

# Improved Regrowth Salt Tolerance in Alfalfa

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## INTRODUCTION

Salinity may severely limit alfalfa yield in many growing regions throughout the southwest. Approximately 30% of the agricultural land in Arizona is now affected by salinity, and nearly 6% of this land has salinity levels that may significantly decrease alfalfa yield (Doerge, 1985). Reductions in yield and stand life have become severe enough to cause cropland to be abandoned in some highly saline areas. Fortunately, alfalfa breeders may be able to develop varieties bred to remain productive in the presence of moderate levels of salinity.

Alfalfa is affected by salinity at all stages of development. Significant salt tolerance at germination and during seedling growth have been observed in improved populations of alfalfa at salinity levels approaching sea water (Dobrenz et al., 1989). However, salt tolerance at different growth stages may not be related. We have developed a selection method to identify plants with the ability to germinate, establish, and regrow under salinity levels similar to those actually encountered in typical Arizona hayfields (Johnson et al., 1988).

The goal of our research is to improve tolerance to moderate levels of salinity at all growth stages. The objectives of the present study were to determine the: (1) salinity level that should be used in breeding for regrowth salt tolerance in alfalfa, (2) criteria that should be used to identify tolerant plants in order to obtain maximum response in breeding, and (3) relationships between salt tolerance at three developmental stages in alfalfa. The results presented in this paper summarize our findings after completing one cycle of selection for regrowth salt tolerance in alfalfa.

## MATERIALS AND METHODS

A selection method was developed and used to identify plants with the ability to germinate, establish, and regrow under saline irrigation. 'Sonora' and its parent cultivar 'African' were grown in soil-filled conetainers (tapered plastic tubes, 25 mm in diam. and 123 mm long) in a greenhouse. Four stress levels were imposed on the plants by adding different amounts of NaCl to irrigation water; the concentrations used were 0, 30, 60, and 80 mM NaCl. Salt levels were regularly monitored since salts accumulate over time in the soil. Solution (effluent) flowing from 20 randomly selected conetainers per salt level was collected weekly and the osmotic potential determined. To minimize salt accumulation, all conetainers were flushed with non-saline irrigation water every 28 days. This was followed by irrigation with the appropriate NaCl solution. Yield of each plant was measured for five harvests (made at the 8, 15, 20, 24, and 29 week). Selection was based on mean yields of individual plants for the third, fourth, and fifth harvests. The 20 highest-yielding plants in each salt level were selected and interpollinated. Seed was harvested from each plant separately to obtain half-sib families.

Equal amounts of seed from each half-sib family from each salt level and germplasm source were bulked to create populations for testing. Two randomly selected control populations from Sonora and African were produced using the same procedures to determine if selection for regrowth salt tolerance was successful. The yield of all the selected populations (designated: A0, A30, A60, A80, S0, S30, S60, and S80), control populations (designated: AC1, AC2, SC1, and SC2), and original populations (designated: A and S) were then retested under all salt levels (0, 30, 60, and 80 mM NaCl). Individual plant yield was measured at the 8, 12, 16, 21, and 27 week in this trial. Percent gain in yield was determined for each selected population, relative to the control populations at each salinity level.

A separate study was conducted to determine the relationships between salt tolerance at three developmental stages (germination, seedling establishment, and regrowth) in African alfalfa. Remnant seed from 14 half-sib families, selected for their ability to regrow under 80 mM NaCl, and 14 unselected families were irrigated with 0 or 80 mM NaCl. Individual plant performance was measured at germination and the 6, 10, and 14 week. Genotypic and phenotypic correlations between performance at each growth stage was estimated for each salt level.

## RESULTS AND DISCUSSION

The osmotic potential (a measure of salinity) of the irrigation effluent increased during the 29-week selection period a mean of 3, 67, 103, and 83% for the 0, 30, 60, and 80 mM NaCl irrigation treatments, respectively (Fig. 1). Flushing with non-saline irrigation water every 28 days reduced the osmotic potential of the irrigation effluent samples to levels comparable to the irrigation water. The mean osmotic potentials of the irrigation effluent samples were 6, 87, 72, and 71% higher than the mean osmotic potentials of the water used for irrigation of the 0, 30, 60, and 80 mM NaCl treatments during the 29 week selection period (Fig. 2).

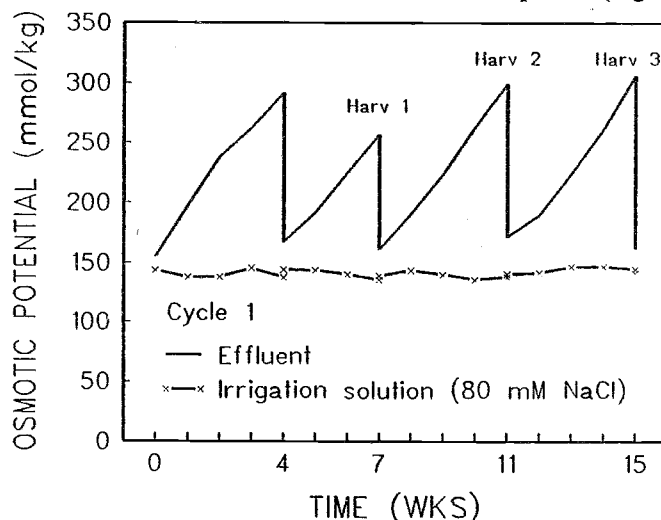


Fig. 1. Osmotic potential of effluent from containers irrigated with 80 mM NaCl and the 80 mM irrigation solution over 15 weeks.

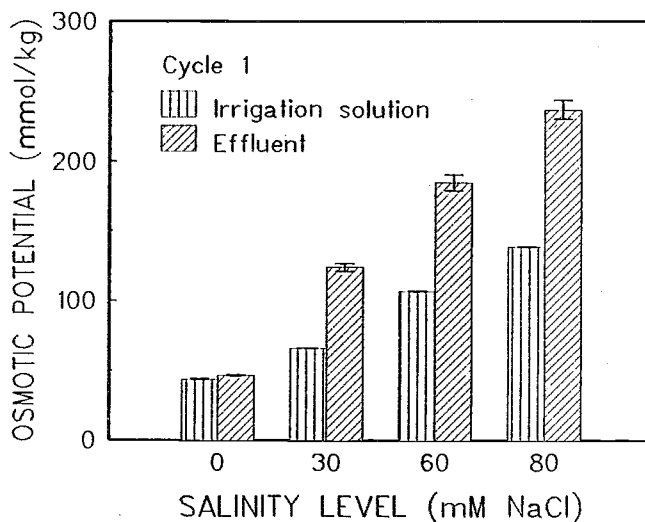


Fig. 2. Mean salinity levels of the irrigation solution and effluent at four salinity levels.

Selection for forage yield during regrowth currently requires evaluating yield for several harvests (up to five) before accurate identification of superior parents can be made. Nevertheless, the time and cost involved in making multiple harvests on large numbers of plants may drastically reduce breeding efficiency. The efficiency of breeding for regrowth salt tolerance could be improved if tolerant plants could be identified in a single harvest, preferably at an early stage of development. However, in our research, forage yield for individual harvests would not result in reliable identification of those plants with highest yields over all five harvests (Table 1). Selection based on mean plant yields for harvests 2 and 3 would result in the identification of 75% of those plants that would have been selected, based on yields in all five harvests. Therefore, improved breeding efficiency for regrowth salt tolerance in alfalfa may result if selection is based on two regrowth harvests.

Table 1. Percentage of plants that would be correctly identified if selection was based on yield in individual harvests or in regrowth harvests instead of mean yield over harvests 1 to 5.

Salinity level (mM NaCl)	Harvest no.					2+3	3-5	1-5
	1	2	3	4	5			
	-----%-----							
0	45	65	55	80	75	75	80	100
30	35	50	75	55	50	80	90	100
60	20	65	65	40	50	70	75	100
80	30	65	65	55	40	75	75	100

Performance immediately following germination (measured by radicle length at 7 days) was not highly correlated with seedling or regrowth forage yield (Table 2). Seedling yields (harvest 1) also were not well correlated with regrowth yields. Genotypic correlation coefficients between forage yields were all positive in populations selected for regrowth at 80 mM NaCl, but were generally negative for unselected control populations (Table 3). This indicates that selection for salt tolerance at any of these developmental stages will generally not decrease tolerance at other stages.

Table 2. Estimates of phenotypic correlations for radicle length and forage yield at three harvests in unselected (AC1) and selected (A80) populations of alfalfa grown at two salinity levels.

Pop.	Salinity level (mM NaCl)	Developmental stage / harvest					
		rl-y1 <sup>†</sup>	rl-y2	rl-y3	y1-y2	y1-y3	y2-y3
AC1	0	.01	.04	.10	.59	.39	.70
	80	-.04	-.06	.04	.52	.39	.69
A80	0	-.06	-.09	-.17	.59	.46	.76
	80	.02	.13	.01	.31	.33	.62

<sup>†</sup> rl=radical length at 7 days, y1-y3=yields harvests 1 to 3

Table 3. Estimates of genotypic correlation coefficients for forage yield for harvests 1 to 3 in unselected (AC1) and selected (A80) populations of alfalfa grown at two salinity levels.

Population	Salinity level (mM NaCl)	Harvest nos.		
		1 - 2	1 - 3	2 - 3
AC1	0	-0.88	1.02	-0.51
	80	-0.03	-0.07	-0.36
A80	0	0.26	0.32	0.23
	80	0.35	0.26	0.28

Positive gains in yield were seen when populations that were selected at a particular salinity level were evaluated at the same salinity level (Table 4). Selection at 0 mM NaCl lead to little gain in salt tolerance, whereas selection at 60 mM NaCl lead to highest mean gains in all environments. Negative gains were observed under low- to non-saline environments in populations selected at 80 mM NaCl. These results indicate highest mean gains in yield may be associated with selection under moderate NaCl stress.

Table 4. Average percent gain in yield of alfalfa populations selected for increased regrowth forage yield for one cycle under four salinity levels. Populations developed were tested under all salinity levels.

Salinity level selection environment (mM NaCl)	Salinity level test environment (mM NaCl)				x
	0	30	60	80	
	-----%				
0	14.4	0.8	-0.2	8.2	5.8
30	12.2	3.6	-2.5	3.6	4.2
60	20.0	-4.5	5.3	17.8	9.7
80	-9.2	-5.3	3.1	6.0	-1.4

## CONCLUSIONS

These findings indicate that regrowth salt tolerance in alfalfa could be improved in a single cycle of selection. A second cycle of selection has been completed and we are currently evaluating this material for additional progress. In addition, we have conducted two cycles of selection for increased regrowth yield under moderate salinity levels (60 mM NaCl) in a composite population of alfalfa. This improved germplasm will be available in the spring of 1990 so that plant breeders may incorporate regrowth salt tolerance in newly released cultivars of alfalfa.

## REFERENCES

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