.6
21996 (p)	23	44	14	7	6	6	42.5	21.8
22058 (e)	84	58	13	41	29	35	37.2	18.5
22082 (s)	79	82	58	69	37	53	48.4	20.0
22087 (p)	27	51	17	15	4	10	40.3	17.8
22090 (s)	44	47	10	10	5	7	41.1	17.5
22285 (s)	43	75	42	32	21	27	38.7	20.4
22327 (s)	41	62	33	20	21	21	48.4	19.3
Mean	70	78	29	60	42	51	39.0	20.9
Range	15-146	21-122	4-58	0-184	1-91	1-135	31-51	16-24

Genetic analysis by molecular markers (AFLPs): Samples of 5 g of young leaves were taken of all *C. argentea* and *F. macrophylla* accessions and DNA was been extracted and quantified. To identify efficient primers for the AFLP analysis, 2 supposedly genetically contrasting accessions of each *F. macrophylla* and *C. argentea* (CIAT 21990 and 21529, and CIAT 18672 and 18516 respectively) were tested with different primer combinations and the resulting polymorphic bands were counted (Table 90).

Table 90. Polymorphic bands of different primer combinations for *Flemingia macrophylla* (accessions CIAT 21990 and 21529) and *Cratylia argentea* (accessions CIAT 18672 and 18516).

Primer combination	Polymorphic bands		
	F. macrophylla	*C. argentea*	Total
E-AAC / M-CAA	n.a.	n.a.	n.a.
E-AAG / M-CAA	n.a.	n.a.	n.a.
E-AAG / M-CAT	28 / 24	2 / 4	58
E-ACA / M-CAT	18 / 20	7 / 9	54
E-ACA / M-CTG	15 / 8	4 / 5	32
E-ACT / M-CTG	13 / 8	4 / 5	30
E-ACC / M-CAG	20 / 15	9 / 8	52
E-ACG / M-CAG	16 / 24	2 / 21	62
E-ACG / M-CAC	26 / 24	19 / 12	81
E-AGC / M-CTA	11 / 18	3 / 3	35
E-AGG / M-CTC	24 / 21	9 / 12	66
E-AAC / M-CTT	45 / 20	18 / 3	86

3.3.3 Agronomic characterization of a collection of *Rhynchosia schomburgkii*

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

Rationale

From the evaluation of a range shrub legumes with tolerance to cool temperatures *Rhynchosia schomburgkii* emerged as one of the most promising species for higher altitude hillsides. Thus, we were interested in characterizing its potential feed value (Photo 16).

Photo 16. *Rhynchosia schomburgkii* at Quilichao

Materials and Methods

A total of 13 accessions of *Rhynchosia schomburgkii*, mostly originating from Colombia, were planted at Quilichao. Plants were transplanted into single-row plots, with 4 replications. Dry matter yield, drought tolerance and forage quality are the main parameters being measured.

Results and discussion

Results from last year had indicated that of the 13 accessions evaluated, CIAT 17918, 22134, 918 and 19235 showed the highest yields. This year we were interested in measuring quality parameters in the collection of *R. schomburgkii* as affected by seasonal variation.

Results indicated that during the season with maximum rainfall, there were differences among accessions for IVDMD but not for CP (Table 91). In the drier period no significant (P>0.05) differences among accessions in terms of quality were recorded. However, season had a large effect on digestibility, but the effect was not the same for all accessions.

Table 91. Fodder quality of accessions in a collection of *Rhynchosia schomburgkii* grown in Quilichao in Minimum and Maximum precipitation.

Accession	Season					
	Minimum		Maximum			
	IVDMD	CP	IVDMD	CP	Tannins	
					Soluble	Bound
8582	42	22	42	20	4.81	0.78
19235	39	22	40	22	3.68	0.40
20800	38	21	38	21	2.49	0.84
17918	38	19	49	22	3.06	0.73
20456	38	19	52	23	3.31	0.68
22134	37	20	42	22	3.69	4.55
7389	36	22	44	23	2.95	0.67
7810	36	20	41	22	5.23	0.72
18490	36	21	40	22	3.44	0.92
918	31	19	38	22	5.85	0.72
LSD	NS	NS	5.7 (P <0.001)	NS		

The concentration of condensed tannins measured in the wet season was relatively low (2.5 to 5.8%) and not as variable as IVDMD and CP.

In general, our results show that, in the small collection of *R. schomburgkii* evaluated there is limited variability in CP and IVDMD which limits the scope for selecting genotypes based on quality.

Activity 3.4 Selection of legumes for multipurpose use in different agroecosystems

Highlights

- Accessions of *Vigna unguiculata* with specific adaptation to acid or neutral soil and more broadly adapted accessions identified

- Research on *Vigna unguiculata* carried to Honduras and Nicaragua, Participatory evaluations in preparation
- *Lablab purpureus* accessions with outstanding performance on neutral soils identified

Progress towards achieving milestones

- **Suitability of *Vigna unguiculata* for acid and neutral soils defined**
  Accessions were identified with specific and broad adaptation to variable soil pH and fertility conditions.

- **List of accessions of *Vigna unguiculata* for use as feed and/or green manure in Central America**
  A core collection of *Vigna unguiculata* from IITA is now in Honduras and Nicaragua, for participatory evaluation. Seed multiplication of promising accessions is underway in Costa Rica.

- **Results on characterization of a core collection of *Lablab purpureus* in acid and neutral soils**
  The *Lablab purpureus* accessions evaluated were well adapted to acid low fertility soils, eventhough productivity was much lower than on neutral higher fertility soils. However, there is intra-specific variation in adaptation to soil and climate conditions. Some accession have more specific adaptation while other accessions showed a more broad adaptation. The next step is to carry out more detailed studies with a limited number of accessions, focusing on small farmers in Colombia and Central America. For comparison, we are trying to obtain seed of available commercial cultivars (cv. Endurance Rongai, Highworth and Koala) from Australia.

3.4.1 Evaluation of a core collection of *Vigna unguiculata* for multipurpose uses in Colombia, Nicaragua and Honduras

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

CIAT projects: PE-2, PE-3

Quilichao and Palmira

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. Work of CIAT with a limited number of accessions had indicated potential of cowpea for soil improvement, 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.

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. It remains to be seen if cultural traditions allow for the inclusion of cowpeas in the daily menu of people in Central America.

A) Evaluation of cowpea in Quilichao and Palmira, Colombia

Materials and Methods

A core collection of 15 cowpea accessions was obtained from Dr. B.B. Singh, cowpea breeder of IITA and complemented with two local accessions from Colombia (cultivar Sinu) and Brazil (cultivar Verde Brasil). After initial experiments on acid soils (Annual report 2000), these accessions were again planted at CIAT's Quilichao Research Station. Accessions were evaluated for grain and forage yield and their value as green manure for a succeeding maize crop (Photo 17).

Photo 17. *Vigna unguiculata* in grass production phase at Quilichao

Results and Discussion

In the Quilichao site, all accessions established rapidly, reaching soil covers of >80%, 8 weeks after planting. At the time of incorporation into the soil (9 weeks after planting) all accessions were well established and vigorous. No significant differences ($P>0.05$) were found among accessions for DM yields (Table 92).

However, significant differences ($P<0.05$) were found in maize dry matter production and in grain yield following the incorporation of cowpea accessions. Highest maize yields were recorded after green manuring with IT93K-573/5, with yields being 3.6 t/ha grain and almost 9 t of dry matter. In contrast, with no N grain and dry matter yields were 1.5 t and 4.1 t, respectively. Fertilizations higher than 80 kg N had a negative effect on maize grain and dry matter yields.

Results confirm data obtained in the initial experiments (AR 2000). All green manure treatments except IT96D-759 led to higher maize yields than obtained with any level of nitrogen fertilizer applied.

Table 92. Dry matter yield (kg/ha) of cowpea green manure herbage and grain before soil incorporation and grain and dry matter yield of a following maize crop in Quilichao, 2nd phase.

Accessions	Cowpea	Maize	
	Herbage	Grain	DM Total
	(kg/ha)		
IT93K-573/5	3180	3619	8882
IT90K-284/2	2187	3558	8442
IT89KD-391	2387	3382	7576
IT95K-1088/4	2033	3350	7765
IT86D-716	2293	3290	8433
IT95K-1088/2	2213	3255	7779
IT86D-715	3313	3280	8308
IT6D-733	2867	3192	8993
IT96D-740	1940	3104	7321
IT90K-277/2	1913	3067	7520
IT93K-503/1	2047	2868	7219
IT86D-719	2393	2803	8238
IT93K-637/1	2613	2636	6338
IT89KD-288	3513	2558	6728
IT96D-759	1047	2331	5194
80N	-	2405	5213
160N	-	2104	4337
200N	-	2094	4360
0N	-	1487	4105
40N	-	1478	3577
120 N	-	1330	3785
LSD (P<0.05)	NS	1862	3804

Forage quality of cowpea accessions in terms of CP, lignin, digestibility, P and Ca concentrations varied among accessions (Table 93). Nevertheless, with CP concentrations of 14-21 % and a digestibility of dry matter of 80% or more cowpea is also an excellent fodder for livestock (Table 93).

Table 93. Fodder quality in accessions of *Vigna unguiculata* (cowpea) grown in Quilichao.

Accessions	Forage					Grain		
	Protein	IVDMD	Lignin	P	Ca	N	P	K
				%				
IT86D-715	21	80	4.5	0.14	2.1	4.19	0.36	1.22
IT90K-277/2	19	82	2.5	0.12	2.1	3.00	0.28	1.00
IT93K-573/5	19	82	2.7	0.13	1.5	3.71	0.30	1.13
IT96D-740	18	83	5.9	0.13	1.6	3.20	0.33	1.20
IT90K-284/2	18	81	2.4	0.13	1.6	3.41	0.36	1.28
IT96D-733	17	84	4.4	0.12	1.5	3.37	0.33	1.22
IT86D-719	17	83	4.2	0.11	1.8	3.58	0.35	1.25
IT93K-673/1	17	85	2.7	0.13	1.5	3.47	0.34	1.25
IT93K-503/1	17	83	2.1	0.12	1.3	3.16	0.31	1.17
IT95K-1088/2	16	85	4.5	0.11	1.4	3.39	0.35	1.18
IT89KD-391	16	82	1.7	0.10	2.1	3.28	0.33	1.27
IT95K-1088/4	16	84	3.4	0.14	1.6	3.42	0.36	1.27
IT89KD-288	15	85	2.6	0.09	1.4	3.47	0.30	1.16
IT86D-716	14	86	5.6	0.10	1.3	3.78	0.35	1.3
LSD (P<0.05)	3.1	2.66	1.2	0.02	0.61			

In a 3rd phase, cowpea accessions were sown in the same season in Quilichao and Palmira to compare the effect of climate and soil on performance and possibly identify accessions with broad adaptation, which is key for Central American Hillsisdes with highly variable soil and climatic conditions.

The establishment of the accessions of cowpea included in the trial was slower in Palmira than in Quilichao, due to higher incidence of insects and weeds. In Quilichao, the incidence of pest and diseases was minimal, with the exception of a localized incidence of ants.

Results showed that no differences in DM yields among accessions in the two sites (Table 94). However, mean dry matter yield in Quilichao (2229 kg/ha) was 30% higher than in Palmira (1752 kg/ha). In addition, we observed a G x E interaction in performance of accessions tested.

For example, accessions IT86D-715 and IT89KD-391 had high DM yields on the acid soils in Quilichao, but were among the lowest yielding accessions on the more neutral fertile soils in Palmira. However, other accessions such a IT95K-1088/4 had high dry matter yields in the two sites.

Table 94. Dry matter yield (kg/ha) of cowpea green manure herbage and grain before soil incorporation en Quilichao and Palmira, 2001.

Accessions	Quilichao	Palmira
	DM Herbage (kg/ha)	
IT86D-715	3147	1280
IT89KD-288	2653	1873
IT6D-733	2567	1627
IT89KD-391	2413	1187
IT95K-1088/4	2373	2480
IT93K-503	2353	1627
IT96D-740	2230	1947
IT90K-277/2	2220	1307
IT86D-716	2187	1807
IT90K-284/2	2080	1900
IT93K-573/5	1993	1493
IT95K-1088/2	1827	2040
IT93K-637/1	1813	1927
IT86D-719	1773	2326
LSD (P<0.05)	1,130	1,252

B) Evaluation of cowpea in Nicaragua

Materials and Methods

A core collection of 19 accessions of *Vigna unguiculata* (Table 95) was established at the SOL SECO site (the Spanish acronym for Supermarket of technologies for hillsides – dry) in San Dionisio, Matagalpa, Nicaragua. The accessions were replicated three times in a randomized block design. Plots measured 5 x 2.5 m and seeds were sown at a distance of 0.25 m within a row and 0.5 m between rows.

Table 95. Accessions of *Vigna unguiculata* sown in San Dionisio/Nicaragua and Yorito/Honduras and Nicaragua as green manures for maize-based systems; *Lablab purpureus* DICTA was sown in Honduras only as a local check.

Accessions	Accessions
Vigna unguiculata IT86D-277/2	*Vigna unguiculata* IT86D-715
Vigna unguiculata IT90K-284/2	*Vigna unguiculata* IT96D-740
Vigna unguiculata IT89KD-391	*Vigna unguiculata* IT95K-1088/4
Vigna unguiculata IT86D-716	*Vigna unguiculata* IT89KD-288
Vigna unguiculata IT93K-503/1	*Vigna unguiculata* IT93K-573/5
Vigna unguiculata IT93K-637/1	*Vigna unguiculata* CIDDICO1
Vigna unguiculata IT86D-719	*Vigna unguiculata* CIDDICO2
Vigna unguiculata IT95K-1088/2	*Vigna unguiculata* CIDDICO3
Vigna unguiculata IT6D-733	*Lablab purpureus* DICTA

Evaluations will include seed emergence, ground cover, plant height, plant vigour, biomass/grain production flowering patterns, and incidence of pest and disease. Local farmers will be invited to participate in the evaluation of the core collections and soil fertility enhancement effects will be measured through the planting of a maize crop at the onset of the next wet season and comparing maize yields with N-fertilized plots.

Expected results

The selection of superior accessions based on agronomic performance on a farmer criteria will provide a clear indication on the potential of *Vigna unguiculata* in farming systems found in Hillsides of Central America. Further evaluations with the selected accessions will be necessary in order to optimize management techniques.

C) Evaluation of cowpea in Honduras

In 2001, an experiment was established in the SOL Yorito to evaluate a core collection of cowpea (Table 96). In this case the experiment was complemented with the addition of *Lablab purpureus* DICTA as a control.

In Yorito, the focus is again on selecting cowpeas for green manures in maize-based systems for soils with neutral to alkaline pH. Significant ($P<0.0001$) differences among accessions were found for DM yield. The highest biomass production was recorded with CIDICCO3 (6.2 t of DM/ha) and IT90K-284/2 (6.1 t of DM/ha).

In general, the ranking of accessions compares favourably with results obtained on neutral soils in Palmira though yields are much higher in Honduras.

Table 96. Dry matter yields of *Vigna unguiculata* (cowpea) genotypes before soil incorporation before a maize crop in Yorito, Honduras.

	DM yield	
	Soil cover (%)	kg/ha
CIDICCO3	93	6212
IT90K-284/2	90	6123
CIDICCO1	83	5282
Lablab purpureus DICTA	85	5230
IT96D-740	72	5112
IT93K-637/1	72	5101
IT86D-716	67	4944
IT95K-1088/2	72	4042
IT95K-1088/4	68	3923
IT6D-733	50	3827
IT93K-503/1	60	3672
CIDICCO2	78	3521
IT93K-573/5	50	2926
IT89KD-391	43	2867
IT89KD-288	53	2734
IT86D-719	38	2381
IT86D-715	37	2175
IT90K-277/2	33	1754
LSD (P<0.05)		2014

3.4.2 Evaluation of core collection of *Lablab purpureus* for multipurpose uses (Quilichao and Palmira)

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

Rationale

Lablab purpureus is a free seeding, fast growing or 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 lower altitudes and 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 to evaluate the Lablab collection in acid and neutral soils to define niches of Lablab for 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 total of 44 accessions of *Lablab purpureus* were initially sown on an acid soil (pH 4.0) in the Quilichao Research Station for seed multiplication. In 2001, 42 and 25 accessions were planted for agronomic evaluation in neutral (Palmira), and acid soil (Quilichao), respectively (Photo 18).

Photo 18. Seed of *Lablab purpureus* at Quilichao

Results and Discussion

Results of the agronomic evaluations are shown in Tables 97 and 98. In Quilichao (Table 97), accessions 14442, I 14411, I 14437, 76996, 21603 and 99985 were the fastest to establish, with soil cover of >95 % and vigour ratings of 4 to 5, 12 weeks after sowing. Of the 25 accessions sown 15 (60%) had a soil cover above the mean of the experiment (87% soil cover). As expected, early flowering accessions were less productive than late flowering accessions.

The highest yields were recorded with accessions CIAT 34777, 52535 and 21603, and the lowest yields were recorded for T 52508, 17192, I-6536 and I-11613. However, it is interesting to note that accession I-6536, which had very low yields at 12 weeks, became one of the most productive accessions, 4 weeks later.

Plant vigour and yields of Lablab in Palmira (Table 98) were higher than in Quilichao. In this site significant ($P<0.05$) differences among accessions were found for DM yield and soil cover. The accessions with fastest soil cover and ability to compete with weeds measured 8 weeks after planting were 14442, I-11630, I-14437, 29398, 76996, 34777, I-6533, I-14411, L-987 and 106494.

In general, the accessions with the best adaptation across different soil and climate conditions were 34777, 96924, 21603, I-11630, I-14411, I-14441, 67639 and 52535.

Table 97. Dry matter yield (kg/ha) and soil cover (%) of *Lablab purpureus* herbage in Quilichao, 2001.

Accessions	Vigour	Cover (%)		DM (kg/Ha)	
	1 a 5	12 Weeks	16 Weeks	12 Weeks	16 Weeks
34777	4	83	50	2447	2153
52535	4	77	57	2440	1973
21603	5	97	90	2327	2367
I-11632	3	73	50	2067	1453
76998	4	93	77	2060	1607
99985	5	95	97	2027	1927
106494	5	87	93	2013	1153
36903	3	73	53	1920	1453
I-14411	5	100	93	1913	1573
100602	4	80	87	1887	1627
I-14437	4	98	83	1853	2447
I-11630	5	70	100	1840	2093
14442	5	100	97	1840	2667
67639	4	93	80	1820	2313
CQ-2975	4	93	87	1807	2046
106548	4	90	93	1793	1833
81626	3	80	60	1707	2073
106500	4	93	90	1640	1560
76996	5	98	90	1560	1873
I-14441	4	90	73	1493	2067
I-6533	3	77	70	1426	1273
I-11613	4	92	93	1280	1387
I-6536	5	93	97	1213	1980
17192	2	80	77	1160	1060
52508	3	67	50	1147	1053
22183				.	1453
LSD (P<0.05)		22.5	16.4	878	NS

Table 98. Dry matter yield (kg/ha) and soil cover (%) of *Lablab purpureus* herbage in Palmira, 2001.

Treatment	Vigour	cover(%)		DM kg/ha	
	1 a 5	8 weeks	13 Weeks	8 Weeks	13 Weeks
35894	2	93	70	3493	9067
I-14437	4	97	97	3280	7760
I-11615	2	87	60	3293	7340
34777	4	97	77	3380	6933
96924	2	83	77	2840	6927
21603	4	93	100	3760	6847
I-11630	5	100	100	4807	6740
2160	3	87	77	2707	6707
I-14411	4	97	93	3080	6587
I-14441	4	87	95	2420	6547
67639	4	87	97	2780	6533
29398	4	97	93	3447	6527
L-987	5	77	100	2367	6407
52535	3	93	73	3360	6340
I-6533	4	97	87	3193	6300
52544	3	87	83	2413	6187
106494	4	97	100	2440	6120
100602	3	87	90	2113	5660
L-1683	5	77	100	2367	5500
76998	4	93	93	3433	5480
76996	4	97	93	3213	5440
81626	2	83	53	2487	5433
CQ-2975	4	80	90	2387	5407
17197	3	57	80	1560	5193
I-6536	3	73	90	2000	5173
I-11613	4	87	90	2780	5027
I-11632	2	83	63	2793	5007
36903	3	93	60	2660	4900
106548	4	77	90	2067	4747
14442	5	100	97	2367	4640
99985	3	90	63	3113	4587
I-6930	2	83	57	2247	4527
52508	2	77	47	2473	3927
22183	4	83	93	2100	3853
106500	3	73	100	1373	3827
69498	2	63	73	1387	3747
17196	2	50	77	867	2987
51564	2	40	73	500	2880
17193	1	43	60	680	2020
17192	1	23	43	393	1147
17195	1	17	73	187	1140
17189	1	20	20	247	853
LSD (P<0.05)		35.2	47.3	2514	5557