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Quantitative trait loci for root morphology traits under aluminum stress in common bean (*Phaseolus vulgaris* L.)

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Abstract Aluminum (Al) toxicity is a major limiting factor of crop production in acid soils, which are found mostly in developing countries of the tropics and sub-tropics. Common bean (*Phaseolus vulgaris* L.) is particularly sensitive to Al toxicity; and development of genotypes with better root growth in Al-toxic soils is a priority. The objectives of the present study were to physiologically assess root architectural traits in a recombinant inbred line (RIL) population of common bean that contrasts for Al resistance (DOR364 × G19833) and to identify quantitative trait loci (QTL) controlling root growth under two nutrient solutions, one with 20 μM Al concentration and the other without Al, both at pH 4.5. A total of 24 QTL were found through composite interval mapping analysis, 9 for traits under Al treatment, 8 for traits under control treatment, and 7 for relative traits. Root characteristics expressed under Al treatment were found to be under polygenic control, and some QTL were identified at the same location as QTL for tolerance to low phosphorous stress, thus, suggesting cross-links in genetic control of adaptation of common bean to different abiotic stresses.

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Abbreviations

ARD	Average root diameter
DTZ	Distal part of the transition zone
EZ	Elongation zone
LG	Linkage group
NRT	Average number of root tips
RDW	Root dry weight
RIL	Recombinant inbred line
SRL	Specific root length
TRE	Tap root elongation rate
TRL	Total root length

Introduction

Aluminum (Al) toxicity is a major abiotic stress affecting crop production over a wide swath of acid soils in tropical and subtropical environments (von Uexküll and Mutert 1995). Al toxicity occurs when soil pH is less than 5.0 and Al dissolves from non-toxic Al oxides and silicates into the phytotoxic form Al^{3+} (Kochian 1995). Aluminum cations limit root growth and increase the risk of plants succumbing to drought and mineral deficiencies, therefore limiting plant growth and production (Foy 1984). Root elongation in plants susceptible to Al is inhibited rapidly upon exposure to the element (Kochian 1995; Llugany et al. 1995; Zheng and Yang 2005). In maize, for example, Ryan et al. (1993) demonstrated that only 3 mm of tissue behind the area of root quiescence, the area that includes the root meristem, needs to be exposed to toxic Al compounds to result in inhibited root growth. Sivaguru and Horst (1998) demonstrated that in Al-susceptible maize, the distal part of the transition zone (DTZ, 1–2 mm from the root tip) succumbs first to Al cations, because the cells of this area are in a preparatory phase for the DTZ's rapid elongation. Moreover, if

Al is applied over a short-period to either the DTZ or the entire root meristem, root elongation in the principal elongation zone (EZ, 2.5–5.0 mm from the root tip) will be inhibited to the same extent, whether the maize genotype is resistant or susceptible to Al toxicity (Horst et al. 1999; Sivaguru et al. 1999) suggesting that under conditions of Al stress, a hormone transport signaling pathway may be activated in the root apex through a flow of information between the DTZ (where Al is perceived) and the EZ (where growth is inhibited) (Kollmeier et al. 2000).

Common bean (*Phaseolus vulgaris* L.) is an important nutritional component in the diet of more than 300 million people around the world, especially in developing countries. Total production exceeds 23 million metric tons per year of which 7 million are produced in Latin America and Africa (Broughton et al. 2003). Production on these continents occurs mainly on small farms, where about 40% of the production area is affected by Al toxicity resulting in a 30–60% reduction in production (Rao 2002). Al toxicity also occurs in temperate regions, affecting other legumes, or on large-scale bean farms in the Cerrados of Brazil, however, in those cases liming is frequently used to correct pH and diminish toxicity effects. Applying soil amendments to correct Al toxicity is impractical for most hillside bean farms in the developing world due to high transport costs and erosion risks (Rao et al. 1993; Samac and Tesfaye 2003). Therefore, the development of Al-resistant common beans is the most viable alternative for confronting this global problem (Thung and Rao 1999).

Exudation of organic acids was identified early on in beans as a possible mechanism and associated effect of resistance to Al toxicity: Lee and Foy (1986) found that Al stress reduced root extract organic acid concentration to a greater extent in Al-sensitive than in Al-resistant genotypes; while Miyasaka et al. (1991) demonstrated that other Al-resistant genotypes of common bean exuded eight times more citrate than susceptible genotypes during prolonged exposure. After these initial studies in beans, exudation of different organic acids was correlated with Al resistance in other plants, including citrate in maize (*Zea mays* L.) (Pellet et al. 1995; Arruda and Jorge 1997; Ishikawa et al. 2000; Kidd et al. 2001; Kollmeier et al. 2001; Piñeros et al. 2002), and sorghum [*Sorghum bicolor* (L.) Moench] (Magalhaes et al. 2007); malate in wheat (*Triticum aestivum* L.) (Rincón and Gonzales 1992; Tice et al. 1992; Delhaize et al. 1993) and *Arabidopsis thaliana* (L.) Heynh. (Hoekenga et al. 2003); and oxalate in buckwheat (*Fagopyrum esculentum* Moench) (Ma et al. 1997; Zheng et al. 1998a, b) and taro [*Colacasia esculenta* (L.) Schott] (Ma and Miyasaka 1998). Two models for activating the exudation of organic acids have been proposed by Ma et al. (2001): the first is represented by plants such as wheat, where the exudation of malate is rapidly activated on exposure to Al; however, with no increase in the rate of malate efflux over time, perhaps because in this species, Al activates a transporter that is already expressed, and hence, gene activation plays no role in resistance. The second model is represented by species such as rye (*Secale cereale* L.), senna (*Cassia tora* L.), and common bean. In this model, a difference in time can be observed between activation of the exudation of organic acids and the increase in the rate of exudation during the first 12–24 h of exposure and consequently an important mechanism of gene activation may operate in the exudation of these compounds. In both *Arabidopsis* (Kobayashi and Koyama 2002; Hoekenga et al. 2003; Kobayashi et al. 2005) and sorghum (Magalhaes et al. 2004), inheritance of Al resistance appears to be associated with organic acid transporters and involve a mixture of strong and weak tolerance QTL (quantitative trait loci).

The objectives of the present research were (1) to evaluate a recombinant inbred line (RIL) population of common bean from the cross DOR364 × G19833 for root growth traits under hydroponic screening with and without Al stress, and (2) to identify QTL for root architectural traits that were related to Al resistance. Our results show that common bean has polygenic inheritance of resistance to Al and that some Al resistance QTL co-localize with QTL for tolerance to low phosphorous (P) (Liao et al. 2004; Yan et al. 2004; Beebe et al. 2006), suggesting cross-links between different mechanisms of abiotic stress adaptation in common bean.

Materials and methods

Plant materials

A common bean population of 87 RILs generated at the International Center for Tropical Agriculture (CIAT, its Spanish acronym) from the cross DOR364 × G19833 as described in Blair et al. (2003) and Beebe et al. (2006) was used for this study. The bean genotype G19833 is a large-seeded Peruvian landrace, Chaucha Chuga, which belongs to the Andean gene pool that is classified as Al-resistant, while DOR364 is a small-seeded advanced line that was released as a variety in several countries of Central America, which belongs to the Mesoamerican gene pool and is classified as intermediate in Al resistance (Rangel et al. 2005). The seed for the experiment was in the $F_{7:11}$ and was used along with the parents of the population to screen for resistance to Al as described below.

Screening for Al resistance

Experiments were carried out in a greenhouse at CIAT during July and August 2006 where relative humidity averaged 72%, temperatures averaged 29°C and the maximum photon flux density during the day was 1,100 μmol $m^{-2} s^{-1}$. The hydroponic screening technique was implemented based on that of Rangel et al. (2005). To begin the experiment, we germinated 40 seeds on peat moss (pH 5.5) for 3 days taking care to plant seeds with their micropyles facing downwards so that the radicles would emerge vertically. Seedlings were carefully removed and gently washed with deionized water to eliminate any peat moss still remaining on the roots. A total of eight seedlings were then selected for uniform root elongation and suspended over the hydroponic solution. This was done by placing their hypocotyls through small circular foam pads held in place by multi-well floating plastic trays placed in tanks supplied with 20 L of a simple nutrient solution (5 mM $ClCa_2$, 0.5 mM KCl, and 8 μM H_3BO_3 at pH 5.5) and continuous aeration. Two Al treatments recommended by Rangel et al. (2007) for distinguishing resistant genotypes were applied, one with 20 μM $AlCl_3$ (+Al) and one without Al (−Al) considered as a control. Mononuclear Al (Al_{mono}) concentration was measured colorimetrically using the aluminon or pyrocatechol violet method according to Kerven et al. (1989). Aluminum treatment of 20 μM resulted in 16 ± 2 μM Al_{mono} after 24 h. To prevent growth inhibition through pH shock, the seedlings were given a period of adaptation for 1 day at pH of 5.5 after which pH in the growth solution was decreased using 1 N HCl to pH 5.0 for 8 h, and then to pH of 4.5 for the remainder of the treatment period. After 3 days, hydroponic solutions were changed and Al treatment was applied to the appropriate tanks for 48 h. Experimental design was a completely randomized design with four repetitions per treatment.

Data collection and analysis

To determine the tap root elongation rate, the roots were measured for tap root length (in mm) at the beginning and end of the treatment at which point whole roots and aerial parts were collected. The roots were washed and scanned in a TIF format, using an Epson flatbed type scanner (STD 1600) at a resolution of 300 pixels per inch in gray scale. The resulting images were analyzed with WinRHIZO 2003b® software (Regent Instruments, Quebec, Canada) to determine total root length (TRL), average root diameter (ARD), and number of root tips (NRT). Finally, root systems were dried in an oven at 65°C for 48 h and then weighed using an analytical balance to estimate root dry weight (RDW) and to calculate the specific root length based on the ratio between TRL and RDW. After collecting the primary data, relative variables were derived by dividing values for the variable under Al treatment by those for the variable under control treatment and multiplying by 100 to express the level of Al resistance as a percentage. Analyses of variance were performed for each variable obtained directly using the SAS Institute (1999) and a critical range value (CRV) calculated based on the Ryan–Einot–Gabriel–Welsch multiple range test. Broad-sense heritability (h_b^2) values were calculated on an entry mean basis ($r = 4$) using the formula: where σ_g^2 was the genetic variance of the recombinant inbred lines and σ_e^2 was the environmental variance. QTL were identified with QTL Cartographer v.2.5 (Wang et al. 2006) using the genetic map for DOR364 × G19833 from Beebe et al. (2006) which was also used in the QTL analyses by Liao et al. (2004) and Yan et al. (2004), allowing the comparison of results from each of these studies. Map associations were identified with composite interval analysis, using the standard model (6), a window size of 10 cM (centimorgans), a walk speed of 1 cM, and five background loci. Marker inclusion or exclusion was evaluated with a significance level of 0.05 for partial F tests and the probability of a QTL being present was expressed according to a likelihood ratio (LR) scale. The level of significance was determined with 1000 permutations for each trait, with a confidence level of 95% (Churchill and Doerge 1994). Coefficients of determination were reported for each QTL separately (R^2), and for the QTL and background markers (TR^2). Furthermore, the source of the QTL (parent contributing the QTL) was determined, as was the additivity.

Results

Evaluation of primary and derived traits

The primary traits with highly significant genotype (RIL) effects, treatment effects and RIL × treatment interactions from the analyses of variance were: (1) tap root elongation rate; (2) total root length; (3) average root diameter; (4) number of root tips; (5) root dry weight and (6) specific root length (Fig. 1). These six primary root traits were used to create six additional derived traits as a measure of common bean Al resistance, which we referred to as relative traits (Table 1). Below we describe the results found for each primary root trait.

Tap root elongation

The parental genotype G19833 was observed to have a higher TRE than DOR364 in both the control and +Al treatment. The range for the RILs was wider than the differences between the parents in both control and +Al treatment. The normal distribution of the RILs in the +Al treatment suggested that resistance to Al was inherited quantitatively in the population. Furthermore, high transgressive segregation was observed in the control solution, and was both positive (22%) and negative (12%). We can therefore deduce that

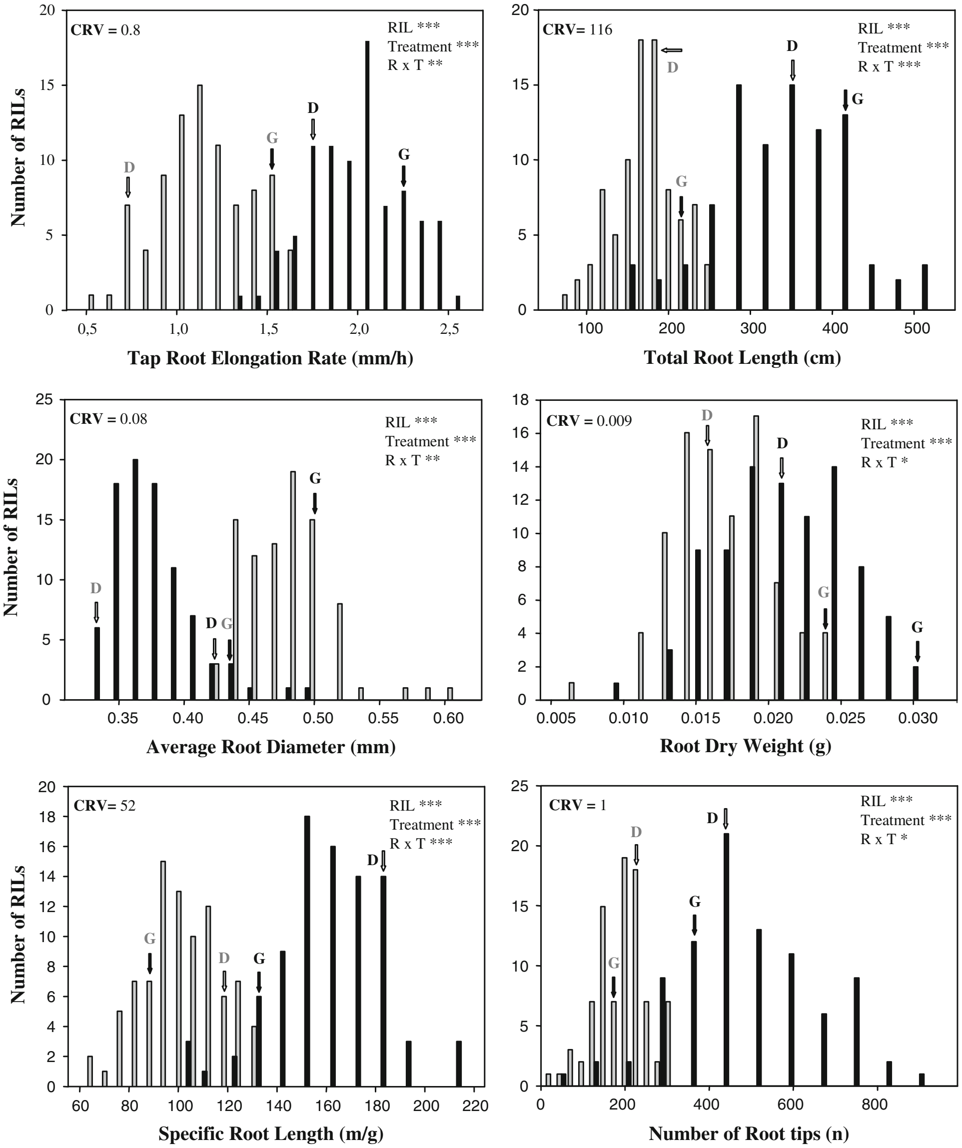

Fig. 1 Population distribution histograms for the common bean recombinant inbred lines (RILs) from the cross of DOR364 (D, intermediate level of Al resistance) by G19833 (G, Al-resistant) for tap root elongation rate, total root length, average root diameter, root dry weight, specific root length and number of root tips in nutrient solutions with (*light bars*) and without (*dark bars*) aluminum treatments. Analysis of variance effects of 87 RIL at significance levels of $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***) shown within each subfigure, the critical range value (CRV) cited correspond to the maximal critical value to protect against type I error at 95% experimental wise significance based on the Ryan–Einot–Gabriel–Welsch multiple range test

the combination of parental alleles from different gene pools (G19833, Andean; and DOR364, Mesoamerican) helps broaden the genetic base for TRE and that the segregation of these alleles in the RILs enables the generation of novel highly Al-resistant or Al-sensitive genotypes. In contrast, the TRE values for the RILs in the +Al treatment solution concentrated mainly between those of the parents, and less transgressive segregation was observed.

Total root length

Differences among the parents for TRL in both the control and +Al treatment solutions were less notable. Meanwhile, the RIL population showed a broader range in the control than in the +Al treatment solution. Interestingly, on observing the degree of transgressive segregation for TRL, the percentage of negative transgressive segregation was high in both control and +Al treatment while positive transgressive segregation was low. Furthermore, when the distribution of the TRL in the RIL population was compared across the two solutions, the effect of Al on TRL was observed to reduce the variability of plant response. This implied that the number of loci controlling Al resistance is smaller than that controlling TRL. The population distribution in Al compared to control conditions was also observed to displace towards the left, indicating the negative effect of Al on root elongation.

Average root diameter and specific root length

The parents presented significantly contrasting ARD in both control and +Al treatments. The parent DOR364 showed a system of fine roots, whereas G19833 showed thick roots. Compared with the above-mentioned traits, the

Table 1 Means of the parents DOR364 and G19833; range and average for the recombinant inbred lines population and broad-sense heritability (h_b^2) of eight root architectural traits in nutrient solutions with (+Al, 20 μM) and without (−Al) aluminum treatments

Traits	Treatment	Parents[a]		RILs[a]		
		DOR364	G19833	Range	Average	h_b^2
Tap Root Elongation Rate (mm/h)	−Al	1.73 ± 0.10	2.22 ± 0.07	0.29−3.81	1.20 ± 0.02	0.94
	+Al	0.74 ± 0.03	1.54 ± 0.22	0.15−2.00	1.18 ± 0.02	0.95
Total Root Length (cm/plant)	−Al	361 ± 58	405 ± 29	81−644	341 ± 5	0.98
	+Al	180 ± 32	219 ± 13	13−283	170 ± 2	0.97
Average Root Diameter (mm)	−Al	0.33 ± 0.01	0.44 ± 0.02	0.30−0.51	0.37 ± 0.01	0.98
	+Al	0.43 ± 0.04	0.51 ± 0.02	0.37−0.67	0.47 ± 0.01	0.96
Specific Root Length (m/g)	−Al	181 ± 29	131 ± 12	82−351	163 ± 1	0.96
	+Al	119 ± 19	91 ± 12	53−157	103 ± 1	0.96
Number of Root Tips	−Al	427 ± 91	393 ± 51	79−1157	494 ± 10	0.97
	+Al	237 ± 43	179 ± 33	16−448	197 ± 4	0.97
Root Dry Weight (g/plant)	−Al	0.020 ± 0.003	0.031 ± 0.004	0.004−0.037	0.021 ± 0.001	0.97
	+Al	0.015 ± 0.002	0.024 ± 0.003	0.001−0.033	0.017 ± 0.001	0.96

[a] Means of 4 replicates ± SD

percentage of transgressive segregation was smaller and the RILs presented trait values between those of the parental genotypes, both in the control and +Al treatments. The distribution curves tended to be normal and unlike for the other traits, Al displaced the population distribution towards the right. This indicated an increase in average root diameter of the genotypes due to Al treatment compared with the control with radial root swelling being a well characterized sign for Al intoxication of roots (Kochian et al. 2004). Meanwhile, the parents showed significant differences for SRL; however, under +Al treatment values were lower than under control treatment. Again for the RILs, not only was the SRL highly reduced by Al treatment, but the variability of response was also reduced. The percentages of transgressive segregation were relatively low in both the control (21% positive and 8% negative) and Al treatments (16% positive and 18% negative).

Number of root tips and root dry weight

NRT included root tips found on basal roots, tap root, and secondary roots and showed significant differences between the parents both in the control and Al treatments, and RILs showed high percentages of transgressive segregation: positive at 64% and negative at 25% in the control solution, and positive at 27% and negative at 38% in the Al treatment solution. Meanwhile, for RDW parental genotypes in the control and +Al treatment solutions differed significantly and the RILs had distribution ranges showing low positive, but high negative transgressive segregation (43 and 37% in the control and Al + treatment solutions, respectively). The effect of Al not only reduced RDW, but also diminished its variability.

QTL identification

A total of 24 QTL (defined as one or more adjacent markers that showed associations with root architectural traits as measured in the control and +Al treatments, or as relative variables) were found in 17 regions across the common bean genome (Fig. 2). Of these QTL, nine were identified only for variables measured in the +Al treatment, eight only in the control treatment, and seven for the relative variables (Table 2). A total of 12 QTL were identified for those traits directly related to resistance to Al given that they were identified for RILs grown in the +Al treatment or through analysis of the relative values comparing Al and control treatments. These QTL will be highlighted in the following paragraphs.

The largest number and arguably most important QTL were found for the TRL and NRT traits while no QTL were found for TRE. Among the QTL for TRL, three loci were classified as Al responsive QTL, one (*Trl9.1*) was identified on linkage group b09, in the same region where QTL for the relative depth of surface roots, dry weight of the taproot, and length of root hairs on the taproot had been detected in trials under low P hydroponic or field conditions with this same population (Liao et al. 2004; Yan et al. 2004; Beebe et al. 2006) indicating that root traits expressed under +Al treatment may be related to root traits expressed under low P conditions. This makes this region of b09 important, especially as this QTL explained the largest percentage of phenotypic variation observed for a relative variable ($R^2 = 0.29$).

The other two QTL for TRL (*Trl11.1* and *Trl11.2*) were located on linkage group b11, with lower coefficients of determination. These QTL were associated with previously

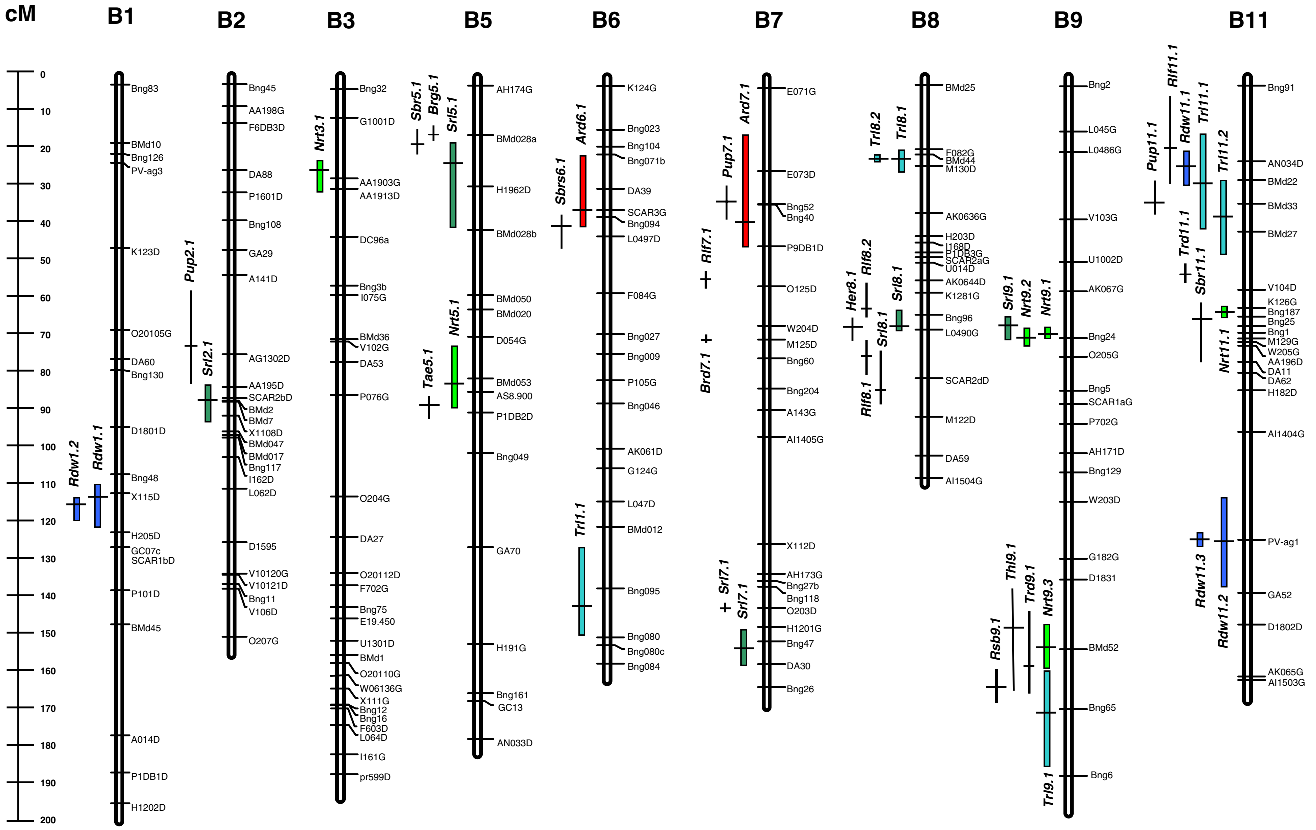

Fig. 2 Location of quantitative trait loci (QTL) for root architectural traits related to Al resistance in the DOR364 × G19833 genetic map from Beebe et al. (2006). QTL as named in Table 2 are represented as thick vertical bars with a short horizontal line across the QTL indicating the position of highest LR peak within individual locus. QTL identified under conditions of low P stress from the studies of Beebe et al. (2006), Liao et al. (2004) and Yan et al. (2004) are shown with thin vertical lines with horizontal cross marks

identified QTL for P acquisition efficiency (Liao et al. 2004), and for root length in the field (Beebe et al. 2006) suggesting further associations between root growth under Al toxicity and under low P levels. For NRT, we identified three QTL for the trait in the Al treatment (*Nrt3.1*, *Nrt5.1* and *Nrt11.1*) and two QTL for the relative variable (*Nrt9.3* and *Nrt9.3*). This last QTL was located in the same region as QTL for shallow basal root length, total root hair length and taproot dry weight under low P conditions (Liao et al. 2004; Yan et al. 2004; Beebe et al. 2006) and *Trl9.1*, the QTL for TRL described earlier. *Nrt11.1* was the only QTL co-segregating with a QTL for seed weight from the study by Beebe et al. (2006) mapping to the same location as *Swf11.1* from the previous study.

For root biomass and the related traits of average root diameter and specific root length, another set of Al resistance QTL were identified some of which overlapped with root length QTL and others that did not. For relative RDW, *Rdw11.1* contributed by the Al-resistant parent (G19833) was identified on linkage group b11 explaining 17% of phenotypic variation, and was located in the same region where *Rlf11.1* was identified for root length in the field during a trial on low P tolerance (Beebe et al. 2006). Meanwhile, for ARD, two QTL were identified, the first of these was located on linkage group b06 (*Ard6.1*, $R^2 = 0.18$), in the same region where a QTL was found for the length of shallow basal roots by Liao et al. (2004). The basal roots, together with the taproot, are the bean plant's thickest roots. Basal roots allow the plant to explore a broad area for nutrient acquisition in the surface soil horizons; however, these roots are more susceptible to Al than the taproot due to lower root exudation of organic acids (Shen et al. 2004). Consistent with these findings, the parent contributing the thick root system (G19833) was the one contributing the QTL we identified here. The second QTL for relative ARD was identified on linkage group b07 (*Ard7.1*) in the region of a QTL for P acquisition efficiency identified by Liao et al. (2004). In both cases, DOR364 was the parent that contributed to fine root systems. *Ard7.1* also stands out as the second most important quantitative locus, explaining 28% of the phenotypic variation observed for a relative variable. Finally, for SRL, two QTL were identified in the Al treatment solution: one (*Srl2.1*) in linkage group b02 and the other (*Srl7.1*) in linkage group b07, with $R^2 = 0.15$ and 0.13, respectively.

Table 2 Quantitative trait loci (QTL) for root architectural traits in nutrient solutions with (+Al, 20 μM) and without (-Al) aluminum treatments for the recombinant inbred line (RIL) population from the cross DOR364 × G19833

Trait	QTL name	LG[a]	Treatment	Nearest marker	Marker interval	Source	Significance[b]			Additivity
							LR	R^2	TR^2	
Total root length (cm)	*Trl6.1*	6	−Al	Bng95	BMd12–Bng95	G19833	19.55	0.20	0.51	36.95
	Trl8.1	8	−Al	M130D	AK0636G–M130D	DOR364	16.62	0.15	0.32	31.74
	Trl8.2	8	+Al/−Al	M130D	AK0636G–M130D	G19833	14.81	0.12	0.37	2.43
	Trl9.1	9	+Al/−Al	Bng6	Bng6–Bng65	G19833	18.85	0.29	0.66	3.89
	Trl11.1	11	+Al/−Al	BMd22	Bng91–BMd33	G19833	16.86	0.15	0.39	2.79
	Trl11.2	11	+Al	BMd33	BMd22–BMd27	G19833	22.14	0.22	0.38	18.2
Average root diameter (mm)	*Ard6.1*	6	+ Al	SCAR3G	Bng71b–Bng94	G19833	25.66	0.18	0.47	0.02
	Ard7.1	7	+Al/−Al	P9DB1D	P9DB1D–E073D	DOR364	29.15	0.28	0.5	2.08
Specific root length (m/g)	*Srl2.1*	2	+Al	SCAR2bD	AG1302D–Bng117	DOR364	23.68	0.15	0.55	6.03
	Srl5.1	5	−Al	BMd28a	BMd28a–H1962D	G19833	25.17	0.22	0.55	11.09
	Srl7.1	7	+Al	DA30	Bng26–Bng47	G19833	19.63	0.13	0.57	5.47
	Srl8.1	8	−Al	L0490G	L0490G–Bng96	DOR364	17.6	0.13	0.47	8.88
	Srl9.1	9	−Al	Bng24	0205G–Bng24	DOR364	18.25	0.15	0.5	9.16
Number of root tips (*n*)	*Nrt3.1*	3	+Al	AA1903G	AA1913D–AA1903G	G19833	15.46	0.14	0.52	23.19
	Nrt5.1	5	+Al	BMd53	DO54G–AS8.900	G19833	25.41	0.16	0.47	23.22
	Nrt9.1	9	−Al	Bng24	0205G–Bng24	DOR364	17.29	0.14	0.4	65.72
	Nrt9.2	9	+Al/−Al	Bng24	0205G–Bng24	G19833	14.31	0.12	0.4	3.34
	Nrt9.3	9	+Al/−Al	BMd52	Bng65–BMd52	G19833	19.61	0.16	0.4	4.25
	Nrt11.1	11	+Al	K126G	V104D–K126G	G19833	21.36	0.15	0.47	23.04
Root dry weight (g)	*Rdw1.1*	1	−Al	X115D	Bng48–SCAR1bD	DOR364	23.71	0.18	0.5	0.0019
	Rdw1.2	1	+Al	X115D	Bng48–SCAR1bD	DOR364	16.27	0.10	0.52	0.0011
	Rdw11.1	11	+Al/−Al	AN034D	Bng91–AN034D	G19833	18.36	0.17	0.44	3.88
	Rdw11.2	11	+Al	PV-ag1	AI1404G–PV-ag001	G19833	18.51	0.13	0.54	0.0012
	Rdw11.3	11	−Al	PV-ag1	AI1404G–PV-ag001	G19833	14.74	0.11	0.48	0.0014

Values represent QTL significance (LR) and determination coefficients explained by each QTL (R^2 and TR^2)

[a] LG: Linkage group according to Blair et al. (2003)

[b] LR: Likelihood ratio test statistic for H_0:H_1, were H_0 is the hypothesis of no QTL effect at test position and H_1 is the hypothesis of QTL effect at the test position; R^2: proportion of variance explained by QTL at test site; TR^2: for the QTL and the background markers

The 17 remaining QTL were defined as QTL unrelated to Al resistance, as they were identified in the control solution alone, control and Al treatments together, or for the control solution and relative variable, but were not differentially expressed when exposed to Al. For example, for TRL, two QTL were identified in linkage group b08: one (*Trl8*.1) in the control solution; and the other (*Trl8.2*) for the relative variable. For NRT, three QTL were identified in linkage group b09 (*Nrt9.1*, *Nrt9.2* and *Nrt9.3*) for the control solution and also for the relative variable. For RDW, four QTL in two regions were identified in both the control and treatment solutions located on linkage groups b01 (*Rdw1.1* and *Rdw1.2*) and b11 (*Rdw11.2* and *Rdw11.3*). Those QTL may not be related to Al resistance as they were expressed in both treatments but rather could be related to adaptation to low pH in the nutrient solution used in the experiment. This could be consistent with high susceptibility of common bean to proton toxicity as demonstrated by Rangel et al. (2005), and also from the study of Yan et al. (2004). Among the QTL for SRL that were unrelated to Al resistance, the locus *Srl5.1* was identified on linkage group b05 located near QTL for length of basal roots in the surface and basal root growth identified by Liao et al. (2004); *Srl8.1* was found on linkage group b08 located in the same region as QTL for root length and organic acid exudation from Beebe et al. (2006) and Yan et al. (2004); and *Srl9.1*, identified on linkage group b09, but with low R^2 values.

Discussion

The hydroponic screening protocol used in this study was found to be very reliable. To measure this, broad-sense heritabilities (h_b^2) were calculated for all the absolute variables, both in the control and Al treatments for the DOR364 × G19833 population. All the evaluated traits were found to be highly heritable, with broad-sense heritability (h_b^2) values varying from 0.94 to 0.98 (Table 1), indicating that the experimental conditions and physiological response were very repeatable and validating the precision of our QTL analysis. The controlled nature of our hydroponic growth experiments probably increased our heritability values and decreased sources of environmental error that would not be controlled in a field experiment where heritabilities are usually lower (Alonso-Blanco and Koornneef 2000). This confirms results from Beebe et al. (2006) and Yan et al. (2004) where hydroponic screenings were also used in genetic analysis of this population.

From our QTL analysis, it was notable that the Al-resistant parent G19833 contributed the most positive alleles for variables under exposure to Al. Parent DOR364 in contrast, contributed most of the alleles for other QTL not involved with Al resistance or for variables under control conditions. Furthermore of the seven QTL for relative variables, six were contributed by G19833 and one by DOR364. These findings agree with physiological analysis of the parents in our study and by Rangel et al. (2005), where G19833 was observed to show higher level of Al resistance as compared to DOR364. Transgressive segregation observed in the population distributions for each trait also suggests that DOR364 contributes positive alleles for some traits. The difference between G19833 and DOR364 in inhibition of root elongation with exposure to Al is typical of the contrast between other Mesoamerican and Andean genotypes according to Rangel et al. (2005) and the DOR364 × G19833 population has been extensively analyzed for root traits under low phosphorus stress (Beebe et al. 2006).

Based on the differential response in control and +Al treatments, the traits TRE, TRL, NRT, RDW, ARD and SRL were selected as suitable parameters to assess Al resistance with all of these except the first useful for the identification of QTL in this study with a total of 12 QTL being associated with Al resistance and 12 QTL being Al non-specific. These latter QTL may have been more involved with constitutive traits for root architecture or adaptation to low pH, than with response to Al resistance. However, because some of these QTL co-localized with QTL for low P tolerance (Beebe et al. 2006; Liao et al. 2004; Yan et al. 2004) they might be valuable for marker-assisted selection. The inheritance of Al resistance is likely to be genetically simpler than that of acid soil tolerance because of other factors such as low pH, low P and low Ca also influence acid soil tolerance (Kochian et al. 2004).

Meanwhile, the 12 QTL involved in specific mechanisms of Al resistance were those that detected only for the variables measured in the Al treatment specifically (*Ard6.1*, *Nrt3.1*, *Nrt5.1*, *Nrt11.1*, *Srl2.1* and *Srl7.1*); for the relative variables (*Ard7.1*, *Rdw11.1*, *Trl9.1*, and *Nrt9.3*); or for both (*Trl11.1*, and *Trl11.2*). The polygenic control of root characteristics under exposure to Al in common bean from this study are in agreement with studies in *Arabidopsis* (Kobayashi and Koyama 2002; Hoekenga et al. 2003; Kobayashi et al. 2005); sorghum (Magalhaes et al. 2004) and rice (*Oryza sativa*) where combinations of major and minor QTL each contribute in a small proportion to improve Al resistance, indicating that different pathways of Al resistance can be operating at the same time (Wu et al. 2000; Nguyen et al. 2001; Ma et al. 2002; Nguyen et al. 2002; Nguyen et al. 2003; Xue et al. 2006).

Another finding of our study was that several QTL controlling root morphology under Al treatment overlapped with QTL for root architecture parameters under low-P stress from previous studies (Beebe et al. 2006; Liao et al. 2004; Yan et al. 2004). QTL in common for root morphological characteristics under these two major abiotic stresses were found in five regions of the bean genome namely on linkage groups b02, b06, b07, b09 and b11. This finding implies common mechanisms of common bean root response under conditions of Al or low P stresses, potentially mediated by organic acid exudation, which would agree with recent results from Liao et al. (2006) in soybean where P efficient genotypes appear to be more Al-resistant than P inefficient genotypes and the P efficient genotypes secrete Al chelators such as malate differentially from the tip of the taproot in split chamber experiments.

In this regard, a QTL identified in this study for number of root tips under Al stress (*Nrt5.1*) and an overlapping QTL controlling total acid exudation (*Tae5*.1) from Yan et al. (2004) are interesting given that both Miyasaka et al. (1991) and Shen et al. (2004) found that Al-resistant common bean genotypes exude more organic acids than sensitive genotypes when exposed to Al. Rangel et al. (2007) predicted an Al exclusion mechanism in common bean based on accumulation of Al in the root tips of an Al-resistant genotype ICA Quimbaya decreasing over time of Al exposure while in the Al-sensitive genotype VAX 1 accumulation increases. Further studies by these authors have shown that this exclusion mechanism is based on differential citrate, malate, oxalate or succinate exudation (A. F. Rangel, I. M. Rao, Z. Yang, A. Stass and W. J. Horst, unpublished results), results which classify common bean as a type II response according to Ma et al. (2001) and indicate that gene expression response occurs upon exposure to Al.

In conclusion, our results show that inheritance of Al resistance in common bean is quantitative and related to differential response of root architecture traits to exposure to Al and that some common QTL exist for resistance to Al and tolerance to low P stress. Furthermore, transgressive segregation was high in this inter-genepool cross population and may be promising for crop improvement. QTL for total root length and number of root tips that are common for Al and low P stress conditions indicate the importance of size and branching of root system under these often coexisting abiotic stress conditions that enables greater acquisition of P through exploration of greater volume of soil under infertile acid, high-Al soil conditions.

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