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Effect of Salinity (NaCl) on Germination, Growth, Ion Accumulation, and Protein Synthesis in Alfalfa (*Medicago sativa* L.)

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EFFECT OF SALINITY (NACL) ON GERMINATION, GROWTH, ION ACCUMULATION, AND PROTEIN SYNTHESIS IN ALFALFA (*MEDICAGO SATIVA* L.)

by

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To my parents

who taught me honesty, patience and virtue in working hard.

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ABSTRACT

Effect of Salinity (NaCl) on Germination, Growth, Ion Accumulation, and Protein Synthesis in Alfalfa (*Medicago sativa L.*)

by

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To study the effect of NaCl stress on gene expression in alfalfa (*Medicago sativa* L.), greenhouse and laboratory experiments were conducted with 22 cultivars during germination and post-germination growth. The ability of alfalfa cultivars to germinate at the different NaCl concentrations was not related to their postgermination performance (salt tolerance) under those conditions.

Genetic effects were evident for Na and Cl ion uptake and accumulation in alfalfa shoots and roots. The strategies of alfalfa cultivars to cope with NaCl stress includes exclusion of Na from shoots and Cl from roots or from the whole plant depending on cultivar and NaCl level. The reduction in shoot dry weight was not related to water stress or reduction in Ca, Mg, or chlorophyll concentrations. Results of this study indicated that the reduction in K ion uptake and more directly

the toxic effects of high Na and Cl in plant tissues contributed to the reduction in shoot dry weight. The possible subpartitioning of Na and Cl ions between different tissues, cell types, cell components, and/or the different changes in protein structure and enzyme activity at the high Na and Cl concentrations, independent of total concentration of ions, might also contribute to differences in salt tolerance (shoot dry weight) among cultivars. The factors determining shoot dry weight in alfalfa grown under the different NaCl treatments of this study were not the same for all cultivars.

Sodium chloride stress induced qualitative and quantitative changes in shoot and root proteins of alfalfa. These changes were dependent on cultivar and salt concentration, as well as length of exposure to salt stress.

(136 pages)

CHAPTER I

INTRODUCTION

Salinity has been a relevant factor in crop and food production throughout the history of mankind. Many ancient civilizations, such as Mesopotamia, have floundered due to a build-up of salinity in productive lands, thereby causing yield reduction.

Two main factors make research work in the area of salinity of special importance. First, cumulative improper water management has resulted in an increasing salt concentration in agricultural lands, making vast areas unfit for agriculture. Salinity currently affects one-third of all irrigated land on earth (Maas and Hoffman, 1977; Epstein et al., 1980; Boyer, 1982). In addition to poor water management, salt load in the water used for irrigation is of great importance as a significant source of salinity, e.g., 10,000,000 tons of salt are delivered in the water used in California each year; 90% of this water is used for irrigation (Norlyn, 1980). Second, increasing human population in the world increases the demand for agricultural products. Also the utilization of more land area for housing and industrial activities forces agriculture onto marginally productive areas, characterized by salinity.

Scientists have dealt with the salinity problem by improving the environment of the plant through reclamation procedures, which have included land leveling, surface and subsurface drainage, application of soil amendments, and

improved irrigation practices to provide leaching. However, reclamation procedures are not universally effective, as they are expensive and require continuous management control. As a result, reclamation is not complete. This realization logically leads to the idea of providing more salt-tolerant crops, which will improve yields on saline soils not fully reclaimed, and provide farmers with another management option to use in conjunction with reclamation programs.

Although some progress has been made in selection for salt tolerance, there still is a lack of knowledge about the mechanisms of tolerance available for plant breeders in the selection processes. An understanding of the molecular basis of tolerance mechanisms by which plants and plant cells adjust to saline environments is important to all programs attempting to develop salt-tolerant crop species. The identification of specific characteristics related to salt tolerance, such as specific proteins, specific enzymes, amino acids, etc., will provide potential biological markers useful in the identification and genetic manipulation of salt-tolerant plants and plant cells.

In order to face the increasing salinity problem by providing more salt-tolerant species, we should emphasize first the important crops on which humans and their animals depend. Alfalfa (*Medicago sativa* L.) is considered a primary agricultural crop and the most important forage crop species in the world. It is often called "Queen of the Forages"

because it is widely adapted, the most effective source of biological nitrogen (N_2) fixation, the most energy-efficient crop to grow, the most important source of protein yield ha^{-1}, and an attractive source of nectar for honey bees. Also, it is an excellent source of vitamins and minerals, and it is important for improving soil properties (Barnes et al., 1988). According to the latest estimations, alfalfa is grown on about 32 million ha around the world: 10.5 to 10.9 million ha of this area is in USA and 4.9 to 5.7 million in Canada (Barnes et al., 1988).

Salinity has direct harmful effects on alfalfa. It suppresses top and root growth, imposes significant restriction on nodule dry weight, affects specific nodule activity, and exerts negative effects on total acetylene reduction activity in the plants (Keck et al., 1984). Salinity could also affect alfalfa plants indirectly through disrupting symbiotic N_2-fixing systems. Salts can decrease nodule formation by reducing Rhizobium populations in the soil or by limiting their ability to infect root hairs (Pillai and Sen, 1966; Mohammad et al., 1989).

Based on the above information, it is clear that alfalfa production will be severely affected by an increasing salinity problem throughout the world. The present study was, therefore, conducted to deal with the following objectives:

Major Objective:

Determine whether sublethal levels of NaCl induce changes in alfalfa protein synthesis that affect its growth and performance.

Secondary Objectives:

1. Determine the differences among alfalfa cultivars in response to NaCl stress during germination and post germination growth.
2. Identify salt-tolerant and salt-sensitive cultivars to be used in studies of gene expression.
3. Determine the genetic effects on ion accumulation in alfalfa shoots and roots under NaCl stress.

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CHAPTER II

LITERATURE REVIEW

EFFECT OF NACL ON GROWTH, PHYSIOLOGICAL PROCESSES, AND ION ACCUMULATION IN PLANTS

Extensive research has been devoted to the study of plant responses to salinity and the various responses of salt-tolerant vs. salt-sensitive species (Staples and Toenniessen, 1984). Also, salt tolerance variation between and within crop species has been documented (Greenway and Munns, 1980; Maas, 1986). The literature indicates that, under saline conditions, salt-tolerant genotypes frequently contain lower concentrations of ions in shoots compared to salt-sensitive ones.

No differences in root-Cl contents were observed between salt-tolerant and salt-sensitive soybean [*Glycine max* (L.) Merr.] cultivars but shoot Cl content was prominent only in the salt-sensitive ones (Abel, 1969). Ahmad et al. (1981) reported that a salt-tolerant ecotype of *Agrostis stolonifera* L. accumulated fewer Na and Cl ions than a sensitive one. Greenway (1965) noted that shoot Na and Cl contents were greater in a sensitive barley (*Hordeum vulgare* L.) cultivar than a tolerant one. Ashraf et al. (1986) reported that *Trifolium alexandrinum* L. genotypes, which had been selected for salinity tolerance, showed greater Na accumulation in roots. Walker (1986) observed that storage of Na in roots and

basal stems of *Poncirus trifoliata* L. appeared to be responsible for the low leaf Na concentrations, and thus for its salt tolerance compared to *Citrus reticulata* Blanco. Jacoby (1964) found that storage of Na in root parenchyma cells was less than the limited Na transported into *Phaseolus vulgaris* L. shoots.

McKimmie and Dobrenz (1991) investigated the physiological basis of variability within alfalfa populations in response to salinity stress. They compared two groups of a population of alfalfa seedlings that differ in vigor under 0.7 MPa salinity. They found that shoots of the more vigorous group contained lower concentrations of Na and Cl while roots of the same group contained higher concentration of Na. The vigorous group also contained more total ions ($Na^+ + Cl^+ + K^+$) per plant but fewer total ions on a dry weight basis. They concluded that salt tolerance appeared to be related to the ability of the vigorous plants to maintain lower ion concentrations, particularly in the leaves, and to their greater growth rates, which may have helped to dilute ions.

Two alfalfa populations (HP 32 and M69-33), which were developed by selection for increased growth and N assimilation in nonsaline conditions, produced more forage than the original cultivars when exposed to moderate salt stress after seed germination. The superior growth of HP 32 under salt stress was associated with substantial exclusion of Na and Cl from the leaves (Kapulnik et al., 1989).

Van Steveninck et al. (1982) investigated the distribution of K, Na, and Cl in various tissues of two species of lupine (*Lupinus luteus* L. and *L. angustifolius* L.) under moderate NaCl stress (20 and 50 mM). They reported that *L. luteus* was more tolerant to salt but less efficient in excluding Na and Cl from its aboveground parts than *L. angustifolius*. *L. luteus* showed severe necrosis and leaf abscission when NaCl concentration increased to 100 mM.

Ashraf and Karim (1990) found a considerable amount of intercultivar variability in blackgram (*Vigna mungo* L.) in response to 40 mM NaCl. The nine cultivars/lines used in their study showed significant differences in their leaf area, shoot and root Na content, yield, and yield components under NaCl stress.

Wallace et al. (1948) observed that the addition of Na to alfalfa (cv. Ranger) increased both K and Na contents and that most of the accumulated Na was in the stem and a little in leaf tissue. In contrast, Dow and James (1970) reported the presence of a reciprocal relationship between K and Na concentration in plants, i.e., if one of them increased, the other decreased.

Genetic manipulation of plants to magnify biological tolerance to saline environments is an essential factor in limiting the salinity threat to agriculture in irrigated farming (Stavarek and Rains, 1983). Biological manipulation of plants includes the identification of high salt-tolerant

genotypes and the incorporation of their salt-tolerant traits into crop plants. Thus, identification of specific characteristics related to salt tolerance will provide biological markers useful in selecting salt-tolerant crops.

Avoidance, exclusion, and physiological tolerance are the three possible mechanisms that have been identified among plants growing in saline environments (Stavarek and Rains, 1983). Physiological tolerance, which includes compartmentation and osmotic adjustment utilizing inorganic and organic components, has been found to be the most significant mechanism for dealing with salt. Storey and Wyn Jones (1979) proposed that *Atriplex spongiosa* and *Suaeda monoica* employed glycinebetaine with K salts as a major cytoplasmic osmoticum and Na salt as the vacuolar osmoticum.

Grattan and Maas (1985) examined the relative contribution of scion and rootstock to control leaf P and Cl accumulation under salinity stress in soybean. They included in their study four cultivars that showed different potential to exclude or accumulate leaf P and Cl from saline solutions including $\geq$ 0.25 mM P. They found that soybeans excluded Na from their leaves and that the differences in leaf Mg concentrations were analogous to those for leaf Ca concentrations. Leaf K concentrations were highest in scions on Clark 63 (a cultivar that accumulates P and Cl in leaf when growing in saline solution with high P rootstocks) and lowest in scions on Lee (excludes P and Cl from leaf when growing in

saline solution with high P) and Lee 74 (accumulates P and excludes Cl in saline solution with high P) rootstocks. Phosphorus-sensitive cultivars accumulated more P in their roots in saline solutions than P-tolerant cultivars. Leaf-Cl excluders contained more Cl in their roots than leaf-Cl accumulators. The genotype of scions did not appear to influence root Cl. The leaf-P and Cl accumulation were controlled predominantly by roots, and the ion transport mechanism in the roots that controlled translocation to the leaves differed between elements. Leaf P was apparently controlled by both root uptake and translocation of P. Thus, leaf-P accumulators contained more P in both their leaves and roots than leaf-P excluders. Leaf-Cl, on the other hand, did not appear to be controlled by Cl uptake by the roots as much as by restricted translocation of Cl from the roots to the leaves. Therefore, leaf-Cl excluders contained more Cl in their roots than leaf-Cl accumulators. This effect has been observed by other workers (Lauchli and Wieneke, 1979; Roeb et al., 1983; Grattan and Maas, 1985). Scions from Cl-excluder cultivars maintained lower leaf Cl concentrations than Cl accumulator scions on leaf-Cl accumulator rootstocks. McCaw (1972) concluded that the scion modified leaf Cl concentration.

Seemann and Critchley (1985) studied the effects of salt stress on the growth, ion relations, and photosynthetic performance of *P. vulgaris* (sensitive species to NaCl). They

reported that the relationship between leaf ion concentration and external NaCl concentration is indicative of a Na excluder, with internal Cl concentration increasing linearly (up to 250-300 mM) with external increase in Cl for plants grown at 150 mM external NaCl, and Na showing no considerable increase until a relatively high external Na concentration (75-100 mM). Although Ca and Mg contents showed no appreciable change by changing the external NaCl level, K concentrations in leaves increased linearly with increasing external NaCl. The change in K and Na concentrations provided most of the cations required to balance altered Cl concentrations. A large difference in Cl concentration was found between control and 150 mM NaCl plants for both the chloroplast-cytoplasm and vacuolar contents. Differences between NaCl treatments for other ions were not significant. The high leaf Cl concentration decreased the ambient photosynthetic rate per unit area by approximately 75%. The reduction in photosynthesis rate was associated with a large reduction in stomatal conductance with increasing leaf Cl content. Since concentrations of Cl were essentially equal (250-300 mM) in the chloroplast-cytoplasm and vacuolar compartments, it is evident that the effective compartmentation failed in *P. vulgaris* plants grown at high salt concentrations. These ion concentrations would be enough to inhibit enzyme activity markedly (Greenway and Osmond, 1972; Osmond and Greenway, 1972; Pollard and Wyn Jones, 1979). Finally, these studies

demonstrated that a substantial portion of the reduction in growth could be related to the reduction in carbon allocation to photosynthetic organs relative to roots that accompanied salt stress in *P. vulgaris*.

In halophytes, Na and Cl concentrations are relatively low in the cytoplasm as compared with the vacuole (Flowers et al., 1977; Greenway and Munns, 1980; Robinson et al., 1983; Storey et al., 1983). In glycophytes (most of the crop plants), the sensitivity of many species to salt may be a result of a failure to keep Na and Cl out of the cytoplasm (Flowers et al., 1977; Osmond, 1976). Yeo (1983) estimated the energy costs of ion movement in halophytes and glycophytes. He reported that, in some cases, the glycophyte ion transport is, in fact, more costly than that of the halophyte.

Subbarao et al. (1990) investigated the nature of inheritance of salinity tolerance in pigeonpea [*Cajanus cajan* (L.) Millse.]. They constructed reciprocal crosses between a very salt-sensitive cultivar (ICP 3783) of pigeonpea and *Atylosia albicans* (L.) Mirrel, one of the most salt-tolerant wild relatives of pigeonpea. Their results demonstrated the feasibility of transferring salinity tolerance from *A. albicans* to *C. cajan*. They concluded that the physiological characteristics granting salinity tolerance in *A. albicans* and the F_1 hybrids, which involves Na and Cl retention in the roots and limited translocation to the shoots, high K selectivity, and maintenance of transpiration rate under

saline conditions, are heritable and controlled by a dominant gene or genes.

Lauter and Munns (1987) designed two experiments to determine whether salt stress of *Cicer arietinum* L. (salt-sensitive legume) was associated with water stress or with ion excess. They found that the relative growth rate of stressed plants declined to a steady low value about 10 d after exposure to salt. The decline in growth rate corresponded with changes in shoot Na and Cl concentrations, and not with the changes in shoot water and osmotic potentials that occurred soon after the addition of Na salts. Shoot Na and Cl concentrations increased gradually until the growth rate of the salt exposed plants diverged from the untreated ones near day 10. Shoot dry weight correlated closely with leaf and shoot Na content. Shoot Cl, shoot water potential, or transpiration rate did not correlate well with growth. Data from this study suggest that salt primarily inhibits growth of *C. arietinum* through accumulation of Na in shoots.

Akhavan-Kharazian et al. (1991) reported that in *P. vulgaris*, increasing NaCl level significantly decreased shoot and root dry weight, chlorophyll concentration, and leaf K/Na ratio. Leaf Na, K, Ca, and Mg increased with increasing NaCl concentration.

Subbarao et al. (1990a) found a positive response by salt-tolerant and salt-sensitive pigeonpea genotypes to a decreasing Na/Ca ratio under constant salinity. They reported

that a reduction in Na/Ca ratio in the medium enhanced K uptake, thereby increasing the K/Na ratio in shoots. This ratio was greater in shoots of the tolerant genotype than in the sensitive genotype. Shoot Cl and K increased while Na decreased with decreasing Na/Ca ratio in the medium. Shoot and root Ca contents increased with decreasing Na/Ca ratios in the medium at both 60 and 80 mM salinity levels. The salt-tolerant genotype was more efficient in excluding Na and Cl from and keeping higher K/Na ratios in shoots than the salt-sensitive genotype.

Taleisnik (1989) studied sodium accumulation in the *Pappophorum* genus under saline conditions. The study included *Pappophorum philippianum* (facultative halophytic species) and *Pappophorum pappiferum* (glycophytic species). He observed that at low salinity (50 mM NaCl), *P. Pappiferum* accumulated less Na in shoots compared to *P. philippianum* species; this response was not related to Na uptake. He concluded that recirculation of Na from shoots to roots and increased loss of recently accumulated Na, rather than limitation in uptake or upward transport, could contribute to the lower concentration of Na found in shoots of *P. pappiferum*.

Matsushita and Matoh (1991) investigated the growth, Na content, and kinetics of ^{22}Na uptake and translocation in salt-tolerant reed plants (*Phragmites communis* Trinius) and salt-sensitive rice plants (*Oryza sativa* L. cv. Kinmaze) in salinized nutrient solutions up to 50 mM NaCl. They noticed

that when both plants were grown under the same salt level, Na concentration in shoots was lower in reed plants, whereas that of the roots was quite similar. In both species, the shoot base region accumulated more Na than the leaf blades. The results of this study suggested that the lower Na transport rate from root to shoot might limit excessive Na accumulation in the reed shoot and that the lower transport rate of Na to the shoot of reed plants was possibly due to a net downward Na transport from shoot base to root.

Francois et al. (1989) investigated the response of rye to saline conditions. In a two-year field experiment, they found that rye was unaffected up to 114 mM soil salinity and each unit increase in salt above that level reduced yield by 10.8%. This response places rye in the salt-tolerant category. The two cultivars included in the study showed slightly less salt tolerance during plant emergence than during the following growth stages. Salinity reduced plant height, grain yield, and straw yield. Sodium and Cl concentrations increased moderately in leaf tissues with increasing soil salinity. Calcium concentration increased slightly in one year but decreased slightly in the other year of the study. Potassium was increased moderately with increasing salinity. Rye plants showed Na exclusion from leaves. Also, there was considerable linear reduction in leaf Mg contents with soil salinity level, while phosphorus contents decreased slightly with increasing salinity.

Jeschke et al. (1992) investigated the effects of NaCl on phloem and xylem flows of C, N, K, Na, and Cl and on their partitioning in the whole plants of white lupine (*Lupinus albus* L.). The K uptake and transport in both xylem and phloem was severely inhibited under saline conditions. The uptake and flow of Na and Cl were substantially increased in the presence of salt. Shoot Na and Cl contents were high in salt treated plants. The contents of K, Mg, and Ca, specially K and Mg in leaflets, were decreased by the presence of salt in the medium. This indicates the replacement of K, Mg, and to a lesser extent Ca by Na, although not in their function. Sodium chloride has relatively little effect on Ca leaf content. The results of this study suggested that the restriction of K supply, by diminished K uptake and reduced K cycling within the plant, and the strongly increased Na translocation in phloem were contributing to the unfavorable effects of NaCl in white lupin.

Wolf et al. (1991) investigated the distribution of Na and K within barley grown in the presence of 100 mM NaCl with special emphasis on the role of stems for partitioning of nutrients in shoots. Concentration of K in leaves increased from older to younger leaves while those of Na decreased remarkably. All organs showed a net increase in Na during the study period. There was a massive accumulation of Na and a considerable loss of K in lower leaves and lower Na accumulation and loss of K in the basal internodes. The

results showed the ability of barley roots to limit Na entry to the shoot and maintain the supply of K; moreover, recirculation contributes more than 50% to the amount of K transported from root to shoot and the stem has an apparent role in reducing the flow of Na and elevating K transport to young leaves.

Huang and Van Steveninck (1989) studied the vacuolar localization of Na and Cl in the different cell types of the blade and sheath of two barley cultivars (California Mariout and Clipper) after exposure of seedlings to different levels of salt solution. The mesophyll cells of the blade maintain a low concentration of Cl after exposure to 50 or 100 mM NaCl for 1 d or to 50 mM for 4 d, whereas Cl concentration in the epidermis and mesophyll of the sheath increased dramatically. The distinctive accumulation of Cl in the sheath would diminish the effect of salinity on photosynthesis in the leaf blades. The high Cl concentration in the blade mesophyll cells of Clipper suggests that the lower resistance of this cultivar to salt is directly related to the degree of Cl exclusion by these cells. Results of the study suggested that barley plants are able to regulate ion balance in leaves to a certain degree by partitioning ions into different plant parts and into different cell types in these parts.

To determine whether Na or Cl is more toxic to wheat, two selected lines of bread wheat (*Triticum aestivum* L.) were included in a comparative experiment (Kingsbury and Epstein,

1986). Wheat plants were grown in isosmotic solutions of different ionic compositions (Na-Hoagland, Hoagland-Cl, NaCl, and Seawater). Neither Na nor Cl had any significant effect on the resistant line, whereas Na had a significant negative effect on the sensitive line. The chemical analysis showed high Cl contents in the resistant line growing in Hoagland-Cl solution, high Na contents in the sensitive line growing in Na-Hoagland solution, and low Ca:Mg ratios in both lines in seawater. The investigators proposed that the differential effects of salinity on growth rates and photosynthesis might be related to specific ion toxicities.

Abdul Karim et al. (1992) reported that in hexaploid triticale, the salt-sensitive cultivar Welsh accumulated more Na and Cl in shoots compared to the salt-tolerant cultivar, Currency, when both were exposed to 25 and 50 mM NaCl stress. Under salt conditions, K, Ca, and Mg did not show the same accumulation trend as Na and Cl in the different parts of the plant in either one of the cultivars. Therefore, salt tolerance between the two cultivars is related to differences in Na and Cl accumulation in shoots of these cultivars. Results of this study supported the idea that the regulation of Na and Cl ions transported to the shoot is located in the roots, and that roots of salt-tolerant cultivars have higher regulating capacity than the salt-sensitive ones.

Low Na and high K concentration in leaves as a characteristic of the enhanced K/Na discrimination character

was recorded in *Aegilops squarrosa*, other *Aegilops* species containing the D genome, hexaploid wheat, diploid wheat species, GGAA genome tetraploid wheats, rye, and triticale (Gorham, 1990a, 1990b, and 1990c). Analysis of amphiploid hybrids suggested that this character was dominant in crosses including *A. squarrosa* or *Aegilops umbellulata* with other species lacking this trait. Bar-Tal et al. (1991) reported that the increase in K contents and a drop in the Na/K ratio in plant tissue by adding potassium fertilization did not eliminate the deleterious effects of salinity on corn (*Zea mays* L.) yield.

Fidgore et al. (1989) found no relationship between Na substitution capacity of five tomato [*Lycopersicon esculentum* (L.) Merr.] cultivars, selected as differing in K efficiency and Na substitution capacity, grown under low-K stress and their tolerance to toxic Na levels under adequate K conditions. Differences in dry weight among strains grown under low-K stress were due primarily to differences in the added Na rather than in the added Cl. Heritable differences in the ability to substitute Na for K have been reported in tomatoes (Figdore et al., 1989; Makmur et al., 1978), sugarbeets (Marschner et al., 1981) and in pasture species (Mundy, 1983) grown under low-K stress.

Sodium has been postulated to substitute for K in a non-specific function in which K is involved such as in the replacement of vacuolar K (Marschner, 1971). More Na

substitution is likely to occur in plants that allow more Na to be translocated to and accumulated in the shoots (Flowers and Lauchli, 1983).

Chow et al. (1990) reported that excessive K was required for spinach shoot growth under high rather than low salinity conditions, and that the elevated concentration of Na in the leaves may help to maintain turgor, but it cannot substitute for adequate K levels in the leaves, presumably because K is particularly required for protein synthesis.

Cramer and Arthur (1986) found no significant differences in Na concentrations in shoots and roots between two lettuce (*Lactuca sativa* L.) cultivars that differed in their tolerance to increasing NaCl concentrations. Chloride was about twice the concentration of Na in both of the cultivars. No significant differences in K, Ca, and Mg of shoots or roots were found between cultivars. The lack of significant differences in ion concentration between shoots of the two cultivars indicate that, at least, in the shoot, the differences in salt tolerance were unlikely due to a difference in ion toxicity. Roots of the cultivar, Climax, showed a greater Cl concentration in roots and greater shoot growth at 40 mM NaCl than observed in the cultivar Calmar. It seems that the higher Cl concentration in the root of Climax at this level may have helped in maintaining its normal root water status, which was obvious from the unchanged fresh

weight/dry weight ratio. The reduced water content in roots of Calmar indicated that the root was under water stress.

Marschner et al. (1981) found considerable genotypic variations in salt tolerance of sugar beet (*Beta vulgaris* L.) and reported a positive correlation between salt tolerance and accumulation of Na and Cl in shoots. The increasing NaCl supply decreased K and Ca and increased the accumulation of Na and Cl steeply, especially in shoots.

ALTERATION OF GENE EXPRESSION BY SALT STRESS

Studies of the effects of environmental stress conditions, such as salt, flooding, high temperature, and intense UV light, on plants showed that plants responded to the stress via altering their gene expression by synthesizing novel proteins and repressing at least some of the normally expressed ones. The investigation of the stress-induced proteins and the genes encoding them may lead to the engineering of crop plants more resistant to normally encountered stress conditions. The latter task is conceivable with the massive advancement in plant transformation techniques (Sachs and Ho, 1986).

Ramagopal (1986) exposed maize callus to media containing different levels of NaCl (0 to 3%) and mannitol (0 to 18.2%) for a 4-week period to study their affect on protein synthesis. He found that, in general, the overall mean inhibition of cellular uptake of amino acids and protein

synthesis in the first 2-week period was approximately 50% for 1% NaCl. Three new proteins (74, 28.5, and 26.2 kDa) were induced *de novo* under both NaCl and mannitol treatments whereas four other proteins (39.5, 39.0, 30.0, and 16.5 kDa) showed increasing or decreasing level of synthesis. NaCl above 0.5% did not alter the pattern of newly synthesized proteins and all the alteration in the expression of the newly made proteins in the maize callus occurred only after a week of exposure to salinity or osmotic stress. Altered protein expression coincided with the cell growth phase.

Ramagopal (1987a) investigated the variation in gene expression during NaCl stress in barley. He indicated that NaCl induces distinct protein changes in root and shoot tissues of California Mariout (salt-tolerant) and Prato (salt-sensitive) barley cultivars. Both cultivars are reported to be biochemically quite homogeneous in terms of their protein patterns and mRNAs. Roots of both cultivars show identical responses to salinity. Sodium chloride modulated (elevated or depressed) the synthesis of some proteins and induced the synthesis of new proteins in roots of both cultivars. Six new proteins (24 to 27 kDa) were induced by NaCl in roots. Although NaCl inhibited the synthesis of the majority of proteins in shoots, it induced the synthesis of cultivar specific proteins. Five new proteins of molecular weights between 20 to 24 Kd were induced by NaCl in shoots, three of which were unique to the salt-sensitive Prato. In general, the

new proteins induced in shoots and roots were unique to each tissue and their induction appeared to be regulated coordinately during exposure to NaCl stress.

Ramagopal (1988) reported that approximately 8% of the total proteins expressed in barley seedlings was regulated quantitatively by salinity (NaCl). These quantitative changes were selective and salinity left over 90% of the proteins unaffected. Also, salinity affects the qualitative expression of a small fraction, about 1% of the total, of newly synthesized proteins in seedlings. The proteins of this category were specifically induced only under salinity stress and were detected in both shoots and roots of barley seedlings.

Hashim and Campbell (1988) studied the induced proteins in several legume seedlings. They reported that distinct low molecular weight proteins unique to root and shoot tissues of alfalfa seedlings were induced by NaCl stress. The induction of the new proteins appeared to be regulated during salinity stress.

Chen and Tabaeizadeh (1992) investigated the effect of drought and NaCl stress on polypeptide levels in roots and shoots of tomato plant seedlings using two-dimensional polyacrylamide gel electrophoresis. They found that in roots, both drought and salt treatments caused high accumulation of two proteins, whereas only the NaCl treatment induced stress-specific proteins in roots. Leaves of NaCl-treated plants did

not synthesize any salt-stress specific polypeptides, whereas drought stress resulted in considerable changes in leaf protein pattern. The results suggested that the quantitative and qualitative changes in protein synthesis may contribute to stress-resistant or stress-injury mechanisms in tomato plants.

Ostrem et al. (1987) reported that *Mesembryanthemum crystallinum* L. plants responded to salt stress [by switching from C_3 photosynthesis to Crassulacean acid metabolism (CAM)]. The switching occurred by increasing the level of phosphoenolpyruvate carboxylase (PEPCase) protein in leaf and shoot tissues via a stress-induced increase in the steady-state level of translatable mRNA for this enzyme. The mRNA amounts for PEPCase and for other CAM enzymes, such as pyruvate orthophosphate dikinase, increased between day 2 and 3 after stress induction (Michalowski et al., 1989).

Claes et al. (1990) demonstrated that, in rice (*Oryza sativa* L.), the pattern of Na accumulation matches the expression pattern of *salT*, a characterized rice gene showing organ-specific expression in response to salt stress and drought. After exposure to salt, the mRNA levels for this gene were highest in the sheath and lowest in the leaf lamina. The rice sheath acquired 4 times more Na than the leaf lamina (Yeo and Flowers, 1982).

Hurkman and Tanaka (1987) investigated the effects of NaCl on the *in vivo* protein synthesis in barley roots (cv. California Mariout 72) using two-dimensional polyacrylamide

gel electrophoresis. Although NaCl treatment altered the relative amounts of a number of *in vivo* synthesized proteins, it did not induce noticeable qualitative differences in the protein pattern of roots. Quantitative changes occurred in a heterogeneous group of proteins having a wide range of molecular weights and pIs. Of the abundant changes, the amounts of 4 polypeptides with pIs of 6.3 and 6.5 and mol wt of 26 and 27 kDa increased significantly in response to NaCl treatments. The changes in net protein synthesis induced by NaCl may be related to the varying degrees of inhibition or stimulation of mRNA translation by increased cytoplasmic NaCl contents (Hurkman and Tanaka, 1987).

By assaying the poly(A)$^+$ RNA in an *in vitro* translation system, Hurkman et al. (1989) showed that the levels of translatable mRNAs in barley roots (cv. CM 72 and Prato) changed with NaCl treatments and that these changes were associated with the changes in protein synthesis under salt conditions.

Gibson et al. (1984) reported that, in an *in vitro* translation experiment of mRNAs isolated from salt-tolerant and salt-sensitive plants, protein synthesis was inhibited by substitutions of Na for K or of Cl for acetate at concentrations above 80 mM.

To determine if salt-induced changes in barley were time-dependent, Robinson et al. (1990) examined the effect of salt on the levels of polypeptides and translatable mRNAs following

exposure of roots of seedlings to a series of short-term and a long-term NaCl treatment. They illustrated that salt appears to affect gene expression quantitatively, qualitatively, and temporally at the levels of transcription and translation. Since most of the changes in both polypeptides and translation products seen at 24 h are also seen at 6 days of salt stress, it is possible that these changes enabled barley to adapt to saline conditions.

Hurkman et al. (1991) isolated and identified the 26 kDa, isoelectric point 6.3 (Gs1), and 6.5 (Gs2) polypeptides that increase in barley roots during exposure to salt stress. Antibodies were raised against the purified polypeptides and used to probe blots of polyacrylamide gels to determine their distribution among barley organs and cell-free fractions. The results showed that Gs1 and Gs2 are glycoproteins that are protease resistant. Both proteins were present in roots and coleoptiles but absent in leaves and they increased in roots and decreased in coleoptiles in response to NaCl stress.

McCue and Hanson (1992) reported that exposure of sugar beets to 500 mM NaCl level increased the betaine aldehyde dehydrogenase (BADH) activity in the expanded leaves approximately 3-fold. The increase in the activity was associated with an increase in BADH mRNA, which was illustrated by the immunoprecipitation of the *in vitro* translation products, using antibodies raised against spinach BADH enzyme.

Sadka et al. (1991) reported a 150 kDa protein (p150) as a salt-induced protein required for proliferation of green alga (*Dunaliella salina*) in saline media. The p150 is found to be a detergent-soluble glycoprotein located at the *D. salina* cell surface.

To investigate the effects of NaCl on gene expression at the transcriptional level, Ramagopal (1987b) exposed the seedling of a salt-tolerant (California Mariout) and a salt-sensitive (Prato) genotype of barley to NaCl stress (2% NaCl for 18 h). Root and shoot translatable mRNAs were isolated from tissues and *in vitro* translated in a reticulocyte cell-free system. Results of the *in vitro* translated products on two-dimensional polyacrylamide gels showed that, quantitatively, the functional mRNAs in unstressed seedlings were almost indistinguishable in the two genotypes. During exposure to salinity stress, NaCl triggered the transcription of different specific mRNAs, which depend upon genotype and tissue. Twelve new mRNAs in roots and nine new mRNAs in shoots were induced by NaCl stress. These induced mRNAs encoded proteins of 21 to 34 kDa in roots and 18 to 50.5 kDa in shoots. Although few of the new mRNAs were specific to each genotype, the salt-tolerant genotype preferentially accumulated unique mRNAs in roots, whereas the unique mRNAs of shoots were accumulated in the salt-sensitive genotype. The researcher suggested that both transcriptional and post-

transcriptional mechanisms regulate gene expression in barley grown under salinity stress conditions.

Gulick and Dvorak (1985) investigated the molecular basis of high salt tolerance in the artificial amphiploid *T. aestivum* cv. Chinese Spring (salt-sensitive) X *Elytrigia elongata* (salt-tolerant). They isolated the poly(A^+) mRNAs from roots of amphiploid plants grown in nutrient solution without salt and three days after progressively increased salinity to 250 mM NaCl. The *in vitro* translation of the mRNA populations showed that, at least, four major proteins were induced in roots by exposure to NaCl and these proteins were absent in the nontreated control. They suggested that *E. elongata* salt tolerance depended upon the expression of genes located on several *Elytrigia* chromosomes. They hypothesized that the genetic system controlling salt tolerance in this plant is induced by salt stress.

Gulick and Dvorak (1987) studied the effect of NaCl on mRNAs species in salt-sensitive bread wheat (Chinese Spring) and in the artificial amphiploid Chinese Spring X *E. elongata* (highly tolerant to salinity). They compared the poly(A^+) RNA populations isolated from roots, expanding leaves, and old leaves containing each of the two genomes prior to and after exposure to high levels of NaCl in solution cultures. The *in vitro* translation results, using two-dimensional gel electrophoresis, showed that 10 mRNA species induced or enhanced and eight mRNA species repressed in the amphiploid

root tissue during exposure to NaCl. The 18 mRNAs, which were affected by NaCl, were detected also in wheat roots but only four of them were similarly regulated. NaCl stress causes marked changes in the expression level of 34 mRNAs in Chinese Spring wheat roots. Twenty-six of them were detected in the amphiploid, but only six of these were regulated as in the amphiploid. Leaves, meristematic crowns, and unexpanded leaves of the amphiploid showed no differences in gene expression by exposure to NaCl.

Winicov et al. (1989) used the *in vitro* translation technique to compare mRNA populations isolated from the salt-tolerant HG2-N1 and parent salt-sensitive HG2 diploid alfalfa cell lines grown in the presence and absence of 171 mM NaCl. They found an increase and a decrease in specific polypeptides in HG2-N1 as compared to HG2. Many of the enhanced polypeptides in the salt-tolerant HG2-N1 cell line were constitutively expressed (with and without the presence of salt), while the expression of few polypeptides was dependent on the presence of NaCl.

Winicov and Seemann (1990) studied the bases for salt tolerance of the diploid alfalfa cell line (HG2-N1) derived by selection from a salt sensitive line (HG2). The salt tolerant cell line showed an 11-fold increased chlorophyll content over that of the salt-sensitive parent cell line with a further two-fold increase in chlorophyll accumulation when cells were grown in 1% NaCl. The chlorophyll accumulation and response to

salt were associated with large increases in the two photosynthetic mRNAs, *rbcL* [ribulose-1,5-bisphosphate carboxylase (Rubisco) large subunit] and *rbcS* (Rubisco small subunit) and substantial increases in the activity of the holoenzyme. The increase in Rubisco activity demonstrates that the accumulated rbcL and rbcS mRNAs were translated into Rubisco's polypeptides (large and small subunits) and then assembled into functional enzyme. The results implied that the salt-induced increase in mRNA and protein buildup involved in photosynthesis may play a significant role in the salt tolerant competence of HG2-N1 alfalfa cells.

Narasimhan et al. (1991) isolated a cDNA clone encoding the 70 kDa subunit of tobacco (*Nicotiana tabacum* cv. Wisconsin) tonoplast ATPase. Using a labeled probe produced from this clone, they studied the gene encoding the subunit. They reported that the gene was expressed constitutively throughout the growth cycle in the unadapted and the 428 mM NaCl adapted cells. The levels of its mRNA are regulated, moderately, by NaCl and this regulation occurs during the initial period of response to salt but is not evident after cell adaptation to or growth in the saline environment. It is not clear if transcription or stability of the tonoplast ATPase message is enhanced by NaCl. Four genes encoding the 70 kDa subunit were detected in this study.

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CHAPTER III

RESPONSE OF ALFALFA CULTIVARS TO NACL DURING GERMINATION AND POST-GERMINATION GROWTH

ABSTRACT

During postgermination growth, alfalfa (*Medicago sativa* L.) cultivars may exhibit differential salinity tolerance from that expressed during germination. To explore this possibility, 86 cultivars and experimental populations of alfalfa were exposed to 9 levels of NaCl ranging from 0 to 342.2 mM during germination. Cultivars that exhibited the highest (14 cultivars) and the lowest (8 cultivars) NaCl tolerance from the germination study were exposed to 0, 88, and 132 mM NaCl after the completion of germination under 0 NaCl to determine postgermination NaCl tolerance. There were no significant correlations between percent germination of these cultivars at 88 or 132 mM NaCl and their subsequent growth parameters. The IC_{50} (NaCl concentration that inhibited germination of 50% of the viable seeds) of the cultivars was negatively correlated only with shoot:root ratio when the plants were exposed to 132 mM NaCl after germination. Post-germination performance of cultivars that needed high NaCl concentration to inhibit 50% of their seed germination (high IC_{50} group) was not significantly different from the low IC_{50} group under 88 or 132 mM NaCl treatments. This study showed that the ability of alfalfa cultivars to germinate at high

NaCl concentrations was not related to their postgermination performance under salt stress, except for the negative correlation between IC_{50} and shoot:root ratio at 132 mM.

INTRODUCTION

Salinity threatens irrigated agriculture in many semiarid and arid regions of the world (Epstein, 1980; Norlyn, 1980; Staples and Teonnissen, 1984). Alfalfa is considered moderately sensitive to salinity (Maas and Hoffman, 1977) and is a particularly important crop in many of these regions. It can symbiotically fix N and extract water from the soil profile when the surface soil layers are dry (Hanson et al., 1988). Considerable variation in salt tolerance appears among and within alfalfa populations (Allen et al., 1985; Mohammad et al., 1989; Rumbaugh and Pendery, 1990). Many researchers have focused on alfalfa germination under salt conditions (Allen et al., 1985; Dobrenz et al., 1983, 1989). Salt tolerance at germination, however, appears not to be related to tolerance during postgermination growth in limited populations selected for their high germination under salt conditions (Allen, 1984; Johnson, 1991). Our experiments were conducted to study the NaCl tolerance of 86 alfalfa cultivars and experimental populations during germination and its relationship to postgermination growth parameters in 22 of these cultivars.

MATERIALS AND METHODS

Seventy cultivars and 16 other populations, hereafter referred to as cultivars of alfalfa (*Medicago sativa* L.) were exposed to 0, 42.3, 85.6, 128.3, 171.1, 213.9, 256.7, 299.4, or 342.2 mM NaCl during germination using standard test procedures (Rumbaugh, 1991). A single sample of seed of each cultivar was obtained from the originating company or public agency. It is not known whether the environment during seed development affects the ability of the seeds to germinate in saline solutions. Two replications of 25 scarified seeds of each cultivar were placed in 100 mm disposable petri dishes containing a single Whatman No. 2 filter paper and 4.5 mL of the NaCl solution. IC_{50} values were determined for each cultivar by probit analysis (SAS, 1987) of the germination percentages. The IC_{50} value is the NaCl concentration in mM that inhibits germination of 50% of the viable seeds for a particular cultivar (Rumbaugh and Pendery, 1990).

Twenty-two alfalfa cultivars, 14 with the highest IC_{50} values (203-233 mM NaCl) and 8 with the lowest IC_{50} values (76-160 mM NaCl), were further tested for NaCl tolerance. Plastic containers, 164 cm^3 (Stuewe and Sons, Inc. Corvallis, Oregon 97333), were filled with 150 cm^3 coarse sterilized sand (Silica #20). Five seeds of each cultivar were planted in each container and irrigated with tap water for 14 d until emergence was complete; plants were then thinned to one per

container and inoculated with *Rhizobium meliloti* (USDA strain #1031) diluted in 0.25-strength Hoagland's (Hoagland and Arnon, 1938) solution (1 L of rhizobial culture to 10 L of 0.25-strength Hoagland's solution). Plants were inoculated three times at 7 d intervals. Plants were irrigated thereafter with 0.25-strength Hoagland's solution daily.

When the plants were 49 d old, 36 containers with plants of a uniform size (height) were chosen from each cultivar and divided randomly into nine groups of four containers (plants) each. Each group was considered one experimental unit. Each day, 25 mL of the NaCl treatments of 0, 88 and 132 mM in 0.25-strength Hoagland's solution were applied to each container. The 198 experimental units were arranged in a 3 x 22 factorial (3 NaCl treatments x 22 alfalfa cultivars) in a completely randomized design with 3 replications on a greenhouse bench. Ambient temperature in the greenhouse was 25/20 °C with supplemental light 500 W m^{-2} 16/8 h day/night regime.

Twenty-eight days after NaCl irrigation began, plant heights (3 cm above the soil surface) and number of stems per plant were recorded. Plants were harvested (3 cm above the soil surface) and dried at 70 °C for 48 h to determine shoot dry weight. Dry weight per plant was determined by dividing the total weight by the number of plants in the experimental unit. The second and third harvests were made at 28 d intervals after the first harvest. Dry weight shoot:root (S:R) ratios were determined in the first and third harvest by

randomly selecting one of the harvested plants, from each replication, and adding crown dry weights to the average shoot dry weights determined previously. Root dry weights of the selected plants were determined after washing the roots with tap water for one minute, blotting them with paper-towels, and drying them at 70 °C for 48 h. Data from the three harvests were combined for plant height, number of stems per plant, and dry weights. Since no root samples were collected at the second harvest, data from the first and third harvests were combined to calculate root dry weights and S:R ratios. All data were examined by analysis of variance and correlation procedures (Snedecor and Cochran, 1980).

RESULTS

Seeds of most cultivars germinated well in the lower concentrations of NaCl. Germination in the 85.6 mM NaCl solution averaged 90%. There were highly significant ($p \leq 0.01$) differences in germination among cultivars at NaCl concentrations of 128.3 mM and higher (data not shown). The IC_{50} values ranged from 76 mM NaCl for Centurion, the most sensitive cultivar, to 241 mM NaCl for Condor, the most resistant cultivar.

All alfalfa plants exposed to 88 or 132 mM NaCl were significantly ($P \leq 0.05$) shorter than control (no NaCl) plants (Table 1). Height of six cultivars (WL 516, Crown II, 8737N, Husky, Regen-S, and WL SS) was significantly decreased at 132

Table 1. Effect of NaCl stress on seed germination and growth parameters of 22 alfalfa cultivars.

Cultivar	IC_{50}†	NaCl treat.	Seed germ.	Plant height	Stem plant^{-1}	Dry wt.		
						S	R	S:R‡
	mM		%	cm	no.	g		
CONDOR	241 (66)	0	96	27.32	4.85	1.11	1.49	1.17
		88	94	15.10	4.13	0.45	0.97	0.71
		132	90	15.17	3.70	0.38	1.22	0.57
5364	233 (40)	0	100	24.12	4.63	1.02	1.55	0.87
		88	96	14.22	4.38	0.42	1.02	0.73
		132	94	16.12	3.06	0.35	0.82	0.68
8638C	233 (29)	0	90	24.07	4.88	1.05	1.92	0.81
		88	90	13.98	3.82	0.38	1.03	0.56
		132	80	12.34	2.65	0.26	0.80	0.53
5331	228 (47)	0	100	26.08	5.47	1.16	1.73	0.95
		88	90	12.18	3.61	0.31	0.82	0.49
		132	92	11.57	3.28	0.28	0.54	0.93
WL 516	228 (32)	0	96	24.06	5.12	1.00	1.73	0.84
		88	100	18.91	3.79	0.50	1.04	0.75
		132	86	14.89	3.38	0.34	0.79	0.64
CROWN II	226 (66)	0	90	23.39	4.58	1.17	1.52	0.80
		88	88	15.42	3.60	0.43	1.08	0.82
		132	92	11.27	3.35	0.28	0.64	0.84
SABRE	223 (55)	0	94	24.64	4.47	0.95	1.69	0.91
		88	92	16.20	3.98	0.43	0.97	0.64
		132	76	16.17	3.23	0.37	0.93	0.66
531	223 (47)	0	84	21.94	4.83	0.91	1.90	0.68
		88	76	16.30	3.63	0.45	1.08	0.62
		132	82	13.76	3.27	0.33	0.87	0.62
8737N	221 (47)	0	98	24.63	4.46	1.00	1.58	0.71
		88	92	15.05	4.25	0.42	1.20	0.59

Table 1 continued.

Cultivar	IC_{50}†	NaCl treat.	Seed germ.	Plant height	Stem plant^{-1}	Dry wt.		
						S	R	S:R‡
	mM		%	cm	no.	g		
		132	86	12.31	3.87	0.28	0.70	0.63
HUSKY	221 (10)	0	88	25.07	4.52	0.97	1.61	0.94
		88	90	14.65	3.95	0.38	1.10	0.55
		132	86	11.93	3.31	0.31	0.84	0.72
5929	218 (62)	0	86	19.33	5.40	0.82	1.36	0.89
		88	90	16.81	4.09	0.50	0.97	0.76
		132	68	14.98	3.91	0.35	0.91	0.67
86108	218 (36)	0	100	25.17	4.42	1.00	1.68	0.82
		88	96	13.65	4.06	0.39	0.99	0.57
		132	80	14.22	3.37	0.36	1.04	0.55
REGEN-S	213 (21)	0	90	21.45	4.13	0.84	1.50	0.83
		88	82	14.54	3.47	0.40	0.85	0.67
		132	88	10.02	3.30	0.22	0.49	0.86
WL SS	203 (43)	0	94	23.81	5.44	1.02	1.46	0.94
		88	90	17.09	4.06	0.53	0.98	0.84
		132	71	13.24	3.50	0.38	0.79	0.71
526	160 (17)	0	88	20.98	5.65	0.83	1.35	0.90
		88	80	13.11	4.56	0.35	0.86	0.62
		132	66	11.11	4.15	0.26	0.70	0.72
MECCA	158 (70)	0	80	24.48	3.90	0.91	1.71	0.72
		88	60	13.33	2.81	0.32	0.75	0.92
		132	44	14.30	3.48	0.28	0.82	0.57
ROAMER	152 (36)	0	96	23.06	5.61	1.05	1.84	0.78
		88	64	15.83	5.03	0.49	1.01	0.77
		132	59	13.54	4.12	0.34	0.68	1.01
REGEN-Y	150 (6)	0	94	22.91	4.04	0.82	1.09	1.07
		88	78	15.32	3.90	0.38	0.88	0.72

Table 1 continued.

Cultivar	IC_{50}†	NaCl treat.	Seed germ.	Plant height	Stem plant^{-1}	Dry wt.		
						S	R	S:R‡
	mM		%	cm	no.	g		
RAMBLER	147 (17)	132	76	14.64	3.20	0.37	0.72	0.80
		0	98	22.11	6.08	0.93	1.71	0.74
		88	79	15.32	5.23	0.43	0.88	0.81
3713	137 (6)	132	70	13.46	4.39	0.38	0.80	0.77
		0	98	21.83	5.17	0.85	1.50	1.15
		88	82	13.06	3.75	0.32	0.52	0.76
SPREDOR 2	122 (32)	132	60	12.15	2.62	0.27	0.40	0.95
		0	98	22.22	5.96	0.98	1.45	0.91
		88	55	11.78	5.02	0.31	0.90	0.59
CENTURION	76 (5)	132	51	12.77	3.86	0.44	0.77	0.94
		0	44	26.98	4.79	1.06	1.59	0.97
		88	12	16.69	3.22	0.40	1.07	0.60
LSD (0.05)	---	132	6	14.20	3.46	0.30	0.70	0.70
(0.01)	---	---	---	NS	2.66	0.77	0.14	NS
		---	---	NS	3.49	1.01	0.18	NS

† concentration of NaCl inhibiting germination of 50% of viable seeds in initial experiment. Numbers in parentheses represent the standard error.
‡ S = shoot; R = root; S:R = Shoot:root ratio = (shoot + crown) dry wt./root dry wt.

mM compared with 88 mM NaCl treatment. The height of cultivar 5331 was reduced most by NaCl while that of cultivar 5929 was least affected.

Compared with controls, the 88 mM NaCl significantly reduced the number of stems per plant in 12 cultivars (8638C, 5331, WL 516, Crown II, 531, WL SS, 526, Mecca, Rambler, 3713, Spredor 2, and Centurion) while the 132 mM treatment significantly reduced the number of stems in all except one cultivar (Mecca) (Table 1). The 132 mM NaCl treatment significantly decreased the stem number of seven cultivars (5364, 8638C, 8737N, Roamer, Rambler, 3713, and Spredor 2) compared with the 88 mM treatment. Cultivar 5331 appeared to be the most sensitive to NaCl in terms of number of stems per plant, while 8737N exhibited the least effect of NaCl on stem number. Compared to controls, both NaCl treatments significantly decreased shoot dry weight of all cultivars (Table 1). Compared with 88 mM NaCl treatment, the 132 mM NaCl treatment induced a significant decrease of shoot dry weight in seven cultivars (5929, WL SS, WL 516, 8737N, Crown II, Regen-S, and Roamer). Although Spredor 2 was the only cultivar whose shoot dry weight increased at 132 mM compared with the 88 mM NaCl treatment, the increase, however, was insignificant. Both NaCl treatments did not significantly affect the root dry weight of any cultivar (Table 1).

Compared to nonsaline controls, 88 mM NaCl treatment significantly decreased the S:R ratio of eight cultivars

(5331, 526, Husky, Centurion, Regen-Y, Condor, Spredor 2, and 3713) (Table 1). The 132 mM NaCl significantly decreased the S:R ratio of 8638C and Condor compared to controls. Compared to 88 mM, the 132 mM NaCl treatment significantly increased the S:R ratio in 5331 and Spredor 2 and decreased it in Mecca.

No significant differences were found between the high IC_{50} group (14 cultivars) and the low IC_{50} group (8 cultivars) in any of the growth parameters included in this study (data not shown). There was a significant negative correlation between S:R ratio at 132 mM NaCl and IC_{50} (Table 2).

DISCUSSION

Although NaCl reduced shoot growth of all cultivars, the magnitude of the reduction varied among them. Considerable variation in salt tolerance among plant populations and cultivars of alfalfa has been reported by others (Robinson et al., 1986; Kapulnik et al., 1989; Mohammad et al., 1989).

While plant height was decreased significantly in all cultivars by both NaCl treatments compared to controls, the height of 16 cultivars did not change significantly at 132 mM compared to the 88 mM NaCl treatment. Among these cultivars were Condor, 5364, 8638C, and 5331 exhibiting high salt tolerance during germination (IC_{50} 203-241 mM) and all those with IC_{50} 76-160 mM. These results indicated that plant height was independent of salt tolerance during seed germination. The lack of correlation between plant height and IC_{50} values and

Table 2. Correlation coefficients (r) for 22 alfalfa cultivars between IC_{50}, seed germination percentages at 88, 132 mM NaCl and plant height, stems per plant, shoot and root dry weight, and shoot:root ratio.

	Plant height	Stem plant^{-1}	Dry wt. Shoot	Dry wt. Root	S:R†
			88 mM NaCl		
IC_{50}‡	0.17	-0.13	0.35	0.38	-0.15
Germ. at 88 mM NaCl	0.05	0.14	0.26	0.07	-0.00
Germ. at 132 mM NaCl	-0.00	0.11	0.20	0.21	-0.13
			132 mM NaCl		
IC_{50}	0.06	-0.26	-0.10	0.35	-0.42*
Germ. at 88 mM NaCl	0.02	-0.19	0.01	0.22	-0.23
Germ. at 132 mM NaCl	-0.12	-0.20	-0.06	0.14	-0.11

* Significant at the 0.05 level of probability.
† Dry weight shoot:root ratio.
‡ Concentration of NaCl (mM) inhibiting germination of 50% of the viable seeds. Germ = seed germination.

germination at 88 or 132 mM NaCl supported this conclusion(Table 2). Mohammad et al. (1989) reported that young alfalfa seedlings became shorter as NaCl levels increased. Our data showed the same response at later alfalfa growth stages under 88 mM NaCl level, but at the 132 mM NaCl level, six cultivars showed no further decrease in their height. The latter result may be related to an increased salt tolerance at later growth stages.

The cultivars that showed significant decreases in number of stems per plant at 88 and/or 132 mM NaCl treatment compared to controls belonged to both high and low IC_{50} groups. These results indicated that the branching ability trait in alfalfa cultivars growing under NaCl stress was independent from their NaCl tolerance during germination. The correlation coefficients in Table 2 supported this conclusion. Kapulnik et al. (1989) and Noble et al. (1984) reported that stem production in alfalfa decreased as salinity increased and that salt-tolerant plants had more stems than salt-sensitive plants as salinity increased. Our data indicated that the effect of NaCl stress on branching in alfalfa plants was influenced by both NaCl level and cultivar.

Although shoot dry weight of all cultivars decreased significantly at both NaCl treatments, the degree of reduction varied between cultivars at each salt level. These results were consistent with the results of others (Keck et al., 1984; Mohammad et al., 1989; Kapulnik et al., 1989). Except for the

cultivar Roamer, the low IC_{50} group of cultivars (IC_{50} 76-160) produced the same shoot dry weight at 88 and 132 mM NaCl treatments, while six cultivars of the high IC_{50} group showed a significant decrease in shoot dry weight at 132 compared to 88 mM treatment. These results indicated that shoot dry weight production in alfalfa is not dependent on the germination ability under NaCl conditions.

The lack of significant differences in root dry weights of the high and low IC_{50} groups following exposure to NaCl indicated that root dry weight was independent of NaCl tolerance during germination. Changes in S:R ratios in response to NaCl that were recorded in this study were inconsistent with salt tolerance during germination. For example, compared with controls, the 88 mM NaCl treatment significantly decreased the S:R ratio in Condor, the most salt tolerant cultivar during seed germination (IC_{50} 241 mM), and Centurion, the most salt sensitive cultivar (IC_{50} 76 mM), while it increased the S:R ratio of Mecca (IC_{50} 158 mM). Also, the 132 mM treatment significantly decreased the S:R ratio of Condor (IC_{50} 241 mM) and 8638C (IC_{50} 233 mM) compared to controls and increased it in 5331 (IC_{50} 228 mM) and Spredor 2 (IC_{50} 122 mM) compared to 88 mM treatment. Cultivars that showed no significant change in their S:R ratios (such as Condor, 5364, Centurion, and many others) following exposure to salt may be able to maintain high S:R photosynthate partitioning patterns between shoots and roots. These

partitioning patterns appear not to be dependent on salt tolerance during germination. The size of the small containers used in these experiments might have affected the plant root dry weight, which, in turn, affected the S:R ratios.

Cultivars that showed high average seed germination and shoot dry weight at the two NaCl levels (WL SS, WL 516, Condor) provide promising materials for alfalfa production in saline soils. Other cultivars with superior average shoot dry weight and low seed germination under these salt treatments (Roamer, Spredor 2, and others) may be highly productive under NaCl stress once stands are established. If their germination ability is improved through selection, they can be used successfully in saline soils. Allen et al. (1985) reported that broad sense heritability for alfalfa seed germination at -1.3 MPa increased from 3 to 86% after five cycles of recurrent selection.

The lack of significant differences between the high IC_{50} and low IC_{50} groups of cultivars in all growth parameters, the nonsignificant correlations between any growth parameter at any NaCl level and the IC_{50}, and the independence of postgermination growth parameters from germination ability under salt stress indicate that there is no evidence of a relationship between germination and postgermination performance in alfalfa in this study. Allen (1984) and Johnson (1991) found similar results in limited populations selected for their high germination under salt conditions.

Seed germination processes depend mainly on water imbibition (no specialized root is present) and are affected by the degree of sensitivity of the germination enzymes and/or hormones to the level of toxic ions, i.e., Na^+ and Cl^- in the medium. In postgermination stages, the well-developed root and shoot systems and processes such as photosynthesis, ion transport, accumulation and compartmentation, and photosynthate partitioning might require different mechanism(s) for NaCl tolerance. Flowers et al. (1986) reported that plant tolerance to salt stress during the postgermination stages includes the ability to accumulate and compartment high levels of Na and Cl ions in the vacuole so that cellular metabolism is not inhibited. However, the results of this study showed that the ability of alfalfa cultivars to germinate at high NaCl concentrations was not related to their postgermination performance under salt stress.

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CHAPTER IV

GENETIC EFFECTS ON ION ACCUMULATION IN ALFALFA PLANT COMPONENTS UNDER SALT STRESS AND ITS EFFECT ON SHOOT DRY WEIGHT

ABSTRACT

Differences in toxic ion uptake and partitioning between and within shoots and roots of many glycophytic species account for the salt tolerance among cultivars. In this study, the differences in ion accumulation between and within shoots and roots of 22 alfalfa (*Medicago sativa* L.) cultivars and their relation with shoot dry weight were determined. Alfalfa plants, 49-d-old, growing in 164 cm^3 plastic containers, were irrigated daily with 0.25 strength Hoagland's solution containing 0, 88, and 132 mM NaCl. Shoots were harvested 3 times at 28 d intervals. Data of the third harvest (84 d after salt treatments began) were used in this study. The strategies by which alfalfa cultivars cope with NaCl stress includes exclusion of Na from shoots and Cl from roots or from the whole plant depending on cultivar and NaCl level. The reduction in shoot dry weight was not related to water stress or deterioration in Ca, Mg, or chlorophyll concentration but it was possibly a result of the apparent shortage in K ion uptake and from the toxic effects of high Na and Cl ions (especially Na^+) in shoot and root tissues. The sub-partitioning of Na and Cl ions between different tissues in

shoots and roots might contribute effectively to the differences in salt tolerance (shoot dry weight) between cultivars.

INTRODUCTION

In saline environments, higher plants have adopted two physiological strategies: the plants either reduce the uptake of toxic ions (e.g. Na^+, Cl^- and others) or prevent their accumulation in the aerial part by retention in the roots or stems and in successive-reduction of their transport to the youngest part of the shoot (Erdei and Kuiper, 1979). The determination of plant strategies to cope with saline environments may advance the selection for salt tolerance in crop plants (McKimmie and Dobrenz, 1991).

Subjected to moderate salinities, glycophytes (most crop plants) have been found to exclude salt and to isolate the salt they absorb in the roots and stems, which minimizes the exposure of the leaf cells (the photosynthetic apparatus) to salt. Regarding the latter characteristic, the glycophytes are less active than halophytes in regulating ion fluxes at the cellular level, but more active in partitioning ions at the organ and tissue level (Lauchli and Epstein, 1990).

Alfalfa is considered a moderately sensitive forage crop whose yield is reduced by 7.3 % for each EC unit ($dS\ m^{-1}$) above the threshold value of 2 (Maas, 1990). Salt tolerance in 6-wk-old alfalfa seedlings appeared to be related to the control of

Na and Cl concentrations in the plant, specifically the maintenance of a low concentration of Na in leaves and low concentration of Na and Cl in shoot parts. The phenomenon of maintaining low Na in leaves was associated with increased root-Na concentrations (McKimmie and Dobrenz, 1991).

Two factors must be considered in studying the salt tolerance of alfalfa: (1) alfalfa is a perennial and its sensitivity to salt changes during its developmental and regrowth stages, and (2) alfalfa is an open pollinated autotetraploid plant, thus, variation in salt tolerance between and within alfalfa cultivars is expected. To increase our knowledge of salt tolerance strategies in alfalfa, a greater number of cultivars in various stages of development must be considered.

The objectives of this study were to determine the differences in ion accumulation within and between shoots and roots of 22 alfalfa cultivars exposed to two levels of NaCl stress, and to determine if ion accumulation differences are reflected in shoot dry weight between cultivars.

MATERIALS AND METHODS

Five seeds each of 22 alfalfa (*Medicago sativa* L.) cultivars were planted in 164 cm^3 plastic containers (Stuewe and Sons, Inc., Corvallis, Oregon 97333) filled with coarse sterilized sand (Silica #20) to within 25 mm of the top. Irrigation with tap water continued (14 d) until germination

was completed; plants were then thinned to one per container and inoculated with *Rhizobium meliloti* (USDA strain #1031) in 0.25-strength Hoagland's (Hoagland and Arnon, 1938) solution (1 L of rhizobial culture to 10 L of 0.25-strength Hoagland's solution). Plants were inoculated three times at 7 d intervals. Plants were irrigated with 0.25-strength Hoagland's solution after inoculation. When the plants were 49 d old, 36 containers with plants of a uniform size were chosen from each cultivar and divided randomly into nine groups of four plants each. Each group was considered one experimental unit. NaCl treatments of 0, 88, and 132 mM in 0.25-strength Hoagland's solution were applied until the termination of the experiment. The 198 experimental units were arranged in a 3 x 22 x 3 factorial (3 NaCl treatments x 22 alfalfa cultivars x 3 replications) in a complete randomized design on a greenhouse bench. Ambient temperature in the greenhouse was 25/20 ^{0}C with supplemental light 500 W m^{-2} 16/8 h day/night regime.

Three harvests were taken at 28 d intervals after NaCl irrigation began. No samples were collected from the first and second harvest. In the third harvest (after 84 d exposure to NaCl), shoots of each experimental unit (four plants) were harvested (30 mm above the soil surface). Roots of each experimental unit were cut directly below the crown region, washed with tap water for (approximately) 5 s and blotted with paper-towels. Shoot and root samples were dried at 70 ^{0}C for 48 h. Shoot and root dry weight per plant was determined by

dividing the total dry weight by the number of plants in the experimental unit. The dried samples used for elemental analyses consisted of the combined shoots and combined roots of each experimental unit ground separately using a Cyclone mill with a 0.03 mm (20 mesh) screen.

The plant samples (500 mg each) were digested in a nitric/perchloric acid mixture and the concentration of Na, K, Ca, and Mg were determined using a Perkin-Elmer Model 2380 atomic absorption spectrophotometer. For extraction of Cl from the plant tissues, 0.1 g of dry sample was added to 10 mL deionized water then heated to 90 °C and shaken for 10 min (Cramer and Spurr, 1986). The extract was titrated with 0.03 N $AgNO_3$. Leaf chlorophyll concentration was determined prior to shoot harvest by a nondestructive method using a Minolta dual-wavelength chlorophyll meter (model SPAD-502, Minolta Corp., Ramsey, N. J.; Minolta, 1989).

Data were examined by analysis of variance and correlation procedures (Snedecor and Cochran, 1980). Costat statistical software (Cohort software, P.O. Box 1149, Berkeley, California 94701) was used to determine the best 3 variable model (among 14 variables included in the study) that describes the variation of shoot dry weight of alfalfa cultivars under salt stress and also, the resultant multiple regression.

RESULTS

Shoot Dry Weight

All alfalfa cultivars showed a highly significant reduction ($P \leq 0.01 - 0.001$) in shoot dry weights at the 88 and 132 mM NaCl levels compared to controls (0 NaCl) (Table 3). The reduction in shoot dry weights at 132 mM compared to 88 mM NaCl was significant ($P \leq 0.05$) only in three cultivars (WL SS, Crown II, and WL 516).

Na

Alfalfa cultivars showed highly significant differences ($P \leq 0.001$) in shoot Na concentration at both NaCl levels compared to controls (Table 3). No significant differences in shoot Na concentration were detected between the 88 and 132 mM NaCl treatments. All cultivars exhibited highly significant differences ($P \leq 0.001$) in root Na concentration at 88 and 132 mM NaCl treatments. Significant differences ($P \leq 0.05 - 0.001$) in Na concentration between 88 and 132 mM NaCl treatments were detected in roots of 10 cultivars; three only showed a significant decrease ($P \leq 0.05 - 0.01$) in root Na concentration at the high salt level (132 mM NaCl). All cultivars accumulated high Na concentration ($P \leq 0.001$) in root (0.47 - 0.94 $mmol_c\ g^{-1}$) compared to shoot (0.17 - 0.33 $mmol_c\ g^{-1}$) at the 88 and 132 mM NaCl levels.

Table 3. Effect of NaCl stress on dry weight, Na and K ions concentration in shoots and roots of alfalfa.

Cultivar†	NaCl treat.	Dw.		Na		Cl	
		S	R	S	R	S	R
	mM	g		$mmol_c\ g^{-1}$‡		$\mu mol_c\ g^{-1}$	
5929	0	1.07	1.52	0.02	0.04	0.15	0.15
	88	0.67	1.28	0.27	0.73	7.64	4.15
	132	0.52	1.18	0.28	0.83	14.40	4.40
WL SS	0	1.47	1.87	0.01	0.03	0.15	0.15
	88	0.69	1.35	0.27	0.63	8.40	3.65
	132	0.38	0.95	0.25	0.87	8.15	4.65
5364	0	1.36	1.80	0.02	0.04	0.15	0.15
	88	0.52	1.40	0.25	0.68	0.65	1.65
	132	0.40	1.05	0.27	0.68	11.02	2.90
5331	0	1.72	2.34	0.03	0.04	0.15	0.15
	88	0.32	0.88	0.29	0.83	11.40	5.65
	132	0.31	0.77	0.33	0.73	14.40	3.90
Regen-S	0	1.14	1.90	0.02	0.04	0.15	0.15
	88	0.49	1.04	0.19	0.72	1.28	3.65
	132	0.34	0.58	0.27	0.78	10.65	5.15
526	0	1.03	1.59	0.02	0.04	0.15	0.15
	88	0.47	0.92	0.24	0.90	10.65	3.15
	132	0.38	0.93	0.31	0.74	9.90	4.85
531	0	1.33	2.38	0.02	0.03	0.15	0.13
	88	0.64	1.47	0.19	0.57	3.15	2.65
	132	0.52	1.08	0.27	0.55	7.65	3.28
Regen-Y	0	0.95	1.40	0.02	0.03	0.15	0.15
	88	0.44	1.09	0.21	0.89	10.15	1.90
	132	0.35	0.85	0.23	0.78	9.90	2.15
Crown II	0	1.48	2.26	0.02	0.03	0.15	0.15
	88	0.69	1.30	0.17	0.55	2.02	1.40
	132	0.37	0.78	0.25	0.80	13.90	4.65
Rambler	0	1.08	2.01	0.01	0.03	0.15	0.15
	88	0.53	1.11	0.23	0.74	13.65	0.15
	132	0.46	0.94	0.31	0.82	14.15	3.77
Roamer	0	1.45	2.15	0.01	0.04	0.15	0.15
	88	0.55	1.26	0.18	0.75	9.15	2.15
	132	0.40	0.79	0.29	0.94	17.02	5.15
86108	0	1.33	2.11	0.02	0.04	0.15	0.15
	88	0.49	1.12	0.23	0.66	9.90	1.40
	132	0.50	1.36	0.23	0.66	6.53	0.40
Sabre	0	1.67	2.31	0.02	0.03	0.15	0.15
	88	0.58	1.08	0.20	0.57	2.65	1.40
	132	0.48	1.24	0.21	0.58	2.40	1.90
Condor	0	1.71	1.87	0.01	0.03	0.15	0.15

Table 3 continued.

Cultivar†	NaCl treat.	Dw.		Na		Cl	
		S	R	S	R	S	R
	mM	g		$mmol_c\ g^{-1}$‡		$\mu mol_c\ g^{-1}$	
	88	0.61	1.25	0.28	0.83	8.90	0.40
	132	0.62	1.74	0.23	0.64	10.43	1.90
WL 516	0	1.36	2.29	0.01	0.03	0.15	0.15
	88	0.70	1.30	0.17	0.64	1.52	1.15
	132	0.39	0.83	0.24	0.78	9.15	5.65
Spredor 2	0	1.18	1.84	0.01	0.04	0.15	0.15
	88	0.46	1.05	0.19	0.69	7.65	1.15
	132	0.47	0.96	0.22	0.70	8.90	2.27
Husky	0	1.36	1.50	0.02	0.04	0.15	0.15
	88	0.53	1.39	0.22	0.55	2.65	2.15
	132	0.34	1.16	0.23	0.71	6.37	4.40
8638C	0	1.47	2.48	0.01	0.03	0.15	0.15
	88	0.50	1.18	0.18	0.69	7.27	3.65
	132	0.34	0.99	0.20	0.71	7.65	3.15
Mecca	0	1.38	2.00	0.02	0.03	0.15	0.15
	88	0.34	1.03	0.24	0.81	7.65	5.40
	132	0.35	0.99	0.26	0.71	8.02	4.90
Centurion	0	1.42	1.87	0.02	0.03	0.15	0.15
	88	0.51	1.46	0.24	0.57	9.15	1.65
	132	0.34	0.86	0.26	0.76	14.77	3.40
8737N	0	1.31	1.95	0.02	0.05	0.15	0.15
	88	0.54	1.64	0.20	0.47	2.07	1.15
	132	0.36	0.77	0.25	0.82	8.02	5.15
3713	0	1.02	1.79	0.01	0.03	0.15	0.15
	88	0.29	0.58	0.19	0.90	10.27	3.15
	132	0.35	0.39	0.19	0.68	1.40	3.15
LSD§ 0.05	---	0.29	ns	0.14		1.67	
0.01	---	0.39	--	0.18		2.21	

† Dw. = Dry weight; S = Shoot; R = Root.
‡ Means of 6 replications.
§ LSD values for differences within and between plant parts in case of ions concentration.

Cl

Except for cultivars 5364, Regen-S, and WL 516 at 88 mM, and 3713 at 132 mM, all cultivars showed a significant increase ($P \leq 0.05 - 0.001$) in shoot Cl concentration at 88 and 132 mM NaCl treatments (Table 3). Compared to the 88 mM, the 132 mM NaCl treatment increased shoot Cl concentration significantly ($P \leq 0.001$) in 12 cultivars (5929, 5364, 5331, Regen-S, 531, Crown II, Roamer, 86108, WL 516, Husky, Centurion, and 8737) and decreased it in 1 cultivar (3713). The remainder of the cultivars (WL SS, 526, Regen-Y, Rambler, Sabre, Condor, Spredor 2, 8638C, and Mecca) showed no change in shoot Cl content between the 88 and 132 mM NaCl levels. While nine cultivars at 88 mM and one cultivar at 88 and 132 mM NaCl level showed no change in root Cl concentration, the rest of the cultivars (12) exhibited a significant increase ($P \leq 0.05 - 0.001$) in root Cl concentration at 88 and 132 mM NaCl compared to the controls. The 132 mM NaCl treatment did not increase root Cl concentration significantly in 12 out of 22 cultivars studied. Except for seven cultivars at 88 mM and one cultivar at 132 mM NaCl, all alfalfa cultivars showed significant differences ($P \leq 0.05 - 0.001$) in Cl concentration between shoots and roots. The concentration of Cl was higher in shoots than in roots except for Regen-S at 88 and 3713 at 132 mM NaCl level, which exhibited reversed response.

K

Except for a significant decrease ($P \leq 0.05$) in cultivar 531 at 132 mM NaCl level, no significant differences in shoot K concentration were found between 0, 88, and 132 mM NaCl treatments in all alfalfa cultivars (Table 4). NaCl treatments decreased K in roots significantly ($P \leq 0.05 - 0.001$) compared to control in all cultivars except 531 and Husky at 132 mM, and 86108 and Mecca at 88 and 132 mM NaCl levels in which no significant change in root K concentration occurred. Compared to 88 mM, the 132 mM NaCl treatment increased root K concentration significantly ($P \leq 0.05 - 0.01$) in 5929, 531, and Centurion and decreased it significantly ($P \leq 0.05 - 0.001$) in Regen-Y, Roamer, Spredor 2, and 3713. The rest of the cultivars (15) showed no significant change in their root K concentration between the two NaCl treatments. With only one exception (cultivar 3713 at 132 mM NaCl level), all alfalfa cultivars accumulated significantly ($P \leq 0.05 - 0.001$) higher K in roots than in shoots independent of NaCl treatment.

The analysis of variance showed no significant differences in root dry weight, Ca, Mg, water, and chlorophyll contents of all alfalfa cultivars included in this study (Tables 3, 4, and 5).

Highly significant negative correlation between shoot dry weight and Na in shoots and roots was found in all alfalfa cultivars (Table 6). With very few exceptions, alfalfa shoot

Table 4. Effect of NaCl stress on Ca, Mg, and Cl concentration in shoots and roots of alfalfa.

Cultivar†	NaCl treat.	K		Ca		Mg	
		S	R	S	R	S	R
	mM	mmol$_c$ g^{-1}‡					
5929	0	0.12	0.43	0.75	0.65	0.34	0.31
	88	0.09	0.23	0.32	0.21	0.14	0.18
	132	0.06	0.31	0.30	0.27	0.11	0.25
WL SS	0	0.12	0.49	0.57	0.88	0.26	0.27
	88	0.08	0.27	0.30	0.22	0.10	0.20
	132	0.10	0.26	0.26	0.20	0.10	0.23
5364	0	0.12	0.44	0.71	0.67	0.33	0.31
	88	0.09	0.28	0.32	0.22	0.13	0.20
	132	0.09	0.27	0.29	0.20	0.13	0.17
5331	0	0.13	0.38	0.56	0.65	0.24	0.33
	88	0.10	0.21	0.33	0.34	0.17	0.30
	132	0.08	0.26	0.24	0.24	0.13	0.25
Regen-S	0	0.14	0.44	0.62	0.59	0.29	0.32
	88	0.15	0.29	0.30	0.25	0.12	0.28
	132	0.12	0.27	0.30	0.26	0.15	0.25
526	0	0.12	0.42	0.71	0.75	0.33	0.23
	88	0.08	0.29	0.29	0.34	0.10	0.25
	132	0.10	0.33	0.28	0.20	0.11	0.22
531	0	0.14	0.38	0.55	0.80	0.31	0.26
	88	0.10	0.23	0.24	0.26	0.11	0.19
	132	0.07	0.33	0.19	0.24	0.10	0.19
Regen-Y	0	0.13	0.43	0.73	0.62	0.33	0.33
	88	0.13	0.36	0.37	0.34	0.18	0.25
	132	0.12	0.25	0.28	0.19	0.15	0.22
Crown II	0	0.12	0.40	0.57	0.67	0.24	0.24
	88	0.10	0.24	0.31	0.18	0.12	0.21
	132	0.11	0.22	0.28	0.24	0.16	0.23
Rambler	0	0.12	0.46	0.62	0.78	0.30	0.34
	88	0.11	0.29	0.33	0.25	0.13	0.25
	132	0.10	0.29	0.24	0.24	0.13	0.20
Roamer	0	0.13	0.40	0.64	0.56	0.28	0.31
	88	0.12	0.30	0.36	0.29	0.15	0.28
	132	0.13	0.21	0.30	0.28	0.16	0.27
86108	0	0.11	0.32	0.62	0.77	0.27	0.26
	88	0.10	0.31	0.36	0.21	0.12	0.20
	132	0.09	0.28	0.23	0.19	0.09	0.20
Sabre	0	0.13	0.33	0.64	0.77	0.28	0.30
	88	0.09	0.26	0.28	0.20	0.12	0.20
	132	0.08	0.25	0.20	0.20	0.10	0.22
Condor	0	0.10	0.38	0.61	0.50	0.24	0.26

Table 4. continued.

Cultivar†	NaCl treat.	K		Ca		Mg	
		S	R	S	R	S	R
	mM	$mmol_c\ g^{-1}$‡					
	88	0.06	0.25	0.33	0.33	0.10	0.19
	132	0.07	0.22	0.24	0.16	0.07	0.19
WL 516	0	0.11	0.38	0.62	0.57	0.27	0.21
	88	0.10	0.27	0.32	0.21	0.10	0.20
	132	0.09	0.22	0.24	0.19	0.09	0.19
Spredor 2	0	0.13	0.45	0.79	0.65	0.34	0.35
	88	0.13	0.33	0.43	0.31	0.19	0.25
	132	0.12	0.21	0.30	0.24	0.12	0.22
Husky	0	0.13	0.35	0.80	0.76	0.27	0.33
	88	0.09	0.27	0.29	0.21	0.12	0.18
	132	0.09	0.29	0.28	0.16	0.11	0.23
8638C	0	0.11	0.40	0.61	0.60	0.27	0.26
	88	0.11	0.27	0.34	0.24	0.13	0.19
	132	0.11	0.21	0.23	0.19	0.16	0.21
Mecca	0	0.11	0.13	0.69	0.48	0.27	0.27
	88	0.07	0.28	0.39	0.29	0.13	0.25
	132	0.06	0.30	0.28	0.21	0.09	0.21
Centurion	0	0.12	0.40	0.63	0.50	0.27	0.31
	88	0.08	0.25	0.34	0.22	0.10	0.22
	132	0.11	0.32	0.28	0.17	0.13	0.28
8737N	0	0.11	0.42	0.61	0.65	0.28	0.27
	88	0.08	0.28	0.30	0.19	0.12	0.19
	132	0.11	0.25	0.24	0.25	0.12	0.25
3713	0	0.13	0.42	0.55	0.73	0.28	0.25
	88	0.13	0.33	0.38	0.34	0.19	0.26
	132	0.11	0.17	0.31	0.17	0.15	0.12
LSD§ 0.05	---	0.07		ns		ns	
0.01	---	0.09		--		--	

† S = Shoot; R = Root.
‡ Means of 6 replications.
§ LSD values for differences within and between plant parts.

Table 5. Effect of NaCl stress on water contents and chlorophyll concentration in alfalfa.

Cultivar†	NaCl treat.	H2O		Chlorophyll
		S	R	
	mM	g gDw.$^{-1}$‡		mg m^{-2}
5929	0	3.15	3.79	470
	88	4.23	3.77	505
	132	4.19	4.22	436
WL SS	0	2.84	3.11	539
	88	3.77	4.00	615
	132	3.77	4.06	538
5364	0	2.78	2.93	499
	88	4.58	4.61	495
	132	4.43	3.47	571
5331	0	2.79	3.06	510
	88	4.36	4.23	444
	132	4.73	3.73	518
Regen-S	0	3.43	3.40	615
	88	4.37	4.17	643
	132	5.15	6.20	517
526	0	2.94	3.69	526
	88	4.87	3.93	501
	132	5.41	4.14	573
531	0	3.17	2.26	443
	88	3.53	3.53	599
	132	3.47	3.22	600
Regen-Y	0	2.92	2.72	398
	88	4.75	4.37	516
	132	4.73	3.66	494
Crown II	0	2.54	2.33	550
	88	3.35	3.25	683
	132	5.07	4.29	589
Rambler	0	2.63	2.77	531
	88	4.52	4.29	600
	132	4.73	4.89	464
Roamer	0	2.76	2.83	537
	88	4.41	5.12	621
	132	4.10	4.98	486
86108	0	3.22	2.52	482
	88	4.19	3.96	559
	132	4.25	3.36	499
Sabre	0	2.87	2.42	494
	88	3.68	3.61	583
	132	3.75	3.20	557
Condor	0	2.63	2.80	517

Table 5. continued.

Cultivar†	NaCl treat.	H2O		Chlorophyll
		S	R	
	mM	g gDw.$^{-1}$‡		mg m^{-2}
	88	4.46	4.21	519
	132	3.05	3.09	643
WL 516	0	2.73	2.53	443
	88	3.76	4.35	643
	132	4.26	4.27	562
Spredor 2	0	2.90	3.31	539
	88	5.00	4.41	643
	132	3.38	3.63	585
Husky	0	2.80	3.05	511
	88	4.28	3.11	541
	132	5.29	3.34	623
8638C	0	2.76	2.75	517
	88	3.88	3.56	571
	132	4.67	3.78	530
Mecca	0	2.46	2.78	537
	88	5.15	4.15	606
	132	4.79	3.69	496
Centurion	0	3.04	3.67	455
	88	4.08	3.61	617
	132	4.74	3.65	468
8737N	0	3.03	3.04	493
	88	3.28	2.69	532
	132	4.36	4.12	492
3713	0	3.72	2.19	553
	88	5.09	5.14	527
	132	3.51	5.47	503
LSD§ 0.05	---	ns		ns
0.01	---	—		—

† S=shoot R=root.
‡ Means of 6 replications.
§ LSD values for differences within and between plant parts.

Table 6. Correlation coefficients between shoot dry weight and root dry weight, Na, Cl, K, Ca, Mg, water content, and chlorophyll concentration under NaCl conditions.

	Na		Cl		K	
Cultivar†	S	R	S	R	S	R
5929	-0.80**	-0.70*	-0.72*	-0.69*	0.55	0.52
WL SS	-0.94**	-0.94**	-0.90**	-0.90**	0.53	0.95**
5364	-0.94**	-0.94**	-0.60	-0.78*	0.71*	0.89**
5331	-0.98**	-0.90**	-0.94**	-0.77*	0.84**	0.90**
Regen-S	-0.88**	-0.86**	-0.63	-0.87**	0.02	0.50
526	-0.93**	-0.89**	-0.89**	-0.81**	0.51	0.62
531	-0.92**	-0.91**	-0.79*	-0.93**	0.81**	0.55
Regen-Y	-0.77*	-0.75*	-0.78*	-0.62	-0.23	0.28
Crown II	-0.94**	-0.95**	-0.76*	-0.79*	0.47	0.70*
Rambler	-0.90**	-0.89**	-0.94**	-0.55	0.48	0.77*
Roamer	-0.89**	-0.92**	-0.88**	-0.76*	0.15	0.75*
86108	-0.80**	-0.82**	-0.78*	-0.45	0.10	0.41
Sabre	-0.89**	-0.97**	-0.95**	-0.78*	0.97**	0.61
Condor	-0.88**	-0.91**	-0.61	-0.64	0.72*	0.74*
WL 516	-0.92**	-0.87**	-0.77*	-0.65	0.58	0.82**
Spredor 2	-0.96**	-0.95**	-0.93**	-0.59	0.10	0.77*
Husky	-0.96**	-0.94**	-0.89**	-0.83**	0.86**	0.77*
8638C	-0.91**	-0.95**	-0.94**	-0.88**	0.19	0.93**
Mecca	-0.95**	-0.97**	-0.97**	-0.95**	0.94**	0.42
Centurion	-0.94**	-0.95**	-0.95**	-0.87**	0.35	0.60
8737N	-0.81**	-0.80**	-0.69*	-0.65	0.18	0.55
3713	-0.97**	-0.93**	-0.65	-0.96**	0.16	0.53
All‡	-0.85**	-0.86**	-0.71*	-0.68*	0.32	0.51

† r values of 9 replications for each cultivar; S = Shoot; R = Root; *,** = Significant at the 0.05 and 0.01 probability levels, respectively.

‡ r values of the 198 replication for the 22 cultivars together.

Table 6 continued.

Cultivar†	Ca		Mg	
	S	R	S	R
5929	0.44	0.27	0.61	0.41
WL SS	0.91**	0.89**	0.90**	0.25
5364	0.86**	0.89**	0.84**	0.80**
5331	0.94**	0.82**	0.84**	0.48
Regen-S	0.66	0.72*	0.64	0.51
526	0.86**	0.83**	0.84**	0.01
531	0.93**	0.90**	0.91**	0.78*
Regen-Y	0.54	0.52	0.41	0.26
Crown II	0.92**	0.89**	0.63	0.20
Rambler	0.91**	0.82**	0.87**	0.42
Roamer	0.78*	0.77*	0.72*	0.40
86108	0.57	0.83**	0.66	0.78*
Sabre	0.98**	0.90**	0.99**	0.55
Condor	0.93**	0.78*	0.93**	0.50
WL 516	0.89**	0.92**	0.84**	0.02
Spredor 2	0.93**	0.97**	0.88**	0.54
Husky	0.79*	0.94**	0.94**	0.78*
8638C	0.92**	0.90**	0.75*	0.65
Mecca	0.91**	0.77*	0.89**	0.46
Centurion	0.99**	0.96**	0.87**	0.57
8737N	0.77*	0.46	0.70*	0.41
3713	0.79*	0.85**	0.65	0.25
All‡	0.49**	0.73**	0.68**	0.37**

† r values of 9 replications for each cultivar; S = Shoot; R = Root; *,** = Significant at the 0.05 and 0.01 probability levels, respectively.

‡ r values of the 198 replication for the 22 cultivars together.

Table 6 continued.

Cultivar†	H2O		Root dry wt.	Chlorophyll Concentration
	S	R		
5929	-0.83**	-0.43	0.59	0.38
WL SS	-0.64	-0.55	0.94**	0.08
5364	-0.86**	-0.47	0.69*	-0.05
5331	-0.80**	-0.45	0.96**	0.30
Regen-S	-0.74*	-0.66	0.89**	0.40
526	-0.95**	-0.44	0.81**	-0.16
531	-0.33	-0.55	0.90**	-0.78*
Regen-Y	-0.74*	-0.75*	0.79*	-0.63
Crown II	-0.84**	-0.71*	0.92**	-0.22
Rambler	-0.94**	-0.84**	0.91**	0.04
Roamer	-0.74*	-0.77*	0.91**	0.03
86108	-0.86**	-0.66	0.79*	-0.36
Sabre	-0.92**	-0.93**	0.86**	-0.51
Condor	-0.64	-0.55	0.71*	-0.44
WL 516	-0.85**	-0.84**	0.96**	-0.18
Spredor 2	-0.66	-0.47	0.95**	-0.68*
Husky	-0.88**	-0.30	0.59	-0.23
8638C	-0.83**	-0.66	0.82**	-0.04
Mecca	-0.90**	-0.58	0.88**	0.03
Centurion	-0.86**	0.03	0.72*	-0.26
8737N	-0.71*	-0.56	0.86**	0.03
3713	-0.44	-0.77*	0.84**	0.42
All‡	-0.74**	-0.53**	0.82**	-0.08

† r values of 9 replications for each cultivar; S = Shoot; R = Root; *,** = Significant at the 0.05 and 0.01 probability levels, respectively.

‡ r values of the 198 replication for the 22 cultivars together.

dry weight correlated negatively with shoot and/or root Cl concentration (Table 6). Positive correlations were found between shoot dry weight and shoot and/or root K, Ca, Mg, and root dry weight in many cultivars. Shoot dry weight of most cultivars showed significant negative correlation with shoot and/or root water contents. Chlorophyll concentration correlated negatively with shoot dry weight only in two cultivars.

Table 7 shows the multiple regression equations of the best 3-variables model that describes variation in shoot dry weight in alfalfa cultivars grown under salinity. Not all alfalfa cultivars had the same model for shoot dry weight prediction. For example, the concentration of Cl in shoot, and Na and Mg in root determine 98% of shoot dry weight in cultivar Regen-S, whereas Na, Ca, and Mg concentration in the shoot explained 100% of shoot dry weight variation in cultivar WL SS under NaCl stress conditions. The chlorophyll concentration was included only in the cultivar Roamer model. Potassium concentration of shoot and Cl concentration of root both appear in two models. Other variables were found in models of 3 up to 8 cultivars.

Table 7. Multiple regression analysis for the best 3 variables model which describes shoot dry weight variation in alfalfa cultivars.

Cultivar	Regression equation†	R^2
5929	$\hat{Y}$ = 0.65 − 54.73 x Cl S^{**} + 3.43 x K R^{ns} + 1.62 x Ca R^{**}	0.91
WLSS	" = −0.49 − 0.92 x Ca S^{***} + 4.22 x Mg S^{**} + 0.75 x R^{***}	1.00
5364	" = −1.57 + 28.46 x Cl S^{***} + 5.10 x Mg R^{***} + 0.74 x R^{***}	0.99
5331	" = −0.89 + 2.34 x K R^{**} + 0.81 x Ca R^{**} + 0.51 x R^{***}	0.99
Regen-S	" = 3.97 − 44.84 x Cl S^{***} − 1.32 x Na R^{***} − 8.61 x Mg R^{**}	0.98
526	" = 0.72 + 0.51 x Ca S^{***} + 0.19 x R^{**} − 0.12 x H2OS**	0.99
531	" = 2.34 − 10.02 x Na S^{***} − 9.31 x Mg S^{*} + 5.58 x K R^{***}	0.99
Regen-Y	" = 2.13 − 8.43 x Na S^{***} + 9.54 x K S^{*} − 6.66 x Mg S^{**}	0.96
Crown II	" = 0.03 + 3.75 x Ca S^{***} − 6.08 x Mg S^{**} + 1.15 x Ca R^{ns}	0.98
Rambler	" = 1.31 − 0.61 x Mg R^{***} + 0.25 x R^{***} − 0.20 x H2OS**	0.98
Roamer	" = −0.34 + 1.38 x Ca R^{***} + 0.36 x R^{***} + 0.001 x Chl*	0.97
86108	" = 3.31 − 12.05 x K S^{ns} + 3.13 x Mg S^{**} − 0.47 x H2OS***	0.97
Sabre	" = 1.15 + 3.95 x Mg S^{***} − 1.09 x Na R^{ns} − 1.67 x K R^{**}	1.00
Condor	" = 1.53 − 84.53 x Cl S^{***} + 1.30 x K R^{ns} − 0.11 x H2OR**	0.99
WL 516	" = −0.30 + 0.71 x Ca S^{***} + 1.02 x K R^{**} + 0.37 x R^{***}	0.99
Spredor 2	" = 0.76 − 0.45 x Na R^{***} + 0.94 x Ca R^{***} − 0.06 x H2OS**	0.99
Husky	" = 0.88 − 2.08 x Na S^{***} − 65.24 x Cl S^{**} + 1.55 x Mg R^{*}	0.99
8638C	" = −2.86 + 8.22 x K R^{***} − 92.04 x Cl R^{**} + 0.38 x H2OS^{ns}	0.98
Mecca	" = 1.27 − 2.37 x Na S^{***} − 0.78 x Na R^{***} + 0.07 x H2OR***	1.00
Centurion	" = −0.69 + 2.55 x Ca S^{***} + 0.54 x Ca R^{ns} + 0.06 x H2OR^{ns}	0.99
8737N	" = 0.32 − 3.75 x Na S^{***} + 113.81 x Cl R^{ns} + 0.53 x R^{***}	0.97
3713	" = 1.63 − 5.13 x Na S^{***} + 0.58 x Ca S^{***} − 3.10 x Mg S^{***}	1.00
All‡	" = 0.76 − 1.23 x Na S^{***} − 0.41 x Na R^{***} + 0.29 x R^{***}	0.84

† Fourteen variables (included in table 1), nine replication of each, were included in determining the best models and in their regression analysis; S = Shoot dry weight; R = Root dry weight; Chl. = Chlorophyll concentration; ns = Not significant; *, **, *** = significant at the 0.5, 0.01, and 0.001 probability levels respectively.

‡ Data from 22 cultivars (198 replication) were used in determining the best model and in its regression analysis.

DISCUSSION

Alfalfa cultivars showed different magnitudes of reduction in shoot dry weight by exposure to salt. For example, the reduction (average reduction at 88 and 132 mM NaCl treatments) was 44% in cultivar 5929, 64% in cultivar Rambler, 75% in Mecca, and 81% in cultivar 5331. The reduction in shoot dry weight was the net effect of NaCl stress on the essential growth parameters such as plant height, stems plant^{-1}, and shoot:root ratio (Al-Niemi et al., 1992). The cultivars that showed no significant change in shoot dry weight between the 88 and 132 mM NaCl (19 cultivars) indicated that increasing salt level to 132 mM had no additional significant effect on the physiological processes that affect growth and development. In other words, the mechanism(s) of NaCl tolerance in these cultivars at 88 mM NaCl level were effective also at the higher NaCl treatment.

Exclusion of Na from shoot tissues was evidently the mechanism adopted by all alfalfa cultivars grown under NaCl treatments in this study. Exclusion of Na from alfalfa shoots under salt stress was reported by McKimmie and Dobrenz (1991) and from alfalfa leaves by Kapulnik et al. (1989). It is clear that the accumulation of high Na concentration in roots of all cultivars at both NaCl levels was related to a restricted Na translocation from root to shoot. This notion is supported by the insignificant change in shoot Na concentration albeit the

significant increase in root Na concentration in many cultivars. Restriction in Na translocation from root to shoot was reported in reed (Matsushita and Matoh, 1991) and barley (Wolf et al., 1991) plants. Reduction in root Na concentration at 132 mM compared to 88 mM NaCl was noticed in six cultivars. The decrease was significant in the case of three cultivars (526, Condor, and 3713). Because there was no decrease in shoot Na concentration and no increase in the concentration of other ions that might substitute for Na in the roots of these cultivars, this response might be related to a Na uptake control system located in the root, which was activated at the high NaCl treatment. It is possible that the roots of other cultivars may require more NaCl (above 132 mM) to show the latter response.

Generally, the alfalfa cultivars accumulated higher Cl in shoots than in roots at both NaCl treatments. McKimmie and Dobrenz (1991) found lower Cl concentration in shoots of 8-wk-old alfalfa seedlings. This response was probably related to the length of exposure to salt (8 wk) compared to this study (19 wk) as well as the regrowth following plant harvest (2 regrowths in this study). Kapulnik et al. (1989) reported Cl exclusion from alfalfa leaves. Whether Cl accumulation sites were located in the leaf or stem parts of the shoot was not determined in our study. The insignificant differences in Cl concentration between shoot and root of eight cultivars at 88 mM NaCl level appeared to be related to the active exclusion

of Cl from the whole plant (shoot + root). The increase in Cl uptake by roots at the 132 mM NaCl level resulted in a significant increase in plant Cl contents in these cultivars and at this NaCl level they exhibited significantly higher Cl in shoot compared to root. The results of Cl analysis imply the presence of a restricted Cl concentration in alfalfa roots above which any obtained Cl ions are excluded from roots and accumulated in shoots. The restricted root Cl concentration ranged between 0.15 - 5.65 μmol g^{-1} dry weight at the 88 mM and between 0.40 - 5.65 μmol g^{-1} dry weight at the 132 mM NaCl treatment. Apparently the cultivar Sabre did not absorb enough Cl to reach its restricted root Cl concentration at either the 88 or 132 mM NaCl treatment. Thus, no significant differences in Cl content between its shoot and root were evident at either NaCl treatment. The data indicate that in some cultivars, such as WL SS, 526, and Condor, once the Cl concentration reached a threshold level in the plant (shoot + root), no more Cl uptake occurred albeit the increase in NaCl level. This response suggested that these cultivars might possess a more effective Cl control system that inhibits Cl uptake after it reaches a threshold concentration (which differs among cultivars) in the plant, and that this system was not affected by the increasing Cl concentration in the growing medium. Whether this response occurs at NaCl levels above 132 mM is not known.

The insignificant differences in shoot K concentration and the significant decrease in root K concentration by exposure to NaCl stress suggested that alfalfa plants were maintaining sufficient shoot concentration of K for metabolic processes. Because K uptake by alfalfa plants was decreased significantly under NaCl stress, maintaining adequate K in the shoot was associated with the reduction in its concentration in roots. The high K concentration in alfalfa roots compared to shoots independently of salt treatments indicated that more K is needed in the roots than shoots of alfalfa. The lack of correlation between shoot and/or root water contents and K concentration in roots of most cultivars, together with the negative correlation between these factors in several cultivars, indicates that the high K concentration in roots did not directly influence the shoot or root water status.

The positive correlation between Na and Cl concentration and the negative correlation between them and shoot dry weight indicated that Na and Cl ions reduced shoot dry weight both individually and collectively. The separate effect of Na on shoot dry weight was clear in the cultivars that accumulate very low concentration of Cl in both shoot and root, e.g. cultivars 5364, Sabre, and 8737N at 88 mM and WL 516 at both NaCl levels.

The positive correlation between Na and/or Cl in shoot and/or root and water contents of shoots and/or roots (Table

8) indicated that alfalfa cultivars were using the accumulated Na and Cl in these tissues to regulate their water status.

The most effective factors (see Table 7) in determining shoot dry weight in alfalfa grown under the NaCl treatments of this study were not the same for all cultivars. This fact must be considered in alfalfa breeding programs by emphasizing the factors that increase shoot dry weight under NaCl stress in choosing parental salt tolerant cultivars and in selection criteria for high salt tolerance.

CONCLUSIONS

This study indicated that there was a genetic influence on Na and Cl ion uptake and accumulation in alfalfa shoots and roots. The strategies of alfalfa cultivars to cope with NaCl stress include exclusion of Na from shoots and Cl from roots or from the whole plant depending on cultivar and NaCl level. The reduction in shoot dry weight was not related to water stress or deterioration in Ca, Mg, or chlorophyll concentration. Based on these results, the shortage in K ion uptake and more directly the toxic effects of high Na and Cl in the plant tissues contributed to the reduction in shoot dry weight. The possible subpartitioning of Na and Cl ions between different tissues, cell types, cell compartments, and/or the possible changes in protein content (Al-Niemi et al., 1993) and enzyme activity at the high Na and Cl concentrations, independent of total concentration of ions, might also contribute to

Table 8. Correlation coefficients between shoot and root water content and shoot and root Na and Cl concentrations.

	H2O in shoot			
	Na		Cl	
Cultivar†	S	R	S	R
5929	0.74*	0.59	0.46	0.65
WL SS	0.64	0.40	0.48	0.32
5364	0.97**	0.97**	0.36	0.60
5331	0.83**	0.84**	0.89**	0.84**
Regen-S	0.65	0.75*	0.56	0.59
526	0.92**	0.81**	0.83**	0.72*
531	0.38	0.39	0.11	0.31
Regen-Y	0.81**	0.81**	0.85**	0.42
Crown II	0.81**	0.81**	0.90**	0.89**
Rambler	0.93**	0.92**	0.95**	0.53
Roamer	0.78*	0.78*	0.62	0.55
86108	0.68*	0.61	0.55	0.29
Sabre	0.94**	0.94**	0.94**	0.85**
Condor	0.75*	0.74*	0.34	0.08
WL 516	0.97**	0.94**	0.82**	0.67*
Spredor 2	0.50	0.52	0.48	0.05
Husky	0.92**	0.97**	0.87**	0.91**
8638C	0.87**	0.70*	0.88**	0.63
Mecca	0.95**	0.86**	0.80**	0.86**
Centurion	0.84**	0.84**	0.86**	0.86**
8737N	0.70*	0.76*	0.78*	0.86**
3713	0.25	0.33	0.58	0.22
All‡	0.72**	0.71**	0.61**	0.56**

† r values of 9 replications for each cultivar. S = Shoot; R = Root; *,** = Significant at the 0.05 and 0.01 probability levels, respectively.

‡ r values of the 198 replication for the 22 cultivars together.

Table 8 continued.

Cultivar†	H2O in shoot			
	Na		Cl	
	S	R	S	R
5929	0.17	0.30	0.26	0.36
WL SS	0.52	0.54	0.63	0.53
5364	0.69*	0.71*	0.12	0.14
5331	0.50	0.71*	0.57	0.86**
Regen-S	0.52	0.52	0.65	0.49
526	0.21	0.21	0.20	0.23
531	0.69*	0.81*	0.46	0.64
Regen-Y	0.52	0.53	0.53	0.46
Crown II	0.55	0.76*	0.72*	0.91**
Rambler	0.87**	0.77*	0.79*	0.62
Roamer	0.84**	0.84**	0.72*	0.77**
86108	0.80**	0.78*	0.82**	0.73*
Sabre	0.87**	0.85**	0.89**	0.72*
Condor	0.60	0.62	0.37	0.10
WL 516	0.87**	0.83**	0.55	0.44
Spredor 2	0.43	0.46	0.46	0.03
Husky	0.40	0.43	0.30	0.43
8638C	0.69*	0.72*	0.64	0.58
Mecca	0.72*	0.56	0.42	0.55
Centurion	0.09	0.10	0.00	0.13
8737N	0.40	0.47	0.58	0.68*
3713	0.81**	0.78*	0.43	0.82**
All‡	0.51**	0.58**	0.45**	0.47**

† r values of 9 replications for each cultivar. S = Shoot; R = Root; *,** = Significant at the 0.05 and 0.01 probability levels, respectively.

‡ r values of the 198 replication for the 22 cultivars together.

differences in salt tolerance (shoot dry weight) between cultivars.

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CHAPTER V

SODIUM CHLORIDE STRESS INDUCED PROTEIN DIFFERENCES IN ALFALFA (*MEDICAGO SATIVA* L.)

ABSTRACT

To determine the effect of salt stress on alfalfa (*M. sativa* L.) 10 cultivars with different NaCl tolerances were exposed to 0, 88, and 132 mM NaCl in full strength modified Hoagland's solution. Samples from shoot and root were collected after different periods of exposure to salt. Total protein extracted from shoot and root samples were analyzed by sodium dodecylsulfate-polyacrylamide gel electrophoresis (1D SDS-PAGE) and isoelectric focusing sodium dodecylsulfate-polyacrylamide (2D IEF-SDS-PAGE). Sodium chloride stress induced quantitative and qualitative changes in shoot and root proteins of salt-tolerant cultivars and shoot proteins of salt-sensitive cultivars. These changes were affected by the concentration of NaCl in the growth medium and by the length of exposure to salt stress.

INTRODUCTION

Extensive research has been devoted to the study of plant responses to salinity and the various responses of salt-tolerant vs. salt-sensitive species (Staples and Toenniessen, 1984). Genetic manipulation of plants to magnify biological tolerance to saline environments is an essential factor in

limiting the salinity threat to agriculture in irrigated farming (Stavarek and Rains, 1983). This manipulation includes the identification of high salt-tolerant genotypes and the incorporation of their salt-tolerant traits into crop plants. Thus, identification of specific characteristics related to salt tolerance will provide biological markers useful in selecting salt-tolerant crops.

Avoidance, exclusion, and physiological tolerance are the three possible mechanisms that have been identified among plants growing in saline environments that enable them to cope with the environment (Stavarek and Rains, 1983). Physiological tolerance, which includes compartmentation and osmotic adjustment utilizing inorganic and organic components, has been found to be the most significant mechanism for dealing with salt. Whatever the mechanism of salt tolerance, it most likely involves quantitative and qualitative changes in certain proteins in the plant under stress.

Studies of the effects of environmental stress conditions (such as salt, flooding, high temperature, and intense UV light) on plants showed that the plants responded to the stress by altering gene expression. This included the synthesis of novel proteins and repression of some of the normally expressed ones. Investigation and understanding of the stress-induced proteins and the genes encoding them may lead to the engineering of crop plants more resistant to normally encountered stress conditions. The latter task is

attainable because of the massive advancement in the plant transformation technology (Sachs and Ho, 1986).

The objective of this study was to determine whether NaCl stress induces changes (quantitative and/or qualitative) in alfalfa proteins and to determine the effect of salt concentration, length of exposure, and alfalfa cultivar on these changes.

MATERIALS and METHODS

Production of Plant Materials

Ten alfalfa cultivars, Regen-Y, 5929, Rambler, and Sabre (tolerant at 88 and 132 mM NaCl levels), Mecca (intermediate), Spredor 2 (sensitive at 88 tolerant at 132 mM NaCl level), 8638C, Centurion, 5331, and Crown II (very sensitive to 88 and 132 mM NaCl levels (Al-Niemi et al., 1992) were used in this study. To provide a sufficient number of genetically identical plants, cuttings 10-15 cm in length from 3-week-old tillers were obtained from one plant of each cultivar. Rootone, a compound of Lilly and Miller [(1-Naphthaleneacetamide 0.20%, Indol-3-butyric acid 0.10%), Thiram (tetramethyl thiuramdisulfide 4.04%, and Inert ingredients 95.66%)], was used to facilitate rooting. Clones were grown in 15-cm diameter plastic pots filled with a mixture of 1:1 loam soil and vermiculite. Twenty plants from each variety were produced and kept in a greenhouse. When plants reached suitable size (about 12 weeks old), their shoots were harvested and their

roots washed with tap water and trimmed off uniformly to 10 cm length, then transferred to a full strength modified Hoagland's (Hoagland and Arnon, 1938) solution (Table 9) in 70 L containers (Fig. 1), 18 plants per container. The first full shoot growth (at 50% flowering) in Hoagland solution was harvested and discarded. When the plants reached the second full-shoot growth stage (4 wk from the first harvest), nutrient solutions were replaced with fresh ones. After 48 h in the fresh nutrient solution, shoot and root samples of the control treatments (0 NaCl) were collected and salt treatments were added to the solution. Two NaCl levels, 88 and 132 mM, were added to the nutrient solution. Shoot and root samples were collected after 0, 8, 23, and 50 h exposure to salt in one set of the experiments, and after 0, 48, 104 h or 11 d in the rest. Plant samples were immediately frozen in liquid N_2 and stored at -80 ^{0}C until analyzed.

Protein Extraction and Preparation

Proteins were extracted according to the method of Shuster and Davies (1983), and including the modification by Ramagopal (1987a). Six to 800 mg of frozen tissues were ground extensively under liquid N_2 using a prechilled mortar and pestle, and suspended in 2 ml extraction buffer (0.5 M Tris-HCl, pH 7.5; 0.1 M KCl; 0.05 M Na_2EDTA, pH 7.4; 2% 2-mercaptoethanol, and 0.7 M sucrose). Immediately, an equal volume of water saturated phenol, molecular biology grade, was

Fig. 1. Photograph of alfalfa plants growing in the 70 L container during exposure to NaCl stress.

added to the extract. The mixture was poured into a 15 ml Oak Ridge centrifuge tube and shaken for 15 min at 300 rpm on a Cole Parmer model 51502 shaker. The upper layer (phenol phase) was collected by centrifugation at 10,000 X g for 10 min at room temperature, mixed with equal volume of extraction buffer, shaken for 15 min, and centrifuged. The reextracted phenol phase was collected, mixed with 5 volumes of methanol containing 0.1 M ammonium acetate (NH_4Ac), and placed at -20 ^0C overnight to precipitate proteins. The precipitated proteins were collected by centrifugation at 5000 X g at room temperature, washed three times with 5 ml methanol-NH_4Ac solution, one time with 5 ml cold acetone, and air dried for several min at room temperature. For analysis by one-dimensional sodium dodecylsulfate-polyacrylamide gel electrophoresis (1D SDS-PAGE), purified proteins were suspended in a (0.13 M Tris-Cl PH 6.8, 4% SDS, 20% glycerol, 2% 2-mercaptoethanol), boiled in a water bath for three min, and placed directly on ice to cool. For analysis by two-dimensional isoelectric focusing sodium dodecylsulfate-polyacrylamide (2D IEF-SDS-PAGE), purified proteins were suspended in an O'Farrell (O'Farrell et al., 1975) lysis buffer (9.5 M Urea, 2% Nonidet P-40, 2% Resolyte pH 3.5-10 [BDH] and 5% 2-mercaptoethanol). All protein samples were stored at -80 ^0C until analyzed. Suspended protein samples were clarified in an Eppendorf microcentrifuge (model 5415) for 5 min at 14,000 X g before loading onto gels.

Electrophoresis of Proteins by 1D SDS-PAGE

Proteins were resolved by discontinuous uniform and linear gradient SDS-PAGE slab gels (Laemmli, 1970). Gel type and concentration are described in the figure legends. Vertical slab gel unit SE600 and mini gel unit SE250 of Hoefer scientific were used. Protein concentration was determined by the Bradford method (Bradford, 1976), and equal amounts of protein (~150 μg) were loaded in each well. Two staining methods, Commassie Blue and silver stains (Ausubel et al., 1992, Unit 10.6), were used to visualize protein bands.

Electrophoresis of Proteins by 2D IEF SDS-PAGE

Polyacrylamide isoelectric focusing (IEF) gels containing 5.3% Ampholytes (LKB pH 3.5-10), 2% NP-40, 9 M urea, and 13.3% monomer solution (30% acrylamide/1.8% bisacrylamide) were used in the first dimension. Gels were prefocused for 1 h at 200 V and focused for 11,000-13,000 Vh after loading the protein samples, using 0.085% phosphoric acid anolyte and 0.02 M NaOH catholyte. A SE 125 tube gel unit (Hoefer Scientific) with 12 cm length 1 mm inside diameter tubes was used in the first dimension. The tube gels were equilibrated in 1.5% Tris base (pH 6.8), 2.1% SDS, 10% glycerol, and 0.13% dithiothreitol (DTT) for 25 min with gentle agitation before loading onto the second dimension gels. Vertical discontinuous 6-20% linear

gradient SDS-PAGE (Laemmli, 1970) was used in the second dimension. Gels were stained as described.

RESULTS

Shoots

Salt treatments induced the synthesis of a 41 kilodalton (kDa) polypeptide in shoots of cultivar Regen-Y (Fig. 2) and 11 and 12 kDa in Spredor 2, 5929, and Rambler (Fig. 3). Some shoot proteins disappeared after exposure to salt. A 35 kDa polypeptide disappeared from shoots of cultivar Regen-Y (Fig. 2) and a 106 kDa polypeptide disappeared from shoots of cultivars Spredor 2, Sabre, and Mecca (Fig. 4) and from cultivar 8638C (Fig. 5). The induced changes in shoot protein synthesis were dependent upon NaCl concentration and/or length of exposure. For example, while the 41 kDa polypeptide in cultivar Regen-Y appeared after an 8 h exposure to both salt treatments and continued to be present at 48 and 104 h, the 106 kDa polypeptide in cultivar 8638C (Fig. 5) was induced after 8 h, decreased after 23 h, and disappeared after 50 h at both NaCl levels. A 35 kDa polypeptide in cultivar Regen-Y (Fig. 2) disappeared after 104 h exposure to 88 mM NaCl, but disappeared after only 8 h under the 132 mM NaCl treatment. A 106 kDa polypeptide disappeared in cultivar Spredor 2 and Mecca after 11 days of exposure to 88 mM NaCl and in cultivar Sabre at 132 mM NaCl (Fig. 4). Two proteins of 11 and 12 kDa (Fig. 3) were induced in the cultivar Rambler after 48 h, but

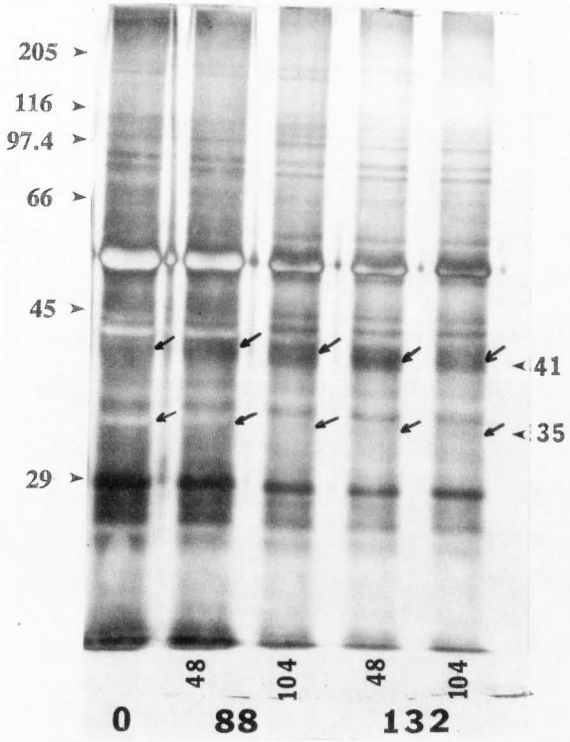

Fig. 2. Silver-stained shoot protein extracts of cultivar Regen-Y separated on 7% SDS-PAGE. The position of protein standards of known molecular weights (kDa) are indicated to the left. The molecular weights of the salt-induced or suppressed proteins, indicated by arrows, are shown to the right. The first and second rows of numbers at the bottom of figure indicate length of exposure to NaCl (h) and NaCl level (mM), respectively.

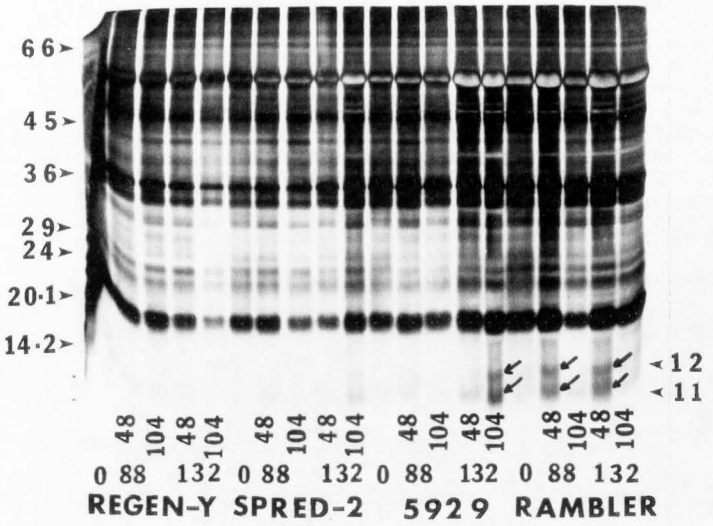

Fig. 3. Silver-stained shoot protein extracts of cultivars Regen-Y, Spredor 2, 5929, and Rambler separated on 15% SDS-PAGE. The position of protein standards of known molecular weights (kDa) are indicated to the left. The molecular weights of the salt-induced proteins, indicated by arrows, are shown to the right. The first and second rows of numbers at the bottom of figure indicate length of exposure to NaCl (h) and NaCl level (mM), respectively.

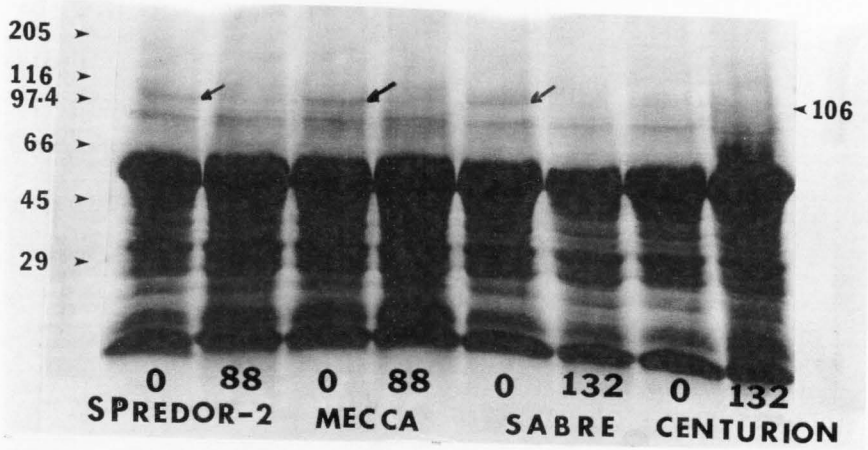

Fig. 4. Commassie Blue-stained shoot protein extracts of cultivars Spredor 2, Mecca, Sabre,and Centurion after 11 days of exposure to NaCl stress. The protein extracts were separated on 6-20% linear gradient SDS-PAGE. The positions of the protein standards of known molecular weights (kDa) are indicated to the left. The molecular weight of the salt-suppressed protein is indicated to the right.

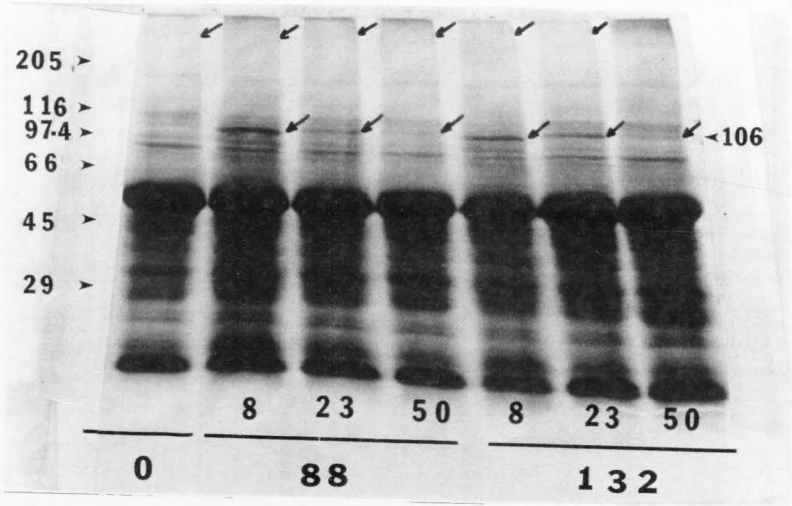

Fig. 5. Commassie Blue-stained shoot protein extracts of cultivar 8638C separated on 6-20% linear gradient SDS-PAGE. The positions of the protein standards of known molecular weights (kDa) are indicated to the left. The molecular weight of the salt-regulated protein is indicated to the right. The first and second rows of numbers at the bottom of figure indicate length of exposure to NaCl (h) and NaCl level (mM), respectively.

disappeared after 104 h at both NaCl treatments. The same proteins were induced in cultivars Spredor 2 and 5929 after 104 h at the 132 mM NaCl level. A 161 kDa polypeptide in cultivars 5331 and Crown II disappeared after 104 h at the 132 mM NaCl level (Fig. 6). The 2D IEF SDS-PAGE of cultivar 5929 shoot (Fig. 7) showed the synthesis of at least 24 new proteins and the termination of more than nine proteins after 11 days exposure to 132 mM NaCl stress.

Roots

Salt treatments induced greater protein changes in roots than in shoots of alfalfa cultivars. In Regen-Y, two polypeptides (23 and 17 kDa) were induced after 104 h at 88 mM NaCl level and four polypeptides (26, 29, 101, and 170 kDa) disappeared after 48 h at 132 mM NaCl level (Fig. 8). In Spredor 2, three polypeptides were induced [67 kDa after 104 h (Fig. 8) and 208 and 133 kDa after 11 days (Fig. 9)] at the 88 mM NaCl level, and three polypeptides (33, 43, and 101 kDa) disappeared after 48 h at the 132 Mm NaCl treatment (Fig. 8). Three polypeptides (97, 133, and 208 kDa) in cultivar Mecca were induced after 11 days at 88 mM NaCl (Fig. 9). In cultivar 5929, two polypeptides were induced (160 and 20 kDa), three polypeptides terminated (16, 95, and 171 kDa) at both NaCl treatments, and one polypeptide (22 kDa) terminated only at the 88 mM NaCl treatment (Fig. 10). One polypeptide (13 kDa) was induced in cultivar Rambler after 104 h at 88 mM NaCl

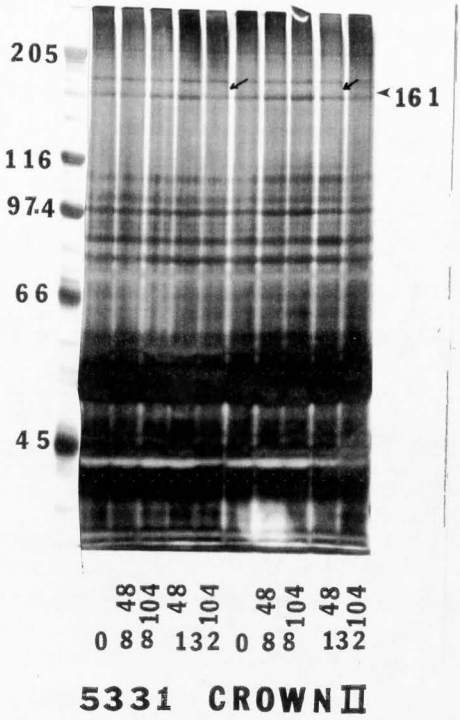

Fig. 6. Silver-stained shoot protein extracts of cultivars 5331, and Crown II separated on 7% SDS-PAGE. Protein standards of known molecular weights (kDa) are indicated in the first lane to the left. The molecular weight of the salt-suppressed protein, indicated by arrow, are shown to the right. The first and second rows of numbers at the bottom of figure indicates length of exposure to NaCl (h) and NaCl level (mM), respectively.

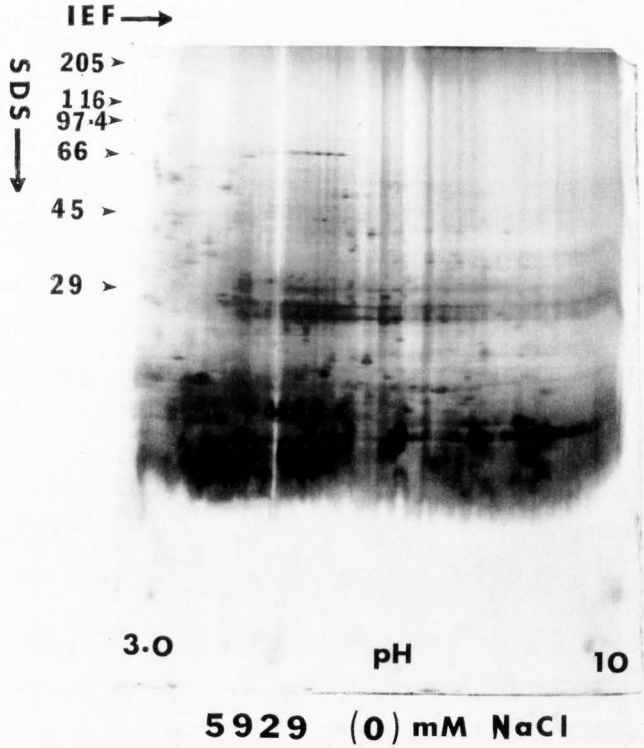

Fig. 7. Two dimensional gel electrophoresis patterns of shoot protein extracts of cultivar 5929. A. protein pattern of control (0 NaCl). B. protein pattern of the 132 mM NaCl treatment after 11 days of exposure. Proteins were separated by IEF gel in the first dimension and by 6-20% linear gradient SDS-PAGE in the second dimension as described in materials and methods. The black arrows indicate the NaCl-induced proteins. The red arrows indicate the NaCl-suppressed proteins. The PH range of the gel and the positions of protein standards of known molecular weights (kDa) are indicated.

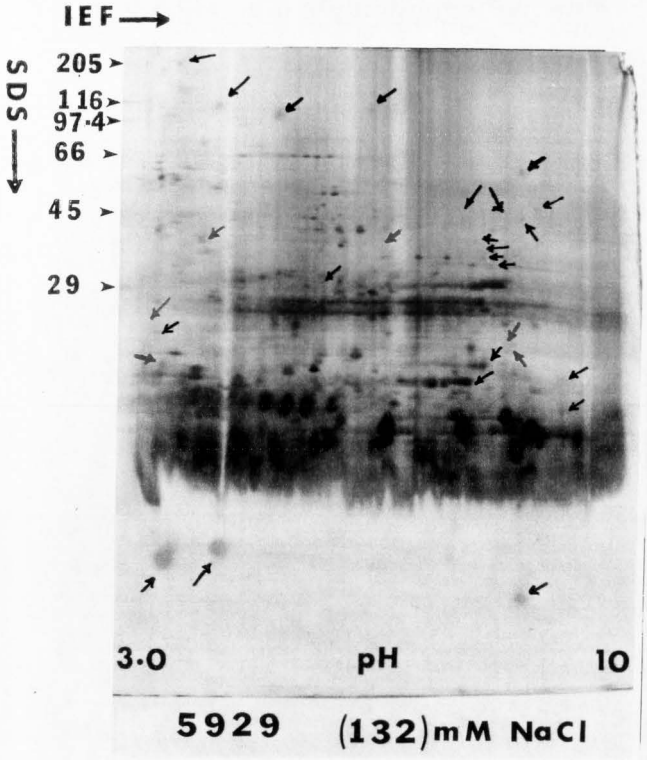

Fig. 7. B.

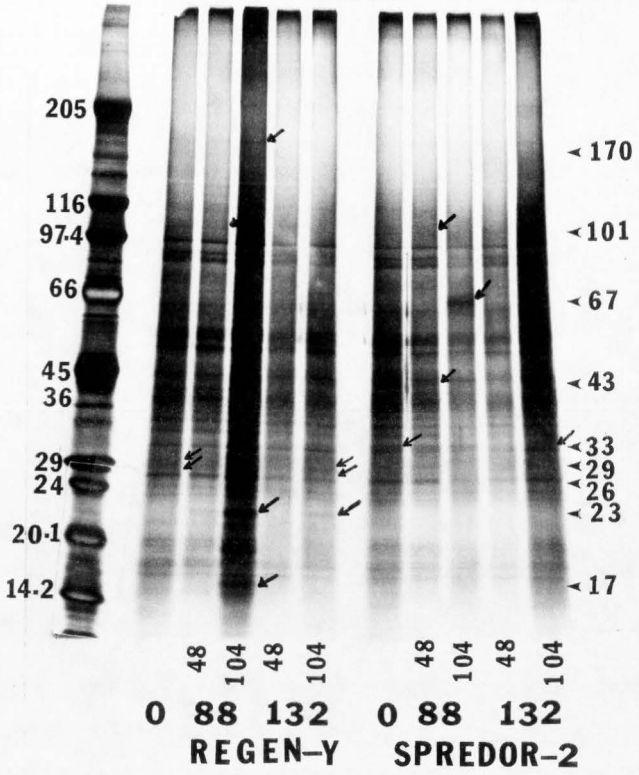

Fig. 8. Silver-stained root protein extracts of cultivars Regen-Y and Spredor 2 separated on 6-20% linear gradient SDS-PAGE. Protein standards of known molecular weights (kDa) are shown in the first lane to the left. The molecular weights of the salt-induced or suppressed proteins, indicated by arrows, are shown to the right. The first and second rows of numbers at the bottom of figure indicate length of exposure to NaCl (h) and NaCl level (mM), respectively.

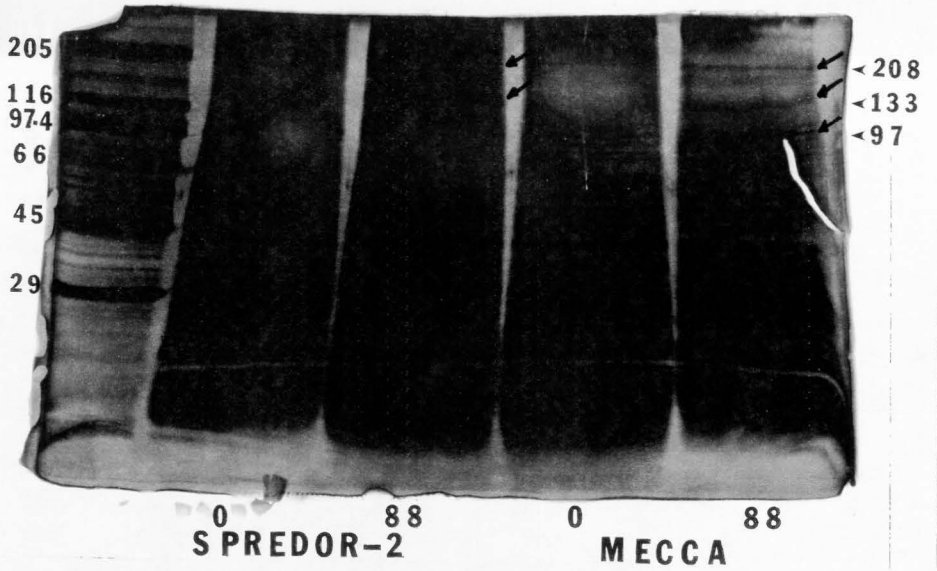

Fig. 9. Silver-stained root protein extracts of cultivars Spredor 2 and Mecca after 11 days of exposure to NaCl stress. The proteins were separated on 6-20% SDS-PAGE. Protein standards of known molecular weights (kDa) are shown in the first lane to the left. The molecular weights of the salt-induced proteins, indicated by arrows, are shown to the right.

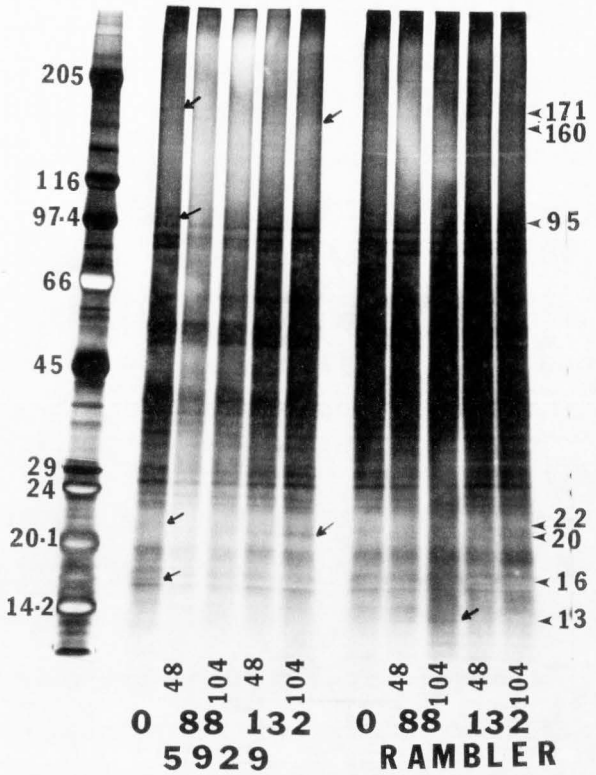

Fig. 10. Silver-stained root protein extracts of cultivars 5929 and Rambler separated on 6-20% linear gradient SDS-PAGE. Protein standards of known molecular weights (kDa) are shown in the first lane to the left. The molecular weights of the salt-induced or suppressed proteins, indicated by arrows, are shown to the right. The first and second rows of numbers at the bottom of figure indicate length of exposure to NaCl (h) and NaCl level (mM), respectively.

(Fig. 10). In cultivar Sabre, a 65 kDa polypeptide was induced after 50 h and a 107 kDa polypeptide was disappeared after 23 h of exposure to 132 mM NaCl (Fig. 11). No detectable protein changes were found in roots of cultivars 5331 and Crown II (Fig. 12).

DISCUSSION

Sodium chloride treatments induced the synthesis of new proteins and reduced or terminated the synthesis of other proteins in alfalfa shoots and roots. This response has been reported in shoots and roots of barley, *Hordeum vulgare* L. (Ramagopal, 1987a, 1987b, and 1988), roots of barley (Hurkman and Tanaka, 1987; Hurkman et al., 1989; Robinson et al., 1990), seedlings of different legumes (Hashim and Campbell, 1988), tomato, *Lycopersicon esculentum* Mill. (Chen and Tabaeizadeh, 1992), and in the artificial amphiploid [*Triticum aestivum* L. (cv. Chinese Spring) X *Elytrigia elongata*] (Gulick and Dvorak, 1987). With few exceptions, the enhanced, reduced, or repressed proteins under salt-stress in our study appeared to be dependent on the length of exposure to salt. A similar response has been reported in barley (Robinson et al., 1990; Ramagopal, 1987b), maize callus (Ramagopal, 1986), and in *Mesembryanthemum crystallinum* L. (Michalowski et al., 1989). Since more than one protein of the same molecular weight might form one band on one-dimensional SDS-PAGE, some bands might consist of more than one protein. Therefore, the total number

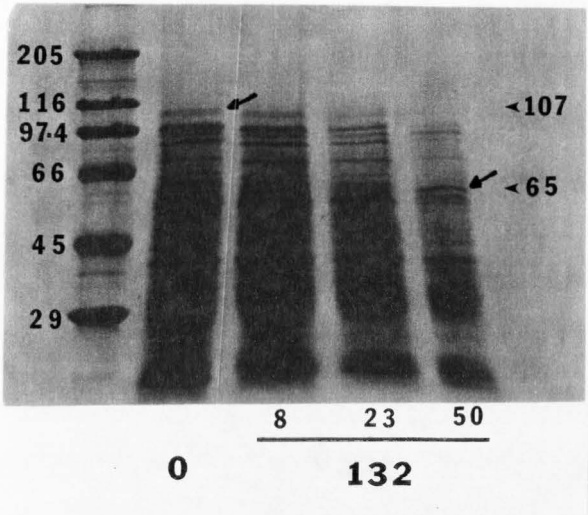

Fig. 11. Commassie Blue stained shoot protein extracts of cultivar Sabre separated on 6-20% linear gradient SDS-PAGE. Protein standards of known molecular weights (kDa) are shown in the first lane to the left. The molecular weights of the salt-induced or suppressed proteins, indicated by arrows, are shown to the right. The first and second rows of numbers at the bottom of figure indicate length of exposure to NaCl (h) and NaCl level (mM), respectively.

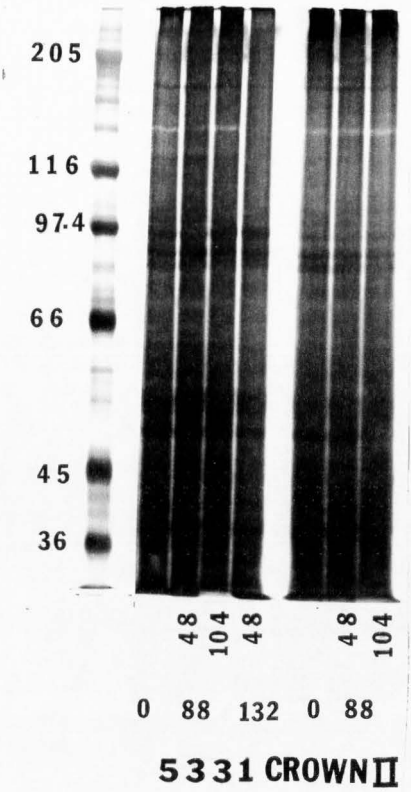

Fig. 12. Silver-stained root protein extracts of cultivars 5331, and Crown II separated on 7% SDS-PAGE. Protein standards of known molecular weights (kDa) are shown in the first lane to the left. The first and second rows of numbers at the bottom of figure indicate length of exposure to NaCl (h) and NaCl level (mM), respectively.

of the salt-induced proteins detected by one-dimensional SDS-PAGE is a minimum estimate of the salt-induced proteins in each treatment. Analyzing the samples by two-dimensional gel electrophoresis (see Fig. 7) will reveal a more exact number of salt-affected proteins. Sodium chloride stress regulates gene expression on the transcriptional level in sugar beets, *Beta vulgaris* L. (McCue and Hanson, 1992), and in the artificial amphiploid between bread wheat X *E. elongata* (Gulick and Dvorak, 1987). In contrast, it was found to regulate both transcription and translation in barley (Robinson et al., 1990), and to regulate the steady-state level of the translatable mRNA in tobacco, *Nicotiana tabacum* L. (Narasimhan et al., 1991), *M. crystallinum* (Ostrem et al., 1987; Michalowski et al., 1989) and barley roots (Hurkman and Tanaka, 1987; Hurkman et al., 1989). Whether NaCl stress regulates gene expression on the transcriptional and/or the posttranscriptional level remains to be determined.

CONCLUSIONS

Sodium chloride stress induced quantitative and qualitative changes in shoot and root proteins of both salt-sensitive and salt-tolerant alfalfa cultivars. These changes were affected by the concentration of NaCl in the growing medium and by the length of exposure to salt stress.

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CHAPTER VI

CONCLUSIONS

The ability of alfalfa cultivars to germinate at the different NaCl concentrations was not related to their postgermination performance (salt tolerance) under those conditions.

This study indicated that there was a genetic influence on Na and Cl ion uptake and accumulation in alfalfa shoots and roots. The strategies of alfalfa cultivars to cope with NaCl stress include exclusion of Na from shoots and Cl from roots or from the whole plant depending on cultivar and NaCl level. The reduction in shoot dry weight was not related to water stress or deterioration in Ca, Mg, or chlorophyll concentration. The shortage in K ion uptake and more directly the toxic effects of high Na and Cl in the plant tissues contributed to the reduction in shoot dry weight. The possible subpartitioning of Na and Cl ions between different tissues, cell types, cell compartments, and/or the changes in protein content and enzyme activity at the high Na and Cl concentrations, independent of total concentration of ions, might also contribute to differences in salt tolerance (shoot dry weight) between cultivars.

Sodium chloride stress induced quantitative and qualitative changes in shoot and root proteins of both salt-sensitive and salt-tolerant alfalfa cultivars. These changes

were affected by the concentration of NaCl in the growing medium and by the length of exposure to salt stress.

APPENDICES

APPENDIX A

MODIFIED HOAGLAND SOLUTION

Table 9. Chemical contents of the modified Hoagland nutrient solution.

Chemical	Conc.	ml L^{-1}	Final conc.	
			nutrient	ppm
$Ca(NO_3)_2$	1M	5	Ca	200.00
KNO_3	1M	5	N	210.00
$MgSO_4$	1M	2	K	234.00
KH_2PO_4	1M	1	S	64.00
			Mg	48.00
			P	31.00
H_3BO_3	500 ppm	1	B	0.50
$MnCl_2.4H_2O$	500 ppm	1	Mn	0.50
$ZnSO_4.7H_2O$	500 ppm	1	Zn	0.05
$CuSO_4.5H_2O$	20 ppm	1	Cu	0.02
MoO_3	10 ppm	1	Mo	0.01
Fe Chelate	4g chelate L^{-1}	10	Fe	2.40

APPENDIX B

ANALYSIS OF VARIANCE TABLES

Table 10. Analysis of variance for alfalfa seed germination percentage.

Source	DF	MS
Blocks	1	2.128E-3
Cultivars	21	1.571E-1**
NaCl levels	2	3.658E-1**
Cultivar x NaCl	42	1.436E-2
Error	65	1.841E-2
Total	131	

** significant at 0.01 probability level.

Table 11. Analysis of variance for alfalfa plant height of the three harvests.

Source	DF	MS
Harvest	2	3656.586**
Cultivar	21	40.659**
NaCl levels	2	6030.768**
Harvest x Cultivar	42	28.971**
Harvest x NaCl	4	28.624**
Cultivar x NaCl	42	22.593**
Harvest x Cultivar x NaCl	84	5.967
Error	396	8.263
Total	593	

** significant at 0.01 probability level.

Table 12. Analysis of variance for number of stems per plant.

Source	DF	MS
Harvest	2	202.302**
Cultivar	21	5.785**
NaCl levels	2	100.842**
Harvest x Cultivar	42	2.544**
Harvest x NaCl	4	22.272**
Cultivar x NaCl	42	1.037*
Harvest x Cultivar x NaCl	84	0.734
Error	396	0.687
Total	593	

*, ** significant at 0.05 and 0.01 probability levels, respectively.

Table 13. Analysis of variance for shoot dry weight of the three harvests.

Source	DF	MS
Harvest	2	0.222E+1**
Cultivar	21	6.519E-2**
NaCl levels	2	2.478E+1**
Harvest x Cultivar	42	2.921E-2
Harvest x NaCl	4	2.320E-2
Cultivar x NaCl	42	4.772E-2**
Harvest x Cultivar x NaCl	84	1.846E-2
Error	396	2.174E-2
Total	593	

** significant at 0.01 probability level.

Table 14. Analysis of variance for shoot dry weight of the third harvest only.

Source	DF	MS
NaCl levels	2	16.655***
Cultivar	21	0.092***
NaCl x Cultivar	42	0.057**
Error	132	0.033
Total	197	

, * significant at 0.01 and 0.001 probability levels, respectively.

Table 15. Analysis of variance for root dry weight of the first and third harvests.

Source	DF	MS
Harvest	1	2.650E+1**
Cultivar	21	2.609E-1**
NaCl levels	2	2.4355E+1**
Harvest x Cultivar	21	8.818E-2
Harvest x NaCl	2	8.204E-1**
Cultivar x NaCl	42	1.367E-1
Harvest x Cultivar x NaCl	42	1.246E-1
Error	264	1.138E-1
Total	395	

** significant at 0.01 probability level.

Table 16. Analysis of variance for root dry weight of the third harvest only.

Source	DF	MS
NaCl levels	2	18.207***
Cultivar	21	0.267*
NaCl x Cultivar	42	0.200
Error	132	0.159
Total	197	

*, *** significant at 0.05 and 0.001 probability levels, respectively.

Table 17. Analysis of variance for the dry weight shoot:root ratio.

Source	DF	MS
Harvest	1	1.847E+1**
Cultivar	21	1.123E-1*
NaCl levels	2	0.141E+1**
Harvest x Cultivar	21	6.648E-2
Harvest x NaCl	2	3.519E-1**
Cultivar x NaCl	42	9.033E-2*
Harvest x Cultivar x NaCl	42	9.880E-2*
Error	264	6.284E-2
Total	395	

*, ** significant at 0.05 and 0.01 probability levels, respectively.

Table 18. Analysis of variance for chlorophyll concentration in leaves of the third harvest.

Source	DF	MS
NaCl levels	2	67454.672**
Cultivar	21	11363.206
NaCl x Cultivar	42	7727.624
Error	132	9406.278
Total	197	

** significant at 0.01 probability level.

Table 19. Analysis of variance for Na concentration in shoots and roots of alfalfa plant.

Source	DF	MS
NaCl levels	2	9.000***
Cultivar	21	0.018***
Plant part	1	10.702***
NaCl x Cultivar	42	0.015***
NaCl x Part	2	2.379***
Cultivar x Part	21	0.011*
NaCl x Cultivar x Part	42	0.011*
Error	264	0.007
Total	395	

*, *** significant at 0.05, and 0.001 probability levels, respectively.

Table 20. Analysis of variance for K concentration in shoots and roots of alfalfa plant.

Source	DF	MS
NaCl levels	2	0.252***
Cultivar	21	0.006***
Plant part	1	4.089***
NaCl x Cultivar	42	0.004***
NaCl x Part	2	0.113***
Cultivar x Part	21	0.003
NaCl x Cultivar x Part	42	0.005***
Error	264	0.002
Total	395	

*** significant at 0.001 probability level.

Table 21. Analysis of variance for Ca concentration in shoots and roots of alfalfa plant.

Source	DF	MS
NaCl levels	2	6.763***
Cultivar	21	0.012
Plant part	1	0.117***
NaCl x Cultivar	42	0.013*
NaCl x Part	2	0.069***
Cultivar x Part	21	0.016**
NaCl x Cultivar x Part	42	0.010
Error	264	0.008
Total	395	

*, **, *** significant at 0.05, 0.01, and 0.001 probability levels, respectively.

Table 22. Analysis of variance for Mg concentration in shoots and roots of alfalfa plant.

Source	DF	MS
NaCl levels	2	0.565***
Cultivar	21	0.007***
Plant part	1	0.406***
NaCl x Cultivar	42	0.003*
NaCl x Part	2	0.100***
Cultivar x Part	21	0.003*
NaCl x Cultivar x Part	42	0.002
Error	264	0.002
Total	395	

*, *** significant at 0.05, and 0.001 probability levels, respectively.

Table 23. Analysis of variance for Cl concentration in shoots and roots of alfalfa plant.

Source	DF	MS
NaCl levels	2	1481.810***
Cultivar	21	23.045***
Plant part	1	1189.101***
NaCl x Cultivar	42	18.836***
NaCl x Part	2	322.756***
Cultivar x Part	21	20.323***
NaCl x Cultivar x Part	42	12.043***
Error	264	1.083
Total	395	

*** significant at 0.001 probability level.

Table 24. Analysis of variance for H_2O content in shoots and roots of alfalfa plant.

Source	DF	MS
NaCl levels	2	68.538***
Cultivar	21	1.726***
Plant part	1	3.839*
NaCl x Cultivar	42	1.133*
NaCl x Part	2	0.953
Cultivar x Part	21	0.578
NaCl x Cultivar x Part	42	0.681
Error	264	0.691
Total	395	

*, *** significant at 0.05, and 0.001 probability levels, respectively.

CURRICULUM VITAE

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(March 1993)

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