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Genetic and phenotypic relationships in response to NaCl at different developmental stages in alfalfa

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Summary. The perennial forage alfalfa (*Medicago sativa* L.) may be affected by salinity at all stages of development. Selection for increased seed germination or seedling growth in saline environments has not resulted in improved forage yield under salt stress. The purpose of this study was to determine genetic and phenotypic relationships between plant performance in the presence of NaCl at three developmental stages in alfalfa. Understanding these relationships may improve the efficiency of breeding programs aimed at increasing crop survival and yields in saline environments. Fourteen half-sib families were randomly chosen from both an experimental alfalfa population produced from two cycles of mass selection for improved forage regrowth yield at 80 m*M* NaCl (A80), as well as from an unselected control population (AC1). In two separate experiments, individual plant performance was measured in these families at seed germination (radicle length at 7 days), and during seedling growth (forage yield at 40 days post-planting) and post-harvest regrowth (forage yield at 67 and 95 days post-planting) in the presence of 0 or 80 m*M* NaCl. Genetic, phenotypic, and family rank correlation coefficients, and broad-sense and narrow-sense heritability estimates were calculated within each growth stage, NaCl level, and population. Radicle length was not highly correlated with seedling or regrowth forage yield within a population or across NaCl levels. Phenotypic correlations between seedling and regrowth yields were also low. Heritability estimates were higher at 0 NaCl in AC1 between all growth stages, but were greater in A80 at 80 m*M* NaCl. Genetic correlations between seedling and regrowth yields were all positive. This suggests that selection for forage yield in saline environments at harvests-1, -2, or -3 should not decrease performance at other stages. Genetic correlations between seedling and regrowth yields were higher in A80 than in AC1 at 80 m*M* NaCl. The results indicate that selection for increased alfalfa forage yield in saline environments at germination may not be optimum. Family selection at germination or during seedling growth may be more effective than individual plant selection at any growth stage in saline environments. The results suggest than selection methods which include each critical growth stage may be required to develop alfalfa cultivars with increased forage yield in saline environments.

Key words: Genetic parameters – Growth stage – Lucerne – *Medicago sativa* – Salinity

Introduction

Response to salinity in crop plants may be affected by developmental stage. Understanding the relationships between plant growth and development and reaction to salinity may facilitate efforts to improve salt tolerance. Usually, crops are as salt tolerant at germination as at later stages (Maas 1986). However, certain species (e.g., sugarbeet, *Beta vulgaris* L.) are more sensitive during germination than at later growth stages (Ayers and Hayward 1948). Salt tolerance at seed germination has not been consistently related to tolerance during emergence, vegetative growth, flowering, or fruit development. Barley (*Hordeum vulgare* L.), maize (*Zea mays* L.), cowpea (*Vigna unguiculata* L.), rice (*Oryza sativa* L.), sorghum (*Sorghum bicolor* L.), and wheat (*Triticum aestivum* L.) are most salt sensitive during early seedling, growth, and become increasingly tolerant during later stages of development (Maas 1986). Although most crop plants generally become more tolerant at later stages of growth, salt

may affect pollination and thus decrease seed set and grain yield (Maas 1986).

Alfalfa is considered moderately sensitive to salinity (Maas and Hoffman 1977). Salinity research in this species has focused primarily on germination (Carlson et al. 1983; Allen et al. 1985; Dobrenz et al. 1983, 1989) and seedling establishment (Noble et al. 1984; Ashraf et al. 1986, 1987; McKimmie and Dobrenz 1987) in the presence of NaCl. Several reports indicate that alfalfa has the genetic potential for improved salt tolerance and that plant breeding may be the solution for increasing yield in saline environments (Noble et al. 1984; Allen et al. 1985; Kapulnik et al. 1989; Mohammad et al. 1989). Alfalfa is a multiple-harvest perennial, and it is possible that different mechanisms of salt tolerance might be involved during seed germination, seedling establishment, and post-harvest regrowth. Selection based on only a single developmental stage may preclude genotypic expression of traits, such as salt tolerance, that may be influenced by ontogenic stage (Shannon 1985). In this case, selection for salt tolerance at early stages of development, such as germination, may not result in expression of salt tolerance at later developmental stages, such as post-harvest regrowth.

Genetic and physiological linkages may exist between factors affecting seed germination or plant growth (seedling or post-harvest regrowth) in the presence of salinity stress (Blum 1988). However, indirect selection for field-relevant levels of salt tolerance in alfalfa at early stages of development (e.g., germination) have generally been unsuccessful (Smith et al. 1989). Apparent salt tolerance at germination does not appear to be related to tolerance during seedling growth (Allen 1984) or mature plant regrowth (Johnson 1990) in the presence of salinity in populations selected for germination salt tolerance. Salt tolerance during seedling growth has been observed in experimental populations of alfalfa selected for increased shoot length at 14 days (Ashraf et al. 1987) or 75 days (Noble et al. 1984) in the presence of 250 m*M* NaCl. Kapulnik et al. (1989) discovered that alfalfa populations selected for increased growth and N assimilation in non-saline conditions produce more forage at 20% bloom than the original cultivars at 200 m*M* NaCl. Johnson et al. (1991) developed selection methodology to identify alfalfa plants with the ability to germinate, establish, and regrow after harvest at salinity levels similar to those typically encountered in agriculture (Burns et al. 1990; McKell et al. 1986).

Breeding efficiency for increased forage yield in saline environments may be improved if high yielding (presumably tolerant) plants could be identified at an early stage of development, reducing the time required per cycle of selection. Reports such as those by Maas (1986) suggest that stage of development × stress level interaction in common. However, little use has been made of estimates of this interaction in developing breeding strategies for improving plant performance in saline environments. The objective of this study was to determine genetic and phenotypic relationships between measures of salt tolerance at the three primary developmental stages in alfalfa: germination, seedling establishment, and post-harvest regrowth. Understanding these relationships may help improve the effectiveness of breeding programs aimed at increasing crop yields in saline environments.

Materials and methods

Genetic materials

An experimental alfalfa population produced from two cycles of mass selection for improved forage regrowth yield at 80 m*M* NaCl (A80) and an unselected control population (AC1) were used in this study. Both A80 and AC1 were derived from the nondormant alfalfa 'African.'

Development of A80. A greenhouse screening system previously described by Johnson et al. (1991) was used to evaluate populations of individual alfalfa plants grown in a soil medium, and irrigated with water containing added NaCl. Osmotic potentials in the screening system were maintained at levels similar to those observed in agricultural areas where salinity is a problem (McKell et al. 1986; Burns et al. 1990). The first cycle of selection for A80 was conducted between 1 September 1987 and 22 March 1988 in a greenhouse under 24 h fluorescent lighting, where mean temperatures ranged from 17 to 30 °C. Ten scarified seeds "of African" were sown in each of 196 individual 40 × 0.2 m cylindrical containers containing 64.0 ± 1.6 g (dry wt., mean ± SE) of a soil medium (2:3:3:4 ratio of peat, perlite, sand, and plotting mix), covered with sand to a depth of 10 mm (Assadian and Miyamoto 1987), and thinned to one plant per container at 14 days. Two replicates, each with 98 plants, were designated. Immediately after sowing, containers were irrigated with 0.035 l of water plus 4.68 g l^{-1} NaCl (80 m*M* NaCl) every 3–5 days. Salt accumulation in the soil medium was estimated weekly with a vapor pressure osmometer, by measuring the osmotic potential of effluent (Noble et al. 1984) flowing from ten randomly selected containers per treatment in each replicate 15 min after irrigation. Forage fresh weight (clipped 50 mm above the crown) was measured for each plant 56, 105, 140, 167, and 204 days post-planting. Fresh weights were used, since moisture contents of plants irrigated with 0 and 80 m*M* NaCl did not differ in a preliminary experiment. Most plants were class 3 or class 4 maturity, using the 0 to 9 scale of Kalu and Fick (1981) at each harvest. Each container was flushed with 0.1 l non-saline water only immediately after each harvest (Noble et al. 1984). This was followed by irrigation with the saline solution. Irrigation treatment maintained mean salinity level of effluent for the 80 m*M* NaCl treatment that was equivalent to 164 ± 8 m*M* NaCl. In March 1988, individual plants were ranked by replicate based on mean forage fresh weight for harvests 3 to 5. Twenty cycle-1 plants, the ten highest ranked in each replicate, were selected ($i = 10\%$), randomly arranged, and interpollinated by hand. Seed was harvested from each plant separately and the population was produced by bulking an equal number of seed from each plant. The second cycle of selection for A80 was conducted between 9 August 1988 and 18 January 1989 as before, except

forage fresh weight was measured for each plant 49, 77, 105, 133, and 162 days post-planting. Seed was harvested from each plant separately to obtain half-sib families. A80 was produced by bulking an equal number of seed from each plant. Mean salinity levels of effluent for the 80 m*M* NaCl treatment was equivalent to 150 ± 7 m*M* NaCl.

Development of AC1. AC1 was developed by two cycles of random selection from 196 African plants established and grown with non-saline irrigation, independent of those plants used to produce A80. Two replicates, each with 98 plants, were designated. all plants were clipped 50 mm above the crown at each harvest and the herbage was discarded. The unselected control population (AC1) was produced by interpollinating 20 plants, 10 randomly chosen from each replicate. Seed was harvested from each plant separately and the population was produced by bulking an equal number of seed from each plant. Seed from each plant was also maintained separately as half-sib families.

Evaluation of A80 and AC1

Individual plant performance was measured at seed germination, and at harvests-1 (seedling growth), -2 and -3 (mature plant forage regrowth) in separate experiments to determine genetic and phenotypic relationships among these growth stages. Fourteen half-sib families were randomly chosen from each of A80 and AC1 and progenies were grown at 0 or 80 m*M* NaCl in both experiments.

Germination. Germination tests were conducted in 10 cm petri dishes containing a single piece of Whatman no. 2 filter paper (Smith and Dobrenz 1987). Initially, 4.5 ml of 0 or 80 m*M* NaCl solution was applied to the paper, seed was added, and the dishes were enclosed in plastic bags containing damp paper towels. The dishes were then placed in the dark at 25 °C. Germination was scored after 7 days by measuring radicle length to the nearest 1 mm. Unimbibed seeds were considered to be hard seeds and were excluded from all analyses. Seed germination involves not only qualitative responses of individuals (i.e., germination/no germination), but also quantitative response characters that may change over time (Scott et al. 1984). Radicle length at 7 days, a quantitative character that reflects speed of germination as well as the number of seeds germinated, was used as a measure of germination in these studies.

Seedling development and post-harvest regrowth. Forage yield was evaluated in a greenhouse between 23 June 1989 and 26 September 1989 under 24 h fluorescent lighting, where temperatures ranged from 22 to 35 °C. Seed from each half-sib family of AC1 or A80 were sown in 40×0.2 m cylindrical containers as before. Two salt levels, 0 and 80 m*M* NaCl, were imposed by irrigating seedlings with 0.035 l of 0.25 strength Hoagland solution (Hoagland and Arnon 1950) plus 0 (0 m*M*) or 4.68 (80 m*M*) g l^{-1} NaCl every 3–5 days. Containers were thinned to one plant at 14 days. Salinity levels for all treatments were imposed, monitored, and controlled as before. Forage fresh weight (clipped 50 mm above the crown) was measured 40, 67, and 95 days post-planting. Mean salinity level of container effluent during the evaluation period was equivalent to 121 ± 7 m*M* NaCl for the 80 m*M* NaCl treatment.

Statistical analyses

Experimental design. In both the germination and seedling growth and regrowth experiments, a randomized complete block design with a split-split-plot arrangement was used with NaCl levels (0 or 80) as main plots, populations (AC1 or A80) as subplots, and half-sib families within populations as sub-subplots. In the germination experiment, each sub-subplot was a single petri dish of seven seeds. In the seedling development and post-harvest regrowth experiment, each sub-subplot was a single row of seven plants, with one plant per container. Sub-subplots were replicated four times in each experiment. This allowed 28 individuals per half-sib family to be tested.

Estimates of genetic parameters. Half-sib families were evaluated in a randomized complete block design with an equal number of plants in each plot. An analysis of variance (Table 1) was computed for radicle length, seedling yield, and for each regrowth yield by salt level for balanced data on an individual plant basis (Hill and Leath 1979; Levings and Dudley 1963; Nguyen and Sleper 1983).

From these estimates, narrow-sense heritabilities (h^2_{NS}) were estimated on an individual plant basis as:

$$h^2_{NS} = \frac{\sigma^2_A}{\sigma^2_P} = \frac{4\sigma^2_F}{\sigma^2_F + \sigma^2 + \sigma^2_W}$$

and broad-sense hertiabilities (h^2_{BS}) were estimated as:

$$h^2_{BS} = \frac{\sigma^2_G}{\sigma^2_P} = \frac{\sigma^2_F}{[(\sigma^2_W)] \div [(rf) + (\sigma^2 \div r) + (\sigma^2_F)]}$$

with σ^2_A = additive genetic variance, σ^2_P = total phenotypic variance, σ^2_F = family variance component, σ^2_W = variance component among individual plants within plots, σ^2 = error variance component (plot-to-plot environmental variance), σ^2_G = total genetic variance, r = number of replicates, and f = number of half-sib families.

Estimates of genetic correlation were calculated to determine the extent to which the measurements of the various growth stages reflected what is genetically the same character. Covariance analyses were conducted for each pair or growth stages in each salt level by partitioning the sums of products according to the source of variation (Falconer 1981). From these estimates additive genetic correlation coefficients (r_A) were calculated as:

$$r_A = \frac{\mathrm{cov}_A X \cdot Y}{(4\sigma^2_F X \cdot 4\sigma^2_F Y)^{0.5}},$$

where cov_A equals the components of additive covariance and X and Y the pair-wise combinations between growth stages. The standard error of estimates of heritability and genetic correlation were calculated according to Falconer (1981).

Table 1. Analysis of variance on an individual plant basis for radicle length, or forage yield at harvests-1, -2, -3 in alfalfa populations grown at 0 or 80 m*M* NaCl[a]

Source	Degrees of freedom	Expected mean squares
Replications	r-1	
Families	f-1	$\sigma^2_w + (n\sigma^2) + (rn\sigma^2_F)$
Error	(r-1) (f-1)	$\sigma^2_w + (n\sigma^2)$
Within plots	(rf) (n-1)	σ^2_w

[a] r = no. of replicates; f = no. half-sib families; n = no. of individual plants per plot (a single row of seven plants, one per container)

Results and discussion

Seed germination, seedling growth, and post-harvest regrowth were all reduced at 80 m*M* NaCl. Mean radicle length at 80 m*M* NaCl was reduced by 42% in AC1, the unselected control population, relative to 0 NaCl, but only 9% in A80, the population selected for improved performance at 80 m*M* NaCl (Table 2). Forage yield at 80 m*M* NaCl was reduced by 45% at harvest-1, 44% at harvest-2, and 45% at harvest-3 in AC1, and 38% at harvest-1, 44% at harvest-2, and 36% at harvest-3 in A80. Because improved radicle length and forage yield during seedling and post-harvest regrowth were observed in A80 in saline environments, confirming its tolerance, this population could be used to establish whether or not the enhanced performance at these growth stages is any way related.

Table 2. Mean radicle length at 7 days, forage yield for seedling growth (harvest-1), and post-harvest regrowth (harvest-2 or -3) in AC1 (unselected control population) and A80 (population selected for improved forage yield at 80 m*M* NaCl) alfalfa grown at 0 or 80 m*M* NaCl

Population	Evaluation environment (m*M* NaCl)	Developmental stage			
		Radicle length	Harvest-1	Harvest-2	Harvest-3
		mm	g plant^{-1}		
AC1	0	29.0±0.9[a]	1.74±0.02	1.79±0.03	2.11±0.04
	80	16.9±0.6	0.96±0.01	1.00±0.01	1.15±0.02
A80	0	30.1±0.1	1.73±0.02	1.83±0.03	2.06±0.04
	80	27.3±0.9	1.08±0.01	1.02±0.02	1.32±0.02

[a] Mean ± standard error

The efficiency of breeding multiple-harvest perennial forages for increased forage yield in saline environments might be improved if tolerant plants could be identified at an early stage of development. However, salt tolerance at different growth stages may not be positively related. In this study, germination success (measured by radicle length) at 0 or 80 m*M* NaCl was not highly correlated with seedling development (forage yield in harvest-1) or mature plant regrowth (forage yield in harvests-2 or -3) in either population (Table 3). Phenotypic correlations between seedling and regrowth yields were also low but were higher at 0 than at 80 m*M* NaCl. Correlations between regrowth harvests were the highest of any two stages of development. Family rank correlations between seedling growth an post-harvest regrowth at 80 m*M* NaCl were generally similar to phenotypic correlations.

Broad-sense and narrow-sense heritability estimates for radicle length were higher in progenies of both AC1 and A80 when evaluated at 0 NaCl compared to 80 m*M* NaCl; however, heritability estimates for radicle length were higher in AC1 at both 0 and 80 m*M* NaCl than in A80 (Table 4). This indicates that the selection for regrowth forage yield in saline environments practiced to produce A80 may have reduced variation for performance at earlier growth stages such as germination. In contrast heritability estimates for seedling forage yield (harvest-1) were higher in progenies of both AC1 and A80 when evaluated at 80 m*M* NaCl compared to 0 NaCl, and heritability estimates for seedling yield were higher in A80 than in AC1 (Table 4). This suggests that the selection for forage yield in saline environments practiced to produce A80 did not reduce variation for seedling yield in saline environments. Heritability estimates for forage yield at 80 m*M* NaCl were highest at harvest-1 and lowest at harvest-3 in both AC1 and A80, suggesting that the greatest potential genetic advance in

Table 3. Estimates of phenotypic and family rank correlations for radicle length (RL) at 7 days and forage yield for harvests-1 ("seedling," H1), -2 (H2), and -3 (H3) in AC1 (unselected control population) and A80 (population selected at 80 m*M* NaCl) alfalfa grown at 0 or 80 m*M* NaCl

Population	Evaluation environment (m*M* NaCl)	Correlation	Developmental stages compared[a]					
			RL–H1	RL–H2	RL–H3	H1–H2	H1–H3	H2–H3
AC1	0	Phenotypic	0.00	0.03	−0.01	0.53**	0.43**	0.73**
		Family rank	0.09	−0.16	−0.13	0.53*	0.78**	0.76**
	80	Phenotypic	0.15**	0.12*	−0.02	0.42**	0.38**	0.44**
		Family rank	0.63*	0.39	0.50	0.42	0.43	0.48
A80	0	Phenotypic	0.09	0.10	0.09	0.65**	0.47**	0.71**
		Family rank	0.35	0.44	0.39	0.66**	0.36	0.67**
	80	Phenotypic	0.10	−0.01	0.05	0.33**	0.13**	0.56**
		Family rank	0.16	0.24	0.17	0.78**	0.16	0.15

[a] RL = radicle length at 7 days; H1, H2, H3 = yields for harvests-1, -2, and -3, respectively
*, ** Indicates significance at the 0.05, and the 0.01 probability levels

Table 4. Estimates of broad-sense (upper line) and narrow-sense (lower line) heritability for radicle length at 7 days and forage yield for seedling growth (harvest-1), and post-harvest regrowth (harvest-2 or -3) in AC1 (unselected control population) and A80 (population selected at 80 m*M* NaCl) alfalfa grown at 0 or 80 m*M* NaCl

Population	Evaluation environment (m*M* NaCl)	Developmental stage				
		h^2	Radicle length	Harvest-1	Harvest-2	Harvest-3
AC1	0	BS[a]	0.79±0.06	0.48±0.04	0.68±0.06	0.59±0.05
		NS	0.88±0.07	0.23±0.02	0.29±0.02	0.21±0.02
	80	BS	0.42±0.03	0.64±0.05	0.65±0.05	0.32±0.03
		NS	0.22±0.02	0.35±0.03	0.27±0.02	0.11±0.01
A80	0	BS	0.65±0.05	0.56±0.05	0.18±0.02	0.38±0.03
		NS	0.44±0.04	0.32±0.03	0.05±0.00	0.11±0.01
	80	BS	0.18±0.01	0.76±0.06	0.67±0.05	0.43±0.03
		NS	0.08±0.01	0.54±0.04	0.27±0.02	0.11±0.01

[a] BS = broad-sense heritability estimate ± standard error; NS = narrow-sense heritability estimate ± standard error

Table 5. Estimates of genetic correlation coefficients for forage yield for harvests-1 ("seedling," H1), -2 (H2), and -3 (H3) in AC1 (unselected control population) and A80 (population selected at 80 m*M* NaCl) alfalfa grown at 0 or 80 m*M* NaCl

Population	Evaluation environment (m*M* NaCl)	Developmental stages compared[a]		
		H1–H2	H1–H3	H2–H3
AC1	0	1.21±0.18[b]	2.03±1.34	1.35±0.34
	80	0.07±0.36	0.31±0.41	0.42±0.40
A80	0	1.42±0.57	1.14±0.14	1.64±1.24
	80	0.33±0.29	0.37±0.36	0.42±0.41

[a] H1, H2, H3 = yields for harvests-1, -2, and -3, respectively
[b] Genetic correlation coefficient estimate ± standard error

saline environments may occur by selection based on harvest-1. Our heritability estimates are similar to those previously reported for alfalfa selected for salt tolerance at germination ($h^2_{BS}=0.50$, Allen et al. 1985), seedling growth at 14 days ($h^2_{NS}=0.52$; $H_{realized}=0.31$, Ashraf et al. 1987), and seedling growth at 75 days ($H_{realized}=0.41$, Noble et al. 1984).

Genetic correlation coefficients (r_A) between forage yield at 0 NaCl for harvests-1 and -2, harvests-1 and -3, and harvests-2 and -3 were all greater than 1.0 in both AC1 and A80 (Table 5), indicating that most of the genetic variation at these growth stages resulted from factors common to the growth stages. However, at 80 m*M* NaCl, genetic correlations between forage yield in A80 indicate that only 11% (r^2_A) of the existing genetic variation at harvests-1 and -2, 14% at harvests-1 and -3, and 18% at harvests-2 and -3 resulted from factors common to these growth stages. These percentages were typically lower in AC1 (0.5% for harvests-1 and -2, 10% for harvests-1 and -3, and 18% for harvests-2 and -3). Genetic correlations were generally highest between harvests-2 and -3. Because genetic correlations between seedling and regrowth yields were all positive, selection for forage yield in saline environments at harvests-1, -2, or -3 should not decrease performance at other stages. These data again indicate that selection for forage yield at harvest-1 may be an efficient way to improve total yield (i.e., throughout the life of the plant) in saline environments, and would eliminate the need to take expensive and time-consuming multiple regrowth harvests.

Our results suggest that alfalfa genotypes that yield well at moderate NaCl levels can be selected from the germ plasm used in this study, and that selection for increased forage yield at 80 m*M* NaCl based on multiple regrowth harvests is possible. Low phenotypic correlations between radicle length and seedling or regrowth yields indicate that responses at germination are not generally indicative of mature plant performance in saline environments. Similarly, Jones (1987) observed little correlation between germination responses and subsequent seedling growth performance in several accessions of *Lycospersicon* grown at 10 m*M* NaCl. Some *Lycospersicon* accessions demonstrated stable responses over a range of stress levels during seedling development, but exhibited low stability in germination responses and vice versa.

Our data suggest that when attempting to improve seedling and mature plant salt tolerance in nondormant alfalfa, it may be reasonable to concentrate on an improved population that is already salt tolerant at germination. Following this approach, tolerances at each growth stage could be concentrated into a single population via tandem selection, leading to a single population having agronomically relevant salt tolerance. The alternative would be to simultaneously select within populations not previously selected for response to salinity, via independent culling levels based on their tolerance at each of the critical growth stages. Tandem selection, as described above, might be the most rapid, since germination salt-tolerant populations already exist (Dobrenz

et al. 1983, 1989) and additional selection for this trait would not be necessary; only seedling and mature plant tolerance would need to be considered. The independent culling levels approach would be preferred if previous selection for salt tolerance at germination had negatively affected yield performance of the salt-tolerant population. Independent culling levels may also be preferred in dormant alfalfas where germination salt tolerance is not yet available. In either strategy, progeny testing may be more effective than mass selection for traits, such as regrowth forage yield, that have low heritability.

In this study, phenotypic and family rank correlations between germination performance and seedling or mature plant regrowth yield were generally not significant. Selection for increased forage regrowth at 80 m*M* NaCl reduced additive genetic variation for germination performance in saline environments. Similarly, selection for enhanced performance at germination in alfalfa may reduce variation for enhanced forage yield in saline environments (Allen 1984; Johnson 1990). This implies that using the tandem selection strategy may not be as efficient as the independent culling levels approach if the goal is to improve forage yield in saline environments. Given the variation in salinity seen between seasons or even within single salt-affected fields, reduced yield in tolerant populations would be unacceptable. Therefore, we have adopted selection for salt tolerance at germination, and during seedling and mature plant growth in a greenhouse screening system (Johnson et al. 1991). Noble et al. (1984) also used a similar approach with success in an experimental breeding program to develop an alfalfa population with salt tolerance at all growth stages.

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