PLANT GENETIC RESOURCES

Aluminum Tolerance Associated with Quantitative Trait Loci Derived from Soybean PI 416937 in Hydroponics

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ABSTRACT

Acid soils with high levels of Al impede root growth, causing increased crop sensitivity to drought and decreased nutrient acquisition. Development of Al-tolerant cultivars may be a cost effective response to the problem. In previous investigations, we identified an Al-tolerant soybean [*Glycine max* (L.) Merr.] plant introduction from Japan (PI 416937), and subsequently determined the heritability of the trait in a cross with Young, a highly productive Al-sensitive cultivar. The objective of the present study was to identify quantitative trait loci (QTL) which condition Al tolerance by a genetic linkage map of 155 restriction fragment length polymorphism (RFLP) marker loci and a hydroponics-based Al response. The 120 F_4-derived progeny from Young × PI 416937 were divided into four sets and evaluated with the parents for tap root extension in 0 and 2 μM Al^{3+} activity solutions (NOAL and HIAL, respectively) employing Al levels as whole plots in a split-plot experimental design. Aluminum tolerance was defined as (i) root extension under HIAL conditions, and (ii) root extension as a percentage of control [PC = (HIAL/NOAL) × 100]. Multiple regression analysis revealed five QTL from independent linkage groups which conditioned root extension under HIAL stress. Three of the five QTL were also detected by PC as the expression of Al tolerance. While most alleles for Al tolerance were derived from the Al-tolerant parent, PI 416937, a RFLP allele from Young (for marker EV2-1) improved Al tolerance expressed as PC and exhibited a similar trend under HIAL stress. At present, it is not known whether the Al tolerance gene from Young, in combination with those from PI 416937, will raise Al tolerance beyond that now observed in the PI. One allele for Al tolerance from PI 416937 (for marker B122-1) may be difficult to capitalize upon, agronomically, because of its association with a detrimental pod dehiscence factor. Further experimentation is needed to distinguish between linkage and pleiotropic effects near this marker. A favorable epistatic effect for Al tolerance was detected between two alleles from the PI 416937. The relationships revealed by marker analysis indicated that marker-facilitated selection may be a viable approach in the breeding of Al-tolerant soybean.

THE INCREASING DEMAND for agricultural lands throughout the world challenges soil scientists, plant breeders, and agronomists alike to improve the productivity of problem soils. The acid soils of the Brazilian Cerrados (Spehar, 1995; Carter et al., 1999) and those of the more temperate southeastern USA (Reich et al., 1981), for example, pose serious problems in terms of phytotoxic Al which undermines crop yield potential and, thus, attempts to implement sustainable agriculture systems. When lime is applied to alleviate Al toxicity near the surface of such soils, toxic subsoil aluminum often remains a barrier to deep rooting and the uptake of water and nutrients. An important consequence is that Al toxicity can accentuate a problem with drought even in well managed soils (Carter and Rufty, 1993; Goldman et al., 1989; Spehar and Galwey, 1996; Carter et al., 1999).

Although soil amelioration and irrigation can minimize harmful Al effects on crop performance, they are often economically unfeasible (Spehar et al., 1993; Alva et al., 1986). Genetic adaptation of plants to Al-toxic soils is an attractive and potentially less expensive alternative. In that regard, soybean is grown on Al-rich soils in many parts of the world (Smith and Huyser, 1987), and the identification of Al-tolerant soybean cultivars has been pursued for many years (Hanson and Kamprath, 1979; Sartain and Kamprath, 1978). However, genetic manipulation of Al tolerance has proven difficult. From the breeder's perspective, the problem can be characterized as a dearth of genetic diversity among improved cultivars and a wealth of discrepancies among screening methods (Campbell and Carter, 1990; Hanson, 1991; Foy et al., 1993; Bennet and Breen, 1991; Foy et al., 1992; Dall'Agnol et al., 1996).

Advances in DNA marker technology have added a new dimension to the study of genetic traits in the past decade, offering hope that marker technology can begin to clarify the genetics of Al tolerance as an aid to practical breeding. In soybean, DNA marker analyses have identified chromosomal regions with genes for pest resistance (Diers et al., 1992a; Weisemann et al., 1992), nitrogen fixation (Landau-Ellis et al., 1991), and important agricultural crop characteristics such as plant height, lodging, and maturity (Diers et al., 1992b; Lee

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Abbreviations: NOAL, no aluminum treatment (0 μM Al^{3+} activity); HIAL, high aluminum treatment (2 μM Al^{3+} activity); PC, percentage of control (HIAL/NOAL × 100); RFLP, restriction fragment length polymorphism; QTL, quantitative trait loci; cM, centimorgan; LOD, Log of the Odds; r_p = phenotypic correlation of genotypic means; r_g = genetic correlation between two traits; POP-LG, population-specific linkage group; USDA/ISU, the USDA/Iowa State Univ. soybean genetic map.

et al., 1996a, c), seed composition (Lee et al., 1996b) and seed weight (Mian et al., 1996b). Riede and Anderson (1996) identified a single major gene for Al tolerance using RFLP marker technology in wheat (*Triticum aestivum* L.). Association between molecular markers and Al tolerance has not been reported in soybean.

In the 1980s, a soybean plant introduction from Japan, PI 416937, was identified as drought tolerant (Sloane et al., 1990). Since its discovery, PI 416937 has been included in numerous studies related to drought stress tolerance in soybean (Mian et al., 1996a; Hudak and Patterson, 1996). Further investigation revealed that the PI 416937 was also Al tolerant (Campbell and Carter, 1990). Heritability of Al tolerance derived from the PI is reported (Bianchi-Hall et al., 1998). The objective of our research was to identify QTLs that control Al tolerance in a F_4-derived soybean population from the cross of Young and PI 416937. Knowledge of the genetic basis for Al tolerance should facilitate transfer of this trait from exotic germplasm to economically important cultivars.

MATERIALS AND METHODS

Genetic Materials and Hydroponics System

A family of 120 randomly F_4-derived lines was developed by the USDA-ARS at North Carolina State Univ. (NCSU). Each line descended from a unique F_2 plant from the cross of Young × PI 416937. Young is a highly productive but Al-susceptible cultivar adapted to the southern USA (Burton et al., 1987). The progeny were evaluated in hydroponics to assess Al tolerance in comparison with the parents. For screening purposes, the 120 lines were sorted into four sets of 30 lines each, which were based on maturity ratings from two locations in Georgia and North Carolina in 1994 (Lee et al., 1996a). Within each set, parents were included as reference types in triplicate, making a total of 36 entries per set.

The hydroponic system consisted of eight individual tanks located in a controlled environment chamber in the Biological Resource Center at NCSU. Details of the hydroponic system and experimental set up were provided elsewhere (Bianchi-Hall et al., 1998). Each tank held 84 L, and continual water movement between the upper and lower reservoirs assured aeration of the system. The growth medium was deionized water with 800 μM Ca_2SO_4 maintained at a pH of 4.3 ± 0.1 which was automatically monitored and adjusted. The aluminum treatments consisted of 0 and 2 μM Al^{3+} activity (NOAL and HIAL, respectively), achieved through the addition of Al Cl_3. Following germination in the dark at 25°C, seedlings corresponding to stage G3 (Muthiah et al., 1994) were transferred into the hydroponics system and roots were submerged in the medium. After 24 h, individual root lengths were measured and then Al treatments were imposed. After 72 h, root lengths were again measured. Linear tap root extension was defined as final minus initial root length for the 3-d period. Experiments were completed during November 1995 and February 1996.

RFLP Linkage Map

The population was used to establish an RFLP linkage map. The DNA isolation and RFLP protocols were conducted at the Univ. of Georgia and have been described elsewhere (Lee et al., 1996a). The linkage map was constructed with marker data using the Kosambi (1944) map function of GMendel (Holloway and Knapp, 1993) taking into account the F_4-derived population structure. For combining markers into population-specific linkage groups (POP-LG), a minimum LOD of 3.0 and maximum distance of 50 cM between linkage markers were used. The genetic map consisted of 155 RFLP markers in 33 linkage groups and covered approximately 973 cM or about half of the soybean genome (Mian et al., 1996a). To facilitate the use of our results, the map was reconciled with the more highly saturated USDA/ISU soybean genetic map using anchor probes and markers (Shoemaker and Specht, 1995). Anchor probes had the same restriction enzyme and an identical banding pattern with the USDA/ISU map.

Table 1. Tap root growth after 3 d in hydroponics culture for soybean cultivar Young, PI 416937, and the F_4-derived progeny in the absence, 0 μM (NOAL), and presence, 2.0 μM (HIAL), of Al^{3+} activities and with Al tolerance expressed as PC (percent of control = HIAL/NOAL × 100).

	NOAL	HIAL	PC
	cm $3d^{-1}$		%
Young	7.1	3.3	46.3
PI 416937	8.6	8.9	104.7
LSD (0.05)	0.65	0.51	13.8
Progeny range†	5.7–11.8	2.6–9.5	30.2–115.8
Progeny mean (120 lines)	8.5	5.8	69.3

† To standardize across the four sets, progeny means were adjusted using the reference parents by the following formula: Adjusted set mean for progeny = Unadjusted set mean for progeny − (Parental mean of set − Parental mean over all sets). This adjustment minimized bias that may have arisen from small weekly differences in experimental conditions from set to set.

Experimental Design and Data Analysis

Experimental Design

Each set of 36 entries (30 F_4-derived lines and 6 entries of parents in triplicate, four sets in total), was evaluated in a split-plot design with replication over time. Successful replications for NOAL treatments ranged from three to six depending on the set employed and for HIAL treatments replications ranged from five to eight. Whole plots (tanks) were assigned to Al treatments and subplots (individual holes in tank lid) to entries. Four seedlings in a foam support constituted the experimental unit. The ANOVA and GLM procedures of SAS were used for statistical analyses (SAS, 1990). In addition to tap root extension per se, Al tolerance was also expressed as percentage of control (PC) which was defined as (growth HIAL /growth NOAL) × 100.

Analyses of variance, heritability, and genetic correlation estimates were reported previously (Bianchi-Hall et al., 1998). Neither heterogeneity of error variance nor deviations of residuals from normality were a factor in this study. Thus, results based only on untransformed data were employed here. Progeny means from the four sets were combined into a single data set for comparison with RFLP data. To standardize across the four sets, progeny means were adjusted from triplicate entries of the parents as a reference by the following formula: Adjusted set mean for progeny = Unadjusted set mean for progeny − (Parental mean of set − Parental mean over all sets). This adjustment minimized bias that may have arisen from small weekly differences in experimental conditions (Table 1).

Single Factor QTL Analysis

Each marker locus was evaluated for linkage to a QTL for Al tolerance by contrasting the mean performance of the

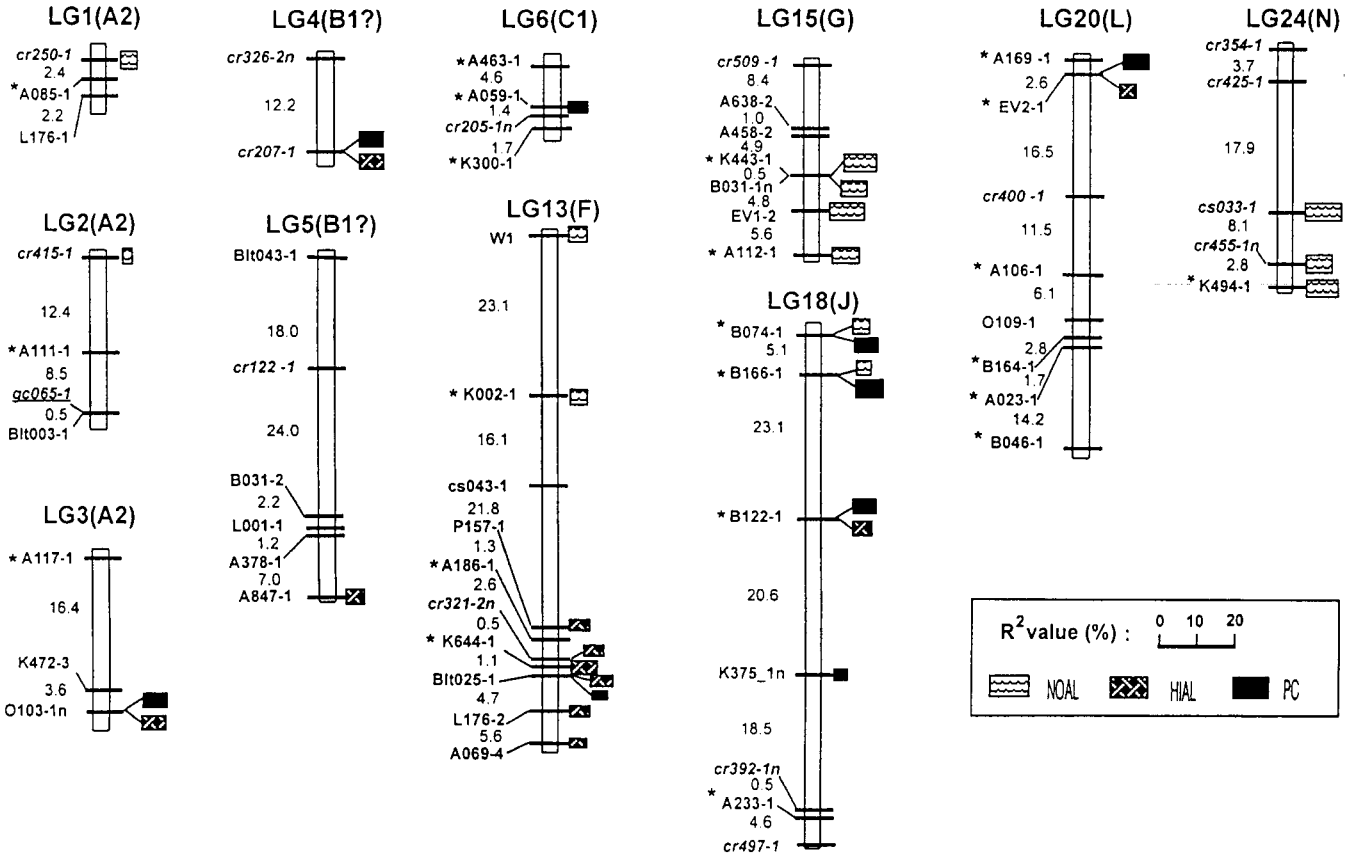

Fig. 1. The RFLP markers associated with tap root extension in the absence, 0 μM (NOAL), and presence, 2.0 μM (HIAL), of Al^{3+} activities and with Al tolerance expressed as PC (percent of control = HIAL/NOAL × 100). Length of bars indicates R^2 values for the loci associated with tap toot extension, where R^2 is defined as the proportion of the phenotypic variation among line means accounted for by a RFLP marker in a single factor analysis. The genetic map consisted of 155 RFLP markers in 33 linkage groups and covering approximately 973 cM. Only those linkage groups with markers associated with tap root extension in this study are presented. The population-specific linkage groups are designated LG. The population-specific map was reconciled with an existing USDA/ISU soybean genetic map using anchor probes and markers. The corresponding USDA/ISU linkage groups are given in parenthesis for comparison. * represents an anchored probe which had an identical banding pattern with the image in Grant et al. (1996). A marker locus is identified by a probe designation and a dashed number suffix, where the latter identifies the specific locus of the two or more loci detected by that probe.

progeny for the two homozygous RFLP classes (SAS, 1990). Only a limited number of heterozygous lines were available for a given marker locus (expected number of 15 = 12.5% of 120 lines), and, thus, the heterozygous class was excluded from this analysis. To establish a framework for declaring a significant relation between marker and phenotype, we grouped markers for testing according to linkage group. The intent was to identify the marker most strongly associated with phenotype for each linkage group. We established an overall α for each linkage group as 0.05. Subsequently, the Bonferroni method was employed to determine significance for each individual test within a linkage group (Senn, 1997). On average, approximately five markers were represented per linkage group, and, thus, significance of each marker was tested at probability level $\alpha/5$ or 0.01. Markers were tested for association with rooting phenotype based upon type III mean squares obtained from the GLM procedure of SAS (SAS, 1990; Fig. 1 and Table 2).

Multiple Factor QTL Analysis

For 155 markers, 11 935 (155 × 154/2) potential two-marker interactions existed, a number too great to examine in practice. Two-marker interactive effects on phenotype were examined in a limited way by subjecting the marker most highly associated with phenotype from a linkage group to pairwise analysis with similar markers from the other linkage groups. Breeding lines which were heterozygous for either marker in the pairwise analysis were excluded from that specific analysis.

Results from the single-factor and interaction analyses were used to construct a multiple regression analysis. Those markers which interacted significantly ($P < 0.01$) were included in a multiple regression analysis along with the *best markers* (i.e., those most strongly associated with phenotype) from single factor analyses within each linkage group with the restriction that the best markers were also significant at $P < 0.01$. In this multiple analysis, exclusion of heterozygous loci was not practical because this would have forced the deletion of all breeding lines which were heterozygous for one or more markers in the regression model. In a multiple analysis of five markers, for example, exclusion of heterozygotes would forfeit as much as one-half of the data set (i.e. probability that a F_4-derived progeny is homozygous at five independent markers is $0.875^5 = 0.51$) and weaken inferences from the analysis. As an alternative, we retained heterozygotes in the regression and then partitioned the two degrees of freedom associated

Table 2. RFLP markers associated with tap root extension in hydroponics based on single factor analysis. The F_4-derived progeny were grown in the absence, 0 μM (NOAL), and presence, 2.0 μM (HIAL), of Al^{3+} activities. Growth as percent of control (PC) was calculated as HIAL/NOAL × 100. Linkage groups are designated both as population specific (POP-LG) and from the USDA-Iowa State Univ. Map (USDA/ISU LG).

RFLP† Locus	Linkage group POP LG	Linkage group USDA/ISU LG	P	Allele mean Young	Allele mean PI 416937
				cm $3d^{-1}$	
		NOAL			
Cr250A-1	LG1	A2	0.0040	8.1	8.9
Cr415-1	LG2	A2	0.0132	8.2	8.8
K002-1‡	LG13	F	0.0069	8.2	8.9
EV1-2‡	LG15	G	0.0003	8.2	9.1
B074-1	LG18	J	0.0085	9.0	8.3
Cs033-1	LG24	N	0.0009	8.2	9.0
		HIAL			
O103-1n	LG3	A2	0.0033	5.4	6.2
Cr207-1‡	LG4	B1	0.0089	5.5	6.3
A847-1‡	LG5	B1	0.0356	5.6	6.3
K644-1	LG13	F	0.0009	5.4	6.4
B122-1‡	LG18	J	0.0054	5.5	6.3
EV2-1‡	LG20	L	0.0503	6.2	5.5
		PC			
O103-1n	LG3	A2	0.0037	64	74
Cr207-1	LG4	B1	0.0094	66	76
Blt025-1	LG13	F	0.0180	66	75
B122-1‡	LG18	J	0.0027	64	75
EV2-1‡	LG20	L	0.0074	74	63

† Putative independent QTL (greater than 50 cM from another marker significantly associated with the corresponding trait).
‡ Markers associated with epistatic interactions at $P < 0.01$ based on pairwise analyses.

with a marker into linear and quadratic effects. Linear corresponded to the contrast of the homozygote effects and quadratic corresponded to the contrast of the heterozygote class to the average of the homozygote classes. The linear × linear interaction corresponded to the two-way interactive effects of homozygote classes for the markers. Linear effects and linear × linear interactions were employed to assess marker effects. The heterozygote vs. homozygote contrast main effects and interactions were generally small and pooled with error for testing.

RESULTS

RFLP Markers Associated with Root Extension under NOAL Conditions

Progeny exhibited significant ($P < 0.05$) transgressive segregation for tap root extension under NOAL conditions, suggesting genetic effects were sufficiently large so that marker analysis had the potential to identify genetic factors conditioning root growth (Table 1). Single factor analysis and a subsequent pairwise analysis of two-way interactions indicated that RFLP markers on six linkage groups were potentially associated with this trait at $P < 0.01$ (Tables 2 and 3). These results were the basis for a multiple regression analysis which incorporated the best marker (i.e., the marker which had the strongest association with phenotype as a main effect or interaction) from each of these six linkage groups. This analysis confirmed a positive association ($P < 0.01$) of three independent RFLP markers with root extension (Table 4), with two alleles derived from PI 416937 and one from Young (for marker BO74-1) (Table 2). The general location of the three putative QTL (K002-1, EV1-2, and B074-1) on the linkage map was confirmed by five additional linked markers which were also associated with root extension albeit to a lesser degree (Fig. 1). Epistatic interaction was found between markers K002-1 (POP-LG13) and EV1-2 (POP-LG15, Tables 3 and 4). The heritability of this root extension under NOAL conditions, which was based on genotypic means of five replicates, was 0.76 (Bianchi-Hall et al., 1998) while the multiple regression analysis accounted for 22% of the phenotypic variation.

Table 3. Epistatic interactions between pairs of RFLP markers based on pairwise analysis of those markers which were associated with tap root extension in a single factor analysis. The F_4-derived progeny were grown in the absence, 0 μM (NOAL), and presence, 2.0 μM (HIAL), of Al^{3+} activities. Growth as percent of control (PC) was calculated as HIAL/NOAL × 100).

Al Treatment or trait	RFLP Locus 1	Allele† at Locus 1	RFLP Locus 2	Allele† at Locus 2	Root‡ extension	P for interaction
NOAL	K001-2	Y	EV1-2	Y	8.1	0.0081
		Y		PI	8.1	
		PI		Y	8.3	
		PI		PI	9.7	
HIAL	A847-1	Y	cr207-1	Y	4.9	0.0051
		Y		PI	6.5	
		PI		Y	6.6	
		PI		PI	6.2	
HIAL	B122-1	Y	EV2-1	Y	5.5	0.0142
		Y		PI	5.4	
		PI		Y	7.2	
		PI		PI	5.5	
PC	B122-1	Y	EV2-1	Y	65	0.0098
		Y		PI	62	
		PI		Y	86	
		PI		PI	63	

† Y indicates allele derived from Young parent; PI indicates allele derived from PI 416937 parent.
‡ cm $3d^{-1}$ for NOAL or HIAL stress and percentage for PC.

RFLP Markers Associated with Root Extension under HIAL Conditions

Progeny differed significantly ($P < 0.01$) for tap root extension under HIAL conditions, showed continuous variation for the trait, but did not exhibit significant ($P < 0.05$) transgressive segregation (Bianchi-Hall et al., 1998); three lines were slightly superior to PI 416937 numerically. Single factor and pairwise analysis of two-way interactions indicated that RFLP markers on six linkage groups were potentially associated this trait ($P < 0.01$, Tables 2 and 3). The best marker from each of these six linkage groups (i.e., the marker which had the strongest association with phenotype as a main effect or interaction) was incorporated into a multiple regression analysis. This analysis confirmed a positive association ($P < 0.01$) of five independent RFLP markers to root extension (Table 4), with all five alleles derived from PI 416937 (Table 2). The general location of one putative QTL (K644-1) on the linkage map was confirmed by five additional linked markers which were also associated with root extension but to a lesser degree

Table 4. Multiple regression analysis of RFLP markers associated with tap root extension. Multiple regression included, initially, markers that (i) interacted significantly ($P < 0.01$) in pairwise analyses or (ii) were the 'best markers' from single factor analyses (i.e., those most strongly associated with a phenotype) within each linkage group ($P < 0.01$). Markers, marker interactions, or pooled marker plus interaction effects which were significant at $P < 0.01$ were retained in the final model. The F_4-derived progeny were grown in absence, 0 μM (NOAL), and presence, 2.0 μM (HIAL), of Al^{3+} activities. Growth as percent of control (PC) was calculated at HIAL/NOAL × 100. Linkage groups are designated both as population specific (POP-LG) and from the USDA-Iowa State Univ. Map (USDA/ISU LG).

RFLP Locus†	Linkage group: POP LG	Linkage group: USDA/ISU LG	*P*§ Adjusted for all other model effects	*P*‡ Pooled interaction and main effects	*P* Overall model	r^2 Overall model
		NOAL				
B074-1	LG18	J	0.0090			
K002-1‡	LG13	F	0.0521			
EV1-2‡	LG15	G	0.0744			
K002-1 × Ev1-2‡			0.0244	0.001	0.001	0.22
		HIAL				
O103-1n	LG3	A2	0.0083			
Cr207-1‡	LG4	B1	0.0976			
A847-1‡	LG5	B1	0.0460			
K644-1	LG13	F	0.0193			
B122-1	LG18	J	0.0041			
Cr207 × A847-1‡			0.0255	0.003	0.0001	0.32
		PC				
EV2-1	LG20	L	0.0038			
O103-1n	LG3	A2	0.0021			
Cr207-1	LG4	B1	0.0117			
B122-1	LG18	J	0.0027		0.0001	0.24

† Putative independent QTL (greater than 50 cM from another marker significantly associated with the corresponding trait).
‡ Markers associated with epistatic interactions at $P < 0.01$.
§ Derived from Type III sums of squares from multiple regression using PROC GLM of SAS.

(Fig. 1). Epistatic interaction was found between markers A847-1 (POP-LG5) and cr207-1 (POP-LG4, Tables 3 and 4). The heritability of root extension under HIAL stress, which was based on the genotypic means of five replicates, was 0.87 (Bianchi-Hall et al., 1998) while the multiple regression analysis accounted for 32% of the phenotypic variation.

RFLP Markers Associated with PC

Progeny differed significantly ($P < 0.01$) for Al tolerance expressed as PC. The PC of the 120 lines showed continuous variation and slight transgressive segregation for this trait, although the highest ranking F_4-derived lines were not significantly different from PI 416937 (Table 1 and Bianchi-Hall et al., 1998). Single factor and pairwise analysis of two-way interactions indicated that RFLP markers on five linkage groups were potentially associated with PC ($P < 0.01$, Tables 2 and 3). The best marker from each of these five linkage groups (i.e., the marker which had the strongest association with phenotype as a main effect or interaction) was incorporated into a multiple regression analysis. This analysis confirmed a positive association ($P < 0.01$) of four independent RFLP markers to PC (Table 4), with three alleles derived from PI 416937 and one from Young (for marker EV2-1) (Table 2). The general location of one putative QTL on the linkage map (B122-1) was confirmed by three additional linked markers which were also associated with PC but to a lesser degree (Fig. 1). The heritability of PC, which was based on genotypic means of five replicates, was 0.76 (Bianchi-Hall et al., 1998) while the multiple regression analysis accounted for 24% of the phenotypic variation.

DISCUSSION

Despite the negative impact of Al toxicity on soybean production, no highly Al-tolerant soybean cultivars have been developed for North America, Asia, or Europe. No soybean germplasm has been discovered with a sufficiently high level of Al tolerance to warrant its use as a sole source in the practical breeding of Al tolerance (Hanson, 1991). No major genes for Al tolerance have been described. The PI 416937 is one of the few soybean germplasm sources reported with measurable levels of Al tolerance both in hydroponics and in the field (Campbell and Carter, 1990; Ritchey and Carter, 1993). Thus, the identification of QTL for Al tolerance from this PI could provide a first practical step in breeding efforts which may eventually pyramid Al tolerance genes from multiple genetic sources to create economically important levels of Al tolerance. In the present study, we searched for Al-tolerance genes in a F_4-derived population of soybean from the hybridization of Al-susceptible Young and Al-tolerant PI 416937. Aluminum tolerance was detected in hydroponics both as root growth in the presence of Al per se and when root growth was expressed as PC. The population was also polymorphic for 155 RFLP markers, and thus, the population lent itself well to an RFLP-based assessment of Al tolerance.

QTL and Al Tolerance

Genotypic variation for root extension was larger under Al stress than for control conditions, suggesting that genes may exist which are specific for Al tolerance in the population (Bianchi-Hall et al., 1998). The QTL

analysis revealed that this is likely the case. Multiple regression analyses identified a total of six independent putative QTL for Al tolerance (expressed as either growth under HIAL stress or as percentage of control), but only two (on POP-LG13 and POP-LG18) were common to linkage groups associated with growth in the non-stress treatment (Table 4). The other four appeared to have a unique role in Al tolerance. Of the two linkage groups affecting growth both in the presence and absence of Al, one (POP-LG18), was likely a carrier of an additional fifth gene specific for Al tolerance as indicated by the following allelic effects. For this linkage group, an allele from PI 416937 (marker B122-1 on linkage group 18) improved growth only in the presence of Al, while an allele from Young (marker B074-1) improved growth only in the absence of Al. It is unlikely that both markers identify a single gene locus responsible for such dissimilar effects. The two-gene hypothesis for this linkage group is further supported by the fact that four widely dispersed markers on POP-LG18 are all associated with PC, while only the one marker (B074-1) was associated with NOAL conditions (Fig. 1).

Because the genetic correlation between HIAL and NOAL treatments was significantly greater than zero (r_g = 0.51**) (Bianchi-Hall et al., 1998), we inferred that, in addition to QTL specific for HIAL conditions, there must be other genes in the population which contribute to general plant vigor, thus enhancing root growth under both HIAL and NOAL conditions. Bouton and Parrott (1997) have suggested that this may also occur in alfalfa (*Medicago sativa* L.). Despite the plausibility of this idea, the theory could not be confirmed by marker results. There was no instance in which an allele from a single marker was positively ($P < 0.01$) associated with growth under both HIAL and NOAL conditions. The only potential example of a single QTL affecting growth under both conditions was provided by POP-LG13. For this linkage group, the PI appeared to possess two linked alleles for growth—one detected only under HIAL and the other only under NOAL conditions (Tables 2 and 4). However, the alleles were from two markers approximately 40 cM apart, indicating a strong possibility that the two marker alleles were linked to two distinct QTL rather than one (Fig. 1).

Al tolerance expressed as growth in Al per se and as PC were highly correlated (r_p = 0.70** based on genotypic means; r_g was not employed here because of inherent correlated errors between Al per se and PC), suggesting that many of the same genes control these traits. In fact, this was the case. Three of the four independent markers associated with PC were also associated with root extension under HIAL stress. Surprisingly, despite the high correlation and similar QTL for the two Al tolerance measures, the distributions of progeny means for HIAL conditions and PC were clearly different (Bianchi-Hall et al., 1998). For HIAL conditions, the distribution fit a normal curve, while for PC the distribution was skewed and bimodal, implying a more qualitative inheritance than for HIAL (Bianchi-Hall et al., 1998). Given the contrasting progeny distributions but similar RFLP marker results, one may suspect that undiscovered QTLs remain present in the population which affected PC more than growth per se under HIAL stress.

Relation of Al Tolerance to Agronomic Traits

A breeder may question whether or not selection for Al tolerance will lead to deleterious effects on other traits. In other work, this population has been evaluated for maturity date, 100-seed weight, lodging, plant height, and seed protein and oil content (Mian et al. 1996b; Lee et al., 1996a, b, c). Aluminum tolerance, whether expressed as growth under HIAL conditions or as PC, was not correlated phenotypically to any of these traits. The most important agronomic trait associated with Al tolerance was pod dehiscence. An allele from PI 416937 for marker B122-1 was associated with both Al tolerance and pod dehiscence (Bailey et al., 1997). Water use efficiency (WUE) may also be associated with Al tolerance. Mian et al. (1996a) identified four QTL associated with water use efficiency (WUE) in the same population, two of which were on the same POP-LG as the B122-1 marker. Both alleles for poor WUE derived from the PI, indicating that selection for Al tolerance has the potential to decrease WUE as well as increase pod dehiscence.

Implications to Breeding

Our results provide the first clear basis for the underlying genetics of Al tolerance in soybean. We detected six putative QTL for Al tolerance under HIAL conditions in hydroponics assay. This estimate of gene number agrees roughly with an earlier estimate of three to five genes for Al tolerance for this same population (Bianchi-Hall et al., 1998). This earlier estimate was based upon an algebraic analysis of parental phenotypes in the progeny and probably underestimated the true number of genes because of a crucial assumption that all positive alleles derive from one parent. We found that while most RFLP alleles for Al tolerance were derived from the PI 416937, an allele from susceptible Young (for marker EV2-1) was associated with Al tolerance expressed as PC. Fulton et al. (1997) reported a similar phenomenon in which a QTL for large fruit was identified in a small fruited relative of the cultivated tomato (*Lycopersicum esculatum* Mill.). In soybean, Hnetkovsky et al. (1996), also found a beneficial QTL derived from a susceptible parent when mapping markers associated with Sudden Death Syndrome disease. In the present study, the Al stress treatments were designed to impose the maximum effect on susceptible cultivar Young without inhibiting root extension of tolerant PI 416937. Thus, it was not possible to detect levels of Al tolerance greater than that of the PI in the progeny. Although this is a tantalizing possibility, the ability of the allele from Young to raise Al tolerance beyond that observed in the PI 416937 could not be ascertained in this study.

The potentially important Al tolerance associated with marker B122-1 in PI 416937 may prove difficult to capitalize upon in applied breeding, because of its association with a detrimental pod dehiscence QTL in

the PI (Bailey et al., 1997). The five most Al-tolerant progeny in this study averaged twice the level of pod dehiscence as the five most Al susceptible (Bailey et al., 1997; data not shown). All five Al-tolerant types above carried the PI 416937 marker allele for B122-1 compared with none of the five Al-sensitive types. Fourteen of the 15 progeny most resistant to pod dehiscence carried the Young allele for marker B122-1, further illustrating the importance of this marker. The one progeny resistant to pod dehiscence and carrying the PI allele for the B122-1 marker was sensitive to Al and thus it is not clear that a linkage between pod dehiscence and Al tolerance was broken. Additional markers on POP-LG18 may help to distinguish between linkage and pleiotropic effects.

One may speculate that Al tolerance is rare among North American cultivars because of linkage effects associated with intense selection against pod dehiscence in the adaptation of soybean to mechanical harvest. Horst and Klotz (1990) screened more than 1000 accessions from the USDA soybean collection and found one accession comparable to the PI 416937 in terms of Al tolerance. This accession was also highly susceptible to pod dehiscence (T.E. Carter, 1995, personal communication). The relation of Al tolerance with other traits in the population studied here indicates that selection for Al tolerance will probably have little impact on agronomic traits such as maturity, lodging, and plant height in practical breeding. Water use efficiency may be decreased somewhat, however. The economic impact of WUE in this population is under current study.

The putative QTL for Al tolerance identified in this study are candidates for applied breeding. Validation of results presented here will be an important component of commercial application. The actual utility of DNA markers in comparison to direct phenotypic screening for Al tolerance will be determined by cost effectiveness. With refined DNA tagging of QTL, marker assisted selection achieved through service companies may pose a viable alternative to development of an *in house* phenotypic assay of Al tolerance, especially where Al tolerance is a secondary rather than primary breeding objective. Presently, no service companies offer assays for Al tolerance. Marker assisted selection may offer the added advantage of dissecting and overcoming breeding bottlenecks such as the detrimental linkage that may exist between Al tolerance and pod dehiscence alleles.

The results provided here may also guide the use of additional Al-tolerant PIs in breeding. As new PIs with Al tolerance are identified, markers may elucidate contrasting genetic mechanisms for tolerance in comparison to the Al-tolerant PI 416937 now in use, leading to strategies for the pyramiding of Al tolerance genes in a practical breeding program.

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Evaluation of Perennial *Glycine* Species for Resistance to Soybean Fungal Pathogens That Cause Sclerotinia Stem Rot and Sudden Death Syndrome

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ABSTRACT

The cultivated soybean [*Glycine max* (L.) Merr.] has a relatively narrow genetic base and most commercial cultivars are susceptible to *Sclerotinia sclerotiorum* (Lib.) de Bary and *Fusarium solani* (Mart.) Sacc. f. sp. *glycines*, which, respectively cause Sclerotinia stem rot (SSR) and sudden death syndrome (SDS). The objective of this study was to screen all the available accessions of the perennial *Glycine* species for resistance to the pathogens that cause SSR and SDS. For SSR evaluations, five seedlings of each of 787 accessions were screened once in a series of eight non-replicated runs. Seedlings were inoculated with an agar plug cut from the edge of a 1-d-old fungal culture by placing the plug next to the stem. Of the 787 accessions, 183 had partial resistance with 144 of these accessions being *G. tabacina* (Labill.) Benth. A selected set of 53 accessions was further screened in two replicated trials with five plants per each of four replications. *Glycine tabacina* had several accessions that were consistently rated as partially resistant. For SDS evaluations, five plants of each of 767 accessions were screened once in a series of eight runs. Plants were inoculated by a layered technique in which infested sorghum seed were placed below the transplanted seedlings. In the initial evaluation of 767 accessions, 134 had partial resistance with 65 of these accessions being *G. tomentella* Hayata. In a replicated set of selected accessions, *G. tomentella* had several accessions that were consistently rated as partially resistant. These perennial *Glycine* species represent potential untapped sources for improving disease resistance in soybean.

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THE GENUS *Glycine* Willd. is composed of two subgenera, *Glycine* and *Soja* (Moench) F. J. Herm. The cultivated soybean and its wild annual progenitor *Glycine soja* Sieb. and Zucc. belong to the subgenus *Soja*. Both species are diploid ($2n = 40$) and are cross compatible. The subgenus *Glycine* contains 16 wild perennial species. They are indigenous to Australia and grow in diverse geographical areas under a wide range of climatic conditions. These species are diploid ($2n = 40$), with aneuploidy ($2n = 38$ and 78) and tetraploidy ($2n = 80$) occurring in *G. tomentella*, *G. tabacina*, and *G. hirticaulis* Tind. and Craven (Tindale and Craven, 1993; Kollipara et al., 1997; Singh et al., 1998). Genomic symbols have been assigned to each species on the basis of cytogenetic, biochemical, and molecular studies (Kollipara et al., 1997).

Useful traits have been identified from accessions of at least some of the perennial *Glycine* species. Some species carry resistance to soybean pathogens like *Heterodera glycines* Ichinohe (Riggs et al., 1998), *Microsphaera diffuse* Cke. & Pk. (Mignucci and Chamberlain, 1978), *Phakopsora pachyrhizi* H. Sydow & Sydow (Hartman et al., 1992; Schoen et al., 1992), *Phytophthora soja* Kaufmann & Gerdemann (Kenworthy, 1989), *Septoria glycines* Hemmi (Lim and Hymowitz, 1987), and yellow mosaic virus (Horlock et al., 1997).

Sclerotinia sclerotiorum on soybean is referred to as

Abbreviations: SDS, sudden death syndrome; SSR, Sclerotinia stem rot.