

INFLUENCE OF SODIUM CHLORIDE ON TEPARY
(PHASEOLUS ACUTIFOLIUS GRAY) AND NAVY
(P. VULGARIS L.) BEANS

by

Nabeel Yonnis Alislail

A Dissertation Submitted to the Faculty of the
DEPARTMENT OF PLANT SCIENCES
In Partial Fulfillment of the Requirements
For the Degree of
DOCTOR OF PHILOSOPHY
WITH A MAJOR IN AGRONOMY AND PLANT GENETICS
In the Graduate College
THE UNIVERSITY OF ARIZONA

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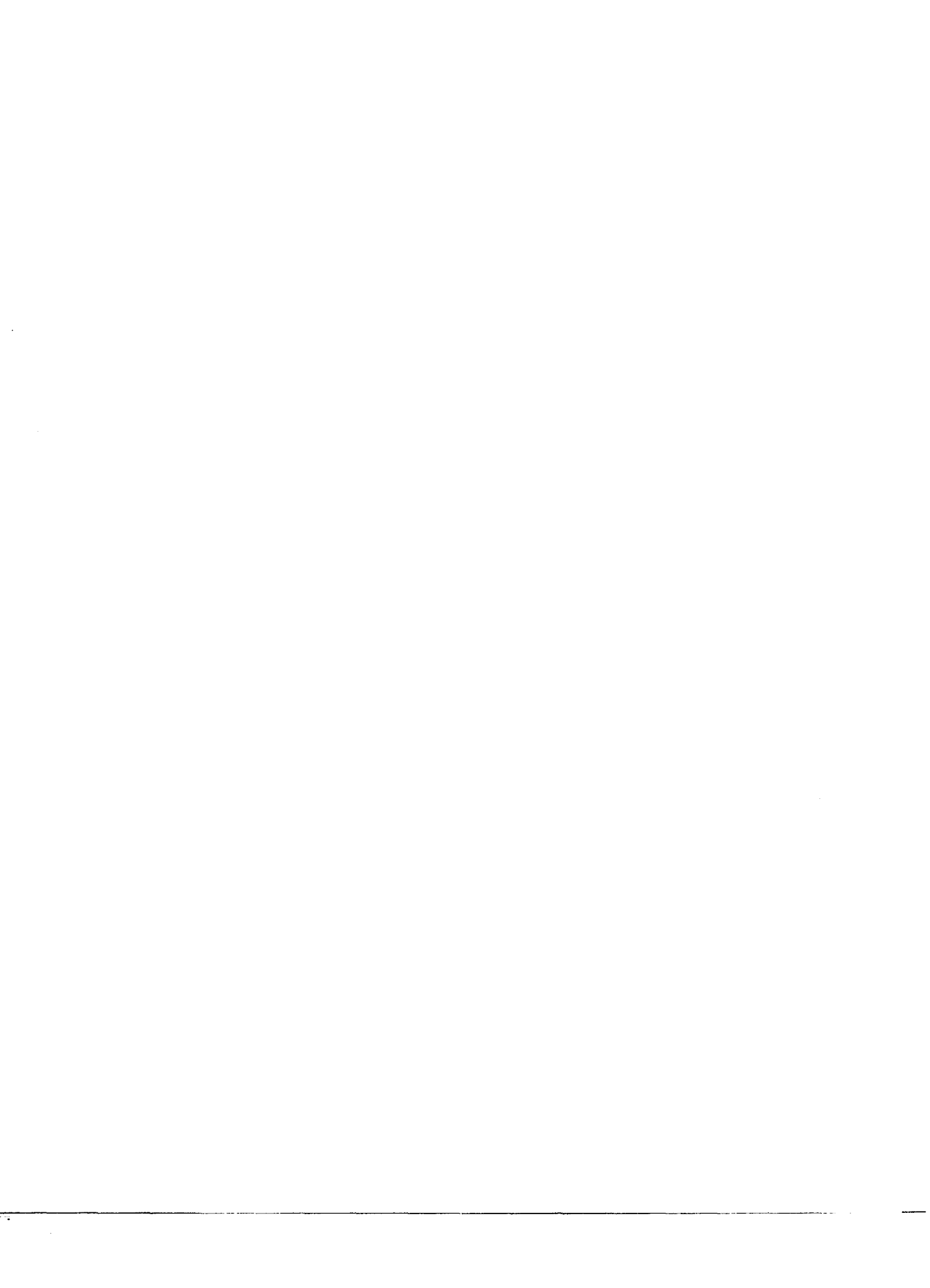
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**Influence of sodium chloride on tepary (*Phaseolus acutifolius* Gray)
and navy (*Phaseolus vulgaris* L) beans**

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The University of Arizona, 1990

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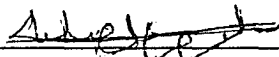
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ABSTRACT

Shoot and root fresh and dry weight, shoot length, leaf area, leaf area index and relative growth rate of 14 day old tepary bean (Phaseolus acutifolius Gray) and navy bean (Phaseolus vulgaris L.) seedlings were reduced following treatment with NaCl solution exhibiting osmotic potential of either -0.25, -0.50, and -0.75 MPa. Salinity reduced the growth of navy bean more than tepary bean. The physiological basis of the adaptive response of tepary bean seedlings to salt stress was explored by determining the water and osmotic potentials, relative water content, free amino acid and sugar concentrations, distribution and levels of inorganic ions within the seedlings and ATPase activity of the root plasma membrane.

Salinity led to an osmotic adjustment in the leaves and the proximal part of the root of tepary bean. Turgor remained almost constant whereas osmotic and water potential and relative water content declined following the salt treatments. The osmotic adjustment of the leaves and proximal part of the roots was -1.7 MPa and -1.2 MPa, respectively, in seedlings treated with -0.75 MPa NaCl solution. Free amino acids and sugars increased under salinity stress in both species but they increased more in the tepary bean. Glucose was the most abundant free sugar. The nonstructural carbon solutes contributed -0.15 MPa to the seedling's osmotic adjustment whereas Na, Cl, K and Ca ion levels contributed -0.85 MPa. However, the levels of these solutes were not large enough to account for the total osmotic adjustment observed in the salt treated seedlings.

The distribution of Na, Cl, K, and Ca ions within the tepary bean and navy bean seedlings showed that these bean species were able to retain Na and Cl ions in the proximal part of the root and base of the stem excluding them from the upper part of the seedlings. This exclusion mechanism was more efficient for the tepary bean than navy bean. Root plasma membrane ATPase activity tripled when tepary bean was treated with -0.50 MPa NaCl but only doubled with -0.75 MPa NaCl, suggesting that ATPase activity was inhibited by higher levels of NaCl. Sodium chloride when applied as a shock treatment to tepary bean roots loaded with ^{14}C -methylglucose caused the leakage of labelled glucose from the roots.

Electron microscope examination showed no structural difference in the cortical cells of tepary bean grown under salinity stress. An increase in the leaf thickness and a reduction in the root and stem diameter of tepary bean seedlings treated with NaCl was observed with light microscope.

This study shows that tepary bean has specific strategies to overcome the impact of salinity through osmotic adjustment and exclusion of Na and Cl ions from the stems and leaves by retaining these ions in the proximal part of root and stem base.

CHAPTER 1

INTRODUCTION

Saline soil can develop either naturally from exposure to sea water and weathering of saline rocks or artificially from irrigation water which contains additional salt and fertilizers. Soil salinity is a very ancient problem. Raloff (1984) reported that the Sumerian culture of the Tigris-Euphrates was affected by salinity when the fertile land was changed into a saline desert land. This region became a food importer, even though it had a smaller population than had previously existed. Salinity problems still exist today, especially in arid and semi-arid regions of the world because of inadequate leaching, high evaporation rate, restricted drainage, and the dependence on irrigation to provide the water needed for crops. All of these factors contribute to salt accumulation in the soil. Thus, salinity may be the ultimate limitation to irrigated agriculture (Rains, 1979). Epstein et al. (1980) reported that approximately 9×10^8 hectares of earth land is affected by salinity, an area about three times greater than all of the land that is presently irrigated. Also, several tens of thousands of hectares are lost every year due to the effect of salt accumulation. As the world demand for food increases, exploiting marginal areas such as arid lands becomes a necessity. These marginal areas are characterized by high salinity in both the soil and the water (Epstein, 1962). Accumulation of high levels of sodium salts in soil changes the soil's physical characteristics. Also, salinity affects the water relations of plants and the

physiological-biochemical functions of plant's cells (Greenway and Munns, 1980).

The impact of salinity on agriculture could be alleviated either by removing the salt from the soil, through adequate drainage and leaching programs which are very expensive, or by using salt-tolerant plant species which can maintain normal growth and metabolism under saline conditions. The later method seems to be a more promising approach (Greenway and Munns, 1980).

One of the promising plant species that may be salt tolerant is tepary bean (Phaseolus acutifolius Gray). Because of the growth characteristics of tepary bean, Nabhan and Felger (1978) suggested that they are suitable for extensive cultivation in arid lands with high salt levels. Numerous reports have documented that tepary bean, a native to the southern United States and Mexico, exhibits drought and heat resistance (Freeman, 1913). Domesticated white tepary bean can produce as much as 800 kg/acre in the Sonoran Desert when supplemented with water whereas they produced more than 1867 kg/acre in areas where climatic conditions are less extreme such as Fresno, California (Hendry, 1918). In contrast, navy bean (Phaseolus vulgaris) is very sensitive to drought, heat, and salinity (Ayers and Westcot, 1985; Meiri and Poljakoff-Mayber, 1970). Tepary bean out-performed navy bean under salinity conditions. Yield was higher for tepary bean grown under 10.8 mmhos/cm soil salinity than for navy bean (Coons and Pratt, 1988). Under salinity conditions, tepary bean germination was 43% while navy bean germination was only 23% (Goertz and Kobriger, 1986a). Also, leaf water potential was higher for

tepary bean (-1.1 MPa) than for navy bean (-1.6 MPa) and plant height was 31 cm for tepary bean and 15 cm for navy bean in a field with 6500 ppm soluble soil salts (Goertz and Kobriger, 1986b).

No research has been published about the mechanisms of salt tolerance in tepary bean. However, some work was conducted to determine the basis for drought tolerance in tepary bean. Tepary bean has certain strategies to deal with drought, among them are (a) closing the stomata at a much higher water potential than those of navy bean, thus delaying dehydration of tissue; (b) deeply penetrating root system which reach up to 89 cm compared to other types of bean (Phaseolus vulgaris L. cvs Pinto) which reaches only 71 cm (Markhart, 1985); (c) short life cycle (Nabhan and Felger, 1978), (d) also, tepary bean exhibits reduced water and osmotic potentials more than navy and pinto beans (Parsons and Howe, 1984).

Plants in nature have evolved several adaptive mechanisms to deal with the presence of salt in their environment. Three adaptive mechanisms have been reported for plants which survive in saline environments. They are: firstly, avoidance of salinity which basically allows a glycophyte to grow at a particular time and/or place and survive when salt level is low; secondly, exclusion of salts, which occurs when ions are prevented from entering into the plant cells or by pumping ions out of the root cells once they enter; and lastly, osmotic adjustment resulting from the synthesis and accumulation of additional solutes (Stavarek and Rains, 1983).

The objectives of this research were to investigate the growth response of tepary bean to various levels of NaCl treatment and to explore the physiological bases of the adaptive response of tepary beans to salt stress by investigating osmotic adjustment, distribution of inorganic ions within the seedlings and the ATPase activity in the root plasma membrane.

CHAPTER 2

LITERATURE REVIEW

Salinity can affect plant growth in three main ways. Firstly, salinity tends to affect plant growth by reducing water availability and water relations of the plant (Rains, 1979). Secondly, excess of quantities of toxic ions like Na and Cl tend to modify plant metabolism (Lagerwerff and Eagle, 1961). Lastly, the predominance of Na and Cl ions in the soil water potentially reduces the uptake of nutrient ions by the roots (Nanawati and Maliwal, 1973).

Any of these effects of salt, impact the energy status of the plant. Plants consume additional respiratory energy to accumulate ions or synthesize solutes for producing osmoticum to lower osmotic potential, and to transport additional ions within the plant under salinity conditions. This expenditure of energy used for saline adaption may result in a reduced growth and therefore reduced yield (Yeo, 1983).

Salinity and Plant Growth

The affect of salinity on plants may vary depending on their stage of development. Sensitivity of plants to salinity may be quite different during germination than at the later stages of development (Bernstein and Hayward, 1958). Lunin et al. (1963) conducted an experiment to determine the effect of salinity at various growth stages on yield of several vegetable crops. Yield was reduced significantly for all crops with increasing salinity. With the exception of beet (Beta vulgaris L.) and

broccoli (Brassica oleracea L.), vegetative growth and reduction of yields were significantly less when plants were salt stressed at more mature growth stages. Tomato (Lycopersicon esculentum L.) and pepper (Capsicum annum L.) were the only crops tested that showed a significant interaction between growth stage and salinity. Such a significant interaction was not found for beet, broccoli, spinach (Spinacia oleracea L.), and onion (Allium cepa L.).

Dumbroff and Copper (1974) found that the early seedling stage of tomato development was the stage most affected by salinity. Growth rates remain severely restricted even after removal of salt stress during this period, but plants stressed at later times resumed growth similar to the control as soon as they were returned to base nutrient solution. Pearson and Bernstein (1959) investigated the effect of soil salinity on three development stages of rice (Oryza sativa L.) and they reported that salinity inhibited growth more severely at earlier stages of growth than at later stages. Salinity treatment during the tillering stage inhibited growth twice as much as during heading.

Bernstein and Hayward (1958) stated that salinity retards vegetative growth and delays flowering. Dumbroff and Cooper (1974) also observed that bud and flower formation in tomato were delayed due to the salt treatment. Other experiments showed that salinity did not affect the time to flowering and first harvest of tomato fruit grown at various salinity levels (Hall, 1983). Stroganov (1964) reported that tomato plants were more sensitive to salinity during the flowering stage than other growth stages. Stroganov's results, however, could not be

attributed totally to the stage of the plant development because the length of the salt treatment period was more than that reported by other researchers (Dumbroff and Cooper (1974).

The salt tolerance of four barley (Hordeum vulgare L.) and two wheat (Triticum aestivum L.) varieties was tested on artificially salinized field plots. Adding salinity during grain development and maturation did not affect the yield of these grains (Ayers et al., 1952). Greenway (1965) imposed salinity on two varieties of Hordeum vulgare during early tillering and continued until grain formation. Relative growth rates and grain formation of barley were not affected by salt treatment.

Salinity also influences plant growth according to plant species. Neiman (1962) studied the growth response of twelve crop species grown over a range of NaCl treatments and reported that fresh weight of salt-sensitive species was severely depressed whereas fresh weight of salt-tolerant species was stimulated by salinity. Salinity stimulated growth of salt tolerant species at low concentration but reduced their growth at high concentration. Milford et al. (1977) reported that NaCl increased sugar beet dry weight and the area, thickness, and succulence of leaves. Acosta-Nunes and Ashton (1981) reported a stimulation of fresh weight and shoot length of lettuce (Lactuca sativa L.) with salinity treatments of 0.23 and 0.30 MPa. They suggested that stimulation occurred because high Cl ion levels in tissues induced high turgor and enzyme stimulation. Jennings (1976) stated that C₄ plants require Na ions in trace amounts for photosynthetic carbon fixation. He also reported that tomato responded

to trace amounts of Na ions by increasing carbon fixation. In bean plants subjected to NaCl treatment Gauch and Waldleigh (1944) reported that Cl ions led to more succulent leaves.

Jeschke and Wolf (1988) exposed castor bean plants (Ricinus communis L.) to different NaCl levels. The addition of NaCl above 40 mol m⁻³ caused slight reduction in bean growth whereas 80 mol m⁻³ inhibited growth by 50%, suppressed branching, and reduced size and epinasty of leaves. The leaf epinasty consisted of curvature of petioles and upward tilting of blades relative to the petiole. Thereby, the blades of leaf one and two from the top were arranged almost parallel to incident light. Ayers and Westcot (1985) indicated that yield of navy bean was reduced by 50% using 2.4 ds/m irrigation water and yield was zero with 4.2 ds/m irrigation water. Yield of wheat was reduced by 50% with irrigation water that contains 8.7 ds/m salts and to zero percent at 13 ds/m salt. Papadopoulos and Rendig (1983) indicated that growth of tomato roots was reduced to a lesser extent than the stems when subjected to high salinity levels. Salama et al. (1981) used sand culture technique to investigate the effect of salinity on tomato and rocket (Hesperis matronalis L.) growth. Salinity significantly reduced the shoot growth of both species. Similar results were obtained by Tompkins and Hung (1981), who reported that dry weight of shoots of both tomato and sugar beet was significantly reduced following salt treatment.

Acosta-Nunes and Ashton (1981) showed that high levels of salinity inhibited growth of tomato and lettuce. High levels of salinity caused an increase of the osmotic potential from -0.30 to -0.96 MPa and decreased

fresh weight, root length, and shoot length in both species. At the highest salinity level, root and shoot elongation were essentially blocked and fresh weight was reduced to 72 and 87% for tomato and lettuce, respectively. When bean plants were grown under saline conditions, growth was depressed and dry weight reduced. The shoot, especially the leaves, were affected more than roots by salinity (Meiri and Poljakoff-Mayber, 1970).

Salinity may also lead to changes in plant color. In Atriplex nummularia grown at salinity levels above 10 meq/L, leaf color changed from dark green to light green (Greenway, 1968). Chlorosis was observed in bean plants grown under various salinity levels (Gauch and Wadleigh, 1944).

In an attempt to overcome growth inhibition due to salinity, O'Leary (1971) suggested growing plants in enclosures to maintain high humidity and applying cytokinins to plant foliage. Foliar application of cytokinin was to offset the lack of hormones transported from the root to leaves when plants are subjected to salinity. Results of Hoffman et al. (1971) supported O'Leary's suggestion. Cotton (Gossypium hirsutum L.) plants grown under high salinity level and in 90% relative humidity resulted in 40% increase in growth as compared to salinity treated plants grown under 25% relative humidity. Wagenent et al. (1983) found that increased frequency of water application increased yield of navy bean at high and low levels of salinity.

Salinity and Leaf Anatomy

The growth processes of leaf initiation, unfolding, and expansion are expressions of the cellular processes of division, expansion, and differentiation. All these processes continue through the development of the leaf, and therefore, all of these processes may be affected by salinity.

Bernstein (1961) found that osmotic potential of both the above- and below-ground plant parts of cotton and pepper plants decreased over a wide range of salinity treatments which would permit plant growth. Leaf enlargement was highly sensitive to water stress and was one of the first growth processes affected by a decrease in leaf water potential. For example, leaf enlargement in corn (Zea mays L.) ceased at a leaf water potential of -0.7 MPa (Acevedo et al., 1971).

Salinity stress will first cause a reduction in the rate of leaf surface additions, followed by a cessation of leaf expansion as the stress intensifies, and lastly, an inhibition of cell division during severe stress. If the stress is not too severe, growth resumption after removing salt treatment is rapid, suggesting a physical process is involved in leaf development (Terry et al., 1983). Jennings (1976) stated that salinity suppresses leaf expansion. Such suppression occurs even with halophytes at high salt concentrations. In their study on bean plants, Meiri and Polojakoff-Mayber (1970) reported that leaf expansion was retarded immediately at the beginning of salinization and retardation was proportional to the level of salinization.

Wignarajah et al. (1975), in their study on bean, noticed a reduction in leaf cell division as a result of being exposed to salinity. Cell division and DNA replication ceased with the onset of increased stress. At severe levels of water deficit, 50% or more loss of tissue water, most cell nuclei showed a reversible aggregation for chromatin (Creveaceous et al., 1976), which presumably would prevent both DNA replication and transcription. Protein synthesis is also inhibited by osmotic stress imposed on excised plant parts. Inhibition of protein synthesis was demonstrated in leaf discs cut from plants subjected to salinity stress (Ben-Zion et al., 1967). Exposure of bean leaves to salt-containing media decreased chlorophyll content, increased the rate of respiratory O_2 uptake, increased the number of mitochondria, and led to abnormal chloroplast fine structure (Siew and Kelin, 1968).

In plants grown at high salinity level, rapid uptake of ions could result in a build-up of high ion concentrations in the cell walls of leaves, particularly when cells reach flux equilibrium. This would cause adverse effects on the water relations of individual leaf cells (Oertli, 1968).

Longstreth and Nobel (1979) studied the effects of salinity on the leaf anatomy of cotton, bean, and Atriplex patula L. They found that increasing salinity led to a higher ratio of mesophyll surface area to leaf area for cotton and bean and to a lesser extent for Atriplex patula L. (salt-tolerant species). Photosynthesis did not increase as a response to the increase in the internal leaf surface because the mesophyll resistance to CO_2 exchange increased with increasing salinity level.

Meiri and Poljakoff-Mayber (1967) investigated the effect of NaCl on growth of bean leaves and reported that salinity reduced leaf area. Growth of leaf area ceased before growth of leaf thickness. Reduction of leaf area seems therefore to be a result of reduction in cell size. Leaves of salt-affected bean plants are often thicker than leaves of non-saline control plants and thickening of the leaf seems to be due mainly to enlargement of the palisade layer. Leaves of the salt-affected plants are smaller than those of the control plants, but there are more cells and stomata per unit leaf area. The epidermal cells are therefore smaller in the salt-affected plants than in the controls.

In contrast to what Meiri and Poljakoff-Mayber (1967) reported, Hayward and Long (1941) found that palisade tissue in the leaves of tomato plants grown in high sodium sulfate cultures was very compact. They also indicated that leaflets of plants grown at high salt concentrations were thicker and more succulent. This was correlated with the loose arrangement of mesophyll cells. Many other investigators have reported an increase in leaf succulence in glycophytes as a response to salinity. Chloride ions were claimed to be responsible for succulence (Gauch and Wadleigh, 1944; Lagerwerff and Eagle, 1961; Milford et al., 1977; and Scanlon and Morgan, 1982).

In halophytes, growth of Atriplex nummularia was optimum at 100 to 200 meq/L of NaCl, with leaf size being largest at 100 meq/L. The leaf and stem water content (percent of dry weight) generally increased with increasing chloride concentration, particularly in the NaCl treatment. Greenway (1968) concluded that Na ions stimulated leaf growth and led to

leaf succulence. In Atriplex halimas, salinity also led to increased leaf area and succulence. This resulted in an increase of leaf area available for transpiration and photosynthesis (Gale and Poljakoff-Mayber, 1970).

Salinity and Accumulation of Compatible Organic Solutes

Salinity induces an accumulation of organic solutes in some glycophytes as well as in halophytes. Flowers et al. (1977) indicated that osmotic adjustment occurs in halophytes so that a reduction in cell water potential will be achieved while cell pressure potential remains relatively constant. In glycophytes, Greenway and Munns (1980) pointed out that osmotic adjustment can occur due to synthesis and accumulation of solute or by loss of cell pressure potential, which will lead to growth reduction.

Bernstein (1961) proposed an explanation for the osmotic adjustment. He indicated that as soil water potential decreases due to increased salinity, the plant water potential must decrease to maintain a negative water potential between the root environment and the plant. Plants can reduce their water potential either by reducing the pressure potential, which has a detrimental effect on plant growth or by decreasing the plant osmotic potential, which is called osmotic adjustment.

Gorham et al. (1985) summarized various hypotheses regarding the principal features of salt tolerant mechanism at the cellular level. They have introduced three interrelated propositions. First, under saline conditions, the large quantities of salt (mainly, but not exclusively, NaCl) are absorbed into the leaves and contribute to osmotic adjustment.

The salts accumulated mainly in the vacuole when tissue concentrations exceed about 200 mol m^{-3} i.e., osmotic pressure greater than 0.9 MPa. Secondly, the concentration of the inorganic ions in the cytoplasm (especially of meristematic cells) is held in the range of 100 to 200 mol m^{-3} , and the cytoplasm shows a strong selectivity for potassium over sodium, magnesium over calcium, and phosphate over chloride or nitrate. And lastly, under hyperosmotic conditions (cell osmotic pressure greater than 0.9 MPa), the maintenance of osmotic (water potential) equilibrium across the tonoplast requires the accumulation of non toxic compatible solutes in the cytoplasm.

Hasegawa et al. (1986) reported that numerous organic solutes accumulated to significant levels in salt-adapted cells. Level of sugars, free amino acids, and proline increased with the level of adaption. Proline accumulation was correlated with osmotic potential changes, both in presence or absence of osmotic stress. Moore (1975), in his study with halophyte plants that were exposed to salt stress, found that proline accounted from 30 to 70% of the free amino acids.

Storey and Wyn-Jones (1977) treated fourteen plant species which include salt-tolerant halophytes, semi-tolerant glycophytes, and salt-sensitive glycophytes with salinity and determined the choline, betaine, and proline levels in these plant species. Their results showed that choline content in salt treated plants was not related to the salt concentration in which the plants were grown. Betaine levels were similarly high in halophytes before and after salinity treatments, whereas betaine was significantly higher in semi-tolerant glycophytes only after

NaCl treatment. In salt-sensitive glycophytes, betaine levels were low before and after NaCl treatment. Proline level increased in all species after exposure to NaCl, with the most salt-tolerant species accumulating the greatest amounts of proline. Weimberg (1987) in his study with wheat found that in the control plants, sucrose and betaine concentrations were 4 and 48 $\mu\text{mol (g dry weight)}^{-1}$, respectively. At -1.2 MPa salt treatment, sucrose increased thirty fold but betaine increased only two and a half fold.

Steward and Lee (1974) pointed out that the primary role attributed to the organic solutes, especially proline is that of osmotic adjustment. Unlike inorganic ions, the compatible organic solutes do not interfere with the cytoplasmic activities. Thompson et al. (1966) suggested that the accumulated solutes, especially proline, may act as a storage compound for energy and reduce nitrogen and carbon compounds during post-stress metabolism.

Tal et al. (1979) investigated proline accumulation in cultivated and two salt and drought-tolerant wild relatives of tomato. Their results showed that the cultivated species accumulated more proline than the wild relatives under both salinity and drought stress. Based on this result, the authors concluded that proline accumulation does not play an important role in either salt or drought tolerance.

Chu et al. (1976) studied specific ion effects on proline accumulation in barley. They pointed out that proline accumulation was inhibited by Na, whereas Cl and K ions had no significant effect on proline accumulation.

Hanson et al. (1979) and Hasegawa et al. (1986) reported that proline accumulation does not initiate salinity adaptation but may accumulate as a result of the initiation of other responses to salinity stress. However, not all plants accumulated proline at the same rate. Hanson et al. (1979) found that the rate of proline accumulation during stress was faster in susceptible cultivars than in the non-susceptible ones. Riazi et al. (1985) found that proline accounted for only 5% of the total osmotic adjustment after 24 hr of stressing barley with -0.8 MPa polyethylene glycol (PEG). When NaCl was used, similar responses were obtained except that proline increase per MPa unit decrease in osmotic potential was two to three times greater than in PEG-stressed tissue. They reached the conclusion that proline appears to be a significant but minor contributor to osmotic adjustment.

Salinity and Water Relations of the Plant

Generally, salinity lowers the water potential of the soil. Plants obtain water by maintaining a more negative water potential than the soil. Plants achieve the reduction in water potential by producing organic osmotica e.g., sugars and amino acids or accumulating ions from the external media, which leads to the build-up of the internal solute sufficient to reduce the water potential and maintain water flow into the plant. Such mechanisms are very expensive in terms of energy utilization by the plant and significant amounts of carbon are consumed that would otherwise be used for growth (Rains, 1979).

Lowering of water potential occurs when either turgor or osmotic potential are reduced (Bernstein, 1961). Hasio (1973) divides water stress into three categories. He considers lowering the water potential by several bars to be a mild stress, and lowering the water potential by more than several but less than -12 to -15 bars to be moderate stress. In both of these cases, plants tend to lower their osmotic potential due to the increase in solute concentration within the cell. The third category is severe stress, which occurs by lowering the water potential by more than -15 bars. Greenway and Munns (1980) discussed the effect of highly saline soil on water potential of plants. They concluded that plants grown under severe stress will lose their turgor pressure, decrease their growth, and possibly die.

O'Leary (1969) found that increasing the salinity of the growth solution by addition of NaCl reduces the permeability of kidney bean roots to water flow. Very little water could be forced through the roots under pressure as compared to roots from plants grown in non-salinized solutions. Relative water content of the bean leaves decreased with increasing salinity. Diffusion resistance of the leaves was considerably higher in plants grown in salinized solutions. O'Leary (1969) suggested that physiological drought will take place even if osmotic adjustment occurs because of the increase in resistance in the water flow pathway from external solution. Hayward and Spurr (1943) conducted an experiment to determine the effect of salinity on the flow of water into corn roots. They found a reduction in water flow into the roots.

Boyer (1965) investigated the effect of salinity on water potential of cotton plants subjected to salinity. Change in water potential of leaf tissue followed changes in the water potential of the root medium, and changes in the osmotic potential of leaf tissue exceeded those of the root medium by a factor of 1.2 to 1.5.

The effect of salinity on water balance of three wild relatives of the cultivated tomato and two tomato cultivars was studied. Unlike bean and corn, salinity induced succulence in all cultivars, especially in stem and leaf of wild cultivars (Tal and Shannon, 1983).

Some reports also suggest that salinity increases water potential in glycophytes. Tal and Shannon (1983) and Milford et al. (1977) reported an increase in water potential for tomato and sugar beet, respectively, when subjected to salinity.

Abel and MacKenzie (1964) investigated salt tolerance of soybean (Glycine max L.) varieties and they found that osmotic adjustment occurred in salt-tolerant rather than salt-sensitive varieties. In a comparative study between cultivated tomato and two wild tomato species, fruit size decreased in the cultivated species and remained unchanged in the wild species following salt treatment, while water content decreased under salinity in both cultivated and wild plants (Tal, 1971).

Bernstein (1961) reported that the osmotic potential of roots and above-ground parts of cotton and pepper plants decreased with increases in the salinity of the growth medium over as wide a range of salinity level that would permit growth. Since osmotic potential differentials between plant parts and root media are maintained, turgor does not de-

crease. The reduction of osmotic potential is suggested as a likely limiting factor for growth under saline conditions.

Gale et al. (1967) studied the water balance of onion bean, and cotton plants grown under various salinity levels. Osmotic potential of onion leaf sap did not adjust to salinity, and consequently water potential and turgor were reduced. Osmotic potential of bean and cotton leaf sap decreased with the salinity treatment and turgor was maintained. Gale et al. (1967) concluded that salinity does affect water balance of plants, and that the nature and degree of the affect will depend upon climatic conditions and may be very different among plant species and in the same species at different periods of their development.

Slatyer (1961) investigated the water balance of tomato plants subjected to different salinity levels. At the beginning of the treatment, water was lost from the plants and plants wilted. However, recovery from the wilting took place in all treatments after 28 hr following salinity treatment. This recovery of water content and maintenance of turgor was associated with the rapid increase in internal osmotic potential. When salinity treatment was removed, osmotic potential of plants declined rapidly due to the increase in plant water content.

Ziska et al. (1989) found that maintenance of leaf water status for Prunus salicina grown under saline conditions was in part a consequence of increased stomatal closure, with a subsequent reduction in leaf transpiration rate. Ziska et al. (1989) reported no decline in osmotic potential with increasing salinity.

Water potential of seeds is also affected by salinity. McDonough (1976) studied the water potential of bromegrass (Bromus inermis Leyss) and alfalfa seeds imbibed on media of various osmotic potential (-2 to -16 bars). Water potentials were lower for seeds grown at lower osmotic potential because of greater restrictions in water uptake during imbibition. Over a 72 hr period, seed water potentials were lower than osmotic potentials of the media. Kurth et al. (1986) found similar results with tomato seeds imbibed at very high salinity. The osmotic potential of the seeds was reduced sharply 4 hr after transferring from nutrient solution to 100% sea water, and osmotic potential continued to decline during the next 3 days, but rose sharply soon after the seeds were returned to non-saline medium.

Greenway (1973) summarized the affect of salinity on the water balance of plants which is supported by other researchers. Greenway (1973) stated that as salt accumulates in the soil around a plant, the water potential is lowered, resulting in an almost inevitable decrease in water potential of the plant tissues. Plants grown in saline conditions may respond in one of the following ways. Firstly, plants may not take up the salt and turgor will be lost and plants will wilt. Secondly, plants may produce and accumulate organic solutes which bring about an osmotic adjustment and restores turgor. This accumulation of organic solutes also may be induced by salt uptake.

Salinity and Accumulation of Inorganic Ions

An excess of potentially toxic ions such as sodium and chloride in the growth media of the plants tend to affect plant metabolism (Greenway and Munns, 1980); and when these salts accumulate in excessive amounts in plant tissue, toxic effect may occur (Greenway, 1973). Smith et al. (1981) studied the response of alfalfa seedlings to increasing levels of chloride salts. Their results showed that as soil salinity increased, so did the Cl concentration in the leaves of alfalfa. Damage of the top growth was associated with the increasing salinity level, which suggests that the plants may be subjected to Cl toxicity.

Many investigators reported the accumulation of inorganic ions, especially sodium and chloride in certain parts of the plants when subjected to salinity. Such preferential accumulation of inorganic ions will protect the more sensitive organs in the plants from the damaging effect of inorganic ions. Aswathappa and Bachelard (1986) studied the distribution of Na, Cl, K, Ca, and Mg ions in individual organs of two highly tolerant and one moderately tolerant species of *Casuarina*. The highly tolerant species (*Casuarina equisetifolia* and *C. glauca*) accumulated less Na and Cl ions in their shoots than roots. The concentration of Na and Cl ions was higher in older needles than in young needles. The same pattern of Cl ions distribution in *C. equisetifolia* was found in seedlings exposed to both short-term (13 days at 100 mol m^{-3} NaCl in solution culture), and long-term (6 months at 250 mol m^{-3} NaCl in sand culture) salinization. The concentrations of Na and Cl ions were much higher in shoots of the moderately tolerant species (*C. cunninghamiana*)

but the concentration differences between old and young needles was not observed. The three species showed little difference in their root ion concentration. A time sequence experiment of Cl ions uptake indicated that the greater exclusion of Cl ions from the shoots of C. equisetifolia than C. cunninghamiana was due to a lower rate of Cl ions uptake and lower net transport into the shoot rather than to its retention in the root, or reabsorption at the proximal end of root or hypocotyl.

Lessani and Larschner (1978), in their study on the relationship between salt tolerance and long-distance transport of Na and Cl ions in sugar beets and beans found that Na and Cl ion concentrations increased similarly in the leaves of sugar beets and beans, as the concentration of NaCl increased in the growth media.

Greenway and Thomas (1965) grew barley, a relatively salt-tolerant crop, in a media which contained a high concentration of NaCl. They studied Cl ions uptake into individual tissues of barley. Chloride ion concentrations increased similarly in shoots and the oldest leaves for the first 5 days after initial salt treatment. Salt concentration leveled off after 5 days in the shoots, but continued to increase linearly in the most mature leaves. The younger, still expanding leaves also accumulated Cl ions, but in lower amounts than the older leaves. The expansion and uptake of water by the younger leaves may have diluted the Cl ion concentration which attributed to lower Cl ion level. Greenway and Thomas (1965) showed that the roots accumulated less Cl ion than either the shoots or leaves as NaCl of the growth media increased.

Different stages of root development of Atriplex amnicola will accumulate different amounts of inorganic ions. A. amnicola was grown in 25, 200, or 400 mol m⁻³ of NaCl and root tissues at various stages of development were analyzed for the concentrations of K, Na and Mg ions and in some cases for Cl ions. In the slightly vacuolated root tips, Na ion level was 40 mol m⁻³ in the root tip in plants treated with an external solution of 400 mol m⁻³ NaCl. The concentration of K ions in the root tip was not affected substantially by external NaCl solution between 25 mol m⁻³ and 400 mol m⁻³. The highly vacuolated root tissues had substantially higher concentrations of K, Na, and Cl ion in plants grown in 25 mol m⁻³ NaCl external solution. The K/Na ratio in recently expanded 12 mm root tips was as high as 1.6 compared with 0.7 for the bulk of the roots (Jeschke et al., 1986).

Eight-day old cotton seedlings were exposed to saline treatments ranging from zero to 250 mM NaCl in the presence of nutrient solutions containing 0.4 or 10 mM Ca. Sodium influx increased proportionally to increasing levels of salinity, but influx was reduced at high external Ca ion levels. Calcium influx increased (especially at high Ca concentration) with increased salinity. Potassium uptake decreased with increasing salinity and was unaffected by external Ca (Cramer et al., 1987). Mass and Grieve (1987) indicated that the addition of 86.5 mM NaCl to the nutrient solution of corn resulted in Ca ion deficiency. Calcium deficiency was noted in the four leaf stage. Free Ca ions of about 10⁻⁷ to 10⁻⁶ M or even higher were required for optimal metabolism in the cells

(Steer, 1988). So, external calcium at the appropriate level must be present to insure proper metabolism in the plant.

Mass et al. (1986), determined the relative salt tolerance of two sorghum (*Sorghum bicolor* L.) cultivars at three stages of growth and development in a greenhouse. The three stages were vegetative, reproductive, and maturation. Both cultivars were most sensitive to salinity during the vegetative stage and least sensitive during maturation. Mineral analysis of the first leaf below the flag leaf at maturation indicated that both cultivars tended to exclude Na ions from the upper leaves. Calcium and Cl ion concentrations increased with increased salinity in sorghum cultivars salinized during the maturation stage, but salinization at vegetative or reproductive stages decreased Ca ions concentration of this upper leaf at harvest and had no effect on the final Cl ions concentration. Phosphate and K ion concentrations decreased when plants were salinized during the maturation stage, but increased when plants were salinized during the vegetative and reproduction stages. Magnesium was unaffected by salinization during the first and last stage but decreased when plants were salinized during the reproductive stage.

Some tissues were found to act as an alternative sink for Na and Cl ions to protect the more sensitive tissues. Boursier et al. (1987) found more accumulation of Cl ion in the sheaths than in the leaf blades of sorghum, maize, wheat and barley when subjected to salinity. At 3.5 mM of soil Cl ion level, mature sorghum sheath accumulated 1018.7 μM of Cl ion/g dry weight whereas mature blades accumulated only 433.5 μM of Cl ion/g dry weight.

Huang and Van Stevenink (1989) found that in barley, the concentration of Cl ions in the mesophyll cells of the blade remained at a low level after exposure to 50 or 100 mM NaCl for 1 day or 50 mM for 4 days, while at the same time in the sheath, the concentration of Cl ions in epidermal and mesophyll tissue was much higher. The first mesophyll layer of the blade contained 4.1 mM Cl while the sheath first mesophyll layer contained 110 mM Cl.

The mesocotyl of 13 day old corn plants subjected to 1 to 10 mM labelled $^{22}\text{NaCl}$ accumulated 6 to 19% of ^{22}Na ions. At 100 mM $^{22}\text{NaCl}$ mesocotyl hold only 3 to 8% of ^{22}Na ions. Therefore, mesocotyl appears to act as an alternative sink for Na at low but not high NaCl levels (Drew and Lauchli, 1987).

The long term effect of NaCl on castor bean mature plants was studied by Jeschke and Wolf (1988). Sodium concentration was 50 $\mu\text{M/g}$ fresh weight in petiole and was 25 $\mu\text{M/g}$ fresh weight in leaf blades of plants treated with 80 mM NaCl. Potassium concentration remained constant in leaf, but decreased in petiole from 110 to 50 $\mu\text{M/g}$ fresh weight with 160 mM external NaCl treatment.

Huang and van Stevenink (1988) treated barley plants with 50 mM NaCl and determined Cl ion concentration in various cell types 1 to 50 mm from the root tip by the use of X-Ray microanalysis. Higher concentrations of Na and Cl ions were found in late metaxylem elements (LMX) than in the surrounding cells (71 μM Cl ions in LMX compared to 43 μM Cl ions in second layer of cortex). This finding suggests that living

xylem elements may assist in alleviating salinity stress in the meristemic region of barley root tips.

Harvey et al. (1985) in their study with Plantago coronopus L. found that in roots grown in saline conditions (115 to 125 mM NaCl) the parachlyma cells surrounding the xylem vessels had very uneven wall thickenings and corrugations. In another study with corn subjected to salinity, Yeo et al. (1977) showed that Na ions were usually less concentrated in the root than the S and Cl ions (in the lumen of the vessels), but Na ions were concentrated markedly relative to either Cl or S in the adjoining xylem parenchyma cells.

Sodium accumulates in the proximal parts of the roots of Phaseolus and the lower parts of the stem when the plants are treated with NaCl (Jacoby, 1965). Kramer et al. (1977) studied the effect of NaCl and Na₂SO₄ salinity on Phaseolus coccineus. They found that in the proximal region of the root, xylem parenchyma cells differentiated into transfer cells with well developed wall protuberances adjacent to the half bordered pits of vessels. They concluded that transfer cells may act in reabsorption of Na ions from the xylem vessel because of uneven distribution of Na ions between the xylem vessel and transfer cells in roots.

The energy required for the exclusion of Cl and Na ions in plants grown under salinity conditions is low. In rapidly expanding cells, Na ions extrusion requires only 1 to 2% of the energy normally produced in respiration (Greenway et al., 1983).

Some generalization have been proposed by Greenway and Munns (1980) to explain the pattern of accumulation of inorganic ions in the various

plant tissues. They stated that halophytes, which accumulate Na and Cl ions, store these ions in the vacuole of the leaf cells. Such storage will separate the high ion concentration from the salt-sensitive enzymes and organelles located in the cytoplasm. Synthesis of compatible organic solutes in cytoplasm maintains a sufficiently low cytoplasmic osmotic potential for uptake of water even when the plants are grown in saline soil with a low water potential. Glycophytes that accumulate ions and are salt sensitive do not compartmentize the ions within the leaf cells. This leads to accumulation of ions in the cytoplasm that will interfere with enzyme activities and the metabolic process as that takes place in the cytoplasm.

Salinity and Plasma Membrane

Salt tolerant plants have been classified into salt excluders or salt includers. Salt inclusion is a characteristic of halophytes, whereas salt tolerant glycophytes are mostly salt excluders (Greenway and Munns, 1980). Exclusion of salt in the salt tolerant glycophyte plants requires some mechanism for preventing the salt entry into the plants. This may require some discrimination mechanism at the cortical plasma membrane (Yeo et al., 1977). Therefore, a functional and intact plasma membrane is required for salt tolerant glycophytes to withstand saline conditions (Stuiver et al., 1978).

Intactness of plasma membrane is required to maintain a favorable ionic composition in the plant cell cytoplasm (Kramer, 1983). Salinity also induces a lowering osmotic potential which may severely damage the

plasma membrane. As membranes undergo dehydration, extensive lateral-phase separations of membrane phospholipid, cholesterol, and intramembrane particles are readily seen with freeze-fracture microtechnique. In addition to the obvious morphological damage, these plasma membrane vesicles possess no ability to accumulate Ca ions (Crowe and Crowe, 1986). Some plant cells adapt to salinity by developing a cell wall-plasma membrane labyrinth which regulates ion transport into the symplasm (Kramer, 1983). Such morphological changes in the cell wall plasma membrane may also help to prevent leakage of organic osmoticum (Alder and Liljenberg, 1981).

Salinity and Membrane Calcium

Calcium is a critical factor for the maintenance of membrane integrity (Lauchli and Epstein, 1970). However, increasing sodium chloride level will lead to a reduction in Ca ions uptake and to symptoms of Ca ion deficiency (Mass and Grieve, 1987). Kent and Lauchli (1985) studied salinity and calcium interaction in cotton. In the presence of complete nutrient solution, cotton seed germination was delayed when 200 mol m⁻³ NaCl was added and fresh weight of seedlings 7 to 9 days old was greatly reduced. The addition of supplemental Ca ion to the medium did not improve the germination, but did to a large degree offset the reduction in seedling root growth caused by NaCl. In addition, roots grown in the high salt medium without supplemental Ca appeared infected with microbes. Lahaye and Epstein (1969) also conducted an experiment which shows the protective role of calcium. They subjected bean plants

to a sodium chloride concentration about one-tenth that of seawater for 1 week. Bean plants suffered no damage if the calcium concentration of the nutrient solution was 1 mM or higher, but at lower calcium concentration, damage was severe. They concluded that the damage may be due to massive breakthrough of Na ions into the leaves.

Kent and Lauchli (1985), reported that the content of K and Ca ions were reduced in both roots and shoots by the NaCl treatments. Supplement Ca ions partially offset this effect for K ions in the root and for Ca ions in both shoots and roots. Sodium content was not affected by the supplemental Ca. Therefore, Kent and Lauchli (1985) concluded that the beneficial effect of high Ca ion concentration on root growth of cotton seedlings in a saline environment may be due to maintenance of high K/Na ratio and adequate Ca ion status in root. Lynch and Lauchli (1985) proposed a mechanism for the salinity induced Ca ion reduction from their findings that the reduction of Ca ion content in the salt tolerant barley cultivar is less than in the salt sensitive one. They proposed that the inhibition was not due to the effect of NaCl on Ca ions influx into the root, but rather that NaCl inhibits Ca ions transport from root to shoot by interfering with the release of Ca ions into the root xylem. The active loading of Ca ions into xylem vessels appear to be the site where NaCl inhibits Ca ion release. They further speculated that the reduction of Ca ion content of root tissue caused by salt stress may reflect a displacement of Ca ions by Na ions from apoplastic cation exchange site. However, this displacement had no effect on Ca ion influx into the

symplasm, therefore will have little influence on symplastic Ca ion movement into the stem.

A displacement of Ca ions by Na ions from membrane sites has been reported by Cramer et al. (1985) when plants were subject to increasing level of salinity. Therefore Cramer et al. (1985) concluded that Ca ions appear to protect membrane from adverse effects of Na ions and thereby maintaining membrane integrity and minimizing leakage of cytosolic K ions. In trying to determine the site of Ca ions displacement, the authors proposed a binding site other than those of phospholipids, perhaps at site of protein. These data have been analyzed later on in a separate study (Cramer and Lauchli, 1986). A computer program was used to analyze the data. In their analysis, they showed that displacement of Ca by Na ions in the presence of low and high Ca ion levels in the external solution, appeared to take place at two different classes of membrane binding sites. One site had the properties of a high affinity Ca-binding site, possibly being a protein. The other site did not have high affinity for Ca ion, and was occupied by Ca ion only at high concentration of Ca ion. This site may not be a protein. At low Ca ion concentration, Na ion will displace Ca ion at the protein site leading to high K ion efflux, but at high Ca ions concentration, displacement of Ca ion will be at the non-protein site, and will minimize K leakage.

Lynch et al. (1987), have reported that salinity reduced membrane-associated Ca ion on root protoplast plasma membrane, but they believed

that loss of Ca ion is not specific to Na or Cl ions, but may be due to increased ionic strength of the treatment solution.

Salinity and Leakage of Plasma Membrane

Salinity will induce the leakage of substances like protein from the barley root cells and will inhibit the subsequent uptake of orthophosphate (Pi). The inhibition of Pi uptake may be caused by the loss of protein required for active Pi transport (Mass et al., 1979).

In a study with Zea mays, Grunwaldt et al. (1978) found that osmotic shock induces protein release from roots into the medium. Damage of plasma membrane was observed by the uptake of Evans Blue. Sensitivity of root cells to osmotic stress increases with differentiation and vacuolation.

Sterols and Salinity

Sterols differ in their effect on permeability of red beet cell membrane with cholesterol and campesterol being most effective, stigmasterol being intermediate and sitosterol being least effective (Grunwald, 1968). Free sterols play a significant role in the salt exclusion mechanism which is related to salt tolerance (Kuiper, 1984). The level of free sterols in the roots of the halophytic Plantago maritima and P. coronopus is maintained upon exposure of the plants to salt, whereas their level is decreased in the salt-sensitive P. media (Erdei, et al., 1980). Higher content of sterol is found in the salt resistant species like sugar beet than in the salt-sensitive bean (Stuiver et al., 1978). In barley, upon exposure to 100 mM NaCl there was no change in the

mole percent of sterols, but there was slightly higher percentage (14% in plasma membrane fraction) of stigmasterol in salt treated plants than in the control (11% in plasma membrane fraction) (Brown and Dupont, 1989).

Glycolipids and Salinity

In a study with five grape root stocks which differ markedly in the extent to which they permit Cl ions accumulation in leaves, Kuiper (1968) found that monogalactose oliglycerides do not contribute positively in the regulation of ion membrane permeability, but increase in percentage of monogalactose diacylglyceride was correlated with Cl ion accumulation.

Polyethyleneglycol (PEG) does not bring about the same effect on glycolipid as salinity. By comparing the effect of NaCl and PEG on the lipids of barley chloroplast, Muller and Santarius (1978) observed that the PEG does not produce changes in these lipids, whereas the NaCl decreases the amount of glycolipids in these organelles. Muller and Santarius (1978) suggested that this is caused by an ionic effect. As to the decrease of glycolipids, salts may inhibit the enzymes involved in the biosynthesis of these lipids, namely the galactosyl-transferase and the acyl CoA-1 acylglycerol-3 phosphate acyltransferase. This inhibition is probably due to the accumulation of ions in the leaf cells. Muller and Santarius (1978) suggested that the decrease in content of galactolipids in biomembranes is one of the factors causing increased salt resistance in barley plants which are adapted to extreme salinity.

Salinity and Other Lipids

In the roots of Plantago media L. (salt sensitive), the level of phospho-lipids decreased strongly with increased NaCl concentration, indicating decreased control of permeability of the root cell membrane. In the root of P. maritima and P. coronopus (salt tolerant) the level of most lipid classes was maintained or even increased in 75 mM NaCl, but a decreased at higher NaCl concentrations. The decreased lipid level in the salt sensitive species may be due to reduced synthesis or to accelerated degradation of the lipids by NaCl. In the salt tolerant species, phospholipid synthesis may be stimulated by a minor salt stress (Erdei et al., 1980).

Fatty acid analysis of olive (Olea europaea L.) total chloroplast lipids showed that high NaCl concentration in the growth media led to a decrease in the percentage of linoleic acid and an increase of linolenic acid. There was also a slight increase of oleic acid level. Except for phosphatidylinositol and phosphatidic acid, which showed increased levels, contents of different chloroplast lipid decreased with increasing salinity. A decrease in linolenic acid level in membranes would modify their permeability properties for Na and K ions. In fact, the increase of unsaturated fatty acid proportion of membrane lipids increases their Na and K permeability (Zarrouk and Cherif, 1984).

In the sunflower (Helianthus annuus L.) leaf, four lipid classes vary remarkably when the salinity increases. The phosphatidyl choline (PC) rate decreased whereas the neutral lipid level increased. In roots the sulfoquinovosyl diacylglycerol (SQDG) increased while the

monogalactosyl diacylglyceride (MGDG) decreased, whereas PC decreased. The decrease in PC may be due to NaCl inhibition of its synthesis, or because the choline is used for the biosynthesis of glycinebetaine. The SQDG increase would be related to (Na + K) stimulated ATPase activity because this lipid is known to be essential for the function of the microsomal ATPase. The MGDG decrease and the neutral lipids increase may be the consequence of leaf senescence, which is an indirect effect of salt. In the leaves, salinity tends to decrease unsaturated lipids, while it increases unsaturated lipids ratio in roots (Gharalli and Cherif, 1984).

The idea that salinity induced senescence is responsible for the change in lipid content is supported by data from Ferguson and Simon (1973). In their study, phospholipid levels at senescence were reduced resulting in destruction of membrane integrity and allows leakage of cellular components.

In barley plants, the total lipid and phospho-lipid concentration in the roots tends to increase slightly with increasing salinity. This led to the conclusion that lipid synthesis is probably not a specific factor in salt induced growth suppression. However, the relative concentration of the various phospholipid classes was altered by increasing NaCl concentration (Ferguson, 1966).

Not all salt classes affect lipid composition the same. In sunflower leaves, the total lipid content, as well as the percent of linolenic acid are decreased with NaCl and increased with CaSO₄ (Bettaleb et al., 1980).

Salinity and ATPase Activity

Atriplex memmularia Lindl plants were grown in the presence of 400 mM NaCl and plasma membrane vesicles were isolated from roots of the halophyte. The proton-translocating activity per mg of plasma membrane protein was increased as compared to control, i.e., increasing ATPase activity (Braun et al., 1986). The effect of Mg, Na, K, ions and pH on ATPase activity of purified membrane fractions enriched in plasma membrane fragments from Hordeum vulgare L. (Glycophyte) and Haloceum strobilaceum L. (halophyte) was studied by Vakmistrov et al. (1982). Plasma membrane ATPases from both plants were synergistically activated by K and Na ions in the presence of Mg ions. The maximum activity of the enzymes was observed at a Na/K ratio of 2 to 3. The ATPases of Hordeum strobilaceum L. had a single pH optimum (pH 8), but that of the Haloceum had two optimal pH (6 and 8). The authors concluded that higher ATPase activity of the halophyte compared to that of the glycophyte suggest the involvement of ATPase in the extrusion of Na ions from the cytoplasm of cells of both plants.

In a study with Plantago species, there was a limit for salinity induced ATPase activity, after which ATPase activity was reduced (Erdei et al., 1980). In this study 150 mM NaCl and higher decreased Ca and Mg stimulated ATPase activity of the salt tolerant species. In the salt sensitive species, all salinity levels used decreased the activities of the ATPase enzyme.

Not all investigators agree that salinity will lead to an increase in ATPase activity, Braggemann and Janiesh (1988) found no change in

ATPase activity after growing Plantago crassifolia (halophyte) in 150 mM NaCl for 6 weeks. This result was obtained for ATPase in the root and leaves, which accumulated Na and Cl ions.

An inverse relation between the activation energy of the plasma membrane ATPase activity and the phospholipid to sterol ratio of the same plasma membrane preparation was observed by Kuiper (1984). A single inverse linear relationship was found in all genotypes and all salt treatments investigated. Clearly, changes in fluidity of the membrane as induced by free sterols, modulate ion-stimulated ATPase activity of the plasma membrane of root cells (Kuiper, 1984).

CHAPTER 3

MATERIALS AND METHODS

Plant Material

White tepary bean, (Phaseolus acutifolius Gray var latifolius) and navy bean, (Phaseolus vulgaris CV. NA sanilac) were used in this research. Navy bean was used only in part to compare its performance with the tepary bean. The tepary bean seeds were obtained from Dr. Janice Coons, Department of Plant Sciences, University of Arizona, Tucson, and the navy bean seeds were from Rogers Brother Seed Company, Boise, Idaho.

Before germination, seeds were sterilized with a solution containing 1% (v/v) bleach and 0.5% (v/v) tween 20 for 30 sec and then washed with water for 5 min. Seeds were germinated in vermiculite in plastic trays and grown for 5 days in a growth chamber at 18 °C day/15 °C night (± 2 °C) with 13 hr photoperiod and irrigated with tap water when needed. Irradiance was 400 $\mu\text{mole m}^2 \text{S}^{-1}$ at canopy level measured by Licor model LI-185A Quantum/Radiometer/Photometer.

Seedlings were carefully removed from vermiculite after 5 days. Roots were gently washed and 20 seedlings with an average length of 9 cm were transferred to racks with roots immersed in an aerated modified Hoagland's solution (Hoagland and Arnon, 1938) in plastic trays. Seedlings were grown at room temperature (25 °C ± 3) under photoperiod of 13 hr with light intensity of 400 $\mu\text{mole m}^2 \text{S}^{-1}$ at canopy level measured by Licor model LI-185A Quantum/Radiometer/Photometer.

Plants were treated with various concentrations of NaCl solution 24 hr after transplanting. The salt was added to nutrient solution in increments of -0.25 MPa/day until the desired levels of -0.25, -0.50, and -0.75 MPa were obtained. The solutions osmotic potentials were verified with Wescor model 5100 C vapor pressure osmometer. The solution volume in each plastic tray was maintained at 700 ml level by adding Hoagland's solution to replace the loss of water caused by evapotranspiration.

Unless otherwise stated, all treatments in each experiment were replicated three times and repeated over time. The experimental design was a randomized complete block design (RCB). Analysis of variance (ANOVA) and mean separation were performed for all data.

Plant Growth

Plant height, shoot fresh and dry weights and leaf area were measured to determine the effect of salt solution on plant growth. Measurements were conducted on three plants selected randomly from the trays. All measurements were conducted every 3 days. Shoot and root fresh and dry weights were also measured 12 days after the desired NaCl level was reached.

Plant height was measured to the nearest millimeter. Leaf area was measured with Licor LI-3100 area meter immediately after harvest. Leaf area index was calculated by measuring leaf area and dividing that by the ground area. The shoot and root (fresh and dry) weights were measured with an electronic balance model Mettler A30. To determine the dry weight of the shoots and roots, each plant was placed in a marked container in

an oven at 65 °C for 48 hr to dry both shoots and roots. Relative growth rate was measured by measuring the change in dry weight over a week time period.

Free Amino Acids

Amino acid analysis was conducted on seedlings treated with 0.75 MPa NaCl and the control. Twenty-four, 48 and 72 hr after the -7.5 bars level was reached, samples from 10 plants were harvested for measurement of amino acids content. The roots were rinsed for 30 sec in distilled water to remove surface salts and then blotted with kimwipes paper before the fresh weight was taken of the selected part of the root. Samples were also harvested from shoot meristem, mature true leaf, and from the root (3 cm section taken 1 cm away from the shoot/root junction). Sample fresh weight were measured and then plant tissues were ground with mortar and pestle in liquid nitrogen, put in plastic weighing dishes, and placed quickly in the freezer (-21°C) for storage.

Amino acids were extracted using the method of Bielęski and Turner (1966) with methanol/chloroform/water (MCW) (12/5/3, v/v/v) solvent system. About 1 g of plant tissue was extracted with 20 ml of cold (4°C) MCW for 5 min, and the mixture shaken periodically. The homogenate was filtered through Whatman #1 filter paper and the filtrate saved. The residue on the filter paper was re-extracted with cold MCW for 5 min, and the filtrate was added to the first filtrate and the final volume was recorded. To the MCW extract was added 1 volume of chloroform and then 1 volume of water. This resulted in a two-phase mixture which was

separated with a separatory funnel. The bottom chloroform layer was discarded and the upper aqueous layer was saved. A 1 ml aliquot of the aqueous phase was used for quantitative analysis of amino acids as described by Moore and Stein (1948). One ml of the aqueous solution is added to 2 ml of ninhydrin reagent in a test tube. Then the tube was shaken with a vortex. The test tubes with the reagents were heated for 15 min in a boiling water bath. Next, 8 ml of 50% (v/v) ethanol was added to each tube. The tubes were then allowed to cool to room temperature, shaken, and read with a spectrophotometer at 570 nm. A standard curve prepared with amino acid leucine was used to estimate the quantity of amino acid in the samples.

This experiment was repeated over time. A -0.5 MPa treatment was included and the harvested tissues were lyophilized with a freeze dryer for 72 hr.

Free Sugars

Seven day old seedlings were transplanted into the Hoagland's solution. One salt level was used in this experiment (-0.75 MPa) plus the control. Twenty-four, 48, and 72 hr after the -0.75 MPa level was reached, samples from 10 plants were harvested for measuring free sugar content. The roots were rinsed for 30 sec in distilled water to remove surface salt and then blotted with kimwipe paper before the fresh weight was taken of the selected part of the root. Samples were taken from shoot meristem, mature true leaf, and from the root, and 3 cm section of the root was taken 1 cm away from the shoot/root junction. Fresh weight of

each harvested sample was measured. Next, plant samples were homogenized with mortar and pestle in liquid nitrogen, put in plastic dishes and quickly placed in the freezer (-21°C). To extract free sugar, about 1 g of plant sample was added to 20 ml of 95% (v/v) cold ethanol (4 °C) for 5 min. with periodic shaking. The homogenate was filtered through Whatman #1 filter paper and the filtrate saved. The residue on the filter paper was re-extracted with 80% (v/v) cold ethanol for 5 min and the filtrate was added to the first filtrate and the final volume was recorded. One volume of chloroform and 1 volume of water was added to the ethanol extract. This results in a two-phase mixture which was then separated with a separatory funnel. The bottom chloroform layer was discarded whereas the aqueous phase was saved with final volume recorded. A 1 ml aliquot of this aqueous solution was used for quantitative analysis of free sugar as described by Dubois et al. (1956). One ml of the aqueous sugar solution was added to a test tube and followed by addition of 0.5 ml of 5% (v/v) phenol. Then 2.5 ml of concentrated H₂SO₄ was added rapidly to the tubes. The tubes were shaken and left at room temperature for 20 min. The tubes were then shaken again and read with spectrophotometer at 490 nm. The total free sugars were determined using a standard curve prepared with glucose.

This same experiment was repeated over time. In the second experiment, 5 day old seedlings were transplanted into the Hoagland solution and -0.5 MPa treatment was included. The harvested tissues were lyophilized with a freeze dryer for 72 hr. Seventy-two hr after the -0.75 MPa treatment was reached, leaf samples were harvested for qualitative

analysis of the free sugars with HPLC using method of Miller (1989). Also, in the second experiment, free sugars were extracted with a solution of methanol/chlorophorm/water 12/5/3 (v/v/v) instead of the 95 (v/v) and 80% (v/v) ethanol.

Osmotic Potential

The experiment was repeated three times over time. Only a control and -0.75 MPa NaCl treated seedlings were used in experiment one and two. In the third experiment, the -0.5 MPa treatment was included. Seventy-two hours after the -0.75 MPa level was reached, 10 plants were removed from the Hoagland's solution, rinsed in distilled water for 30 sec and blotted with kimwipe paper.

The osmotic potential of fully expanded, mature leaves, 3 cm segments of the root located 1 cm from the shoot/root junction, and the remaining part of the root were performed by a modification of the method of Chapman and Fischer (1988). The samples were placed in 10 ml syringes which had a cloth filter in the bottom of the syringe. The syringe was dipped in liquid nitrogen for 45 sec to disrupt the tissues. The tissues were allowed to thaw by putting the syringe under running hot water for 2 min. The cell sap was expressed from cell debris by pressing the cell sap through the filter with the syringe plunger.

The osmotic potential readings of cell sap were taken with a Wescor 5100 C Vapor pressure osmometer. Also, frozen and thawed samples from the control and the -0.75 MPa treated plant parts were centrifuged at 500 x g for 10 min and osmotic potential of cell sap supernatant was determined

with osmometer to ascertain if there was a difference in the readings between the two procedures. No differences were found.

Water Potential

The water potential measurements of leaf and root samples were conducted 72 hr after the -0.75 MPa level was reached. All water potential measurements were taken early in the morning between 6:30 to 7:30 a.m., using Merrill Thermocouple Psychrometer (model 75-11C) and a Wescor HP-115 Water Potential Data System.

Two 6 mm diameter leaf disks were taken from each plant with a paper punch from the middle leaflets of the upper most fully expanded leaf, for each plant. For the root water potential, the roots were dipped in distilled water for 10 sec to remove surface salt, then blotted gently with kimwipe paper. Four 0.25 cm sections of the roots were cut with a sharp razor blade from the area 1 cm from the root/stem junction and all samples were placed immediately in a psychrometer.

The psychrometers were left to run for 24 hr in a styrofoam chamber to maintain constant temperature, and the readings were taken after 12 hr, after equilibration was reached.

The same experiment with the same procedure was repeated over time, except in the second time the -0.5 MPa treatment was included and only two replications were done. Also, in the second experiment, osmotic potential was measured by dipping the psychrometers in liquid nitrogen for 20 sec. The psychrometers were left at room temperature overnight, then put in styrofoam chambers for 24 hr and the readings were taken.

To obtain the data stored in the Wescor HP-115 Water Potential Data System, the data system was connected into a computer with a communication program Procomm. To convert the mv data to MPa, a computer program (Wescor) that has the psychrometer calibration curves was used.

Relative Water Content

To determine relative water content (RWC), a modification of the method of Ibarra-Caballero et al. (1988) was followed. Seventy-two hr after the -0.75 MPa level was reached, 1 cm wide segments obtained from 10 leaves were cut. The fresh weight was measured quickly for the leaf segments. The leaf segments were put in a plastic container having a water saturated environment.

After 6 hr, the leaf segments were blotted and turgor weight determined. The same segments were put in the oven to dry at 65°C for 48 hr. Relative water content was calculated with the following formula:

$$RWC = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Saturated weight} - \text{Dry weight}} \times 100$$

The same experiment was repeated over time, using the method of Munns and Weir (1981), and a -0.5 MPa treatment was included.

Inorganic Ions

The Ca, Na, K and Cl ions were measured in whole stem, mature true leaf, and root (3 cm section cut 1 cm from the shoot/root junction). The plants were treated with -0.75 MPa NaCl and the harvest was conducted 72 hr after the -0.75 MPa level was reached.

The plant tissues were oven dried at 65°C, weighed and ground with a cyclone sample mill, UD Corporation, Boulder, CO. For analysis of Ca, Na, and K ions, the samples were wet-oxidized in nitric-perchloric acid by the method of Ganje and Page (1974). The digest was then analyzed for Ca, Na, and K ions using atomic absorption spectrophotometry and for Cl ions with a solid state chloride ion specific electrode (Islam et al., 1983).

This experiment was repeated over time. In this experiment, the -0.5 MPa treatment was added. The plant parts which were sampled were mature leaf, stems (2 cm segment away from the root/stem junction), the rest of the stem, root (3 cm segment taken 1 cm from the root/shoot junction), and the rest of the root excluding the meristem.

Seventy-two hr after the -0.75 MPa treatment was reached, 10 plants were harvested and divided to the same parts used in the second experiment to determine Na, Ca and K ions concentration. In this experiment, the inorganic ions content in the free space were removed before measuring ions concentration by the method of Shennan et al. (1987). The ions in the free space were removed by placing plants in mannitol solutions that had the same osmotic potential as salt treatment solution (-0.5 and -0.75 MPa). The mannitol solutions were cooled to 4°C for 20 min before the plants were put in them.

Anatomy

About 4 mm root samples were taken 1 cm from the root/stem junction. The samples were fixed in 1% (v/v) glutaraldehyde with sodium

cacodylate buffer 50 mM, pH 6.8 for 3 hr at room temperature. After thorough washing in the same buffer for 2 hr, specimens were post fixed in 1% (v/v) OsO₄ (pH 6.8) for 2 hr. The specimens were dehydrated in graded acetone series, transferred to propylene oxide and embedded in Spurr's (1969) resin. Ultrathin sections were cut with a glass knife on a Sorval MT-1 Porter Blum ultramicrotome, collected on uncoated copper grids and examined in Hitachi H-500 electron microscope after post staining with uranyl acetate and basic lead citrate (Hayat, 1986).

ATPase Activity

Three treatments were used in this experiment (i.e. Control, -0.5 and -0.75 MPa NaCl). About 40 g of roots from each treatment were used for the determination of ATPase activity. The method of Hodges and Leonard (1974) was used for plasma membrane isolation and for determination of ATPase activity. Protein content of plasma membrane fractions was determined by Lowry et al. (1951). The experiment was repeated two times.

Plasma Membrane Leakage

Seven day old tepary bean plants which were grown in Hoagland's solution for 24 hr were used in this experiment. Three beakers were filled with 150 ml Hoagland's solution and 2,200,000 dpm (10 ul) of methyl-o-[¹⁴C] glucose (obtained from New England Nuclear, Boston, MA) was added to each one. Six tepary bean plants were put in each beaker. Four hours later, the plants were removed, washed three times with Hoagland's solution, and put in Hoagland's solution for 15 min.

The plants were put in three beakers (150 ml), each with either 150 ml of distilled water, -0.5, or -0.75 MPa NaCl solution. One hundred μ l samples of each solution were taken 15, 30, 60, and 120 min after the beginning of the treatments and added to 15 ml of scintillation cocktail and the radioactivity of the samples were determined with liquid scintillation counter.

CHAPTER 4

RESULTS AND DISCUSSION

Plant Growth

Analysis of growth and development of tepary and navy beans grown in saline solution showed that tepary bean tolerate the salt treatment to a greater extent than navy bean. Shoot length, shoot fresh and dry weights, root fresh and dry weights, leaf area, leaf area index (LAI) and relative growth rate of tepary and navy beans were all affected by salinity. These growth parameters were reduced more at the higher rate (-0.75 MPa) of salts than with the lower rate (-0.25 MPa) treatment and navy bean was affected to a greater degree than tepary bean by salinity at high concentration (Table 1). These findings indicate that tepary bean is more tolerant to salt stress than navy bean and tepary bean may have adaptive mechanisms to salt.

All salinity treatments significantly reduced shoot length for both species (Table 1). However, navy bean shoot length was affected more than tepary bean. The -0.75 MPa salt treatment on the ninth day reduced shoot length to by 52% as compared to navy bean control, but only 40% of the tepary bean control.

Shoot fresh and dry weights were also measured every 3 days. The result obtained followed the same trend as that obtained for shoot length with tepary bean being affected less by the high salinity concentration than navy bean (Tables 2 and 3). Twelve days after the salinity treatment

Table 1. Shoot length of tepary and navy bean over time at various NaCl levels.

	Solution Osmotic Potential (MPa)	Days after treatment		
		3	6	9
		(----- cm -----)		
Navy bean	0	18.7 c ^z	28.9 c	49.8 d
	-0.25	17.6 b	27.6 c	41.7 c
	-0.50	17.3 b	23.8 b	32.3 b
	-0.75	15.8 a	21.3 a	23.8 a
Tepary bean	0	17.8 c	28.1 c	34.3 d
	-0.25	17.4 c	26.7 b	29.5 c
	-0.50	16.4 b	19.1 a	25.3 b
	-0.75	15.9 a	18.4 a	20.7 a

^zMeans within a column for a given genotype followed by a different letter are significantly different at the 0.05 level (LSD).

both fresh and dry weights of shoots and roots of both genotypes were measured. Tepary bean was again less affected than navy bean. The navy bean root dry weight of -0.75 MPa treatment was reduced by 46% compared to the control but for tepary bean root dry weight was reduced by only 33% as compared to the control (Table 4).

Leaf area and leaf area index (LAI) were both measured over time. Salinity treatments reduced navy bean leaf area by 62% whereas tepary bean leaf was only 47% as compared to the control (Table 5). Leaf area of control navy bean was higher than control tepary bean. In contrast, the LAI was similar for control navy and tepary bean, but salinity treatment (-0.75 MPa) caused a reduction of LAI for navy bean to 0.32 and only to 0.39 for tepary bean (Table 6).

When relative growth rate (RGR) was calculated, salinity at -0.75 MPa reduced RGR of navy bean from 0.42 to 0.12 (Table 7), while the same treatment reduced tepary bean RGR from 0.33 to 0.21, which is twice as high as that of navy bean (Table 7).

Analysis of variance of the data indicated that salinity significantly affected all of the morphological and growth characteristics measured.

These results agree with the results of other investigators in that tepary bean out-performed navy bean under salinity conditions (Goertz and Kobriger, 1986b; Coons and Pratt, 1988). The slow growth rate (RGR) after the addition of salt may represent the reallocation of cellular energy from growth to solute synthesis and accumulation of solutes for osmotic adjustment or ion-exclusion and the necessary growth maintenance for

Table 2. Shoot fresh weight of tepary and navy bean over time at various NaCl levels.

	Solution Osmotic Potential (MPa)	Days after treatment			
		3	6	9	12
Navy bean	0	1.5 d ^z	2.0 d	2.3 d	4.0 d
	-0.25	1.3 c	1.5 c	2.0 c	3.7 c
	-0.50	1.2 b	1.3 b	1.7 b	3.2 b
	-0.75	1.1 a	1.1 a	1.3 a	2.7 a
Tepary bean	0	.9 b	1.2 b	1.3 c	2.7 b
	-0.25	.9 b	1.1 b	1.1 b	2.5 b
	-0.50	.7 a	.8 a	.9 a	1.9 a
	-0.75	.7 a	.8 a	.8 a	1.8 a

^zMeans within a column for a given genotype followed by a different letter are significantly different at the 0.05 level (LSD).

Table 3. Shoot dry weight of tepary and navy bean over time at various NaCl levels.

	Solution Osmotic Potential (MPa)	Days after treatment			
		3	6	9	12
Navy bean	0	0.09 c ^z	0.12 b	0.15 d	0.27 c
	-0.25	0.08 cb	0.11 b	0.12 c	0.23 b
	-0.50	0.08 b	0.08 a	0.11 b	0.20 a
	-0.75	0.07 a	0.07 a	0.09 a	0.19 a
Tepary bean	0	0.08 c	0.09 c	0.09 c	0.17 c
	-0.25	0.07 bc	0.08 bc	0.08 bc	0.13 b
	-0.50	0.06 ab	0.07 ab	0.08 ab	0.11 a
	-0.75	0.05 a	0.06 a	0.07 a	0.11 a

^zMeans within a column for a given genotype followed by a different letter are significantly different at the 0.05 level (LSD).

Table 4. Root fresh and dry weight of tepary and navy bean after being treated with various NaCl levels for 12 days.

	Solution Osmotic Potential	Root Fresh Weight	Root Dry Weight
	(MPa)	(----- g -----)	
Navy bean	0	1.72 d ^z	.056 c
	-0.25	1.52 c	.044 b
	-0.50	1.34 b	.034 a
	-0.75	1.23 a	.030 a
Tepary bean	0	1.75 c	.051 b
	-0.25	1.57 b	.047 b
	-0.50	1.42 a	.041 ab
	-0.75	1.38 a	.034 a

^zMeans within a column for a given genotype followed by a different letter are significantly different at the 0.05 level (LSD).

Table 5. Leaf area of tepary and navy bean at different time intervals at various NaCl levels.

	Solution Osmotic Potential (MPa)	Days after treatment		
		3	6	9
		(----- g -----)		
Navy bean	0	35.3 d ^z	56.4 d	78.1 d
	-0.25	29.5 c	44.1 c	61.8 c
	-0.50	25.0 b	34.8 b	42.9 b
	-0.75	19.6 a	26.2 a	29.2 a
Tepary bean	0	25.2 d	38.2 b	48.4 d
	-0.25	23.6 c	37.0 b	43.1 c
	-0.50	20.7 b	22.3 a	28.5 b
	-0.75	18.7 a	19.7 a	25.0 a

^zMeans within a column for a given genotype followed by a different letter are significantly different at the 0.05 level (LSD).

Table 6. Leaf Area Index (LAI) of tepary and navy bean at different time intervals at various NaCl treatments

	Solution Osmotic Potential (MPa)	Days after treatment		
		3	6	9
Navy bean	0	0.59 d ^z	0.71 d	0.77 a
	-0.25	0.49 c	0.55 c	0.61 c
	-0.50	0.42 b	0.43 b	0.42 b
	-0.75	0.32 a	0.33 a	0.32 a
Tepary bean	0	0.55 d	0.63 b	0.76 d
	-0.25	0.51 c	0.62 b	0.67 c
	-0.50	0.45 b	0.37 a	0.45 b
	-0.75	0.41 a	0.33 a	0.39 a

^zMeans within a column for a given genotype followed by a different letter are significantly different at the 0.05 level (LSD).

Table 7. Relative growth rate (RGR) of tepary and navy bean at various NaCl levels.

	Solution Osmotic Potential	RGR
	(MPa)	(g.g.wk)
Navy bean	0	.42 a ^z
	-0.25	.27 b
	-0.50	.20 c
	-0.75	.12 d
Tepary bean	0	.33 a
	-0.25	.24 b
	-0.50	.25 c
	-0.75	.21 d

^zMeans within a column for a given genotype followed by a different letter are significantly different at the 0.05 level (LSD).

seedling establishment (Meyer and Boyer, 1981). Leaf area reduction under salinity conditions may be a response to declining water potential in crop plants (Curtis and Lauchli, 1986), which would result in reduction in the rate of cell division in the leaf meristem followed by cessation of cell expansion (Terry et al., 1983).

Water (ψ), Osmotic (ψ_{π}) and Turgor (ψ_p) Potential

Growth of tepary bean was reduced by salt treatment, possibly because cellular energy was utilized to synthesize and accumulate organic solutes and to exclude Na and Cl ions. Tepary bean which exhibited less growth inhibition than navy bean may be able to adapt to salinity stress by osmotic adjustment or exclusion of inorganic ions. To determine if this occurred, a study was performed to ascertain if NaCl induced osmotic adjustment (osmotic potential, water potential, and relative water content), and if inorganic ions distribution modified ATPase activity.

Both water and osmotic potential were both significantly reduced when measured 72 hr after subjecting the plants to -0.75 MPa NaCl treatment. Tepary bean seedlings underwent a significantly more negative reduction in ψ and ψ_{π} , while turgor (ψ_p) stayed almost constant in leaves and proximal roots when treated with various salinity levels (Table 8). Both -0.5 and -0.75 MPa treatment reduced ψ in leaves and proximal roots, but the reduction was greater in the leaves, perhaps to keep the water moving into the leaves. Also osmotic potential was reduced more in the leaves, than roots which might be due to the manufacturing of organic solutes and/or to the deposition of inorganic ions in the leaves.

Table 8. Water potential (ψ), osmotic potential (ψ_{π}) and turgor (ψ_p) potential, in leaves and roots of tepary bean 72 hr after being treated with various levels of NaCl.

Solution Osmotic Potential (MPa)	ψ	ψ_{π}	ψ_p
----- MPa -----			
<u>LEAVES</u>			
0	-0.45 c ^z	-0.80 c	0.35 a
-0.50	-0.90 b	-1.27 b	0.37 a
-0.75	-1.35 a	-1.68 a	0.33 a
<u>PROXIMAL ROOTS</u>			
0	-0.29 c	-0.61 b	0.33 a
-0.50	-0.62 b	-0.81 b	0.30 a
-0.75	-0.94 a	-1.20 a	0.26 a

^zMeans within a column for a given plant part followed by a different letter are significantly different at the 0.05 level (LSD).

These results indicated that ψ_p was not affected by salinity treatment which agrees with the result of Ziska et al. (1989) and Matsuda and Riazi (1981) in that ψ_p would stay almost constant when the plants are subjected to stress. In contrast, Parsons and Howe (1984) reported a reduction in tepary bean ψ_p under water stress.

Relative Water Content (RWC)

The relative water content between the control and the -0.5 or the -0.75 MPa treatment of tepary bean leaves 72 hr after being treated with NaCl was significantly different (Table 9). Relative water content of seedlings treated with -0.5 MPa NaCl was reduced to 9% of the control and the -0.75 MPa NaCl caused 24% reduction of the control.

Osmotic Adjustment

Salinity treatment of -0.5 and -0.75 MPa led to a significant increase in osmoticum in leaves and proximal roots. The proximal root section did not show a significant difference between the -0.5 and -0.75 MPa treatment (Table 10). Salinity significantly increased osmoticum in all plant parts measured.

Osmotic adjustment in leaves can be accounted for by changes in total fresh weight/dry weight (changes in cell volume), variation in the proportion of bound water, and accumulation of organic and/or ions solutes. I assumed the bound water to be unchanged due to salinity stress, and calculated the osmotic adjustment at full turgor to determine if osmotic adjustment was due to less water uptake (since salinity caused

Table 9. Relative water content (RWC) in leaves of tepary bean after being treated with various NaCl levels for 72 hr.

Solution Osmotic Potential (MPa)	RWC
0	94.9 c ^z
-0.50	86.1 b
-0.75	72.8 a

^zMean followed by a different letter are significantly different at the 0.05 level (LSD).

Table 10. Apparent osmoticum (mM) in tepary bean leaves, proximal part of the root (3 cm) and the remaining part of the root after being treated with various NaCl levels for 72 hr.

Solution Osmotic Potential	Apparent Osmotic adjustment		
	Leaves	Proximal part of the root (3 cm)	Remaining part of the root
	(----- mM -----)		
0	249 a ^{zx}	204 a	208 a
-0.50	431 b	350 b	336 ab
-0.75	598 c	364 b	379 b

^zMeans within a column for a given plant part followed by a different letter are significantly different at the 0.05 level (LSD).

^xThe numbers obtained here are the mean of nine values from three experiments (three for each). The three experiments are exactly the same but have been repeated over time. Time was insignificant at the 0.01 (LSD).

significant difference in RWC), or if it is a true osmotic adjustment phenomena caused by accumulation of solutes.

The osmotic adjustment at turgor was calculated using the formula of Siraramakrishnan et al. (1988).

$$OP_{100} = OP(RWC-AWC)/(100-AWC)$$

OP_{100} = osmotic potential at turgor

OP = osmotic potential

AWC = Apoplastic water content; (assumed to be 12.8%).

The calculations were conducted with the data on Table 10 (for the leaves) and Table 9. The plants used for both experiments were grown at the same time under the same conditions. The results showed that osmotic adjustment at full turgor did happen (Table 11). There was a significant increase in osmoticum due to salinity which shows a true osmotic adjustment phenomena.

Free Amino Acids

Because osmotic adjustment occurred in the salt treated tepary bean seedlings, an experiment was conducted to determine which solutes contributed to the osmotic adjustment. Analysis for the free amino acids in the shoot meristem, leaves, and proximal part of the root over time, indicated that salinity led to a significant increase in free amino acids level in all parts of navy and tepary bean seedlings (Table 12). Free amino acids increased 24 and 48 hr after salinity treatment, but dropped to a level less than that at 72 hr. This might be due to the wilting and

Table 11. True osmoticum concentration (mM) at full turgor for leaves of tepary bean 72 hr after being treated with various NaCl levels.

Solution Osmotic Potential (MPa)	True Osmoticum concentration (mM)
0	233 a ^z
-0.50	263 b
-0.75	418 c

^zMeans followed by a different letter are significantly different at the 0.05 level (LSD).

Table 12. Free amino acids in various plant parts of navy and tepary bean at three daily intervals under NaCl treatment.

	Solution	24 hr	48 hr	72 hr	72 hr
	Osmotic Potential (MPa)	(-ug/g fresh weight-)			(mM)
		<u>SHOOT MERISTEM</u>			
Navy bean	0	1482 a ^z	1915 a	1689 a	
	-0.75	1828 b	3513 b	2499 b	
Tepary bean	0	2398 a	2854 a	2158 a	19 a ^x
	-0.50			2682 b	24 b
	-0.75	3859 b	3669 b	3624 c	37 c
		<u>LEAVES</u>			
Navy bean	0	887 a	1241 a	825 a	
	-0.75	1603 b	2380 b	1396 b	
Tepary bean	0	1707 a	1681 a	1148 a	9 a
	-0.50			1920 b	16 b
	-0.75	2635 b	2835 b	2210 c	19 c
		<u>PROXIMAL ROOTS (3 cm)</u>			
Navy bean	0	362 a	326 a	386 a	
	-0.75	463 b	753 b	452 a	
Tepary bean	0	400 b	468 a	337 a	2.5 a
	-0.50			410 b	3.2 b
	-0.75	526 b	574 b	463 c	4.0 c

^zMeans within a column for a given genotype in a given plant part followed by a different letter are significantly different at the 0.05 level (LSD).

^xThe values obtained for tepary bean 72 hr after NaCl treatment are from two experiments. In the second experiment only tepary bean was used -0.50 MPa treatment was included and the mM concentration of amino acids was calculated.

abscission of the cotyledons 48 hr after the start of the treatment. Cotyledons may have contributed to the increase in free amino acids in the 24 and 48 hr measurements.

Free amino acids were much higher in shoot meristem than in leaves and they were higher in leaves than in roots for both genotypes. The level of free amino acids was much higher for tepary bean than for navy bean (Table 12).

Significant increase in free amino acids induced by salinity was found in all plant parts which indicated that the increase in the level of free amino acids increases with salinity level.

Free amino acids level in leaves of salinity treated tepary bean increased significantly from 9 mM to 19 mM (Table 12), which indicates that free amino acids are minor contributors to total osmotic adjustment. Free amino acids contributed only about 5% to the total osmotic adjustment in the leaves (Table 11). Such finding is in agreement with Chapman and Fischer (1988) results which indicated that the increase in osmotic adjustment may not be the result of accumulation of plant assimilates.

Free Sugar

Free sugars level was measured to determine the contribution of free sugars to osmotic adjustment in navy and tepary bean. The results obtained for free sugars followed the same trend found with free amino acids with salinity causing a significant increase in free carbohydrate levels in shoot meristem, leaves and proximal roots in tepary and navy bean seedlings. In general, tepary bean accumulated more free sugars in

shoot meristem and leaves than navy bean (Table 13). Seventy-two hr after treating the seedlings with -0.75 MPa NaCl free sugars level in navy bean shoot meristem was 164% higher than the control and in treated tepary bean shoot meristem free sugars were 362% higher than the control. In treated tepary bean leaves, the increase in free sugars was not as high as that for shoot meristem. Free sugar levels in leaves of treated tepary bean were 253% higher than the control, whereas in treated navy bean leaves free sugar level was only 163% higher than the control. Free sugar level was similar in the treated seedling in the proximal roots of tepary and navy beans.

An analysis of free sugars showed that all sugars significantly increased in response to salinity, and glucose and fructose were most abundant sugars in the leaves of salt treated tepary seedlings (Table 14). Sucrose level did not increase to the same extent as glucose or fructose. The increase of glucose and fructose may be an artifact due to break down of sucrose during tissue preparation for sugar extraction. HPLC chromatographs showed two peaks which might be raffinose and stachyose because they had the same retention time as standard raffinose and stachyose. Both raffinose and stachyose levels significantly increased following salinity treatment with stachyose increasing two times as much as raffinose.

Free sugars level in leaves of tepary bean increased significantly from 14 mM to 37 mM following -0.75 MPa NaCl treatment (Table 14). The contribution of free sugars to total osmotic adjustment in leaves of salt

Table 13. Free sugars in different plant parts of navy and tepary bean at three daily intervals under NaCl treatment.

	Solution Osmotic Potential	24 hr	48 hr	72 hr
	(MPa)	(----- μgg^{-1} fresh weight-----)		
		<u>SHOOT MERISTEM</u>		
Navy bean	0	643 a ^z	1261 a	1379 a
	-0.75	1413 b	2250 b	2262 b
Tepary bean	0	770 a	630 a	648 a ^x
	-0.50			1720 b
	-0.75	1195 b	2472 b	2345 c
		<u>LEAVES</u>		
Navy bean	0	1130 a	1261 a	1433 a
	-0.75	2431 b	2046 b	2336 b
Tepary bean	0	1067 a	1033 a	1053 a
	-0.50			1516 a
	-0.75	1928 b	2569 b	2660 c
		<u>PROXIMAL ROOTS</u>		
Navy bean	0	725 a	696 a	711 a
	-0.75	1082 b	1199 b	1087 b
Tepary bean	0	677 a	612 a	603 a
	-0.50			1117 b
	-0.75	1122 b	1097 b	1199 c

^zMeans within a column for a given genotype in a given plant part followed by a different letter are significantly different at the 0.05 level (LSD).

^xThe values obtained for tepary bean 72 hr. after NaCl treatment are from two experiments. In the second experiment only tepary bean was used, and -0.50 MPa treatment was included.

Table 14. Free sugar levels in leaves of tepary bean 72 hr after being treated with various NaCl levels.

Solution Osmotic Potential (MPa)	Fructose (----- mM -----)	Glucose	Sucrose	Raffinose	Stachyose
0	3.4 a ^z	3.4 a	3.8 a	2.0 a	1.5 a
-0.50	6.5 b	7.0 b	5.1 b	3.3 b	4.0 b
-0.75	9.5 c	11.5 c	5.2 b	3.4 b	7.0 c

^z Means within a column followed by a different letter are significantly different at the 0.05 level by least significant difference (LSD).

treated bean seedling (Table 11) was only 10% which was twice as great as the contribution of free amino acids.

An increase in the level of free sugars as a response to salinity agrees with findings of Weimberg (1987). The HPLC results showed that glucose and fructose were the most abundant sugars that increased in response to salinity. These findings agree with Riazi et al. (1985) because their results showed that glucose contributed most to organic osmotic adjustment.

Ions and Osmotic Adjustment

Ions accumulation may contribute to osmotic adjustment. Ion levels were measured to determine if they had an impact on solute levels.

In tepary bean seedlings grown for 72 hr in -0.75 MPa saline solution, the Na and Cl ions significantly increased and the Ca and K ions declined in leaves, base of the stem (2 cm), the remaining part of the stem, proximal part of the root (3 cm) and the remaining part of the root (Table 15). Table 15 indicated that inorganic ions in the leaves of tepary bean seedlings treated with -0.75 MPa totaled 337 mM which is equal to -0.85 MPa (~50% of osmotic adjustment) (Munns and Weir, 1981). Such contributions of inorganic ions will help in maintaining favorable water potential in tepary bean seedlings stressed with salinity.

Osmotic adjustment might be an adaption for surviving stress rather than for growing during stress. The solutes which account for osmotic adjustment must be diverted from essential processes such as protein and

Table 15. Inorganic ions in different parts of tepary bean after being treated with NaCl for 72 hr.

Solution Osmotic Potential	Ions			
	Na	Ca	K	Cl
MPa	(----- mM -----)			
	<u>LEAVES</u>			
0	13 a ^z	37 b	97 a	14 a
-0.50	66 b	27 a	106 b	72 b
-0.75	96 c	26 a	114 c	101 c
	<u>BASE OF THE STEM (2 cm)</u>			
0	39 a	13 b	62 a	41 a
-0.50	176 b	12 ab	64 a	193 b
-0.75	190 b	11 a	61 a	210 c
	<u>THE REMAINING PART OF THE STEM</u>			
0	9 a	15 b	89 a	9 a
-0.50	145 b	14 ab	86 a	159 b
-0.75	174 c	11 a	83 a	176 c
	<u>PROXIMAL ROOT (3 cm)</u>			
0	51 a	15 b	84 c	55 a
-0.50	93 b	6 a	62 b	105 b
-0.75	121 c	5 a	34 a	109 b
	<u>THE REMAINING PART OF THE ROOT</u>			
0	14 a	14 b	94 c	10 a
-0.50	62 b	6 a	70 b	83 b
-0.75	79 c	5 a	39 a	91 c

^zMeans within a column for a given plant part followed by a different letter are significantly different at the 0.05 level (LSD).

tissues such as the shoot apex and keep turgor above zero even when the mature leaves die. The importance of solute increases in the dehydrated plant may be in maintaining cell volume at critical value and metabolite concentrations as much as maintaining turgor (Munns, 1988).

Inorganic Ions Distribution

Salt tolerance may also be the result of ion exclusion, so ion distribution study in different parts of navy and tepary bean was conducted to test if tepary bean exhibited ion exclusion.

In navy and tepary bean plants grown for 72 hr in -0.75 MPa saline solution, the Na and Cl ions content significantly increased and the K and Ca ions content declined in stem, leaves and proximal part of the roots (3 cm) Table 16). Navy bean accumulated more Cl and Na ions than tepary bean in all parts measured except for the proximal part of the roots.

In the second experiment, tepary bean seedlings were divided into leaves, base of the stem (2 cm), the remaining part of the stem, proximal root (3 cm), and the remaining part of the root. The results showed a significant increase of Na and Cl ions per g dry weight in salinity treatments of -0.50 and -0.75 MPa and a significant reduction of Ca and K ions as compared to control (Table 17).

Another experiment was conducted to determine the concentration of inorganic ions excluding the apoplastic ion concentration (Table 18). Sodium, Ca, and K ions concentrations were similar to those obtained in Table 17 which indicates that most of these ions are within the cells and apoplastic ions do not contribute much to total concentration of ions.

Table 16. Inorganic ions in different parts of navy and tepary bean after treatment with NaCl for 72 hr.

Solution Osmotic Potential	MPa	Ions			
		Na	Ca	K	Cl
		(----- $\mu\text{mol/g}$ Dry Weight -----)			
				<u>STEM</u>	
Navy bean	0	836 a ^z	254 b	1223 b	906 a
	-0.75	2462 b	174 a	1028 a	2479 b
Tepary bean	0	646 a	240 b	1261 b	723 a
	-0.75	2084 b	156 a	1005 a	2129 b
				<u>LEAVES</u>	
Navy bean	0	157 a	448 b	1225 b	168 a
	-0.75	997 b	235 a	1086 a	1145 b
Tepary bean	0	156 a	448 b	1251 a	164 a
	-0.75	807 b	255 a	1150 a	922 b
				<u>PROXIMAL ROOTS (3 cm)</u>	
Navy bean	0	942 a	404 b	1620 b	930 a
	-0.75	2052 b	198 a	542 a	2151 b
Tepary bean	0	1318 a	416 b	1808 b	1381 a
	-0.75	2671 b	132 a	536 a	2508 b

^zMeans within a column for a given genotype in a given plant part followed by a different letter are significantly different at the 0.05 level (LSD).

Table 17. Inorganic ions in different parts of tepary bean after being treated with NaCl for 72 hr.

Solution Osmotic Potential MPa	Ions			
	Na	Ca	K	Cl
	(----- $\mu\text{mol/g Dry Weight}$ -----)			
	<u>LEAVES</u>			
0	154 a ^z	450 c	1181 b	169 a
-0.50	653 b	264 b	1055 a	722 b
-0.75	827 c	233 a	1007 a	888 c
	<u>BASE OF THE STEM (2 cm)</u>			
0	623 a	206 c	993 c	652 a
-0.50	2412 b	168 b	874 b	2614 b
-0.75	2559 b	144 a	814 a	2788 c
	<u>THE REMAINING PART OF THE STEM</u>			
0	134 a	232 c	1278 c	143 a
-0.50	1865 b	175 b	1149 b	2051 b
-0.75	2130 b	140 a	1033 a	2145 b
	<u>PROXIMAL ROOTS (3 cm)</u>			
0	1240 a	358 b	2046 c	1364 a
-0.50	2098 b	127 a	1384 b	2364 b
-0.75	2602 c	109 a	720 a	2471 b
	<u>THE REMAINING PART OF THE ROOT</u>			
0	349 a	350 b	2764 c	261 a
-0.50	1498 b	136 a	1686 b	2012 b
-0.75	1815 c	127 a	894 a	2087 c

^zMeans within a column for a given plant part followed by a different letter are significantly different at the 0.05 level (LSD).

The increase in Cl ion concentration in various parts of the seedlings following salinity treatment agrees with the results of Harvey et al. (1985) and Schubert and Lauchli (1986) who showed a differential increase in Cl ion concentration in different plant parts due to salinity treatment.

Sodium and chloride ions were retained in the proximal roots of both tepary and navy bean. However, these ions were retained to a higher extent in tepary bean (Tables 16). When tepary bean plants were further divided, Na and Cl ions were concentrated in the base of the stem and in the proximal part of roots (Table 17). These results are consistent with the results of Kramer et al. (1977), who indicated a retention of Na ions in the proximal part of Phaseolus coccineus roots. Jacoby (1965) in his study with Phaseolus vulgaris found that Na accumulated both in the proximal part of the root and in the base of the stem.

The fact that tepary bean accumulated more Na and Cl ions in the proximal roots than navy bean indicates a better exclusion mechanism of these ions from the rest of the plant. This mechanism, as well as osmotic adjustment, may help tepary bean in tolerating higher salt concentration than navy bean.

Anatomy Study

Electron and light microscope studies investigated the effect of salinity on tepary bean seedlings, root and stem xylem tissue and on leaf thickness 72 hr after salinity treatment. The sections examined with the electron microscope were for cortical root cells. They showed no

Table 18. Inorganic ions levels in tepary bean following removal of apoplastic ions after being treated with NaCl for 72 hr.

Solution Osmotic Potential MPa	Ions		
	Na	Ca	K
	(----- $\mu\text{mol/g}$ Dry Weight -----)		
	<u>LEAVES</u>		
0	145	456	1174
-0.50	581	248	1096
-0.75	785	233	1031
	<u>BASE OF THE STEM (2 cm)</u>		
0	639	193	1018
-0.50	2318	151	876
-0.75	2380	135	802
	<u>THE REMAINING PART OF THE STEM</u>		
0	146	248	1295
-0.50	803	168	1152
-0.75	2025	123	1073
	<u>PROXIMAL ROOTS (3 cm)</u>		
0	1221	368	2012
-0.50	1973	125	1386
-0.75	2482	106	696
	<u>THE REMAINING PART OF THE ROOT</u>		
0	332	368	2785
-0.50	1452	119	1706
-0.75	1718	116	924

No replication has been done (no statistical analysis).

differences in the shape of the cortical cells from -0.75 MPa treatment and the control seedlings (Figs. 1, 2, and 3).

Examination of plant tissues with the light microscope indicated no difference in root and stem xylem when treated with salinity (Figs. 4 through 9). Salinity did not cause any change in the arrangement and shape of the cells in the xylem tissue of roots and stems. Also, no specific cells were lost in xylem tissue due to salinity treatment. No transfer cells were observed with the light microscope, maybe higher magnification is required for observing transfer cells. In the leaf tissues, salinity lead to longer palisade tissue and a more free space in the spongy tissue (Figs. 10 through 12). Measurements of the thickness of the leaf palisade and spongy tissues indicated an increase in thickness following salinity treatment (Table 19). These results are consistant with the result of Meiri and Poljakoff-Maybar (1967) who reported an increase in thickness of bean leaves when treated with salinity. The increase in thickness of leaves may be due in part to the increase in leaf succulence. Largerwerff and Eagle (1961) attributed the increase in succulence under salinity stress to Cl ions by improving water balance of the leaf by acting as osmoticum. However, Cl may not be the only ions acting as osmoticum. Table 16 showed that Na ions level also increased in leaves of salt treated plants.

The stem pith diameter and the root diameters were reduced by salinity treatments. This reduction may be due to less water uptake induced by salinity and hence less cell expansion and division.

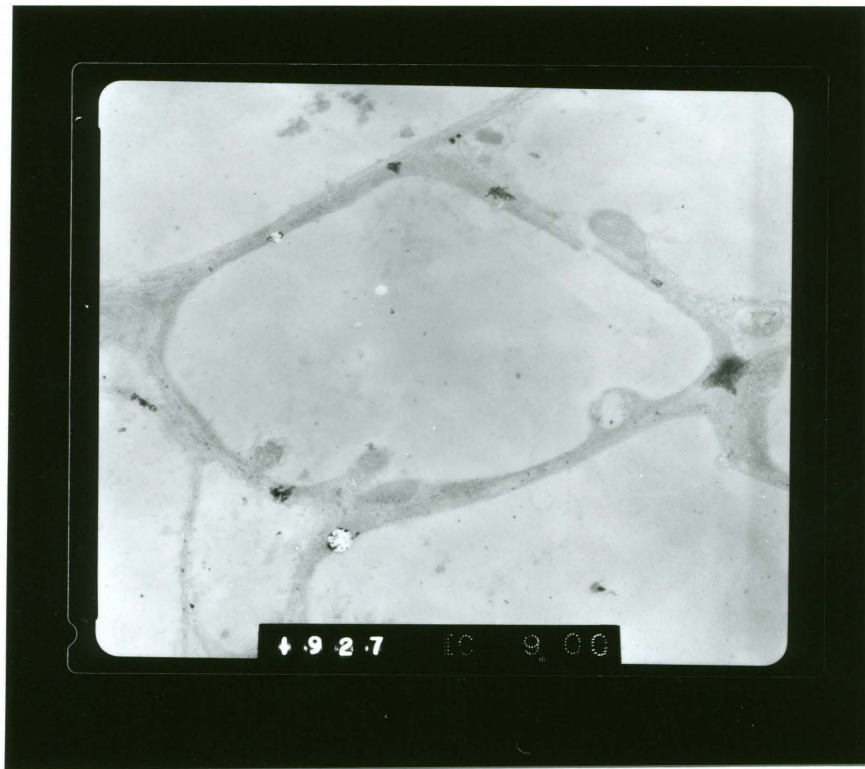


Fig. 1. Electron micrograph of cortical cell in the proximal part of the root of tepary bean grown in Hoagland's solution in the absence of salt ($x = 900$).



Fig. 2. Electron micrograph of cortical cell in the proximal part of the root of tepary bean treated with -0.75 MPa NaCl ($x = 480$).



Fig. 3. Electron micrograph of cortical cell in the proximal root of tepary bean treated with -0.75 MPa NaCl ($x = 900$).

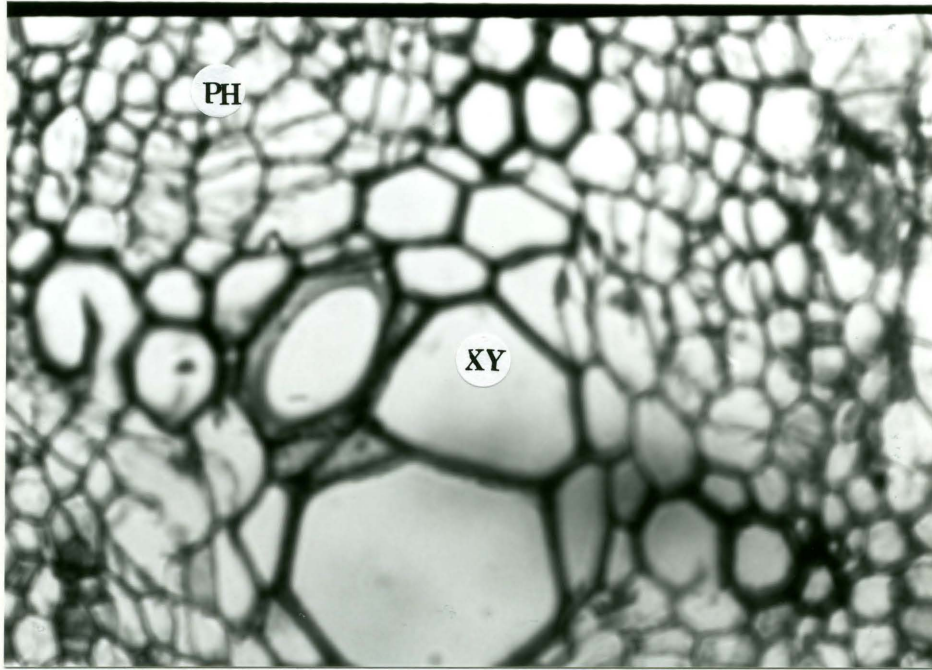


Fig. 4. A cross-section in the proximal part of the root of tepary bean grown in the absence of NaCl. XY, xylem; PH, phloem.

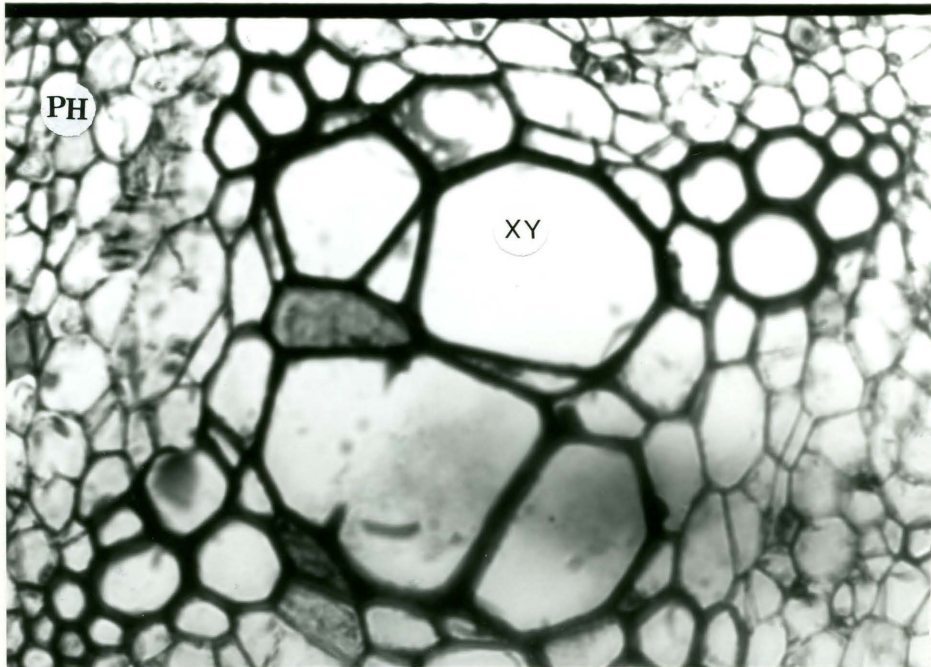


Fig. 5. A cross-section in the proximal part of the root of tepary bean treated with -0.50 MPa NaCl. XY, xylem; PH, phloem.

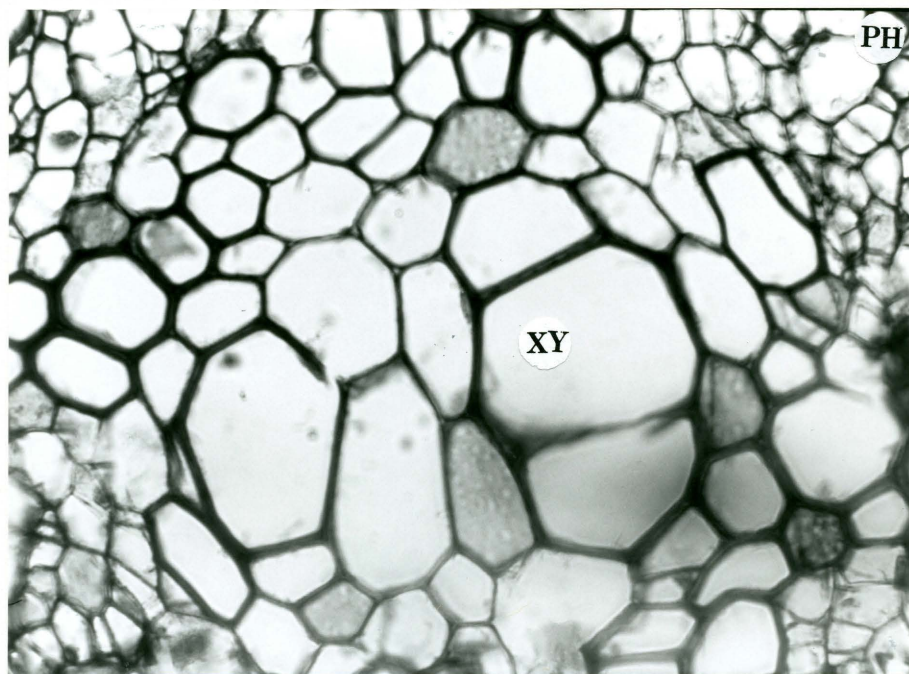


Fig. 6. A cross-section in the proximal part of the root of tepary bean treated with -0.75 MPa NaCl. XY, xylem; PH, phloem.

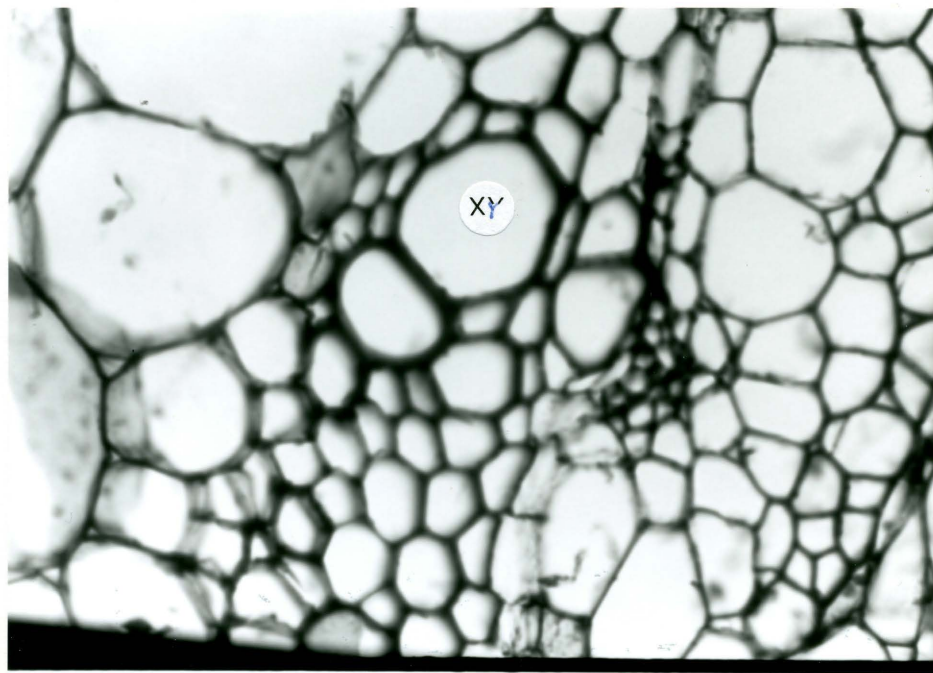


Fig. 7. A cross-section in the base section of the stem of tepary bean grown in the absence of NaCl. XY, xylem.

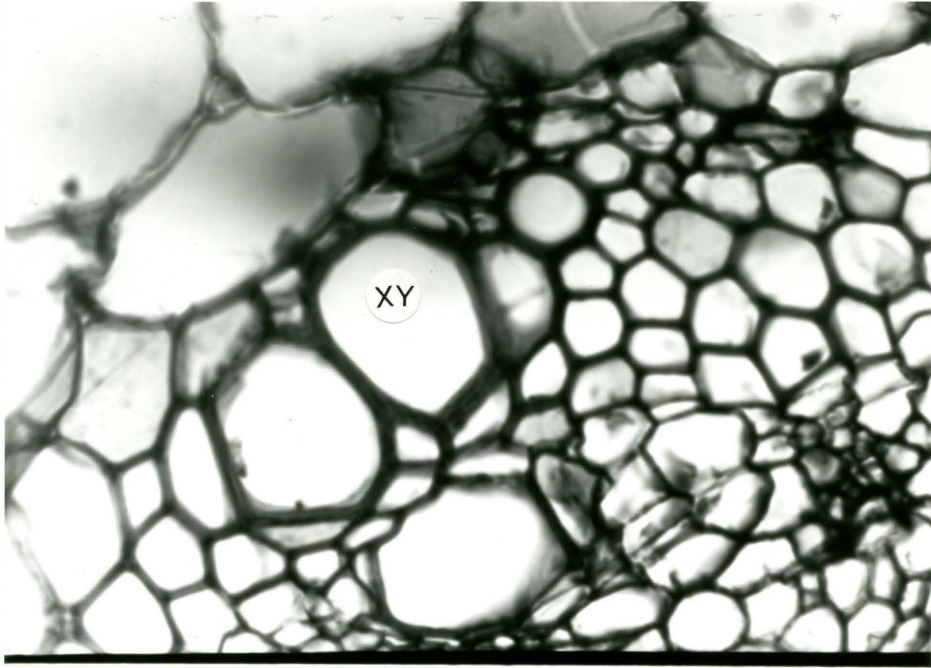


Fig. 8. A cross-section of the base section of the stem of tepary bean treated with -0.50 MPa NaCl. XY, xylem.

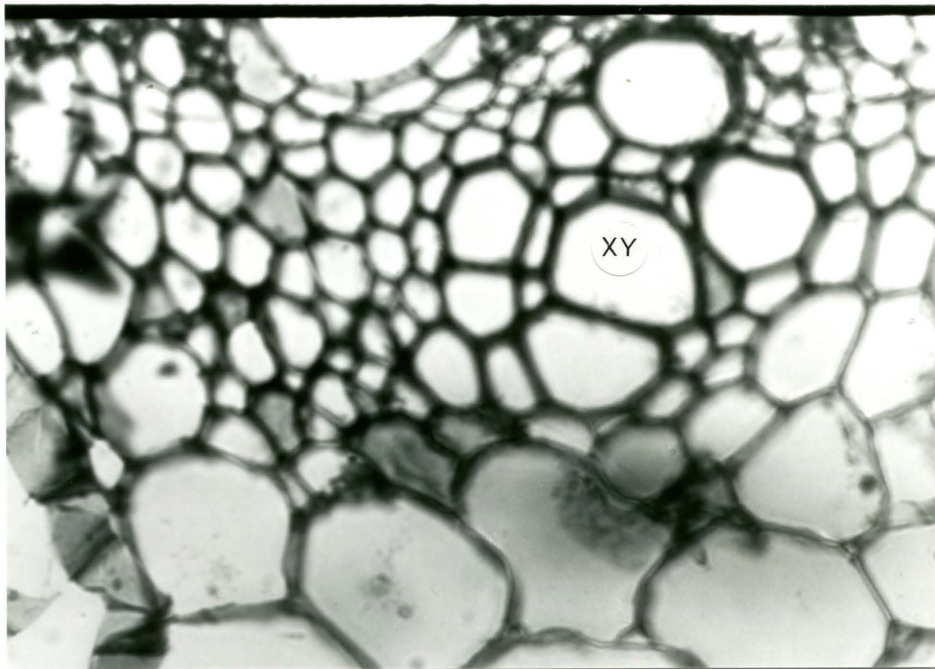


Fig. 9. A cross-section of the base section of the stem of tepary bean treated with -0.75 nPa NaCl. XY, xylem.

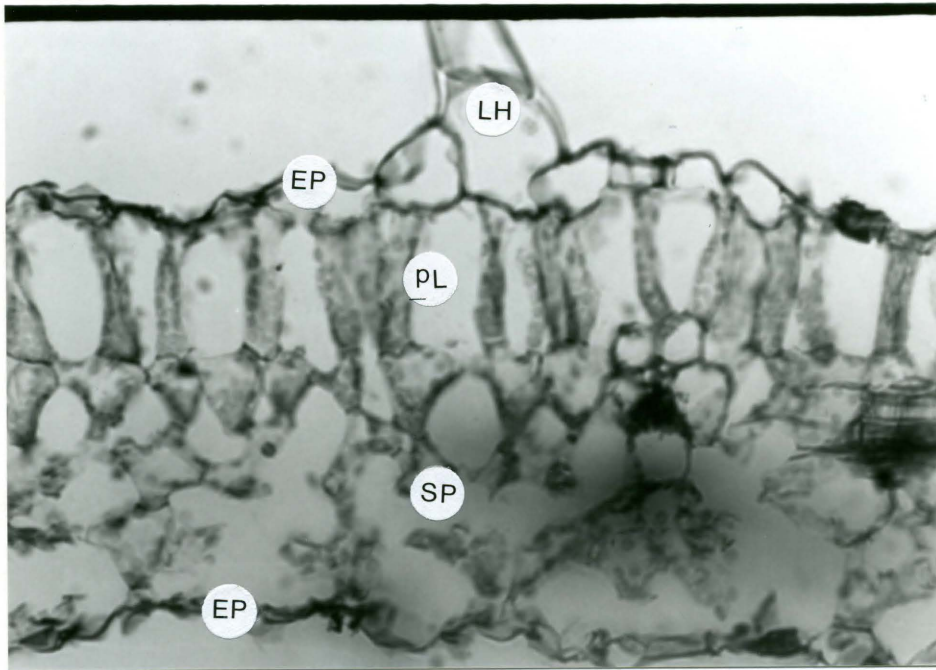


Fig. 10. Leaf cross-section of tepary bean grown in Hoagland's solution in the absence of NaCl. EP, epidermis; LH, leaf hair; PL, palaside layer; SP, spongy layering.

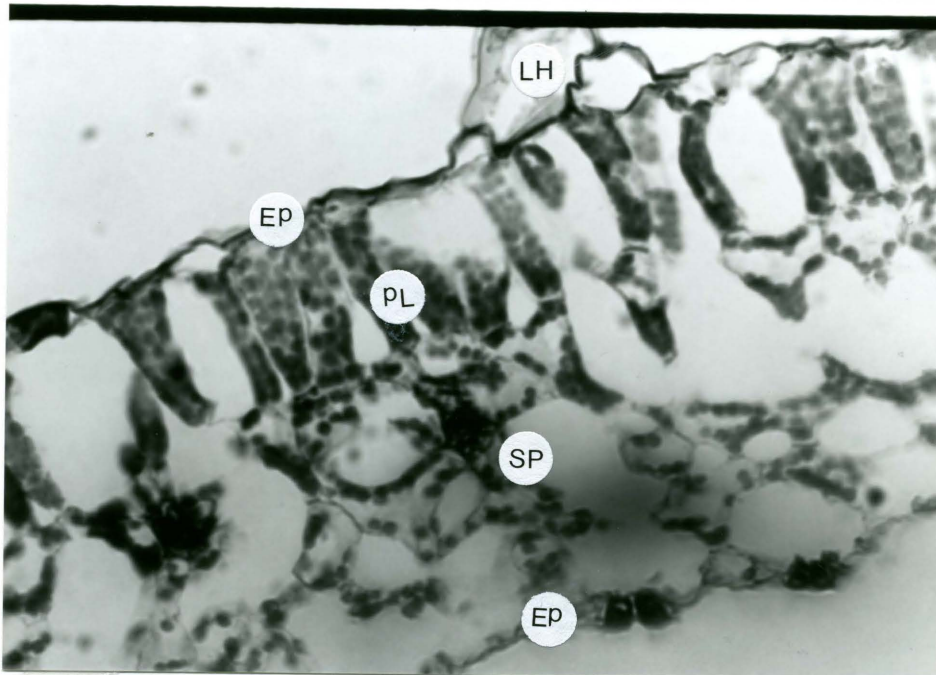


Fig. 11. Leaf cross-section of tepary bean treated with -0.50 MPa NaCl. Ep, epidermis; LH, leaf hair; PL, palaside layer; SP, spongy layer.

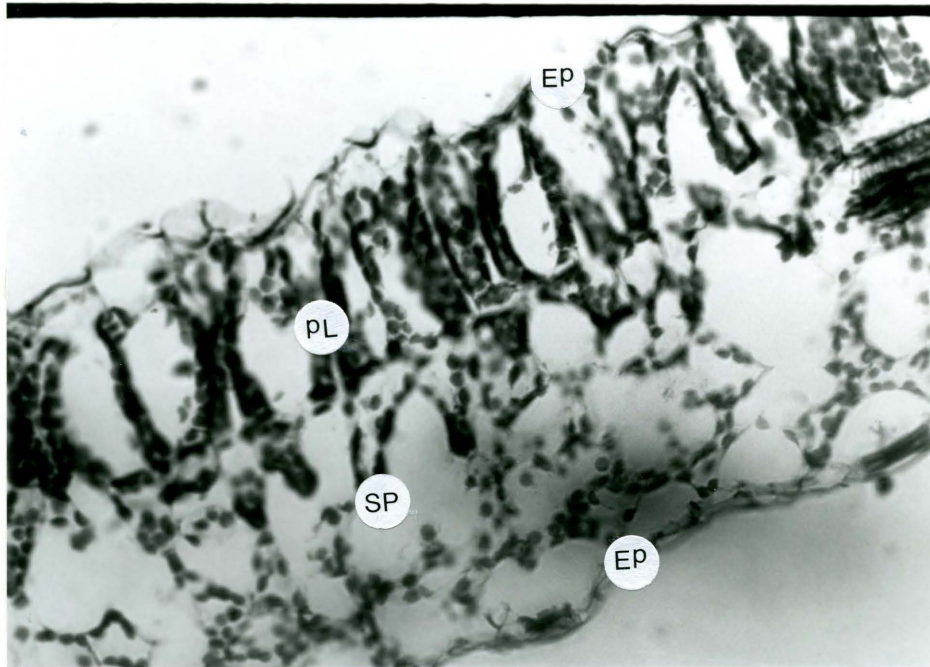


Fig. 12. Leaf cross-section of tepary bean treated with -0.75 MPa NaCl. EP, epidermis; PL, palaside layer; SP, spongy layer.

Table 19. Thickness of leaf palisade and spongy layers, roots, stem pith and the remaining stem width in tepary bean 72 hr after being treated with various salinity levels.

Solution Osmotic Potential	Thickness				
	Leaf palisade layer	Leaf spongy layer	Root diameter	Stem pith diameter	Remaining stem width
MPa	(----- um -----)				
0	20.3 a ^z	34.7 a	119.5 c	85.1 c	85.4 a
-0.50	24.5 b	37.5 b	101.5 b	76.4 b	82.0 a
-0.75	27.8 c	42.5 c	96.0 a	64.7 a	82.0 a

^zMeans within a column followed by a different letter are significantly different at the 0.05 level (LSD).

ATPase Activity

ATPase activity was measured in roots of tepary bean seedlings to determine if there is an involvement of the enzyme in the exclusion of Na and Cl ions.

ATPase activity tripled 72 hr after treating tepary bean with -0.50 MPa NaCl and doubled when the plants were treated with -0.75 MPa NaCl (Table 20). The increase of ATPase activity under salinity treatment was significantly different than the control. The rise in the ATPase activity may indicate the involvement of energy requiring process in the roots, possibly for Na and Cl ion exclusion. However, the reduction seen in the ATPase activity at -0.75 MPa suggests an inhibition of the enzyme at high salinity level which may reduce the exclusion of Na and Cl ions.

The results agree with those of Erdei et al. (1980) who reported that salinity (up to -0.80 MPa) increase ATPase activity of *Plantago*; however, higher levels of salinity will reduce the activity of the enzyme.

Plasma Membrane Leakage

The highest efflux of cellular ^{14}C material from the roots was in the -0.75 MPa treated seedlings followed by the -0.50 MPa treatment and least in the control (Fig. 13). These results indicate that plasma membrane was injured due to salinity shock treatment and the injuries increase with increasing the salinity level.

Salinity treatment significantly led to an increase in the discharge of the radioactive material from the roots in all time sequences

Table 20. ATPase activity in roots of tepary bean 72 hr after NaCl treatment.

Solution Osmotic Potential	ATPase Activity
MPa	($\mu\text{mol phosphate/hr/mg protein}$)
0	10.72 a ^{zx}
-0.50	35.15 c
-0.75	22.26 b

^zMeans followed by a different letter are significantly different at the 0.05 level by least significant difference (LSD).

^xThe numbers obtained here are the mean of six values from two experiments (three for each), the two experiments are exactly the same but have been repeated over time. Time was insignificant at the 0.01 level by LSD.

measured (except for 30 min.) (Table 21). These results are consistent with the results of Mass et al. (1979) who showed that salinity induces leakage of cellular substances like protein from barley plants.

The high discharge of ^{14}C material in the -0.75 MPa treatment and the relative moderate leakages of ^{14}C material from the -0.50 MPa treatment may somehow explain the differences in the ATPase activity between -0.50 and -0.75 MPa treatments. The reduction in the activity of the enzyme with the -0.75 MPa treatment may be due to greater damage caused by salinity to the plasma membrane at the -0.75 MPa than at the -0.50 MPa treatment. This is supported by the higher leakage observed with the -0.75 MPa treatment.

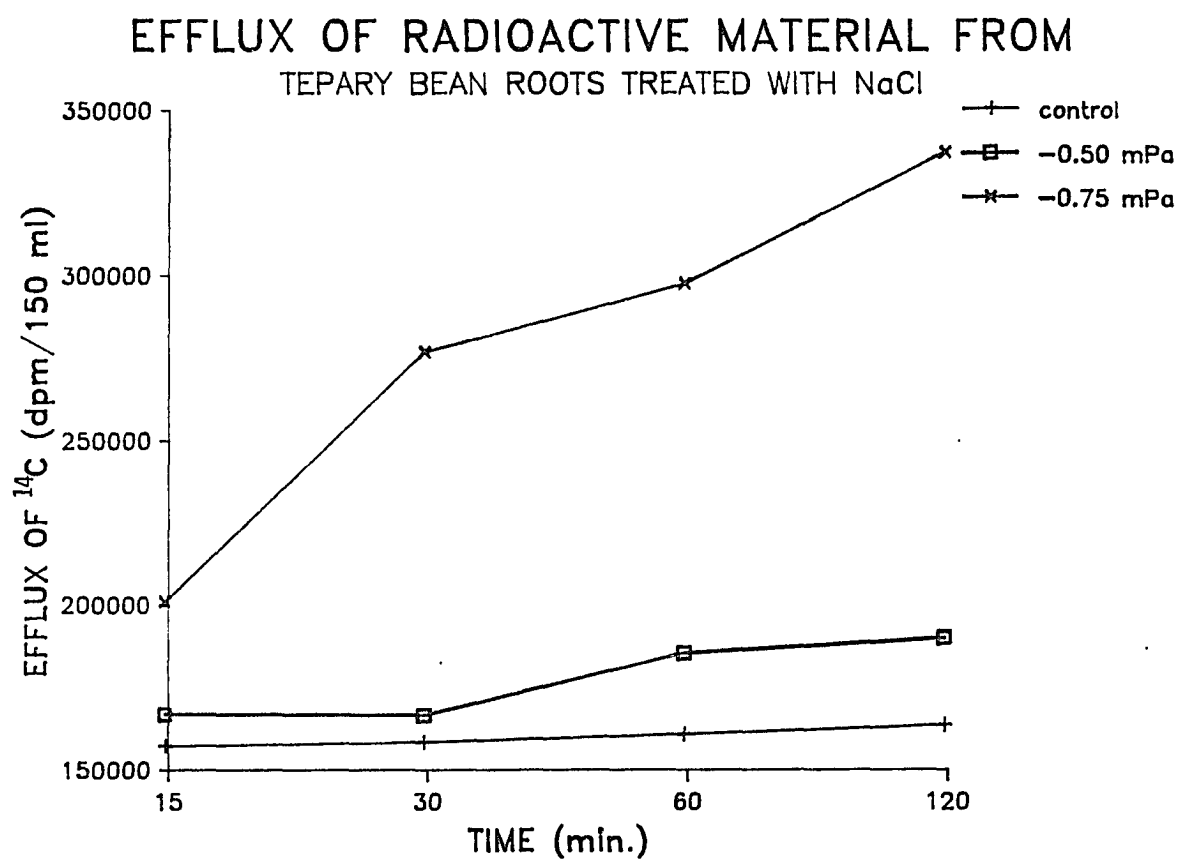


Fig. 13. Efflux of radioactive material over time from tepary bean roots treated with various NaCl levels.

Table 21. Efflux of ^{14}C material from tepary bean roots treated with NaCl for various time periods.

Solution Osmotic Potential		Leakage (dpm/150ml)
	<u>15 min.</u>	
0		156900 a ^z
-0.50		166400 b
-0.75		200400 c
	<u>30 min.</u>	
0		158100 a
-0.50		166100 a
-0.75		276200 b
	<u>60 min.</u>	
0		160500 a
-0.50		185100 b
-0.75		297000 c
	<u>120 min.</u>	
0		163300 a
-0.50		189700 b
-0.75		336700 c

^zMeans within a column for a given time period followed by a different letter are significantly different at the 0.05 level (LSD).

CHAPTER 5

SUMMARY AND CONCLUSIONS

Sodium chloride adversely affect the growth and yield of plants. Successful growth and reproduction of plants under saline conditions requires changes in the metabolism of plants to cope with the salinity stress, such changes include lowering water potential, osmotic adjustment, and exclusion of inorganic ions in some plants (Cheesman, 1988). Not all plants have the ability to alter their metabolism or morphology to deal with salt stress.

Research was performed to explore the effect of salinity on tepary and navy bean. Salinity significantly reduced shoot and root fresh and dry weight, shoot length, leaf area, leaf area index, and relative growth rate for both beans. However, the effect of salinity was more pronounced on navy bean.

Osmotic adjustment in plants is an adaptive response to salinity stress. In tepary bean seedlings, salinity led to an osmotic adjustment in different parts of the seedlings. In the leaves, the osmoticum increased 380 mM for the -0.75 MPa treated seedlings which is equal to about -1 MPa. Water and osmotic potential of leaves and proximal part of the roots of tepary bean were both reduced by salinity while turgor potential stayed almost constant.

Relative water content in leaves of tepary bean was significantly reduced by salinity. Calculation of osmotic potential at full turgor showed a significant rise in osmoticum under salinity treatment.

Free sugars and amino acid, both increased in navy and tepary bean when treated with salinity and they increased to a greater degree in tepary bean. When individual free sugars were measured in the leaves of tepary bean, glucose and fructose were the most abundant sugars increased by salinity. The summation of individual free sugars added up to 38 mM which is about -0.1 MPa. Munns and Weir (1981) assumed that 40 mM free sugars equal to 0.1 MPa. Free amino acids increased in tepary bean to 19 mM following salinity treatment which is about -0.05 MPa. Organic solutes appear to contribute only for a small part of the total osmotic adjustment. This agrees with the view of Hasegawa et al. (1986).

Sodium, Cl, and K ions in the leaves of tepary bean seedlings treated with -0.75 MPa increased by 174 mM which is equal to about 0.45 MPa. This is consistent with the view of Binzel et al. (1983), who found that inorganic ions are the major contributor to the osmotic adjustment.

In summary, the values obtained from the organic solutes and the inorganic ions added up to 0.6 MPa which is 0.4 MPa less than the value obtained when measuring the osmotic adjustment. Such differences may be accounted for by the inorganic ions and other organic solutes like betaine which were not measured.

Tepary and navy bean have an exclusion mechanism for Na and Cl ions and they hold the ions in the proximal part of the root and the base of the stem. Sodium and Cl ion concentration increased while potassium and calcium decreased in different parts of navy and tepary bean plants when treated with NaCl. The increased Na and Cl ions and the decrease in Ca and K ions was more evident for navy bean than for tepary bean. However,

in the proximal part of the roots, tepary bean showed higher accumulation of Na and Cl ions than navy bean which indicates a better ion exclusion mechanism. When apoplastic ions were excluded, the inorganic ion concentration was similar to that obtained with apoplastic ions present.

The electron microscope examination indicated no change in the proximal roots cortical cell under salinity treatment. Light microscope study showed that salinity led to an increase in the thickness of the leaves and a reduction in the diameter of roots and stems.

ATPase activity tripled when tepary bean was treated with -0.50 MPa NaCl and doubled under -0.75 MPa NaCl. When tepary bean seedlings were loaded with radioactive methyl-o-glucose and then treated with NaCl, a tremendous leakage of radioactive material occurred in the root. The outflow of the ^{14}C material increased with time.

In conclusion, tepary bean has certain mechanisms to deal with salt stress like osmotic adjustment, lowering of water potential, maintaining turgor, and exclusion of Na and Cl ions. These adaptive responses will make tepary bean a better candidate to be grown under moderate salt conditions than navy beans.

I suggest for future work that further measurements of inorganic ions should be conducted. Inorganic ions such as NO_3^- , P, S, and betaine be determined to evaluate their contribution to the osmotic adjustment. Also, the partitioning of the inorganic ions between different parts of the cell should be investigated. An anatomical study, especially in the proximal part of the root and in the base of the stem should be conducted

to investigate the affect of salinity on xylem parenchyma cell and whether they develop into transfer cells.

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