

*Full Length Research Paper*

# Strain crossing to transfer germination and seedling salt tolerance in alfalfa (*Medicago sativa*)

A. A. Al-Doss

Department of Plant Production, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia.  
E-mail: aaldoss@ksu.edu.sa.

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**This study was conducted to evaluate the potential of strain crossing, transferring seedling salt tolerance into elite cultivars of alfalfa (*Medicago sativa* L.) without additional selection. Alfalfa cultivars (Mesilla, Moapa 69 and CUF 101) and two donor populations ('AZ-GERM SALT-II' (AZ-GS-II) and AZ-GS-EM) were used in this study. The experiment was conducted during season 2005 at the College of Food and Agriculture Sciences, King Saud University, Experimental Research Station, at Deirab near Riyadh (24oN,46oE), Saudi Arabia. Six strain crosses were produced between the two donor populations and three alfalfa cultivars. The performance of strain crosses for salt tolerance at germination (250 mM NaCl) was between 7.0 and 52.4% higher than the non-tolerant parental population. Seedling emergence was uniformly high for all populations with non-saline irrigation in the glasshouse (78 to 90%) and no significant differences in emergence percentage were observed between parental or strain-cross populations. The ratio of performance with saline irrigation to that under non-saline conditions, of strain crosses averaged 10.6% greater than that of the non-tolerant population. Relatively little variation in single-plant forage yield was observed among populations in either irrigation regime. With saline irrigation, significant low-parent heterosis for survival (+33.2%) was observed, only for the strain cross whose parents differed significantly in survival (AZ-GS-II X CUF 101). These results indicate that strain crossing could be used to achieve a rapid improvement in seedling emergence under salt stress in alfalfa. Strain crossing did not have a consistent effect on seedling survival or forage yield under salt stress (100 mM NaCl).**

**Key words:** Lucerne, forage crop, salt tolerance.

## INTRODUCTION

Salinity is one of the most significant problems of agriculture worldwide. It affects more than 50% of worldwide arable lands, mainly in third world countries, due to fresh water scarcity and inadequate irrigation methods, and is recognized as the major reason of desertification. Most known agricultural crops are salt-sensitive and do not produce economically at high salinity levels. Increased soil salinity is one of the natural detrimental factors that have a negative effect on plant growth and development (Flowers, 2004). About one billion hectares of lands are saline and this constitutes a serious threat for farmers (Flowers and Flowers, 2005). With an increasing area of arable land turning saline (Szabolcs, 1994) accompanied by increasing food demand from the growing human population, the need to

develop salt-tolerant crops and to identify the degree of salinity tolerance in crops, is becoming more important.

Crop performance under abiotic stress typically involves a complex interaction between environmental factors, physiological reaction to stress and expression of inherent yield potential (Richards, 1983; Bradshaw and Hardwick, 1989) which complicates breeding for salt tolerance. However, because reclamation and drainage are very expensive, improving salt tolerance represents one of the most cost-effective means of maintaining crop production in salt-affected areas (Blum, 1988).

Alfalfa is known to be moderately tolerant to salinity stress (Noble et al., 1984), however, investigations into its response to salt stress has shown different results (Khan et al., 1998; Esehie and Rodriguez, 1999; Rogers

et al., 2003; Boughanmi et al., 2005). Selection has been conducted in alfalfa (*Medicago sativa* L.) to improve germination percentage (Dobrenz et al., 1989) and seedling (Noble et al., 1984) and regrowth yield (Johnson et al., 1991) under saline conditions. Comparable screening and selection procedures are available for other crop species (Blum, 1988). These procedures are frequently costly and time consuming because of the complexity of response to salinity and the low heritability of tolerance. Moreover, the genetic or physiological basis of tolerance may differ during different growth stages (Allen, 1984) complicating selection projects. Generally, the most critical stages for salt stress are germination and seedling growth stage but sensitivity at germination or during early seedling stage does not indicate that the plant species will show similar sensitivity at a mature plant (Zapat et al., 2004).

Selection for salt tolerance may therefore only be feasible for a small number of populations. In these instances, improvement of salt tolerance would be simplified, if it were possible to rapidly transfer tolerance from a highly tolerant source into otherwise elite populations without direct selection for tolerance. Strain crossing (uncontrolled hybridization between two or more self-fertile populations with seed of the populations combined to produce a "strain cross" population) represents a potentially valuable alternative procedure for exploiting heterosis in this species.

A common strategy used for character transfer in alfalfa improvement involves hybridization between two or more distinct germplasm sources, in order to incorporate positive dominant traits into a single population (Rumbaugh et al., 1988; Brummer, 1999). This process is referred to as cultivar or strain crossing. It has been widely used because it represents a potentially valuable alternative procedure for exploiting heterosis and offers the opportunity of rapidly improving many characteristics in two or more parental populations with complementary expression of important traits.

Strain crosses have shown potential as a method of incorporating multiple-pest resistance into alfalfa (*M. sativa* L.) cultivars (Rummy et al., 1987). It has been employed most successfully to combine tolerance to insects and diseases where phenotypic recurrent selection has produced high rates of tolerance to different pests in parental populations (Elgin et al., 1983; Miller et al., 1987). Some strain crossing programmes have also attempted to exploit heterosis to improve forage yield, although generally only a small proportion of strain crosses exhibit significant mid-parent heterosis for this trait (Kehr, 1960; Hanson et al., 1964; Hill, 1983; Smith and Fairbanks, 1989).

Strain crosses may also be useful for transferring tolerance to abiotic stresses to elite alfalfa populations with specific adaptation. Such crosses might be made between otherwise unimproved, but stress-resistant (donor) populations, which may be very diverse

genetically (Johnson et al., 1991) and more narrowly-based elite populations having the majority of economically important traits that are stress susceptible. Strain crossing could offer many practical advantages in these situations since the specialized and expensive selection procedures used to improve stress tolerance would need only be conducted on a small number of donor populations. However, the complexity of expression of tolerance to abiotic stresses could confound prediction of the expected performance of strain-cross populations. The use of strain crosses to transfer abiotic stress tolerance will be most effective when tolerance is dominant and may be ineffective if tolerance is due primarily to additive gene action (Busbice et al., 1972).

The objective of this research was to determine the potential of strain crossing, for transferring tolerance to salinity from improved donor populations of non-dormant alfalfa into different elite cultivars.

## MATERIALS AND METHODS

### Plant materials

Three alfalfa cultivars and two donor populations were used in this study. The experiment was conducted during season 2005 at the College of Food and Agriculture Sciences, King Saud University, Experimental Research Station, at Deirab near Riyadh (24°N, 46°E), Saudi Arabia. The cultivars were one semi-dormant variety, 'Mesilla', and two non-dormant cultivars 'Moapa 69', and 'CUF 101' (Barnes et al., 1977). The populations were 'AZ-GERM SALT-II' (AZ-GS-II) and AZ-GS-EM. Both populations are non-dormant with improved performance under saline conditions. The first population 'AZ-GERM SALT-II' (AZ-GS-II) resulted from nine cycles of phenotypic recurrent selection within the non-dormant cultivar 'Mesa-Sirsa' for germination at concentrations of NaCl that increased with each cycle (to 600 mM NaCl in cycle 9) (Dobrenz et al., 1989). This population was considered tolerant to NaCl at germination and emergence. The second population 'AZ-GS-EM' was produced after two cycles of selection for germination, emergence and seedling establishment under irrigation with 160 mM NaCl (McKimmie and Dobrenz, 1987). AZ-GS-EM exhibits some tolerance to NaCl during forage regrowth. In addition, the population 'AZ-GERM SALT-I' (Dobrenz et al., 1983), which was the fifth cycle population in the selection program that led to AZ-GS-II, was used to initiate selection that led to AZ-GS-EM.

### Strain crosses development

Six strain crosses (Table 1) were produced between the two donor populations (AZ-GS-II and AZ-GS-EM) and three alfalfa cultivars (Mesilla, Moapa 69 and CUF 101). Between 50 and 60 plants from each parental source of the strain crosses were randomly paired and hand pollination carried out between plants of the two parents without emasculation in the glasshouse. The same plants from each of the five parental populations were also randomly intercrossed by hand to produce representative samples of the parental populations for evaluation. An equal number of seeds from each parental plant were bulked to form the parental population in each of the strain-cross populations. Seed from both reciprocal strain crosses for each pair of parents were bulked independently and evaluated as separate populations.

**Table 1.** Mean ratio of performance ( $\pm$  SE) with saline irrigation to that under non-saline conditions (saline/non-saline = salt tolerance) of parental and strain-cross populations at four growth stages.

Parent/strain cross	Germination +	Emergence $\ddagger$	Survival $\S$	Forage yield $\P$
Mesilla	0.24 $\pm$ 0.02	0.46 $\pm$ 0.05	0.96 $\pm$ 0.09	0.81 $\pm$ 0.06
Moapa 69	0.57 $\pm$ 0.02	0.39 $\pm$ 0.04	0.89 $\pm$ 0.02	0.81 $\pm$ 0.04
CUF 101	0.61 $\pm$ 0.03	0.40 $\pm$ 0.05	0.56 $\pm$ 0.09	0.86 $\pm$ 0.06
AZ-GS-II	0.96 $\pm$ 0.04	0.72 $\pm$ 0.05	1.14 $\pm$ 0.05	---#
AZ-GS-II X Mesilla	0.75 $\pm$ 0.02	0.57 $\pm$ 0.04	0.80 $\pm$ 0.04	---
AZ-GS-II X Moapa 69	0.65 $\pm$ 0.01	0.61 $\pm$ 0.05	0.69 $\pm$ 0.03	---
AZ-GS-II X CUF 101	0.75 $\pm$ 0.01	0.39 $\pm$ 0.05	0.78 $\pm$ 0.03	---
AZ-GS-EM	---	---	---	0.93 $\pm$ 0.02
AZ-GS-EM X Mesilla	---	---	---	0.80 $\pm$ 0.02
AZ-GS-EM X Moapa 69	---	---	---	0.85 $\pm$ 0.03
AZ-GS-EM X CUF 101	---	---	---	0.88 $\pm$ 0.03

+ Seeds germinated after 7 days; 250 mM NaCl solution in saline treatment.  $\ddagger$  Seedlings emerged and established at 14 days; 100 mM NaCl solution in saline treatment.  $\S$  Survival after six harvests (190 days); 100 mM NaCl solution in saline treatment.  $\P$  Mean forage yield per plant in six harvests; 100 mM NaCl solution in saline treatment. # Not included as AZ-GS parental population did not exhibit significantly higher tolerance than cultivars.

### Seedling response to NaCl

Germination of all parental and strain-cross populations was evaluated in 250 mM NaCl solutions, as well as under non-saline (control) conditions. Thirty mechanically scarified seeds per population were incubated on filter paper in Petri dishes in each of five replications. Tests were conducted in a dark growth chamber at 25°C. Seeds with radicals >2 mm long after 7 days were considered germinated.

Germination, establishment and regrowth, under saline and non-saline irrigation conditions were measured in plants grown in soil-filled containers (tapered plastic 25 X 123 mm tubes, "containers") in a glasshouse. Both irrigation solutions contained 0.25 Hoagland's solution (Hoagland and Arnon, 1950); the saline treatment also contained 5.84 gL<sup>-1</sup> (100 mM) NaCl. Plants were irrigated individually with 35 ml of the appropriate solution every 3 to 5 days. To minimize salt accumulation, all tubes were flushed with 0.1 l non-saline water every 28. This was immediately followed by irrigation with the appropriate irrigation solution. The experiment was conducted in a greenhouse under 24 h fluorescent lighting where mean temperatures ranged from 17 to 30°C.

Seedling emergence was recorded 14 days after sowing and reported as a percentage of seeds germinated and established. Forage yield per plant (5 cm stubble) was measured for six harvests beginning 42 days after sowing and at approximately 28 day intervals thereafter. Survival was recorded following the sixth harvest (190 days after sowing) and was expressed as a percentage of the number of established seedlings after thinning. Tolerance at each growth stage was determined.

### Design and statistical analysis

A split-plot design with four replications was used with irrigation treatments (100 mM NaCl and a non-saline control) as main plots and populations as subplots. Three seeds were sown in each of seven tubes for each population and irrigation treatment in each

replication. These were randomly thinned to one plant per tube 14 days after sowing. The data presented for each treatment in this study is presented as mean of samples with standard deviation ( $X \pm S.D.$ ) calculated by standard statistical methods (Steel and Torrie, 1980).

### RESULTS

Laboratory germination under non-saline conditions was 95% and did not differ between any of the parental or strain cross populations. As expected AZ-GS-II had significantly higher NaCl tolerance ( $P \leq 0.01$ ) than the elite cultivars at germination and emergence (Table 1). Under saline conditions, significant differences in germination percentage were observed between reciprocal strain crosses made with the germination-tolerant population (AZ-GS-II) (Table 2). In each cross, germination was higher when the tolerant population was used as the seed parent. For a population, such as Mesilla, with particularly low NaCl tolerance (measured by the saline: non-saline germination percentage ratio, Table 1), both reciprocal strain crosses had germination that exceeded the mean of the two parents (Table 2). Mean germination of the reciprocal crosses was between 13.0 and 224.9% higher than the non-tolerant cultivar. Mean germination of strain crosses in saline condition was less than the mid-parent value in those crosses where germination of the non-tolerant population exceeded 55%. Importantly, salinity tolerance of strain crosses was between 7.0 and 52.4%, higher than that of the non-tolerant parental population (Table 2).

**Table 2.** Mean germination, emergence percent, and heterosis ( $\pm$  SE) for parental and strain cross populations under saline (100 mM NaCl) irrigation.

Cross (A X B)	Germination+							Emergence $\pm$				
	Parents		Strain cross					Parents		Strain cross		
	A	B	AXB	BXA	Cross mean	Mid-parent heterosis	Low-parent heterosis	A	B	cross mean	Mid-parent heterosis	Low-parent heterosis
	-----%-----											
AZ-GS-II X Mesill	96.0	23.3*	84.0	67.3*	75.7	+27.0 $\pm$ 4.5	+224.9 $\pm$ 2.3	60.7	35.7	50.6	+4.9 $\pm$ 15.0	+41.7 $\pm$ 6.2
AZ-GS-II X Moapa 69	96.0	54.0*	70.0	52.0*	61.0	-18.6 $\pm$ 2.7	+13.0 $\pm$ 2.1	60.7	35.7	47.6	+1.2 $\pm$ 11.6	+33.3 $\pm$ 3.3
AZ-GS-II X CUF 101	96.0	58.0*	85.3	57.3*	71.3	-7.4 $\pm$ 2.7	+22.9 $\pm$ 1.3	60.7	27.4	30.3	-31.1 $\pm$ 4.6	+10.6 $\pm$ 3.1

+ Germination 95% for all parental and strain cross populations with non-saline irrigation.  $\pm$  Emergence ranged from 78 to 90% and did not differ significantly among parental and strain cross populations with non-saline irrigation. \* Significant differences ( $P \leq 0.05$ ) between means of parental or reciprocal strain cross populations based on LSD.

Seedling emergence was uniformly high for all populations with non-saline irrigation in the glasshouse (78 to 90%) and no significant differences in emergence percentage were observed between parental or strain cross populations. With saline irrigation, significant differences in emergence occurred only among reciprocal crosses between the tolerant population, AZ-GS-II and CUF 101 (Table 2). Mean emergence of both reciprocal strain crosses was between 10.6 and 41.7%, higher than for the non-tolerant parent with saline irrigation. Strain cross emergence under NaCl stress, did not exceed the mid-parent value for any of the strain crosses. Nevertheless, tolerance, the ratio of performance with saline irrigation to that under non-saline conditions, of strain crosses averaged 10.6% greater than that of the non-tolerant population (Table 1).

Relatively little variation in single-plant forage yield was observed among populations in either irrigation regime (Table 3). Under non-saline conditions, Moapa 69 did produce significantly more forage than the most salt-tolerant population AZ-GS-EM. With saline irrigation, yields of the parental populations did not differ significantly. Forage yields of reciprocal strain crosses also did

not differ under either non-saline or saline irrigation (data not shown). The cross AZ-GS-EM X Moapa 69 was the only strain cross that exhibited significant mid-parent heterosis under non-saline conditions (Table 3). Over all, strain crossing resulted in little or no increase in tolerance to salinity relative to that of the non-tolerant parent for forage yield.

Salt tolerance, expressed as survival under saline irrigation, was significantly higher in AZ-GS-II than in Moapa 69 and CUF 101 (Table 1). Survival after 190 days under non-saline conditions was uniformly high (82 to 96%) and did not differ significantly among the parental populations and strain crosses, nor between reciprocal strain crosses. With saline irrigation, significant low-parent heterosis for survival (+33.2%) was observed only for the strain cross whose parents differed significantly in survival (AZ-GS-II X CUF 101). Mean survival did not differ between AZ-GS-II and Moapa 69 or Mesilla (Table 4), or between reciprocal strain crosses (data not shown). Tolerance to salinity in this trait was not affected consistently in strain crosses. In Mesilla and Moapa 69, which exhibited relatively high survival under saline irrigation, tolerance was higher in the parental populations than in the

strain crosses Mesilla and Mopa 69 (+15.7 and +19.2%, respectively). Alternatively, tolerance in the AZ-GS-II X CUF 101 strain cross was 22.4% higher than that of the least-tolerant parental population CUF 101.

## DISCUSSION

Salinity affects the major processes such as germination growth (Parida et al., 2004). Germination percentage is one of the most worldwide spread methods for determination of plant tolerance to salts (Dantas et al., 2005). This study demonstrates that strain crossing represents a useful method of improving germination and emergence under saline conditions in non-tolerant populations of alfalfa when a highly tolerant donor population (e.g. AZ-GS-II) is available. Both germination and emergence were lower when the non-tolerant population was the seed parent in the strain cross, implying that either some self-pollination occurred when strain crosses were made, or some maternal effect has contributed to the difference between reciprocal crosses. However, mean germination of reciprocal strain crosses, which represents the most likely

**Table 3.** Mean single-plant forage yield and heterosis ( $\pm$  SE) for parental and strain cross populations harvested six times under non-saline and saline (100 mM NaCl) irrigation.

Cross (A X B)	Non-saline					Saline				
	Parents		Strain cross			Parents		Strain cross		
	A	B	Reciprocal cross mean	Mid-parent heterosis	Low-parent heterosis	A	B	Reciprocal cross mean	Mid-parent heterosis	Low-parent heterosis
	g plant-1			----- % -----		g plant-1			----- % -----	
AZ-GS-EM X Mesilla	1.03	1.13	1.09	+1.0 $\pm$ 4.6	+5.8 $\pm$ 6.5	0.94	0.92	0.95	+2.0 $\pm$ 3.0	+3.3 $\pm$ 4.6
AZ-GS-EM X Moapa 69	1.03	1.16*	1.15	+5.5 $\pm$ 4.5	+11.7 $\pm$ 6.0	0.94	1.03	1.03	+4.0 $\pm$ 2.0	+9.6 $\pm$ 3.4
AZ-GS-EM X CUF-101	1.03	1.12	1.04	-3.5 $\pm$ 2.7	+1.0 $\pm$ 1.0	0.94	0.95	0.95	+2.0 $\pm$ 2.0	+3.3 $\pm$ 4.8

\* Significant differences ( $P \leq 0.05$ ) between parental populations based on LSD.

**Table 4.** Mean percent survival and heterosis values ( $\pm$  SE) for parental and strain cross populations after 190 day growth with saline (100 mM NaCl) irrigation.

Cross (A X B)	Parents			Strain cross	
	A	B	Reciprocal- cross mean	Mid-parent heterosis	Low-parent heterosis
AZ-GS-II X Mesilla	100.0	75.0	73.2	-16.3 $\pm$ 6.1	-24.0 $\pm$ 7.2
AZ-GS-II X Moapa 69	100.0	82.1	66.1	-27.3 $\pm$ 4.7	-19.5 $\pm$ 4.0
AZ-GS-II X CUF-101	100.0	53.6*	71.4	-5.4 $\pm$ 7.1	+33.2 $\pm$ 10.4

\* Significant differences ( $P \leq 0.05$ ) between parental populations based on LSD.

strain cross constitution to be used in alfalfa breeding (Rumney et al., 1987), was significantly higher than that of the non-tolerant parent. It is significant that improved salt tolerance was not associated with any decrease in germination or emergence under non-saline conditions. Using precursor populations of AZ-GS-II, Allen et al. (1985) estimated that, germination salt tolerance in alfalfa had a broad-sense heritability of 0.50, however, estimates for specific types of gene action were not presented. Improved salt tolerance in strain crosses suggests that, tolerance at germination in AZ-GS-II is due at least partially to dominant gene action.

Results were less conclusive for post-emergence tolerance to salinity. Strain crosses, on average, exhibited slightly higher tolerance in

forage yield than their non-tolerant parent; however, differences in performance between the tolerant and intolerant parents were comparatively small. Even though AZ-GS-II exhibited very high survival under NaCl stress, this trait was only improved significantly in strain crosses, when the non-tolerant parent displayed exceptionally low survival. The inconsistent performance of parental and strain-cross populations in survival under salt stress suggests that numerous characteristics other than NaCl tolerance, such as resistance to root and crown disease, may interact with NaCl tolerance to affect seedling survival under salt stress.

Findings for forage yield and survival are not unexpected, since neither of the AZ-GS populations had been directly selected for improved NaCl

tolerance following emergence. Similar studies with populations having higher levels of post-emergence tolerance to NaCl (Johnson et al., 1991) will be needed to determine the efficiency of transfer of tolerance via strain crosses. Ashraf et al. (1987) estimated that narrow-sense heritability of seedling shoot length in alfalfa under salt stress was 0.52. This suggests that non-additive genetic effects may be relatively unimportant in influencing tolerance to NaCl during shoot growth. In this case, unlike with germination and emergence, strain crosses would not be expected to result in significant improvement in NaCl tolerance over non-tolerant parental populations.

Recently, salinity tolerance and management for alfalfa have been investigated (Sanden and Sheesley, 2007). They reported that vegetative

forage production is basically a linear function of plant transpiration. Open stomata with lots of water vapor leaving the plant (transpiration) allows for maximum carbon dioxide uptake to build plant carbohydrates and biomass. Excessive salinity in the crop root zone creates osmotic stress that reduces root uptake of water and crop transpiration. The added stress then reduces forage yield.

The interest for improving crop tolerance to salinity has increased in recent years, using either traditional improvement and selection methods or genetically modified organisms. Different alternatives can be implemented to improve the aptitude of the saline soils. Most of them are extremely expensive and do not solve the problem permanently. In contrast, the biological approach has gained considerable recognition due to the evolution of tolerance to salinity in different ecotypes of plant species (Pearen et al., 1997). On the other hand, there is an important series of information about the effects of salinity on cultivar performance, which supplies a cohesive dissection of the physiological, biochemical and molecular components (Flowers, 2004). On the basis of the results obtained, it is evident that strain crossing could be used to achieve a rapid improvement in seedling emergence under salt stress in alfalfa. Strain crossing did not have a consistent effect on seedling survival or forage yield under salt stress (100 mM NaCl).

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