

# New genetic sources of resistance in the genus *Phaseolus* to individual and combined aluminium toxicity and progressive soil drying stresses

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**Abstract** Bean species and genotypes show wide phenotypic variability in relation to aluminium (Al) resistance and progressive soil drying. The objective of this study was to identify and characterize sources of resistance to Al toxicity and progressive soil drying among six genotypes of common bean (*Phaseolus vulgaris*), four of runner bean (*P. coccineus*), and one of tepary bean (*P. acutifolius*), using hydroponic and soil cylinder screening methods. One experiment on hydroponic screening of Al resistance was carried out using a basal nutrient solution with and without 20  $\mu$ M Al. Two experiments were carried out using two oxisols in 80 cm long soil cylinders with high Al (HA) and low Al (LA) saturation treatments. The three experiments showed an average of 36.9–53.5% inhibition of root growth with HA compared with LA treatments. Differences in root development and distribution were observed

among genotypes and species. Two accessions of *P. coccineus* (G35346-2Q, G35464-5Q) and one Andean common bean genotype (ICA Quimbaya) were outstanding in root and shoot growth in the HA treatments. *P. coccineus* accession (G35346-3Q) was outstanding under combined stress of Al-toxic acid soil and progressive soil drying. Accessions of *P. coccineus* may represent unique sources of Al resistance for the improvement of common bean through interspecific crosses.

**Keywords** Abiotic stress · Acid soil · Aluminium resistance · Root growth · Screening methods · Water stress

## Abbreviations

HA	High aluminium soil saturation
LA	Leaf area
LAI	Low aluminium soil saturation
MRD	Mean root diameter
NRT	Number of root tips
RDW	Root dry weight
REGWQ	Ryan–Einot–Gabriel–Welsh Multiple Test
R:S	Root to shoot
SDW	Shoot dry weight
SRL	Specific root length
TPRL48h	Tap root length at 48 h of exposure to with and without aluminium in solution
TPRL120h	Tap root length at 120 h of exposure to with and without aluminium in solution

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TRER	Tap root elongation rate
TRL	Total root length
VRD29d	Visual rooting depth at 29 days
VRD33d	Visual rooting depth at 33 days
WS	Water stress
WW	Well watered

## Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most important grain legumes for direct human consumption in the world. The crop is mainly produced on small-scale farms in developing countries in Latin America and Africa where both biotic and abiotic stress factors limit production. About 60% of the bean growing area is affected by drought while about 40% of the bean growing area is affected by aluminium (Al) toxicity, resulting in yield reductions from 30 to 60% (Wortmann et al. 1998; Thung and Rao 1999). Abiotic stress resistance is by its nature more complex physiologically, typically subject to large environmental effects and has been less well studied than biotic stress resistance in common bean (Rao 2001).

Aluminium is one of the most abundant minerals in the soil, comprising approximately 7% of soil mass. At neutral or weakly acidic pH, it exists in insoluble forms of alumino-silicate or oxide. However, in acidic soils, it is solubilised into a phytotoxic form. Toxic Al levels damage roots, restrict plant size, and lower yield in most crops (Villagarcia et al. 2001). Aluminium toxicity results in inhibition of root elongation (Mossor-Pietraszewska 2001; Horst et al. 2010). The toxic effects of Al in soil can be overcome by adding appropriate soil amendments such as lime (Pandey et al. 1994; Villagarcia et al. 2001). However, lime application usually must be repeated over several years and is not affordable to most smallholders in developing countries that grow beans. Developing bean genotypes tolerant to acid soil conditions is an ecologically friendly, energy-conserving, and economical solution for resource-poor farmers in the tropics (Rangel et al. 2005).

Genotypic differences in seed yield of common bean germplasm accessions and breeding lines have been observed in field screening on Al-toxic acid soils that were amended with or without lime (65%

Al saturation) (Rao 2001; Rao et al. 2004). These genotypic differences in seed yield could be related to differences in resistance to Al, and acquisition and utilization of nutrients for transport of photoassimilates to developing seeds. Significant genotypic differences in Al resistance in common bean were reported based on Al-inhibited root elongation in nutrient solution (Foy 1988; Massot et al. 1999; Rangel et al. 2005, 2007; Manrique et al. 2006). Differential genotypic response to Al stress contributes to identification of new sources of Al resistance as well as improved understanding of mechanisms of Al resistance in common bean (Rangel et al. 2005, 2007, 2009, 2010; Blair et al. 2009; López-Marín et al. 2009). Resistance to Al in common bean is attributed to the release of citrate by the root apex (Rangel et al. 2010) and the expression of a citrate transporter *MATE* (multidrug and toxin extrusion family protein) gene is crucial for citrate exudation (Eticha et al. 2010). Although the *MATE* gene expression was a prerequisite for citrate exudation and Al resistance, genotypic difference in Al resistance in common bean was mainly dependent on the capacity to sustain the synthesis of citrate for maintaining the cytosolic citrate pool that enables exudation (Rangel et al. 2010; Eticha et al. 2010). The initial Al-induced inhibition of root elongation in both Al-resistant (ICA Quimbaya) and Al-sensitive (VAX 1) genotypes was correlated with the expression of the *ACCO* (1-aminocyclopropane-1-carboxylic acid oxidase) gene (Eticha et al. 2010).

Improving Al resistance in plants has been limited by inadequate screening methodologies (Villagarcia et al. 2001). Screening for Al resistance based on field data would be strengthened by complimentary evaluation of specific phenotypic and physiological traits. Hydroponic systems have been used with success in evaluation of Al resistance for many crops and are an attractive alternative. They allow evaluation of a large number of genotypes quickly and have been used to identify parental stock for soybean breeding (Bianchi-Hall et al. 1998; Campbell and Carter 1990; Carter and Rufty 1993; Spehar 1994; Bianchi-Hall et al. 2000; Silva et al. 2001). Common bean and soybean both pertain to the tribe Phaseoleae, much more closely related to each other than they are to Lotus, Medicago, or other grain legumes such as lentils or chickpeas (Choi et al. 2004). This opens the possibility of common genes and/or

mechanisms between the species, and suggests that the application of methods used in soybean might serve to discriminate genetic differences in common bean as well. Hydroponic systems for evaluation of genetic materials provide a strict control of nutrient availability and are widely used in genetic studies. To complement the hydroponic screening system, researchers at CIAT developed a greenhouse screening method using vertical soil cylinders with high Al (HAl) saturation to quantify genotypic differences in root development and distribution in Al-toxic soil conditions (CIAT 2008). Soil-based systems offer a medium that is more similar to field conditions. This methodology also permits characterization of the root system, in terms of rooting depth and branching of fine roots in a soil with known bulk density.

Drought is a major abiotic stress in many parts of the world (Johansen et al. 1994). There is an urgent need for developing high yielding drought resistant cultivars that use water efficiently, reduce dependence on irrigation water and associated production costs, increase and stabilize yield in drought-prone environments, and increase profit margins for producers (Muñoz-Perea et al. 2006). Selection for drought resistance based on yield alone may not recover all specific physiological attributes as these might be expressed differentially under distinct conditions (Subbarao et al. 1995). Rooting pattern, especially greater root length in lower soil strata, is an important drought resistance mechanism for common bean (Sponchiado et al. 1989). Drought resistant bean genotypes could extend their roots to 1.2 m depth in drought environments, whereas the sensitive genotypes could not extend their roots beyond 0.8 m; and these differences in rooting depths were reflected in overall shoot growth and yield (White and Castillo 1988). Root systems show considerable architectural variation among species, among genotypes of given species, and even with different parts of a single root system (Lynch 1995). Wild relatives in many legumes possess deep rooting capability that could be transferred to cultivated legumes. A number of *Phaseolus* species, such as *P. acutifolius*, *P. retensis*, and *P. coccineus*, have deep and/or tuberous primary root attributes (Singh and White 1988).

Understanding the genetic and physiological mechanisms by which plants cope with changes in environmental conditions is critical for creating efficient strategies to develop stress-resistant cultivars

for sustainable production systems (Rao 2001). Abiotic stress factors often co-occur in farmers' fields. Roots that are stunted by Al toxicity are inefficient in absorbing both nutrients and water (Mossor-Pietraszewska 2001). Al-resistant plants may be more drought tolerant and require lower inputs of lime and P fertilizer than less resistant genotypes (Little 1988). Yang et al. (2010) characterized the combined Al toxicity and drought stress on root growth, with special emphasis on Al/drought interaction in the root apex of common bean. Using polyethylene glycol (PEG) to simulate osmotic stress (OS) or drought stress, they found that OS enhances Al resistance by inhibiting Al accumulation in the root apices of the Al-sensitive genotype (VAX 1). This alleviation of Al toxicity was found to be related to the alteration of cell wall porosity resulting from PEG-induced dehydration of the root apoplast.

Improving resistance to two complex stresses such as Al toxicity and water stress (WS) in common bean requires identifying new sources of resistance among *P. vulgaris* accessions and in sister species including *P. coccineus* and *P. acutifolius*. *P. coccineus* is placed within the secondary gene pool and can be crossed readily with common bean. *P. acutifolius* is considered to form part of the tertiary gene pool, but can also be crossed to common bean using embryo rescue. The objective of this work was to identify potential parents based on phenotypic differences among bean genotypes in root development and root distribution under individual and combined stress factors of HAl toxicity and progressive soil drying.

## Materials and methods

Three greenhouse trials were conducted at CIAT headquarters in Palmira (Lat. 3°29'N; Long. 76°21'W, Altitude 965 m) using hydroponic and soil cylinder systems. For purposes of these studies, "Al resistance" refers to the response of a genotype to toxic Al in the hydroponic system, and tolerance to Al-toxic acid soil conditions refers to tolerance to HAl saturation in acid soil together with low availability of nutrients. A single hydroponic screening (Trial 1) employed a low ionic strength nutrient solution to evaluate root traits of seedlings grown with or without 20  $\mu$ M Al in a basal nutrient solution (Rangel et al. 2005, 2007). One soil cylinder experiment (Trial 2) compared plant response

in two oxisols with HAl and low Al (LAl) saturation. A second soil cylinder experiment (Trial 3) was conducted to evaluate plant response to individual and combined stress factors of acid soil (HAl and LAl saturation) and two levels of soil moisture (well watered (WW) and water stress (WS) induced by progressive soil drying) in a factorial design.

#### Plant materials

Eleven bean genotypes were selected for the study from our previous work (CIAT 2005; S. Beebe, unpublished data), including four runner bean (*P. coccineus*) accessions (G35066-1Q, G35346-2Q, G35346-3Q and G35464-5Q); six common bean genotypes including two large seeded beans of the Andean gene pool (ICA Quimbaya and IJR, indeterminate Jamaica red) and four lines of the Mesoamerican gene pool (VAX1, VAX3, VAX6, SER16); and one tepary bean (*P. acutifolius*) accession (G40159). The *P. coccineus* accessions had been identified in a field screening of 155 entries of *P. coccineus* and *P. polyanthus* in an Al toxic field site in Santander of Quilichao, Colombia, based on shoot vigor (S. Beebe, unpublished results). *P. acutifolius* is a drought resistant desert species and one of its accessions, G40159 had been identified as especially drought resistant (Rao et al. 2007). The VAX lines had been selected for common bacterial blight resistance in Santander of Quilichao during their development, and VAX1 had expressed good shoot vigor in Al toxic soils. These plant materials were evaluated for their phenotypic differences under individual and combined stresses of Al and drought. For the hydroponic experiment, germinated seeds were transferred to small pots containing sterile sand for root development and were carefully removed from the sand after 3 days. Seedlings with uniform vigour and tap root length were chosen for evaluation in hydroponic system. For the two soil cylinder experiments, seeds were surface sterilized with sodium hypochlorite (1% for 5 min) and washed with abundant deionised water. Seeds were germinated on filter paper for 2–3 days before planting in soil tubes.

#### Evaluation for Al resistance using hydroponic system

The hydroponic experiment (Trial 1) was conducted during November and December 2007. Plants were grown in a greenhouse with an average temperature

of 31.1/22.3°C (day/night), a relative humidity of 48.0/67.3% (day/night), and with a maximum photosynthetic active radiation of 1,100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  photon flux density at noon. Seedlings with uniform root length (5–7 cm) were selected for evaluation with nutrient solution composed with 5 mM  $\text{CaCl}_2$ , 0.5 mM KCl and 8  $\mu\text{M}$   $\text{H}_3\text{BO}_3$  at pH 4.5, with or without 20  $\mu\text{M}$   $\text{AlCl}_3$  (Rangel et al. 2007; López-Marín et al. 2009). Mononuclear Al ( $\text{Al}_{\text{mono}}$ ) concentration was measured colorimetrically using aluminon or pyrocatechol violet method according to Kerven et al. (1989). Aluminium treatment of 20  $\mu\text{M}$  resulted in  $16 \pm 2 \mu\text{M}$   $\text{Al}_{\text{mono}}$  after 24 h. Twenty liter plastic tanks were filled with 16 l of nutrient solution. Each seedling was placed in an individual compartment in a tray floating on the solution, and the nutrient solution was permanently aerated with a compressor during the evaluation. Acclimation to low pH before applying Al treatment was made by adjusting the solution pH to 5.5 for 0 h, followed by pH 4.9 for 18 h and lastly by pH 4.5 for 24 h (Rangel et al. 2007). Nutrient solutions were renewed every third day. Plants with the same root length were distributed in pairs in each treatment after measuring the length of tap root with a ruler. The experimental design was a randomized complete block with five to ten replications.

Root morphological attributes were evaluated. Tap root length (TPRL) at 48 h (TPRL48h) and at 120 h (TPRL120h) was recorded. Tap root elongation rate (TRER) was determined at 48 h (TRER48h) and after 120 h (TRER120h) with and without Al stress based on the initial measurement of tap root length. TRER was defined as the difference between the initial and final tap root length during the treatment period; and Al-induced inhibition of TRER was calculated according to Rangel et al. (2005):

#### Inhibition of root elongation (%)

$$= [(\text{TRER}_{\text{control}} - \text{TRER}_{\text{Al}}) / \text{TRER}_{\text{control}}] \times 100$$

At harvest, roots were separated from the rest of the plants, saved in plastic bags and refrigerated at 4°C while proceeding to analyze images using a flatbed colour scanner, Epson Expression1680 Scanner. Differences in root morphological attributes among genotypes including total root length (TRL), mean root diameter (MRD), and number of root tips (NRT) were analyzed using WinRhizo<sup>®</sup> software program.

Specific root length (SRL, root length per unit dry weight) was calculated, and the root dry weight (RDW) was determined by drying roots at 65°C in an oven for 48 h.

#### Evaluation for individual and combined stress in soil

Two soil cylinder experiments were carried out, each arranged as three randomized complete blocks. The first soil cylinder experiment (Trial 2) compared plant response in Oxisols with LAI and HAI saturation and was conducted during June–July 2007 in a greenhouse in Palmira (CIAT/Colombia) with an average temperature of 29.4/23.1°C (day/night), relative humidity of 57.2/79.4% (day/night), and maximum photosynthetic active radiation of 1,100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  photon flux density at noon.

Stress of Al-toxic acid soil was simulated using soils collected from Santander de Quilichao, Cauca Department (3°06'N lat., 76°31'W long; 990 m altitude), Colombia. Soil used in the Al stress treatment (HAI) was characterized by a pH of 4.11 and 76% Al soil saturation (0–10 cm) for top-soil (top 10 cm of the cylinder) and 83% Al saturation for subsoil (10–75 cm) with pH 4.14 (Table 1). This treatment did not receive any additional fertilizer application to simulate HAI with low nutrient availability soil conditions that are typical of Al-toxic acid soils. Root and shoot growth of bean genotypes under this treatment was visually (based on symptoms) restricted by both Al-toxicity and low availability of P. Soil used for LAI treatment was characterized by a pH of 4.45 and 28% Al saturation (0–10 cm) for top-soil and 58% Al saturation for subsoil (10–75 cm) with pH 4.29.

The soil cylinders for LAI treatment were packed with Quilichao soil (described in Table 1), previously fertilized with adequate amendments ( $\text{g kg}^{-1}$  soil) for top soil (0–10 cm): 3.69 N (urea), 5.30 P (triple superphosphate), 5.30 Ca (triple superphosphate), 4.08 K (KCl), 6.36 Ca ( $\text{CaCO}_3$ ), 6.36 Mg ( $\text{MgCO}_3$  or dolomite lime), 0.49 S (elemental sulphur), 0.09 Zn ( $\text{ZnCl}_2$ ), 0.11  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.01 B ( $\text{H}_3\text{BO}_3$ ) and 0.01 Mo ( $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ ); and for subsoil (10–75 cm) 14.76 N (urea), 21.2 P (triple superphosphate), 21.21 Ca (triple superphosphate), 16.32 K (KCl), 25.45 Ca ( $\text{CaCO}_3$ ), 25.45 Mg ( $\text{MgCO}_3$  or dolomite lime), 1.97 S (elemental sulphur), 0.36 Zn ( $\text{ZnCl}_2$ ), 0.46  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.05 B ( $\text{H}_3\text{BO}_3$ ) and 0.02 Mo ( $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ ). This level of fertilizer application was designed to provide adequate supply of nutrients, and it did not affect Al saturation and pH of the amended soil. The polyethylene cylinders were inserted into PVC pipes and were maintained at 80% field capacity by weighing each cylinder every 3 days and applying water to the soil at the top (Polanía et al. 2009).

Shoot and root attributes were evaluated on plants in soil tubes. Total chlorophyll content (SPAD) was measured every week using SPAD-502 Chlorophyll meter (Minolta camera Co., Ltd, Japan). Visual rooting depth (VRD) was determined at 29 days after planting. At the time of harvest (29 days after planting), leaf area (LA) was determined by scanning leaves of each genotype using a LI-3100 Area meter (LI-COR Biosciences). Shoot dry weight (SDW) was measured after drying leaves, stems and pods in an oven at 70°C for 72 h. Each soil cylinder was sliced into six layers representing different soil depths (0–5, 5–10, 10–20, 20–40, 40–60, 60–75 cm), soil and roots were collected, and roots washed and cleaned to separate living plant roots from organic debris before

**Table 1** Chemical characteristics of two oxisols with HAI or LAI saturation from Santander of Quilichao used for evaluating acid soil tolerance

Al saturation	Soil depth (cm)	pH	Al ( $\text{cmol kg}^{-1}$ soil)	Ca ( $\text{cmol kg}^{-1}$ soil)	Mg ( $\text{cmol kg}^{-1}$ soil)	K ( $\text{cmol kg}^{-1}$ soil)	Al sat. (%)	SOM (%)	Available P ( $\text{mg kg}^{-1}$ )
High	0–10	4.11	4.60	0.94	0.30	0.18	76	5.96	8.80
High	10–20	4.14	4.40	0.69	0.16	0.07	83	4.94	3.30
Low	0–10	4.45	1.65	3.32	0.89	0.26	28	5.38	9.70
Low	10–20	4.29	3.02	1.63	0.25	0.28	58	4.56	4.30

SOM Soil organic matter

scanning. Root length and biomass distribution were determined for each profile, but cutting of soil cylinders at different depths did not permit measuring NRT. Differences in distribution of root length and root biomass within soil cylinder among genotypes were estimated by Gale and Grigal (1987) asymptotic equation:

$$Y = 1 - \beta^d$$

where  $Y$  is the fraction of root length/biomass accumulated from the soil surface to depth  $d$  (cm), and  $\beta$  is a parameter that describes the shape of the cumulative distribution with depth. Higher  $\beta$  values (closer to 1) indicate a greater proportion of root length/biomass deeper in the soil profile. Lower  $\beta$  values (e.g.  $\beta = 0.920$ ) imply a greater proportion of root length/biomass nearer to the soil surface.

In a second soil cylinder trial (Trial 3), individual and combined stress of Al and progressive soil drying were evaluated in September 2008 under greenhouse conditions at an average temperature of 30.7/23.3°C (day/night), a relative humidity of 49.0/68.3% (day/night), and at an average photosynthetic photon flux density of 820  $\mu\text{mol m}^{-2} \text{s}^{-1}$  during the day. Plants were grown in transparent tubes inserted in PVC pipes as previously described for Al screening with the same soil type from Santander of Quilichao. The LAI saturation treatment was fertilized with adequate supply of nutrients as described above for top soil and subsoil. The experimental design was a randomized complete block with three repetitions and with two levels of Al saturation in soil (high and low) and two levels of water supply (WW and WS due to progressive soil drying) in a factorial design. Cylinders of genotype-soil-moisture combinations were randomized within each block. Each cylinder was packed with two types of soil (top-soil and sub-soil); and maintained at 80% of field capacity by weighing every 3 days (4,780 g for HAI saturation treatment cylinders and 4,910 g for the low saturation). WS was imposed to simulate progressive soil drying after 10 days of initial growth while for the WW treatment, water was applied to the top of cylinders to maintain them at 80% of field capacity (Polanía et al. 2009). At harvest, shoots and roots of 33 day-old plants (23 days without water application in the WS treatment) were separated, and LA measured by scanning leaves. Shoot biomass was determined after drying leaves and stems in an oven. Roots were

processed in the same way as for previous soil cylinder trial (Trial 2) with individual stress treatment of Al-toxic acid soil alone and the same parameters were determined.

### Statistical analysis

Analysis of variance was performed by the ANOVA statistical procedure of SAS (SAS 9.1, 2002–2003; SAS institute Inc.; SunOS 5.9 platform). The means were compared using Ryan–Einot–Gabriel–Welsh (REGWQ) Multiple Test. This test controls the type I experiment-wise error rate. Means for each dependent variable were grouped, and means with the same letter are not significantly different ( $P < 0.05$  level). Differences between genotypes were analyzed with the least significant difference (LSD). Correlation coefficients were calculated (PROC CORR) for all pairs of genotypic means across all replications with each treatment and for all three experiments. Regression analysis was carried with the PROC REG procedure.

## Results and discussion

The response of root attributes (TRL, MRD, SRL, VRD) to Al stress was used to assess resistance of beans to Al toxicity and Al toxic acid soil. Variation among the 11 genotypes was found in this study using both hydroponic (Tables 2, 3) and soil cylinder (Tables 4, 5, 6, 7) screening systems. Relationships between traits were considered based on expectations of which combinations of traits would confer a favourable reaction to Al toxicity. High variability in root attributes was observed between genotypes except for TPRL at 48 h of treatment (TPRL48h) with 20  $\mu\text{M}$  Al in hydroponic system. Since *P. coccineus* accessions did not show any difference in the hydroponic system between treatments with and without Al in solution for 48 h, the exposure time was extended to 120 h where significant genotypic variation ( $P < 0.001$ ) in Al resistance was observed.

### Evaluation of Al resistance in hydroponic system

Al stress in hydroponic screening (Trial 1) affected root growth characteristics of all 11 genotypes tested. Variation in response to Al stress (20  $\mu\text{M}$  Al) among



**Table 2** Influence of Al-stress (with (20  $\mu\text{M}$  Al) and without (0  $\mu\text{M}$  Al)) on total root length (TRL), tap root length (TPRL), tap root length (TPRL) at 48 h and 120 h, root dry weight (RDW), mean root diameter (MRD), specific root length (SRL) and number of root tips (NRT) of 11 bean genotypes from three *Phaseolus* species grown in hydroponic system (Trial 1)

Genotype	TRL (m)		TPRL48h (cm)		TPRL120h (cm)		RDW (g)		MRD (mm)		SRL ( $\text{m g}^{-1}$ )		NRT	
	20 $\mu\text{M}$ Al	0 $\mu\text{M}$ Al	20 $\mu\text{M}$ Al	0 $\mu\text{M}$ Al	20 $\mu\text{M}$ Al	0 $\mu\text{M}$ Al	20 $\mu\text{M}$ Al	0 $\mu\text{M}$ Al	20 $\mu\text{M}$ Al	0 $\mu\text{M}$ Al	20 $\mu\text{M}$ Al	0 $\mu\text{M}$ Al	20 $\mu\text{M}$ Al	0 $\mu\text{M}$ Al
G 35464-5Q <sup>a</sup>	8.4	11.7	22.4	22.3	30.8	31.1	0.13	0.12	0.57	0.5	68.2	91.4	926	1924
G 35346-2Q <sup>a</sup>	8.1	11.0	17.3	20.0	26.4	28.7	0.15	0.16	0.69	0.61	53.6	72.8	668	1307
G 35346-3Q <sup>a</sup>	5.5	9.7	16.7	19.6	24.0	27.1	0.11	0.14	0.62	0.57	49.3	70.5	472	1395
G 35066-1Q <sup>a</sup>	4.2	8.8	16.0	18.4	20.9	22.6	0.1	0.12	0.7	0.55	41.2	75.3	374	1325
ICA Quimbaya <sup>b</sup>	3.2	5.9	17.4	19.2	20.2	24.2	0.07	0.09	0.63	0.54	44.3	62.6	364	1221
IUR <sup>b</sup>	2.8	4.7	16.4	18.6	18.9	23.9	0.06	0.09	0.6	0.55	53.7	61.6	396	965
VAX 1 <sup>b</sup>	2.5	6.4	16.4	21.0	17.0	26.2	0.04	0.06	0.57	0.45	59.8	114.6	284	1110
SER 16 <sup>b</sup>	2.0	3.7	15.8	18.0	17.0	23.0	0.03	0.05	0.58	0.51	64.1	75.9	228	561
VAX 3 <sup>b</sup>	2.0	4.9	16.1	20.3	18.2	26.5	0.03	0.06	0.56	0.48	63.2	91.4	218	802
VAX 6 <sup>b</sup>	1.9	3.3	13.0	15.9	14.3	19.7	0.04	0.06	0.63	0.58	49.9	56.0	177	422
G 40159 <sup>c</sup>	2.4	4.0	16.6	20.5	17.8	23.5	0.03	0.04	0.47	0.42	86.1	111.4	306	707
Mean	3.5	6.3	16.5	19.4	19.6	24.8	0.07	0.08	0.6	0.52	56.3	80.5	360	1028
LSD <sub>0.05</sub>	0.3	0.4	0.6	0.7	0.8	0.8	0.01	0.02	0.03	0.02	15.3	25.7	4.8	7.0

<sup>a</sup> *P. coccineus*<sup>b</sup> *P. vulgaris*<sup>c</sup> *P. acutifolius*

genotypes was found for TRL, TPRL48h, TPRL120h, RDW, MRD, SRL and NRT (Table 2). Three *P. coccineus* accessions (G35464-5Q, G35346-2Q, G35346-3Q) were highly resistant to Al, whereas three Mesoamerican common bean genotypes (VAX6, VAX3, SER16) were more sensitive. Genotype  $\times$  treatment (Level of Al) interaction were found for SRL, NRT, MRD and TRL (Table 3). TRL was highly correlated with TPRL48h ( $r = 0.77^{**}$ ), with TPRL120h ( $r = 0.92^{***}$ ), with RDW ( $r = 0.91^{***}$ ), and with NRT ( $r = 0.96^{***}$ ). This suggests that the tap root response to Al toxicity (TPRL5d) reflects the reaction of the rest of the root system. In contrast López-Marín et al. (2009) found that while bean genotype G19833 showed a higher TRER than DOR364 in both control and Al treatment solutions, differences in TRL between the two genotypes were less notable.

Correlation was also found between TPRL48h and NRT ( $r = 0.78^{**}$ ), and between TPRL48h and TPRL120h ( $r = 0.74^{**}$ ) (Table 3). This latter

correlation was perhaps lower than expected, considering that it reflects measurements of the same trait on the same plants over a 72 h period. Perhaps different plant responses were being expressed over this period, as noted by Rangel et al. (2007, 2010) in other trials.

TRER can be monitored easily and is assumed to be an indicator of Al resistance. Al strongly affected TRER with means varying from 0.15 to 1.0 mm h<sup>-1</sup>. TRER of three *P. coccineus* accessions (G35464-5Q, G35346-2Q and G35346-3Q) and ICA Quimbaya (an Andean common bean) were high ( $>0.6$  mm h<sup>-1</sup>) compared to other genotypes (Fig. 1A). The major Al toxicity symptom observed in plants is inhibition of root elongation rate calculated from the comparison of root elongation rate with and without toxic Al (Marschner 1991; Ryan et al. 1993; Delhaize and Ryan 1995). TRER was inhibited by 21.3–60.7%, with an average of 42.7%. Three genotypes (VAX3, VAX1, G35066-1Q) were sensitive to Al with over 50%; six genotypes including SER16, IJR, G35346-

**Table 3** Correlation coefficients and mean squares for total root length (TRL), tap root length (TPRL) at 48 h and 120 h, root dry weight (RDW), mean root diameter (MRD), specific

root length (SRL), and number of root tips (NRT) of 11 bean genotypes grown under hydroponic system with two level of Al (20  $\mu$ M Al and 0  $\mu$ M Al) (Trial 1)

Traits/source	Al level/DF	TRL	TPRL48h	TPRL120h	RDW	MRD	SRL	NRT
TRL	0	1						
	20	1						
TPRL48h	0	0.55 (ns)	1					
	20	0.77**	1					
TPRL120h	0	0.78**	0.74**	1				
	20	0.92***	0.74**	1				
RDW	0	0.85***	0.13 (ns)	0.58 (ns)	1			
	20	0.91***	0.59 (ns)	0.83**	1			
MRD	0	0.14 (ns)	-0.59 (ns)	-0.04 (ns)	0.61*	1		
	20	0.29 (ns)	-0.13 (ns)	0.24 (ns)	0.57 (ns)	1		
SRL	0	0.17 (ns)	0.72*	0.26 (ns)	-0.34 (ns)	-0.84***	1	
	20	-0.23 (ns)	0.07 (ns)	-0.20 (ns)	-0.46 (ns)	-0.87***	1	
NRT	0	0.95***	0.43 (ns)	0.66*	0.86***	0.16 (ns)	0.04 (ns)	1
	20	0.96***	0.78**	0.91***	0.84***	0.20 (ns)	-0.11 (ns)	1
Level of Al	1	12.34***	4.54*	10.93***	0.0062***	0.0494***	11894***	5158***
Rep. (Al level)	4	0.099 (ns)	0.46 (ns)	0.68 (ns)	0.0002 (ns)	0.0008 (ns)	518.5 (ns)	34.97 (ns)
Genotype	10	4.93***	0.86*	2.33***	0.0157***	0.0184***	5894***	525.5***
Gen. $\times$ Al level	10	2.23*	0.1 (ns)	0.45 (ns)	0.0001 (ns)	0.0015*	1713***	73.77**
Error	236	0.12	0.37	0.53	0.0002	0.0007	377.5	30.3

ns Non significant

Statistical significance at the \* 0.5, \*\* 0.01, and \*\*\* 0.001 probability levels, respectively



**Table 4** Influence of acid soil stress (HAI high aluminium saturation, LAI low aluminium saturation) on total root length (TRL), mean root diameter (MRD), specific root length (SRL)and VRD at 29 days (VRD29d) for 29 days-old plants of 11 bean genotypes from three *Phaseolus* species grown in soil cylinders under “WW conditions” (Trial 2)

Genotype	TRL (m)		MRD (mm)		VRD29d (cm)		SRL (m g <sup>-1</sup> )	
	HAI	LAI	HAI	LAI	HAI	LAI	HAI	LAI
G 35464-5Q <sup>a</sup>	40.0	66.1	0.34	0.33	68.0	66.7	96.1	89.8
G 35346-2Q <sup>a</sup>	62.0	76.8	0.33	0.35	74.3	71.3	88.8	88.6
G 35346-3Q <sup>a</sup>	40.1	73.0	0.35	0.37	75.0	75.0	84.8	79.3
G 35066-1Q <sup>a</sup>	24.6	47.1	0.30	0.29	57.0	64.3	65.8	111.8
I. Quimbaya <sup>b</sup>	22.8	55.1	0.31	0.31	62.0	65.7	63.1	90.4
IJR <sup>b</sup>	18.1	57.2	0.32	0.31	55.8	75.0	61.9	113.0
VAX 1 <sup>b</sup>	23.0	56.1	0.24	0.24	40.8	62.7	61.1	126.2
SER 16 <sup>b</sup>	15.4	53.1	0.26	0.28	37.7	66.3	71.3	109.5
VAX 3 <sup>b</sup>	15.0	59.5	0.26	0.27	45.0	64.7	67.1	114.9
VAX 6 <sup>b</sup>	19.1	50.0	0.28	0.27	55.0	57.0	71.6	106.5
G 40159 <sup>c</sup>	21.3	55.5	0.25	0.27	53.0	69.0	134.3	135.7
Mean	27.4	59.0	0.29	0.30	56.7	67.1	78.7	106.0
LSD <sub>0.05</sub>	22.6	32.0	0.09	0.07	24.2	17.02	45.7	35.1

<sup>a</sup> *P. coccineus*<sup>b</sup> *P. vulgaris*<sup>c</sup> *P. acutifolius*

3Q, VAX6, ICA Quimbaya, and G40159 were intermediate with inhibition between 37.5 and 44.0%, whereas *P. coccineus* accessions G35346-2Q and G35464-5Q were resistant with inhibition of 30.8 and 21.3%, respectively (Fig. 1B), confirming the variability observed previously between the two bean species (CIAT 2005), and the level of Al resistance reported in ICA Quimbaya (Rangel et al. 2007).

Fine and extensive roots that continue to grow and that thicken less in the presence of toxic Al should be able to explore Al toxic soil more efficiently (Eisenstat 1992; Villagarcia et al. 2001). Genotypes grown in presence or absence of Al for 120 h also showed differential response to Al for the increase of MRD, ranging from 8.1 to 20.5% (Fig. 1C). VAX6, G35346-3Q, G40159, and ICA Quimbaya presented less increase of root diameter (<9.43%). Five genotypes from different bean species and gene pools (G35464-5Q, G35346-3Q, G40159, ICA Quimbaya and VAX6) were outstanding for minimizing inhibition of TRER and increase of root diameter (Fig. 1). In contrast, Al sensitive line VAX1 presented inhibition of TRER and increase of MRD of 60.3 and 20.2%, respectively. Similar results were found

before in evaluation of Al resistance among 52 genotypes of common bean (CIAT 2005). Villagarcia et al. (2001) reported that number of basal roots and branching from tap root were clearly reduced by Al in all soybean genotypes. We found similar results for NRT (root branching) and for SRL which is affected by number of fine branches. As expected, SRL exhibited a strong negative correlation with average root diameter in the hydroponic system. However, the ranking of genotypes by SRL did not appear to correspond to ranking by other traits.

#### Evaluation for Al-toxic acid soil tolerance

Aluminium-toxic acid soil in Trial 2 affected all root parameters except for MRD (Tables 4, 5). Average values for LAI and HAI treatments, respectively were: TRL, 59 and 27.4 m; MRD, 0.30 and 0.29 mm; VRD29d, 67.1 and 56.7 cm; and SRL, 106.0 and 78.7 m g<sup>-1</sup>. Genotypic differences were highly significant for TRL, MRD, SRL and VRD29d at 29 days. Acid soil tolerant genotypes G35346-2Q, G35346-3Q and G35464-5Q maintained good root structure in acid soil. Genotype × Al level interaction was significant for SRL and VRD29d at 29 days. In

**Table 5** Correlation coefficients and mean squares for total root length (TRL), mean root diameter (MRD), specific root length (SRL), VRD at 29 days (VRD29d), leaf area (LA), shoot dry weight (SDW) and root:shoot ratio (R:S ratio) of

29 days-old plants of 11 bean genotypes evaluated under conditions of high Al saturation (HAl) and low Al saturation (LAl) in soil cylinders under “WW conditions” (Trial 2)

Traits/source	Al level/DF	TRL	MRD	SRL	VRD29d	LA	SDW	R:S ratio
TRL	LAl	1						
	HAl	1						
MRD	LAl	0.53 (ns)	1					
	HAl	0.58 (ns)	1					
SRL	LAl	−0.39 (ns)	−0.79**	1				
	HAl	0.33 (ns)	0.27 (ns)	1				
VRD29d	LAl	0.65*	0.77**	−0.33 (ns)	1			
	HAl	0.78**	0.92***	0.34 (ns)	1			
LA	LAl	0.15 (ns)	0.35 (ns)	0.02 (ns)	0.64*	1		
	HAl	0.52 (ns)	0.14 (ns)	0.22 (ns)	0.31 (ns)	1		
SDW	LAl	0.64*	0.57 (ns)	−0.26 (ns)	0.82**	0.69*	1	
	HAl	0.50 (ns)	0.38 (ns)	0.46 (ns)	0.47 (ns)	0.81**	1	
R:S ratio	LAl	0.47 (ns)	0.68*	−0.79**	0.24 (ns)	−0.29 (ns)	−0.02 (ns)	1
	HAl	0.81**	0.70*	0.05 (ns)	0.71*	0.15 (ns)	0.11 (ns)	1
Level of Al	1	16524***	0.0007 (ns)	12236***	1774***	811056***	19.58***	0.34***
Rep. (Al level)	4	28.77 (ns)	0.0009 (ns)	299.3 (ns)	420.9 (ns)	2991 (ns)	0.19 (ns)	0.008 (ns)
Genotype	10	776.2***	0.009***	1342***	3747***	3235 (ns)	0.28*	0.06***
Gen. × Al level	10	100.3 (ns)	0.0003 (ns)	986.2**	1815*	8193*	0.18 (ns)	0.012*
Error	40	138.9	0.0012	301.1	79.23	3773	0.119	0.004

ns Non significant

Statistical significance at the \* 0.5, \*\* 0.01, and \*\*\* 0.001 probability levels, respectively

HAl soil, TRL was correlated with only two root traits, VRD29d at 29 days ( $r = 0.78^{**}$ ) and root to shoot ratio (R:S) ratio ( $r = 0.81^{**}$ ); whereas MRD was highly correlated with VRD29d of 29 day-old plants ( $r = 0.92^{***}$ ) (Table 5). This implies that thicker roots were penetrating deeper into soil, and reflects the fact that *P. coccineus* presented high values of both traits. However, thicker roots did not necessarily represent less efficient use of biomass, as indicated by values of SRL. Whereas HAl soil induced serious reductions in SRL in common bean in Trial 2, three of the runner bean accessions maintained their values of SRL. In Trial 3, G35346-2Q likewise showed a minimal reduction in SRL in acid soil. In both soil trials runner bean presented thicker roots than common bean under Al stress, and yet runner bean presented SRL that was greater than (in Trial 2) or comparable (in Trial 3) to common bean in Al stress, especially compared to the Mesoamerican genotypes SER 16, VAX 3 and VAX 6. This implies that the roots of runner bean

are less dense than roots of common bean. This may be an expression of aerenchyma development in runner bean, which would be a very interesting trait to introgress to common bean. The tendency to thicker roots in *P. coccineus* was more evident in the soil experiments than in the hydroponic trials.

#### Tolerance to combined stress in soil

Aluminium levels × water regimes in Trial 3 effects were large for TRL and moderate for SRL. As expected, combined stress of Al-toxic soil and WS was the most inhibitory to TRL, followed by WS alone, and then by Al alone, based on the treatment averages (Table 6). However, as expected, Al-stress alone was more inhibitory than WS to SRL and VRD33d. Genotypes differed for all root traits considered (TRL, MRD, SRL, and VRD33d) (Table 7). Two sister lines of *P. coccineus*, G35346-3Q and G35346-2Q, were the most tolerant to combined stress, presenting the highest values of

**Table 6** Influence of individual and combined stress factors of acid soil (HAI high aluminium, LAI low aluminium) and water stress (WW well watered, WS water stress) on total root length (TRL), mean root diameter (MRD), VRD at 33 days (VRD33d) and specific root length (SRL) of 33 days-old plants of 11 bean genotypes from three *Phaseolus* species grown in soil cylinders (23 days under WS) (Trial 3)

Genotype	TRL (m)			MRD (mm)			VRD33d (cm)			SRL (m g <sup>-1</sup> )		
	LAI– WW	LAI– WS	HAI– WW	HAI– WS	LAI– WW	LAI– WS	HAI– WW	HAI– WS	LAI– WW	LAI– WS	HAI– WW	HAI– WS
G 35464 5Q <sup>a</sup>	42.3	26.7	35.7	19.9	0.35	0.37	0.43	0.4	60.5	61.4	69.2	59.8
G 35346 2Q <sup>a</sup>	56.9	28.6	66.5	37.7	0.36	0.36	0.37	0.37	62.9	59.0	68.0	73.0
G 35346 3Q <sup>a</sup>	73.7	30.4	38.7	42.5	0.38	0.35	0.42	0.39	72.2	56.6	66.8	75.0
G 35066 1Q <sup>a</sup>	25.6	18.8	31.7	27.8	0.35	0.34	0.38	0.38	45.8	59.0	67.8	62.0
I. Quimbaya <sup>b</sup>	32.4	17.2	29.2	22.5	0.36	0.41	0.39	0.39	68.1	65.5	66.0	69.0
IJR <sup>b</sup>	44.0	28.4	22.7	21.9	0.38	0.38	0.41	0.4	75.0	75.0	72.0	71.0
VAX 1 <sup>b</sup>	34.0	25.4	25.4	16.4	0.33	0.33	0.35	0.34	58.5	71.6	59.5	65.7
SER 16 <sup>b</sup>	58.9	21.9	14.6	11.9	0.36	0.34	0.35	0.37	70.3	72.5	60.0	48.3
VAX 3 <sup>b</sup>	42.7	19.7	15.3	12.2	0.36	0.34	0.34	0.35	67.7	68.2	46.9	49.0
VAX 6 <sup>b</sup>	29.9	16.7	12.1	11.8	0.35	0.35	0.3	0.41	68.2	66.7	44.2	42.9
G 40159 <sup>c</sup>	54.1	22.9	20.5	15.1	0.32	0.34	0.33	0.33	75.0	71.5	64.5	57.0
Mean	45.0	23.3	28.4	21.8	0.35	0.36	0.37	0.37	65.8	66.1	62.3	61.2
LSD <sub>0.05</sub>	45.7	11.4	14.0	9.4	0.07	0.05	0.07	0.08	22.6	10.0	14.9	18.1

<sup>a</sup> *P. coccineus*<sup>b</sup> *P. vulgaris*<sup>c</sup> *P. acutifolius*

**Table 7** Correlation coefficients and mean squares for total root length (TRL), mean root diameter (MRD), specific root length (SRL), VRD at 33 days (VRD33d), leaf area (LA),

shoot dry weight (SDW), root:shoot ratio (R:S ratio) of 11 bean genotypes grown under combined stress of Al and water stress (23 days under WS) (Trial 3)

Traits/source	DF	TRL	MRD	VRD33d	SRL	LA	SDW	R:S ratio
TRL	–	1						
MRD	–	0.43*	1					
VRD33d	–	0.79***	0.27 (ns)	1				
SRL	–	–0.2 (ns)	–0.62***	–0.11 (ns)	1			
LA	–	0.62***	0.56***	0.58***	–0.39*	1		
SDW	–	0.82***	0.55***	0.59***	–0.42*	0.62***	1	
R:S ratio	–	0.57***	0.19 (ns)	0.59***	–0.06 (ns)	0.32 (ns)	0.10 (ns)	1
Rep	2	347.1*	0.004**	12.7	344.6***	1.45	0.013	0.040
Water	1	6582.4*	0.000	6.0	909.5	405.55**	12.597**	0.749
Rep × water	2	390.2*	0.001	212.0*	67.1	4.53	0.061	0.101*
Al	1	2700.9***	0.009**	598.0**	3444.6***	417.00***	9.855**	0.804**
Water × Al	1	1867.5***	0.000	15.4	246.0*	170.83***	3.437*	0.016
Rep × water × Al	4	5.8	0.000	10.8	24.9	1.63	0.211	0.030
Genotype	10	1023.5***	0.005***	313.9***	1244.2***	13.55**	0.435*	0.355***
Al × genotype	10	386.7***	0.001	463.6***	128.4***	24.11***	0.819***	0.088**
Water × genotype	10	150.2	0.001	57.9	48.2	9.78	0.238	0.026
Water × Al × genotype	10	184.1	0.002	104.1*	21.2	17.74***	0.241	0.060
Error	80	113.5	0.001	52.6	31.4	5.23	0.219	0.032

ns Non significant, W<sub>r</sub> water regime

Statistical significance at the \* 0.5, \*\* 0.01, and \*\*\* 0.001 probability levels, respectively

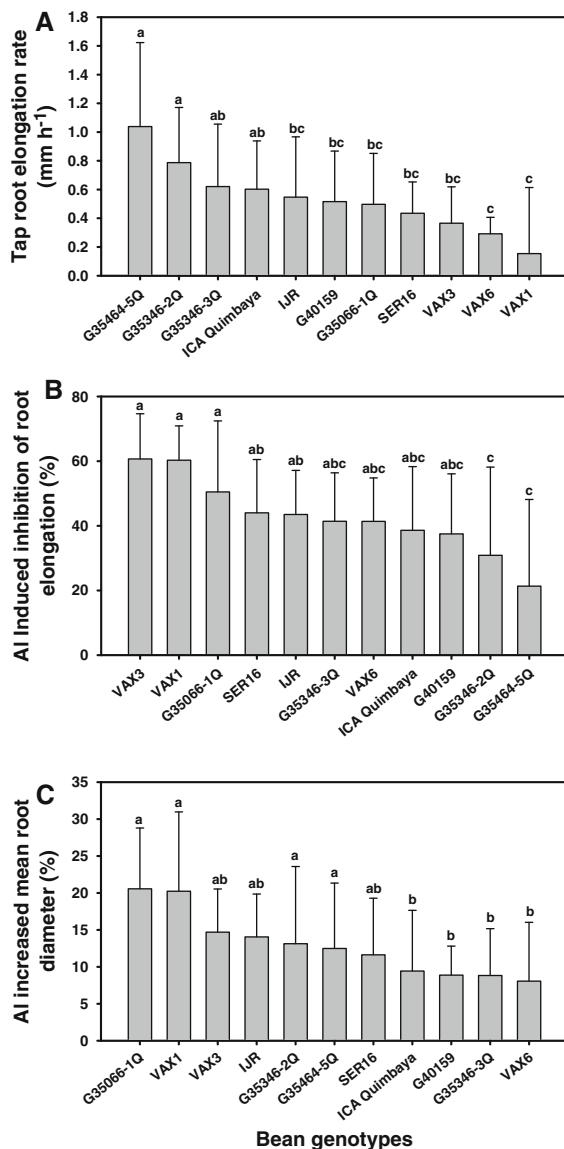
TRL and maintaining a deeper root system (Tables 6, 8). They were also the two best under Al-stress alone but showed difficulties to develop a deep rooting system under WS alone. Other relatively tolerant genotypes in combined stress were G35066-1Q and ICA Quimbaya. Interactions of genotype × Al treatment were highly significant for TRL, SRL, and VRD (Table 7). Although combined stress of Al and WS is generally more damaging than each stress considered separately, in this study an unexpected interaction between these two stress factors was observed. *P. coccineus* accessions (G35346-3Q, G35346-2Q, and G35066-1Q) and to lesser extent ICA Quimbaya developed more roots, and showed deeper rooting based on VRD33d with the combined Al and WS than with WS alone (Table 6). TRL was correlated with MRD ( $r = 0.43^*$ ), and with root depth ( $r = 0.79^{***}$ ) in the combined stress treatment, whereas MRD was negatively correlated with SRL ( $r = -0.62^{***}$ ) as expected (Table 7).

### Root length and dry weight distribution

Results on genotypic differences in rooting strategies estimated by the values of the extinction coefficient  $\beta$  for root length distribution in Trials 2 and 3 showed that the accessions of *P. coccineus* had greater proportion of root length at depth than the other genotypes except for the Andean genotype IJR under HAl treatment in both WW as well as WS conditions (Table 8). Similar effects were observed on  $\beta$  values for root biomass distribution (Table 9).

### Root and shoot attributes

Shoot traits were measured only in the soil cylinder trials. If an improved root system with Al resistance is to result in yield improvement, it is important that it contribute to enhanced shoot development. Estimation of shoot vigor of plants grown in acid soil was made based on LA development and SDW under individual (Trial 2) and combined stress of Al and WS (Trial 3).



**Fig. 1** Root parameters of 11 bean genotypes grown under hydroponic system under two levels of Al (20  $\mu$ M Al and 0  $\mu$ M Al) at pH 4.5 (Trial 1). **A** TRER in mm per hour under HAl (20  $\mu$ M Al); **B** Al-induced inhibition of TRER in per cent; **C** Al-induced increase of MRD in per cent. Bars represent means  $\pm$  SD, with 4 replicates. Different letters indicate differences between at  $P < 0.05$  (REGWQ test)

G35346-2Q was the best genotype under Al-stress alone for combining an extensive root system, large leaves and vigorous seedlings. G35346-3Q was found to be the best with similar potential under combined stress of Al and WS, but was intermediate for Al alone for both traits, and was very poor for WS alone

(Table 10). Genotype  $\times$  Al level interaction was significant for LA and R:S ratio and a highly significant interaction was observed for genotype  $\times$  (Al and water regime) for LA and R:S ratio and SDW (Table 7). Larger LA development was accompanied by strong shoot biomass investment in stems, branches and petioles. *P. vulgaris* genotypes SER16 and VAX1, and *P. acutifolius* G40159 expressed relatively higher values of LA and shoot biomass under drought stress, but were strongly affected by Al-toxic acid soil, and were more sensitive in shoot parameters under combined stress factors of Al and WS (Table 10), indicating that their capacity to acquire nutrients and water for shoot growth was reduced.

The relationships between shoot traits (LA and SDW) and TRL in Al stress alone (Trial 2) and in combined stress of Al and WS (Trial 3) in soil cylinder system were analysed. Linear regression of LA on TRL in Al stress for the 11 genotypes showed positive relationship ( $r = 0.81^*$ ) (Fig. 2A), and a similar relationship was observed between SDW and TRL ( $r = 0.83^*$ ) (Fig. 2B) in Al stress. Relationship between SDW and TRL in combined stress of Al and WS also showed a strong positive relationship ( $r = 0.81^*$ ) (Fig. 2C). The accessions of *P. coccineus* in most cases presented the highest values and influenced the regression values heavily. The genotypes G35346-2Q, G35346-3Q and G35464-5Q were outstanding under Al stress expressing a deeper root system (Table 4) and maintaining above average shoot development. All *P. vulgaris* cultivars and the *P. acutifolius* accession performed poorly in root development and LA production with the exception of an Andean bean type IJR that was intermediate (Fig. 3A). Roots can exert indirect control on leaf growth which depends on the supply of cytokinins and water from roots (Lambers et al. 1995). Results from short-term experiments indicated that at low nutrient availability some species with a high potential root growth rate still grow faster than those with low potential root growth rate and have greater capacity to acquire nutrients (Chapin 1980; Lambers and Poorter 1992; Ryser and Lambers 1995).

#### Root traits across experiments

TRL was analysed at two levels of Al treatment both in hydroponic and soil cylinder experiments. There was a strong linear relationship between TRL per

**Table 8** Influence of individual and combined stress factors of acid soil (HAI high aluminium, LAI low aluminium) and water stress (WW well watered, WS water stress) on root length distribution estimated by root length extinction coefficient  $\beta$ ,

that describes the shape of the cumulative root length distribution with depth, of 11 bean genotypes from three *Phaseolus* species grown in soil cylinders (Trial 3)

Genotype	Individual Al stress		Combined Al and water stress			
	HAI	LAI	WW		WS	
			HAI	LAI	HAI	LAI
G 35464 5Q <sup>a</sup>	0.9609	0.9607	0.9664	0.9599	0.9640	0.9555
G 35346 2Q <sup>a</sup>	0.9628	0.9611	0.9666	0.9615	0.9642	–
G 35346 3Q <sup>a</sup>	0.9507	0.9642	0.9685	0.9691	0.9672	0.9616
G 35066 1Q <sup>a</sup>	0.9411	0.9569	0.9578	0.9452	0.9584	0.9206
ICA Quimbaya <sup>b</sup>	0.9562	0.9623	0.9599	0.9520	0.9529	0.9550
IJR <sup>b</sup>	0.9516	0.9684	0.9660	0.9690	0.9663	0.9759
VAX 1 <sup>b</sup>	0.9397	0.9514	0.9486	0.9486	0.9479	0.9589
SER 16 <sup>b</sup>	0.9372	0.9563	0.9480	0.9680	0.9479	0.9644
VAX 3 <sup>b</sup>	0.9369	0.9574	0.9442	0.9610	0.9386	0.9617
VAX 6 <sup>b</sup>	0.9492	0.9495	0.9230	0.9466	0.9381	0.9596
G 40159 <sup>c</sup>	0.9504	0.9672	0.9498	0.9603	0.9512	0.9714
Mean	0.9488	0.9596	0.9544	0.9583	0.9542	0.9585
LSD <sub>0.05</sub>	NS	0.101*	0.0168***	NS	0.0167*	0.0179*

<sup>a</sup> *P. coccineus*

<sup>b</sup> *P. vulgaris*

<sup>c</sup> *P. acutifolius*

plant under 20  $\mu$ M Al and the control with 0  $\mu$ M Al in hydroponic system ( $r = 0.94$ ) (Fig. 3A). For soil cylinder system, the linear relationship between TRL under LAI and HAI saturation was also strong ( $r = 0.84$ ) (Fig. 3B). Four *P. coccineus* accessions (G35464-5Q, G35346-2Q, G35346-3Q, and G35066-1Q) showed greater values of TRL both with and without Al in hydroponic system, and in soil cylinder studies, three of these (G35346-2Q, G35346-3Q, and G35464-5Q) maintained good root development with either LAI or HAI saturation. The two screening methods were highly correlated ( $r = 0.85^{***}$ ) for TRL in HAI soil and in nutrient solution with 20  $\mu$ M Al (Fig. 3C), although correlation was driven in large part by the values of the *P. coccineus* accessions. A significant but lower correlation ( $r = 0.69^*$ ) was found between TRL in LAI soil and nutrient solution without Al in hydroponic system (Fig. 3D). Several authors have examined the relationship of response with different screening methods with varying results. Narasimhamoorthy et al. (2007) compared three methods including hydroponics, soil, and root staining for evaluation of Al tolerance in *Medicago*

*truncatula* (Barrel Meic) germplasm and found a weak correlation, suggesting that each technique is distinct and cannot be substituted for each other. A large discrepancy between hydroponics-based ratings of seedlings and sand-culture-based ratings of soybean plants was found when Al tolerance was expressed as percentage of controls, and correlations between sand culture and hydroponics-based results were found to be low (Villagarcia et al. 2001). Horst and Klotz (1990) compared 31 soybean genotypes using hydroponics and soil systems and detected a positive though non-significant relationship ( $r = 0.79$ ). In contrast, Campbell and Carter (1990) demonstrated in their experiments a good agreement between Al tolerance ratings determined in solution culture and pots with soil in the greenhouse when expressed as percentage of the control treatment. Recent research on peanut indicated that root characteristics of plants grown in hydroponics were closely related with those of plants grown with soil in small pot conditions (Girdthai et al. 2010). Hydroponic evaluation identified the soybean cultivar Perry as Al sensitive even though it had been found to



**Table 9** Influence of individual and combined stress factors of acid soil (HAI high aluminium, LAI low aluminium) and water stress (WW well watered, WS water stress) on root biomass distribution estimated by root biomass extinction coefficient  $\beta$ ,

that describes the shape of the cumulative root biomass distribution with depth, of 11 bean genotypes from three *Phaseolus* species grown in soil cylinders (Trial 3)

Genotype	Individual Al stress		Combined Al and water stress			
	HAI	LAI	WW		WS	
			HAI	LAI	HAI	LAI
G 35464 5Q <sup>a</sup>	0.9560	0.9574	0.9635	0.9535	0.9591	0.9513
G 35346 2Q <sup>a</sup>	0.9603	0.9583	0.9639	0.9593	0.9628	–
G 35346 3Q <sup>a</sup>	0.9467	0.9594	0.9649	0.9663	0.9637	0.9578
G 35066 1Q <sup>a</sup>	0.9401	0.9545	0.9537	0.9397	0.9528	0.9001
ICA Quimbaya <sup>b</sup>	0.9495	0.9572	0.9575	0.9470	0.9495	0.9431
IJR <sup>b</sup>	0.9474	0.9606	0.9642	0.9675	0.9616	0.9720
VAX 1 <sup>b</sup>	0.9416	0.9516	0.9505	0.9406	0.9465	0.9546
SER 16 <sup>b</sup>	0.9544	0.9544	0.9485	0.9637	0.9400	0.9581
VAX 3 <sup>b</sup>	0.9321	0.9540	0.9516	0.9572	0.9356	0.9573
VAX 6 <sup>b</sup>	0.9467	0.9451	0.9249	0.9417	0.9327	0.9526
G 40159 <sup>c</sup>	0.9448	0.9647	0.9468	0.9559	0.9497	0.9673
Mean	0.9472	0.9561	0.9536	0.9538	0.9504	0.9514
LSD <sub>0.05</sub>	NS	NS	0.0131***	NS	0.0168**	0.0163*

<sup>a</sup> *P. coccineus*

<sup>b</sup> *P. vulgaris*

<sup>c</sup> *P. acutifolius*

be tolerant in soil-based assays with older plants (Armiger et al. 1968; Foy et al. 1969, 1992; Devine et al. 1979; Sapra et al. 1982; Horst and Klotz 1990). VAX1 which was found to be Al sensitive in hydroponic system was found to be acid soil tolerant under field conditions because of its abundant adventitious root system that helped avoid Al toxicity.

In our experience with the hydroponic system and in Al-toxic acid soil, each screening method permitted evaluation of different aspects of root behaviour. For example, the hydroponic system enabled quantification of NRT (the most Al-sensitive part of the root) which was not possible with soil cylinders after cutting the cylinders at different soil depths, whereas soil based screening revealed rooting ability to penetrate Al-toxic soil. In this sense the two methods were complementary. A strong relationship was observed between Al resistance in hydroponics and acid soil tolerance for some specific root traits. Significant correlations were found between three root traits from hydroponic evaluation (TRL, TPRL120h and SRL) and three other root traits from soil cylinder evaluation (TRL, VRD and MRD).

Villagarcia et al. (2001) concluded that some genetic sources will lend themselves well to hydroponics-based screening while others may not. Of these traits, TRL and VRD in soil are those that we interpret as being the most relevant measures of root health in an Al toxic environment.

On the other hand, no significant correlation was found between SRL in hydroponic and soil cylinder experiments, and genotypic ranking based on SRL in nutrient solution with 20  $\mu\text{M}$  Al did not agree with the ranking in Al-toxic acid soil. While some traits express similarly in the two systems, others apparently do not. An unusual relationship was observed between SRL and MRD. In the hydroponic system the correlation of SRL and MRD was highly negative as expected:  $-0.84^{***}$  at 0  $\mu\text{M}$  Al and  $-0.87^{***}$  with 20  $\mu\text{M}$  Al. With LAI stress in soil, the correlation of SRL and MRD presented a similar value:  $-0.79^{***}$ . But in Al-toxic acid soil, the correlation was slightly positive (0.27; NS). This difference reflects a contrasting response of roots in soil as compared to the hydroponic system that seems to be due to *P. coccineus*.

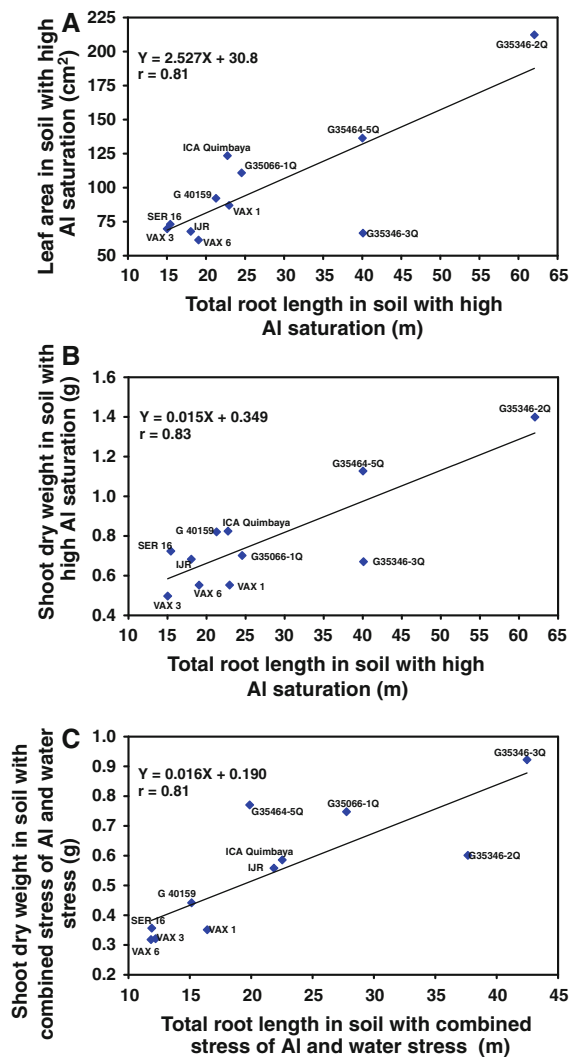
**Table 10** Leaf area (LA), shoot dry weight (SDW) and root to shoot ratio (R:S ratio) of 11 bean genotypes including 4 *P. coccineus* accessions, 1 *P. acutifolius* and 6 *P. vulgaris* grown under individual and combined stress of Al (HAI high aluminium, LAI low aluminium) and water stress conditions (WW well watered, WS water stress) (Trials 2 and 3)

Genotypes	Individual stress of Al (Trial 2)						Individual and combined stress of Al and WS (Trial 3)														
	LA (cm <sup>2</sup> )			SDW (g)			R:S ratio			LA (cm <sup>2</sup> )				SDW (g)				R:S ratio			
	HAI	LAI		HAI	LAI		HAI	LAI		LAI- WW	LAI- WS	HAI- WW	HAI- WS	LAI- WW	LAI- WS	HAI- WW	HAI- WS	LAI- WW	LAI- WS	HAI- WW	HAI- WS
G 35464-5Q <sup>a</sup>	136.4	304.9		1.13	2.10	0.56	0.35		164.5	123.1	189.8	91.0	1.21	0.91	1.19	0.77	0.49	0.51	0.63	0.62	
G 35346-2Q <sup>a</sup>	212.2	300.3		1.40	1.90	0.52	0.48		317.4	93.2	152.8	106.8	1.66	0.72	0.86	0.6	0.45	0.75	1.08	1.03	
G 35346-3Q <sup>a</sup>	66.6	399.1		0.67	2.25	0.71	0.42		356.6	78.0	132.0	136.6	1.69	0.52	0.96	0.92	0.75	0.99	0.72	0.8	
G 35066-1Q <sup>a</sup>	110.8	330.2		0.70	1.54	0.48	0.28		125.9	153.6	137.5	83.8	0.96	0.36	0.88	0.75	0.44	1.18	0.65	0.66	
I. Quimbaya <sup>b</sup>	123.5	245.3		0.83	1.58	0.43	0.37		178.3	36.3	98.9	56.6	1.24	0.52	0.87	0.59	0.4	0.61	0.57	0.74	
IUR <sup>b</sup>	67.8	375.6		0.68	2.22	0.45	0.25		365.2	91.1	108.4	95.1	1.97	1.17	0.82	0.56	0.34	0.43	0.53	0.85	
VAX 1 <sup>b</sup>	87.0	290.4		0.55	1.37	0.49	0.29		169.9	150.7	127.1	69.8	0.98	0.77	0.63	0.35	0.45	0.46	0.55	0.73	
SER 16 <sup>b</sup>	73.1	341.7		0.73	1.86	0.33	0.25		564.1	157.4	60.9	62.3	3.13	0.98	0.6	0.36	0.24	0.32	0.4	0.58	
VAX 3 <sup>b</sup>	69.7	326.8		0.50	1.91	0.37	0.24		350.2	134.9	70.9	29.6	1.89	0.67	1.09	0.32	0.3	0.39	0.5	0.68	
VAX 6 <sup>b</sup>	61.5	291.7		0.55	1.76	0.44	0.25		260.5	105.1	55.2	40.2	1.57	0.68	0.62	0.32	0.29	0.36	0.34	0.62	
G 40159 <sup>c</sup>	92.2	333.4		0.82	2.07	0.19	0.19		418.7	136.7	109.5	43.1	2.49	1.14	0.69	0.44	0.2	0.25	0.34	0.42	
Mean	100.1	321.8		0.78	1.87	0.45	0.31		297.4	114.6	113.0	74.1	1.71	0.77	0.84	0.54	0.4	0.57	0.57	0.7	
LSD <sub>0.05</sub>	148.6	140.0		0.75	0.87	0.18	0.12		318.7	84.1	56.6	61.6	1.9	0.57	0.92	0.25	0.3	0.6	0.29	0.37	

<sup>a</sup> *P. coccineus*

<sup>b</sup> *P. vulgaris*

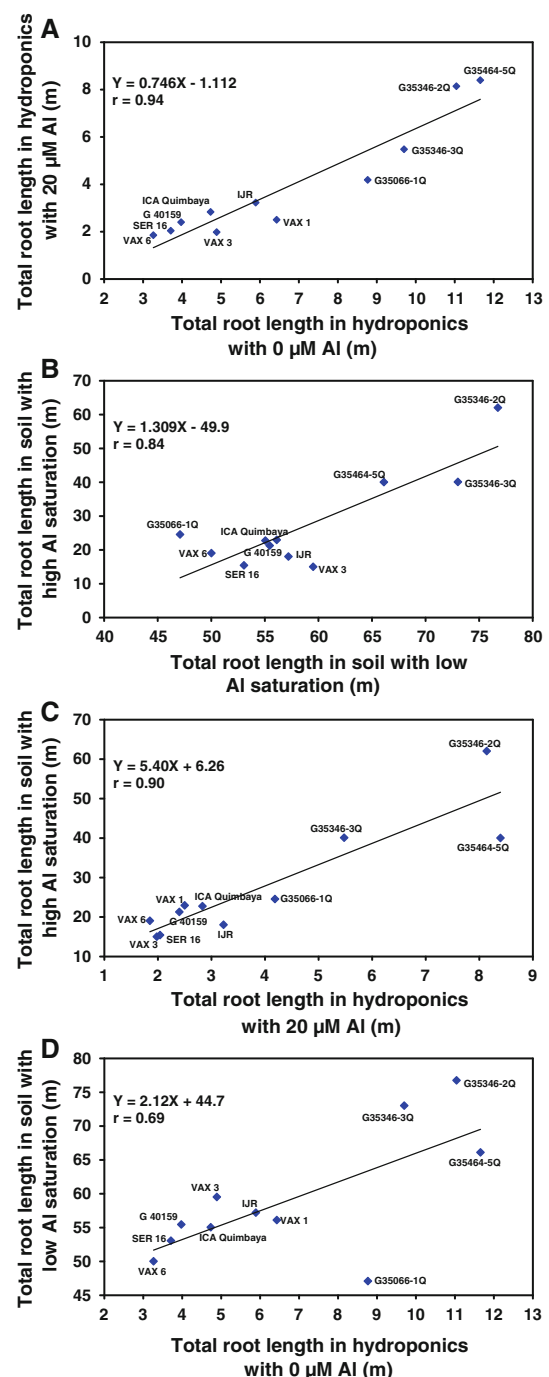
<sup>c</sup> *P. acutifolius*



**Fig. 2** Relationship between TRL and shoot attributes of 11 bean genotypes under individual and combined stress of Al and WS (Trial 3). **A** LA as influenced by TRL under Al toxic acid soil stress; **B** SDW as influenced by TRL under Al toxic acid soil stress; **C** SDW as influenced by TRL under combined stress of Al toxic acid soil and WS

## General discussion

The nature of response to Al toxicity may be either non-specific and constitutive or specific. A reaction that is specific to Al ought to be associated with an interaction term of Genotype  $\times$  Al level in the analysis of variance. Comparing TRL with and without Al in hydroponic evaluation (Fig. 3A) we found that the correlation between them was high



**Fig. 3** Relationship between TRL of 11 bean genotypes in Al treatment in hydroponics with 0 and 20  $\mu\text{M}$  Al (Trial 1), and soil cylinders with LAI and HAI saturation soil (Trial 2): **A** TRL in hydroponics with 0 and 20  $\mu\text{M}$  Al; **B** TRL in soil with LAI and HAI saturation soil; **C** TRL in hydroponics with 20  $\mu\text{M}$  Al versus HAI saturation in soil; **D** TRL in hydroponics with 0  $\mu\text{M}$  Al versus LAI saturation in soil

( $r = 0.94^{***}$ ). Genotype  $\times$  Al level interaction was also significant but at  $P < 0.05$ . Effects of both Al and genotype were highly significant. In contrast genotype  $\times$  Al treatment was not significant in soil cylinder system suggesting that whatever effect of Al that resulted in much less TRL was not an effect for which lines express differential resistance for Al. The relationship of TRL with and without Al stress for soil-based screening (Fig. 3B) was also strong ( $r = 0.84$ ), and three Al resistant *P. coccineus* accessions form a separate group from all the other genotypes. The high relationship of stressed and unstressed treatments implies that differences among genotypes were largely constitutive. Urrea-Gómez et al. (1996) suggested that constitutive morphological characteristics such as vigorous rooting could be advantageous in the breeding of Al-resistant cultivars.

One underlying issue that may influence plant response is the initial vigour of the seedlings, reflecting differences in seed size. Accessions of *P. coccineus* have larger seed size than *P. vulgaris*, and Andean beans are larger than Mesoamerican types. While this effect can not be discounted, *P. coccineus* displays traits that are independent of vigour per se and that set it off from *P. vulgaris*, such as: higher R:S ratio; increase of TRER at the 120 h measurement compared to the 48 h measurement; the maintenance of SRL in spite of Al toxicity; a positive response to combined stress of Al toxicity and WS in soil compared to WS alone. These observations argue in favour of unique mechanisms in *P. coccineus* that could make a valuable contribution to the improvement of common bean.

Although closely related to common bean, *P. coccineus* appears to have evolved for adaptation in different environments, and with different strategies. Many accessions originate in high rainfall areas on volcanic soils, possibly with low pH and some degree of Al stress, leading to adaptation to Al toxic soil. On the other hand, its root system is especially poor under WS in the soil cylinders, with less penetration than any of the common bean lines. In the moist environment in which it evolved a deep rooting trait was not an advantage. The resistance to Al toxicity in *P. coccineus* illustrates the value of the broad genetic variability of the *Phaseolus* genus, and especially the secondary gene pool of *P. vulgaris* that can be crossed readily with common bean. *P. coccineus*, and *P. dumosus* have been employed in interspecific

crosses for the improvement of common bean before, but in most cases for disease resistance, given their adaptation to humid environments where disease pressure is intense. Their importance could increase under the threat of climate change and increased rainfall in some tropical regions. However, this is the first report that we are aware of, identifying sources for abiotic stress resistance in these species. While readily crossable to common bean, progenies of interspecific hybridizations are often of poor agronomic value. We are investigating ways to take better advantage of these species through interspecific crosses. Given the superior reaction of G35346-3Q in the treatment of combined Al and WS treatment, it was chosen for crossing with common bean.

These studies also confirmed the superiority in Al response of Andean common beans compared to Mesoamerican types for several important traits under Al toxicity in the hydroponic system (e.g., TPRL120h, or TRL) or acid soil stress (e.g., TRL or VRD). This confirms previous reports on Andean and Mesoamerican genotypes (Rangel et al. 2005, 2007; Blair et al. 2009; López-Marín et al. 2009). Only one accession of *P. acutifolius* was included in this study, but it was distinguished by its consistently greater values of SRL and correspondingly thinner roots, in both the hydroponic system and in soil cylinders, and under either stress. This species evolved in a semi-arid to arid environment in northwest Mexico and southwest United States in which Al toxicity would not be a problem. It also presented good VRD in several treatments in the soil cylinder experiments.

## Conclusions

The greater level of Al-resistance found in *P. coccineus* accessions (G35346-2Q and G35464-5Q) offers the opportunity to obtain much better Al resistance in common bean through interspecific crosses. Another *P. coccineus* accession G35346-3Q identified in this study showed the ability to tolerate combined stress factors of Al and WS. Given that abiotic stresses often co-occur in farmers fields, the use of this genotype in common bean improvement for resistance to these two stress factors is likely to be more productive than considering resistance to stresses in isolation. Populations created from multiple stress resistance donors could be more stable and

capable to produce grain under stress in the face of climate change.

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