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Effect of Salinity, Mineral Ions and Organic Solutes Contents on *Vicia faba* L.

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Abstract: The effect of different concentrations of NaCl on growth, mineral ions and organic solutes content of *Vicia faba* were investigated. Experimental measurements were made after 50 days of growth in vessels containing Hoagland nutrient solution under controlled climate conditions. The NaCl salinity induced changes in the growth criteria of the *Vicia faba* plant. However there is a gradual decline in these parameters by increasing salinity in the culture media. This reduction was more pronounced at the higher doses of the salt. It was also observed that proline, saccharide and soluble protein contents increased in the shoot juice of the investigated plant. Glycolic and citric acids were slightly affected by increasing salinity in the nutrient solution, whereas oxalic, succinic and fumaric acids were significantly increased up 0.0, 4.3 and 2.5 times of the control samples.

Key words: Growth, mineral ions, organic solutes, salinity, *Vicia faba*

INTRODUCTION

Saline soils are very common in regions of arid and semi-arid climate, due to the high evaporation process, a well as due to the low transport of soluble salts to the areas and/or rivers as a result of low rainfall. Saline conditions have reported to affect nodule intiation and growth as well as mineral ions and organic solutes of host plants (Sprent and Sprent, 1990). A condition of ion excess within the plant has been considered an important factor that limits growth under saline conditions. Studies effects of an excessive ion concentration on growth, mineral ions and organic solutes contents and Onkware, (2000) have shed some light on the mode of salinity injury. Gorham *et al*. (1985) attributed the inhibitory effects of salinity on plant growth to water stress, resulting from low osmotic potentials of the soil water as well as to ion toxicity or nutritional imbalance. Ionic balance, enzymatic activity and hormonal balance were also disturbed by salt stress (Paraksh *et al*., 1982; Cramer *et al*., 1986; Ben-Hayyim *et al*., 1987).

The present study aims to know the extent to which *Vicia faba* plants can tolerate the salt stress.

MATERIALS AND METHODS

Seeds of *Vicia faba* of the same size and color were germinated in November 2003 in quarz sand. One week old seedlings of uniform size and appearance were transferred into Hoagland nutrient solution containing NaCl to maintain osmotic potential-0.05, -0.1, -0.2, -0.4, -0.8, -1.6 and -2 MPa (by using osmometer 800 Slamed). Nutrient solution in black vessels, (1.5 L) were replaced every four days initially and every two days after two weeks culture. Nutrient solution pH 6.8 was checked every two days and adjusted by using buffer if necessary (Fig. 1). The vessels ere aerated thoroughly all over the experimental period and kept under temperature of about 30°C during the day and about 20°C at the night with relative humidity fluctuating between 50 and 80%.

Light intensity, light duration was nearly similar to the conditions of natural habitats, 16 h light 8 h dark. There were 16 replicates for each treat and the

Fig. 1: The effect of different concentrations of NaCl solution on the growth of *Vicia faba* L.

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experiment was arranged in a randomized design. Plants were harvested after 7 weeks. Roots were washed with distilled water. Shoot and root lengths were measured. Dry mass of shoots and roots were determined, osmotic potentials by using osmometer, proline contents were analyzed following the procedure of Bates *et al.* (1973) and the contents of saccharides organic acids were determined by using gas chromatography, Proline, Suger and Orgnic acids were determined in the juice of the squeezed fresh shoot system. Tissues were dried in a forced air oven at 80°C for 48 h and ground in Wiley Mill (40 mesh). Samples were ashed for 3 h at 500°C HCl and filtered (Whatman No. 42). The sample was diluted further with glass-distilled water and mixed 10:1 with La_2O_3 HCl solution. The concentrations of calcium, magnesium, sulphur and Sodium were determined by atomic absorption spectrophotometry and of potassium by flame emission. Phosphorus concentration was determined from the same sample using a molybdovanadate reaction (Greweling, 1976).

Nitrogen was determined in micro-kjeldahl digest of dried and round plant material using an Orion ammonia ion-specific electrode (model 95-10) Power. Choloride was measured by titration method after. Values for all nutrient analyses were converted to milliequivalent dry weight of plant material.

RESULTS AND DISCUSSION

The NaCl salinity induced mostly significant changes in the growth criteria of the studied plant increasing levels of salinity affected total dry weight; both shoot and root lengths as well as on lateral root initial (Table 1).

At -0.4 MPa the dry mass of shoots and roots was reduced by 30 and 24% with respect to control, respectively. At -2.0 MPa shoot and root dry mass were reduced to 12 and 10% of the control, respectively. It is to be mentioned that all NaCl concentrations resulted in a decrease of growth, at the same time there is a significant changes increase in the nutrient solution (Table 1).

The preservation of normal number of lateral root initials might increase essential supply of cytokinins which improves its salt resistance (Waisel, 1991).

The decrease in dry weight of roots should be related to the disturbance of metabolic activity. Spyropoulos (1986) as well as El-Sharkawi and Springuel (1977) suggested that *Trigonella foenomgraecum* seeds its osmotic potential through reduction in the imbibition of salt solution. Flowers *et al.* (1977) revealed that increased succulence as a protective adaptive response against salt stress. Current data from shoots that increasing salinity had a high effect on shoot to root ratio (Table 1). He and Cramer (1993), Amin (1999) and Ali (1998) as well as Al-Khateeb (2006) and Flowers (2004) have shed some light on the mode of salinity injury, but the exact mechanism by which an excessiveion concentration suppresses plant growth is not clear.

NaCl has also been shown to cause extensive damage to membrane integrity, resulting in severe injury to the cells (Houle *et al.*, 2001); (Barnett and Naylor, 1966). Table 2 shows that there is a positive correlation between osmotic values of the irrigation solution and the concentrations of the organic solutes in plant shoots, the increase was greater especially in shoots subjected to high values of salt stress -1.6 and -2.0 MPa.

It has been known that water and salt stress induce the accumulation of proline, (Batanouny *et al.*, 1991; Batanouny and Ebeid, 1981). The role of proline accumulated in the plant cells as a result of salt stress could be considered either as a help for resistance against water and salt stress (Barnett and Naylor, 1966), or as an indicator of water and salt status in the plant cells. Several other roles have been suggested for proline accumulation. These include a role as storage compound for carbon and nitrogen, (Schobert, 1977; Singh *et al.*, 1973) has suggested that proline is involved in preserving the hydration of proteins in dehydrated tissues, therefore contributed to the survival of cellular functions. The biochemical mechanism by which proline

Table 1: Effect of NaCl on shoot and root dry weights, shoot and root lengths and shoot/root ratio of *Vicia faba* after 7 weeks treatment

NaCl	Shoot dry (wt. g plant^{-1})	Root dry (wt. g plant^{-1})	Shoot length (cm)	Root length (cm)	Shoot/root ratio	No. of root initials
0.0	1.91±0.12	1.73±0.12	38.3±1.3	17.8±0.8	2.15	23±2.5
-.05	1.94±0.11	1.75±0.14	32.1±2.2	16.8±1.1	1.91	43±-3.1
-0.1	1.34±0.10	1.23±0.13	27.9±1.3	14.9±1.0	1.87	68±3.6
-0.2	1.14±0.04	1.11±0.12	17.2±1.2	10.1±0.7	17.0	77±3.8
-0.4	0.55±0.06	0.41±0.04	6.8±0.27	6.6±0.30	1.03	71±3.1
-0.8	0.42±0.04	0.35±0.04	4.0±0.32	5.2±0.30	0.77	58±3.0
-1.6	0.27±0.03	0.25±0.03	2.8±0.26	3.9±0.18	0.72	---
-2.0	0.23±0.03	0.19±0.02	1.0±0.19	1.7±0.22	0.64	

Table 2: The effect of salt stress on water content and accumulation of organic solutes in *Vicia faba* shoot system

NaCl OP (MPa)	Water content (%)	Proline aemoles (g D. wt.)	Total soluble protein (mg g D.wt.)	Soluble sugars (mg g D. Wt.)
0.0	62.43±2.7	3.8±0.21	5.1±1.7	1.7±0.1
-0.05	59.83±2.4	3.5±0.20	5.5±0.24	1.95±0.12
-0.1	55.71±1.9	4.6±0.23	5.3±0.28	2.46±0.14
-0.2	50.65±2.0	6.71±0.31	7.2±0.3	3.19±0.15
-0.4	43.87±1.7	8.4±0.39	8.6±0.29	4.34±0.17
-0.8	38.97±1.5	11.8±0.6	9.1±0.34	5.98±0.18
-1.6	36.74±1.3	13.1±0.62	11.3±0.41	6.55±0.27
-2.0	34.81±1.4	19.0±0.9	14.2±0.52	7.28±0.31

Table 3: Organic acid contents (milliequivalent) in the shoot Juice of *Vicia faba* under salt stress on dry weight bases

NaCl O P. (MPa)	Glycolic acid	Oxalic acid	Succinic acid	Fumaric acid	Tartaric acid	Citric acid
0.0	8.28±0.42	17.76±0.86	15.07±0.75	40.06±2.5	16.0±0.8	8.4±0.32
-0.4	9.03±0.43	31.40±1.7	15.91±0.76	65.60±3.28	19.28±0.83	8.39±0.37
-0.8	10.12±0.43	63.21±2.65	32.01±1.09	71.82±4.20	29.90±1.44	9.28±0.41
-1.6	11.30±0.45	121.21±5.06	39.38±2.29	83.51±4.49	41.18±1.81	9.63±0.39
-2.0	11.70±0.44	136.40±7.13	66.04±3.18	98.43±5.81	75.47±0.66	9.43±0.40

Table 4: Effect of salt stress on element contents {Milliequivalent) (d.m.)} in *Vicia faba* shoots and roots

Organ treatment Ψ_s (Mpa)	N	P	S	Ca	Mg	K	Na	Cl
Shoot								
0.0	235	35.5	268.2	60.9	38.2	99.36	14.35	53.5
-0.05	450	48.7	286.9	60.7	34.4	80.38	19.14	118.3
-0.1	599	55.8	299.4	58.9	29.4	77.03	29.1	132.4
-0.04	728	62.3	324.3	53.4	25.2	70.8	39.6	163.4
-0.8	771	72.0	417.9	40.3	20.9	51.3	58.3	177.5
Root								
0.0	97	46.5	168.4	55.8	30.4	102.2	8.7	39.4
-0.05	86	49.4	180.9	43.9	26.1	97.1	17.8	64.8
-0.1	124	30.7	212.1	36.9	21.0	80.36	38.3	90.1
-0.4	189	80.3	424.2	30.4	20.3	62.1	54.3	121.1
-0.8	197	91.6	299.4	27.9	17.3	45.4	76.5	160.6

is accumulated in plants is not understood. Studies by Singh *et al.* (1973) on barley and Boggess and Stewart (1976) on vean, indicates that water stress may enhance the synthesis from glutamic acid. Jaeger and Meyer (1977) has suggested that the proline accumulated within water-stressed plants may be involved in osmotic adjustment as a compatible solute.

The total soluble protein and soluble sugar were greatly increased nearly three times and four times of the control respectively. It is well known that injury by salinity affects greatly the protoplasmic proteins and/or sugar synthesis. This increase in both components might play a positive role in osmoregulation in turn exert a positive role in the alleviation of the imposed salt stress (Osmond, 1976; Handa *et al.*, 1983) using cultured tomato and pepper cells, respectively adapted to water stress, found that the concentration of sugars in the cells increased with the degree of adaptation to salinity concentration as high as 600 mM in the cells.

Small but significant increases in the free pools of amino acids found predominantly in proteins (cytokine, valine, isoleucine, leucine, tyrosine and phenylalanine) may indicate that severe stress has intered with protein synthesis or turnover, as suggested in other studies on bean (Lopez-Berenguer *et al.*, 2004) cotton (Mcmichael and El-More, 1977) and sunflower (Lawlor and Fock, 1977) but evidently not in barley leaves (Singh *et al.*, 1973; Keiffer and Ungar, 1997).

Organic acids compositions for the plant under study were also affected by salt stress condition. Table 3 shows that the large increase (nearly 8.0 fold in oxalic acid and 4.5 fold in succinic acid and 2.5 folds in fumaric acid) with stress.

Organic acids were accumulated in under salt stress, this may be due to the deviation in the main cycles such as Krab cycle or glycolysis pentose phosphate and glycolate cycles.

It can be suggested that the increase in organic acids in could be on the exense of anabolism (biomass gain) and used as compatible solutes beside proline and soluble sugars to keep the water balance between the cytoplasm and vacuole, (Boggess and Stewart, 1993). Oxalic acid, succinic acid and Fumaric acids are the more dominant acids in the plant cells. The amounts of glyconic and citric were comparatively smaller, but in all cases the values, under stress, dramatically increased (at lest two times).

The results of Table 4 reveal, that under salt stress, the N and P contents in both shoots and roots slightly increased S has up two folds increased, Na and Cl contents in both shoots and roots were significantly increased, whilst the Ca and Mg contents in both shoots and roots were reduced to more than 50% of the control, also K was significantly reduced.

Reduction of plant growth under salt stress is usually attributed to osmotic stress due to a lowering of external water potential or to specificion effect on metabolic processes in the cell (Bennett and Adams, 1970; Lynch *et al.*, 1985) suggested that it is the (Ca)/(total cation) ratio in nutrient solution that influences growth and Ca sufficiency. Cramer *et al.* (1986) suggest that it is the interaction between Ca and Na activities in nutrient solution that is significant in determining root growth of cotton in saline solution.

The effect of salinity was significant concerning Cl, Na, Mg and P of *Crotolaria aegyptiaca*.

Increasing salinity in irrigation was almost associated with increased accumulation of Cl, Na and P.

On the other hand, there is growing evidence that salt stress inhibits the uptake and transport of Ca in nonhalophytes (Lahaye and Epstein, 1969). Ca is essential to the selectivity of membrane transport Processes, a function that (Lynch *et al.*, 1985) proposed for its role in improving salt tolerance (Kabir *et al.*, 2004) demonstrated that the ionic component of salt stress displace Ca from membrance of corn root cells and associated with this a loss of K from the plant tissues and an alterating of K\Na selectivity (Kabir *et al.*, 2004) .

The increase of total ions content under severe salt stress, suggests that vicia faba plant depends on the accumulation of mineral ions together with the organic solutes in its cytoplasmic osmoregulation.

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