Advances in developing screening methods and improving aluminum resistance in common bean and *Brachiaria*

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ABSTRACT

We summarize progress made towards the development of tools and knowledge that aid the genetic improvement of aluminum (Al) resistance in two of CIAT's (International Center for Tropical Agriculture) commodity crops; common bean (*Phaseolus vulgaris* L.) and brachiariagrasses (*Brachiaria* spp. Grisib). Approximately 40% of common bean production is on acid soils where Al toxicity limits root development. Improving Al resistance, therefore, is an important breeding objective. We compared a group of 53 common bean genotypes with differing levels of adaptation to acid soils to identify Al resistant genotypes using a hydroponic screening method. Four different root traits (percent inhibition of root elongation, percent increase of average root diameter, total root length per plant and total number of root tips per plant) were identified as useful indicators for Al resistance. Application of this method to 30 accessions of scarlet runner bean (*P. coccineus* L.) identified three Al-resistant genotypes that could be used for introgressing Al resistance into cultivated germplasm. Work is in progress to characterize the physiological mechanisms underpinning Al resistance in common bean. This will facilitate developing simplified screening procedures and identifying quantitative trait loci (QTLs) contributing to Al resistance. Compared to common bean, brachiariagrasses tend to be better adapted to acid soils. Yet edaphic adaptation is an important component of CIAT's and EMBRAPA's (Empresa Brasiliera de Pesquisa Agropecuária) breeding programs because one of the three parental genotypes is poorly adapted. We developed and incorporated into our breeding program a solution-culture technique that uses rooted stem cuttings to screen for Al resistance and root vigor, both of which are segregating in breeding populations. Physiological research into the remarkably high level of Al resistance of signalgrass (*B. decumbens* Stapf) has ruled out a significant contribution of 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. Once taken up into roots, Al appears to be complexed by low-molecular-weight ligands such as citrate. Other adaptive traits may counteract secondary effects of Al toxicity on phosphorus (P) nutrition through stimulation of P uptake or use efficiency.

INTRODUCTION

The highly weathered acid soils of the tropics are characterized by a combination of nutrient deficiencies and mineral toxicities (Rao et al., 1993; Rao and Cramer, 2003). It is estimated that over 50% of the world's potentially arable lands are acidic with pH < 5.5 (von Uexkull and Mutert, 1995; Bot et al., 2000). Up to 60% of the acid soils in the world are in developing countries, where food production is particularly critical. Al phytotoxicity is the primary limitation to agricultural production on these acid soils. At soil pH values of 5 or below, toxic forms of Al are solubilized into the soil solution. These inhibit root growth and function and thus reduce crop and forage yields.

The primary and earliest symptom of Al toxicity is a rapid (within minutes) inhibition of root elongation (Ryan et al., 1993; Sivaguru and Horst, 1998; Kollmeier et al., 2000). The distal part of the transition zone in the root apex was identified as the primary site of action of toxic Al ions (Sivaguru and Horst, 1998). Research conducted over the past two decades on the physiology, genetics and molecular biology of plant Al resistance and toxicity has shown that Al resistance can be achieved by mechanisms that exclude Al from the root apex (Al exclusion) and/or by mechanisms that enable plants to tolerate Al in the symplasm (Al tolerance) (Barcelo and Poschenreider, 2002; Garvin and Carver, 2003; Kochian et al., 2004; Kochian et al., 2005; Ma and Furukawa, 2003; Ma et al., 2001; Matsumoto, 2000; Matsumoto, 2005; Vitorello et al., 2005).

Existing information clearly indicates that long-term, sustainable crop and forage production on acid soils requires both Al-resistant cultivars and appropriate agronomic practices. Significant progress has been made in increasing Al resistance to improve crop production on acid soils (Garvin and Carver, 2003; Hede et al., 2001). Most genetic and physiological studies have focused on major cereal crops such as wheat, rice and maize (Kochian et al., 2005). In wheat, Al resistance is genetically simple, with a single major gene accounting for most of the resistance. In rice and maize, however, Al resistance is quantitatively inherited and involves a number of different genes and possibly a variety of physiological mechanisms.

Aluminum resistance is usually assessed in seedling-based assays, either by quantifying root elongation or apical callose concentrations, or by staining root apices with hematoxylin (Kerridge and Kronstad, 1968; Polle et al., 1978; Ruiz-Torres et al., 1992;

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Llugany et al., 1994; Cançado et al., 1999). Before incorporating any of these assays into a breeding program, it is important to validate their efficacy in identifying adapted genotypes and to establish the relative importance of Al resistance compared to other acid-soil constraints (e.g., phosphorus or nitrogen deficiencies). In cases where Al resistance is genetically complex and based on physiologically distinct traits encoded by different loci, breeding programs are likely to benefit from a better understanding of physiological mechanisms because only then can physiologically-based selection strategies be designed to enrich for the desired alleles at individual loci. Such a dissection of Al resistance into 'physiological components' could also facilitate the identification of quantitative trait loci (QTL) and candidate genes associated with Al-resistance, and could help to predict the long-term impacts of Al-resistant cultivars. Our review summarizes the progress made toward achieving some of these goals for two of CIAT's commodity crops: common bean and brachiariagrasses.

COMMON BEAN

Common bean is the world's most important food legume, with an annual production value in excess of US$10 billion. It is produced mainly on small-scale farms in developing countries in Latin America and Africa (80% of dry bean production). About 40% of the bean-growing area is affected by Al toxicity, resulting in decrease of grain yield between 30% and 60% (Thung and Rao, 1999; Rao, 2001a). The crop needs significant improvement in Al resistance to reduce the dependence of small farmers on lime and fertilizer inputs.

Genetic variation in acid-soil adaptation

Genetic variation exists for acid soil adaptation among common bean genotypes (Rao et al., 2004). Field screening of 5,000 germplasm accessions and bred lines in Al-toxic soils with and without lime (70% Al saturation) and participatory evaluation with farmers have resulted in identification of several promising genotypes. Field screening for Al resistance would seem to be the most desirable approach, because it best approximates the intended cropping environment. In practice, however, reliable ranking of genotypes in the field can be difficult. This is mainly because exchangeable Al levels may not be uniform and also environmental factors could interact with soil Al to mask the expression of Al resistance. Thus it is necessary to combine field screening with greenhouse screening techniques based on physiological traits of Al resistance.

The most frequently measured effect of excess Al is inhibition of root elongation. Nutrient solution studies are better suited for determining more precisely the Al activity that is influencing root elongation, exclusive to other associated acid-soil-stress constraints. In order to identify genotypes resistant to Al, additional ions in solution that may be toxic, such as H^+, and ions that may help alleviate Al toxicity, such as Ca^{2+}, must be controlled (Rangel et al., 2005). Nutrient solution culture allows evaluation of a large number of genotypes quickly and could be very useful for identification of (i) parental genotypes with contrasting root architecture for bean breeding, (ii) contrasting genotypes for physiological analysis, (iii) QTLs related to Al resistance, and (iv) candidate genes associated with Al resistance in common bean.

A solution-culture technique for evaluating Al resistance

We developed a greenhouse screening method that uses low-ionic-strength nutrient solutions to evaluate genotypic differences in Al resistance based on root traits of seedlings (CIAT, 2003, 2004, 2005; Manrique et al., 2006). Seeds were germinated on filter paper for 2 to 3 days, and seedlings with uniform root lengths (5-7 cm) were selected for evaluation in nutrient solutions composed of (in μM): 286 $CaSO_4$, 300 KNO_3, 150 NH_4NO_3, 2.5 NaH_2PO_4, 150 $MgCl_2$, 14 $CaCl_2$, 5 $FeCl_3$, 5 Na_2EDTA, 1 $MnCl_2$, 1 $ZnCl_2$, 0.2 $CuCl_2$, 6 H_3BO_3, 5 Na_2SiO_3, 0.001 $NaMoO_4$, 57.5 NaCl and with or without 50 μM $AlCl_3$ (pH 4.5). Changes in root elongation caused by Al were monitored by measuring root elongation during the first three days of the Al treatment. After harvest, the root systems were stained and scanned to determine total root length, average root diameter, number of root tips and total root volume (CIAT, 2003).

The hydroponic screening method was used to evaluate a group of 52 common bean genotypes (landraces and breeding lines) and 66 recombinant inbred lines (RIL) derived from the cross G 5273 × MAM 38. We identified three Andean genotypes (G 19833, BRB 191 and G 5273), eight Mesoamerican genotypes (G 1261, MAR 1, DOR 714, FEB 190, G 11015, G 3513, A 774 and G 855) based on the relationship between the % inhibition of total root length and the % of increase of average root diameter per plant (Fig. 1). Genotypes that showed lower values of % inhibition of total root length and % increase of average root diameter were identified as Al resistant. We also identified four RIL which had a higher level of Al resistance than the other RIL (CIAT, 2003, 2004). This study also indicated that percent inhibition of root elongation, percent increase of average root diameter, total root length per plant and total number of root tips per plant could serve as screening parameters to identify Al-resistant common bean genotypes (Manrique et al., 2006).

During a screening of 30 germplasm accessions of scarlet runner bean we identified three accessions (G 35341, G 35266, G 35025) with a high level of Al resistance (CIAT, 2005). These accessions could serve as donor parents for introgressing Al resistance into common bean (CIAT, 2005).

Physiological mechanisms of Al resistance

Physiological studies conducted at the University of Hannover, Germany, showed that common bean is very sensitive of low pH (4.3) with large genotypic differences in proton sensitivity (Rangel et al., 2005). Therefore, proton toxicity prevented screening of common bean genotypes for Al resistance using the protocol established for maize (0.5 mM $CaCl_2$, 8 μM H_3BO_3, pH 4.3). Increasing the pH to 4.5, the Ca^{2+} concentration to 5 mM, and adding 0.5 mM KCl fully prevented proton toxicity in all cultivars and allowed us to identify differences in Al resistance among 28 cultivars by quantifying the inhibition of root elongation at 20 μM Al during 36 h as parameter of Al injury. As in maize, Al treatment induced callose formation in root apices of common bean. Al-induced callose formation reflected well the alleviating effect of Ca^{2+} ions on Al intoxication as revealed by root-growth inhibition. Comparing 28 bean genotypes differing in Al resistance after 36 h of Al treatment, Al-induced callose formation in root apices was positively correlated with Al-induced inhibition of root elongation and the Al content of root apices. The relationship, however, was less than previously reported for maize. Also, after 12 h of Al treatment, neither callose formation nor the Al content of root apices reflected the difference in Al resistance between two contrasting cultivars, which indicated a different mode of expression of Al toxicity and resistance in common bean (Rangel et al., 2005).

Recent studies showed that common bean has a pattern II of organic-acid exudation (A. Rangel and W. Horst, unpublished results). A lag period of 4 to 6 h was observed between addition of Al

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and release of organic anions from root tips. The main anions exuded were succinate, malate, citrate, oxalate, and pyruvate. Aluminum resistance was associated with higher organic-anion contents in root tips. Work is in progress to further characterize physiological mechanisms of Al resistance. We are following a scheme that combines physiological analyses, QTL mapping for specific traits, and quantification of edaphic adaptation in the field, to link component traits with overall Al resistance and to determine the relative importance of individual traits.

BRACHIARIAGRASSES

Perennial brachiariagrasses (*Brachiaria* spp. Griseb.) are the most widely sown forage grasses in tropical America (Miles et al., 2004). 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 (Miles et al., 2004; Miles et al., 2006). 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.

Aluminum toxicity and other acid-soil constraints

In the past, edaphic adaptation of brachiariagrasses was exclusively evaluated by quantifying forage yield and pasture persistence in field trials (Rao et al., 1996; Miles et al., 2004). These trials have resulted in the release of several well-adapted cultivars such as cv. Basilisk (signalgrass), Tully, and Llanero (Miles et al., 2004). 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 (Rao et al., 1998). 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) (Rao et al., 1996; Li et al., 1997; Rao et al., 1998, 1999a, b; Rao, 2001a, b; Wenzl et al., 2001, 2002a, b, 2003; Ishitani et al., 2004; Miles et al., 2004; Nanamori et al., 2004; Wagatsuma et al., 2005a, b; Wenzl et al., 2006; Begum et al., 2006; Watanabe et al., 2006; Haussler et al., 2006). It was not clear whether there was genetic variation among brachiariagrass genotypes for Al resistance *per se*.

In an attempt to disentangle the various stress factors affecting growth of brachiariagrasses 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 (Wenzl et al., 2003). 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 (Wenzl et al., 2001). 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.

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 simultaneously assessment of a set of genotypes for a variety of traits including edaphic adaptation, insect and disease resistance, nutritional quality and seed production (Miles et al., 2004). 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 (Wenzl et al., 2001) into a format that was suitable for the adventitious roots of stem cuttings, by increasing the concentration of Al (200 µM $AlCl_3$, 200 µM $CaCl_2$, pH 4.2) and simultaneously quantifying the intrinsic root vigor of each genotype in a solution containing only 200 µM $CaCl_2$ (pH 4.2) (Wenzl et al., 2006). 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) segregants was more than eight times longer than that of poorly-adapted ruzigrass (Wenzl et al., 2006). 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 (Rao et al., 1996). 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 (Rao et al., 2006). In 2002, we identified two sexual hybrids (SX01NO/3178) and SX01NO/7249) and one apomictical hybrid (BR99NO/4132) with superior Al resistance/root vigor 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 (Fig. 2). Results

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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 (Miles et al., 2004; Rao et al., 2006).

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 (Wenzl et al., 2001; Wagatsuma et al., 2005a). However, secretion of organic acids and phosphate at root apices, a widespread mechanism of Al exclusion in plants (Kochian et al., 2004), 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 30-fold 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 (Wenzl et al., 2004). 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 (Wagatsuma et al., 2005a, b). 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 (Wagatsuma et al., 2005b), and a short-term Al treatment seemed to permeabilize the PM of root apices of signalgrass less than those of ruzigrass (Wagatsuma et al., 2005a). 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 (Wagatsuma et al., 2005a).

Internal Al detoxification

Both Al-resistant signalgrass and less resistant ruzigrass accumulated high concentrations of Al in roots (Wenzl et al., 2002a).

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) (Watanabe et al., 2006). 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 (Wenzl et al., 2000). These ligands may constitute a sink for Al ions in mature roots because very little Al was translocated to shoots (Wenzl et al., 2002a).

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 brachiariagrass apices (the most Al-sensitive site), they do not fully account for the superior resistance level of signalgrass (Wenzl et al., 2002a).

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 (Rao et al., 1993; Rao and Cramer, 2003). Chemical interactions between Al and P within plant tissues are commonly considered an important secondary effect of Al toxicity. In brachiariagrasses, 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 (Wenzl et al., 2002b).

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 (Begum et al., 2006). 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 (Nanamori et al., 2004).

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THE WAY FORWARD

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 and RIL of beans 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 crop and forage germplasm would be immense in terms of increased food production, more efficient use of purchased inputs, and improved integration of crop-livestock systems.

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REFERENCES

BARCELO, J.; POSCHENRIEDER, C. Fast root growth responses, root exudates, and internal detoxification as clues to the mechanisms of aluminum toxicity and resistance: A review. **Environmental and Experimental Botany**, v.48, p.75-92, 2002.

BEGUM, H. H.; OSAKI, M.; NANAMORI, M.; WATANABE, T.; SHINANO, T.; RAO, I.M. Role of phospho*enol*pyruvate carboxylase in the adaptation of a tropical forage grass, *Brachiaria* hybrid, to low phosphorus acid soils. **Journal of Plant Nutrition**, v.29, p.35-57, 2006.

BOT, A.J.; NACHTERGAELE, F.O.; YOUNG, A. Land Resource Potential and Constraints at Regional and Country Levels. **Food and Agricultural Organization of the United Nations**, Rome, 2000, 114 p.

CANCADO, G.M.A.; LOGUERCIO, L.L.; MARTINS, P.R.; PARENTONI, S.N.; PAIVA, E.; BOREM, A.; LOPES, M.A. Hematoxylin staining as a phenotypic index for aluminum tolerance selection in tropical maize (*Zea mays* L.). **Theoretical and Applied Genetics**, v.99, p.747–754, 1999.

CIAT. 2003. Annual Report 2003 Project IP-1. Bean improvement for the tropics. p.24-27. CIAT, Cali, Colombia.

CIAT. 2004. Annual Report 2004 Project IP-1. Bean improvement for the tropics. p.27-29. CIAT, Cali, Colombia.

CIAT. 2005. Annual Report 2005 Project IP-1. Bean improvement for the tropics. p.13-18. CIAT, Cali, Colombia.

GARVIN, D.; CARVER, B. Role of the genotype in tolerance to acidity and aluminum toxicity. In: RENGEL, Z.; DEKKER, M. (Ed.). **Handbook of Soil Acidity**. New York.

HAUSSLER, K.; RAO, I.M.; SCHULTZE-KRAFT, R.; LATE MARSCHNER, H. Shoot and root growth of two tropical grasses, *Brachiaria ruziziensis* and *B. dictyoneura* as influenced by aluminum toxicity and phosphorus deficiency in a sandy loam Oxisol of the eastern plains of Colombia. **Trop. Grasslands**, (in press), 2006.

HEDE, A.; SKOVMAND, B.; LEPEZ-CESATI, J. Acid soils and aluminum toxicity. In REYNOLDS, M. (Ed.). **Application of Physiology in Wheat Breeding**. CIMMYT, Mexico, D.F., 2001.

p.172-182.

ISHITANI, M.; RAO, I.; WENZL, P.; BEEBE, S.; TOHME, J. Integration of genomics approach with traditional breeding towards improving abiotic stress adaptation: drought and aluminum toxicity as case studies. **Field Crops Res**, v.90, p.35-45, 2004.

KERRIDGE, P.C.; KRONSTAD, W.E. Evidence of genetic resistance to aluminum toxicity in wheat (*Triticum aestivum* Vill., Host) **Agron. J,** v.60, p.710–711, 1968.

KOCHIAN, L.V.; HOEKENGA, O.A.; PINEROS, M.A. How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorus efficiency. **Annual Review of Plant Biology**, v.55, p.459-493, 2004.

KOCHIAN, L.V.; PINEROS, M.A.; HOEKENGA, O.A. The physiology, genetics and molecular biology of plant aluminum resistance and toxicity. **Plant and Soil**, v.274, p.175-195, 2005.

KOLLMEIER, M.; FELLE, H.H.; HORST, W.J. Genotypical differences in aluminum resistance of maize are expressed in the distal part of the transition zone. Is reduced basipetal auxin flow involved in inhibition of root elongation by aluminum? **Plant Physiology**, v. 122, p. 945-956, 2000.

LI, M.; OSAKI, M.; RAO, I.M.; TADANO, T. Secretion of phytase from the roots of several plant species under phosphorus-deficient conditions. **Plant and Soil**, v.195, p.161-169, 1997.

LLUGANY, M.; MASSOT, N.; WISSEMEIER, A.H.; POSCHENRIEDER, C.; HORST, W.J.; BARCELO, J. Aluminum tolerance of maize cultivars as assessed by callose production and root elongation. **Z. Pflanzenern. Bodenk**, v.157, p.447-451, 1994.

MA, J.; FURUKAWA, J. Recent progress in the research of external Al detoxification in higher plants: a minireview. **Journal of Inorganic Biochemistry**, v.97, p.46-51, 2003.

MA, J.; RYAN, P.; DELHAIZE, E. Aluminum tolerance in plants and the complexing role of organic acids. **Trends in Plant Science**, v.6, p.273-278, 2001.

MANRIQUE, G.; RAO, I.; BEEBE, S. Identification of aluminum resistant common bean genotypes using a hydroponic screening method. Paper presented at the 18TH WORLD CONGRESS OF SOIL SCIENCE, Philadelphia, USA. July 9-15, 2006.

MATSUMOTO, H. Cell biology of aluminum toxicity and tolerance in higher plants. **International Review of Cytology**, v.200, p.1-46, 2000.

MATSUMOTO, H. Molecular aspect of Al tolerance in crop plants: Novel Al-activated malate transporter gene in wheat roots. **Soil Science and Plant Nutrition**, v.51 (5), p. 613-615, 2005.

MILES, J.W.; DO VALLE, C.B.; RAO, I.M.; EUCLIDES, V.P.B. Brachiariagrasses. In: MOSER, L.; BURSON, B.; SOLLENBERGER, L. E. (Ed.). **Warm-season grasses.** ASA-CSSA-SSSA, Madison, WI, USA, 2004. p.745-783.

MILES, J.W.; CARDONA, C.; SOTELO, G. Recurrent selection in a synthetic brachiariagrass population improves resistance to three spittlebug species. **Crop Science**, v.46, 1088-1093.

NANAMORI, M.; SHINANO, T.; WASAKI, J.; YAMAMURA, T.; RAO, I.M.; OSAKI, M.. Low phosphorus tolerance mechanisms: Phosphorus recycling and photosynthate partitioning in the tropical forage grass, *Brachiaria* hybrid cultivar Mulato compared with rice. **Plant Cell Physiol**, v.45, p.460-469, 2004.

POLLE, E.; KONZAK, C.F.; KITTRICK, J.A. Visual detection of aluminum tolerance levels in wheat by hematoxylin staining of seedling roots. **Crop Science**, v.18, p.823–827, 1978.

RANGEL. A.F.; MOBIN, M.; RAO, I. M.; HORST, W.J. Proton toxicity interferes with the screening of common bean (*Phaseolus vulgaris* L.) for aluminum resistance in nutrient solution. J. **Plant Nutr, Soil Sci**, v.168, p.607-616, 2005.

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R. Bras. Agrociência, Pelotas, v.14, n.4-4, p.01-07, out-dez, 2008

RAO, I. M. Role of physiology in improving crop adaptation to abiotic stresses in the tropics: The case of common bean and tropical forages. In: PESSARAKLI, M. (Ed.). **Handbook of Plant and Crop Physiology.** Marcel Dekker, Inc., New York, USA, 2001a. p.583-613.

RAO, I. M. Adapting tropical forages to low-fertility soils. *In:* GOMIDE, J.A.; MATTOS, W.R.S.; DA SILVA, S.C. (Ed.). **Proceedings of the XIX International Grassland Congress**. Brazilian Society of Animal Husbandry, Piracicaba, Brazil. 2001b. p.247-254.

RAO, I.; CRAMER, G. Plant nutrition and crop improvement in adverse soil conditions. In: CHRISPEELS, M.; SADAVA, D. (Eds.). **Plants, Genes, and Crop Biotechnology**. Published in partnership with the American Society of Plant Biologists and ASPB Education Foundation. Jones and Bartlett Publishers, Sudbury, Massachusetts, USA, 2003. p.270-303.

RAO, I.M.; ZEIGLER, R.S.; VERA, R.; SARKARUNG, S. Selection and breeding for acid-soil tolerance in crops: Upland rice and tropical forages as case studies. **BioScience, v.**43, p.454-465, 1993.

RAO, I.M.; MILES, J.W.; GRANOBLES, J.C. Differences in tolerance to infertile acid soil stress among germplasm accessions and genetic recombinants of the tropical forage grass genus, *Brachiaria*. **Field Crops Res**, v.59, p.43-52, 1998.

RAO, I.M.; MILES, J.W.; RICAURTE, J. Selección de hibridos de *Brachiaria* con resistencia a aluminio. **Pasturas Tropicales**, v.28, p.20-25, 2006.

RAO, I.M.; KERRIDGE, P.C.; MACEDO, M. Adaptation to low fertility acid soils and nutritional requirements of *Brachiaria. In*: MILES, J.W.; MAASS, B.L. AND DO VALLE, C.B. (Ed.). **The Biology, Agronomy, and Improvement of Brachiaria**. CIAT, Cali, Colombia, 1996. p.53-71.

RAO, I.M.; FRIESEN, D.K.; OSAKI, M. Plant adaptation to phosphorus-limited tropical soils. In: PESSARAKLI, M. (Ed.). **Handbook of Plant and Crop Stress**. Marcel Dekker, Inc., New York, USA, 1999a. pp. 61-96.

RAO, I.M.; FRIESEN, D.K.; HORST, W.J. Opportunities for germplasm selection to influence phosphorus acquisition from low-phosphorus soils. **Agroforestry Forum 9**, p.13-17, 1999b.

RAO, I.M.; BEEBE, S.; RICAURTE, J.; TERAN, H.; SINGH, S. Common bean (*Phaseolus vulgaris* L.) genotypes tolerant to aluminum-toxic soils in the tropics. In: PROCEEDINGS OF THE 6TH INTERNATIONAL SYMPOSIUM ON *PLANT-SOIL INTERACTIONS AT LOW PH* (PSILPH) held in Sendai, Japan from 31 July to 5 August, 2004. Japanese Society of Soil Science and Plant Nutrition, Sendai, Japan, 2004. p. 272-273.

RUIZ-TORRES, N.A.; CARVER, B.F.; WESTERMAN, R.L. Agronomic performance in acid soils of wheat lines selected for hematoxylin staining patterns. **Crop Sci,** v.32, p.104–107, 1992.

RYAN, P.; DITOMASSO, J.; KOCHIAN, L. Aluminum toxicity in roots: An investigation of spatial sensitivity and the role of the root cap. **Journal of Experimental Botany**, v. 44, p.437-446, 1993.

SIVAGURU, M.; HORST, W. The distal part of the transition zone is the most aluminum-sensitive apical root zone of maize. **Plant Physiology**, v.116, p.155-163, 1998.

THUNG, M.; RAO, I.M. Integrated management of abiotic stresses. In: SINGH, S.P. (Ed.). **Common Bean Improvement in the Twenty-First Century**. Kluwer Academic Publishers, Dordrecht, The Netherlands, 1999. p.331-370.

VITORELLO, V.A.; CAPALDI, F.R.; STEFANUTO, V.A. Recent advances in aluminum toxicity and resistance in higher plants. **Brazilian Journal of Plant Physiology**, v.17 (1), p. 129-143, 2005.

VON UEXKULL, H.R.; MUTERT, E. Global extent, development and economic impact of acid soils. **Plant and Soil**, v.171, p.1-15, 1995.

WAGATSUMA, T.**;** RAO, I.M.; WENZL, P.; KHAN, M.S.H.; TAWARAYA, K.; IGARASHI, K.; MURAYAMA, T.; KAWAMURA, T.; ISHIKAWA, S.; UEMURA, M. Plasma membrane lipid layer plays a key role in high level of aluminum resistance in signalgrass (*Brachiaria decumbens*): A new aspect on aluminum resistance. In: LI, C.J.; ZHANG, F.S.; DOBERMANN, A.; LAMBERS, H.; LI, X.L.; MARSCHNER, P.; MAENE, L.; MCGRATH, S.; OENEMA, O.; PENG, S.B.; RENGEL, Z.; SHEN, Q.R.; WELCH, R.; VON WIREN, N.; YAN, X.L.; ZHU, Y.G. (Ed.). **Plant Nutrition for Food and Security, Human Health and Environment Protection.** XV International Plant Nutrition Colloquium, Beijing, China, 2005. p.650-651.

WAGATSUMA, T.; KHAN, M.S.H.; RAO, I.M.; WENZL, P.; TAWARAYA, K.; YAMAMOTO, T.; KAWAMURA, T.; HOSOGOE, K.; ISHIKAWA, S. Methylene blue stainability of root-tip protoplasts can serve as an indicator of aluminum tolerance in a wide range of plant species, cultivars and lines. **Soil Science and Plant Nutrition,** v.51, p.991-998, 2005b.

WATANABE, T.; OSAKI, M.; YANO, H.; RAO, I.M. Internal mechanisms of plant adaptation to aluminum toxicity and phosphorus starvation in three tropical forages. **J. Plant Nutrition**, v.29, p.1243-1255, 2006.

WENZL, P.; CHAVES, A.L.; MAYER, J.E.; RAO, I.M.; NAIR, M.G. Roots of nutrient-deprived *Brachiaria* species accumulate 1,3-di-0-trans-feruloylquinic acid. **Phytochemistry**, v.55, p.389-395, 2000.

WENZL, P.; PATIÑO, G.M.; CHAVES, A.L.; MAYER, J.E.; RAO, I.M. The high level of aluminum resistance in signalgrass is not associated with known mechanisms of external detoxification in root apices. **Plant Physiology,** v.125, p.1473-1484, 2001.

WENZL, P.; CHAVES, A.L.; PATIÑO, G.M.; MAYER, J.E.; RAO, I.M. Aluminum stress stimulates the accumulation of aluminum-detoxifying organic acids in root apices of *Brachiaria* species. **J. Plant Nutrition and Soil Science**, v.165, p.582-588, 2002a.

WENZL, P.; MAYER, J. E.; AND RAO, I. M. Inhibition of phosphorus accumulation in root apices is associated with aluminum sensitivity in *Brachiaria*. **Journal of Plant Nutrition,** v.25, p.1821-1828, 2002b.

WENZL, P.; MANCILLA, L.I.; MAYER, J. E.; ALBERT, R.; RAO, I.M. Simulating infertile acid soils with nutrient solutions and the effects on *Brachiaria* species. **Soil Sci. Soc. Am. J,** v.67, p.1457-1469, 2003.

WENZL, P.; CHAVES, A.L.; RAO, I.M. Aluminum resistance coincides with differential resistance to trivalent lanthanide cations in *Brachiaria*. PROCEEDINGS OF THE 6TH INTERNATIONAL SYMPOSIUM ON PLANT-SOIL INTERACTIONS AT LOW PH (PSILPH) held in Sendai, Japan from 31 July to 5 August, 2004. p.262-263. Japanese Society of Soil Science and Plant Nutrition, Sendai, Japan.

WENZL, P.; ARANGO, A.; CHAVES, A.L.; BUITRAGO, M. E.; PATIÑO, G.M.; G.M. MILES, G.M.; RAO, I.M. A greenhouse method to screen brachiariagrass genotypes for aluminum resistance and root vigor. **Crop Sci**, v.46, p.968-973, 2006.

Figure legends

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Fig. 1. Relationship between the % inhibition of total root length and the % of increase of average root diameter per plant of 52 common bean (*Phaseolus vulgaris* L.) genotypes as affected by Al (50 µM) in nutrient solution. The values of % inhibition were obtained from [(total root length without Al – total root length with Al) / (total root length without Al)] x 100. The values of % increase in average root diameter were obtained from [(average root diameter with Al – average root diameter without Al) / (average root diameter without Al)] x 100
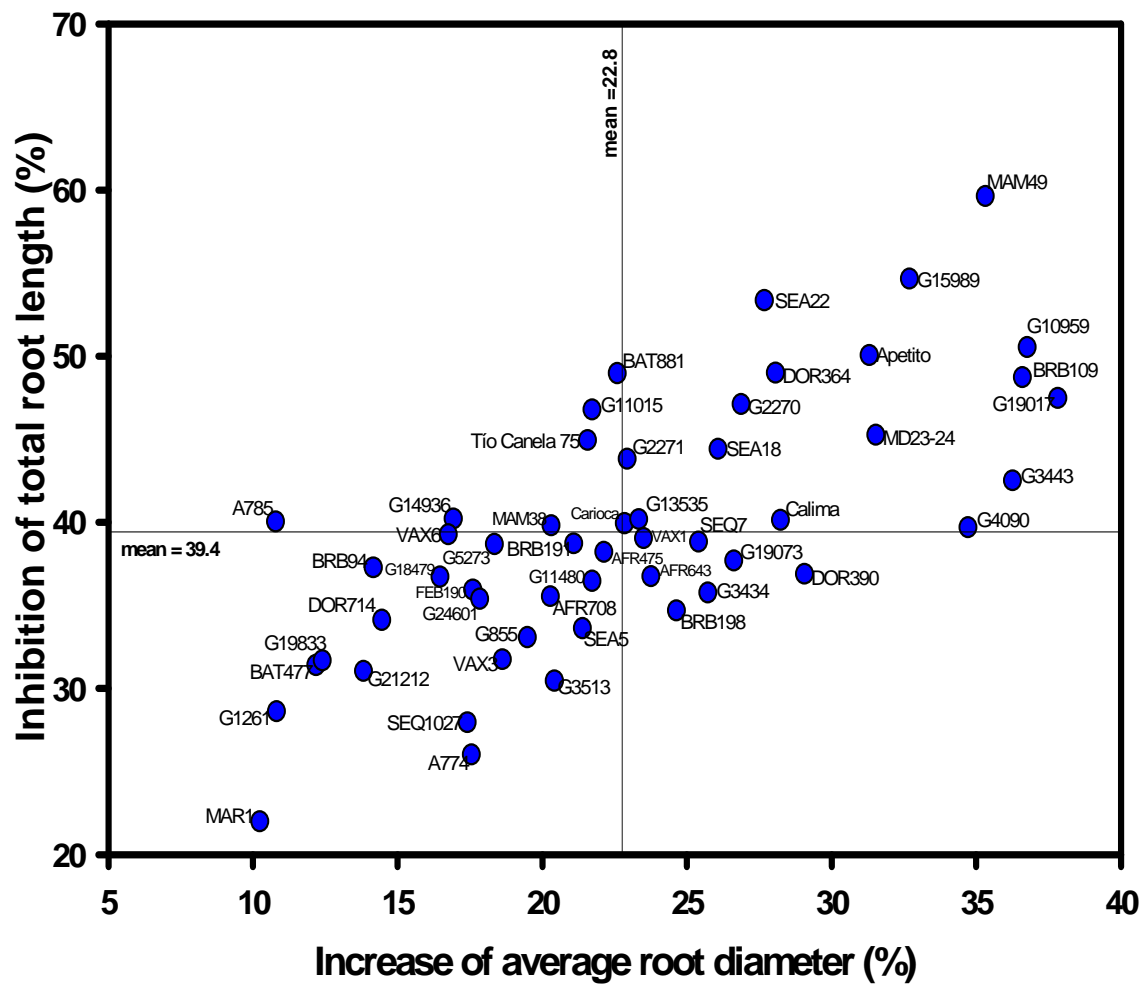

Fig. 2. Relationship between total root length with Al and total root length without Al in solution of 149 genotypes including 139 brachiariagrass (*Brachiaria* spp. Grisib) apomictic/sexual hybrids of the BR04NO population, 3 parents (CIAT 606, BRUZ/44-02, CIAT 6294) and 7 checks. Gentoypes that developed greater root length under both conditions were considered as Al resistant and were identified in the upper box of the right hand side.

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