Genotypic Rankings for Aluminum Tolerance of Soybean Roots Grown in Hydroponics and Sand Culture

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ABSTRACT

Screening methodology remains a practical barrier in the breeding of Al-tolerant soybean [*Glycine max* (L.) Merr.]. Our objectives were to (i) develop a repeatable sand-media culture method for Al tolerance screening of plants, (ii) compare Al response of genotypes in sand culture to a standard hydroponics-based seedling culture, and (iii) establish a practical guide for the use of hydroponics and sand-culture screening methods in the selection of Al-tolerant soybean. We developed a sand-media culture method and imposed 0 and 450 μM Al^{3+} activity treatments upon 10 diverse soybean genotypes. The experiment employed a randomized complete block design with nine replications. Root weight and relative root surface area (RRSA) were determined at 18 d after transplanting (DAT). In hydroponics, the genotypes were compared for taproot elongation after 3 d of exposure to 0, 2, and 5 μM Al^{3+} activity treatments in a split plot design with six replications. Aluminum stress was imposed successfully (approximately 57% of the growth in control) in hydroponics and sand culture, but discrepancies between methods were apparent. The hydroponics-based seedling screen produced an inflated range of genotypic response and altered Al tolerance rankings in comparison with sand culture. 'Perry', which was tolerant to Al in sand culture, was remarkably sensitive to Al in hydroponics. Despite the discrepancies, seedling-based screening successfully identified three (PI 417021, PI 416937, and Biloxi) of the four genotypes that were most tolerant to Al in sand culture. Results suggested that seedling screens can play a practical role in breeding. However, their application to a specific breeding population should be validated with older plants and solid media. The RRSA appeared to be a promising measure of A1 tolerance for soybean roots.

Aluminum is the third most abundant element in the earth's crust and a formidable phytotoxic barrier to crop production in acidic soils (40% of the world's arable lands) (Kochian, 1995). Toxic Al levels damage roots, restrict plant size, and lower yield in most crops. Although the toxic effects of Al in soil can be overcome by addition of appropriate soil amendments, the economic costs can prohibitive in many parts of the world (Pandey et al., 1994). A cost effective alternative is the fitting of Al-tolerant cultivars to problem soils (Brown and Jones, 1977; Foy, 1988).

In soybean, Al tolerance has been studied for many years. However, practical breeding for Al tolerance has been limited by inadequate screening methodologies. Most soybean cultivars and germplasm accessions in the world's collections (more than 25 000 genotypes) have never been rated for Al tolerance (Palmer et al., 1996). The few soybean germplasm evaluations to date indicate that soybean can be screened for tolerance to Al-rich acid soil with some degree of success (Sartain and Kamprath, 1978; Hanson and Kamprath, 1979; Campbell and Carter, 1990; Horst and Klotz, 1990; Foy et al., 1992, 1993b; Spehar, 1994). However, genotypic rankings for Al tolerance often vary among soil types.

Aluminum saturation, the percentage of cation-exchange capacity occupied by Al, has been employed in recent decades to classify the potential for Al toxicity in soils, but it has not helped soybean breeders to predict changes in genotypic rankings for Al tolerance that may occur from one soil type to the next (Kamprath, 1984; Fageria et al., 1988). Researchers have attributed discrepancies in genotype rankings to the different concentrations of Al, P, Ca, Mg, organic acids, and other soil components which greatly affect and potentially mask the expression of genetic tolerance to Al toxicity (Kamprath, 1984; Foy, 1984; Fageria et al., 1988; Blamey et al., 1991). The complexity of soil-based screening for Al-tolerance has been sufficiently great that researchers often exercise caution and describe their screening efforts in terms of acid-soil tolerance rather than Al tolerance per se, even though Al may be the major phytotoxic problem (Nyborg and Hoyt, 1978; Noble et al., 1984; Edmeades et al., 1995). For such reasons, no breeder in North America or Asia is using a soil-based approach in practical cultivar improvement of Al tolerance in soybean. In Brazil, new cultivars are often evaluated for Al tolerance in pot studies, but no breeder actively selects superior Al-tolerant breeding lines from a segregating population (Carter et al., 1999).

Hydroponics is an attractive alternative to soil-based screening for Al tolerance. It allows evaluation of a large number of genotypes quickly and has been used to identify parental stock for soybean breeding (Campbell and Carter, 1990; Carter and Rufty, 1993; Spehar, 1994; Bianchi-Hall et al., 1998; Bianchi-Hall et al., 2000; Silva et al., 2001). However, no commercial soybean breeder is using hydroponics-based screening for Al tolerance to our knowledge. Breeders have not adopted hydroponics screening, because it is usually limited to seedling assays, and there is a question of how well rankings of seedling Al tolerance apply to the field. Two observations gleaned from the published literature underscore this point. First, hydroponics screening has consistently demonstrated that the soybean plant introduction (PI) 416937 is very Al tolerant (Campbell and Carter, 1990; Bianchi-Hall et al., 1998). Yet, examination of the Al tolerance of PI 416937 in the field or in large pots has

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Abbreviations: RRSA, relative root surface area; PC, percent of control; DAT, days after transplanting.

indicated a potentially lower level of tolerance than that predicted by hydroponics assays (Fountain, 1990; Low, 1990; Hanson, 1991; Ritchey and Carter, 1993; Bushamuka and Zobel, 1998; Ferrufino et al., 2000). Second, hydroponics screening identified the soybean cultivar Perry as Al sensitive even though it had been found to be tolerant in soil-based assays with older plants (Armiger et al., 1968; Devine et al., 1979; Sapra et al., 1982; Horst and Klotz, 1990; Foy et al., 1969 and 1992). While these observations are drawn from a wide range of literature and do not provide positive proof that seedling-based hydroponics screening is unreliable for breeding, they do suggest the need for a methodology that supplements hydroponics screening in the accurate identification of Al tolerance.

Soil-based rankings for Al tolerance may be soil-type dependent and, thus, not easily reproducible across wide geographical areas. Sand culture is a potentially viable alternative as a supplement to hydroponics screening for Al tolerance. It has the advantage of allowing examination of Al tolerance with somewhat older plants than for current hydroponics approaches, and as a solid substrate, it is more similar to field conditions, physically, than hydroponics. Because nutrients and Al are applied in solution form and because sand is almost inert, the level of Al applied to plants can be regulated and reproduced with consistency. To date, however, the complex interaction of nutrients with Al has restricted the use of sand culture screening to seedlings. In seedlings, nutrient applications and their interactions can be avoided because stored nutrients in the cotyledon can sustain growth for the first days after germination (Horst and Klotz, 1990). With soybean, it has just been recognized that Mg modifies Al tolerance. The presence of relatively low amounts of mg in soil or nutrient solution can reduce Al sensitivity and lead to inaccuracies in rankings of Al tolerance (Silva et al., 2001). Thus, a viable sand-culture screen for Al tolerance that compares plants beyond the seedling stage must include a protocol that maintains healthy plants through nutrient additions, avoids Mg deficiencies, and minimizes the interactions of Mg and Al.

Screening methodology remains a practical barrier in the breeding of Al-tolerant soybean cultivars. To address this problem, our objectives were to (i) develop and evaluate a repeatable sand-media culture method for Al tolerance screening, (ii) compare Al response of genotypes in sand-media culture at 18 DAT with that in a hydroponics-based seedling culture, and (iii) establish a practical guide for the use of hydroponics and sand-culture screening methods in the selection of Al-tolerant soybean.

MATERIALS AND METHODS

Hydroponics

Ten genotypes were grown in hydroponics and subjected to three Al treatments—0, 2, and 5 μM Al^{3+} activities—for 3 d by means of a technique similar to that employed by Bianchi-Hall et al. (1998) (Table 1). The Al^{3+} activities were estimated by GEOCHEM (Parker et al., 1994). Seedlings were germinated for 68 h in moist paper towels in a dark germination chamber and transferred to foam supports suspended over 50-L, continuous flow hydroponics tanks located in a controlled environment chamber in the North Carolina State University Phytotron. The chamber was maintained at 26°C and light was provided during a daily 12/12 h light/dark cycle at 650 μmol m^{-2} s^{-1} by a combination of fluorescent and incandescent lights. The initial nutrient solution contained only 800 μM $CaCO_4 \cdot 2H_2O$. Following a 24-h acclimation period, primary root lengths were measured as the distance from the root tip to the engorged region between the root and hypocotyl, and Al was added to the solution. After 72 h of exposure to Al, final primary root lengths were measured. The pH of the solution was maintained at 4.3 ± 0.1 by continuous monitoring and automatic additions of 0.025 M H_2SO_4. The experiment was conducted during June 1999.

The experimental design was a split-plot with two replications employed each week and repeated during 3 wk for a total of six observations per genotype. The Al treatments were

Table 1. Means of 10 diverse soybean genotypes grown in the presence and absence of Al as seedlings in hydroponics and 18-d-old plants in sand-culture in the greenhouse. Al treatments were 0, 2, and 5 μM Al^{3+} in hydroponics and 0 and 450 μM Al^{3+} in sand culture.

	Hydroponics					Sand culture								
	Taproot extension					Root dry weight			RRSA‡			Shoot dry weight		
	μM Al^{3+}					μM Al^{3+}			μM Al^{3+}			μM Al^{3+}		
Entry	0	2	5	PC2†	PC5†	0	450	PC†	0	450	PC†	0	450	PC†
	cm $(3d)^{-1}$					g $(plant)^{-1}$			g $(plant)^{-1}$			g $(plant)^{-1}$		
PI 417021	10.8	10.1	6.7	93.3	61.4	0.59	0.43	72.4	8.5	4.9	57.9	0.83	0.55	66.2
PI 416937	11.3	10.8	6.1	96.6	54.4	0.47	0.33	71.3	6.9	3.9	56.4	0.55	0.35	63.1
Biloxi	9.8	10.3	4.9	106.5	51.1	0.56	0.40	71.2	6.6	4.0	59.8	0.65	0.42	64.8
Davis	9.3	4.8	2.9	52.0	31.7	0.46	0.29	62.9	6.5	3.1	48.0	0.47	0.31	65.9
N95-7424	9.8	7.8	2.7	81.4	28.1	0.57	0.37	64.7	8.4	4.5	54.0	0.67	0.41	61.5
Lee 74	7.7	5.6	1.9	76.1	23.9	0.39	0.25	63.4	5.8	2.6	44.4	0.49	0.29	59.8
Essex	6.4	4.0	1.2	59.4	22.7	0.50	0.31	62.3	7.0	3.4	48.1	0.54	0.32	58.5
Tokyo	10.9	8.5	2.4	78.4	22.0	0.54	0.39	71.9	9.1	4.2	46.2	0.66	0.38	57.5
Perry	10.3	7.4	2.0	72.4	19.4	0.40	0.31	77.8	5.5	3.4	61.6	0.45	0.30	67.7
Cook	9.5	3.6	1.6	39.0	17.5	0.48	0.36	73.4	8.2	4.1	50.1	0.53	0.34	62.9
Mean	9.6	7.3	3.2	75.6	33.2	0.50	0.34	69.1	7.3	3.8	52.5	0.58	0.37	62.8
$LSD_{0.05}$	1.2	1.3	0.9	13.8	10.1	0.79	0.04	11.8	0.1	0.5	8.9	0.07	0.04	10.6

† Al tolerance expressed as percent of control (PC) and defined as (growth in presence of Al/growth in absence of Al) × 100. PC2 and PC5 refer to percentage of control for the 2 and 5 μM Al^{3+} treatments respectively.

‡ Relative root surface area (RRSA) was measured by immersing air-dried roots in a viscous 36.6 M $Ca(NO_3)_2$ solution for 10 s, suspended out of solution for 20 s, and weight of $Ca(NO_3)_2$ removed from solution was recorded (i.e., that adhering to root surface).

randomly assigned to whole plot (tanks) and genotypes to subplots (individual holes in a tank lid). Three seedlings in a foam support constituted the experimental unit.

Sand Culture

The 10 genotypes were grown in the greenhouse and subjected to Al-stress and Al-free treatments (450 and 0 μM Al^{3+}) for 11 d beginning at 7 DAT. Seed were germinated for 72 h in the same manner used for the hydroponics experiments, and then transferred to 45-cm length, 20-cm diameter polyvinylchloride cylinders (hereafter referred to as pots) containing builders grade washed sand which had been fitted with 1-mm mesh bottoms. Plants were thinned to three per pot at 6 DAT. During the first week, all pots in the greenhouse were supplied daily with 500 mL of a complete nutrient solution composed of the following to promote vigorous and plant growth: 4 mM $CaSO_4 \cdot 2H_2O$, 250 mM KH_2PO_4, 2 mM KNO_3, 18 μM $FeSO_4 \cdot 7H_2O$, 18.9 μM KCl, 9.3 μM H_3BO_3, 0.9 μM $MnSO_4 \cdot H_2O$, 0.9 μM $ZnSO_4 \cdot 7H_2O$, 0.18 μM $CuSO_4 \cdot 5H_2O$, 0.18 μM $(NH_4)_6Mo_7O_{24}$, and 250 μM $MgSO_4 \cdot 7H_2O$. The macronutrients composition of this nutrient solution was a modification of that employed by McClure and Israel (1979), and the micronutrients composition was a modification of that described by Ahmed and Evans (1960). The pH of the solution was adjusted to 6.2 with 1.0 M KOH. Weak supplementary lighting was provided to establish an 18-h photoperiod and prevent flowering using four 1000-W halide bulbs.

The Al treatment solutions were prepared daily and applied in the morning (~0900 h). Pots were first flushed with 1.2-L of deionized water (adjusted to pH 4.3) to leach excess salts accumulated in the sand media. After draining for approximately 30 min, each pot received 1.2-L of the assigned 450 μM Al^{3+} solution (plus 800 μM $CaSO_4 \cdot 2H_2O$) or otherwise 1.2-L of the $CaSO_4 \cdot 2H_2O$ solution adjusted to pH 4.3 with no Al. Each afternoon (~1600 h), the pots were flushed again with 1.2-L deionized water, allowed to drain 30 min, and then received 800 mL of complete nutrient solution adjusted to pH 4.3. To establish an Al^{3+} activity of 450 μM in treatment solution, 0.8 M $AlCl_3$ was dissolved in 50 mL of distilled water with pH adjusted to 4.3 with 1 M HCl to form a stock solution. Twenty-four milliliters of the Al stock solution were mixed with 18-L of 800 μM $CaSO_4 \cdot 2H_2O$, at pH 4.3. The speciation of Al^{3+} in the treatment solution was calculated as 43% by GEOCHEM (Parker et al., 1994).

At 18 DAT, the shoot of each plant was removed, and the roots were carefully separated from the sand by washing gently onto a wire mesh. Clean intact roots were floated in shallow water in a plastic tray and spread so that overlapping of roots was minimal. The root system was then air-dried for 2 d and weighed. Relative root surface area was estimated by the gravimetric method (Carley and Watson, 1966). The separated air-dried roots were immersed for 10 s in a container with 1.0-L of a viscous 36.6 M $Ca(NO_3)_2$ solution held on an analytical balance. The roots then were removed and allowed to drip into the solution for 20 s. The weight of $Ca(NO_3)_2$ removed from solution was determined (i.e., that adhering to root surfaces). Shoots were oven dried at 68°C for 48 h and then weighed.

The experimental design for the sand-culture experiments was a randomized complete block, and the treatment design was a factorial combination of the 10 genotypes and two Al levels. The experiment was replicated over time in a series of four runs (November 1998 to March 1999), with two replications completed in each of the first three runs and three replications completed in the fourth for a total of nine replications.

Data Analysis

Data were subjected to analysis of variance by the GLM and ANOVA procedures of SAS (SAS, 1990). Genotype and Al were considered fixed effects and runs and replications as random effects in the statistical model. Aluminum tolerance was also expressed as percentage of control (PC) for each trait [defined as (growth in the presence of Al/growth in the absence of Al) $\times$ 100]. Whole plots were paired by replicate for statistical analysis of PC. No significant heterogeneity of error variances were detected within experiments based on the F-max test, indicating that no transformation of the data was needed (David, 1952).

Repeatability was estimated for Al tolerance expressed as growth and as PC for each trait. Repeatability (t^2) of genotypic effects is analogous to heritability of a trait, and is calculated as $t^2 = [M_1/(M_1 + M_2/r + M_3/rn)]$, where M_1 represents the genotypic component of variance, M_2 the Run $\times$ Genotype component of variance, M_3 the error mean square, r the number of runs in each genotypic mean, and n the number of observations per genotype in each run (Falconer, 1981; Campbell and Carter, 1990). Run is roughly analogous to environment in the analysis of heritability in field studies and refers to a repeated experiment in the greenhouse. The PROC CORR from SAS was used to calculate the correlation of genotypic means (SAS, 1990). The probability that a correlation coefficient was greater than zero was taken from the output of PROC CORR.

RESULTS AND DISCUSSION

Selection of Genetic Materials

To compare Al-tolerance screening methods and detect discrepancies between them effectively, one must employ genotypic materials with a wide range of response to Al. Unfortunately, published data on Al tolerance of soybean germplasm are limited and often contradictory, making the choice of appropriate test material difficult. Securing seed of putative tolerant types can be difficult as well. As an example, Foy et al. (1993b) described Al-tolerant soybean genotypes from the former USSR, which are no longer available for study and are not part of the USDA-ARS soybean collection. Cultivars from Brazil have been described as Al-tolerant but are not widely available outside Brazil and are not a part of the USDA-ARS collection (Spehar, 1994).

To minimize these problems, we selected 10 diverse genotypes on the basis of availability of seeds from the USDA-ARS collection and evidence (or suspicion) of tolerance to Al. The cultivar Essex was employed as the Al-sensitive control because it has been cited consistently as sensitive in hydroponics and Al-rich soil (Smith and Camper, 1973; Ritchey and Carter, 1993; Campbell and Carter, 1990; Foy et al., 1993b; Bushamuka and Zobel, 1998). The genotypes Perry, PI 416937, and 'Davis' were included because they were reported to have some level of tolerance in Al-rich soil (Caviness and Walters, 1966; Foy et al., 1969, 1992, 1993a,b; Sapra et al., 1982; Horst and Klotz, 1990; Goldman et al., 1989; Ritchey and Carter, 1993; Bushamuka and Zobel, 1998). Both Davis and PI 416937 have also been cited as having prolific roots as compared to typical soybean cultivars (Brown et al., 1985; Hudak and Patterson, 1995; Panta-

lone et al., 1996). The genotypes PI 417021, PI 416937, 'Biloxi', and 'Lee 74' have been cited as Al-tolerant in hydroponics (Sartain and Kamprath, 1978; Horst and Klotz, 1990; Spehar, 1994; Bianchi-Hall et al., 1998). The genotype 'Cook', although not rated for Al tolerance, is a modern high yielding cultivar currently grown on acid soils in the southeastern USA (Boerma et al., 1992). The breeding line N95-7424, developed by USDA-ARS, but not a part of the USDA-ARS collection at present, was included because it produces a 25% larger-than-normal canopy when grown on clay-rich soils at pH 5.5 to 6.0 (based on fresh weight at 40 d after planting, T.E. Carter, Jr. 1998, unpublished data). 'Tokyo' and Davis are parents of N95-7424.

Genotypic Response

Hydroponics

To ensure appropriate characterization of Al tolerance, genotypes were subjected to both moderate and high Al levels (2 and 5 μM Al^{3+}) in solution culture for 3 d (Fig. 1). The higher Al treatment produced a more severe stress, as expected, and both levels of Al produced changes in genotypic ranking relative to the control treatment (Table 1). However, there was good agreement between the two Al treatments in the ranking of genotypes. The genotypes PI 417021, PI 416937, and Biloxi were the most Al tolerant in both Al treatments whether tolerance was expressed as absolute root extension or PC (Table 1). All other genotypes were clearly less tolerant. The good agreement between Al tolerance expressed as root extension and as PC (r = 0.93** for the high Al treatment) indicated that changes in ranking of genotypes from imposition of Al stress were large and that innate differences in rooting vigor among genotypes were relatively small (Bianchi-Hall et al., 1998).

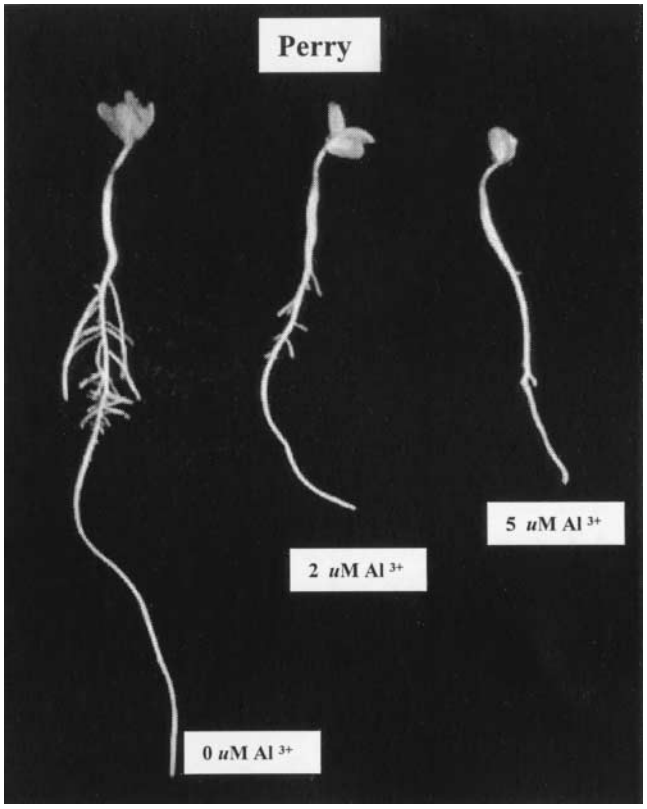

Fig. 1. Roots of soybean seedlings exposed to 0, 2, and 5 μM Al^{+3} in hydroponics for 72 h.

Sand Culture

The sand-media culture was developed through a series of pilot studies. Initial experiments confirmed findings by Silva et al. (2001) that confounding effects of Mg on Al sensitivity were pronounced when the two were present in the same nutrient solution. In later experiments, it was found that Mg–Al interactions were minimized by temporal separation of nutrient and Al solution applications, with pots flushed with water between applications. This innovation produced healthy plants in the control pots, allowed an evaluation of Al toxicity effects separate from interactions with Mg, and was an essential component of the finalized sand-media culture employed in this study.

In the sand-culture, taproot length was not affected greatly by Al treatments. However, the basal roots and branches from the taproot were clearly reduced in all genotypes (Fig. 2). Mean shoot and root weight and RRSA for the Al treatment were 63, 69, and 53% of that observed under Al-free control conditions (Table 1). Thus, Al stress was successfully imposed, with shoots and roots affected to approximately the same degree. A significant Al × genotype interaction indicated genotypic variation in response to the imposition of Al stress for RRSA and shoot and root dry weights (Table 2).

Aluminum tolerance ratings were expressed as growth in the presence of Al and as PC. However, absolute growth and PC ratings did not agree well for any trait on the basis of phenotypic correlation of genotypic means (Table 3). Ratings based on PC tended to reflect changes in genotypic ranking which resulted from Al stress, while ratings based upon absolute growth in the presence of Al reflected not only the effects of Al stress, but more general constitutive genotypic differences in plant vigor as well. The overall genotypic differences in vigor were large relative to changes in ranking from Al stress, and illustrated by the fact that genotypic growth

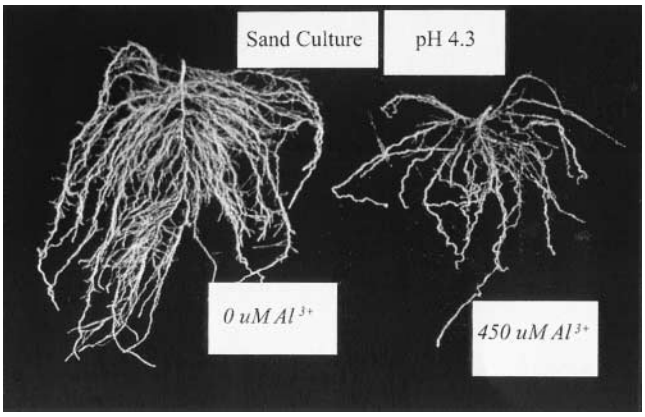

Fig. 2. Roots of soybean (PI 416937) exposed to 0 and 450 μM Al^{+3} for 18 d in sand culture.

Table 2. Analysis of variance for 10 diverse soybean genotypes grown in the presence and absence of Al as seedlings in hydroponics and as 18-d-old plants in sand-culture in the greenhouse. Al treatments were 0, 2, and 5 μ*M* Al^{3+} in hydroponics, and 0 and 450 μ*M* Al^{3+} in sand culture.

	Hydroponics				Sand culture			
	Taproot extension		Root dry weight		Shoot dry weight		RRSA†	
Source‡	df	Mean square	df	Mean square	df	Mean square	df	Mean square
Run	2	5.8812	3	0.4299	3	1.1746	3	61.6020
Rep (Run)	3	7.0571	5	0.0028	5	0.0112	5	1.7703
Al	2	615.1632**	1	1.0120*	1	2.0068*	1	514.4244*
0 Al vs. other	1	740.6400**						
2 Al vs. 5 Al	1	491.5820**						
Run × Al	4	1.3625	3	0.0821**	3	0.2171**	3	30.0456**
Rep × Al (Run)	6	2.1443						
Genotype	9	62.8122**	9	0.0674**	9	0.1369**	9	14.5682**
Run × Genotype	18	0.9656	27	0.0096**	27	0.0107*	27	2.1573NS
Al × Genotype	18	8.2880**	9	0.0047†	9	0.0089*	9	2.5339*
(Al vs. other) × Genotype	9	9.6580**						
(2 Al vs. 5 Al) × Genotype	9	6.9180**						
Run × Al × Genotype	36	0.8875	27	0.0024	27	0.0039	27	1.0020**
Residual	80	0.8295	90	0.0035	90	0.0038	92	0.4889

* Indicates significance at $P = 0.05$.
** Indicates significance at $P = 0.01$.
† Relative root surface area (RRSA) was measured by immersing air-dried roots in a viscous 36.6 *M* $Ca(NO_3)_2$ solution for 10 s, suspended out of solution for 20 s, and weight of $Ca(NO_3)_2$ removed from solution was recorded (i.e., that adhering to root surface).
‡ Hydroponics experiments were analyzed as split-plot experimental design with Al levels associated with whole plots and genotypes with subplots. Sand culture experiments were analyzed as randomized complete block design.

under control conditions was more highly correlated to growth under Al-stress conditions than was PC (Table 1). Also, genotypic variation was larger, and the range of genotypic means wider under Al-free than under Al-stress conditions (Table 1), supporting the concept that innate differences in genotypic vigor had important effects. Genotypic variation in plant vigor has been observed previously in soybean (Hanson and Kamprath, 1979; Hanson, 1991). Urrea-Gomez et al. (1996) suggested that constitutive morphological characteristics such as vigorous rooting could be advantageous in the breeding of Al-tolerant cultivars. Thus, Al tolerance expressed as growth under Al-stress conditions, and as PC, though different, are both potentially valuable ratings in the breeding of Al-tolerant soybean.

When Al tolerance was expressed as absolute growth in the presence of Al, all three indicators of Al response (shoot and root weight and RRSA) identified the cultivars Lee 74, Davis, Essex, and Perry as the most sensitive (consistently below the treatment mean) and genotypes PI 417021, N95-7424, Biloxi, Tokyo, and Cook as the most tolerant (consistently above the treatment mean). Numerical differences between an individual from the sensitive group and one from the contrasting tolerant group were always larger than the LSD values ($P > 0.05$). When tolerance was expressed as PC, all three indicators of Al response (shoot and root weight and RRSA) identified the cultivars Lee 74 and Essex as Al sensitive and Perry, PI 417021, and Biloxi as Al tolerant. For RRSA and shoot weight only, the PC values also suggested Al tolerance in PI 416937. Consistent with our observations, Essex and Biloxi have often been em-

Table 3. Phenotypic correlation of genotypic means for 10 diverse soybean genotypes grown in the presence and absence of Al as seedlings in hydroponic and 18-d-old plants in sand-culture in the greenhouse. Correlations were computed with and without the cultivar Perry to show its effect on agreement among Al tolerance ratings. Al treatments were 0, 2, and 5 μ*M* Al^{3+} in hydroponics, and 0 and 450 *M* Al^{3+} in sand culture.

			Sand culture											
			Root dry weight				RRSA‡				Shoot dry weight			
			450 μ*M* Al^{3+}		PC†		450 μ*M* Al^{3+}		PC†		450 μ*M* Al^{3+}		PC†	
Screening system	Trait	Al treatment	No Perry	With Perry	No Perry	With Perry	No Perry	With Perry	No Perry	With Perry	No Perry	With Perry	No Perry	With Perry
Hydroponics	Taproot extension§	PC†	0.46	0.50	0.40	0.15	0.41	0.45	0.84**	0.53	0.68*	0.70*	0.67*	0.41
Sand culture	Root dry weight	450 μ*M* Al^{3+}			0.74**	0.47	0.93**	0.94**	0.67*	0.45	0.87**	0.88**	0.28	0.13
		PC†					0.67*	0.43	0.50	0.65*	0.53	0.25	0.25	0.46
Sand culture	RRSA‡	450 μ*M* Al^{3+}							0.64*	0.43	0.85**	0.85**	0.26	0.12
		PC†									0.71*	0.43	0.65*	0.74*
Sand culture	Shoot dry weight	450 μ*M* Al^{3+}											0.44	0.22

* Indicates significance at $P = 0.05$.
** Indicates significance at $P = 0.01$.
† Al tolerance expressed as percent of control (PC) and defined as (growth in presence of Al/growth in absence of Al) × 100.
‡ Relative root surface area (RRSA) was measured by immersing air-dried roots in a viscous 36.6 *M* $Ca(NO_3)_2$ solution for 10 s, suspended out of solution for 20 s, and weight of $Ca(NO_3)_2$ that adhered to roots and was removed from solution was recorded (i.e., that adhering to root surface).
§ The correlation between Al tolerance expressed as growth under 0 and 5 μ*M* Al^{3+} conditions was 0.63. The correlation between mean growth in the presence of Al and PC was 0.97. Therefore only data for PC are presented here.

ployed as Al-sensitive and -tolerant controls (Campbell and Carter, 1990; Hanson, 1991; Spehar, 1994).

The clearest case for Al tolerance comes when genotypes are rated similarly by both PC and absolute growth in the presence of Al. Applying this criterion to all three traits measured (root and shoot weight, and RRSA), Lee 74 and Essex genotypes were consistently the most sensitive to Al while PI 417021 and Biloxi were most tolerant. The cultivar Perry was strikingly inconsistent for the two measures of Al response, however. It was the most Al-tolerant genotype in the study based upon PC and one of the most sensitive based upon absolute growth for all three traits measured. The cultivar Perry was also the smallest genotype under Al-free conditions, suggesting that its small size may be related to its high rating for PC.

Discrepancies between the Two Methods

In comparison with hydroponics-based screening, an approximate 100-fold increase in Al concentration was required to inhibit root growth of plants in sand culture to a comparable degree (Table 1). While the exact reason for the higher Al requirement in sand culture is unclear, it was probably due to exposure of plants to Al for only part of the day as a result of the imposed treatment regimen. It is also conceivable that physiological factors were involved, such as increased formation of a pH gradient at the root surface leading to precipitation of Al or enhanced presence of root exudates which bind and inactivate Al (Horst and Klotz, 1990). Despite the imposition of stress to approximately the same degree in hydroponics and sand culture, the genotypic variation in Al tolerance was much greater in hydroponics as evidenced by the following: (i) much greater Al × genotype interaction, (ii) increased genotypic variation in response to stress, (iii) a much wider range in Al tolerance expressed as PC, (iv) a lower correlation between genotypic means for Al-free and Al-stress treatments, and (v) a greater correlation between ratings under Al-stress conditions and PC.

Screening at the seedling stage in hydroponics identified three Al-tolerant genotypes, PI 417021, PI 416937, and Biloxi. Two of the genotypes, PI 417021 and Biloxi, were also the only two identified as Al tolerant in sand culture when tolerance was expressed both as growth under Al-stress conditions and as PC for all three variables measured (root and shoot weight, and RRSA). Thus, there was clearly a positive association between Al tolerance in seedlings and plants at 18 DAT. However, there was also a large discrepancy between hydroponics-based ratings of seedlings and sand-culture-based ratings of plants when Al tolerance was expressed as PC. The cultivar Perry was the most tolerant genotype on the basis of PC in sand, but was overall the most sensitive one in hydroponics.

The PI 416937 exhibited a modest level of Al tolerance in sand culture based on RRSA and shoot weight while appearing more tolerant in hydroponics-based screening. A modest level of field Al tolerance has been observed previously for PI 416937 and was clearly reflected more accurately in sand culture than in hydroponics (Low, 1990; Ritchey and Carter, 1993). The discrepancies in Al tolerance ratings for Perry and PI 416937 documented here confirm the initial observations that led to this study.

To quantify further the relation between Al tolerance of seedlings in hydroponics and plants in sand at 18 DAT, we examined the phenotypic correlation of genotypic means between the two techniques and the impact of Perry on that relation by computing correlations with and without the inclusion of Perry (Table 3). Shoot weight under Al-stress conditions in 18-d-old plants was significantly correlated to seedling ratings of Al tolerance (r = 0.70*), while root weight and RRSA were associated to a lesser degree (r = 0.50 and 0.45, respectively). The deletion of Perry had only minimal effects on these correlations because it appeared to be sensitive to Al, both in hydroponics and in terms of absolute growth in sand culture. When tolerance was expressed as PC in sand culture rather than as absolute growth, however, correlations between sand culture and hydroponics-based results were lower numerically (Table 3). Correlation analysis indicated that Perry was at least partially responsible for the relatively poor association between Al tolerance expressed as PC for plants at 18 DAT and that observed in seedlings. The deletion of Perry from the computation greatly improved the agreement between Al tolerance ratings in hydroponics and PC in sand culture, with the highest association achieved when RRSA was expressed as PC in sand culture and with Perry deleted from the data set (r = 0.84**) (Table 3). These correlation results (i) confirm the general notion that Perry was responsible for much of the discrepancy between hydroponics and sand-based screening, and (ii) suggest that PC for RRSA may be a useful indicator of Al tolerance in soybean.

Relative Root Surface Area as an Indicator of Al Tolerance in Sand Culture

Employment of RRSA is rare in Al-tolerance studies. A brief discussion of its application may be useful to researchers working in this area. In that regard, a difficulty in soil-based identification of Al tolerance is the quantification of root response to Al. Aerial plant response is often used as the measure of Al tolerance because it is much easier to score than root response, and agreement has often been observed between the two traits (Foy et al., 1993b). However, the available evidence suggests that root growth inhibition is potentially the most sensitive measure of Al tolerance. Hanson (1991) demonstrated that Al toxicity reduced root weight of 18-d-old soybean genotypes by more than 50%, compared with the control, while shoot weight was unaffected when the soil medium was composed of shallow surface non-toxic soil layer covering a deeper layer of Al toxic soil. Sapra et al. (1982) and Foy et al. (1969) showed that root dry weight could be a better index of cultivar differences in Al tolerance than top dry weight.

Although the dry weight of roots has been used to differentiate Al tolerance among genotypes, it does not draw a distinction between large thick roots with limited

surface area and small finer roots with greater surface area. Fine roots are believed more important than thick roots in nutrient and water absorption, and therefore, more important in terms of Al tolerance (Eisenstat, 1992). Results in this study suggested that RRSA was indeed a better discriminator of Al tolerance than was root weight. Although the genotypic means for RRSA, root weight, and shoot weight, were all highly correlated for the Al-stress treatment (all r values >0.84, Table 3), they were somewhat less so (all r values <0.74) when Al tolerance was expressed as PC (only 0.47 between root and shoot weight for PC). The PC for RRSA appeared to discriminate among soybean genotypes best, by exhibiting a wider range of sensitivity and a lower LSD (Table 1). Additionally, the genotype $\times$ Al interaction was proportionally greater for RRSA and the correlation between Al-free and Al-stress treatment means was correspondingly lower than for shoot and root weight, further indicating that RRSA may be the better discriminator of Al tolerance (Table 2 and Table 3).

Repeatability (analogous to heritability) of RRSA was greater numerically than for root weight under Al-stress conditions (0.81 versus 0.60), and greater than either shoot or root weight when expressed as PC (0.57 versus 0.27 or 0), based upon the mean of four runs and two replications per run. One may speculate that genetic differences in root morphology existed in the genotypes studied here and that they were reflected by RRSA more so than by root dry weight. The method for RRSA has been used successfully as a diagnostic screen for prolific rooting among soybean genotypes (Pantalone, 1996).

The greatest practical problem with the use of RRSA versus root dry weight is the increased time and labor cost required for spreading the fresh roots after excavation from pots, to minimize overlapping and clumping of roots that would limit root surface area exposed to the $Ca(NO_3)_2$ solution. The roots from a single plant at 18 DAT required approximately 20 min to spread effectively.

Implications to Breeding

Generally, plant breeders conduct selection programs in the target field environment when possible, because the economic impact of results is unmistakable. A field selection program would be the safest approach to Al-tolerance breeding if it could be accomplished effectively. Unfortunately, large uniform Al-toxic field sites are usually unavailable, especially in the USA. Thus, one must ask how a breeder would best use available screening methods, such as those described here.

Hydroponics-based assay of Al tolerance with seedlings and the sand-media nutrient-solution-based assays with somewhat older plants may both have a role in breeding. The hydroponics assay is more repeatable, more easily accomplished, and more cost effective than the sand-culture method. Thus, it lends itself better to the large scale screening efforts inherent to breeding (Bianchi-Hall et al., 1998). The sand-culture method, however, probably predicts field results more accurately. On the basis of PC, the sand-culture method reproduced the high level of Al tolerance in Perry in soil noted by Foy et al. (1969, 1992) and the modest level of Al tolerance of PI 416937 in soil noted by Low (1990) and Ritchey and Carter (1993).

Results of this study suggest that not all genetic sources of Al tolerance will appear so in hydroponics and, conversely, false positive ratings can occur. The implication is that some genetic sources (and populations derived from them) will lend themselves well to hydroponics-based screening while others may not. For most breeders, the sand-culture method described here is prohibitively expensive as a sole screening method for practical breeding. However, it may prove valuable as part of a validation technique for hydroponics-based screening prior to initiating a commercial breeding program, thus protecting the economic investments of the breeder and his/her institution.

As breeding for Al tolerance becomes common place over a wide array of Al-rich soils, complex genotype $\times$ soil interactions are likely to be identified. That is, genotypes that are tolerant in an Al-rich soil from one area may not exhibit tolerance in a soil from another area. The physical nature of a soil, its particular chemical properties, along with climatic factors may interact to influence genotypic response. These effects of various soil types in masking Al tolerance may limit the broad use of a single Al-tolerant cultivar and affect the ultimate utility of any particular screening technique. For example, although Davis and Lee 74 have been cited as Al tolerant in previous studies, they did not appear to be highly Al tolerant in the present study. The breeding line N95-7424, although possessing an apparent ability to grow fast in vegetative stages on Al-rich clay soils, did not exhibit a high degree of Al tolerance in sand and hydroponic culture. The sand-culture methodology as described here may help offset some of the practical problems of breeding for Al tolerance by serving as a reproducible standard that can be used by virtually any research group, regardless of location.

The sand-culture method allows for easy excavation of intact roots from pots for quantification of root characters using RRSA, as in the present study or by computer imaging of roots (Villagarcia et al., 1998; Wright et al., 1999). Quantification of roots may be especially important to breeders in comparing multiple genetic sources of Al tolerance and developing strategic plans for their use in population development. Scott and Fisher (1989) and Baligar et al. (1993) have noted an additional Al-tolerance screening problem in that selection upon high PC alone in breeding may result in plants which grow poorly under stress free conditions rather than plants which excel under stress, simply because slow-growing plants may be less sensitive to Al. A practical screening program should monitor this potential problem. The sand-culture approach may be useful for this purpose also.

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Major and Minor Genes for Stimulation of *Striga hermonthica* Seed Germination in Sorghum, and Interaction with Different Striga Populations

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ABSTRACT

The parasitic angiosperms *Striga hermonthica* (Del.) Benth. and *S. asiatica* (L.) Kuntze severely constrain cereal production in sub-Saharan Africa. A resistance mechanism to these root parasites in sorghum [*Sorghum bicolor* (L.) Moench] is low exudation of striga seed germination stimulants. The trait is controlled by a single recessive gene in the sorghum × *S. asiatica* interaction, but information is lacking for *S. hermonthica*. Objectives of this investigation were to study the inheritance of stimulation of *S. hermonthica* seed germination in three F_2 and two $F_{3:5}$ recombinant inbred populations of sorghum, and to determine the effects of striga populations from Mali, Niger, and Kenya on the effectiveness of the low-stimulant character. An agar-gel assay was employed for this purpose. In this laboratory assay, the maximal distance between sorghum rootlets and germinated striga seed ("maximal germination distance") reflects the magnitude of germination stimulation. Bimodal frequency distributions supported the hypothesis of one recessive gene with a major effect for low maximal germination distance in progenies from crosses of low-stimulant lines (Framida, IS 9830) with a high-stimulant line (E 36-1), tested with striga from Mali or Niger. However, low- versus high-stimulant classes were not always clearly distinct, indicating that additional minor genes modified maximal germination distance in the progenies. The Kenyan striga population led to higher maximal germination distances and larger overlap of low- and high-stimulant classes than striga from Mali or Niger. Minor genes seemed therefore more important with Kenyan striga seed. The general involvement of minor genes in stimulating *S. hermonthica* seed germination was also evident from the heritable, quantitative variation observed in $F_{3:5}$ lines derived from a cross of the high-stimulant lines N 13 and E 36-1. Because of the higher sensitivity of Kenyan striga to germination stimulation, the low-stimulant character may be less effective in Kenyan fields.

Parasitic flowering weeds of the genus *Striga* (Scrophulariaceae) cause great losses to cereal production in sub-Saharan Africa. The economically most important species are *S. hermonthica* and *S. asiatica*. Two-thirds of the acreage used for cereal production in 17 sub-Saharan countries are estimated to be infested by the parasite (Kim et al., 1998). Most cultivars planted are highly susceptible to striga.

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Resistant sorghum cultivars could become a major component of integrated striga control packages if effective host-plant resistance were incorporated into adapted, productive cultivars. The best-described mechanism of resistance in sorghum to the weed is low production of root exudates required by the striga seed to germinate. The major germination stimulant in sorghum is sorgolactone (Hauck et al., 1992), whereas sorgoleone and strigol seem to be of minor importance (Ejeta et al., 1992). Other putative mechanisms of resistance to striga include mechanical barriers, inhibition of germ tube exoenzymes by root exudates, phytoalexine synthesis, post-attachment hypersensitive reactions, antibiosis (e.g., unfavorable phytohormone supply by the host), insensitivity to a postulated striga toxin, and avoidance through root growth habit (Ejeta et al., 1992; Ejeta and Butler, 1993; Berner et al., 1995; Heller and Wegmann, 2000). Absence of a haustorial inducer in root exudate is an improbable resistance mechanism since the haustorial inducer 2,6-dimethoxy-parabenzoquinone is thought to be produced by all plants under striga attack (Frick et al., 1996).

The agar-gel assay developed by Hess et al. (1992) is an excellent tool for screening host genotypes in the laboratory for the low-stimulant character (Vogler et al., 1996). Low stimulation of *S. asiatica* seed germination was shown to be inherited as a single recessive gene in the sorghum cultivars Framida, 555, SRN 6496 (Ramaiah et al., 1990), and SRN 39 (Vogler et al., 1996). However, in the study of Vogler et al. (1996), distinction of low- and high-stimulant groups was not very clear. The authors suggested that the F_2 distributions reflected the presence of the three stimulant compounds, sorgolactone, sorgoleone, and strigol. Either the major stimulant, or the three separately or in combination, could induce germination in a 1 low: 3 high ratio in the tested F_2 populations.

Quantitative variation with predominance of additive gene effects for maximal germination distance of *S. hermonthica* in the agar-gel assay was reported on the basis of a 9-by-9 diallel-cross study in sorghum (Haussmann et al., 2000). In the same experiment, different general combining ability effects of Framida and line 555 indicated that the low-stimulant genes (or alleles) may differ in these two lines.

While the genetics of stimulation of *S. asiatica* seed

Abbreviations: RIP, recombinant inbred population.