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PHYSIOLOGY OF SALT TOLERANCE IN ALFALFA

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PHYSIOLOGY OF SALT TOLERANCE IN ALFALFA

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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Stephen Gregory Allen

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ABSTRACT

The application of fertilizers and saline irrigation water have resulted in increased soil salinity and the removal of large land areas from crop production. One method to overcome the effects of soil salinity is to increase the salt tolerance of crops. The objective of this research was to investigate the physiological, genetic, and agronomic differences between alfalfa (Medicago sativa, L.) bred for increased salt tolerance and salt sensitive alfalfa. The materials used in these studies were the result of five cycles of selection for germination NaCl tolerance, AZST 1978 to 1982, and the source population, 'Mesa-Sirsa'.

All salt-tolerant cycles and Mesa-Sirsa were evaluated for ability to germinate in NaCl, $NaNO_3$, KCl, KNO_3, mannitol and polyethyleneglycol (PEG) solutions ranging from -1.0 to -1.6 MPa of osmotic potential and a control of distilled water. Germination in the lower osmotic potentials of all germination medias was significantly higher with each succeeding cycle of selection for germination NaCl tolerance. Selection for tolerance to NaCl during germination also resulted in increased tolerance to the other salts as well as mannitol and PEG. Germination in mannitol was higher than in any of the salt solutions. This suggests that ion toxicity also inhibits germination.

There was no significant difference between Mesa-Sirsa and AZST 1982, the most salt-tolerant cycle, in seed respiration in NaCl

solutions or in uptake of tritiated NaCl solution during germination. Broadsense heritability of germination NaCl tolerance was estimated at 49 %.

All the Arizona Salt Tolerant cycles and Mesa-Sirsa were evaluated for several mature plant characteristics under non-saline field conditions. There were no significant differences among germplasm sources in forage yield, apparent photosynthesis, transpiration, or diffusive resistance.

Seedlings of Mesa-Sirsa and AZST 1982 were grown in NaCl solutions ranging from 0 to 18000 ppm NaCl in the greenhouse. The plants were evaluated for several plant growth characteristics to determine whether selection for germination NaCl tolerance resulted in increased salt tolerance at more mature plant growth stages. There was no evidence that germination salt tolerance is related to salt tolerance at later growth stages in alfalfa. Salt tolerance during germination and later growth stages may be controlled by different physiological and genetic mechanisms.

INTRODUCTION

Agricultural science has made tremendous contributions in terms of increasing crop productivity on arable lands. Improved crop cultivars and management practices, and the discovery and application of effective herbicides and pesticides have all led to significant increases in yield for all major crop species.

Yet an ever increasing world population dictates a need for even greater food production in the future. One way to meet this need is to bring marginally productive and presently non-arable land, much of which is affected by salinity, under crop production. It has been estimated that between 400 X 10^6 (Ponnamperuma, 1977) and 950 X 10^6 hectares (Massoud, 1974) are adversely affected by soil salinity. Most saline soils are found in arid and semiarid regions of the world, but the problem also exists in some subhumid areas (Epstein et al., 1980).

The problem of saline soils is increasing. Approximately 40,000 hectares of the Indian sub-continent are annually removed from crop production due to increasing soil salinity (Chapman, 1975). An estimated 2 % of the agricultural lands in Western Australia have been lost to crop production due to increasingly saline soils in recent years (Marshall, 1977).

Saline irrigation water, as well as the application of fertilizers, are the factors most responsible for increasing soil salinity (Epstein et al., 1980). Agriculture in the arid and semiarid

regions of the world is dependent on irrigation to supply adequate moisture for crop production. Unfortunately, irrigation waters often contain large quantities of dissolved salts. Evaporation of water from canals, reservoirs, and fields tends to increase the concentration of salts in irrigation water. An estimated 230 X 10^6 hectares are irrigated world wide (Wittwer, 1979), and nearly a third of this area is affected by excess salinity (Eckholm, 1975; Kelley et al., 1979).

In highly productive areas under intensive crop management, it has been economically possible to desalinize soils. In the Imperial Valley of California, for example, extensive areas of land have been underlain with drainage tile and pipe so that the salty leachates of the irrigation water can be drained off into the Salton Sea (Kelley et al., 1979). For most agricultural areas the cost of soil desalinization is prohibitive.

Another approach to utilizing saline soils is to grow crops which exhibit salt tolerance. A number of crop species such as barley (Hordeum vulgare, L.) and sugar beet (Beta vulgaris, L.) have relatively high levels of tolerance to salinity (Kelley et al., 1979; Lessani and Marschner, 1978).

Other species generally considered to be salt sensitive carry genetic variability for salt tolerance (Venables and Wilkins, 1978; Kelley et al., 1979). Some crop species, tomato (Lycopersicon esculentum, L.), for example, have closely related species with a high degree of salt tolerance. Crosses between related species have resulted in increased salt tolerance in commercial cultivars (Kelley

et al., 1979).

Breeding efforts to utilize intraspecific and interspecific genetic variability should produce a wide variety of salt tolerant crops for the future. At the present time our knowledge of the mechanisms which govern salt tolerance is limited; in fact, there is evidence that these mechanisms may differ widely among species (Greenway and Munns, 1980).

Alfalfa (Medicago sativa, L.) is the most important forage crop in Arizona and the arid and semiarid Southwestern United States. Much of this region is affected by saline soils, and efforts at the University of Arizona have resulted in the development of alfalfa germplasm that is salt tolerant during seed germination (Stone et al., 1981; Dobrenz et al., 1982; Smith et al. 1981a; Dobrenz et al., 1983). Although these breeding efforts have proven successful, the physiological basis of salt tolerance in alfalfa remains largely unknown.

The objectives of this research were to explore the physiological basis of salt tolerance in alfalfa and to determine the relationship between salt tolerance during seed germination and later growth stages.

LITERATURE REVIEW

Soils with a high salt content can effect plants in two main ways. One of these effects is to change the water balance of the plant and the soil in which it grows. This response is due to the osmotic effects of salts in the soil solution. The other salt effect is a specific ion or toxic effect on the plant. Inorganic ions may directly interfere with plant metabolic processes, or interefere with the uptake and transport of other ions by the plant (Lagerwerff and Eagle, 1961).

These two plant responses to salinity are not necessarily mutually exclusive, which often makes it difficult to study the effects of salts on a given plant species. Osmotic effects are often confounded with specific ion effects because high total salt concentrations are usually associated with unbalanced ionic concentrations with respect to plant nutrition (Lagerwerff and Eagle, 1961).

Greenway (1973) observed that, in general, salt-sensitive species are more susceptible to specific ion toxicity while salt-tolerant species are more likely to suffer from osmotic effects.

Inorganic Ion Accumulation

Toxic effects of salts occur when salts accumulate in excess amounts in plant tissue (Greenway, 1973). In comparing soybean (Glycine max, L.) cultivars, for example, Abel and Mackenzie (1964)

found that leaves of salt-sensitive cultivars accumulated 25 times more Cl (50,000 ppm) than salt-tolerant cultivars (2,000 ppm) when grown in high NaCl concentrations. Approximately 15 times as much Cl (30,000 ppm) accumulated in the stems of the salt-sensitive cultivars. The Cl levels of leaves and stems of the salt-tolerant cultivars did not change appreciably as the salt concentration of the external medium was increased. Cl levels in the roots of both the sensitive and tolerant cultivars increased to approximately 10,000 ppm in the highest salt treatment.

Greenway and Thomas (1965) studied Cl regulation in individual tissues of barley, a relatively salt-tolerant crop, when grown in media with a high NaCl concentration. Cl concentrations increased similarly in shoots and the oldest leaves for the first 5 days after salt treatment. After this period, salt concentrations leveled off in the shoots, but continued to increase linearly in the most mature leaves.

The younger, still expanding, leaves also showed linear increases in Cl accumulation, but at a much slower rate than the older leaves. The expansion and uptake of water by the younger leaves was thought to have a dilution effect upon Cl concentration which was responsible for the apparent slow rate of Cl accumulation. The roots accumulated less Cl than either the shoots or leaves as the NaCl concentration of the root media was increased.

Lessani and Marschner (1978) found that Na and Cl concentrations increased similarly in the leaves of sugar beets, a salt-tolerant crop species, and beans (Phaseolus vulgaris, L.), a

highly salt-sensitive species, as the NaCl concentration of the root media was increased. Marschner et al. (1981) observed ion accumulation in sugar beet genotypes representing a range of tolerance to salinity. The more tolerant genotypes were accumulated more Na and Cl in the shoots, while the less tolerant genotypes accumulated fewer ions in the shoot, but more in the roots, than the tolerant genotypes. These results are directly opposed to the previously discussed findings of Abel and MacKenzie (1964) with soybeans.

In halophytes, the most salt-tolerant of all plants, ion accumulation nearly always results when the plants are grown in a highly saline environment (Flowers et al., 1977). The relationship between ion accumulation or exclusion and salt tolerance is confusing, with apparently contradictory experimental results having been reported. Greenway and Munns (1980) have made some generalizations in an attempt to explain the situation. They maintain that halophytes, which accumulate Na and Cl ions, store these inorganic ions in the vacuole of the leaf cells. This separates the high ion concentration from both the salt-sensitive metabolic apparatus located in the cytoplasm and the organelles present in the cytoplasm. Synthesis of compatible organic solutes in the cytoplasm maintains a sufficiently low cytoplasmic osmotic potential for uptake of water even when the plants are grown in saline soils with a low water potential.

Alternately, salt-sensitive glycophytes, many of which are also ion accumulators, are thought to have inadequate compartmentation of ions within the leaf cells. This causes high ion concentrations in the cytoplasm which may inactivate enzymes and interfere with metabolic

processes.

The more salt-tolerant glycophytes are generally considered to be ion excluders by Greenway and Munns (1980). Ion exclusion may result in leaf cells deficient in solutes for osmotic adjustment, leading to decreased water absorption from saline soils with low water potential.

Ion accumulation could have significant applications for selection and breeding of salt-tolerant crop cultivars. For dryland crops grown on saline soils a certain amount of ion accumulation may be necessary to create a sufficiently low osmotic potential and cellular water potential to maintain water absorption from the soil. A high cellular water potential would reduce water uptake and consequently reduce pressure potential of the cells resulting in reduced cell extension and growth.

Irrigated crops grown on saline soils are also subject to physiological drought conditions when salt in the soil reduces soil water potential, but not to the same degree as crops grown on drier saline soils. Consequently, irrigated crops may be able to grow and survive better without osmotic adjustment and ion accumulation, indicating that ion excluding crops may be better suited to irrigated agriculture.

Smith et al. (1981a) showed that as soil salinity increased so did the Cl concentration in the leaf tissue of alfalfa. Increasing soil salinity was also associated with damage to the plant top growth. However, root and crown tissue appeared to be less severely affected. These results indicate that ion accumulation in alfalfa is

detrimental. It does not necessarily indicate that ion exclusion would result in increased salt tolerance, as is the case with soybeans (Abel and MacKenzie, 1964). It is possible that salt-tolerant alfalfa may still accumulate inorganic ions, but that other physiological adaptations allow the plants to tolerate high internal ion concentrations which have been reported for other salt-tolerant glycophytes (Lessani and Marschner, 1978; Greenway and Thomas, 1965).

The results of Smith et al. (1981a) suggest that alfalfa seedlings are subject to Cl toxicity. The high rates of ion accumulation found in alfalfa seedlings reduce the probability that osmotic effects of the salt in the soil were the primary cause of damage to the plants. Osmotic effects cannot be ruled out completely, however.

Synthesis and Accumulation of Compatible Organic Solutes

There have been numerous studies during the past decade to determine if there is a relationship between environmental stress and the synthesis and accumulation of compatible, organic solutes in plants. The primary role most often attributed to compatible solutes in salt stressed plants is that of an osmoticum for intracellular osmotic adjustment (Flowers et al., 1977; Stewart and Lee, 1974).

The organic compounds most often implicated in stress metabolism are primary organic acids, nitrogen compounds, and carbohydrates. The accumulation of such material has been found in halophytes as well as glycophytes, and in response to a number of stress situations including water deficits, winter hardening, and salt

stress (Flowers et al., 1977). Recent research has focused on the relationship between compatible solutes and salt stress.

In halophytes, Na and Cl ions are accumulated in high concentrations to maintain cell turgor and plant turgidity (Greenway and Munns, 1980). The level of ion concentrations found in halophytes has been shown in some studies to disrupt activity of in vitro cytoplasmic enzymes (Osmond and Greenway, 1972; Flowers et al., 1977). Several researchers maintain that inorganic ions are stored in the vacuole where they will not effect cellular metabolic processes, and compatible solutes are synthesized and accumulated in the cytoplasm where they balance the lowered osmotic potential of the vacuole (Stewart and Lee, 1974; Flowers et al., 1977). Unlike inorganic ions the compatible, organic solutes do not interfere with the activity of cytoplasmic enzymes (Pollard and Wyn Jones, 1979).

Jennings (1976) warns that the above scenario for halophytes is not supported by sufficient evidence of cellular compartmentation of ions and compatible solutes. He also feels there is insufficient evidence of salt-induced damage to cytoplasmic enzymes. His apprehension is supported by several studies which have shown cytoplasmic enzyme activity to be unaffected by relatively high concentrations of NaCl (Greenway and Osmond, 1972; Weimberg, 1970).

Thompson et al. (1966) have suggested that accumulated solutes, particularly proline, may act as storage compounds for energy, and reduced nitrogen and carbon compounds for post-stress metabolism.

The role of organic solutes is even less clear when considering glycophytes, some of which store inorganic ions and some of which do

not. Storey and Wyn Jones (1975) measured choline, betaine, and proline levels in the shoots and roots of one cultivar of tomato and three cultivars of barley under salt stressed (100 to 150 mM NaCl) and nonstressed conditions. Choline levels in all plants sampled were not significantly affected by a saline growth media, and betaine levels were unchanged in roots and shoots of tomato. In barley, however, there were varietal differences in NaCl-induced betaine accumulation. One cultivar, 'Zephyr', showed no appreciable accumulation, while 'California Mariout' responded with a two-fold increase in shoot betaine content. 'Arimar' barley, however, contained eight times as much betaine in both roots and shoots of salt stressed plants.

Proline levels increased significantly in both tomato and barley plants treated with NaCl. There were differences among barley cultivars for proline accumulation. The authors speculated that the quaternary ammonium compounds, betaine and proline, may function as nontoxic osmotic agents in certain plants.

Tal et al. (1979) studied proline accumulation in cultivated tomato and two salt-and drought-tolerant wild relatives (Lycopersicon peruvianum, Mill. and Solanum pennelli, Cor.) under NaCl-induced salt stress and polyethylene glycol (PEG)-induced drought stress. Free proline levels increased in all three species under both PEG and salt-induced stresses. The cultivated species accumulated more proline than the wild relatives under both stresses. This led the authors to conclude that proline accumulation does not play an important role in either salt or drought tolerance.

Chu et al. (1976) measured proline accumulation in 12 day old

'Prior' barley seedlings growing in iso-osmotic solutions of PEG (molecular weight 4,000), KCl, and NaCl. The seedlings grown in NaCl solutions accumulated less proline than those grown in PEG or KCl at equal osmotic potentials. The results indicated that Na ions inhibit proline accumulation; whereas Cl and K ions had no significant effect. The PEG-grown plants had the highest levels of proline, suggesting that osmotic stress stimulates proline accumulation. Specific ion effects appear to alter proline metabolism in some cases.

In 1977, Storey and Wyn Jones investigated choline, betaine, and proline levels in 14 plant species, including salt-tolerant halophytes, semi-tolerant glycophytes, and salt-sensitive glycophytes, representing a wide range of salt tolerance. Choline contents did not increase significantly in any species as NaCl levels increased. Betaine levels were similarly high in halophytes before and after salinity treatments, whereas betaine was significantly higher in semi-tolerant glycophytes only after NaCl treatment. Betaine levels were low in all salt-sensitive glycophytes before and after NaCl treatment. Proline content increased in all species after exposure to NaCl, with the most salt-tolerant species accumulating the greatest amounts of proline.

Choline content in plants has seldom been shown to be related to the salt concentration in which the plants were grown. Betaine and proline, however, appear to be associated with salt stress in some cases, but with different responses occurring among species and even among cultivars within a species. The inconsistency of the results for organic solute accumulation may reflect different mechanisms of salt

tolerance if organic compounds actually function to relieve salt-induced stress. Also, it is not clear whether these compatible solutes are synthesized as a protection mechanism to prevent stress, or as a result of stress.

The Effect of Salinity on Plant Water Balance

As soil water potential decreases due to increasing salinity, the water potential of the plant cells must decrease to maintain a negative water potential gradient between the root environment and the plant vascular system. Cellular water potential may be reduced either by decreasing the pressure potential, which can result in detrimental effects on plant growth, or by decreasing the cell osmotic potential, which is called osmotic adjustment (Bernstein, 1961).

As previously indicated, cell osmotic potential of some plants may be influenced by the accumulation of inorganic ions from saline growing media, or to a lesser degree by the synthesis and accumulation of organic solutes. Osmotic adjustment occurs in the halophytes so that cell water potential decreases while cell pressure potential remains relatively contsant (Flowers et al., 1977).

Salt tolerance in some glycophytes is attributed to the ability of plants to exclude potentially toxic ions (Greenway, 1973; Abel and MacKenzie, 1964). In these plants, partial osmotic adjustment can occur due to synthesis of organic solutes, or else the plants may lose pressure potential to reduce cellular water potential. When these plants are grown in highly saline soils, especially if coupled with low soil moisture conditions, the result will be a loss of turgor,

decreased growth, and possibly death of the plant (Greenway and Munns, 1980)

In any case, whether the plants are ion accumulators or ion excluders, the water balance and the components of water potential are usually affected by growth under saline conditions.

Bernstein (1961) found that osmotic potentials of both the above and below ground plant parts decreased in cotton (Gossypium hirsutum, L.) and pepper plants (Capsicum annum, L.) over as wide a range of salinity as would permit plant growth. Growth inhibition occurred for both species with increasing salinity, but plant turgor remained constant due to increased osmotic concentration and osmotic adjustment. The results indicated that growth inhibition was not due to lowered pressure potential. Bernstein feels that osmotic adjustment may inhibit growth. The cellular organelles, such as plastids and mitochondria, might not adjust to decreasing cellular osmotic potentials, resulting in dehydration and reduced activity.

Gale et al. (1967) investigated the effect of NaCl on the water relations of onion (Allium cepa, L.), bean, and cotton plants. Osmotic adjustment of leaf sap occurred in bean and cotton, but not in onion plants grown in NaCl solutions. The water potential, pressure potential, transpiration, and stomatal conductance were all reduced in onion plants. Osmotic adjustment of the bean and cotton plants maintained nearly constant pressure potentials as salinity increased. Stomatal conductance decreased causing a reduction in transpiration in both species. The authors believed that stomatal closure and subsequent decreases in transpiration may have been due to a lack of

osmotic adjustment in the guard cells, or to a toxic effect on the biochemical processes involved in stomatal regulation.

Other studies with bean plants have shown that osmotic adjustment of leaf tissue was due primarily to increasing accumulation of Cl in the leaves when plants were grown in media containing high levels of NaCl (Meiri and Poljakoff-Mayber, 1969). Osmotic adjustment, or decreased leaf osmotic potential, lagged behind the decreasing leaf water potential when the plants were placed in NaCl solutions, resulting in an initial loss of turgor.

After Cl accumulation occurred in the leaves, turgor returned to nonsaline control levels. Relative water content of the leaves also began declining immediately after the plants were placed in a saline media but returned to control levels after osmotic adjustment was completed. The plants appeared to suffer from a disturbed water balance when placed in the saline environment. The effects were not permanent, and when returned to nonsaline media, normal water relations within the plant resumed. The adverse effects of the NaCl were partly negated by osmotic adjustment when plants remained exposed to salinity for prolonged periods (Meiri and Poljakoff-Mayber, 1969).

Meiri and Poljakoff-Mayber (1970) investigated bean plants grown under various fluctuating regimes of NaCl salinity in order to simulate irrigated field conditions. Growth was inhibited at all treatment levels and transpiration was reduced in proportion to salinity. When the plants were returned to non-saline conditions, growth and transpiration increased, but not to the level of non-salinized control plants. This may have been the result of a

permanent toxic effect of the NaCl.

O'Leary (1969) studied the effect of NaCl on root permeability, relative water content of leaves, and stomatal conductivity in bean plants. Root permeability was measured by forcing water under pressure through excised roots. The rate of water movement through roots of non-salinized control plants was significantly higher than the plants grown in NaCl solutions of -2 and -4 bars osmotic potential. Leaf relative water content and stomatal conductance of water vapor also decreased significantly for the plants grown in salt solutions. The lower water content indicated a loss of turgor and a water stress condition.

O'Leary (1969) feels that physiological drought can result when plants are grown in saline media even when osmotic adjustment occurs. This results from the increased root resistance to water flow, which reduces the water delivery to the leaves causing partial stomatal closure. A decreased net photosynthetic rate, which lowers the food reserves delivered to the roots, results in less root growth and root area available for water absorption. This cycle results in an overall reduction of water uptake and plant growth.

The effect of NaCl on the water status of sugar beets, a much more salt-tolerant species than beans, has also been studied. Unlike beans, sugar beets grown in NaCl solutions developed higher relative water contents than non-salinized control plants (Milford et al., 1977). Ion accumulation in the sugar beets resulted in leaf expansion and greater succulence. The authors hypothesized that the increased leaf growth and associated water uptake diluted the accumulated ions

causing a relatively stable osmotic potential and pressure potential in the growing plant, thus the water balance was largely unaffected by salinity.

These results seem contradict O'Leary's (1969) findings that NaCl induced decreased root permeability to water in beans. It may be that root permeability is unaffected in sugar beets, or even increased, and might be at least one of the causes of the high degree of salt tolerance found in sugar beets.

A recent study by Sanchez-Dias et al. (1982) examined NaCl effects on the water relations of alfalfa. Transpiration, predawn leaf water potential, and stomatal response were measured in two alfalfa cultivars, 'Tierra de Campos' and 'Aragon', at three NaCl stress levels, 0.05, 0.30, and 0.50 MPa. Leaf diffusive resistance increased and transpiration rates decreased as salinity increased. Predawn leaf water potentials were not significantly affected by salinity, due to ion uptake and osmotic adjustment according to the authors. This conclusion appears to be incorrect. Osmotic adjustment results in a lowered leaf water potential due to an increased solute concentration. The lower leaf water potential is necessary to maintain a negative water potential gradient between the growth media and the plant tissue.

The effect of NaCl on the water potential of germinating alfalfa seeds was examined by McDonough (1976). The seeds were germinated in NaCl solutions ranging from -2 to -16 bars osmotic potential. For the first 10 hours after treatment, the seed water potentials roughly reflected the water potential of the corresponding

germination media. After 72 hours the water potentials of seeds in all salinity treatments were similar, ranging from -15 to -18 bars.

The results of experiments on a number of different species indicate that plant water balance is generally affected in some way by the osmotic effects of salt. The first effect of salt is to lower soil water potential which causes a reduction in plant water potential and pressure potential. In most species some ion accumulation leads to at least partial osmotic adjustment and turgor maintenance, although growth inhibition still results in most non-tolerant species.

When returned to non-saline growth media most plants resume growth indicating that osmotic effects, or a disturbed water balance, is at least partially responsible for growth inhibition. One would not expect a rapid recovery from an ion toxicity condition.

The different effects of NaCl on water balance observed among species may reflect different mechanisms involved in salt tolerance, or possibly just different levels of salt tolerance.

The Effect of Salinity on Photosynthesis and Respiration

As previously indicated, salinity can theoretically affect plants in two primary ways, by specific ion toxicity and by osmotic effects. Many researchers have reported experimental results indicating that photosynthesis and respiration are influenced by salinity. In most cases the results have not been able to show conclusively whether ionic or osmotic effects of salt are responsible for the observed changes in metabolic processes.

Nieman (1962) measured photosynthesis and respiration in leaf

disks of 12 crop species representing a wide range in salt tolerance. Measurements were taken in a Warburg apparatus on plants grown in NaCl solutions of -1 to -4 atmospheres osmotic potential.

The rate of photosynthesis per unit of leaf area was not significantly influenced by salinity at any of the treatment levels for any of the species tested. Photosynthesis measured per unit of chlorophyll decreased with increasing salinity for the four most salt-sensitive species. Respiration was stimulated in leaf disks of all species except peas (Pisum sativum, L.). Nieman believes that supra-optimal growth temperatures probably inhibited respiration of the peas in this experiment. Caution should be used when interpreting Nieman's data. His measurements of metabolic processes in excised leaf disks may not accurately reflect the effect of salinity on whole plants. Also, all of the plants in his experiment were grown in the same light, temperature, and humidity conditions. Considering the wide range of plant species used in this experiment, it is not likely that these environmental conditions were equally conducive for the growth of all species involved.

Photosynthesis was measured in onions, beans, and cotton grown under saline conditions (5.4 atm. osmotic potential of NaCl solution) by Gale et al. (1967). Salinity reduced net photosynthesis of onions, but if the plants were returned to non-saline conditions net photosynthesis increased to control levels. Net photosynthesis of beans was reduced by 30 % when grown in the NaCl solution. This reduction was thought to be caused by partial loss of stomatal conductance because raising the CO_2 concentration in the air caused an

increase in net photosynthesis.

Net photosynthesis also decreased in cotton, but stomatal closure in the salinized plants had very little effect on photosynthesis. CO_2 enrichment of the atmosphere did not increase the net photosynthesis. Salinity was thought to reduce photosynthesis by interfering with the light reaction of photosynthesis. It was concluded that NaCl affects net photosynthetic rates differently for different species.

Boyer (1965) found similar results for the affect of NaCl on cotton photosynthesis. The cotton plants were grown in NaCl solutions ranging from -0.5 to -12.5 bars osmotic potential for 4 weeks. Stomatal resistance to CO_2 diffusion remained stable as salinity increased. Yet net photosynthesis decreased. Respiration also decreased slightly with increasing salinity.

In general, salinity appears to have a detrimental effect on photosynthesis. Photosynthetic activity has decreased in response to salinity in nearly all crop species examined.

Greenway and Munns (1980) feel that reduction of photosynthesis may, in some cases, be a secondary response to salinity. The primary response could be a reduction of the growth rate due to osmotic stress and a subsequent reduction in the utilization of photosynthate. Osmotic stress could also produce a decrease in stomatal conductance to CO_2 exchange. Either of these effects would create an inhibition of photosynthesis. In other instances, mesophyll resistance to CO_2 assimilation has accounted for reduced photosynthesis, indicating that the primary salinity effect was on the photosynthetic mechanism

(Downton, 1977).

Respiration rates have been reported to both decrease and increase in response to salinity. Increased leaf respiration could be due to an energy demand created by compartmentation of ions within leaf cells and the necessary active movement of ions against a concentration gradient. Decreased respiration rates could occur in leaf tissue of plants which exclude ions at the soil-root interface. Reduced leaf expansion due to salinity would result in lower photosynthate utilization and lower respiration rates. In these plants respiration rates may be very high in the roots where metabolic energy would have to be expended to exclude the inorganic ions. No reliable data are available to support this hypothesis.

The Effects of Salinity on Plant Growth and Development

The effects of salinity on plant growth and development are well documented. Many researchers have studied the effects of salts on such characteristics as seed germination, plant growth parameters, and leaf anatomy. The growth responses to saline environments differ widely between species and few generalizations can be made concerning the effects of salinity on overall growth and development.

Seed Germination

Along with proper temperature and light conditions, moisture is one of the most important factors which influences seed germination (Doneen and MacGillivray, 1943). Therefore, the most obvious effect of salt on seed germination would be a reduction in the water

potential of the germination media to a level low enough to prevent or alter the absorption of water by the seed. Germination may also be influenced by toxic effects of the ions present in the germination media, or by a combination of osmotic and toxic effects.

McDonough (1975) measured the water potential of germinating seeds of a number of different species including alfalfa. Measurements of water potential were made with a thermocouple psychrometer periodically between 10 and 120 hours after imbibition was begun in distilled water. Alfalfa seeds reached a water potential maximum of -2 bars after the initial imbibition period and then declined to a stable plateau of -11 bars after 80 hours, when germination was completed.

McDonough (1975) believes that water potential decreases during germination due to the solubilization of reserve compounds in the seed resulting in a lower osmotic potential and the redistribution of water within the seed to areas with a higher matric capacity. He also feels that germination may be inhibited if the water potential of the germination media falls below that of the seed at any stage of the germination process. This would suggest that alfalfa seeds would most likely be affected by salinity during the initial imbibition period when seed water potentials were at their highest levels. If this is true, germination could be inhibited by media with water potentials of -2 bars or less.

McDonough (1976) found in a later experiment that the maximum water potential of alfalfa seed germinating in distilled water was -4 bars. When seeds were placed in NaCl solutions with water potentials

less than -4 bars, germination was significantly reduced. Germination was not significantly affected when the germination media had osmotic potentials equal to or greater than -4 bars. He also germinated alfalfa seeds in PEG solutions ranging from -2 to -16 bars water potential. Germination was reduced from 100 % in distilled water to 42 percent at -4 bars. No germination occurred at less than -4 bars osmotic potential.

Germination was depressed more by PEG than NaCl under iso-osmotic conditions. This may have been due to better osmotic adjustment of seeds germinated in the NaCl solutions. These results indicate that germination was probably inhibited by osmotic rather than toxic effects.

Several experiments have been conducted specifically to determine whether toxic or osmotic influences of salinity are responsible for germination inhibition. Uhvits (1946) measured germination of alfalfa seeds in iso-osmotic solutions of NaCl and mannitol ranging from -1 to -15 atmospheres osmotic potential. Germination percentage decreased with decreasing osmotic potential of both types of media. NaCl decreased germination more than mannitol at equal osmotic potentials.

Uhvits (1946) maintained that these results indicated a possible toxic effect of NaCl. Mannitol may act as a compatible solute to increase osmotic adjustment in germinating seed and may even be used as a respiratory substrate within the seed to enhance germination (Long, 1943).

Redman (1974) compared the percent germination of alfalfa seeds

in iso-osmotic solutions of NaCl, PEG, and mannitol ranging from 0 to -15 bars osmotic potential. He found that NaCl reduced germination less than either mannitol or PEG, which were similar in their inhibition of germination. Redman also measured germination recovery after treatment with the osmotic agents. Seeds in the mannitol and PEG treatments had significantly higher recovery rates when placed in distilled water as compared to seeds from the NaCl treatment. Redman interprets these results as evidence of a toxic effect of the NaCl during germination.

The results of these experiments indicate that NaCl salinity consistently results in an inhibition of alfalfa seed germination. Osmotic inhibition has been established in most experiments. It is difficult to determine whether or not toxic ion effects contribute substantially to germination inhibition. If toxic effects occur, it is probably at osmotic potentials lower than those responsible for osmotic inhibition. The toxic effects are therefore masked by the osmotic effects and are difficult to measure.

Plant Growth

Salinity affects plant growth of different species in different ways. The halophytic species generally require a moderate level of soil salinity to maintain optimal growth conditions. Most halophytes reach optimum growth rates with 200 to 500 mM of NaCl in the root environment (Greenway and Munns, 1980)

Glycophytes, in contrast, generally reach their maximum growth rates in non-saline conditions, but the glycophytes represent a broad

range of susceptibility to salinity. Sugar beets, for instance, have exhibited increased fresh weights, dry weights, and sugar yield in response to NaCl applications (Milford et al., 1977; Draycott and Farley, 1971). Ulrich and Ohki (1956) found maximum growth stimulation of sugar beets to occur at 25 to 30 meq NaCl l^{-1} in the external growth medium. Eaton (1942) has shown that tomato, another relatively salt-tolerant glycophyte, also exhibits growth stimulation by small concentrations of Cl salts up to 10 meq l^{-1}.

In his study involving 12 crop species grown in a range of NaCl treatments, Nieman (1962) found that growth response, measured as fresh weight increase, ranged from stimulation of the most salt-tolerant species to severe depression and death of the most sensitive species. Bean plants, a salt-sensitive species, respond to even small concentrations of salt with growth retardation (Meiri and Poljakoff-Mayber, 1970).

O'Leary (1971) suggested that salt-induced growth inhibition can be altered by changing the relative humidity of the environment surrounding the plant. This hypothesis is supported by data presented by Hoffman et al. (1971). Shoot growth of cotton was 40 % greater at 90 % relative humidity than at 65 % relative humidity. The shoot to root ratio was also doubled at the higher relative humidity.

Salinity also affects the growth of alfalfa plants. Smith (1975) indicated that high applications of KCL fertilizer caused burning of the leaflets and shoot death of alfalfa. This was thought to be caused by the Cl ion since alfalfa requires high levels of K for optimal growth. LaCroix (1969), as cited by Smith and Struckmeyer

(1977), found that high plant mortality occurred when Cl concentrations in alfalfa reached 5 %.

In a series of greenhouse experiments using potted alfalfa seedlings, Smith et al. (1981a) determined that Cl was much more toxic than Na in reducing plant growth. Replacing NaCl with KCl treatments did not influence plant growth, but replacing NaCl with Na_2SO_4 resulted in much greater plant growth. Growth in K_2SO_4 was also higher than in NaCl solution. They also found that while damage occurred in top growth and crown tissue at high Cl treatments, the roots remained relatively unaffected. The root tissue also accumulated significantly less Cl than the above ground tissues.

Plants which were initially exposed to non-lethal concentrations of Cl appeared to develop a greater tolerance to subsequent higher application rates of Cl. This result indicated that alfalfa plants may become acclimated to high levels of salinity. It also reveals a potential problem in the methodology of studying salt tolerance in alfalfa.

Very little information is available concerning crop yields under field conditions and controlled salinity treatments, probably due to the difficulty in controlling such an experiment. The results of most growth chamber and greenhouse studies indicate that salt-sensitive crops, such as alfalfa, would suffer yield depression under saline soil conditions.

Brown and Hayward (1956) did conduct a yield trial of six alfalfa cultivars over a 3-year period in field plots treated with irrigation water containing 0, 3000, and 9000 ppm of a one to one

ratio of NaCl and $CaCl_2$ salts. The average yields over all cultivars were reduced to 79 and 42 % of the control at the 3000 and 9000 ppm salt treatments, respectively.

Leaf Anatomy

One of the most salient effects of salinity is on leaf anatomy. In general, salinity causes increased leaf succulence and a decrease in leaf expansion. These observations appear true for a wide variety of plant species, including many of the halophytes as well as most salt-sensitive glycophytes (Jennings, 1976).

The bulk of the available literature indicates that the Cl ion is primarily responsible for increased leaf succulence in glycophytic plants grown in saline conditions. This response to Cl has been observed in tomato (Hayward and Long, 1941), beans (Lagerwerff and Eagle, 1961; Gauch and Wadleigh, 1944), as well as other species. Yet, in several of the halophytes, Atriplex nummularia, Lindl. and Atriplex halimus, L., Na has been indicated as a cause of increasing leaf succulence by Greenway (1968), and Gale and Poljakoff-Mayber (1970), respectively.

Meiri and Poljakoff-Mayber (1967) studied the effect of Cl on area and thickness of bean leaves. Cl caused reduced leaf area and increased leaf thickness compared to non-saline control plants. Leaf thickening was caused by enlargement of cells in the palisade layer of the mesophyll. The epidermal cells, in contrast, were smaller in response to the Cl treatment, which the authors felt was responsible for the smaller leaf area. Wignarajah et al. (1975) observed similar

results in their study of the effects of NaCl on bean leaves.

Meiri and Poljakoff-Mayber (1970) studied the effects of fluccuating salinity treatments, at various Cl levels, on leaf expansion of bean plants. Changes in leaf area were very sensitive to Cl concentration. Faster rates of salinization also appeared to inhibit leaf expansion more than the slower rates of salinization, and the length of exposure to Cl often had a stronger effect on leaf expansion than the final level of Cl.

Gausman and Cardenas (1968) examined leaves of cotton plants grown in a field containing areas of high and low levels of soil salinity. Their data showed that leaves of plants grown on highly saline soils were thicker and contained fewer stomata and epidermal cells per unit of leaf area. Unfortunately, leaf areas were not measured.

Longstreth and Nobel (1979) examined the consequences of NaCl-induced changes in leaf anatomy on photosynthesis in beans, cotton, and Atriplex patula, L., a salt-tolerant halophyte. Exposure to high concentrations of NaCl significantly increased the ratio of mesophyll surface area to leaf area for cotton and beans, and to a lesser degree for Atriplex patula, L. Net photosynthesis per unit of leaf area did not increase as the mesophyll resistance to CO_2 exchange increased with increasing salinity.

Heritability of Salt Tolerance

Only a small amount of data is available concerning the heritability of salt tolerance in plants. Although, there have been

numerous studies conducted with alfalfa and other crop species to determine differences in tolerance among cultivars.

Brown and Hayward (1956) measured forage yields of six alfalfa cultivars grown in three salt concentration treatments. 'California Common' and 'India' gave the highest yields under the lowest salt treatment, while 'Turkestan' and 'Atlantic' produced the most forage at the medium salinity level. There were no significant yield differences among cultivars at the highest salt treatment. The cation content of the top growth was not significantly affected by any of the salinity treatments, suggesting to the authors that osmotic rather than toxic salt effects were influencing forage yield.

Dotzenko and Dean (1959) studied the ability of seed of six alfalfa cultivars to germinate in mannitol solutions representing a range in osmotic potential. Significant differences occurred among cultivars for ability to germinate at -7 bars osmotic potential.

Dotzenko and Haus (1960) reported the results of an experiment to determine if alfalfa genotypes could be selected within cultivars for their ability to germinate in low osmotic potentials and to examine the heritability of this characteristic. Selections were made within each cultivar for high and low ability to germinate in mannitol solution of -12 bars osmotic potential. The high and low selections within each cultivar were cross pollinated. Three phenotypes appeared in the F_2 generation with respect to germination ability at low osmotic potential. Two of the crosses had germination percentages intermediate to their parents, three crosses resulted in dominance for high germination, and one cross expressed heterosis for ability to

germinate at low osmotic potential. Although no estimates of heritability were calculated from the data, the authors concluded that ability to germinate under conditions of low osmotic potential was highly heritable. Smith et al. (1981b) observed cultivar differences in alfalfa seedling salt tolerance, indicating that selection potential for this trait may also exist.

Successful selection of salt-tolerant alfalfa cell lines has been accomplished using cell suspension culture techniques with a 1 % NaCl media (Croughan et al., 1978). The selected salt-tolerant lines performed poorly in non-saline media, suggesting a salt requirement similar to halophytes.

Differences among sugar beet cultivars for salt tolerance have also been demonstrated (Marschner et al., 1981). Sugar beet salt tolerance was positively associated with Na and Cl accumulation in the shoot.

Abel (1969) has shown that soybean cultivars differ widely in their ability to exclude inorganic ions from the top growth. Unlike sugar beets, the soybean cultivars which were most salt-tolerant were those which were the most successful ion excluders.

These reports indicate that salt tolerance may be successfully selected for in plant breeding programs. The same reports also suggest that mechanisms of salt tolerance differ widely among species. It is important, therefore, to characterize the mechanism of salt tolerance for a given species. This will give the plant breeder a better understanding of which physiological and morphological parameters should be used as selection criteria.

The Relationship Between Salt Tolerance and Drought Tolerance

Much of the literature previously cited in this review has shown that salts in the soil or growth media are capable of producing a physiological drought condition by lowering the soil water potential. There are also numerous reports which indicate that salinity affects are osmotic rather than toxic in nature.

It seems possible that plants or cultivars which exhibit a high degree of salt tolerance might also have greater drought tolerance, especially if salinity affects the species in question primarily by osmotic stress. Yet there are no indications in the literature that breeding and selection of plant materials for salt tolerance has any relationship with selection for drought tolerance.

There have been a number of studies conducted to compare the effects of salts, such as NaCl, and inert osmotic agents such as PEG on seed germination and plant growth (Sanchez-Dias et al., 1982; Parmar and Moore, 1968; Jarvis and Jarvis, 1963; Wiggans and Gardner, 1959). The results of these studies have established that both NaCl and PEG inhibit seed germination and plant growth, often in a very similar manner. Yet, as previously indicated, it is very difficult to distinguish between the confounding osmotic and toxic effects of salts.

Probably the only way to establish a relationship between salt and drought tolerance is to make selections for one of these traits and test the resulting selected lines for the other trait. An investigation by Allen and Dobrenz (1982) with alfalfa indicates a

possible relationship between selection for seed germination salt tolerance and germination drought tolerance. Four cycles of mass selection for germination salt tolerance in NaCl solutions resulted in significant increases in tolerance to NaCl (Dobrenz et al., 1982). The same material was also tested for ability to germinate in PEG solutions. Germination was inhibited more by PEG than NaCl at iso-osmotic concentrations, but the most salt-tolerant lines were also the most tolerant for germination in the PEG solutions.

These results are somewhat contradictory to those of Smith et al. (1981a). They suggest that alfalfa is affected more by Cl toxicity than osmotic effects, at least in the seedling growth stage. Perhaps seed germination is less affected by ion toxicity than seedling growth.

MATERIALS AND METHODS

Germplasm

The alfalfa germplasm used in these studies represented five cycles of mass selection for germination salt tolerance. The source population for the initial cycle of selection was 'Mesa-Sirsa', a high yielding, non-dormant alfalfa cultivar with resistance to several biotypes the spotted alfalfa aphid (Therioaphis maculata, Buckton) found in Arizona. Mesa-Sirsa was developed by personnel from the Arizona Agricultural Experiment Station and the Forage and Grain Insects Research Division of the United States Department of Agriculture. It originated from selections out of P.I. 235736, from India, and was released in 1966 (Schonhorst et al., 1966).

The germination salt-tolerant cycles are designated Arizona Salt Tolerant (AZST) 1978, 1979, 1980, 1981, and 1982, with each succeeding cycle of selection being more salt-tolerant. These lines represent a wide range of tolerance to NaCl during seed germination. Approximately a 10 to 15 fold increase in germination salt tolerance has been realized after five selection cycles (Dobrenz et al., 1982)

Germination Studies

Several seed germination studies were conducted to determine the physiological basis of germination salt tolerance, and to determine the manner by which salts inhibit seed germination.

Germination in NaCl, $NaNO_3$, KCl, KNO_3, Mannitol, and PEG

Mesa-Sirsa and the germination salt-tolerant alfalfa, AZST 1978 through 1982, were evaluated for ability to germinate in six different germination media, NaCl, $NaNO_3$, KCl, KNO_3, mannitol, and PEG 6000. Each of the media was prepared at five concentrations, 0.375, 0.430, 0.485, 0.540, and 0.595 osmoles. The osmolarity of each solution was verified with a Wescor model 1500-c vapor pressure osmometer and all germination solutions were accurate to within plus or minus 0.002 osmoles.

Seventy five percent streptomycin sulfate was added to all germination media at a rate of .01 g l^{-1} to control bacterial contamination, and 1.5 g l^{-1} of 50 % active Captan (active ingredient N[(trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide) were added to each solution to control contamination by fungus.

Individual experimental units consisted of 13.5 by 13.0 by 3.5 cm covered plastic containers which contained 50 seeds sandwiched between two pieces of highly absorbent blotter paper. The pieces of blotter paper were moistened by soaking them in the appropriate germination medium. The germination boxes were placed in a darkened growth chamber at 26 C for a period of 5 days. At the end of this time percent germination was determined. Seeds were considered successfully germinated if their radicle was at least 2 mm in length. Each treatment was replicated four times.

Germination results for a given germplasm source were calculated as a percentage of the germination of that germplasm in the control medium of distilled water. This was done to correct for any

inherent differences in germination potential among the different germplasm entries.

An estimate of the broadsense heritability of germination NaCl tolerance was calculated according to Allard (1960), where heritability equals the genetic variance divided by the sum of the genetic and environmental variances. An analysis of variance of the germination results in the NaCl solutions was used to estimate the genetic and environmental variances. The genetic variance was represented by the mean square for germplasm sources. The environmental variance was estimated by the sum of the mean square values for osmotic concentration, osmotic concentraton by germplasm interaction, and error.

Seed Respiration

Four replications of Mesa-Sirsa and AZST 1982 alfalfa seed were germinated in NaCl solutions of -.60, -1.20, -1.80, -2.40, and -3.00 MPa osmotic potential, and a control solution of distilled water (0.00 MPa osmotic potential). Seed respiration of these samples was measured at 3, 6, 12, 24, and 48 hours after germination was begun.

Each sample consisted of 500 seeds, which were weighed, and placed on four 9.0 cm diameter fine, ashless filter paper disks in 100 by 15 mm plastic petri dishes. After 6.0 ml of the germination medium was applied to the filter papers the petri plates were placed in a darkened growth chamber at 26 C. All germination solutions also contained .10 g l^{-1} streptomycin sulfate (74 % active) and 1.5 g l^{-1} of 50 % active Captan to prevent contamination by bacteria and fungi,

respectively.

Seed respiration was measured with a Beckman Model 865 infrared gas analyzer (IRGA). The top of each petri plate was removed and the petri plate placed in a glass chamber which was connected to the IRGA in an airtight system. The air in the system, which occupied a volume of 774 cm , was pumped through the IRGA at a rate of 2050 cm min^{-1}. The IRGA measured the increase in CO_2 concentration in the system as the respiring seed released CO_2. Results are reported as change in ppm CO_2 min^{-1} g^{-1} of seed.

Water Uptake

Uptake of water was measured in Mesa-Sirsa and AZST 1982 seeds during germination to determine if there are differences between the two seed sources for ability to absorb water and to determine if the addition of NaCl to the germination media influenced water uptake.

A small aliquot of tritiated water was diluted in 10 ml of distilled deionized water. The solution was divided into three 3-ml and one 1-ml portions. The 1-ml aliquot was used to estimate the activity of the solution. One 3-ml aliquot was used as a control germination solution; the other 3-ml aliquots had NaCl added to them to produce .26 and .40 M solutions, respectively.

Twelve lots of 25 seeds of each germplasm were weighed and placed in 12 by 75 mm stoppered test tubes. A .3 ml portion of each of the three solutions was added to four replications of each germplasm. Two replications of each germplasm in each salt concentration were removed from the solutions at 12 and 24 hours. The

seeds were rinsed in distilled water and blotted dry. The seeds were then crushed and placed in liquid scintillation vials with 12 ml of Triton X liquid scintillation cocktail solution. Two 10-ul samples of each tritiated germination solution were also placed in Triton X sclutions and were used to determine tritium activity of the germination media. The NaCl did not significantly influence the activity of the solutions.

The amount of tritium in the standards and the samples was determined by a Beckman model LS 8000 liquid scintillation counter. The liquid scintillation counter provided counts per minute (cpm) and H# data for each sample. Efficiency (E) for each sample was determined from the H# and a standard curve [E = .325 + -.001(H#)] provided by Dr. Paul Bartels, Department of Plant Sciences, University of Arizona. Results are reported as disintegrations per minute (dpm) per milligram of seed weight; dpm were calculated as cpm/E.

Seed Sodium and Chloride Analysis

Na and Cl ion concentrations were measured in Mesa-Sirsa and AZST 1982 seeds germinating in NaCl solutions of -.6, -1.2, and -1.8 MPa osmotic potential and a control solution of distilled water. The NaCl concentrations of the germination solutions were verified with a vapor pressure osmometer, as previously described. Each experimental unit consisted of 500 seeds germinated between blotter papers in closed plastic boxes for 48 hours in a growth chamber at 26 C. After 48 hours the seeds were removed and rinsed in distilled water to remove any excess salts adhering to the seed coats. The seeds were

next oven dried for 48 hours at 80 C then ground in a UD Corporation Cyclone model sample mill. The samples were analyzed for Na and Cl ion content by the Soil and Plant Tissue Testing Laboratory of the Department of Soils, Water, and Engineering at the University of Arizona. Two replications of each treatment were analyzed and each replication consisted of four subsamples which were bulked together. The results were analyzed using an analysis of variance.

Field Studies

Forage yield and several physiological characteristics of Mesa-Sirsa, the five AZST germplasm sources, Large Leaflet (a large leaflet alfalfa germplasm under development), and AZST 1979 syn II were evaluated in bordered, replicated plots at the USDA Plant Materials Center in Tucson, Arizona during 1983. The plant characteristics measured were forage yield, apparent photosynthesis, leaf transpiration, leaf diffusive resistance, and the difference between leaf and air temperature.

The soil type at the Plant Materials Center was a Comoro fine sandy loam. Soil samples were taken from 30, 60, and 90 cm depths for analysis on 5 August 1982. The analysis was performed by the Department of Soils, Water, and Engineering Soil and Plant Tissue Testing Laboratory at the University of Arizona. Results are in Appendix Table A1. The plots were planted in sudangrass (Sorghum sudanense (Piper) Stapf.) during the summer of 1982 and plowed under 3 weeks before planting the alfalfa. Nitrogen fertilizer, as urea, and P_2O_5 were applied at a rate of 67.3 and 336.3 kg ha^{-1}, respectively,

on 14 October 1982. Tolban (active ingredient N(cyclopropylmethyl)--trifluoro-2,6-dinitro-N-propyl-p-toluidine) was applied as a preplanting herbicide on 15 October 1982.

The plots were planted on 19 October 1982. A plot consisted of four 6.1 m rows, with 30.5 cm row spacing. The seed was planted with a single row Planet Junior planter at a rate of 390 seeds m^2. Six replications were planted with 1.2 m alleys between replications. The plots were inoculated with Rhizobium bacteria which were incorporated into the first irrigation on 20 October 1982. Two subsequent flood irrigations were applied in the fall of 1982 and continued again in March of 1983. Irrigations occurred at approximately 14-day intervals until the last harvest in July.

Weeds were controlled by hoeing as needed during the 1983 growing season. Two of the replications were destroyed by rabbits and ants, so measurements were made only on the four remaining replications.

Forage Yield

Forage yield was harvested from the center 4.9 m of the middle two rows of each plot five times during 1983 (13 April, 20 May, 15 June, and 6 and 27 July) at approximately the 10 % bloom stage of growth. The plants were harvested with hand clippers about 4 to 6 cm above the soil surface. The forage yield of each plot was weighed immediately in the field.

A small subsample of each plot was also weighed in the field to obtain a fresh weight. The subsamples were then oven dried for 48

hours at 80 C and dry weights measured. Percent moisture was calculated [(fresh weight - dry weight)/fresh weight] for each subsample and used to calculate the dry forage yield of each plot.

Forage yields at each harvest were analyzed using an analysis of variance, and a combined analysis for the last four cuttings was calculated using a repeated measures procedure. The first cutting was not included in the combined analysis because rabbit damage only allowed the harvest of two replications.

Physiological Studies

Apparent photosynthesis, transpiration, diffusive resistance, and temperature differential between air and leaf temperature were measured twice during the 1983 growing season (14 June and 25 July).

Photosynthesis was measured on two intact stems of a single plant from a border row in each plot. The two stems were sealed in an airtight 1.96 l clear plexiglass cylinder. The chamber was equipped with a small fan to mix the air and two septums for extracting gas samples with syringes. Soft clay was used to seal the area of the chamber where the stems entered. Two 5 ml gas samples were taken from the chamber; the first immediately after the chamber was sealed and the second 30 s later. The stems were cut off and returned to the laboratory where the leaf area of each sample was measured with a Licor model LI 3100 area meter.

The gas samples were also returned to the laboratory and were analyzed for CO_2 concentration on the IRGA. The difference in CO_2 concentration between the two gas samples for each plot was used to

calculate the net CO_2 consumed during photosynthesis. Results are reported as mg CO_2 dm^{-2} hr^{-1} according to the formula by Muramoto et al. (1967).

$$mgCO_2\ dm^{-2}\ hr^{-1} = \frac{(ppm\ CO_2/10^6)(F)(K)}{A}$$

where F = system flow rate in 1 hr^{-1}
K = constant (1651)
A = leaf area (dm^2)

Transpiration, diffusive resistance, and temperature differential were measured on the middle leaflet of the uppermost fully expanded leaf of two plants in each plot. The measurements were made with a Licor model LI 1600 steady state porometer equipped with a 0.6 cm^2 apperature.

Each physiological characteristic at each sampling date was analyzed using an analysis of variance, and a combined analysis over both sampling dates was calculated for each trait using a repeated measures procedure. A correlation matrix was also calculated to determine the relationships among all of the physiological characteristics measured.

Mature Plant Studies

Mesa-Sirsa and AZST 1982 plants were grown in NaCl solutions of -.6, -1.2, and -1,8 MPa osmotic potential as well as a control solution of distilled water. Each solution also consisted of one-third strength Hoagland's solution (Hoagland and Arnon, 1950). The plants were started from seed in 20 by 3.5 cm diameter plastic cones filled with fine-grained vermiculite. Three seeds were planted

in each cone and thinned to one plant per cone after 1 week of growth. The cones were placed into holes in the top of black plexiglass boxes (55 by 43 by 20 cm). The bottom 2 to 4 cm of the cones were suspended in the treatment solutions which were aerated with small air stones attached to a pump.

Four boxes were used in this experiment. Each box contained 16 l of one-third strength Hoagland's solution during the first week of the experiment to insure uniform germination and emergence of at least one seedling in each cone. After 1 week the solution in each box was changed so that each box contained one of the four treatment solutions. Forty plants of each germplasm, Mesa-Sirsa and AZST 1982, were randomly placed within each box.

The boxes were placed in a greenhouse and were shaded by netting which allowed 53 % light penetration. This was necessary to reduce the temperature at the surface of the vermiculite while the seedlings were emerging. The greenhouse temperature was monitored with a thermograph and ranged from 25 to 32 C during the course of the experiment.

Plant water balance characteristics, an elemental analysis, and several plant growth characteristics were measured for Mesa-Sirsa and AZST 1982 plants grown in each growth media. There were no randomized replications of the salinity treatments, so no statistical analysis could be performed to compare responses among the different growth media. However, measurements of all traits, except the elemental analysis, were replicated within each salt treatment, allowing a comparison of the two germplasm sources within each salinity level.

Leaf water potential and osmotic potential were measured using Merrill thermocouple psychrometers (model 75-11C) and a Wescor HP-115 Water Potential Data System. Pressure potential was calculated from the water potential and osmotic potential measurements.

Calibration of the psychrometers was accomplished using standard NaCl solutions of -1.0, -2.0, -3.0, and -4.0 MPa osmotic potential. Solution concentrations were verified with a Wescor 1500-C vapor pressure osmometer. A 6 mm filter paper disk was placed inside of each psychrometer sample chamber and wetted with 8 ul of the appropriate NaCl solution. The chambers were sealed immediately after applying the NaCl solution.

The psychrometer chambers were placed into holes drilled in a foam rubber block which was inside of a styrofoam ice chest. The holes were covered with strips of foam rubber, and the lid of the ice chest sealed with masking tape. The protruding psychrometer lead wires were attached to the Wescor Water Potential Data System.

This aparatus provided very constant temperature regulation as indicated by low offset voltage readings which were consistently in the range of 0.0 to 0.3 uV. High offset voltage values are associated with temperature flucuations which may influence water potential readings.

Water was condensed on each measuring junction by applying an 8 mA current for 12 s. Wet bulb microvolt output and ambient temperature were recorded after a delay of 4 s. Microvolt readings were corrected to a temperature of 25 C using the formula corrected uV

= observed uV / [.325 - .027(observed temperature)] (Wescor, Inc., 1982). Microvolt output and ambient temperature readings were taken every 15 minutes for 24 hours. Microvolt output and temperature readings were stabilized after approximately 3 hours. Microvolt output from 3 to 6 hours were averaged and used for the calibration. Microvolt standard errors for the period ranged from 0.1 to 0.3 uV.

Three replications of each NaCl solution were measured in each psychrometer. There was a strong linear relationship between megapascals and microvolt output for all psychrometers, with r^2 values ranging from 0.975 to 0.999. The linear regression equations were used to estimate leaf water potentials and osmotic potentials, in megapascals, for the leaf samples.

Two 6-mm-diameter leaf disks were taken from the middle leaflets of the uppermost fully expanded leaves of each plant sampled using a paper punch. The two disks were placed in a sample chamber, abaxial side facing the junction. The chambers were quickly sealed to prevent evaporation from leaf disk margins. Care was taken to avoid damaging the leaf disks during this procedure. Psychrometers were located approximately 2 mm above the leaf disks after the chambers were sealed.

Leaf water potential values were measured every 15 minutes for 24 hours, and appeared to stabilize between 8 and 12 hours. Leaf water potential values were obtained by averaging the values from 12 to 18 hours.

After 24 hours the same leaf samples, still sealed in the chambers, were emersed in liquid nitrogen (-196 C) for 5 minutes in

order to rupture cell membranes and release the cell sap (Walker, et al., 1983). Readings were then taken at 15 minute intervals for another 24 hours to obtain leaf osmotic potential measurements. Osmotic potential readings between 12 and 18 hours were averaged to estimate osmotic potential for each leaf sample.

Pressure potential for each leaf sample was estimated according the the formula for water potential (water potential = osmotic potential + pressure potential), using the measured values for water potential and osmotic potential.

Five subsamples of each germplasm in each treatment were sampled over an 8-day period beginning 5 weeks after the plants were placed in the treatment solutions. The plants were approximately 10 to 25 cm tall during this period. Samples were taken between 1000 and 1030 MST. The temperature and relative humidity ranges at the time of sampling were 27 to 30 C and 61 to 68 %, respectively.

Plant Growth and Development

Eight of the remaining Mesa-Sirsa and AZST 1982 plants in each NaCl treatment were evaluated for several plant growth characteristics. The traits measured were plant height, leaf area, leaf and stem fresh and dry weight, and percent moisture. These traits were evaluated after the plants were 12 weeks old.

Plant height was measured to the nearest millimeter from the top of the longest stem to the surface of the vermiculite. The plants were cut off at the surface of the vermiculite and placed in plastic bags to avoid dehydration. On the same day the leaves were removed

and the leaf area of each plant measured with a LiCor LI 3100 area meter. Leaf and stem fresh weights were measured to the nearest .01 mg.

The leaf and stem samples were then oven dried for 48 hours at 26 C and measured for dry weight. Percent moisture for leaves, stems, and whole plants was calculated. An analysis of variance was used to compare the responses of Mesa-Sirsa and AZST 1982 in each salinity treatment.

Elemental Analysis

The remaining plants of each germplasm in each treatment were harvested and ground with a UD Corporation Cyclone sample mill in preparation for an elemental analysis. The elements measured were Cl, Na, K, N (as NO3), P, Ca, Mg, Fe, Mn, Cu, and Zn. The analysis was performed by International Minerals and Chemical Corporation, Terre Haute, Indiana. All elements are reported as parts per million. Only one replication of each germplasm source in each treatment was evaluated, so no statistical analysis could be performed.

Photosynthesis and Respiration

Two replications of plants in each of the four treatments were grown in a growth chamber at 26 C for a period of 6 weeks. The light level in the growth chamber was approximately 500 uE m^{-2} s^{-1}. After 6 weeks of growth two plants in each treatment combination were evaluated for apparent photosynthesis and respiration using the closed system IRGA. The plant stems were sealed in a 1.5 l plexiglass cylinder, placed beneath a bank of lights, and connected to the IRGA.

The CO_2 level in the closed system was recorded for several minutes until a stable CO_2 uptake rate was established. Then the lights were switched off and the cylinder with the plant inside was covered by a cloth to prevent light from reaching the plant. The CO_2 level in the system was measured again for several minutes to establish the increase in CO_2 concentration associated with dark respiration.

RESULTS AND DISCUSSION

Germination Studies

Germination in NaCl, $NaNO_3$, KCl, KNO_3, Mannitol, and PEG

Mesa-Sirsa and all five cycles of the AZST alfalfa (AZST 1978 through 1982) were germinated in NaCl, $NaNO_3$, KCl, KNO_3, mannitol, and PEG 6000 solutions of -1.00, -1.15, -1.30, -1.45, and -1.60 MPa osmotic potential and a control solution of distilled water.

Due to the size of the experiment and the time necessary to prepare each experimental unit, the experiment was broken into six parts. Germination in each of the six mediums was conducted at different times. Each treatment combination was repeated four times, but there was no randomization. The environmental factors, temperature and humidity, and experimental procedures were closely controlled during the experiment. The osmotic potentials of the germination solutions were monitored closely and were no more 0.002 osmoles from the desired values for all germination solutions. There was also a control germination solution of distilled water included in each of the six germination periods. Germination results in the control solutions were not significantly different within any individual germplasm source among the six germination periods. There were differences in germination among the six germplasm sources. To correct for inherent differences among seed sources in germination ability due to seed age and deterioration, the germination results are reported as percent of germination in distilled water for each

germplasm. For the reasons stated above, the results were analyzed as if the experiment were a completely randomized design in order to make comparisons among the different germination medias.

Percent germination of each germplasm source decreased in each germination medium as the osmotic potential of the medium decreased (Table 1). Germination within each osmotic concentration of each germination medium increased, however, as the number of cycles of selection for NaCl tolerance increased. AZST 1982, for instance had the highest percent germination in all types and concentrations of germination solutions. An analysis of variance (Appendix Table A2) revealed highly significant differences ($P < .01$) among germplasm sources, types of germination media, and osmotic levels of germination media.

Mean separations were performed using the least significant difference method (Table 2). The mean percent germination of all germplasm sources, averaged over all media types and osmotic levels, were significantly different except Mesa-Sirsa and AZST 1978. Except for AZST 1978, the average percent germination increased with each successive cycle of selection for NaCl tolerance. The AZST 1978 seed used in this experiment produced only 50.7 % germination in distilled water, much lower than all other seed sources, which were all greater than 80 %. Germination of AZST 1978 was also slower and less vigorous in distilled water than the other seed sources. The AZST 1978 seed was approximately 5 years old when this experiment was conducted and may have begun to deteriorate. The decreased vigor may have resulted in a decreased ability to express its genetic potential for

Table 1. Mean adjusted* percent germination of Mesa-Sirsa and AZST 1978 through 1982 in six types of germination media at five levels of osmotic potential.

Water Potential (MPa)	Germplasm	Mean Adjusted Percent Germination — Germination Medium					
		NaCl	$NaNO_3$	KCl	KNO_3	Mannitol	PEG
-1.00	Mesa-Sirsa	52.1	51.7	81.3	53.7	74.9	4.0
	AZST 1978	51.0	47.3	40.0	50.0	70.4	5.6
	AZST 1979	86.9	63.6	67.5	48.5	87.9	11.4
	AZST 1980	88.7	74.7	74.7	62.4	97.0	17.4
	AZST 1981	90.7	83.9	74.1	59.1	101.7	21.0
	AZST 1982	99.4	90.3	93.1	69.8	102.8	28.0
-1.15	Mesa-Sirsa	8.8	59.9	37.1	34.7	68.2	0.6
	AZST 1978	14.7	45.1	22.6	17.7	61.1	1.1
	AZST 1979	60.0	44.2	33.1	24.6	67.3	6.3
	AZST 1980	71.7	68.8	57.4	42.1	92.3	9.0
	AZST 1981	79.5	74.1	59.4	38.7	93.3	10.0
	AZST 1982	104.3	85.8	81.6	54.4	101.1	24.4
-1.30	Mesa-Sirsa	3.1	4.1	22.9	21.6	13.4	0.0
	AZST 1978	12.8	9.9	18.3	21.6	19.4	0.0
	AZST 1979	37.7	31.2	35.0	26.3	34.6	0.0
	AZST 1980	61.0	50.0	40.1	25.3	62.5	0.0
	AZST 1981	74.5	52.9	48.8	24.7	87.7	1.1
	AZST 1982	85.9	71.6	54.6	37.9	97.8	9.3
-1.45	Mesa-Sirsa	0.6	1.7	6.5	6.3	2.2	0.0
	AZST 1978	1.0	4.4	7.8	15.7	18.5	0.0
	AZST 1979	10.0	6.5	10.0	15.8	23.0	0.0
	AZST 1980	40.3	20.6	15.4	24.2	58.3	0.0
	AZST 1981	54.0	28.2	16.5	23.1	75.4	0.0
	AZST 1982	63.2	40.3	32.2	29.1	92.3	0.5
-1.60	Mesa-Sirsa	0.0	0.0	2.4	1.6	2.8	0.0
	AZST 1978	1.0	1.8	1.7	2.9	4.6	0.0
	AZST 1979	0.8	5.2	5.0	2.9	17.6	0.0
	AZST 1980	18.9	7.7	8.6	12.4	42.9	0.0
	AZST 1981	23.0	14.9	15.3	11.3	66.5	0.0
	AZST 1982	48.5	26.1	24.1	13.7	90.6	0.0

* Mean germination values are reported as percent of germination in distilled water for each germplasm.

Table 2. Mean separations of germplasm, germination media, and media water potential for germination results of Mesa-Sirsa and AZST 1978 through 1982 in six types of germination media at five levels of osmotic potential.

Germplasm Source	Mean*
Mesa-Sirsa	20.5 a
AZST 1978	18.9 a
AZST 1979	28.8 b
AZST 1980	41.5 c
AZST 1981	46.8 d
AZST 1982	58.4 e

Germination Media	Mean
PEG 6000	5.0 a
KNO	29.1 b
KCl	36.2 c
NaNO	38.9 c
NaCl	44.8 d
Mannitol	60.9 e

Media Osmotic Potential (MPa)	Mean
-1.00	63.2 a
-1.15	48.7 b
-1.30	33.3 c
-1.45	20.7 d
-1.60	13.2 e

* Means within a column followed by a different letter are significantly different at the .01 level by Least Significant Difference.

germination salt tolerance at the lower osmotic potentials.

Germination among all osmotic concentrations, averaged over all germplasms and all media types, was significantly different ($P < .01$). Germination decreased from 63.2 % at the -1.00 MPa level to 13.2 % at the -1.60 MPa concentration.

The AZST germplasm were selected only on the basis of NaCl tolerance during germination. A comparison of the mean germination percentage in all six types of germination media reflects this (Table 2). Germination was significantly greater ($P < .01$) in the NaCl solutions than any of the other salt solutions. Germination was intermediate and similar in the KCl and $NaNO_3$ solutions. Both of these solutions contained one of the ions, either Na or Cl, that were used in screening the AZST material for salt tolerance. There was no selection for either the K or NO_3 ion and germination in the KNO_3 solutions was significantly lower than in the other salt solutions.

Figure 1 also illustrates the effects of selection for NaCl tolerance by comparing Mesa-Sirsa and AZST 1982 germination in all six media types at -1.3 MPa osmotic potential. Germination of AZST 1982 is greater in all media types than Mesa-Sirsa. The difference in germination between Mesa-Sirsa and AZST 1982 was greatest in the NaCl solution than any of the other salts; the least difference in KNO_3. This suggests that selection for NaCl tolerance results in a tolerance to the specific ion effects of Na and/or Cl.

The selection process also resulted in increased tolerance to the other salts, but not to the same degree as the tolerance to NaCl. This means that another factor is involved in salt tolerance other

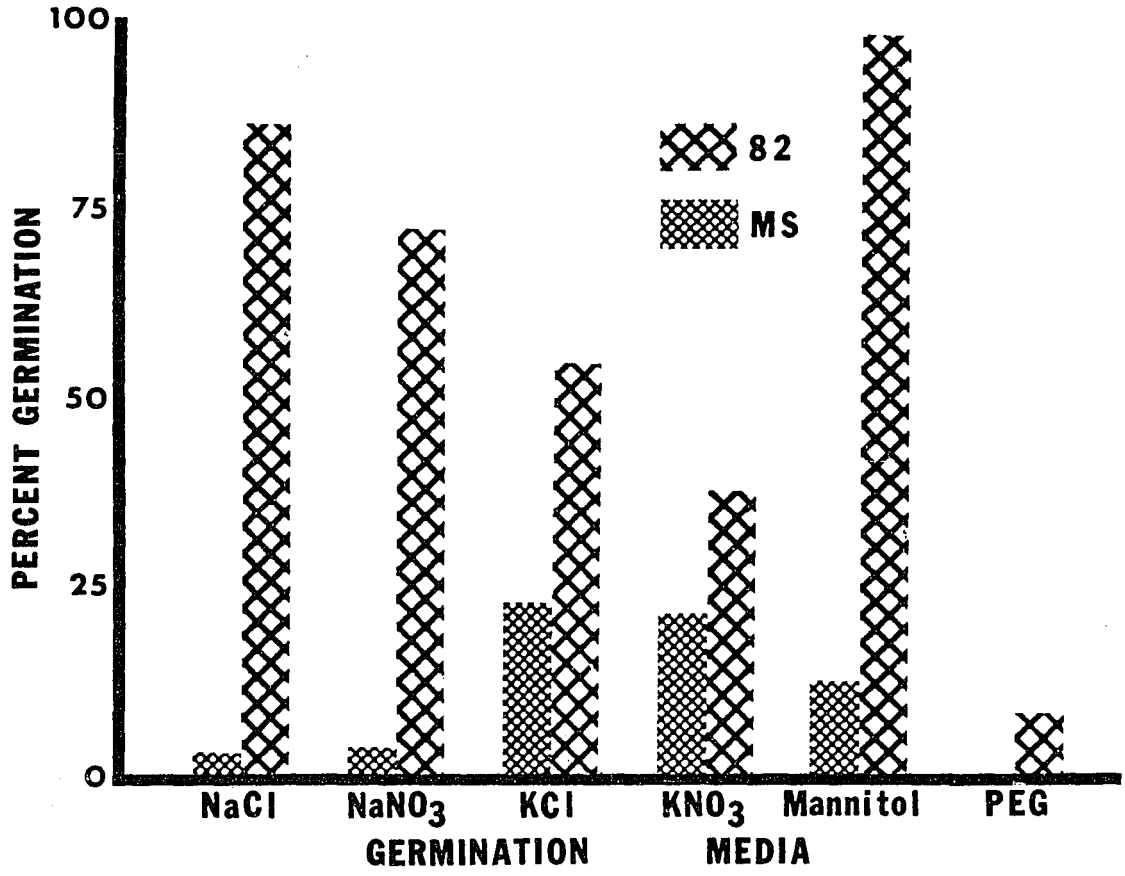

Figure 1. Percent germination of Mesa-Sirsa and AZST 1982 alfalfa in six types of germination media at -1.3 MPa osmotic potential.

than tolerance of specific ion effects. This other factor is probably tolerance to a decrease in osmotic potential of the germination media. A comparison of germination of Mesa-Sirsa and AZST 1982 in mannitol solution supports this conclusion. AZST 1982 had its highest germination in the mannitol solution (Fig. 1). Mannitol is a metabolically inert osmotic agent that functions only to lower the osmotic potential of the germination media. It has no toxic ionic effects as do the salt solutions. Since selection for NaCl tolerance resulted in a successful increase in tolerance to mannitol, then osmotic stress is probably an important factor that inhibits seed germination in saline environments. This result also indicates that successful selection for germination NaCl tolerance results in an increased tolerance to drought conditions during germination.

Germination in PEG 6000, also a biologically inert osmotic agent, would be expected to produce results similar to those with mannitol at iso-osmotic concentrations. PEG 6000 is composed of high molecular weight (mw = 6000) ethylene glycol polymers which form a much more viscous solution than the lower molecular weight salts or mannitol at iso-osmotic concentrations. Visual observations indicated that seeds germinating in the viscous PEG 6000 solutions became coated with the material, and water uptake by the seeds may have been physically inhibited beyond the expected osmotic effects. The higher molecular weight of PEG 6000 would also inhibit its absorption into the seed which would eliminate the potential for osmotic adjustment to low osmotic potential.

It appears that the Na ion is more responsible for inhibiting

germination of the unselected Mesa-Sirsa than the Cl ion (Fig. 1). Germination of Mesa-Sirsa in NaCl and $NaNO_3$ was significantly lower than the other two salts in which Na was replaced by K. No such effect was apparent when replacing Cl with NO_3.

This result is in apparent contradiction to the results of Smith et al. (1981a). They found, in a similar experiment with seedling alfalfa plants, that the Cl ion was responsible for inhibiting growth. The opposing results of these two experiments may indicate that alfalfa plants at different growth stages may be affected differently by exposure to salt and salt tolerance during germination and at later growth stages may be controlled by different physiological mechanisms. This also suggests that more than one selection criterion for salt tolerance, employed at several different growth stages, may be required to produce salt tolerance that extends throughout the entire life cycle of an alfalfa crop.

A second analysis of variance was calculated for the germination results of all six germplasm sources at all five osmotic levels of the NaCl solutions (Appendix Table A3). This analysis was used to produce an esitmate of the broadsense heritability of germination salt tolerance. The analysis was calculated with only the germination results in NaCl since selection of the AZST cycles was conducted using NaCl and not any of the other germination medias used in this experiment. The broadsense heritability estimate was 49.9%.

Seed Respiraton

Mesa-Sirsa and AZST 1982 were germinated in NaCl solutions of

-0.6, -1.2, -1.8, -2.4, and -3.0 MPa osmotic potential and a control of distilled water. Seed respiration was measured for these plants after 3, 6, 12, and 24 hours of germination.

An analysis of variance was calculated using the five germination periods as repeated observations (Appendix Table A4). Seed respiration (ppm CO_2 min^{-1} g^{-1} of seed) was significantly different among NaCl treatment levels and among germination periods. Respiration decreased with increasing salt concentration and increased with increasing germination time, as shown by the treatment means in Table 3. There was no significant difference in respiration between Mesa-Sirsa and AZST 1982. There was a significant salt concentration by germplasm interaction. As the salt concentration increased, respiration of the Mesa-Sirsa seed decreased in relation to the AZST 1982 seed.

In general, Mesa-Sirsa showed respiration greater than or equal to that of AZST 1982 in all germination time by salt concentration treatment combinations, but AZST 1982 was more stable over all salt concentrations as indicated by the significant germplasm by NaCl level interaction (Appendix Table A4).

It is difficult to speculate whether or not respiration is a factor in salt tolerance. As salinity increased, the greater stability of AZST 1982 for respiration suggests that basic metabolic processes, such as respiration, may be involved in salt tolerance. On the other hand, Mesa-Sirsa exhibited respiration rates greater than or equal to AZST 1982 throughout the experiment. High absolute respiration values, therefore, are probably not a requirement for

Table 3. Mean seed respiration of Mesa-Sirsa and AZST 1982 after 3, 6, 12, 24, and 48 hours of germination in six NaCl solutions ranging from 0.0 to -3.0 MPa osmotic potential.

NaCl Level (MPa)	Germplasm*	Respiration (ppm CO_2 min^{-1} g^{-1}) Germination Period (hours) 3	6	12	24	48	Mean
0.0	Mesa-Sirsa	2.5	4.3	5.9	19.3	11.7	
	AZST 1982	1.2	2.0	4.5	13.7	13.4	
	Mean	1.9	3.1	5.2	16.5	12.5	7.8
-0.6	Mesa-Sirsa	1.7	3.1	4.7	5.9	13.8	
	AZST 1982	1.2	1.4	3.1	7.6	13.7	
	Mean	1.4	2.3	3.9	6.7	13.8	5.6
-1.2	Mesa-Sirsa	1.4	2.3	4.1	4.3	8.4	
	AZST 1982	0.9	1.1	3.0	5.0	15.1	
	Mean	1.2	1.7	3.6	4.7	11.7	4.6
-1.8	Mesa-Sirsa	1.0	1.8	3.5	3.7	4.4	
	AZST 1982	0.4	0.7	2.6	2.8	6.0	
	Mean	0.7	1.3	3.1	3.3	5.2	2.7
-2.4	Mesa-Sirsa	1.0	1.5	2.9	3.0	4.4	
	AZST 1982	0.7	0.7	2.0	2.7	3.6	
	Mean	0.8	1.1	2.4	2.8	4.0	2.3
-3.0	Mesa-Sirsa	1.0	1.3	1.8	2.6	3.3	
	AZST 1982	0.7	0.6	1.7	2.3	3.4	
	Mean	0.9	1.0	1.8	2.5	3.3	1.9
	Mean	1.2	1.7	3.3	6.1	8.4	

* There were no significant differences among germplasm sources at any treatment level.

LSD (.05) values for mean seed respiration among NaCl treatment levels and germination periods are 1.0 and 3.6 ppm CO_2 min^{-1} g^{-1} of seed, respectively.

germination salt tolerance.

Water Uptake

Mesa-Sirsa and AZST 1982 seeds were evaluated for water uptake during germination using tritiated water solutions with 0.00, 0.26, and 0.40 M NaCl concentrations. These solutions without seeds yielded average values of 5411, 5834, and 5340 dpm, respectively. These results indicated no obvious effect of NaCl on the background counts of the germination media. Therefore, no adjustment was made to correct for the possibility of NaCl interference.

An analysis of variance of dpm mg^{-1} of seed indicated that the most significant variation in seed hydration occurred among the salt concentrations (Appendix Table A5), with the control solution (0.00 M NaCl) resulting in the greatest amount of water absorption by both Mesa-Sirsa and AZST 1982 seed (Table 4). There was no significant difference between Mesa-Sirsa and AZST 1982 seed in water uptake at any NaCl level. These results indicate that the difference in germination salt tolerance between Mesa-Sirsa and AZST 1982 may not be due to differences in ability to absorb water in saline germination media.

The initial hydration of germinating seeds is a purely mechanical process which will occur even with dead seed. During this phase of germination the matric potential, or imbibitional forces, in dry seed can be as high as 200 MPa. Water uptake at this early stage of germination is due primarily to the ability of the cell wall and protein matrices to be hydrated and bind water. This type of water

Table 4. Mean absorption of tritiated water, as disintegrations min^{-1} mg^{-1} of seed, by Mesa-Sirsa and AZST 1982 after 6 and 12 hours of germination in .00, .24, and .40 M NaCl solutions.

NaCl Level (M)	Germination Period (hours)	Disintegrations min^{-1} mg^{-1} seed		
		Germplasm*		
		Mesa-Sirsa	AZST 1982	Mean
.00	6	4960	4308	4634
.00	12	5617	5839	5728
	Mean	5288	5073	5181
.26	6	3358	3554	3456
.26	12	3513	3952	3732
	Mean	3435	3753	3594
.40	6	3258	3801	3529
.40	12	3960	3670	3815
	Mean	3609	3735	3672

* No significant differences were found among germplasm sources at any treatment level.

LSD (.05) value for mean water uptake among NaCl treatment levels is 709.5 disintegrations min^{-1} mg^{-1} seed.

uptake may result in a two- to three-fold increase in the seed fresh weight. After the initial imbibitional uptake of water osmotic uptake becomes more important in viable seed. Osmotic water absorption is controlled by the osmotic potential and pressure potential of the cells, and results in a slower rate and smaller amount of water absorption than matric forces during the early stages of germination (Bewley and Black, 1978; Kozlowski, 1971).

It is possible that this experiment reflected primarily imbibitional or matric water uptake and not osmotic uptake. If so, physiological differences among germplasm such as rate of ion absorption and cell solute concentration which influence osmotic water uptake would be masked. The results do suggest that there is probably no significant difference between the Mesa-Sirsa and AZST 1982 seed in their water absorption due to matric forces.

Seed Sodium and Chloride Analysis

Mesa-Sirsa and AZST 1982 seeds were germinated in distilled water and NaCl solutions of -0.6, -1.2, and -1.8 MPa osmotic potential and analyzed for Na and Cl ion content.

A separate analysis of variance was calculated for the Na ion and Cl ion results (Appendix Table A6). In both analyses there was a significant ($P < .01$) increase in seed ion content as the NaCl concentration of the germination media increased (Table 5). Seed Na concentrations ranged from 367.7 to 5902.2 ppm when germinated in distilled water and -1.8 MPa of NaCl, respectively. Seed Cl levels ranged from 1729.4 to 10916.8 ppm when germination took place in

Table 5. Mean sodium and chloride content of Mesa-Sirsa and AZST 1982 seeds germinated in NaCl solutions ranging from 0.0 to -1.8 MPa osmotic potential.

NaCl Level (MPa)	Germplasm*	Elemental Concentration (ppm)	
		Sodium	Chloride
0.0	Mesa-Sirsa	368	1729
	AZST 1982	290	1730
	Mean	329	1730
-0.6	Mesa-Sirsa	3244	6128
	AZST 1982	4134	7762
	Mean	3689	6945
-1.2	Mesa-Sirsa	3761	7465
	AZST 1982	4065	8048
	Mean	3913	7756
-1.8	Mesa-Sirsa	5902	10917
	AZST 1982	5086	9200
	Mean	5494	10059

* No significant differences were found among germplasm sources for sodium or chloride concentration at any treatment level.

LSD (.05) values for mean sodium and chloride concentration among NaCl treatment levels are 638 and 1127 ppm, respectively.

distilled water and -1.8 MPa of NaCl, respectively. There was no statistically significant difference between Mesa-Sirsa and AZST 1982 in Na or Cl ion accumulation.

The germplasm by germination media concentration interaction was significant at the .10 level. Na and Cl accumulation was very similar in the distilled water treatment for both seed sources. AZST 1982 had slightly higher ion concentrations when germinated in two lower NaCl concentrations (-0.6 and -1.2 MPa) and Mesa-Sirsa accumulated higher Na and Cl levels when germinated in the highest NaCl concentration (-1.8 MPa). The magnitude of the differences in ion accumulation between the two germplasm sources does not appear great enough to be associated with the differences in germination salt tolerance found between AZST 1982 and Mesa-Sirsa. It appears that the ability of germinating seeds to accumulate or exclude inorganic ions is not an important factor in alfalfa salt tolerance during seed germination.

Field Studies

Forage yield, apparent photosynthesis, leaf transpiration, leaf diffusive resistance, and the temperature differential between leaf and air were measured in replicated field plots during 1983. The objective of the field study was to determine if the selection process for seed germination salt tolerance resulted in changes in other plant characteristics when grown under non-saline field conditions.

Forage Yield

Forage yield was measured five times durng the 1983 growing

season, 13 April, 20 May, 15 June, 6 July, and 27 July. An analysis of variance was calculated for the results of each harvest. Only on the second harvest date was there a significant difference among germplasm sources for forage yield ($P < .01$); AZST 1980 exhibited a significantly higher yield than the other entries. A combined analysis of variance for the second through fifth harvest dates also resulted in no significant differences among germplasm sources (Appendix Table A7). There was a significant yield difference among harvest dates ($P < .01$). Yields steadily decreased throughout the summer, a common occurrence in Arizona as a result of increasing temperatures.

The yield results indicate that no significant changes in yield potential in non-saline environments resulted from selection for germination NaCl tolerance (Table 6). Mesa-Sirsa and AZST 1982 exhibited similar germination results in non-saline, high osmotic potential conditions. Under drought or saline field conditions AZST 1982 might possibly be expected to produce a greater forage yield than Mesa-Sirsa due to a greater ability to germinate and produce a stand.

Physiological Studies

Photosynthesis, transpiration, diffusive resistance, leaf temperature, and temperature differential were measured twice during 1983, on 6 June and 25 July. An analysis of variance was calculated for each physiological characteristic on each sampling date (Appendix Table A8). There was no significant difference among germplasm sources for any trait measured on either sampling date.

Table 6. Forage yield of eight alfalfa lines at the Plant Material Center, Tucson, Arizona on five cutting dates during 1983.

	Mean Forage Yield (kg ha^{-1})				
	Cutting Date				
Germplasm*	Apr 13	May 20	Jun 15	Jul 6	Jul 27
Mesa-Sirsa	2739	2748	2419	2314	1860
Large Leaflet	2353	2772	2300	2314	1767
AZST 1979 syn II	2635	2634	2243	2200	2080
AZST 1978	2394	2408	2410	2360	1967
AZST 1979	2800	2478	2149	2289	2119
AZST 1980	2957	3026	2619	2565	1788
AZST 1981	2674	2726	2304	2344	1651
AZST 1982	2811	2689	2540	2545	1951
Mean	2671	2685	2373	2366	1898

* There were no significant differences among germplasm sources for forage yield on any cutting date.

LSD (.05) value for mean forage yield among cutting dates is 192 kg ha^{-1}.

A combined analysis of variance, over both sampling dates, was also calculated for each trait (Appendix Table A8). Again, there were no significant differences among germplasm sources for any of the physiological characteristics, but for each trait there was a significant difference ($P < .01$) among sampling dates.

The mean values (Table 7) for each trait at each sampling date were used to calculate linear correlations among all physiological traits (Table 8). The mean values of each germplasm at each sampling date were used to calculate the coefficient of determination because not all characteristics were measured on the same plants and the number of subsamples measured per plot was not the same for each trait.

Apparent photosynthesis was positively correlated with leaf transpiration and negatively related to leaf diffusive resistance ($P < .01$). Transpiration was also negatively associated with diffusive resistance ($P < .01$). Lower diffusive resistance rates probably allowed a greater exchange of gasses between the leaf and the atmosphere resulting in increased rates of photosynthesis and transpiration.

Diffusive resistance was significantly negatively related to both ambient and leaf temperature ($P < .01$). Higher temperatures, at least in the range measured in this study, resulted in lower gas exchange rates, as evidenced by the negative relationship between leaf and air temperature and transpiration ($P < .01$), and leaf and air temperature and apparent photosynthesis ($P < .05$).

The temperature differential was also closely related to both

Table 7. Mean values for physiological traits of eight alfalfa lines, grown at the Plant Material Center, Tucson, Arizona, measured on two sampling dates during 1983.

Germplasm Source*	Apparent Photosynthesis (mg CO_2 dm^{-2} hr^{-1})	Leaf Transpiration (ug H_2O cm^{-2} s^{-1})	Leaf Diffusive Resistance (s cm^{-1})	Temperature Differential (air - leaf) (C)	Ambient Temp (C)	Leaf Temp (C)
			14 June 1983			
Large Leaflet	14.9	41.9	0.5	3.6	33.3	29.7
AZST 1979 syn II	21.9	36.5	0.6	3.4	33.2	29.8
Mesa-Sirsa	20.2	41.8	0.5	3.4	33.3	29.9
AZST 1978	19.1	42.1	0.5	3.5	33.1	29.7
AZST 1979	20.9	70.8	0.5	3.5	33.5	29.9
AZST 1980	20.4	41.4	0.5	3.4	33.2	29.8
AZST 1981	16.3	42.1	0.5	3.4	33.1	29.7
AZST 1982	20.6	40.3	0.5	3.2	33.1	29.9
			25 July 1983			
Large Leaflet	12.7	5.0	2.5	3.0	34.6	31.6
AZST 1979 syn II	17.3	5.2	2.2	2.7	34.0	31.3
Mesa-Sirsa	14.1	5.2	2.0	3.4	34.1	31.0
AZST 1978	13.2	5.2	2.1	2.7	33.8	31.1
AZST 1979	16.5	5.2	2.2	2.9	34.8	31.9
AZST 1980	16.5	5.4	1.9	3.1	34.2	31.4
AZST 1981	14.9	5.3	2.3	2.8	34.4	31.6
AZST 1982	18.4	5.4	2.2	2.5	34.5	31.9

* There were no significant differences among germplasm sources for any physiological trait on either sampling date.

Table 8. Correlation matrix for physiological traits of eight alfalfa lines measured on two sampling dates at the Plant Material Center, Tucson, Arizona during 1983. N=16.

	Coefficient of Determination				
Physiological Characteristic	Leaf Transpiration	Diffusive Resistance	Temperature Differential	Ambient Temperature	Leaf Temperature
Apparent Photosynthesis	.6525**	-.6729**	.3717	-.5946*	-.5607*
Leaf Transpiration		-.9894**	.8163**	-.9191**	-.9591**
Diffusive Resistance			-.8356**	-.9390**	-.9701**
Temperature Differential				-.7341**	-.8603**
Ambient Temperature					.9730**

*, ** Coefficient of determination significant at .05 and .01 level, respectively.

transpiration and diffusive resistance ($P < .01$). As the resistance to gas exchange increased the rate of transpiration decreased, resulting in less evaporative cooling of the leaf and a smaller temperature differential between the leaf and the air.

The results of the field studies indicated that there were no apparent differences among the eight germplasm sources for any of the characteristics measured when the plants were grown under relatively non-saline conditions (less than 1000 ppm soluble salts). Apparently, the selection process for seed germination salt tolerance did not result in measureable genetic changes in forage yield or any of the physiological characteristics under non-saline field conditions. It has yet to be established whether the AZST alfalfa and Mesa-Sirsa will perform similarly under saline field conditions.

Mature Plant Studies

Mesa-Sirsa and AZST 1982 plants were grown in a greenhouse in one third strength Hoagland's solution with treatments of 0, 6000, 12000, and 18000 ppm NaCl. A number of plant characteristics were measured to determine if selection for seed germination NaCl tolerance resulted in greater tolerance to NaCl during the seedling growth stage as well.

There were no randomized replications of the salt treatments, so no statistical comparisons could be made for plant performance among the NaCl treatment levels. Replications of Mesa-Sirsa and AZST 1982 were included within each NaCl treatment. Consequently, comparisons could be made between Mesa-Sirsa and AZST 1982 within each

NaCl treatment level. No germplasm source by NaCl level interactions could be calculated since there were no replications of the NaCl treatments. These limitations of the statistical analyses were not a serious hindrance in making the desired comparisons between Mesa-Sirsa and AZST 1982.

The plant characteristics measured were leaf water potential, osmotic potential, and pressure potential; an elemental analysis; plant height; leaf area; and leaf and stem fresh weight and dry weight.

Plant Water Balance

There were no statistically significant differences between Mesa-Sirsa and AZST 1982 for leaf water potential, osmotic potential, or pressure potential when grown in any of the four treatment solutions. Both entries did show steady increases in leaf water potential and osmotic potential as the NaCl concentration of the growth solution increased from 0 to 18000 ppm (Table 9). This was probably a result of accumulation of Na and Cl ions in the plant tissue. The ion accumulation would be a source of osmotic adjustment to allow the plants to maintain a water potential less than the growth solution so that absorption of water could continue.

Leaf pressure potential was calculated from the water potential and osmotic potential data for each sample (pressure potential = water potential - osmotic potential). Leaf pressure potential of both Mesa-Sirsa and AZST 1982 decreased as the growth solution increased from 0 to 6000 ppm NaCl, then stabilized as the NaCl concentration of

Table 9. Mean values for leaf water potential, osmotic potential, and pressure potential of Mesa-Sirsa and AZST 1982 alfalfa seedlings grown in solutions containing 0 to 18000 ppm NaCl.

Germplasm*	NaCl Level (ppm)	Water Potential (MPa)	Osmotic Potential (MPa)	Pressure Potential (MPa)
Mesa-Sirsa	0	-0.66	-1.44	0.78
AZST 1982	0	-0.67	-1.38	0.71
Mesa-Sirsa	6000	-1.54	-1.80	0.27
AZST 1982	6000	-1.44	-1.78	0.34
Mesa-Sirsa	12000	-1.75	-2.10	0.34
AZST 1982	12000	-1.72	-2.39	0.69
Mesa-Sirsa	18000	-1.92	-2.46	0.54
AZST 1982	18000	-2.25	-2.78	0.53

* No significant differences were found among germplasm sources for water potential, osmotic potential, of pressure potential.

the growth solution increased from 6000 to 18000 ppm NaCl. The magnitude of the change in pressure potential with changing salinity levels was considerably less than the change in water potential or osmotic potential, although not supported statistically. This is further evidence of the occurrence of osmotic adjustment in order for the plants to maintain turgor.

There were no statistically significant differences between Mesa-Sirsa and AZST 1982 for any trait measured in this study. This suggests that selection for germination salt tolerance had no influence on the water balance characteristics of seedling alfalfa plants when grown in saline or non-saline conditions.

Plant Growth and Development

Eight replications of 12-week-old Mesa-Sirsa and AZST 1982 seedlings were evaluated for several plant growth and development characteristics (Table 10). The plants were grown in solutions containing 0, 6000, 12000, and 18000 ppm NaCl. There were, for several traits, significant differences between Mesa-Sirsa and AZST 1982. In particular, Mesa-Sirsa exhibited significantly greater ($P < .05$) leaf fresh and dry weight than AZST 1982 when grown in the control solution of one third strength Hoagland's solution. AZST 1982 produced lower stem dry weight than Mesa-Sirsa in the 6000 ppm NaCl treatment ($P < .05$) and Mesa-Sirsa showed a higher stem moisture percentage than AZST 1982 in the 6000 ppm NaCl treatment.

In general, there were no obvious trends which indicated that Mesa-Sirsa and AZST 1982 performed differently from each other in

Table 10. Mean values of 13 plant growth characteristics of 12 week old Mesa-Sirsa and AZST 1982 seedlings grown in solutions containing 0 to 18000 ppm NaCl.

Germplasm	NaCl Concentration (ppm) 0	6000	12000	18000
		Plant Height (cm)		
Mesa-Sirsa	29.8 a	21.3 a	12.9 a	9.8 a
AZST 1982	27.2 a	20.7 a	13.4 a	10.7 a
		Leaf Area (cm^2)		
Mesa-Sirsa	30.2 a	18.4 a	10.0 a	10.1 a
AZST 1982	22.9 a	17.2 a	12.3 a	9.9 a
		Leaf Fresh Weight (mg)		
Mesa-Sirsa	395.7 a	224.7 a	134.2 a	149.2 a
AZST 1982	268.1 b	194.7 a	159.6 a	150.8 a
		Stem Fresh Weight (mg)		
Mesa-Sirsa	491.5 a	218.7 a	118.2 a	124.8 a
AZST 1982	321.5 a	198.1 a	132.5 a	118.1 a
		Leaf Dry Weight (mg)		
Mesa-Sirsa	104.7 a	64.9 a	34.4 a	42.7 a
AZST 1982	78.2 b	60.5 a	36.7 a	31.9 a
		Stem Dry Weight (mg)		
Mesa-Sirsa	101.3 a	49.9 a	17.1 a	23.4 a
AZST 1982	70.7 a	32.2 b	21.5 a	18.0 a
		Leaf % Moisture		
Mesa-Sirsa	73.5 a	70.2 a	74.5 a	70.8 a
AZST 1982	70.4 a	69.8 a	76.6 a	79.2 a
		Stem % Moisture		
Mesa-Sirsa	79.8 a	77.0 a	82.3 a	81.7 a
AZST 1982	78.4 a	84.8 b	84.1 a	93.1 b

Table 10. Continued.

Germplasm	NaCl Concentration (ppm) 0	6000	12000	18000
	Leaf Fresh Weight/Stem Fresh Weight			
Mesa-Sirsa	.95 a	1.02 a	1.13 a	1.20 a
AZST 1982	.89 a	.96 a	1.22 a	1.49 a
	Leaf Dry Weight/Stem Dry Weight			
Mesa-Sirsa	1.21 a	1.32 a	6.45 a	2.07 a
AZST 1982	1.22 a	1.98 b	2.24 b	4.60 b
	Top Growth Fresh Weight (mg)			
Mesa-Sirsa	887.3 a	443.4 a	252.4 a	274.0 a
AZST 1982	589.6 a	392.8 a	292.1 a	268.9 a
	Top Growth Dry Weight (mg)			
Mesa-Sirsa	206.0 a	114.7 a	41.5 a	66.1 a
AZST 1982	148.9 a	92.7 a	58.2 b	39.9 b
	Top Growth % Moisture			
Mesa-Sirsa	77.1 a	73.7 a	83.7 a	75.9 a
AZST 1982	74.8 b	77.3 a	79.9 b	85.2 b

Means within a column followed by different letters are significantly different at the .05 level by t-test.

response to changes in the NaCl level of the growth solution. Increases in the NaCl level of the growth solution did influence the growth of Mesa-Sirsa and AZST 1982 in a similar manner. Plant height, leaf area, fresh weight, and dry weight of both entries decreased as the NaCl level increased, while leaf to stem ratios using fresh weight and dry weight increased with increasing salinity. These observed differences in plant growth characteristics in response to changes in salinity were not supported statistically. Percent moisture in Mesa-Sirsa and AZST 1982 top growth was erratic and showed no apparent trend related to the NaCl level of the growth solution.

Elemental Analysis

Treatments for the elemental analysis were not replicated and no statistical analysis was possible. Several trends in the results are apparent (Table 11). The Na and Cl concentration in the top growth of both Mesa-Sirsa and AZST 1982 seedlings increased as the NaCl concentration of the growth media increased. There were no apparent differences in Na or Cl content between Mesa-Sirsa and AZST 1982 at any of the NaCl treatment levels. The plants grown in 18000 ppm NaCl solution contained approximately 22 times as much Cl and 60 times as much Na as the plants grown in the control solution with no NaCl.

There were no striking differences in accumulation patterns between Mesa-Sirsa and AZST 1982 for any of the elements measured. Some of the elements were accumulated at apparently different rates as the amount of NaCl in the growth solution varied. K, Ca, Mg, and Fe

Table 11. Elemental analysis of top growth of 12 week old Mesa-Sirsa and AZST 1982 seedlings grown in solutions containing 0 to 18000 ppm NaCl.

Germplasm	NaCl Level (ppm)	Elemental Concentration (ppm)										
		Cl	Na	K	N	P	Ca	Mg	Fe	Mn	Cu	Zn
Mesa-Sirsa	0	1600	400	47900	28800	6200	22700	7600	362	135	20	10
AZST 1982	0	1600	300	51500	30700	7200	21500	7500	310	152	17	10
Mesa-Sirsa	6000	9900	6400	35600	35900	8200	14800	5500	150	170	18	13
AZST 1982	6000	11800	6600	36100	36200	10100	14900	6100	163	181	18	9
Mesa-Sirsa	12000	17200	11500	30400		8200	13700	5700	135	167	20	11
AZST 1982	12000	18500	9900	34200	37700	8800	14100	6100	157	182	21	11
Mesa-Sirsa	18000	33800	22400	28100		6500	9600	3800	127	135	16	10
AZST 1982	18000	36000	19200	26900	35600	7500	10900	4400	126	153	16	11

content, averaged over both Mesa-Sirsa and AZST 1982 top growth, decreased by 45, 54, 46, and 62 % as the NaCl concentration in the growth media increased from 0 to 18000 ppm. Absorption of these elements, all of which are absorbed as cations, were inhibited by the higher NaCl concentrations. The higher Na ion content in the growth solution may have competed with the other cations in the solution for absorption by the plant roots (Greenway, 1973).

The content of the other elements measured in the plant top growth, N (as NO_3), P, Mn, Cu, and Zn, were not influenced by the NaCl concentration of the growth media.

Photosynthesis and Respiration

Mesa-Sirsa and AZST 1982 plants were grown for 6 weeks in a growth chamber in solutions containing 0, 6000, 12000, and 18000 ppm NaCl. All solutions contained one third strength Hoagland's solution. The experiment was replicated twice, and two subsamples in each treatment were evaluated for apparent photosynthesis and dark respiration using a closed system IRGA.

The results showed a wide range of values for both apparent photosynthesis and dark respiration on an individual plant basis. This variation was as great within the individual treatments as between treatments. An analysis of variance revealed that there were no significant effects of the NaCl level upon photosynthesis or respiration for either Mesa-Sirsa of AZST 1982 (Appendix Table A9). Also, there was no significant difference between Mesa-Sirsa and AZST 1982 in their photosynthesis or respiration responses at any salinity

level.

A third parameter, the photosynthesis/respiration ratio, was also evaluated. There were no significant differences between Mesa-Sirsa and AZST 1982 for this trait, but there was a significant increase in the photosynthesis/respiration ratio as the NaCl level increased. The ratio tended to mask the greater variability found within germplasms and salinity treatments for photosynthesis and respiration since these traits tended to change similarly for any particular plant.

The results suggest that photosynthesis and respiration may be influenced by salinity, but individual plant responses to salinity vary so much that the effect was difficult to isolate. The photosynthesis/respiration ratio results indicate that the rate of apparent photosynthesis increases relative to the dark respiration rate as salinity increases. Observation of the means (Table 12) would suggest that respiration may have increased with increasing NaCl concentration of the growth media, even though this observation is not supported statistically.

The series of experiments conducted with Mesa-Sirsa and AZST 1982 alfalfa in the seedling growth stage have shown that both germplasms responded similarly for all of the traits which were evaluated in non-saline as well as in saline conditions. It appears, therefore, that selection for salt tolerance during seed germination may not result in increased salt tolerance at later stages of growth. The breeding of a salt tolerant alfalfa cultivar may require several selection processes at different growth stages.

Table 12. Mean values for apparent photosynthesis (PHS), respiration (RSP), and photosynthesis/respiration ratio of six week old Mesa-Sirsa and AZST 1982 seedlings grown in solutions containing 0 to 18000 ppm NaCl.

Germplasm*	NaCl Level (ppm)	mg CO_2 dm^{-2} hr^{-1} PHS	RSP	PHS/RSP
Mesa-Sirsa	0	20.6	13.2	2.0
AZST 1982	0	11.6	7.1	1.7
Mean		16.1	10.2	1.9
Mesa-Sirsa	6000	17.5	6.6	2.8
AZST 1982	6000	14.1	6.4	2.3
Mean		15.8	6.5	2.5
Mesa-Sirsa	12000	19.7	7.0	2.8
AZST 1982	12000	20.2	6.4	3.5
Mean		19.9	6.7	3.2
Mesa-Sirsa	18000	13.9	4.4	3.1
AZST 1982	18000	15.3	4.6	3.5
Mean		14.6	4.5	3.3

* No significant differences were found among germplasm sources for PHS, RSP, or PHS/RSP ratio.

LSD (.05) values for mean PHS, RSP, and PHS/RSP ratio are 7.6, 6.4, and 1.0 mg CO_2 dm^{-2} hr^{-1}, respectively.

CONCLUSION

These studies have provided several results which may be beneficial to the understanding of the mechanisms involved in salt tolerance in alfalfa. The series of experiments involving seed germination indicated that germination in NaCl solutions was inhibited in at least two ways; by toxic effects of the specific ions, and by lowering the osmotic potential of the germination media which inhibits absorption of water.

The results also indicated that the Na ion is more responsible for inhibiting seed germination than the Cl ion. Smith (1981a) found that the Cl ion was more responsible than Na for inhibiting growth of non-salt-tolerant seedling alfalfa plants. These seemingly inconsistent results may indicate that NaCl inhibits seed germination and plant growth by affecting different plant processes. If this is so, selection for NaCl tolerance during germination may not be related to salt tolerance during later growth stages.

The AZST germplasm had higher germination percentages than Mesa-Sirsa in mannitol solutions. Mannitol is a non-toxic chemical which functions to lower the osmotic potential of the germination media, mimicking drought conditions. It appears that selection for NaCl tolerance during seed germination also results in increased tolerance to drought conditions.

Field studies were conducted in relatively non-saline soil conditions to compare Mesa-Sirsa and the AZST alfalfa under normal

growing conditions. There were no statistically significant differences among the germplasm sources in forage yield or any of several physiological characteristics evaluated. This result showed that selection for germination salt tolerance did not result in inadvertant selection for undersirable genetic changes in field performance characteristics.

Mesa-Sirsa and AZST 1982 seedlings were evaluated for several characteristics after being grown in solutions containing 0 to 18000 ppm NaCl. Carbon exchange, plant water status, elemental analysis, and plant growth traits such as plant height, leaf area, fresh weight, dry weight, and percent moisture were evaluated.

Most of the traits measured were influenced by changes in the NaCl level of the growth media, but there was no evidence that Mesa-Sirsa and AZST 1982 performed differently from each other for any of the characteristics measured. This result is further evidence that germination salt tolerance may be governed by different mechanisms than salt tolerance of whole alfalfa plants. Development of an alfalfa cultivar which is salt tolerant from germination through harvest may require selection for salt tolerance at several different growth stages.

APPENDIX A

STATISTICAL ANALYSES

Appendix Table 1. Results of field plot soil analysis[1] at the Plant Material Center, Tucson Arizona. Samples, taken during August 1982, represent a composite of eight subsamples taken from each depth.

		Saturation Extract					CO_2 Extraction		
Sampling Depth (cm)	pH	EC ($x10^3$)	Soluble Salts (ppm)	Na (meq/l)	K (meq/l)	ESP	NO_3 (ppm)	PO_4 (ppm)	Mg (ppm)
30	7.9	1.18	826	7.8	0.8	7.3	15.8	27.9	18.4
60	7.9	1.44	1008	9.3	0.7	7.5	28.6	11.2	17.2
90	8.0	2.00	1428	13.0	0.7	8.4	23.8	4.7	22.8

[1] Soil samples analysed by the Department of Soils and Water Engineering Soils and Plant Tissue Testing Laboratory, University of Arizona, Tucson, Arizona, 85721.

Appendix Table 2. Analysis of variance for percent germination of Mesa-Sirsa and AZST 1978 through 1982 in six types of germination media at five levels of osmotic potential.

Source	DF	SS	MS	F-ratio
Treatments	179	714,540.52		
Germplasm	5	147,789.87	29,557.97	293.11**
Media	5	206,068.35	41,213.67	408.70**
Osmotic Potential (OP)	4	239,984.25	59,996.06	594.95**
Germplasm x Media	25	51,082.22	2,043.29	20.26**
Germplasm x OP	20	7,939.35	396.97	3.94**
Media x OP	20	34,297.39	1,714.87	17.01**
Germplasm x Media x OP	100	27,379.09	273.79	2.72**
Error	540	54,454.50	100.84	
Total	719	768,995.02		

** Significant at .01 level.

Appendix Table 3. Analysis of variance for adjusted[1] percent germination of Mesa-Sirsa and AZST 1978 through 1982 in five NaCl solutions ranging from -1.00 to -1.60 MPa osmotic potential.

Source	DF	MS	F-ratio
Germplasm	5	14,889.32	93.32**
NaCl Level	4	14,254.88	89.34**
Germplasm x NaCl level	20	520.63	3.27**
Error	90	159.96	

1 Adjusted percent germination calculated as percent of germination in distilled water for each germplasm.

** Significant at .01 level.

Appendix Table 4. Analysis of variance for seed respiration of Mesa-Sirsa and AZST 1982 in six NaCl solutions ranging from 0.0 to -3.0 MPa osmotic potential after 3, 6, 12, 24, and 48 hours of germination.

Source	DF	SS	MS	F-ratio
Subplots (SP)	239	4458.23		
Main Plots (MP)	19	1233.58	26.25	
Blocks	3	76.96	25.65	5.37*
Germplasm (G)	1	11.05	11.05	2.31
NaCl Level (N)	5	1073.92	214.78	44.93**
G x N	1	36.15	36.15	7.56*
MP Error	15	71.65	4.78	
Sampling Time (T)	1	1793.30	1793.30	34.03**
C x T	1	59.72	59.72	1.13
N x T	4	1055.47	263.87	5.01*
SP Error	6	316.16	52.69	

*, ** Significant at .05 and .01 level, respectively.

Appendix Table 5. Analysis of variance for uptake of tritiated water in .00, .26, and .40 M NaCl solution after 6 and 12 hours of germination.

Source	DF	MS	F-ratio
Replications	1	274,846.63	.66
NaCl Level	2	6,401,223.68	15.40**
Time	1	1,827,114.65	4.40*
Germplasm	1	35,017.94	.08
NaCl level x Time	2	440,421.89	1.06
NaCl level x Germplasm	2	145,427.91	.35
Time x Germplasm	1	13,517.08	.03
Time x NaCl x Germplasm	2	372,407.41	.90
Error	11	415,642.24	

*,** Significant at .05 and .01 levels, respectively.

Appendix Table 6. Mean square values from analysis of variance for sodium and chloride content of Mesa-Sirsa and AZST 1982 seed germinating in NaCl solutions ranging from 0.0 to -1.8 MPa osmotic potential.

Source	DF	Mean Square	
		Chloride	Sodium
Blocks	1	334,286.40	3,719,690.83**
Treatments	7	22,071,214.86**	8,312,390.66**
Germplasm	1	62,787.86	22,575.07
NaCl Level	3	49,514,004.52**	18,876,552.42**
Germplasm x NaCl	3	1,964,567.53	511,500.77
Error	7	454,295.07	145,297.19

*,** Significant at .05 and .01 levels, respectively.

Appendix Table 7. Analysis of variance for forage yield of eight alfalfa lines grown at the Plant Material Center, Tucson, Arizona, during 1983.

Source	DF	SS	MS	F-ratio
Subplots	127	30,091,178.17	236,938.41	
Main Plots	31	7,349,861.81	237,092.32	
Blocks	3	1,251,517.30	417,172.43	1.68
Germplasm	7	874,268.84	124,895.55	0.50
MP Error	21	5,224,075.67	248,765.51	
Cuttings	3	10,109,117.14	3,369,705.71	22.76**
Germ x Cut	21	1,974,619.06	94,029.48	0.64
Subplot Error	72	10,657,580.16	148,021.95	

** Significant at the .01 level.

Appendix Table 8. Mean square values from analysis of variance for physiological traits of eight lines of field grown alfalfa on two sampling dates during 1983.

Source	DF	Mean Square				
		Apparent Photosynthesis	Transpiration	Diffusive Resistance	Temperature Differential	Leaf Temperature
Main Plots	31	40.70	13.97	.06	.40	.70
Blocks	3	54.99	54.99	.18	1.29	3.84
Germplasm	7	33.57	7.19	.07	.21	.29
MP Error	21	41.03	10.36	.05	.33	.39
Harvest	1	234.86*	20286.66**	45.72**	4.54**	47.62**
Germplasm x Harvest	7	6.15	7.03	.07	.13	.24
Subplot Error	24	31.47	17.80	.06	.35	1.61

*, ** Significant at the .05 and .01 level, respectively.

Appendix Table 9. Mean square values from analysis of variance for photosynthesis, respiration, and photosynthesis/respiration ratio of Mesa-Sirsa and AZST 1982 seedlings grown in solutions containing 0 to 18000 ppm NaCl.

		Mean Square		
Source	DF	Apparent Photosynthesis	Respiration	Photosynthesis /Respiration
Blocks	3	20.57	15.50	2.44*
Treatments	7	45.47	29.95	1.72*
Germplasm	1	53.79	22.65	.01
NaCl Level	3	42.51	44.19	3.47**
Germplasm x NaCl	3	45.66	18.14	.54
Error	21	27.01	18.80	.50

*, ** Significant at the .05 and .01 level, respectively.

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