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Effect of inbreeding on germination salt tolerance in alfalfa

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EFFECT OF INBREEDING ON GERMINATION
SALT TOLERANCE IN ALFALFA

by

Tateo Morita

A Thesis Submitted to the Faculty of the

DEPARTMENT OF PLANT SCIENCES

In Partial Fulfillment of the Requirements
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In the Graduate College

THE UNIVERSITY OF ARIZONA

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ABSTRACT

The performance of an alfalfa (*Medicago sativa* L.) population has been improved by recurrent selection for germination salt tolerance. However, recurrent selection may lead to increased inbreeding. Since alfalfa is subject to severe inbreeding depression, accumulation of inbreeding during the selection process may negatively affect performance. This experiment was designed to determine the effects of inbreeding on germination performance in alfalfa under saline and non-saline conditions. The germination performance of seed having three different levels of inbreeding was examined. No adverse effects of inbreeding were observed in non-saline conditions. Consistent (but nonsignificant) declining trends were observed in germination percentage in saline conditions as the level of inbreeding increased. Proportionately larger declines were observed between generations in germination speed and early seedling vigor. These results sugest heterozygosity in alfalfa may be maintained simultanioustly while recurrent selection for germination salt tolerance is conducted. Moreover, reducing inbreeding during recurrent selection for germination salt tolerance may be more successful if germination speed index or early seeding vigor are used for the measurement.

INTRODUCTION

The earth's surface is 70% salt water and 30% land, with less than half the land surface available for agricultural use. Moreover, much of the land that is used for agricultural production presents many environmental constraints, such as water and mineral stress (Lewis and Christiansen, 1979). For example, severe water and mineral stress problems are common in arid regions such as Arizona and other Western states. Although the application of irrigation and fertilizers has increased productivity by up to factor of 10 in these arid regions, such application has also accelerated the build-up of soil salinity problems.

Between 60,000 and 81,000 ha of cropland in the United States have been eliminated from production, or have had production significantly reduced by salinity. An estimated additional 8,100 ha are being damaged each year. The major salinity problems are found primarily in arid and semiarid portions of eleven Western states (Wright, 1981). In addition, as a greater emphasis is placed on bringing marginally productive and presently non-arable land under prodution, salinity problems may become more common as much of the world's non-arable land is presently affected by salinity (Allen et al., 1984).

Plant breeding may lead to populations which perform better under stress conditions than germplasm now available making agricultural production practical on marginal lands (Lewis and Christiansen, 1979). Alfalfa (Medicago sativa L.) is only moderately tolerant of saline conditions; however, it is an economically important crop in Arizona and other arid regions where salinity can be a severe problem. Developing salt tolerant alfalfa germplasm has been an important goal for plant breeders in Arizona. Dobrenz, et al. (1983) at the University of Arizona, developed germination salt tolerant alfalfa. Their results indicate that germination salt tolerance is highly heritable and quantitatively inherited in alfalfa. Although selection has been shown to improve salt tolerance in alfalfa, recurrent selection per se may lead to a loss of heterozygosity. Because alfalfa is subject to severe inbreeding depression, loss of heterozygosity during selection may seriously limit productivity.

This study was designed to determine whether inbreeding has any effect on germination salt tolerance in alfalfa. The objective of this experiment was to relate the level of inbreeding with germination salt tolerance by comparing the germination responses of three generations of inbred alfalfa under saline and non-saline conditions. The germination responses measured in this experiment were: germination percentage, germination speed, and early seedling vigor. Data from this experiment may be used to explain the potential problems or benefits of inbreeding as it relates to recurrent selection for germination salt tolerance.

LITERATURE REVIEW

Alfalfa was introduced to the Southwestern United States from Mexico around 1850. It proved to be well-suited to the sunny and dry climate and the irrigated soils of the Southwestern states. Production has increased constantly since this time. In 1984, over 900 million kg of alfalfa was produced on 69 thousand ha of farmland in Arizona. Its value was over 100 million dollars. The production and acreage of alfalfa were the second largest in the state, next to cotton, and the value was third behind cotton and lettuce (Arizona Agricultural Statistics, 1985). From these figures, it is obvious that alfalfa is economically one of the most important crops in Arizona.

Alfalfa is well adapted to the sunny and dry Arizona climate. However, in this region the summer temperature can reach 50°C. Therefore, high levels of commercial alfalfa production in this environment are only possible with intensive irrigation (WGBH, 1987). Farmers use as much as 2 meters per hectare of irrigation water each year and much of this water may be saline.

Soil Salinity Problems in Agriculture

Salinity is usually associated with saline outcrops or with irrigation water containing dissolved salts (MaKell et al., 1986). Salinity inhibits plant performance mainly in two ways. It lowers the osmotic potential, inducing drought-like conditions,andit causes toxic ion effects. Salinity has been significant in many agricultural areas throughout history. Robinson (1986) cites Gelburd's (1985) discussion of field records showing a significant decline in grain yield in the ancient civilization of Mesopotamia, which had one of the oldest irrigation systems in the world. Archaeologists believe that salinity was the major cause for this drop in yield (Jacobsen and Adams, 1958). Another example of the effects of salinization can be found in the Arizona. More than 500 years ago, Hohokum Indians built canals for irrigation. They turned the desert into cropland, but their irrigated fields slowly accumulated salt until crops could no longer be produced. Salinization of their croplands forced the Indians to migrate. Phoenix, Arizona got its name because the city arose from the ruins of such an Indian settlement (WGBH, 1987).

Climate is a major factor affecting the salinization process (McKell et al., 1986). Saline soils are more likely to occur where precipitation is not adequate to leach excess soluble salts. Also, cropland in dry region requires more frequent irrigation to attain maximum crop production. Irrigation water usually contains some level of salts.

In Arizona, ground water and three major rivers--the Colorado, the Salt, and the Gila--are major sources of irrigation water. Ground water in Arizona typically contains from 92 mg/l to 5033 mg/l of dissolved salts (White and Garrett, 1982). The Colorado, Salt, and Gila Rivers contain approximately 890, 1070, and 1955 mg/l dissolved salt, respectively (Shainberg and Oster, 1978). Whenever irrigation water from these sources is applied to a field, it may lead to increases in the salt concentration of the soil because as water evaporates from the surface salt builds up in the soil. The remainder sinks into the earth, which may slowly makes the ground water more salty (WGBH, 1987). In addition, moisture uptake by plants can increase salt accumulation in the root zone (Saleh and Troeh, 1982). Since there usually is not enough precipitation to wash the built-up salt away in arid regions, farmers may be forced to apply additional irrigation water simply to leach accumulated salt into the ground water. This process can cause salty ground water to rise into the root zone, however. (WGBH, 1987). A recent report commissioned by the Council on Environmental Quality (Sheriden, 1981) attributes the downward trend in productivity of irrigated land to the following factors: (a) over-drawing of ground water without sufficient regard for the rate of recharge, (b) irrigation with saline water which increases soil salinity, and (c) inadequate leaching and drainage systems to remove salts.

Approximately 7.5 percent of the cropland in Arizona contains soil which is designated saline (McKell et al., 1986) where electrical conductivities of the soil saturation extract (ECe) exceed the standard value of 4 ds/m described in U.S.D.A.

Handbook 60 (Richards, 1954). The average ECe value for farmland in the state is 2.8 ds/m, which is 40% higher than the threshhold value for alfalfa, as determined by the U.S Salinity Laboratory (Doerge, 1985). No report has been published concerning the actual yield losses due to salinity in Arizona (Robinson, 1986). Alfalfa yields are predicted to decline at the rate of 7.3% per ds/m when salinity exceeds 2 ds/m (Maas and Hoffman, 1977). Each ds/m increase in salinity could amount to more than 5 million dollars per year lost to alfalfa growers in Arizona with over one million tons of production at an average price of $87.60 per ton (1984-1985 price). Moreover, salt stress encourages premature flowering of alfalfa (Hoffman et al., 1975), leading to premature decline in quality and even greater declines in crop value. Francois (1981) indicated that poorly drained soils do not reduce yield until salts begin to accumulate within about one meter of the soil surface. Therefore, accumulation of salt may not result in immediately visible adverse effects since the soil system has some level of tolerance. But alfalfa growers in Arizona are probably experiencing an invisible reduction in yield much like ancient Mesopotamians did (Robinson, 1986). In order to avoid any serious problems in the future, salt tolerant alfalfa germplasm should be developed as one solution to this increasing problem.

There are several ways to alleviate soil salinity problems. Installation of underground drainage pipes is one solution. Farms in the Imperial Valley in the California have installed more than 45,000 km of drainage pipe to remove salts at a cost of over 2 million dollars (Raloff, 1984). A drainage pipe built in Welton-Mohawk area of Arizona removed the salt from local farms, but poured the salt back into the Colorado river just north of the Mexican border. Because the Mexican government protested, a large desalinization plant is now under construction at Yuma, Arizona to reduce the river's salt content. It will cost over 200 million dollars to complete by 1989, with cost of operation estimated at twenty million dollars a year (WGBH, 1987).

Reclamation using procedures such as laser leveling or the use of improved irrigation systems, such as drip irrigation, are other alternatives in dealing with salinity that cost less than building drainage systems or desalinization plants. But even these cheaper alternatives usually cost so much that they are only possible with government subsidies. According to Epstein (1976), the problem of soil salinity is solved primarily by reclamation and drainage projects, but an easier, admittedly short-term solution would be to develop salt tolerant crops which are better able to remain productive under saline conditions. It seems that developing salt tolerant germplasm is the only feasible alternative currently available in many regions where capital intensive reclamation or desalinization are not possible.

Salt tolerant crops may in fact require less irrigation because their reaction to salt is highly correlated with field drought resistance (Tanimoto and Nickell, 1965). This means that salt tolerant crops may not only increase productivity on marginal farmland, but also may slow the soil damage from salt accumulation by irrigation. Also, farmers may not have to leach accumulated salts in their fields as often as with non-tolerant crops because salt tolerant crops can survive in a higher concentration of salt. Significant amounts of water may therefore be conserved by growing salt tolerant crops. Water is a scarce resource in the dry Southwestern states, and competition from residental and other industrial uses always restricts the availavility of water for agricultural uses in this region.

Breeding for Salt Tolerance

Social, physiological, and ecological factors have shaped the geographic distribution of crop plants (Klages, 1947). In other words, plants are grown where they fit the environment. Each species has certain adaptations, such that if environmental conditions change, it may be better to turn to a species more suited to the environment than to attempt to grow unadapted plants (Lewis and Christiansen, 1979). Plant species differ widely in their salinity tolerance. Salt sensitive plants are negatively affected by water exceeding 1 mmhol/cm (=1ds/m, 640mg/l) (Neiman and Shannon, 1976). Yields of even the most salt tolerant glycophytes are affected when irrigation water exceeds 7-10 mmhos/cm. Many halophytes can grow with water having salt concentrations as high as 0.5 M NaCl (35,000 mg/l). Some economically important glycophytes are fairly salt tolerant. Maas (1986) analyzed an extensive data base to determine the relative tolerance of plants to salts and boron. His results indicate that crops such as barley (Hordeum vulgare L.) and cotton (Gossypium hirsutum L.) are relatively salt tolerant. Fifty percent yield reduction is observed at 18 ds/m and 17 ds/m EC of saturated soil extract from the root zone for each of these crops, respectivly. Alfalfa is only moderatly salt tolerant, exhibiting the same yield reduction at roughly half the salt concentration as barley. Maas also notes that the salt tolerance of plants not only varies considerably among species, but also depends heavily upon the cultural conditions under which the crop is grown.

Yields of several crops under saline conditions have been studied by many reseachers. Ballantyne (1962) measured the growth of wheat and barley, and observed no reduction in yield at up to 3 mmhos/cm and 4.5 mmhos/cm, respectively. The effect of different frequencies of irrigation on yield on saline site was studied by Sharma et al. (1977). They measured the yield of wheat and their results indicate that an irrigation frequency of 10 days produced 47 percent more yield than a normal frequency of 25 days on a saline sodic soil. Study of the effects of saline irrigation water and exchangeable sodium on alfalfa growth showed the yield of alfalfa did not decrease from water containing 3,000 ppm (0.3 MPa) dissolved salts unless the sodium content of the water, soil or soil water was high (Chang, 1961). These results and others from similar studies may provide valuable informations for farmers making decisions and evaluating their farming strategies when confronted with salinity problems.

Many species generally considered to be salt sensitive have been shown to possess significant intraspcific genetic variability for salt tolerance (Kelly et al., 1979; Venables and Wilkins, 1978). Therefore, it has been possible to select salt tolerant genotypes from otherwise salt sensitive species. Because of the severity of salinity in many regions of the world, improvement of salt tolerance may be necessary for many of the economically important crops, even for crops like barley which are categorized as salt tolerant species.

The response of plants to stress environments may be different at each stage of growth. For example, the level of salt tolerance during germination is not always identical to that at a later stage of growth. Maliwal and Paliwal (1969) found that the tolerance of crops varies from the germination to later growth stages because the criteria for the evauation of salt tolerance may be entirely different at different growth stages. The physiology of seeds and plants in different growth stages are also different so that the responses to a similar stress condition may not always the same. Therefore, germination tests should be done along with mature plant tests to measure total salt tolerance. Although the responses to salinity at germination and later growth stages are not identical, many plant breeders frequently use germination tests for selection since germination is one of the simplest responses to measure which is related to survivability. In addition, germination performance may provide a good measure of total performance of the plant under salt stress. However, most grain crops, such as wheat (Triticum aestivum L.), barley, corn (Zea mays L.), sorghum (Sorghum bicolor L.), and rice (Oryza sativa L.), are more tolerant at germination than at other growth stages (Ayers et al., 1952; Francois et al,. 1984; Maas et al., 1983; Iyengar et al. 1977; Pearson et al., 1966). In contrast, safflower (Carthamus tinctorius L.), sugarbeet (Beta vulgaris L.), guayule (Parthenium argentatum L.) and various halophytic plant species are reported to be more susceptible to salt stress during germination than at later growth stages (Francois and Bernstein, 1964; Francois and Goodin, 1972; Miyamoto et al., 1984; O'Leary, 1984). All of these studies also reported that crops exhibit specific or varietal differences under salinity stress at different growth stages.

Different seed treatments may lead to differences in salt tolerance. Pre-soaking seeds with salt and/or hormone solutions has been shown to improve germination and later stages of growth in most saline treatments. Pre-soaking seed in distilled water, Na2SO4, MgC12, IAA and IBA increased the germination, height, tillering, grain and straw yield of wheat (Chippas and Lal, 1978). Chippas and Lal (1976), John and Chinoy (1971) and Khan and Khan (1978) reported simmilar results for different crops. Pre-soaking seed in distilled water also improved the germination rate of alfalfa in saline solution (S.E. Smith, pers. comm.).

Breeding Alfalfa for Salt Tolerance

Biotechnological techniques, such as somatic-cell hybridization and gene trasfer are possible methods of improving salt tolerance. However, the yielding ability of a crop is generally influenced by the cumulative effect of many genes, so such procedures may have limited applicability with geneticaly complicated traits such as salt tolerance (Cocking and Riley, 1979).

Genetic variation for salt tolerance among existing genotypes of a species or a related species represents the most important source of salt tolerant genotypes for use in selection and breeding (Noble et al., 1984). Noble et al. concluded that two generations of recurrent selection for tolerance significantly increased the mean whole plant salt tolerance without decreasing production under non-saline conditions in the alfalfa cultivar CUF101. They used a score based on percentage of leaf damage and length of main the shoot as their evaluation criteria, and showed that whole plant salt tolerance had a reasonably high heritability ($h^2 = 0.41$). Rush and Epstein (1976) backcrossed a highly salt tolerant tomato ecotype of *Lycopersicon cheesmanii* L. with a commercial cultivar of *L. esculentum* L. to improve the salt tolerance of the commercial type without decreasing its yield or quality.

Research on salt tolerance in alfalfa in Arizona has concentrated mostly on seed germination since the seed is less susceptible to environmental interactions than either seedlings or mature plants during regrowth (Robinson, 1986). This author also stated that there are strong agronomic justifications for trying to improve alfalfa germination under saline conditions. Alfalfa is more sensitive to salt at germination than at later growth stages (Uhvits, 1946; Ayers and Hayward, 1948; Forsberg, 1953; Chang, 1961). The alfalfa seed is also very sensitive to other environmental stresses at germination. Disease and inadequate moisture can be problems in Southwestern regions of the U.S. at the time of sowing and stand establishment. Thus, growers commonly sow two to five times the number of seeds required for good stands even though alfalfa is one of the most vigorous forage species for seedling establishment in the field (Barnes and Scheaffer, 1985; Blaser et al., 1956).

Salt tolerant alfalfa populations have been developed by Dobrenz et al. (1983), using a simple germination selection method. Germination in 1.30 MPa NaCl was increased from 3% for source population, (Mesa-Sirsa), to 86% for the fith cycle of selection, the germplasm °Arizona Salt Tolerant 1982' (AZST82). Many genes seem to involved in germination salt tolerance in alfalfa (Allen et al., 1985). An average heritability of 49.9 percent was estimated from the results of the germination test averaged over all salt concentrations and over all five cycles of selection (Allen et al., 1985). Germination increased in a 1.15

MPa NaCl solution over the control for AZST82, while all others decreased. This may indicate that repeated selection for NaCl tolerance during germination resulted in an NaCl requirement for maximum germination. Since the concentration of salt required to achieve 1% germination has increased at a nearly constant rate in Dobrenz work, further gain with selection should be possible before a plateau is reached. In fact, in the eighth cycle of selection the salt concentration required to inhibit germination in 99% of the population tested increased to 0.14 MPa (Robinson, 1986).

The physiolosical responses of salt tolerant AZST82 and nontolerant Mesa-Sirsa under salt stress were described by Allen et al. (1985). They observed that seed respiration was inhibited less by NaCl in AZST82 than in Mesa-Sirsa during germination, probably due to differences in rate of germination with increasing NaCl concentrations. Ion exclusion and imbibitional water absorption did not appear to be influenced by selection for NaCl tolerance. Allen (1984) reported that there were no significant differences between Mesa-Sirsa and cycles 1 to 5 of their selection program in forage yield, apparent photosynthesis, transpiration, or diffusive resistance. He evaluated the plants for several other growth characteristics to determine whether selection for NaCl tolerance resulted salt tolerance at more mature growth stages. His results indicate there is no evidence that germination salt tolerant alfalfa is also tolerant of salinity at later growth stages.

Effects of Inbreeding in Alfalfa

Allen (1984) concluded from his results that there was no significant differences in yield between Mesa-Sirsa, and populations derived from five cycles of selection, AZST78 to 82 in a non-saline environment. Results of experiments conducted by Smith et al. (1987) indicate a significant reduction in the yield on non-saline site of germination salt tolerant materials relative to Mesa-Sirsa over seven harvests in 1986. The highest average yield was observed in Mesa-Sirsa (14.93 grams/plot), while AZST81 was lowest (13.02 grams/plot). A fitted regression line over the eight selected cycles indicated yield may be significantly reduced as generation of selection increases. This yield reduction may have been caused by the accumulation of inbreeding during the selection process.

The basic objectives of recurrent selection are to increase the frequency of superior genes in the gene pool and to increase the chances of genetic recombination (Briggs and Knowles, 1967). However, when recurrent selection is conducted, effective population size may become reduced. This may be due to reduction in the number of parents intermated following selection or to unequal contributions of seed from each parent following intermating. If either of these are the case, recurrent selection has a tendency to lead to increases in population inbreeding.

A large number of deleterious qualitative characteristics appear in the early generations following inbreeding experiments in alfalfa. Alfalfa is subject to severe inbreeding depression even though it is an autotetraploid and the theoretical rate of approach to homozygosity is slower than in a diploid. Wilsie (1958) reported that one generation of selfing resulted in an average loss of 80 to 90 percent in self fertility and 20 to 30 percent in vegetative vigor. Kirk (1927), Williams (1931) and Tysdal et al. (1942) reported similar results from their experiments with alfalfa. Busbice (1968) related seed yield in alfalfa to the coefficient of inbreeding. He proposed that reduced seed yield with inbreeding in alfalfa resulted primarily from a loss of heterozygosity per se rather than from the action of accumulated deleterious recessive loci. It is important to note that heterozygosity is not completely restored with a single generation of outcrossing in autotetraploids as it is in diploids. Bingham (1980) illustrated that maximum heterozygosity and heterosis do not occur in the F1 or single cross generation when parents are inbred but occurs in the segregating double cross or even later generations.

In addition to overall productivity, the survival rate of inbred alfalfa in competitive situations has been studied by Veronesi and Lorenzetti (1982). They observed no differences in establishment between S1 and F1 seedlings, but fall survival percentages of S1 plants were significantly lower than that of the F1's. This data suggest selective elimination of S1 plants may have occured the seedling year.

MATERIALS AND METHODS

A germination test utilizing two generations of inbred and one generation of non-inbred alfalfa seed was performed under saline and non-saline conditions to evaluate the effect of inbreeding on germination salt tolerance.

The seeds used were derived from three populations: the cultivars Lew, and Mesa-Sirsa (MS) and the germplasm Arizona Salt Tolerant 84 (AZST84). Lew was derived from 'African' and 'Indian' germplasm (Schonhorst et al., 1981). It is adapted to the low desert valleys of Arizona and is highly tolerant to the stem nematode (*Ditylenchus dipsaci*, Kuhn Filipjeu) and the Ent-H biotype of the spotted alfalfa aphid (*Therioaphis maculata*, Buckton) (Smith et al., 1981). Mesa-Sirsa, a non-dormant cultivar, was developed from a selection from PI235736, introduced from India and has Resistance to the spotted alfalfa aphid (Allen et al., 1985). AZST84 was derived from Mesa-Sirsa by Dobrenz et al. through seven cycles of selection for germination salt tolerance. The selection methodology used in the development of this germplasm is described by Allen et al. (1985).

The source material from each of three populations used in this experiment were randomly selected S0 plants which were considered completely non-inbred (Table 1). Each of the S0 plants was self-pollinated to produce S1 seeds in the greenhouse. Self-pollination was performed by gently rolling or squeezing racemes between the fingers. This procedure is as effective as other self-polination methods, such as tripping flowers with a toothpick or a folded cardboard sheet, but is about three times more efficient than any other method (Barnes and Stephenson, 1971). Harvested self-seed were cleaned and mechanically scarified with sandpaper before they were sown in artificial soil in a styrofoam flat. The flats were kept in a growth chamber with 24 hours of light and temperature at $26 \pm 4°$ C. When the seedlings were about 10 cm tall, they were transplanted into 2 l pots. S2 plants were produced from S1 plants by using exactly the same procedure used to produce S1 plants from S0 plants.

When S2 plants were established, all three generations of plants were synchronized for flowering. Each of the plants was treated as a male and used to pollinate the male sterile clone 'Southern Arizona Male Sterile 1' (SAMS1), which was derived from the cross of '6-4' x 'CUF101-A'. 6-4 is a male sterile from 'Saranac' provided by E.T. Bingham of the University of Wisconsin. CUF101-A is a breeding clone in the Arizona program derived from the non-dormant cultivar CUF101.

Aging seed generally results in significunt declines in germination percentage and in germination score in saline as well as control conditions (Smith and Dobrenz, 1987). Therefore, seed production for all three generations was performed over a one

Table 1. Randomly selectedalfalfa clones which were used for seed production.

Population	Generation/level	Clone no.
Lew	S0/A	3* 6* 7* 11* 14* 15 16* 18* 19*
Lew	S1/B	3* 6* 7* 11* 14* 15* 16* 18* 19*
Lew	S2/C	3* 6* 7* 11 14* 16 18* 19
MS +	S0/A	16 19* 23* 38* 42* 43 45* 46* 47* 50 52 53* 54* 55 56* 83
MS	S1/B	16* 19* 23* 34* 38* 42* 43* 45* 46* 47* 50* 52* 53* 54* 55* 56* 83*
MS	S2/C	16 19 23 42* 43 45* 46 47* 50 52* 53 54* 55 56* 83
AZST84	S0/A	6* 22* 30 31 34* 36* 40* 43* 44 54* 66* 67* 72* 76* 78* 84*
AZST84	S1/B	6* 22* 30* 31* 34* 36* 40* 43* 44* 54* 66* 67* 72* 76* 78* 81* 84*
AZST84	S2/C	6 22 30* 31* 34 40 43* 54 66 67 72 76* 84*

* Clone which produced hybrid seed in crosses

+ 'Mesa-Sirsa'

month period in order to minimize seed age differences. Because alfalfa is autotetraploid, heterozygosity is not completely restored with a single generation of outcrossing (Hanson and Davis, 1972). For clarity, seeds produced from cross-pollination between SAMS1 and S0, S1 or S2 plants are referred to as "S0", "S1" or "S2" seed even though the level of inbreeding of these hybrids is less than that of their inbred parents. Approximate coefficients of inbreeding for hybrids resulting from S0 x S0, S0 x S1, and S0 x S2 crosses are 0, 0.028 and 0.051, respectively.

A preliminary test was made to determine the appropriate NaCl concentration for the germination test. Two S0, two S1, and one S2 family were selected, one each from MS, Lew, and AZST84. NaCl concentrations used for the preliminary test were 0, 182, 222, and 263 mM for Lew and MS (Lew-MS), and 263, 303, and 344 mM for AZST84. Five seeds from each family were planted on a petri plate for this test. All the seed were mechanically scarified with sandpaper before they were planted. No. 2 Whatman filter paper was placed in the plate and 3 ml of each NaCl solution added. The plates were then enclosed in plastic bags to avoid evaporation. Wet paper towels were placed in the plastic bags to maintain adequate moisture. The plates were placed in a dark growth chamber kept at a temperature of 26$\pm$1°C.

Little germination was observed after 5 days even in the plate with the lowest NaCl concentration, while 100 percent germination was observed in the controls. The appropriate NaCl concentrations were determined as 142 mM for Lew and MS, and

222 mM for AZST84 from the results of the preliminary test (Table 2).

The final germination tests were performed under exactly the same conditions as the preliminary test, except that 15 seeds per entry were planted in two petri plates, one containing a saline solution, the other distilled water. If less than 30 seeds were available, one half was planted in each plate. Double distilled water was used as a solvant for the NaCl solutions. 0.25 g/l of fungicide (Benlate) was added to prevent fungal growth during the germination test. Osmotic potentials were measured using a Wescor Model 5500 vapor pressure osmometer, which indicated -6.98 and -10.47 bars for the two NaCl solutions.

Germination was evaluated 24, 36, 48, 72, 96 and 120 h after initiation. Seeds were defined as germinated if they showed radical growth equal or greater than the seed length (about 2 mm). Early seedling vigor scores were measured at 72 and 120 h. They were measured using the same criteria described by Smith and Dobrenz (1987). Their scale indicates the score "5" was given only to those seedlings with radicals longer than 2.5 cm at 72 h. All remaining seeds and seedlings were scored at 120 h. At this time seedlings with radicals longer than 2.5 cm, 1.0-1.5 cm, or 0-1.0 cm were scored "3", "2", or "1", respectively. Seeds which were obviously imbibed but did not have a visible radical were scored "0". The same formula used by Robinson (1986) was used to calculate Germination Speed Index (GSI) in this experiment. The formula is as follows:

Table 2. Results of preliminaly germination test at 24 and 120 h after initiation.

Lew - MS

Clone	NaCl concectration (mM)			
	0	182	222	263
24h				
Lew 11 S0	5/5 *	0/5	0/5	0/5
Lew 19 S0	5/5	0/5	0/5	0/5
MS 47 S1	5/5	0/5	0/5	0/5
MS 52 S1	5/5	0/5	0/5	0/5
MS 42 S2	5/5	0/5	0/5	0/5
120h				
Lew 11 S0	---	0/5	0/5	0/5
Lew 19 S0	---	2/5	0/5	0/5
MS 47 S1	---	1/5	0/5	0/5
MS 52 S1	---	2/5	0/5	0/5
MS 42 S2	---	3/5	0/5	0/5

AZST84

Clone	NaCl concentration (mM)			
	0	263	303	344
24h				
AZST84 22 S0	5/5	0/5	0/5	0/5
AZST84 72 S0	5/5	0/5	0/5	0/5
AZST84 34 S1	5/5	0/5	0/5	0/5
AZST84 78 S1	5/5	0/5	0/5	0/5
AZST84 30 S2	5/5	0/5	0/5	0/5
120h				
AZST84 22 S0	---	0/5	0/5	0/5
AZST84 72 S1	---	1/5	0/5	0/5
AZST84 34 S1	---	1/5	0/5	0/5
AZST84 78 S1	---	2/5	0/5	0/5
AZST84 30 S2	---	3/5	0/5	0/5

* Number seeds germinated / Number seeds planted

$$GSI = (N1/D1 + N2/D2 \ldots Nn/Dn) / P$$

where N is number of seeds which germinate each consecutive day following initiation, D is day and P is the cumulative number of seeds to germinate by the last day of measurement. This formula was modified by Robinson (1986) from an index described earlier by Guneyli et al. (1969).

RESULTS AND DISCUSSION

General Observation

The plant materials were divided into six groups based on the salt concentration they were tested at and the generations represented (e.g. S0, S1 and S2 or S0 and S1 etc.) in order to process them using one-way analysis of variance (Table 3).

Inbreeding generally produces adverse effects on plant performance in alfalfa; however, the results from this experiment (Tables 6-11) indicate that there apparently was not an inordinately large effect of inbreeding on germination of alfalfa seed under non-saline conditions. Specifically, no significant differences were observed in germination percentage, speed index or early seedling vigor score between the three generations evaluated for any of the groups tested. Indeed, the results suggest some favorable effects on germination performance as the level of inbreeding increases. For example, the mean value of germination percentage increased by as much as 8.0 percent between levels A and B (S0 and S1) in group 4 (Table 7), early seedling vigor increased 12.4 percent between levels A and B in group 2 (Table 9), and germination speed increased 9.8 percent between levels A and B in group 5 (Table 11). None of those

Table 3. Description of groups based on availability of clones in each generation.

Group no.	Generation Level	Population	Clone no.
1 +	S0, S1, S2 / A, B, C	Lew MS	3, 6, 7,14,18 42,45,47,54,56
2	S0, S1 / A, B	Lew MS	11,16,19 19,23,38,46,53
3	S0, S1, S2 / A, B, C	AZST84	43,76,84
4	S0, S1 / A, B	AZST84	6,22,34,36,40,54,67,72 78
5	S0, S1 / A, B	Lew MS	3, 6, 7,11,14,16,18,19 19,23,38,42,45,46,47,53 54,56
6	S0, S1 / A, B	AZST84	6,22,31,34,36,40,43,54 67,72,76,78,84

+ Group 1 = Population Lew and MS, each clone represented by three generations: S0, S1, and S2.

2 = Population Lew and MS, each clone represented by two genereations: S0 and S1.

3 = Population AZST84, each clone represented by three generations: S0, S1 and S2.

4 = Population AZST84, each clone represented by two generations: S0 and S1.

5 = Population Lew and MS, group 1 and 2 were combined, then S2 were deleted from the group.

6 = Population AZST84, group 3 and 4 were combined, then S2 were deleted from the group.

figures are statistically significant at 5% level, however a trend is suggested in these data.

In contrast, the results in saline conditions indicate a consistent downward trend in germination performance as the level of inbreeding increases. However, again differences between generations are not statistically significant (Table 4). Taken together, data from non-saline and saline conditions suggest that inbreeding depression may indeed exacerbate the effects of salt stress at germination in alfalfa. The lack of significant differences in germination performance between generations may have been due to the relatively small range in inbreeding represented in the generations tested. Given this, it is certainly encouraging to see evidence of trends in the data. Examination of materials having coefficients of inbreeding less than 0.1 may provide statistically significant differences between generations which needed to totally verify the significance of the phenomenon observed in this experiment.

Under non-saline conditions, most seed completed their germination by 36h after initiation of the test. The majority of those seeds that did not germinate within 48h rarely produced any signs of germination (Table 5). Moreover, they often began to decay after a few days. On the other hand, germination in saline conditions typically began 36h after initiation. Decay of ungerminated seeds was less obvious in the saline treatments.

Table 4. F values from analysis of variance of the effects of generation on germination percentage, early seedling vigor and germination speed.

Group no.	Germination percentage		Early seedling vigor		Germination speed	
	F	Prob. > F	F	Prob. > F	F	Prob. > F
1	0.38	0.69	0.65	0.53	2.64	0.08
2	0.04	0.85	0.00	0.97	0.00	0.97
3	0.71	0.51	1.53	0.25	0.36	0.70
4	0.42	0.52	0.14	0.71	1.05	0.31
5	0.02	0.88	0.89	0.35	1.42	0.24
6	0.05	0.82	0.04	0.85	0.81	0.37

Table 5. Germination percentage at each observation time.

Population	Treatment	Hours after initiation					
		24	36	48	72	96	120
Lew	Non-saline	83	95	98	99	99	99
	Saline	7	57	74	82	86	90
MS	Non-saline	69	94	97	97	98	98
	Saline	1	27	65	76	79	86
AZST84	Non-saline	88	98	99	99	99	99
	Saline	0	32	49	60	64	74

Germination percentage

The results of measures of germination percentage are shown in Tables 6 and 7. In general, germination was quite high under non-saline conditions. The lowest mean value for germination percentage was 92.6% observed in group 4 level A (4-A) (Table 6). Seed from many clones exhibited 100% germination in the non-saline control solution. Under saline conditions, germination percentage ranged from 82.2% in group 2-B to 94.3% in group 1-A (Table 6). germination of the AZST84 materials ranged from 64.4% (group 3-C, Table 6) to 78.9% (group 4-A, Table 6).

Total proportional changes in mean germination percentage were calculated by representing the difference between germination percentages for each level of inbreeding as a percent of the value of the least inbred plant. Proportional changes in the mean germination percentage values declined consistently in saline conditions as the level of inbreeding increased except for the changes between B and C in group 1 (Table 7), in which the mean value increased by 2.4%.

There was an approximate 5 to 10 percent decline in mean germination percentage observed between levels A and B in Lew and MS groups, while about a 10 percent decline between levels A and B was observed in the AZST84 materials (Table 7). Larger proportional declines between non-inbred and inbred materials were observed in AZST84 than in Lew or MS. This may be due to the higher levels of inbreeding which already existed in

Table 6. Mean germination percentage in saline andnon-saline conditions for inbred and non-inbred alfalfa.

Group no.	Treatment (mM NaCl)	Level of Inbreeding *		
		A	B	C
1	0	96.7±1.6	99.5±0.5	98.9±0.8
	142	94.3±2.7	87.1±3.5	89.2±2.2
		(- 2.5) +	(-12.5)	(- 9.8)
2	0	96.7±2.5	100.0±0.0	---
	142	84.2±4.9	82.2±4.3	---
		(-12.9)	(-17.8)	(---)
3	0	100.0±0.0	100.0±0.0	100.0±0.0
	222	77.0±7.0	69.0±7.0	64.4±4.4
		(-23.0)	(-31.0)	(-35.6)
4	0	92.6±4.9	100.0±0.0	---
	222	78.9±5.9	76.8±4.0	---
		(-14.8)	(-23.2)	(---)
5	0	96.7±1.5	99.7±0.3	---
	142	88.9±3.1	85.2±2.7	---
		(- 7.8)	(-14.5)	(---)
6	0	94.4±3.8	100.0±0.0	---
	222	78.4±4.6	74.4±3.5	---
		(-16.9)	(-25.6)	(---)

* A = S0 x SAMS1; F = 0.
B = S1 x SAMS1; F = 0.028.
C = S2 x SAMS1; F = 0.051.

\+ Control-salt difference as % of control.

Table 7. Proportional changes in mean value for germination percentagebetween alfalfa of different levels of inbreeding (%).

Group no.	Treatment (mM NaCl)	Levels of inbreeding compared *		
		A,B	B,C	A,C
1	0 142	+ 2.9 - 7.6 (-10.5)+	- 0.6 + 2.4 (+ 3.0)	+ 2.3 - 5.4 (- 7.7)
2	0 142	+ 3.4 - 2.4 (- 5.8)	--- --- (---)	--- --- (---)
3	0 222	0.0 -10.4 (-10.4)	0.0 - 6.7 (- 6.7)	0.0 -16.4 (-16.4)
4	0 222	+ 8.0 - 2.7 (-10.7)	--- --- (---)	--- --- (---)
5	0 142	+ 3.1 - 4.2 (- 7.3)	--- --- (---)	--- --- (---)
6	0 222	+ 5.9 - 5.1 (-11.0)	--- --- (---)	--- --- (---)

* A = S0 x SAMS1; F = 0.
B = S1 x SAMS1; F = 0.028.
C = S2 x SAMS1; F = 0.051.

+ Total change.

AZST84. AZST84 is the germplasm from the seventh cycle of recurrent selection the pressure to acquire some levels of inbreeding existed during the selection process. The largest total proportional decline in germination performance (16.4%) was observed between levels A and C in AZST84 (Table 7).

Mean germination percentages were higher in the Lew-MS materials than in AZST84 under saline conditions, but this does not indicate Lew and MS are more salt tolerant than AZST84 since these two groups were evaluated under different NaCl treatments (1.94 mM and 2.90 mM, respectively).

Early Seedling Vigor

Data for early seedling vigor (Tables 8 and 9) show similar results as seen for germination percentage except that larger proportional changes in early seedling vigor values were observed between inbred and non-inbred materials in Lew and MS than for AZST84 (see especially groups 2 and 4, Table 9). All the scores in the control were above 4.0 where the possible highest score was 5.0. Mean values ranged from 2.00 in group 2-B to 2.53 in group 1-A for Lew and MS and from 0.78 in group 3-C to 1.29 in group 4-A (Table 8) for AZST84 in saline conditions. Total proportional changes in mean value declined as much as 32.7% for Lew and MS and 30.6% for AZST84 between levels A and B in group 2 and A and C in group 3 (Table 9), respectively.

F values in analysis of variance tables indicate that the

Table 8. Mean score of early seedling vigor in saline and non-saline conditions for inbred and non-inbred alfalfa.

Group no.	Treatment (mM NaCl)	Level of Inbreeding *		
		A	B	C
1	0	4.15±0.15	4.62±0.05	4.51±0.09
	142	2.53±0.21	2.35±0.12	2.35±0.12
		(-39.0)+	(-49.1)	(-47.9)
2	0	4.04±0.13	4.54±0.12	---
	142	2.51±0.24	2.00±0.11	---
		(-37.9)	(-56.0)	(---)
3	0	4.67±0.33	4.44±0.08	4.39±0.21
	222	1.23±0.16	1.10±0.21	0.78±0.09
		(-73.7)	(-75.2)	(-82.2)
4	0	4.27±0.18	4.50±0.05	---
	222	1.29±0.11	1.15±0.11	---
		(-69.8)	(-74.4)	(---)
5	0	4.09±0.10	4.59±0.06	---
	142	2.51±0.16	2.21±0.09	---
		(-38.6)	(-51.9)	(---)
6	0	4.38±0.16	4.88±0.04	---
	222	1.27±0.09	1.13±0.10	---
		(-71.0)	(-76.8)	(---)

* A = S0 x SAMS1; F = 0.
B = S1 x SAMS1; F = 0.028.
C = S2 x SAMS1; F = 0.051.

\+ Control-salt difference as % of control.

Table 9. Proportional changes in mean value for early seedling vigor between alfala of different levels of inbreeding (%).

Group no.	Treatment (mM NaCl)	Levels of inbreeding compared *		
		A,B	B,C	A,C
1	0	+11.3	- 2.4	+ 8.7
	142	- 7.1	- 0.0	- 7.1
		(-18.4)+	(+ 2.4)	(-15.8)
2	0	+12.4	---	---
	142	-20.3	---	---
		(-32.7)	(---)	(---)
3	0	- 4.9	- 1.1	- 6.0
	222	-10.6	-29.1	-36.6
		(- 5.7)	(-28.0)	(-30.6)
4	0	+ 5.4	---	---
	222	-10.9	---	---
		(-16.3)	(---)	(---)
5	0	+12.2	---	---
	142	-12.0	---	---
		(-24.2)	(---)	(---)
6	0	+11.4	---	---
	222	-11.0	---	---
		(-22.4)	(---)	(---)

* A = S0 x SAMS1; F = 0.
B = S1 x SAMS1; F = 0.028.
C = S2 x SAMS1; F = 0.051.

\+ Total change.

differences between generations for early seeding vigor values were also not statistically significant. Early seedling vigor data do however suggest a generally consistent decline in mean value as the level of inbreeding increased in saline conditions, a decline which was not evident under non-saline conditions. This result is very similar to what was seen with germination percentage, which indicates a consistent declining trend in germination performance as the level of inbreeding increases. However, proportinal changes in early seedling vigor were much larger than those observed for germination percentage, so the declining trend was more evident in early seedling vigor than germination percentage. This may mean that early seedling vigor is a more sensitive indicator of the effect of such negative factors as inbreeding during seed germination than is germination percentage.

Germination Speed Index

The results of measurement of germination speed index are presented in Tables 10 and 11. All mean germination speed index values in control conditions were above 0.8 (1.0 represents the maximum value). In saline conditions, mean values for Lew and MS ranged from 0.319 in group 2-B to 0.388 in group 1-A (Table 10). For AZST84, values ranged from 0.210 in group 3-C to 0.305 in group 3-A (Table 10). Trends in proportional changes in germination speed index between generations were similar to those

Table 10. Mean germination speed in saline and non-saline conditions for inbred and non-inbred alfalfa.

Group no.	Treatment (mM NaCl)	Level of Inbreeding *		
		A	B	C
1	0	0.818±0.033	0.904±0.015	0.822±0.031
	142	0.388±0.020	0.372±0.026	0.354±0.017
		(-52.6)+	(-58.9)	(-56.9)
2	0	0.810±0.046	0.875±0.024	---
	142	0.381±0.028	0.319±0.026	---
		(-53.0)	(-63.5)	(---)
3	0	0.917±0.083	0.971±0.014	0.941±0.032
	222	0.305±0.054	0.240±0.035	0.210±0.035
		(-66.7)	(-75.3)	(-77.7)
4	0	0.865±0.056	0.935±0.018	---
	222	0.287±0.032	0.283±0.019	---
		(-66.8)	(-69.7)	(---)
5	0	0.813±0.028	0.893±0.013	---
	142	0.384±0.017	0.351±0.064	---
		(-52.8)	(-60.7)	(---)
6	0	0.878±0.046	0.946±0.014	---
	222	0.291±0.026	0.269±0.017	---
		(-66.9)+	(-71.6)+	(---)

* A = S0 x SAMS1; F = 0.
B = S1 x SAMS1; F = 0.028.
C = S2 x SAMS1; F = 0.051.

+ Control-salt differenc as % of control.

Table 11. Proportinal changes in mean value for germination speed between alfalfa of differentlevels of inbreeding (%).

Group no.	Treatment (mM NaCl)	Levels of inbreeding compared *		
		A,B	B,C	A,C
1	0	+10.5	- 9.1	+ 0.5
	142	- 4.1	- 4.8	- 8.8
		(-14.6)+	(+ 4.3)	(- 9.3)
2	0	+ 8.0	---	---
	142	-16.3	---	---
		(-24.3)	(---)	(---)
3	0	+ 5.9	- 3.1	+ 2.6
	222	-21.0	-12.5	-31.1
		(-26.9)	(+ 9.4)	(-33.7)
4	0	+ 8.1	---	---
	222	- 1.4	---	---
		(- 9.5)	(---)	(---)
5	0	+ 9.8	---	---
	142	- 8.6	---	---
		(-18.4)	(---)	(---)
6	0	+ 7.7	---	---
	222	- 7.6	---	---
		(-15.3)	(---)	(---)

* A = S0 x SAMS1; F = 0.
B = S1 x SAMS1; F = 0.028.
C = S2 x SAMS1; F = 0.051.

+ Total change.

observed for germination percentage and early seedling vigor.

Statistical significance was not observed in the results from germination speed index either. However, the same trend as was seen with germination percentage and early seedling vigor was observed with this trait. Specifically, proportional declines in mean germination speed values increased in saline conditions as level of inbreeding increased, while little or no decline was observed in non-saline conditions.

Relationships between germination performance and seed weight

Seed weight did not differ between any of the groups tested (Table 12) was not significantly related to germination performance in this experiment. Correlation coefficients between seed size and germination percentage, early seedling vigor and germination speed were uniformly low (Table 13). Extremely small seed such as was observed in Lew 18-S2 (0.83 mg/seed where average S2 seed weighed approximately 2.50 mg/seed), exhibited poor germination performance. But such seed were very uncommon and no significant general relationships were observed between seed size and germination behavior.

Table 12. Mean seed weight for each population and generation.

Population	Generation/level	Mean seed weight (mg/seed)
Lew	S0/A	2.52 ± 0.23
	S1/B	2.52 ± 0.39
	S2/C	2.43 ± 0.76
MS	S0/A	2.60 ± 0.20
	S1/B	2.62 ± 0.31
	S2/C	2.40 ± 0.31
AZST84	S0/A	2.73 ± 0.25
	S1/B	2.84 ± 0.25
	S2/C	2.74 ± 0.14

Table 13. Correlation coefficients between seed weight and germination percentage, early seedling vigor and germination speed.

	Germination percentage	Early seedling vigor	Germination speed
R^2	0.079	0.005	0.098
Prob >¶R¶	0.226	0.944	0.133

SUMMARY AND CONCLUSIONS

Two generations of inbred alfalfa plants were derived from randomly selected (S0) clones from two cultivars, Lew and Mesa-Sirsa and the germplasm AZST84. Progeny resulting from one and two generations of self-pollination of these clones were treated as S1's and S2's, respectively. Three generations of hybrid seed were produced using these S0, S1 and S2 plants as male parents in crosses with the male sterile clone SAMS1. Germination tests were conducted with these hybrid seeds which represented three different levels of inbreeding with the goal of comparing the effects of inbreeding on germination under saline and non-saline conditions. The objective of this study was to determine whether inbreeding exacerbates the effects of salt stress during germination.

Germination percentage, early seedling vigor and germination speed were measured in this experiment. No inbreeding depression was observed for any of these measures of germination performance under non-saline conditions. Although F values indicated that differences between generations were statistically insignificant, consistent declining trends in germination performance were observed as the level of inbreeding increased under saline conditions. Veronesi and Lorenzetti

(1983) reported that the level of inbreeding had no effect on alfalfa during germination stage, but increasing stress in the research reported here did lead to indications of inbreeding depression. Less proportional decline between generations (i.e. inbreeding depression) was observed for germination percentage than for early seedling vigor and germination speed. This suggests that inbreeding primarily delays germination and reduces early seedling growth rather than inhibiting germination under salt stress. Therefore, the apparent heterozygosity-maintaining effects of selection for salt tolerance at germination would apparently only be realized if selection is based on germination speed or early seedling vigor. The seed utilized in this experiment had relatively low coefficients of inbreeding (F for S0 = 0, S1 =0.028 and S2 = 0.051). These values may have been too low to observe significant effects of inbreeding on germination. Further studies are needed by using seed with higher levels of inbreeding in order to demonstrate significant inbreeding effect during germination under salt stress.

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