GENETIC CONTROL AND AGRONOMIC BENEFIT OF ALUMINUM TOLERANCE IN HARD RED WINTER WHEAT

By

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TABLE OF CONTENTS

LIST OF TABLES

Table Page

Chapter II

Chapter III

LIST OF FIGURES

Figure Page

Chapter II

Chapter III

INTRODUCTION

Each chapter in this thesis conforms to the Publications Handbook and Style Manual of the American Society of Agronomy. Chapters will be submitted for publication in Crop Science, a Crop Science Society of America publication.

CHAPTER I

RELATION OF ALUMINUM TOLERANCE TO ACID SOIL TOLERANCE

LITERATURE REVIEW

Aluminum toxicity causes short, stunted roots in wheat, reducing water and nutrient uptake. Symptoms in the plant tops may include stunted dark green leaves, leaf purpling and wilting, and delayed maturity. Aluminum toxicity may also induce calcium deficiency (Foy, 1976). These plants are not suitable for grazing, because they not only lack sufficient forage, but the roots are so poorly anchored that the plants are easily pulled out of the ground. Severe injury may result in lower grain yield or even death of the plant early in the growing season.

Various studies, ranging from field trials to laboratory assays, have been completed on the genetic control of Al tolerance. Genes for Al tolerance have been disproportionately located on the A, B, and D genomes of Chinese Spring wheat (Carver and Ownby, 1995). More genes are located on D chromosomes and fewest on B. Several of these genes could play a role in Al tolerance at any one time during vegetative or reproductive growth. Perhaps a single major gene is influenced by or masks several minor genes (Aniol, 1990). Aluminum tolerance may also be influenced by the background genotype (Ruiz-Torres and Carver, 1992). It has been generally reported that Al tolerance is a dominant trait (Carver and Ownby, 1995).

Researchers at Oklahoma State University studied the effects of Al tolerance on spike production, biomass, and grain yield (Ruiz-Torres et al., 1992), and found that grain yield is mostly limited by reduced tiller number under Al-toxic conditions. They also found Al tolerance to be under complex genetic control (Ruiz-Torres and Carver, 1992). Bona et al. (1994) found that Al tolerance in wheat is not simply inherited and that gene interactions may exist. One example is that gene expression appeared to be influenced by the concentration of Al in the nutrient solution. Assuming multiple genes for Al tolerance, different mechanisms of tolerance may be activated by different genes at different Al stress levels. Briggs and Taylor (1994) also found responses of different tolerance sources that were not explained by simple genetic models. They too suggested more complex inheritance of several genes with only a few acting at any one time in response to a given Al stress level.

One physiological explanation of Al tolerance is that the plant may control its rhizosphere pH, preventing Al in the soil to be absorbed (Boman et al., 1992). However, conflicting reports indicate no significant change in pH near the root (Miyasaka et al., 1989). Another more likely hypothesis is that Al-tolerant plants exclude Al from the root via the plasma membrane. Wright (1989) proposed two types of tolerance, external exclusion and internal detoxification. Rincon and Gonzales (1992) also cited several mechanisms of Al tolerance, including chelation of Al, sequestering Al in organelles, immobilizing Al in the mucigel, inducing less Al sensitive enzymes, and transport of Al out of the root tissue. Data on Al accumulation in the root is conflicting. Whether tolerant cultivars do not accumulate Al as do susceptible cultivars, or are simply more tolerant of equal accumulations, is still debated (Rincon and Gonzales, 1992).

Acid soil tolerance is measured indirectly by a variety of laboratory bioassays for Al tolerance. Hematoxylin staining of the roots is most often used to quickly identify Al tolerance (Polle et al., 1978). Seedlings are grown for about one week in a nutrient solution culture. The hematoxylin stain leaves dark bands where Al is conjugated in the root tips of susceptible plants (Ownby, 1993). This test has shown moderate correlation with measurements of field tolerance (Ruiz-Torres et al., 1992). This method is being used by wheat breeders at several public and private institutions.

Aluminum-tolerant germplasm lines were developed at Oklahoma State University using the hematoxylin stain bioassay. They were formed by making three backcrosses of Chisholm or Century as susceptible recurrent parents with Atlas 66 as a tolerant donor parent (Carver et al., 1993). The tolerant lines are theoretically 94% identical to their recurrent parents and resemble their recurrent parents for milling and flour quality (Carver et al., 1993). In 1991 on an acid field ($pH<5.5$), a heterogeneous population from which tolerant Chisholm isolines were derived produced 35% more grain and 31% more biomass than Chisholm. A similar population in the Century background produced 68% more grain, 66% more biomass, and 17% heavier kernels than Century (Carver et al., 1993). More extensive experiments are needed using the specific isolines to more accurately determine agronomic relevance. Cultivars currently available with a high degree of Al tolerance, 2163 and 2180, offer potential for acid soils in the Great Plains (Krenzer and Hodges, 1993). There is also genetic variation available in genotypes from Brazil, Europe, and the eastern United States to use for future plant improvement (Carver and Ownby, 1995).

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CHAPTER II

GENETIC CONTROL OF ALUMINUM TOLERANCE IN WHEAT SELECTED FOR HEMATOXYLIN STAIN PATTERN

Genetic Control of Aluminum Tolerance in Wheat Selected for Hematoxylin Stain Pattern

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ABSTRACT

It is widely accepted that aluminum (Al) tolerance in wheat (Triticum aestivum L.) contributes to acid soil tolerance. However, much is not known about the number of genes which condition Al tolerance, nor the effects of variable Al stress or genetic background on the degree of tolerance expressed in the plant. Genetic stocks were produced in two hard red winter (HRW) backgrounds ('Chisholm' and 'Century') which are near-isogenic for gene(s) governing Al tolerance in solution culture, based on the hematoxylin stain assay. Our objectives were to characterize actual Al tolerance based on dose-response curves for root growth of the isolines, and two check cultivars with opposite hematoxylin staining patterns (TAM 105 and 2180), and to determine inheritance of the gene(s) transferred from Atlas 66. The tolerant isolines showed a linear decline in root growth across Al concentrations of 0.09 to 0.72 mM, whereas the decline for the susceptible parents was curvilinear. The effect of tolerance transferred from Atlas 66 was more dramatic in the Century background than in Chisholm. Segregation for stain intensity was characterized in F_2 and backcross populations derived from crosses of each tolerant isoline with its recurrent parent and with 2180. Trends in segregation ratios were consistent across Al concentrations although less tolerance (more staining) was observed at each incremental increase of Al concentration. A single major gene was transferred to Chisholm and Century from Atlas 66 that was

independently inherited from the gene(s) expressed in 2180. Expression of the tolerance gene was influenced by the genotype of the recurrent parent, which could limit its effectiveness in breeding programs.

INTRODUCTION

One approach to reducing the impact of acid-soil stress on wheat production in the southern Great Plains is to genetically improve the tolerance in adapted varieties. While tolerance to soil acidity is generally regarded as complex with several component traits (e.g., Al tolerance, manganese tolerance, phosphorus-use efficiency, drought tolerance), certain genes with major effects on root growth can be selected in solution media containing known quantities of Al (Carver and Ownby, 1995). Aluminum tolerance genes normally have dominant effects, but additive effects have also been reported (Bona, 1994; Ruiz-Torres and Carver, 1992; Aniol, 1990). The number and location of chromosomes believed to be involved in Al susceptibility or tolerance have been determined in wheat (Aniol and Gustafson, 1984; Aniol, 1990; Takagi et al., 1983).

Expression of Al tolerance has been shown to be background-dependent. Ruiz-Torres and Carver (1992) crossed six tolerant or intermediate cultivars with three susceptible cultivars. The F_1 progeny usually resembled the tolerant parent for relative root length, but sometimes resembled the mid-parent or susceptible parent depending on the parents. Expression of Al tolerance is also not stable across variable levels of toxicity. Bona et al. (1994) found a higher frequency of susceptible genotypes in solution culture at higher Al concentrations, resulting in different modes of inheritance.

Tolerant near-isolines of the variably sensitive HRW cultivars, Chisholm and Century, were developed by introgressing tolerance from the soft red winter cultivar, Atlas 66 (Carver et al., 1993). Gene detection was accomplished indirectly by the hematoxylin stain assay (Polle et al., 1978; Carver et al., 1988). Differences in actual root growth under Al stress have not yet been quantified for these isolines, although they have been used to refute prominent hypotheses of Al tolerance. Ryan and Kochian (1993) found that reduction in root growth of the susceptible Century isoline vs. the tolerant isoline was not caused by inhibition of Ca uptake. They reported a greater decrease, about 12% at two Al concentrations, in root elongation for the susceptible isoline compared to the tolerant isoline of Century in 0.02 and 0.05 mM Al.

Evidence accumulated during the development of these near-isolines indicated that more than one gene may control tolerance. While segregation in consecutive BCF_1 generations was generally 1:1 (tolerant:susceptible), variation within each class was apparently caused by environmental effects or the expression of modifying genes. Atlas 66 is believed to possess at least two genes governing Al tolerance (Aniol, 1990; Berzonsky, 1992).

Our primary objective was to describe the genetic basis for Al tolerance in the near-isolines of Century and Chisholm. This material is also being used in a companion study to determine the contribution of the Atlas 66 gene, or possibly two genes, to agronomic performance in acidic soil (Johnson and Carver, 1995). Second, Chisholm and Century differ in their sensitivity to Al tolerance (Carver et al., 1988) and, therefore, provide potentially diverse genetic backgrounds for the expression of the introgressed Al tolerance gene(s). Modifying effects of the recurrent parent will be examined. Our final objective was to determine if the tolerance transferred from Atlas 66 to Chisholm and Century differs genetically (gene number and action) from that already present in 2180, a tolerant cultivar recommended for acidic soils of the southern Great Plains.

MATERIALS AND METHODS

Dose-Response Experiment

Laboratory experiments were conducted using two sets of near-isolines, and three check cultivars (Table 1). The Al-tolerant/susceptible near-isolines were BC_3-F_2 derived sister lines in the F_6 generation. TAM 105 and 2180 were included to reference extreme levels of susceptibility and tolerance, respectively, currently known in HRW wheat. Atlas 66 was the original donor parent used in crosses with Chisholm and Century.

Seedlings were grown in solution culture as described by Carver et al. (1988). The 11 genotypes were arranged in a randomized complete block design with three replicates for each of six Al concentrations (0, 0.09, 0.18, 0.36, 0.54, and 0.72 mM Al). The length of the primary root was measured on four plants of each genotype per replication after a 24-h exposure of 3-d-old seedlings to Al. Root growth was then expressed as a percentage of the root length in a given Al treatment relative to root length in the absence of Al (relative root length, RRL). The roots were also stained with hematoxylin (Polle et al., 1978) and scored on a 1 (no stain) to 7 (intense root tip and mid-root staining) scale (Ruiz-Torres and Carver, 1992).

Statistical analysis emphasized the regression of RRL and staining score (SS) on Al concentration. Data for each pair of tolerant isolines were averaged for the regression due to similar responses. Linear and curvilinear models were considered in maximizing R^2. Best-fit curves were generally found with linear and logarithmic models.

Genetic Analysis Experiment

One tolerant near-isoline of Chisholm (Chisholm-T[1]) and of Century (Century-T[2]) was crossed with their corresponding recurrent parent to produce F_1, F_2, and backcross (BCF_1) populations. The near-isolines were also crossed with 2180, another tolerant genotype with a potentially different gene source for Al tolerance than Atlas 66, to produce F_1, F_2, and BCF_1 populations. Seed stocks of all parents used in crosses were confirmed for homogeneity of tolerance or sensitivity based on the hematoxylin assay.

Seedlings were grown in solution culture as described by Carver et al. (1988). Plants of each population were arranged without randomization for convenience of rating. Populations with all tolerant parents were treated with 0.36, 0.72, and 0.90 mM Al. Populations with a susceptible parent were treated with 0.18, 0.36, and 0.72 mM Al. The latter were less tolerant of higher Al concentrations and thus were challenged at lower Al concentrations.

Tolerant and susceptible ratings were given based on 24-h exposure of 3-d-old seedling roots to three concentrations of Al and subsequent staining with hematoxylin. The hematoxylin stain assay was used to determine tolerant and susceptible ratings because it was the assay used to select the isolines. The roots were scored on the same scale described above. Every attempt was made to score a given stain intensity consistently across genotypes and Al concentrations. Therefore, a value of two may indicate the same degree of tolerance as a value of one at a lower Al concentration. Phenotypic classes (tolerant vs. susceptible) were demarcated on the numerical scale for each cross and Al concentration based on the ratings of the parents of that cross.

Segregation ratios were formed on the hypothesis that a single dominant gene was transferred into Chisholm and Century from Atlas 66. We also hypothesized that 2180 possessed a different gene from that transferred from Atlas 66. Segregation ratios were tested for goodness of fit using Chi-squared values.

RESULTS AND DISCUSSION

Dose-Response Experiment

The dose-response curves confirmed differences in Al tolerance as predicted by the hematoxylin assay. A curvilinear model provided the best fit for the susceptible cultivars, TAM 105 (Fig. 1), Chisholm (Fig. 2), and Century (Fig. 3), as the rate of decline in RRL decreased with increasing Al concentration. Century and TAM 105 had almost identical logarithmic relationships, whereas Chisholm appeared less sensitive. This coincides with unpublished field observations that Chisholm is susceptible to acid-soil stress, but not to the same degree as Century, TAM 105, and a host of other HRW cultivars.

The tolerant isolines, in contrast, showed a linear decline in RRL across the range of Al concentrations, consistently maintaining a higher RRL than their recurrent parent (Fig. 2 and 3). Although the tolerant cultivar, Atlas 66, showed a unique curvilinear response (Fig. 1), its RRL was similar to the tolerant isolines at the higher Al concentrations.

Comparison of the susceptible isolines with their corresponding recurrent parent indicates that the original dose responses of Chisholm and Century were not completely restored. The Chisholm-S isoline showed a logarithmic decline in RRL, like Chisholm, but its response was more erratic (Fig. 2). Similar RRL values were found for Century vs. Century-S, but the decline in RRL was curvilinear for Century and linear for Century-S (Fig. 3). The effect of tolerance gene(s) transferred from Atlas 66 was more dramatic in the more susceptible Century background (Fig. 3) than in the Chisholm background (Fig. 2). The RRL of Chisholm and Chisholm-T were indistinguishable at the extremely low and high Al stress levels. These results suggest that differences between Chisholm and Chisholm-T might be difficult to detect under Al-toxic field conditions, depending on the level of Al toxicity.

Genetic Analysis Experiment

Chisholm-T contains one major dominant gene for Al tolerance, contributed by Atlas 66, and possibly some minor genes from its recurrent parent. This is demonstrated by the 3:1 (tolerant:susceptible) ratio of phenotypes in the F_2 progeny of the Chisholm-T/Chisholm cross (Table 2). It is also demonstrated by the 1:1 ratio (tolerant:susceptible) in the backcross to Chisholm, and uniformly tolerant progeny in the backcross to Chisholm-T (Table 2). Century-T, like Chisholm-T, contains one dominant gene for Al tolerance contributed from Atlas 66. The F_2 progeny of the Century-T/Century cross segregated in a 3:1 (tolerant:susceptible) phenotypic ratio (Table 3). The backcross to Century produced a 1:1 (tolerant:susceptible) ratio of phenotypes while the backcross to Century-T produced all tolerant plants.

2180 has a different gene(s) for Al tolerance than the gene transferred to the Chisholm and Century near-isolines from Atlas 66. This was demonstrated by the 15:1 (tolerant:susceptible) segregation ratio in the F_2 generation (Tables 4 and 5), indicating two genes segregating with duplicate dominant epistasis. No segregation was expected among F_1 progeny. All F_1 plants were tolerant. However, in the backcrosses of 2180/Chisholm-T//2180 and 2180/Chisholm-T//Chisholm-T, the plants segregated for tolerance at all Al concentrations. We expected these plants to resemble the F_1 or either parent if tolerance genes in Chisholm-T and 2180 had equal effects. The staining scores of the backcross plants were ≤ 3 (0.36 mM Al), ≤ 4 (0.72 mM Al), and ≤ 5 (0.90 mM Al). In comparison, the staining scores for Chisholm at each concentration were 6,7, and 7, respectively. Even though the plants were classed as susceptible, they were more tolerant than the moderately susceptible Chisholm.

In conclusion, the hematoxylin assay assisted in moving a major gene governing root growth in Al-toxic solution culture into two susceptible backgrounds. Gene expression differed between backgrounds, as Al-induced root inhibition was more pronounced in the Century background. The Al tolerance gene in 2180 and in the tolerant near-isolines is dominant. The Al tolerance gene in 2180 is different from the Al tolerance gene in the tolerant near-isolines transferred from Atlas 66, and their expression does not appear to be additive when co-segregating. These genes must eventually show agronomic benefit under acidic field conditions if they are to be widely deployed.

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Table 1. Genetic materials used to generate dose-response curves in response to a range of Al concentrations in solution culture.

Entry	PI number	Aluminum tolerance rating[a]
Chisholm	PI486219	Moderately susceptible
Chisholm-T(1)	PI561722	Tolerant
Chisholm-T(2)	PI561723	Tolerant
Chisholm-S	PI561726	Moderately susceptible
Century	PI502912	Very susceptible
Century-T(1)	PI561724	Tolerant
Century-T(2)	PI561725	Tolerant
Century-S	PI561727	Very susceptible
TAM 105	CI17826	Susceptible
2180	---	Tolerant
Atlas 66	CI12561	Tolerant

[a] Based on ratings reported by Carver et al. (1993).

Table 2. Reaction of Chisholm, Chisholm-T, F_1, F_2, and backcross progeny to hematoxylin staining at three Al concentrations.

Al Concentration	Genotype[a]	Plant reaction		χ^{2c}	Observed P value
		Tolerant[b]	Susceptible[b]		
mM		no.			
0.18	Chisholm-T/Chisholm F_1	11	1		
	Chisholm-T/Chisholm F_2	123	45	0.1984	0.50-0.75
	Chisholm-T/Chisholm//Chisholm F_1	10	20	2.7000	0.10-0.25
	Chisholm-T/Chisholm//Chisholm-T F_1	21	3		
0.36	Chisholm-T/Chisholm F_1	13	0		
	Chisholm-T/Chisholm F_2	110	36	0.0000	0.995
	Chisholm-T/Chisholm//Chisholm F_1	20	10	2.7000	0.10-0.25
	Chisholm-T/Chisholm//Chisholm-T F_1	24	0		
0.72	Chisholm-T/Chisholm F_1	11	0		
	Chisholm-T/Chisholm F_2	126	42	0.0079	0.90-0.95
	Chisholm-T/Chisholm//Chisholm F_1	15	9	1.0417	0.25-0.50
	Chisholm-T/Chisholm//Chisholm-T F_1	28	0		

[a] Based on a 1-7 scale at 0.18, 0.36, and 0.72 mM Al, respectively, reactions of parents were 3,5,7 (Chisholm); and 1,1,3 (Chisholm-T).

[b] Breakpoints on a 1-7 scale for tolerant and susceptible reactions are stain ratings of 1-2 (0.18), 3-4 (0.36), and 4-5 (0.72), respectively.

[c] Ratio tested in F_2 = 3:1 (tolerant:susceptible); Ratio tested in F_1 = 1:1 (tolerant:susceptible).

Table 3. Reaction of Century, Century-T, F_1, F_2, and backcross progeny to hematoxylin staining at three Al concentrations.

Al Concentration	Genotype[a]	Plant reaction		χ^2[c]	Observed P value
		Tolerant[b]	Susceptible[b]		
mM		no.			
0.18	Century-T/Century F_1	11	2		
	Century-T/Century F_2	101	38	0.2902	0.50-0.75
	Century-T/Century//Century F_1	18	21	0.1026	0.50-0.75
	Century-T/Century//Century-T F_1	33	0		
0.36	Century-T/Century F_1	16	0		
	Century-T/Century F_2	108	34	0.0376	0.75-0.90
	Century-T/Century//Century F_1	13	22	1.8286	0.10-0.25
	Century-T/Century//Century-T F_1	24	2		
0.72	Century-T/Century F_1	13	0		
	Century-T/Century F_2	105	39	0.2315	0.50-0.75
	Century-T/Century//Century F_1	17	14	0.1290	0.50-0.75
	Century-T/Century//Century-T F_1	30	0		

[a] Based on a 1-7 scale at 0.18, 0.36, and 0.72 mM Al, respectively, reactions of parents were 4,7,7 (Century); and 1,2,3 (Century-T).

[b] Breakpoints on a 1-7 scale for tolerant and susceptible reactions are stain ratings of 1-2 (0.18), 4-5 (0.36), and 4-5 (0.72), respectively.

[c] Ratio tested in F_2 = 3:1 (tolerant:susceptible); Ratio tested in F_1 = 1:1 (tolerant:susceptible).

Table 4. Reaction of 2180, Chisholm, F_1, F_2, and backcross progeny to hematoxylin staining at three Al concentrations.

Al Concentration	Genotype[a]	Plant reaction Tolerant[b]	 Susceptible[b]	χ^2 15:1	Observed P value
mM		no.			
0.36	2180/Chisholm-T F_1	28	0		
	2180/Chisholm-T F_2	145	9	0.0017	0.95-0.975
	2180/Chisholm-T//2180 F_1	17	16		
	2180/Chisholm-T//Chisholm-T F_1	12	22		
0.72	2180/Chisholm-T F_1	28	0		
	2180/Chisholm-T F_2	138	12	0.5138	0.25-0.50
	2180/Chisholm-T//2180 F_1	4	28		
	2180/Chisholm-T//Chisholm-T F_1	7	24		
0.90	2180/Chisholm-T F_1	25	0		
	2180/Chisholm-T F_2	141	2	4.9459	0.025-0.05
	2180/Chisholm-T//2180 F_1	30	3		
	2180/Chisholm-T//Chisholm-T F_1	24	10		

[a] Based on a 1-7 scale at 0.36, 0.72, and 0.90 mM Al, respectively, reactions of parents were 1,2,2 (2180); and 1,2,3 (Chisholm-T).

[b] Breakpoints on a 1-7 scale for tolerant and susceptible reactions are stain ratings of 1-2 (0.36), 2-3 (0.72), and 3-4 (0.90), respectively.

Table 5. Reaction of 2180, Century, F_1, F_2, and backcross progeny to hematoxylin staining at three Al concentrations.

Al Concentration	Genotype[a]	Plant reaction: Tolerant[b]	Plant reaction: Susceptible[b]	χ^2 15:1	Observed P value
mM		no.			
0.36	2180/Century-T F_1	9	0		
	2180/Century-T F_2	149	1	7.0560	0.005-0.01
	2180/Century-T//2180 F_1	21	0		
	2180/Century-T//Century-T F_1	29	0		
0.72	2180/Century-T F_1	10	0		
	2180/Century-T F_2	152	3	4.2155	0.025-0.05
	2180/Century-T//2180 F_1	23	1		
	2180/Century-T//Century-T F_1	29	2		
0.90	2180/Century-T F_1	12	0		
	2180/Century-T F_2	137	13	1.1111	0.25-0.50
	2180/Century-T//2180 F_1	25	0		
	2180/Century-T//Century-T F_1	24	7		

[a] Based on a 1-7 scale at 0.36, 0.72, and 0.90 mM Al, respectively, reactions of parents were 1,2,2 (2180); and 2,2,3 (Century-T).

[b] Breakpoints on a 1-7 scale for tolerant and susceptible reactions are stain ratings of 2-3 (0.36), 3-4 (0.72), and 4-5 (0.90), respectively.

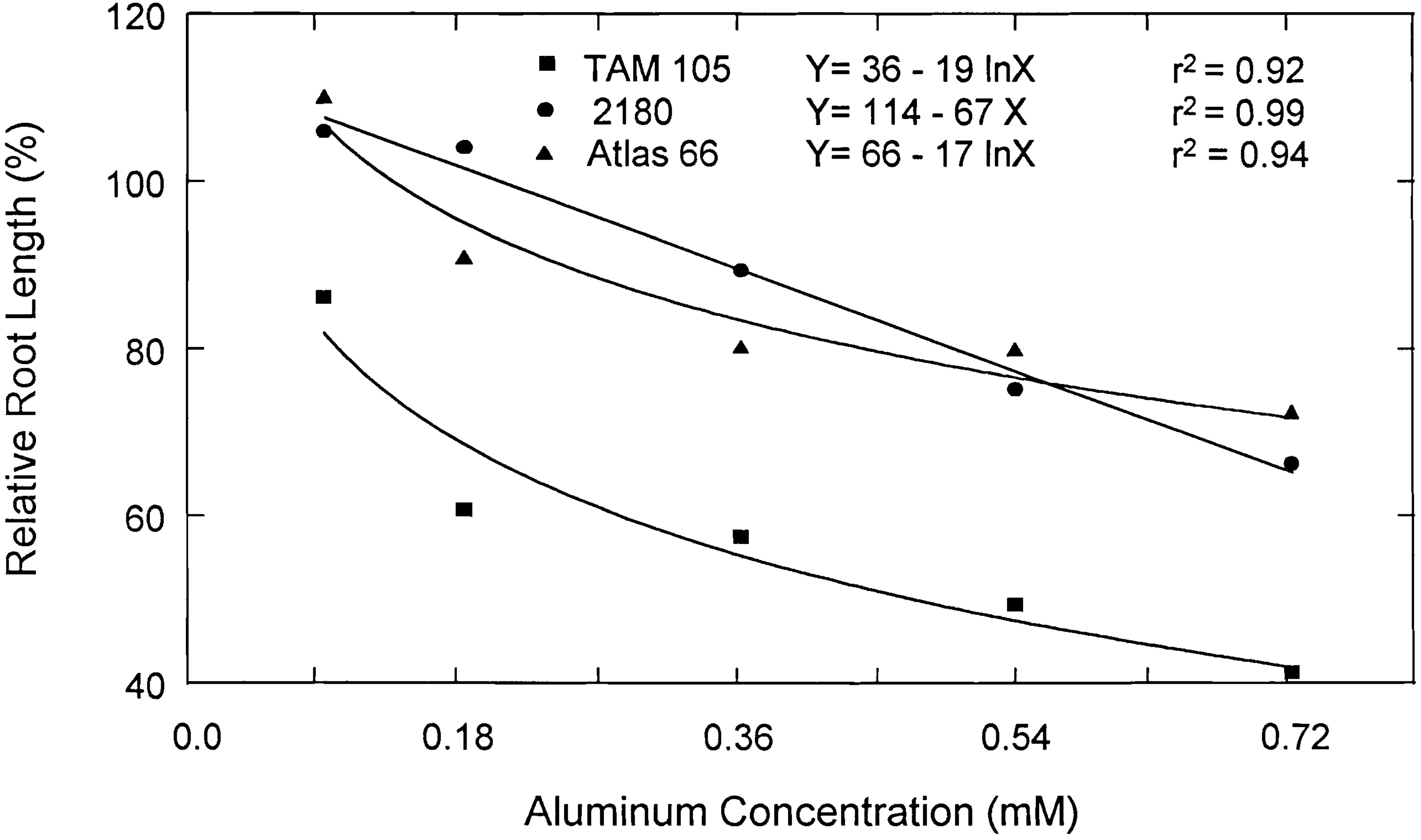

Figure 1. Plot of relative root length vs. Al concentration for three check cultivars.

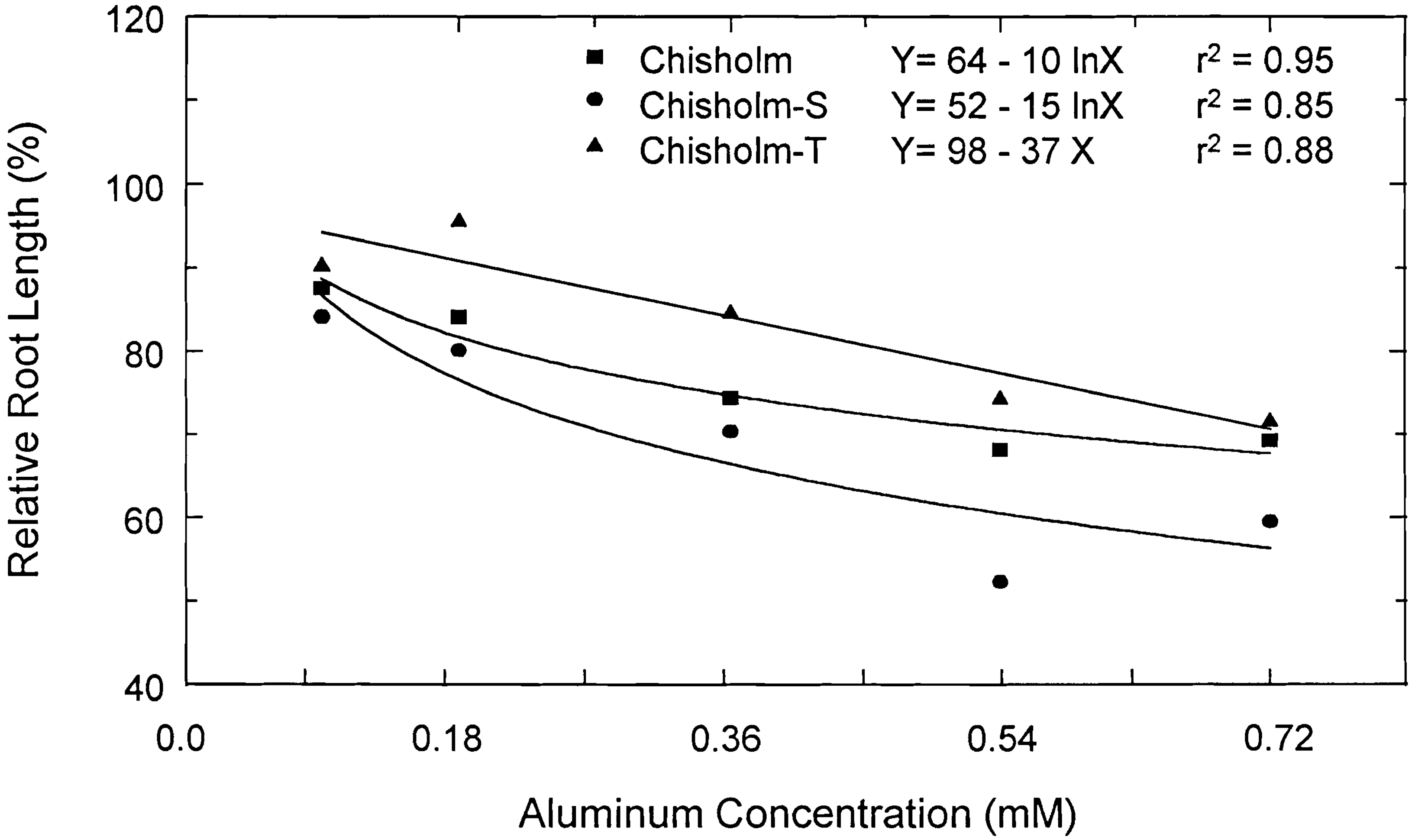

Figure 2. Plot of relative root length vs. Al concentration for Chisholm, its Al-susceptible near-isoline, and the average of its two Al-tolerant near-isolines (Chisholm*4/Atlas 66).

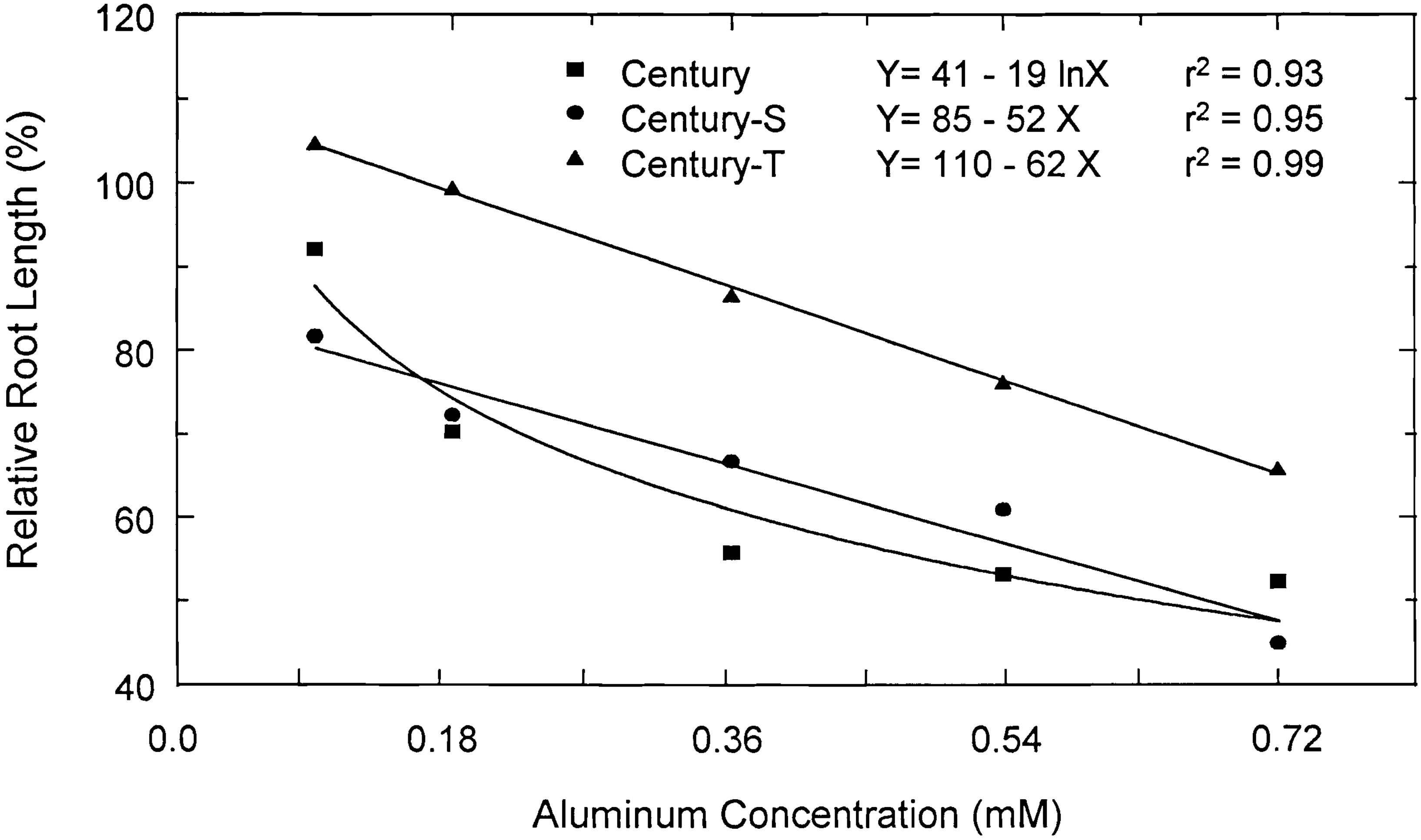

Figure 3. Plot of relative root length vs. Al concentration for Century, its Al-susceptible near-isoline, and the average of its two al-tolerant near-isolines (Century*4/Atlas 66).

CHAPTER III

AGRONOMIC BENEFIT OF ALUMINUM TOLERANCE IN ACIDIC SOILS OF OKLAHOMA

Agronomic Benefit of Aluminum Tolerance in Acidic Soils of Oklahoma

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ABSTRACT

Approximately 0.5 million hectares of hard red winter (HRW) wheat (Triticum aestivum L.) in Oklahoma suffer potential production losses due to soil acidity ($pH<5.5$). Soil acidity in the Great Plains reduces forage and grain yields primarily by increased Al toxicity. Although HRW cultivars are variable in their response to Al toxicity, it is not known to what extent Al tolerance genes enhance forage and grain production in acidic soil. Genetic stocks were produced in two HRW backgrounds ('Chisholm' and 'Century') which are near-isogenic for a gene governing Al tolerance in nutrient solution culture, based on the hematoxylin stain assay. This material was evaluated under surface-acidic conditions in three Oklahoma field environments, with two check cultivars having opposite hematoxylin staining patterns (TAM 105, susceptible; 2180, tolerant). Field responses of the check cultivars were consistent with laboratory assessments. This was demonstrated by almost immeasurable forage and grain yield of TAM 105 in the low pH sites vs. normal yields from 2180 in both the low and normal pH sites. Forage and grain yields were inconsistently enhanced by the presence of aluminum tolerance genes in either background. Grain yield increased in the Chisholm background at Haskell, and forage yield increased in the Century background at Lahoma. There were no significant increases in either background at Stillwater. The agronomic benefit derived

from the Al tolerance mechanism selected in this material is strongly influenced by the environment and/or the genetic background in which it is expressed.

INTRODUCTION

Wheat is the most widely grown cereal crop in the world and the most economically valuable crop in Oklahoma. Miyasaka et al. (1989) estimated that 40% of all cultivated land is acidic. An increasing amount of land under wheat cultivation is also becoming acidic in Oklahoma (Johnson, 1992). According to a 1985 survey by the Oklahoma Cooperative Extension Service, 30% of the samples tested had a $pH < 5.5$ (Johnson et al., 1988). In addition to managing soils to reduce the rate of acidification, HRW wheat must be bred for increased tolerance to acid soils and inherently toxic levels of Al. Soluble Al in the soil becomes toxic to wheat at a $pH < 5.5$ and increases in severity by 1000-fold for every unit decrease in pH (Johnson, 1992).

As land is farmed over time it becomes more acidic depending on parent materials. This is caused by removal of bases with the crop, acid rain, leaching of bases, weathering of acid parent material, and decay of organic matter in the soil (Johnson, 1992). Where vegetative material is removed, as well as grain, the acidification is accelerated because of the increased removal of Ca, Mg, K, and Na. This is especially true for grazing-plus-grain management systems in Oklahoma. The increased use of nitrogen fertilizer also contributes to accelerated soil acidification by increasing net production of H^+ via nitrification of NH_4^+ to NO_3^- (Westerman, 1987). Soil acidification can be corrected with the addition of agricultural lime. However, lime is not always a feasible option under leased-land arrangements or affordable because of high transportation costs.

Another method of reducing the toxic effect of acid soils is genetic improvement of Al tolerance, practiced for several years in the traditionally acid-soil regions of the southeastern USA or of South America. More HRW cultivars should be developed with the objective of improving aluminum tolerance, in addition to traditional objectives like grain and forage yield improvement, disease and insect resistance, and bread-making quality. This is critical to maintaining current yield levels for grain and forage, but may also reduce the lime requirement to a more affordable level. As researchers learn more about the genetic control and agronomic benefit of Al tolerance, they will be more effective in selecting promising lines to use for cultivar improvement.

The main objective of this research was to determine the impact of Al tolerance on grain and forage production under naturally acidic field conditions in Oklahoma. Precise achievement of this objective requires the evaluation of Al tolerant vs. susceptible near-isolines, now available in two HRW wheat backgrounds, Chisholm and Century. The genetic basis for tolerance may by more easily described using these pairs than unrelated cultivars (Carver et al., 1993). Lines were selected by the hematoxylin staining method (Polle et al., 1978; Carver et al., 1988) and evaluated in three Oklahoma field environments.

MATERIALS AND METHODS

Field experiments were established in the 1993-1994 crop season using two pairs of near-isolines, previously described by Carver et al. (1993), and two check cultivars (Table 1). Each pair of Al-tolerant near-isolines from Chisholm and Century were BC_3-F_2 derived sister lines, tested in the F_6 generation. TAM 105 and 2180 were included as a reference for extreme levels of susceptibility and tolerance, respectively, currently known in HRW wheat. The susceptible isolines of Chisholm and Century were included but their data were not reported.

Experiments were conducted at three locations where acid-soil conditions persist: Haskell, Lahoma, and Stillwater, OK. A "normal pH" field site (considered not yield limiting) was also established at each location by applying lime prior to 1989, to raise the soil pH (1:1 soil:H_2O basis) at least one unit. Therefore, each location had two treatments, limed ($pH > 5.0$) and unlimed ($pH < 5.0$), which were applied in separate areas of the field. The soil types consisted of a Taloka silt loam (Haskell), a Grant-Pond Creek silt loam complex (Lahoma), and a Bethany-Kirkland silt loam complex (Stillwater). The plots were replicated three times at each location in a randomized complete block design for each treatment. Date and rate of planting conformed to standard production practices for a forage-plus-grain management system at Lahoma and Stillwater.

At Haskell, planting was delayed by excessive rainfall until October 5, 1993. Plot size was 3.6 m^2 with 5 rows at all locations.

Forage was hand-clipped at Stillwater and Lahoma from each plot at least once in the fall (more than 6 wk after planting) and once in the spring before jointing. Clipping ceased before growing points of any entry in the test reached cutting height. The forage was taken from a 0.6 m^2 interior portion of the plot, when canopy height in the low-pH sites exceeded ca. 5 cm above the soil surface. The remainder of the plot was mowed to a uniform 5-cm height after each harvest. Forage samples were oven-dried at 45°C for more than 3 d and weighed. Fall and spring measurements were combined to estimate total forage yield prior to jointing. Aboveground biomass at maturity, grain yield, and grain yield components were determined from the three interior rows. Number of kernels per spike were determined from 15 spikes randomly sampled from the interior rows of each plot. Spike density (number of spikes per meter squared) was determined in a random 50-cm section of two interior rows.

The data were analyzed using Statistical Analysis System procedures (SAS Inst., 1985). Pre-planned comparisons were made between each susceptible parent and the corresponding tolerant isolines and between TAM 105 and 2180. Data for the two isolines from each cultivar were combined due to similarity in response. Spatial variation in pH was closely monitored in the low-pH plots. Three soil cores (top 15-20 cm) were collected from each plot to determine pH and provide a covariate in the analysis of variance. For those trait-location combinations where the covariate provided a significant reduction in the experimental error variance, analysis of covariance was used where pH accounted for a significant portion of error variance within the model. This was done for grain yield at Haskell (low pH) and for all traits at Lahoma (low pH). Least-squares means were reported in these cases.

RESULTS AND DISCUSSION

The transfer of Al tolerance from Atlas 66 to Chisholm and Century provided significant agronomic benefit under low-pH conditions at Haskell, without changes in performance under normal-pH conditions (Table 2). Aluminum tolerance resulted in a 40% increase in grain yield in the Chisholm background. The 30% increase in the Century background could not be declared significant (observed $\underline{P}=0.19$). Similar changes were noted in aboveground biomass at harvest. The increased biomass and grain yield mostly resulted from increased spike production, not from increased kernel weight (Table 2) or seeds per spike (data not shown). Spike production in the tolerant Century isolines was almost doubled compared to Century. Increased spike production of Al-tolerant genotypes is consistent with previous observations of Carver et al. (1993) and Ruiz-Torres et al. (1992), who found increases of 30% and 12%, respectively, in tolerant sister lines.

The same trends were not observed at the other locations. At Lahoma, forage production was increased 75% by the transfer of Al tolerance to Century, but no change occurred in Chisholm (Table 3). The increased forage yield was not accompanied by improvements in aboveground biomass, grain yield, or spike number at harvest in either background. Acid-soil stress was considered very severe at Lahoma based on early-season growth of the sensitive genotypes. Hence, the selected gene(s) apparently did not provide measurable protection for grain production at that stress intensity. In the 1994-1995 crop season, forage production increased over 5-fold in the Chisholm background and almost 4-fold in the Century background (Appendix Table 3). Gene expression was apparently modified by genetic background and/or by other environmental factors confounded with soil acidity.

At Stillwater, the Al-tolerant isolines provided no detectable advantage for forage and grain yield under low-pH conditions (Table 4). Acid-soil stress was considered moderate at this location based on comparisons of forage yield and spike density between the low-pH and normal-pH sites. In the 1994-1995 crop season, forage yield in Century was increased 27% with the addition of Al tolerance, but there was no improvement in Chisholm (Appendix Table 3). Soil acidity was observed to be more severe at Stillwater in the fall of 1994, evidenced by the photographs in Fig. 1. Visual effects of acid-soil stress were reduced juvenile plant growth first evident at emergence, and restricted tiller formation. Visual differences between the tolerant isolines and their recurrent parent were more noticeable in the Century background (Fig. 2). Differences were less noticeable as the season progressed.

Yield performance of 2180 at Lahoma and Stillwater did not accurately reflect their genetic potential due to poor emergence with early planting. Coleoptile length of 2180 is greatly reduced under warmer soil temperatures encountered with early planting (pers. commun., E.G. Krenzer, Jr.). 2180 would normally exceed Chisholm and Century in forage and grain production under acid-soil conditions (Carver et al., 1993). The sensitive check TAM 105, on the other hand, produced almost immeasurable yields in low-pH trials at Haskell (Table 2) and Lahoma (Table 3). Based on relative forage or grain yields of TAM 105, the intensity of acid-soil stress among locations was Lahoma > Haskell > Stillwater. Suppression of grain yield under normal pH relative to low pH was attributed to i) higher incidence of root rot diseases (*Rhizoctonia* sp. and *Gaeumannomyces graminis* var. *tritici*) in the normal-pH sites (Krenzer and Singleton, 1995), and ii) proportionately greater canopy removal, without removal of growing points, during forage harvest under normal pH, particularly at Lahoma.

In conclusion, agronomic benefits of Al-tolerance were found in forage production prior to jointing and grain yield, predominately a result of increased spike production per unit area. Gene effects were generally neutral in the absence of acid-soil stress. The magnitude of the agronomic benefit is influenced by the environment and by the genetic background in which gene effects are assessed. Field sites with similar pH may produce entirely different results depending on inherent soil chemical properties. Some breeding progress may be expected from selection of "major" genes for Al tolerance (such as the gene transferred from Atlas 66), but further selection is necessary under low-pH field conditions to maximize genetic gains in the target soil environment.

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Table 1. Genetic materials, and their Al tolerance rating, tested in field experiments.

Entry[a]	PI number	Hematoxylin rating[b]	Aluminum tolerance rating
Chisholm	PI486219	P,C,C	Moderately susceptible
Chisholm-T(1)	PI561722	N,N,P	Tolerant
Chisholm-T(2)	PI561723	N,N,P	Tolerant
Century	PI502912	C,C,C	Very susceptible
Century-T(1)	PI561724	N,N,P	Tolerant
Century-T(2)	PI561725	N,N,P	Tolerant
TAM 105	CI17826	C,C,C	Susceptible
2180	---	N,N,P	Tolerant

[a] T(1) and T(2) are tolerant sister lines from selected BC_3F_2 plants as described by Carver et al. (1993).

[b] Hematoxylin staining scores at 0.18, 0.36, and 0.72 mM Al, respectively, according to procedure by Carver et al. (1988).

Table 2. Agronomic traits for two check cultivars, Chisholm, Century, and their Al-tolerant near-isolines under low pH and normal pH soil conditions at Haskell, OK, 1993-1994.

	Low pH[a]				Normal pH[a]			
Genotype or Contrast	Grain yield[b]	Biomass	Spikes m^{-2}	1000-kernel wt.	Grain yield	Biomass	Spikes m^{-2}	1000-kernel wt.
	------ g m^{-2} ------		no.	g	------ g m^{-2} -------		no.	g
Chisholm	161	682	338	29.6	238	922	504	29.1
Chisholm-T(1)	234	862	453	28.1	203	837	532	29.1
Chisholm-T(2)	215	948	516	27.5	224	910	557	32.0
Csm vs. Csm-T(1,2)	**	**	*	NS	NS	NS	NS	NS
Century	144	708	327	24.9	131	755	570	20.1
Century-T(1)	171	893	670	25.5	143	775	678	22.2
Century-T(2)	190	936	596	24.7	111	688	626	23.8
Cty vs. Cty-T(1,2)	NS	**	*	NS	NS	NS	NS	NS
TAM 105	48	486	221	25.2	173	900	607	30.1
2180	221	815	535	25.0	233	771	449	28.4
TAM 105 vs. 2180	**	**	**	NS	*	NS	*	NS
LSD (0.05)	54	186	146	3.7	54	143	149	5.6

*, ** F-test significant at P = 0.05 and 0.01, respectively; NS = not significant.

[a] Low pH (H_2O) = 4.2; Normal pH (H_2O) = 5.3.

[b] Adjusted means based on pH as a covariate.

Table 3. Agronomic traits for two check cultivars, Chisholm, Century, and their Al-tolerant near-isolines under low pH and normal pH soil conditions at Lahoma, OK, 1993-1994.

	Low pH[a]					Normal pH[a]				
Genotype or Contrast	Forage yield[b]	Grain yield[b]	Biomass[b]	Spikes m^{-2}[b]	1000-kernel wt.[b]	Forage yield	Grain yield	Biomass	Spikes m^{-2}	1000-kernel wt.
	------------ g m^{-2}		-------------	no.	g	------------ g m^{-2}		------------	no.	g
Chisholm	80	215	681	654	27.5	240	117	621	902	24.1
Chisholm-T(1)	81	179	645	641	27.9	251	93	594	972	20.7
Chisholm-T(2)	94	191	625	560	26.3	315	98	569	855	23.5
Csm vs. Csm-T(1,2)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Century	63	216	734	710	25.3	245	121	678	876	21.3
Century-T(1)	99	239	778	769	30.0	257	111	636	1108	20.6
Century-T(2)	113	197	707	692	26.5	278	94	544	870	19.5
Cty vs. Cty-T(1,2)	*	NS	NS	NS	NS	NS	NS	*	NS	NS
TAM 105	18	9	170	49	21.5	338	120	841	1095	18.1
2180	89	149	499	752	24.9	193	126	454	743	22.4
TAM 105 vs. 2180	**	**	**	**	NS	**	NS	**	**	**
LSD (0.05)	40	53	144	188	4.8	62	36	96	227	2.8

*, ** F-test significant at P = 0.05 and 0.01, respectively; NS = not significant.

[a] Low pH (H_2O) = 4.2; Normal pH (H_2O) = 5.0.

[b] Adjusted means based on pH as a covariate.

Table 4. Agronomic traits for two check cultivars, Chisholm, Century, and their Al-tolerant near-isolines under low pH and normal pH soil conditions at Stillwater, OK, 1993-1994.

	Low pH[a]					Normal pH[a]				
Genotype or Contrast	Forage yield	Grain yield	Biomass	Spikes m^{-2}	1000-kernel wt.	Forage yield	Grain yield	Biomass	Spikes m^{-2}	1000-kernel wt.
	------------ g m^{-2}		-------------	no.	g	------------ g m^{-2}		------------	no.	g
Chisholm	87	177	631	808	26.2	120	122	492	975	22.5
Chisholm-T(1)	71	167	613	654	26.9	96	130	510	879	24.7
Chisholm-T(2)	95	172	637	801	27.0	86	134	494	800	24.8
Csm vs. Csm-T(1,2)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Century	63	176	681	839	24.1	107	159	671	893	21.9
Century-T(1)	72	141	643	788	26.1	109	147	614	855	24.7
Century-T(2)	93	123	608	781	21.6	144	92	503	848	19.7
Cty vs. Cty-T(1,2)	NS	NS	NS	NS	NS	NS	*	NS	NS	NS
TAM 105	52	166	680	778	22.5	81	152	671	980	22.4
2180	92	173	572	673	23.9	108	117	385	749	22.1
TAM 105 vs. 2180	*	NS	NS	NS	NS	NS	NS	**	*	NS
LSD (0.05)	41	49	122	196	2.3	35	43	155	217	2.8

*, ** F-test significant at P = 0.05 and 0.01, respectively; NS = not significant.

[a] Low pH (H_2O) = 4.9; Normal pH (H_2O) = 6.2.

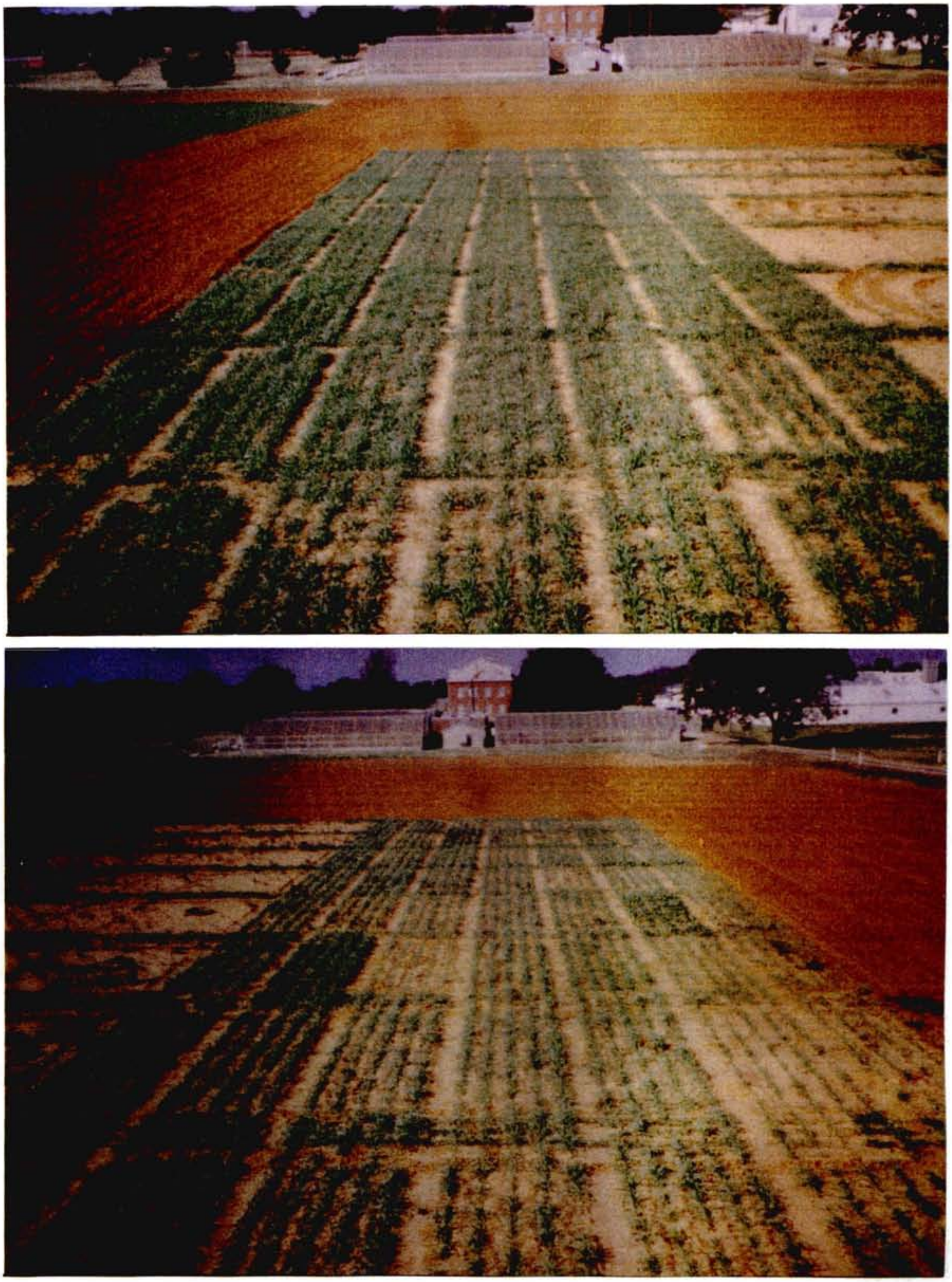

Figure 1. Early-season growth of plots under normal-pH (top) and low-pH (bottom) conditions at Stillwater, OK in October, 1994.

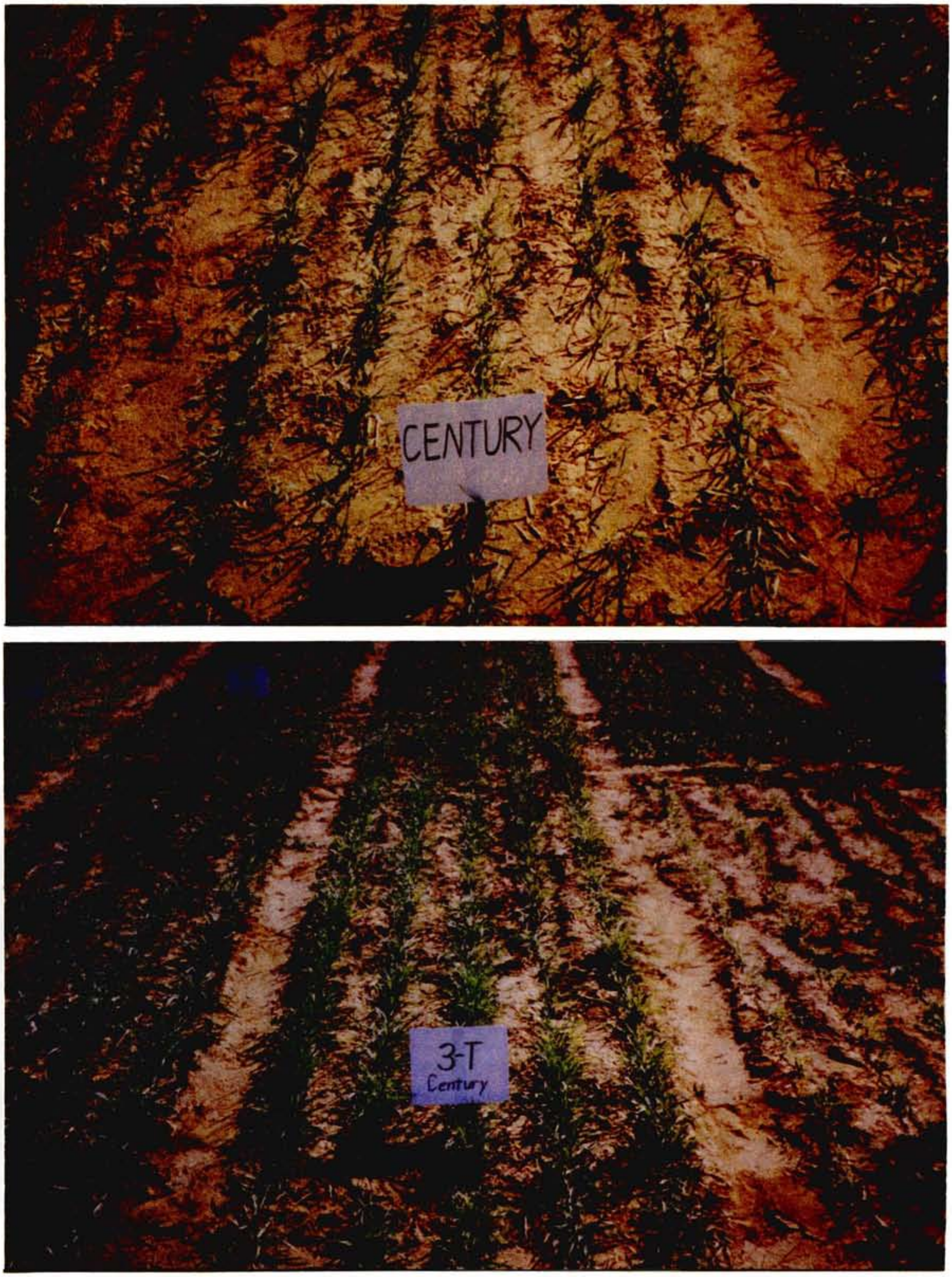

Figure 2. Early-season growth of plots of Century (top) and Century-T (bottom) under low-pH conditions at Stillwater, OK in October, 1994.

APPENDIX

Table 1. Reaction of 2180, Chisholm-S, Century-S, F_1, and F_2 progeny to hematoxylin staining at three Al concentrations.

Al Concentration	Genotype[a]	Plant reaction		χ^2 3:1	Observed P value
		Tolerant[b]	Susceptible[b]		
mM		no.			
0.18	Chisholm-S/2180 F_1	2	4		
	Chisholm-S/2180 F_2	120	32	1.0614	0.25-0.50
	Century-S/2180 F_1	7	1		
	Century-S/2180 F_2	118	35	0.2636	0.50-0.75
0.36	Chisholm-S/2180 F_1	7	0		
	Chisholm-S/2180 F_2	125	30	2.3419	0.10-0.25
	Century-S/2180 F_1	8	0		
	Century-S/2180 F_2	119	34	0.4902	0.25-0.50
0.72	Chisholm-S/2180 F_1	5	0		
	Chisholm-S/2180 F_2	125	35	0.6750	0.25-0.50
	Century-S/2180 F_1	6	0		
	Century-S/2180 F_2	122	25	4.5918	0.025-0.05

[a] Based on a 1-7 scale at 0.18, 0.36, and 0.72 mM Al, respectively, reactions of parents were 1,2,2 (2180); 2,6,7 (Chisholm-S); and 3,7,7 (Century-S).

[b] Breakpoints on a 1-7 scale for tolerant and susceptible reactions are stain ratings of 1-2 (0.18), 2-3 (0.36), and 3-4 (0.72) (Chisholm-S) and 4-5 (0.72) (Century-S), respectively.

Table 2. Selected chemical and physical properties of low-pH soils similar to those used in the study.

Property[a]	Taloka	Pond Creek	Bethany
pH (1:1 soil:H_2O)	4.1	4.0	5.0
CEC, cmol kg^{-1}	3.2	4.2	5.2
Exchangeable bases, cmol kg^{-1}			
Ca	0.67	0.60	1.38
Mg	0.15	0.29	0.57
K	0.22	0.68	0.28
Na	0.16	0.13	0.10
Exchangeable Al, cmol kg^{-1}	0.32	0.49	0.27
Mn, %	0.014	0.011	0.012
Fe, %	0.397	0.513	0.976
Texture, %			
Sand	30	23	45
Silt	58	62	33
Clay	12	15	22

[a] As reported by Sloan (1994).

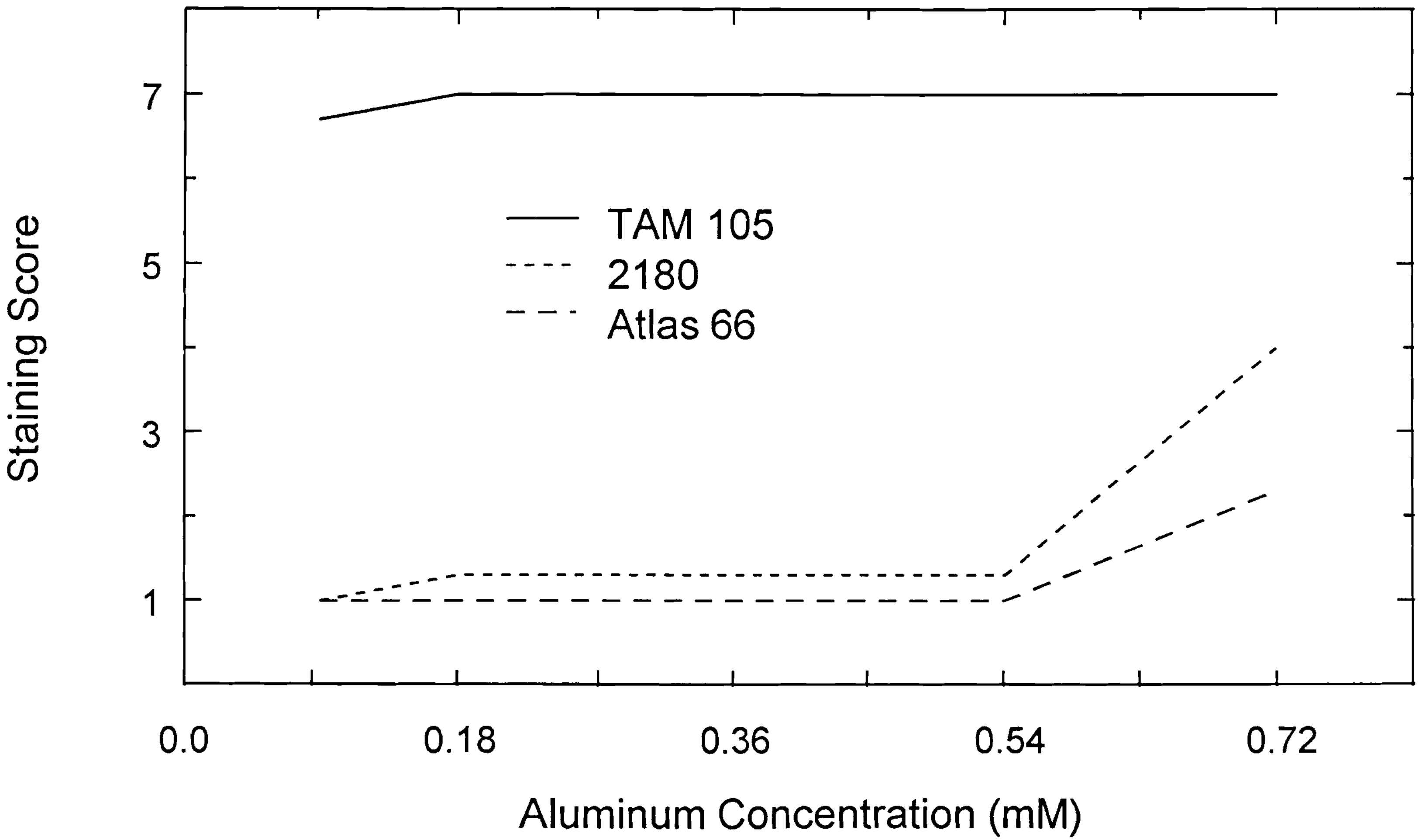

Figure 1. Plot of hematoxylin staining score vs. Al concentration for three check cultivars.

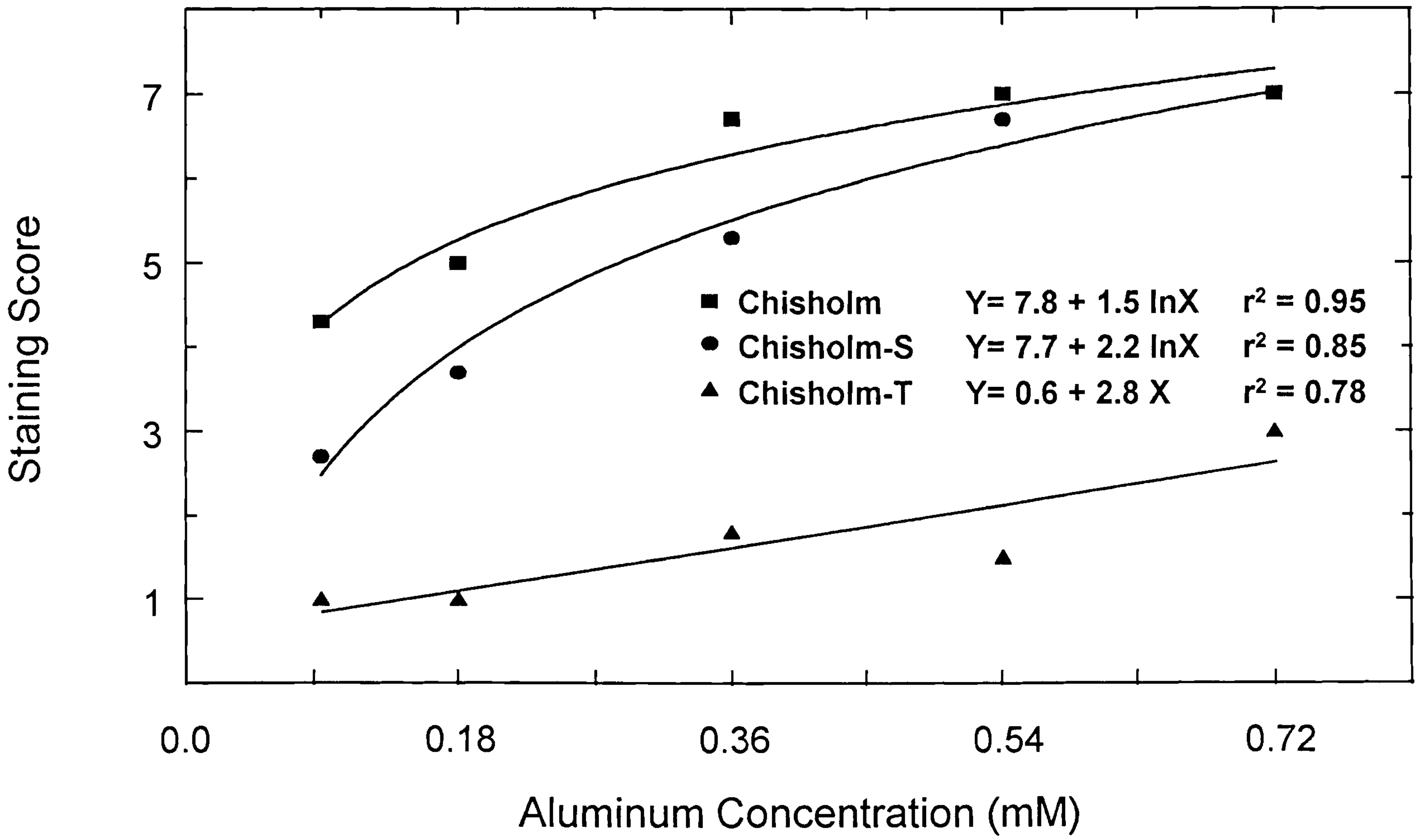

Figure 2. Plot of hematoxylin staining score vs. Al concentration for Chisholm, its Al-susceptible near-isoline, and the average of its two Al-tolerant near-isolines (Chisholm*4/Atlas 66).

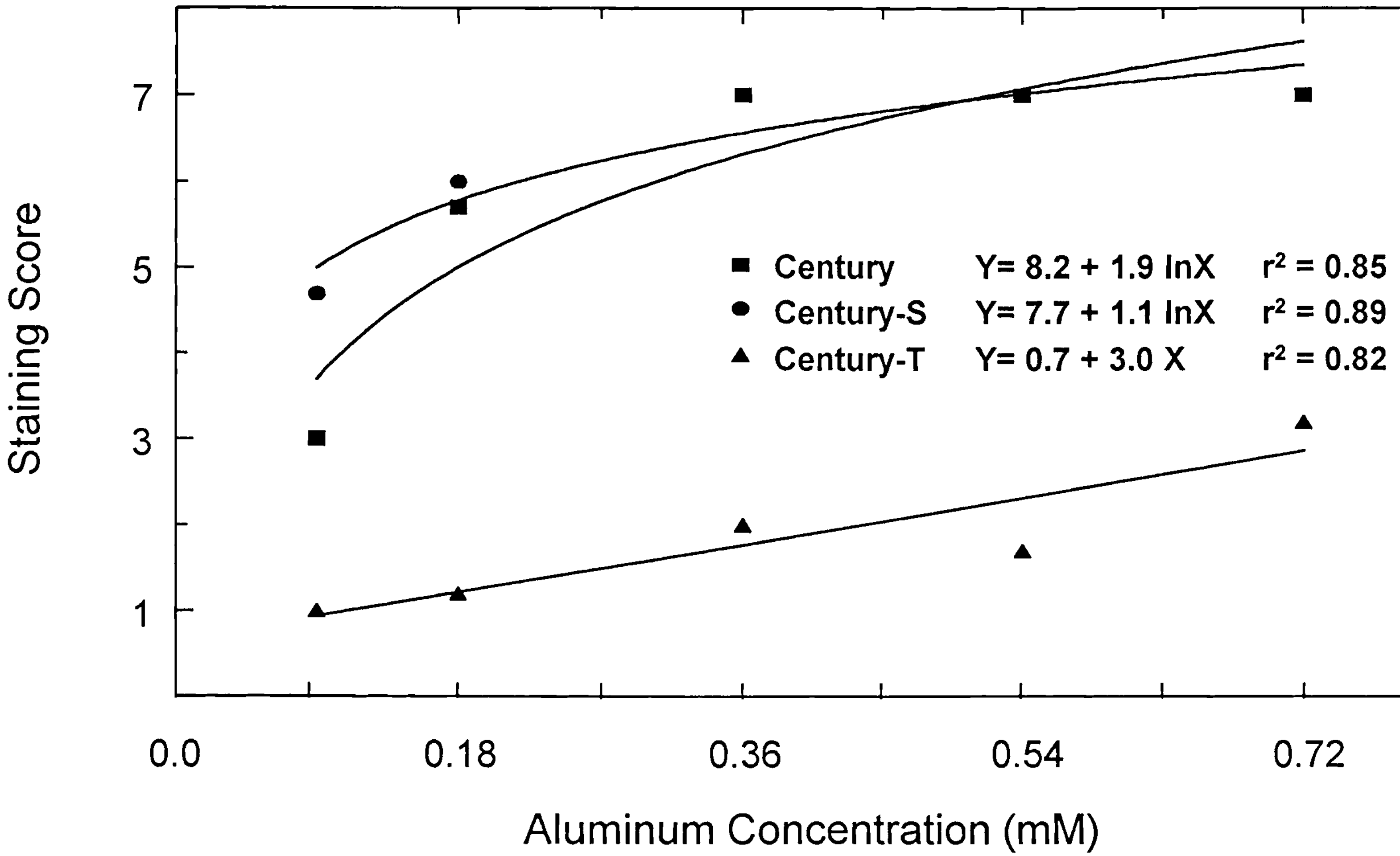

Figure 3. Plot of hematoxylin staining score vs. Al concentration for Century, its Al-susceptible near-isoline, and the average of its two Al-tolerant near-isolines (Century*4/Atlas 66).

VITA

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