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Calcium Amelioration of Salinity (Sodicity) on Nitrogen Fixation, Stomatal Resistance, Potassium/Sodium Ratio and Total Nitrogen of Phaseolus vulgaris L.

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CALCIUM AMELIORATION OF SALINITY (SODICITY) ON NITROGEN FIXATION, STOMATAL RESISTANCE, POTASSIUM/SODIUM RATIO AND TOTAL NITROGEN OF *Phaseolus vulgaris* L.

by

Mahmood Akhavan-Kharazian

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DEDICATION

To my mother and father

and

to my lovely wife and children

who are my happiness and hope.

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Mahmood Akhavan-Kharazian

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ABSTRACT

Calcium Amelioration of Salinity (Sodicity) on Nitrogen Fixation, Stomatal Resistance, Potassium/Sodium Ratio, and Total Nitrogen of Phaseolus vulgaris L.

by

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Department: Plant Science

Extreme salinity is one of the most common environmental constraints with which legume/rhizobia symbionts must deal in arid and semi-arid regions of the world. In some areas, with good management, it has been economically possible to ameliorate the saline soil with calcium. The objective of this study, therefore, was to investigate calcium amelioration of salinity (sodicity) on nitrogen fixation, stomatal resistance, potassium/sodium ratio, and total nitrogen of Phaseolus vulgaris L. Seeds of snapbeans were grown in pots under green house conditions and were irrigated with NaCl concentrations of 0, 0.4, 0.8 or 1.2 S m^{-1} combined with $CaSO_4.2H_2O$ or $CaCl_2.2H_2O$, each at concentrations of 0, 4, and 8 mM.

The results show that increasing NaCl concentration decreased leaf water potential, total leaf chlorophyll, shoot and root dry weight, and nitrogen fixation but increased stomatal diffusive resistance. At the highest level of NaCl, addition of $CaSO_4$ to NaCl

increased leaf water potential via increasing stomatal diffusive resistance. Such effects were not observed with the addition of $CaCl_2$ to NaCl.

Addition of $CaSO_4$ to all levels of NaCl increased total leaf chlorophyll. The shoot and root dry weight and nitrogen fixation was also increased when $CaSO_4$ was added to 0.4 and 0.8 S m^{-1} NaCl. Again, such effects were not observed with the addition of $CaCl_2$ to NaCl.

Furthermore, analysis of leaf mineral composition showed that leaf Ca^{2+}, Mg^{2+} and K^+ were increased with each increase in NaCl concentration, whereas the K^+/Na^+ ratio was decreased. Also, the total leaf nitrogen increased with 0.4 and 1.2 S m^{-1} NaCl as well as with all levels of $CaSO_4$.

Neither $CaSO_4$ nor $CaCl_2$ had any significant effect on leaf K^+, Na^+, or Mg^{2+} of the plant when they were added to different levels of NaCl. However, leaf Ca^{2+} increased with an increase in concentration of $CaSO_4$ or $CaCl_2$, but only $CaSO_4$ exhibited an interaction when combined with NaCl.

Speciation modeling showed that a considerable amount of SO_4 was complexed as the $CaSO_4^o$ and $NaSO_4^-$ species. In spite of this, $CaSO_4$ treatment had ameliorating effect on NaCl induced salinity symptoms in snapbeans.

(131 pages)

INTRODUCTION

Cultivation of legumes to maintain the nitrogen balance in the biosphere through biological nitrogen fixation is of fundamental importance to farmers, as the cost and availability of chemically combined nitrogen often limits primary production in many areas of the world. Plants of the legume family, in addition to fixing nitrogen, are rich in protein and have played a leading role in the advancement of civilization. The proteins of legumes are found in both seeds and vegetative portions of the plants. The proteins are synthesized largely from both available nitrogen in the soil and that provided through biological conversion of atmospheric nitrogen by microsymbionts, namely rhizobia.

Successful initiation of nodulation and nitrogen fixation in legumes is adversely affected by soil salinity (Singleton and Bohlool, 1984). In fact, extremes of salinity are among the most common environmental constrains with which legumes/rhizobia symbionts must deal in arid and semi arid regions of the world. Salinity and water quality can preclude symbionts' establishment, growth, plant dry weight and eventually crop yield (Bishnoi and Pancholy, 1980; Campbell et al., 1983, 1985; Jurinak, 1981; James et al., 1982). Retardation of new nodules and a drop in efficiency of nitrogen fixation might be expected because plants may close the stomates at the time of stress and reduce accumulation of reserves upon which nodules are dependent (Gale et al., 1967; Wilson, 1970; Huang et al, 1975).

Several investigators have shown that salinity stresses adversely affect the symbiotic process and legumes grown in saline environments exhibited reduced yield potential and reduced number and weight of

root nodules (Wilson, 1970; Lahshmi-Kumari et al., 1974; Lauter et al., 1981; and Singleton and Bohlool, 1984). Investigations by Balasubramanian and Sinha (1976) indicated that growth of leaves, stems and roots of both Mung beans and cowpeas was reduced because of salinity stress. Campbell et al. (1985) have shown that salinity and water quality significantly reduced dry weight, pod and seed number of snapbean, Phaseolus vulgaris L. cv Earliwax.

Wilson (1970) showed that symbiotic performance in Glycine wightii was limited by sensitivity of host and not by nodule formation or function because nodules remain remarkably resistant upon addition of NaCl and recovered soon after its removal. Wilson (1970) also noted that symbiosis, when well established, was quite adaptable to fluctuation in substrate salinity, but the ability of young seedlings to initiate nodulation and effective nitrogen fixation at high salinity was limited.

Recent literature has indicated the regulatory role of $CaSO_4$ in growth, development, and adaptation to environmental pertubations such as salinity (Lahaye and Epstein, 1971; Leopold, 1977). It has been shown that addition of Ca^{2+} may "neutralize" the harmful effect of sodium on some plants. Hyder and Greenway (1965) stated that adverse effects of high exchangeable Na^{+} in plant growth was due to low Ca^{2+}/Na^{+} ratios. Lahaye and Epstein (1971) reported that dry weight of leaves, stems and roots of plants increased as Ca^{2+} concentration of the solution increased. They stated that the presence of an appropriate concentration of Ca^{2+} increased the ability of an otherwise susceptible species to withstand the effects of high concentration of NaCl.

Devitt et al. (1984) stated that under sodic saline conditions, the mineral nutrient of most plants can be expected to be detrimentally affected. For example, increasing salt concentration of soil solutions, may decrease K^+ concentration of the leaves. Since K^+ is the major solute in turgid guard cells, K^+ deficiency may result in stomatal closure thereby limiting CO_2 uptake (Peoples and Koch, 1979). In saline soils where plants are subjected to high osmotic potential (large negative number), stomatal regulation becomes even more critical. Even though the K^+ concentration in the leaf may be above the so-called "critical level" for a given species, the proportion of the total K^+ that is actively capable of fulfilling the role in stomatal regulation may be inadequate when Na^+ is present in high amounts (Devitt et al., 1984). Reviews of the literature have shown that relatively little work has been done on Ca^{2+} amelioration of NaCl effects on nitrogen fixation. Thus, it seems valid to investigate the effects of NaCl on nitrogen fixation, stomatal resistance, K^+/Na^+ ratio, percent leaf nitrogen and biomass of snapbeans (P. vulgaris) to which various Ca^{2+} forms were applied.

LITERATURE REVIEW

The effects of salt devastation are apparent in many areas of the world and are becoming increasingly prevalent and serious as population grows, land use intensifies and water becomes more limiting and increasingly polluted with soluble salts (Donahue et al., 1977). Large amounts of formerly arable lands are being removed from crop production every year due to increasing soil salinity and improper water management (Chapman, 1975; Campbell et al., 1983; Allen et al., 1985). Saline irrigation water, fertilizer application and improper crop management are among the factors most responsible for increasing soil salinity (Epstein et al., 1980; Jurinak, 1981; James et al., 1982).

Extreme salinity is one of the most common environmental constraint with which legumes/rhizobia symbionts must deal in arid and semi arid regions of the world. Researchers have found that legumes grown in saline environments exhibit reduced yield potential and reduced numbers and weight of root nodules (Wilson, 1970; Lahshmi-Kumar et al., 1974; Lauter et al., 1981; Singleton and Bohlool, 1984). Longstreth and Nobel (1979) have shown that salinity induced changes in leaf anatomy of P. vulgaris and led to substantially higher ratios of mesophyll surface area to leaf area.

Singleton and Bohlool (1984) found that the process of nodule initiation in soybeans, Glycine max L. Merr., was extremely sensitive to sodium chloride. Even low concentrations (26.6 mM NaCl) caused significant reduction in nodule number and weight, however, development of nodule tissue following infection is more resistant to salinity. Lahshmi-Kumari et al. (1974) investigated the adverse

effects of NaCl on nodulation and found that: 1) root hair infections were reduced to a minimum (even at 0.2% NaCl) accompanied by a reduction in number of nodules, 2) the number of meristematic primordia increased at higher levels of NaCl, which, however, did not lead to any increased lateral root nodule formation, 3) increasing salinity led to a reduction in the number of root hairs and the formation of a mucilaginous layer around the roots. Even the sparse numbers of root hairs present appeared to be short, stubby and bulbous, and 4) root hairs did not show curling, deformation or a shepherd's crook formation characteristic of the early phase of modulation.

Wilson (1970) suggested that the development of nodular tissue of G. wightii. appeared closely associated with the growth of laminae, which was strongly retarded by salt. This might be expected, because salinity reduces photosynthesis (Gale et al. 1967) and the nodules depend on the plant for their source of energy. Wilson (1970) showed that the symbiosis, when well established, has proven quite adaptable to fluctuations in substrate salinity. However, the ability of young seedlings to initiate nodulation and effective nitrogen fixation at high salinity would seem limited. Pate (1966), in studying field peas, Pisum arvense L., has shown that it is mostly the older leaves that export carbohydrates to the root and nodules and those are the leaves that accumulate the highest concentration of salt and show the most injury (Wilson et al. 1970).

Devitt et al. (1984) stated that under saline conditions, the mineral nutrients of most plants can be expected to be detrimentally affected. For example, increasing salt concentration of soil

solutions may decrease K^+ concentration of the leaves. Since K^+ is the major solute in turgid guard cells, K^+ deficiency may result in stomatal closure, thereby limiting CO_2 uptake (Peoples and Koch, 1979).

In some areas of the world, desalinization of the soil is economically feasible and recommended. Jurinak (1981) stated that the management of salt-affected soils depends on the chemistry of the soil solution. Presence of high exchangeable sodium percentage (ESP$\geq$ 15) or sodium adsorption ratio (SAR $\geq$ 13) deteriorates physical structure of the soil, causing swelling and deflocculation of the clay minerals and retards both air and water entry into the soil. Jurinak (1981) and James et al. (1982) reported that reclamation of saline soils must involve replacement of Na^+ by Ca^{2+}, increasing soil hydrolic conductivity and leaching of sodium salts from the system. Hanson (1983) reviewed the biochemical effects of Ca^{2+} on plants and reported that a concentration of 1 mM Ca^{2+} was required in the soil solution for healthy growth of plants in the field.

Several investigators have reported on the regulatory role of Ca^{2+} in growth, development, and adaptation to environmental perturbations (Hyder and Greenway, 1965;Lahaye and Epstein 1971; Leopold, 1977). A so-called "antagonism between Na^+ and Ca^{2+} was noted as far back as 1902 when early studies showed that addition of Ca^{2+} would "neutralize" the harmful effects of Na^+ on some plants.

Lahaye and Epstein (1971), in studying the Ca^{2+}/Na^+ relationship, have shown that soybean plants cultured in the presence of 50 mM NaCl and 10 mM $CaSO_4$ for 6 weeks flowered and set fruit normally. The roots remained healthy and white-colored without any ill effects. The

trifoliate leaves remained healthy and showed no sign of salt damage. Without the addition of Ca^{2+}, plants absorbed and translocated Na^{+} into the leaves. The leaves were slimy and necrotic, and a general breakdown of the roots was observed.

Hyder and Greenway (1965) showed that NaCl reduced growth of Hordeum vulgare L. more strongly at 1/40 of Hoagland's nutrient solution than at 1/10. Growth was restored to that of the 1/10 nutrient medium when Ca^{2+} was added to the 1/40 solution. They believed that adverse effects of high exchangeable Na^{+} on plant growth was due to low Ca^{2+}/Na^{+} ratio.

The role of Ca^{2+} in wall structure is cell-to-cell adhesion. The "glue" is usually identified as the calcium-pectate of the middle lamella laid down during cytokinesis. Calcium removal from root tips by acid treatments or chelating agents will lower cell adhesion (Ginzburg, 1961; Brown, 1963). The most conspicuous role for Ca^{2+} in the apoplast lies within the integrity of the plasma membrane. Lahaye and Epstein (1971) propose that Ca^{2+} is an integral part of the plasmalemma, governing normal impermeability to and transport of ions. A deficiency of Ca^{2+}, they proposed, leads to an impairment of the membrane structure, increasing cell permeability. Zubay (1983) stated that the permeability of gap junction pores of membrane protein in eukaryotes is regulated by cytoplasmic concentration of Ca^{2+}. Low concentrations (less than 10^{-7} M) lead to open channels and effect the communication between the cells, while higher concentrations tend to close the channel in a graded manner.

In plants, concentrations of 1 to 5 mM Ca^{2+} are required to screen the plasma membrane from the deleterious effects of low pH,

salinity, toxic ions, and nutrient imbalance. Without such protection the membranes leak, and fail to discriminate between ions, the proton pump appears to become disfunctional and senescence is accelerated (Hanson, 1983; Lahaye and Epstein, 1971). Poovaiah and Leopold (1973) have provided convincing evidence that Ca^{2+} can delay senescence and leaf abscission. It has been observed that Ca^{2+} is required for H^+ pumping. Increased H^+ pumping in the presence of Ca^{2+} accounts for the observed promotion of solute uptake (Cohen and Nadler, 1976).

Review of the literature has shown that relatively little work has been done on Ca^{2+} amelioration of salinity on nitrogen fixation, stomatal resistance, K^+/Na^+ ratio and percent total leaf nitrogen of P. vulgaris L. to which various Ca^{2+} forms have also been applied.

MATERIALS AND METHODS

Snapbean, Phaseolus vulgaris L. cv Contender, seeds were inoculated with commercial Rhizobium phaseoli L. obtained from the Nitragin Company, Milwaukee, WI, 53209 and planted in 120 x 150 mm styrofoam pots, each with appropriate drainage. Two seeds were planted per pot. The pots were kept under greenhouse conditions with a day/night regime of 14/10 h at 25/18^{o} C. The growth medium was sand:peat (3:1) by volume.

The pots were placed in a randomized complete block design (RCB), with four replications. All pots received equal amounts of water for the first 14 days of germination and seedling growth. Six days after seedling emergence, when unifoliate leaves were expanded, plants were thinned to one healthy plant per pot. Modified 1/2 strength Hoagland's solution minus nitrogen was provided to the plants at 10 days after germination and thereafter every week (Appendix A).

Salt Treatments

Salt treatments were commenced on the 14th day after germination when the inoculated plants were actively fixing nitrogen. Two sources and two concentrations of calcium ($CaSO_4 \cdot 2H_2O$ at 0, 4 and 8 mM and $CaCl_2 \cdot 2H_2O$ at 0, 4 and 8 mM) were added to NaCl solution with initial concentrations of 0, 0.4, 0.8 and 1.2 siemens per meter (Sm^{-1}) (0.1 Sm^{-1} = 1 dS m^{-1} = 1 mmhos cm^{-1} = 640 mg L^{-1} = 10 mM NaCl) in a factorial arrangement (Table 1). Hereafter the two hydrated forms of calcium will be referred to as $CaSO_4$ and $CaCl_2$. The electrical conductivity (EC) and pH of all salt solutions were measured before salt treatments commenced. All pots received the salt treatments 3

Table 1. Combination of sodium chloride and different calcium compounds.

NaCl S m^{-1}	$CaSO_4 \cdot 2H_2O$ (mM)	$CaCl_2 \cdot 2H_2O$ (mM)		
0	0	0	4	8
	4	0	4	8
	8	0	4	8
0.4	0	0	4	8
	4	0	4	8
	8	0	4	8
0.8	0	0	4	8
	4	0	4	8
	8	0	4	8
1.2	0	0	4	8
	4	0	4	8
	8	0	4	8

$(NaCl)(CaSO_4)(CaCl_2)$(Replications) Treatments

4 x 3 x 3 x 4 = 144

Total Treatments = 144

times a week (gravimetrically) with a volume of mixed electrolyte solution to saturate the soil and to maintain the sand and peat mixture close to field capacity (14 to 16% soil water content). The pots were then allowed to drain out freely when irrigated.

Stomatal Diffusive Resistance Measurement

Stomatal response was measured the first and second weeks after salt treatments commenced. Midday measurements of leaf resistance were accomplished with a diffusion porometer (model Lambda LI-65). The instrument was kept in the greenhouse 5 h before each measurement so as to be equilibrated with the temperature of the greenhouse. At the time of measurement, the instrument cup was clamped onto leaf veins so as to not break the foam seal. Each count was noted when the instrument had stabilized. An appropriate calibration graph of diffusion resistance vs. count was developed to determine the leaf stomatal resistance each time the instrument was used. The unit of stomatal diffusive resistance was expressed as seconds per meter (s m^{-1}).

Leaf Water Potential

Ten days after salt treatments were applied, leaf xylem pressure potential of all the plants was measured with a pressure bomb as a close approximation of leaf water potential. (Assuming osmotic potential is near zero, water potential is generally the same magnitude as xylem pressure potential with the opposite sign). Therefore, one of the unifoliate leaves of each plant was selected, wrapped in a plastic bag, and the petiole was cut smoothly at a 45 degree angle (Wiebe and Welkie, 1979). The petiole of the leaf was

inserted promptly into the stopper of the pressure chamber lid and secured onto the chamber. The chamber was then gently pressurized with nitrogen gas. As soon as the sap exudate had reached the cut surface of the petiole, the gas was shut off and the pressure recorded in mega- pascals (MPa).

Nitrogen Fixation Meaurement

Ten days after salt treatment, the plants were harvested at the soil surface. The above ground portions of each plant were dried at 70 C, for 48 hours, and saved for additional studies. Soil samples, (250 g) from each pot were placed in individual plastic bags and saved for soil extract and pH analysis. In addition, the roots of each plant were gently washed and analyzed for acetylene reduction (Hardy et al., 1968). The detached roots were placed in 60 mL syringes and acetylene was injected into each syringe to create an atmosphere of air/acetylene (90/10%). After 60 minutes, gas samples were withdrawn, stored in 10 mL vacutainers until nitrogen fixation analysis could be carried out using a gas chromatograph (model HP 5880A). A standard graph was established based on area vs. ppm ethylene in nitrogen quantifying acetylene reduction of the plants. The measurements were expressed in microliters per plant per hour (ul $plant^{-1}$ h^{-1}). In addition, all nodules were separated from the root system of each plant. Roots and nodules were dried for 48 hours at 70 C and weighed.

Total Leaf Nitrogen Analysis

Fifty mg of dried leaf material were placed in Kjeldahl flasks, mixed with 1.8 g of Kjeldahl catalyst (with the following proportions: 2 mL of distilled water, 5 mL of concentrated H_2SO_4 and boiling

granules) and digested at 370 C for 1 h until the mixture reached a bluish color. Following digestion, the flask was cooled at room temperature for 1 h and five drops of 30% hydrogen peroxide (H_2O_2) were added to the flask to oxidize any remaining traces of organic matter adhering to the neck of the flask. Ten mL of distilled water were then added to the sample and the flask was attached to a micro-Kjeldahl unit designed and prepared for distillation of samples (Appendix B). To each flask, 30 mL of 40% NaOH were added until the mixture reached a brownish color. The suspension was steam-distilled and the distillate collected in individual flasks containing 5 mL of 2% boric acid (H_3BO_3) and 2 drops of Tashior's indicator (Appendix C). The distillation was continued for 5 minutes until 30 mL of distillate was collected from each sample (Bremner, 1965). The distillates collected in boric acid were treated with 0.01 M H_2SO_4. The amount of nitrogen content was then calculated and all measurements were reported as millimole per plant per kilogram (mmole Plt^{-1} kg^{-1}):

1 mg N gr^{-1} = (mL titrated - mL blank) x (normality of acid, mmole mL^{-1}) x (nitrogen atomic number, 14 mg $mmole^{-1}$)/ (grams of sample).

Plant Analysis

Leaves of the plants harvested and dried at 70 C were used for analysis of K^+/Na^+ ratio. Fifty mg of each sample were ground in mortar and pestle and placed into 50 mL flasks. Eight mL of nitric acid (HNO_3) and 3 mL of perchloric acid ($HClO_4$) were added to each flask and covered with a small pyrex funnel. The flasks were put on a hot plate at 60^o C for 15 minutes or until the reaction had subsided, then heated at 120^o C for 75 minutes (Ganje and Page, 1974) until

complete dissolution occurred, brown smoke disappeared and approximately 4 ml of solution remained in each flask.

The flasks were then cooled to room temperature and the volume of the solution was brought to 10 mL using deionized double-distilled water. The solutions were kept in individual 20 mL scintillation vials until they were analyzed for Ca^{2+}, K^{+}, Mg^{2+} and Na^{+} using an atomic absorption spectrophotometer (model Perkin-Elmer 2380) at wave lengths equal to 422 nm for Ca^{2+}, 285.2 nm for Mg^{2+}, 766.5 nm for K^{+} and 589 nm for Na^{+} (Appendix D). A standard curve was developed for each element to convert the plant readings to mg kg^{-1} and soil reading to mg L^{-1}.

Saturation Extract Measurements

Two hundred fifty mL of the Soil samples (sand and peat mixture) collected from each pot at harvest time were used for preparation of soil saturation extract and electrical conductivity (EC) measurements. The soil was moistened with distilled water to saturation, transferred to a Buchner funnel fitted with a Whatman No. 1 filter. The funnel was connected to a side arm flask in which a test tube was placed for collection of the extract. The flask was connected to a vacuum pump for 5 minutes, during which time, 25 mL of soil saturation extract was collected.

The extract was further purified by centrifugation (model Sorvall SS-3), at 8000 G for 5 minutes. The following measurements were made on the saturation extracts:

1. Electrical conductivity of the soil extracts (EC_e) was measured at 25^{o} C using a Beckman Conductivity Bridge (model R016 C). The EC in this study was expressed in S m^{-1}.

2. The saturation extract was used to obtain the soil pH. Measurement was done by a Beckman pH meter (model 60).
3. The saturation extract was anaylzed for K^+, Na^+, Ca^{2+} and Mg^{2+} by atomic absorption spectrophotometer (model Perkin-Elmer 2380) (Appendix D). The units of measurements were expressed in mg kg^{-1} for plants and mg L^{-1} for soil.
4. Finally, the saturation extract analyses were used to calculate sodium adsorption ratio (SAR) using the following formula:

$$SAR = [Na^+]/[Ca^{2+} + Mg^{2+}]^{1/2}$$

Where all concentrations are in mmole L^{-1}. The units for SAR are (millimole L^{-1})$^{1/2}$.

Chlorophyll Estimation

The leaves were finely ground in mortar and pestle and chlorophyll was extracted. Fifty mg units of plant leaves were weighed and placed in 20 mL glass jars, to which was added 10 mL of methanol (CH_3OH) (99.9% purity). Each jar was covered tightly and stored in the dark for 24 h at 25 C. Absorbance of the samples were read at wavelengths of 645 and 663 nm using a Sargent-Welch 6-450 UV/VIS Spectrophotometer. Chlorophyll a, chlorophyll b, and chlorophyll a+b were calculated using the following equations (Bruinsma, 1963; Mughrabi, 1983):

$$\text{Chlorophyll } a = 12.7\ A_{663} - 2.7\ A_{645}$$

$$\text{Chlorophyll } b = 22.9\ A_{645} - 4.7\ A_{663}$$

$$\text{Chlorophyll } a+b = 20.2\ A_{645} + 8.0\ A_{663}$$

where A_{645} and A_{663} are the absorbance readings at wavelengths of 645 and 663 nm, respectively. The chlorophyll content was expressed in mg $100g^{-1}$ of dry weight.

RESULTS AND DISCUSSION

Leaf Water Potential

Sodium chloride concentrations of 0.4, 0.8 and 1.2 S m^{-1} induced a significant decrease in leaf water potential of P. vulgaris L. with increasing NaCl compared to the control plants (Table 2 and Fig. 1). The leaf water potential was equal to -0.20, -0.24, -0.27 and -0.28 MPa for NaCl concentration of 0, 0.4, 0.8, and 1.2 S m^{-1}, respectively. Furthermore, the leaf water potential at a NaCl concentration of 0.4 S m^{-1} significantly differed from 0 and 1.2 S m^{-1}, but not from 0.8 S m^{-1}.

The interaction of NaCl and $CaSO_4$ is shown in Table 2 and Figure 2. Leaf water potential was at its highest value (-0.17 MPa) when 4 mM of $CaSO_4$ were mixed with the control solution. A solution of 8 mM $CaSO_4$ decreased the water potential to a value (-0.22 MPa) lower than the control (-0.20 MPa), however, the effect was not significant.

When the NaCl concentration was increased from the control level to 0.4 S m^{-1} and then mixed with 4 or 8 mM of $CaSO_4$, the leaf water potential decreased from -0.21 MPa at control to -0.25 and -0.26 MPa for 4 and 8 mM $CaSO_4$, respectively. However, the effect was significant only at 8 mM $CaSO_4$.

Similarly, when the NaCl concentration was increased to 0.8 S m^{-1} and mixed with 4 or 8 mM of $CaSO_4$, the leaf water potential decreased from -0.24 MPa at control to -0.28 and -0.29 MPa for 4 and 8 mM $CaSO_4$, respectively. At this level of NaCl, only the effect of 8 mM of $CaSO_4$ was significant.

Finally, a NaCl concentration of 1.2 S m^{-1} mixed with 4 mM of $CaSO_4$ significantly increased the leaf water potential from -0.31 MPa

Table 2. Effects of sodium chloride and calcium compounds on leaf water potential in Phaseolus vulgaris L.

Sources	DF	MS	Obs. F	Prob. F
Block	3	0.548	1.744	0.163
NaCl	3	4.330	13.786	0.000 **
$CaSO_4$	2	0.521	1.660	0.195
$CaCl_2$	2	0.083	0.263	0.769
NaCl x $CaSO_4$	6	0.895	2.849	0.013 *
NaCL x $CaCl_2$	6	0.528	1.681	0.134
$CaSO_4$ x $CaCl_2$	4	0.858	2.732	0.033 *
NaCl x $CaSO_4$ x $CaCl_2$	12	0.203	0.646	0.797
Error	100	0.314	-----	-----

* Significant at $P < 0.05$
** Significant at $P < 0.01$

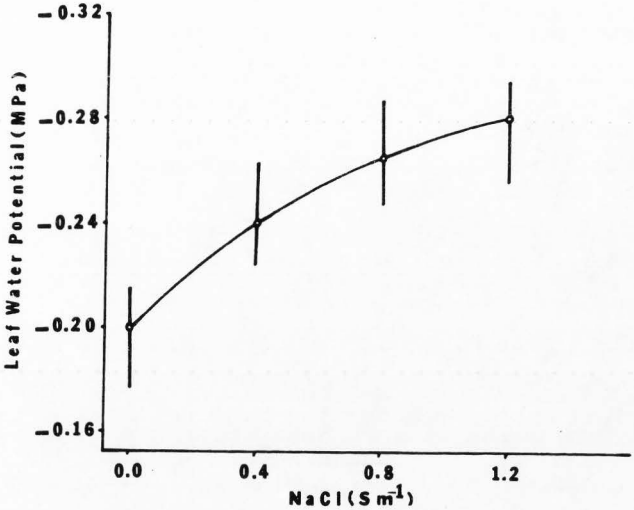

Figure 1. Effects of sodium chloride on leaf water potential in Phaseolus vulgaris L. (vertical bars denote confidence intervals).

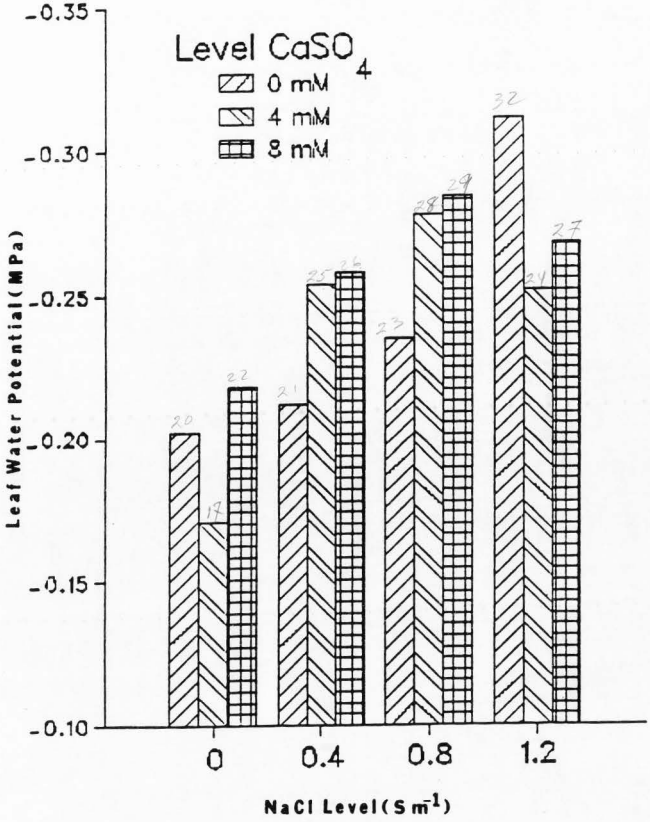

Figure 2. Effects of sodium chloride and calcium sulfate on leaf water potential in Phaseolus vulgaris L.

at control to -0.25 MPa with 4 mM $CaSO_4$. At this level of NaCl, the addition of 8 mM of $CaSO_4$ had no significant effect (Fig. 2).

The interaction of $CaSO_4$ and $CaCl_2$ is shown on Table 2 and Figure 3. An insignificant increase in leaf water potential was observed when 4 mM $CaCl_2$ was compared to control. Leaf water potential of the control solution was -0.25 MPa while that with 4 mM $CaCl_2$ was -0.22 MPa. When 4 mM of $CaSO_4$ was mixed with 4 or 8 mM of $CaCl_2$, the leaf water potential decreased (although not significantly) to -0.25 and -0.24 MPa as compared to the example when no $CaCl_2$ was added, i.e. -0.22 MPa.

A highly significant difference in leaf water potential was noticed when 8 mM of $CaCl_2$ was mixed with 8 mM $CaSO_4$. In this case, leaf water potential was increased from -0.28 MPa without the addition of $CaCl_2$ to -0.24 MPa with the addition of 8 mM $CaCl_2$. With the addition of 4 mM $CaCl_2$ to 8 mM $CaSO_4$, the leaf water potential increased (-0.26 MPa) but not significantly (Fig. 3).

The results indicated that any NaCl concentration above the control reduced the leaf water potential. This could be due to the fact that the presence of salt in the soil solution decreased the osmotic potential of soil water, which in turn, increased the energy the plant must expend to extract water from the soil, i. e., decreasing soil moisture content was associated with increasing osmotic pressure of the tissue fluid or decreasing water potential in leaves of P. vulgaris L. (Wadleigh and Ayers, 1945; Lagerwerff and Eagle, 1961; Hanks and Ashcroft, 1980; Jurinak, 1981; James et al., 1982).

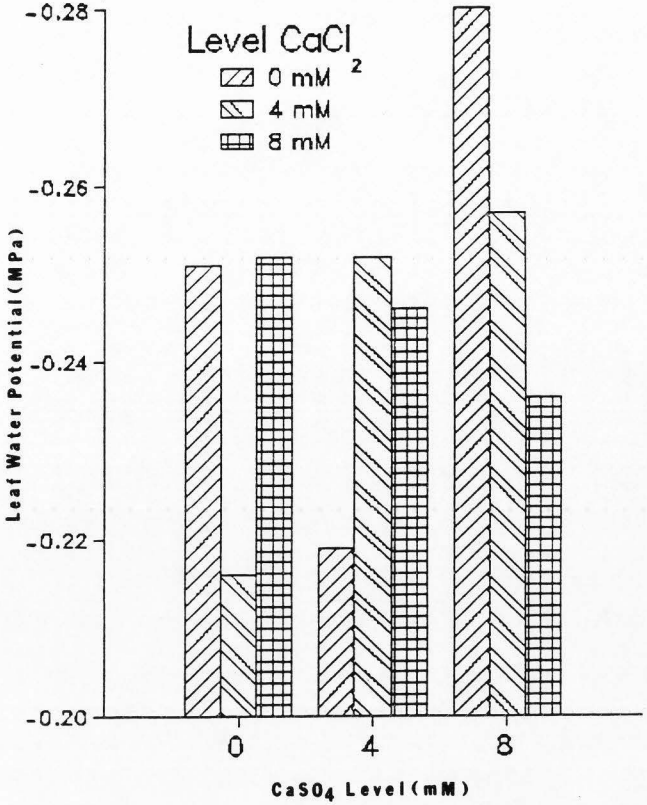

Figure 3. Effects of caclium sulfate and calcium chloride on leaf water potential in Phaseolus vulgaris L.

Also, the results showed that 4 and 8 mM of $CaSO_4$ were able to ameliorate the hazard of NaCl and increase the leaf water potential of P. vulgaris at a NaCl concentration of 1.2 S m^{-1}. In addition, a mixture of 8 mM of $CaCl_2$ and 8 mM of $CaSO_4$ was more effective in increasing leaf water potential than lower concentration combinations.

Stomatal Diffusive Resistance

Week One After Salt Treatment. The stomatal diffusive resistance of P. vulgaris increased significantly with increased NaCl concentration (Table 3 and Fig. 4). The stomatal diffusive resistance was 1880, 2200, 3050 and 3720 s m^{-1} for NaCl concentrations of 0, 4, 8, and 1.2 S m^{-1}, respectively.

The interaction of NaCl and $CaSO_4$ on stomatal diffusive resistance is shown in Table 3 and Figure 5. The stomatal diffusive resistance was at its lowest value (1700 s m^{-1}) with 0 NaCl and 0 mM $CaSO_4$. Addition of 4 or 8 mM $CaSO_4$ to the solution without NaCl slightly increased the stomatal diffusive resistance to 1800 and 2140 s m^{-1}, respectively, compared to 1700 s m^{-1} for the control. When 0.4 S m^{-1} of NaCl was mixed with a solution of 8 mM $CaSO_4$, the stomatal diffusive resistance decreased insignificantly to 1950 s m^{-1} compared to the NaCl solution by itself with a stomatal diffusive resistance of 2320 s m^{-1}. At this NaCl level (0.4 S m^{-1}), 0 and 4 mM of $CaSO_4$ had no significant effect (Fig. 5).

When the NaCl concentration was increased from 0.4 to 0.8 S m^{-1} and the latter was mixed with 4 mM $CaSO_4$ the stomatal diffusive resistance was significantly reduced to 2600 s m^{-1} as compared to 3450 s m^{-1} by the NaCl itself. At this NaCl level (0.8 S m^{-1}), 8 mM of $CaSO_4$ also ameliorated the effects of NaCl and reduced the stomatal

Table 3. Effects of sodium chloride and calcium compounds on leaf diffusive resistance (for the first week after salt treatments) in Phaseolus vulgaris L.

Sources	DF	MS	Obs. F	Prob. F
Block	3	9584.355	7.444	0.000 **
NaCl	3	70928.482	55.093	0.000 **
$CaSO_4$	2	2423.94	1.883	0.157
$CaCl_2$	2	760.313	0.591	0.556
NaCl x $CaSO_4$	6	5614.845	4.361	0.001 **
NaCl x $CaCl_2$	6	446.483	0.347	0.910
$CaSO_4$ x $CaCl_2$	4	470.561	0.366	0.833
NaCl x $CaSO_4$ x $CaCl_2$	12	795.44	0.618	0.823
Error	104	1287.442	-----	-----

** Significant at $P < 0.01$

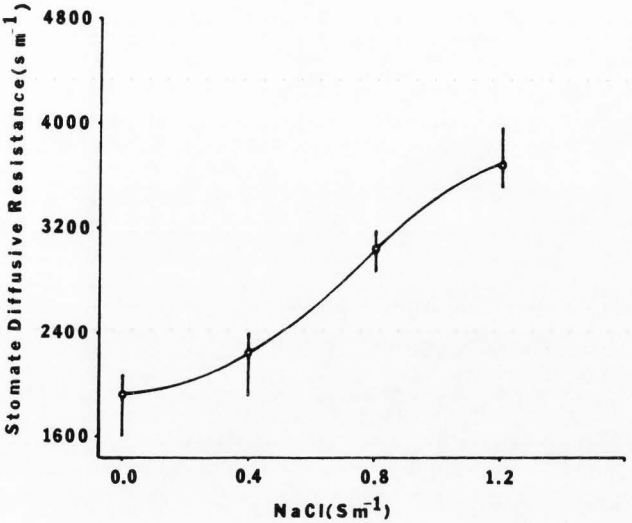

Figure 4. Effects of sodium chloride on stomate diffusive resistance (week one of treatment) in Phaseolus vulgaris L. (vertical bars denote confidence intervals).

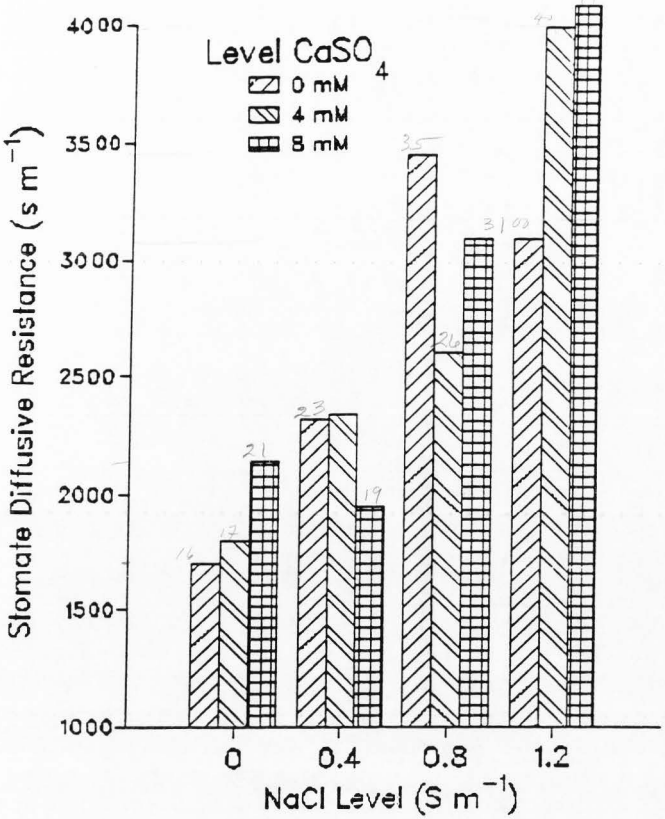

Figure 5. Effects of sodium chloride and calcium sulfate on stomate diffusive resistance (first week of treatment) in Phaseolus vulgaris L.

diffusive resistance to 3090 s m^{-1}, but again the effect was not significant (Fig. 5).

When the NaCl concentration was increased to 1.2 S m^{-1} a highly significant increase in stomatal diffusive resistance was observed with the addition of 4 or 8 mM $CaSO_4$. At this level of NaCl, the stomatal diffusive resistance was increased from 3090 s m^{-1} by NaCl alone to 3990 and to 4070 s m^{-1} by the addition of the 4 and 8 mM $CaSO_4$, respectively (Fig. 5).

Week Two After Salt Treatment. The stomatal diffusive resistance of P. vulgaris increased significantly with each increase in NaCl concentration (Table 4 and Fig. 6). The stomatal diffusive resistance was 4400, 9700, 1260, and 1470 s m^{-1} for NaCl concentrations of 0, 4, 8, and 1.2 S m^{-1}, respectively.

The interaction of NaCl and $CaSO_4$ is shown in Table 4 and Figure 7. Stomatal diffusive resistance was at its lowest value (3700 s m^{-1}) with 0 NaCl and 0 mM $CaSO_4$. Addition of 4 or 8 mM of $CaSO_4$ to the control solution without NaCl increased the stomatal diffusive resistance slightly from 3700 s m^{-1} for control to 4400 and 5100 s m^{-1}, respectively. In another case, when NaCl concentration was increased from 0 to 0.4 S m^{-1} and mixed with 4 mM $CaSO_4$, the stomatal diffusive resistance increased significantly to 11300 s m^{-1} compared to 8200 s m^{-1} for the NaCl. At this level of NaCl (0.4 S m^{-1}), 8 mM of $CaSO_4$ increased the stomatal diffusive resistance insignificantly (Fig. 7).

When the NaCl concentration was increased from 0.4 to 0.8 S m^{-1} and the latter mixed with 4 mM of $CaSO_4$ stomatal diffusive resistance was significantly decreased to 10600 s m^{-1} compared to 15100 s m^{-1} for

Table 4. Effects of sodium chloride and calcium compounds on leaf diffusive resistance (for the second week after salt treatments) in Phaseolus vulgaris L.

Sources	DF	MS	Obs. F	Prob. F
Block	3	405.745	9.852	0.000 **
NaCl	3	2450.808	59.512	0.000 **
$CaSO_4$	2	39.475	0.959	0.387
$CaCl_2$	2	23.484	0.570	0.567
NaCl x $CaSO_4$	6	218.084	5.296	0.000 **
NaCl x $CaCl_2$	6	82.038	1.992	0.073
$CaSO_4$ x $CaCl_2$	4	40.913	0.993	0.415
NaCl x $CaSO_4$ x $CaCl_2$	12	37.709	0.916	0.534
Error	104	41.182	-----	-----

** Significant at $P < 0.01$

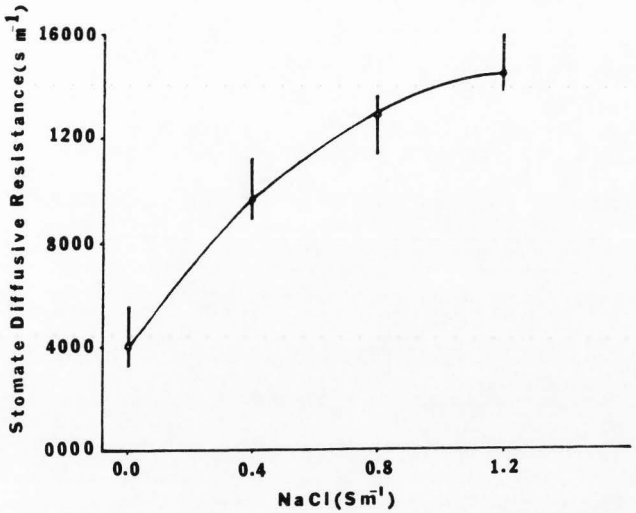

Figure 6. Effects of sodium chloride on stomate diffusive resistance (week two of treatment) in Phaseolus vulgaris L. (vertical bars denote confidence intervals).

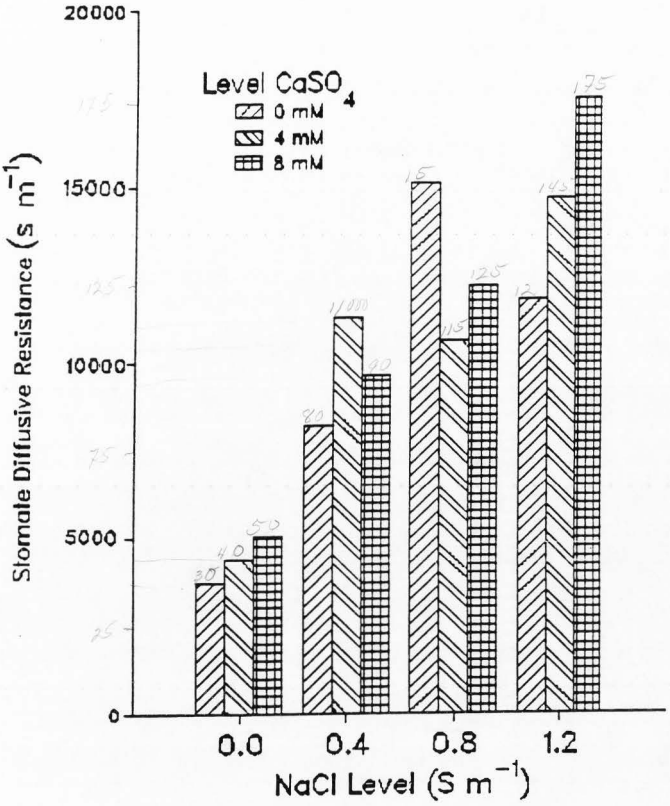

Figure 7. Effects of sodium chloride and calcium sulfate on stomate diffusive resistance (second week of treatments) in Phaseolus vulgaris L.

the NaCl. At the 0.8 S m^{-1} NaCl level, 8 mM of $CaSO_4$ also reduced the stomatal diffusive resistance from 15100 to 12200 s m^{-1}, but the effect was not significant (Fig. 7).

When the NaCl concentration was increased to 1.2 S m^{-1}, a highly significant increase in stomatal diffusive resistance was noted with the addition of 8 mM $CaSO_4$. At this level of NaCl, 8 mM $CaSO_4$ increased the leaf stomatal diffusive resistance from 11800 s m^{-1} for 1.2 S m^{-1} NaCl to 17500 s m^{-1} for the 8 mM $CaSO_4$. At this NaCl level, 4 mM of $CaSO_4$ had no significant effect on stomatal diffusive resistance (Fig. 7).

Comparison of the results of stomatal diffusive resistance for the first and second week of salt treatments (Figs. 4, 5, 6, and 7) showed that the stomata apparatus was closed more during the second week of salt treatments than during the first week. As stress progressed, the plants increased the stomatal diffusive resistance to reduce transpiration (Huang et al., 1975; Finn and Brun, 1980; Sanchez-Diaz et al., 1982; and Halterlein, 1983). This could be a survival mechanism for the plants, since the plants may close the stomates to avoid further stress and possibly adjust the cell turgor pressure (Fitter and Hay, 1983).

The results also showed that 4 and 8 mM of $CaSO_4$ interacted with 0.8 S m^{-1} of NaCl and lowered the stomatal diffusive resistance of P. vulgaris more effectively than when no $CaSO_4$ was added. This may have allowed the plants to transpire, photosynthesize, and effectively metabolize at moderately high levels of NaCl.

Leaf Chlorophyll

Chlorophyll a. Sodium chloride, at concentrations of 0.4, 0.8 and 1.2 S m^{-1}, significantly decreased the chlorophyll a of P. vulgaris compared to the control (Table 5 and Fig. 8). The mean chlorophyll a for the control was 4.4 mg $100g^{-1}$ dry weight, while with NaCl solutions of 0.4, 0.8 and 1.2 S m^{-1}, the mean chlorophyll a was reduced to 2.9, 2.7 and 2.9 mg $100g^{-1}$ dry weight, respectively.

The effects of NaCl and $CaSO_4$ on chlorophyll a are shown in Table 5 and Figure 9. Chlorophyll a was at its highest value (5.1 mg $100g^{-1}$ dry weight) for the control plants. Without NaCl, 4 and 8 mM $CaSO_4$ significantly reduced chlorophyll a from 5.1 mg $100g^{-1}$ dry weight to 4.0 and 4.1 mg $100g^{-1}$ dry weight with 4 and 8 mM $CaSO_4$, respectively. Mixing 0.4 S m^{-1} NaCl with 4 or 8 mM $CaSO_4$ resulted in an increase in chlorophyll a compared to the situation where no $CaSO_4$ was added. In this case, chlorophyll a was increased insignificantly from 2.63 mg $100g^{-1}$ dry weight by the NaCl to 3.1 and 2.8 mg $100g^{-1}$ dry weight with 4 and 8 mM $CaSO_4$, respectively.

The effect of $CaSO_4$ on chlorophyll production in the presence of NaCl was more obvious when NaCl concentration was increased from 0.4 to 0.8 S m^{-1}. In this case, 8 mM $CaSO_4$, when mixed with 0.8 S m^{-1} of NaCl, significantly increased chlorophyll a from 2.4 mg $100g^{-1}$ dry weight for the NaCl alone to 3.1 mg $100g^{-1}$ dry weight when mixed with 8 mM $CaSO_4$. When 4 mM of $CaSO_4$ was mixed with 0.8 S m^{-1} of NaCl, the increase in chlorophyll a was insignificant (Fig. 9).

Finally, when the NaCl concentration was increased to 1.2 S m^{-1}, 4 mM of $CaSO_4$ significantly increased chlorophyll a from 2.6 mg $100g^{-1}$ dry weight with NaCl alone to 3.3 mg $100g^{-1}$ dry weight by the 4 mM

Table 5. Effects of sodium chloride and calcium compounds on chlorophyll a in Phaseolus vulgaris L.

Sources	DF	MS	Obs. F	Prob.F
Block	3	3.885	5.493	0.002 **
NaCl	3	21.446	30.323	0.000 **
$CaSO_4$	2	0.148	0.210	0.811
$CaCl_2$	2	0.235	0.332	0.718
NaCl x $CaSO_4$	6	2.903	4.105	0.001 **
NaCl x $CaCl_2$	6	0.986	1.395	0.224
$CaSO_4$ x $CaCl_2$	4	0.299	0.422	0.792
NaCl x $CaSO_4$ x $CaCl_2$	12	0.584	0.826	0.623
Error	104	0.707	-----	-----

** Significant at P<0.01

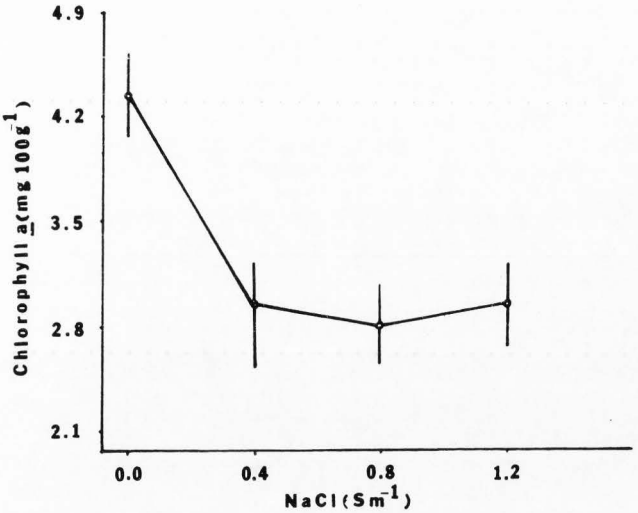

Figure 8. Effects of sodium chloride on chlorophyll a in Phaseolus vulgaris L. (vertical bars denote confidence intervals).

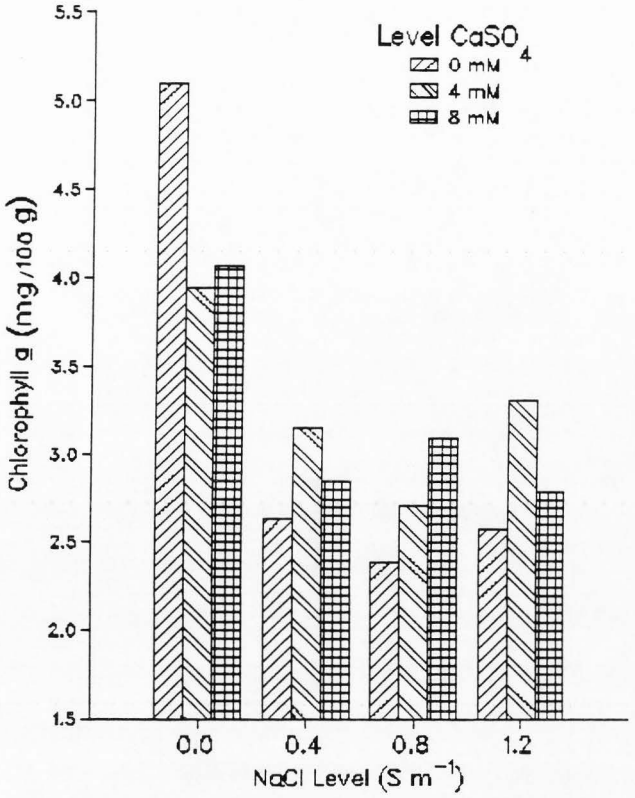

Figure 9. Effects of sodium chloride and calcium sulfate on chlorophyll a in Phaseolus vulgaris L.

$CaSO_4$. At 1.2 S m^{-1} of NaCl, 8 mM of $CaSO_4$ did not significantly increase chlorophyll a (Fig. 9).

Chlorophyll b. Sodium chloride, at concentrations of 0.4, 0.8 and 1.2 S m^{-1}, significantly decreased chlorophyll b in P. vulgaris compared to the control (Table 6 and Fig. 10). The mean chlorophyll b in control plants was 2.9 mg $100g^{-1}$ dry weight. However, with NaCl concentrations of 0.4, 0.8 and 1.2 S m^{-1}, chlorophyll b was reduced to 1.9, 1.9 and 1.8 mg $100g^{-1}$ dry weight, respectively.

The effect of $CaSO_4$ on leaf chlorophyll b is shown in Table 6 and Figure 11. Calcium sulfate, at a concentration of 4 mM, significantly increased leaf chlorophyll b of P. vulgaris compared to the controls and compared to those plants receiving 8 mM of $CaSO_4$. The mean chlorophyll b was equal to 2.1, 2.3 and 2.1 mg $100g^{-1}$ dry weight for $CaSO_4$ concentrations of 0, 4, and 8 mM, respectively.

The interactive effects of NaCl and $CaSO_4$ on chlorophyll b are shown on Table 6 and Figure 12. Chlorophyll b was at its highest value at control. The addition of 4 or 8 mM of $CaSO_4$ alone significantly reduced chlorophyll b to 2.8 and 2.6 mg $100g^{-1}$ dry weight (as compared to the control 3.4 mg $100g^{-1}$) for $CaSO_4$ concentration of 4, and 8 mM, respectively.

Chlorophyll b was significantly increased (as compared to the control) when 0.4 S m^{-1} NaCl was added to 4 mM $CaSO_4$. In this case, chlorophyll b was increased from 1.6 mg $100g^{-1}$ dry weight for the 0 NaCl to 2.2 mg $100g^{-1}$ dry weight for the 4 mM $CaSO_4$. A concentration of 8 mM $CaSO_4$, when mixed with 0.4 S m^{-1} NaCl, slightly increased the chlorophyll b to 2.0 mg $100g^{-1}$ dry weight, but the effect was not significant (Fig. 12).

Table 6. Effects of sodium chloride and calcium compounds on chlorophyll b in Phaseolus vulgaris L.

Sources	DF	MS	Obs. F	Prob. F
Block	3	6.144	24.403	0.000 **
NaCl	3	9.610	38.167	0.000 **
$CaSO_4 2$	2	0.809	3.213	0.044 *
$CaCl_2$	2	0.320	1.271	0.285
NaCl x $CaSO_4$	6	1.334	5.299	0.000 **
NaCl x $CaCl_2$	6	0.323	1.281	0.272
$CaSO_4$ x $CaCl_2$	4	0.186	0.738	0.568
NaCl x $CaSO_4$ x $CaCl_2$	12	0.256	1.015	0.441
Error	104	0.252	-----	-----

* Significant at P<0.05
** Significant at P<0.01

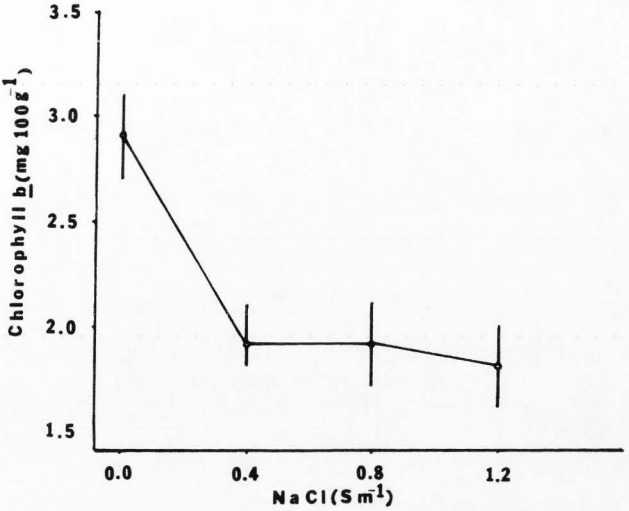

Figure 10. Effects of sodium chloride on chlorophyll b in Phaseolus vulgaris L. (vertical bars denote confidence intervals).

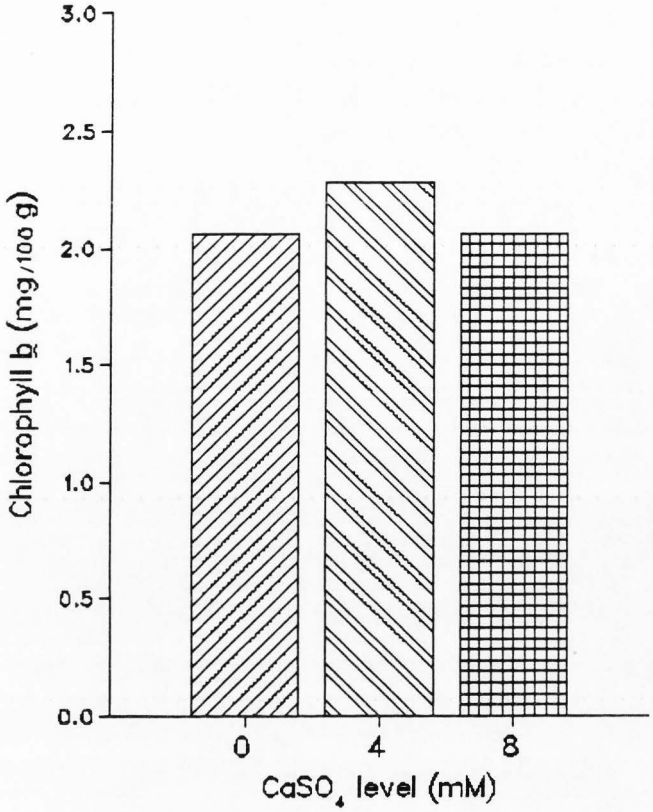

Figure 11. Effects of calcium sulfate on chlorophyll b in Phaseolus vulgaris L.

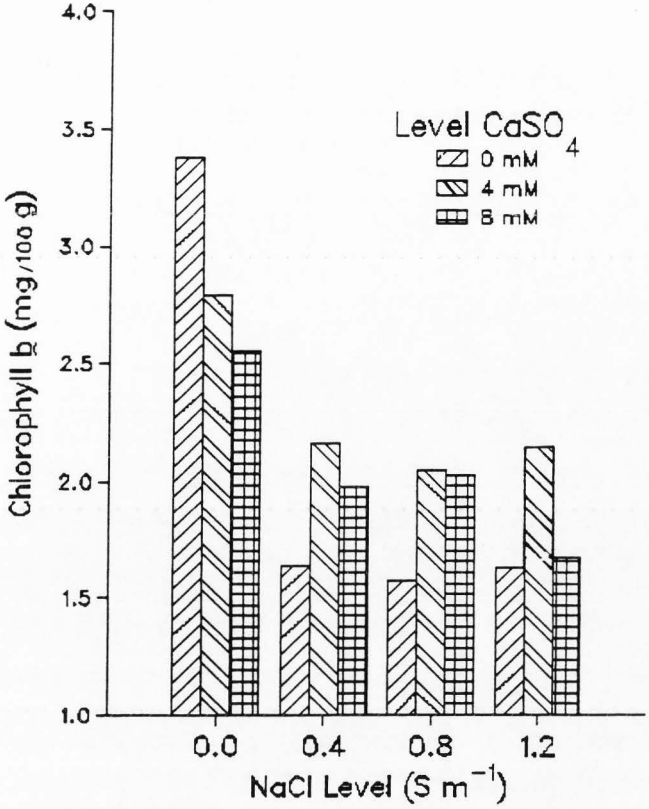

Figure 12. Effects of sodium chloride and calcium sulfate on chlorophyll b in Phaseolus vulgaris L.

When the NaCl concentration was increased from 0.4 to 0.8 S m^{-1}, the addition of 4 and 8 mM $CaSO_4$ significantly increased chlorophyll b from 1.6 mg $100g^{-1}$ dry weight with NaCl alone to 2.1 and 2.0 mg $100g^{-1}$ dry weight for 4 and 8 mM $CaSO_4$, respectively. However, at 1.2 S m^{-1} NaCl, only 4 mM $CaSO_4$ significantly increased chlorophyll b. In this case, chlorophyll b was increased from 1.6 mg $100g^{-1}$ dry weight by the NaCl treatment to 2.1 mg $100g^{-1}$ dry weight with the 4 mM $CaSO_4$ (Fig. 12).

Chlorophyll a+b. Sodium chloride, at concentrations of 0.4, 0.8, and 1.2 S m^{-1}, significantly decreased chlorophyll a+b in P. vulgaris compared to the control plants (Table 7 and Fig. 13). The mean chlorophyll a+b for the control plants was 7.3 mg $100g^{-1}$ dry weight. With NaCl concentrations of 0.4, 0.8 and 1.2 S m^{-1}, the mean chlorophyll a+b was reduced to 4.8, 4.6, and 4.7 mg $100g^{-1}$ dry weight, respectively.

The effects of NaCl and $CaSO_4$ on chlorophyll a+b are shown in Table 7 and Figure 14. Chlorophyll a+b was at its highest value of 8.4 mg $100g^{-1}$ dry weight for the control plants. Without the presence of NaCl, 4 and 8 mM $CaSO_4$ significantly reduced chlorophyll a+b from 8.5 mg $100g^{-1}$ dry weight to 6.7 and 6.6 mg $100g^{-1}$ dry weight, respectively.

Chlorophyll a+b was significantly increased (as compared to the control) when 0.4 S m^{-1} of NaCl was mixed with 4 mM $CaSO_4$. In this case, chlorophyll a+b was increased from 4.3 mg $100g^{-1}$ dry weight for NaCl alone to 5.3 mg $100g^{-1}$ dry weight with 4 mM $CaSO_4$. A concentration of 8 mM $CaSO_4$ when mixed with 0.4 S m^{-1} of NaCl slightly

Table 7. Effects of sodium chloride and calcium compounds on chlorophyll a+b in Phaseolus vulgaris L.

Sources	DF	MS	Obs. F	Prob. F
Block	3	17.902	10.992	0.000 **
NaCl	3	59.461	36.510	0.000 **
$CaSO_4$	2	1.634	1.003	0.370
$CaCl_2$	2	1.075	0.660	0.519
NaCl x $CaSO_4$	6	8.010	4.918	0.000 **
NaCl x $CaCl_2$	6	2.282	1.401	0.221
$CaSO_4$ x $CaCl_2$	4	0.900	0.553	0.697
NaCl x CaSO4 x $CaCl_2$	12	1.555	0.955	0.496
Error	104	1.629	-----	-----

** Significant at $p < 0.01$

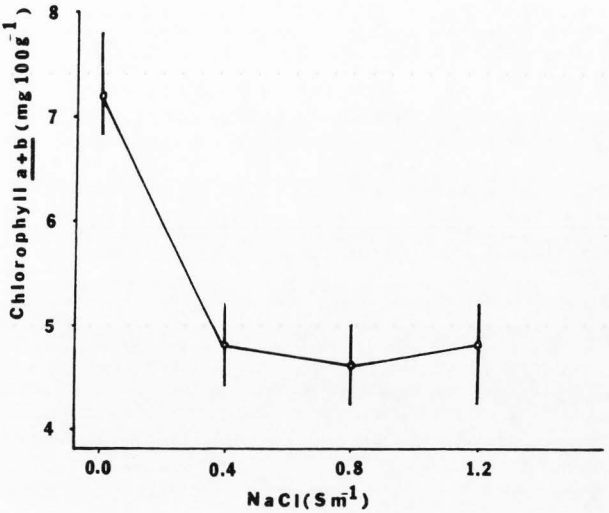

Figure 13. Effects of sodium chloride on total chlorophyll a+b in Phaseolus vulgaris L. (vertical bars denote confidence intervals).

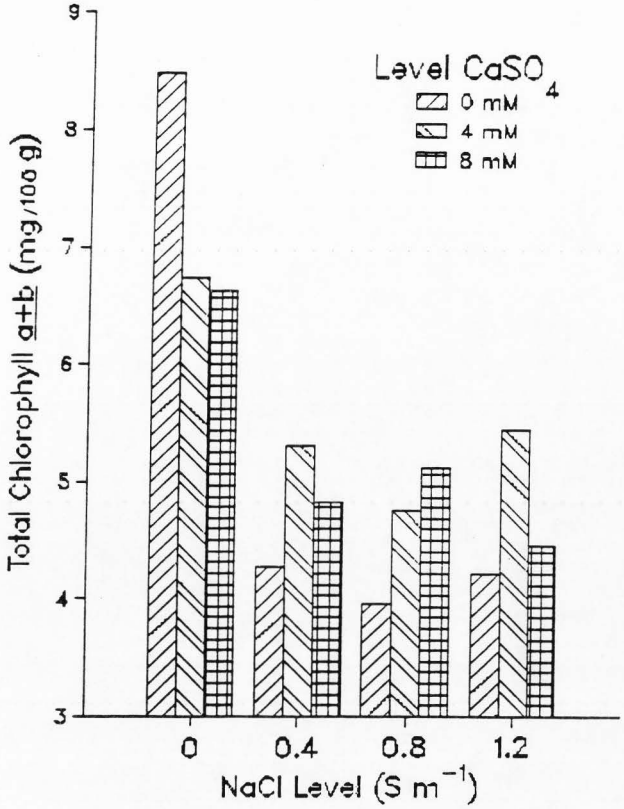

Figure 14. Effects of sodium chloride and calcium sulfate on total chlorophyll a+b in Phaseolus vulgaris L.

increased chlorophyll a+b to 2.0 mg $100g^{-1}$ dry weight. This effect, however, was not significant (Fig. 14).

The effect of $CaSO_4$ on the production of chlorophyll a+b in the presence of NaCl was more obvious when the NaCl concentration was increased from 0.4 to 0.8 S m^{-1}. In this case, 8 mM $CaSO_4$, when mixed with 0.8 S m^{-1} of NaCl, significantly increased chlorophyll a+b from 3.9 mg $100g^{-1}$ dry weight NaCl alone to 5.1 mg $100g^{-1}$ dry weight with the 8 mM $CaSO_4$. At the 0.8 S m^{-1} level of NaCl, 4 mM of $CaSO_4$ increased chlorophyll a+b to 4.7 mg $100g^{-1}$ dry weight, but the effect was not significant (Fig. 14).

When the NaCl concentration was increased from 0.8 to 1.2 S m^{-1} and the latter was mixed with 4 mM $CaSO_4$, chlorophyll a+b was significantly increased from 4.2 mg $100g^{-1}$ dry weight with the NaCl alone to 5.5 mg $100g^{-1}$ dry weight for the 4 mM $CaSO_4$. At this level of NaCl (1.2 S m^{-1}), 8 mM of $CaSO_4$ did not significantly increase the chlorophyll a+b. That is, the increase was only up to 4.5 mg $100g^{-1}$ dry weight (Fig. 14).

Comparison of the results of chlorophyll a, b, and total chlorophyll a+b showed that NaCl at any concentration above the control reduced leaf chlorophyll significantly. This could be due to the fact that the presence of NaCl in the substrate is associated with decreasing availability of moisture to the plant, increasing osmotic potential of the tissue (decreasing water potential), increasing stomatal diffusive resistance and a concomitant decrease in photosynthesis (Huang et al., 1975; Finn and Brun, 1980; and Dejong and Phillips, 1982).

By contrast, $CaSO_4$, especially a concentration of 4 mM significantly increased leaf chlorophyll b. Even when $CaSO_4$ was added to different levels of NaCl such as 0.8 and 1.2 S m^{-1}, the leaf chlorophyll was higher than in the control plants (i.e., when no $CaSO_4$ was added to the NaCl). This could indicate that at high levels of salinity having more chlorophyll, compared to controls, is a result of $CaSO_4$ addition which was effective in keeping the stomates open for more photosynthesis and synthesis of chlorophyll.

Whole Plant Effects

Effects On Shoot Dry Weight. Sodium chloride, at concentrations of 0.4, 0.8, and 1.2 S m^{-1}, significantly decreased the shoot dry weight of P. vulgaris when compared to the control plants (Table 8 and Fig. 15). The mean shoot dry weight for control plants was 0.68 g, while those for the NaCl concentrations of 0.4, 0.8 and 1.2 S m^{-1} were 0.56, 0.51, and 0.50 g, respectively.

The interaction of NaCl and $CaSO_4$ is shown in Figure 16. Shoot dry weight was at its highest value of 0.74 g with control plants. In the absence of NaCl, 8 mM of $CaSO_4$ significantly reduced the shoot dry weight from 0.74 g for control plants to 0.62 for those receiving 8 mM $CaSO_4$. At 0 NaCl, 4 mM of $CaSO_4$ reduced the shoot dry weight slightly to 0.69 g.

A pattern of increase in shoot dry weight was noticed when 0.4 S m^{-1} of NaCl was mixed with 4 or 8 mM $CaSO_4$. In this case, shoot dry weight increased from 0.53 g for NaCl alone to 0.59 and 0.55 g with 4 and 8 mM $CaSO_4$, respectively.

The pattern of increase in shoot dry weight was more obvious when the NaCl concentration was increased from 0.4 to 0.8 S m^{-1}. In this

Table 8. Effects of sodium chloride and calcium compounds on shoot weight in Phaseolus vulgaris L.

Sources	DF	MS	Obs. F	Prob. F
Block	3	0.073	4.362	0.006 **
NaCl	3	0.256	15.273	0.000 **
$CaSO_4$	2	0.007	0.421	0.657
$CaCl_2$	2	0.013	0.788	0.457
NaCl x $CaSO_4$	6	0.026	1.560	0.166
NaCL x $CaCl_2$	6	0.012	0.731	0.625
$CaSO_4$ x $CaCl_2$	4	0.013	0.782	0.539
NaCl x $CaSO_4$ x $CaCl_2$	12	0.021	1.229	0.275
Error	105	0.017	-----	-----

** Significant at $P < 0.01$

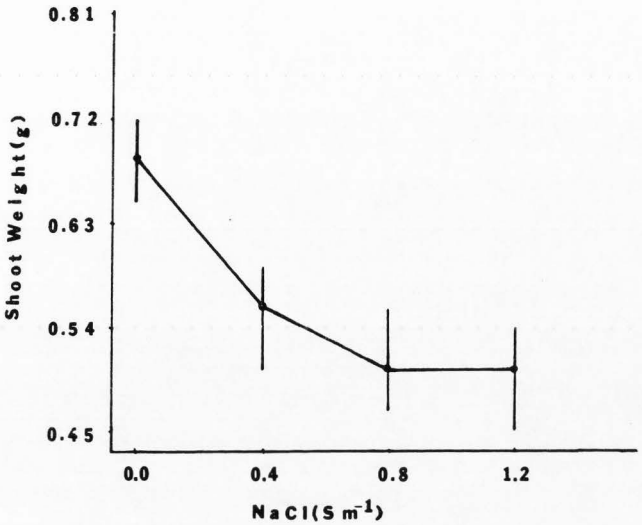

Figure 15. Effects of sodium chloride on shoot weight in Phaseolus vulgaris L. (vertical bars denote confidence intervals).

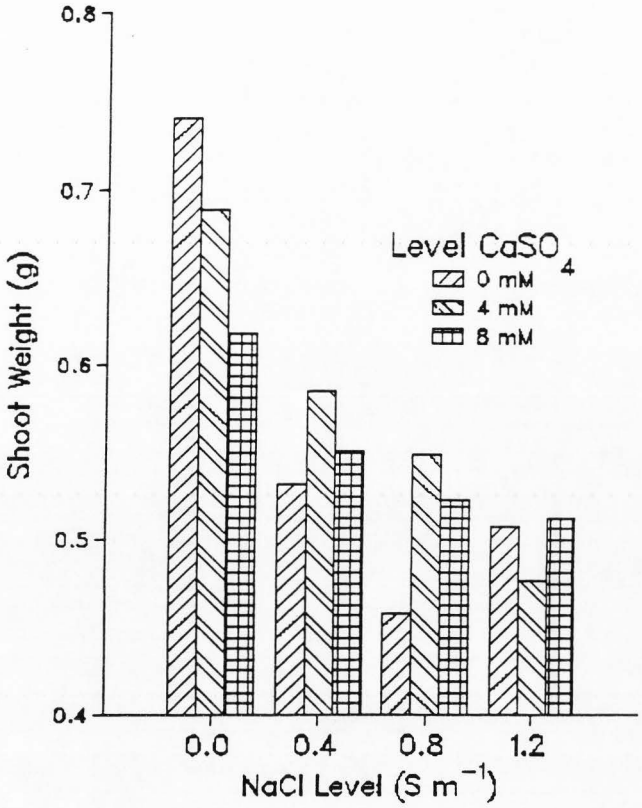

Figure 16. Effects of sodium chloride and calcium sulfate on shoot weight in Phaseolus vulgaris L.

case, 4 and 8 mM of $CaSO_4$ mixed with the NaCl solution increased the shoot dry weight from 0.46 g for the NaCl alone to 0.55 and 0.52 g with 4 and 8 mM $CaSO_4$, respectively. At a NaCl concentration of 1.2 S m^{-1}, the shoot dry weight showed no increase with 4 mM of $CaSO_4$, but increased slightly with 8 mM, i.e. shoot dry weight increased from 0.50 g for control plants to 0.51 g for those receiving 8 mM $CaSO_4$ (Fig. 16).

Effects On Root Dry Weight. Sodium chloride, at concentrations of 0.4, 0.8, and 1.2 S m^{-1}, significantly reduced the root dry weight of P. vulgaris compared to the control plants (Table 9 and Fig. 17). The mean root dry weight for control plants was 0.35 g while with NaCl concentrations of 0.4, 0.8 and 1.2 S m^{-1} the mean root dry weights were 0.30, 0.29 and 0.28 g, respectively. The mean root dry weight at NaCl concentration of 0.4 and 0.8, 0.4 and 1.2, and 0.8 and 1.2 S m^{-1} were not significantly different from each other.

The effects of NaCl and $CaSO_4$ on root dry weight in P. vulgaris are shown in Table 9 and Figure 18. Root dry weight was at its highest value of 0.38 g for control plants. In the absence of NaCl, 4 and 8 mM of $CaSO_4$ reduced the root dry weight insignificantly from 0.38 g to 0.34 and 0.33 g with 4 and 8 mM $CaSO_4$, respectively.

There was a pattern of increase in root dry weight when 0.4 S m^{-1} NaCl was mixed with 4 mM $CaSO_4$. In this case, root dry weight increased from 0.29 g for NaCl alone to 0.33 g when mixed with 4 mM $CaSO_4$. The concentration of 8 mM $CaSO_4$ decreased the root dry weight to 0.28 g and the effect was highly significant compared to the 4 mM $CaSO_4$ (Fig. 18).

Table 9. Effects of sodium chloride and calcium compounds on root weight in Phaseolus vulgaris L.

Sources	DF	MS	Obs. F	Prob. F
Block	3	0.004	1.000	0.396
NaCl	3	0.035	9.269	0.000 **
$CaSO_4$	2	0.001	0.376	0.687
$CaCl_2$	2	0.001	0.343	0.711
NaCl x $CaSO_4$	6	0.008	2.223	0.046 *
NaCL x $CaCl_2$	6	0.004	1.162	0.332
$CaSO_4$ x $CaCl_2$	4	0.006	1.531	0.199
NaCl x $CaSO_4$ x $CaCl_2$	12	0.006	1.654	0.088
Error	105	0.004	-----	-----

* Significant at $P < 0.05$
** Significant at $P < 0.01$

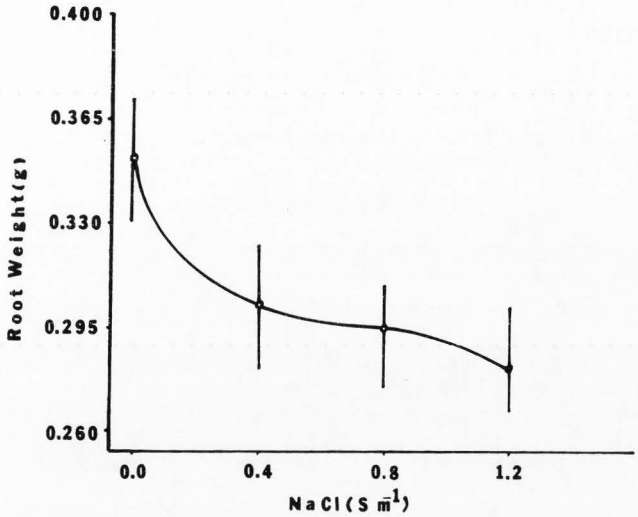

Figure 17. Effects of sodium chloride on root weight in Phaseolus vulgaris L. (vertical bars denote confidence intervals).

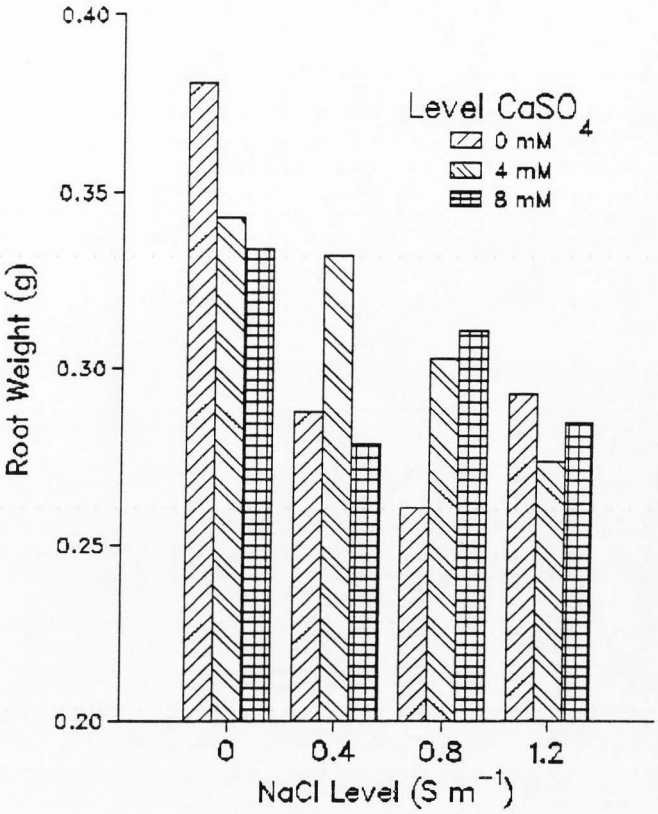

Figure 18. Effects of sodium chloride and calcium sulfate on root weight in Phaseolus vulgaris L.

However, when NaCl concentration was increased from 0.4 to 0.8 S m^{-1} and the latter was mixed with 4 or 8 mM $CaSO_4$, the root dry weight increased from 0.26 g for NaCl alone to 0.30 and 0.31 g when mixed with 4 and 8 mM $CaSO_4$, respectively. When NaCl was increased beyond 0.8 S m^{-1} to 1.2 S m^{-1} and the latter was mixed with 4 or 8 mM of $CaSO_4$, the root dry weight decreased from 0.29 for NaCl alone to 0.27 and 0.28 g when mixed with 4 and 8 mM $CaSO_4$, respectively (Fig. 18).

The results of this study are in agreement with findings of Wadleigh and Ayers (1945); Porath and Poljakoff-Mayber (1964); Weimberg (1970); Greenway (1973); and Halterlein (1983), who found that the presence of excess salts in the substrate adversely affected the growth and development of shoot and roots in most agronomic plants. In addition, these data showed that the shoot dry weight decreased sharply and continuously up to a NaCl concentration of 0.8 S m^{-1} and then leveled off with any increase in NaCl concentration (Fig. 15).

By contrast, the root dry weight of the plants decreased sharply up to a NaCl concentration of 0.4 S m^{-1} and then decreased smoothly as the NaCl concentration increased (Fig. 17), which may indicate that above-ground portions of the plants were more severly affected by NaCl than were roots (Halterlein, 1983). This could also be an adaptative or survival mechanism for the plants. Evidence for such a mechanism was demonstrated by Sosebee and Wiebe (1971), who stated that, in periods of stress, plants do not expend energy on the production of new leaves and shoot growth, but rather will accumulate photosynthate, and possibly grow roots that can be used when environmental conditions become more favorable. In other words, in periods of stress, roots

may grow to explore the soil for water with less salt or water with higher water potential.

These results, in part, are also in agreement with findings of Lahaye and Epstein (1971), who found that shoot and root dry weight of soybeans increased as calcium concentration of the solution increased. However, these data also indicate that at low concentrations of NaCl (0 to 0.8 S m^{-1}) low levels of $CaSO_4$ (4 mM) could increase the shoot dry weight of P. vulgaris. At a high concentration of NaCl (1.2 S m^{-1}), however, low levels of $CaSO_4$ were not sufficient to increase shoot and root dry weight and high concentrations of $CaSO_4$ (8 mM) was required.

Nitrogen Fixation

Sodium chloride, at concentrations of 0.4, 0.8 and 1.2 S m^{-1}, significantly decreased nitrogen fixation of P. vulgaris when compared to the control plants (Table 10 and Fig. 19). The mean nitrogen fixation for control plants was 590 ul $plant^{-1}$ h^{-1}, while with NaCl concentrations of 0.4, 0.8 and 1.2 S m^{-1}, the mean nitrogen fixation was 304, 149 and 103 ul $plant^{-1}$ h^{-1}, respectively. Also, the mean nitrogen fixation at NaCl concentrations of 0.4 and 0.8 S m^{-1} and 0.4 and 1.2 S m^{-1} were significantly different.

The interactions of NaCl and $CaSO_4$ on nitrogen fixation are shown in Table 10 and Figure 20. Nitrogen fixation for the control plants was 633 ul $plant^{-1}$ h^{-1}, while the addition of 4 mM of $CaSO_4$ to the solution without NaCl decreased nitrogen fixation to 399 ul $plant^{-1}$ h^{-1}. Increasing $CaSO_4$ concentration from 4 mM to 8 mM significantly increased nitrogen fixation from 399 ul $plant^{-1}$ h^{-1} for 4 mM of $CaSO_4$ to 738 ul $plant^{-1}$ h^{-1} for 8 mM $CaSO_4$.

Table 10. Effects of sodium chloride and calcium compounds on nitrogen fixation in Phaseolus vulgaris L.

Sources	DF	MS	Obs. F	Prob. F
Block	3	497834.088	6.244	0.001**
NaCl	3	1735311.077	26.567	0.000**
$CaSO_4$	2	9904.737	0.152	0.859
$CaCl_2$	2	142505.245	2.182	0.118
NaCl x $CaSO_4$	6	16018.799	2.542	0.024*
NaCl x $CaCl_2$	6	35111.813	0.538	0.779
$CaSO_4$ x $CaCl_2$	4	37229.669	0.570	0.685
NaCl x $CaSO_4$ x $CaCl_2$	12	52695.175	0.807	0.643
Error	105	65318.617	-----	-----

** Significant at P<0.01
* Significant at P<0.05

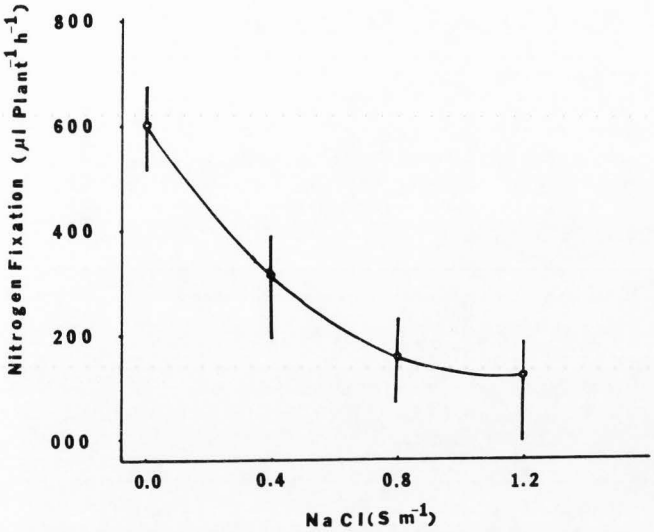

Figure 19. Effects of sodium chloride on nitrogen fixation in Phaseolus vulgaris L. (vertical bars denote confidence intervals).

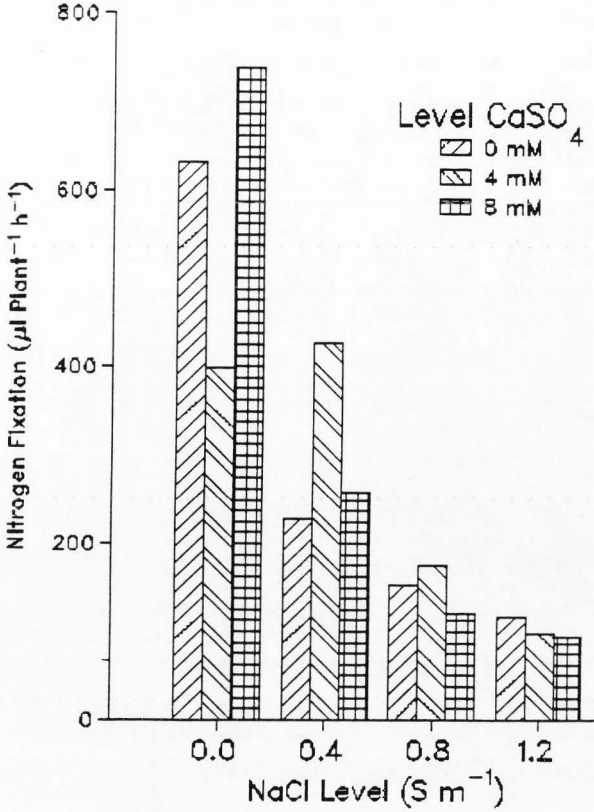

Figure 20. Effects of sodium chloride and calcium sulfate on nitrogen fixation in Phaseolus vulgaris L.

A pattern of increase in nitrogen fixation was noticed when the NaCl concentration was increased to 0.4 S m^{-1} and then mixed with 4 or 8 mM of $CaSO_4$. In this case, nitrogen fixation increased from 229 ul $plant^{-1}$ h^{-1} for NaCl alone to 426 and 258 ul $plant^{-1}$ h^{-1} for 4 and 8 mM of $CaSO_4$, respectively (Fig. 20).

Similarly, when the NaCl concentration was increased to 0.8 S m^{-1} and then mixed with 4 mM of $CaSO_4$, acetylene reduction substantially increased to 175 ul $plant^{-1}$ h^{-1} as compared to 152 ul $plant^{-1}$ h^{-1} for NaCl alone. At this level of NaCl, 8 mM of $CaSO_4$ slightly decreased nitrogen fixation to 120 ul $plant^{-1}$ h^{-1} as compared to the 152 ul $plant^{-1}$ h^{-1} for the NaCl alone (Fig. 20).

When the NaCl concentration was increased to 1.2 S m^{-1} both 4 and 8 mM of $CaSO_4$ reduced nitrogen fixation to 99 and 95 ul $plant^{-1}$ h^{-1}, respectively, as compared to 117 ul $plant^{-1}$ h^{-1} for the NaCl alone (Fig. 20).

These data agree with results of Sprent (1972) and Sanchez-Diaz et al., (1982), who found that a decrease in acetylene reduction activity was associated with an increase in NaCl concentration of the soil solution. These results also showed that a decrease in acetylene reduction could be correlated with an increase in stomatal diffusive resistance and a decrease in shoot and root dry weight in P. vulgaris. Reduction in nitrogen fixation could be due to the fact that the increase in stomatal diffusive resistance reduced photosynthesis as it is known that photosynthesis is required for the reduction of nitrogen fixation (Sprent 1971; Huang et al., 1975; Patterson et al. 1979; Finn and Brun, 1980; Dejong and Phillips, 1982; and Sanchez-Diez et al., 1982).

These results also showed that $CaSO_4$ at 4 and 8 mM concentration interacted with 0.4 and 0.8 S m^{-1} NaCl and substantially increased the amount of acetylene reduction compared to situations where no $CaSO_4$ was added to the NaCl solutions.

Nodule Dry Weight

Sodium chloride, at concentrations of 0.4, 0.8 and 1.2 S m^{-1}, significantly decreased the nodule dry weight of P. vulgaris as compared to those on the control plants (Table 11 and Fig. 21). The mean nodule dry weight for control plants was 0.103 g, but with addition of NaCl concentrations of 0.4, 0.8 and 1.2 S m^{-1}, the nodule dry weight decreased to 0.086, 0.065 and 0.052 g, respectively. The mean nodule dry weight at NaCl concentrations of 0.4 and 0.8, 0.4 and 1.2, and 0.8 and 1.2 S m^{-1} were significantly different from one another.

The interactions of NaCl and $CaSO_4$ on nodule dry weight in P. vulgaris are shown in Figure 22. Nodule dry weight was at its highest value of 0.108 g for the control plants. In the absence of NaCl, 4 and 8 mM of $CaSO_4$ reduced the nodule dry weight insignificantly from 0.108 g for the NaCl alone to 0.103 and 0.097 g for 4 and 8 mM $CaSO_4$, respectively.

There was a pattern of increase in nodule dry weight when 0.4 S m^{-1} of NaCl was mixed with 4 and 8 mM $CaSO_4$. In this case, the nodule dry weight increased from 0.085 g for the NaCl alone to 0.088 and 0.086 g when mixed with 4 and 8 mM $CaSO_4$, respectively (Fig. 22).

When the NaCl concentration was increased from 0.4 to 0.8 S m^{-1} and the latter was mixed with 4 and 8 mM of $CaSO_4$, the nodule dry weight decreased slightly from 0.068 g for NaCl to 0.061 and 0.067 g

Table 11. Effects of sodium chloride and calcium compounds on nodule weight in Phaseolus vulgaris L.

Sources	DF	MS	Obs. F	Prob. F
Block	3	0.001	2.161	0.097
NaCl	3	0.018	34.934	0.000 **
$CaSO_4$	2	0.000	0.798	0.453
$CaCl_2$	2	0.000	0.663	0.518
NaCl x $CaSO_4$	6	0.000	0.423	0.862
NaCL x $CaCl_2$	6	0.000	0.456	0.840
$CaSO_4$ x $CaCl_2$	4	0.000	0.232	0.920
NaCl x $CaSO_4$ x $CaCl_2$	12	0.000	0.781	0.668
Error	104	0.000	-----	-----

** Significant at $P < 0.01$

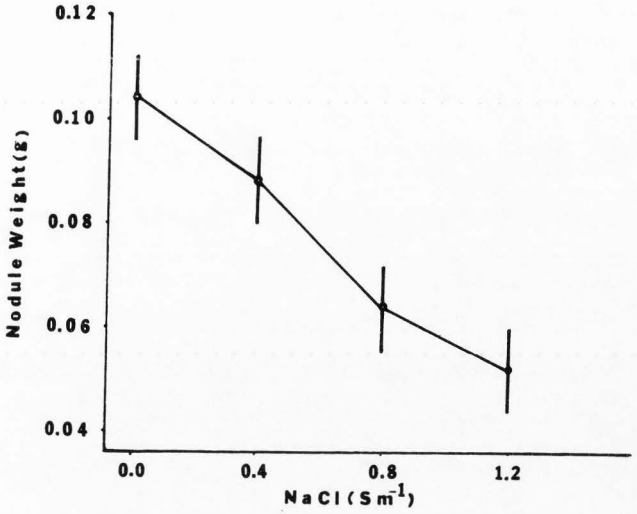

Figure 21. Effects of sodium chloride on nodule weight in Phaseolus vulgaris L. (vertical bars denote confidence intervals).

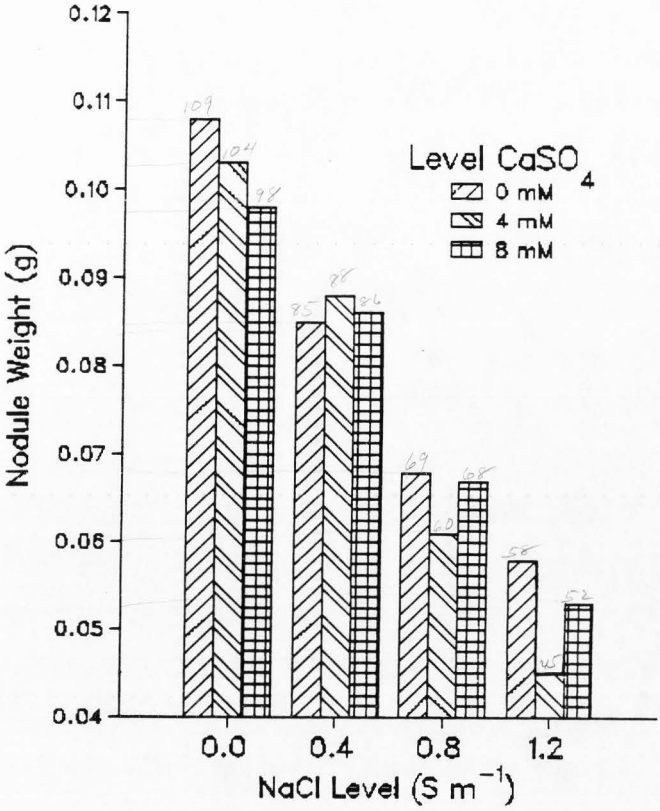

Figure 22. Effects of sodium chloride and calcium sulfate on nodule weight in Phaseolus vulgaris L.

when mixed with 4 and 8 mM $CaSO_4$, respectively. Increasing the NaCl concentration to 1.2 S m^{-1} and mixing with 4 or 8 mM of $CaSO_4$ decreased the nodule dry weight substantially from 0.058 g for the NaCl to 0.045 and 0.520 g with 4 and 8 mM $CaSO_4$, respectively (Fig. 22).

These data showed that as NaCl concentrations increased, nodule dry weight of P. vulgaris decreased. However, an increasing pattern in nodule dry weight was noticed when 4 and 8 mM of $CaSO_4$ was added to low concentrations of NaCl (0.4 S m^{-1}). Beyond a concentration of 0.4 S m^{-1} NaCl, nodule dry weight decreased with any addition of $CaSO_4$. This reduction in nodule dry weight could be due to direct effects of salts on the activity of Rhizobium in symbiotic nitrogen fixation.

Total Leaf Nitrogen

Sodium chloride at concentrations of 0.4, 0.8, and 1.2 S m^{-1} caused a significant increase in the total leaf nitrogen of P. vulgaris compared to the control (Table 12 and Fig. 23). The mean total leaf nitrogen for control was 665 mmole kg^{-1}, while at NaCl concentrations of 0.4, 0.8 and 1.2 S m^{-1} the mean total leaf nitrogen was increased to 795, 751 and 792 mmole kg^{-1}, respectively.

The effects of $CaSO_4$ on total leaf nitrogen are shown in Table 12 and Figure 24. Calcium sulfate, at concentrations of 4 and 8 mM, caused a significant increase in the total leaf nitrogen compared to the control. However, the effect of 4 mM $CaSO_4$ was not significantly different from 8 mM. Total leaf nitrogen was 673, 795 and 784 mmole kg^{-1} for concentrations of 0, 4, and 8 mM $CaSO_4$, respectively.

The results show that total leaf nitrogen increased with 0.4 and 1.2 S m^{-1} NaCl. The evidence for such a mechanism is still under

Table 12. Effects of sodium chloride and calcium compounds on plant total nitrogen in Phaseolus vulgaris L.

Sources	DF	MS	Obs. F	Prob. F
Block	3	287528.843	13.652	0.000 **
NaCl	3	131881.880	6.262	0.001 **
$CaSO_4$	2	217566.694	10.330	0.000 **
$CaCl_2$	2	940.528	0.045	0.956
NaCl x $CaSO_4$	6	27693.991	1.315	0.257
NaCL x $CaCl_2$	6	15354.713	0.729	0.627
$CaSO_4$ x $CaCl_2$	4	16958.819	0.805	0.525
NaCl x $CaSO_4$ x $CaCl_2$	12	28571.282	1.357	0.199
Error	105	21061.567	-----	-----

** Significant at $P < 0.01$

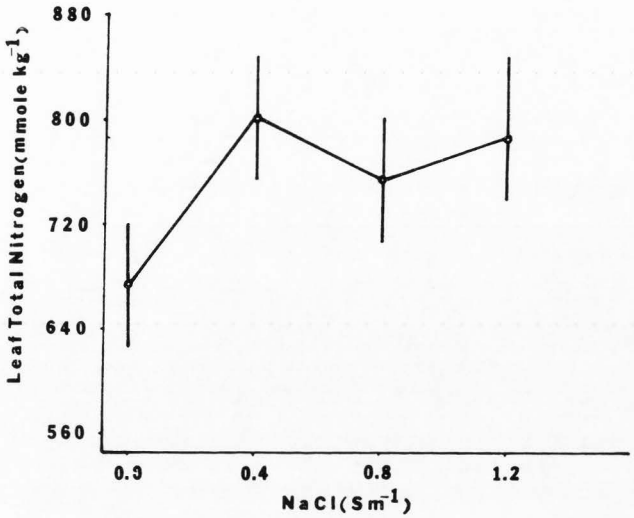

Figure 23. Effects of sodium chloride on leaf total nitrogen in Phaseolus vulgaris L. (vertical bars denote confidence intervals).

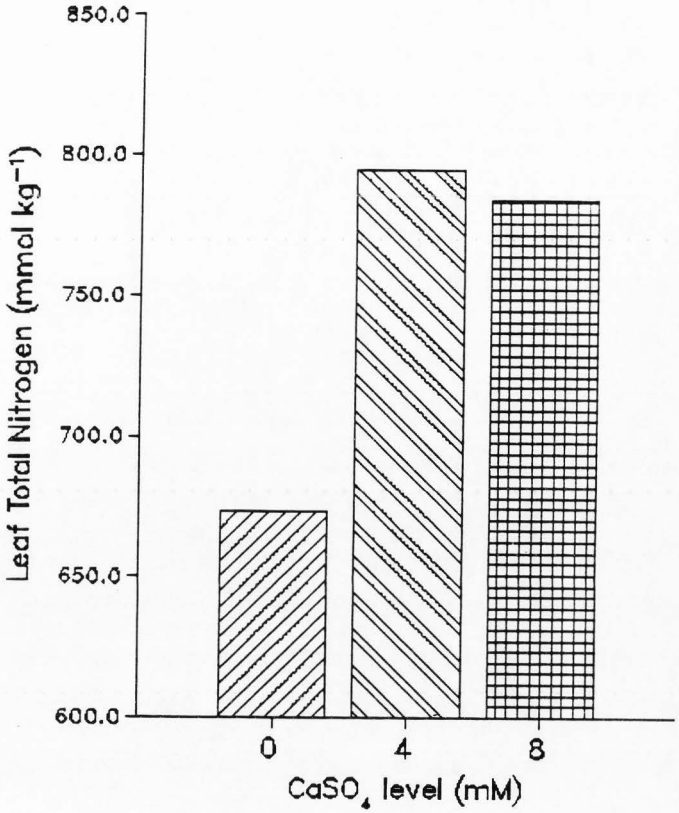

Figure 24. Effects of calcium sulfate on leaf total nitrogen in Phaseolus vulgaris L.

investigation. Wadleigh and Ayers (1945), Gale et al. (1967) and Halterlein (1983) have shown that salinity reduced net photosynthesis. Porath and Poljakoff-Mayber (1964) found that as the level of NaCl in the growth medium increased, glucose consumption and the C_6/C_1 ratio decreased. Thus, growth and development of plants decreased due to a depletion of carbohydrates (Mengel and Kirkby, 1982). Furthermore, Weimberg (1970) observed that specific activities of enzymes (such as malate dehydrogenase) in leaves, stems and roots of pea seedlings grown in a saline medium were not altered by salinity. We may, therefore, conclude that excess salt reduces carbon/cytoplasm ratio and since cytoplasm is proportional to protein of the cell, any increase in cytoplasmic concentration due to a reduction in cell size would increase the protein content per unit dry weight.

Similarly, $CaSO_4$ may increase the total leaf nitrogen in two ways: 1) by a direct effect of Ca^{2+} on the integrity of cell wall structure and cell membrane permeability and/or 2) by an indirect effect on cysteine and methionine. These are two important sulfur containing amino acids and essential constituents of ferridoxin. The reduced form of this highly negative redox potential is the ultimate source of electons in the nitrogen reduction cycle (Mengel and Kirkby, 1982).

Leaf Sodium

Sodium chloride, at concentrations of 0.4, 0.8 and 1.2 S m^{-1}, caused a significant increase in the leaf Na^+ of P. vulgaris compared to the control (Table 13 and Fig. 25). The mean leaf Na^+ for control plants was 6 mg kg^{-1} while for the NaCl concentrations of 0.4, 0.8, and 1.2 S m^{-1} the mean leaf Na^+ were 23, 47, and 82 mg kg^{-1},

Table 13. Effects of sodium chloride and calcium compounds on leaf sodium Phaseolus vulgaris L.

Sources	DF	MS	Obs. F	Prob. F
Block	3	3097.842	3.234	0.025 *
NaCl	3	38850.825	40.555	0.000 **
$CaSO_4$	2	261.243	0.273	0.762
$CaCl_2$	2	557.905	0.603	0.549
NaCl x $CaSO_4$	6	138.113	0.144	0.990
NaCl x $CaCl_2$	6	678.495	0.708	0.644
$CaSO_4$ x $CaCl_2$	4	992.330	1.036	0.392
NaCl x $CaSO_4$	12	1255.911	1.311	0.223
Error	105	957.990	-----	-----

** Significant at P<0.01
* Significant at P<0.05

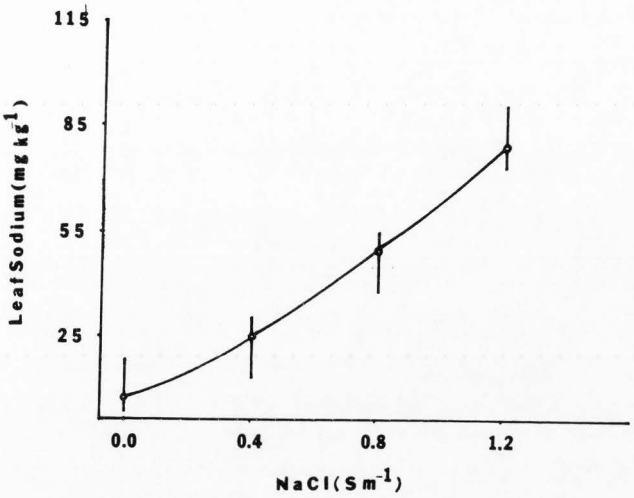

Figure 25. Effects of sodium chloride on leaf sodium (Na^+) in Phaseolus vulgaris L. (vertical bars denote confidence intervals).

respectively. The mean leaf Na^+ at NaCl concentrations of 0.4 and 0.8, 0.4 and 1.2, and 0.8 and 1.2 S m^{-1} were significantly different from each other.

The interactions of NaCl and $CaSO_4$ on leaf Na^+ in P. vulgaris are shown in Figure 26. The leaf Na^+ was at its lowest value of 4.3 mg kg^{-1} with the control solutions of NaCl and $CaSO_4$. In the absence of NaCl, 4 and 8 mM of $CaSO_4$ caused a slight increase in the leaf Na^+ to 7 and 8 mg kg^{-1} respectively. A decrease in leaf Na^+ was noticed when NaCl concentration of 0.4 S m^{-1} was mixed with either 4 or 8 mM $CaSO_4$. In both cases, the leaf Na^+ decreased (although not significantly) from 26 mg kg^{-1} for the NaCl alone to 22 and 21 mg kg^{-1} for 4 and 8 mM $CaSO_4$, respectively (Fig. 26).

Furthermore, when NaCl concentration of 0.8 S m^{-1} was mixed with 4 or 8 mM of $CaSO_4$ the leaf Na^+ declined slightly from 50 mg kg^{-1} for NaCl alone to 44 and 48 mg kg^{-1} for the 4 and 8 mM $CaSO_4$, respectively. Finally, when NaCl concentration was increased to 1.2 S m^{-1} and mixed with 4 or 8 mM of $CaSO_4$ the leaf Na^+ decreased from 89 mg kg^{-1} for NaCl alone to 78 and 77 mg kg^{-1} for 4 and 8 mM $CaSO_4$, respectively (Fig. 26).

The results of this study show that an increase in leaf Na^+ was associated with an increase in NaCl concentration of soil solution. Many investigators (Greenway, 1973; Mengel and Kirkby, 1982; Fitter and Hay, 1983) reported that the nutrient concentation at the root surface directly controls nutrient uptake. Mengel and Kirkby (1982) indicated that soil with a higher nutrient level has a steeper concentration gradient, and therefore the rate of diffusion to the plant root is greater. A higher nutrient level in a bulk soil also

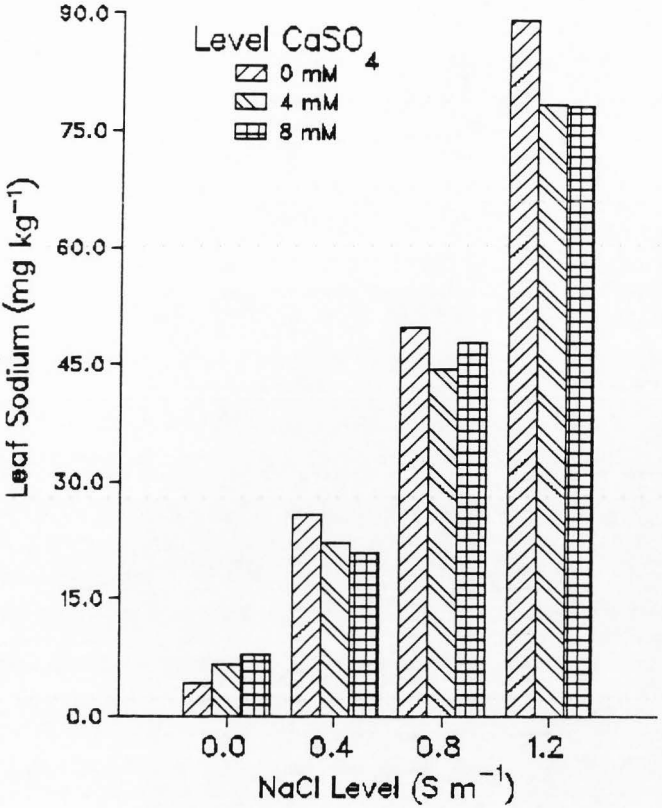

Figure 26. Effects of sodium chloride and calcium sulfate on leaf sodium in Phaseolus vulgaris L.

gives a higher concentration at the root surface that causes a more rapid uptake rate and a larger gradient allows this to be maintained.

The detrimental effects of excess Na^+ on plants have been explained by several researchers. Greenway (1973) stated that soils with high levels of Na^+ can affect plants in at least 3 ways: 1) it changes water relations of the plants, 2) specific ion effects, and 3) effects on transport of solutes. The presence of NaCl depresses water potential of the nutrient medium and hence restricts water uptake by plant roots. This effect, to some extent, is counterbalanced by an osmotic adjustment, because the higher salt concentration in the nutrient medium leads to an increase in the rate of ion uptake. This lowers the water potential in the plant roots and stimulates water uptake, which raises cell turger and the turgidity of plant tissue increases (Mengel and Kirkby, 1982).

Adequate turgor of P. vulgaris growing under sodic conditions may imply that detrimental effects of NaCl on plant growth result from salt induced physiological disorders rather than osmotic effects per se (Mengel and Kirkby, 1982). They reported that toxicity begins with an imbalance of ions in the plant tissues, often with a large excess of sodium. The plants can cope to some degree with excess Na^+ by excluding its uptake or secreting it into vacuoles. This regulatory process requires an additional amount of energy and for this reason plants subject to saline conditions show higher respiration rates and deplete storage carbohydrates to a greater extent than plants grown under non-saline conditions. Thus, plants suffering from salinity may also be poor in energy status (Mengel and Kirkby, 1982). Lack of

energy as a consequence of salinity then may affect various energy requiring processes such as CO_2 assimilation and nitrogen fixation.

Furthermore, these data showed that leaf Na^+ can decrease with an addition of $CaSO_4$ in different levels of NaCl (Fig. 26). The regulatory role of calcium (Ca^{2+}) in growth, development, and adaptation to environmental perturbation was investigated by several researchers (Hyder and Greenway, 1965; Lahaye and Epstein, 1971; and Leopold, 1977). Apoplastic movement of water through the cell walls and xylem moves calcium into the foliage with the bulk of it being found in the apoplast (Hanson, 1983). The most conspicuous role for Ca^{2+} in the apoplast lies within the integrity of the plasma membrane. Lahaye and Epstein (1971) proposed that Ca^{2+} was an integral part of the plasmalemma, governing normal impermeability to transport ions. A deficiency of Ca^{2+}, they proposed, leads to an impairment of the membrane structure, increasing cell permeability. Zubay (1983) stated that the permeability of gap junction pores of membrane protein in eukaryotes is regulated by cytoplasmic concentrations of Ca^{2+}. Low concentrations of calcium (below 10^{-7}M) opens channels and affects communication between the cells, while higher concentrations close channels and tend to increase the cell permeability.

Leaf Potassium

Sodium chloride, at concentrations of 0.4, 0.8 and 1.2 S m^{-1}, significantly increased leaf K^+ in P. vulgaris compared to the control plants (Table 14 and Fig. 27). The mean leaf K^+ for control plants was 51 mg kg^{-1} while with the addition of NaCl concentrations of 0.4, 0.8, and 1.2 S m^{-1}, the mean leaf K^+ increased to 81, 110 and 119 mg kg^{-1}, respectively. The mean leaf K^+ for NaCl concentrations of 0.4

Table 14. Effects of sodium chloride and calcium compounds on leaf potassium in Phaseolus vulgaris L.

Sources	DF	MS	Obs. F	Prob. F
Block	3	1002.102	6.477	0.000 **
NaCl	3	34017.074	219.855	0.000 **
$CaSO_4$	2	1356.149	8.765	0.000 **
$CaCl_2$	2	133.747	0.864	0.424
NaCl x $CaSO_4$	6	192.201	1.242	0.291
NaCl x $CaCl_2$	6	276.517	1.787	0.109
$CaSO_4$ x $CaCl_2$	4	38.277	0.247	0.911
NaCl x $CaSO_4$ x $CaCl_2$	12	141.085	0.912	0.538
Error	105	154.725	-----	-----

** Significant at $P < 0.01$

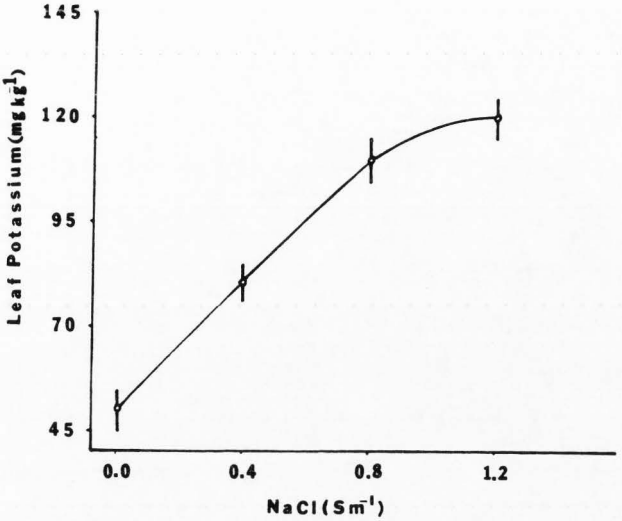

Figure 27. Effects of sodium chloride on leaf potassium (K^+) in Phaseolus vulgaris L. (vertical bars denote confidence intervals).

and 0.8, 0.4 and 1.2, and 0.8 and 1.2 S m^{-1} were significantly different from each other.

The effects of $CaSO_4$ on leaf K^+ are shown in Table 14 and Figure 28. Leaf K^+ at $CaSO_4$ concentrations of 4 and 8 mM were increased significantly compared to those of the control. Leaf K^+ was 84, 93, and 94 mg kg^{-1} for $CaSO_4$ concentration of 0, 4, and 8 mM, respectively.

The data show that an increase in leaf K^+ was associated with an increase in NaCl concentration of the soil solution. This may be attributed to a release of K^+ ions from the cation exchange site in the soil solution by the addition of high concentrations of Na^+. Higher K^+ in the soil solution could result in a higher uptake rate by the plants (Jurinak, 1981, Mengel and Kirkby, 1982).

In addition, the data show that leaf K^+ increased with addition of $CaSO_4$. This could be due to: 1) a direct effect of Ca^{2+} replacing K^+. Therefore, high levels of K^+ ions are in the soil solution available for uptake, and/or 2) an indirect effect via increasing membrane permeability by the Ca^{2+} that decreases K^+ leakage through the membrane (Mengel and Kirkby, 1982).

Leaf Potassium/Sodium Ratio

Sodium chloride, at concentrations of 0.4, 0.8 and 1.2 S m^{-1}, resulted in a significant decrease in the leaf K^+/Na^+ ratio of P. vulgaris plants compared to the control plants (Table 15 and Fig. 29). The mean leaf K^+/Na^+ ratio for control plants was 24, while with the NaCl concentrations of 0.4, 0.8 and 1.2 S m^{-1} the mean leaf K^+/Na^+ ratio dropped to 6.8, 5.5, and 2.2, respectively. The mean leaf K^+/Na^+ ratio at NaCl concentration of 0.4 and 0.8, 0.4 and 1.2, and

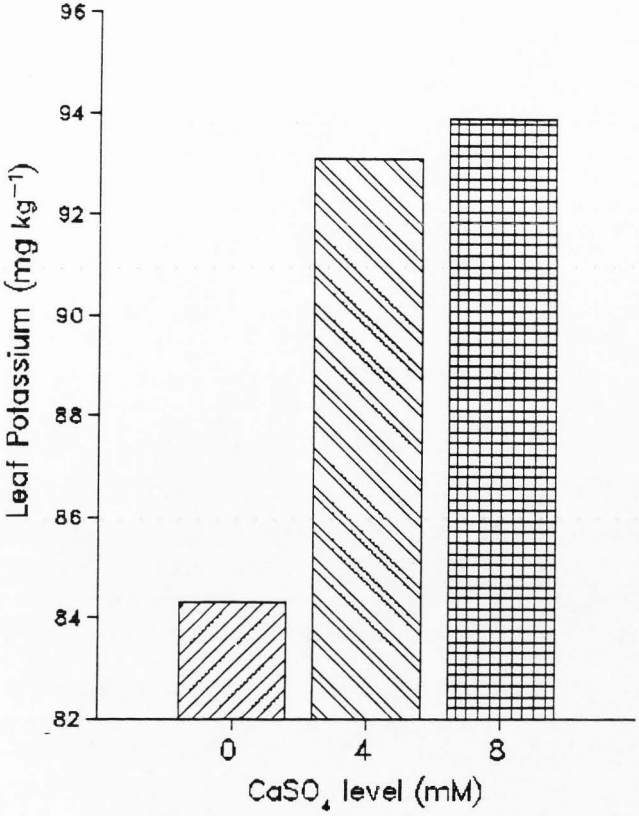

Figure 28. Effects of calcium sulfate on leaf potassium in Phaseolus vulgaris L.

Table 15. Effects of sodium chloride and calcium compounds on K^+/Na^+ ratio in Phaseolus vulgaris L.

Sources	DF	MS	Obs. F	Prob. F
Block	3	2259.368	4.749	0.004**
NaCl	3	3339.336	7.018	0.000**
$CaSO_4$	2	672.266	1.413	0.248
$CaCl_2$	2	299.236	0.629	0.535
NaCl x $CaSO_4$	6	603.108	1.268	0.279
NaCl x $CaCl_2$	6	178.662	0.375	0.893
CaSO4 x $CaCl_2$	4	194.053	0.408	0.803
NaCl x $CaSO_4$ x $CaCl_2$	12	166.249	0.349	0.977
Error	105	475.798	-----	-----

** Significant at P<0.01

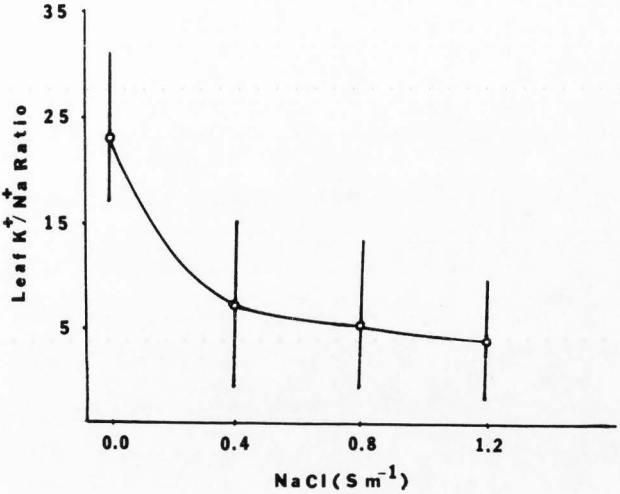

Figure 29. Effects of sodium chloride on leaf potassium sodium ratio (K^+/Na^+) in Phaseolus vulgaris L. (vertical bars denote confidence intervals).

0.8 and 1.2 S m^{-1} were not significantly different from each other (Fig. 29).

The interactions of NaCl and $CaSO_4$ on leaf K^+/Na^+ ratio in P. vulgaris are shown in Figure 30. The K^+/Na^+ ratio for the control solution, i.e., water, had a value of 10. In the absence of NaCl, 4 mM of $CaSO_4$ significantly increased the leaf K^+/Na^+ ratio to 38 compared to 10 for the control. A concentration of 8 mM $CaSO_4$ in the solution increased the leaf K^+/Na^+ ratio to 23 but this effect was not significant compared to the control.

An increase in the leaf K^+/Na^+ ratio was noticed when the NaCl concentration was increased to 0.4 S m^{-1} and mixed with either 4 or 8 mM $CaSO_4$. In both of these cases, leaf K^+/Na^+ ratio increased from 4.6 for control to 5.3 and 11 with 4 and 8 mM $CaSO_4$, respectively (Fig. 30). However, when the NaCl concentration was increased to 0.8 S m^{-1}, 8 mM of $CaSO_4$ caused a slight increase in the leaf K^+/Na^+ ratio (7) compared to 5 for the control.

Finally, at a NaCl concentration of 1.2 S m^{-1} alone the leaf K^+/Na^+ ratio sharply declined to 1.7 compared to ratios of 2.4 and 2.3 when 4 and 8 mM of $CaSO_4$ were added to the NaCl solution (Fig. 30).

The data showed that a decrease in the leaf K^+/Na^+ ratio of P. vulgarus was associated with an increase in NaCl concentration of the soil solution. Although soil salinity increased the leaf Na^+ and K^+ (Figs. 25and 27), however, the increase in leaf Na^+ was much greater than K^+. Therefore, the K^+/Na^+ ratio decreased with an increase in salinity, and that by itself could be attributed to cation exchange capacity and concentration of Na^+ in the soil solution.

In addition, the data showed that the addition of 8 mM $CaSO_4$ resulted in a slight increase in leaf K^+/Na^+ ratio at all levels of NaCl compared to situations where no Ca^{2+} were added (Fig. 30). This could be attributed to: 1) a direct effect of Ca^{2+} on the ion exchanger, and replacement of Ca^{2+} for Na^+ and/or 2) an indirect effect via increasing membrane permeability by Ca^{2+}. Thus, lesser amounts of Na^+ were passed through membranous systems of the plant and into the cytoplasm.

Leaf Calcium

Sodium chloride at concentrations of 0.4, 0.8 and 1.2 S m^{-1} caused a significant increase in the leaf Ca^{2+} of P. vulgaris compared to the control treatment (Table 16 and Fig. 31). The mean leaf Ca^{2+} for the control treatment was 217 mg kg^{-1} while those for the NaCl concentrations of 0.4, 0.8 and 1.2 S m^{-1} were 302, 346 and 352 mg kg^{-1}, respectively. The mean leaf Ca^{2+} for NaCl concentrations of 0.4 and 0.8, and 0.4 and 1.2 S m^{-1} were signficantly different.

The effects of $CaSO_4$ on leaf Ca^{2+} in P. vulgaris are shown in Table 16 and Figure 32. Calcium sulfate at concentrations of 4 and 8 mM significantly increased the leaf Ca^{2+} compared to that in the control and compared to one another. Leaf calcium was 286, 311, and 315 mg kg^{-1} for 0, 4, and 8 mM of $CaSO_4$, respectively.

Similarly, the effects of $CaCl_2$ on leaf Ca^{2+} are shown in Table 16 and Figure 33. Leaf Ca^{2+} increased significantly with an increase in $CaCl_2$ concentration. Leaf Ca^{2+} was 283, 301, and 328 mg kg^{-1} corresponding to $CaCl_2$ concentrations of 0, 4, and 8 mM, respectively.

Interactions of NaCl and $CaSO_4$ on leaf calcium in P. vulgaris are shown in Table 16 and Figure 34. The leaf Ca^{2+} was at its lowest

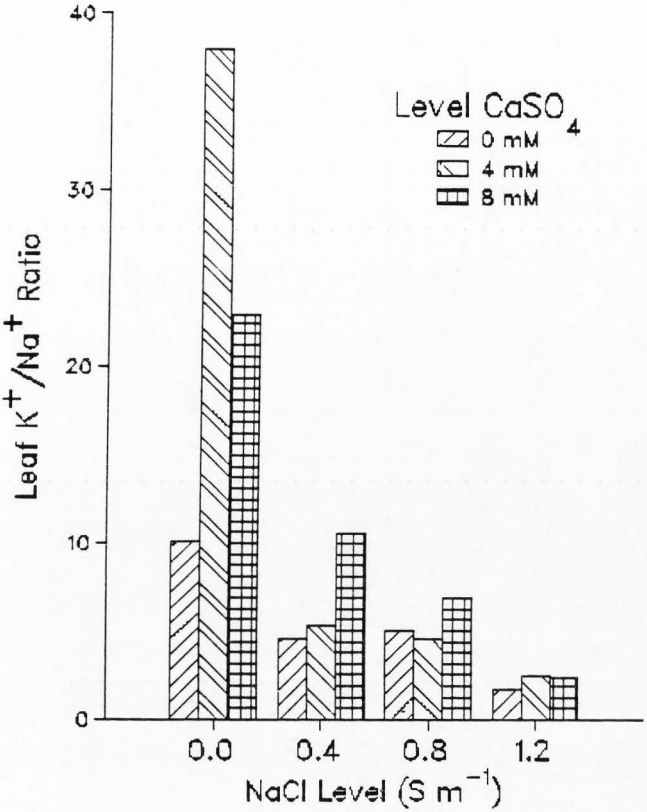

Figure 30. Effects of sodium chloride and calcium sulfate on leaf K^+/Na^+ ratio in Phaseolus vulgaris L.

Table 16. Effects of sodium chloride and calcium compounds on leaf calcium in *Phaseolus vulgaris* L.

Sources	DF	MS	Obs. F	Prob. F
Block	3	34386.866	23.764	0.000 **
NaCl	3	138681.457	95.840	0.000 **
$CaSO_4$	2	11787.031	8.146	0.001 **
$CaCl_2$	2	15490.557	17.616	0.000 **
NaCl x $CaSO_4$	6	6553.375	4.529	0.000 **
NaCl x $CaCl_2$	6	744.949	0.515	0.796
$CaSO_4$ x $CaCl_2$	4	466.127	0.322	0.863
NaCl x $CaSO_4$ x $CaCl_2$	12	1234.044	0.853	0.597
Error	105	1447.009	-----	-----

** Significant at $P<0.01$

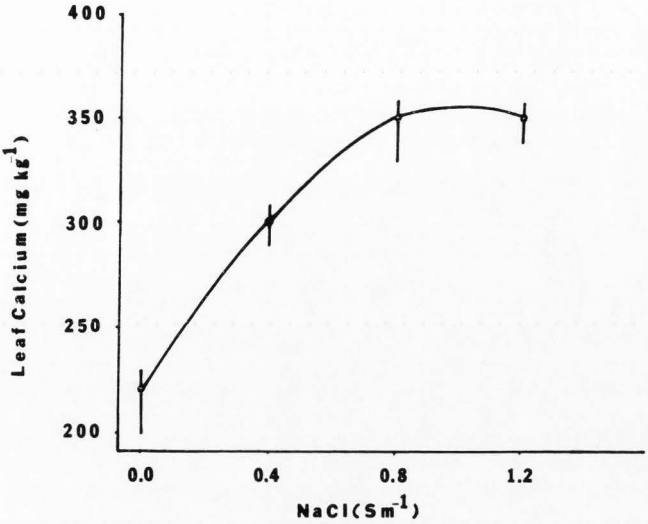

Figure 31. Effects of sodium chloride on leaf calcium (Ca^{2+}) in Phaseolus vulgaris L. (vertical bars denote confidence intervals).

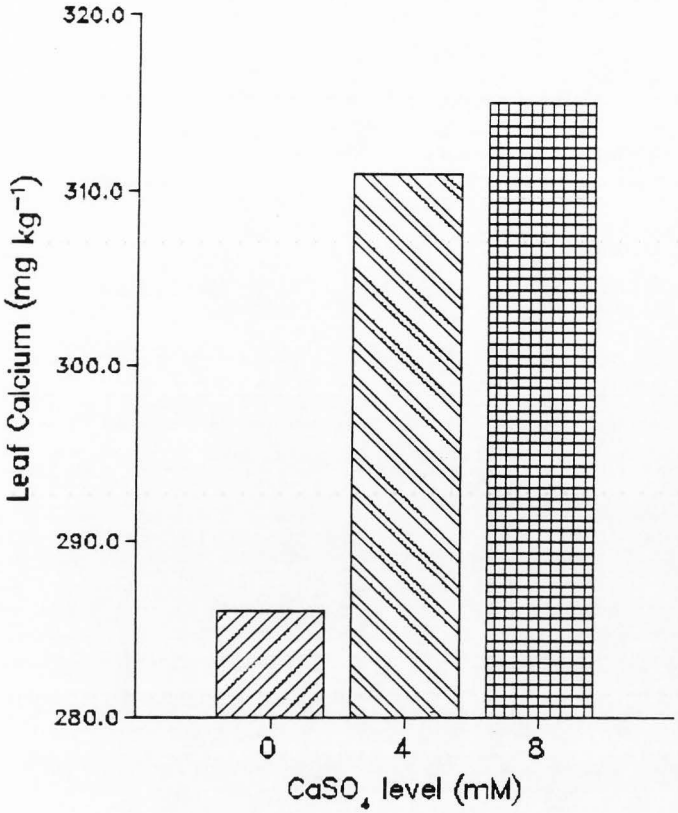

Figure 32. Effects of calcium sulfate on leaf calcium in Phaseolus vulgaris L.

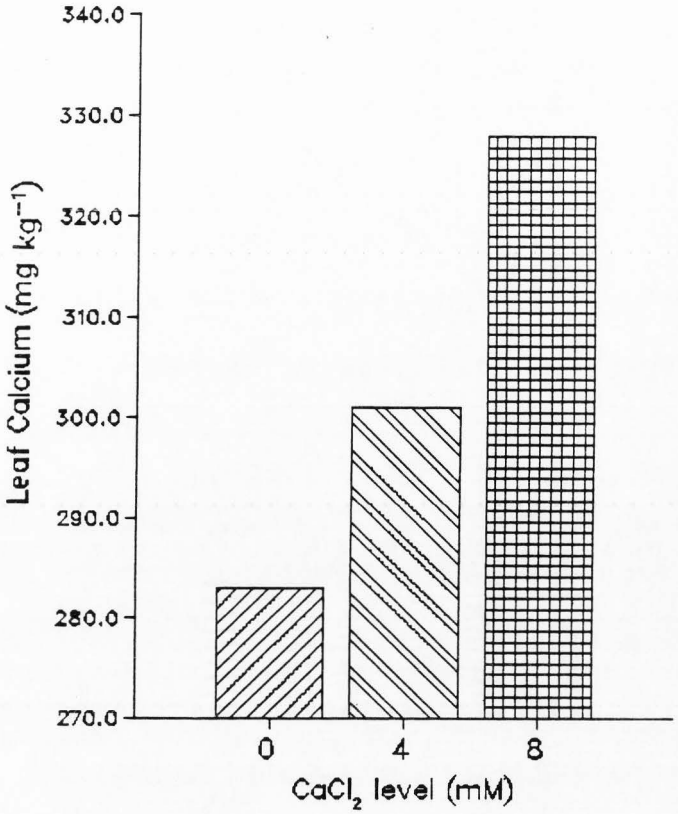

Figure 33. Effects of calcium chloride on leaf calcium in Phaseolus vulgaris L.

value of 203 mg kg^{-1} when the control solution was applied. In the absence of NaCl, 4 and 8 mM $CaSO_4$ resulted in a slight increase in the leaf Ca^{2+} to 229 and 220 mg kg^{-1} respectively.

An increase in leaf Ca^{2+} was noticed when 0.4 S m^{-1} of NaCl was mixed with either 4 or 8 mM $CaSO_4$. In both of these cases, leaf Ca^{2+} was increased from 290 mg kg^{-1} for the control treatment to 300 and 315 mg kg^{-1} for 4 and 8 mM $CaSO_4$, respectively (Fig. 34).

A significant increase in leaf Ca^{2+} was observed when 0.8 S m^{-1} of NaCl was mixed with 4 and 8 mM $CaSO_4$. In this case, leaf Ca^{2+} was increased from 307 mg kg^{-1} to 387 and 343 mg kg^{-1} for 4 and 8 mM $CaSO_4$, respectively (Fig. 34). At 0.8 S m^{-1} of NaCl, the effect of 4 mM $CaSO_4$ was significantly greater than 8 mM of $CaSO_4$ (Fig. 34).

When the NaCl concentration was increased to 1.2 S m^{-1}, 8 mM of $CaSO_4$ significantly increased the leaf calcium to 382 mg kg^{-1} compared to NaCl alone with a leaf Ca^{2+} content of 344 mg kg^{-1}. At the 1.2 S m^{-1} level of NaCl, 4 mM of $CaSO_4$ slightly reduced the leaf Ca^{2+} to 330 mg kg^{-1} compared to 344 ppm for NaCl alone (Fig. 34).

These results show that an increase in leaf Ca^{2+} of P. vulgaris was associated with an increase in NaCl concentration of the soil. This could be attributed to the presence of a high concentration of Na^{+} ions in the soil that replace 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 (Jurinak, 1981; and Mengel and Kirkby, 1982).

Furthermore, this study showed that an increase in leaf Ca^{2+} was associated with an increase in Ca^{2+} concentration of the soil. This could be due to: 1) direct effects of Ca^{2+} ions which increased their

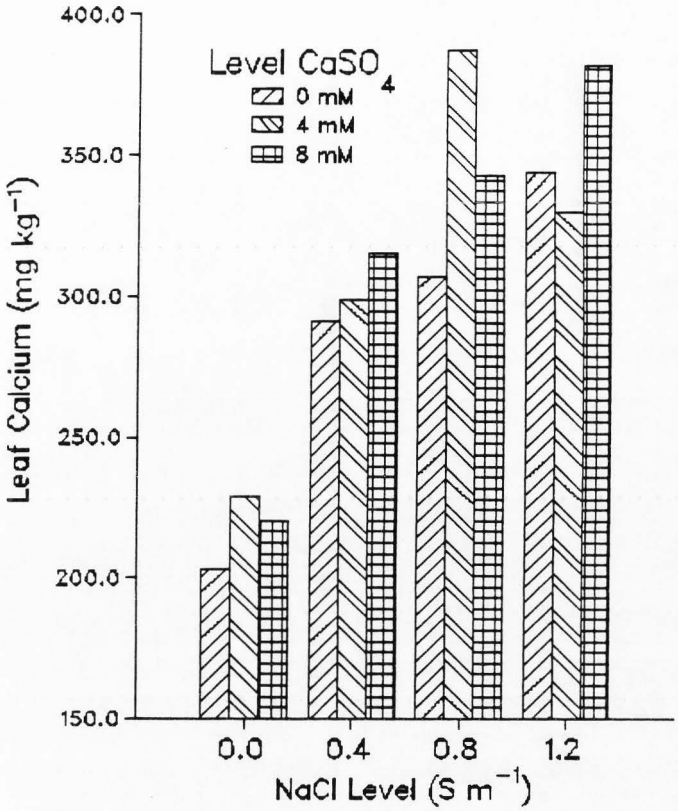

Figure 34. Effects of sodium chloride and calcium sulfate on leaf calcium in Phaseolus vulgaris L.

concentration in the soil solution and raised the uptake rate of the plants and 2) indirect effects via increasing the integrity of the plasma membrane by Ca^{2+}, thereby reducing ion leakage through the membrane (Lahaye and Epstein, 1971; Leopold, 1977; and Hanson, 1983).

Leaf Magnesium

Sodium chloride, at concentrations of 0.4, 0.8 and 1.2 S m^{-1}, resulted in a significant increase in the leaf Mg^{2+} of P. vulgaris compared to the control treatment (Table 17 and Fig. 35). The mean leaf Mg^{2+} for control plants was 215 mg kg^{-1} while values for the NaCl treatments of 0.4, 0.8 and 1.2 S m^{-1} were 250, 313 and 335 mg kg^{-1}, respectively. The mean leaf Mg^{+2} for NaCl concentrations of 0.4 and 0.8, and 0.4 and 1.2 S m^{-1} NaCl treatments were significantly different from each other.

Interactions of NaCl and $CaSO_4$ on leaf Mg^{2+} in P. vulgaris are shown in Figure 36. The leaf Mg^{2+} was at its lowest value of 186 mg kg^{-1} for 0 NaCl and 0 mM $CaSO_4$. Presence of 8 mM $CaSO_4$ in solution without NaCl caused a signficant increase in leaf Mg^{2+} to 246 mg kg^{-1} compared to 186 mg kg^{-1} for control plants. In the absence of NaCl, 4 mM of $CaSO_4$ increased leaf Mg^{+2} to 213 mg kg^{-1}, but this effect was not significant compared to the control treatment (Fig. 36).

With a NaCl concentration of 0.4 S m^{-1}, 8 mM of $CaSO_4$ resulted in a slight increase in the leaf Mg^{2+} to 266 mg kg^{-1} compared to NaCl alone with a leaf Mg^{2+} of 249 mg kg^{-1}. At this level of NaCl, 4 mM of $CaSO_4$ caused a slight reduction in the leaf Mg^{2+} to 236 mg kg^{-1} compared to the NaCl alone (Fig. 36).

When 0.8 S m^{-1} of NaCl was added to 8 mM of $CaSO_4$, leaf Mg^{2+} increased slightly to 328 mg kg^{-1} compared to 313 mg kg^{-1} for NaCl

Table 17. Effects of sodium chloride and calcium compounds on leaf magnesium in Phaseolus vulgaris L.

Sources	DF	MS	Obs. F	Prob. F
Block	3	649648.020	196.597	0.000 **
NaCl	3	109577.170	33.160	0.000 **
$CaSO_4$	2	5849.338	1.770	0.175
$CaCl_2$	2	1337.787	0.405	0.668
NaCl x $CaSO_4$	6	5695.187	1.723	0.123
NaCl x $CaCl_2$	6	1133.269	0.343	0.913
$CaSO_4$ x $CaCl_2$	4	1551.560	0.470	0.758
NaCl x $CaSO_4$ x $CaCl_2$	12	717.845	0.217	0.997
Error	105	3304.470	-----	-----

** Significant at $P < 0.01$

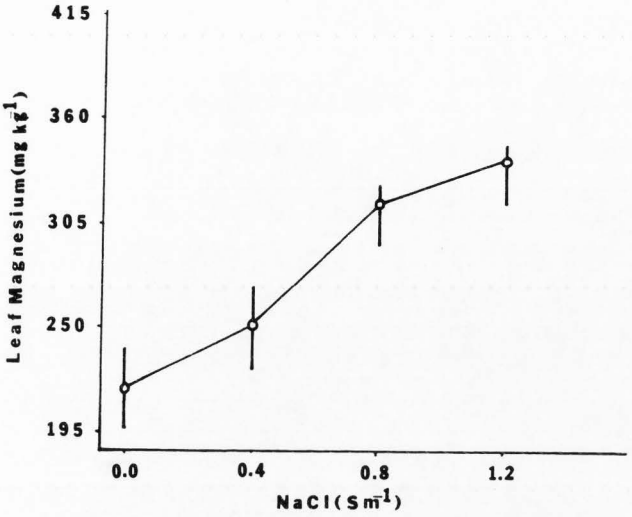

Figure 35. Effects of sodium chloride on leaf magnesium in Phaseolus vulgaris L. (vertical bars denote confidence intervals).

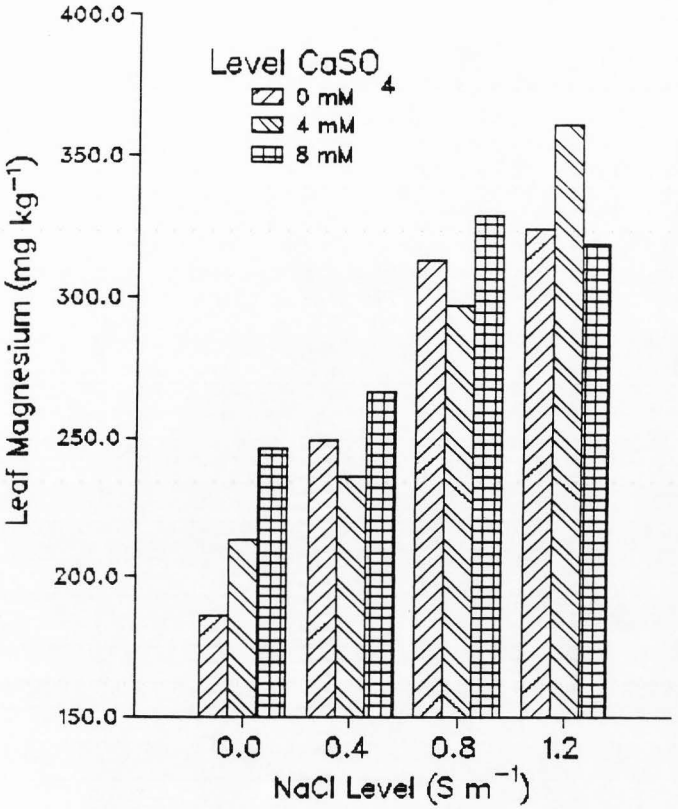

Figure 36. Effects of sodium chloride and calcium sulfate on leaf magnesium in Phaseolus vulgaris L.

alone. At this level of NaCl, 4 mM of $CaSO_4$ slightly reduced leaf Mg^{2+} to 297 mg kg^{-1} (Fig. 36).

Increasing the salt mixture to 1.2 S m^{-1} NaCl and 4 mM of $CaSO_4$ increased the leaf Mg^{2+} from 324 mg kg^{-1} for NaCl alone to 361 mg kg^{-1} when mixed with 4 mM $CaSO_4$. When this level of NaCl was mixed with 8 mM of $CaSO_4$, the leaf Mg^{2+} was reduced to 319 mg kg^{-1}. At this level of NaCl, both effects of 4 and 8 mM of $CaSO_4$ were considered insignificant (Fig. 36).

Results of this study showed that the increase in leaf Mg^{2+} of P. vulgaris was associated with an increase in NaCl concentration of the soil. This could be attributed to the presence of a high concentration of Na^+ that replaced the Mg^{2+} ion and released it into the soil solution. A higher concentration of Mg^{2+} ions in the soil solution, therefore, could result in a higher uptake rate by the plants. Furthermore, these data showed that leaf Mg^{2+} increased when 8 mM of $CaSO_4$ was added to 0.4 and 0.8 S m^{-1} NaCl. This could be due to: 1) direct effects of high concentration of Ca^{2+} replacing Mg^{2+}. Hence, more Mg^{2+} was available at the root surface which was taken-up by the plant, and/or 2) indirect effects via increasing membrane permeability by the Ca^{2+} ions, reducing Mg^{2+} leakages (Mengel and Kirby, 1982).

Electrical Conductivity of Irrigation Water.

The electrical conductivity of irrigation water (EC_{iw}) with various concentrations of NaCl and Ca compounds are shown in Table 18 and Figure 37. The electrical conductivity varied with salt types and concentration. EC_{iw} was at its lowest value of 0.01 S m^{-1} with the control treatments of NaCl, $CaSO_4$ and $CaCl_2$. As concentrations of NaCl increased, EC_{iw} also increased. This could be attributed to the

Table 18. Electrical conductivity (EC) and pH of irrigation water for the different salt treatment combinations.

	Treatment Combinations			Irrigation Water	
	NaCl ($S\ m^{-1}$)	$CaSO_4$ (mm)	$CaCl_2$ (mM)	Ec_{iw} ($S\ m^{-1}$)	pH
1.	0	0	0	0.01	7.73
2.			4	0.12	7.90
3.			8	0.20	7.60
4.		4	0	0.08	8.15
5.			4	0.17	7.90
6.			8	0.26	8.00
7.		8	0	0.14	8.25
8.			4	0.22	8.12
9.			8	0.30	7.79
10.	0.4	0	0	0.40	8.29
11.			4	0.50	8.14
12.			8	0.57	7.80
13.		4	0	0.47	8.26
14.			4	0.54	7.96
15.			8	0.61	7.90
16.		8	0	0.52	7.95
17.			4	0.60	7.88
18.			8	0.67	7.96
19.	0.8	0	0	0.81	8.15
20.			4	0.91	8.19
21.			8	1.00	7.88
22.		4	0	0.89	8.24
23.			4	0.94	8.19
24.			8	1.05	8.13
25.		8	0	0.94	8.24
26.			4	1.03	8.20
27.			8	1.08	7.89
28.	1.2	0	0	1.22	8.03
29.			4	1.27	8.03
30.			8	1.34	8.10
31.		4	0	1.25	8.26
32.			4	1.33	8.25
33.			8	1.40	7.95
34.		8	0	1.33	8.25
35.			4	1.38	8.23
36.			8	1.46	8.12

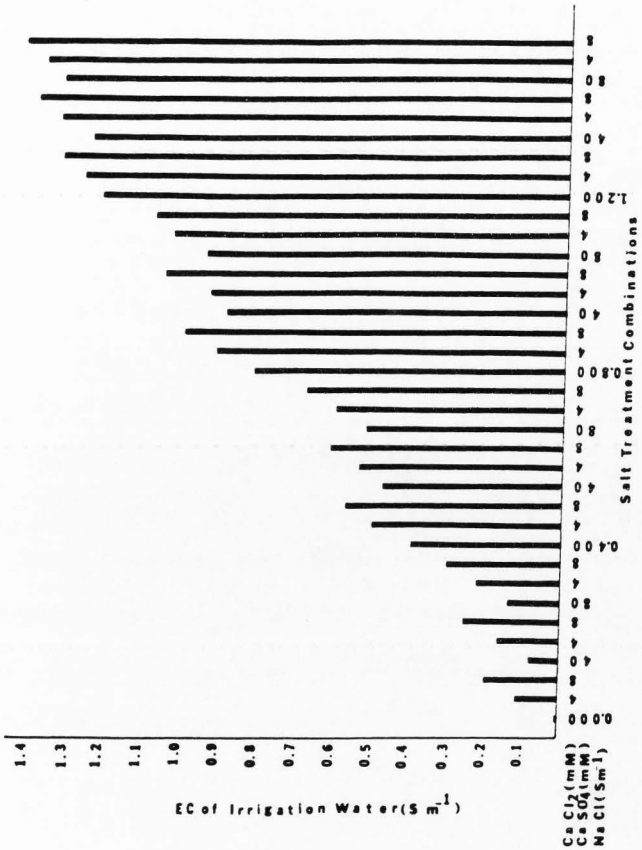

Figure 37. Electrical conductivity (EC) of irrigation water used for different salt treatment combinations.

fact that ions carry electric current, and the greater the concentration of ions the greater is the current conducting capacity or electrical conductivity of the solution (Jurinak, 1981).

At a given concentration of $CaSO_4$ or $CaCl_2$ the EC_{iw} was higher with $CaCl_2$ than it was with $CaSO_4$. For example, EC_{iw} corresponding to 8 mM of $CaCl_2$ was equal to 0.20 S m^{-1}, while EC_{iw} for 8 mM of $CaSO_4$ was 0.14 S m^{-1}. The reason for this difference is attributed to the different degree of ionic dissociation of these two salts when dissolved in water. When $CaSO_4$ (gypsum) is dissolved, a large fraction of the cations and anions are so attracted to one another thus they behave as if un-ionized, that is, forming the ion pair, $CaSO_4^o$. The salt $CaCl_2$ does not form ion pairs to the extent that $CaSO_4$ does (Adams, 1971; Jurinak, 1981, 1984). The presence of the ion-pair $CaSO_4^o$ reduces the current conducting capacity of the solution. Assuming that molar solubility of gypsum is 15.3 mM L^{-1} the activity of ion-pair $CaSO_4^o$ is estimated to be 4.7 mM L^{-1}. In other words, about 1/3 of all Ca^{2+} and SO_4^{2-} ions found in a saturated gypsum solution exist as ion-pair and the other 2/3 (10.6 mM L^{-1}) is Ca^{2+} and SO_4^{2-}.

An estimate of free cations and anions concentration (Table 19) using GEOCHEM or speciation modeling (Jenne, 1979) have shown that a solution of 8 mM of $CaSO_4$ and $CaCl_2$ and 120 mM of NaCl (1.2 S m^{-1}) will contain 80% free cation Ca^{2+} and 93% Na^+ as well as 56% free anion SO_4^{2-} and 94% Cl^-. The complex forms of $CaSO_4^o$, $CaCl^+$ and $NaSO_4^-$ make up the remaining electrolyte concentrations. Indicating that plants at the highest concentration of salts not only had access to free Ca^{2+}, Na^+, SO_4^{2-}, and Cl^- but also complex species involving

Table 19. Primary distribution of metals and ligands with highest concentration of NaCl (1.2 S m^{-1} NaCl, 8 mM $CaSO_4$ and 8 mM $CaCl_2$)

Ca			
	As a free metal (Ca^{2+})	(12.78 mM)	79.9 percent
	Bound with SO_4 ($CaSO_4^o$)	(1.16 mM)	7.3 percent
	Bound with Cl ($CaCl^+$ and $CaCl_2^o$)	(2.03 mM)	12.7 percent
Na			
	As a free metal (Na^+)	(111 mM)	92.5 percent
	Bound with SO_4 ($NaSO_4^-$)	(2.64 mM)	2.2 percent
	Bound with Cl (NaCl)	(6.36 mM)	5.3 percent
SO_4			
	As a free ligand (SO_4^{2-})	(4.52 mM)	56.5 percent
	Bound with Ca ($CaSO_4^o$)	(1.16 mM)	14.6 percent
	Bound with Na ($NaSO_4^-$)	(2.31 mM)	28.9 percent
Cl			
	As a free ligand (Cl^-)	(119.93 mM)	93.7 percent
	Bound with Ca ($CaCl^+$ and $CaCl_2^o$)	(1.92 mM)	1.5 percent
	Bound with Na (NaCl)	(6.14 mM)	4.8 percent

these four ions. Ion speciation always result in the total free ion concentration being less than the treatment concentration.

In addition, comparison of Table 19 (with high NaCl concentration) and Table 20 (with no NaCl) indicates that the amount of free Ca^{2+} in both treatments were the same (approximately 13 mM). Also, the SO_4^{2-} concentrations were similar, i.e., 4.5 and 5.3 mM respectively. The major differences were the amount of free anion concentration Cl^- as well as the ratio of Na^+/Ca^{2+}. There was much more Cl^- (approximately 120 mM) and higher ratio of Na^+/Ca^{2+} in the NaCl system than without NaCl. Indicating that the physiological differences noticed in the plants were probably attributed to presence of SO_4^{2-}, Cl^- and Na^+.

For interested readers, GEOCHEMICAL analysis for the different salt treatment combinations and data of saturated extract analysis of plants growing medium are summarized in Appendix E and F, respectively.

Table 20. Primary distribution of metals and ligands with lowest concentration of NaCl (0.0 mM NaCl, 8 mM $CaSO_4$ and 8 mM $CaCl_2$)

Ca			
	As a free metal (Ca^{2+})	(13.04 mM)	81.5 percent
	Bound with SO_4 ($CaSO_4^o$)	(2.64 mM)	16.5 percent
	Bound with Cl ($CaCl^+$ and $CaCl_2^o$)	(0.36 mM)	2.3 percent
SO_4			
	As a free ligand (SO_4^{2-})	(5.3 mM)	67.1 percent
	Bound with Ca ($CaSO_4^o$)	(2.6 mM)	32.9 percent
Cl			
	As a free ligand (Cl^-)	(7.84 mM)	98.0 percent
	Bound with Ca ($CaCl^+$ and $CaCl_2^o$)	(0.16 mM)	2.0 percent

POSSIBLE MECHANISMS

A brief review of the results show that increasing NaCl concentration was associated with a decrease in leaf water potential, total leaf chlorophyll, shoot and root dry weight, and nitrogen fixation and an increase in stomatal diffusive resistance. At the highest level of NaCl, additions of $CaSO_4$ ameliorated the effects of NaCl and resulted in an increase in leaf water potential via increasing stomatal diffusive resistance. Also, adding $CaSO_4$ to different levels of NaCl (0.4 and 0.8 S m^{-1}) resulted in an increase in total leaf chlorophyll, as well as an increase in shoot and root dry weight and nitrogen fixation. Such effects were not observed with the addition of $CaCl_2$ to NaCl in any of the parameters analyzed (i.e., the interactions of NaCl x $CaCl_2$ were insignificant - Appendix G).

Although no detailed anatomical or cytological studies were conducted, however, speciation modeling (GEOCHEM) in Appendix E is helpful for explaining some possible mechanisms for the results. The speciation modeling shows that the percentage of free Na^+ and Ca^{2+} in each of the NaCl categories (for example, 1.2 S m^{-1} NaCl, 0 mM $CaSO_4$ and 8 mM $CaCl_2$ as compared to 1.2 S m^{-1} NaCl, 8 mM $CaSO_4$ and 0 mM $CaCl_2$) is approximately the same. The major differences in them are the amounts of free SO_4^{2-} and Cl^-. The treatment that received 8 mM of $CaSO_4$ had high amounts of free SO_4^{2-} (61%) available for plant uptake. Treatments that received 8 mM of $CaCl_2$ had high amounts of free Cl^- (94.1%), which probably is beyond the requirements of the plant. Under normal conditions, the physiological requirements of the plants for Cl^- is low and is in order of a few mg kg^{-1}. Usually, limited amounts of Cl^- is required for the photosynthetic process.

Chloride may also act as a counter-ion in rapid potassium fluxes and contributes to turgor pressure of the leaves. The effect of excess Cl^- in some plants is a more serious problem and plants may show symptoms of Cl^- toxicity. In that case plants may have burning of leaf margins, bronzing, premature yellowing or abscision of leaves. Beans are generally considered a chlorophobic crop and reduction in yield and quality in crops is associated with approximate tissue levels of 0.5-2% Cl^- (Reisenauer et al., 1973).

In contrast to Cl^-, the physiological requirements of most plants for sulfur is high. Sulfur is an essential element that has a major effect on the properties and structural conversions of the protein molecules as well as the redox reactions of the cell (Mercado and Gollek, 1973; Mengel and Kirkby, 1982). Among proteins, cysteine is considered one of the most important amino acids formed by the organic precursor serine and inorganic sulfide. Cysteine plays an important role in metabolism of plant cells (Mercado and Gollek, 1973). It does initiate synthesis of various sulfur amino acids such as methionine, cysteic acid, and glutathione, to mention a few. Mengel and Kirkby (1982) have shown that organic compounds containing the sulfhydryl group can protect plants against several important environmental stresses such as irradiation, low temperature and drought. The sulfate reduction mechanisms are reported to be in chloroplast and mitochondria and the photosynthetic electron transfer system provides electrons for the reduction of sulfate in the chloroplast.

Thus, we speculate that the plants that received $CaSO_4$ treatments did better than those that received $CaCl_2$ mainly because of high amounts of free SO_4^{2-} that were available for plant uptake. Plants

may have used SO_4^{2-} for synthesis of peptides and certain energy-rich derivatives (co-enzyme A) required for metabolism and were more able to cope with the salt stress. As a result, plants lowered the stomatal diffusive resistance, photosynthesized effectively, sent more assimilates to root nodules and fixed more nitrogen which again was used for the growth and development of the whole plant.

Another possibility is that synergetic effects of SO_4^{2-} and Ca^{2+} might have been effective for better growth of plants that received $CaSO_4$ treatment. The role of Ca^{2+} in wall structure is cell-to-cell adhesion. The most important effect of Ca^{2+} in the apoplast lies within the integrity of the plasmamembrane (Lahaye and Epstein, 1971). Ca^{2+} is an integral part of the plasmalemma, and its presence with SO_4^{2-} might have been more helpful in governing normal impermeability to the transport of ions, especially the Na^+ and Cl^-. One can also speculate that there was antagonistic effects between the presence of Cl^- and Ca^{2+} which led to an impairment of the membrane structure, and increased the cell permeability. Thus, all the plants that received $CaCl_2$ treatment had toxic ion effects of Cl^- and/or Na^+. Therefore, there was burning of leaf tips, yellowing and reduced yield.

The presence of SO_4^{2-} alone or with Ca^{2+} may also have been effective in helping the plant to survive in saline conditions, but not high levels of NaCl. One reason is that beans are a sensitive crop and physiological requirements of the plant for Na^+ or Cl^- are low. High levels of NaCl may contribute to impairment of membranes and inhibit enzyme systems participating in the conversion of sulfate to sulfide and synthesis of sulfur amino acids. The other reason

might be that the presence of NaCl depresses water potential of the medium and hence restricts water uptake by plant roots.

Finally, high levels of NaCl may have induced physiological disorders. Since toxicity can begin with an imbalance of ions in the tissue (especially with Na^+), the plants might have been able to cope to some degree with excess Na^+ by excluding its uptake or channelling it into vacuoles. However, this regulatory process required an additional amount of energy and for this reason plants subjected to high levels of NaCl had higher respiration rates and depleted the storage carbohydrate to a greater extent than those under low levels of salinity (0.4 S m^{-1}). Thus, the plants grown under high levels of salinity were poor in energy status compared to lower levels and compared to control. Lack of energy as a consequence of salinity then may have contributed to another energy requiring process such as the nitrogen fixation or whole plant growth.

SUMMARY

The effects of NaCl and various forms of calcium on nitrogen fixation, stomatal diffusive resistance, potassium/sodium ratio, percent leaf nitrogen, leaf chlorophyll, biomass and saturated extract analysis on Phaseolus vulgaris L. were investigated and are summarized as follows:

1. EC_{iw} was at its lowest value with the control treatments of NaCl, $CaSO_4$, and $CaCl_2$. As the concentration of NaCl increased, EC_{iw} also increased, which could be due to a greater concentration of ions increasing current conductivity capacity.
2. At a given concentration of $CaSO_4$ or $CaCl_2$, the EC_{iw} was higher with $CaCl_2$ than it was with $CaSO_4$, which related to the current carrying capacity as affected by the different degree of ionic association of the two salts.
3. Increasing NaCl concentration was associated with a decrease in leaf water potential, total leaf chlorophyll, acetylene reduction activity, nodule dry weight and leaf K^+/Na^+ ratio. Increasing the NaCl concentration also caused an increase in the stomatal diffusive resistance.
4. The stomatal apparatus was closed more during the second week of salt treatment than during the first week (i. e., as stress progressed, the plants increased the stomatal diffusive resistance to reduce transpiration).
5. Addition of $CaSO_4$, especially at the highest level of NaCl, caused an increase in the stomatal diffusive resistance. The interaction of $CaCl_2$ and NaCl in this regard was insignificant.

6. At the highest level of NaCl, closure of the stomates was associated with a decrease in leaf water potential.
7. Ash analysis of leaves showed that an increase in concentration of $CaSO_4$ increased the leaf K^+ as well as total leaf nitrogen. Similar effects were not observed with $CaCl_2$.
8. Neither $CaSO_4$ nor $CaCl_2$ had any significant effect on leaf K^+, Na^+, or Mg^{2+} of snapbeans when they were added to different levels of NaCl.
9. Addition of $CaSO_4$ to the NaCl solutions, however, increased the leaf K^+/Na^+ ratio, total leaf chlorophyll, shoot and root dry weight, and acetylene reduction activity of snapbeans when compared to control at each NaCl level.
10. Leaf Ca^{2+} was increased with an increase in concentration of $CaSO_4$ or $CaCl_2$, but only $CaSO_4$ exhibited an interaction when combined with NaCl.
11. GEOCHEM analysis showed that in the NaCl treatments the free Na^+ ion varied from 92 to 98% for all three different levels. The concentration varied from 39 to 112 mM free Na^+ depending on treatment.
12. GEOCHEM analysis showed that the free Ca^{2+} concentration varied from about 3 to 12 mM, depending on treatment. From 14 to 20% of the total Ca was complexed as $CaSO_4$ and $CaCl^-$.
13. GEOCHEM analysis showed that free SO_4^{2-} concentration varied from about 2 to 6 mM, depending on treatment. From 19 to 44% of the total SO_4 was complexed as $CaSO_4^0$ and $NaSO_4^-$.
14. GEOCHEM analysis showed that in the NaCl treatments, the free Cl^- ion varied from 94 to 98% for all three treatment levels. The

concentration varied from 39 to 120 mM free Cl^- depending on treatment.

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APPENDICES

Appendix A. Modified Hoagland Solution

Table 21. Modified Hoagland Solution

	Stock Soln. Conc.	Mol. Wt.	PPM of Nutr. in Final Soln.	mL Nutr. G L^{-1} Stock	mL Nurt. L^{-1} $D.H_2O$ Full Strength	mL Nurt. L^{-1} $D.H_2O$ 1/2 Strength
Major Nutrients:						
$KH_2 PO_4$	1 M	136.04	P 31	136.04	1	0.5
$K_2 SO_4$	1 M	174.30	K 234	174.30	5	2.5
Ca CO_3	1 M	100.09	Ca 200	100.04	5	2.5
MG SO_4, $7H_2O$	1 M	246.48	Mg 48 S 64	246.48 ------	2	1.0
Micro Nutrients:						
H_3BO_3	500 ppm	61.83	B 0.5	2.860	1.0	0.5
M_nCl_2, $4H_2O$	500	169.01	Mn 0.5	1.810	1.0	0.5
Zn SO_4, $7H_2O$	500	287.56	Zn 0.5	2.20	1.0	0.5
Cu SO_4, H_2O	20	249.64	Cu 0.02	.078	1.0	0.5
Na Mo O_4, $2H_2O$	10	241.95	Mo 0.01	.025	1.0	0.5
Fe Chelate	4 g L^{-1}	------	Fe 2.4	4.000	10.0	5.0
KOH	4 g L^{-1}	------	-------	-----	----	---

The pH of the final solution was adjusted with KOH to 6.2.

Appendix B. Micro Kjeldahl Unit
Used for Analysis of Total Nitrogen

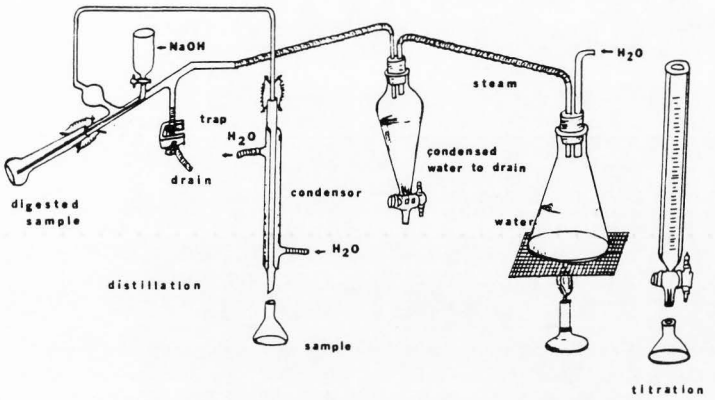

Figure 38. Micro Kjeldahl unit used for analysis of total nitrogen.

Appendix C. Reagents for Total Nitrogen Analysis

Table 22. Reagents for total nitrogen analysis.

Kjeldahl catalyst:
100 parts K_2SO_4
10 part $CuSO_4$, $5H_2O$
1 part selenium metal (Se)
Tashiyo's indicator:
Methylene blue 0.248 g
Methylene red 0.375 g
Ethanol 300 ml.
NaOH, 40% (10 M):
(add 40 grams of NaOH to distilled H_2O and bring the volume to 100 mL).

Appendix D. The Procedure Used to Set Up the Absorption Spectophotometer for Emission Reading

The procedure used to set up the absorption spectophotometer for emission reading.

1. Signal switch on "EM"
2. Mode on "CONT"
3. Recorder on ABS
4. BG Corrector on "AA"
5. Turn "Lamp" and "Gain" switch counterclockwise
6. Acetylene tank to 10-15 psi
7. Turn on Air Tank to 40 psi
8. Turn on Fuel switch
9. Turn on the knob to air
10. Turn on machine power
11. Push the ignite switch for flame
12. Adjust the following for the desired elements:

Element	Slit	Wavelength
K	2.0	766.5 nm
Na	0.2	589.0 nm

13. With a standard solution and some energy (50-75 units), set wavelength to maximum reading (do not change the wavelength again)
14. Set gain to zero
15. Put in time interval (punch 1 then "t" for interval 1 second, punch 3 then "AVE" for average of 3 intervals
16. Put tubing in the highest standard, adjust "Gain" to about 75 (do not change it again)

17. Take the tube out of standard solution and put it into distilled water. Then push "AZ" to adjust for zero.
18. Put tubing in different standards and read values for standard curve. Set a standard curve (ppm vs. readings)
19. Put tubing in samples and read corresponding values
20. Compare the readings to the standard curve to quantify the values.

Appendix E. Primary Distribution of Metals and Ligands (GEOCHEM) for the Different Salt Treatment Combinations

Table 23. Primary distribution of metals and ligands (GEOCHEM), for the different salt treatment combinations.

Treatments														
			Ca			Na			SO_4			Cl		
Na Cl S m^{-1}	Ca SO_4 mM	Ca Cl mM	Free Ca^{2+} %	Bound to SO_4 %	Bound to Cl %	Free Na^{+} %	Bound to SO_4 %	Bound to Cl %	Free SO_4^{2-} %	Bound to SO_4 %	Bound to Cl %	Free Cl^{-} %	Bound to SO_4 %	Bound to Cl %
0.0	0	0	---	---	---	---	---	---	---	---	---	---	---	---
0.0	0	4	98.2	---	1.8	---	---	---	---	---	---	99.1	0.9	---
0.0	0	8	97.0	---	3.0	---	---	---	---	---	---	98.5	1.5	---
0.0	4	0	81.1	19.0	---	---	---	---	81.1	19.0	---	---	---	---
0.0	4	4	84.4	14.2	1.4	---	---	---	71.7	28.3	---	98.6	1.4	---
0.0	4	8	86.8	10.7	2.5	---	---	---	66.2	33.8	---	98.0	2.0	---
0.0	8	0	75.3	24.7	---	---	---	---	75.3	24.7	---	---	---	---
0.0	8	4	79.4	19.5	1.1	---	---	---	69.1	30.9	---	98.3	1.7	---
0.0	8	8	81.5	16.5	2.0	---	---	---	67.1	32.0	---	98.0	2.0	---
0.4	0	0	---	---	---	97.8	---	2.2	---	---	---	97.8	---	2.2
0.4	0	4	93.1	---	6.9	97.1	---	2.1	---	---	---	97.1	0.5	2.3
0.4	0	8	92.1	---	7.9	96.5	---	3.5	---	---	---	96.8	1.0	2.2
0.4	4	0	86.2	8.9	4.9	96.1	1.5	2.3	76.5	8.9	14.6	97.2	0.5	2.3
0.4	4	4	86.9	7.4	5.7	95.8	1.3	2.9	72.6	14.8	12.6	96.8	0.9	2.3
0.4	4	8	88.0	6.6	5.5	96.0	1.2	2.9	68.0	20.7	11.2	96.4	1.4	2.3
0.4	8	0	79.7	15.4	4.9	95.0	3.1	2.0	70.4	15.4	14.2	97.0	1.0	2.0
0.4	8	4	80.9	13.2	5.6	94.9	2.7	2.4	66.4	20.9	12.7	96.6	1.5	1.9
0.4	8	8	81.4	11.6	7.0	94.5	2.4	3.0	65.2	23.2	11.6	96.3	1.8	1.9

Table 23. Primary distribution of metals and ligands (GEOCHEM), for the different salt treatment combinations (continued).

Treatments														
			Ca			Na			SO_4			Cl		
Na Cl S m^{-1}	Ca SO_4 mM	Ca Cl mM	Free Ca^{2+} %	Bound to SO_4 %	Bound to Cl %	Free Na^{+} %	Bound to SO_4 %	Bound to Cl %	Free SO_4^{2-} %	Bound to SO_4 %	Bound to Cl %	Free Cl^{-} %	Bound to SO_4 %	Bound to Cl %
0.8	0	0	---	---	---	96.1	---	3.1	---	---	---	96.1	---	3.9
0.8	0	4	89.7	---	10.3	95.7	---	4.3	---	---	---	95.6	0.5	3.9
0.8	0	8	89.3	---	10.7	95.3	---	4.7	---	---	---	95.2	0.9	3.9
0.8	4	0	85.2	6.0	8.7	94.7	1.4	4.0	69.3	6.0	24.7	95.6	0.4	---
0.8	4	4	85.4	5.4	9.1	94.5	1.2	4.3	66.6	10.8	22.6	95.2	0.8	3.9
0.8	4	8	85.6	4.9	9.5	94.2	1.1	4.7	64.0	15.4	20.6	94.8	1.3	3.9
0.8	8	0	81.6	10.1	8.3	93.3	2.4	4.4	68.1	10.1	21.7	95.3	0.8	4.0
0.8	8	4	80.6	9.5	9.9	93.3	2.4	4.3	63.5	14.7	21.8	95.2	1.2	3.5
0.8	8	8	81.1	9.2	9.7	93.6	2.3	4.2	60.8	18.3	20.9	95.0	1.6	3.5
1.2	0	0	---	---	---	94.8	---	5.2	---	---	---	94.8	---	5.2
1.2	0	4	86.2	---	13.8	94.6	---	5.4	---	---	---	94.4	0.4	5.1
1.2	0	8	85.7	---	14.3	94.3	---	5.7	---	---	---	94.1	0.8	5.1
1.2	4	0	83.1	4.5	12.4	93.5	1.3	5.2	61.9	4.5	33.6	94.4	0.4	5.2
1.2	4	4	83.2	4.2	12.7	93.4	1.2	5.4	60.0	8.3	31.7	94.0	0.8	5.2
1.2	4	8	83.0	3.8	13.2	93.1	1.1	5.8	58.1	12.1	29.8	93.6	1.2	5.1
1.2	8	0	80.6	8.0	11.5	92.4	2.3	5.2	60.9	8.0	31.1	94.0	0.8	5.2
1.2	8	4	80.9	7.4	11.7	92.4	2.2	5.5	59.0	11.7	29.3	93.6	1.2	5.2
1.2	8	8	79.9	7.3	12.7	92.5	2.2	5.3	56.5	14.6	28.9	93.7	1.5	4.8

Appendix F. Mean Values of Saturated Extract Analysis

Table 24. Mean values of saturated extract analysis.

Treatments									
NaCl (S m^{-1})	$CaSO_4$ (mM)	$CaCl_2$ (mM)	EC (S m^{-1})	pH	K^+ (mg L^{-1})	Na^+ (mg L^{-1})	Ca^{2+} (mg L^{-1})	Mg^{2+} (mg L^{-1})	SAR (mmole L^{-1})$^{1/2}$
0.0	0	0	0.05	8.10	7	25	46	8	0.89
0.0	0	4	0.01	7.97	9	26	190	12	0.52
0.0	0	8	0.13	7.90	8	26	265	17	0.42
0.0	4	0	0.07	8.10	5	18	70	23	0.47
0.0	4	4	0.13	8.16	13	105	70	12	3.04
0.0	4	8	0.19	7.86	10	55	395	23	0.72
0.0	8	0	0.09	8.06	5	15	85	23	0.37
0.0	8	4	0.16	8.05	9	45	195	30	0.79
0.0	8	8	0.20	7.98	11	13	345	37	0.18
0.4	0	0	0.39	8.14	32	620	220	37	10.15
0.4	0	4	0.40	7.99	27	350	347	45	4.68
0.4	0	8	0.45	8.00	34	640	550	30	7.18
0.4	4	0	0.40	8.11	26	623	190	37	10.91
0.4	4	4	0.43	8.00	25	623	343	45	8.38
0.4	4	8	0.47	7.98	33	645	552	54	7.00
0.4	8	0	0.42	8.12	32	623	192	45	10.48
0.4	8	4	0.43	8.00	32	618	265	54	9.02
0.4	8	8	0.44	7.93	31	600	437	45	7.29

Table 24. Mean values of saturated extract analysis (continued).

Treatments									
NaCl ($S\ m^{-1}$)	$CaSO_4$ (mM)	$CaCl_2$ (mM)	EC ($S\ m^{-1}$)	pH	K^+ ($mg\ L^{-1}$)	Na^+ ($mg\ L^{-1}$)	Ca^{2+} ($mg\ L^{-1}$)	Mg^{2+} ($mg\ L^{-1}$)	SAR ($mmole\ L^{-1})^{1/2}$
0.8	0	0	0.58	8.05	33	1125	345	64	14.56
0.8	0	4	0.73	8.03	41	1325	548	64	14.24
0.8	0	8	0.74	8.04	42	1320	718	54	12.77
0.8	4	0	0.56	7.96	33	1075	550	55	11.68
0.8	4	4	0.81	8.00	42	1375	570	73	14.36
0.8	4	8	0.76	7.87	39	1320	720	74	12.50
0.8	8	0	0.69	8.03	34	1280	260	86	17.53
0.8	8	4	0.79	8.00	39	1325	531	87	14.03
0.8	8	8	0.80	8.00	42	1320	720	73	12.50
1.2	0	0	0.94	8.05	47	1775	540	74	18.95
1.2	0	4	0.95	7.98	43	1770	575	64	20.30
1.2	0	8	1.16	7.98	52	2100	1100	98	16.24
1.2	4	0	0.92	7.93	46	1765	390	86	21.02
1.2	4	4	0.97	7.97	47	1768	540	74	18.88
1.2	4	8	1.13	7.96	43	2050	1050	86	16.32
1.2	8	0	1.13	7.91	48	2100	530	111	21.60
1.2	8	4	1.14	7.94	51	2080	385	98	24.42
1.2	8	8	1.19	7.90	52	2050	390	98	23.96

Appendix G. Significant Effects of Sodium Chloride and Calcium Compounds on Selected Variables in Phaseolus vulgaris L.

Table 25. Significant effects of sodium chloride and calcium compounds on selected variables in *Phaseolus vulgaris* L.

Measurements	Probability of F							
	Main Effects				Interaction Effects			
	Block	NaCl	$CaSO_4$	$CaCl_2$	NaCl x$CaSO_4$	NaCl x$CaCl_2$	$CaSO_4$ x$CaCl_2$	NaClx$CaSO_4$ x$CaCl_2$
Nitrogen Fixation	0.001**	0.000**	0.859	0.118	0.024*	0.779	0.685	0.643
Nodule Weight	0.097	0.000**	0.453	0.518	0.862	0.840	0.920	0.668
Shoot Weight	0.006**	0.000**	0.657	0.457	0.166	0.625	0.539	0.274
Root Weight	0.396	0.000**	0.687	0.711	0.046*	0.332	0.199	0.088
Stomate Diffusive Resistance Week 1	0.000**	0.000**	0.387	0.567	0.000**	0.073	0.415	0.534
Stomate Diffusive Resistance Week 2	0.000**	0.000**	0.157	0.556	0.001**	0.910	0.833	0.823
Xylem Water Potential	0.163	0.000**	0.195	0.769	0.013*	0.134	0.033*	0.797
Chlorophyll a	0.002**	0.000**	0.811	0.718	0.001**	0.224	0.792	0.623
Chlorophyll b	0.000**	0.000**	0.044*	0.285	0.000**	0.272	0.568	0.441
Chlorophyll a+b	0.000**	0.000**	0.370	0.519	0.000**	0.221	0.697	0.496
Plant K^+	0.000**	0.000**	0.000**	0.424	0.291	0.109	0.911	0.538
Plant Na^+	0.025*	0.000**	0.762	0.549	0.990	0.644	0.392	0.223
Plant K^+/Na^+	0.004**	0.000**	0.248	0.535	0.279	0.893	0.803	0.977
Plant Ca^{+2}	0.000**	0.000**	0.001**	0.000**	0.000**	0.796	0.863	0.597
Plant Mg^{+2}	0.000**	0.000**	0.175	0.668	0.123	0.913	0.758	0.997
Plant Total Nitrogen	0.000**	0.001**	0.000**	0.956	0.257	0.627	0.525	0.199
Soil Ec_e	0.266	0.000**	0.000**	0.000**	0.079	0.012*	0.448	0.957
Soil pH	0.000**	0.000**	0.790	0.000**	0.188	0.060	0.195	0.101
Soil K^+	0.621	0.000**	0.199	0.002**	0.349	0.191	0.319	0.670
Soil Na^+	0.215	0.000**	0.418	0.021**	0.049*	0.043*	0.606	0.824
Soil Ca^{+2}	0.952	0.000**	0.057	0.000**	0.025*	0.000**	0.180	0.128
Soil Mg^{+2}	0.588	0.000**	0.000**	0.498	0.391	0.246	0.851	0.389
Soil SAR	0.621	0.000**	0.849	0.000**	0.593	0.414	0.970	0.710

*Significant at P<0.05; **Significant at P<0.01