Phenotypic evaluation of interspecific recombinant inbred lines (RILs) of *Phaseolus* species for aluminium resistance and shoot and root growth response to aluminium–toxic acid soil

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Abstract Aluminium (Al) toxicity limits common bean productivity in acid soil regions of the tropics. To improve Al resistance of common bean, Al-sensitive *Phaseolus vulgaris* (SER16) was crossed to Al-resistant *P. coccineus* (G35346-3Q) to create 94 $F_{5:6}$ recombinant inbred lines (RILs) of the pedigree SER16 × (SER16 × G35346-3Q). RILs were characterized for resistance to Al in a hydroponic system with 0 and 20 μM Al in solution, and for shoot and root growth response to Al-toxic infertile acid soil in 75 cm long soil cylinder system using an oxisol of low Al- (12.5%; pH 4.6; fertilized) and high Al-saturation (77%; pH 4.1; unfertilized). G35346-3Q increased its taproot elongation rate by 3.5% between 24 and 48 h under 20 μM Al in solution, while the best RIL, Andean genotype ICA Quimbaya, and sensitive genotype VAX1 expressed reductions of 2.6, 12.5, and 69.5%, respectively. In the acid soil treatment the correlation between leaf area and total root length was highly significant under high Al saturation ($r = 0.70^{***}$). Genotypes that were Al resistant in the hydroponic system were not necessarily tolerant to Al-toxic acid soil conditions based on shoot and root growth responses. Phenotypic evaluation using both systems allows the identification of genotypes with Al resistance combined with acid soil adaptation. Two genotypes (ALB88 and ALB91) emerged as lines with multiple traits. Results suggest that inheritance of Al resistance and acid soil tolerance in G35346-3Q is complex. Results from this work will be useful for identification of molecular markers for Al resistance in *Phaseolus* species and to improve acid soil adaptation in common bean.

Keywords Abiotic stress · Aluminium resistance · Common bean · Leaf area · Root growth · Screening methods

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Abbreviations

HAl	High aluminium soil saturation
LA	Leaf area
LAl	Low aluminium soil saturation
MRD	Mean root diameter
NRT	Number of root tips
PC	Percent of control
RDW	Root dry weight
R:S	Root to shoot ratio
SDW	Shoot dry weight
SRL	Specific root length

TRER24h	Tap root elongation rate between 0–24 h
TRER24-48h	Tap root elongation rate between 24–48 h
TRER48h	Tap root elongation rate between 0–48 h
TRL	Total root length
VRD34d	Visual rooting depth at 34 days

Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important food legume for direct human consumption worldwide. Annual production, including both dry and snap bean, exceeds 21 million metric tons (Miklas et al. 2006). A majority of bean production occurs under low input agriculture on small-scale farms in developing countries particularly in Latin America and Africa. Aluminium toxicity in acid soils is one of the major constraints to crop production worldwide (Rao et al. 1993; Shen et al. 2004). Aluminium constitutes the third most abundant element in the earth's crust and a formidable phytotoxic barrier to crop production in acidic soils which represent 40% of the world's arable lands (Kochian 1995). Aluminium is present in all soils, but its toxicity is manifested only in acidic conditions, in which the phytotoxic form Al^{3+} predominates (Rout et al. 2001). Soils with high Al-saturation are often associated with a complex of factors (including P and Ca deficiency and Mn toxicity) that affect the ranking of genotypes for Al tolerance (Campbell and Carter 1990).

Common bean proved to be very sensitive of low pH (4.3), with large genotypic differences in proton sensitivity (Rangel et al. 2005). The low pH in acid soils itself is not so much the cause of problems, but the fact that the solubility of specific metals such as Al depends on pH (Kochian et al. 2005). When the soil pH is lower than 5.0, Al is solubilized in the soil solution and absorbed by plant roots. Inhibition of root elongation rate has been widely recognized as the most striking symptom of Al toxicity on plants (Clarkson 1965; Foy 1988). Total root length (TRL), surface area, and branching patterns have been shown to influence nutrient uptake (Raper et al. 1978). Aluminium sensitivity is located specifically at the root apex and absorbed Al inhibits root elongation severely

within an hour(s). Al-sensitive plants absorb more Al than do Al-resistant plants (Rangel et al. 2009), and thus the exclusion mechanism of Al is thought to be the major mechanism for Al resistance (Matsumoto 2000). The toxic effects of Al in soil can be overcome by adding appropriate amendments to acid soil such as lime (Pandey et al. 1994), but lime application must be repeated over time in most acid soils and is not affordable to most households in developing countries that grow beans.

Mechanisms of Al toxicity and resistance are complex and have not yet been fully characterized (Kochian et al. 2004; Ryan and Delhaize 2010; Horst et al. 2010). 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). The initial Al-induced inhibition of root elongation in both Al-resistant (ICA Quimbaya) and Al-sensitive (VAX1) genotypes was correlated with the expression of the *ACCO* (1-aminocyclopropane-1-carboxylic acid oxidase) gene (Eticha et al. 2010). Genotypic and phenotypic differences for Al resistance exist among plant species including beans (Rangel et al. 2005; Blair et al. 2009; López-Marín et al. 2009) but reliable ranking in field for acid soil tolerance is still a problem for breeders. Plant resistance to Al stress is a key component of an appropriate and effective integrated approach for farmers with low income in Africa and Latin America. Along with diverse germplasm and an appropriate breeding program, a reliable screening procedure for Al stress is one of the most important tools required to effectively develop Al-resistant cultivars (Butare et al. 2011). Despite the existence of many Al tolerance screening methods, the use of a single method to identify Al tolerant genotypes may lead to misleading results due to the complexities involved in each method (Narasimhamoorthy et al. 2007).

Previous research at CIAT has shown that some accessions of *P. coccineus* (runner bean) are more resistant to Al in solution and to Al-toxic acid soil than common bean (CIAT 2005; Butare et al. 2011). Significant genotypic differences in Al resistance in common bean were also reported based on Al-inhibited root elongation in nutrient solution (Massot et al. 1999; Rangel et al. 2005; Manrique et al. 2006; Rangel et al. 2007; Blair et al. 2009; López-Marín

et al. 2009). Breeding for resistance to Al toxicity is therefore an alternative that should be explored.

The best option for plant breeders whenever possible will be to conduct screening in the target field. However, in practice, reliable ranking of genotypes in the field can be difficult, because exchangeable Al levels may not be uniform and because environmental factors interact with soil Al to mask the expression of Al-toxic acid soil tolerance (Goldman et al. 1989). Nutrient solution cultures to simulate acid soil solutions were used by many plant physiologists in screening for Al resistance (Wenzl et al. 2003). These methods of assessing root growth in nutrient solutions are more attractive than soil assays (Villagarcia et al. 2001), as they provide controlled forms of Al toxicity, are easily repeatable, and many plants can be evaluated rapidly in a small space. Few breeders have adopted hydroponic screening system because it is usually limited to seedling assays (Villagarcia et al. 2001), but it has been used widely to screen cultivars of soybean (*Glycine max* L.) for Al resistance (Sartain and Kamprath 1978; Horst et al. 1992, 1997). Greenhouse soil-based rankings for Al resistance could be soil-type dependent and thus may not be easily reproducible across wide geographical areas. However, use of a combination of hydroponic and greenhouse soil-based evaluations could identify acid soil tolerant genotypes. At CIAT, a technique using plastic cylinders with high Al saturation soil was developed to rank genotypes for Al-toxic acid soil tolerance based on differences in root development and distribution (Butare et al. 2011). The technique allows evaluation of effects of Al toxicity on plant growth (shoot and root development) in conditions similar to the field. The methodology is reproducible since it employs a known acid soil with high Al saturation from the target area. It permits characterizing the depth of the primary root penetration, extraction of roots for quantification of root distribution across soil depth and TRL in soil with a uniform bulk density. Computer-assisted electronic image analyses have made root analysis less time-consuming and allowed more accurate and less subjective measurement of root characteristics than the human eye is capable of making (Box 1996).

The objective of this research work was to conduct phenotypic evaluation of a population of recombinant inbred lines (RILs) of *Phaseolus* species using hydroponic and soil cylinder systems to identify superior progenies with traits of the Al-resistant parent, and to make a comparative analysis of these two experimental methods to identify Al resistant and acid soil tolerant genotypes based on root and shoot traits.

Materials and methods

Plant materials

A high level of Al resistance was observed in G35346-3Q, an accession of *P. coccineus* which is a sister species of common bean that is characterized by an aggressive vine with great biomass and low harvest index. In contrast, Al-sensitive Mesoamerican small red drought tolerant common bean, SER16 has an erect type II upright indeterminate bush growth habit (Beebe et al. 2008), maturing in under 70 days and with excellent remobilization of photosynthate to grain (CIAT 2007; Butare et al. 2011). A backcross of the F_1 hybrid to the recurrent common bean parent was pursued to recover the desirable plant and seed type of *P. vulgaris*. Ninety-four RILs from the $F_{5:6}$ generation of SER16 × (SER16 × G35346-3Q) were developed by single seed descent. This study was conducted using 102 bean genotypes including the 94 RILs, both parents, and six checks (four from the Mesoamerican gene pool (VAX1, 'Tio-Canela75', DOR390 and G21212), an elite Andean cultivar ('ICA Quimbaya'), and one *P. acutifolius* accession (G40159)). Twenty-five seeds (15 for hydroponics and 10 for soil cylinders) from each genotype were surface sterilized by sodium hypochlorite (1%) for 5 min; rinsed with abundant distilled water and pre-germinated in a sandwich system using filter paper and Styrofoam soaked with tap water in an upright position (Rangel et al. 2007) for 48 h. For evaluation in nutrient solution, germinated seedlings were maintained in sandwiches for 24 h more to allow further root development before transferring them to nutrient solution (Butare et al. 2011). For the soil cylinder system, uniform seedlings with emerged radicals were transplanted to soil cylinder (one seedling per cylinder) in the center of each cylinder (Butare et al. 2011). For purposes of this paper, Al resistance refers to the reaction of genotype to toxic Al in the hydroponic system, whereas tolerance to Al-toxic acid soil refers to tolerance to high Al saturation in acid soil conditions characterized with low nutrient availability (Butare et al. 2011).

Phenotypic evaluation in hydroponic system

The 102 bean genotypes described above were evaluated under hydroponic system. After germination, seedlings with well developed uniform roots were transferred to a tray floating in nutrient solution (López-Marín et al. 2009; Butare et al. 2011) before applying Al treatment (0 or 20 μM Al as AlCl$_3$). The experimental design was a randomized complete block with three replications and the trial was repeated three times and the mean values are reported. Monitoring of the pH was done at 6 h intervals and maintained at 4.5. The greenhouse day/night temperature was on average 29.5/22.8°C; relative air humidity was 50.4/72.6% (day/night); and the maximum photosynthetic photon flux density at noon was 1,100 μmol m^{-2} s^{-1}. Al resistance was assessed by measuring root elongation of tap roots after 24 and 48 h with a ruler from a point initially marked at 3 cm behind the root apex, to calculate tap root elongation rate (TRER at 24 h and also at 48 h) and inhibition of root elongation (Rangel et al. 2007). At harvest, roots were separated from shoots and transferred to plastic bags and refrigerated at 4°C while root image analysis was carried out using a flatbed color scanner "Epson Expression 1680 Scanner." Root attributes, including TRL, mean root diameter (MRD), and number of root tips (NRT) were quantified using a computerized software program WinRhizo® (Butare et al. 2011). Root growth inhibition and the change in MRD, and specific root length (SRL) were calculated. Root dry weight (RDW) for each plant was determined after drying the entire root system in an oven at 60°C for 48 h.

Phenotypic evaluation in soil cylinder system

A greenhouse evaluation to quantify phenotypic differences among 94 RILs, two parents and six checks was carried out in transparent plastic cylinders at two levels of Al saturation (Butare et al. 2011). Soil was collected from Santander de Quilichao, Department of Cauca in Colombia (Lat. 3° 06′ N; Long. 76° 31′ W; Altitude 990 m) at soil layer of 0–20 cm from surface. The soil was characterized as an oxisol (very fine kaolinitic, isohypothermic, plinthic Kandiudox) with pH of 4.1, bulk density of 1.13 g cm^{-3} and high Al saturation of 77%. This treatment did not receive any additional fertilizer application to simulate high Al 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 restricted (based on symptoms) by both Al-toxicity and low availability of P. Soil for the low Al treatment presented pH of 4.6, bulk density of 1.26 g cm^{-3} and an Al saturation of 12%. The soil cylinders for low Al treatment were packed with Quilichao soil (Butare et al. 2011), previously fertilized with adequate amendments (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 (CaCO$_3$), 6.36 Mg (MgCO$_3$ or dolomite lime), 0.49 S (elemental sulphur), 0.09 Zn (ZnCl$_2$), 0.11 Cu (CuCl$_2$.2H$_2$O), 0.01 B (H$_3$BO$_3$) and 0.01 Mo (NaMoO$_4$·2H$_2$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 (CaCO$_3$), 25.45 Mg (MgCO$_3$ or dolomite lime), 1.97 S (elemental sulphur), 0.36 Zn (ZnCl$_2$), 0.46 CuCl$_2$. 2H$_2$O, 0.05 B (H$_3$BO$_3$) and 0.02 Mo (NaMoO$_4$·2H$_2$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).

The experiment was planted as a randomized complete block design with three replications. Each cylinder was filled uniformly with soil (5.01 kg for low Al saturation treatment and 4.76 kg for high Al saturation). The experiment was conducted in a greenhouse at an average temperature of 29.5/22.8°C (day/night), relative air humidity of 43.7/60.9% (day/night), and a maximum photosynthetic photon flux density of 1,100 μmol m^{-2} s^{-1}. Plants were harvested at 34 days after planting as described previously (Butare et al. 2011).

Shoot and root attributes

At harvest, leaf area (LA) of 34 day-old plants was determined by scanning leaves of each genotype using a LI-3100 leaf area meter (LI-COR Biosciences, USA). Shoot dry weight (SDW) was measured after drying leaves, stems and pods in an oven at 70°C for 72 h. Root attributes were determined by image analysis with the WinRhizo software program (Regent Instruments Inc., Canada): TRL, MRD, root volume,

and root length by category of root diameter (<0.5, 0.5–1.0 mm). It was not possible to determine TRER and NRT in the soil cylinder experiment.

Statistical analysis

For statistical analysis of data, PC SAS was used. Analysis of variance was performed using the ANOVA procedure (PROC GLM) of statistical program SAS 9.1 (SAS institute Inc. SunOS 5.9 platform). Data of checks were excluded from the analysis of variance but data of parents were included to permit statistical comparisons of parents and progenies. Correlation coefficients were calculated by the PROC CORR procedure using means across treatments and replications for the two experiments. Correlations were calculated only over data of RILs, to observe the tendencies reflected in genetic segregation. Significant differences (*, **, and ***) were detected at the 0.05, 0.01, and 0.001 probability levels, respectively.

Relationships between the two Al-screening methods and between levels of stress within methods for genotypic characteristics (root and shoot traits) were determined. Differences between genotypes were analyzed with the least significant difference (LSD) at $P < 0.05$.

Results

Progeny means for some specific root and shoot traits in both Al treatment and control were compared to the two parents G35346-3Q and SER16, and the implications for inheritance of these traits were identified (Table 1). The range of progeny means exceeded that of their parents both in hydroponics for several traits (with and without Al) and in soil cylinder screening for a few traits (with high and low Al saturation in soil) suggesting transgressive segregation for these plant phenotypes.

Table 1 Trait values of parents of interspecific populations (G35346-3Q and SER16) evaluated under 0 and 20 μM Al treatments in hydroponics, and in low and high Al saturation in soil cylinder screening, and range of their recombinant inbred lines for specific plant traits

Hydroponics	0 μM Al treatment				20 μM Al treatment			
	G35346-3Q	SER16	Range	LSD$_{0.05}$	G35346-3Q	SER16	Range	LSD$_{0.05}$
TRER24 h	1.52	1.82	1.42–**2.25**	0.21	1.41	1.38	**0.88**–1.75	0.20
TRER24-48 h	1.49	1.64	**1.15**–1.86	0.26	1.46	1.04	**0.18**–1.69	0.24
TRER48 h	1.50	1.73	1.31–**2.04**	0.19	1.44	1.21	**0.55**–1.72	0.18
TRL	4.22	4.10	**1.48**–5.51	1.13	2.82	2.30	**0.84**–2.90	0.57
MRD	0.47	0.32	0.29–0.47	0.03	0.54	0.40	**0.35**–0.54	0.04
NRT	720	962	**394**–**1316**	259	457	441	**172**–513	105
SRL	121.1	245.9	121–281	37.2	95.3	170.5	95–**279**	48.0
Soil cylinder	Low Al saturation soil				High Al saturation soil			
	G35346-3Q	SER16	Range	LSD$_{0.05}$	G35346-3Q	SER16	Range	LSD$_{0.05}$
TRL	79.4	64.8	**36.1**–97	24.2	42.9	16.6	8.81–42.9	7.8
MRD	0.43	0.32	0.30–0.43	0.03	0.46	0.40	0.34–0.47	0.07
SRL	60.2	98.1	60.2–**123**	18.1	62.1	72.1	51.3–**89.1**	15.5
VRD34d	53.6	61.5	50–75	17.6	53.6	33.2	22.6–55.3	11.2
LA	444	548	269–**854**	189	259	106	48.8–271	88.0
SDW	2.6	3.46	1.62–**5.2**	1.12	1.25	0.81	**0.26**–1.43	0.37
R:S ratio	0.54	0.21	0.13–0.54	0.08	0.59	0.29	0.14–**0.79**	0.18

TRER Tap root elongation rate (mm h^{-1}), *TRL* total root length (m plant^{-1}), *MRD* mean root diameter (mm), *NRT* number of root tips, *SRL* specific root length (m g^{-1}), *VRD34d* visual rooting depth at 34 days (cm), *LA* leaf area (cm^2 plant^{-1}), *SDW* shoot dry weight (g plant^{-1}), *R:S ratio* root:shoot ratio. Values in *bold* fall significantly outside of the range of the parental values

Phenotypic differences in Al resistance in hydroponic system

Tap root elongation rate (TRER)

A hydroponic system with two levels of Al (0 and 20 μM Al) was used to detect genotypic differences in Al resistance. Analysis of variance revealed highly significant genotype (RIL) effects, and genotype × Al ($P < 0.001$) interaction among the 94 RILs in TRER under Al toxicity at 0–24, 24–48 and 0–48 h (Table 2). Mean value for TRER of RILs in our nutrient solution screening between 24 and 48 h was 0.96 mm h^{-1} for Al treatment (20 μM Al) and 1.60 mm h^{-1} for the control (0 μM Al) treatment. G35346-3Q actually increased its TRER slightly by 3.5% with 20 μM Al-stress between 24 and 48 h, while SER16 presented an inhibition of TRER of 31.1%, and RILs presented reductions from 6.7 to 88.8%. Both positive and negative transgressive segregation for TRER was observed, both with and without Al in solution (Table 1), implying ample genetic variability among the two parents.

The range of TRER among RILs in 20 μM Al increased from 0.88 to 1.75 mm h^{-1} at 24 h to 0.18–1.69 mm h^{-1} at 24–48 h. TRER at 0–24 h correlated to TRER at 24–48 h ($r = 0.49$***; Table 2), although this correlation was lower than might have been expected, considering that the data were taken on the same plants in the same environment. This suggests that measurements of TRER at 0–24 h may not be fully representative of TRER at 24–48 h. Genotype ranking varied between the two periods of time. G35346-3Q ranked 53rd among 102 genotypes for TRER at 24 h but 5th at 24–48 h. G35346-3Q was the only genotype with greater TRER in the second period (1.46 mm h^{-1}) compared to the first (1.41 mm h^{-1}). TRER values among RILs at 0–24 h were correlated to MRD with a significant but weak correlation, $r = 0.37$***, but not at 24–48 h ($r = 0.07$). The positive correlation implies that fast growing genotypes in the first 24 h have a thicker tap root. A significant but weak negative correlation was found also between TRER at 24 h and NRT ($r = -0.24$***).

TRL in RILs

A highly significant genotype effect was found on TRL, NRT, SRL and MRD in solution culture, while genotype × Al treatment interaction was significant for NRT and for TRL (Table 2; Fig. 1A). TRL and NRT were highly correlated among RILs (Table 2)

Table 2 Correlation coefficients between root characteristics of 94 RILs of beans grown with 20 μM Al in a hydroponic system and mean squares from combined ANOVA of 0 and 20 μM Al treatments for 94 RILs and two parents (G35346-3Q and SER16)

Variable/source	df	TRER24 h (mm h^{-1})	TRER48 h (mm h^{-1})	TRER24–48 h (mm h^{-1})	TRL (m plant^{-1})	MRD (mm)	NRT	SRL (m g^{-1})
TRER 48 h	_	0.83***						
TRER24–48 h	_	0.49***	0.88***					
TRL	_	−0.31***	−0.17**	−0.02 (ns)				
MRD	_	0.37***	0.25***	0.07 (ns)	−0.80***			
NRT	_	−0.24***	−0.09 (ns)	0.04 (ns)	0.89***	−0.81***		
SRL	_	−0.19**	−0.08 (ns)	0.03 (ns)	0.66***	−0.80***	0.72***	
Level of Al	1	49.95***	53.64***	57.57***	504.1***	1***	28955056***	661867***
Rep. (Al level)	4	2.73***	0.91***	0.25***	78.3***	0.32***	1882195***	54286***
Genotype	95	0.12***	0.15***	0.21***	2.53***	0.01***	119760***	2464***
Genotype × Al level	95	0.03***	0.05***	0.11***	0.43*	0 (ns)	33006***	645 (ns)
Error	376	0.02	0.01	0.02	0.31	0.24	14936	704

TRER Tap root elongation rate at 0–24 h, at 0–48, and 24–48 h, *TRL* Total root length, *MRD* mean root diameter, *NRT* number of root tips, *SRL* specific root length, and *ns* not significant

* Significant at the 0.05 probability level

** Significant at the 0.01 probability level

*** Significant at the 0.001 probability level

Fig. 1 Relationship between **A** total root length in hydroponics with 0 μM Al (m plant^{-1}) and total root length in hydroponics with 20 μM Al (m plant^{-1}), and **B** total root length in soil with low Al saturation (m plant^{-1}) and total root length in soil with high Al saturation (m plant^{-1}) of 102 bean genotypes including 94 RILs, two parents (G35346-3Q and SER16), and six checks. The values of r are for 94 RILs. Parents and checks were included for comparison. ***, ** Significant at the 0.001, 0.01 probability level, respectively
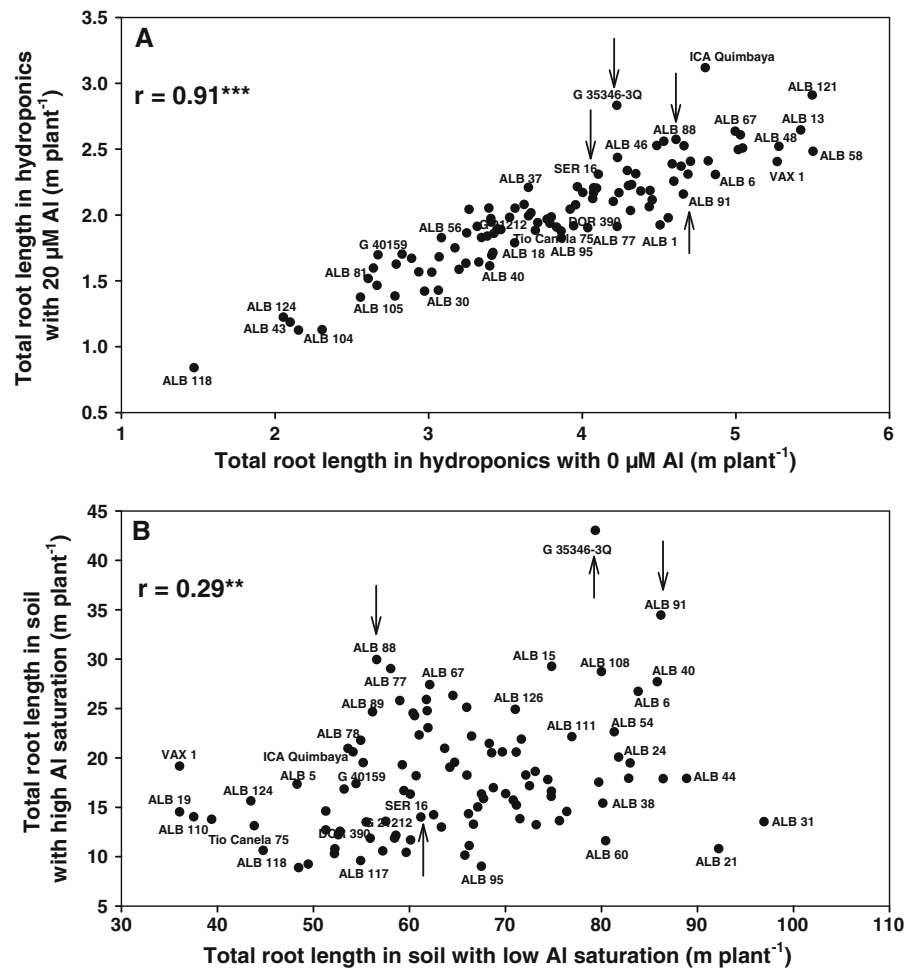
($r = 0.89$***). Checks ICA Quimbaya and VAX1, parent SER16, and several RILs including ALB88 were characterized by an extensive root system with greater values of NRT. A negative association was found between TRL and MRD ($r = -0.80$***), and between NRT and MRD ($r = -0.81$***). SER16, Al sensitive checks VAX1 and DOR390, and ALB46, 88 and 121 presented high TRL and low MRD, with less increase in MRD under Al toxicity.

There was a negative relationship between TRL and TRER at 24 h ($r = -0.31$***) that diminished at 48 h ($r = -0.17$**), suggesting that the tap root behaved differently from the rest of the root system in this system and at these points in time (Table 2). ALB43 and ALB106 actually expressed high TRER and low TRL. ICA Quimbaya was the only genotype which combined high TRL (extensive root system) and high TRER at 24 h.

Phenotypic differences in acid soil tolerance in soil cylinder system

Visual rooting depth and TRL in soil

The major Al toxicity symptom observed in plants is inhibition of root growth that affects directly the distribution of roots in soil profiles. VRD of 34 day-old plants (VRD34d) showed significant differences between the two levels of Al treatment, replications (Al level), genotype effects, and Genotype × Al interaction (Table 3). Mean value of VRD34d in soil cylinders was 63.9 cm in low Al treatment and 37.2 cm for high Al treatment. Roots of G35346-3Q penetrated to 53.6 cm deep in both the high and low Al treatment soils while sensitive genotype DOR390 reached only 25.2 cm. The best RIL (ALB 70) reached 55.3 cm. At 38 days G35346-3Q actually increased

Table 3 Correlation between root and shoot characteristics of 94 RILs of beans grown in a soil cylinder system with high Al saturation soil and mean squares from a combined ANOVA of high and low Al treatments for 94 RILs and two parents (G35346-3Q and SER16)

Variable/source	df	TRL (m plant^{-1})	MRD (mm)	SRL (m g^{-1})	VRD34d (cm)	LA (cm^2 plant^{-1})	SDW (g plant^{-1})	R:S ratio
MRD	–	−0.43***						
SRL	–	0.40***	−0.69***					
VRD34d	–	0.53***	−0.16**	0.15*				
LA	–	0.70***	−0.32***	0.26***	0.39***			
SDW	–	0.66***	−0.25***	0.19**	0.34***	0.72***		
R:S ratio	–	0.22***	0.06 (ns)	−0.20***	0.17**	−0.09 (ns)	−0.42***	
Level of Al	1	324648***	0.89***	100314***	100142***	26110141***	1080***	3.17***
Rep. (Al level)	4	3532***	0.16***	10947***	644***	58744***	2.27***	0 (ns)
Genotype	95	332***	0***	268***	124**	17383***	0.8***	0.03***
Genotype × Al level	95	197**	0 (ns)	197***	127**	15992***	0.59***	0.01***
Error	380	125	0	109	84.4	8427	0.27	0.01

TRL Total root length, *MRD* mean root diameter, *SRL* specific root length, *VRD34d* visual rooting depth of 34 day-old plants, *LA* leaf area, *SDW* shoot dry weight, *R:S ratio* root:shoot ratio, and *ns* not significant

* Significant at the 0.05 probability level

** Significant at the 0.01 probability level

*** Significant at the 0.001 probability level

root penetration in high Al saturation soil cylinders by 12% compared to the low Al-saturation treatment (data not shown). VRD34d was reduced by 46% for recurrent parent SER16, and by as much as 65% in RIL. Significant differences among RILs reflected introgression of genes from G35346-3Q into SER16. VRD34d was highly correlated with some plant traits (Table 3) of soil cylinder experiment with high Al saturation soil: TRL ($r = 0.53***$), LA ($r = 0.39***$), and SDW ($r = 0.34***$). There were also significant correlations with MRD ($r = -0.16**$), and with SRL ($r = 0.15*$).

Interaction between root and shoot traits

Effects on LA and SDW were highly significant for genotype, and genotype × Al treatment interaction (Table 3). Correlations between these two shoot traits and TRL under high Al saturation soil were highly significant, $r = 0.70***$ for LA and $r = 0.66***$ for SDW (Table 3.). Rating based on the combination of TRL and SDW revealed not only the effects of Al-stress in the soil but biomass accumulation in shoot that could translate into yield in the reproductive phase. Genotypes characterized by an extensive and deep root system, enhanced exploratory capacity, and superior SDW were G35346-3Q, and ALB15, 40, 77, 88, 91, and 119. In this regard ALB91 was the RIL that most closely approximated the behavior of G35346-3Q (Figs. 1B, 2A). ALB88 was able to produce more LA with less biomass which is an advantage in a competitive situation, and it has also high NRT in hydroponics.

Comparison of traits in hydroponic and soil cylinder systems

In nutrient solution a close relationship ($r = 0.91***$) was found between TRL of RILs with and without Al treatment (Fig. 1A). In contrast, in the soil cylinder system the relationship between TRL under high and low Al saturation treatment was poor ($r = 0.29***$) (Fig. 1B). TRL discriminated genotypes for their Al response in acid soil better than in hydroponics. In a comparison of methods, TRL was positively correlated in the control treatments (low Al soil with 0 μM Al nutrient solution; $r = 0.29**$), and also in the stress treatments (high Al soil with 20 μM Al solution; $r = 0.40***$) (Fig. 2B). The two techniques

revealed some common traits associated with Al toxicity as well as some other unique traits.

Several weak correlations were identified between parameters of RILs for the two methods (Table 4). TRL in acid soil correlated with NRT in Al toxic hydroponic solution ($r = 0.24***$). SRL in soil presented negative correlations with TRER and MRD in hydroponics, and positive correlations with TRL ($r = 0.23*$), NRT ($r = 0.25*$), and SRL ($r = 0.41**$). MRD in high Al saturation soil gave a response that was opposite to that of SRL, presenting negative correlations with TRL ($r = -0.36***$) and with NRT ($r = -0.31**$) in nutrient solution with 20 μM Al. Several RILs and especially ALB88 presented low MRD in soil cylinders and maintained high TRL and NRT in nutrient solution. NRT could not be evaluated effectively in soil due to cutting the soil cylinders. Based on correlations between root characteristics under Al stress in both hydroponics and soil cylinder systems, the response of RILs and two parents in terms of MRD and SRL was similar ($r = 0.36***$ and $0.41***$, respectively) (Table 4).

ALB88 and ALB91 emerged as lines with multiple traits (Table 5). Comparison of these two elite progenies with recurrent parent SER16 and the *P. coccineus* donor G35346-3Q for root and shoot characteristics showed that both progenies combined the Al resistance characteristics of G35346-3Q with fine root development characteristics of SER16.

Discussion

The unique response of G35346-3Q under high Al in solution and in Al toxic soil that was observed previously (Butare et al. 2011) was confirmed in this study. G35346-3Q maintained an excellent response and root elongation under Al stress in the hydroponic system, and when plants were subjected to Al stress in soil cylinder screening system, G35346-3Q was better than any progeny in TRL and VRD34d. G35346-3Q actually responded to stress by increasing its VRD34d under Al-toxic acid soil stress, and was the only genotype that increased its TRER between 24 and 48 h in the hydroponic system with 20 μM Al. Doncheva et al. (2005) concluded that Al-induced inhibition of root elongation rate, measurable after 45 min in the Al-sensitive maize variety HS16X36, must be attributed to an Al-induced decrease of root cell expansion rather

than to fast inhibition of root cell division. Over longer periods of time (more than 24 h), processes of both cell elongation and cell division are inhibited (Matsumoto 2000; Stass et al. 2007). On the other hand, Bennet et al. (1991) concluded that Al acts by releasing the root meristem of maize from growth inhibition originating in the root cap, and that the recovery of root growth rates from Al treatment are initially faster than normal, suggesting that the early phases of recovery may involve growth stimulation. If different reactions are expressed at different times after exposure, these may have come into play in the observed differences between 0–24 and 24–48 h in our study, especially if some such stimulus mechanism were associated with the increase in TRER of G35346-3Q at 24–48 h, in contrast with other genotypes. However, this trait was not readily transferred to the progenies through the single backcross that created the progenies.

Magnitude of effects of Al on TRER were similar to those reported elsewhere for sensitive genotypes SEA5 and VAX1 (74 and 85%, respectively) (Rangel et al. 2005). López-Marín et al. (2009) have likewise shown with a mapping population of bean in hydroponic screening that genotype (RIL) effects, Al treatment, and the interactions RIL × treatment were highly significant for TRER, TRL, MRD, NRT, RDW and SRL.

Doncheva et al. (2005) showed that Al caused not only a reduction in the length of the main root, but also changes to the entire root architecture. In our study inhibition of TRER was accompanied by an increase of MRD (data not shown) from 15 to 36% among RILs over 48 h. In the hydroponic system, TRL and NRT correlated positively, and MRD correlated negatively with TRL and NRT, as would be expected. Greater root length ought to be associated with more NRT and

Table 4 Correlation coefficients between root and shoot characteristics of 94 RILs of beans grown under Al-stress using both hydroponics (20 μM Al) and soil cylinder screening (high Al saturation soil) systems

Hydroponics/soil cylinders	TRL (m plant^{-1})	MRD (mm)	SRL (m g^{-1})	VRD34d	LA (cm^2 plant^{-1})	SDW (g plant^{-1})	R:S ratio
TRER24 h (mm h^{-1})	0.22*	0.01 (ns)	−0.14	0.15 (ns)	0.06 (ns)	0.07 (ns)	0.23*
TRER48 h (mm h^{-1})	0.19 (ns)	0.16 (ns)	−0.21*	0.06 (ns)	0.09 (ns)	0.08 (ns)	0.14 (ns)
TRER24–48 h (mm h^{-1})	0.15 (ns)	0.23*	−0.22*	0.002 (ns)	0.08 (ns)	0.07 (ns)	0.07 (ns)
TRL	0.29**	−0.36***	0.23*	0.12 (ns)	0.14 (ns)	0.17 (ns)	0.06 (ns)
MRD	−0.11 (ns)	0.36***	−0.42***	0.02 (ns)	−0.04 (ns)	−0.19 (ns)	0.15 (ns)
NRT	0.24*	−0.31**	0.25*	0.09 (ns)	0.16 (ns)	0.20*	−0.03 (ns)
SRL	0.04 (ns)	−0.31**	0.41**	0.02 (ns)	−0.007 (ns)	0.09 (ns)	−0.09 (ns)

Root and shoot characteristics included *TRER* tap root elongation rate, *TRL* total root length, *MRD* mean root diameter, *SRL* specific root length, and *VRD34d* visual rooting depth of 34 day-old plants, *LA* leaf area, *SDW* shoot dry weight and *R:S ratio* root:shoot ratio. ns Not significant

* Significant at the 0.05 probability level

** Significant at the 0.01 probability level

*** Significant at the 0.001 probability level

thinner roots (higher SRL values). Unexpectedly, MRD correlated positively with TRER, implying that thicker roots grew faster in the first 24 h after exposure. The relationship between TRER, MRD, TRL and NRT might be explained by root apical dominance which could be stronger in G35346-3Q than in SER16. Al-resistant parent G35346-3Q was characterized by low NRT and large TRL value. Apical dominance would repress lateral roots, reducing NRT and partitioning photosynthate for the rapid growth of larger roots including the tap root. This could increase TRER and MRD at the expense of NRT, while the effect on TRL could be less dramatic. Thus, focusing on the tap root in populations where apical dominance is segregating may produce results that are not representative of the rest of the root system. In this study TRL and TRER were not correlated or presented negative correlation, suggesting that the tap root growth in fact did not reflect the vigor of entire root system.

Conclusions about the nature of Al resistance depend on the method of reference. A close relationship ($r = 0.91***$) was found between TRL with and without Al treatment in nutrient solution (Fig. 1A). This suggests that whatever mechanism that resulted in greater TRL in solution culture could be constitutive. Urrea-Gómez et al. (1996) suggested that constitutive morphological characteristics such as vigorous rooting could be advantageous in the breeding of

Al–tolerant cultivars. However, we found that in acid soil the correlation between stress and non-stress treatments was low ($r = 0.29***$). Based on experience in soil in our study, some resistance mechanisms would not appear to be constitutive. In light of the low correlation between TRL under stress in the two systems, that correlation could be due to the constitutive traits observed in the hydroponic system, while additional traits are expressed in the soil system. In contrast, Villagarcia et al. (2001) found that the genotypic variation in Al tolerance and genotype × treatment interactions were much greater for several traits in hydroponics than in sand culture, and were accompanied by a lower correlation between Al-free and Al-stress treatments.

Some authors have found positive relationships between solution culture and soil methods of evaluation (Gahoonia and Nielsen 1997; Horst and Klotz 1990), while others report little or no correspondence in plant response between the two sorts of methods (Noble et al. 1987; Villagarcia et al. 2001; Sartain and Kamprath 1978; Sapra et al. 1982). We found a modest correlation ($r = 0.40**$) between TRL from soil cylinder system and hydroponics for Al stress treatments. This correlation contrasts with those of our previous study using a limited number of genotypes in hydroponics and soil cylinder screening methods, showing that TRL under stress was highly correlated in the two methods (Butare et al. 2011). That

Table 5 A comparison of two elite progenies (ALB 88 and ALB 91) of an interspecific cross between the common bean recurrent parent SER 16 and the *P. coccineus* donor G35346-3Q for root and shoot characteristics

	Soil cylinders						Hydroponics				
	TRL (m plant^{-1})	MRD (mm)	SRL (m g^{-1})	VRD34d (cm)	LA (cm^2)	SDW (g plant^{-1})	TRER 24–48 h (mm h^{-1})	TRL (m plant^{-1})	MRD (mm)	NRT	SRL (m g^{-1})
Low Al											
ALB 88	56.6	0.34	82.1	75	586.9	3.62	1.53	4.61	0.34	993	234
ALB 91	86.3	0.33	93.1	73.7	623.4	3.64	1.73	4.66	0.33	1097	235
SER 16	64.8	0.32	98.1	61.5	548	3.46	1.64	4.10	0.32	962	246
G35346-3Q	79.4	0.43	60.2	53.6	443.6	2.61	1.49	4.22	0.47	720	121
LSD (0.05)	24.2	0.03	18.1	17.6	189	1.12	0.26	1.13	0.03	259	37.2
High Al											
ALB 88	29.8	0.35	78.3	41.5	235	1.23	0.73	2.57	0.40	435	158.6
ALB 91	34.3	0.37	83.4	50.2	211	1.10	0.64	2.15	0.42	382	146.8
SER 16	16.6	0.40	72.1	33.2	106	0.81	1.04	2.30	0.40	441	170.5
G35346-3Q	42.9	0.46	62.1	53.6	259	1.25	1.46	2.82	0.54	457	95.3
LSD (0.05)	7.8	0.07	15.5	11.2	88.0	0.37	0.24	0.57	0.04	105	48

TRL Total root length, *MRD* mean root diameter, *SRL* specific root length, *VRD34d* visual rooting depth of 34 day-old plants, *LA* leaf area, *SDW* shoot dry weight, *TRER* tap root elongation rate and *NRT* number of root tips

correlation was apparently driven by the presence of several accessions of *P. coccineus* which have multiple Al resistance traits. Genetic segregation has separated these traits in the RILs, such that some RILs are superior in hydroponics, and others in acid soil.

There may be several reasons for differences in results between soil and solution culture. Soil offers resistance to root elongation that is absent in solution culture. Al toxicity inhibits cell wall expansion and cell elongation that are necessary to drive roots through soil. The combination of Al-induced inhibition of root elongation and soil resistance to root penetration may require additional mechanisms of resistance than those revealed by solution culture alone. Many Al-tolerant plant species release organic acid anions such as citrate, malate or oxalate from root apices that chelate Al cation and ameliorate Al-induced inhibition of root elongation (Delhaize et al. [1993]; Kidd et al. [2001]; Ma et al. [2001]; Kikui et al. [2005]; Rangel et al. [2010]). Solution culture may dilute the organic acids and reduced their effect. Other potential differences between methods could be in the nutritional status of the plants. Seedlings in nutrient solution are evaluated for a short period (for several hours or days) while for soil cylinder evaluation, plants are grown for several days or even a month or more. P diffuses freely to the root surface in solution culture (Clarkson [1991]), whereas in soil, diffusion of P to the root surface is rate limiting and the zone of soil close to the root is depleted uniformly due to the geometrical arrangement of root hairs on root (Gahoonia and Nielsen [1991]). Calcium availability can also be limiting in acid soil. Ryan et al. ([1994]) found that low concentrations of Al could inhibit both root growth and Ca uptake.

To successfully select Al-resistant lines that perform well in acid soil, Al-resistance could not be deduced based only on the inhibition of root growth but must take into account other multiple traits reflected in the plant response. The hydroponic system used in this study is suitable for selection for Al resistance alone based only on root development, and for NRT. NRT could not be evaluated effectively in soil due to cutting the soil cylinders, but this trait could be important for acquisition of Ca from infertile acid soil (Hausler et al. [2006]). The soil cylinder system revealed differences in root distribution, acquisition of soil resources and pattern of biomass allocation in leaves, stems, and roots. Screening methods and Al concentrations that reveal differences in shoot development could probably improve correlations with yield performance in acid soil, and selection for Al resistance.

The population of RILs was created from a single backcross of the F_1 of G35346-3Q $\times$ SER16 to SER16. Differences among RILs and between RILs and the recurrent parent SER16 reflect introgression from the *P. coccineus* parent. Segregation of several traits suggested quantitative inheritance and often transgressive segregation, especially in the hydroponic system, implying that both parents possessed complementary genes for some traits. This was not a surprise considering that SER16 was quite good for TRL and NRT in the hydroponic system. For example, the two parental lines were virtually equal for TRER 24 h in the 20 µM Al at about 1.4 mm h^{-1} but the progenies ranged from less than 1–1.75 mm h^{-1}. Other traits for which the RILs presented transgressive segregation were MRD in 20 µM Al and SRL in low Al saturation soil. López-Marín et al. ([2009]) also found transgressive segregation in beans for TRER in the control solution and NRT in control and Al treatment, although the percentage of negative transgression was higher than positive transgression in both control and Al treatments. Transgressive segregation has also been reported in soybean for tap root extension under 0 µM Al in a hydroponic system (Bianchi-Hall et al. [2000]), and for a number of root traits in a study of Al tolerance in rice (Nguyen et al. [2002], [2003]).

Inheritance of Al resistance and/or acid soil tolerance in G35346-3Q appears to be complex, and no line received the full complement of the *P. coccineus* genes through backcross and selfing. Given the several traits for which G35346-3Q is superior, some traits likely have independent genetic control. The multiple traits of G35346-3Q evidently were separated through genetic segregation that produced the RILs. These observations on quantitative and/or transgressive segregation confirm the complexity of traits for Al resistance or acid soil tolerance, and suggest that recovering the full complement of genes from G35346-3Q would be difficult, especially through a backcross. In the case of multiple traits that contribute to Al resistance, correlations of individual traits with the overall response are likely to be low, and in proportion to their relative contribution to resistance.

Correlations may be indicative of useful traits but not fully informative. Examining the behavior of individual lines may reveal more about the specific traits that combine to give better response. Furthermore, it may be necessary to select those genotypes that combine two or more favorable traits and that appear as outliers, and to use these in a recurrent selection scheme to continue to pyramid genes and traits.

ALB88 and ALB91 emerged as superior for several traits (Table 5), and could be candidates for crossing with each other or with other lines. Both lines behaved similarly to parent SER16 in the hydroponic system for most parameters, both with and without Al. The only exception was TRER24–48 h with high Al, for which both lines were inferior to both parents. Compared to SER16 in the soil system without stress, ALB88 was marginally inferior for SRL (NS), and was similar for other parameters. However, it was superior to SER16 under acid soil stress for TRL, LA and SDW. It appears to have recovered genes from *P. coccineus* that permit better performance in acid soil. ALB91 presented a pattern similar to ALB88 but with one important difference. In soil without stress it was superior to ALB88 for TRL, presenting the same TRL as G35346-3Q. This perhaps gave ALB91 a wider advantage over SER16 in acid soil compared to ALB88, for example, in greater TRL and VRD34d compared to SER16. ALB91 expressed more influence of *P. coccineus* than ALB88, and was the RIL that most approached the performance of G35346-3Q in combining excellent TRL with good SDW. However, both ALB88 and ALB91 derived positive traits from SER16 as well. Compared to G35346-3Q, each presented a tendency for higher values of NRT in hydroponics without stress, and higher SRL and lower MRD in acid soil. Fine roots reflected in SRL are considered to be more important than thick roots in nutrient and water absorption, and therefore, more important in terms of Al resistance (Eisenstat 1992; Liu et al. 2010).

Conclusions

Our data reaffirm that the runner bean genotype, G35346-3Q, is an Al resistant material and is a very good source for improving acid soil tolerance in common bean. The response of G35346-3Q in Al treatments versus controls, resulting in no reduction in

TRER in hydroponics, and greater VRD34d in soil cylinders, testifies to the unusual nature of its Al resistance which ought to be studied in more detail. Compared to SER16, tolerance of derived lines to Al-toxic acid soil was improved by the introduction of G35346-3Q traits, which are apparently quite complex. Several root parameters evaluated using the soil cylinder system were associated with superior shoot development and may contribute to shoot biomass accumulation of the best lines. The introgression of different traits and/or mechanisms into different RILs could be a tool to study the role of each of these traits or mechanisms under different conditions. The use of both soil and hydroponic systems could contribute to evaluation of breeding materials to identify genotypes that combine Al resistance with acid soil tolerance. This knowledge will contribute to understanding the physiological basis of differences in ranking of genotypes under acid soil field conditions across seasons and years. The results from this work will be useful for identification of molecular markers for Al resistance in *Phaseolus* species and to improve acid soil adaptation in common bean.

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