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DEVELOPMENT OF ALFALFA STRAINS WITH DIFFERENTIAL TOLERANCE TO ALUMINUM TOXICITY

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SUMMARY

Aluminum toxicity limits root growth in acid subsoils that are difficult to lime. An alternative to subsoil liming is the development of plants having greater tolerance to Al. Alfalfa (*Medicago sativa* L.) is considered an Al-susceptible species. Preliminary studies indicated that alfalfa cultivars differ in Al tolerance, but the extreme plant-to-plant variation within cultivars prevented the establishment of clearcut cultivar differences.

Tolerant and susceptible plants were selected from each of six cultivars ('DuPuits,' 'Atlantic,' 'Team,' 'Buffalo,' 'Grimm,' and 'Sirsa 9') grown on an Al-toxic Bladen soil at pH 4.1 to 4.3. The tolerant selections were repotted and interpollinated to form one population of polycross seed. Susceptible selections were treated similarly to form a second population. These two populations, tolerant and susceptible, were subjected to an additional cycle of recurrent phenotypic selection for tolerance and susceptibility, respectively, to Al-toxic Bladen soil at pH 4.6.

Plants from the population selected for tolerance to the acid Bladen soil were significantly higher in both root and top vigor on Al-toxic Tatum soil than plants from the population selected for susceptibility. The results indicated that Al tolerance is a heritable trait in these alfalfa populations and that recurrent selection can be used effectively to develop strains having differential tolerance to Al-toxic soils. The observation that only 2% of the plants from the tolerant population were in the most tolerant class suggests a good opportunity for more progress in selecting toward Al tolerance.

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is characterized by the ability to produce satisfactory yields of forage, despite summer drought stresses that limit the productivity of other perennial forage species. This drought tolerance is attributed in part to the deep rooting system of

alfalfa, which in suitable soils often penetrates to depths of more than 4 m [4] and permits the plants to draw on moisture reserves in the lower soil horizons. It has been suggested that in the eastern United States, the high level of soluble aluminum in acid subsoils is toxic to the root development of several crop species [1 6]. Root growth restricted to the shallow surface soil increases the vulnerability of the crop to summer droughts. Lime applied to the plow layer does not neutralize subsoil acidity, and liming the subsoils to detoxify the Al is not economically feasible. An alternative solution is the development of varieties having greater tolerance to Al. A recent review by Foy [3] cites reports of varietal differences in response to Al concentration in ryegrass, cotton, wheat, barley, soybeans, rice, potato, and peanuts. Analysis by Reid has shown a dominant gene conditioning tolerance to Al in barley [6]. As a species, alfalfa is extremely sensitive to Al [5], but Ouellette and Dessureaux [4] have reported clonal differences in tolerance. Selfed progeny of alfalfa clones have also been studied for Al response [2]. Simpson [8] recently found that alfalfa clones within cultivars differed significantly in root penetration of acid subsoil in Australia.

Our study was undertaken to determine the feasibility of developing alfalfa strains with contrasting responses to toxic levels of Al. Such strains would be expected to prove useful as germplasm for variety development and as experimental materials for physiological studies of the mechanism of Al toxicity.

MATERIALS AND METHODS

A preliminary greenhouse pot experiment indicated that DuPuits, Atlantic, and Team cultivars of alfalfa were significantly more tolerant to acid (pH 4.6), Al-toxic Bladen soil[3] than were Buffalo, Grimm and Sirsa-9 (unpublished data). However, subsequent experiments with different samples of Bladen soil or with Al in nutrient culture failed to establish clear-cut differences in Al tolerance between these cultivars. These difficulties, also met later by Simpson[7], suggested that there was high plant-to-plant variation in Al tolerance within cultivars. For example, we observed that we reduced variability within a cultivar by liming the soil to about pH 5.1.

To study this problem in greater detail, we grew DuPuits, Atlantic, Team, Buffalo, Grimm, and Sirsa 9 cultivars in triplicate 1.81-kg pots of Bladen soil at pH 4.1 to 4.3. The soil was fertilized with 100, 109, and 137 ppm of N, P, and K, respectively, added as NH_4NO_3 and KH_2PO_4. From each cultivar, the 12 plants with the most vigorous top growth were selected and

repotted in greenhouse potting soil for random interpollination to produce polycross seed. Similarly, 12 plants showing least growth were selected from each cultivar for interpollination. Thus, two populations were initiated, one selected for tolerance and the other for sensitivity to Al-toxic Bladen soil.

For the next cycle of selection, the polycross seeds of the two populations were seeded in wooden flats, 30 × 60 × 7.5 cm. The progeny of each maternal parent was planted as a separate entry. Each entry consisted of three replications of a single row plot of seven seeds. Sixty-four entries were planted for each of the two populations. The rooting medium was Bladen soil, adjusted to pH 4.5 by addition of $CaCO_3$ and fertilized with 100, 109, and 137 ppm of N, P, and K, respectively, added as NH_4NO_3 and KH_2PO_4. The soil was covered with 0.6 cm of 2-Q-ROK* sand. Seeds were placed on this surface and covered with 1.4 cm of 4-Q-ROK graded quartzite sand. The flats were watered with distilled water and then treated with Zineb (zinc ethlenebis [dithiocarbarmate]) to control pathogens. Four weeks after seeding, 209 plants with superior secondary and tertiary root branching and vigorous growth were selected from 1,344 progeny of the plants selected for tolerance. At least two plants were selected from each of 64 progeny. These plants were repotted in greenhouse soil and intercrossed to produce the next generation of the tolerant strain. Similarly, 174 plants with the most restricted root branching and poor vigor were selected for intercrossing from 1,344 progeny of the plants selected for susceptibility to the acid soil. About equal amounts of seed were harvested from each of 161 tolerant plants and bulked. Similarly, equal amounts of seed were bulked from each of 155 susceptible plants.

To test the progress in two cycles of selection, the tolerant and susceptible strains were planted in flats of Al-toxic Tatum soil, adjusted to pH 4.6 with $CaCO_3$ and fertilized with 100, 109, and 137 ppm of N, P, and K, respectively added as NH_4NO_3 and KH_2PO_4. This soil is an acceptable Al-screening medium[6] and was used because it is more readily available to our laboratory than Bladen (Va *vs* Ga). Seeds were planted in rows 3.8 cm apart; each row contained 15 seeds spaced at 2-cm intervals. Eighty replications of three row plots were planted. Strains selected for tolerance or susceptibility were seeded in alternate plots within a flat. Two weeks after seeding, the plots were rated for vigor of top growth on a scale of 1 to 5, with 1 meaning most vigorous and 5 meaning least vigorous. Plants were then washed out of the soil and scored individually for degree of root development, on a scale of 1 to 5, with 1 meaning the best growth and branching and 5 meaning the poorest (Fig. 1).

* Use of Company or Product name by the Department of Agriculture does not imply approval or recommendation of the product to exclusion of others which may also be suitable.

RESULTS

The top growth score of the Al-tolerant strain (2.69) was significantly greater than that of the susceptible strain (4.00) at the 1% level of probability.

Data for the root scores of the tolerant and susceptible strains are reported in Table 1. Figure 1 illustrates the root-score classes.

TABLE 1

Response of two alfalfa populations to recurrent selection for tolerance and susceptibility to Al toxicity as determined by frequency distribution of plants in root score classes and mean root score after 2 weeks' growth on Al-toxic Tatum soil at pH 4.6

Entry	Plants in root score classes* (%)					Mean score
	1	2	3	4	5	
AT 3 (Tolerant strain)	1.75	6.36	31.33	41.41	19.16	3.70
AS 3 (Susceptible strain)	0.19	1.11	11.32	49.09	38.29	4.24
LSD (.01)						0.06
(.01)						0.08

* 1 = vigorous growth, secondary and tertiary branching;
5 = severely stunted growth.

Class 1 was characterized by a well-developed tap root with numerous secondary roots. The longest secondary roots were $\frac{1}{3}$ the length of the tap root. Some tertiary roots had been initiated. Class 2 was characterized by a prominent tap root, but with fewer secondary roots than those in class 1. A few tertiary root initials were present. Class 3 was characterized by a distinct tap root, but with sparse secondary root development. The secondary roots were reduced in length, and some had enlarged root tips. The class-4 root system was severely shortened. The main root was $\frac{1}{4}$ the length of the class 1 taproot. The secondary roots were merely short protrusions from the main axis. All root tips were enlarged, and some were abnormally curved and discolored. In class 5, the primary root and all branch roots were very short and discolored. Some root tips were necrotic.

The root mean score indicates that the root system of the tolerant strain was significantly more vigorous than that of the susceptible strain. The frequency distribution indicates a shift in frequency in all score classes. The tolerant strain showed a higher frequency than

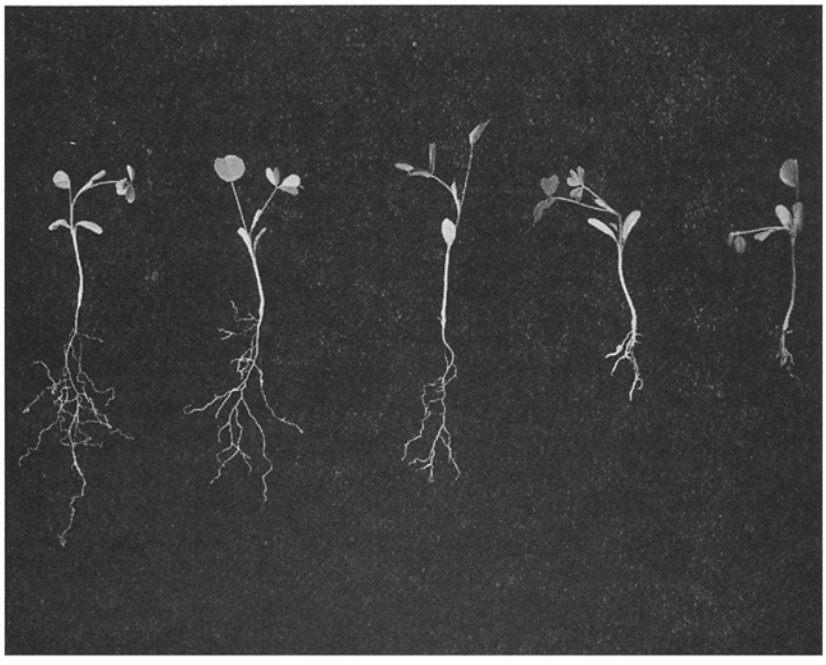

Fig. 1. Differential tolerances of alfalfa seedlings to an acid (pH 4.6) Al-toxic Tatum soil 2 weeks after seeding.
Left to right: Classes 1 to 5 used in Table 1.

the susceptible strain in score classes 1, 2, and 3 and a lower frequency in classes 4 and 5.

The plot top-growth vigor score was correlated with the plot mean-root score. The correlation coefficient was 0.706, with a significance of $P = 0.0001$ for 158 df.

DISCUSSION

These data indicate that there are heritable differences in alfalfa for tolerance to Al toxicity and that recurrent selection can be used effectively to develop alfalfa strains with contrasting responses to Al stress. The strong correlation between the visual top-growth vigor score and the root score suggests that effective selection could be based on the top-growth evaluation alone. Further studies are planned to determine whether equivalent progress is made in selec-

tion for tolerance and susceptibility. Although Al-toxic Bladen soil was used for both cycles of initial selection, the differential responses of the tolerant and susceptible strains were measurable also on an Al-toxic Tatum soil. Therefore, the differential response of these strains is not unique to a Bladen soil, but is a response to the common property of both soils, *i.e.* a high level of soluble Al.

The two strains undergoing selection in this program were selected from diverse parental populations. Because alfalfa is a tetraploid species, and the parental varieties used were both heterozygous and heterogeneous, a broad spectrum of variation would be expected to segregate from the original crosses and be available for selection. The frequency distribution of root-score classes after two cycles of selection indicates that a broad range of variation remains in both populations. Less than 40 per cent of the susceptible strains are in the most-susceptible class 5. Lethality would obviously restrict selection progress toward susceptibility. That less than 2 per cent of the plants in the tolerant strain are in the most-tolerant class 1 would indicate a good opportunity for more progress in selection toward tolerance.

Aluminum-tolerant strains of alfalfa would be expected to utilize subsoil moisture and produce higher yields during frequent droughts in the eastern United States. Advanced strains of alfalfa developed in this program on Al-toxic soils in a growth chamber will be tested later in field plots of acid Al-toxic soils.

In our studies to date, the alfalfa seeds have been used without Rhizobium inoculation, and adequate nitrogen has been supplied in the fertilizer. However, eventually the effects of Al on rhizobial activity, also, should be considered in developing varieties for better tolerance to acid soils.

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