3.3 Genotypes of Brachiaria with adaptation to poorly drained soils

Highlights

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

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

3.3.1 Genotypic variation in waterlogging tolerance of *Brachiaria* genotypes

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

Rationale

In the tropics, *Brachiaria* pastures during the rainy season occasionally face waterlogging conditions that severely limit pasture productivity and animal performance. Waterlogging or flooding reduces the availability of soil oxygen to the plant. In 2005, we developed a screening method to evaluate waterlogging tolerance of 15 *Brachiaria* hybrids and 5 checks. Waterlogging for 21 days resulted in senescence and death of a great proportion of shoot biomass of majority of hybrids and affected development of adventitious roots in some hybrids. The hybrid BR02NO/1245 was particularly outstanding in its ability to maintain green leaf area and to develop adventitious roots.

Results on living leaf area and living leaf biomass production per pot indicated that one of the 5 checks, *B. humidicola* CIAT 6133, was outstanding in its level of tolerance to waterlogging. Two other checks, *B. humidicola* CIAT 679 and *B. brizantha* CIAT 26110 also showed greater level of waterlogging tolerance in terms of green leaf area and green leaf biomass. Among the 15 hybrids tested BR02NO1245 was markedly superior in waterlogging tolerance than the other hybrids. In late 2005, we screened 60 *Brachiaria* genotypes with an objective to quantify genotypic variation in waterlogging tolerance in *Brachiaria*.

Material and Methods

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

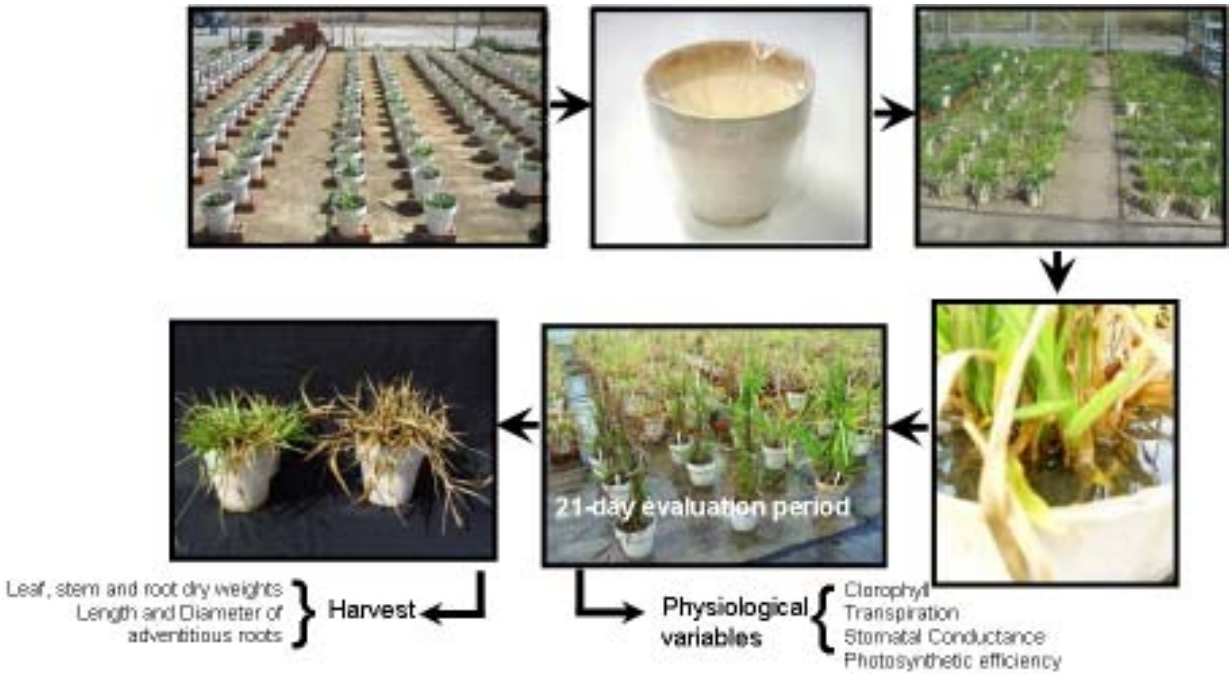

Photo 5. Different steps involved for inducing waterlogging treatment for 21 days and identification of waterlogging tolerant genotypes.

The trial was planted as randomized complete block with 3 replications. Each experimental unit consisted of one pot filled with 3.5 kg of fertilized top soil (0-20 cm) from Santander de Quilichao's Oxisol and sown with two vegetative propagules (stem cuttings). An adequate amounts of fertilizer were supplied (kg ha^{-1}: 80 N, 50 P, 100 K, 66 Ca, 28 Mg, 20 S, 2 Zn, 2 Cu, 0.1 B and 0.1 Mo) to soil at the time of planting. Plants grew for 50 days under 100% field capacity of soil moisture.

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

Results and Discussion

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

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

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

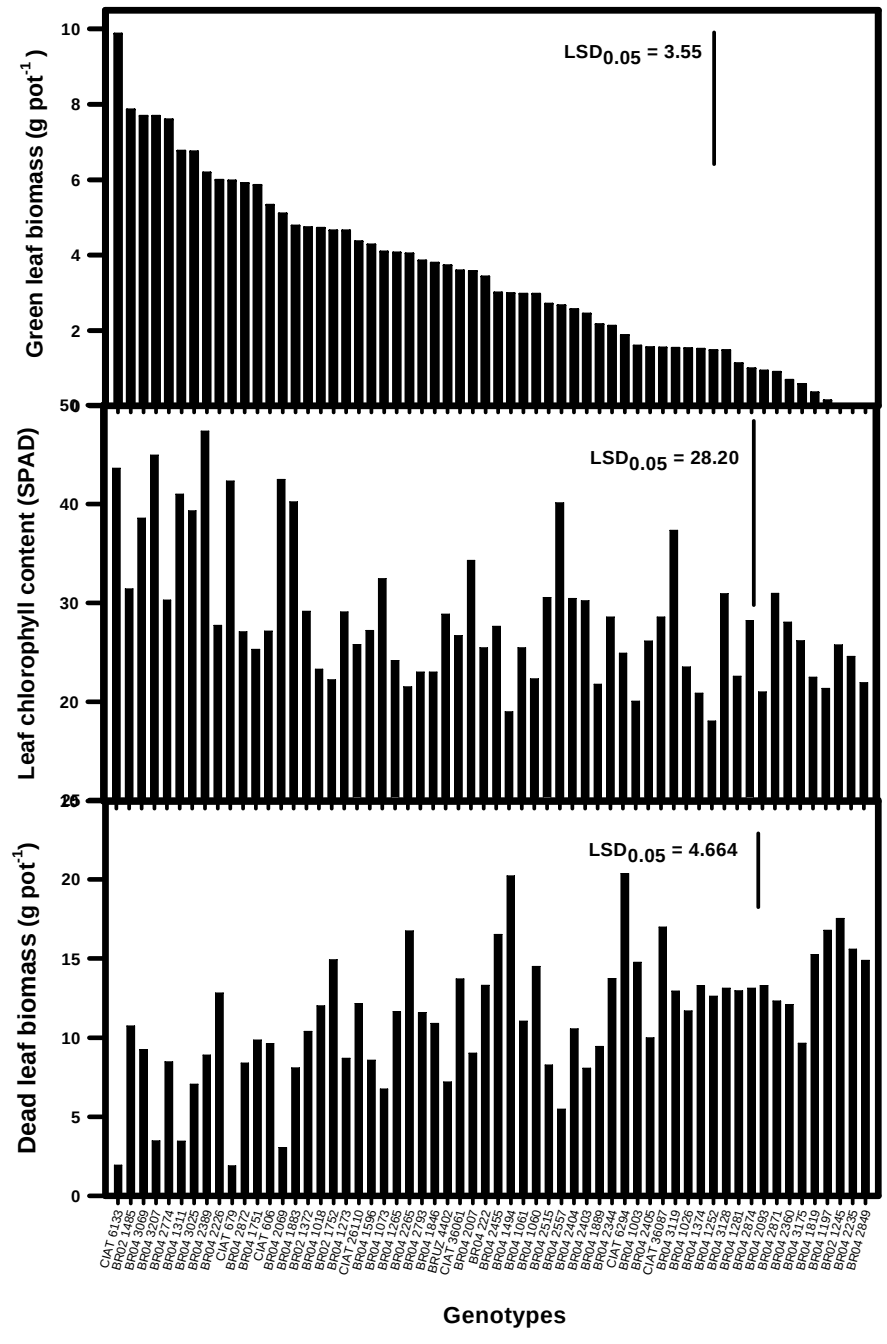

Figure 27. Influence of waterlogging on genotypic variation in green leaf biomass production, leaf chlorophyll content (SPAD) and dead leaf biomass (g pot^{-1}) of three parents (CIAT 606, 6294, *B.ruziziensis* 44-02), nine checks (CIAT 6133, 679, 26110, 36061 and 36087, BR02NO/1485, 1752, 1245, 1372) and 48 hybrids, of population BR04 of *Brachiaria*, grown in an Oxisol from Santander de Quilichao, Colombia. Plant attributes were measured at 21 days after waterlogging. LSD values are at the 0.05 probability level.

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

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

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

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

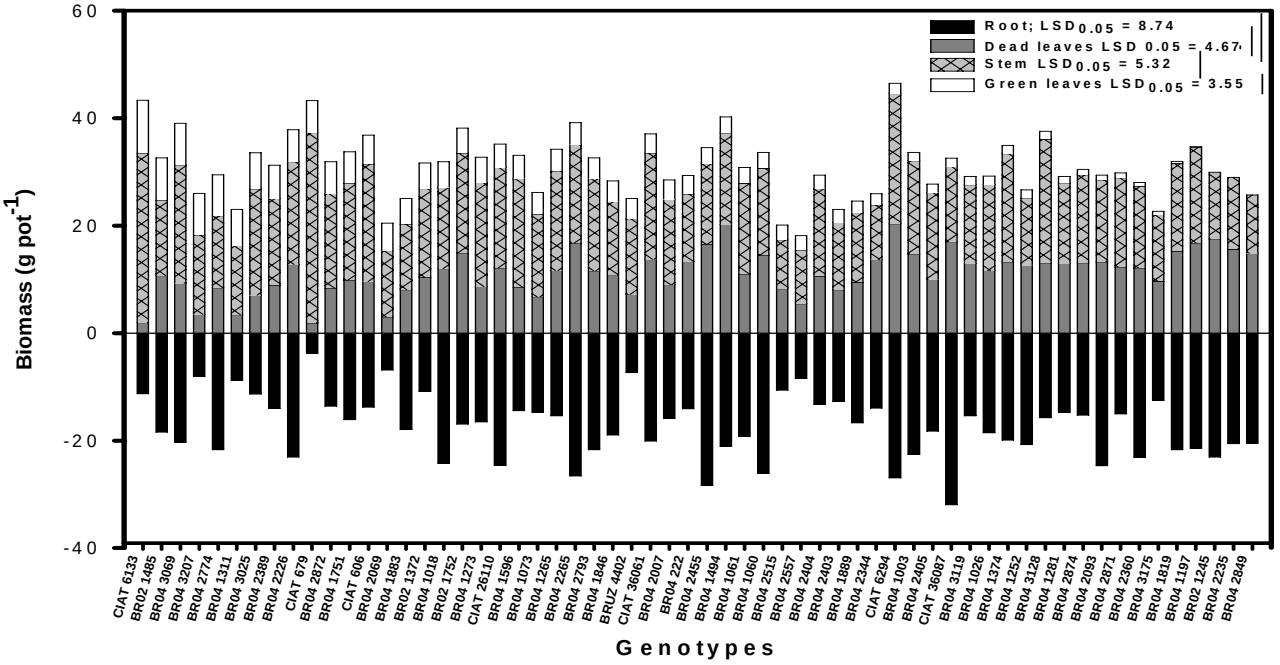

Figure 28. Influence of waterlogging on genotypic variation in biomass distribution among leaves, stems and root production of three parents (CIAT 606, 6294, *B.ruziziensis* 44-02), (CIAT 6133, 679, 26110, 36061 and 36087, BR02NO/1485, 1752, 1245, 1372) and 48 hybrids, of population BR04 of *Brachiaria*, grown in an Oxisol from Santander de Quilichao, Colombia. Plant attributes were measured at 21 days after waterlogging. LSD values are at the 0.05 probability level.

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

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

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

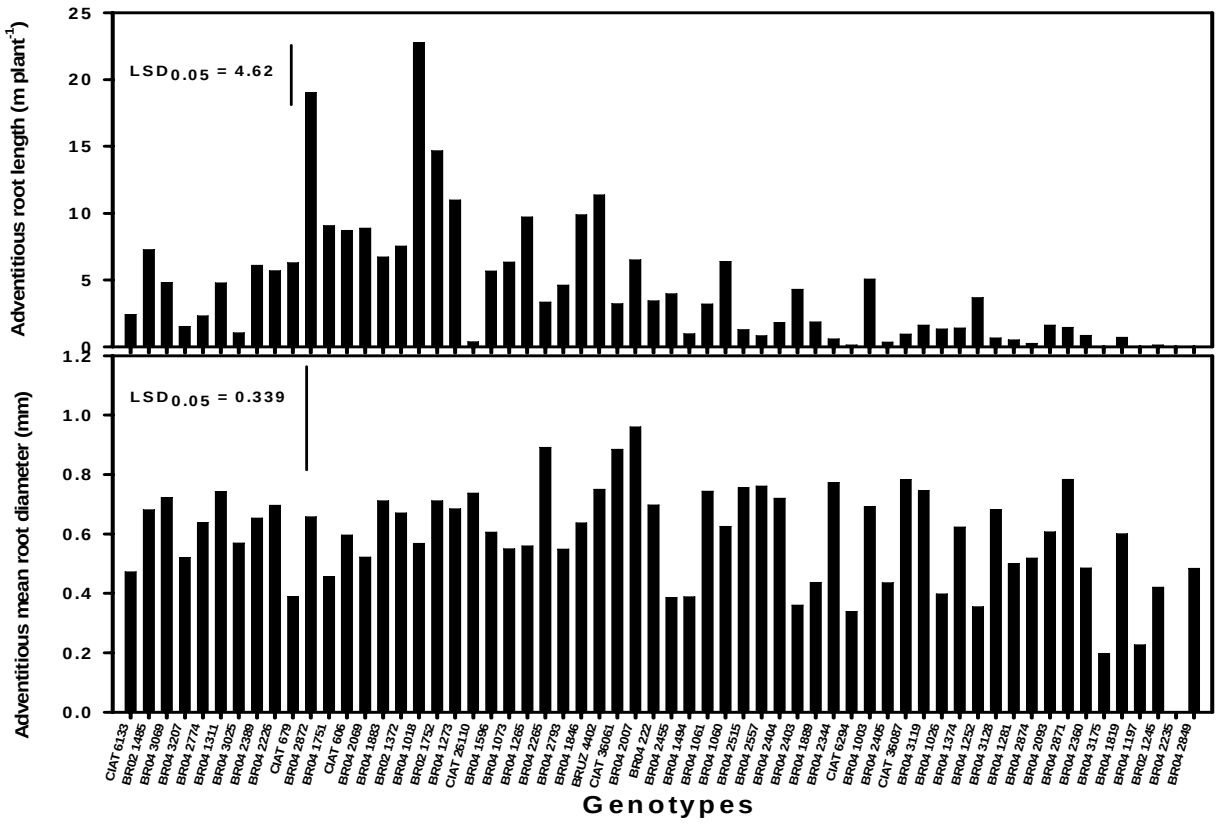

Figure 29. Influence of waterlogging on genotypic variation in adventitious root length production (m pot^{-1}) and mean root diameter of three parents (CIAT 606, 6294, *B.ruziziensis* 44-02), (CIAT 6133, 679, 26110, 36061 and 36087, BR02NO/1485, 1752, 1245, 1372) and 48 hybrids, of population BR04 of *Brachiaria*, grown in an Oxisol from Santander de Quilichao, Colombia. Plant attributes were measured at 21 days after waterlogging. LSD values are at the 0.05 probability level.

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

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

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

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

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

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

Conclusions

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

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

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

Rationale

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

Material and Methods

A pot experiment was conducted outside in the Forages patio area of CIAT Palmira between May 26 to June 20, 2006 to determine differences in tolerance to waterlogging among 121 *Brachiaria* genotypes (109 hybrids – SX05NO series; 3 parents - *B. decumbens* CIAT 606; *B. ruziziensis* 44-02; *B. brizantha* CIAT 6294; and 9 checks – *B. humidicola* CIAT 679; *B. humidicola* CIAT 6133; *B. brizantha* CIAT 26110; Brachiaria hybrid cv. Mulato CIAT 36061; Brachiaria hybrid cv. Mulato II CIAT 36087; BR02NO/1485; BR02NO/1372; BR02NO/1245; BR02NO/1752). The trial was planted as completely randomized block with 3 replications. Each experimental unit consisted of one pot filled with 3.5 kg of fertilized top's soil (0-20 cm) from Santander de Quilichao's Oxisol and sown with two vegetative propagules (stem cuttings). An adequate fertilizer was supplied (kg ha^{-1}: 80N, 50P, 100K, 66Ca, 28Mg, 20S, 2Zn, 2Cu, 0.1B and 0.1Mo) to soil at the time of planting. Plants were grown for 50 days under field capacity conditions. Waterlogging treatment was imposed by an excessive water supply (5 cm over soil surface) for 24 days. After inducing waterlogging treatment, leaf chlorophyll content (SPAD), stomatal conductance (mmol $m^{-2}s^{-1}$), transpiration rate (mmol $m^{-2}s^{-1}$) were measured at weekly intervals on a full-expanded young leaf that was marked at the initiation of waterlogging treatment. At the end of the 24 days of treatment, green leaf area (cm^2 $plant^{-1}$), green leaf biomass (g $plant^{-1}$), total shoot and root biomass (g $plant^{-1}$), root volume (cm^3), root length (m $plant^{-1}$) and mean root diameter (mm) of adventitious roots were measured.

Results and Discussion

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

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

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

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

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

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

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

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

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

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

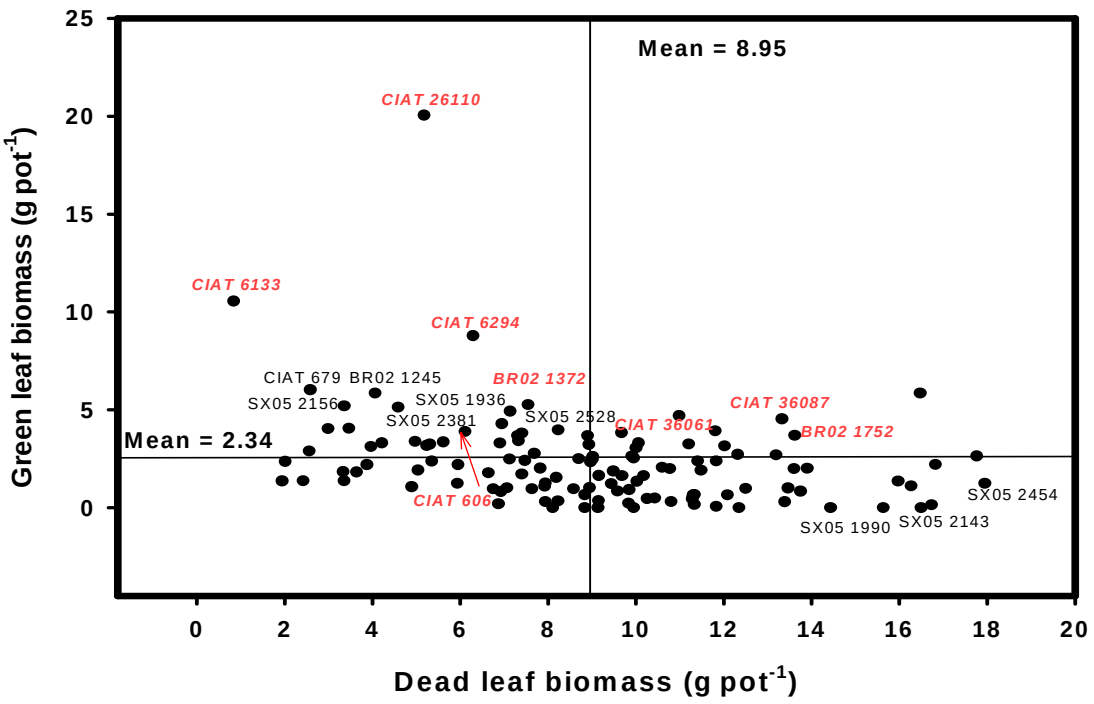

Figure 30. Relationship between green leaf biomass and dead leaf biomass of 109 sexual hybrids, 3 parents and 9 checks of *Brachiaria* after 24 days of waterlogging in an Oxisol from Santander de Quilichao, Colombia.

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

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

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

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

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

Conclusions

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

3.4 Biological Nitrification Inhibition (BNI) in tropical grasses

Highlights

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

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

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

3.4.1 Biological nitrification inhibition (BNI) is a widespread phenomenon

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

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

conclusively demonstrate *in situ* inhibition of nitrification by chemicals released in the plant-soil system, due to lack of an appropriate methodology.

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

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

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

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

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Rationale

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

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

Materials and Methods

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

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

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

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

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

Results and Discussion

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

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

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

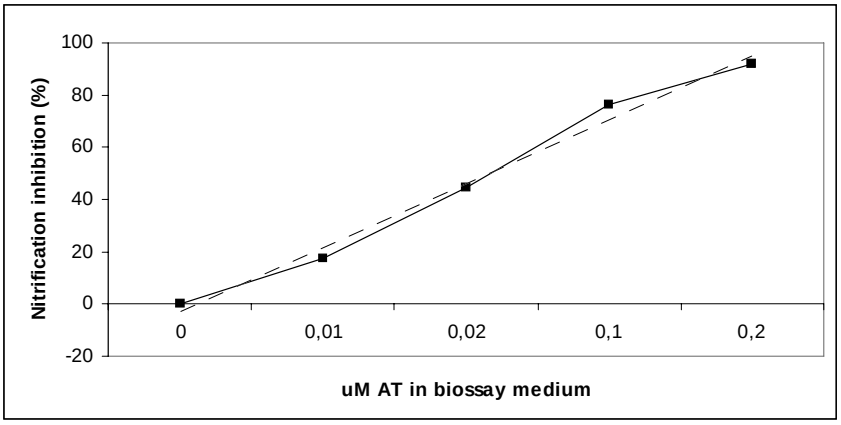

Figure 31. Transgenic *Nitrosomonas europea* response to synthetic nitrification inhibitor, allylthiourea (AT) in the bioassay medium.

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

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

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

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Rationale

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

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

Materials and Methods

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

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

inhibitory activity using a specific bioassay developed at JIRCAS.

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

Results and Discussion

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

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

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

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

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

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

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

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

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

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

Conclusions

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

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

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

Rationale

A range of Biological Nitrification Inhibition (BNI) activity has been observed for diverse accessions of *B. humidicola* and other tropical grasses under glasshouse conditions, as part of collaborative research between JIRCAS and CIAT. As a continuation of these research efforts, a long term field experiment was planned to validate the phenomenon of BNI under field conditions and test the hypothesis that the BNI activity is a cumulative factor in soils under species that release the BNI activity from root exudates. Given the vast areas that are currently grown with tropical grasses, an understanding of the BNI process and the possibility of managing it

to improve N use efficiency, reduce nitrate pollution of surface and ground waters as well as reduce net impact on global warming through reduced emissions of nitrous oxide, could have potentially global implications. Various tropical grasses showing a varying degree of BNI activity were selected for the experiment and a soybean crop and a tropical grass (*P. maximum*) that lacks the BNI activity were selected as controls.

Materials and Methods

The field experiment was initiated in September 2004 at CIAT-HQ at Palmira, Colombia on a fertile clayey Vertisol (pH 6.9), and with an annual rainfall of 1000 mm and mean temperature of 25 ^{0}C. Two accessions of *B. humidicola* were used: the commercial reference material CIAT 679, which has been used for most of our previous studies, and the high NI activity *B. humidicola* accessions CIAT 16888. The *Brachiaria* Hybrid cv. Mulato was included for having moderate NI activity and *Panicum maximum* var. common was used as a negative non-inhibiting control. Soybean (var. ICAP34) is also used as a negative control due to its known effect on promoting nitrification. A plot without plants is used as an absolute control.

Treatments were placed in plots of 10 m x 10 m with three replications and distributed in a completely randomized block design. Soybean was planted from seeds and the grasses were propagated from cuttings. Soybean was inoculated with the *Rizhobium* strain CIAT 13232 to favor biological nitrogen fixation. Irrigation was provided to the field as required and two applications of broadcast fertilization were made at 30 and 60 days after planting on each plot, except within two 1 m^2 subplots demarcated in each plot, where the same levels of fertilizer were applied in solution to favor a more homogeneous distribution of the applied nutrients within the soil. Each application consisted of an equivalent dose of (kg ha^{-1}): 48N, 24K, 8P, 0.2Zn, 0.2B. The nitrogen source was ammonium sulfate. Weed control was done using Glyphosate in the bare soil plots and in the soybean plots before planting. During the soybean growing cycle manual weeding was done in such plots.

At harvest, soybean plants including roots were removed from the field when they had reached full maturity and the grain was already dry. The plants were separated into roots, shoots and grain, and a representative subsample taken for measuring dry matter content and N analysis. Plants of *P. Maximum* were cut at approximately 20 cm height twice during the crop cycle. From each cut a representative subsample collected for dry weight and N analysis. The *Brachiaria* Hybrid cv.Mulato was cut at 20 cm height while the *B.humidicola* accessions were cut at 10 cm height. Similar procedure used for cv. Mulato was used for *P.maximum*. At harvest time, soil was carefully collected in the rizhopane of all species with an auger from the top 10 cm of the soil within each subplot. Five samples were collected in each subplot and pooled to obtain a composite sample. Samples were carefully managed and only the soil adhered to the roots was removed and used for soils analysis. Once the rhizosphere soil was collected, it was allowed to air dry and then was finely ground to < 0 – 1 mm mesh. Soil was analyzed for nitrate and ammonium content using KCl extracts and colorimetric determination. Gas samples for measuring N_2O fluxes were collected monthly.

Results and Discussion

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

In Figure 32 we show the differences in total shoot biomass harvested during the fourth crop cycle (March – July 2006). The results between treatments presented significant differences (LSD, $p<0.001$). Total shoot biomass yields of *P. maximum* and the *B. humidicola* accessions were similar but greater than that of the biomass

from cv. Mulato and soybean. The decreased vigor of cv. Mulato was found to be due to reduced N availability in soil. Soybean had a total shoot biomass markedly lower than that of *B. humidicola* 679 and *B. humidicola* 16888. The growth of the *B. humidicola* accessions had been stimulated with the ammonium sulfate application as nitrogen source.

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

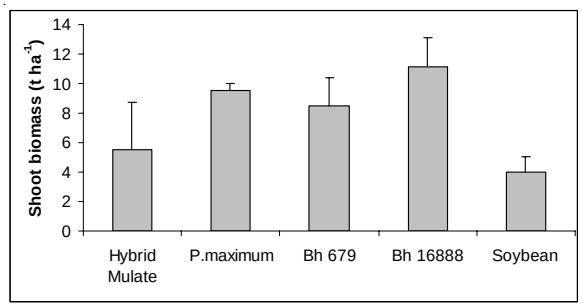

Figure 32. Shoot biomass production during the fourth cropping cycle (March -August 2005).

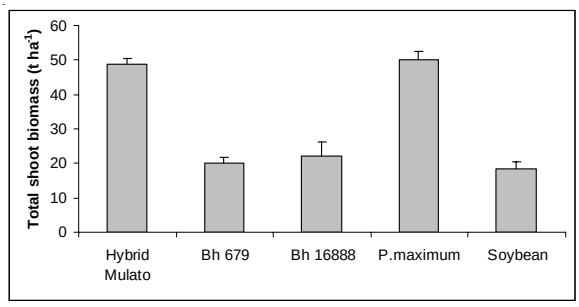

Figure 33. Total dry biomass production over the period of September 2004 and July 2006.

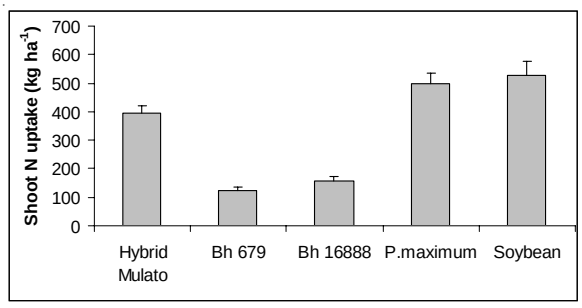

Figure 34. Total shoot nitrogen uptake for the period September 2004 and July 2006.

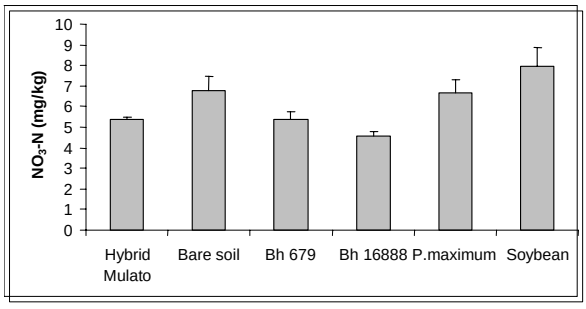

Figure 35. Nitrate levels in the top soil (0-10 cm) at harvest time of the fourth cropping cycle.

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

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

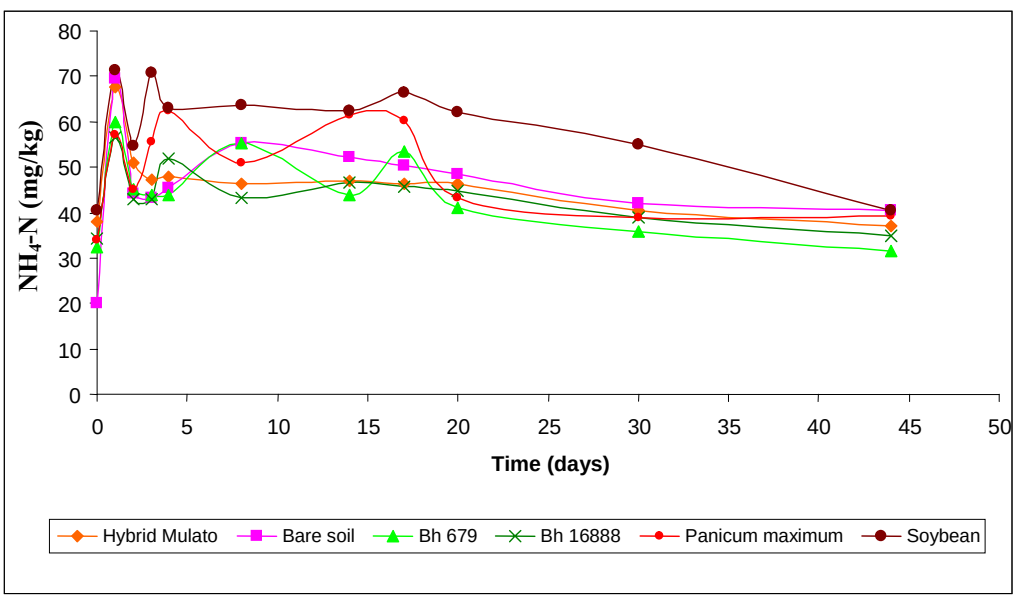

Figure 36. Ammonium to nitrate ratios in soil.

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

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

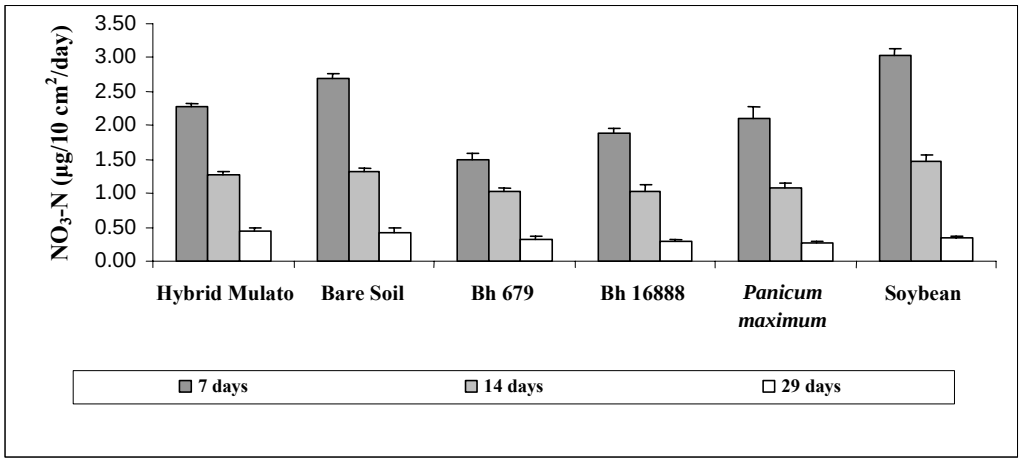

Figure 37. Nitrate retained in ion exchange resins over a 7, 14 and 29 days period after fertilizer application.

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

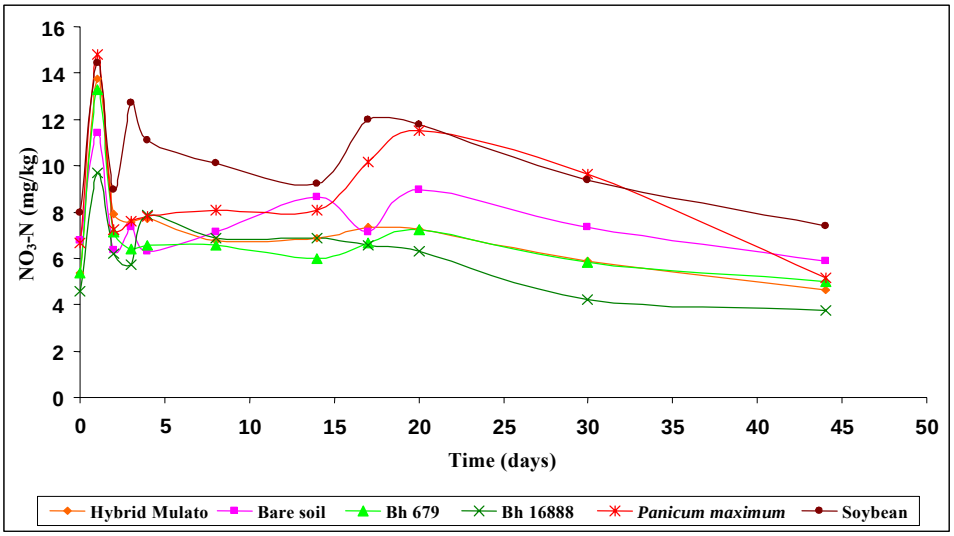

Figure 38. Nitrate levels in the top soil (0 – 10 cm) after fertilizer application.

Nitrous oxide emissions: Results on the behavior of the net fluxes of N_2O during 30 days after fertilizer application (March 31/2006 – May 4/2006) are shown in Figure 39. The highest concentrations were obtained at 1 and 3 days after fertilizer application while the lowest concentrations were observed at 20 days after fertilizer application. The fluxes were highest in the bare soil plots. These results support the view that B. *humidicola* is effectively inhibiting the nitrification process. However, P.*maximum* also showed lower net emissions of N_2O but this may be attributable to the much higher nitrogen uptake by the plants which may limit the total amounts of N available for nitrification, assuming that the grass is able to take up N from the soil in ammonium form.

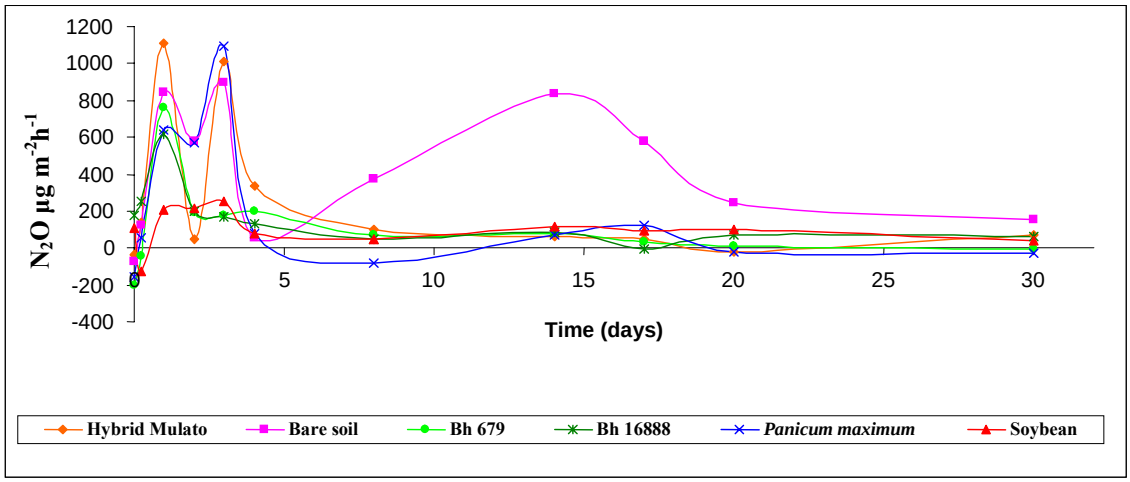

Figure 39. Net fluxes of N_2O during 30 days after fertilizer application.

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

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

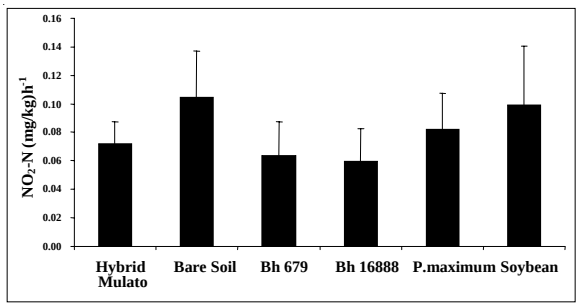

Figure 40. Average nitrification rates from incubated soils during 30 days after fertilizer application.

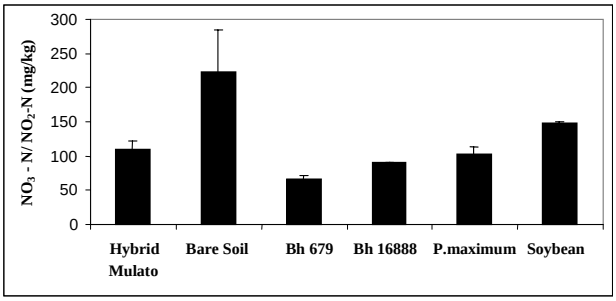

Figure 41. Nitrate to nitrite ratios in soil.

Conclusions

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

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

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

Rationale

In soil, nitrogen (N) available to plants in the form of nitrates (NO_3^-) does not bind to soil particles due to its negative charge. Therefore, this form of N is highly susceptible to leaching and thereby it is lost from the system. Nearly 70% of the N fertilizer applied to agricultural soils is lost due to the nitrification process (oxidation of the relatively immobile ammonium - NH_4^+ - into the

highly mobile nitrate - NO_3^- -N). In the same way, the loss of N from soil to the atmosphere in the form of other compounds, cause a negative impact on the environment by contributing to worldwide global warming and the greenhouse effect. Moreover, the soil N that is lost by leaching and/or denitrification can be substantial, which promotes pollution of ground water.

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

Materials and Methods

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

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

Results and Discussion

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

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

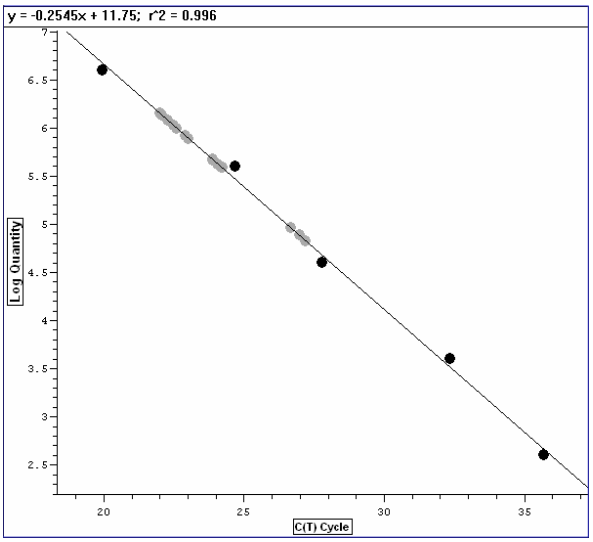

Figure 42. Standard curve generated from *E. coli* gDNA to quantify the copy number of the bacteria *amoA* gene.

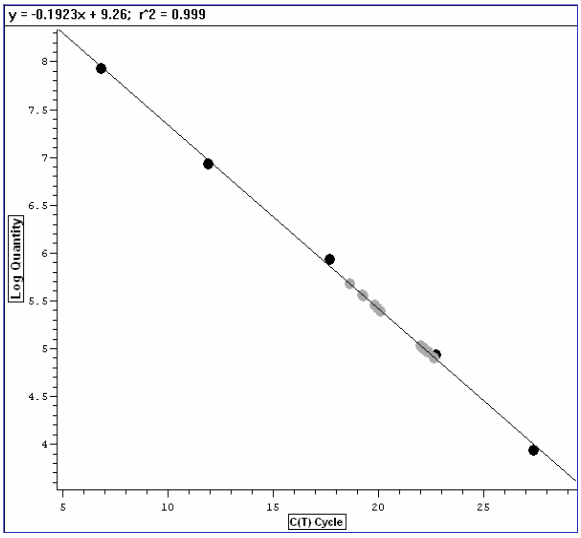

Figure 43. Standard curve generated from plasmid DNA to quantify the copy number of the *Archaea* 16S rRNA gene.

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

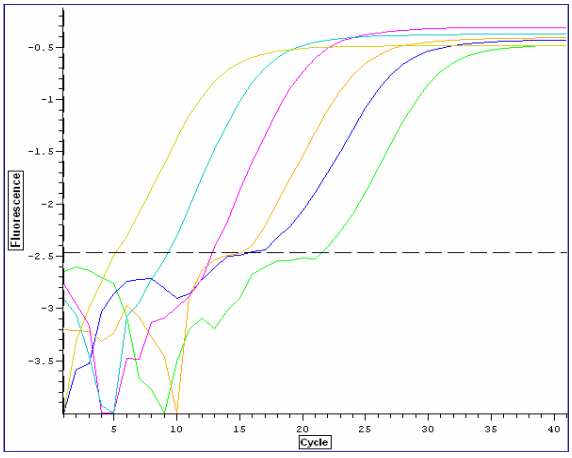

Figure 44. Fluorescence plot of PCR products of bacteria *amoA* gene using diluted *Nitrosomonas* genomic DNA

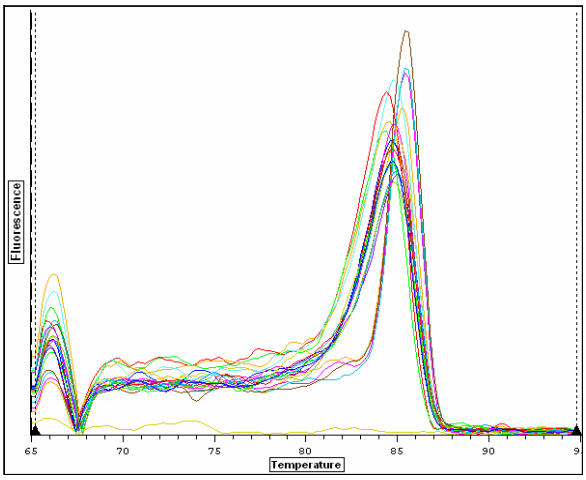

Figure 45. Melting curve of PCR products using a primer set for Archaea 16S rRNA gene
The DNA template used for this experiment was a diluted plasmid DNA containing Archaea 16S rRNA.

Our results suggest that the PCR technique developed is precise and reproducible for monitoring soil bacterial populations. For the soil samples, we have isolated good quality DNA from the soil samples that were described above. Currently, we are implementing this technique along with a detailed statistical analysis to monitor effects of BNI in the field conditions on bacterial populations of ammonium oxidizing bacteria and *Archaea*.

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

Highlight

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

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

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

Rationale

The work of CIAT on shrub legumes focuses on development of materials to be utilized as a feed supplement during extended dry seasons. Tropical shrub legumes of high quality for better soils are readily available, but germplasm with similar characteristics adapted to acid, infertile soils is scarce. On the other hand, shrub legumes so far selected for acid soils do not perform well in mid altitudes (1200-2000 m.a.s.l.). For example, *C. argentea* is well adapted to acid soils but normally does not perform well in altitudes above 1200 m.a.s.l. One alternative for sites with acid soils and mid altitudes is *Flemingia macrophylla*. However its use has been limited due to poor quality and palatability. The research reported in this section had two main objectives: 1) Define the diversity available within the genus *Flemingia* and 2) Identify new, forage genotypes with superior agronomic traits and forage quality parameters.

Material and Methods

A collection of 63 accessions of *Flemingia* spp., originating in Asia was established in 2004 at CIAT's research station in Santander de Quilichao together with 3 control accessions (CIAT 21083, 21090 and 21092) which had been evaluated previously (Photo 6).

Passport information of all accessions was presented in the annual report 2004. Accessions were planted in jiffy pots in the green house and inoculated with adequate *rhizobium* strains; transplanting into the field was done after 8 weeks. Six months after transplanting plants were well established with good vigor and low incidence of pests and diseases. A standardization cut to 40 cm above soil was done, but some accessions did not reach cutting height during the establishment phase.

Results and Discussion

Accessions were grouped according to growth habit: (a) e = erect (24 accessions) and (b) se = semi-erect (42 accessions). For the semi-erect accessions the number of branches varied between 2 and 14, while for the erect accessions 3 to 17 branches per plant were counted (Table 31). Vigor varied strongly among accessions and also among plants within accessions

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

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

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

Accession No. CIAT	Vigor	Height	Diameter	Branches	Accession No. CIAT	Vigor	Height	Diameter	Branches
	1-5	cm		No.		1-5	cm		No.
	Semi - Erect					Erect			
21556	4	63	125	14	21093	5	141	143	17
19392	5	101	122	13	22060	4	95	96	16
19803	5	95	114	13	21117	5	101	129	15
17406	4	101	121	13	21112	5	121	134	15
17401	3	44	57	12	21113	4	106	108	14
21492	4	78	104	12	21116	5	124	137	14
19802	4	99	110	12	17403	5	90	137	13
20629	4	90	110	12	21115	5	123	136	13
22047	3	55	107	12	22094	4	88	127	13
17408	5	97	109	11	21094	4	117	123	13
17410	4	94	107	11	21090	4	102	104	12
20628	5	112	121	11	22057	4	98	108	12
21104	3	58	78	11	21118	4	79	115	12
19393	4	85	109	11	22063	5	108	125	11
7966	5	85	105	9	21111	5	121	111	10
17453	4	90	112	9	22169	4	107	101	10
19455	4	61	117	9	21084	4	105	101	9
22083	3	60	100	9	21109	4	104	91	7
19796	4	86	108	9	21091	4	95	61	6
21100	2	43	67	9	21083	4	112	93	6
20627	3	69	100	9	22058	5	119	67	5
21101	3	62	90	9	21096	3	77	55	4
22043	4	48	129	9	21089	3	72	46	3
19456	4	59	100	8	21092	2	59	39	3
21098	3	79	66	8					
21103	3	66	86	8					
21106	3	53	85	8					
21099	3	58	72	8					
21102	3	60	86	7					
19458	3	55	90	7					
21097	3	45	73	6					
22064	3	46	60	6					
21105	3	55	78	5					
22092	2	48	57	5					
19625	1	25	73	5					
21107	2	39	54	5					
19626	1	31	44	4					
19627	1	24	41	4					
17402	1	49	40	3					
19623	1	25	49	3					
22084	2	72	46	3					
18046	1	27	20	2					
Range	1-5	24-112	20-125	2-14		2-5	59-141	39-143	3-17

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

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

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

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

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

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

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

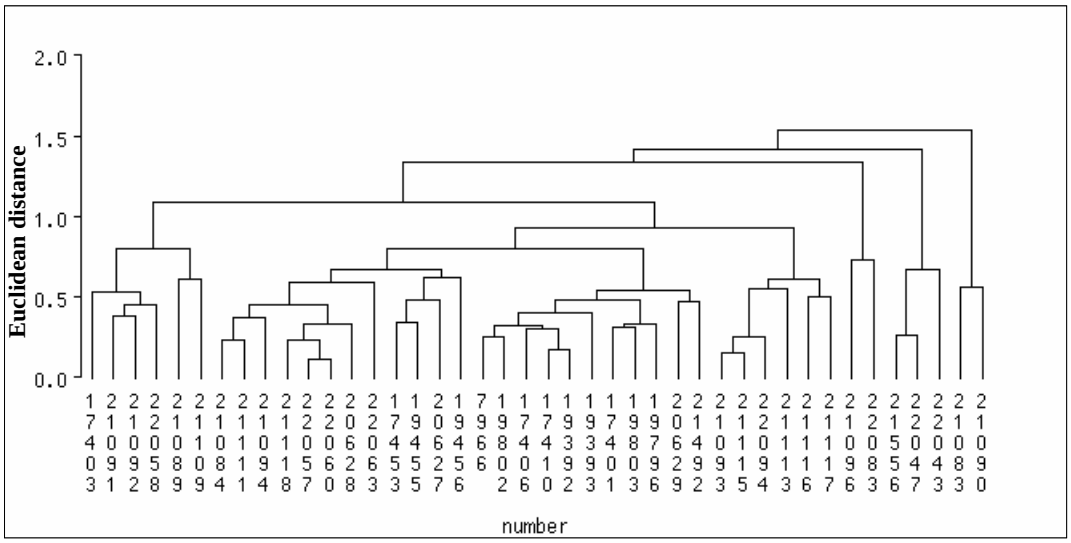

Figure 46. Dendrogram of a collection of *Flemingia* spp.

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

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

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

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

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

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

Rationale

Previous research had shown *Leucaena diversifolia* and *Leucaena trichandra* are well adapted to acid and infertile soils and mid altitudes (up to 2000 m.a.s.l.), in contrast to the widely used *Leucaena leucocephala* that does not perform well under these conditions. However, the evaluation of *L. diversifolia* and *L. trichandra* up to now has been restricted to

only few accessions. A collection (61 accessions) of the two species, was put together in order to screen for genetic diversity in agronomic traits and nutritive value. Moreover, the study aims to find morphological differences both between the two species as well as among accessions.

Materials and Methods

The collection of *L. diversifolia* (50 accessions) and *L. trichandra* (11 accessions) was planted in jiffy pots in the green house. After eight weeks, seedlings were transplanted (June, 2005) to the CIAT station in Santander de Quilichao (Photo 7). A randomized block with four replications was employed; three replications were used for agronomic and forage quality evaluation, and one for morphological characterization. Each replication consisted of five plants per accession. The plants were spaced 1 m, with 1.5m between rows. Fertilization was (kg/ha) P40, K50, Mg20, and S20. *Rhizobium* bacteria were applied in the field as a mixture of water and bacteria, given to each plant directly into the soil. During the first eight weeks after transplanting plants were irrigated when necessary to safeguard the establishment of the trial. Equally pesticides were applied when pests appeared to threaten the survival of the plants.

Agronomic evaluation included regular assessment every four weeks of plant vigor and symptoms of nutritional deficiencies, pests and diseases. Three months after planting, plant height, and diameter and growth habit were recorded. The first cut in three replications was done on October, 2005, at a height of 0.5m above the ground. In the fourth replication morphological evaluation was carried out four month after transplanting, and comprised the assessment of leaf morphology (form, color, pubescence, and glands), leaf area, and flowering and growth habit.

Results and Discussion

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

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

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

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

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

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

Continues…..

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

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

* CIAT 17503 check

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

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

Based on the available results, accessions CIAT 17248 and 17249 appear to be of highest interest as they combine good DM yield and forage quality. Both originate from the state of Chiapas

in Mexico, characterized by dry environments with a precipitation of 850 mm rain per year. In view the high forage digestibility in contrast to other *Leucaena diversifolia/L. trichandra* accessions it would be necssary to study the composition of the tannins. Accessions for higher altitudes need to be defined.

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

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

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

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

Rationale

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

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

Materials and Methods

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

A randomized block with four replications was employed; three replications were used for agronomic and forage quality evaluation, and one for morphological characterization. Each replication consisted of five plants per accession. The plants were spaced 1 m, with 1.5 m between rows. Fertilization was (kg/ha) P40, K50, Mg20, and S20. During the first eight weeks after transplanting plants were irrigated when necessary to safeguard the establishment of the trial. Equally pesticides were applied when pests appeared to threaten the survival of the plants.

Agronomic evaluation included regular assessment every four weeks of plant vigor and symptoms of nutritional deficiencies, pests and diseases. Five months after planting, plant height, plan diameter and growth habit were recorded (Table 37).

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

Results and Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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