

IDENTIFICATION OF ALUMINUM RESISTANT COMMON BEAN GENOTYPES USING A HYDROPONIC SCREENING METHOD



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Introduction

Common bean is the world's most important food legume, with an annual production value of over US\$10 billion. It is produced mainly on small-scale farms (80% of dry bean production) in developing countries in Latin America and Africa. About 40% of the bean-growing area is affected by aluminum (Al) toxicity, resulting in decrease of grain yield from 30% to 60%. It needs significant improvement in Al resistance to reduce the dependence of small farmers on lime and fertilizer inputs. Genetic variation exists for acid soil adaptation (Rao et al., 2004) and Al resistance (Rangel et al., 2005) among common bean genotypes. The most frequently measured effect of excess Al is inhibition of root elongation (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. The main objective of this study was to quantify genotypic differences in Al resistance in common bean.

Materials and Methods

We developed a hydroponic screening methodology using low ionic strength nutrient solutions to evaluate the effect of Al on root traits of common bean seedlings grown under greenhouse conditions (Figure 1). Seeds were germinated using a filter paper for 2 to 3 days and seedlings with uniform root length (5-7 cm) were selected for evaluation with nutrient solution, composed of (in μM) 286 $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, 300 KNO_3 , 150 NH_4NO_3 , 2.5 $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 150 $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 14 $\text{CaCl}_2 \cdot \text{H}_2\text{O}$, 5 $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 5 $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$, 1 $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 1 ZnCl_2 , 0.2 $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 6 H_3BO_3 , 5 $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$, 0.001 $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ and 57.5 NaCl . We used 2 treatments (pH 4.5 without Al and pH 4.5 with Al of 50 μM). Changes in root elongation as influenced by Al treatment were monitored by measuring the length of the primary root at 0 and 3 days after Al treatment. Seedlings were harvested and root systems were scanned using a software program, WINRHIZO and a flatbed scanner. Root attributes including total root length, mean root diameter, number of root tips and root volume were recorded. Shoot and root biomass were also recorded after drying the tissue in an oven at 65 °C for 2 days.

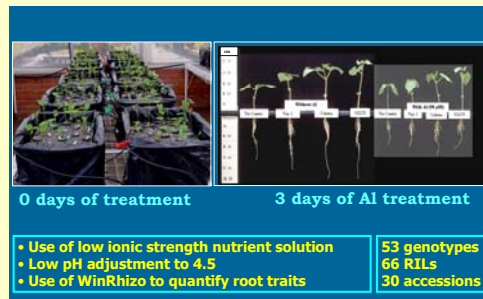


Figure 1. Common bean seedlings grown in low ionic strength nutrient solution at pH 4.5 with the presence (50 μM) or absence (0 μM) of Al in solution for 3 days in the greenhouse.

Results

Using this hydroponic screening method, we evaluated the rate of elongation of the primary root and the root morphology of 53 bean genotypes (landraces and breeding lines) and 66 RILs (Recombinant Inbred Lines) of G 5273 x MAM 38 to identify Al resistant genotypes. We identified 3 Andean genotypes (G19833, BRB 191 and G 5273), 8 Mesoamerican genotypes (G 1261, MAR 1, DOR 714, FEB 190, G 11015, G 3513, A 774 and G 855) and 4 RILs of G 5273 x MAM 38 (HF14137-19, HF14137-26, HF14137-98 and HF14137-88) with greater level of Al resistance (Figures 2 and 3). We also found that four 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 could serve as screening tools to identify Al resistant common bean genotypes.

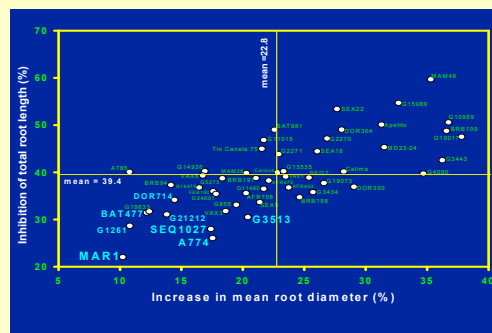


Figure 2. Relationship between percent increase in mean root diameter and percent inhibition of total root length of 52 genotypes of common bean grown in nutrient solution with (50 μM) or without (0 μM) Al for 3 days in the greenhouse.

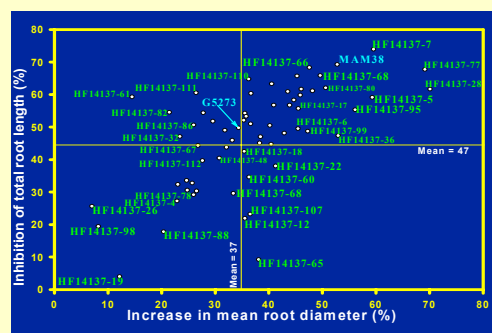


Figure 3. Relationship between percent increase in mean root diameter and percent inhibition of total root length of 66 RILs of G 5273 x MAM 38 of common bean grown in nutrient solution with (50 μM) or without (0 μM) Al for 3 days in the greenhouse.

Aluminum toxicity has significant effects on root development in common bean (Rangel et al., 2005). This was clearly evident through decrease in root elongation, increase in mean root diameter, decrease in number of root tips and decrease in specific root length (Figures 2 and 3). Root biomass, shoot biomass and total biomass were not significantly affected by Al treatment for 3 days.

Screening of 30 germplasm accessions from a sister species, *Phaseolus coccineus*, resulted in identification of 3 accessions with high level of Al resistance. These accessions could serve as parents to incorporate high level of Al resistance in common bean through genetic enhancement. Work is in progress to further

characterize physiological mechanisms of Al resistance, a prerequisite for development of simplified screening procedures and identification of quantitative trait loci (QTLs) for Al resistance in common bean. We are following a scheme that combines physiological analysis, QTL mapping for specific traits, and field phenotypic expression of acid soil tolerance, to associate traits with tolerance and to determine the relative importance of different traits (Figures 4 and 5).

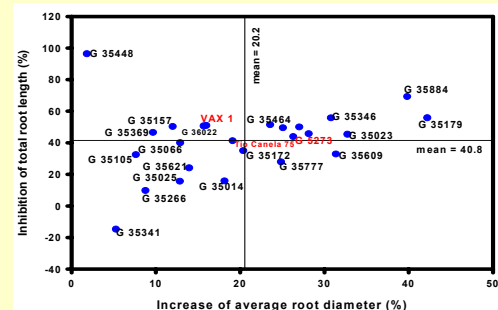


Figure 4. Relationship between percent increase in mean root diameter and percent inhibition of total root length of 30 accessions of *P. coccineus* grown in nutrient solution with (50 μM) or without (0 μM) Al for 7 days in the greenhouse.



Figure 5. Higher level of aluminum resistance in *P. coccineus* compared with *P. vulgaris*.

Conclusions

We implemented a hydroponic screening method and identified 3 Andean genotypes, 8 Mesoamerican genotypes and 4 recombinant inbred lines of common bean with greater level of Al resistance. We also found that four 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 could serve as screening tools to identify Al resistant common bean genotypes. Screening of 30 germplasm accessions from a sister species, *Phaseolus coccineus*, resulted in identification of 3 accessions with high level of Al resistance. These accessions could serve as parents to incorporate high level of Al resistance in common bean through genetic enhancement.

References

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