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Physiological Aspect of Nacl-salt Stress Tolerant among Cucurbitaceous Cultivars

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Abstract: Experiment was conducted in the experimental farm of Agricultural Botany Department, Faculty of Agriculture, Ain Shams Univ. during the two successive seasons of 2008 and 2009 to study the responses of four cucurbitaceous cultivars on salinity effects: Shintosa Supreme pumpkin (Cucurbita maxima x C. moschata) 'Major', Bottle gourd (Lagenaria siceraria), Gourd Black Seed (Cucurbita maxima) and pumpkin (Cucurbita moschata). Four salinity levels were applied through irrigation water containing 0, 2500, 3500 and 4500 PPM NaCl. Samples were taken at six weeks after planting to determine plant growth, leaf electrolyte leakage percentage (Membrane Permeability) and salt injury index (SII). As well as, the distribution of chloride, sodium and potassium ions in all plant parts. The dry and fresh weights of stems and roots, plant height, leaf area and root length in the 4 cultivars decreased gradually with increasing NaCl concentration comparing with control plants. Shintosa Supreme pumpkin showed the best salt tolerance at 3500 ppm as indicated by plant height, leaf area and root length, fresh and dry weights of stems and roots. With increasing NaCl concentrations, the salt injury index of all cultivars increased significantly. There was insignificant difference in the salt injury index between pumpkin and Bottle gourd cultivars under different levels of salinity. Gourd Black Seed showed significant increase in salt injury index comparing with other cultivars at the 3500 and 4500 ppm NaCl. Salt injury index of Shintosa Supreme pumpkin was lower than that of other cultivars. The membrane permeability of the four cultivars increased significantly with increasing concentrations of NaCl; the membrane permeability of Shintosa Supreme pumpkin was lower than that of other cultivars. However, a difference between the cultivars in response to the salinity was apparent. In addition, the contents of Na+ in the root and stem of Shintosa Supreme pumpkin cultivars increased significantly but in the leaf decreased, whereas the contents of K+ versa decreased significantly, resulting in an increase in the Na+/K+ ratio when NaCl concentrations increased.

Key words: Cucumber; Cucurbita rootstock; Salt stress; Physiological response; Mineral nutrition; Sodium; Chloride

INTRODUCTION

Salinity is one of the most significant factors limiting crop productivity. Approximately 20% of the world's cultivated land and nearly half of all irrigated lands are affected by salinity (Zhu, 2001). Plant species and cultivars within a crop greatly differ in their responses to salinity (Dasgan *et al.* 2002). Salinity and drought are becoming particularly widespread in many regions, and may cause serious salinization of more than 50% of all arable lands by the year 2050. Drought, salinity, extreme temperatures and oxidative stress are often interconnected, and may induce similar cellular damages (Wang *et al.* 2003).

Salinity affects almost every aspect of the physiology and biochemistry of plants and signi?cantly reduces yield. As saline soils and saline waters are common around the world, great effort has been devoted to understanding physiological aspects of tolerance to salinity in plants, as a basis for plant breeders to develop salinity-tolerant genotypes. In spite of this great effort, only a small number of cultivars, partially tolerant to salinity, have been developed (Cuartero, *et al* , 2006).

Understanding to the physiology of salt tolerance in plants became important for the effective solution of the problem of salinity in agricultural and horticultural soils (Larcher, 1994). A wide range of environmental stresses (such as high and low temperature, drought, alkalinity, salinity, UV stress and pathogen infection) are potentially harmful to the plants (Van Breusegem *et al.* 2001).

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Attempts to increase salinity tolerance of sensitive crops by traditional breeding have not been very successful due to the physiological and genetic complexity of salt tolerance in plants (Flowers, 2004).

Salt stress has been reported to cause an inhibition of growth and development, reduction in photosynthesis, respiration and protein synthesis in sensitive species (Boyer, 1982; Meloni, *et al*., 2003; Pal, *et al*., 2004). Salt stress, like many abiotic stress factors, also induces oxidative damage to plant cells catalyzed by reactive oxygen species (Mittler, 2002; Demidchik *et al*., 2003; Azevedo-Neto, *et al*., 2006).

Vegetable production is threatened by increasing soil salinity particularly in irrigated croplands which provide 40% of the world's food (FAO 2002). Vegetables are often salt-sensitive plants (Shannon and Grieve 1999) and the ability to improve the salt tolerance of vegetables using traditional breeding programmes is limited over the short term (Flowers 2004). On the other hand, using salt-tolerant rootstock is an alternative effective method for increasing plant productivity under salinity stress. The synthesis and accumulation of compatible solutes is a ubiquitous mechanism for osmotic adjustment in plants (Trajkova, *et al.*, 2006). Therefore grafted cucumber on salt tolerance root stocks can improve cucumber adaptation to salt stress (Yang *et al*. 2006). The main target of this study is to investigate tolerance potential of four cucurbitaceous cultivars to salt stress. Investigations extend also to select the most salt tolerance among cultivars to use as rootstocks for grafted salt-sensitive cucumber scions. So, by this way could cucumber plant avoid deleterious effect of salinity and achieve promising yield (El-Shraiy, *et al* 2011; in publication).

MATERIALS AND METHODS

1. *Plant Material:*

Pots experiment was conducted at the Experimental Farm of the Agricultural Botany Department, Faculty of Agriculture, Ain Shams University, at Shoubra El-Kheima, Kalubia, Egypt during the two cultivated seasons of 2008 & 2009. Four cucurbitaceae cultivars: (1) Shintosa Supreme pumpkin (Cucurbita maxima x C. moschata), (2) Bottle gourd (Lagenaria siceraria), (3) Gourd Black Seed (Cucurbita maxima), (4) pumpkin (Cucurbita moschata); seeds were sown in plastic pots 40 cm diameter and 45 cm depth in September 10th in both seasons, each pot was filled with 12 kg of sandy soil with 3 seeds/pot. Pots were arranged in randomized complete design with four replicates. Each replicate consisted of three pots with three plants per pot.

2. *Treatments:*

Four concentrations of NaCl-Salt treatments; (0, 2500, 3500 and 4500 PPM) were applied with irrigation water. Plants were irrigated 6 times a week with Hogland solution (containing standard nutrient additional salt) according to Kong *et al*., (2005).

3. *Growth Parameters:*

Six weeks after sowing, three plants from each replicate were harvested, and growth parameters; such as plant height, stem and root fresh weight, stem and root dry weight, leaf area and root length were recorded. The plant material for dry weight was dried at 70? C for 48 hours. Also, leaves samples were taken to determine leaf electrolyte leakage percentage (EL), salt injury index (SII). Mineral concentrations (Na, K and Cl) in stem, root and leaves were determined.

4. *Measurement of Electrolyte Leakage Percentage (Membrane Permeability)*

For measurement of electrolyte leakage, 20 leaf discs (10mm in diameter) from the young fully expanded leaves from two plants per replicate were placed in 50 mL glass vials, rinsed with distilled water to remove electrolytes released during leaf disc excision. Vials were then filled with 30mL of distilled water and allowed to stand in the dark for 24 h at room temperature. Electrical conductivity (EC1) of the bathing solution was determined at the end of incubation period. Vials were heated in a temperature-controlled water bath at 95? C for 20 min, and then cooled to room temperature and the electrical conductivity (EC2) was measured. Electrolyte leakage was calculated as percentage of EC1/EC2 (Shi, *et al*., 2006).

5. *Calculation of the Salt Injury Index*:

The plants were grown for 30 days under different salt stress conditions and then classified for their salt tolerance by visual appearance; classification of the standard and calculation of the salt injury index followed the method of Zhang *et al*. (2003). The classified criteria of salt injury are: 0 level (non-sufferable injury), 1 level (one- third of the leaf edge suffered injury), 2 level (two-thirds of the leaf edge suffered injury), 3 level (full leaf edge suffered injury or one-third of the lamina desquamated), 4 level (two-thirds of the lamina desquamated), 5 level (full lamina desquamated).

The salt injury index was calculated by using the equation:

Salt injury index (%) = Σ (level value × plant number) × 100/ (the highest level value × total plant number).

6. *Mineral Analysis:*

The dried stem, root and leaves (six leaf from the top) were used for determination of Na+, K+, and Cl^- concentrations, following the methods described by Xu *et al*. (2006). Na+ and K+ concentrations were determined by atomic absorption spectrometry (Varian Spectra AA 220, USA). Cl^- concentrations were determined by silver nitrate (AgNO3) titration using a neutral indicator agent containing 4.2% (w/v) K2CrO4 and 0.7% (w/v) K2Cr2O7.

7. *Statistical Analysis:*

Growth Parameters (plant height, leaf area, stem and root fresh and dry weights , root length , membrane permeability (%), salt injury index and the average of two season (2008 and 2009) for ion concentrations in cucurbitaceous cultivars were determined by analysis of variance using SAS software (SAS Institute, Cary NC). Differences between means treatment were separated by the least signi?cant difference (L.S.D.) test at a 0.05 probability level.

RESULTS AND DISCUSION

Growth Parameters:

Data in Tables (1 and 2), four cucurbitaceous cultivars at different concentrations of NaCl showed differences in the efficiency of growth parameters as compared with untreated plants. Stem and root dry weights, plant height, leaf area and the root length of the cultivars decreased significantly with increasing levels of NaCl concentration in both seasons. The highest values of plant height, leaf area, shoot fresh and dry weights , root fresh and dry weights and root length were obtained by Shintosa Supreme pumpkin (Cucurbita maxima x C. moschata) at 3500 ppm NaCl which recorded 212 cm of plant height, 250.93 cm2 leaf area, 141.4 gm stem fresh weight, 45.6 gm stem dry weight, 42.9 gm root fresh weight, 30.8 gm root dry weight and 63 cm root length comparing with pumpkin (Cucurbita moschata) plants which recorded 70 cm of plant height, 105.73 cm2 leaf area, 71 gm stem fresh weight, 16 gm stem dry weight, 15 gm root fresh weight, 11 gm root dry weight and 38 cm root length respectively, followed by, Bottle gourd(Lagenaria siceraria) and Gourd Black Seed (Cucurbita maxima) in both seasons respectively. The results indicated that growth inhibition caused by NaCl was more severe in Gourd Black Seed and Bottle gourd than Pumpkin and Shintosa Supreme pumpkin plants.

Table 1: Effect of Nacl concentrations on growth parameters of cucurbitaceous cultivars during the season of 2008.

Cucurbitaceous Cultivars	Nacl con.	Shoot fresh Wt. (g)	Shoot dry Wt. (g)	Plant Height (cm)	Leaf area (cm2)	Root fresh Wt. (g)	Root dry Wt. (g)	Root Length (cm)
Gourd Black Seed	0 ppm	111.6	34.32	140	197.69	30.25	20.30	53
(Cucurbita maxima)	2500 ppm	88.44	19.56	99	190.21	24.12	9.12	47
	3500 ppm	65.52	18.66	70	118.93	11.04	5.86	35
	4500 ppm	43.2	11.7	30	111.89	10.9	6.90	27
pumpkin	0 ppm	142.8	51.91	230	184.49	45	23.97	61
(Cucurbita moschata)	2500 ppm	107.52	24.6	165	182.85	29.96	18.76	55
	3500 ppm	99.48	20.28	109	121.57	20.88	14.08	44
	4500 ppm	71.3	16	70	105.73	15.5	11.00	38
Shintosa Supreme pumpkin	0 ppm	154.56	54.24	240	286.13	54.2	34.53	75
(Cucurbita maxima x C. moschata)	2500 ppm	156.72	63.36	235	282.17	51.8	36.30	75
	3500 ppm	141.36	45.59	212	250.93	42.92	30.82	63
	4500 ppm	121.44	37.56	189	217.49	39.8	30.63	60
Bottle gourd	0 ppm	109.68	28.32	92	154.13	37.76	17.6	57
(Lagenaria siceraria)	2500 ppm	104.28	35.43	89	155.89	31.56	21.66	55
	3500 ppm	91.32	19.79	65	143.57	25.45	15.59	48
	4500 ppm	51.84	18.52	43	121.57	17.08	10.81	40
LSD. NaCl Con. at 0.05		1.12	0.98	1.49	1.21	1.01	0.98	1.34
LSD. Cucurbit. Cult.. at 0.05		2.32	1.08	2.22	2.89	1.33	1.03	1.88
LSD. Cucurbit. Cult.X NaCl Con.. at 0.05		2.87	1.11	1.76	1.44	1.21	1.09	1.56

Table 2: Effect of Nacl concentrations on growth parameters of cucurbitaceous cultivars during the season of 2009.

Cucurbitaceous Cultivars	Nacl con.	Shoot fresh Wt. (g)	Shoot dry Wt. (g)	Plant Height (cm)	Leaf area (cm2)	Root fresh Wt. (g)	Root dry Wt. (g)	Root Length (cm)
Gourd Black Seed (Cucurbita maxima)	Cont.	122.8	41.2	170	214.8	33.3	22.3	57.5
	2500 ppm	97.3	21.5	112.4	209.2	26.5	10	52.4
	3500 ppm	72.1	22.4	92	131.4	14.4	6.4	38.5
	4500 ppm	47.5	12.9	40.3	123.1	12	7.6	29.7
pumpkin (Cucurbita moschata)	0 ppm	157.1	62.3	281	201.8	49.5	26.4	66.4
	2500 ppm	118.3	27.1	195.6	200.9	33	20.6	61.2
	3500 ppm	109.4	24.3	113.4	133.7	23	15.5	48.1
	4500 ppm	78.4	17.6	90	116.3	17.1	12.1	41.8
Shintosa Supreme pumpkin (Cucurbita maxima x C. moschata)	0 ppm	170	65.1	295	310.7	59.6	39.5	83.9
	2500 ppm	172.4	69.7	265.4	304.5	57	38.4	82.5
	3500 ppm	155.5	54.7	245	276	47.2	33.9	69.3
	4500 ppm	133.6	41.3	214	239.5	43.8	33.7	65.8
Bottle gourd (Lagenaria siceraria)	0 ppm	120.6	34	110.4	168.7	41.5	19.4	62.7
	2500 ppm	114.7	39	99	171.5	34.7	23.8	60.1
	3500 ppm	100.5	23.7	84.5	157.9	28	17.1	52.7
	4500 ppm	57	20.4	55.9	132.4	18.8	11.9	44.1
LSD. NaCl Con. at 0.05		2.02	1.08	2.31	1.41	1.43	1.32	1.82
LSD. Cucurbit. Cult.. at 0.05		3.63	2.11	3.30	3.28	2.03	2.13	2.01
LSD. Cucurbit. Cult.X NaCl Con.. at 0.05		2.56	2.01	1.58	2.32	1.81	1.54	1.93

Reduction in growth in response to salinity is usually attributed to either ion toxicity or low external osmotic potential (Munns and Termaat, 1986). The effect of salinity on cultivars is a reduction in growth. Salinity affects almost every aspect of the physiology and biochemistry of plants and significantly reduces growth Zhu Jin. *et al.* (2008a). Huang, *et al.* 2009 showed that 100 mmol L-1 NaCl (salt stress) significantly decreased plant dry weight by 41%, also NaCl stress decreased plant growth Wei GuoQiang *et al.* (2004); Zhu Jin and Bie ZhiLong (2007). Whereas, Sacaa, *et al.*(2008) mentioned that under salt stress decrease in dry weight of cucumber shoots was more pronounced than in fresh weight.

Leaf Electrolyte Leakage Percentage (Membrane Permeability):

Environmental stress often damages the plant organic membrane permeability and integrality, induces all kinds of water-solubility matters including electrolyte exuded, and also increases the plant conductance. Therefore, according to the relative conductance, we can understand the damaged degrees. Membrane lipid peroxidation often happens in the course of plant organ aging and environmental stressing. (Wang *et al.* 2007).

Lipid peroxidation has been associated with the damage provoked by a variety of environmental stresses and is often used as an indicator of salt-induce oxidative damage (Elkahoui *et al.* 2005). Malondialdehyde is the decomposition product of polyunsaturated fatty acids in the biomembranes. It has been used as an indicator of lipid peroxidation and tends to show greater accumulation under salt stress (Zhu *et al.* 2008). Salt stress also leads to oxidative stress through an increase in ROS, such as superoxide (O2-), H2O2 and hydroxyl radicals (OH-) (Mittler 2002), and it can injure the plant cell if it is not eliminated in time (Zhang *et al.* 2005). Some evidence suggests that resistance to oxidative stress may, at least in part, be involved in salt stress tolerance (Ashraf 2009; Badawi *et al.* 2004; Mittova *et al.* 2002).

Rout and Shaw (2001) reported that salt-tolerant plants should have a better anti-oxidant defense system for the effective removal of ROS, including anti-oxidant enzymes such as SOD, POD and CAT.

The membrane permeability of the four cucurbitaceous cultivars increased significantly with increasing concentrations of NaCl; the membrane permeability of Shintosa Supreme pumpkin (Cucurbita maxima x C. moschata) was lower than that of Bottle gourd (Lagenaria siceraria), Gourd Black Seed (Cucurbita maxima) and pumpkin (Cucurbita moschata) (Figure 1-a and 1-b). There were significant differences in the rates of increase in the membrane permeability between the Shintosa Supreme pumpkin cultivar and the rest of cultivars at 3500 ppm and 4500 ppm. For instance, the membrane permeability of Shintosa Supreme pumpkin and Gourd Black Seed in the 3500 ppm NaCl treatment decreased by 23.53 % and 31.32 %, respectively, compared to the controls and other cultivars.

Membrane permeability is a sensitive test to determine salt stress and tolerance (Mansour and Salama 2004). Cell membrane stability has been widely used to differentiate stress tolerant and susceptible cultivars of some crops. In some cases, higher membrane stability could be correlated with abiotic stress tolerance (Meloni *et al.* 2003; Sudhakar *et al.* 2001). Zhu, *et al* 2008 mentioned that membrane permeability significantly increased with salt stress for salt-tolerant cultivar. Similar results were observed on mulberry (Sudhakar *et al.* 2001), tomato (Alpaslan and Gunes 2001), cotton (Meloni *et al.* 2003) and Catharanthus roseus (Elkahoui *et al.* 2005).

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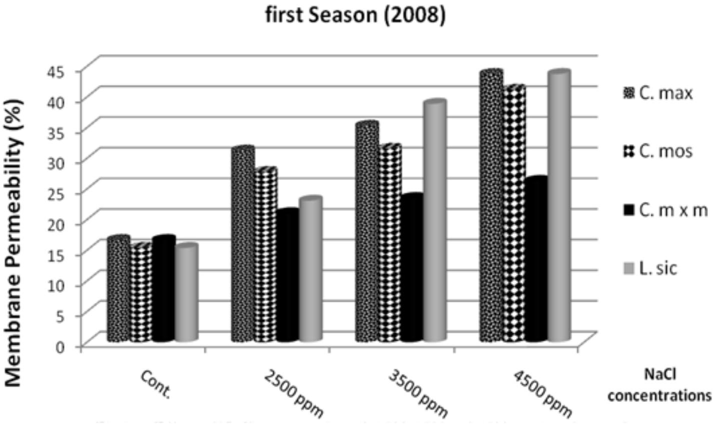

Fig. 1a: Effect of NACl concentrations (0,2500,3500 and 4500 ppm) on the membran pereability (%) of four cucurbitaceous cultivars during the season of 2008.

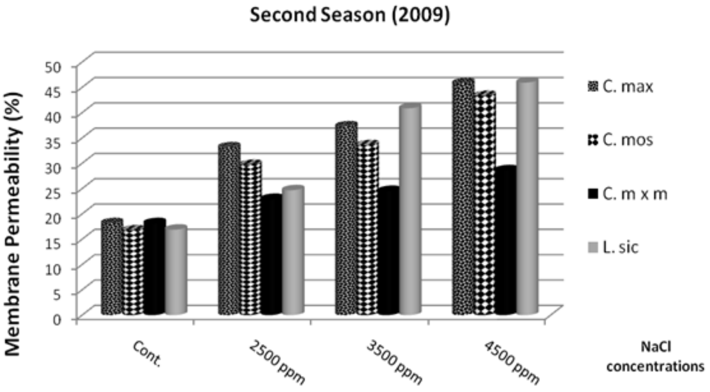

Fig. 1b: Effect of NaCl concentrations (0,2500,3500 and 4500 ppm) on the membran pereability (%) of four cucurbitaceous cultivars during the season of 2009.

Salt Tolerance of Four Cucurbitaceous Cultivars:

Salt injury index is qualitative description of morph visible symptom on plant. However, salinity stress caused nutrient unbalance. Where Na & Cl were dominant against K, Ca, P either on cell membrane or inside cells. Therefore, NaCl salinity stress could be considered as abiotic disease resulted from physiological disorder. Life disease severity index % was applied under salinity stress to reveal the degree of salt sensitive and tolerant plant.

With increasing concentrations of NaCl, the salt injury indices of the Shintosa Supreme pumpkin (Cucurbita maxima x C. moschata), pumpkin (Cucurbita moschata) and Bottle gourd (Lagenaria siceraria) cultivars significantly increased (Figure 2-a and 2- b). There was insignificant differences in the salt injury index between the pumpkin and Bottle gourd cultivars at the 3500 and 4500 ppm NaCl treatment, but the salt injury index of Gourd Black Seed (Cucurbita maxima), was significantly higher values than that of other cultivars at the 3500 and 4500 ppm NaCl treatments, indicating that the salt tolerance of Shintosa Supreme pumpkin (Cucurbita maxima x C. moschata) was lower values than that of other cultivars, so Shintosa Supreme pumpkin cultivars showed the best salt tolerant. The plasma membrane is the part of the cytoplasm that first encounters the salt and this may be the primary site of salt injury. Plant species and cultivars within a crop species differ greatly in their responses to salinity (Dasgan *et al.* 2002).

Mineral Analysis:

The concentrations of Na+ in the root of Shintosa Supreme pumpkin (Cucurbita maxima x C. moschata) cultivar increased significantly with increasing concentrations of NaCl, while the concentrations of Na+ in the stem and leaf decreased. Potassium (K+) showed an opposite trend. Also Cl^{-} increased in the leaf, (Figure 3).

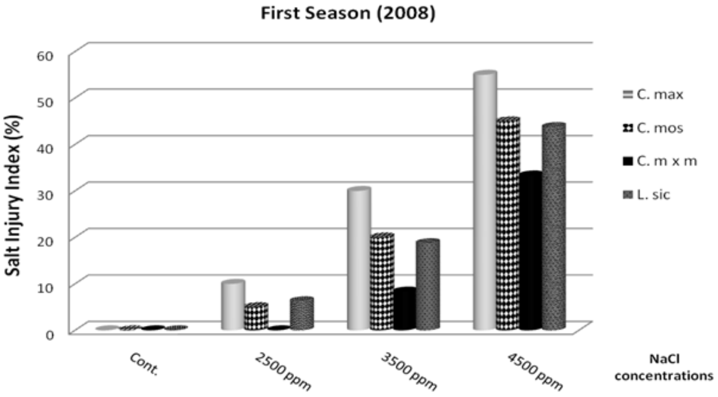

Fig. 2a: Effect of Nacl concentrations (0,2500,3500 and 4500 ppm) on thesalt injury index (%) of four cucurbitaceous cultivars during the season of 2008.

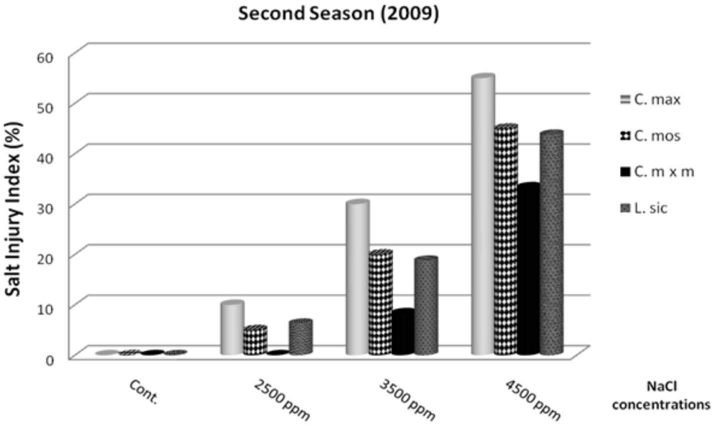

Fig. 2b: Effect of Nacl concentrations (0,2500,3500 and 4500 ppm) on thesalt injury index (%) of four cucurbitaceous cultivars during the season of 2009.

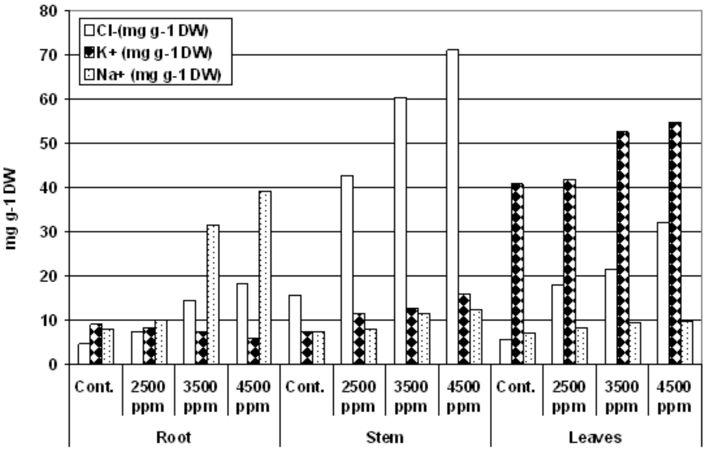

Fig. 3: Effect of NaCl concentration (0,2500,3500 and 4500 ppm) on the ion concentrations (mg g^{-1} DW) of Shintosa Supreme Pumpkin *(Cucurbita maxima x Cucurbita moschata)*

On the contrary, Gourd Black Seed (Cucurbita maxima) cultivar significantly increased Na+ concentrations in leaves than stem and decreased it in root with increasing concentrations of NaCl. The concentration of Cl^{-} increased in leaves (Figure 4). Also, both pumpkin (Cucurbita moschata) and Bottle gourd (Lagenaria siceraria) cultivars increased in Na+ concentrations in stem than leaves and root, and significantly decreased Cl^{-} concentrations in root (Figures 5 and 6).

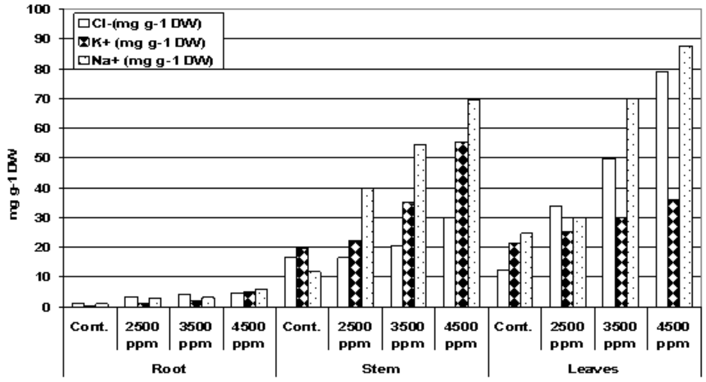

Fig. 4: Effect of NaCl concentration (0,2500,3500 and 4500 ppm) on the ion concentrations (mg g^{-1} DW) of Gourd Black Seed (*Cucurbita maxima*).

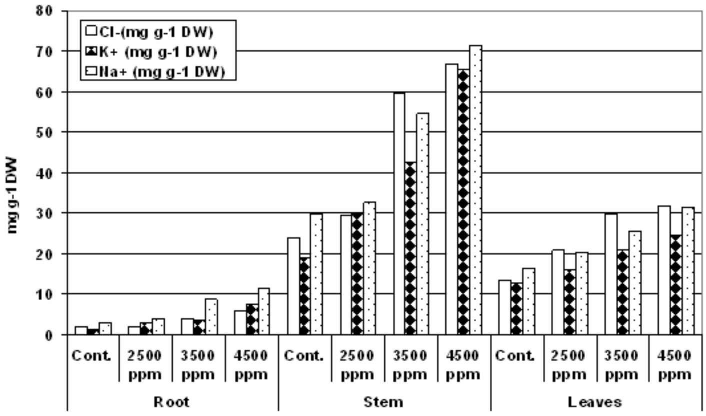

Fig. 5: Effect of NaCl concentration (0,2500,3500 and 4500 ppm) on the ion concentrations (mg g^{-1} DW) of Pumpkin *(Cucurbita maxima x Cucurbita moschata)*

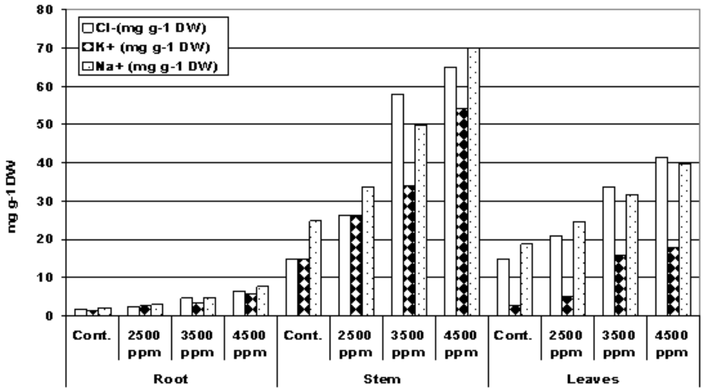

Fig. 6: Effect of NaCl concentration (0,2500,3500 and 4500 ppm) on the ion concentrations (mg g^{-1} DW) of Bottle gourd *(Lagenaria siceraria)*

To clarify the varaiation of Na+ and K+ concentrations in root, stem and leaves this may be due to Na+-K+ pump which export of sodium from the cell provides the driving force for several secondary active transporters membrane transport proteins, which import glucose, amino acids, and other nutrients into the cell by use of the sodium gradient. The pump, with bound ATP, binds 3 intracellular Na+ ions and conformational change in the pump exposes the Na+ ions to the outside. The phosphorylated form of the pump has a low affinity for Na+ ions, so they are released. The pump binds 2 extracellular K+ ions. This causes the dephosphorylation of the pump, reverting it to its previous conformational state, transporting the K+ ions into the cell (Li, *et al* 2009). This result is in agreement with the ?ndings of Alfocea *et al*. (1993) who suggested that K nutrition is not affected by excessive Na in salt tolerant tomato plants, while K uptake is strongly inhibited by the ionic competition of Na with K in salt sensitive tomato plants. Antogonistic relations between Na and K or negative effect of salinity on K uptake in different plants were reported by (Carjaval *et al*., 2000; Grieve and Poss, 2000).

It is well-known that Na+, Cl¯and K+ are the principal inorganic solutes under salt stress (Munns and Tester, 2008). In some salt-tolerant crops, the inorganic solutes rather than organic solutes make the greatest contribution to osmotic adjustment (Rodriguez, *et al* 1997, Meloni, *et al*, 2001, Ghoulam, *et al* 2002).

***Conclusions*:**

It is concluded from this work that Shintosa Supreme pumpkin (Cucurbita maxima x C. moschata) cultivar was the highest salt tolerant as compared to other cucurbitaceous cultivars. It was also realized that NaCl has a destructive effect on growth parameters of cucurbitaceous cultivars (pumpkin, Bottle gourd and Gourd Black Seed); also, salt injury index % and membrane peramability showed higher values when compared to Shintosa Supreme pumpkin (Cucurbita maxima x C. moschata) cultivars. Therefore Shintosa Supreme pumpkin cultivar could be recommended as rootstocks for cucumber particularly under saline condition.

According to salt susceptible and tolerant among four cucurbitaceous cultivars, the previous results revealed great variation under NaCl- salt stress. The tested cultivars could be arranged to the following orders: (I) high salt tolerant e.g. Shintosa Supreme pumpkin (Cucurbita maxima x C. moschata), (II) moderate salt tolerant e.g. pumpkin (Cucurbita moschata) and Bottle gourd (Lagenaria siceraria) and (III) low salt tolerant e.g. Gourd Black Seed (Cucurbita maxima).

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