The Effect of Sudden Sodium Chloride Stress on the Ion Composition and the Mechanism of Osmotic Adjustment in *Vicia faba*

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Abstract: Salt stressed *Vicia faba* L. FAAU 900-3 plants had a greater increase in Na^+ and Cl^- contents than in other ions to compensate for increases in the external NaCl concentration. Leaves had more accumulated K^+ than roots and stems. Most ions in roots were lower than control under salt treatment. At higher salinity Na in the root decreased potassium, Cl^- and PO_4^{3-} in the uptake increased under stress. All ions except SO_4^{2-} and K^+ showed a strong correlation between their contents in stems and salinity, while K^+, Na^+ and Cl^- showed a strong correlation in leaves, which indicates their importance in osmotic adjustment.

Key words: Osmotic adjustment, salt stress, salt avoidance, *Vicia faba*, ionic composition

Introduction

Today approximately one-third of the world's irrigated lands suffer some degree of salinization (Epstein *et al.*, 1980). Overuse of fertilization and poor drainage leads to the accumulation of high concentrations of salts in soils, especially in arid and semiarid regions (Leidi and Saiz, 1997; Sibole *et al.*, 1998). Legumes are not considered as a protein source in the diet of humans and livestock but they play important role in fixing the atmospheric nitrogen and improving the physical and chemical characteristics of the soil (Hoshikawa, 1991). Legumes are considered to be the main food in most Mediterranean countries. The need for water increases with population growth, so water deficiency will become a great problem especially in the Middle East and North Africa. The use of saline water will be a necessary demand. So, a better understanding of plant tolerance is required against salinity (Materon, 1988).

To overcome the external stress plant cells tend to readjust their osmotic potential to prevent water losses that can be achieved either by uptake of inorganic ions from the external, or by *de novo* synthesis of compatible solute solutes as amino acids and soluble sugars acting as osmolytes (Jones and Pritchard, 1989; Delauney and Verma, 1993; Bohnert *et al.*, 1995; Bohnert and Shen, 1998; Serrano *et al.*, 1999). The osmotic readjustment is quickly induced by the changes in ion fluxes than the synthesis of compatible solutes (Jones and Pritchard, 1989; Lew, 1996).

When entration of sodium chloride increases in the external medium, it competes with mineral nutrients needed for optimal growth. An important cause of NaCl induced growth inhibition, is the difficulty in uptake of mineral nutrients due to competition with Na^+ (Solovev, 1969). The effect of Na^+ on K^+ uptake is two fold. At low concentrations, Na^+ may increase K^+ uptake, though decreasing it at higher concentration (Rains and Epstein, 1967; Nimbalker and Joshi, 1975; Paricha *et al.*, 1975 Orton, 1980; Lynch and Lauchli, 1984). Calcium uptake and growth were inhibited at high Na^+ concentration (Poonia *et al.*, 1972; Lohaus *et al.*, 2000). Decreases in Mg^{2+} and K^+ occur (Okusanya and Ungar, 1984; Nakamura *et al.*, 1990), although N, P and Ca^{2+} increased at higher external Na^+ concentration (Ansari, 1972; and Matar *et al.*, 1975). Chloride is accumulated more rapidly by the plant from NaCl solutions than Na^+ (Levitt, 1980). Salt accumulation facilitates osmotic adjustment but may lead to ion toxicity and nutritional imbalances (Greenway and Munns, 1980; and Jones, 1981; Kinraide, 1999). Salinity severely affects Ca^{2+} uptake and transport from roots to shoots (Lynch and Lauchli, 1985; Cramer *et al.*, 1988; Ehret *et al.*, 1990; Francois *et al.*, 1991). Drew *et al.* (1988) reported that NaCl salinity in the soil decreased the rate of K^+ transport to the shoots, which in turn suppressed the accumulation of potassium in different parts of glycophytic plants (Jeschke, 1984; Gorham *et al.*, 1988) and consequently inhibited plant growth (Drew *et al.*, 1988). Increasing NaCl salinity of the rooting medium resulted in an increase in Na^+ and Cl^- in leaf sap (Demmig and Winter, 1986; Khan *et al.*, 2001). The adverse effects of salinization were attributed to the ion excess effect that lowered the internal K^+/Na^+ ratio (Greenway and Munns, 1983; Lynch and Lauchli, 1984). Cytoplasmic damage results from high cytoplasmic Na^+: K^+ ratios (Flowers *et al.*, 1977; Winter and Preston, 1982; and Jones *et al.*, 1979) and high Na^+ concentrations may interfere with K^+ acquisition (Greenway, 1965; Rains and Epstein, 1967; Orton, 1980; Lynch and Lauchli, 1984). Salinity increases K^+ loss and decreases K^+/Na^+ selectivity (Cramer *et al.*, 1987). Correlations have been found between Na^+ content in plants and in the soil in which they grow (Hansen and Weber, 1975; Tiku, 1975; and Hansen *et al.*, 1976).

Beans were able to adjust osmotically the Cl^- salinity (Gale *et al.*, 1967; Shabala *et al.*, 2000). Bean plants show a lag in adjustment, due to a rapid drop in water potential and leaf turgor, followed by a slower increase in cell sap concentrations (Puscas *et al.*, 1966; Meiri and Mayber, 1969). Bean plants tolerated NaCl 6 g/l all the way to flowering; due to osmotic adjustment by absorption of Cl^- and K^+ with exclusion of Na^+ (Hamza, 1973).

It is well known that most of legume plants are ranged from sensitive to moderate sensitive to salinity. The aim of this study was to determine the effect of salt stress on the ionic composition and to understand the mechanism of osmotic readjustment in the different organs of the stressed *Vicia faba*.

Materials and Methods

Vicia faba L. FAAU 900-3 plants were cultivated (two replicates) in sand-clay (2:1 respectively) soil at field capacity without stress for four weeks. The first group was left as control and irrigated with tap water. The other five groups irrigated with 85, 170, 255, 340, 425 mM NaCl. The salt used was extracted from sea water. The NaCl solutions contained 9,18,27,36 and 45 mM of potassium respectively. The plants survived under stress for another four weeks until flowering. During the experiment, maximum temperature ranged between 33 and 20 °C, minimum temperature ranged between 15 and 4°C, relative humidity from 30 to 85% and average day length was 13 hours. At harvesting, all groups of roots were rinsed in their own treatment then immersed in distilled water and dried by a filter paper. Roots, stems and leaves of each group were dried in a drying oven at 95°C. After drying, the water content was calculated as a percent of fresh weight. Then dried parts were ground into a fine powder and analyzed, after extraction in water (El-Sharkawi and Michel, 1977).

Sodium and potassium were measured by flame photometry as described by Williams and Twine (1960). Calcium and magnesium were determined volumetrically by the Versene method (Johnson and Ulrich, 1959). Phosphate was determined colourimetrically according to Woods and Mellon (1941). Chloride was determined volumetrically according to Jackson (1958). Sulphate was determined turbidimetrically according to Bremner *et al.* (1965).

Correlation between soil and plant contents was computed according to the simple linear correlation procedure.
Sodium/potassium selectivity in root, stem and leaves was calculated as:

$S_{K:Na\ (root)}$ = $(K_r)(Na_{ext.})/(Na_r)(Kext.)$ (1)
$S_{K:Na\ (Stem)}$ = $(K_s)(Na_r)/(Na_s)(K_r)$ (2)
$S_{K:Na\ (leaf)}$ = $(K_l)(Na_s)/(Na_l)(K_s)$ (3)

Where the subscripts ext., r, s and l indicate ion concentration in the external solution, in root, in stem and in leaves. Significance of differences between the treatments was analyzed by ANOVA.

Results

Growth and water content: Stressed plants showed a distinctive decrease in growth. The more stressed plants were the most dwarfed and had the smallest leaf area (data not shown).
The water content (Table 1) decreased with increasing NaCl stress in all plant organs. Water content correlated negatively with external salinity (Table 2) and was significant in stem and leaf.

Anions (Cl^-, PO_4^{3-} and SO_4^{2-}): The data of estimated anions showed that chloride was the most accumulated anion (Fig. 1). The chloride was accumulated in high concentrations in the stems, in comparison with other organs, at the lower stresses but the leaves tended to accumulate the chloride in high concentrations at the higher stresses.
Phosphate was accumulated in leaves and stems to higher concentration than in the roots (Table 1), but leaves had the highest concentration. Phosphate concentration correlated positively with the external NaCl stress (Table 2). This correlation was significant in the root and stem but was stronger in stem.
Sulphate was the lowest accumulated anion. The stem had the lowest SO_4^{2-} concentration while root had the highest concentration. Also leaves accumulated sulphate more than stem (Table 1). SO_4^{2-} concentration correlated negatively in the root and positively in stems and leaves with the external NaCl stress (Table 2).

Cations (Na^+, K^+, Ca^{2+} and Mg^{2+}): Data showed high accumulation of sodium cation. It was accumulated in stems at a higher concentration than in root or leaves (Fig. 2). Both stems and leaves increased their content of Na^+ with the increasing external NaCl. Roots tended to decrease or at least stabilize their content of Na^+. Root Na^+ content was correlated negatively with the external NaCl stress (Table 2) but not significant. The same
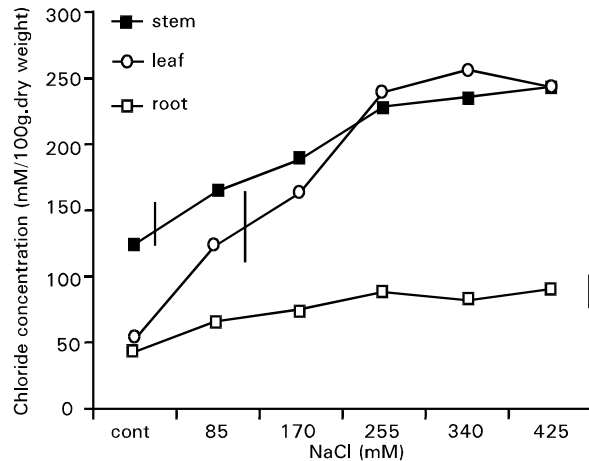

Fig. 1: Chloride ion concentrations in root, stem and leaves of stressed *Vicia faba*, vertical lines represent LSD at 0.01.
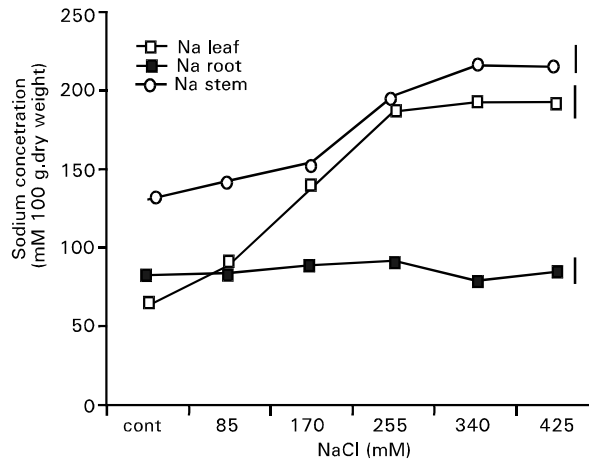

Fig. 2: Sodium ion concentration in root, stem and leaves of stressed *Vicia faba*, vertical lines represent LSD at 0.05

Table 1: Water content (WC) as percentage of fresh weight, and plant content of phosphate, sulphate, calcium and magnesium (mM/100 g. dry weight) under different treatments of salt stress in root (r), stem (s) and leaves (l)

Treatments	Water content %			PO_4^{3-}			$SO4^{2-}$			Ca^{2+}			Mg^{2+}		
	r	s	l	r	s	l	r	s	l	r	s	l	r	s	l
Control	91.4	90.2	87.5	2.77	5.00	6.40	1.93	0.93	1.00	5.61	4.07	9.06	22.83	13.29	8.64
85 mM NaCl	89.3	89.3	86.9	2.98	5.49	6.01	1.44	0.65	1.00	4.99	4.99	9.11	16.04	12.67	8.02
170 mM NaCl	88.9	88.6	86.1	4.03	6.40	6.67	1.56	0.61	1.21	6.86	5.31	9.98	10.28	15.14	5.88
255 mM NaCl	87.5	88.4	86.2	3.38	6.01	7.68	2.00	0.77	0.98	6.86	9.36	13.72	16.37	17.89	7.40
340 mM NaCl	81.6	85.7	82.2	3.30	6.62	6.40	1.53	0.85	1.09	4.07	9.36	10.30	13.29	17.89	10.20
425 mM NaCl	82.6	85.7	82.3	4.25	6.98	6.84	1.74	0.91	1.01	5.31	11.32	12.18	14.19	16.99	11.11

Table 2: Correlation coefficient of water and ion content with the external salt concentration in Root, stem and leaf

Parameters	Root	Stem	Leaf
Water content	-0.571	-0.953*	-0.918**
Chloride	0.899*	0.967**	0.937*
Phosphate	0.894*	0.934*	0.413
Sulphate	0.051	-0.272	0.063
Calcium	-0.144	0.978**	0.659
Magnesium	-0.576	0.856*	0.575
Sodium	-0.248	0.965**	0.928*
Potassium	0.127	-0.712	0.991**

** significant at 1% . * significant at 5%

Table 3: Ionic ratios, Na^+, K^+, Cl^-:Na^+ + K^+, Ca^{2+}:Mg^{2+}, SAR under different NaCl stresses

Treatment (NaCl)	Na^+/K^+			Cl^-/Na^+ + K^+			Ca^{2+}/Mg^{2+}			SAR		
	r	s	l	r	s	l	r	s	l	r	s	l
Control	4.14	6.84	2.17	0.40	0.83	0.54	0.25	0.31	1.10	1.36	2.55	1.56
85 mM	3.36	6.00	2.89	0.62	0.99	1.04	0.30	0.40	1.20	1.68	2.80	2.05
170 mM	3.99	10.17	4.17	0.75	1.11	0.98	0.41	0.38	1.70	1.46	2.65	3.03
255 mM	3.70	11.03	5.20	0.77	1.08	1.16	0.43	0.46	1.90	1.71	2.97	3.40
340 mM	3.71	18.48	5.08	0.84	1.05	1.12	0.31	0.53	1.00	1.74	3.09	3.60
425 mM	3.76	14.07	4.43	0.86	1.04	1.09	0.37	0.66	1.20	1.71	3.07	3.44

Table 4: Stem Cl^-: root Cl^-, leaf Cl^-: stem Cl^-, stem Na^+ : root Na^+ and leaf Na^+ : stem Na^+ ratios in stressed *Vicia faba* plants

Treatments	Stem Cl^- / Root Cl^-	Leaf Cl^- / Stem Cl^-	Stem Na^+ / Root Na^+	Leaf Na^+ / Stem Na^+
Control	2.999	0.990	1.540	0.480
85 mM	2.500	0.750	1.540	0.620
170 mM	2.500	0.870	1.740	0.890
255 mM	2.600	1.050	2.140	0.950
340 mM	2.900	1.090	2.760	0.900
425 mM	3.900	0.990	2.540	0.900

Table 5: Sodium/potassium selectivity in soil-root, root-stem and stem-leaf at different NaCl stresses

Treatments	$S_{Na:K}$ whole Plant	$S_{Na:K}$ Root	$S_{Na:K}$ Stem	$S_{Na:K}$ Leaf
Control	0.250	0.24	0.62	3.12
85 mM	2.395	2.82	0.57	2.05
170 mM	1.764	2.36	0.40	2.40
255 mM	1.560	2.56	0.34	2.08
340 mM	1.396	2.57	0.21	3.41
425 mM	1.539	2.54	0.27	3.06

Table 6: Calcium/sodium ratio in root, stem and leaves under different NaCl stresses

Treatment	Ca/Na root	Ca/Na stem	Ca/Na leaf
Control	0.068	0.031	0.146
85 mM	0.060	0.035	0.103
170 mM	0.077	0.035	0.073
255 mM	0.075	0.048	0.074
340 mM	0.052	0.044	0.054
425 mM	0.063	0.053	0.063

correlation was positive and significant in stem and leaves. Potassium came after Na^+ in its accumulation. It was accumulated in leaves to a greater extent than in the root or stem. Potassium content in leaves was increased gradually paralleled with the external increase of NaCl concentration. Both the stems and roots were tended to avoid the accumulation of K^+, the roots were the most avoider than stems.

A decrease in the accumulated amount of calcium in the roots at 85 mM NaCl stress, the stems and leaves increased their content of Ca^{2+} directly with the application of stress (Table 1). At 340 mM NaCl stress some disturbance in the rate of calcium accumulation was occurred in roots and leaves. The lowest accumulated amounts of Ca^{2+} were in the roots. Magnesium accumulated in the root and stem to a greater extent than in leaves (Table 1). In all parts, Mg^{2+} concentration decreased at 85 mM. NaCl stress, while Mg^{2+} concentration in stem and leaves exceeded the control at higher stresses, but not in root. Correlations between Mg^{2+} concentration and external stress was similar to Ca^{2+} correlation; negative in root, significantly positive in stem and not significantly positive in leaves.

Ratios (Na^+/K^+, Cl^-:Na^+ + K^+, Ca^{2+}/Mg^{2+} and SAR): Calculations showed that all values of Na^+ : K^+ ratio was higher than one, stem had the highest values (Table 3). In stems and leaves Na^+ : K^+ ratio increased with increasing salinity. The stems had highest values. In roots the Na^+ : K^+ ratio decreased from control and tends to stabilize. With respect to Cl : Na^+ + K^+, it was less than one in root but reached one or little more in stem and leaves under stress. Ca^{2+} : Mg^{2+} ratio was less than one in root and stem. In leaves,
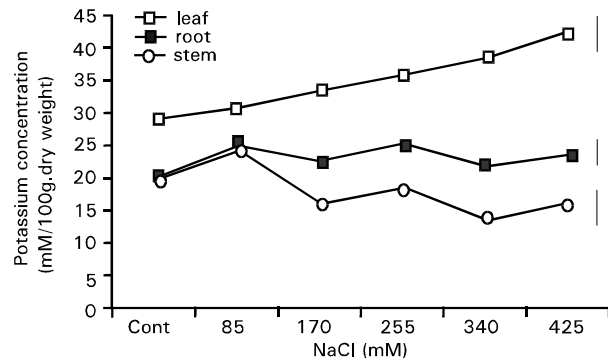

Fig. 3: Potassium ion concentration in root, stem and leaves of stressed *Vicia faba*, vertical lines represent LSD at 0.01.

this ratio increased gradually from 1.1 in the control to 1.9 in 255 mM NaCl treatment and then decreased to one in 340 mM NaCl treatment, increasing again in 425 mM NaCl treatment to 1.2. Sodium adsorption ratio (SAR) was higher in stem controls and 85 mM NaCl treatment; but at greatest stress, leaves had the higher values. The lowest SAR values were found in roots (Table 3).

As shown in Table 4, stem chloride/root chloride ratios indicate that the stem chloride concentration is more than two fold than in roots at all external NaCl stresses. Also, sodium was accumulated in stem to a greater extent than in roots and leaves. In leaves these ratios were lower than in stem, except for the Cl^- ratio at 255 and 340 mM NaCl. The leaves showed a tendency to avoid the accumulation of Na^+ in comparison with Cl^- accumulation.

As shown in Table 5, the potassium/sodium selectivity ($S_{K:Na}$ whole plant) was low in control (0.25) and increased to 2.4 at 85 mM NaCl treatment and decreased to 1.76, 1.56, 1.4 in 170, 255, 340 mM NaCl treatments respectively, but increased to 1.54 at 425 mM NaCl treatment. Potassium/sodium selectivity within plants was divided into three classes. The first to indicate the $S_{K:Na}$ in roots at different stresses. The second indicates the selectivity in stem, with taking in consideration that sodium and potassium ions in roots represent an external media to stem. The third class indicates the selectivity in leaves; stem is external medium to leaves. Root potassium/sodium selectivity increased seven fold than control. In stems $S_{K:Na}$ decreased gradually with an increase in external salinity, but leaves were tended to readjust the sodium/potassium selectivity. To understand the role of calcium in osmotic readjustment and due to its ameliorative effect, calcium/sodium ratio was computed in Table 6. The stems showed a gradual increase in Ca/Na ratio values while leaves tended to decrease Ca/Na ratios. This ratio was disturbed in root. It may be due to the transport to stem inside plant or to the competition with the continued increase of sodium chloride in the external medium.

Discussion

It is clear that all the plants when be lied under salt stress they try to readjust their osmotic pressure to maintain their water content. The osmotic readjustment is dependent mainly on the

accumulation of inorganic and organic solutes (Jones and Pritchard, 1989; Lew, 1996). The continued increase of the accumulated inorganic solutes will cause toxicity (Kinraide, 1999). The response of different organs will differentiate according to their functions and their ability to undergo stress.

The results presented here were used in a try to determine the mechanism of osmotic readjustment inside *Vicia faba* plant lied under sudden salt stress. As shown in the current data there is a negative relationship between water content and external stress; this reflects the sensitivity of bean plant toward salinity (Levitt, 1980).

The main strategy to overcome the external NaCl stress is the accumulation of the most available ions, Na^+ and Cl^- to regulate the internal concentration of ions lies in need to keep tissue water potentials more negative than those in the external medium (Flowers *et al.*, 1986b). It is clear that Na^+ and Cl^- play the main role in osmotic adjustment and were accumulated in higher concentrations than any ion (Flowers *et al.*, 1977; Tal *et al.*, 1978; Ford and Wilson, 1981). In the same time plants must avoid the toxicity produced from the high accumulated Na^+ and Cl^- (Greenway and Munns, 1980; and Jones, 1981; Kinraide, 1999). The exclusion (Hamza, 1973) of whether Na^+ or Cl^- will lead to decreasing the osmotic pressure inside plant cells. So, plants must tend to absorb the ions which can ameliorate the harmful effects (Kinraide, 1998). Both stems and leaves (Fig. 1 and 2) tended to increase their rate of Na^+ and Cl^- accumulation (Seemann and Critchley, 1985) to overcome the stress. The rate of chloride accumulation was more than sodium (Fig. 1 and 2), this was agreed with Levitt opinion (1980). *Vicia faba* roots exhibit poor accumulation rate for the estimated ions. There was more than one reason. First, the root cell vacuoles represent low per cent of the total plant volume and so are of insufficient capacity for transport across root tonoplasts to play other than a temporary role in the long-term ion relations of the shoot (Flowers *et al.*, 1986b). Second, the exclusion of some ions as Na^+ (Hamza, 1973) and/or deficiency in other ions due to high external concentration of Na^+ (Ansari, 1972; Matar *et al.*, 1975; Kinraide, 1999) which compete other ions. The third reason is high transport rate of ions to stem and leaf to maintain their turgidity. Now it is clear that there is a decrease in ionic composition in roots whether due to exclusion or quick transportation, it may alleviate the toxicity. On the other hand, stems and leaves are compelled to accumulate Na^+ and Cl^- to overcome the external stress to maintain their turgidity. So, there is a necessity to accumulate other ions to play a role in osmotic adjustment and in the same time alleviate the Na^+ and Cl^- toxicity and consequently the continuity of biological processes. Sudden stress lead both stems and leaves to increase their content of inorganic solutes as a quick response (Jones and Pritchard, 1989; Lew, 1996). There were two different mechanisms used by stem and leaf. The similarities between them were in their success to readjust the ratios of stem Cl^- : root Cl^- and leaf Cl^- : stem Cl^- (Table 4) to ensure the osmotic gradient. To prevent the Cl^- toxicity both stems and roots fixed the Cl^- : $Na^+ + K^+$ value (Table 3) near the value 1 to avoid the disturbances in the biological processes as protein synthesis (Flowers *et al.*, 1986b). Avoiding to Na^+ toxicity at higher stress, the leaves were depended on potassium more than other cations and gradually readjusted their $S_{K:Na}$ (Table 5) while stems selectivity decreased. So, leaf Cl^- concentrations were greater than those of Na^+ (Flowers *et al.*, 1986a). The potassium accumulation in leaves may due to the ability of guard cells to accumulate and release high quantities of K^+ for stomatal aperture. K^+ ions were accumulated in high concentration in the uppermost leaves in cotton under stress (Leidi and Saiz, 1997). The preferential K^+ accumulation in leaves might be related to the high K^+ requirement for growth (protein synthesis, cell expansion)and as a vacuolar osmoticum (Leigh and Jones, 1984; Marschner, 1995). The continued increase of K^+ ions (Fig. 3) in *Vicia faba* leaves decreased the Na^+/K^+ ratio (Table 3) at higher stress and this reflects the sensitivity of *Vicia faba* to the higher salt stress. Adaptation to salinity was related to the higher

Na^+/K^+ ratio in leaves of two species of *Atriplex* (Glenn *et al.*, 1994). According to Kinraide (1998) high levels of Na^+ in the plant tissues are not toxic unless Ca^{2+} is also deficient. Current results showed increase in Ca^{2+} and Mg^{2+} in stems and leaves. The decrease of calcium content in leaves, at the highest NaCl stresses, opposite a decrease in magnesium content in stems. This reflects the dependence of stems upon calcium in osmotic adjustment while leaves were dependant more on magnesium. The stems Ca^{2+}/Mg^{2+} values increased with the salt stress (Table 3) to avoid sodium toxicity while leaves readjusted the Ca^{2+}/Mg^{2+} may be avoiding to the disturbances in biological processes in the leaves cells. Although the increase in sodium absorption ratio SAR, there were increases in both Ca^{2+}/Mg^{2+} and Ca^{2+}/Na^+ (Table 6) ratios. This reflects the dependency of stems on Ca^{2+} to alleviate Na^+ toxicity. On the other hand, there were continued decrease in Ca^{2+}/Na^+ in leaves with the increase in their SAR values (Table 3), may be due to their dependence on compatible organic solutes. It can be concluded that *Vicia faba* plants when be subjected to sudden salt stress they tend to readjust their osmotic pressure using mechanism depending on alleviating effect of calcium to prevent Na^+ toxicity in stems but the leaves tended to accumulate potassium to decrease the rate of sodium uptake. The stressed leaves were tended to readjust the ratios of different accumulated ions to avoid injury. Approximately, all the plant organs were tried to stabilize their water status especially at the highest stresses (Table 1), which represent a sure danger, to insure the biological processes.

References

Ansari, R., 1972. Effect of salinity on some brassica oilseed varieties Pak. J. Bot., 44: 55-63.

Bohnert, H.J., D.E. Nelson and R.G. Jensen, 1995. Adaptation to environmental stresses. The Plant Cell, 7: 1099-1111.

Bohnert, H.J. and B. Shen, 1999. Transformation and compatible solutes. Scientia Horticulturae, 78: 237-260.

Bremner, J.M., 1965. Nitrogen Availability Indexes. In: Methods of Soil Analysis, Part 2, Black, C.A. (Ed.). Amercian Society Agronomy, Madison, WI., USA., pp: 1324-1345.

Cramer, G.R., J. Lynch, A. Lauchli and E. Epstein, 1987. Influx of Na^+, K^+ and Ca^{2+} into roots of salt stressed cotton seedlings. Pl. Physiol., 83: 510-516.

Cramer, G.R., E. Epstein and A. Lauchli, 1988. Kinetic of root elongation of maize in response to short-term exposure to NaCl and elevated calcium concentration. J. Expl. Bot., 39, 1513-1522.

Delauney, A.J. and D.P.S. Verma, 1993. Proline biosynthesis and osmoregulation in plants. The Plant J., 4: 215-223.

Demmig, B. and K. Winter, 1986. Sodium, potassium, chloride and proline concentration of chloroplasts isolated from a Halophyte, *Mesembryanthemum crystallinum* L. Planta, 168: 421- 426.

Drew, M.C., J. Guenther, A. Lauchli, 1988. The combined effects of salinity and root anoxia on growth and net Na^+ and K^+ accumulation in *Zea mays* growth in solution culture. Ann. Bot., 61: 41-53.

Ehret, D.L., R.E. Redmann, B.L. Harvey and A. Cipywnyk, 1990. Salinity-induced calcium deficiencies in wheat and barley. Plant and Soil, 128: 143-151.

El-Sharkawi, H.M. and B.E. Michel, 1977. Effects of soil water metric potential and air humidity on CO_2 and water vapour exchange in two grasses. Photosynthetica, 11: 176- 182.

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

Flowers, T.J., P.F. Troke and A.R. Yeo, 1977. The mechanism of Salt Tolerance in Halophytes. Ann. Rev. Pl. Physiol., 28: 89- 121.

Flowers, T.J., S.A. Flowers and H. Greenway, 1986a. Effect of sodium chloride on tobacco plants. Pl. Cell Env., 9: 845-651.

Flowers, T.J., M.A. Hajibagheri and N.J.W. Clipson, 1986b. Halophytes. The Quart. Rev. Biol., 61: 313-337.

Ford, C.W. and J.R. Wilson, 1981. Changes in levels of solutes during osmotic adjustment to water stress in four tropical pasture species. Aust. J. Pl. Physiol., 8: 77-91.

Francois, L.E., T.J. Donovan and E.V. Maas, 1991. Calcium deficiency of artichoke buds in relation to salinity. Hortscience, 26: 549-553.

Gale, J., H.C. Kohl and R.M. Hagan, 1967. Changes in the water balance and photosynthesis of onion, bean and cotton plants under saline conditions. Physiol. Plkant., 20: 408- 420.

Glenn, E.P., M. Olsen, R. Frye, D. Moore and S. Miyamoto, 1994. How much sodium accumulation is necessary for salt tolerance in subspecies of the halophyte *Atriplex canescens*? Pl. Cell Env., 17: 711 719.

Gorham, J., O.S. Tomar, Wyn, R.G. Jones, 1988. Salinity induced changes in the chemical composition of *Leucaena leucocephala* and *Sesbania bispinosa*. J. Pl. Physiol., 132: 678-682.

Greenway, H., 1965. Plant respond to saline substrates. VII. Growth and ion uptake throughout plant development in two varieties of *Hordeum vulgare*. Aust. J. Biol. Sci., 18: 763- 779.

Greenway, H. and R. Munns, 1980. Mechanisms of salt tolerance in non-halophytes. Annu. Rev. Pl. Physiol., 31: 149-190.

Greenway, H. and R. Munns, 1983. Interaction between growth, uptake of Cl⁻ and Na⁺ and water relations of plants in saline environment. II. Highly vacuolated cells. Pl. Cell Env., 6: 567-574.

Hamza, M., 1973. The effect of gradual increase in sodium chloride concentration in the medium on the osmotic adjustment in *Phaseaalus vulgaris* L. C. R. Acad. Sci., 276: 1997-2000

Hansen, D.J. and D.J. Weber, 1975. Environmental factors in relation to the salt contents of *Salicornia pacifica* var. Utahensis Great Bas. Nature, 35: 86-96.

Hansen, D.J., Dayanandan, P., P.B. Kaufman and J.D. Brotherson, 1976. Ecological adaptations of salt marsh grass, *Distichlis spicata* (Gramineae) and environmental factor affecting its growth and distribution. American J. Bot., 63: 635-650.

Hoshikawa, K., 1991. Significance of legume crops in improving the productivity and stability of cropping systems. In: Phosphorus Nutrition of legumes in the Semi-Arid Tropics. Johanse, C., K.K. Lee and K.L. Sahrawat (Eds.) ICRISAT. Ind., pp: 173-182.

Jackson, M.L., 1958. Soil chemical analysis. Printice-Hall, Inc., Englewood Cliffs. N.J., 1st ed.

Jeschke, W.D., 1984. K⁺, Na⁺ exchange at cellular membranes, intracellular compartmentation of cations and salt tolerance. In: Salinity tolerance in plants: Strategies for Crop Improvement, Staples, R.C. and G.H. Toenniessen (Eds.), John Wiley and Sons, New York, pp: 37-66.

Johnson, G.M. and A. Ulrich, 1959. Analytical methods for use in plant analysis US Dept. Agric., Calif. Univ., Agric. Inform, Bull. 766.

Jones, R.G.W. and J. Pritchard, 1989. Stresses Membranes and Cell Walls. In: Plants Under Stress Biochemistry Physiology and Ecology and their Application to Plant Improvement, Jones H.G., T.J. Flowers and M.B. Jones (Eds.). Cambridge University Press, Cambridge, MA., pp: 95-114.

Jones, R.G.W., 1981. Salt Tolerance. Butterworth, London, pp: 271-292.

Jones, R.G.W., C.J. Brady and J. Speirs, 1979. Ionic and Osmotic Relations in Plant Cells. In: Recent Advances in the Biochemistry of Cereals, Laidman, D.L. and R.G.W. Jones (Eds.). Academic Press, London, pp: 63-103.

Khan, M.A., B. Gul and D.J. Weber, 2001. Effect of salinity on the growth and ion content of *Salicornia rubra*. Pl. Anal., 32: 1532-2416.

Kinraide, T.B., 1998. Three mechanisms for the calcium alleviation of mineral toxicities. Pl. Physiol., 118: 513-520

Kinraide, T.B., 1999. Interactions among Ca²⁺, Na⁺ and K⁺ in salinity toxicity: quantitative resolution of multiple toxic and ameliorative effects. J. Exp. Bot., 50: 1495-1505.

Leidi E.O. and J.F. Saiz, 1997. Is salinity tolerance related to Na accumulation in upland cotton (*Gossypium hirsutum*) seedlings. Plant and Soil, 190: 67-75.

Leigh R.A. and R.G. Wyn Jones, 1984. A hypothesis relating critical potassium concentrations for growth to the distribution and functions of this ion in the plant cell. New Phytol., 97: 1-13.

Levitt, J., 1980. Responses of plants to environmental stress. Vol II. Academic Press London. New York, pp: 365-453.

Lew, R.R., 1996. Pressure regulation of the electrical properties of growing *Arabidopsis thaliana* L. root hairs. Pl. Physiol., 112: 1089-1100.

Lohaus, G., M. Hussmann, K. Pennewiss, H. Schneider, J. Zhu and H. Sattelmacher, 2000. Solute balance of a maize (*Zea mays* L.) source leaf as affected by salt treatment with special emphasis on phloem retranslocation and ion leaching. J. Exp. Bot., 51: 1721-1732.

Lynch, J. and A. Läuchli, 1984. Potassium transport in salt stressed barley roots. Planta, 161: 295-301

Lynch, J. and A. Läuchli, 1985. Salt stress disturbs the calcium nutrition of barley (*Hordeum vulgare* L.) New Phytol., 99: 345-354.

Marschner, H., 1995. Mineral nutrition of higher plants. 2nd ed. London: Academic Press.

Matar, Y., H.W. Doering and H. Marschner, 1975. Effect of NaCl and Na₂SO₄ on dry matter production, mineral content and organic compounds of spinach and lettuce. Z Pflanzeneraehr. Bodenkd., 3: 295-307.

Materon, L.A., 1988. Maximizing biological nitrogen fixation by forage and pasture legumes in semi-arid areas. In: Nitrogen fixation in Mediterranean agriculture. D.P. Bech and L.A. Materon (Eds.). Int. Centre Agric. Res. Dry Areas, Aleppo, Syria, pp: 131-143.

Meiri, A. and A.P. Mayber, 1969. Effect of variations in substrate salinity on the water balance and ionic composition of bean leaves. Isr. J. Bot., 18: 99-112.

Nakamura, Y., K. Tanaka, E. Ohta, M. Sakata, 1990. Protective effect of external Ca²⁺ on elongation and the intracellular concentration of K⁺ in intact mungbean roots under high NaCl stress. Plant and Cell Physiology, 31: 815-821.

Nimbalker, J.D. and G.V. Joshi, 1975. Effect of increasing salinity on germination, growth and mineral metabolism of sugarcane. Var. Co. 740. J. Biol. Sci., 18: 55-63.

Okusanya, O.T. and I.A. Ungar, 1984. The growth and mineral composition of three species of *Spergularia* as affected by salinity and nutrients at high salinity. Amer. J. Bot., 71:439-447.

Orton, T.J., 1980. Comparison of salt tolerance between *Hordeum vulgare* and *H. jubatum* in whole plants and cultures. Z Pflanzenphysiol., 98: 105-118.

Paricha, P.C., G.J. Patra and P. Sahoo, 1975. Effect of synthetic sea water on growth and chemical composition of rice at different stages of development. J. Indian Soc. Soil Sci., 23: 344-348.

Poonia, S.R., S.M. Virmani and D.R. Bhumbla, 1972. Effect of ESP(exchangeable sodium percentage) of the soil on the yield, chemical composition and uptake of applied calcium by wheat. J. Indian Soc. Soil Sci., 20: 183-185.

Puscas, M., T. Stoiciu, V. Baia, A. Olarasanu and A. Pascasu, 1966. Characteristics of some physiological processes in bean under the toxic action of some salts. Inst. Agron. Timisoora, Lucrstiint. Ser. Agron., 9: 295-309.

Rains, D.W. and E. Epstein, 1967. Sodium absorption by barley roots: its mediation by mechanism 2 of alkali cation transport. Pl. Physiol., 42: 319-323.

Seemann, J.R. and C. Critchley, 1985. Effects of salt stress on the growth, ion content, stomatal behaviour and photosynthetic capacity of a salt-sensitive species, *Phaseolus vulgaris* L. Planta, 164: 151-162.

Serrano, R., J.M. Mulet, G. Rios, J.A. Marquez, I. de Larrinoa, M.P. Leube, I. Mendizabal, A. Pascual-Ahuir, M. Proft, R. Ros and C. Montesinos, 1999. A glimpse of the mechanisms of ion homeostasis during salt stress. J. Exp. Bot., 50: 1023-1036.

Shabala, S., O. Babourina and I. Newman 2000. Ion-specific mechanisms of osmoregulation in bean mesophyll cells. J. Exp. Bot., 51: 1243-1253.

Sibole, J.V., E. Montero, C. Cabot, C. Poschenrieder and J. Barcelo, 1998. Role of sodium in the ABA-mediated long-term growth response of bean to salt stress. Physiologia Plantarum, 104: 299-305.

Solovev, V.A., 1969. Plant growth and water and mineral nutrient supply under NaCl salinization conditions. Fiziol. Rast., 16: 870-876.

Tal, M., H. Heikin and K. Dehan, 1978. Salt tolerance in the wild relatives of the cultivated tomato: Responses of callus tissue of *Lycopersicon esculentum, L. peruvianum* and *Solanum pennellii* to high salinity. Z Pflanzenphysiol. 86: 231-240.

Tiku, B.L., 1975. Exophysiological aspects of halophyte zonation in saline sloughs. Plant and Soil, 43: 355-369.

Williams and Twine, 1960. Flame photometric method for sodium, potassium and calcium. In: Modern methods of plant analysis Paech, K. and Tracey, M. V., (Eds.) Vol. 5, pp: 3-5 Springer-Verlag, Berlin.

Winter, E. and J. Preston, 1982. Salt tolerance of *Trifolium alexandrinum*. L. IV Ion measurements by x-ray microanalysis in unfixed, frozen hydrated leaf cells at various stages of salt treatment. Aust. J. Pl. Physiol., 9: 251-259.

Woods and Mellon, 1941. Chlorostannous-Molybdophosphoric blue colour method in sulfuric acid system. In: Soil chemical analysis by Jackson, M.L. (1958). Prentice Hall International, Inc. London.