Development and validation of a hydroponic screening method to identify acid soil-adapted genotypes of the tropical forage grass *Brachiaria*

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Introduction

The highly weathered acid soils of the South American savannas are characterized by a combination of nutrient deficiencies (phosphorus, calcium [Ca], magnesium, molybdenum; sometimes nitrogen, potassium) and mineral toxicities (aluminum [Al]; occasionally manganese) (Rao et al., 1993). Perennial brachiariagrasses (*Brachiaria* spp.) are the most widely sown forage grasses in these areas, occupying up to 70 million hectares. The Centro Internacional de Agricultura Tropical (CIAT) and the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) are developing apomictically reproducing interspecific hybrids to combine traits of three parental species: acid-soil adaptation of *B. decumbens* and spittlebug resistance of *B. brizantha* (both tetraploid apomicts), and sexual reproduction of a tetraploidized, sexual biotype of *B. ruziziensis*, which lacks both agronomic traits (Miles et al., 2004). Efficient screening methodologies are required to recover the desired traits through stepwise accumulation of favorable alleles in subsequent cycles of recombination and selection. There is a need to develop a greenhouse-based method to assess edaphic adaptation of large segregating populations (Wenzl et al., 2006). Edaphic adaptation is difficult to assess because it is only manifest in the persistence of pastures over several growing seasons.

Our main objective was to establish and validate a high-throughput hydroponic screening method to evaluate the edaphic adaptation of breeding materials using vegetative propagules (stem cuttings) grown in solution culture. The screening procedure was designed to quantify two key component traits: root vigor (which determines a plant's nutrient-foraging ability) and Al resistance.

Materials and Methods

The three main parents of the *Brachiaria* breeding program (*B. decumbens* cv. Basilisk, *B. brizantha* cv. Marandú, tetraploid *B. ruziziensis* clone 44-02) and a group of 38 *B. ruziziensis* × *B. decumbens* hybrids were used to validate the capability of the screening method to quantify root vigor and Al resistance. Root vigor was assessed by measuring growth of adventitious roots during 21 days in 200 µM CaCl2 (pH 4.2) (solution 1). Aluminum resistance was evaluated by comparing root growth in solution 1 with root growth in an identical solution that also contained 200 µM AlCl3 (solution 2). Scanned root images were analyzed with WinRHIZO software to measure total root length (RL) and the average root diameter (RD) for each individual root system. The pooled RL and RD data were log-transformed and adjusted, by linear regression, for harvest mean and the dry weight of stem cuttings. Aluminum resistance was quantified after regressing the adjusted logarithms of the RL (or RD) values from the Al treatment (solution 2) on those from the basal treatment (solution 1) to remove the variance component reflecting differences in root vigor among the hybrids.

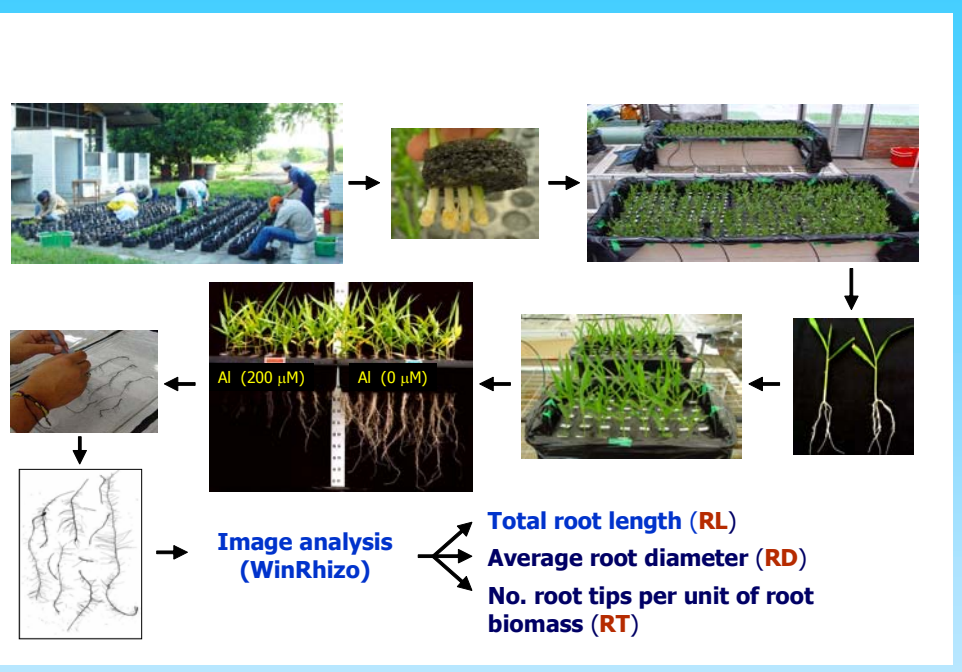

Figure 1. Procedure to identify acid-soil-adapted *Brachiaria* genotypes. Plants were propagated in a mixture of 1:3 (sand and soil). Vegetative propagules (stem cuttings), taken from the plants, were floated at the surface of a low-ionic-strength nutrient solution to produce adventitious roots. After nine days, pairs of rooted stem cuttings were selected for homogeneity. One propagule of each pair was transferred to solution 1 (200 µM $CaCl_2$, pH 4.20), the other to solution 2 (200 µM $CaCl_2$, 200 µM $AlCl_3$ pH 4.20). Twenty-one days after transfer, roots were separated from stems, stained and scanned on a flatbed scanner to determine total root length (RL) and average root diameter (RD). Genotypes with vigorous root growth were identified based on RL in solution 1. Aluminum-resistant genotypes were identified based on RL in solution 2 after removing the variance component that was due to differences root-growth as determined in solution 1.

Results

Roots of *B. decumbens* and *B. brizantha* continued to elongate in solution 1 for the entire period of evaluation (three weeks). Those of *B. ruziziensis*, by contrast, ceased to elongate after approximately one week and were considerably shorter. Presence of Al in solution 2 strongly inhibited root elongation of *B. brizantha*, but had only little effect on roots of *B. decumbens*. Root growth of *B. ruziziensis* in this solution was negligible. For both traits (RL, RD), the acid soil-adapted parent (*B. decumbens*) was significantly superior to the less-adapted parent (*B. ruziziensis*). Both traits segregated in the group of 38 *B. ruziziensis* × *B. decumbens* hybrids, with root vigor but not Al resistance showing transgressive segregation.

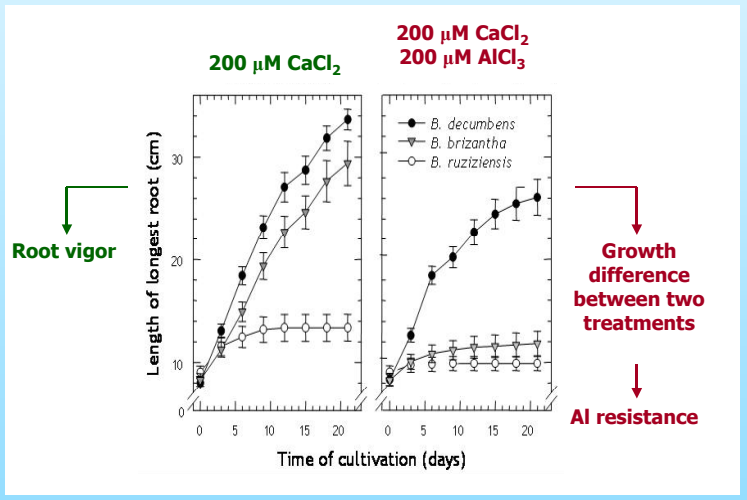

Figure 2. Initial test of the two treatments (± Al) with the three parental genotypes of the *Brachiaria* breeding program. The length of the longest root was recorded for up to 21 days of growth in the basal treatment (left panel) and the Al treatment (right panel).

Although seedling-based assays have been successfully applied to brachiariagrass seedlings, poor germination of *Brachiaria* seeds at the surface of nutrient solutions and the poor viability of hydroponically-grown seedlings upon transplantation to soil limit their applicability in a breeding program. The Al-resistance screen based on stem cuttings circumvents the transplantation problem and enables the concurrent assessment of root vigor of plants at a physiologically mature, vegetative stage as a second component trait contributing to edaphic adaptation. Vegetative propagation also permits simultaneous assessment of a single genotype (clone) for other traits such as insect or disease resistance, nutritional quality and seed production.

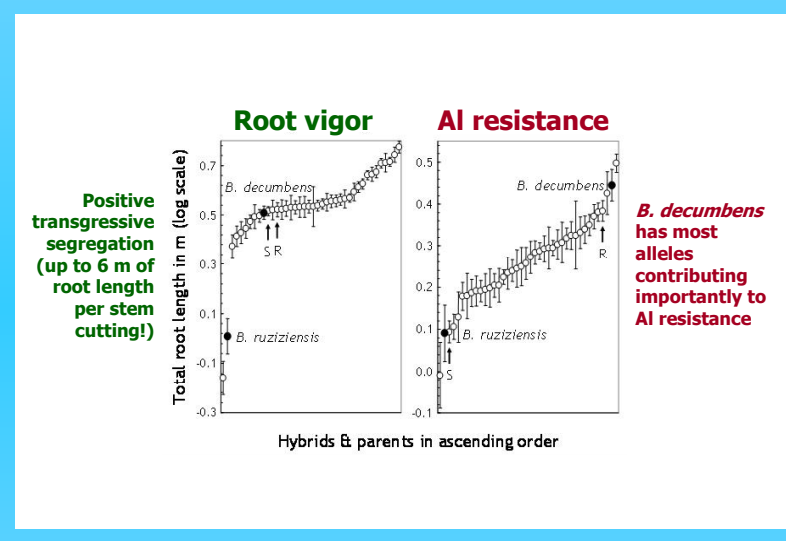

Figure 3. Segregation of root vigor (left panel) and Al resistance (right panel) in a group of 38 *B. ruziziensis* × *B. decumbens* F1 hybrids. Root vigor is the total root length (RL) in solution 1 (200 µM $CaCl_2$, pH 4.20), adjusted for the effects of stem-cutting biomass and harvest. Aluminum resistance is the stem-cutting-biomass/harvest-adjusted RL in solution 2 (200 µM $CaCl_2$, 200 µM $AlCl_3$ pH 4.20), after removing the variance component reflecting differences in root vigor. The two parents are highlighted by black symbols. The two hybrids with contrasting levels of Al resistance that were used for the hematoxylin-staining test (Fig. 4) are designated as "R" for Al-resistant and "S" for Al-sensitive.

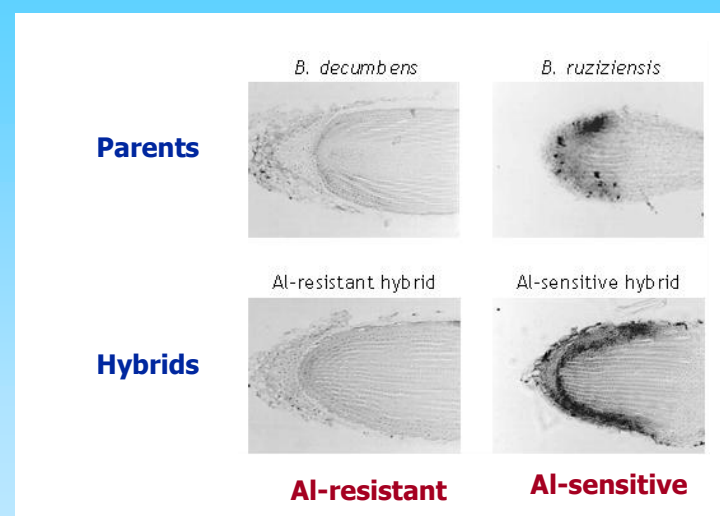

Figure 4. Hematoxylin-staining patterns for root apices of genotypes identified by the screen as Al-resistant (left side) and Al-sensitive (right side). The two *B. ruziziensis* × *B. decumbens* hybrids used for this test are also highlighted in Fig. 3.

Conclusions

Implementation of a simplified version of this hydroponic screening method, which allows simultaneous assessment of both traits (root vigor and aluminum resistance), has facilitated progress toward identifying promising apomictic hybrids that combine insect resistance with aluminum resistance from the *Brachiaria* breeding program at CIAT.

References

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2. Miles, J.W., C.B. do Valle, I.M. Rao, and V.P.B. Euclides. 2004. *Brachiaria* grasses. p. 745−783. *In* L.E. Sollenberger, L. Moser, and B. Burson (eds.) Warm-season grasses. ASA-CSSA-SSSA, Madison, WI.
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