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

3.1 Genotypes of *Brachiaria* with adaptation to edaphic factors

Highlights

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

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

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

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

3.1.1 Edaphic adaptation of *Brachiaria*

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

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Perennial brachiaria grasses (*Brachiaria* spp. Griseb.) are the most widely sown forage grasses in tropical America. CIAT and EMBRAPA are developing interspecific hybrids to combine traits of three parental species: acid-soil adaptation of signalgrass (*B. decumbens*) and spittlebug resistance of palisadegrass [*B. brizantha* (A. Rich) Staff], both tetraploid apomicts, and sexual reproduction of a tetraploidized, sexual biotype of ruzigrass (*B. ruziziensis* Germain &Evard), which lacks both agronomic traits. Efficient screening methodologies are required to recover the desired traits through stepwise accumulation of favorable alleles in subsequent cycles of selection and recombination. Edaphic adaptation is particularly difficult to select for because it is only manifest in the persistence of pastures over several growing seasons. The following is the summary of research accomplishments for the past few years towards

the development of tools and knowledge that aid the genetic improvement acid soil tolerance in brachiariagrasses.

Aluminum toxicity and other acid-soil constraints

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

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

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

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

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

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

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

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

Immobilization of P by Al within plant tissues could be prevented through compartmentalization of Al in vacuoles (roots, shoots) or inhibition of root-to-shoot translocation of Al (shoots; see previous section). Alternatively, a range of metabolic adjustments and accelerated P recycling could increase the efficiency with which P is taken up and/or used in Al-stressed plants. A recent study showed that, when grown on a low-P soil, the *Brachiaria* hybrid cv. Mulato had a higher P use efficiency (PUE: biomass produced per amount of P taken up) than wheat or rice. In contrast to wheat and rice, PUE of the *Brachiaria* hybrid increased under more severe P deficiency and soil acidity. The *Brachiaria* hybrid synthesized larger amounts of organic acids such as oxalate and fumarate in leaves. Phosphorus deficiency further increased organic acid accumulation, presumably as a consequence of a twofold increase in leaf phospho*enol*pyruvate carboxylase (PEPC) activity and a decreased the malate inhibition ratio of the enzyme. This PEPC stimulation was even more pronounced in *Brachiaria* roots,

where it could play a role in the synthesis of organic acids exuded by roots to aid P acquisition from poorly soluble sources such as Al phosphate. Phosphorus deficiency also induced phosphohydrolases in the *Brachiaria* hybrid and enhanced the partitioning of photosynthate into amino acids and organic acids at the expense of carbohydrates. These metabolic adjustments appeared to generate a larger pool of inorganic P than in other species such as rice, which apparently stimulated P turnover and enabled the hybrid to use P more efficiently.

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

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

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

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

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

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

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Rationale

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

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

The objective of this study as part of an output from a special project funded by ZIL-SDC, Switzerland was to investigate a possible link between the P nutritional status and physiological markers for P stress in *B. decumbens* (signalgrass - well adapted to P deficient acid

soils) and *B. ruziziensis* (ruzigrass - less adapted to P deficient acid soils). In terms of root function, we examined the contribution of both organic acid exudation and acid phosphatase secretion during the development of P deficiency in plant tissue. In addition to these biochemical markers, we also evaluated changes in growth (biomass and root length production) as well as root biomass allocation which might be considered as morphological markers for P stress.

Materials and Methods

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

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

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

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

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

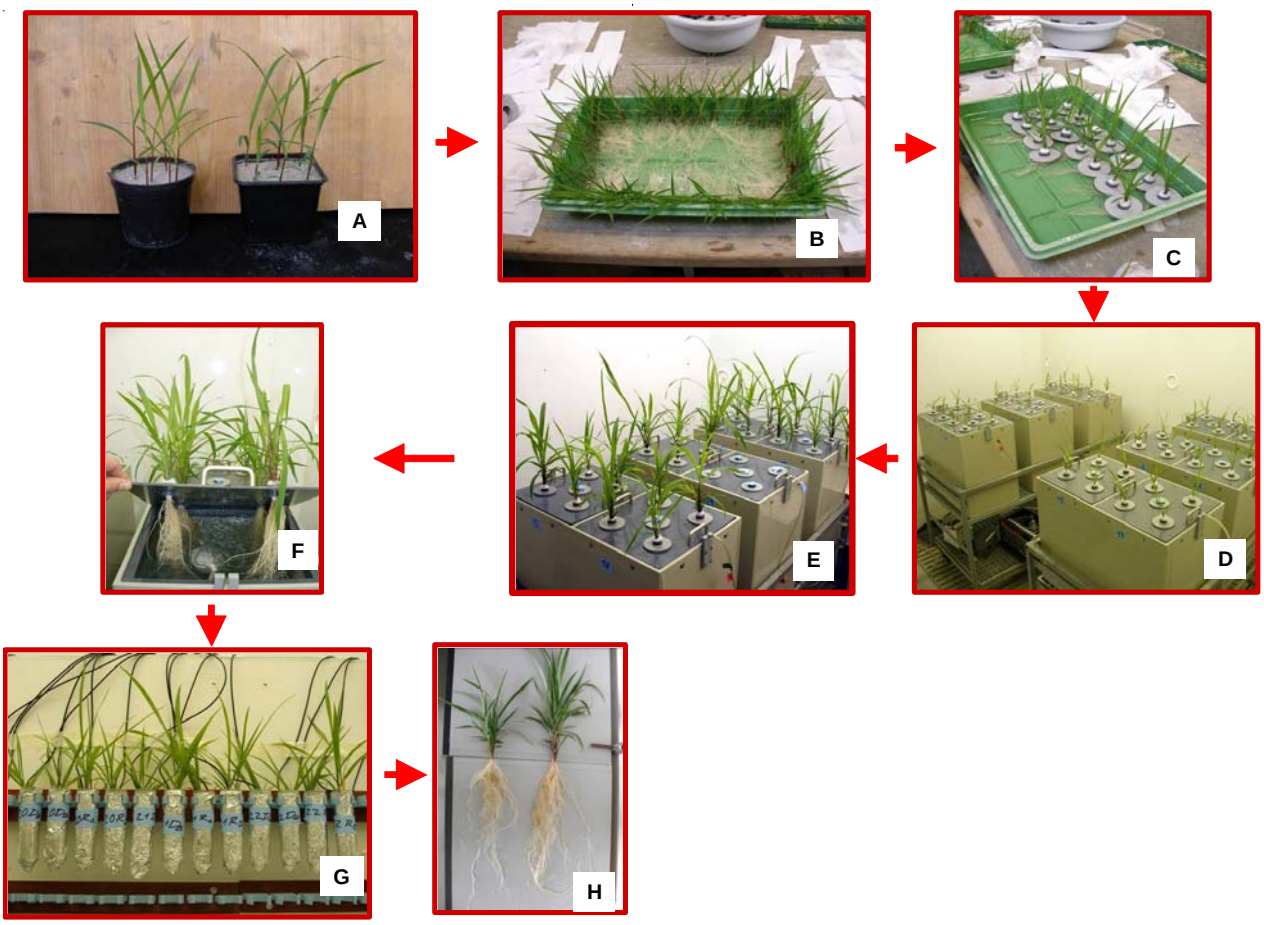


Photo 3. A scheme showing different steps involved for the growth of *Brachiaria* plants in hydroponic conditions and the collection of root exudates: **A.** Pre-grown seedlings (10-12 days old) in sand culture under minimal fertilization; **B.** Washed seedlings ready for selection and seedlings are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

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

Results and Discussion

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

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

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

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

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

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

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

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

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

Role of oxalate exudation in improving acid soil adaptation: Recently, more attention has been focused on the role of oxalate exudation in improving plant abiotic stress resistance, such as detoxification of aluminum and other heavy metals and overcoming P deficiency. Oxalate exudation in response to P deficiency has been

reported to dominate in plant species that are considered to be extreme oxalate accumulators, e.g., sugar beet and spinach. However, recently a strong relationships between the exudation of oxalic acid and plant P deficiency was demonstrated in other plant species, e.g., in soybean and in lowland rice. Furthermore, several lines of evidence indicate that the exogenous application of oxalate to P deficient soils or rock phosphate increases P solubility and therefore P acquisition. Our results on brachiaria grasses are supportive of a role for oxalic acid in the P nutrition of both *Brachiaria* grass species.

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

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

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

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

Conclusions

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

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

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

3.1.1.3 Screening of *Brachiaria* hybrids for resistance to aluminum

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

Rationale

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

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

Materials and Methods

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

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

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

Only two incomplete sets of SX05 plants were evaluated in the presence of Al in solution. Mean values from all the experiments are reported. Stem cuttings of hybrids and checks were rooted in a low ionic strength nutrient solution in the glasshouse for nine days. Equal numbers of stem cuttings with about 5 cm long roots were transferred into a solution containing 200 µM $CaCl_2$ pH 4.2 (reference treatment) and a solution containing 200 µM $CaCl_2$ and 200 µM $AlCl_3$ pH 4.2 (Al treatment).

The solutions were changed every second day to minimize pH drifts. At harvest, on day 21, after transfer, root systems were harvested. Roots were stained and scanned on a flatbed scanner. Image analysis software (WinRHIZO) was used to determine root length and average root diameter.

Results and Discussion

As reported for the past 5 years (see IP- 5 AR), Al resistant hybrids combine greater values of total root length per plant with lower values of mean root diameter relative to the mean values of the population when exposed to 21 days with toxic level of Al in solution.

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

The relationship between root length with Al vs root length without Al, root diameter with Al vs root diameter without Al, root length with Al vs root diameter with Al and root length without Al vs root diameter without Al are shown in Figures 6 to 9. Among these nine hybrids, BR05NO/0406 was markedly superior to *B. decumbens* in terms of fine root production as revealed by total root length and the lower value of mean root diameter in the presence of Al.

Root vigor in terms of root length in the absence of Al in solution was greater for 26 hybrids (BR05NO/0406, BR05NO/0563, BR05NO/0334, BR05NO/0830, BR05NO/0120, BR05NO/0048, BR05NO/0537, BR05NO/0549, BR05NO/0707, BR05NO/0351, BR05NO/0117, BR05NO/1019, BR05NO/1467, BR05NO/0577, BR05NO/0760, BR05NO/0267, BR05NO/0746, BR05NO/1435, BR05NO/0150, BR05NO/0377, BR05NO/0159, BR05NO/0265, BR05NO/0605, BR05NO/0990, BR05NO/0115 and BR05NO/0586) compared with *B. decumbens*.

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

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

Continues…

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

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

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

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

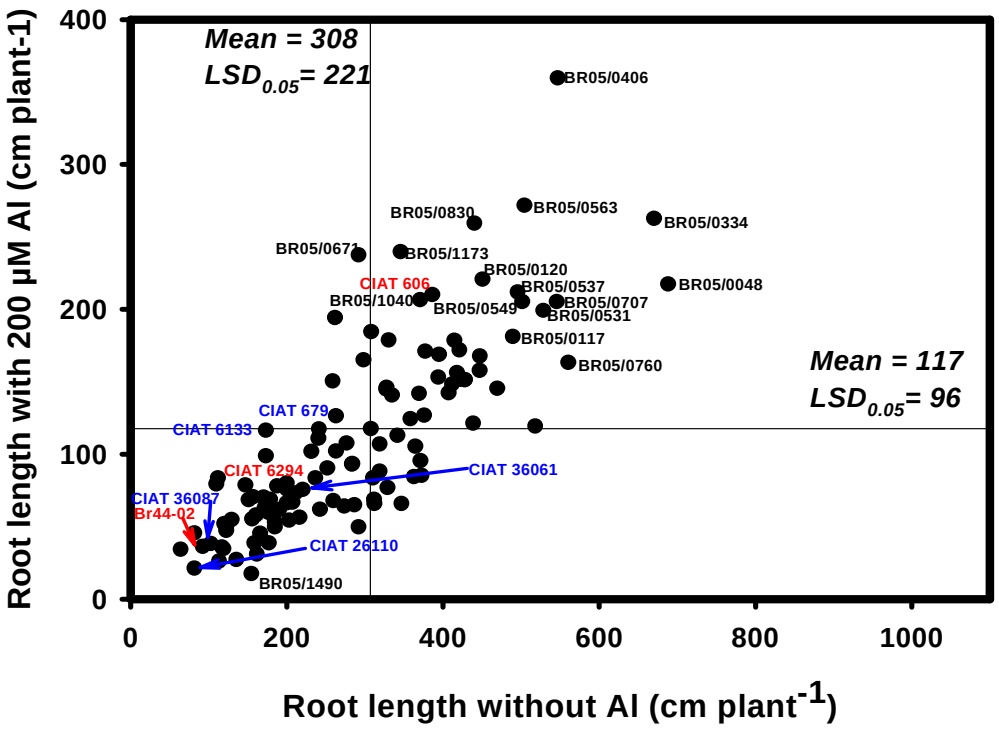


Figure 6. Relationship between total root length with Al and total root length without Al in solution of 103 hybrids, 3 parents and 8 checks of *Brachiaria*. Genotypes that developed greater root length under both conditions were identified in the upper, right hand quadrat.

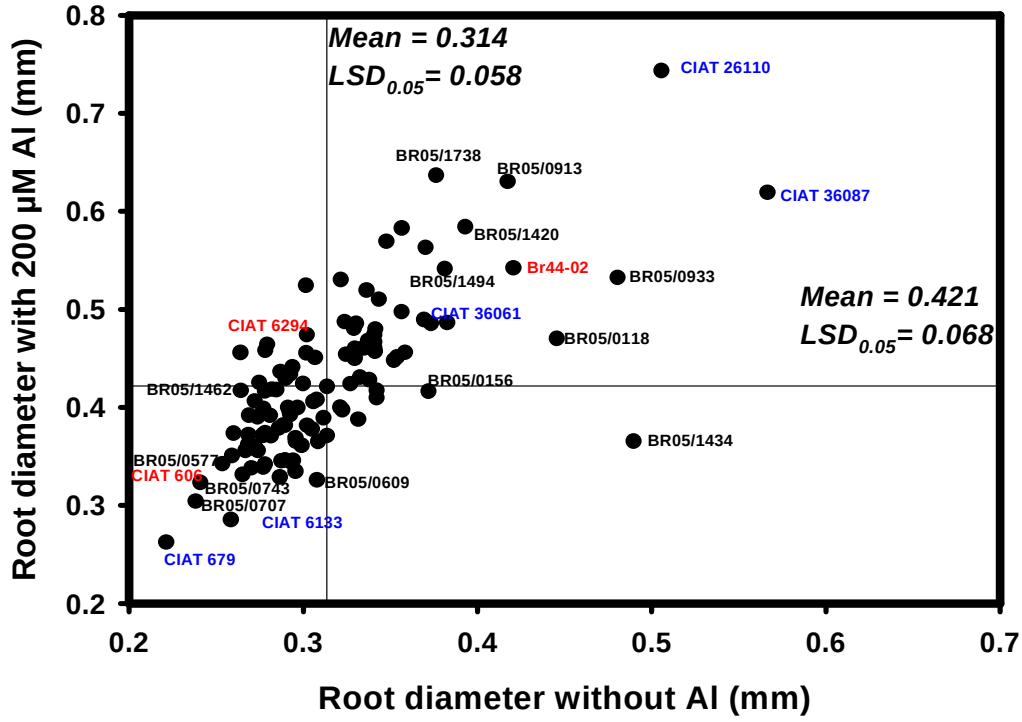


Figure 7. Relationship between mean root diameter with Al and mean root diameter without Al of 103 hybrids, 3 parents and 8 checks of *Brachiaria*. Genotypes that developed finer roots under both conditions were identified in the lower, left hand quadrat.

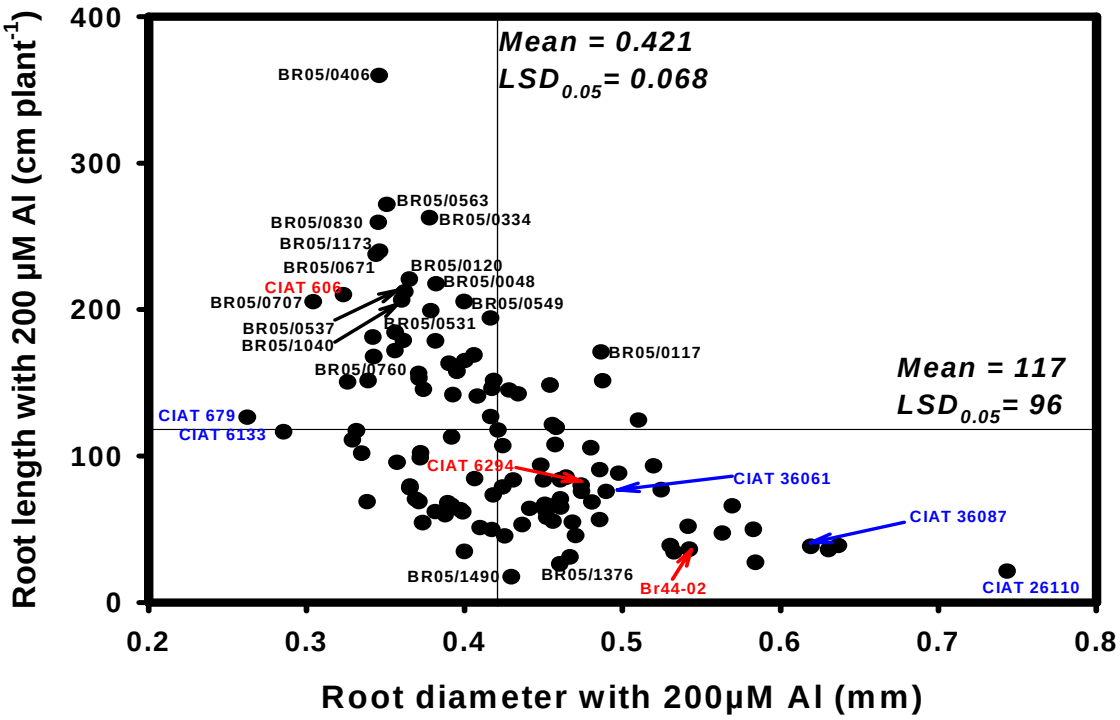


Figure 8. Relationship between total root length and mean root diameter of 103 hybrids, 3 parents and 8 checks of *Brachiaria* with presence of aluminum in solution. Genotypes that develop finer longer roots were identified in the upper, left hand quadrat.

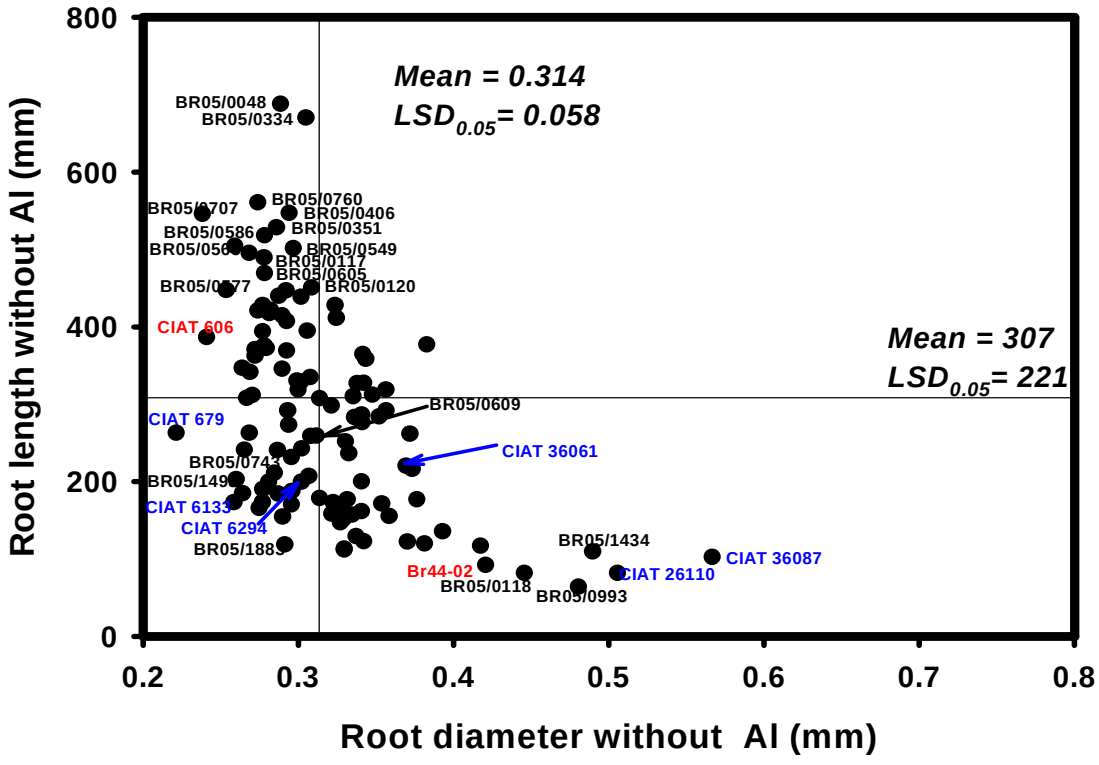


Figure 9. Relationship between total root length and mean root diameter of 103 hybrids, 3 parents and 8 checks of *Brachiaria* with absence of aluminum in solution. Genotypes that develop finer longer roots were identified in the upper, left hand quadrat.

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

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

Relationships between root length with Al vs root length without Al, root diameter with Al vs root diameter without Al, root length with Al vs root diameter with Al and root length without Al vs root diameter without Al are shown in Figures 10 to 13. Root vigor in terms of root length in the absence of Al in solution was similar or slightly superior for 2 hybrids (RZ05NO/3158 and RZ05NO/3378) compared with *B. decumbens*.

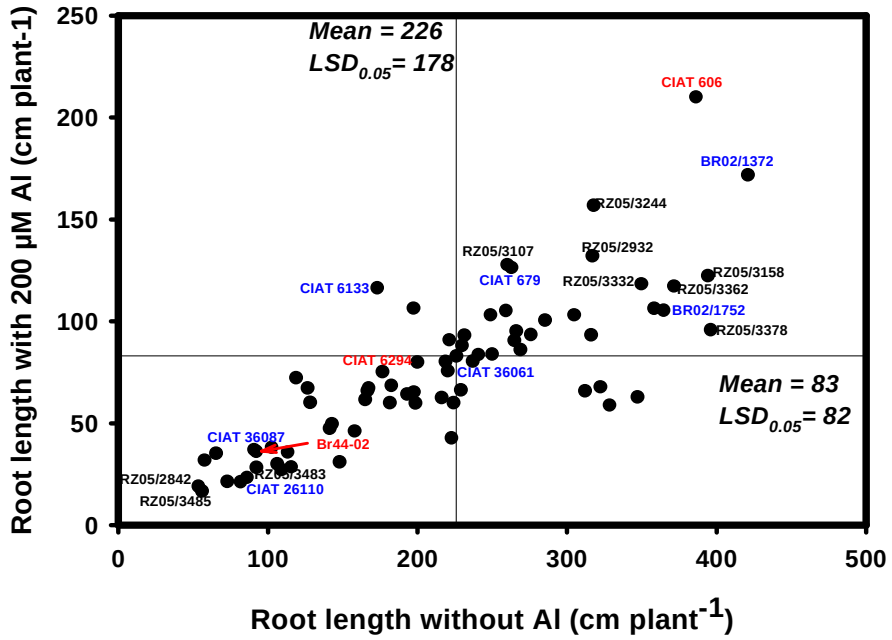


Figure 10. Relationship between total root length with Al and total root length without Al in solution of 60 hybrids, 3 parents and 8 check genotypes of *Brachiaria*. Genotypes that developed greater root length under both conditions were identified in the upper, right hand quadrat.

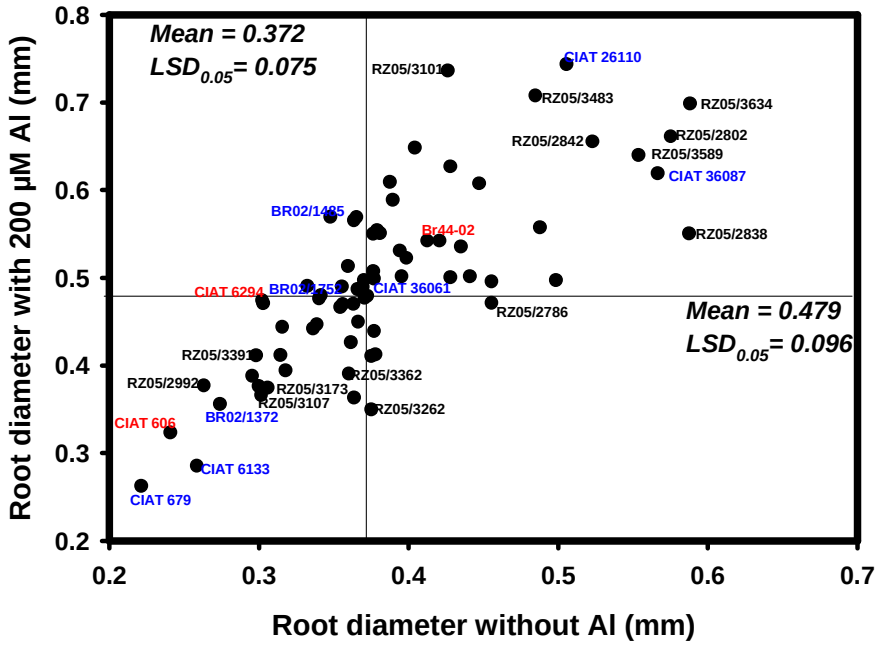


Figure 11. Relationship between mean root diameter with Al and mean root diameter without Al of 60 hybrids, 3 parents and 8 check genotypes of *Brachiaria*. Genotypes that developed finer roots under both conditions were identified in the lower left, hand quadrat.

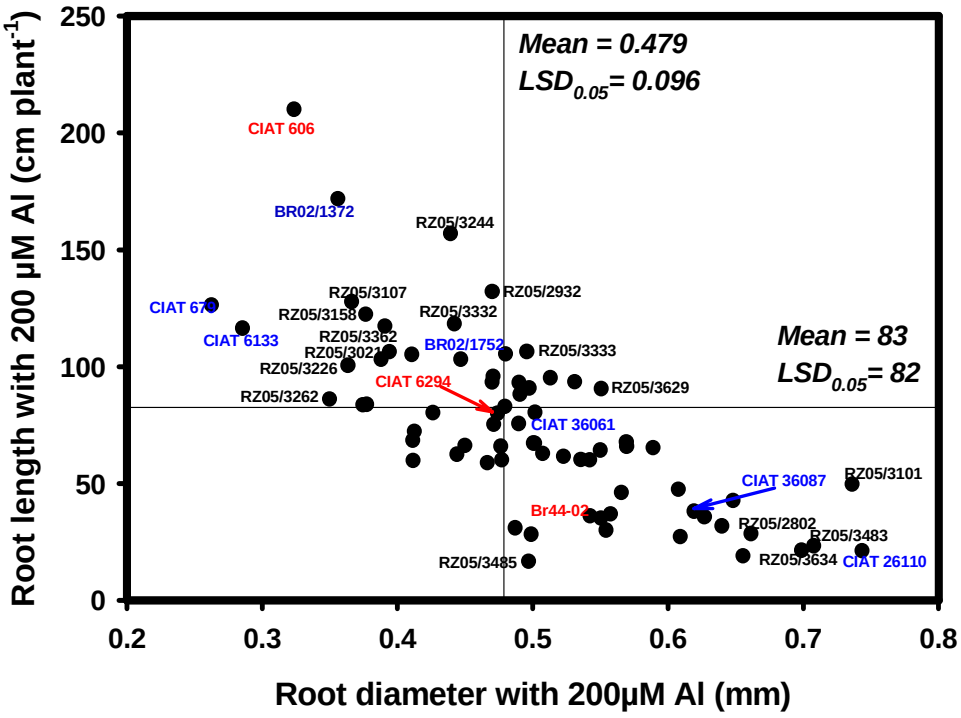


Figure 12. Relationship between total root length and mean root diameter of 60 hybrids, 3 parents and 8 check genotypes of *Brachiaria* with presence of aluminum in solution. Genotypes that develop finer longer roots were identified in the upper, left hand quadrat.

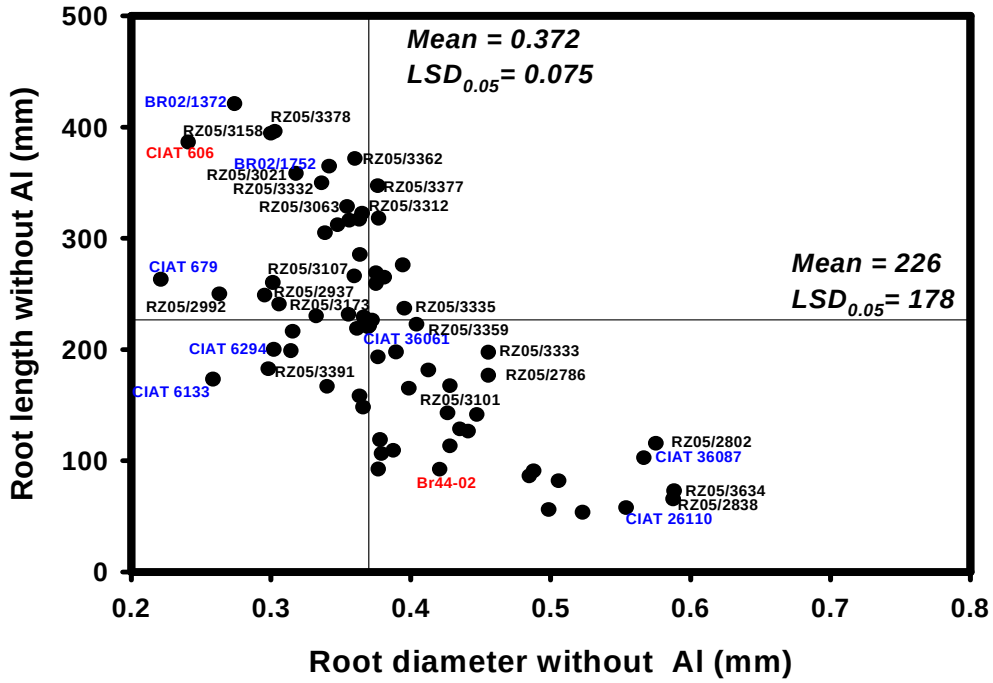


Figure 13. Relationship between total root length and mean root diameter of 60 hybrids, 3 parents and 8 check genotypes of *Brachiaria* with absence of aluminum in solution. Genotypes that develop finer longer roots were identified in the upper, left hand quadrat.

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

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

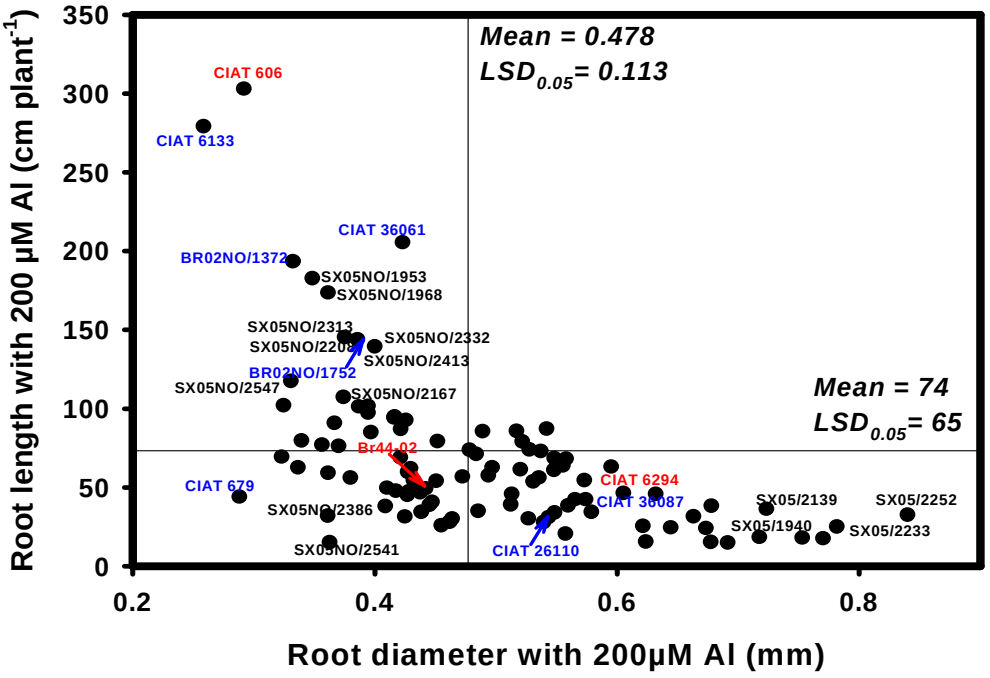


Figure 14. Relationship between total root length and mean root diameter of 88 SX05 hybrids, 3 parents and 8 check genotypes of *Brachiaria* with presence of aluminum in solution. Genotypes that develop finer longer roots with Al were identified in the upper, left hand quadrat.

Conclusions

We screened 103 apomictic/sexual hybrids of BR05 population of *Brachiaria* using hydroponic screening method and identified 9 hybrids (BR05NO/0406, BR05NO/0563, BR05NO/0334, BR05NO/0830, BR05NO/1173, BR05NO/0671, BR05NO/0120, BR05NO/0048, and BR05NO/0537) that were superior to *B. decumbens* parent in terms of aluminum resistance. Three of the 9 Al resistant hybrids (BR05NO/0334, BR05NO/0537, BR05NO/0563) also combined resistance to four spittlebug species and exhibited apomictic mode of reproduction. Results from this BR05NO population in Al resistance, as observed last year, clearly indicated that the level of Al resistance is improving for each breeding cycle illustrating the genetic gain from a very efficient recurrent selection program.

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

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

Rationale

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

Materials and Methods

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

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

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

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

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

Results and Discussion

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

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

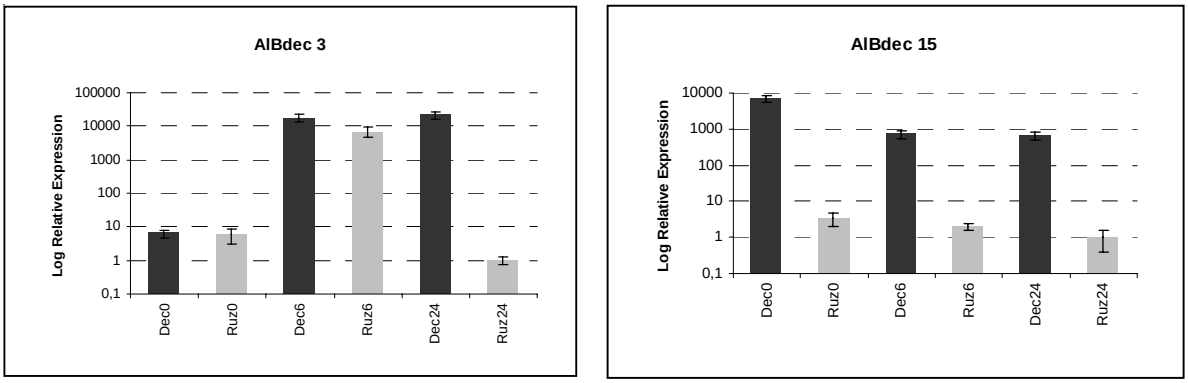


Figure 15. Transcript analysis of *AlBdec3* and *AlBdec15* at 0, 6 and 24 h of Al treatment. Black bar: *B. decumbes*; Grey bar: *B. ruziziensis*. The relative expression level was shown in a logarithmic scale.

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

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

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

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

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

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

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

Rationale

Field evaluation of a number *Brachiaria* hybrids under infertile acid soil conditions with low or high amounts of initial application of fertilizer and application of maintenance fertilizer with half of the amounts of initial applications at 2 year intervals improved the persistence of several hybrids and not allowed to distinguish differences between moderately adapted cultivars (cv. Marandu) and the promising hybrids. Therefore a field experiment was established in 2004 with 15 hybrids along with the 3 parents and 4 checks with low amounts of initial application of fertilizer and no maintenance fertilizer application to select hybrids that persist and produce green forage under acid infertile soil conditions. In 2005 evaluation, we showed that among the 15 *Brachiaria* hybrids tested, BR02NO/1794 and BR02NO/0465 were more productive than the other hybrids in terms of green forage (leaf + stem) yield in rainy season at 13 months after establishment with low initial fertilizer application and this adaptation seems to be closely associated with lower amounts of stem N content. In 2006, we tested the performance of these hybrids in the rainy season at 26 months after establishment under no maintenance fertilizer application.

Materials and Methods

A field trial was established at Matazul farm on 29 June of 2004. The trial included 15 *Brachiaria* hybrids (BR02NO/0465; BR02NO/0768; BR02NO/ 0771; BR02NO/ 0799; BR02NO/1245; BR02NO/1372; BR02NO/1452; BR02NO/1485; BR02NO/1718; BR02NO/1720; BR02NO/1728; BR02NO/1747; BR02NO/1752; BR02NO/1794 and BR02NO/ 1811), three parents (*B. decumbens* CIAT 606 and *B. brizantha* CIAT 6294 and *B.ruziziensis* 44-02) and four CIAT checks (*B.brizantha* CIAT 26110, *B.brizantha* CIAT 26646, *Brachiaria* hybrids CIAT 36061- Mulato and CIAT 36087- Mulato II). The trial was planted as a randomized block with 4 replications. One low level of initial fertilizer application was applied (kg/ha of 20P, 20K, 33Ca, 14 Mg, 10S) at the time of establishment. Maintenance fertilizer was not scheduled for application in order to identify genotypes that are bettter adapted to infertile acid soil conditions. The plot size was 5 m x 2.5 m. A number of plant attributes including forage yield, dry matter distribution and nutrient (N and P) uptake were measured in the rainy season at 26 months after establishment (October 2006).

Results and Discussion

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

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

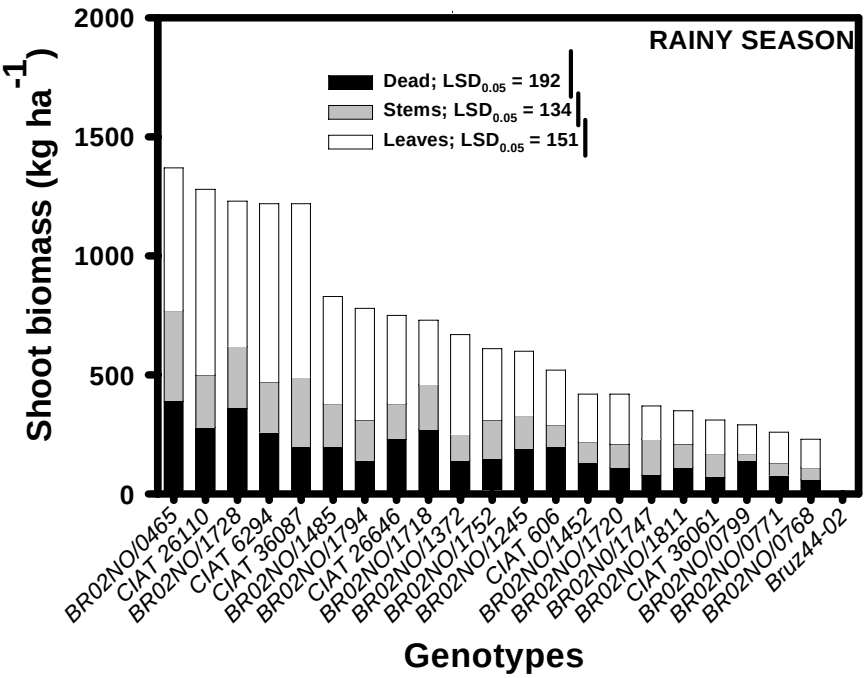


Figure 16. Genotypic variation in shoot biomass production (forage yield) of three parents (CIAT 606, 6294 and *B.ruziziensis* 44-02), four CIAT's accesions (26110, 26646, 36061 and 36087) and 15 genetic recombinants (BR02NO/0465; 0768; 0771; 0799; 1245; 1372; 1452; 1485; 1718; 1720; 1728; 1747; 1752;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

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

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

Table 21. Correlation coefficients (r) between green forage yield (t/ha) and other shoot traits of *Brachiaria* genotypes grown with low initial fertilizer application in a sandy loam oxisol in Matazul, Colombia.

Shoot traits	Low fertilizer
Total (live + dead) shoot biomass (t/ha)	0.94***
Dead shoot biomass (t/ha)	0.58***
Leaf biomass (t/ha)	0.96***
Stem biomass (t/ha)	0.85***
Leaf N content (%)	-0.03
Leaf P content (%)	0.10
Stem N content (%)	-0.31**
Stem P content (%)	-0.25**
Shoot N uptake (kg/ha)	0.95***
Shoot P uptake (kg/ha)	0.89***

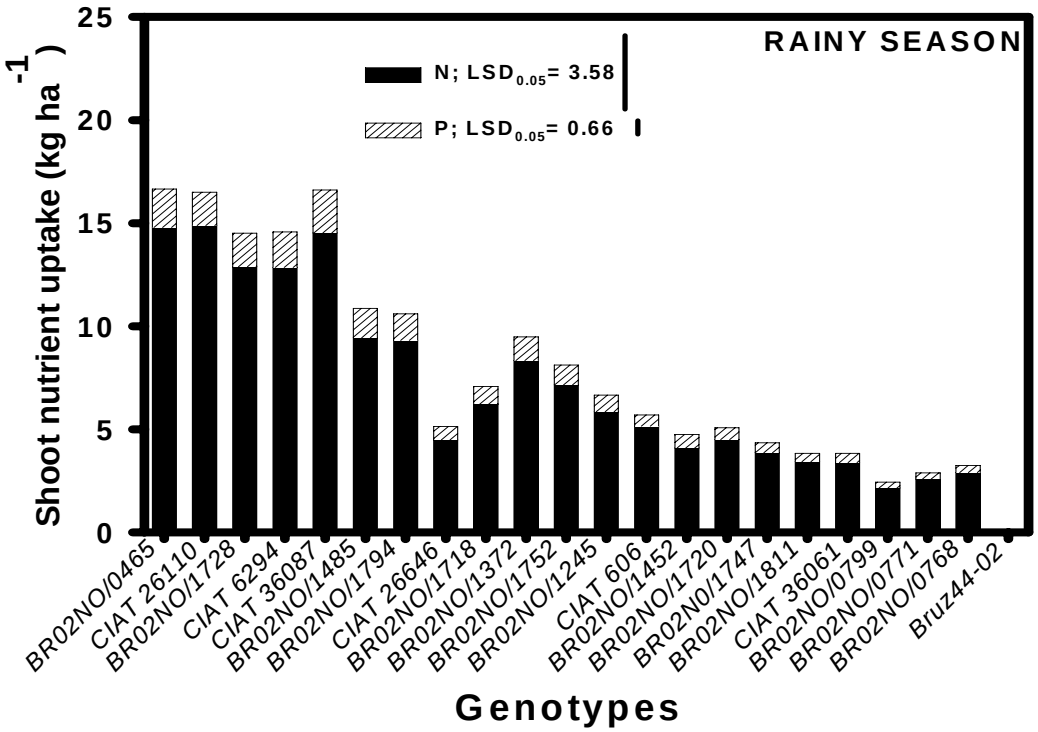


Figure 17. Genotypic variation in living shoot nutrient uptake (N, P, K, Ca and Mg) of three parents (CIAT 606, 6294 and *B.ruziziensis* 44-02), four CIAT's accesions (26110, 26646, 36061 and 36087) and 15 genetic recombinants (BR02NO/0465; 0768; 0771; 0799; 1245; 1372; 1452; 1485; 1718; 1720; 1728;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

Conclusions

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

3.2 Genotypes of *Brachiaria* with dry season tolerance

Highlights

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

- Showed that among the 15 *Brachiaria* hybrids tested under acid soil conditions in the Llanos of Colombia, the hybrids BR02NO/1794 and BR02NO/1718 were more productive than the other BR02NO hybrids in terms of green forage (leaf + stem) yield in dry season at 19 months after

establishment with low initial fertilizer application and no maintenance fertilizer application. But neither of these hybrids was superior to cv. Mulato II. The superior adaptation of the two hybrids seems to be associated with lower amounts of stem and leaf N and P contents and greater uptake of nutrients.

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

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

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

Rationale

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

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

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

Materials and Methods

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

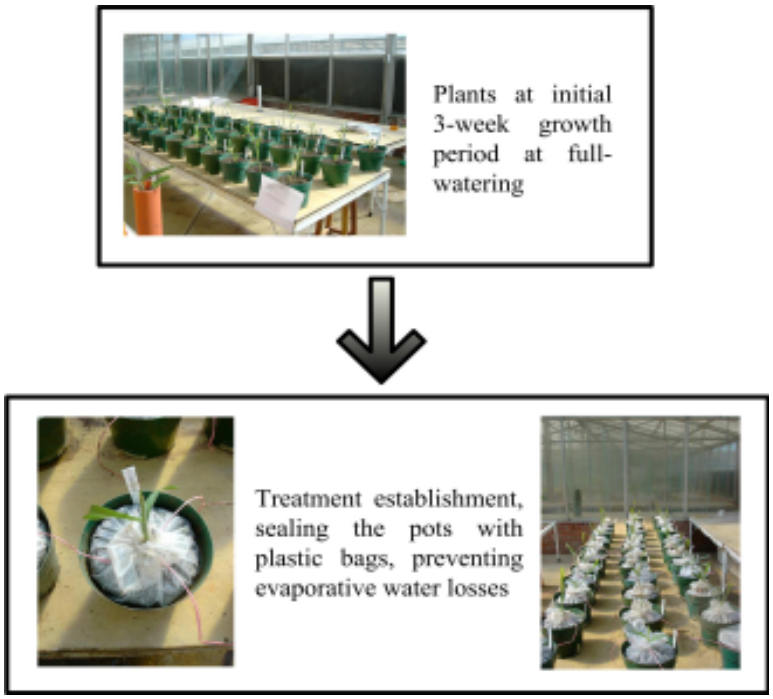


Photo 4. Methodology employed for determining FTSW response curves.

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

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

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

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

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

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

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

Results and Discussion

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

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

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

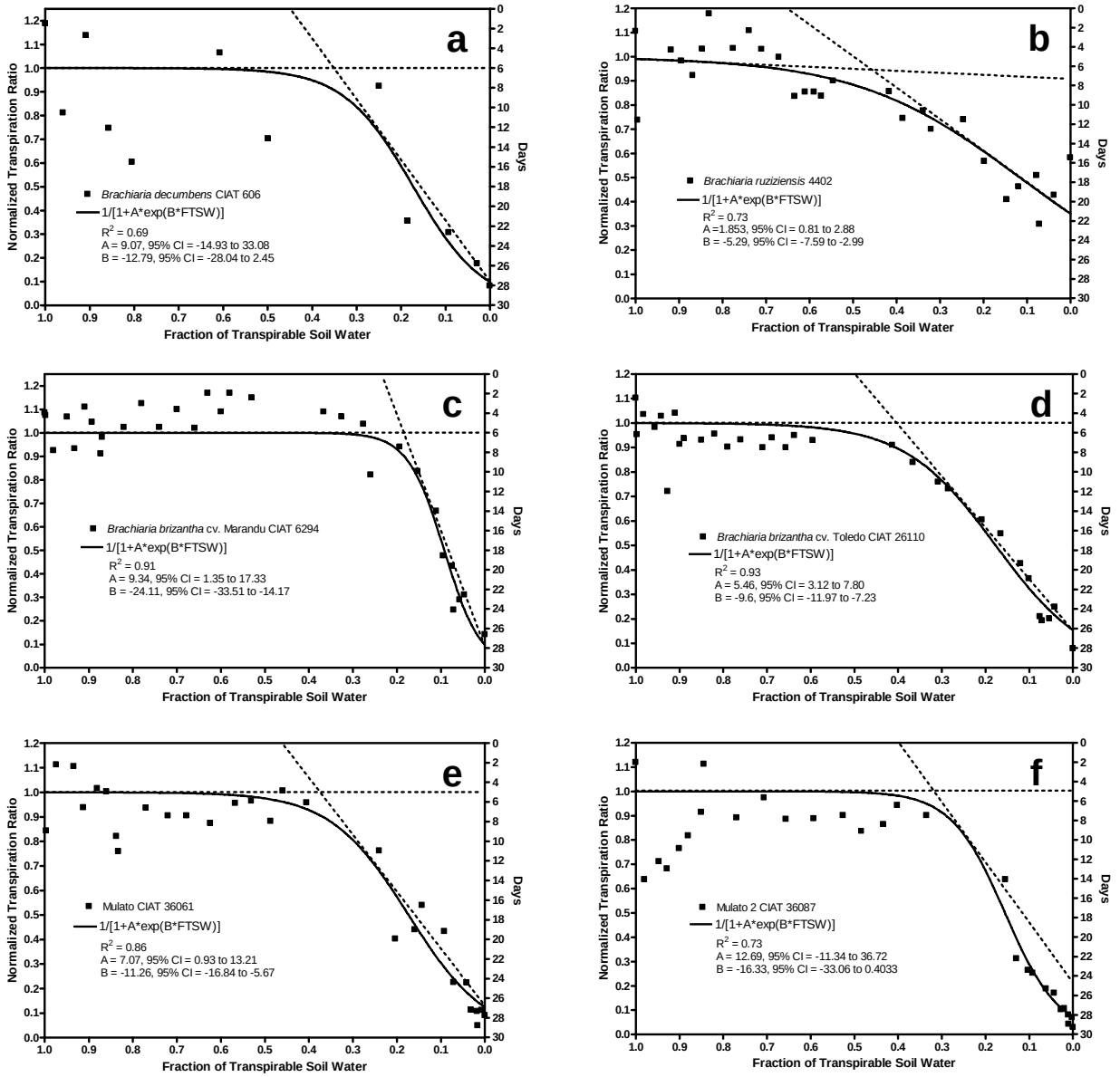
Cultivars Mulato and Toledo also showed delayed response compared with *B. ruziziensis* which showed stomatal sensitivity to soil drying. The response of cv. Toledo to soil drying seems to be somewhat different to the other genotypes, based on the time it took for cv. Toledo to achieve the endpoint (Table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

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

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

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

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



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

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

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

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

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

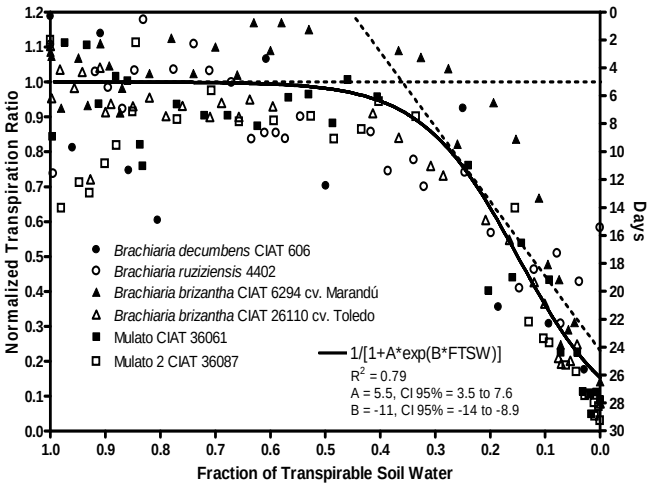


Figure 19. The relationship between the normalized transpiration ratio (NTR) and the daily values of the fraction of transpirable soil water (FTSW) for 6 genotypes of *Brachiaria* during terminal drought stress. The solid line represents the composite fit of all the data to equation 2. The intersection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

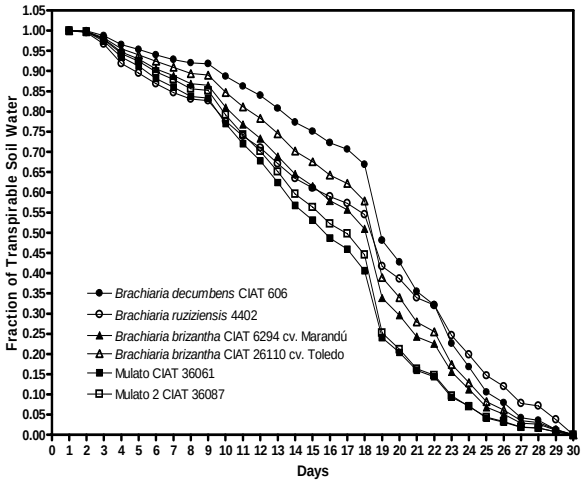


Figure 20. Differences in FTSW values over time for 6 genotypes of *Brachiaria* during soil drying.

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

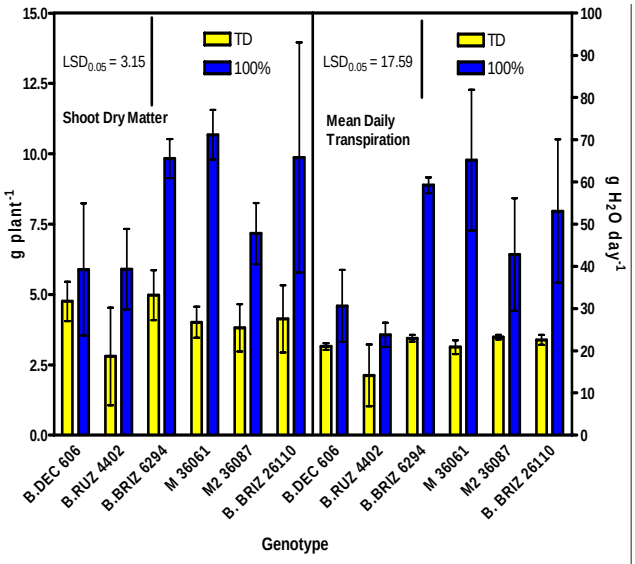


Figure 21. Differences in shoot dry matter and mean daily transpiration among 6 *Brachiaria* genotypes subjected to 100% FC (well watered) and terminal drought stress (TD).

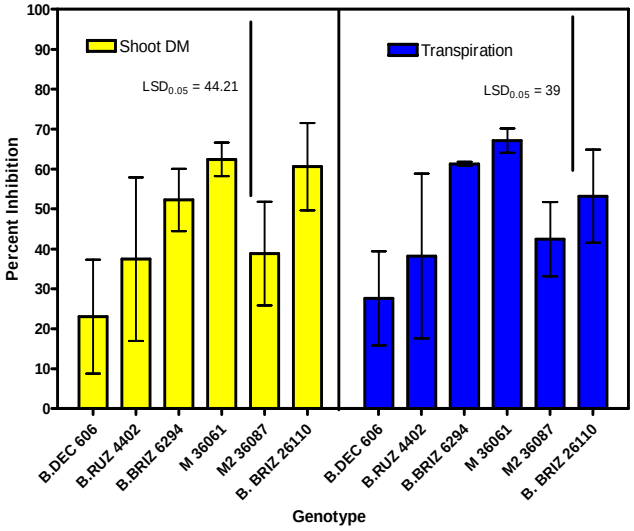


Figure 22. Effect of terminal drought stress on % inhibition of shoot dry matter accumulation and mean daily transpiration of 6 *Brachiaria* genotypes.

Conclusions

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

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

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

Rationale

Field evaluation of a number *Brachiaria* hybrids under infertile acid soil conditions with low or high amounts of initial application of fertilizer and application of maintenance fertilizer with half of the amounts of initial applications at 2 year intervals improved the persistence of several hybrids and not allowed to distinguish differences between moderately adapted cultivars (cv. Marandu) and the promising hybrids in dry season tolerance. Therefore a field experiment was established in 2004 with 15 hybrids along with the parents and checks with low amounts of initial application of fertilizer and no maintenance fertilizer application to select hybrids that persist and produce green forage under drought and acid infertile soil conditions. In 2005 evaluation at 9 months after establishment, we showed that among the 15 *Brachiaria* hybrids tested, BR02NO/1811 was more productive than the other hybrids in terms of green forage (leaf + stem) yield in the dry season and this adaptation seems to be closely associated with lower amounts of stem P content. In 2006, we tested the performance of these hybrids in the dry season at 19 months after establishment under no maintenance fertilizer application.

Materials and Methods

A field trial was established at Matazul farm on 29 June of 2004. The trial included 15 *Brachiaria* hybrids (BR02NO/0465; BR02NO/0768; BR02NO/ 0771; BR02NO/0799; BR02NO/1245; BR02NO/1372; BR02NO/1452; BR02NO/1485; BR02NO/1718; BR02NO/1720; BR02NO/1728; BR02NO/1747; BR02NO/1752; BR02NO/1794 and BR02NO/1811), three parents (*B. decumbens* CIAT 606 and *B. brizantha* CIAT 6294 and *B.ruziziensis* 44-02) and four CIAT checks (*B.brizantha* CIAT 26110, *B.brizantha* CIAT 26646, *Brachiaria* hybrids CIAT 36061 and CIAT 36087).

The trial was planted as a randomized block with 4 replications. One low level of initial fertilizer application was applied (kg/ha of 20P, 20K, 33Ca, 14 Mg, 10S) at the time of establishment. Maintenance fertilizer was not scheduled for application in order to identify genotypes that are bettter adapted to infertile acid soil conditions. The plot size was 5 m x 2.5 m. A number of plant attributes including forage yield, dry matter distribution and nutrient (N, P, K, Ca and Mg) uptake were measured at the end of the 3 month dry season at 19 months after establishment (March 2006).

Results and Discussion

At 9 months after establishment CIAT 606, CIAT 36061, CIAT 36087, BRO2NO/1452 , BRO2NO/1485, BRO2NO/1720, BRO2NO/ 1747, BRO2NO/1752 and BRO2NO/1794 were affected by bacterial infection. Among the hybrids, BR02NO/1752 was relatively more affected. The dead shoot biomass was greater with 2 hybrids (BR02NO/1811 and BR02NO/ 1728) and a check (CIAT 26110) (Figure 23).

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

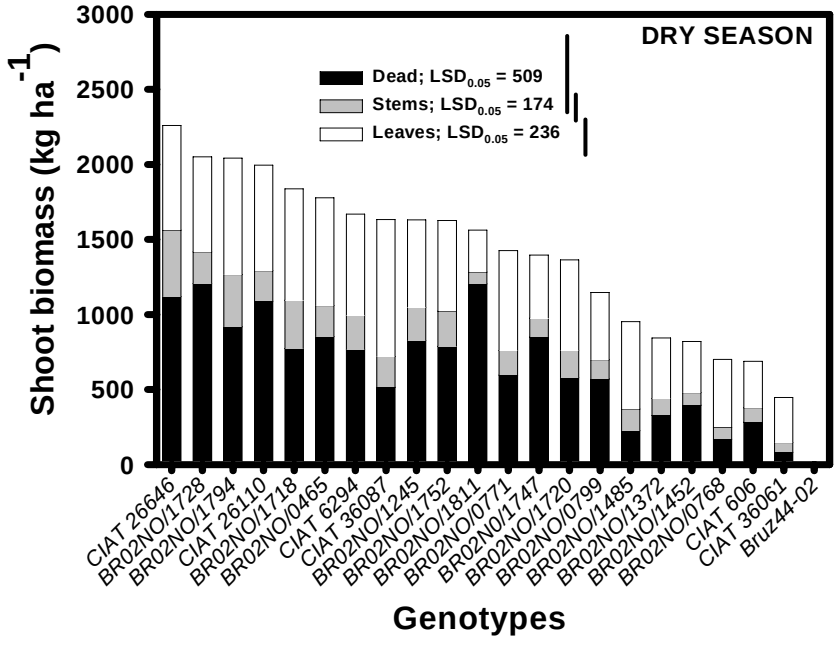


Figure 23. Genotypic variation in shoot biomass production (forage yield) of three parents (CIAT 606, 6294 and *B.ruziziensis* 44-02), four CIAT's accesions (26110, 26646, 36061 and 36087) and 15 genetic recombinants (BR02NO/0465; 0768; 0771; 0799; 1245; 1372; 1452; 1485; 1718; 1720; 1728; 1747; 1752;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

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

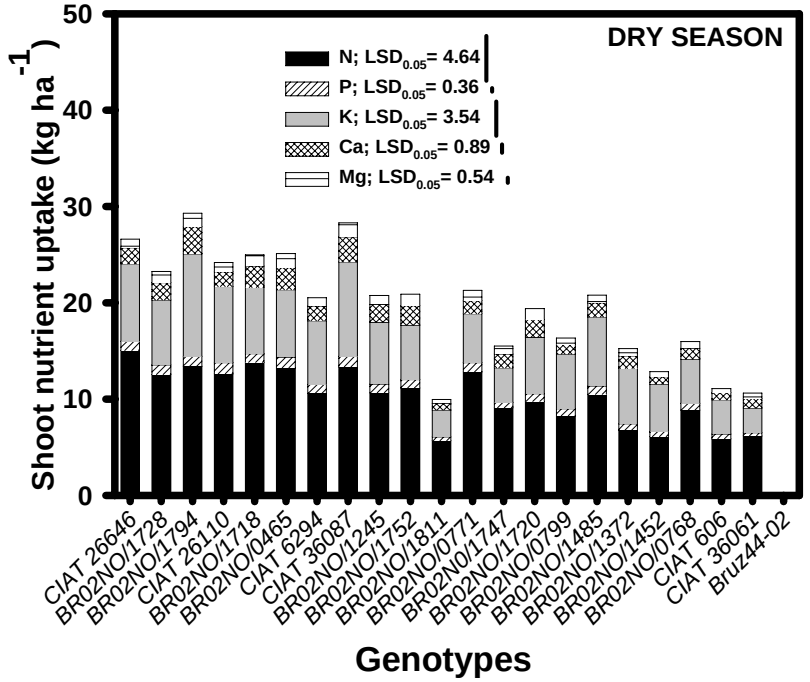


Figure 24. Genotypic variation in living shoot nutrient uptake (N, P, K, Ca and Mg) of three parents (CIAT 606, 6294 and *B.ruziziensis* 44-02), four CIAT's accesions (26110, 26646, 36061 and 36087) and 15 genetic recombinants (BR02NO/0465; 0768; 0771; 0799; 1245; 1372; 1452; 1485; 1718; 1720; 1728;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

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

Correlation coefficients between green forage yield and other plant attributes indicated that greater nutrient acquisition contributed to superior performance (Table 23).

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

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

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

Conclusions

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

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

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Rationale

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

Materials and Methods

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

Results and Discussion

Differences in production of live shoot biomass and total shoot biomass among the six *Brachiaria* grasses are shown in Figure 25. Significant differences were not observed in shoot biomass production among the 6 *Brachiaria* genotypes that were evaluated at 3 and 10 months after establishment. Mean value of live (forage) shoot biomass production was 5136 and 1979 kg ha^{-1} at 3 months after establishment for initial fertilizer applications of high and low, respectively. These values decreased to 860 and 601 kg ha^{-1} for high and low fertilizer application, respectively at 29 months after establishment. Later, the apomictic natural accession *B. brizantha* CIAT 6294 and the

apomictic hybrid cultivar Mulato 2 (CIAT 36087) had significantly more live and total shoot biomass, followed by the apomictic natural accession of *B. decumbens* CIAT 606 and the hybrid BR99NO/4015. Two hybrids, BR99NO/4132 and BR98NO/1251, had markedly lower production of shoot biomass over time when compared to the other genotypes (Figure 25).

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

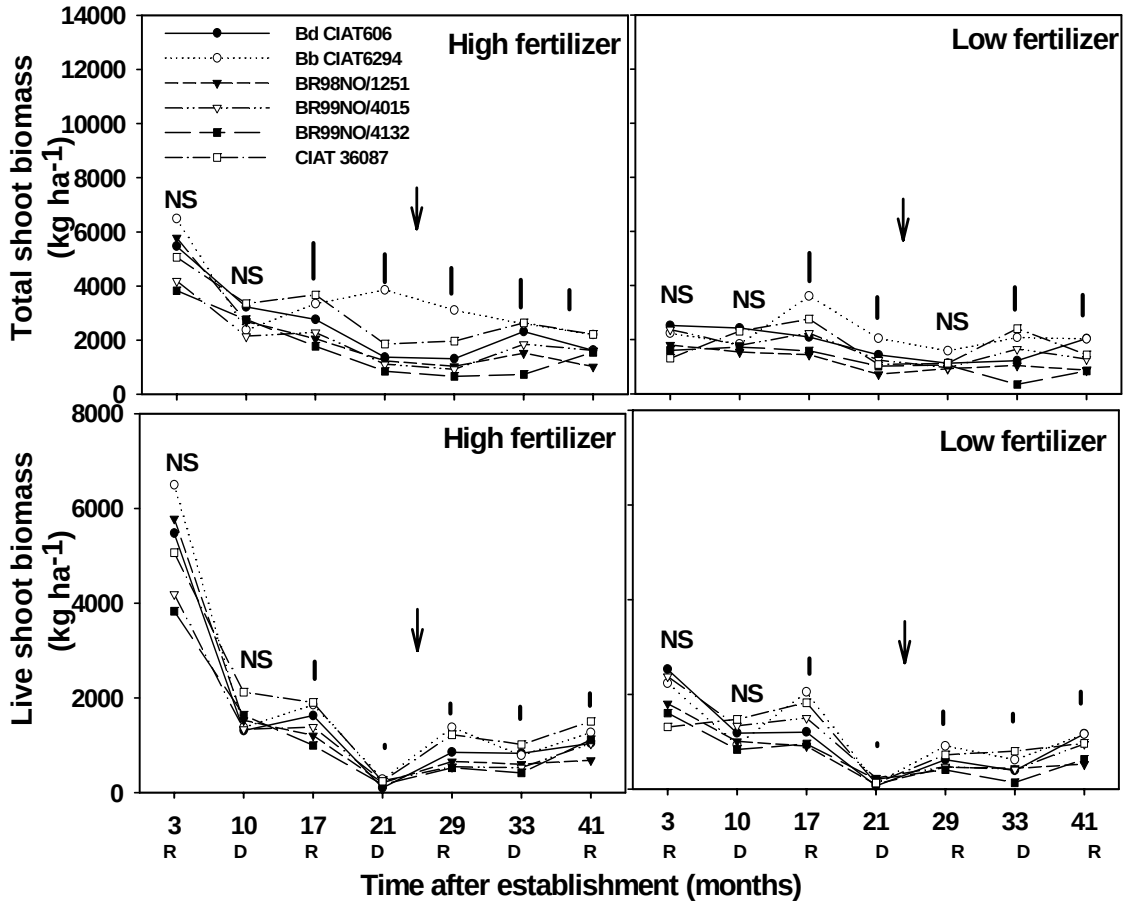

Figure 25. Total (live + dead) and live shoot biomass production (kg ha^{-1}) in 6 *Brachiaria* genotypes evaluated for 4 years. Measurements were made at the end of dry (D) and rainy (R) seasons in each year in an Oxisol of "Altillanura" in Puerto López, Meta (Colombia) with high (High F.) and low (Low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

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

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

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

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

* The least significant difference (LSD) values or not significant (NS) differences with 95% of probability.
** Mean soil bulk density at 0-40 cm depth.

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

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

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

Conclusions

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

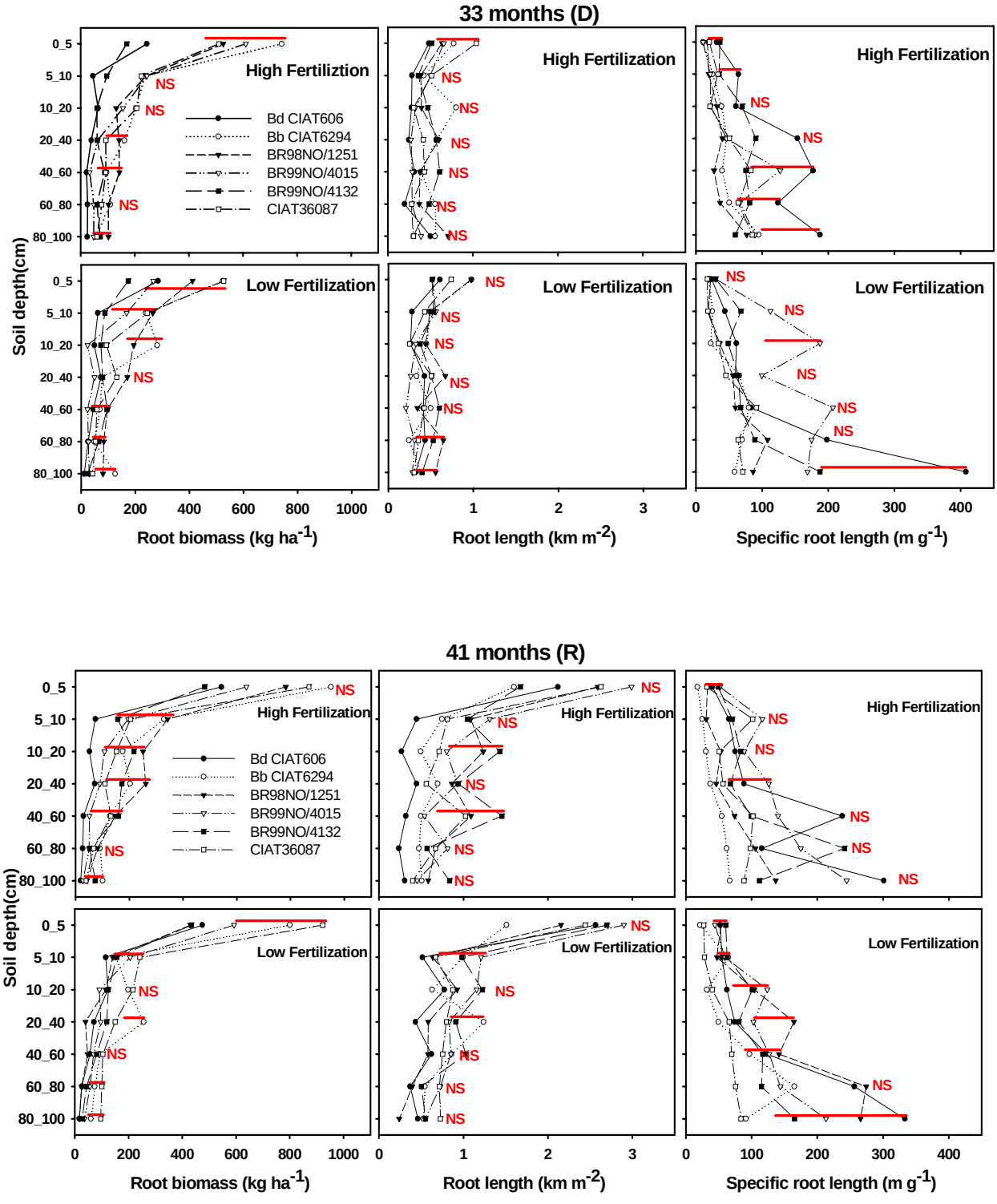

Figure 26. Root biomass (kg ha^{-1}), root length (km m^{-2}) and specific root length (m g^{-1}) distribution across soil profile of 6 *Brachiaria* genotypes evaluated at 33 (dry season - D) and 41 (rainy season - R) months after their establishment with high and low amounts of initial fertilizer application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