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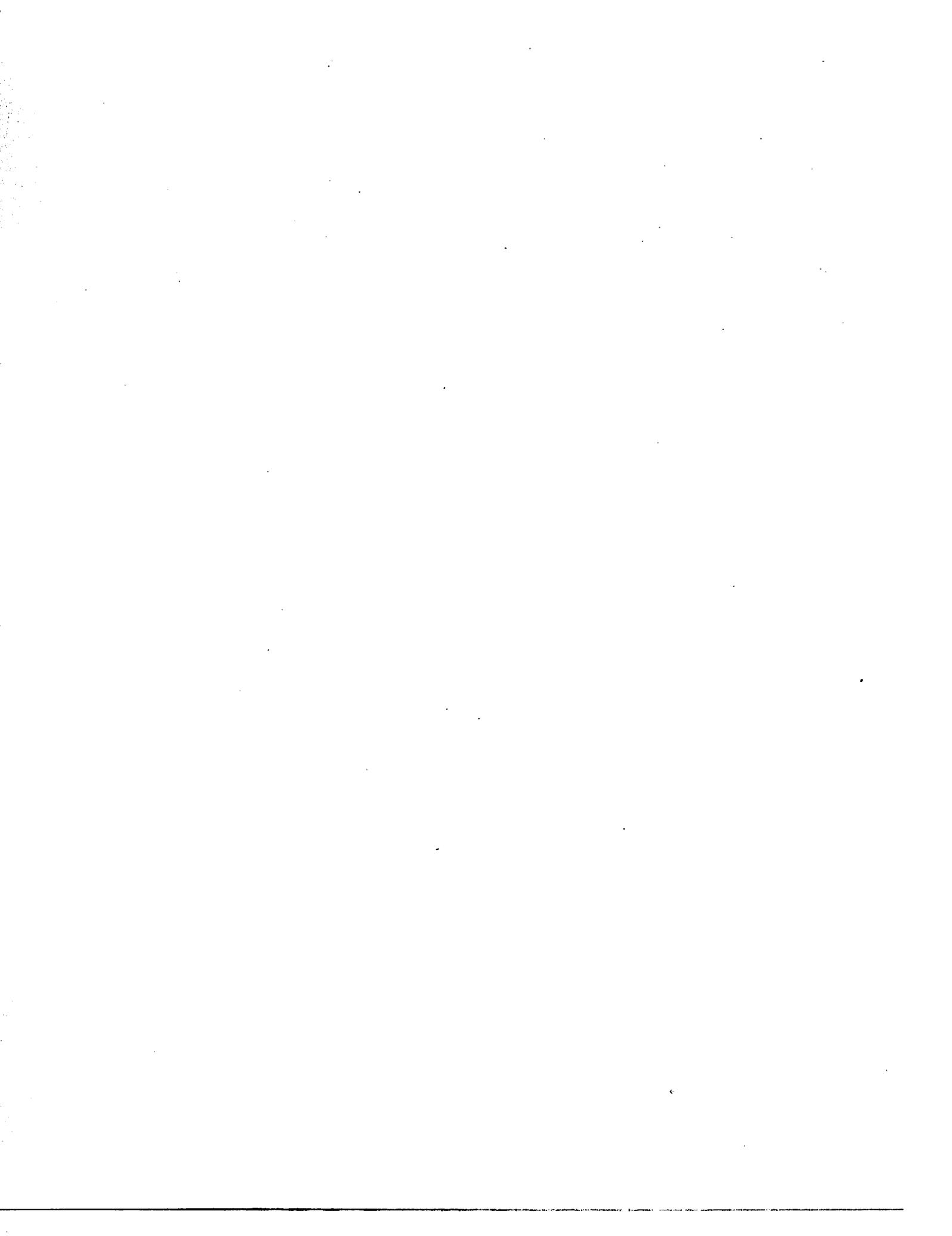
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CHARACTERIZATION OF SALT TOLERANCE IN ALFALFA (MEDICAGO
SATIVA L.)

The University of Arizona

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CHARACTERIZATION OF SALT TOLERANCE IN ALFALFA
(MEDICAGO SATIVA L.)

by

Timothy Irving McKimmie

A Thesis Submitted to the Faculty of the
DEPARTMENT OF PLANT SCIENCES
In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE
In the Graduate College
THE UNIVERSITY OF ARIZONA

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ABSTRACT

A line of alfalfa (Medicago sativa L.), AZ GERM SALT-1, was used to investigate the physiology of salt tolerance. Plants were selected which showed superior vigor under saline conditions at germination, emergence, and establishment. Selections were made using a hydroponic system inside a greenhouse. Selections were intercrossed in the field and the progeny (Cycle 1) were tested against the parent under the original conditions. Plant vigor was greater in Cycle 1. Other parameters measured were proline concentration, water potential and germination.

Seven other cultivars from the southwest were compared to Cycle 1. All were less vigorous under salinity but great variability existed among the cultivars. Sodium content (ppm/gDW), and Na^+/K^+ ratio were lower in the more vigorous seedlings. Plant response to salinity varied over the course of establishment and the early stages appeared to be the most sensitive. Variation in response to salinity within the germplasm has been shown to exist.

INTRODUCTION

It has been estimated that salinity limits crop production on one half to one third of the world's irrigated cropland (Norlyn, 1980; Raloff, 1984a). Though irrigated land accounts for only 15% of the world's total cropland, it produces 30% of the world's food supply (Wittwer, 1979) and there is a worldwide trend toward a saltier water supply. As population rises so does the demand for water. Long term solutions involve the improvement of water resource management technique by regulation of surface and ground watersheds. From an agricultural standpoint we can improve irrigation efficiency and increase crop production efficiency by exploring and utilizing the range of salt tolerance in crops.

Plant response to salinity has not been extensively studied but as the problem becomes more severe and economically important more research is being done. Plant response is variable but osmotic adjustment and consequent maintenance of turgor must occur if plant growth is to continue in a saline environment. This can occur by ion uptake or the manufacture of organic solutes. While osmotic adjustment is common to all plants, the mechanisms vary from species to species and under different environments. Other adaptations to stress such as specialized anatomical

features, metabolic pathways, morphologies, etc., also vary widely. The end result is a myriad of plant responses to salinity at the levels of both root and shoot. The response of the entire plant, not just isolated systems, are what determine survival and growth capabilities and what the plant scientist must ultimately try to understand.

Alfalfa (Medicago sativa L.) is the second largest crop in acreage in Arizona and salinity problems are increasingly a concern in the state. Alfalfa is considered moderately salt tolerant and variability in salt tolerance at several growth stages has recently been shown but no salt tolerant lines are commercially available. The objectives of this research were to 1) characterize and further delineate the response to and mechanisms of salt tolerance in alfalfa seedlings, 2) incorporate salt tolerance in emergence and establishment into an already established germination salt tolerant material and 3) review some plant strategies for coping (or not coping) with salinity.

REVIEW OF LITERATURE

Salinity

Salinity is the most important problem of irrigated agriculture in the world (Kelley, Norlyn, and Epstein, 1979), and generally is restricted to arid and semi-arid areas. Low annual precipitation which is inadequate to move salts down into the soil profile can result in saline soils. Soils of arid lands however are often high in fertility and highly productive when sufficient water is available for plant growth. The use of irrigation has turned many desert areas into lush, green croplands. The major problem however is that all irrigation water, whether from stream diversions or subterranean aquifers, carries salts. The concentration can vary from less than a hundred to several thousand parts per million (about $-.01$ to $-.5$ MPa). Agricultural damage begins when the salt concentration in irrigation water reaches about 750 ppm (El-Ashry, van Schilfgaarde, and Schiffman, 1985), but poor management can lead to soil salinity problems using less saline irrigation water. Salt concentrations of 2,000-3,000 ppm are usually toxic to crops (Raloff, 1984).

The type and degree of salinity varies as does the plant response to it. Ocean water (about 35,000 ppm or 3.0 MPa) may contain many essential nutrients but the dominant

ions are Na^+ which is usually not essential for plants and Cl^- which is needed only in minute amounts. Some irrigation sources as well as saline lands may contain toxic amounts of boron or other ions (Maas and Hoffman, 1977b) or disproportionate amounts of ions resulting in limitations in uptake of important ions such as Ca^{++} . Soil salinity can vary over a wide range in a single field. Salinity effects can be both specific (ion toxic) and osmotic (Redmann, 1974; Gonzalez-Murua et al., 1985).

Evapotranspiration results in the removal of pure water so that salts delivered in the irrigation water become more concentrated. Salts will build up in the field unless excess water is used for leaching. If the water table is high, periodic fluctuations may bring these salts back to the surface and the plant root zone may become waterlogged. In large areas of southern California drainage tiles have been installed below the root zone to carry away the excess (saline) water (Kelley et al., 1979). This technique was used because the area contains some of the most productive ground in the world but the lack of similar resources and technology has caused some areas of the world such as Pakistan and Iraq to suffer large scale losses of production due to salt accumulation. The Sumerian culture of the Tigris-Euphrates had a very productive irrigated agriculture dating back 6,000 years. As salinity built up, wheat (Triticum aestivum L.) was replaced by the salt tolerant barley (Hordeum vulgare L.) and over the next 1,000 years

the yields of barley dropped 65%. Most of this land was abandoned as not suitable for farming and remains so today. According to one estimate at least 50% of all irrigated land will be destroyed by salinity before the end of the century (Raloff, 1984a). As population demand grows, good quality irrigation water is becoming more scarce and more expensive.

In the arid southwestern United States, irrigation has permitted some of the best crop yields in the nation, eg. cotton (Gossypium hirsutum L.), lettuce (Lactuca sativa L.), barley, wheat, and alfalfa. In Arizona virtually all cropland, over one million acres, is irrigated and salinity problems are increasingly a concern for farmers. The Colorado River carrying about 800 ppm salts irrigates nearly a million acres of Arizona and California farmland. The leaching fraction constitutes a major portion of the return flow (that irrigation water which flows back to the river) and means that downstream users have to contend with an increasing salt content. Return flow accounts for 37% of the salt content in its lower basin and the Colorado River (lower basin) will increase in salinity 42% from 1960 to 2010 (Young, Franklin, and Nobe, 1975). Just below our border, in Mexico's Colorado River delta, some 14% of the land is considered too saline for cultivation (Eckholm, 1975). Mexico has protested that the quality of the water crossing the border is unacceptable. As a result, hundreds of millions of dollars are being spent on desalinization plants in the area.

In California about 1.2 million acres are so severely affected by salts as to be of no use to agriculture (Kelley et al., 1979). Of ten million irrigated acres three million suffer from salt damages. Due to high water tables much of this land must be drained. The Central and Imperial Valleys supply nearly one half of the nations fruits, nuts and vegetables. The Central Valley which contains about 4.5 million acres could lose 1.5 million acres to salinity by the year 2000 (Maranto, 1985). In the San Joaquin Valley where drainage is necessary but outlets are often nonexistent, many farmers must use part of their acreage for evaporation ponds. As much as 5-10% of their land may be lost in this way, perhaps permanently. In the Imperial Valley excess water is piped to the Salton Sea. Drainage can amount to one half acre foot per acre per year.

Arizona cropland generally has good drainage except for some areas along the Colorado River where much of the irrigation water ends up as return flow. Some areas of central Arizona may experience increased salinization of groundwater due to deep percolation. Groundwater supplies about two thirds of Arizona's irrigation water as well as a large portion of municipal water. Tucson is the largest city in the world which is totally dependent on ground water. Recent laws require that Arizona groundwater discharge shall not exceed the recharge. Conservation is therefore mandated. The newly arrived Central Arizona project will provide 1.2 million acre feet (MAF) or about

10% of the total flow of the Colorado River to central Arizona (Central AZ Water Conservation District Fact Sheet, Oct. 25, 1985). This will be the largest such allocation project but other states, particularly in the upper basin, will soon develop new projects for their uses. Population and industrial growth will certainly demand a larger share of the water and agriculture will be pressured to reduce consumption. Marginal land will be lost but salt tolerant crops could reduce this loss.

Few crop plants now tolerate salinity. In fact there are presently no commercially available crop lines which have been specifically bred for salt tolerance according to Emanuel Epstein, plant physiologist (AZ Daily Star, Dec. 15, 1984). The more salt tolerant lines of crops such as barley do not provide economic yields (Raloff, 1984b). Various approaches to studying the problem of plant response to salinity include ion transport and inclusion/exclusion, root permeability, enzyme action and sensitivity, use of osmotica for adjustment of water potential, cell anatomy, and the use of phylogenetic relationships. This review focuses particularly on proline, which commonly appears following salt stress in plants, and on the transport and compartmentation of inorganic ions. The lack of salt tolerant germplasm is the major roadblock to elucidation of the mechanisms of salt tolerance in plants. This material is needed for comparison of closely related individuals which may differ in only a few traits. The current interest

in salt tolerance promises to deliver more of such lines in the near future.

Proline

The increase in cell proline content following stress is widely known and can be due to salinity, drought, temperature, flooding and other stresses. The role of proline however has not been clearly delineated and perhaps it can play several roles. It is thought by many to moderate the effects of stress and by others to be merely a byproduct of stress indicating senescence or failure of metabolic systems.

Clearly, osmotic adjustment is of primary importance. Proline can increase more than 100 fold in some plants under stress. Proline is both osmotically active and highly soluble and apparently has no detrimental effect on enzyme activity (Shevyakova, 1984). The increased osmotic activity of proline in the cytoplasm of sorghum (Sorghum bicolor L.) has been estimated to be sufficient to balance the vacuolar osmotic potential and permit the maintenance of a negative water potential gradient between the growing media and the plant (Weimberg, Lerner, and Poljakoff-Mayber, 1982).

Imaml Huq and Larher (1985) concluded that proline was not an indicator of salt tolerance in two species of legume. Despite the fact that both accumulated proline, the species with less accumulation was in fact the more salt tolerant. Tal et al., (1979) found less proline

accumulation in wild tomato species than in cultivated tomato (Lycopersicon esculentum Mill.) despite the fact that the wild species are known to inhabit saline soils. Proline accumulation in potato (Solanum tuberosum L.) occurred too late to overcome the effects of stress and wilting (Levy, 1983).

Riazi, Matsuda, and Arslan (1985) using water stressed barley, found larger and more rapid increases in proline in the growing region than in mature leaves. This would result from the fact that the cells of the growing region are in greater contact with the xylem stream. The picture is complex but glucose appears more important initially as an osmoticum since proline concentration rose after the increase in glucose. Sucrose concentration dropped indicating the method of glucose formation. The appearance of proline following glucose may indicate its role in enzyme protection, but proline may play a significant role in osmotic adjustment if it is confined to the cytoplasm. Though proline is found in vacuoles, its accumulation under water stress has been found to be greater in the cytoplasm than the vacuole (Pahlich, Kerres, and Jager, 1983).

Story and Wyn Jones (1977) felt that proline, despite its accumulation is not as good a relative indicator of salt tolerance as glycine betaine. Upadhyaya et al., (1982) found that added glycine betaine could reduce the

salt induced proline increase in Phaseolus aconitifolius Jacq..

Indications of the positive role of proline in salt tolerance seem to outnumber those arguing against it. At the bacterial level, strains of Salmonella typhimurium (Loeffler) Castellani and Chalmers, have been isolated which produce large quantities of proline and show higher degrees of salt tolerance. The DNA fragment responsible has been transferred to other bacteria conferring salt tolerance to them (Rains et al., 1982). Pandey and Ganapathy (1985) developed a NaCl tolerant callus line of Cicer arietinum L.. This line accumulated proline while the sensitive line did not.

Tal et al. (1979) found that the highly salt tolerant jojoba (Simmondsia chinensis (Link) Schneid) accumulated large amounts of proline following salt stress and returned to control levels following stress relief. Singh and Gupta (1983) felt that proline accumulation could be used as an index for screening soybean (Glycine max Merrill) cultivars for drought tolerance. Proline accumulation in water stressed barley leaves was found to subside with relief of stress in viable tissue but remain high in drought killed tissue (Hanson and Nelson, 1978).

In a massive study covering seven years and 95 species from 26 families of the Namib desert, Treichel et al. (1984) found that the proline content in many species corresponded directly to increasing or decreasing drought.

Proline content in ten of eleven halophytes (Stewart and Lee, 1974) was found to be between 31-73% of the total amino acid pool. This is higher than most glycophytes studied. In addition a coastal population of Armeria maritima Willd. accumulated much higher amounts of proline than an inland population.

Liu and Hellebust (1976a) found that "an increase of external salinity appears to directly increase the rate of proline synthesis and to decrease its rate of catabolism as well as incorporation into protein" in Cyclotella cryptica Reimann, Lewin and Guillard, a diatom. Liu and Hellebust (1976b) found light to be necessary in proline formation and that feedback inhibition of proline biosynthesis is relaxed when cells of C. cryptica are subject to water stress. Kalinka (1985) has postulated that photorespiration may lead to increased proline synthesis via the glycolate pathway.

Buhl and Stewart (1983) found large increases in conversion of (labelled) glutamate to proline in salt treated barley. Proline utilization, by oxidation or protein formation, was cut in half. Fukotoku and Yamada (1984) studied the source of proline in water stressed soybean. They found protein degradation to be stimulated by stress and protein synthesis to be inhibited. Large increases in proline and asparagine resulted from protein degradation. The increase in (labelled) proline was found to be three times as much as the amount of proline present in the (labelled) protein indicating synthesis from other

amino acids.

Shevykova (1984) in a review article presented perhaps the best summary of how proline metabolism might be controlled and activated to provide salt tolerance. He states that proline's possession of both hydrophobic and hydrophilic parts, its high solubility and ability to form aggregates may protect cellular structure and enzymes by maintaining an intact hydration sphere. Formation of aggregates may partly explain why feedback inhibition of proline synthesis enzymes is relaxed. Further lowering of feedback inhibition could be due to alteration of enzyme steric properties under stress. This scientist cites evidence that several enzymes of the proline synthetic pathway have enhanced activity in the presence of NaCl in sharp contrast to most other plant enzymes. ABA concentration can also rise during stress and may induce proline formation in addition to effecting stomate closure (Huber, 1974).

Proline degradation is slowed in several ways (Shevykova, 1984). Pyrroline-5-carboxylate reductase (on the proline degradation or oxidizing pathway) is highly sensitive to inorganic ions. Furthermore the mitochondria, the predominant site of proline degradation, may show altered (lowered) membrane permeability to proline in the presence of drought or salt stress (Bogess, Aspinall, and Paleg, 1976).

Although proline metabolism is commonly associated

with mitochondria, chloroplasts may have a major role during salt stress. Nitrate reduction as well as glutamate synthesis occurs in the chloroplast. NADPH may be more effective than NADH in the conversion from glutamate to proline (Liu and Hellebust, 1976b). The final enzyme in this pathway has been shown to be salt tolerant. ABA synthesis also occurs in chloroplasts and may enhance proline synthesis. It seems likely that salt stress can induce membrane changes and consequent redirectioning of substrates. Asparagine concentration often rises in conjunction with proline and may act as nitrogen storage (Fukotoku and Yamada, 1984).

There is a wealth of information concerning proline metabolism and its regulatory systems. Further studies will clarify areas where less work has been done. One of the major problems facing workers is that plants seem to have so many different roles (or non-roles) for proline due to their varying responses to salinity. This prevents generalization across the plant kingdom which is important in making broad theories.

Ions, Selectivity, Compartmentation, Enzymes

Plants must maintain an osmotic potential which assures water movement from the growing media (soil) to the plant as well as maintenance of turgor necessary for growth. Failure would mean wilting and eventually death. In saline environments inorganic ions such as Na^+ , K^+ , and Cl^- are a

freely acquired means of solutes. Halophytes can absorb large amounts of salts and certainly utilize such ions in maintenance of water balance. Glycophytes are often thought of as ion excluders but this is a relative term. Ions are necessary for growth and exclusion mechanisms and effects are variable.

Despite the fact that inorganic ions permit a lowered water potential, the cellular machinery in the cytoplasm can function effectively only in a fairly narrow range of ion concentration. In general, plants maintain about 100 mM K^+ and 10 mM Na^+ in the cytoplasm (Wyn Jones, Brady, and Spiers, 1979). As little as a twofold increase or decrease can begin to disrupt metabolic systems. The K^+ requirements of various enzymes suggest a cofactor relationship. Under higher cytoplasmic salt concentrations enzyme sensitivity (with the exception of halophilic bacteria) is common. Most plant enzymes are sensitive to salts and this holds true for halophytes as well as glycophytes (Flowers, 1971; Flowers, Hall, and Ward, 1976; Greenway and Osmond, 1972). It is probable that ions affect the structure and activity of enzymes of halophytes and glycophytes in the same manner. Protein synthesis is also maximal at around 100 mM K^+ (Wyn Jones, 1979).

Since plant cells can be as much as 95% vacuole the problem of osmotic adjustment becomes less formidable. Ion compartmentation in vacuoles is a major plant strategy. The small volume of cytoplasm means that accumulated organic

osmotica can serve a proportionately larger role.

Despite the need for a high K^+/Na^+ ratio, Na^+ uptake is often increased at the expense of K^+ uptake (Lessani and Marschner, 1978; Imami Huq and Larher, 1983) under conditions of decreasing external water potential caused by salinity. Mechanisms for dealing with ion uptake include inclusion and exclusion, selective absorption and reabsorption from xylem and retranslocation. Selective and active transport has been noted at both the plasmalemma and the tonoplast (Jeschke, 1984). Preferential uptake of K^+ has been shown for sorghum (Weimberg et al., 1982). Jeschke and Stelter (1973) demonstrated a K^+ dependent Na^+ efflux mechanism at the plasmalemma of root cortical cells in barley. Barley and wheat are considered salt tolerant as crop species and the more tolerant cultivars are more efficient sodium excluders, though barley takes up relatively more salt than many other glycophytes (Jeschke, 1984; Jeschke and Wolf, 1985). Lessani and Marschner (1978) showed that labelled Na^+ applied to leaves of several species could be observed as Na^+ efflux at the roots.

Halophytes may be less discriminate in allowing Na^+ penetration at the root level, thereby permitting more Na^+ accumulation in shoots. However, their efficient compartmentation in vacuoles as well as mechanisms for sequestering salt in shoots such as in salt glands, generally means that halophytic cytoplasm is no more exposed to salinity than glycophyte's. Halophytes can maintain

sufficient K^+ uptake at high salinities in contrast to many glycophytes (Jeschke, 1984).

Jeschke's excellent chapter (1984) on K^+ - Na^+ exchange describes K^+ movement in both directions across the tonoplast while generally Na^+ movement is unidirectional. Vacuolar stored K^+ can therefore serve as a buffer for exchange when Na^+ enters the cytoplasm. This is especially important for growing tissue. Phloem sap can be rich in K^+ providing young tissue with a reservoir which can later be utilized when xylem delivers less K^+ and more Na^+ . The salt tolerance of barley cultivars may be due to efficient K^+ transport to growing areas (Jeschke and Wolf, 1985). Conversely plant growth rates may be limited by lack of ion transport. The salt sensitive bean (Phaseolus vulgaris L.) has a low rate of ion transport to the shoot (Lessani and Marschner, 1978).

The presence of calcium is necessary both in K^+ - Na^+ exchange across membranes and K^+ selectivity at the roots (Jeschke, 1984). Calcium supply may be limited under saline conditions. Lynch and Lauchli (1985) found Ca^{++} transport to barley shoots was decreased by NaCl and the most sensitive cultivar had the least Ca^{++} transport. They suggest a breakdown in the xylem loading mechanism since Ca^{++} did enter the roots. Use of a constant external Na^+ - Ca^{++} ratio helped alleviate some adverse affects of salinity in cowpea (Imaml Huq and Larher, 1984).

Chloride levels may parallel those of Na^+ or be

higher, acting as anion for K^+ . Cytoplasmic levels of Cl^- are kept low by use of organic anions. Chloride is not retranslocated as readily as Na^+ and root efflux of Cl^- is low (Lessani and Marschner, 1978). Tal et al. (1979a) found greater Cl^- accumulation in wild tomato species under salt stress than cultivated tomato. This is in line with the view that ion transport may be limiting in salt sensitive species, and that halophytes are ion accumulators. Jojoba, which is salt tolerant, accumulates large amounts of Cl^- as well as Na^+ under high salinity (Tal et al., 1979).

Plants then have numerous and varied ways of dealing with salt stress and solute accumulation, and there is a wide continuum between ion inclusion and exclusion. Perhaps plants exposed to periodic salinity or drought can afford to restrict ion entry until the stress is relaxed. On the other hand, plants which routinely incur water deficits might find it strategic to acquire ions more readily.

Crops and Selection

Large expanses of arid and semiarid land (needing irrigation for crop growth) exist worldwide. The quality of the water available for irrigation ranges from only mildly saline to ocean water. Salt tolerant crops however are generally not available and their development has been slow. Despite the existence of true halophytes, their use as cultivated crops has not been demonstrated to be practical at this time. A range of salt tolerance in glycophytes

exists but their selection may never result in growth approaching that of halophytes under saline conditions.

Differences in salt tolerance at the species level or below have been noted for many but not all crops. A given species may have limited options for change. The greatest variability between and among species or cultivars is known in grasses and legumes (Maas and Hoffman, 1977b). Barley for example, can be salt sensitive or tolerant depending on the cultivar. Many salt sensitive species such as lettuce show little variability in salt tolerance. Variability is necessary for selection procedures to produce more salt tolerant cultivars. Lacking this, plant breeders would be dependent on gene introduction (splicing) techniques, mutations, or crossings with wild (salt tolerant) species. Selection permits development of plants with unique properties with respect to the norm if they exist. Anatomical, morphological and physiological considerations may limit all approaches. Genetics, plant growth stage, soil fertility, soil water and aeration, climate, and cultural practices influence plant response to salinity. The number of possible interactions between plants and their environments due to differential plant adaptations is therefore enormous. Most salinity research is done under controlled conditions and little data is available on actual yield declines in the field due to salinity. Since NaCl is the most commonly encountered salt in irrigation water it is the salt most commonly discussed and used in research

dealing with salinity. While specific croplands may have individual problems with other salts, many generalizations can be made about NaCl and its effects.

The varying sensitivity of plants to stress at different growth stages from germination, emergence, establishment and flowering/fruitleting has been noted by many authors (Ayers and Hayward, 1948; Abel and McKenzie, 1963; Maas and Hoffman, 1977a; Gonzalez-Murua et al., 1985). Sugarbeets (Beta vulgaris L.) and barley are both considered salt tolerant crops. They differ however in their response at various growth stages. Sugarbeets are relatively salt sensitive at germination while barley is somewhat salt sensitive during early seedling growth (Maas and Hoffman, 1977a; Maas, 1986).

Plant selection beyond the germination stage has been successful with several crops. Noble, Halloran, and West (1984) used percent leaf damage (less than 10%) to select alfalfa plants for salinity tolerance. After two generations of selection the mean population tolerance was increased and yield under non-saline conditions remained unchanged. Shoot levels of both Na^+ and Cl^- were lower in the tolerant plants though root levels were similar. Noble suggested that ion exclusion to the shoot could therefore be used as a selection technique. Hodson, Opik, and Wainwright (1985) stressed the variation in accumulation of ions in different parts of the plant. They found the more salt tolerant clone of Agrostis stolonifera L. to have lower

concentrations of Na^+ and Cl^- under salt stress. They noted that this phenomenon is also followed by many other (but not all) species. Abel (1969) found that Cl^- transport to the shoots of soybean was heritable (and controlled by a single gene) and that the most tolerant plants were also the most efficient excluders. Dewey (1962) used artificially salinized basins to select for salt tolerance in crested wheat grass (Agropyron desertorum Fisch.). In some cases he was able to use simple survival as a selection technique since most of the plants did not complete a life cycle. He also found plant vigor to be useful in selection.

Norlyn (1980) working with barley, wheat and tomato has developed salt tolerant lines of all three crops. Barley plants which germinate and emerge in 90% seawater faster than California Mariout (a local salt tolerant cultivar) were selected. Seed were produced from these in the absence of salt and the progeny were retested at 50% seawater through the entire life cycle. Selections were made and seed from these sown in plots and irrigated with 100% seawater. Several lines have completed their life cycles and produced seed under these conditions. They plan to screen the entire world collection of 22,000 barley entries! The same lab has screened wheat under similar conditions. Since wheat is more sensitive the final selections are made under 50% rather than pure seawater but several lines (out of 5,000 tested) show outstanding salt tolerance. Using tomato, the group crossed a salt sensitive

cultivar with a wild salt tolerant relative, (L. cheesmanii, ssp. minor (Hook.) C.H. Mull.), which produces unedible fruit. After additional screening and backcrossing they have developed a plant which can survive and produce edible tomatoes when irrigated with as much as 70% seawater.

Noble (1983) stressed the need to compare the physiology of closely related lines or cultivars which differ in salt tolerance. Some crops such as lettuce or beans have failed to show much variation. Others such as alfalfa, soybean, and barley exhibit intraspecies variation and are now being bred and developed and will be available for study. Plant anatomy, morphology and physiology may differ so widely that comparisons between species at the whole plant level become almost meaningless. Note the wide range of responses in ion transport or proline accumulation, neither of which show linear relationships to salt tolerance. Studies of cultivar variation within the same species permits isolation and comparison of specific plant systems. In addition to comparison of sensitive and tolerant cultivars, comparisons can be made within cultivars under different levels of salinity to denote where changes begin to occur.

In spite of all the research which has been done, few crop plant cultivars are commercially available with a high degree of salt tolerance. From the standpoint of market demand and yield under saline conditions virtually none are economically competitive. Basic research should and will

continue. Maas (1986) has reviewed the literature and compounded data concerning the salt tolerance of nearly 80 agricultural crops and many horticultural species. He notes that there is usually no yield decrease until a salinity threshold is reached, when yields begin to decrease approximately linearly as salinity increases. This is useful information for crop selection under varying conditions of soil and irrigation water quality. He also discusses the variable effects of salinity on yield components such as grain and fruit, roots, and vegetative material. Salinity effects on barley for example, decrease the yield of the harvested product, the grain, much less than the straw.

Alfalfa is moderately sensitive to salts but there is variability among cultivars. Variation in germination ability and its heritability have been established (Dotzenko and Haus, 1961; Carlson et al., 1983; Allen et al., 1985) and a line of germination salt tolerant alfalfa has been released (Dobrenz et al., 1983). This material however was not more salt tolerant than the parent at later growth stages (Allen, 1984). Noble et al. (1984) selected alfalfa plants at a later growth stage and after two generations isolated a group of plants which are more tolerant to salinity during early growth. Development of a salt tolerant alfalfa cultivar would allow use of marginally saline land and provide forage and nitrogen fixation.

MATERIALS AND METHODS

The germplasm selected for this research was AZ-GERM SALT-I (Dobrenz et al., 1983) which had been developed for germination salt tolerance. This material was derived from 'Mesa-Sirsa', a high yielding, non dormant cultivar with resistance to the spotted alfalfa aphid, Therioaphis maculata, Buckton (Schonhorst et al., 1968). To achieve the objective of increasing salt tolerance, salinity treatment was administered both following germination and at germination through emergence and establishment. Solution culture (hydroponics) was employed because of the uniformity which could be provided between treatments, the relative ease of measurement of solution water potential and the ease with which it could be changed. Two different approaches, conetainers immersed in solution, and a 'salt box' (nursery flats watered from a tank) were used. In both cases the most rapid selection procedure possible was desired, so as to screen the maximum number of plants that time allowed, and keep the space requirements from getting prohibitively large. Greenhouses on the University of Arizona campus were used for the selection procedures. Temperatures ranged from 25-32 C.

Selection from Conetainers

Covered plexiglass boxes, 55 x 43 x 20 cm, which held 22 liters of solution were used. The boxes were painted black and the tops consisted of 80 holes through which were placed plastic cones 20 cm deep and 3.5 cm in diameter (available from Cone/tainer Nursery, Canby, Oregon). The cones were filled with fine vermiculite and four seeds sown in each. They were then immersed in the solution which consisted of a one third Hoaglands mixture. Within the first week each cone was thinned to one seedling. After two weeks (at which time all plants had at least two trifoliate leaves) the solution was changed to NaCl and Hoaglands (- 2.5 MPa). Solution water potential was measured with a Wescor model 1500 C Vapor Pressure Osmometer.

Because of dilution by the residual nutrient solution in each cone, salt induced effects were not evident for several days, but by 5-6 days many plants showed salt burn on the oldest leaves. By 14 days following salt treatment a few of the plants had died. Selection pressure continued for about 21 days. At this time the most vigorous plant from each box was selected, removed and transplanted to a four liter pot where it was watered with one third Hoaglands solution. A total of 13 plants were selected and four cuttings from each were rooted to increase the population. These selections were transplanted to an isolated crossing block at the USDA Plant Materials Center

in Tucson. A beehive was placed nearby for pollination and seed (Cycle 1) was harvested in the fall of 1984.

The harvested seed and the Salt-I seed (AZ GERM SALT-1) were germinated in nutrient solution and changed to - 2.0 MPa NaCl after two weeks. At 15 days following salt treatment several parameters were measured. Each was replicated three times. Plant heights were measured to the nearest millimeter. Leaf area was measured with a Licor LI 3100 leaf area meter. Leaf water potential was measured using Merrill thermocouple psychrometers (model 75-11C) and a Wescor HP 115 Water Potential Data System. Samples from the fully expanded trifoliate leaves were allowed to equilibrate for four hours in a sealed, insulated styrofoam container. Proline content was determined by harvesting all trifoliate leaves, freezing immediately in liquid nitrogen, and lyophilizing. Free proline was extracted by boiling in 5 ml water for 30 minutes in a tightly covered test tube. The acid ninhydrin procedure as modified by Bates, Waldron, and Teare (1973) was used for analysis. Germination in distilled water and -1.3 MPa NaCl was done in petri dishes in a growth chamber with a constant temperature of 25 C. One hundred seeds were placed on filter paper and 5 ml of solution was added. Germinated seeds were counted after three days. Analysis of variance was performed using the MSUSTAT statistical software package in a completely randomized design.

Germination, Emergence and Establishment

In order to increase the capacity for selection a 1.2 x 2.4 x 0.1 meter plywood/fiberglass box ('salt box') was built into which a saline/Hoaglands solution was pumped (Fig. 1). This was regulated by an automatic timer. The solution was held in a 250 liter plastic tank until needed and into which drainage from flats would collect. Seed were sown into 12 nursery flats 54 x 28 x 6 cm containing fine vermiculite. Each flat held 8 inserts with 12 planting sections and each section was planted with four seed. This amounted to approximately 6150 seed that were screened per trial. A Hoagland/NaCl solution of -0.8 MPa was pumped once per day to saturate the vermiculite. After approximately six weeks of growth in this media, the most vigorous plants were selected and prepared for seed production in the field. A total of 79 plants were selected in this manner. The crossing block was located on the University of AZ farm in Marana, AZ. during the summer of 1985.

Seed from each of the 79 selected clones in Cycle 1 was harvested individually. This material was compared to SALT-I under the same experimental conditions in the greenhouse. Survivorship and clonal vigor (leaf area and plant height) were measured weekly for six weeks. Plants greater than 4 cm tall or with more than 4 cm² leaf area were counted. A composite score was compiled for each clone (seed producing plant), by using the vigor score supplemented by one to three points for those clones which

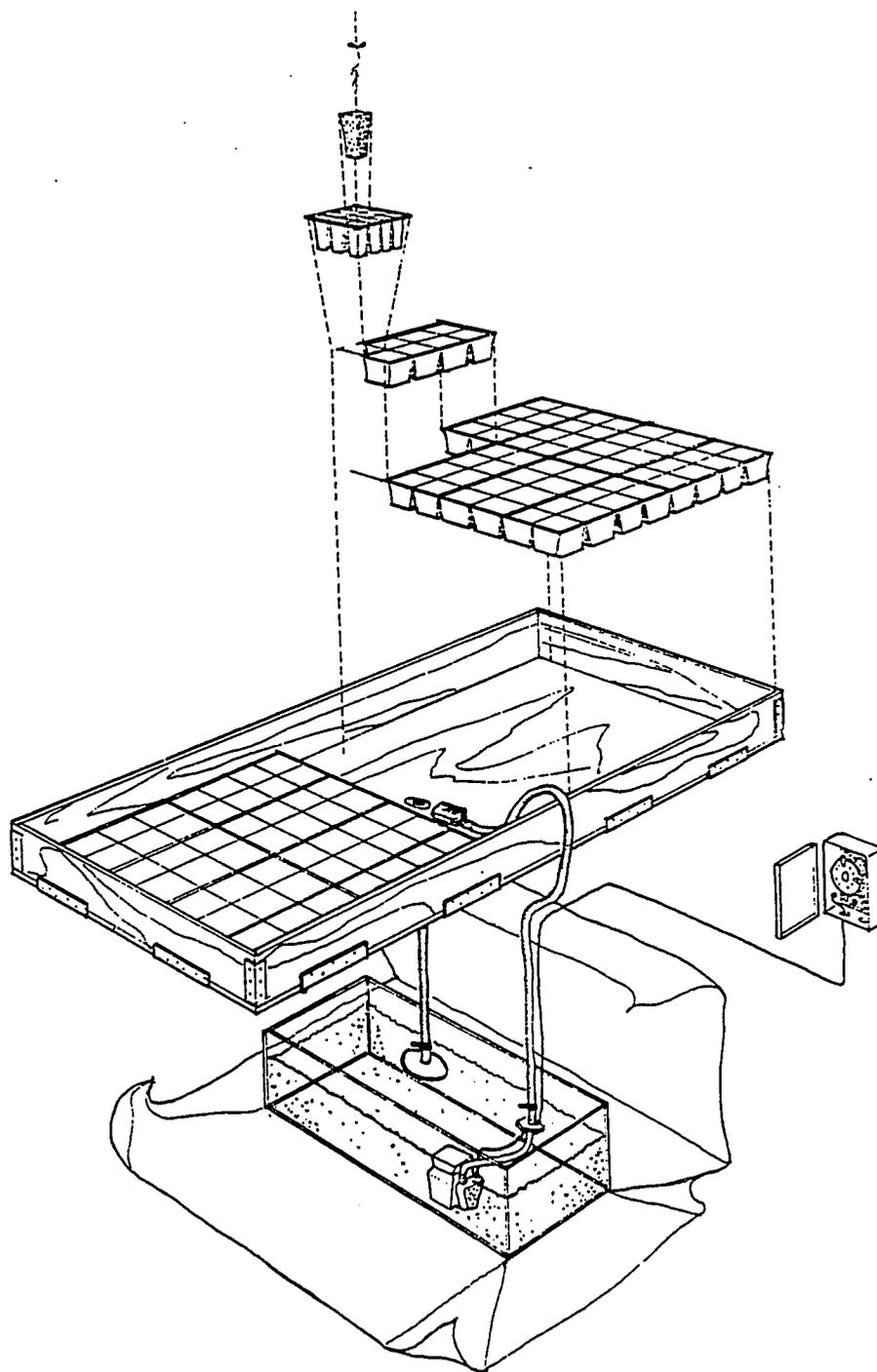


Fig. 1: Diagram of the (salt box) system used to select plants for germination, emergence, and establishment using a solution of -0.8 MPa NaCl and Hoaglands.

had greater than 40% survival. Selections for Cycle II were made from the most vigorous clones, generally those with a score of six or higher.

Bulk seed from the 79 plants of Cycle 1 was also sown in the 'salt box' and grown for five weeks under -0.8 MPa solution. Plants were harvested and divided into three size categories on the basis of height and leaf area and further divided into roots and shoots. The largest size category was comparable to the plants selected as the most salt tolerant (Cycle 1, 2). Ion content (Na^+ , K^+ , and Cl^-) of each of these groups was determined by the Soil and Plant tissue testing Laboratory of the University of Arizona.

RESULTS AND DISCUSSION

Conetainers

The means of six different parameters were measured and compiled for Cycle 1 and SALT-1 (Table 1). Analysis of variance determined that only plant height was significantly different (4.8 vs 3.6 cm respectively). Leaf area was greater in Cycle 1 (6.9 vs 4.6 cm²) and it appears that Cycle 1 seedlings were more vigorous than SALT-1.

Germination in distilled water was slightly lower for Cycle 1 and may have resulted from harvesting technique or from lack of seed aging. Germination in NaCl was reduced for Cycle 1 and this was one reason for instituting a new selection system. The method of selection, whereby plants were not germinated in NaCl, may have been responsible for this reduction.

Water potential was lower for Cycle 1 than SALT-1 (-2.85. vs. -2.67 MPa respectively). Plant water potential must be maintained below the solution water potential (-2.0 MPa) for growth to occur. Both groups had water potentials below this value and Cycle 1 with the lowest value may have been more adept at obtaining water. The difference however was not significant.

Proline content, a possible indicator of osmotic adjustment was not significantly higher in Cycle 1 (39.7

Table 1. Morphological and physiological characteristics of Cycle 1 and SALT-1 alfalfa grown at -2.0 MPa salinity in conetainers

	Cycle 1	SALT-1
Leaf Area (cm ²)	6.9 a	4.6 a
Height (cm)	4.8 a	3.6 b
Z Germination (control)	83 a	86 a
Z Germination (NaCl) (1.3 MPa)	13 a	21 a
Water Potential (MPa)	-2.85 a	-2.67 a
Proline (umol/g DW)	39.7 a	34.6 a

umol/gDW) than SALT-1 (34.6 umol/gDW). Proline increased over non stressed controls where it was also found in measurable amounts (about 2 umol/gDW, results not shown) in unstressed roots, leaves, stems and flowers. These results are comparable to other measurements found in the literature. Proline was found in highest quantities in halophytes and xerophytes, eg. Triglochin maritima L. accumulated 325 umol/gDW after 10 days in a -1.0 MPa NaCl treatment (Stewart and Lee, 1974). Glycophytes accumulated significant amounts of proline though less than halophytes eg. 36 umol/gDW after 2 days in -0.8 MPa PEG (Riazi et al., 1985).

Though one cycle of selection in a cross pollinated tetraploid is not enough to shift complicated characters involved in salt tolerance, the results do suggest that variation exists and that selection for salt tolerant characters is possible. Cycle 1 appears to be more vigorous under saline conditions and is perhaps more able to maintain water balance, however, germination ability under saline conditions was lower.

Salt Box

Seed from the 79 clones selected in Cycle 1 which had germinated, emerged and established at -0.8 MPa in the "salt box" (Fig. 1), was used to repeat the experiment. A wide variation existed among the 79 clones in the composite seedling score, (Fig. 2), as well as it's components,

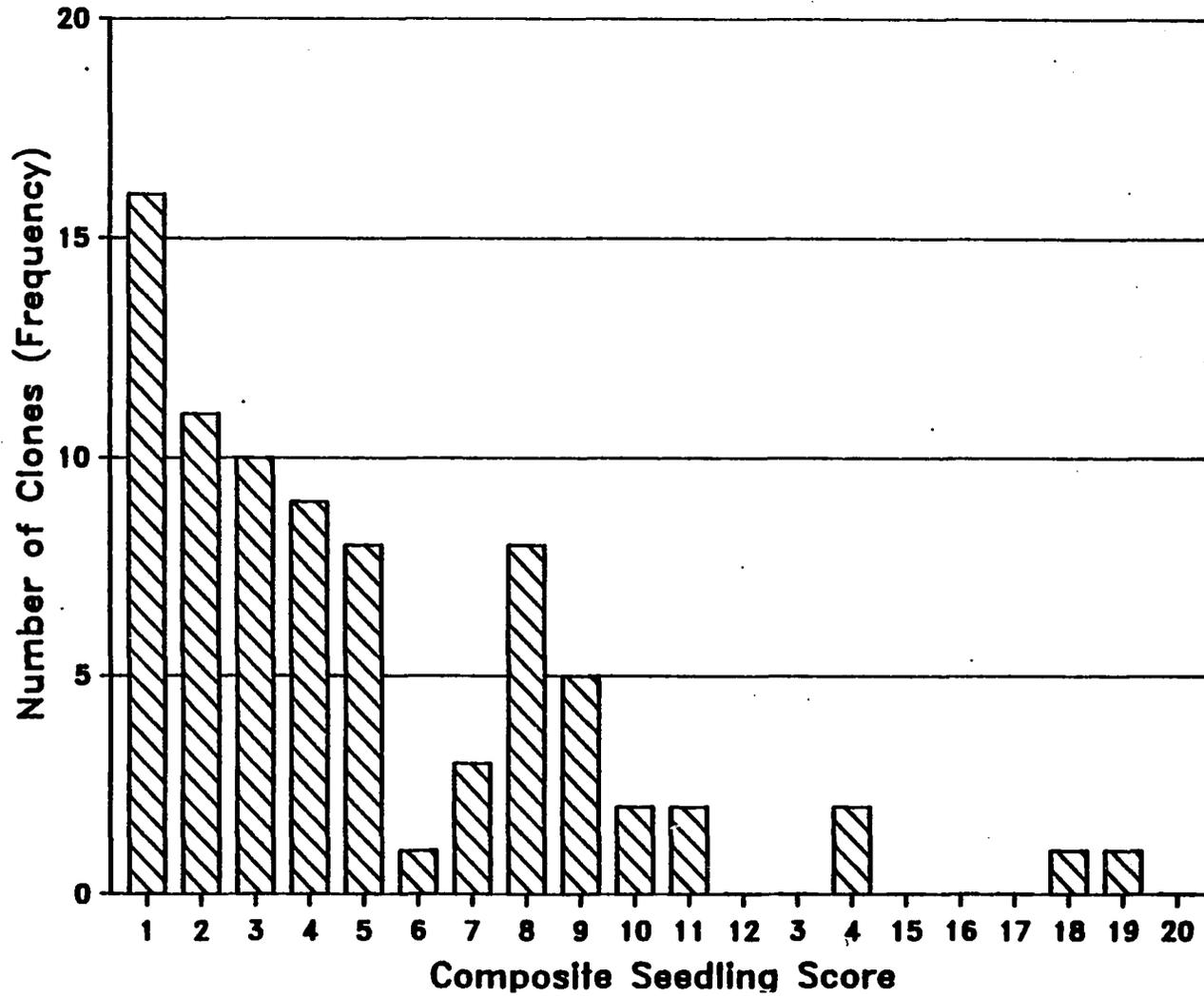


Fig. 2: Composite scores for Cycle 1 seedlings in the salt box at -0.8 MPa.

survivorship and vigor (Fig. 3 and 4). Selections for Cycle 2 were made from the most vigorous seedlings which included those with a composite score of six or greater. This resulted in a final population for Cycle 2 of 51 plants. These plants are now in the field for seed production (summer 1986).

Differences were found in the ion content of roots and shoots (Table 2). These measurements were made to help determine whether alfalfa is a salt includer or excluder. The tall plants harvested corresponded to a similar group of plants which had been selected as salt tolerant in Cycles 1 and 2. These plants contained less total ions as well as a lower Na^+ - K^+ ratio in both shoots and roots on a dry weight basis. Sodium is known to be damaging to cytoplasmic functions, whereas K^+ is not. Potassium content was relatively stable in all groups. It was the Na^+ concentration which varied (the Cl^- content seemed to vary similarly). The tall plants may be able to exclude Na^+ (and Cl^-) in a way superior to shorter plants which are obviously less salt tolerant based on plant vigor. There was nearly a twofold range between the tall and short plants grown in - 0.8 MPa (48.6 vs. 85.4 ppt Na^+ respectively). Our selection of tall plants may result in a population of superior ion excluders. This complex issue will need further work however since on a whole plant basis (not measured here) the tall plants may not have been better excluders. However it can be said that individual tissues were less exposed to

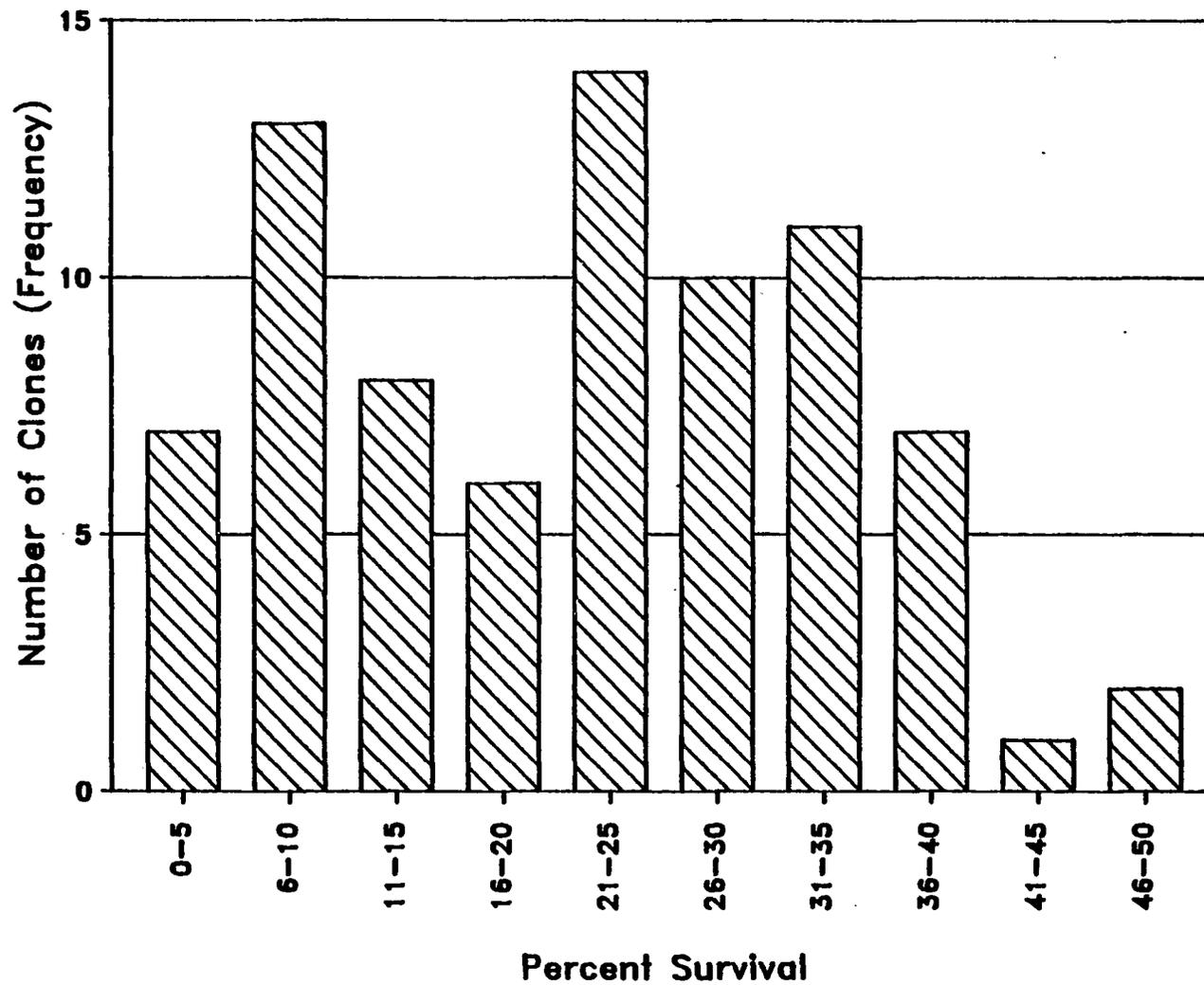


Fig. 3: Survival percentage for Cycle 1 seedlings in the salt box at -0.8 MPa.

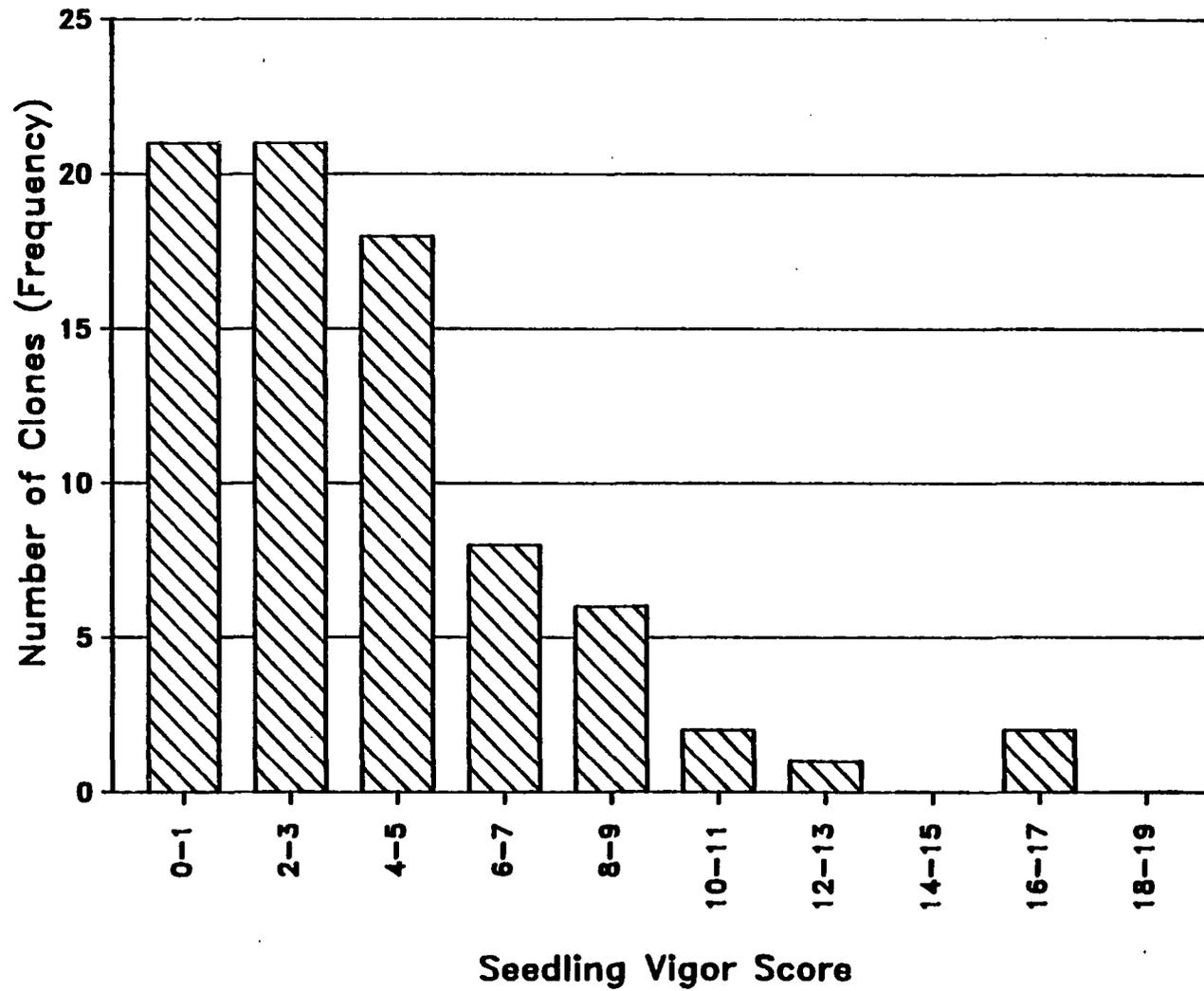


Fig. 4: Vigor scores for Cycle 1 seedlings in the salt box at -0.8 MPa.

Table 2. Ion Content (ppm x 1000) of the shoots and roots of short, medium, and tall alfalfa seedlings grown in the salt box at -0.8 MPa

	Na^+	K^+	Cl^-	Na^+/K^+
Control Root	1.5	24.4	4.0	0.06
NaCl Shoot				
Short	85.4	22.6	113.6	3.8
Medium	59.8	18.2	106.6	3.3
Tall	48.6	20.6	70.9	2.4
NaCl Root				
Short	26.8	11.4	39.8	2.4
Medium	22.2	12.2	29.3	1.8
Tall	19.0	12.8	27.6	1.5

sodium in the progeny (Cycle 1) of selected plants.

By harvesting individual clones and testing their progeny against each other (and SALT-1) we have shown the degree of diversity in the population. We have insured that the next cycle is as diverse as possible since the female parents are identified, and every effort was made to select from as many females as possible. This procedure may be advantageous over a bulk method of seed harvest where the parent plants are unknown. The shape of the curve in Figure 4 indicates a diminishing number of clones in the most vigorous categories. Figure 3 on the other hand indicates that survivorship patterns follow nearly a bell shaped pattern. Such information will assist one in developing selection techniques by maximizing the extent of superior germplasm as well as minimizing the degree of inbreeding.

In a separate experiment seed from seven alfalfa cultivars common in the southwest were sown in the 'salt box' at -0.8 MPa. Two of the cultivars (Table 3), CUF 101 and UC Salton, showed good survival ability. These two also had the highest vigor score but none of the seven had a high enough composite score (six or higher) to have been chosen for Cycle 2. SALT-1 also had a score of less than six. It should be noted however, that these comparisons are made using bulk seed in the case of SALT-1 and the seven southwestern cultivars but using seed from individual clones in the case of Cycle 1.

The salt content of the growing media in the 'salt

**Table 3. Scores in 'Salt Box' for seven cultivars and SALT-1
at -0.8 MPa**

Cultivar	% Survival	Vigor	Composite
SALT-1	24	3	4
CUF 101	38	4	5
UC Salton	36	3	4
Pierce	4	0	0
Lew	14	3	3
WL 605	2	0	0
Madera	8	0	0
UC Cibola	2	1	1

box' increased over time. The osmotic potential of the vermiculite was found to decrease at approximately -0.03 MPa per day. This resulted in about a -0.2 MPa decrease per week and -1.1 MPa over the six week growing period. This occurred due to the watering method where little leaching occurred, since the box itself was flooded up rather than watering individual flats from above. Though watering from the top may have prevented such increase in salinity (due to leaching), for our purposes watering from below appears to have been beneficial. This is because the germination and emergence phase was more sensitive to salts. In a pilot trial few seedlings emerged from a -1.0 MPa solution. At -0.8 MPa however, about 75% of the seed germinated and emerged. During the establishment period over the following five weeks plants were progressively affected by salinity until at the end of six weeks it was possible to select the 6-10 most vigorous survivors (approximately one plant for each 1000 seed sown). If the soil solution had remained at -0.8 MPa, it is likely that many more weeks or months would have been necessary to begin to see selection pressure working. Selection methods therefore can take advantage of the difference in salt tolerance at various growth stages.

Though we can not expect to see great gains in one cycle of selection our results show that variability exists in plant survival and vigor under saline conditions. Plant germination and emergence appear to be the most sensitive stage. It also appears that our selection procedure is

acting to identify those individuals which are more adept at exclusion of sodium. These ideas must be tested and related to yield by using actual field plots under saline conditions in the future.

Other basic questions to be answered are as follows. What is/are the physiological bases for salt tolerance in alfalfa? Are there anatomical differences which are responsible for ion selectivity? Is yield under saline conditions economical? What are the limits of tolerance to salinity for alfalfa, eg. how far can breeding go? Can rainfall be used to the optimum in plant establishment (often a sensitive stage) and then supplemented by (saline) irrigation water. Arizona has a scarce water supply and is dependent on irrigation. Salinity will become a major problem and salt tolerant agronomic and horticultural species will become important in the future.

SUMMARY

Alfalfa is a crop exhibiting variability in tolerance to salinity at all growth stages. The nature of this variability was investigated. Use of two hydroponic growing systems in a greenhouse allowed selection of plants which showed the greatest vigor in a saline environment, consisting of -2.5 MPa stress applied at 2 weeks after germination or -0.8 MPa stress during germination, emergence and establishment.

The selected clones were placed in the field for seed production and the progeny were tested against each other and against the parent under the original conditions of selection. Plant vigor and survival measurements were improved for the selected plants. The earliest stages of plant growth appear the most sensitive and selection for salt tolerance in alfalfa during plant establishment appears feasible. Proline concentration, water potential and percent germination were not significantly different between the original population and the Cycle 1 progeny.

Ion content (K^+ , Na^+ , Cl^-) was measured on large, medium, and small plants that were able to germinate, emerge, and establish at -0.8 MPa. The largest seedlings had the smallest Na^+ concentration and the lowest Na^+/K^+ ratio. This indicates that alfalfa plants which were more salt tolerant also excluded salts more effectively.

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