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Amelioration Effects of Calcium Amendments on the Growth of *Phaseolus vulgaris* L. Under Sodium Stress

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AMELIORATION EFFECTS OF CALCIUM AMENDMENTS ON THE GROWTH OF PHASEOLUS VULGARIS L. UNDER SODIUM STRESS

by

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Salam Awada

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ABSTRACT

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on the Growth of Phaseolus vulgaris L.
under Sodium Stress

by

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Two greenhouse experiments were conducted to determine the amelioration effect of Ca salts ($CaSO_4$ and $CaCl_2$) on the growth of snapbeans (Phaseolus vulgaris L.) under sodium stress and to determine the effect of ion speciation on the uptake of Ca, Na, SO_4, and Cl by snapbeans.

In Experiment 1, the seeds were grown in styrofoam pots, with a growing medium of sand and vermiculite at a volume ratio of 3:1. The treatment solutions were 0 (Hoagland's solution), 20, 40, 60, and 80 $mmol_c/L$ NaCl or Na_2SO_4.

Statistical analysis (ANOVA) showed that NaCl treatments depressed the growth of snapbeans more than corresponding Na_2SO_4 treatments. Also NaCl treatments increased the uptake of Na and Ca as compared to Na_2SO_4 treatments. Sodium uptake appeared to be related to the concentrations of complex

species rather than to free Na ion, whereas Ca uptake strongly correlated with free Ca^{2+} concentration.

In Experiment 2, the seeds were grown in a sand growing medium. The treatment solutions were 0 (Hoagland's solution), 15, 30, 45, and 60 $mmol_c/L$ NaCl or Na_2SO_4, combined with $CaSO_4.2H_2O$ or $CaCl_2.2H_2O$ at concentrations of 15 or 30 $mmol_c/L$. In addition, one replicate was planted in PVC cells (with electrodes) in order to monitor the electrical conductivity of the media using the four probe.

The results showed that addition of $CaSO_4$ to NaCl or Na_2SO_4 was associated with a better amelioration of Na stress than $CaCl_2$. Also the presence of $CaSO_4$, with NaCl or Na_2SO_4, decreased the uptake of Ca and Na ions relative to $CaCl_2$ treatment. Ion speciation data suggested that Na complexation was more important than free ion concentration in affecting Na uptake by the plant, whereas Ca, SO_4, and Cl uptake were correlated to free ion concentrations.

With respect to EC_a determinations, the four probe was used to monitor salinity during the whole experiment period. Ion speciation data showed that Na_2SO_4 treatments had a lower EC than NaCl at the same molar concentration levels. Also, $CaSO_4$ treatments, regardless of the Na salt, had lower EC values than $CaCl_2$ treatments of equal concentration.

(98 pages)

INTRODUCTION

High salt concentrations in the soil can depress plant growth (Lessani and Marschner, 1978) either by osmotic effects (water stress) or ion-specific effects (ion imbalance or toxicity). Any of these factors may dominate depending upon the composition and concentration of a salt, upon the plant species, and upon varieties and environmental conditions.

It is estimated that on a worldwide basis there are between 400 and 950 million hectares of salt-affected soils (Epstein et al., 1980). These soils are primarily located in arid or semiarid regions, where the problem may become exacerbated by the presence of appreciable quantities of exchangeable Na ions (Gupta and Singh, 1988). The presence of Na, especially as NaCl, in large quantities is a significant problem for crop production in many soils. Excessive levels of Na in the soil solution result in an exchange with Ca and a Na-dominated cation exchange complex. Exchangeable Na can deteriorate the physical structure of the soil by causing swelling and deflocculation of the clay minerals. These processes reduce infiltration rate, reduce aeration, and reduce root penetration (Bresler et al., 1982). Chloride ions in soil solutions can be toxic for certain plants.

Gypsum ($CaSO_4.2H_2O$) is the most common source of Ca used to reclaim sodic soils. The importance of adequate levels of Ca in alleviating the deleterious effects of sodicity on plant growth has been reported (Epstein, 1961). This ameliorating

effect is due to the so-called "antagonism" between Na and Ca ions; that is, the addition of Ca as gypsum will "neutralize" the harmful effects of Na to plants (Lahaye and Epstein, 1971). However, the mechanisms involved in the reduction of Na stress by gypsum are not completely understood.

Little work has been done to investigate why one Ca salt is more effective than another in ameliorating Na stress in plants. A study was done by Akhavan-Kharazian (1986) on bean plants (Phaseolus vulgaris L.) grown under conditions of Na stress. He showed that gypsum was more efficient than $CaCl_2.2H_2O$ in ameliorating the stress. In his study, Akhavan-Kharazian was concerned about the effect of these two different salts on plant properties. He suggested that ion-pairs may play a role, but he did not thoroughly investigate the role of ion speciation in the relative effectiveness of the two Ca sources. This study is focussed on the effect of ion speciation on the uptake of solution ions Ca, Na, Cl, and SO_4.

OBJECTIVES

The principle objectives of this study are the following:

1. To determine if the anion of the sodium salt is significant in inducing sodium stress.

2. To determine if there is any significant difference in ameliorating effects between $CaSO_4$ and $CaCl_2$.

3. To determine the cause of these differences: a) To determine if ion-pair formation has positive effects on uptake of solution calcium; b) to determine if ion-pair formation has negative effects on uptake of solution sodium; c) to determine if ion-pair formation has negative effects on uptake of solution chloride; and d) to determine if ion-pair formation has negative effects on uptake of solution sulfate.

4. To monitor salinity and apparent electrical conductivity (EC_a) variation, using the four-probe configuration, during growth.

5. To determine the effects of ion-pair formation on the apparent electrical conductivity.

REVIEW OF LITERATURE

Increasing salinity in soil and water and its effect on crop production have become vast problems for agriculture in arid and semiarid areas that depend on irrigation (Smillie and Nott, 1982). Further, salinity problems are not confined to irrigated fields; large areas of formerly arable lands are being removed from crop production. Thus, salinity restricts economic utilization of available soil resources and threatens the world's food production capacity and the progress of agriculture (Shokohifard et al., 1989).

Salinity effects on plant growth response, nutrient uptake and utilization differ greatly among species. However, the effects of salinity upon plants are due to depression of the solution water potential, or to the specific ions' effects. Depression of the solution osmotic potential, by high salt concentrations, below that of the cell water potential results in osmotic dessication or a decrease in the water available to plants (Maas and Nieman, 1978). With respect to specific ion effects, the plants response to salinity results from mineral nutrition disorder. For example, high Na concentrations may cause deficiencies of other elements such as Ca or K. Also certain ions, such as Cl, may have toxic effects, which may not always be clearly distinguished from deficiencies of other elements. Specific ion effects may alter growth, composition, and yield of some crops (Pasternak, 1987).

The deleterious effect of salinity on plant growth has been repeatedly reported. Lauchli and Wieneke (1979) observed that a concentration of 10 mM NaCl inhibited the growth of salt-sensitive varieties of soybeans. Higher salinity levels of 80 and 120 mM NaCl were also reported to reduce the growth of barley plants (Helal et al., 1975). In broad beans, Vicia faba L., Ayers and Eberhard (1960) observed a decrease in the growth of plant tops with the increase in salinity levels to 6 dS/m. A similar trend was noted by Maas et al. (1972), who found that the growth of soybean, Glycine max L., tops was depressed as a linear function of NaCl concentration in nutrient media containing up to 100 mM NaCl. In addition, Rabie et al. (1985) reported that the growth of wheat, Triticum aestivum L., tops was decreased at a soil salinity level of 6 g/L and was markedly decreased at the level of 9 g/L.

It is interesting to note that the contents of the nutrient elements in various plant parts are differently affected by salinity. Lauchli and Wieneke (1979) found that a salinity level of 50 mM NaCl decreased the K concentration in soybean roots, but that the reverse was true for leaves. However, an opposite trend was observed for Na. Wieneke and Lauchli (1980) also reported that, at a salinity level of 66.5 mM, Na was retained in the proximal roots and stems of soybeans and that the increase of the level of salinity substantially reduced the Ca uptake by the plants. Rabie and

Kumazawa (1988) reported that salinity generally increased K, Ca, and Mg concentrations in the leaves and decreased them in the roots. However, short-term salinization decreased the total uptake of K, Ca, and Mg by plants. Sameni et al. (1980) studied the effects of N fertilization on the growth and mineral composition of Phaseolus vulgaris L. grown under saline stress. They found that growth and N uptake by bean plants generally decreased with increased salinity in irrigation water but increased Cl and Na uptake by the plants.

Many methods have been used to mitigate saline problems. Among these methods is the reclamation of sodium-affected soils using Ca amendments, that is, replacement of exchangeable Na^+ with Ca^{2+}. Among the amendments used to provide soluble Ca, gypsum is the most commonly used as a soil amendment for sodic soil reclamation and as a water amendment to reduce the Na hazard of irrigation water (Tanji, 1969).

The regulatory role of Ca^{2+} in development and growth of plants under saline conditions is well established. Calcium is known for the important role that it plays in cell membrane permeability and transport. Sodium ions have been shown to cause disturbances in Ca regulatory functions. Nutritional imbalances involving other ions may be linked to the effects of salinity on the metabolism and movement of Ca. When solution Ca concentrations are high, they may alleviate the effects of salinity; but high ratios of Na to Ca in the medium tend to be harmful and may adversely affect membrane

permeability and growth within minutes (Epstein, 1961; Lauchli and Epstein, 1970; Cramer et al., 1988). Different types of plants have been observed to have widely different responses due to Na-Ca interactions.

Lahaye and Epstein (1969) reported that when salt-sensitive bean plants were grown in a 50 mM NaCl solution and 1 mM or less $CaSO_4$ concentrations, growth of the beans was retarded during the one-week experiment. Adding 3 or 10 mM Ca^{2+} protected the plants from the salinity effects, however. Similar findings were obtained by Lahaye and Epstein (1971). Their study showed that soybean plants grown in the presence of 50 mM NaCl and 10 mM $CaSO_4$ flowered and set fruit normally and their roots remained white colored and healthy. Cramer et al. (1985, 1986) and Lynch et al. (1987) reported that increasing Na levels caused a displacement of Ca^{2+} by Na^{+} from root membrane sites of cotton and corn plants. They also found that an increase in external Ca concentration (10 mM) alleviated the displacement. Cramer and Spurr (1986a) studied the effect of Ca on the growth and mineral nutrition of lettuce, Lactuca sativa L., in the presence of Na. They reported that high external Ca decreased the absorption of Na but did not affect Cl tissue concentration nor shoot or root growth.

A study by Maas and Grieve (1987) stressed the importance of different Na/Ca ratios on the growth and chemical composition of corn, Zea mays L.. In their study, they found

that at a 34.6:1 molar Na/Ca ratio the plants suffered from Ca deficiency, whereas at a ratio of 5.7 or less no injury symptoms were evident and the Ca concentration was sufficient to overcome the apparent Na-induced Ca deficiency. Also they found that the Cl was readily concentrated in the shoot when salinized, and Cl concentrations in the tissue generally increased as the solution Na/Ca ratio increased to about 1. In a similar experiment with corn, Plaut and Grieve (1988) found that at low Na/Ca ratio in the media, the rate of CO_2 fixation (photosynthesis) decreased and the possible cause was, in part, Ca-induced Mg deficiency.

This review of literature has shown that very little work has been done to determine the role of ion speciation of treatment solution in ameliorating Na stress on plants. A brief discussion on ion speciation follows.

Complete ion dissociation occurs with some salts dissolved in water, but complete dissociation is not universal. For example, a large fraction of the cations and anions of certain strong electrolytes are so attracted to one another in the soil solution that they behave as if un-ionized. Ions associated in this manner are called ion-pairs or complexes; these soluble associations can be neutral, positively, or negatively charged (Adams, 1971). The importance of these ion-pairs is that they reduce the effective free ion salt concentration which, in turn, reduces the current carrying capacity of the soil solution.

Consequently, ion-pairing reduces the electrical conductivity (EC) and the osmotic potential of a given solution (Jurinak, 1988).

In a soil solution, an ion may be present as several different species due to ion-pair formation. For example, soil solution Ca may be present as Ca^{2+}, $CaSO_4^{\circ}$, $CaHPO_4^{\circ}$, $CaH_2PO_4^{+}$, and $CaHCO_3^{+}$ (Adams, 1971). However, many analytical techniques only determine the total concentration of Ca in solution making no distinction among the species. Concentrations of the free ionic species are often needed so that ion activities may be calculated (Dudley and Coray, 1989; Cramer and Lauchli, 1986; Amacher, 1984). This is possible through application of a thermodynamic model of ion speciation to the total concentration data. This approach has been described in the literature (Stumm and Morgan, 1981; Lindsay, 1979; Sposito and Mattigod, 1979). However, the following information should be available (Sposito, 1989):(1) measured metal and ligand total concentrations, along with the pH values, and (2) conditional formation constants for all possible complexes of the metals and H^{+} with the ligands (Appendix A).

The following general principles apply to ion-pairing of common soil solution cations and anions (Adams, 1971; Jurinak, 1988):

1. Ion-pairing with SO_4^{2-} is general; it is slight with univalent cations but extensive with multivalent cations.

2. Ion-pairing with Cl^- is only slight for univalent cations but it is significant with multivalent cations especially at high concentrations.

3. Ion-pairing between HCO_3^- and univalent cations is not significant; ion-pairing of multivalent cations with HCO_3^- is significant at high pH or above normal atmospheric pressure.

The focus of this research was on the effect of ion speciation on the uptake of certain solution ions (Ca, Na, Cl, and SO_4) by snapbean plants.

MATERIALS AND METHODS

Experiment One

Three snapbean seeds, Phaseolus vulgaris L. cv contender, were inoculated with commercial Rhizobia Phaseoli and planted in one liter styrofoam pots. The plants were grown under greenhouse conditions with a day/night regime of 14/10 hours at 25/28°C. The growth medium was sand and vermiculite at 3:1 ratio, respectively, by volume. The saturation water content on a mass basis of the growth medium was 0.31.

The pots were placed on a greenhouse bench in a randomized block design with eight replications. The plants were thinned to two healthy plants per pot one week after seedling emergence.

Stock solutions were NaCl and Na_2SO_4 salts at five different concentrations of 0, 20, 40, 60, and 80 $mmol_{(c)}/L$ in a background of one-quarter strength Hoagland's solution (Hoagland and Arnon, 1950). The electrical conductivity (EC) of all stock solutions were between 0.87 to 8.85 dS/m.

Salt treatments were started on the fourteenth day after germination, each pot received 200 ml (including a 0.25 leaching fraction, LF) of the assigned solution and the plants were irrigated every other day for four weeks.

Experiment Two

Three snapbean seeds were planted in one liter styrofoam pots. The growth medium was sand and the plants were grown

under the same greenhouse conditions as in Expeiment # 1. The pots were placed in a randomized block design with nine replicates. In addition to the nine replicates, one replicate was planted in 1.5 liter PVC pots. The PVC pots had, at 9 cm depth, six equally spaced electrodes that were used to determine bulk electrical conductivity (EC_a), using the four probe, during the growing period. The saturation water content (on a mass basis) of the growth medium was 0.46 and the bulk density was about 1.45 Mg/m^3.

Stock solutions were NaCl and Na_2SO_4 salts at five different concentrations: 0, 15, 30, 45, or 60 $mmol_{(c)}/L$. These solutions were mixed with $CaSO_4.2H_2O$ or $CaCl_2.2H_2O$ salt at two different concentrations, 15 or 30 $mmol_{(c)}/L$. The background for each treatment was Hoagland's solution at one-tenth strength. In addition, the control treatment was Hoagland's solution at one-tenth strength for a total of 37 treatments. The EC of all stock solutions was between 0.152 and 9.468 dS/m. Treatments started on the fourteenth day after germination. Each styrofoam pot received 300 ml (including a 0.20 LF), while PVC pots received 450 ml (including 0.20 LF) of the assigned solution. Plants were irrigated once every four days for four weeks.

The plants of Experiment # 1 and five replicates of Experiment # 2 were harvested six weeks after planting. The shoots were collected, dried in the oven at 60°C for 5 days, weighed, and ground. 0.5 grams of the dried material was

digested (see appendix B for digestion procedure) with HNO_3 and H_2O_2 (Luh Huang and Schulte, 1985).

Soil saturation extracts were prepared for two replicates of Experiment # 1 and three replicates of Experiment # 2. The EC and pH of each of these extracts were then determined (Appendix C).

Calcium and Mg concentrations were determined by atomic absorption spectrophotometry (Baker and Suhr, 1982). Sodium and K concentrations in soil and plant tissues (Appendix D) were determined by emission spectrophotometry. Sulfate concentrations were determined using an ion analyzer (Anonymous, 1987)

Chloride was extracted from 100 mg of dry plant material in 10 ml of deionized distilled water heated to 90°C and shaken for ten minutes (Cramer and Spurr, 1986b). Chloride was determined, in both soil and plant extracts, by titration with standardized $AgNO_3$ solution (Adraino and Dones, 1982; Appendix C).

The program, SPECO2, was used to compute the speciation among Ca, Na, Cl, and SO_4 ions (Dudley and Coray, 1989; see Appendix A for Thermo.Dat File).

Four-Probe Calibration

Geophysicists have used a four-electrode configuration to determine subsurface strata depth by means of a resistance measurement. Shea and Luthin (1961) and others found this

method was applicable to saline soils. Measuring soil conductivity requires an electric source and resistance meter, four electrodes (metal), and connecting wires. Rhoades et al. (1977) used a four-electrode array with coring technique to measure soil resistance at discrete depth increments. However, this device is not convenient for field measurements. But the higher accuracy possible with four-electrode array makes them useful for calibrating soil salinity against soil electrical conductivity (EC_a).

In this study, thirty seven pipes (cells) were constructed from PVC irrigation pipe. Six bolts evenly spaced around the sides of the cell functioned as electrodes to obtain resistance readings. The cell constant (K) was obtained for each of the four cells by filling each cell with a solution of known electrical conductivity at 25°C (EC_{25}) and measuring the cell resistance (Call, 1979). Four electrodes were involved in each reading. Each electrode was connected to the resistance meter by an insulated wire. The inner pair of electrodes were used to measure soil resistance while a current passed between the outer pair. All four wires were moved one electrode clockwise between each reading for a total of three readings per cell.

The following equation was used to determine the cell constant K (Rhoades et al., 1977):

$$K = EC_{25} * R_t * f_t^{-1} \qquad [1]$$

where f_t is a temperature correction factor for conductivity

and resistance data (see page 90, U.S. Salinity Laboratory Staff, 1954). Table 1 gives the K and R_t values that were measured for each cell.

The cells were then filled with soil, and their EC_a was calculated from the resistance measured, temperature of the soil, and cell constant (K) using the following equation:

$$EC_a = K.f_t.R_t^{-1} \quad [2]$$

Table 1. Measured R_t (ohms) and calculated K values for four-electrode cells.

	Cell No.			
	1	2	3	4
Average R_t	902.7±13.6	924.3±17.3	866.7±16.8	897.7±14.2
Average K	35.1	36.0	33.8	35.1

The EC of saturation extract (EC_e) was then determined for the soil samples for which EC_a had been calculated as mentioned above. An EC_a-EC_e calibration curve was then plotted for the desired range of salinity as shown in Fig. 1.

During the greenhouse experiment, a resistance measurment was taken 24 hours after each application of treatment solutions. One measurement was taken each week during the five weeks. Then EC_a was subsequently calculated from the measured resistance using equation [2] above.

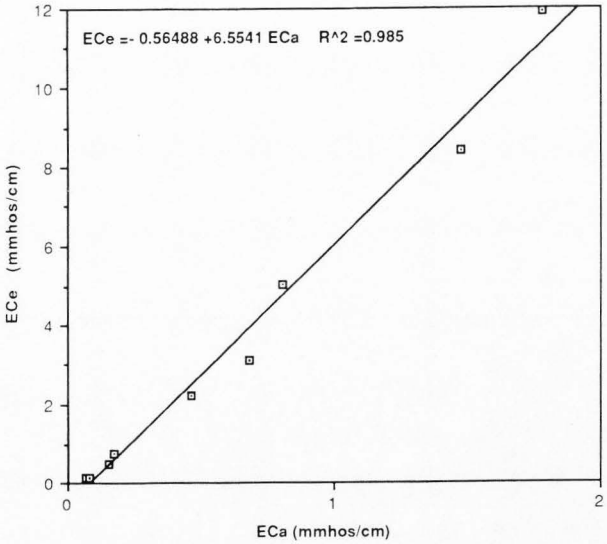

Fig. 1. EC_e-EC_a calibration curve using the four-probe.

RESULTS AND DISCUSSION

Experiment One

Ion Speciation Effect on Sodium Uptake: Plant Na content significantly increased with the increase in applied salt concentrations (Fig. 2). Application of the factorial analysis of variance (ANOVA, Table 2) to the data indicated that a significant difference (α=0.05) in Na content existed between the control plants (Hoagland's solution treatments) and the salt treated plants (Fig. 2). Also the analysis showed that there was a significant difference in Na uptake between the two types of Na salts and among the different levels applied. This difference was especially true at higher concentrations (60 and 80 $mmol_{(c)}/L$). Data in Fig. 2 show that Na uptake by NaCl treated plants was almost double the amount taken up by Na_2SO_4 treated plants. Results of the ANOVA also indicated that there was a significant interaction between Na salt type and concentration applied (S*L) which implies that salt type and concentration were dependent on each other in affecting Na uptake by the plants.

Ion speciation data (Tables 3 and 4) suggested that Na uptake by the plant might have been dependent on the complexes it formed in soil solution. Soil solution total Na concentrations, at each level, were almost the same for the two types of salts. Also free Na ion concentrations (Figs. 3a and 3b) were about 95 percent of the total Na concentrations

for the two different Na salts. However, the Na species were different in each solution due to the presence of different ligands. The data indicated that Na complex was more important than the free ion in determining Na uptake by snapbean plants. Data in Figs. 3a and b show that free ion concentrations were more or less the same for the different solutions, but more Na was taken up from the NaCl solution treatments. It is possible that the $NaCl^{o}$ complex had a significant role in increasing Na uptake relative to the Na-SO_4 complexes. This might indicate that Na-ligand complex form could either increase ($NaCl^{o}$) or decrease (Na-SO_4) Na uptake by the plants. However, further research is still needed to understand the importance of complexation in determining Na uptake.

Besides ion speciation effects on the uptake of Na, the anion (Cl or SO_4) might have played an important role in the physiological processes of the plants grown under sodic conditions. Mengel and Kirkby reported (1982) that detrimental effects of NaCl on plant growth resulted from salt induced physiological disorder. They also reported that toxicity would begin with an imbalance of ions in the plant tissues, often with a large excess of Na. The plant could regulate, to some degree, the excess Na^{+} either by inhibiting its uptake, or by secreting it into vacuoles. However, these regulatory processes would require an additional amount of energy. Sodium and Cl ions also have detrimental effects on

Table 2. Factorial analysis of variance for Na ion.

SV	DF	MS	F
Blocks	7	97575181	1.02
Treats.	8	4677670600	48.78**
Control vs Na salts	1	11922201000	124.32**
Among Na salts	7	3642737600	37.98**
Salt	1	8033064800	33.77**
Level	3	5445353500	56.78**
SxL	3	376679040	3.93*
Error	56	95898609	
Total	71		

* significant at $\alpha = 0.05$.
** significant at $\alpha = 0.01$.

Table 3. Sodium and ligands distribution (SPECO2) for the different salt solution extracts.

NaCl	Na(%)			Na_2SO_4	Na(%)		
$mmol_{(c)}/L$	Free Na^+	$Na-SO_4$	Na-Cl	$mmol_{(c)}/L$	Free Na^+	$Na-SO_4$	NaCl
0	99.85	0.04	0.09	0	99.85	0.04	0.09
20	96.67	----	3.22	20	95.51	4.40	0.01
40	94.92	----	5.03	40	95.23	4.65	0.06
60	94.22	----	5.78	60	94.46	5.51	0.02
80	93.27	----	6.71	80	93.47	6.48	0.04

Table 4. Sodium and ligands distribution (SPECO2) for the different solution treatments.

Salt	Na			
$mmol_c/L$	Total ($mmol_c/L$)	Free(%)	SO_4(%)	Cl(%)
40 NaCl	40	97.4	0.01	2.57
80 NaCl	80	95.5	0.01	4.43
40 Na_2SO_4	40	96.22	3.76	----
80 Na_2SO_4	80	93.88	6.08	----

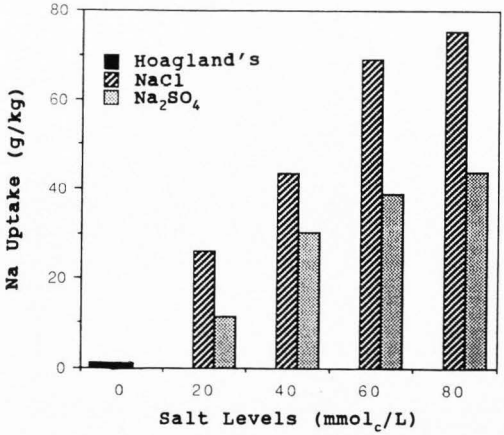

Fig. 2. Phaseolus vulgaris L. shoot Na content resulting from irrigation with Na_2SO_4 and NaCl solutions.

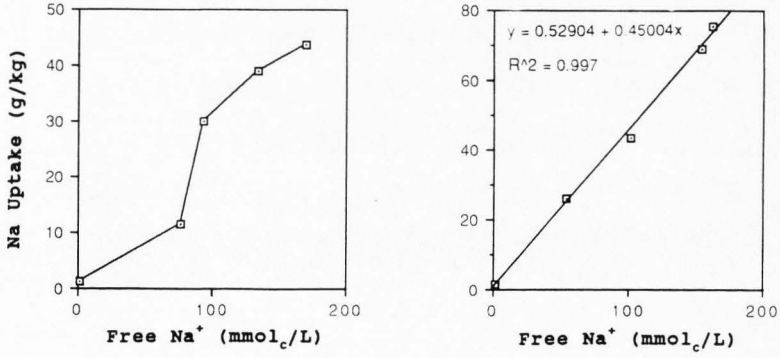

Fig. 3. The relationship between Na plant uptake and the free Na^+ ion concentration in (a) Na_2SO_4 and (b) NaCl solutions.

the enzymatic and photosynthetic activities (Akhavan-Kharazian, 1986). In contrast to NaCl, plants grown under Na_2SO_4 conditions would have a better energy status due to the important effect of S on the properties and structural conversions of the protein molecules as well as the redox reactions of the cells (Mercado and Golleck, 1973; Mengel and Kirkby, 1982). Also, SO_4 ion might have important role in governing normal membrane impermeability to the transport of ions (Akhavan-Kharazian, 1986). As a result, these plants in SO_4 treatments would be more able to exclude Na ion and minimize its uptake as compared to NaCl treated plants.

Ion Speciation Effect on Calcium Uptake: Calcium uptake by snapbean plants was significantly affected by the type of Na salt applied (Fig. 4). Factorial analysis of variance (Table 5) showed that there was a significant difference (α=0.05) in Ca uptake among the nine treatments. Sodium sulfate treated plants showed a decrease in Ca content as compared to the control plants, whereas NaCl treated plants increased Ca content. This indicated that there was a significant effect on Ca uptake between the two types of Na salts. Also the analysis, along with Table 8, indicated that different levels of Na_2SO_4 did not effect Ca content of the plant, but the trend for increasing NaCl concentrations had been to decrease Ca uptake.

Ion speciation data (Tables 6 and 7) showed that Ca

uptake, unlike Na, increased with an increase in free Ca^{2+} ion in soil solution. Although total Ca concentration (Table 6) in Na_2SO_4 soil solutions was significantly higher than those of NaCl solutions, there was more Ca uptake from NaCl than Na_2SO_4 soil solutions at the same treatment level. In Na_2SO_4 solutions, free Ca^{2+} ion was about 60 percent of the total Ca concentration at all sodium levels, and the complexes, mainly as $CaSO_4^o$, were about 40 percent. Whereas, in the control and NaCl solutions, free Ca^{2+} ion was about 99 percent of total Ca. Nearly all of the Ca available for plant uptake was free Ca^{2+} ion (Table 8). This might imply that SO_4 complexation with Ca may decrease Ca uptake by the plant. Also the data showed that with the increase in Na concentrations in treatment solutions, complexed and free Ca^{2+} increased in soil solution. However, this increase in free Ca^{2+} did not increase the uptake of Ca^{2+} due to the competition between Na and Ca for the same sites on the carrier and/or because of absorption of Ca at slower rates in the presence of high Na concentrations under NaCl salinization (Lessani and Marschner, 1978). Furthermore, the increase in NaCl concentrations increased the stress on the growth, shoot production, and yield of the plant; consequently, less Ca ions was present in the plant at higher levels of NaCl.

Chloride Uptake: Chloride uptake increased linearly with the increase in NaCl concentrations. Chloride content

Table 5. Factorial analysis of variance for Ca ion.

SV	DF	MS	F
Blocks	7	83786074	2.38*
Treats	8	488631140	13.89**
Control vs Na salts	1	193688	0.01
Among Na salts	7	558407920	15.88**
Salt	1	3769308000	107.17**
Level	3	17576813	0.50
SxL	3	28938991	0.82
Error	56	35171455	
Total	71		

* significant at $\alpha = 0.05$
** significant at $\alpha = 0.01$

Table 6. Calcium and ligands distribution (SPECO2) for the different salt solution extracts.

NaCl	Ca(%)			Na_2SO_4	Ca(%)		
$mmol_{(c)}/L$	Free Ca	$Ca-SO_4$	Ca-Cl	$mmol_{(c)}/L$	Free Ca	$Ca-SO_4$	Ca-Cl
0	98.22	0.80	0.01	0	98.22	0.80	0.01
20	99.64	0.00	0.28	20	61.96	37.96	0.00
40	99.39	0.00	0.47	40	61.84	37.77	0.00
60	99.46	0.00	0.51	60	60.17	39.76	0.00
80	99.24	0.00	0.69	80	57.82	42.12	0.00

Table 7. Calcium and ligands distribution (SPECO2) for the different solution treatments.

Salt	Ca			
$mmol_c/L$	Total ($mmol_c/L$)	Free (%)	SO_4	Cl(%)
40 NaCl	2	99.5	0.19	0.23
80 NaCl	2	99.4	0.12	0.43
40 Na_2SO_4	2	60.2	39.72	----
80 Na_2SO_4	2	56.5	43.49	----

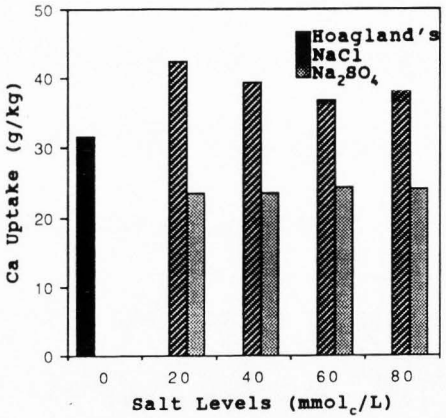

Fig. 4. Phaseolus vulgaris L. shoot Ca content resulting from irrigation with Na_2SO_4 and NaCl solutions.

Table 8. Calcium uptake as affected by different Na salt treatments.

Sodium Treatments.	Free Ca^{2+} in NaCl	Ca uptake	Free Ca^{2+} in Na_2SO_4	Ca uptake
$mmol_c/L$		g/kg	$mmol_c/L$	g/Kg
0	5.96	31.61	5.96	31.61
20	5.54	42.27	4.78	23.52
40	7.30	39.38	7.00	23.44
60	10.70	36.62	8.04	24.22
80	7.91	38.22	7.20	23.92

increased from 0.7 g/kg (dry weight basis) at 0 salt level up to 147 g/kg at 80 $mmol_{(c)}$/L NaCl. Factorial analysis of variance (Table 9), indicated that there was a significant difference in the uptake of solution Cl among the nine treatments, but no significant difference existed between the control and Na_2SO_4 treated plants. However, the most significant difference was due to the salt type (NaCl) and the level applied, as the data in Fig. 5 show. Also, ANOVA indicated that salt type, especially Na_2SO_4, and the level applied (S*L interaction) were highly dependent on each other in affecting chloride uptake.

The results of this experiment were in agreement with findings of Cramer and Spurr (1986a and 1986b); Seeman and Critchley, 1985) who found that the internal Cl ion concentration increased linearly with external Cl (solution) up to 150 mM NaCl. Many investigators (Greenway, 1973; Mengel and Kirkby, 1982) reported that the nutrient concentration at the root surface directly controls nutrient uptake. Mengel and Kirkby (1982) indicated that soil with a high nutrient level had a steeper concentration gradient; consequently, the rate of diffusion to the plant root would be greater. A higher nutrient level in soil solution would also give a higher concentration at the root surface that could cause more rapid uptake, and a larger gradient would allow this to be maintained. Chloride ion content of the plants as a function of the NaCl concentrations at which the plants were grown is shown in Fig. 6. The data show that there is a linear

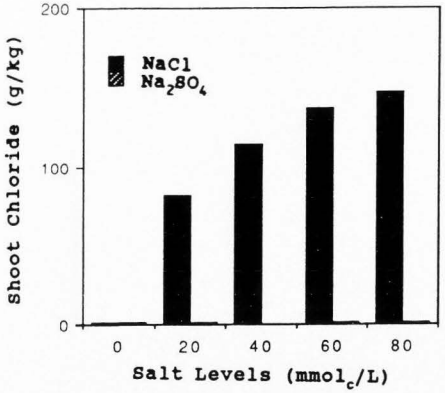

Fig. 5. Phaseolus vulgaris L. shoot Cl content resulting from irrigation with Na_2SO_4 and NaCl solutions.

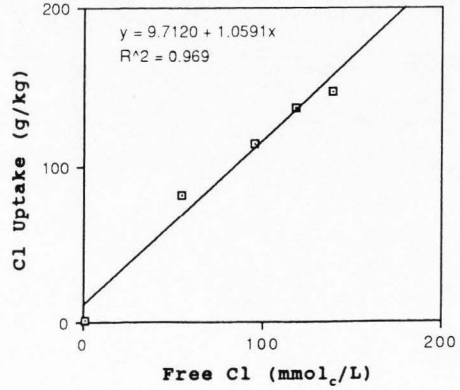

Fig. 6. The effect of varying external NaCl concentrations on the uptake of Cl by Phaseolus vulgaris L.

Table 9. Factorial analysis of variance for Cl ion.

SV	DF	MS	F
Blocks	7	113610270	1.77
Treatsments	8	34161077000	532.22**
Control vs Na salts	1	25215664000	392.85**
Among Na salts	7	35438992000	552.12**
Salt	1	228117760000	3553.98**
Level	3	3323384800	51.78**
SxL	3	3328343700	51.85**
Error	56	64186591	
Total	71		

** significant at $\alpha = 0.01$.

relationship between plant Cl and external NaCl concentration.

Sulfur Uptake: Sulfur uptake by snapbeans increased with the SO_4 solution concentration at low Na_2SO_4 levels (20 and 40 $mmol_{(c)}/L$) and only slightly increased at the higher concentrations (60 and 80 $mmol_{(c)}/L$). As data in Fig. 7 show, S content in plant tissues increased from 1.6 g/kg (dry wt. basis) at 0 Na_2SO_4 treatments and up to 30 g/kg at 80 $mmol_{(c)}/L$ Na_2SO_4. Statistical analysis (Table 10) indicated that a significant difference existed between the nine treatment solutions, but no significant difference existed between the control and NaCl treated plants. However, the most significant difference was at low levels of Na_2SO_4 applied.

Unlike Cl, the uptake of S increased linearly with the increase in Na_2SO_4 only up to 40 $mmol_{(c)}/L$. Beyond this level, there was only a slight increase in uptake. This indicates that the presence of SO_4 may help regulate and control the uptake of the ions by maintaining the normal selective

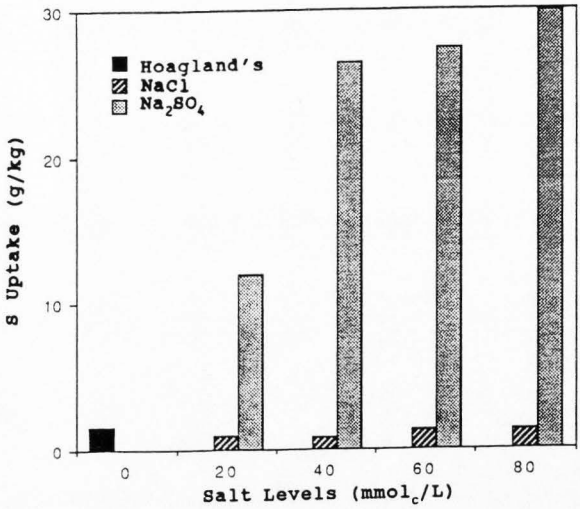

Fig. 7. Phaseolus vulgaris L. shoot S content resulting from irrigation with Na_2SO_4 and NaCl solutions.

Table 10. Factorial analysis of variance for SO_4 ion.

SV	DF		MS	F
Blocks	7		44339246	1.35
Treatments	8		1341792800	40.73**
Control vs Na salts		1	844686280	25.64**
Among rest		7	1412808000	42.88**
Salt		1	8316386100	252.42**
Level		3	268763500	8.16**
SxL		3	255659710	7.70**
Error	56		32946792	
Total	71			

** significant at α = 0.01.

membrane permeability.

Significance of the Anion in Ameliorating Sodium Stress:

Plant shoot weight obtained in this study are shown in Fig. 8. The graph shows that there was a difference in shoot weight between NaCl and Na_2SO_4 treated plants. Both salts decreased shoot dry weight as compared to the control plants. The mean dry weight for control plants was 3.41 g, whereas those of Na_2SO_4 plants were 3.1 g at 20 $mmol_{(c)}/L$ and decreased to 1.2 g at 80 $mmol_{(c)}/L$. As for NaCl treated plants, shoot dry weight decreased from 2.36 g at 20 $mmol_{(c)}/L$ to 1.24 g at 80 $mmol_{(c)}/L$.

Application of ANOVA (Table 11) to the data showed that a significant difference existed among the mean shoot weights of the different treatments. The larger difference existed was between the control and NaCl treated plants. Also ANOVA results indicated that there was a significant difference between the two Na salts and among the levels applied, especially at low Na levels. Again the data indicated that the

Table 11. Factorial analysis of variance for the biomass.

SV	DF		MS	F
Blocks	7		0.237	3.07**
Treatments	8		5.349	69.30**
Control vs Na salts		1	18.160	235.23**
Among Na salts		7	3.520	45.60**
Salt		1	2.485	32.19**
Level		3	6.981	90.43**
SxL		3	0.404	5.23**
Error	56		0.077	
Total	71			

** significant at $\alpha = 0.01$

interaction between the salt type and the level applied was highly significant.

The results of this experiment, suggest that the anion of the Na salt had an effect on ameliorating Na stress. The presence of SO_4 was associated with reduced Na uptake relative to Cl treatments. It is possible that this effect could be a result of complexation of Na ion as ion speciation data suggested.

In addition to the role of ion speciation, the anion itself might have played an important role in the physiology of the plant. One of the reasons for the difference in shoot weight is that bean is a sensitive crop to Na and Cl ions. Under normal conditions, the physiological requirement of the plant for Cl ion is low and in order of a few mg/kg. Usually, a limited amount of Cl is required for the process of photosynthesis (Mengel and Kirkby, 1982). The effect of excess Cl may contribute to impairement of membrane functions and cause physiological disorder in the plant's system. The

effects of excess Cl in some plants is a more serious problem and plants may show symptoms of toxicity and the plant's energy status may be affected. In our experiment, plants exhibited the symptoms of Cl toxicity as burning of leaf margins, bronzing, and premature yellowing. Thus, plants grown under high salinity levels might have a poor energy status as compared to lower levels and control plants. Consequently, energy used to cope with salinity stress was unavailable for other processes resulting in a depression in quality, yield, and whole plant growth (Reisenauer et al., 1973; Akhavan-Kharazian, 1986).

In contrast to Cl, the physiological requirement of most plants for S is high. Sulfur is an essential element that has a major effect on the properties and structural conversion of protein molecules as well as the redox reactions of the cell. It also increases the chlorophyll content of the plant's chloroplast (Bhivare and Nimbalkar, 1984). Thus, the presence of SO_4, in the Na_2SO_4 solution, may have been effective in improving the energy status (photosynthesis) of the plant. Consequently, the plant was able to cope with Na stress (up to 60 $mmol_{(c)}/L$ Na_2SO_4) and produce more shoot weight than NaCl treated plants.

Experiment Two

Ion Speciation and Na Uptake in Presence of Ca Salts: As in Experiment One, Na uptake increased with the increase in

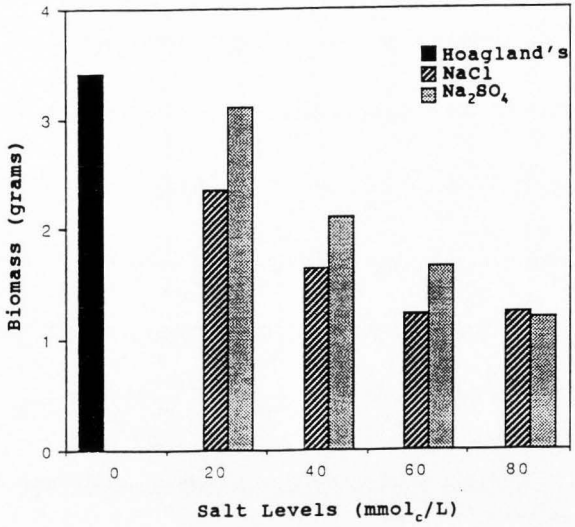

Fig. 8. Phaseolus vulgaris L. shoot weight resulting from irrigation with Na_2SO_4 and NaCl solutions.

treatment Na (Figs. 9 and 10). The ANOVA indicated that Na uptake by snapbeans significantly differed among the 37 treatments (Table 12), and the greater significant difference was due to the type and concentration of Na salt applied. Also factorial analysis showed that Ca salt type, applied with Na salt, affected Na uptake; but there was no significant difference due to the two Ca levels (15 and 30 $mmol_{(c)}/L$). However, there was a significant difference at different salt treatments due to the interaction between Na and Ca salts. The least significant difference test (LSD) showed that there was no significant difference among the treated plants at 0 salt levels.

Ion speciation data (Table 16) again suggested that Na uptake was dependent on the solution complex formed and uptake significantly increased as the $NaCl^{\circ}$ concentration in the soil solution increased. Data in Figs. 11a, b, c and d show that the anion of Ca salts played an important role in determining Na uptake by the beans. At a given Na_2SO_4 level, total Na concentrations and free ion concentrations (Figs. 11a and b) in soil solutions were almost the same for Na_2SO_4 mixed with $CaSO_4$ or $CaCl_2$. However, there was more Na uptake by the plants treated with Na_2SO_4-$CaCl_2$ than those treated with Na_2SO_4-$CaSO_4$. At a given Na_2SO_4 level, the data showed that there was more Na uptake at 30 $mmol_{(c)}/L$ $CaCl_2$ or $CaSO_4$ than at 15 $mmol_{(c)}/L$ $CaCl_2$ or $CaSO_4$ (Fig. 9). This increase in uptake might have

been due to the increase in Na complex form in treatment solution (Table 13).

The effect of ion speciation was better evidenced in the effects of NaCl treatments combined with Ca salts. The increase in Na uptake was linear (Fig. 12a) with increasing NaCl and the greatest uptake was at 60 $mmol_c/L$ combined with 30 $mmol_c/L$ $CaCl_2$. Also at a given NaCl concentration there was more Na uptake at 30 $mmol_c/L$ than at 15 $mmol_c/L$ $CaCl_2$ (Fig. 10). Treatments of NaCl combined with $CaSO_4$ also showed a similar trend, but to a lesser extent than the Cl treatments. Less Na uptake by the plants treated with NaCl combined with $CaSO_4$ salts might have been due to the SO_4 ion complexation with Na decreasing the uptake of Na as compared to $NaCl^o$ complex. Also at each level of NaCl, there was more Na uptake at 15 $mmol_c/L$ $CaSO_4$ than at 30 $mmol_c/L$ $CaSO_4$. This might have been due to a decrease in the concentration of the $NaCl^o$ complex at the higher $CaSO_4$ level.

Sodium chloride combined with $CaSO_4$ resulted in greater Na uptake than occured in Na_2SO_4 - $CaCl_2$ treatments. This might have been due to the formation of greater concentrations of $NACl^o$ complex in the NaCl combined with $CaSO_4$ that increased the uptake; whereas, in the Na_2SO_4 mixed with $CaCl_2$ treatments, there was an increase in Na-SO_4 complex formation that decreased Na uptake.

The above mentioned observations, along with those of Experiment 1, may suggest that ion complexation has an

Table 12. Factorial analysis of variance for the effect of Na and Ca salts on Na uptake.

SV	DF	MS	DF
Blocks	4	218469000	5.95**
Treatments	36	813519650	22.17**
Control vs rest	1	757180000	20.64**
Among the rest	35	815129340	22.21**
Among Na	8	2940440800	80.13**
Na control vs rest	1	3481402000	94.86**
Salt	1	5717694000	155.82**
Level	3	4228559600	115.24**
SxL	3	546246290	14.89**
Among Ca	3	627513000	17.10**
Type(T)	1	2094576000	57.08**
Concentration(C)	1	3146088	0.08
TxC	1	20257829	0.55
Na x Ca	24	130144210	3.55*
Error	144	36694248	
Total	184		

*,** Significant at the 0.05 and 0.01 probability levels, respectively.

Table 13. Sodium distribution with ligands (SPECO2) for different treatment solutions.

Solution treatments $mmol_c/L$	Na Free ($mmol_c/L$)	Na SO_4 (%)	Na Cl (%)
30 Na_2SO_4 +15 $CaSO_4$	28.90	3.68	---
30 Na_2SO_4 +30 $CaSO_4$	28.70	4.17	---
60 Na_2SO_4 +15 $CaSO_4$	56.86	5.19	---
60 Na_2SO_4 +30 $CaSO_4$	56.65	5.55	---
30 Na_2SO_4 +15 $CaCl_2$	28.98	2.46	0.91
30 Na_2SO_4 +30 $CaCl_2$	28.86	2.02	1.74
60 Na_2SO_4 +15 $CaCl_2$	56.98	4.19	0.81
60 Na_2SO_4 +30 $CaCl_2$	56.83	3.68	1.57
30 NaCl + 15 $CaSO_4$	29.07	1.33	1.85
30 NaCl + 30 $CaSO_4$	28.86	2.02	1.74
60 NaCl + 15 $CaSO_4$	57.35	1.06	3.33
60 NaCl + 30 $CaSO_4$	56.99	1.81	3.18
30 NaCl + 15 $CaCl_2$	29.20	0.03	2.79
30 NaCl + 30 $CaCl_2$	28.90	0.02	3.48
60 NaCl + 15 $CaCl_2$	57.40	0.03	4.19
60 NaCl + 30 $CaCl_2$	57.10	0.02	4.78

important role in regulating the uptake of Na ion by bean plants; that is, $NaCl^{o}$ complex form helps in increasing Na uptake. Whereas, the anionic Na-SO_4 complex decreases Na uptake.

Another possibility is that the ions may have played a role in regulating the physiological activities of the beans; consequently, in determining the Na ion uptake.

In general, as in Experiment one, the presence of Cl ion in soil solution increased the uptake of Na; whereas, the presence of SO_4 ion in soil solution was associated with a decrease in Na uptake. In the presence of Cl ion (Fig 12a) in both salts (NaCl-$CaCl_2$ treatments), the average Na content ranged from 7 g/kg at the lowest NaCl level and up to 40 g/kg at the highest NaCl level. However, in the absence of Cl ion in soil solutions (Fig. 12b), Na_2SO_4-$CaSO_4$ treatments, the average was 1 g/kg at the lowest Na_2SO_4 level and increased to 13 g/kg at the highest Na_2SO_4 level.

Furthermore, at 30 and 45 $mmol_{(c)}$/L NaCl, the Na content significantly decreased with an increase in $CaSO_4$ from 15 to 30 $mmol_{(c)}$/L. Conversely, at 15 and 60 $mmol_{(c)}$/L NaCl, there was a slight increase (although not significant) in Na uptake with the increase in $CaSO_4$ level from 15 to 30 $mmol_{(c)}$/L. These results are in agreement with Akhavan-Kharazian (1986), who found that at 0.4 and 1.2 $dS.m^{-1}$ NaCl, the leaf Na decreased slightly with increase in $CaSO_4$ level from 4 to 8 m<u>M</u>; and at 0.8 $dS.m^{-1}$ NaCl concentration, there was a slight increase in

leaf Na with increasing $CaSO_4$ level (from 4 to 8 mM).

In addition, this study showed that increasing the concentration of $CaSO_4$ or $CaCl_2$ from 15 to 30 $mmol_{(c)}/L$, at the same level of Na_2SO_4 (Fig. 9), increased Na uptake (although not significantly for $CaSO_4$, but significant for $CaCl_2$ at 30 and 45 $mmol_c/L$ Na_2SO_4). This may suggest that the presence of $CaSO_4$ in salt solution is not always correlated with a decrease in Na uptake, but rather an increase in the uptake.

In addition to the role that SO_4 ion may have played in bean's physiology and Na uptake, Ca ion also influenced Na uptake. Calcium ion is known for the important role it plays in the integrity of cell membrane structure. The function of Ca^{2+} in membranes, in part, is to minimize ion diffusion, maintain selective ion transport mechanisms, and decrease membrane permeability of ions (Cramer and Lauchli, 1986; Hyder and Greenway, 1965; Leopold, 1977). Consequently, a deficiency of Ca^{2+} leads to an impairment of the cell membrane structure that leads to an increase in ion permeability.

Comparing Experiment 1 (Fig. 2) with Experiment 2 (Figs. 9 and 10) for Na uptake, the results indicated that the plants treated with NaCl only, Na uptake increased from 25 g/kg at 20 $mmol_{(c)}/L$ to 70 g/kg at 60 $mmol_{(c)}/L$ NaCl. Whereas, in the presence of $CaCl_2$, Experiment 2, Na uptake was 7 g/kg at 15 $mmol_{(c)}/L$ and increased up to 40 g/kg at the highest level of NaCl (values reported as averages of 15 and 30 $mmol_{(c)}/L$ $CaCl_2$ at 60 $mmol_{(c)}/L$ NaCl). Also for Na_2SO_4 treatments in Experiment

1, the uptake increased from 11.5 g/kg at 20 $mmol_{(c)}/L$ Na_2SO_4 up to 39 g/kg at 60 $mmol_{(c)}/L$. But in the presence of $CaCl_2$, the uptake was only 2.4 g/kg at the lowest level of Na_2SO_4-$CaCl_2$ treatments (reported as averages of 15 and 30 $mmol_{(c)}/L$ $CaCl_2$ at 15 $mmol_{(c)}/L$ Na_2SO_4) and increased to 17.5 g/kg at the highest level.

Also the interaction between SO_4 and Ca may have been effective in determining Na uptake. Ca is a part of the cell membrane, and it's presence with SO_4 might have been more helpful in governing normal impermeability to the transport of ions, especially the Na. Again comparing Experiment 1 to Experiment 2, the results showed that Na uptake decreased more in the presence of $CaSO_4$, in Na treatments, than in the presence of $CaCl_2$. At 15 $mmol_c/L$, in the presence of $CaCl_2$ (given as the averages of 15 + 30 $mmol_c/L$ $CaCl_2$); the uptake was 7.2 g/kg and decreased to 1.6 g/kg in the presence of $CaSO_4$. At the highest NaCl level (60 $mmol_c/L$ NaCl average of $CaCl^2$ levels), the uptake of Na was 41 g/kg and decreased to 33 g/kg in the presence of $CaSO_4$.

Ion Speciation and Ca Uptake in Presence of Ca Salts: Calcium uptake was affected by the different salt solution treatments (Figs. 13 and 14). Statistical analysis (Table 14) showed that Ca content differed significantly among the 37 treatments. The greatest difference was due to the different Na salt solutions. Also at different concentrations, Na salt

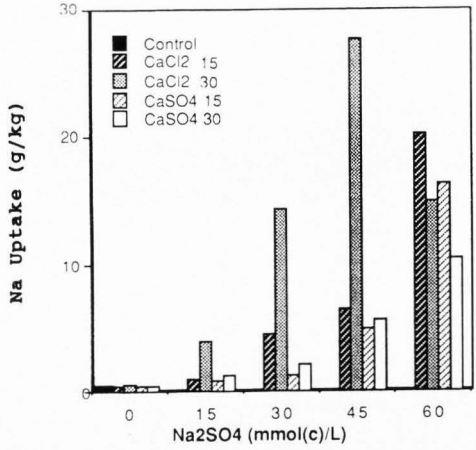

Fig. 9. Phaseolus vulgaris L. shoot Na content resulting from irrigation with Na_2SO_4 and Ca salts solutions.

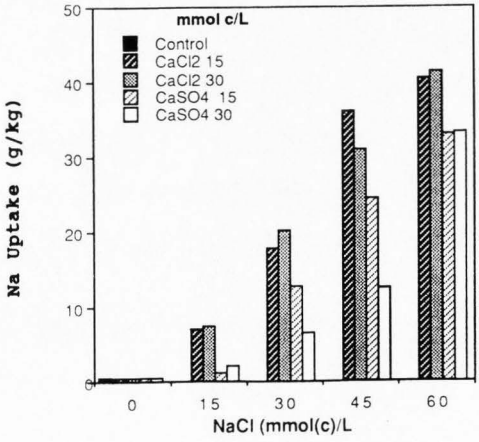

Fig. 10. Phaseolus vulgaris L. shoot Na content resulting from irrigation with NaCl and Ca salts solutions.

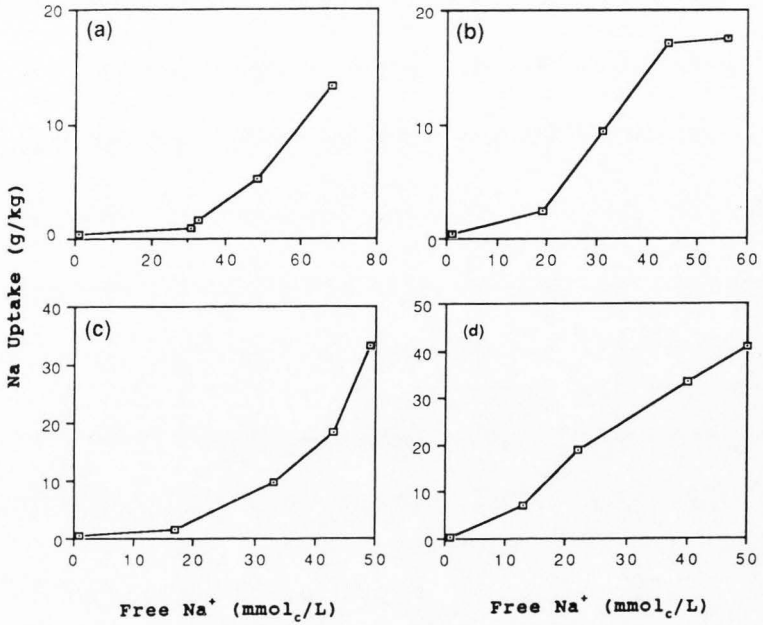

Fig. 11. The effect of varying free Na^+ concentration on the uptake of Na from (a) Na_2SO_4-$CaSO_4$ (b) Na_2SO_4-$CaCl_2$ (c) NaCl-$CaSO_4$ and (d) NaCl-$CaCl_2$ soil solutions.

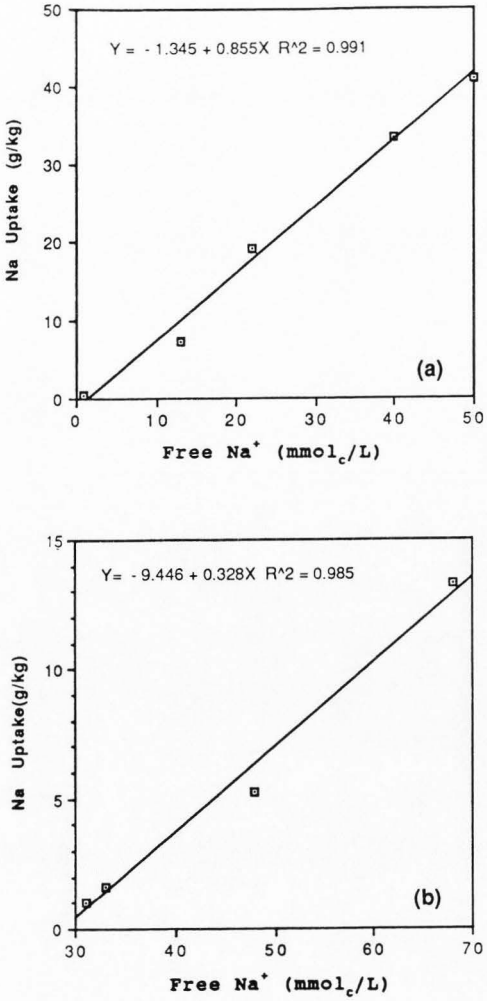

Fig. 12. The relationship between Na uptake and the free Na^+ ion concentration from (a) NaCl-$CaCl_2$ and (b) Na_2SO_4-$CaSO_4$ soil solutions.

treatments significantly affected Ca uptake by snapbean plants. Sodium chloride combined with Ca salts showed an increase in Ca uptake as compared to Na_2SO_4 solution treatments. The results indicated that Ca salt, along with the level applied, influenced Ca uptake. There was significant interaction between Na salt and Ca salt.

Ion speciation data (Tables 15 and 16), as in Experiment 1, indicated that Ca uptake increased with an increase in free Ca^{2+} concentration in the soil solution. Data in Figs. 15a and b showed that, at 0 Na salt level, the highest Ca uptake was at 30 $mmol_c/L$ $CaCl_2$ and this treatment contained the highest free ion concentration. Whereas, at 30 $mmol_c/L$ $CaSO_4$, 26.6 percent of total Ca was complexed as $CaSO_4^\circ$ and this may have decreased Ca uptake as compared to $CaCl_2$ salt and Hoagland's solution (control) treatments.

With respect to the Na_2SO_4 - $CaSO_4$ treatments (Fig. 15a), results indicated that complex formation was associated with decreased Ca uptake especially at 45 and 60 mmol $_c/L$ Na_2SO_4. The NaCl - $CaSO_4$ treatments (Fig. 15c) also showed the trend of increasing Ca uptake with increasing free Ca^{2+} (Table 15). However, with the increase in NaCl concentrations, free Ca^{2+} concentrations increased proportionally up to 45 $mmol_{(c)}/L$. This increase in free Ca^{2+} concentration was probably due to a decrease in the concentration of $CaSO_4^\circ$ in the presence of NaCl (Indifferent ion effect); consequently, more free Ca^{2+} was available to be taken by the plant. These results are in

Table 14. Factorial analysis of variance for the effect of Na and Ca on Ca uptake.

SV	DF	MS	F
Blocks	4	145192400	4.23**
Treatments	36	974026530	28.38**
Control vs rest	1	355622000	10.36**
Among rest	35	991695230	28.89**
Among Na	8	3272863700	95.36**
Na control vs rest	1	178249210	5.19**
Salt	1	15267306000	444.82**
Level	3	3429508600	99.92**
SxL	3	149609260	4.36**
Among Ca	3	1021125800	29.75**
Type	1	267858000	7.80**
Concentration	1	1817643200	52.96**
TxC	1	5408132	0.16
Na x Ca	24	227626930	6.63**
Error	144	34322241	
Total	184		

** significant at $\alpha = 0.01$.

Table 15. Calcium distribution with ligands (SPECO2) for different treatment solutions.

Solution treatments mmol$_c$/L	Ca Free (mmol$_c$/L)	Ca SO_4 (%)	Ca Cl (%)
30 Na_2SO_4 + 15 $CaSO_4$	9.66	38.84	---
30 Na_2SO_4 + 30 $CaSO_4$	18.52	39.91	---
60 Na_2SO_4 + 15 $CaSO_4$	8.94	43.42	---
60 Na_2SO_4 + 30 $CaSO_4$	17.32	43.83	---
30 Na_2SO_4 + 15 $CaCl_2$	11.02	30.20	0.05
30 Na_2SO_4 + 30 $CaCl_2$	23.20	24.65	0.10
60 Na_2SO_4 + 15 $CaCl_2$	9.70	38.63	0.03
60 Na_2SO_4 + 30 $CaCl_2$	20.16	34.56	0.08
30 NaCl + 15 $CaSO_4$	12.94	17.94	0.12
30 NaCl + 30 $CaSO_4$	23.20	24.65	0.10
60 NaCl + 15 $CaSO_4$	13.52	14.23	0.25
60 NaCl + 30 $CaSO_4$	24.00	20.86	0.21
30 NaCL + 15 $CaCl_2$	15.68	0.55	0.21
30 NaCl + 30 $CaCl_2$	30.60	0.39	0.31
60 NaCl + 15 $CaCl_2$	15.68	0.43	0.39
60 NaCl + 30 $CaCl_2$	30.60	0.33	0.45

agreement with Akhavan-Kharazian (1986), who found that increasing NaCl from 0.45 dS/m to 1.2 dS/m increased Ca content of the plant's leaves. However, his explanation was based on the assumption that the presence of high concentrations of Na^+ in the soil replaced Ca^{2+} on the ion exchanger and released it into the soil solution. As a result, more Ca^{2+} came in contact with the root surface and was taken up by the plant. If Akhavan-Kharazian's explanation holds true, then the uptake of Ca^{2+} from the Na_2SO_4 soil solution should also be high due to the replacement of Na for the Ca ion. However, this study used sand as a medium that has a very limited exchange capacity.

The results shown in Fig. 15c indicated that Ca uptake decreased at 60 $mmol_{(c)}$/L NaCl - $CaSO_4$ treatments. Although ion speciation data indicated that this treatment corresponded to the greatest concentration of free Ca^{2+}, the decrease in uptake was probably due to the plant death.

The Na_2SO_4-$CaCl_2$ treatments (Fig. 15b), showed the same trend for Ca uptake as Na_2SO_4-$CaSO_4$ treatment. Free ion concentrations decreased with the increase in Na_2SO_4 levels due to the complexation of Ca with SO_4. At 30 $mmol_{(c)}$/L $CaCl_2$ and Na_2SO_4, Ca uptake and free Ca concentration were greater than Ca uptake and free Ca concentration at the 15 $mmol_c$/L $CaCl_2$ level. With respect to the NaCl-$CaCl_2$ treatments, the results showed (Fig. 15d) that the greatest Ca uptake corresponded to the greatest concentration of free Ca ions. Again, the

decrease in Ca uptake at high NaCl (60 $mmol_{(c)}/L$) level might have been due to the plant death.

Comparing the results of $NaCl-CaSO_4$ to $NaCl-CaCl_2$, ion speciation data of the saturation extract (Table 16) showed that there was more free Ca^{2+} in the $NaCl-CaSO_4$ than in the $NaCl-CaCl_2$; but there was more Ca uptake by the plants treated with $NaCl-CaCl_2$. However, ion speciation data of the treatment solution (Table 15) showed the highest fraction of free Ca^{2+} was in the $NaCl-CaCl_2$ solutions. The reason for this difference in free Ca concentrations was probably due to the higher uptake of Ca^{2+} from the $NaCl-CaCl_2$, that the plant depleted Ca of the soil solution and/or more leaching of Ca from the $NaCl-CaCl_2$ growing media at the end of the experiment.

Ion Speciation and Cl Uptake in Presence of Ca Salts: Statistical analysis (Table 17) showed that Cl uptake differed significantly among the 37 treatments, and the most significant difference was between the Na salts and the levels applied. Also the interaction between Na salt and the level applied was significant in affecting Cl uptake by the plant. Also ANOVA indicated that there was an important interaction between the Na and Ca salts; i.e, Na and Ca salts were dependent on each other in affecting Cl uptake by the plants.

Ion speciation data (Table 20) indicated that Na complexation with Cl as $NaCl^{\circ}$ was greater than Ca complexation

Table 16. Sodium and calcium distribution with ligands (SPECO2) for the different salt solution extracts.

Solution treatments mmol(c)/L	Na(%)			Ca(%)		
	Free	SO4	Cl	Free	SO4	Cl
Hoagland's solution	99.76	0.20	0.04	94.93	5.04	0.00
15 mmol(c)/L CaCl2	98.64	0.10	1.26	98.03	1.87	0.10
30 mmol(c)/L CaCl2	97.48	0.06	2.46	98.74	1.06	0.20
15 mmol(c)/L CaSO4	98.25	1.71	0.04	76.16	23.84	0.00
30 mmol(c)/L CaSO4	97.89	2.06	0.05	73.37	26.62	0.00
15 Na2SO4+ 15 CaSO4	96.51	3.42	0.07	64.87	35.13	0.00
15 Na2SO4+ 30 CaSO4	96.16	3.80	0.04	63.22	36.77	0.00
30 Na2SO4= 15 CaSO4	96.16	3.80	0.04	61.70	38.29	0.00
30 Na2SO4+ 30 CaSO4	95.71	4.20	0.09	61.10	38.89	0.00
45 Na2SO4= 15 CaSO4	95.59	4.36	0.05	59.72	40.28	0.00
45 Na2SO4+ 30 CaSO4	94.64	5.30	0.06	56.98	43.02	0.00
60 Na2SO4+ 15 CaSO4	95.27	4.61	0.02	60.33	39.46	0.00
60 Na2SO4+ 30 CaSO4	94.34	5.61	0.03	56.24	43.83	0.00
15 Na2SO4+ 15 CaCl2	96.92	1.74	1.34	77.76	22.16	0.08
15 Na2SO4+ 30 CaCl2	96.62	1.37	2.01	82.18	17.69	0.13
30 Na2SO4+ 15 CaCl2	96.11	2.82	1.07	68.56	31.39	0.05
30 Na2SO4+ 30 CaCl2	96.17	2.03	1.80	76.74	23.16	0.10
45 Na2SO4+ 15 CaCl2	95.04	4.22	0.74	62.00	37.90	0.03
45 Na2SO4+ 30 CaCl2	95.49	2.92	1.59	69.69	30.23	0.08
60 Na2SO4+ 15 CaCl2	94.75	4.68	0.57	60.16	39.82	0.02
60 Na2SO4+ 30 CaCl2	94.64	4.11	1.25	63.15	36.79	0.06
15 NaCl + 15 CaSO4	97.22	1.73	1.05	77.02	22.91	0.06
15 NaCl + 30 CaSO4	96.77	2.08	1.15	74.85	25.09	0.06
30 NaCl + 15 CaSO4	96.59	1.66	1.75	78.55	21.33	0.11
30 NaCl + 30 CaSO4	95.82	2.18	2.00	75.46	24.42	0.12
45 NaCl + 15 CaSO4	95.80	1.66	2.54	79.62	20.20	0.17
45 NaCl + 30 CaSO4	95.85	2.19	1.96	75.05	24.83	0.11
60 NaCl + 15 CaSO4	95.96	1.43	2.61	81.61	18.20	0.18
60 NaCl + 30 CaSO4	95.39	2.10	2.51	76.49	23.36	0.15
15 NaCl + 15 CaCl2	97.91	0.08	2.28	98.36	1.37	0.19
15 NaCl + 30 CaCl2	97.90	0.05	2.03	98.94	0.83	0.17
30 NaCl + 15 CaCl2	97.41	0.07	2.48	98.45	1.18	0.21
30 NaCl + 30 CaCl2	96.70	0.05	3.11	98.86	0.86	0.27
45 NaCl + 15 CaCl2	96.03	0.05	3.91	98.85	0.82	0.36
45 NaCl + 30 CaCl2	96.94	0.04	2.84	99.11	0.63	0.25
60 NaCl + 15 CaCl2	96.26	0.06	3.70	98.78	0.88	0.33
60 NaCl + 30 CaCl2	96.20	0.04	3.77	99.02	0.67	0.34

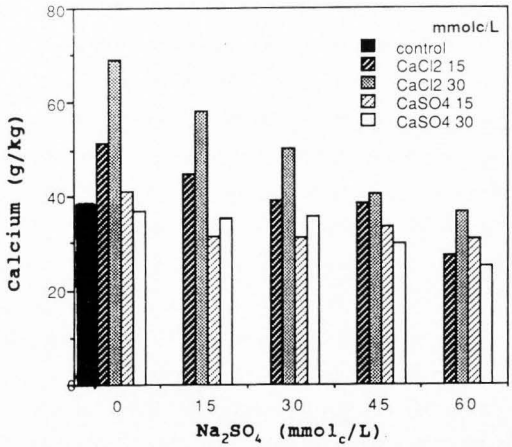

Fig. 13. Phaseolus vulgaris L. shoot Ca content resulting from irrigation with Na_2SO_4 and Ca salts solutions.

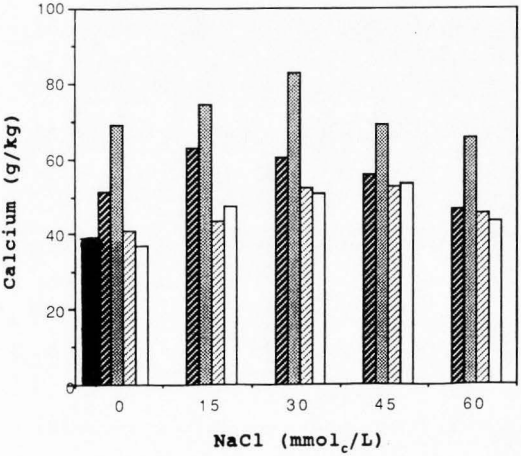

Fig. 14. Phaseolus vulgaris L. shoot Ca content resulting from irrigation with NaCl and Ca salts solutions.

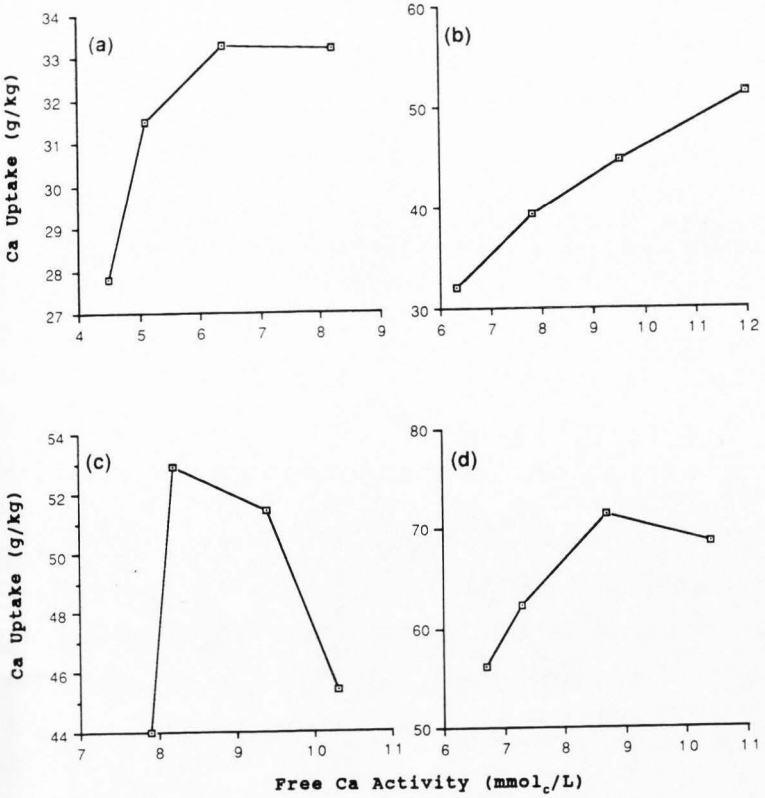

Fig. 15. The relationship between Ca plant uptake and the free Ca^{2+} activity in (a) Na_2SO_4-$CaSO_4$, (b) Na_2SO_4-$CaCl_2$, (c) NaCl-$CaSO_4$ and (d) NaCl-$CaCl_2$ soil solutions.

with Cl. The Na_2SO_4-$CaSO_4$ treatments showed that no significant uptake of Cl was found by the plants because no Cl source was included in the treatment solutions. The Na_2SO_4-$CaCl_2$ salt treatments showed (Fig. 16a) that with increasing Na_2SO_4 levels, Cl uptake decreased, although not significantly, at both levels of $CaCl_2$. The data showed that with increasing Na_2SO_4 levels, Na stress increased and depressed the growth, shoot production, and yield of the plant; consequently, less Cl was taken up by the plant at higher levels of Na_2SO_4. Comparing treatments of $CaCl_2$, at 0 sodium level, to treatments containing Na_2SO_4-$CaCl_2$; the amount of Cl in the plant tissue decreased from 58 g/kg in the absence of Na_2SO_4 (given as the average of 15 $mmol_c$/L + 30 $mmol_c$/L $CaCl_2$) to about 47 g/kg in the presence of Na_2SO_4 (reported as average of all Na_2SO_4 + $CaCl_2$ treatments). Also the data showed that there was more Cl uptake at 30 $mmol_c$/L than at 15 $mmol_c$/L $CaCl_2$, at a given level of Na_2SO_4, due to the higher availability of Cl at higher $CaCl_2$ level.

In addition, ANOVA showed that there was a significant effect of the Ca salts species and the level applied on Cl uptake, but no interaction between these two factors was suggested. With respect to NaCl-$CaSO_4$ treatments (Fig. 16b), the data showed that with increasing NaCl concentrations, there was more Cl uptake by the beans. However, at a given NaCl level there was more Cl uptake (not significant statistically) at 30 $mmol_{(c)}$/L $CaSO_4$ than at 15 $mmol_{(c)}$\L $CaSO_4$.

Table 17. Factorial analysis of variance for the effect of Na and Ca salts on Cl uptake.

SV	DF	MS	F
Blocks	4	140729210	3.17*
Treatments	36	9138564700	205.61**
Control vs rest	1	13660290000	307.30**
Among rest	35	9009372600	297.63**
Among Na	8	34599544000	778.46**
Na control vs rest	1	13228706000	297.63**
Salt	1	170536960000	3836.92**
Level	3	26214589000	589.80**
S x L	3	4795642200	107.90**
Among Ca	3	2431721600	54.71**
Type	1	2876348200	64.72**
Concentration	1	5932147400	133.47**
T x C	1	19800711	0.44
Na x Ca	24	1301521700	29.28**
Error	144	44446310	
Total	184		

*,** Significant at the 0.05 and 0.01 probability levels, respectively.

This difference was may have been due to the difference in shoot production and growth between the 2 levels of $CaSO_4$; that is, at 30 $mmol_{(c)}/L$ $CaSO_4$ there was better growth, shoot production, and yield. Consequently, more Cl was taken up by the plant (Fig. 16b).

Sodium chloride combined with $CaCl_2$ treatments increased the uptake of Cl due to the excess of Cl^- in the soil solutions. The uptake was increased linearly with the increase in the free Cl ion concentration in soil solution (Fig. 17).

In addition, the presence of gypsum might have played a role in the plant physiological activities and in determining Cl uptake. In the absence of $CaSO_4$, in soil solution (Experiment 1), the amount of Cl uptake was 114 g/kg (at 40

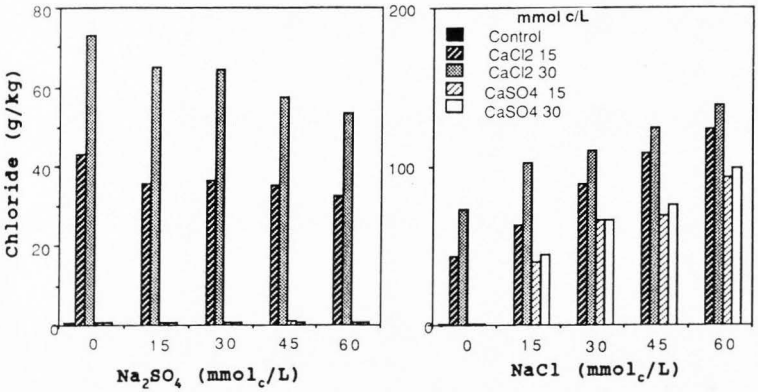

Fig. 16. Phaseolus vulgaris L. shoot Cl content resulting from irrigation with (a) Na_2SO_4, or (b) NaCl mixed with Ca salts.

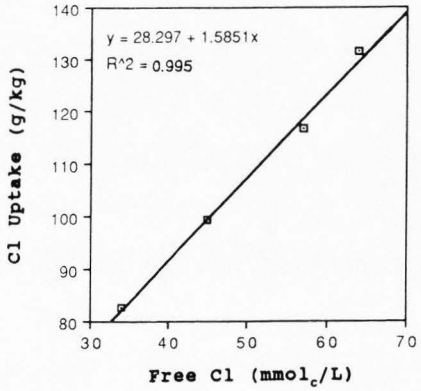

Fig. 17. The relationship between Cl uptake and the free Cl ion concentration in NaCl-$CaCl_2$ solutions.

$mmol_{(c)}$/L NaCl) and increased to 137 g/kg at 60 $mmol_{(c)}$/L NaCl. Whereas, in the presence of $CaSO_4$ (given as the average of 15+ 30 $mmol_c$/L $CaSO_4$), Cl uptake decreased to 70 g/Kg at 45 $mmol_c$/L NaCl; and to 96 g/kg at 60 $mmol_c$/L NaCl. This indicated that the presence of SO_4 with Ca might have helped the plant to maintain selective permeability of the membrane and controlled the amount of Cl uptake (to certain extent). However, this obervation contradicted the findings of Cramer and Spurr (1986a), who had found that increasing Ca concentration in soil solution had not affected Cl^- concentration in lettuce tissue.

Ion Speciation and S Uptake in Presence of Ca Salts:

Data in Figs. 18a and b showed that S uptake by snapbeans increased significantly with the SO_4 level in soil solution. The ANOVA (Table 18) indicated that a significant difference in S uptake existed among the solution treatments. The highest significant differences in S uptake were due to the Na source and to the level applied. Also the analysis showed that Ca salt, along with the level applied, influenced the amount of sulfur in plant tissue. The highest sulfur concentration in the plant tissues was at 60 $mmol_c$/L Na_2SO_4 combined with 15 $mmol_c$/L $CaSO_4$ due to the high source of SO_4 in the treatment solutions.

Ion speciation data (Tables 19 and 20) showed that S uptake increased with an increase in free SO_4^{2-} ion

concentration in soil solution. Data from the Na_2SO_4 - $CaCl_2$ treatments (Figs. 19a and b) showed the importance of complexation in decreasing the uptake of S by the plant. With increasing $CaCl_2$ concentrations, at a given Na_2SO_4 level; sulfur uptake decreased due to the decrease in the fraction of free SO_4 ion. In Na_2SO_4-$CaSO_4$ treatment, there was a significant increase in S uptake at 30 compared to 15 $mmol_c/L$ $CaSO_4$, at a given Na level, and this was mainly due to the increase in SO_4 concentrations in the soil solutions (Fig. 20).

The NaCl - $CaCl_2$ treatments did not show (Fig. 18a and b) any significant uptake of S due to the low SO_4 concentrations in the soil solutions. However, in the NaCl-$CaSO_4$ treatments, increasing NaCl concentration resulted in a small increase in free SO_4^{2-} ion concentration in soil solution. The data indicated that S uptake can be depressed more by Ca than by Na at equal molar levels because Ca forms the stronger complex with SO_4.

Anion Significance on Biomass Production in the Presence of Na and Ca Salts: Plant biomass data obtained in this experiment are shown in Figs. 21 and 22. Application of the ANOVA to the data showed (Table 21) that there was a significant difference in dry biomass production among the 37 treatments. Also the results indicated that there was a significant difference between Na salts and levels applied on the dry weight produced. As the data in the graph show (Figs.

Table 18. Factorial analysis of variance for the effect of Na and Ca salts on SO_4 uptake.

SV	DF		MS	F
Blocks	4		10137760	0.99
Treatments	36		170971930	16.63**
Control vs rest		1	150221300	14.62**
Among rest		35	171564800	16.69**
Among Na		8	636261440	61.90**
Na control vs rest		1	483679760	47.06**
Salt		1	2030389900	197.80**
Level		3	653290020	63.56**
S x L		3	205383980	19.98**
Among Ca		3	84143370	8.19**
Type		1	153489610	14.93**
Concentration		1	40587139	3.95**
T x C		1	59849953	5.82**
Na x Ca		24	27593604	2.68*
Error	144		10278260	
Total	184			

*,** Significant at the 0.05 and 0.01 probability levels, respectively.

Table 19. Sulfate distribution with metals (SPECO2) for different treatment solutions.

Solution treatments mmol$_c$/L	SO_4 Free (mmol$_c$/L)	SO_4 Ca (%)	SO_4 Na (%)
30 Na_2SO_4 + 15 $CaSO_4$	18.42	13.53	4.83
30 Na_2SO_4 + 30 $CaSO_4$	22.69	20.38	4.14
60 Na_2SO_4 + 15 $CaSO_4$	31.00	9.11	8.26
60 Na_2SO_4 + 30 $CaSO_4$	34.98	14.96	7.37
30 Na_2SO_4 + 15 $CaCl_2$	12.01	15.71	4.85
30 Na_2SO_4 + 30 $CaCl_2$	10.74	24.99	3.98
60 Na_2SO_4 + 15 $CaCl_2$	24.53	10.11	8.32
60 Na_2SO_4 + 30 $CaCl_2$	22.57	17.64	7.30
30 NaCl + 15 $CaSO_4$	5.86	18.44	4.78
30 NaCl + 30 $CaSO_4$	10.74	24.99	3.98
60 NaCl + 15 $CaSO_4$	5.90	14.63	8.27
60 NaCl + 30 $CaSO_4$	10.90	20.86	7.14
30 NaCl + 15 $CaCl_2$	0.15	21.76	4.63
30 NaCl + 30 $CaCl_2$	0.13	30.38	3.69
60 NaCl + 15 $CaCl_2$	0.15	16.81	8.13
60 NaCl + 30 $CaCl_2$	0.14	25.23	6.77

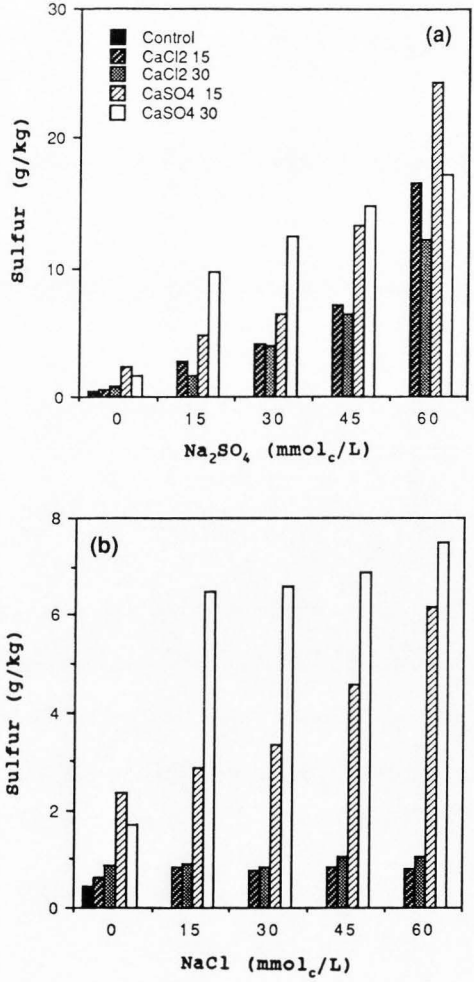

Fig. 18. Phaseolus vulgaris L. shoot S content resulting from irrigation with (a) Na_2SO_4 or (b) NaCl mixed with Ca salts.

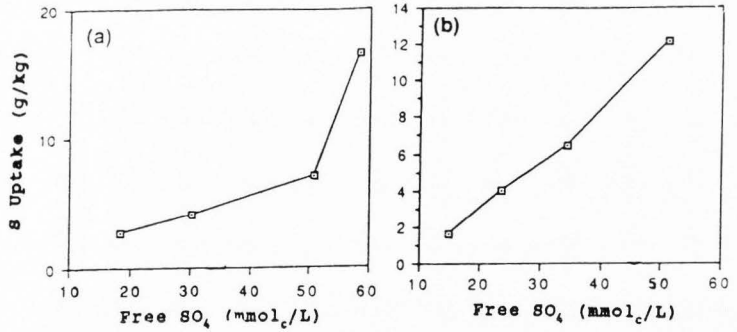

Fig. 19. The relationship between shoot S content and the free SO_4 ion concentrations in Na_2SO_4 solutions mixed with (a) 15 $mmol_c$/L $CaCl_2$ or (b) 30 $mmol_c$/L $CaCl_2$.

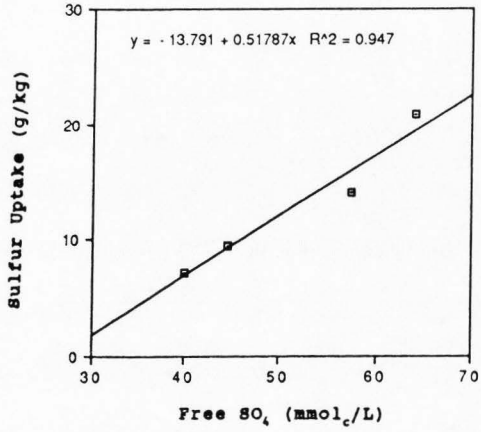

Fig. 20. The relationship between shoot S content and the free SO_4 ion concentration in Na_2SO_4-$CaSO_4$ solutions.

Table 20. Chloride and sulfate distribution with metals (SPECO2) for the different salt solution extracts.

Solution treatments mmol(c)/L	Cl(%)			SO_4(%)		
	Free	Ca	Na	Free	Ca	Na
Hoagland's solution	99.89	0.01	0.07	76.18	14.37	0.23
15 mmol(c)/L CaCl2	99.84	0.08	0.08	61.46	38.38	0.16
30 mmol(c)/L CaCl2	99.83	0106	0.05	58.73	37.28	0.09
15 mmol(c)/L CaSO4	99.87	0.06	0.05	61.40	34.54	0.11
30 mmol(c)/L CaSO4	99.84	0.07	0.06	61.39	35.01	0.13
15 Na2SO4+ 15 CaSO4	98.31	0.04	1.63	69.67	21.86	3.64
15 Na2SO4+ 30 CaSO4	97.97	0.04	1.97	70.20	21.45	4.39
30 Na2SO4+ 15 CaSO4	98.22	0.03	1.73	75.64	17.22	4.26
30 Na2SO4+ 30 CaSO4	97.79	0.04	2.15	73.06	18.87	4.95
45 Na2SO4+ 15 CaSO4	97.57	0.02	2.41	79.73	14.29	5.98
45 Na2SO4+ 30 CaSO4	96.99	0.03	2.97	77.23	13.57	7.09
60 Na2SO4+ 15 CaSO4	95.86	0.02	4.12	75.24	11.17	9.90
60 Na2SO4+ 30 CaSO4	96.69	0.03	3.28	77.02	12.10	7.83
15 Na2SO4+ 15 CaCl2	98.53	0.06	1.39	66.28	26.52	3.00
15 Na2SO4+ 30 CaCl2	98.97	0.08	0.93	62.22	32.93	1.84
30 Na2SO4+ 15 CaCl2	98.25	0.04	1.69	73.15	19.58	3.99
30 Na2SO4+ 30 CaCl2	97.87	0.07	2.04	64.81	28.16	4.14
45 Na2SO4+ 15 CaCl2	97.21	0.03	2.74	74.37	16.66	6.35
45 Na2SO4+ 30 CaCl2	97.66	0.05	2.28	69.96	22.62	5.01
60 Na2SO4+ 15 CaCl2	96.50	0.03	3.45	76.70	13.00	8.21
60 Na2SO4+ 30 CaCl2	97.13	0.04	2.84	73.10	18.48	6.09
15 NaCl + 15 CaSO4	98.80	0.04	1.14	67.85	25.12	2.57
15 NaCl + 30 CaSO4	98.94	0.06	0.98	64.55	29.41	2.04
30 NaCl + 15 CaSO4	97.92	0.04	2.02	70.84	20.61	4.67
30 NaCl + 30 CaSO4	98.00	0.07	1.91	65.82	27.02	3.93
45 NaCl + 15 CaSO4	97.29	0.05	2.64	70.59	20.06	5.92
45 NaCl + 30 CaSO4	97.64	0.05	2.29	69.04	21.38	4.98
60 NaCl + 15 CaSO4	96.96	0.04	2.98	73.32	16.67	7.06
60 NaCl + 30 CaSO4	97.27	0.06	2.65	68.40	23.36	5.65
15 NaCl + 15 CaCl2	98.81	0.06	1.11	68.12	26.38	2.48
15 NaCl + 30 CaCl2	99.33	0.07	0.58	64.49	32.57	1.24
30 NaCl + 15 CaCl2	98.45	0.05	1.48	73.96	22.84	3.54
30 NaCl + 30 CaCl2	98.65	0.07	1.26	66.48	27.25	2.75
45 NaCl + 15 CaCl2	96.93	0.04	3.02	70.71	16.61	7.11
45 NaCl + 30 CaCl2	98.20	0.05	1.73	68.62	24.73	3.95
60 NaCl + 15 CaCl2	96.80	0.03	3.15	74.47	14.76	7.72
60 NaCl + 30 CaCl2	97.22	0.06	2.72	47.88	21.78	6.11

Table 21. Factorial analysis of variance for the effect of Na and Ca salts on biomass of Phaseolus vulgaris L.

SV	DF	MS	F
Blocks	4	0.106	3.11*
Treatments	36	0.613	17.97**
Control vs rest	1	1.955	57.32**
Among rest	35	0.574	16.84**
Among Na	8	1.858	54.48**
Na control vs rest	1	4.334	127.10**
Salt	1	2.772	81.29**
Level	3	2.426	71.15**
S x L	3	0.159	4.65**
Among Ca	3	0.326	9.54**
Type	1	1.425	41.79**
Concentration	1	0.077	2.25
T x C	1	0.188	5.50**
Na x Ca	24	0.178	5.21**
Error	144	0.034	
Total	184		

*,** Significant at the 0.05 and 0.01 probability levels, respectively.

21 and 22), for the same Ca type and concentration, NaCl depressed biomass weight more than Na_2SO_4 did. This again indicates that Cl ion has no effect or a negative effet on biomass production. Whereas, SO_4 ion might enhance or would have no effect on production. The data in Fig. 21 show that $CaSO_4$, at 0 Na level, increased the biomass produced, although the increase was not significant, as compared to the Hoagland's treated plants. Whereas, $CaCl_2$, at both levels, significantly depressed biomass production as compared to Hoagland's solution.

The Least significant difference test (LSD, Appendix E), along with the factorial analysis, indicated that a significant difference existed in dry mass production between

$CaSO_4$ and $CaCl_2$ treatments for both Na salts. In general, no significant difference existed between the two levels of each Ca salt. The data in the graph show, for the same Na salt type and concentration, $CaCl_2$ depressed the growth more than $CaSO_4$ did. The ANOVA also indicated that there was a significant interaction between Na salt, Ca type, and the concentrations applied in biomass production.

The results of both experiments indicated that Na affected growth and biomass production and that the accompanying anion played a crucial role in determining the degree of growth depression. Chloride was associated with decreased shoot production and growth as compared to SO_4 treatments. This might have been due to the role that SO_4 ion played in decreasing Na uptake as compared to Cl. The $NaCl^{\circ}$ complex was more readily taken up by the plant than the $Na\text{-}SO_4$ complex. Also, Cl ion was toxic to plants in sufficient concentration; whereas, S applied in this study as SO_4 ion was required by leguminous crops (snapbean) for physiological activities of the cells, protein, and seed production. With respect to Ca ion, Ca complexation with Cl was not significant and it did not appear to affect Cl uptake by the plant. Sulfate played an important role in complexing the Na ion and decreased its uptake as compared to Na-Cl form.

Effect of Ion Speciation on EC_e: The electrical conductivity of the saturated extracts at various concentrations of Na and Ca salts are shown in Appendix C.

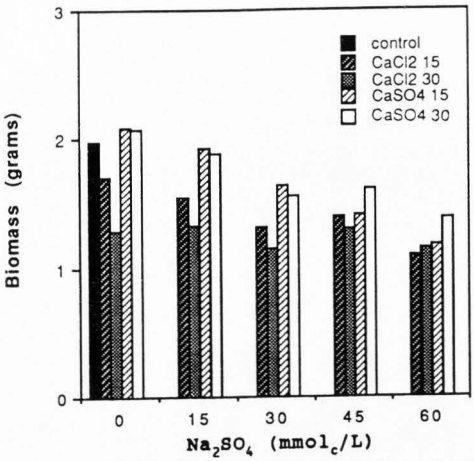

Fig. 21. Phaseolus vulgaris L. shoot weight resulting from irrigation with Na_2SO_4 and Ca salts solutions.

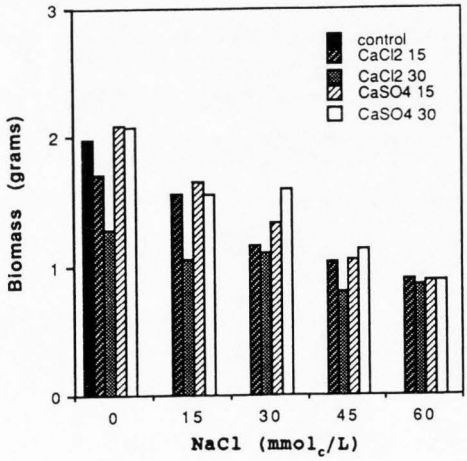

Fig. 22. Phaseolus vulgaris L. shoot weight resulting from irrigation with NaCl and Ca salts solutions.

The bulk soil electrical conductivity (EC_a) varied with salt type and concentration. As the concentration of the salt increased, EC_a also increased. This increase could be due to the fact that ions had carried electric current, and the higher the concentration of ions were, the greater the current conducting capacity or electrical conductivity of the solution would be (Jurinak, 1988).

Results of the calibration procedure outlined in the Materials and Methods section showed that there was a good correlation between apparent electrical conductivity (EC_a) determined by the four-electrode probe resistance reading and between EC_e measured in saturation extracts.

Data in the graphs (Fig. 23) showed that at low salt levels (control treatments), EC_a values were more or less stable over the whole period of the experiment; whereas, at different levels of Na salts, EC_a increased between the second and third week and then decreased through the end of the experiment. This change in EC_a values might have been due to an increase in plant growth between the second and third week corresponding to absorption of most of the available water from the soil. In the later stages of the experiment, the osmotic potential of the soil solution decreased, due to the excess salt, and this decreased the availability of water for plant uptake. Consequently, EC_a decreased due to salt dilution with the excess water.

The effect of ion speciation on EC_a is shown in Figs. 23, 24 and 25. Data in the graphs showed (Fig. 23) that Hoagland's solution had the lowest EC_a value due to the low salt conentration in the treatment solution. However, comparing $CaCl_2$ and $CaSO_4$ at 0 Na level, the EC_a of $CaCl_2$ was much higher than that of $CaSO_4$ although they had the same initial concentrations in the original solution treatments. The reason for this difference was due to the different degrees of ionic dissociation of these two Ca salts. When $CaSO_4$ was dissolved in water, a large fraction of the Ca and SO_4 were attracted to one another, behaving as if they were un-ionized; i.e. forming $CaSO_4^\circ$ complex. The presence of $CaSO_4^\circ$ complex reduced the current conducting capacity of the solution. In a saturated solution of gypsum (15.3 mM/L), $CaSO_4^\circ$ was estimated to be 1/3 of the molar solubility of gypsum (Adams, 1971; Jurinak, 1988). The results of ion speciation (Tables 16 and 20) showed that $CaCl_2$ had about 98% dissociation to free ions; whereas, $CaSO_4$ had about 70% of the total salt as free ions and the rest was $CaSO_4^\circ$. Due to the dependence of EC_a on the electric current (charge) of the solution (Jurinak, 1988), EC_a reading was decreased by ion complexation. The data show that EC_a of $CaSO_4$ was about 60% of that of $CaCl_2$ at 0 sodium level.

Data in Fig. 24a showed that increasing concentrations of NaCl with $CaCl_2$ or $CaSO_4$ increased EC_a values. However, NaCl-$CaCl_2$ treatments had much higher EC_a values than NaCl-$CaSO_4$

treatments, especially at higher NaCl concentrations. Ion speciation data (Tables 16 and 20) showed that in NaCl-$CaSO_4$ treatment extracts there were a number of significant complexes, especially as $CaSO_4^o$, that lowered the EC_a values. Whereas, NaCL-$CaCl_2$ treatment extracts had more free species Na^+, Ca^{2+}, and Cl^-.

With respect to Na_2SO_4 mixed with either $CaSO_4$ or $CaCl_2$ (Fig. 24b), the results showed that there was an increase in EC_a values with increasing Na_2SO_4 concentrations. However, the EC_a of Na_2SO_4-$CaCl_2$ was much higher than that of Na_2SO_4-$CaSO_4$ soil extracts. Ion speciation data showed that Na_2SO_4-$CaSO_4$ soil solution had a higher percentage of SO_4 complexation with the cations (Ca and Na); whereas, Na_2SO_4-$CaCl_2$ had more free ions.

Comparing the effect of ion speciation on EC_a for the different Na salts (NaCl vs Na_2SO_4) regardless of the Ca salt species, the results showed (Figs. 25a and b) that NaCl had more free ions than Na_2SO_4 soil solution; consequently, EC_a values were higher for NaCl salt than Na_2SO_4 EC_a values.

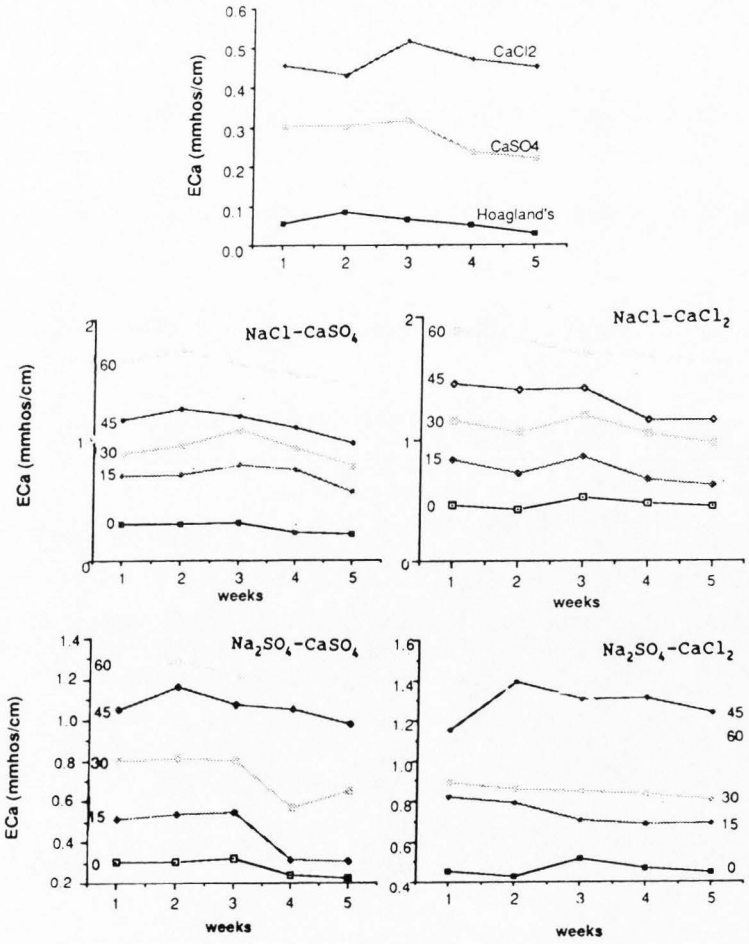

Fig.23 Variation of EC_a with time for different concentrations of sodium salts mixed with $CaCl_2$ or $CaSO_4$ (average of 15 and 30 $mmol_c/L$ Ca treatments).

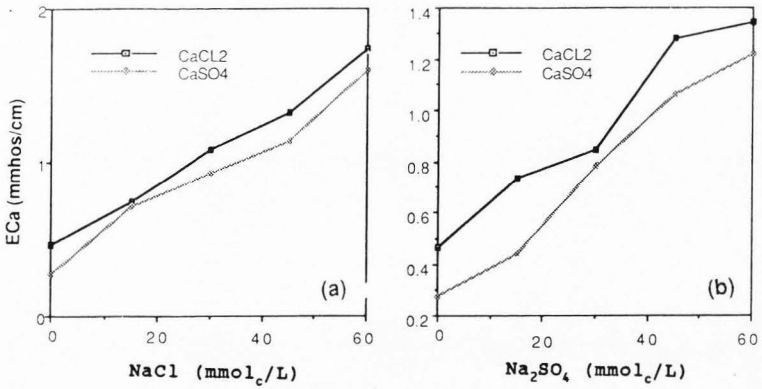

Fig. 24. EC_a variation with the increase in Na concentrations in (a) NaCl-Ca salts and (b) Na_2SO_4-Ca salt solutions.

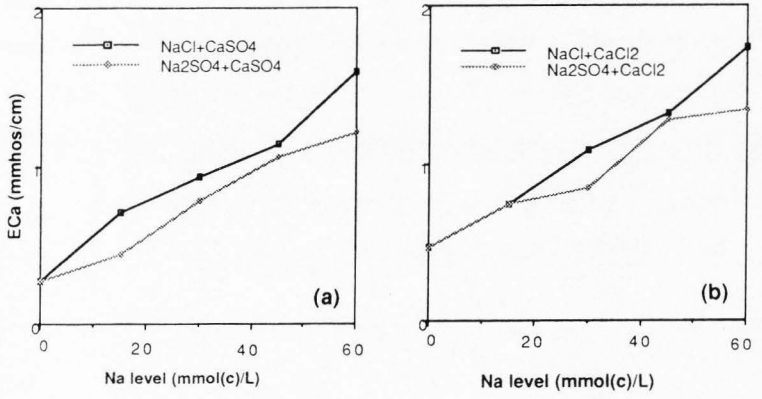

Fig. 25. EC_a variation with increasing concentration of NaCl or Na_2SO_4 mixed with (a) $CaSO_4$ or (b) $CaCl_2$ salts.

SUMMARY

A greenhouse study was conducted to determine the role of ion speciation in the Ca amelioration of Na stress in beans (Phaseolus vulgaris L.).

In Experiment 1, the growing medium was sand:vermiculite at 3:1 volume ratio. Bean seeds were planted and grown in styrofoam pots placed in a randomized block design. Treatment solutions were NaCl and Na_2SO_4 at concentrations of 0, 20, 40, 60 and 80 $mmol_c/L$. The plants were irrigated every other day, and each pot received 200 ml (including a 0.25 leaching fraction, L. F.) of the assigned solution.

In Experiment 2, the growing medium was sand. The pots were placed in a randomized block design. Treatment solutions were NaCl and Na_2SO_4 at concentrations of 0, 15, 30, 45, and 60 $mmol_c/L$ mixed with $CaCl_2$ or $CaSO_4$ at concentrations of 15 and 30 $mmol_c/L$. The plants were irrigated every four days and each pot received 300 ml (including a 0.20 L.F.). In addition, one replicate was planted in 1.5 L. PVC pots designed for electrical conductivity measurements during the growing period. Each of these pots received 450 ml (including a 0.20 L. F.) of the assigned solution.

Plants were harvested 6 weeks after planting. The shoots were oven dried, weighed, and digested. The concentrations of Ca, Na, SO_4 and Cl were determined in the digested material.

Saturation extracts of the growing media were prepared for 2 and 3 replicates of Experiments 1 and 2 respectively.

The concentrations of Na, Ca, Mg, K, SO_4, and Cl and pH and EC in the extracts were determined.

The results were that increasing NaCl concentrations decreased biomass production more than the equal concentrations of Na_2SO_4 did. The NaCl treatments were associated with greater uptake of Ca and Na than corresponding Na_2SO_4 treatments.

In Experiment 2, the presence of $CaSO_4$ with the Na salts was associated with a better amelioration and growth than in the presence of $CaCl_2$ salt. Also in the presence of $CaSO_4$, there was less ion uptake (Ca, Na) than in the presence of $CaCl_2$ at an equilvalent concentration of Na salt. With respect to SO_4 and Cl, their uptake was increased by an increase in their free molar concentration in treatment solutions.

Ion speciation data suggested that complexation of Na ion with SO_4 or Cl was more important than the free ion concentration in affecting sodium uptake. Whereas, for the other ions, (Ca, SO_4, and Cl) the opposite was true; that is, the presence of more free ions in the treatment solution were associated with more uptake by the beans.

Finally, a four-probe arrangement in PVC pots was used to monitor salinity of the growing media during Experiment 2. Ion speciation data showed that the presence of SO_4 in Na or Ca salts was linked with lower EC_a than in the presence of Cl. This difference was primarily due to the formation of the uncharged $CaSO_4^\circ$ complex.

CONCLUSIONS

The following conclusions were reached as a result of this study:

1. A significant difference (α=0.05) in shoot production existed between the NaCl and Na_2SO_4 treatments.

2. A significant difference in shoot production existed between $CaSO_4$ and $CaCl_2$ treatments.

3. Data analysis showed that the presence of SO_4, in Ca or Na form, decreased cation (Ca and Na) uptake. Whereas, in the presence of Cl salts; cation uptake increased.

4. SPECO2 analysis showed that Na as free ion was less important than Na complexes in affecting Na uptake. $NaCl^{\circ}$ increased the uptake; whereas, Na-SO4 decreased the uptake.

5. SPECO2 analysis showed that Ca free ion concentration was more important than complex concentrations in affecting Ca uptake.

6. SPECO2 analysis showed that SO_4 and Cl free ion concentrations were more important than complexes concentrations in affecting their uptake.

7. At the same concentrations ($mmol_c/L$) of Na and Ca salts, the presence of SO_4 was associated with a lower EC than in the presence of Cl.

LITERATURE CITED

Adams, F. 1971. Ionic concentrations and activities in soil solutions. Soil Sci. Soc. Amer. Proc. 35:420-426.

Adraino, D. C., and H. E. Dones. 1982. Bromine, chlorine, and flourine. p. 449-483. In A. L. Page, R. H. Miller, and D. R. Keeney. (ed.) Methods of Soil Analysis. Part 2, no. 9. Amer. Soc. Agronomy, Madison, Wi.

Akhavan-Kharazian, M. 1986. Calcium amelioration of salinity (sodicity) on nitrogen fixation, stomatal resistance, potassium/sodium ratio and total nitrogen of Phaseolus vulgaris L. Ph.D. diss. Utah State University, Logan.

Amacher, M. C. 1984. Determination of ionic activities in soil solutions and suspensions: Principle limitations. Soil Sci. Soc. Amer. J. 48:519-524.

Anonymous. 1987. Ion analysis catalog # 107. Wescan Instruments, Inc. Deerfield, Illinois.

Ayers, A. D., and D. L. Eberhard. 1960: Response of edible broad beans to several levels of salinity. Agronomy J. 52:110-111.

Baker, D. A., and N. H. Suhr. 1982. Atomic absorption and flame emission spectrometry. p. 13-27. In A. L. page, R. H. Miller, and D. R. Keeney. (ed.) Methods of Soil Analysis. Part 2, no. 9. Amer. Soc. Agronomy, Madison, Wi.

Bhivare, V. N., and J. D. Nimbalkar. 1984. Salt stress effects on growth and mineral nutrition of french beans. Plant and Soil. 80:91-98.

Bresler, E., B. L. McNeal, and D. L. Carter. 1982. Saline and sodic soils. Springer-Verlag, New York.

Call, J. A. 1979. Soil salinity measurements as compared to yield. M.S. Thesis. Utah State University, Logan.

Cramer, G. R., E. Epstein, and A. Lauchli. 1988. Kinetics of roots elongation of maize on response to short-term exposure to NaCl and elevated calcium concentration. J. Exp. Botany. 39:1513-1522.

Cramer, G. R., and A. Lauchli. 1986. Ion activities in solution in relation to Na^{+}-Ca^{2+} interaction at the plasmalemma. J. Exp. Botany. 37:321-330.

Cramer, G. R., A. Lauchli, and E. Epstein. 1986. Effects of NaCl and $CaCl_2$ on ion activities in complex nutrient solutions and root growth of cotton. Plant Physiology. 81:792-797.

Cramer, G. R., A. Lauchli, and V. S. Polito. 1985. Displacement of Ca^{2+} from the plasmalemma of root cells. A primary response to salt stress. Plant physiology. 79:207-211.

Cramer, G. R., and A. R. Spurr. 1986a. Salt response of lettuce to salinity. II. Effects of calcium on growth and mineral status. J. Plant Nutrition. 9:131-142.

Cramer, G. R., and A. R. Spurr. 1986b. Responses of lettuce to salinity. I. Effects of NaCl and Na_2SO_4 on growth. J. Plant Nutrition. 9:115-130.

Dudley, L. M., and C. S. Coray. 1989. Computing the equilibrium composition of aqueous systems: An iterative solution at each step in Newton-Raphson. Environ. Sci. Technol. 23:245-247.

Epstein, E. 1961. The essential role of calcium in selective ion transport by plant cells. Plant Physiology.36:437-444.

Epstein, E., J. D. Norlyn, D. W. Rush, R. W. Kingsbury, D. B. Kelley, G. A. Cunningham, and A. F. Wrona. 1980. Saline culture of crops: A genetic approach. Science. 210: 399-404.

Greenway, H. 1973. Salinity, plant growth, and metabolism. J. Austr. Inst. Agr. Sci. 29:24-34.

Gupta, R. K., and R. R. Singh. 1988. A comparative evaluation of the reclamation efficiency of surface-concentrated versus internally incorporated calcium chloride and gypsum in an alkali soil. Soil Science. 140:277-283.

Helal, M., K. Koch, and K. Mengel. 1975. Effect of salinity and potassium on the uptake of nitrogen and on nitrogen metabolism in young barley plants. Physiol. Plant. 35: 310-313.

Hoagland, D. R., and D. I. Arnon. 1950. The water culture method for growing plants without soil. California Exp. Station, Circular 347, University of Ca., Berkeley.

Hyder, S. Z., and H. Greenway. 1965. Effects of Ca^{2+} on plant sensetivity to high NaCl concentration. Plant and Soil. 23:258-260.

Jurinak, J. J. 1988. Salt Affected Soils. Dept of Soil Sciences, Utah State University, Logan.

Lahaye, P. A., and E. Epstein. 1969. Salt tolerance by plants: enhancement with calcium. Science. 166:395-396.

Lahaye, P. A., and E. Epstein. 1971. Calcium and salt toleration by bean plants. Physiol. Plant. 25:213-218.

Lauchli, A., and E. Epstein. 1970. Transport of K and rubidium in plant roots. The significance of calcium. Plant physiology. 45:639-641.

Lauchli, A., and J. Wieneke, 1979. Studies on growth and distribution of Na^+, K^+, and Cl^- in soybean varieties differing in salt tolerance. Z. Pflanzenernaehr. Bodenkd. 142:3-13.

Leopold, A. C. 1977. Modification of growth regulatory action with inorganic solutes. p. 31-41. In C. A. Stutte (ed.) Plant Growth and Regulators: Chemical Activity, Plant Responses and Economic Potential, no 159. Adv. Chem. Series.

Lessani, H., and H. Marschner. 1978. Relation between salt tolerance and long-distance transport of sodium and chloride in various crop spp. Aust. J. Plant Physiol. 5: 27-37.

Lindsay, W. L., 1979. Chemical equilbria in soils. A Wiley-Interscience Publication. John Wiley and Sons, New York.

Luh Huang, C. Y., and E. E. Schulte. 1985. Digestion of plant tissue for analysis by ICP emission spectroscopy. Comm. Soil Sci. Plant Analysis, 16:943-958.

Lynch, J., G. C. Cramer, and A. Lauchli. 1987. Salinity reduces membrane-associated calcium in corn root protoplasts. Plant Physiology. 83:390-394.

Maas, E. V., and R. H. Nieman, 1978. "Physiology of plant tolerance to salinity". p. 277-299. In C. A. Jung (ed.) Crop Tolerance to Subtropical Land Condition. Amer. Soc. Agronomy, Madison. Wi.

Maas, E. V., and G. M. Grieve. 1987. Sodium-induced calcium deficiency in salt stressed corn. Plant, Cell, and environment. 10:559-564.

Maas, E. V., G. Ogata, and M. J. Garber. 1972. Influence of salinity on Fe, Mn and Zn uptake by plants. Agronomy J. 64:793-795.

Mengel, K., and E. A. Kirkby. 1982. Principles of plant nutrition. Third edition. International Potash Institute, Bern, Switzerland.

Mercado, A., and B. Golleck. 1973. Structure and function of plant cells in saline habitats: New Trends in the study of salt tolerance. John wiley and Sons, New York.

Pasternak, D. 1987. "Salt tolerance and crop production- A comprehensive approach". Ann. Rev. Phytopath. 25:271-291.

Plaut, Z., and C. M. Grieve. 1988. Photosynthesis of salt-stressed maize as influenced by Ca:Na ratios in the nutrient solution. Plant and Soil. 105:283-286.

Rabie, R. K., M. K. Matter, A. A. Khamis, and M. M. Mostafa. 1985. Effects of salinity and moisture content of soil on growth, nutrient uptake,and yield of wheat plant. Soil Sci. Plant Nutr. 31:537-545.

Rabie, R. K., and K. Kumazawa. 1988. Effects of NaCl salinity on growth and distribution of sodium and some macronutrient elements in soybean plant. Soil Sci. Plant Nutr. 34:385-391.

Reinsenauer, H. M., L. M. Walsh, and R. G. Hoeft. 1973. Testing soils for sulfur, boron, Molybdenum and chlorine. p. 173-200. In L. M. Walsh, and J. D. Beaton (Ed.). Soil Testing and Plant Analysis. Soil Sc. Soc. Am. Inc. Madison, Wi.

Rhoades, J. D., M. I. Kadah, A. D. Halvarson, and R. J. Prather. 1977. Establishing soil electrical conductivity-salinity calibration using four electrode cells containing undisturbed soil cores. Soil Science. 123:137-142.

Sameni, A. M., M. Maftoun, A. Basseri, and A. R. Sepaskhah. 1980. Growth and chemical composition of dry beans as affected by soil salinity and nitrogen fertilization. Plant and Soil. 54:217-222.

Seeman, J. R., and C. Critchley, 1985. Effects of salt stress on the growth, ion content, stomatal behavior and photosynthesis capacity of a salt-sensetive species, Phaseolus vulgaris L. Planta. 164:151-162.

Shea, P. E., and J. N. Luthin. 1961. An investigation of the use of the four-electrode probe for measuring soil salinity in situ. Soil Science. 92:331-339.

Shokohifard, G., K. Sakagami, R. Hamada, and S. Matsumoto. 1989. Effect of amending materials on growth of radish plants in salinized soil. J. Plant Nutrition. 12:1195-1214.

Smillie, R. M., and R. Nott. 1982. Salt Tolerance in crop plants monitored by cholrophyll fluorescence in vivo. Plant Physiology. 70:1049-1054.

Sposito, G., and S. V. Mattigod. 1979. Geochem: A computer program for the calculation of chemical equilibria in soil solution and other natural water systems. Dept. of Soil and Environmental Sciences. University Ca. Riverside, California.

Sposito, G. 1989. The chemistry of soils. University of California at Berkeley. New York. Oxford. Oxford University Press.

Stumm, W., and J. J. Morgan. 1981. Aquatic chemistry. An introduction emphasizing chemical equilibria in natural waters. John Wiley and Sons. New York.

Tanji, K. 1969. Solubility of gypsum in aqueous electrolytes as affected by ion association and ionic strength up to 0.15 M and at 25°C. Environmental Sci. and Technology. 656-661.

United States Salinity Laboratory Staff. 1954. Diagnosis and improvement of saline and alkali soils. U. S. Dept. Agric. Handbook 60.

Wieneke, J., and A. Lauchli. 1980. Effect of salt stress on distribution of Na^+ and some other cations in two soybean varieties differing in salt tolerance. Z. Pflanzenernahr. Bodenkd. 143:55-67.

APPENDICES

Appendix A. Thermodynamic Data File (THERMO.DAT) for the Speciation Program SPECO2

Thermodynamic data file (THERMO.DAT) for the speciation program SPECO2.

```
5,4             Line 1 is number of metals (Ca,Mg,Na,K,H),
3.14,1,1,0      number of ligands (CO3,SO4,Cl,OH).
1.12,,1,1,1     line 2 till end: Log Kf, Metal stoichiometry,
0,0,0,0         ligand stoichiometry, H stoichiometry.
0,0,0,0         We can have 6 complexes per Metal-Ligand. If
0,0,0,0         6 complexes do not exist, put zeros.
0,0,0,0         Example: lines 2 to 7 represent complexations
2.31,1,1,0      of Ca with the carbonate ligands.
0,0,0,0         Line 2. Formation constant calculation for
0,0,0,0         CaCO3° formation.                    Log Kf
0,0,0,0         Ca2+ + CO2(g) + H2O <=> CaCO3° + 2H+  -15.01
0,0,0,0         H2CO3          <=>  CO2 + H2O          1.46
0,0,0,0         H+ + HCO3-1    <=>  H2CO3              6.36
-1.0,1,1,0      H+ + CO3-2     <=>  HCO3-1            10.33
0.0,1,2,0       Ca2+ + CO3-2   <=>  CaCO3°             3.14
0,0,0,0         for line 3, also calculate formation constant
0,0,0,0         for CaHCO3+1. Since there are no Ca-Carbonate
0,0,0,0         complexes, the lines 4 to 7 are set
0,0,0,0         to zeros.
-12.7,1,0,-1    Then the formation constants for Ca with the
-27.9,1,0,-2    ligands SO4, Cl, and OH are calculated following
0,0,0,0         the same procedure as with carbonates.
0,0,0,0
0,0,0,0
0,0,0,0
3.23,1,1,0      Then follow the same procedure, for the rest of
1.06,1,1,1      the metals complexing the ligands mentioned
0,0,0,0         above.
0,0,0,0
0,0,0,0
0,0,0,0                                              Log Kf
2.23,1,1,0      Mg2+ + SO4-2 <=> MgSO4°               2.23
0,0,0,0
0,0,0,0
0,0,0,0
0,0,0,0
0,0,0,0
-0.03,1,2,0     Mg2+ + 2Cl-  <=> MgCl2°               -0.03
0,0,0,0
0,0,0,0
0,0,0,0
0,0,0,0
0,0,0,0
-11.45,1,0,-1   Mg2+ + H2O    <=> MgOH+ + H+          -11.45
-27.99,1,0,-2   Mg2+ + 2H2O   <=> Mg(OH)2° + 2H+     -27.99
0,0,0,0
0,0,0,0
0,0,0,0
```

		Log K_f
0,0,0,0		
1.26,1,1,0	$Na^+ + CO_3^{-2} <=> NaCO_3^{-1}$	1.26
0.01,2,1,0	$2Na^+ + CO_3^{-2} <=> Na_2CO_3$	0.01
0.24,1,1,1	$Na^+ + H^+ + CO_3^{2-} <=> NaHCO_3^\circ$	0.24
0,0,0,0		
0,0,0,0		
0,0,0,0		
0.7,1,1,0	$Na^+ + SO_4^{-2} <=> NaSO_4^{-1}$	0.7
0,0,0,0		
0,0,0,0		
0,0,0,0		
0,0,0,0		
0,0,0,0		
0.0,1,1,0	$Na^+ + Cl^- <=> NaCl^\circ$	0.0
0,0,0,0		
0,0,0,0		
0,0,0,0		
0,0,0,0		
0,0,0,0		
-14.2,1,0,-1	$Na^+ + H_2O <=> NaOH^\circ + H^+$	-14.2
0,0,0,0		
0,0,0,0		
0,0,0,0		
0,0,0,0		
0,0,0,0		
-0.02,2,1,0	$2K^+ + CO_3^{-2} <=> K_2CO_3^\circ$	-0.02
0,0,0,0		
0,0,0,0		
0,0,0,0		
0,0,0,0		
0,0,0,0		
0.85,1,1,0	$K^+ + SO_4^{-2} <=> KSO_4^{-1}$	0.85
0,0,0,0		
0,0,0,0		
0,0,0,0		
0,0,0,0		
0,0,0,0		
-0.7,1,1,0	$K^+ + Cl^- <=> KCl$	-0.70
0,0,0,0		
0,0,0,0		
0,0,0,0		
0,0,0,0		
0,0,0,0		
-14.5,1,0,-1	$K^+ + H_2O <=> KOH^\circ$	-14.5
0,0,0,0		
0,0,0,0		
0,0,0,0		
0,0,0,0		
0,0,0,0		
-1.46,0,1,2	$2H^+ + CO_3^{2-} <=> H_2CO_3^\circ$	-1.46
10.33,0,1,1	$H^+ + CO_3^{-2} <=> HCO_3^-$	10.33

0,0,0,0
0,0,0,0
0,0,0,0
0,0,0,0 Log K_f
-5,0,1,1 $H^+ + SO_4^{-2} <=> HSO_4^-$ -5.0
0,0,0,0
0,0,0,0
0,0,0,0
0,0,0,0
0,0,0,0
-20,0,1,1 $H^+ + Cl^- <=> HCl°$ -20.0
0,0,0,0
0,0,0,0
0,0,0,0
0,0,0,0
0,0,0,0
-14,0,0,1 $H^+ + OH^- <=> H_2O$ -14.0
0,0,0,0
0,0,0,0
0,0,0,0
0,0,0,0
0,0,0,0

Appendix B. Nitrogen-Peroxide Digestion Procedure for Plant Tissues.

The procedure used for plant tissue digestion:

1. Add 5 ml concentrated nitric acid to 0.5 g. ground plant tissues.
2. Heat to 60°C for half an hour.
3. Heat at 115-120°C for 3-4 hours.
4. Cool and add 2 ml of 30% H_2O_2 and continue heating at 120°C for 3-4 hours. Use small funnels in the tubes for refluxing. Solution should be a clear yellowish color.
5. Remove the funnels and take to dryness.6
6. Take up in 20 ml distilled -deionized water (DDW) heating to 60°C.
7. Filter through whatman#41 and wash the samples with 2-3 small portions of DDW, making the final volume 50 ml.
8. Carry blanks through procedure and analyze for Ca, Na and SO_4.

Note: The blanks may bump and spatter when the H_2O_2 is added. It may be necessary to run an H_2O_2 blank in water separately.

Appendix. C. Mean Values of Saturated Extract Analysis.

Table 22. Mean values of saturated extract analysis.

NaCl	Na_2SO_4	$CaCl_2$	$CaSO_4$	EC_{25}	pH	Ca	Na	SO_4	Cl
mmol$_c$/L				mmhos/cm		mmol$_c$/L			
0	0	0	0	0.98	8.52	6.07	1.34	0.21	1.2
20	0	0	0	6.46	8.28	5.62	56.27	----	56.32
40	0	0	0	10.83	8.39	7.34	106.40	----	100.95
60	0	0	0	13.58	8.34	10.78	163.00	----	128.02
80	0	0	0	15.92	8.32	7.94	172.98	----	150.82
0	20	0	0	7.80	8.34	7.76	79.48	37.02	0.24
0	40	0	0	8.77	8.34	11.48	98.70	43.04	1.20
0	60	0	0	11.96	8.34	13.36	142.28	56.89	0.38
0	80	0	0	15.00	8.37	12.50	182.38	72.57	0.82
						mmol$_c$/L			
0	0	0	0	0.73	7.72	4.56	0.93	1.60	0.56
0	0	15	0	2.35	7.39	33.70	1.27	1.53	19.33
0	0	30	0	3.42	7.22	37.80	0.77	1.00	40.10
0	0	0	15	2.12	7.43	36.58	0.84	25.20	0.70
0	0	0	30	2.41	7.56	42.00	1.02	32.00	0.90
15	0	15	0	3.85	7.32	21.30	17.57	1.10	36.40
15	0	30	0	3.36	7.53	26.68	9.12	0.68	31.70
15	0	0	15	3.75	7.62	27.62	18.72	25.40	17.20
15	0	0	30	4.23	7.59	40.20	16.75	34.10	19.60
0	15	15	0	4.14	7.57	32.80	23.60	27.40	22.63
0	15	30	0	4.48	7.45	44.80	16.10	24.00	34.86
0	15	0	15	4.25	7.56	33.00	28.22	53.20	1.23
0	15	0	30	4.62	7.67	35.60	35.25	61.00	0.70
30	0	15	0	3.86	7.63	17.10	23.62	0.88	39.60
30	0	30	0	4.85	7.55	24.80	20.89	0.78	51.60
30	0	0	15	6.48	7.69	23.80	34.62	24.60	29.90
30	0	0	30	6.12	7.51	42.80	34.83	38.80	36.40
0	30	15	0	4.66	7.69	25.60	29.09	41.20	18.42
0	30	30	0	6.17	7.76	44.00	36.90	36.20	32.59
0	30	0	15	4.22	7.72	24.00	29.80	53.20	0.64
0	30	0	30	5.46	7.68	32.00	38.78	65.80	1.64
45	0	15	0	6.20	7.79	15.99	53.60	0.80	69.40
45	0	30	0	4.78	7.53	20.80	28.50	0.40	46.90
45	0	0	15	6.04	7.61	26.60	47.80	26.80	46.00
45	0	0	30	6.38	7.81	31.40	41.40	36.80	35.50
0	45	15	0	6.92	7.70	30.00	51.62	68.40	13.90
0	45	30	0	6.82	7.42	36.60	41.99	49.00	29.19
0	45	0	15	4.91	7.86	22.44	43.35	63.19	0.85
0	45	0	30	6.45	7.28	26.80	57.01	85.40	1.10

Cont. Table 22.

NaCl	Na_2SO_4	$CaCl_2$	$CaSO_4$	EC_{25}	pH	Ca	Na	SO_4	Cl
mmol$_c$/L				mmhos/cm		mmol/L			
60	0	15	0	5.84	7.66	13.20	55.20	0.80	64.90
60	0	30	0	6.04	7.69	22.02	48.20	0.68	66.70
60	0	0	15	6.87	7.71	19.80	53.50	21.60	46.90
60	0	0	30	6.85	7.44	36.80	49.60	36.80	46.90
0	60	15	0	7.54	7.70	24.80	66.60	76.00	10.96
0	60	30	0	7.80	7.67	35.20	51.88	70.00	23.95
0	60	0	15	7.00	8.03	20.92	79.40	73.98	0.40
0	60	0	30	6.83	7.51	25.36	64.10	91.72	0.50

Appendix D. Mean Values of Shoot Weight and Ions Content.

Table 23. Mean values of shoot weight and ions content.

NaCl	Na_2SO_4	$CaCl_2$	$CaSO_4$	Biomass	Ca	Na	S	Cl
	mmol$_c$/L					g/Kg		
0	0	0	0	3.41	31.61	1.32	1.60	0.73
20	0	0	0	2.36	42.27	25.93	0.98	81.74
40	0	0	0	1.64	39.38	43.64	0.84	114.61
60	0	0	0	1.22	36.62	69.04	1.30	136.68
80	0	0	0	1.24	38.22	75.28	1.28	146.89
0	20	0	0	3.10	23.52	11.45	11.95	0.64
0	40	0	0	2.10	23.44	30.06	26.44	0.49
0	60	0	0	1.66	24.22	38.99	27.38	0.57
0	80	0	0	1.20	23.92	43.76	29.83	0.61
0	0	0	0	1.98	38.25	0.42	0.45	0.68
0	0	15	0	1.71	51.55	0.39	0.60	43.28
0	0	30	0	1.28	69.05	0.54	0.85	72.87
0	0	0	15	2.08	40.92	0.45	2.35	0.72
0	0	0	30	2.07	36.93	0.46	1.69	0.83
15	0	15	0	1.55	62.73	6.98	0.83	62.78
15	0	30	0	1.05	74.48	7.40	0.91	102.74
15	0	0	15	1.65	43.47	1.15	2.85	39.74
15	0	0	30	1.55	47.33	2.14	6.48	44.49
0	15	15	0	1.54	44.87	0.95	2.74	35.86
0	15	30	0	1.32	58.20	3.92	1.62	65.04
0	15	0	15	1.92	31.23	0.79	4.74	0.77
0	15	0	30	1.88	35.10	1.20	9.64	0.51
30	0	15	0	1.16	60.25	17.85	0.78	89.04
30	0	30	0	1.11	82.79	20.14	0.84	109.94
30	0	0	15	1.34	52.41	12.85	3.34	66.04
30	0	0	30	1.60	50.44	6.59	6.58	66.22
0	30	15	0	1.31	39.10	4.53	4.13	36.45
0	30	30	0	1.14	50.32	14.31	3.94	64.38
0	30	0	15	1.63	30.98	1.24	6.45	0.60
0	30	0	30	1.55	35.57	2.07	12.47	0.58
45	0	15	0	1.04	55.72	36.00	0.83	108.40
45	0	30	0	0.81	68.91	30.94	1.05	124.64
45	0	0	15	1.05	52.53	24.31	4.57	68.74
45	0	0	30	1.13	53.31	12.48	6.87	75.68
0	45	15	0	1.39	38.47	6.49	7.10	34.91
0	45	30	0	1.30	40.37	27.61	6.43	57.60
0	45	0	15	1.40	33.39	4.95	13.29	0.86
0	45	0	30	1.61	29.69	5.54	14.78	0.53

Cont. Table 23.

NaCl	Na_2SO_4	$CaCl_2$	$CaSO_4$	Biomass	Ca	Na	S	Cl
	mmol$_c$/L				g/Kg			
60	0	15	0	0.90	46.75	40.41	0.78	123.38
60	0	30	0	0.86	65.53	41.43	1.06	139.38
60	0	0	15	0.89	45.30	33.02	6.14	93.15
60	0	0	30	0.89	43.45	33.21	7.49	99.12
0	60	15	0	1.09	27.35	20.12	16.49	32.18
0	60	30	0	1.15	36.55	14.79	12.09	53.28
0	60	0	15	1.17	30.63	16.27	24.29	0.81
0	60	0	30	1.38	24.97	10.31	17.21	0.55

Appendix E. Example on LSD Test.

LSD test on biomass.

Among control treatments: Hoagland's, $CaCl_2$ and $CaSO_4$ treatments.

$$LSD = t_{\alpha/2} (2MSE/n)^{1/2}$$
$$= 1.972 (2*0.034/5)^{1/2}$$
$$= 0.23.$$

where MSE = Mean square error.
n = Number of observations in the mean.
$t_{\alpha/2}$= t value from statistical table (df=144, $\alpha = 0.05$)

G_1= Average offshoot weight of 15 $mmol_c/L$ $CaSO_4$ trt.= 2.08 g.
G_2= Average offshoot weight of 30 $mmol_c/L$ $CaSO_4$ trt.= 2.07 g.
H = Average offshoot weight of Hoagland's solution= 1.98 g.
B_1= Average offshoot weight of 15 $mmol_c/L$ $CaCl_2$ trt.= 1.71 g.
B_2= Average offshoot weight of 30 $mmol_c/L$ $CaCl_2$ trt.= 1.28 g.

G_1-B_2 = 2.08 - 1.28 = 0.80 > LSD ==> proceed.
G_2-B_2 = 2.07 - 1.28 = 0.79 > LSD ==> proceed.
$H-B_2$ = 1.98 - 1.28 = 0.70 > LSD ==> proceed.
B_1-B_2 = 1.71 - 1.28 = 0.43 > LSD ==> proceed.

G_1-B_1 = 2.08 - 1.71 = 0.37 > LSD ==> proceed.
G_2-B_1 = 2.07 - 1.71 = 0.36 > LSD ==> proceed.
$H-B_1$ = 1.98 - 1.71 = 0.27 > LSD ==> proceed.

G_1-H = 2.08 - 1.98 = 0.10 < LSD ==> Stop.

The difference which is greater than LSD indicates a significant difference between the two means.