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ABSTRACT

Soybean [*Glycine max* (L.) Merr.] producers in the southeastern USA are often confronted with the problems of drought and toxic levels of subsoil Al. Our objective in this study was to determine the reaction of diverse soybean genotypes to combined effects of these two stress factors. Greenhouse studies were initiated to examine the problem. Two levels of Al saturation in a subsoil layer (6 and 71%), two levels of soil moisture (watered vs. unwatered), three genotypes ('Forrest', breeding line N77–114, and PI 416937), and three sampling dates were used as treatments. Data were collected during a 14-d drying cycle imposed at early to midpodfilling. Control pots were watered daily. Traits measured were relative water content, water potential, transpiration, diffusive resistance, leaf weight, and stem weight. In the combined presence of drought and Al stress, the Plant Introduction (PI) maintained a substantially higher relative water content, water potential, transpiration, and leaf weight than Forrest or N77–114. Under drought stress alone, the water status of the PI was inferior to that of Forrest and N77–114. These data suggest that Al tolerance can impart drought tolerance when subsoil Al is present. In regions where subsoil Al toxicity and drought are common, breeders should consider Al screening of germplasm to efficiently characterize and develop drought tolerant cultivars.

DROUGHT AND AL tolerances have historically been low-priority research objectives in soybean breeding programs. A major reason is that reliable field-screening methods for drought and Al tolerance are difficult to implement in the USA. In addition, genetic variability for Al tolerance is not well characterized, having been recognized only within the past 20 yr in soybean (Armiger et al., 1969). Even so, cultivars that exhibit tolerance to drought and Al would be economically useful. In that regard, drought was perhaps the major factor causing low yield and lost income for soybean farmers in North Carolina in 1980 and 1985 (Dunphy et al., 1980; Dunphy, 1985). Reich et al. (1981) also implicated Al as an agronomic problem in that seed yield and soil Al saturation were negatively correlated in 12 major soybean-producing counties of the North Carolina Coastal Plain.

Drought and Al toxicity may be related because many of the subsoils in the southeastern USA have a high Al saturation, which often hinders root growth and may reduce access to subsoil moisture (Sartain and Kamprath, 1978). This restriction may hasten the onset and increase the severity of plant water deficit during drought conditions. Since Al and drought stress may be related and often occur simultaneously in the Southeast, it is not a trivial matter to know which trait a plant breeder might select in the development of drought-resistant lines for the region.

The effect of Al stress on leaf water status, a subject of current study in soybean, has been documented in several other crop species. In wheat (*Triticum aestivum* L.), Al decreases leaf water potential, transpiration, photosynthesis, and chlorophyll content as compared with healthy plants (Kauffman and Gardner, 1978; Ohki, 1986). Additionally, Horton and Edwards (1976) documented increases in the diffusive resistance of peach (*Prunus persica* L. Batsch) seedlings with corresponding increases in the concentration of Al in a nutrient solution. Krizek and Foy (1981) reported a large reduction in the vegetative growth of the Al-sensitive barley (*Hordeum vulgare* L.) cultivar 'Kearney' grown in the presence of moisture stress and mild soil Al stress, while the Al-tolerant cultivar 'Dayton' was less affected.

Genotypic variability has been described in soybean for response to both Al (Hanson and Kamprath, 1979; Devine et al., 1979; Armiger et al., 1969) and moisture stress (Mederski and Jeffers, 1973; Momen et al., 1979). The interaction of genotypes with the combined effects of both drought stress and soil Al has not been reported in soybean. Employing the method of Hanson and Kamprath (1979), we have identified an Al-sensitive breeding line, N77–114, and an Al-tolerant selection from PI 416937 based upon seedling root elongation in the presence of Al. Field data suggest that both N77–114 and Forrest are somewhat drought sensitive, while PI 416937 demonstrates a degree of drought tolerance in soils with low Al saturation (T.E. Carter, Jr., data unpublished). We hypothesized that Al tolerance may impart a degree of drought resistance in some soybean cultivars because it could allow the plant to more fully utilize subsoil moisture. This study was undertaken to investigate and document the interaction of these soybean genotypes with drought and subsoil Al.

MATERIALS AND METHODS

Plant Culture

A Goldsboro sandy loam (Fine-loamy, siliceous, thermic Aquic Paleudult) soil removed from the Ap horizon at Kinston, NC was used in a greenhouse study. This soil, although acidic (pH 5.4), has a very low Al saturation (6%) (Table 1). To study the combined effects of soil Al and drought stress on plant water status, a portion of the soil was amended with 5 cmol (1/3 Al^{+3}) kg^{-1} soil applied as $Al_2(SO_4)_3$ to

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achieve a soil Al saturation of 71%. This Al saturation level was chosen because Evans and Kamprath (1970) suggested that the amount of Al in soil solution markedly increases at Al saturation levels above 60%. To simulate the highly Al-saturated subsoil layer found in a number of southeastern USA soils, a soil-layering technique was employed. Pots (57L) were filled with a 30.5-cm subsoil layer of amended soil and a 10.2-cm topsoil layer of unamended soil. These were compared to control pots that contained 40.7 cm of unamended soil. The large pot size minimized root binding and allowed for the protracted soil-drying period common in field-drought situations.

Three Maturity Group V genotypes of similar phenology were chosen for this study. These genotypes represent a range of sensitivity to both drought and soil Al. Seeds of a selection from PI 416937, breeding line N77–114, and Forrest were sown in greenhouses at Raleigh, NC on 1 Sept. 1986 and 9 Feb. 1987 and thinned to two plants per pot 2 wk after planting. Directly following thinning, soil was amended with 1.3g K, 1.3g P, and 82.8 mg N pot^{-1} in a water-soluble fertilizer. A soil test from the North Carolina department of Agriculture revealed that micronutrients were adequate in this soil. Soil in which control plants were growing was maintained at near field capacity throughout the experiment. Drought stress was imposed at the onset of growth stage R3 (early to midpodfilling; Fehr and Caviness, 1977) by withholding water from the appropriate pots for 14 d. This growth stage was chosen because severe yield losses in soybean are often experienced when drought occurs at this time (Sionit and Kramer, 1977).

Experimental Design

The experiment was arranged in a split-plot design with three replications. Two replicates were completed in 1986 and a third in 1987. Each replicate corresponded to one greenhouse. Treatments were two moisture regimes (watered and unwatered), two levels of Al saturation (6 and 71%), three genotypes, and three sampling dates. A 2 × 3 factorial arrangement of moisture regime and sampling date was randomly assigned to whole plots. The subplot was ascribed to a 2 × 3 factorial of subsoil Al and genotype.

Sampling Procedure

Traits measured included leaf water potential, transpiration, diffusive resistance, relative water content (RWC), leaf weight, and stem weight. All measurements were taken between 1200 and 1400 h. With the exception of RWC, all physiological measurements were taken on the center leaflet of the uppermost fully expanded leaf (UFEL). The sampling period spanned 2 wk during podfilling. Samples were taken approximately every third day. A fourth sampling (data not shown) was taken 24 h after rewatering. Water potential was determined using one 4.5-cm disk excised from the UFEL of each plant in the whole plot being sampled. This disk was placed abaxial side up in a silicone lubricant-coated isopiestic double-junction thermocouple psychrometer cuvette (Boyer and Knipling, 1977) within 5 sec of excision. Remaining leaf material to be used for the RWC determination was placed in airtight plastic bags, which were then packed in ice.

Psychrometers were transported to the laboratory at ambient air temperatures using insulated coolers. Water potential was measured after 4 h equilibration at 26 °C. Relative water content was concurrently determined by excising six 4.5-cm disks from the remaining leaf material. Following fresh weight determination, disks were arranged on a 2-cm layer of distilled water-saturated cheesecloth and covered with a 5-mm layer of distilled water-saturated cheesecloth. Turgid weight was measured after 4 h equilibration with the distilled water-saturated cheesecloth under ambient laboratory light and temperature conditions. Surface water was removed from the disks before weighing. Dry weight was determined after tissue had been dried overnight at 70 °C. Relative water content was expressed (Catsky, 1965) as:

$$\frac{\text{Fresh wt.} - \text{Dry wt.}}{\text{Turgid wt.} - \text{Dry wt.}} \times 100$$

Transpiration and diffusive resistance were assessed with a Li-Cor 1600 Steady State Diffusion Porometer (Li-Cor Inc., Lincoln, NE) (Kanemasu et al., 1969), which was calibrated prior to each use. Plants were harvested and leaves and stems were separated 24 h after the recovery sampling date. Weights of leaves and stems plus petioles were determined after drying for 48 h at 60 °C.

Statistical Analysis

Although replicates were homogeneous with regard to error variance, the four combinations of two soil moisture and two subsoil Al treatments showed heterogeneous error variances. Error variances of traits measured in the presence of both moisture stress and subsoil Al exhibited up to a 100-fold increase over those of the control treatment. Such heterogeneity can result in inaccurate statistical tests in the conventional analysis of variance. For this reason, a weighted analysis of variance was employed to take heterogeneous errors into account. The weighting was accomplished using the GLM procedure of SAS (1982), in which each observation is multiplied by the inverse of its error variance (Searle, 1971).

Sampling date did not show any significant interaction with treatments; thus, observations for each replicate were averaged over three sampling dates prior to application of the weighted analysis of variance. This approach reduced the model size to an acceptable level for computer analysis without sacrificing any tests of the interaction of genotype with other treatments. All main effects with the exception of replicate were treated as fixed effects. The *F*-tests were performed on the main effects of moisture stress and soil Al as well as on their interaction. Single degree of freedom orthogonal contrasts (PI vs. others and Forrest vs. N77–114) were made for all genotypic interactions.

The recovery date was analyzed separately but in a similar manner for RWC, water potential, transpiration, and diffusive resistance. Plant weights from all pots were taken at the completion of the experiment; thus, sampling date was treated as a blocking effect in the analysis of plant weight data. Follow-up comparisons of means were performed using the procedure developed by Bonferroni and recommended by Dunn (1961) for preselected contrasts. The confidence coefficient for pairwise comparisons of means is 1

Table 1. Chemical properties of Goldsboro sandy loam soil used in greenhouse experiments.

		Exchangeable cations†					
	pH	Al	Ca	Mg	K	H	P‡
		cmol (+) kg^{-1}					mg dm^{-3}
Unamended soil	5.4	0.13	0.77	0.28	0.43	0.52	176.4
Amended soil	5.0	3.83	0.76	0.28	0.36	0.17	175.0

† Exchangeable cations determined as outlined by Kamprath (1970).
‡ Value of P determined by the Agronomic Division of The North Carolina Department of Agriculture.

$-\alpha/m$, where m is the number of contrasts and α is the overall error rate.

RESULTS

The weighted analysis of variance revealed that drought stress resulted in significant reductions of most traits measured (Table 2). Plants grown under drought stress had significantly lower values of RWC, water potential, transpiration rates, leaf weight, and stem weight than control plants. An overall genotypic effect was detected for every trait except stem weight. Orthogonal partitioning of the drought stress × genotype (S × G) sums of squares revealed a significant interaction for all traits when averaged over soil Al treatments. This orthogonal partitioning of the S × G interaction showed that 65, 71, 91, and 98% of sums of squares were due to the PI responding more favorably to moisture stress than Forrest and N77–114 for RWC, diffusive resistance, leaf weight, and stem weight averaged over soil Al treatments.

A significant genotype × subsoil Al interaction was detected for all traits averaged over moisture stress treatments. While the water status of the PI was unaffected by subsoil Al, significant reductions in all traits were measured for Forrest and N77–114 when subsoil Al was present. The orthogonal partitioning of this interaction revealed that over 92% of the sum of squares for every trait was due to the PI responding differently (more favorably) to subsoil Al than Forrest and N77–114.

A significant three-way interaction of genotype, subsoil Al, and drought stress (S × G × A interaction) was detected for every trait except transpiration (Table 2). Again, the S × G × A interaction sum of

Table 2. Mean squares for drought stress, subsoil aluminum, genotype, and their interaction from seven soybean traits.

Source	df	RWC†	WP‡	TRANS§	DR¶	Dry wt. Leaf	Dry wt. Stem
Drought stress (S)	1	319.0**	47.3**	111.2*	23.2	22.1**	11.2**
Error a	10	3.2	1.6	17.3	12.1	2.6	3.6
Genotype (G)	2	9.9**	4.7**	14.9**	13.5**	14.9**	1.3
(PI vs. others)	1	12.1**	4.1*	29.5**	26.8**	29.7**	2.2
(For. vs. 114)	1	7.6**	5.2*	0.3	0.1	0.1	0.2
Al (A)	1	14.9**	16.1**	70.3**	50.3**	240.5**	193.5**
G × A	2	7.4**	8.5**	12.8**	12.7**	4.3*	2.6
(PI vs. others) × A	1	13.6**	15.7**	24.9**	24.9**	8.5**	5.1*
(For. vs. N77-114) × A	1	1.2	1.2	0.5	0.5	0.0	0.0
G × S	2	4.0	3.4*	3.4	3.3	4.0*	1.8
(PI vs. others) × S	1	5.2*	1.3	0.1	4.7*	7.2**	3.6*
(For. vs. N77-114) × S	1	2.8	5.4*	6.6*	1.9	0.7	0.1
S × A	1	14.9**	3.3	6.0*	6.8*	0.0	0.1
S × G × A	2	5.2*	4.7**	0.6	2.3	2.3	1.7
(PI vs. others) × S × A	1	8.2*	7.9**	0.5	4.2*	3.6*	3.4*
(For. vs. 114) × S × A	1	2.1	1.5	0.6	0.4	1.0	0.0
error b	60 (80)	1.5	3.4	1.2	1.3	0.1	0.6

*,** indicate significance at 5 and 1% levels, respectively.
†, ‡, §, and ¶ refer to relative water content, water potential, transpiration, and diffusive resistance, respectively, averaged over 3 sampling dates.
Number in parenthesis refers to error degrees of freedom for dry wt. of leaves and stems.

Table 3. Mean combined effect of drought stress and subsoil Al treatments on six traits of three soybean genotypes during 14-d stress period.†

Genotype	Trait RWC‡	WP§	TRANS¶	DR#	Dry wt. Leaf	Dry wt. Stem
		MPa	$\mu g\ cm^{-2}\ s^{-1}$	$s\ cm^{-1}$	$g\ plant^{-1}$	$g\ plant^{-1}$
		Drought stress = no	Subsoil aluminum = no			
Forrest	84.3a**	−1.0a	13.9a	1.8a	7.1a	5.4a
N77-114	83.5a	−1.0a	16.2a	1.2a	6.7a	5.2a
PI 416937	84.2a	−1.0a	16.0a	1.4a	8.0a	4.8a
		Drought stress = no	Subsoil aluminum = yes			
Forrest	84.2a	−1.4a	5.5a	12.1a	1.7a	1.1a
N77-114	82.5a	−1.3a	7.8a	7.5a	2.0a	1.1a
PI 416937	85.3a	−1.1a	13.1a	1.8a	2.6a	1.1a
		Drought stress = yes	Subsoil aluminum = no			
Forrest	75.3a	−1.3a	8.9a	3.5a	4.6a	3.7a
N77-114	67.4b	−1.6a	6.3a	5.3b	4.5a	3.4a
PI 416937	70.5a	−1.9b	7.2a	4.0ab	6.1a	3.3a
		Drought stress = yes	Subsoil aluminum = yes			
Forrest	61.0b	−2.2ab	1.7b	22.2b	1.1b	0.6b
N77-114	58.9b	−3.2b	1.3b	23.8b	0.9b	0.6b
PI 416937	72.0a	−1.4a	8.5a	3.7a	3.0a	1.4a

** means with the same letter in a column and within a specific combination of drought stress and subsoil Al treatment are not significantly different at 1% based on Bonferroni confidence coefficient of .01 and overall error rate of 10%.
† each mean is based on 9 observations.
‡, §, ¶, and # refer to relative water content, water potential, transpiration, and diffusive resistance, respectively, averaged over sampling dates.

squares for every trait (except transpiration) was primarily due to the tolerant response of the PI to the combined effects of both moisture stress and subsoil Al. The favorable response of the PI was much greater than predicted by the performance of the PI under each stress alone. When exposed to both stress factors, the PI maintained much higher levels of RWC, water potential, and transpiration and a lower diffusive resistance than Forrest and N77–114 (Table 3). The PI also attained greater leaf and stem weight than Forrest or N77–114 under these conditions. In this regard, a change in the rank of the genotypes occurred when the moisture stress treatment was imposed and the subsoil Al treatment was varied. When subsoil Al was not present and a moisture stress was imposed, Forrest maintained a more favorable water status than either N77–114 or the PI. It is clear that much of the overall drought stress × genotype and soil Al × genotype interactions was due to the unusual response of the PI when both drought and subsoil Al were present. Differences among genotypes were not detected after rewatering, indicating that Forrest and N77–114 recovered to levels similar to that of the PI.

DISCUSSION

Both Al and drought tolerance of soybean genotypes were detected in this study. A dramatic three-way interaction among genotypes and the two stress factors were noted as well, indicating a unique and unpredicted tolerance of PI 416937 to the simultaneous imposition of Al and drought stress. This reaction of the PI is principally explained by the substantial Al tolerance found in the PI and the impact of that Al tolerance on water status during drought. Although the water status of the PI was substantially inferior to that of Forrest under drought stress alone, the PI was greatly superior to Forrest and N77–114 in the presence of both drought and Al. Unlike the other genotypes, the PI responded to the combined stresses of drought and subsoil Al by maintaining leaf water potential at a relatively high level while continuing to transpire.

One interpretation of these results is that the Al-tolerant PI was able to extract water more efficiently from the Al-toxic subsoil layer than the other genotypes. It is possible that the PI has a more extensive root system than Forrest or N77–114 when grown in the presence of Al, thus facilitating water extraction from the subsoil layer. This explanation seems likely when one considers that Al tolerance is often defined as root growth in the presence of Al (Sartain and Kamprath, 1978). Genotypic variability in root growth has been reported in soybean (Raper and Barber, 1970).

The inter-relationship between Al and drought tolerance, not reported previously in soybean, may explain in part why drought effects on yield are more severe in the southeastern than in the midwestern USA. Although rainfall is 50% greater in the Southeast than the Midwest in summer, widespread subsoil Al in the Southeast probably limits water availability to the topsoil for most cultivars. Hammel et al. (1985) have shown a drastic effect of subsoil Al toxicity on soybean seed yield in the southeastern USA. Even so, no breeder has selected a soybean cultivar specifically for improved Al tolerance or greater rooting depth.

In summary, Al-tolerant soybean germplasm should be considered for soybean breeding programs where: (i) drought tolerance is a breeding objective, and (ii) subsoil Al toxicity is a common problem. Our current research suggests that the greenhouse can be an effective environment for Al screening. Conversely, drought tolerance is difficult to detect in the greenhouse because of a relatively large pot size required for realistic moisture stress imposition. Although drought and Al tolerance are difficult to identify in field situations, Sloane (1987) demonstrated that PI 416937 adjusts osmotically to drought stress in the field. Such adjustment apparently does not occur in the greenhouse. It is believed that this may explain the inferior response of this PI to drought stress in the absence of Al in our present study.

In areas where subsoil Al and drought are a problem, liming and irrigation may not be cost-efficient solutions. We recommend that breeders consider greenhouse or laboratory methods of Al screening to more efficiently characterize and develop drought-tolerant cultivars.

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REFERENCES

Armiger, W.H., C.D. Foy, A.L. Fleming, and B.E. Caldwell. 1969. Differential tolerance of soybean varieties to an acid soil high in exchangeable aluminum. Agron. J. 60:67–70.

Boyer, J.S., and E.B. Knipling. 1965. Isopiestic technique for the determination of water potential with a thermocouple psychrometer. Proc. Nat. Acad. Sci. USA 54:1044–1051.

Catsky, J. 1965. Leaf disk method for determining water saturation deficit. Arid Zone Res. 25:353-360.

Devine, T.E., C.D. Foy, D.L. Mason, and A.L. Fleming. 1979. Aluminum tolerance of soybean germplasm. Soybean Gen. Newsl. 6:24–27.

Dunn, O.J. 1961. Multiple comparisons among means. J. Am. Stat. Assoc. 56:52–64.

Dunphy, E.J. 1985. Soybean on-farm test report. North Carolina Agric. Ext. Serv., Raleigh, NC.

———, J.R. Anderson, Jr., and D.P. Schmitt. 1980. Soybean on-farm test report. North Carolina Agric. Ext. Serv., Raleigh, NC.

Evans, C.E., and E.J. Kamprath. 1970. Lime response as related to percent Al saturation, solution Al, and organic matter content. Soil Sci. Soc. Amer. Proc. 34:893–896.

Fehr, W.R. and C.E. Caviness. 1977. Stages of soybean development. Iowa Agric. Exp. Stn. Rep. SR-80.

Hammel, J.E., M.E. Sumner, and H. Shahandeh. 1985. Effect of chemical profile modification on soybean and corn production. Soil Sci. Soc. Am. J. 49:1508–1511.

Hanson, W.D., and E.J. Kamprath. 1979. Selection for aluminum tolerance in soybeans based on seedling-root growth. Agron. J. 71:581–586.

Horton, B.D., and J.H. Edwards. 1976. Diffusive resistance rates and stomatal aperture of peach seedlings as affected by aluminum concentration. HortScience 11:591–593.

Kanemasu, E.T., G.W. Thurtell, and C.B. Tanner. 1969. Design, calibration, and field use of a stomatal diffusion porometer. Plant Physiol. 44:881–885.

Kauffman, M.D., and E.H. Gardner. 1978. Segmental liming of soil and its effect on the growth of wheat. Agron. J. 70:331–336.

Krizek, D.T., and C.D. Foy. 1981. Water stress: Role in differential aluminum tolerance of barley genotypes. p. 181. *In* Agronomy abstracts. ASA, Madison, WI.

Mederski, H.J., and D.L. Jeffers. 1973. Yield response of soybean varieties grown at two soil moisture stress levels. Agron. J. 65:410–412.

Momen, N.N., R.E. Carlson, R.H. Shaw, and O. Arjmand. 1979.

Moisture-stress effects on the yield components of two soybean cultivars. Agron. J. 71:86–90.
Ohki, K. 1986. Photosynthesis, chlorophyll, and transpiration responses in aluminum stressed wheat and sorghum. Crop Sci. 26:572–575.
Raper, C.D., Jr., and S.A. Barber. 1970. Rooting systems of soybeans. I. Differences in root morphology among varieties. Agron. J. 62:581–584.
Reich, R.C., E.J. Kamprath, and L.A. Nelson. 1981. Soil and management factors correlated with soybean yields in the southeastern U.S. Coastal Plain. Agron. J. 73:90–95.
Sartain, J.B., and E.J. Kamprath. 1978. Aluminum tolerance of soybean cultivars based on root elongation in solution culture compared with growth in acid soil. Agron. J. 70:17–20.
SAS Institute. 1982. SAS user's guide: Statistics. SAS Inst., Cary, NC.
Searle, S.R. 1971. Linear models. John Wiley & Sons, New York.
Sionit, N., and P.J. Kramer. 1977. Effect of water stress during different stages of growth of soybean. Agron. J. 69:274–278.
Sloane, R.J. 1987. Effect of soil moisture deficit on leaf water relations and nonstructural carbohydrate levels in two soybean genotypes. M.S. thesis. North Carolina State Univ., Raleigh.