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## Agronomic Performance in Acid Soils of Wheat Lines Selected for Hematoxylin Staining Pattern

Norma A. Ruiz-Torres, Brett F. Carver,\* and Robert L. Westerman

### ABSTRACT

Laboratory screening techniques enable detection of varying degrees of Al tolerance during seedling development, but their application to crop improvement programs must be verified in the field. The objective of this study was to compare agronomic responses of wheat (*Triticum aestivum* L. emend. Thell.) lines differing in hematoxylin-staining pattern under naturally acidic and limed field conditions. Experiments were conducted on Bethany-Kirkland (fine, mixed, thermic Pachic-Udertic Paleustolls) or Grant-Pond Creek (fine-silty, mixed thermic Udic-Pachic Argiustolls) silt loam soil complexes. Four sets of three experimental lines were classified as very susceptible (VS), moderately susceptible (MS), intermediate (I), or tolerant (T) based on hematoxylin staining pattern, and evaluated in limed and unlimed plots as separate experiments in 1988 and 1989. The VS group produced the fewest spikes per square meter and the lowest above-ground biomass and grain yield in the unlimed treatments; no such reductions occurred in the limed treatments. Differences in agronomic performance were found between groups of VS and MS lines, with relatively little differentiation among MS, I, and T groups. Comparison of two sister lines from the VS and T groups revealed a 12% increase in spike density, 11% increase in biomass, and a 14% increase in grain yield of the tolerant sib under acidic conditions. The two sibs performed similarly under limed conditions, except the VS line was superior in grain yield. The hematoxylin staining procedure shows promise for identifying wheat genotypes with potentially high sensitivity to acidic soils in the southern Great Plains.

SOIL ACIDITY can be corrected by application of lime. Although lime is a relatively inexpensive material, many wheat producers in the southern Great Plains face potential production losses, if not crop failure, due to soil acidity (pH < 5.2). Those producers either find the added costs of transporting lime economically unfeasible (often \$25 to 30 per ton of ECCE), or they lack landowner support to apply lime to leased land. While the use of acid tolerant cultivars may postpone the need for corrective lime applications, it is the only alternative in some situations (Foy, 1983; Kohli and Rajaram, 1988).

Because soluble Al is usually associated with the toxic effect of soil acidity, the identification of acid tolerant germplasms is often based upon some indicator of Al tolerance (Foy et al., 1965; Carver et al., 1988; Garland-Campbell and Carter, 1990). Lafever et al. (1977) related cultivar responses in acidic soil having high exchangeable Al ( $\approx 140 \text{ mg kg}^{-1}$ ) to measurements of root length and weight, leaf length, and shoot weight of the same cultivars grown in nutrient-solutions containing  $8 \text{ mg kg}^{-1}$  Al. Field responses were most consistent with root length measurements. Aluminum sensitivity in wheat has been attributed to the inability to tolerate acidic conditions in the rhi-

zosphere and a consequent increase in Al solubility (Taylor and Foy, 1985), but the primary cause may directly relate to greater uptake of Al in the root (Foy et al., 1967). Differences in Al uptake were also found in *Hordeum vulgare* L. (MacLean and Chiasson, 1966) and *Gossypium hirsutum* L. (Naidoo et al., 1978). Recently, Zhang and Taylor (1989) suggested an exclusion mechanism could possibly reduce Al uptake into the symplasm of tolerant genotypes.

To visually determine the degree of Al uptake, or level of Al tolerance, wheat seedling roots are grown in nutrient solutions containing Al and stained with a sodium iodate-hematoxylin ( $\text{NaIO}_3\text{-C}_{16}\text{H}_{14}\text{O}_6$ ) solution (Polle et al., 1978). As the intensity of staining increases for a given concentration of Al, the level of tolerance decreases. Cultivars subjected to three Al concentrations (0.18, 0.36, and 0.72 mM Al) can be classified into four tolerance levels, ranging from very susceptible to tolerant, according to their staining characteristics. Staining type is scored from the primary root of 1-wk-old plants as complete, partial, or no staining. A very susceptible genotype shows complete staining at all three concentrations, whereas a tolerant genotype shows either partial or no staining. The hematoxylin staining procedure is simple, facilitates screening large numbers of lines, and minimizes confounding factors inherent to field studies. Yet, its agronomic relevance to acidic field conditions has not been documented to justify its use in a selection program. Our objective, therefore, was to compare agronomic responses of wheat lines differing in hematoxylin staining pattern under naturally acidic and limed field conditions of the southern Great Plains.

### MATERIALS AND METHODS

Test materials consisted of 12 experimental HRW wheat lines from the 1987 Southern Regional Performance Nursery, a uniform testing program conducted annually by USDA-ARS and state experiment stations. Genotypes were selected for varying degrees of Al tolerance based on visual assessment of hematoxylin-stained seedling roots in nutrient-solution culture (Carver et al., 1988). Three lines were chosen to represent each of four tolerance levels or groups: VS, MS, I, and T (Table 1). Lines were also chosen based on parentage to maximize genetic similarity among groups and thereby reduce the confounding effect of genetic differences unrelated to Al tolerance. For example, Amigo appeared in the pedigrees of OK82377 (VS), OK85347 (MS), TX81V5581 (MS), and TX81V66072 (T), while 'TAM W-101' or its closely related counterpart, 'TAM W-103', appeared in the pedigrees of TX78A3345 (VS), OK85347 (MS), OK84349 (I), and TX84A7608 (T). TX78A3345 and TX84A7608 have the same pedigree, but these sib lines showed opposite responses to Al toxicity. Some experimental lines were subsequently released as cultivars, including KS82H144 (PI 506344) as 'Dodge' (Martin et al., 1988) and CO82009 as 'Lamar'.

Field experiments were conducted under limed and unlimed soil conditions on a Bethany-Kirkland silt loam com-

**Abbreviations:** ECCE, effective calcium carbonate equivalent; HRW, hard red winter; I, intermediate; MS, moderately susceptible; T, tolerant; VS, very susceptible.

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Table 1. Four groups of wheat genotypes differing in hematoxylin staining pattern of seedling roots.

Group†	Staining‡ pattern	Entry	Pedigree
VS	CCC	OK82377	Amigo sib/2*Newton
		TX78A3345	MV-69-12/TAM W-103
		CO820026	Mexican Dwarf/Baca//Vona
MS	PCC	OK85347	TAM W-101/Amigo
		TX81V5581	TX73V203*3/Amigo
		CO830027	74CB462/Trapper//Vona
I	PPC	OK84349	Aurora/2*TAM W-101
		CO82009	74F878/Wings//Vona
		CO830034	CO5926//Siete Cierros/Tobari 63/3/Baca
T	NPP	TX84A7608	MV-69-12/TAM W-103
		TX81V66072	TX71A1039-V1*3/Amigo
		KS82H144	Newton sib/Arkan sib

†VS = very susceptible, MS = moderately susceptible, I = intermediate, T = tolerant to Al.

‡Degree of staining listed in order of response to 0.18, 0.36, and 0.72 mM Al in nutrient-solution culture: C = complete staining, P = partial staining, and N = no staining.

plex at the Stillwater Agronomy Research Station in 1988 and 1989, and on a Grant-Pond Creek silt loam complex at the North Central Research Station near Lahoma, OK, in 1989. Those locations were specifically chosen for their prior history of acid-soil stress conditions. Lime (50% ECCE) was applied at a rate of  $\approx 2200$  kg ha<sup>-1</sup> at least 1 yr prior to this study to constitute the limed treatment. The 12 genotypes were assigned to four-row plots (1.2 by 3.0 m) in a randomized complete-block design with four replicates in each of two fields (unlimed and limed). Plots were fertilized prior to planting according to soil-test recommendations, with attention given to consistent nutrient composition between treatments.

Due to suspected soil heterogeneity at Stillwater, three soil cores (top 15–20 cm) were collected from each plot during anthesis. The three cores were combined to determine pH in a 1:1 (w/w) ratio of soil and 1 M KCl (pH<sub>s</sub>) in 1988 and 1989. Extractable Al in 1 M KCl was also measured in 1988 by induction coupled plasma-atomic emission spectroscopy (Thermo Jarrel Ash Corp., Franklin, MA). The total plot area in the unlimed and limed fields at Lahoma were partitioned into six blocks of 46 m<sup>2</sup>. Nine soil cores per block were combined to determine pH using a 1:1 (w/w) ratio of soil and water (pH<sub>w</sub>).

After confirming homogeneity of error variances, analyses of variance were combined over the three location-years (environments) for each treatment. Variation among genotypes was partitioned into components representing differences among groups according to Al tolerance level and lines within groups. Effects of groups and lines were considered fixed. Group means were compared within treatments according to Waller-Duncan's *t*-test with a *k*-ratio of 100:1 (Steel and Torrie, 1980, p. 190–191). Single-df contrasts were also made to compare means of the two sister lines.

Table 3. Selected mean squares from the analysis of variance across three unlimed (acid soil) environments of four groups of wheat genotypes differing in hematoxylin staining pattern.

Source	df	Spike no. m <sup>-2</sup> †	Mean squares				
			Aboveground yield†	Grain yield†	Harvest index	Kernel no. spike <sup>-1</sup>	1000-kernel wt.
Group	3	37.1**	4,054.5**	345.2**	22.2**	11.8	11.1
Line/Group	8	26.0**	1,736.6**	457.9**	45.5**	90.3**	81.9**
Group × Environment (Env)	6	2.8	1,316.4**	278.7**	15.6**	16.2	15.0**
Line/Group × Env	16	7.4**	478.0	127.1**	9.8**	22.0**	8.2
Pooled error	99	2.8	338.4	46.8	3.4	9.3	4.7
CV (%)		12	8	9	6	12	8

\*\*\*Significant at *P* = 0.05 and 0.01, respectively.

†Actual quantities equal reported quantity × 10<sup>3</sup>.

Table 2. Soil analyses ( $\bar{x} \pm$  SD) of unlimed and limed field plots in 1988 and 1989.

Location	Treatment	Year	<i>n</i>	KCl pH†	Al concentration mg kg <sup>-1</sup>				
Stillwater, OK	Unlimed	1988	48	3.8 ± 0.1	10.5 ± 3.3				
					Limed	1989	48	3.1 ± 0.2	—
							1988	48	4.5 ± 0.2
	1989	48	5.0 ± 0.2	—					
	Lahoma, OK	Unlimed	1989	6	3.1 ± 0.1†	40.4 ± 9.4			
						Limed	1989	6	4.5 ± 0.01†

†Estimated values based on pH<sub>w</sub> determinations minus the expected difference between pH<sub>w</sub> and pH<sub>s</sub> (1.3 units) measured in adjacent experimental plots.

## RESULTS AND DISCUSSION

Our intention was to use pH and Al concentration as covariates in the analysis of variance and thereby adjust for possible soil heterogeneity at Stillwater, particularly in the unlimed treatment. Neither covariate, however, significantly reduced the error variance (*P* > 0.05); thus, unadjusted means are reported throughout. In acid soils, the KCl extraction generally produces pH<sub>s</sub> values one or more units less than pH<sub>w</sub> values, depending on soil type. Values of pH<sub>s</sub> at Lahoma were estimated by subtracting the difference between pH<sub>w</sub> and pH<sub>s</sub> measured in other experiments (1.3 units) in the same field (Table 2). Values for extractable Al are actual means from the adjacent unlimed and limed experiments. The limed treatment in each location produced pH values not considered yield limiting (Johnson et al., 1988). Corresponding soil pHs in the unlimed treatments were 0.7 to 1.9 units lower, producing 10-fold increases in Al concentration in Stillwater and Lahoma.

Tiller density, measured by the number of spikes per square meter, was expected to provide an early indicator of differential Al tolerance because symptoms of Al toxicity are expressed early in juvenile growth of wheat (Long and Foy, 1970; Westerman, 1981; Unruh and Whitney, 1986). The analysis of variance across three unlimed environments revealed significant differences (*P* < 0.05) among groups for spike density, as well as for total aboveground yield (biomass), grain yield, and harvest index (Table 3). Group differences in spike density were not accompanied by differences in kernel number per spike and kernel weight. The same analysis of variance in the limed environments (data not shown) did not indicate significant group differences, except for spike density

Table 4. Average agronomic performance across three unlimed (acid soil) and across three limed environments of four groups of wheat genotypes differing in hematoxylin staining pattern.

Treatment	Group†	Spike density	Aboveground yield	Grain yield	Harvest index	Kernel $\bar{s}$ spike <sup>-1</sup>	1000-kernel wt.
		spikes m <sup>-2</sup>	kg ha <sup>-1</sup>		%	no.	g
Unlimed	VS	424	6630	2170	33.1	25.5	27.5
	MS	491	7000	2370	33.7	24.3	27.0
	I	442	7360	2370	32.6	25.5	27.7
	T	483	7310	2370	32.9	25.3	26.5
LSD ( $k = 100$ )		23	250	96	0.8	NS	NS
Limed	VS	594	7410	2270	30.6	24.0	26.0
	MS	612	7290	2150	29.6	23.5	25.0
	I	575	7310	2210	30.3	23.9	25.7
	T	624	7260	2160	29.9	23.4	23.5
LSD ( $k = 100$ )		35	NS	NS	NS	NS	0.8

†VS = very susceptible, MS = moderately susceptible, I = intermediate, and T = tolerant to Al based on hematoxylin staining pattern.

( $P < 0.05$ ) and kernel wt. ( $P < 0.01$ ). Averaged across all traits, 93% of the total phenotypic variation in the limed environments was attributed to lines within groups. That source of variance was still significant ( $P < 0.01$ ) for all traits in the unlimed environments (Table 3), but accounted for a lower average proportion of the total (78%). Acid-soil conditions in the unlimed treatment generally produced differences among lines grouped according to Al tolerance level when those differences did not exist in the limed treatment. Most importantly, the four groups did not differ in yield potential in the limed treatment but did differ in the unlimed treatment.

Group  $\times$  environment interactions in the unlimed treatment accounted for a significant component of genotype  $\times$  environment interactions for several traits (Table 3), making comparisons among group means tenuous. Examination of group means within environments, however, revealed rank changes strictly among the MS, I, and T groups. Furthermore, the VS group consistently produced the fewest spikes per square meter and least biomass and grain yield among the four groups; absolute differences among the MS, I, and T groups were generally smaller in magnitude and non-significant. Thus, remaining discussion will focus on the average response of groups across all environments (Table 4).

The VS group produced the fewest spikes per square meter under acidic conditions (Table 4). Spike densities of the MS and I groups were inconsistent with their Al tolerance level. By adjusting for average spike density in the limed treatment, the greatest change among groups in relative spike density occurred between the VS and MS groups. Ratios were 0.71, 0.80, 0.77, and 0.77 for VS, MS, I, and T groups, respectively. We could not confirm based on these data that the reduced spike density in VS lines resulted from a lower tillering capacity during early vegetative growth, or a higher tiller abortion rate during stem elongation, or both.

The VS group also produced the lowest biomass and grain yield under acidic conditions, but no differences occurred under limed conditions (Table 4). The lower grain yield of VS lines was not related to changes in kernel number per spike or kernel weight. Further increases in biomass of I and T groups compared with MS lines did not translate into higher grain

yields, as those groups possessed lower harvest indices.

One unexpected outcome of this experiment was the higher average grain yield in the unlimed treatment (2320 kg ha<sup>-1</sup>) vs. the limed treatment (2200 kg ha<sup>-1</sup>), despite almost a 25% reduction in average spike density in the unlimed treatment. The lack of replication of treatment units (unlimed and limed) precluded statistical comparison of treatment means. The lower spike density in the unlimed treatment, however, was accompanied by numerically higher kernel number (25 kernels spike<sup>-1</sup>) and 1000-kernel weight (27 g) compared with the limed treatment (24 kernels spike<sup>-1</sup> and 25 g). Climatic conditions in 1988 and 1989 were conducive to this recovery because in no environment was drought stress sufficiently severe to cause plant death. Goldman et al. (1989) recognized the compounding effects of drought stress and Al toxicity on soybean (*Glycine max* [L.] Merr.) growth and seed yield. Another contributing factor to the observed grain yield recovery might be that soil acidity and Al toxicity in semiarid regions of the USA, including Oklahoma, are limited to the top 15 to 20 cm of the soil profile (Westerman, 1987). Finally, increased incidence of take-all (*Gaeumannomyces graminis* var. *tritici*) has been shown to reverse the expected increase in grain yield following lime application (Murray et al., 1987). Root and crown diseases were not monitored during this study, but have since been detected in limed treatments at the same test sites. The confounding effect of this disease pressure is especially difficult to remove due to ineffective chemical control measures and the lack of genetic resistance.

Comparison of sister lines, TX78A3345 (VS) and TX84A7608 (T), provide an assessment of the agronomic relevance of Al tolerance with minimal bias caused by differences in genetic background. Their difference in tolerance was confirmed by differences in root elongation under Al stress in nutrient-solution culture. Four days following pulse-exposure to 0.5 mg Al L<sup>-1</sup>, the VS genotype showed no net root elongation, but the T genotype showed a 75% increase (J.D. Ownby, 1990, personal communication). In this study, the T genotype produced 12% more spikes, 11% more aboveground biomass, and 14% more grain yield than the VS genotype under acid-soil stress (Table 5). The T genotype also produced 25% more ker-

Table 5. Average agronomic performance across three unlimed (acid soil) and across three limed environments of two sib lines differing in hematoxylin staining pattern.

Treatment	Entry†	Spike density	Aboveground yield	Grain yield	Harvest index	Kernels spike <sup>-1</sup>	1000-kernel wt.
		spikes m <sup>-2</sup>	kg ha <sup>-1</sup>		%	no.	g
Unlimed	TX78A3345 (VS)	433	6580	2210	34.0	21.9	29.5
	TX84A7608 (T)	487	7320	2520	35.1	27.3	26.9
	F-test	*	**	**	NS	**	**
Limed	TX78A3345 (VS)	645	8040	2580	31.9	23.5	26.3
	TX84A7608 (T)	624	7750	2370	30.7	25.1	23.9
	F-test	NS	NS	*	NS	NS	NS

\*\*\*Significant at  $P = 0.05$  and  $0.01$ , respectively.

†VS = very susceptible, T = tolerant to Al based on hematoxylin staining pattern.

nels per spike, but that difference was partly negated by a 9% reduction in kernel weight. The VS genotype actually showed higher grain yield potential under limed conditions.

The hematoxylin staining procedure certainly shows promise for identifying genotypes with high sensitivity to acidic soils in the southern Great Plains. A recent survey of Al tolerance based on hematoxylin staining pattern classified 54% of the sampled HRW germplasm pool as very susceptible (Carver et al., 1988), the same classification that proved to be uniquely sensitive to acid-soil conditions in this study. The level of resolution among Al-tolerance levels in the laboratory was not repeated in the field, as differences in agronomic performance were generally found only between VS and MS groups. The lack of differentiation among the MS, I, and T groups could reflect (i) an adequate tolerance level among MS genotypes to withstand surface-soil acidity in the southern Great Plains, (ii) a level of acid-soil stress insufficient in this study to distinguish among the higher tolerance levels, or (iii) factor(s) in addition to Al tolerance that govern plant response to acid-soil conditions. Future research is underway to clarify this enigma.

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