Screening Methods to Develop Alfalfa Germplasms Tolerant of Acid, Aluminum Toxic Soils

M. Dall'Agnol, J. H. Bouton,* and W. A. Parrott

ABSTRACT

Soil acidity and aluminum (Al) toxicity are major problems limiting performance of alfalfa (*Medicago sativa* L.) in many parts of the world, but neither an effective screening procedure nor a tolerant cultivar is available. The objective of this study was to evaluate different screening methods for selection of acid soil tolerant alfalfa germplasms in the greenhouse during 1991-1994. The general screening methods included selection in unlimed soil, selection in unlimed soil containing a limed germination layer, selection for either tolerance or sensitivity to acid soil stress with Al toxicity in tissue culture, selection in unlimed soil with tandem selection for Al tolerance in tissue culture, and selection in unlimed soil containing a limed, fertilized germination layer with tandem selection for Al tolerance in tissue culture. All selected populations and checks were evaluated during 1994 in greenhouse cups containing the following soil treatments: (i) cups filled with unlimed soil, (ii) cups filled with limed soil, and (iii) cups filled with unlimed soil containing a germimation layer of limed soil. Most of the selected populations possessed better root and shoot growth than the original base population (GA-TE) in unlimed soil, but only the population selected in unlimed soil showed better root and shoot growth in unlimed soil with a limed germination layer. No population had poorer performance than GA-TE in limed soil. Selection in cell culture for Al toxicity tolerance did not improve tolerance per se, but selection for Al sensitivity enhanced sensitivity. In terms of success, resources, and time, screening in unlimed soil was the most effective method to improve acid soil stress tolerance.

M.Dall'Agnol, EPAGRI, Caixa Postal 181, 88500, Lages, SC Brazil; J.H. Bouton and W.A. Parrott, Dep. of Crop and Soil Sciences, Univ. of Georgia, Athens, GA 30602-7272. Research supported by state and Hatch funds allocated to the Georgia Agric. Exp. Stn. Received 28 Nov. 1994. *Corresponding author (jbouton@uga.cc.uga.edu).

Published in Crop Sci. 36:64-70 (1996).

SOIL ACIDITY limits plant growth in many parts of the world (Van Wambeke, 1976), with Al and manganese toxicities the most important limiting factors in acid soils (Foy and Fleming, 1978). Sanchez and Salinas (1981) estimated that approximately 55% of the soil in tropical America, 39% in tropical Africa, and 37% in tropical Asia are acid, representing 1.6 billion hectares. In the southeastern USA, Al and manganese toxicity and calcium deficiency are the most limiting soil factors for alfalfa growth (Foy, 1964).

Traditionally, in developed countries, the problem of acid soils has been corrected by liming and fertilization, but an increase in lime and fertilizer costs has reduced their use. Various approaches, such as application of lime, gypsum, and phosphogypsum, have been used to overcome subsoil Al toxicity and promote root development into deeper soil zones (Foy, 1992). However, in most soils, conventional liming of the plow layer does not neutralize subsoil acidity. Surface liming easily can solve the problem of Al toxicity and calcium deficiency in the plow layer, but rooting problems with unamended acid subsoils may limit production (Raij, 1991).

In the past, the approach to soil fertility problems emphasized changing the soil to fit the plant (Foy, 1983a). Tailoring plants to fit the soil may be more economical than changing the soil, and the use of tolerant cultivars could extend the use of sensitive species (Foy 1976, 1983b). Efforts to improve alfalfa adaptation to acid soils have been documented for several years (Ouellette and Dessureaux, 1958; Bouton and Radcliffe, 1989; Smith, 1991; Campbell et al., 1994). Alfalfa is highly sensitive

to acid soils (Bouton and Sumner, 1983), requiring large additions of lime and fertilizer to produce high yields, while increasing production costs. The development of germplasm adapted to these stress conditions could extend alfalfa production and utilization to marginal lands. The reports of Ouellette and Dessureaux (1958) and Dessureaux (1969) showed genetic diversity in alfalfa for the acid soil tolerance trait, as well as the possibility of selecting for Al toxicity tolerance by growing alfalfa in sand culture with excess Al. More recently, several authors (Smith, 1991; Baligar et al. 1993; Campbell et al., 1994) have shown that selection for acid soil stress and/or Al tolerance in alfalfa can be effectively accomplished in acid soil. Similarly, Hartel and Bouton (1989, 1991) reported that alfalfa germplasm selected solely in unlimed soil in the greenhouse possessed good acid stress tolerance in the field, especially when inoculated with an acid soil-adapted strain of *Rhizobium meliloti*.

Tissue culture has been used to select plants for tolerance to acid soils. Campbell et al. (1988) suggested using in vitro selection and regeneration of plants from Al tolerant alfalfa cells as an alternative breeding method. Parrott and Bouton (1990) tested two alfalfa germplasms that differed in acid soil tolerance and reported that callus growth from randomly selected plants within each germplasm differed in their level of Al tolerance when subjected to a modified, Al toxic Blaydes medium. The results indicated tissue culture could be used as a bioassay to screen for cellular Al tolerance. Genotypes identified as possessing good cellular tolerance could then be used as parents to develop more tolerant germplasm. Kamp-Glass et al. (1993) reported a method for improving Al tolerance in alfalfa by using an acid-Al toxic medium to induce callus formation and embryo development. Preliminary results indicated that plants regenerated with this medium were more tolerant than nonselected controls.

In spite of the efforts in screening and developing alfalfa germplasms for acid soil stress and Al toxicity tolerance, no tolerant cultivar or reliable screening procedure is available and widely used. One of the reasons may be the lack of understanding about mechanisms involved in tolerance and the conflicting results sometimes obtained. Campbell et al. (1988, 1989) tested several genotypes of alfalfa for tolerance to toxic levels of Al in nutrient solution and soil and found no relationship between the two. They concluded that tolerance in nutrient solution and soil could involve different mechanisms.

The objective of this study was to evaluate the effectiveness of different soil and cell culture screening techniques for improvement of acid soil tolerance in alfalfa.

MATERIALS AND METHODS

All greenhouse tests were conducted during 1991-1994 with 720-mL styrofoam cups filled with 930 g of soil. The unlimed soil treatment was a Cecil sandy clay loam (clayey, kaolinitic, thermic, Typic Kanhapludults) collected in 1991 from The University of Georgia Plant Sciences Farm, near Athens, GA. The soil had the following characteristics: pH_{water} = 4.7; Al_{KCl} = 0.29 cmol kg^{-1}; Ca = 0.283 cmol kg^{-1}; Mg = 0.073 cmol kg^{-1}; P = 7 kg ha^{-1}; and K = 104 kg ha^{-1}. Lime and nutrients were added to some of the unlimed soil to achieve the following limed soil treatment: pH_{water} = 6.5; Al_{KCl} = 0.0 cmol kg^{-1}; Ca = 1.80 cmol kg^{-1}; Mg = 0.56 cmol kg^{-1}; P = 72 kg ha^{-1}; and K = 240 kg ha^{-1}. All soil was air-dried and sieved through a 3-mm screen before placement into cups. Approximately 20 to 25 seeds were sown in each cup and covered with 50 g of sand. The cups were watered by weight to 75% field capacity with distilled water and rewatered to that level every 2 to 4 d. At sowing and again 4 to 6 d later, a mixture of fungicides, metalaxyl [*N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl) alanine methyl ester] and chlorotalonil (tetrachloroisophthalonitrile) was applied as a foliar and a soil drench at 20 mL per cup at a rate of 300 mg a.i. m^{-2} and 460 mg a.i. m^{-2}, respectively, for control of seedling diseases. One week after sowing, seedlings were randomly thinned to 10 per cup and inoculated with 10 mL of the acid soil tolerant *Rhizobium meliloti* strain 59 (Hartel and Bouton, 1989) at 10^7 cells mL^{-1}. Plants were then grown for an additional 7 wk (approximately 8 wk from time of sowing) before termination. For selection of tolerant plants, all plants in the cup were washed free of soil, and one plant in each cup with the best nodulation and growth of roots and shoots was selected. For testing dry matter yield, the cups were marked 11.25 cm from the base and cut into two portions, labelled "roots" and "shoots," washed free of soil, and dry weight determined after drying at 65°C for 72 h. The roots included all roots below the cutting point. The shoots included the first 3.5 cm of roots, the crown region and the shoots.

The tissue culture procedure was basically a bioassay to evaluate the ability of a single plant to produce Al tolerant calli (Parrott and Bouton, 1990). Calli were induced for 8 wk in a modified Blaydes medium from surface-sterilized petioles from each plant. The resulting calli were divided into equally-sized pieces and transferred to two different media. The first (−Al) was a Blaydes medium modified by reducing the calcium to 100 μ*M*, the phosphorus to 10 μ*M*, and the pH to 4.0. The second medium (+Al) was obtained by adding 400 μ*M* Al^{3+} in the form of $AlCl_3$. The Al activity of this medium was calculated with the MINTEQ computer program (Felmy et al., 1984) and 91 % of the added Al was in the solution as Al^{3+}. Calli were subcultured to fresh medium every two wk. After 6 wk of growth, the fresh weight of calli on both the +Al and −Al medium was determined.

Population Selection and Development

The base population was Georgia-Tifton Elite (GA-TE), an experimental developed from three cultivars that were well adapted to the southeastern USA (Apollo, Florida 77, and Saranac AR) (J.H. Bouton, 1985, unpublished data). The following screening methods were used to select the Cycle 1 populations.

Greenhouse Selection in Unlimed Soil

One hundred cups containing unlimed soil were planted with seed from GA-TE and the best plant in each cup was selected, transplanted to new pots, allowed to flower, and intercrossed by hand. Equal quantities of seed from each plant were bulked to form the *unlimed* population.

Greenhouse Selection in Limed-Unlimed Soil

In this method, 100 cups were initially filled with 730 g of unlimed soil and then covered with 200 g of limed soil. GA-TE was sown and the best plant was selected and intercrossed. Equal quantities of seed were bulked to form the *limed-unlimed* population.

Cell Culture Selection for High or Low Callus Growth Ratio

Initially, 100 plants from GA-TE were randomly planted in a greenhouse. The 15 best plants from the 100 were selected based on the ratio obtained by dividing callus growth on +Al medium by callus growth on −Al medium. These plants were intercrossed by hand and equal quantities of seed from each plant were bulked to form the *high ratio* germplasm. As a control, 15 of the 100 plants with a low ratio were selected and intermated. Equal seed quantites were bulked to form the *low ratio* population.

Tandem Selection Based on Unlimed Soil and High Callus Growth Ratio

Petioles from the same 100 plants selected to produce the unlimed population were also used to initiate and screen calli on the +Al and −Al media. The 10 plants whose calli had the highest +Al/−Al ratios were selected and intercrossed to create the *unlimed-high ratio* population.

Tandem Selection Based on Unlimed-Limed Soil Assay and High Callus Growth Ratio

Petioles from the same 100 plants selected to produce the *limed-unlimed* population were also used to initiate and screen calli on the +Al and −Al media. The 10 plants whose calli had the highest +Al/−Al ratios were selected and intercrossed to create the *limed-unlimed high* ratio population.

Each of these populations were subjected to a second cycle of selection as previously described. However, another criterion, net callus growth, was added as an alternative selection parameter to produce an additional four populations for the callus-based assays. Ten plants with the best net callus growth on +Al medium were selected and intercrossed by hand. Equal amounts of seed from each plant were bulked to form the *high net*, *unlimed-high net*, and *limed-unlimed high* net populations. Likewise, the 10 plants with the lowest net callus growth on +Al medium were selected and intercrossed by hand. Equal amounts of seed from each plant were bulked to form the *low net* population.

Germplasm Testing

Greenhouse

All 10 populations were evaluated for acid soil stress tolerance in the greenhouse during 1994 (Exp. 1). The GA-TE base population, GA-AT, an acid, Al-tolerant population (Bouton and Radcliffe, 1989), and sericea lespedeza, *Lespedeza cuneata* [(Dum. Cours.) G. Don., cv Serala], a legume known for its superior acid soil tolerance (Joost and Hoveland, 1986; Joost et al., 1986), were included as checks. Each alfalfa population and sericea was grown in a factorial combination with the following soil treatments: completely unlimed soil, unlimed soil containing a 200-g germination layer of limed soil, and completely limed soil. These factorialized treatments were replicated six times. Root and shoot yields were recorded on each experimental unit (cup). Analysis of variance was performed with the Procedure GLM (SAS Institute, 1982) procedure, with populations (including checks) and soil treatments considered fixed effects and replications considered a random effect.

The experiment was repeated (Exp. 2) with the same experimental design, but with fewer populations and checks. These were unlimed, limed-unlimed, high net, low net, limed-unlimed high ratio, limed-unlimed high net, GA-TE, and GA-AT.

Tissue Culture

The high net, low net, GA-TE, and GA-AT populations were assessed for their in vitro Al tolerance. The experiment was replicated three times, each rep using callus from 16 randomly selected seedlings of each population and check. Six weeks after callus induction calli were divided and transferred to +Al or −Al media. Calli from individual seedlings were maintained in 20- × 100-mm petri dishes with 35 mL of medium, and incubated at 25°C with a 23-h photoperiod provided by fluorescent lighting (20 μmol photons m^{-2} s^{-1}). Analysis of variance was performed with the Procedure ANOVA (SAS Institute, 1982) procedure. Media and alfalfa populations were considered fixed effects, while replicates and genotypes within populations were considered random effects.

RESULTS

In the greenhouse, unlimed soil and unlimed soil containing a limed germination layer significantly reduced ($P < 0.01$) forage yield of all populations when compared to growth in limed soil (Fig. 1). The average reduction on unlimed soil with a limed germination layer was 24% for shoots and 57% for roots. For completely unlimed soil, the average reduction was 93% for shoot growth and 92% for root growth. The analysis of variance showed a significant ($P < 0.01$) population × soil treatment interaction (data not shown) for both shoots and roots, indicating a differential response to the soil treatments among the populations.

For the checks, sericea lespedeza had the poorest root yield, while GA-TE (base population) was equivalent in shoot and root growth to GA-AT (acid, Al-tolerant population) for all three soil treatments in Exp. 1 (Tables 1, 2, and 3). In unlimed soil, the root yield of all populations, with the exception of unlimed-high ratio, unlimed-high net, and high ratio, was significantly higher than that of GA-TE (Table 1). In addition, all populations (regardless of selection conditions) contained higher shoot yields than GA-TE when grown in the unlimed soil. When tested in unlimed soil containing a limed

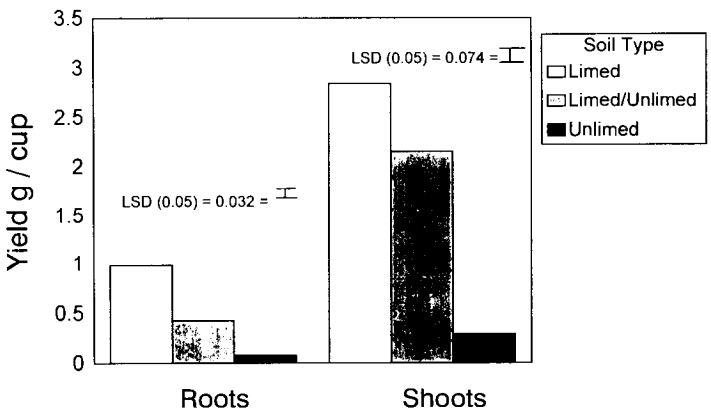

Fig. 1. Average yield of all alfalfa populations in response to different soil treatments. Column means within the error bar are not significantly different ($P < 0.01$) by the LSD test. Shoots represent the upper 3.5 cm (point where the cups were cut) of roots, the crown region and the shoots, while the roots include all roots below the cutting point.

Table 1. Dry matter yield of alfalfa populations selected using different soil and cell culture screening methods when tested in greenhouse cups containing 0.9 kg of unlimed, acid Cecil soil.

Screening method†: Population	Exp. 1		Exp. 2	
	Roots	Shoots	Roots	Shoots
	g cup^{-1}			
Soil:				
Unlimed	0.084 abcd‡	0.236 a	0.112 a	0.226 a
Limed/unlimed	0.090 abc	0.208 abcd	0.105 ab	0.193 abc
Cell culture:				
High ratio	0.071 de	0.216 abcd	–	–
High net	0.086 abcd	0.208 abcd	0.111 a	0.209 ab
Low ratio	0.086 abcd	0.191 d	–	–
Low net	0.095 ab	0.233 ab	0.085 bc	0.142 cd
Tandem soil-cell culture:				
Unlimed-high ratio	0.078 cd	0.209 abcd	–	–
Unlimed-high net	0.072 de	0.182 d	–	–
Limed/unlimed-high ratio	0.080 bcd	0.194 cd	0.110 a	0.157 bc
Limed/unlimed-high net	0.096 ab	0.230 abc	0.100 ab	0.165 bc
Checks:				
GA-TE (base population)	0.059 e	0.140 e	0.072 c	0.135 cd
GA-AT (tolerant population)	0.070 de	0.196 bcd	0.044 d	0.096 de
Sericea lespedeza	0.042 f	0.244 a	–	–
CV (%)	18.1	15.7	23.6	31.1

† Soil: unlimed = no treatment to acid Cecil soil; limed/unlimed = unlimed soil containing a limed, fertilized germination zone; Cell Culture: ratio = calli wt in + Al media/calli wt. in − Al media; net = calli wt. in + Al media.
‡ Means followed by the same letter are not significantly different ($P < 0.05$) according to LSD.

germination layer, only the population selected in unlimed soil produced consistently greater shoot and root yield when compared to the checks (Table 2). Finally, when tested in limed soil, only the population selected in limed/unlimed soil with tandem selection for high ratio in cell culture showed significantly higher root yield than the checks, while the limed-unlimed high ratio, unlimed, low net, and high net populations had higher shoot yields (Table 3). Retesting (Exp. 2) fewer selected populations showed similar trends to those reported above (Tables 1, 2, and 3).

No differences were found between the population whose parents were selected based on high net callus growth in +Al media (high net) and the population whose parents were selected for low net growth (low net) when compared to the two checks evaluated in either +Al or −Al media (Table 4). However, when an Al tolerance ratio was calculated (e.g., callus growth in +Al-callus growth in −Al), the population whose parents were selected based on low net growth in +Al media had a lower ratio than the other populations (Table 4).

DISCUSSION

Among the three soil treatments, the unlimed soil gave the best measure of acid soil stress tolerance. However, the unlimed soil with a limed soil germination layer treatment simulated a more realistic measure of acid soil

Table 2. Dry matter yield of alfalfa populations selected using different soil and cell culture screening methods when tested in greenhouse cups containing 0.7 kg of unlimed, acid Cecil soil overlain with a 0.2 kg germination layer of the same soil limed and fertilized.

Screening method†: Population	Exp. 1		Exp. 2	
	Roots	Shoots	Roots	Shoots
	g cup^{-1}			
Soil:				
Unlimed	0.512 a‡	2.542 a	0.182 a	0.837 abc
Limed/unlimed	0.364 de	2.031 cd	0.208 a	0.847 abc
Cell culture:				
High ratio	0.333 e	2.104 bcd	–	–
High net	0.497 abc	2.288 abc	0.191 a	0.815 bc
Low ratio	0.442 bcd	2.192 bcd	–	–
Low net	0.427 bcd	2.208 bcd	0.202 a	0.874 bc
Tandem soil-cell culture:				
Unlimed-high ratio	0.428 bcd	1.984 d	–	–
Unlimed-high net	0.510 ab	2.194 bcd	–	–
Limed/unlimed-high ratio	0.449 abcd	2.337 ab	0.204 a	0.992 a
Limed/unlimed-high net	0.417 cde	2.329 ab	0.207 a	0.913 ab
Checks:				
GA-TE (base population)	0.422 cd	2.085 bcd	0.174 a	0.695 c
GA-AT (tolerant population)	0.422 cd	2.036 cd	0.203 a	0.785 bc
Sericea lespedeza	0.152 f	1.393 e	–	–
CV (%)	17.9	11.5	24.9	14.3

† Soil: unlimed = no treatment to acid Cecil soil; limed/unlimed = unlimed soil containing a limed, fertilized germination zone; Cell Culture: ratio = calli wt in + Al media/calli wt. in − Al media; net = calli wt. in + Al media.
‡ Means followed by the same letter are not significantly different ($P < 0.05$) according to LSD.

Table 3. Dry matter yield of alfalfa populations selected using different soil and cell culture screening methods and tested in greenhouse cups containing 0.9 kg of limed and fertilized Cecil soil.

Screening method†: Population	Exp. 1		Exp. 2	
	Roots	Shoots	Roots	Shoots
	g cup^{-1}			
Soil:				
Unlimed	1.150 ab‡	3.296 a	0.419 ab	1.463 b
Limed/unlimed	1.119 ab	3.179 abc	0.441 a	1.307 bc
Cell culture:				
High ratio	0.826 de	2.713 ef	–	–
High net	1.101 ab	3.279 ab	0.396 ab	1.257 bc
Low ratio	1.107 bcd	2.724 def	–	–
Low net	1.149 ab	3.172 abc	0.361 bc	1.174 bcd
Tandem soil-cell culture:				
Unlimed-high ratio	1.092 abc	2.823 cdef	–	–
Unlimed-high net	0.982 bcd	2.690 ef	–	–
Limed/unlimed-high ratio	1.279 a	3.413 a	0.402 ab	2.679 a
Limed/unlimed-high net	0.982 bcd	2.690 ef	0.426 ab	1.400 b
Checks:				
GA-TE (base population)	0.989 bcd	2.560 fg	0.322 c	1.013 cd
GA-AT (tolerant population)	0.890 cde	2.480 fg	0.249 d	0.897
Sericea lespedeza	0.241 f	1.776 h	–	–
CV (%)	17.8	12.9	14.2	18.8

† Soil: unlimed = no treatment to acid Cecil soil; limed/unlimed = unlimed soil containing a limed, fertilized germination zone; Cell Culture: ratio = calli wt in + Al media/calli wt. in − Al media; net = calli wt. in + Al media.
‡ Means followed by the same letter are not significantly different ($P < 0.05$) according to LSD.

stress tolerance, since most alfalfa is generally planted in a limed and fertilized acid topsoil but unlimed subsoil. Root growth into the acid subsoil layer is considered an advantageous trait in an acid soil stress breeding program (Bouton and Radcliffe, 1989). The limed soil served as a check to measure the influence of selection on the seedling vigor and forage yield of each population.

For the checks, sericea lespedeza was not a good control because of the short duration of the tests. In this work, alfalfa root yields were much higher than those of lespedeza in all soils (Tables 1, 2, and 3), which is in agreement with the findings of Joost and Hoveland (1986) and Joost et al. (1986). Sericea lespedeza has slow seedling growth relative to that of alfalfa. GA-AT possesses good acid soil stress and in vitro Al tolerance (Hartel and Bouton, 1989; Hartel and Bouton, 1991; Parrott and Bouton, 1990). The base level of acid soil stress and in vitro Al toxicity tolerance of GA-TE was no different from GA-AT (Tables 1 and 2). The reason for equal tolerance is unknown. The performance of individual selections was based primarily on root and shoot yield relative to the base population, GA-TE.

Table 4. Callus weight of different alfalfa populations when tested in tissue culture media with (+) and without (−) Al.

Selection method†: Germplasm	+ Al	− Al	Ratio‡
	mg		
Cell Culture:			
High net	0.796	1.38	0.58 a§
Low net	0.701	1.47	0.48 b
Checks:			
GA-TE	0.792	1.24	0.64 a
GA-AT	0.798	1.24	0.64 a
CV (%)	26.2	24.7	25.8

† Cell culture: ratio = calli wt in + Al media/calli wt. in − Al media; net = calli wt. in + Al media.
‡ Ratio = Calli wt. in medium + Al/calli wt. in medium − Al.
§ Means followed by the same letter are not significantly different ($P < 0.05$) according to LSD.

The population selected solely on the basis of good nodulation and root and shoot growth in unlimed soil demonstrated the best and most consistent acid soil stress tolerance (Tables 1 and 2). Selection in unlimed, unfertilized soil was a cheap and rapid bioassay compared to tissue culture. Different tolerance mechanisms may have been functioning among the populations grown in acid soils (Blum, 1988; Sartain and Kamprath, 1978). When breeding for yield performance in stress environments, direct selection in the target environment is most efficient (Ceccarelli, 1989); however, a separate breeding program may be needed to ensure yield gains in low-yielding systems (Atlin and Frey, 1990). Selection for acid soil tolerance did not negatively affect the root or shoot yield of the populations compared to their performance in limed soil (Table 3). This suggested that increased stress tolerance and yield potential need not be mutually exclusive (Duncan and Baligar, 1990; Ceccarelli and Grando, 1993).

Smith (1991) studied the heritability of several traits associated with acid soil tolerance in alfalfa. The highest narrow sense heritability estimates (0.58) were calculated for net root growth into an acid subsoil layer (e.g., equivalent to selection for the limed-unlimed population). He also reported that shoot growth and the ratio of root yield in unlimed soil to root yield on limed soil had low narrow sense heritability estimates (0.18 and −0.10, respectively). These observations suggested that selecting for tolerance based on net root growth into an acid subsoil layer should be highly effective. Nevertheless, the population selected in limed-unlimed conditions did not show an advantage over its base population and was inferior to the population selected in totally unlimed soil even when evaluated in limed/unlimed conditions. Root growth into a completely unlimed soil may be more effective in screening tolerant genotypes than root growth into an unlimed layer. Selection based on root growth

alone may be insufficient, and selection for shoot growth at pH levels below 5.0 may be more effective (Simpson et al., 1977), especially since root growth is extremely variable in acid soils (Simpson et al., 1979).

Scott and Fischer (1989) proposed a model to explain the differential responses of plants to acid soils and to show the limitations of acid tolerance ratings based on ratios of yield performance in acid stressed soils relative to yield performance in limed soil. They reported that ratios were inappropriate, since they show sensitive types to be more responsive because of their low yield under nonstress conditions. Germplasm that has a high tolerance ratio often has low yield potential and/or lack of general adaptation (Scott and Fischer, 1989). In the present study, if ratios are calculated based on mean yield of each population in the different soil treatments, this point can be illustrated. The population with the highest ratio of root dry wt in unlimed soil to root dry weight in limed soil was sericea lespedeza (ratio = 0.17). This species is acid-tolerant, but low-yielding. In contrast, the population selected in unlimed soil exhibited the best growth in acid soil (Tables 1 and 2) and possessed a ratio (0.07) similar to GA-TE (0.06), which was almost 2.5 times smaller than the sericea ratio. This lack of consistency when ratios are used is in agreement with Smith (1991).

Since a germplasm with good acid soil stress tolerance at the whole plant level was found to possess good Al toxicity tolerance at the cellular level, screening cells from a plant for Al toxicity tolerance in vitro may help identify Al tolerant plants (Parrott and Bouton, 1990). When compared with the approach of regenerating plants from tolerant cell lines, the use of tissue culture in this manner (a bioassay) does not produce negatively associated somaclonal variation (Evans, 1989). However, selection in unlimed soil with tandem selection in cell culture for high net or high ratio callus growth produced less tolerant populations when compared with the populations selected only in unlimed soil (Tables 1 and 2).

When populations were selected solely for low and high net callus growth in tissue culture, a highly significant ($P < 0.001$) effect of medium was found on callus growth, with the +Al medium causing a 32 to 52% reduction in growth, depending on the population (Table 4). Although no significant differences were found between the selected populations for net callus growth in both media, a significant interaction ($P < 0.05$) was detected between the genetic material and the media, indicating a differential response of the populations to the different media. Although not statistically different, the population selected for low net growth in +Al medium had poor growth on this medium, but high callus growth on the −Al medium. This suggested that the mechanisms regulating callus growth in the presence of Al may be different from those affecting callus growth in its absence. The only significant ($P < 0.05$) difference among populations was the callus ratio of the low net population that had a smaller ratio than other populations, demonstrating that in vitro selection for Al sensitivity had been effective (Table 4).

The lack of a detectable growth response of the population subjected to selection for improved growth of cells in +Al medium was probably due to the large variation observed in genotypes within populations. This variation, expressed as the effect of genotypes within populations, was highly significant ($P < 0.001$) in the analysis of variance. Among the total sum of squares in the +Al medium, only 2% was attributed to the effect of populations and 98% was due to the effect of genotypes within populations. Similarly, on the −Al medium, only 5% of the total sum of squares was due to populations. Parrott and Bouton (1990) also observed a large variation among genotypes within populations, and concluded that significant genotypic variability exists for Al tolerance within the populations which were still segregating. This effect would be compounded in an autotetraploid like alfalfa (Rumbaugh et al., 1988). Since the total number of genotypes within a population capable of being evaluated in callus culture is relatively low, and given the resource requirements for in vitro screening in terms of time and capital are high and its success was low, the feasibility of callus culture as a screening bioassay method is limited. Although Al tolerance is expressed at the cellular level, the differences between a cellular manifestation of a phenotype and its expression in the whole plant system in the field are complex (Blum, 1988). Meredith (1984) reported that in vitro selection can be very inefficient because cells within a callus are not uniformly exposed to the stress. Callus cells are in close contact and cross-feeding among them may promote escapes from selection (Meredith, 1984). In the present study, calli were not used to regenerate plants but to assay for acid, Al toxicity tolerance, however. It was hoped the long exposure to the medium (6 wk) in this present study eliminated the effect of escapes.

In conclusion, selection in unlimed soil was the most effective in terms of success, resources, and time. However, further testing under field conditions is needed to assess the agronomic performance of this or any selected germplasm.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Glen Ware for statistical assistance and Donald Wood and Ann Bunce for technical help. M. Dall'Agnol also wishes to acknowledge the financial support of EPAGRI (Empresa de Pesquisa Agropecuária e Difusµo de Tecnologia de Santa Catarina) and the scholarship received from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) in Brazil.

REFERENCES

Atlin, G.N., and K.J. Frey. 1990. Selecting oat lines for yield in low-productivity environments. Crop Sci. 30:556–561.

Baligar, V.C., T. A. Campbell, and R.J. Wright. 1993. Differential responses of alfalfa clones to aluminum-toxic acid soils. J. Plant Nutr. 16:219–233.

Blum, A. 1988. Plant breeding for stress environments. CRC Press, Boca Raton, FL.

Bouton, J.H., and D.E. Radcliffe. 1989. Effects of acid soil selection on agronomically important traits in alfalfa. p. 377–378. *In* Association Française pour la Production Fourragère (ed.) Proc. Intern. Grass. Congress. 16th, Nice, France. 4–11 Oct. 1989. French Grassland Society, Versailles.

Bouton, J.H., and M.E. Sumner. 1983. Alfalfa, *Medicago sativa* L., in highly weathered acid soils. V. Field performance of alfalfa selected for acid tolerance. Plant Soil 74:431–436.

Campbell, T.A., J.H. Elgin, Jr., C.D. Foy, and J.E. McMurtrey III. 1988. Selection in alfalfa for tolerance to toxic levels of aluminum. Can. J. Plant Sci. 68:743–753.

Campbell, T.A., N.J. Nuernberg, and C.D. Foy. 1989. Differential responses of alfalfa cultivars to aluminum stress. J. Plant Nutr. 12:291–305.

Campbell, T.A., Z.L. Xia, P.R. Jackson, and V.C. Baligar. 1994. Diallel analysis of tolerance to aluminum in alfalfa. Euphytica 72: 157–162.

Ceccarelli, S. 1989. Wide adaptation: How wide? Euphytica 40: 197–205.

Ceccarelli, S., and S. Grando. 1993. From conventional plant breeding to molecular biology. p. 533–537. *In* D.R. Buxton et al. (ed.) Int. Crop Sci. I. CSSA, Madison, WI.

Dessureaux, L. 1969. Effect of aluminum on alfalfa seedlings. Plant Soil 30:93–98.

Duncan, R.R., and V.C. Baligar. 1990. Genetics breeding and physiological mechanisms of nutrient uptake and use efficiency an overview. p. 3–35. *In* V.C. Baligar and R.R. Duncan (ed.) Crop as enhancers of nutrient use. Academic Press, San Diego, CA.

Evans, D.A. 1989. Somaclonal variation - genetic vasis and breeding applications. Trends in Genetics. 5:46–50.

Felmy, A.R., D.C. Girvin, and E.A. Jenne. 1984. MINTEQ, a computer model for calculating aqueous geochemical equilibria. NTIS-PB-84157148. Natl. Tech. Inf. Serv., Springfield, VA.

Foy, C.D. 1964. Toxic factors in acid soils of the Southeastern U.S. as related to the response of alfalfa to lime. USDA Prod. Rep. 80. U.S. Gov. Print. Office, Washington, D.C.

Foy, C.D. 1976. Tailoring plants for greater tolerance to mineral toxicities and deficiencies on hill land soil. p. 54–559. *In* I. Luchow et al. (ed.) Proc. Int. Symposium Hill Lands, Morgantown, WV. 3–9 Oct. 1976. West Virginia University Books, Morgantown, WV.

Foy, C.D. 1983a. Plant adaptation to mineral stress in problem soils. Iowa State J. Res. 57:339–345.

Foy, C.D. 1983b. The physiology of plant adaptation to mineral stress. Iowa. State J. Res. 57:355–391.

Foy, C.D. 1992. Soil Chemical factors limiting plant root. Adv. Soil Sci. 19:97–149.

Foy, C.D., and A.L. Fleming. 1978. The physiology of plant tolerance to excess available aluminum and manganese in acid soils. p. 301–328. *In* G.A. Jung (ed.) Crop tolerance to suboptimal land conditions. ASA Spec. Publ. 32. ASA, CSSA and SSSA, Madison, WI.

Hartel, P.G., and J.H. Bouton. 1989. *Rhizobium meliloti* inoculation of alfalfa selected for tolerance to acid, aluminum-rich soils. Plant Soil 116:283–285.

Hartel, P.G., and J.H. Bouton. 1991. *Rhizobium meliloti* inoculation of alfalfa selected for tolerance to acid, aluminum-rich soils. p. 597–601. *In* R.J. Wright et al. (ed.) Proc. Int. Symposium on Plant-Soil Interactions at Low pH, 2nd, Beckley, WV. 24–29 June 1990. Kluwer Academic Publ., Dordrecht, the Netherlands.

Joost, R.E., and C.S. Hoveland. 1986. Root development of sericea lespedeza and alfalfa in acid soils. Agron. J. 78:711–714.

Joost, R.E., C.S. Hoveland, E.D. Donnelly, and S.L. Fales. 1986. Screening sericea lespedeza for aluminum tolerance. Crop. Sci. 26:1250–1251.

Kamp-Glass, M.D. Powell, G.B. Reddy, V.C. Baligar, and R.J. Wright. 1993. Biotechniques for improving acid aluminum tolerance in alfalfa. Plant Cell Rep. 12:590–592.

Meredith, C.P. 1984. Selecting better crops from cultured cells. p. 503–528. *In* J.P. Gustafson (ed.) Gene manipulation in plant improvement. Proc. 16th Stadler Genetics Symposium, Columbia, MI. Plenum Press, New York.

Ouellette, G.J., and L. Dessureaux. 1958. Chemical composition of alfalfa as related to degree tolerance to manganese and aluminum. Can. J. Plant Sci. 38:206–214.

Parrott, W.A., and J.H. Bouton. 1990. Aluminum tolerance in alfalfa as expressed in tissue culture. Crop Sci. 30:387–389.

Raij, B. van, 1991. Fertility of acid soils. p. 159–167. *In* R. J. Wright et al. (ed.) Proc. Int. Symposium on Plant-Soil Interactions at Low pH, 2nd, Beckley, WV. 24–29 June 1990. Kluwer Academic Publ., Dordrecht, the Netherlands.

Rumbaugh, M.D., J.L. Caddel, and D.E. Rowe. 1988. p. 777–808. Breeding and quantitative genetics. In A.A. Hanson et al. (ed.) Alfalfa and alfalfa improvement. Agron. Monogr. 29. ASA, CSSA, SSSA, Madison, WI.

Sanchez, P.A., and J.G. Salinas. 1981. Low-input technology for managing oxisols and ultisols in tropical America. Adv. Agron. 34:279–406.

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. Users guide: statistics. SAS Institute, Cary, NC.

Scott, B.J., and J.A. Fisher. 1989. Selection of genotypes tolerant of aluminum and manganese. p. 167–203. *In* A.D. Robson (ed.). Soil Acidity and Plant Growth. Academic Press, New York.

Simpson, J.R., A. Pinkerson, and J. Luzdovskis. 1977. Effects of subsoil calcium on the root growth of some lucerne genotypes (*Medicago sativa* L.) in acidic soil profiles. Aust. J. Agric. Res. 28:629–638.

Simpson, J.R., A. Pinkerson, and J. Luzdosvskis. 1979. Interacting effects of subsoil acidity and water on the root behavior and shoot growth of some genotypes of lucerne (*Medicago sativa* L.). Aust. J. Agric. Res. 30:609–619.

Smith, S.R., Jr. 1991. Selection and evaluation of alfalfa for grazing tolerance and soil acidity. Ph. D. diss. Univ. of Georgia, Athens (Diss. Abst. 9124339).

Van Wambeke, A. 1976. Formation, distribution and consequences of acid soils in agricultural development. *In* M. J. Wright (ed.) Proc. Conf. Plant Adaptation to Mineral Stress in Problem Soils, Beltsville, MD. 22–23 Nov. 1976. Cornell Univ. Press, Ithaca, NY.