

EFFECT OF SALINITY ON GROWTH, MINERAL IONS AND ORGANIC SOLUTES CONTENTS IN *ARTEMISIA MONOSPERMA*

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The effect of different concentration of NaCl on growth, mineral ions and organic solutes content of *Artemisia monosperma* were investigated. Experimental measurements were made after 70 days of growth in vessels containing Hoagland nutrient solution under controlled climatic conditions. Increased salt stress decreased dry mass, length of shoot and root as well. At the same time, the osmotic potential of NaCl had a great effect on the shoot/root ratio. The root initials also increased and the number of root initials decreased by increasing NaCl concentrations up to 0.4 MPa.

It was also observed that proline, saccharide, and soluble protein contents increased in the shoot sap of the investigated plant. Glycolic, fumaric, tartaric, and succinic acid were clearly affected by increasing salinity in the nutrient solution, whereas oxalic and citric acids were significantly increased up 8.5 and 12 times of the control samples.

Key words: *Artemisia monosperma*, growth, mineral ions, organic solutes, salinity

INTRODUCTION

A condition of ion excess within the plant has been considered an important factor that limits plant growth under saline conditions. Studies effects of an excessive ion concentration on growth, mineral ions and organic solutes contents Greenway and Munns (1980) and Young and Lovett-Doust (1999) and Onkware (2002) 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 (Chavan and Karadge 1980, Cramer *et al.* 1986, Ben Hayyim *et al.* 1987).

Plants increase the tolerance against water or salt stress by decreasing osmotic potential by accumulation of solutes, which allow cell enlargement and plant growth. Many solutes may be used in osmotic adjustment including inorganic ions, such as Na^+ , K^+ and Cl^- (e.g. Ford and Wilson 1981, Wyn Jones and Gorham 1983), and saccharides and amino acids, especially proline (Morgan 1984, Drossopoulos *et al.* 1985, Navari-Izzo *et al.* 1990).

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

MATERIALS AND METHODS

Seeds of *Artemisia monosperma* were collected from plants naturally growing around Cairo Suez desert road 70 km from Cairo and germinated in quartz sand. One-week-old seedlings of uniform size 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 cl Slamed. Nutrient solutions 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. The vessels were aerated thoroughly all over the experimental period and kept in acclimatised chamber under temperature of 35 °C during the day and 20 °C at night with relative air humidity ranging between 50 and 80%.

Light intensity, and light duration was programmed similar to the conditions of natural habitats, 16 hours photoperiod day 8 hours light. There were 16 replicates for each treatment. Plants were harvested after 10 weeks and roots were washed with distilled water. Shoot and root lengths were measured. Dry mass of shoots and roots were determined, osmotic potentials using osmometer, proline contents were analysed following the procedure of Bates *et al.* (1973) and the contents of saccharides according to Umbreit *et al.* (1959). Organic acids were determined using gas chromatography, proline, sugar and organic 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 hours and ground in Wiley Mill (40 mesh). Samples were ashed for 3 hours at 500 °C HCl, and filtered (Whatman No. 42). The sample was diluted with glass-distilled water and mixed 10:1 with $\text{La}_2\text{O}_3/\text{HCl}$ solution. The concentrations of calcium, magnesium, sulphur and sodium were determined by atomic absorption spectro-

photometry to add type and potassium by flame emission. Phosphorus concentration was determined from the same sample using a molybdo-vanadate reaction (Grewling 1976).

Nitrogen was determined in micro-Kjeldahl digest of dried and ground plant material using an Orion ammonia ion-specific electrode (model 95–10). Details of the procedure are given by Powers *et al.* (1981). Chloride was measured by titration method. Values for all nutrient analyses were expressed in percentage per dry weight of plant material.

RESULTS AND DISCUSSION

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 33 and 30% with respect to control, respectively. At -2.0 MPa shoot and root dry mass were reduced to 64 and 58% of the control, respectively. It was mentioned that all NaCl concentrations resulted in a decrease of growth, at the same time there is no significant increase in the number of lateral root initials with increasing the NaCl concentration in the nutrient solution (Waisel 1991). The preservation of the normal number of lateral root initials proves that the plant the essential supply of cytokinins and so improves its salt resistance.

The decrease of root dry weight should be related to the disturbance of metabolic activity, which was significantly affected by salinity. Spyropoulos (1986) as well as El-Sharkawi and Springuel (1977) suggested that *Trigonella foenum-graecum* seeds control its osmotic potential through reduction of salt solution. Presented data of *Artemisia monosperma* showed that increasing salin-

Table 1
Effect of NaCl on shoot and root dry weights, shoot and root lengths and shoot/root ratio of *Artemisia monosperma* after 10 week treatment (mean $\pm$ SE)

NaCl	Shoot (d. wt. g/ plant)	Root (d. wt. g/ plant)	Shoot length (cm)	Root length (cm)	Shoot/ root ratio	No. of root initials
-0.0	0.82 $\pm$ 0.06	0.73 $\pm$ 0.05	8.3 $\pm$ 0.31	7.8 $\pm$ 0.40	1.06	51 $\pm$ 2.5
-0.5	0.98 $\pm$ 0.08	0.95 $\pm$ 0.07	9.1 $\pm$ 0.42	8.8 $\pm$ 0.39	1.09	63 $\pm$ 3.1
-0.1	0.76 $\pm$ 0.07	0.74 $\pm$ 0.06	7.9 $\pm$ 0.33	7.5 $\pm$ 0.37	1.05	68 $\pm$ 3.6
-0.2	0.64 $\pm$ 0.07	0.59 $\pm$ 0.05	7.2 $\pm$ 0.29	6.9 $\pm$ 0.29	1.04	67 $\pm$ 3.8
-0.4	0.55 $\pm$ 0.06	0.51 $\pm$ 0.04	6.8 $\pm$ 0.27	6.6 $\pm$ 0.32	1.03	61 $\pm$ 3.1
-0.8	0.42 $\pm$ 0.04	0.39 $\pm$ 0.04	6.0 $\pm$ 0.32	5.9 $\pm$ 0.30	1.02	58 $\pm$ 3.0
-1.6	0.37 $\pm$ 0.03	0.35 $\pm$ 0.03	5.0 $\pm$ 0.26	4.9 $\pm$ 0.18	1.02	
-2.0	0.30 $\pm$ 0.03	0.31 $\pm$ 0.02	4.3 $\pm$ 0.19	4.4 $\pm$ 0.22	0.98	

ity had not significant effect on shoot to root ratio (Table 1). He and Cramer (1993), Amin (1999) as well as Ali (1998) have shed some light on the mode of salinity injury, but the exact mechanism by which an excessive ion concentration suppresses plant growth is unclear.

NaCl application also confirmed extensive damage of membrane integrity, resulting in severe injury of the cells (Flowers *et al.* 1977, Houle *et al.* 2001). Data presented in Table 2 showed that there was 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 was found that water and salt stress induced the accumulation of proline (Barnett and Naylor 1966, Batanouny *et al.* 1991). The function 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 (Batanouny and Ebeid 1981) or as an indicator of water and salt status in the plant cells. Several other functions for proline accumulation are considered. These include a role as storage compound for carbon and nitrogen. Barnett and Naylor (1966), Schobert (1977) suggested that proline could be involved in preserving the hydration of proteins in dehydrated tissues, and thus could contribute to survive of the cells. The biochemical mechanism by which proline is accumulated in plants is not understood. Results published by Boggess and Stewart (1976) on barley and Jaeger and Meyer (1977) on bean indicated that water stress induced the synthesis of glutamic acid. Osmond (1976) suggested that the proline accumulated within water-stressed plants may be involved in osmotic adjustment as a compatible solute only within the cytoplasm solute only with increase of inorganic ions and carboxylates accounting for osmotic adjustment

Table 2
The effect of salt stress on water content and accumulation of organic solutes in *Artemisia monosperma* shoot system (mean $\pm$ SE)

NaCl (O.P. MPa)	Water content (%)	Proline (mg g moles photoperiod, g d. wt.)	Total soluble protein (mg/g d. wt.)	Soluble sugars (mg/g d. wt.)
-0.0	62.43 $\pm$ 2.7	23.8 $\pm$ 1.7	4.1 $\pm$ 1.7	1.5 $\pm$ 0.1
-0.05	59.83 $\pm$ 2.4	37.5 $\pm$ 1.8	4.5 $\pm$ 0.24	1.8 $\pm$ 0.12
-0.1	55.71 $\pm$ 1.9	42.6 $\pm$ 2.1	5.3 $\pm$ 0.28	2.3 $\pm$ 0.14
-0.2	50.65 $\pm$ 2.0	56.7 $\pm$ 2.3	7.2 $\pm$ 0.3	3.1 $\pm$ 0.15
-0.4	43.87 $\pm$ 1.7	63.4 $\pm$ 2.8	7.7 $\pm$ 0.29	4.2 $\pm$ 0.17
-0.8	38.97 $\pm$ 1.5	71.8 $\pm$ 3.2	9.1 $\pm$ 0.34	6.3 $\pm$ 0.18
-1.6	36.74 $\pm$ 1.3	83.1 $\pm$ 3.6	11.3 $\pm$ 0.41	6.5 $\pm$ 0.17
-2.0	34.81 $\pm$ 1.4	89.0 $\pm$ 3.9	14.2 $\pm$ 0.52	7.2 $\pm$ 0.21

Table 3

Organic acid contents (milliequivalent) in the shoot Juice of *Artemisia monosperma* under salt stress on dry weight bases (mean $\pm$ SE)

NaCl (O.P. MPa)	Glycolic acid	Oxalic acid	Succinic acid	Fumaric acid	Tartaric acid	Citric acid
0.0	8.28 $\pm$ 0.42	17.76 $\pm$ 0.86	15.07 $\pm$ 0.75	50.06 $\pm$ 2.50	0.0	258.4 $\pm$ 12.3
-0.4	9.03 $\pm$ 0.43	31.40 $\pm$ 1.7	15.91 $\pm$ 0.76	65.60 $\pm$ 3.28	9.28 $\pm$ 0.43	388.39 $\pm$ 19.1
-0.8	10.12 $\pm$ 0.43	51.21 $\pm$ 2.65	22.01 $\pm$ 1.09	81.82 $\pm$ 4.20	9.90 $\pm$ 0.44	905.28 $\pm$ 45.8
-1.6	11.30 $\pm$ 0.45	101.03 $\pm$ 5.06	44.03 $\pm$ 2.29	91.51 $\pm$ 4.49	11.18 $\pm$ 0.51	1,680.63 $\pm$ 82.3
-2.0	11.70 $\pm$ 0.44	147.50 $\pm$ 7.13	66.04 $\pm$ 3.18	108.18 $\pm$ 5.81	15.47 $\pm$ 0.66	3,298.43 $\pm$ 166.0

within the vacuole. Wyn Jones and Gorham (1983) have advanced a similar argument for the role of glycine betaine during the osmotic adjustment in halophytes. The total soluble protein and soluble sugars 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. The mechanism by which soluble sugar is accumulated in water-stressed leaves is difficult to establish.

Significant increase in the free pools of amino acids found predominantly in proteins (cytokine, valine, isoleucine, leucine, tyrosine and phenylalanine) could indicate that severe stress has interfered with protein synthesis or turnover, as was suggested for other studies on bean (Jaeger and Meyer 1977) cotton (McMichael and El-More 1977), and sunflower (Lawlor 1970), but evidently not for barley leaves (Singh *et al.* 1973, Keiffer and Ungar 1997).

Organic acids compositions for studied plant were also affected by salt stress condition. Data presented in Table 3 showed dramatic increase of the different organic acids. The large increase (nearly 12.0 fold) in oxalic acid, (5.5 fold) succinic acid and (6.6 fold) citric acid with stress was found.

Organic acids were accumulated in *Artemisia monosperma* under salt stress, which may be due to the deviation in the main cycles such as Krebs' cycle or glycolysis pentose phosphate and glycolate cycles. It could be suggested that the increase of organic acids in *A. monosperma* could be on the expense of anabolism (biomass gain) and used as compatible solutes beside proline and soluble sugars to keep the water balance between the cytoplasm and vacuole (Wyn Jones and Gorham 1983). Oxalic, succinic and citric acids are the more dominant acids in the plant cells. The concentration of glyconic, fumaric and tartaric acids was comparatively smaller, but in all cases the values, under water stress, dramatically increased (at least one three times).

The results in Table 4 showed, that under salt stress, the N and P contents in both shoots and roots significantly decreased. In general S, Na and Cl con-

Table 4
Effect of salt stress on element contents (milliequivalent) (d. m.) in *Artemisia monosperma* shoots and roots

Organ	Treatment (ψ_s MPa)	N	P	S	Ca	Mg	K	Na	Cl
Shoot	0.0	771	75.5	268.2	60.9	38.27	49.36	4.35	53.5
	-0.05	728	68.77	286.9	65.7	44.4	50.38	9.14	118.3
	-0.1	599	65.86	299.4	68.9	49.4	57.03	29.1	132.4
	-0.4	450	52.3	324.3	73.4	55.0	70.8	39.6	163.4
	-0.8	235	32.0	417.9	80.3	50.9	91.3	58.3	177.5
Root	0.0	197	46.5	168.4	35.8	30.4	22.2	8.7	39.4
	-0.05	186	49.4	180.9	43.9	32.1	47.1	17.8	64.8
	-0.1	124	40.7	212.1	46.9	41.0	60.36	38.3	90.1
	-0.4	79	20.3	424.2	50.4	40.3	82.1	54.3	121.1
	-0.8	47	11.6	299.4	57.9	31.3	105.4	76.5	160.6

tents in both shoots and roots of *Artemisia monosperma* increased, contents of potassium in the mentioned plant usually decreased, whilst the Ca and Mg contents in both shoots and roots was not significantly affected.

Reduction of plant growth under salt stress is usually attributed to osmotic stress due to a lowering of external water potential or to specific ion effect on metabolic processes in the cell. Bennett and Adams (1970) suggested that it is the (Ca)/(total cation) ratio in nutrient solution that influences growth and Ca sufficiency. Cramer *et al.* (1986) suggested 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.

It is commonly accepted that competition exists between Na and K leading to a reduced level of internal K at high external NaCl concentrations. On the other hand, there is evidence that salt stress inhibits the uptake and transport of Ca in non halophytes (Lynch *et al.* 1985). Ca is essential for the selectivity of membrane transport processes, a function that LaHaye and Epstein (1969) proposed for its role in improving salt tolerance. Lynch *et al.* (1987) demonstrated that the ionic component of salt stress displaces Ca from membrane of corn root cells, and associated with this a loss of K from the plant tissues and an altering of K\Na selectivity.

The increase of total ions content under severe salt stress, suggested that *Artemisia monosperma* plants depends on the accumulation of mineral ions together with the organic solutes in its cytoplasmic osmoregulation.

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