Evaluation of in Vitro Salt Tolerance in *Cucumis prophetarum* L., A Crop Wild Relative

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ABSTRACT

Salinity is the major abiotic factor that limits plant growth and productivity. In this study, we present an *in vitro* propagation protocol for *Cucumis prophetarum* L. a crop wild relative to cucumber and to compare the effect of salinity on *C. sativus* and *C. prophetarum*. Results showed that Murashige and Skoog (MS) medium supplemented with 1 mg L^{-1} Kin and 0.5 mg L^{-1} NAA was the optimal medium for shoot proliferation and root induction, respectively. Interestingly, *C. prophetarum* plants showed better growth responses than *C. sativus* under low salinity stress. Higher levels of NaCl (100 and 150 mM) were lethal for *C. sativus*. Furthermore, 50 mM NaCl increased proline content in *C. prophetarum* and decreased lipid peroxidation content in *C. sativus*. In addition, *C. sativus* microshoots accumulated high amount of Na and showed higher K/Na ratio than *C. prophetarum*. In conclusion, this study showed that is more salt tolerant than *C. sativus*, which indicates the potential of using *C. prophetarum* to improve salt tolerance traits in cucumber.

Keywords: *Cucumis prophetarum* L., *Cucumis sativus* L., Crop wild relative, in vitro, Salinity.

INTRODUCTION

Drought, salinity, extreme temperatures, chemical toxicity and oxidative stress are abiotic stresses that may restrict plant growth, development and productivity (Debnath, 2008; Ayala-Astorga and Alcaraz-Meléndez, 2010). Abiotic stress is the major reason of crop loss worldwide. It has been shown that the average yield for the major crops is reduced by more than 50% due to abiotic stress. High salinity causes water shortage and ion toxicity to many plant species (Sobhanian et al., 2011). Rainfall shortage, high evaporation, low water management and the use of large quantities of chemical fertilizers raised salts concentrations in the rhizosphere (Colla et al., 2010). Detection and integration of salt tolerancet genotypes into economically useful crops may decrease the adverse effects of salinity on yield (Malik et al., 2010). Under saline stress, plants have defence mechanisms to facilitate their metabolic functions by limiting excess salts in the vacuole or break up the ions into isolated compartments in diverse tissues (Furtana and Tipirdamaz, 2010).

High salt deposition may lower soil water potential and nutrients availability. Na^+, K^+ and Ca^{2+} are the major ions involved in salt stress. The most important effect of NaCl stress is the loss of intracellular water (Mahajan and Tuteja, 2005). Salinity stress may disturb the homeostasis of sodium, calcium and potassium ions (Xue and Liu, 2008). One of the main mechanisms of salinity tolerance is the reduction of sodium loading into

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Received on 2/12/2015 and Accepted for Publication on 16/2/2016.

the xylem (Tavakkoli et al., 2011). Tolerant plants maintain low concentrations of sodium and high concentrations of potassium in cytosol under salinity conditions. The selective uptake of potassium as opposite to sodium is an important physiological salt tolerance mechanism (Ahmadi et al., 2009).

Crop wild relative is defined as any wild taxon related to the same genus of a crop (Maxted et al., 2006). It is important to estimate the degree of CWR relatedness and further provide a more specific meaning of a CWR depending on the Harlan and de Wet (1971) gene pool concept. A close relative found in the primary gene pool, further distant ones in the secondary gene pool, and very distant ones in the tertiary gene pool (Maxted et al., 2006). Secondary gene pool may contain the whole genus of the crop (Harlan and Wet, 1971). CWR are the most important resources for improving agricultural production and for preserving sustainable agroecosystems (Heywood et al., 2007). Conservation of CWR is a significant role to take a part in world food security (Moore et al., 2008). Conservation in seed banks or as live plants is examples of conservation methods. Seed regeneration can be affected by loss of germination potential; plant spread under field conditions may be restricted by specific soil and environmental supplies or by low yield of seed. Therefore, *in vitro* methodologies stand for a significant approach for *ex situ* conservation of plant germplasm (Pacheco et al., 2009). In addition to *in vitro* conservation, plant tissue culture is an excellent way to study the effect of abiotic stress on cell metabolism. Tissue culture is widely used for screening salinity and drought tolerant plant genotypes. The reaction of *in vitro* stressed plants imitates that of the *in vivo* stressed plants (Ztaimeh et al., 2007, Abu Serdane, 2012). Plants tolerant to both biotic and abiotic stresses can be obtained *in vitro* by using selecting agents for example NaCl (for salt tolerance) in the culture media (Rai et al., 2011). Plant tissue culture techniques are being used in cucumber for reducing the cost of hybrid seed production (seeds can be account for more than 30% of the total seedling cost) (Mohammadi and Sovrotepe, 2007).

Cucumis belongs to the Cucurbitoideae, a subfamily of Cucurbitaceae which contains about 700 species (Ghebrtinsae et al., 2007; Kouonon et al., 2009). Cucurbitaceae is an annual group of plants that have high social, cultural and economic values for local communities (Ghebrtinsae et al., 2007; Schaefer and Renner, 2010). *Cucumis* is one of the economically important genera of flowering plants. Almost all cultivated *Cucumis* species are susceptible to several fungal, bacterial, viral, insect diseases and abiotic stress factors. For this reason, a lot of biotechnological studies are in progress to find valuable genes from wild relatives that have better resistance to such stresses (Ghebrtinsae et al. 2007).

The aim of this study was to establish an *in vitro* propagation protocol for *Cucumis prophetarum* L. and compare the responses of *C. prophetarum* Vs its cultivated relative Cucumber (*Cucumis sativus* L.) to salinity stress.

MATERIALS AND METHODS

Plant Material:

Fruits of *Cucumis prophetarum* L. were collected during the period of September, 2012 from wild plants grown in the Dead Sea area/ Jordan (31º73´871"N; 35º57´372"E) (Figure 1 A, B & C). Fruits were dried in the lab, and then seeds were collected and used for various experiments. Seeds of cucumber (*Cucumis sativus* L. variety Qoutah) were purchased from the local market (Irbid/Jordan).

Establishment of in vitro Mother Stock Cultures:

C. prophetarum seeds were cut by scalpel, and then

washed under water flow in a flask containing a few drops of detergent for 20 minutes. Then, under the laminar air-flow cabinet, they were washed with 70% ethanol for 30 seconds, followed by 40 % sodium hypochlorite for 20 minutes, and then washed with 70% alcohol for 1 minute. Finally, seeds were rinsed with sterile distilled water for 3 times (each time for 1 minute).

Sterilized seeds were inoculated into culture tubes (15 ml) containing half strength MS medium (Murashige and Skoog, 1962). The pH of the medium was adjusted to 5.8 using 0.1 N NaOH or 0.1 N HCl. Seven g L^{-1} plant agar was added to solidify the media, 6.0 g L^{-1} sucrose were also added. The media was autoclaved at 121 °C and 1.15 Kg/cm pressure for 20 minutes. Inoculated test tubes were incubated in the growth chamber at 24 ± 2°C and under 16 hr light/8 hr dark.

In vitro Propagation of C. sativus:

In vitro culture of *C. sativus* was established from seeds. Seeds were sterilized and germinated as mentioned for *C. prophetarum*. Shoot tips were excised aseptically and inoculated on full MS medium containing 30 g L^{-1} sucrose and 1.0 mg L^{-1} Kinetin (Kin).

In vitro Shoot Proliferation:

After 2 weeks of seeds inoculation, shoot tips were excised aseptically from germinated seeds and inoculated onto full MS medium supplemented with either, Benzyl adenine (BA) or Kin, at 0.0, 1.0, 2.0 and 3.0 mg L^{-1}. Cultures were incubated in growth chamber at 24 ± 2°C and under 16 hr light (80-100 $\mu E.m^{-2}.s^{-1}$ fluorescent light)/8 hr dark. Six weeks after subculture, number of microshoot, leaves, roots and microshoots length formation was recorded.

In vitro Root Formation:

Microshoots were inoculated on full MS medium supplemented with (0.0, 0.5, 1.0 and 2.0 mg L^{-1}) IBA (indole-3-butyric-acid) or NAA (1- naphthalene acetic acid) containing 7 g L^{-1} agar and 30 g L^{-1} sucrose. Explants were incubated in the growth chamber under 16 h light (80-100 $\mu E.m^{-2}.s^{-1}$ fluorescent light)/8 h dark at 24 ± 2°C. Data were reported after six weeks on root induction %, number of roots and roots length.

Salinity Stress:

Microshoots of both species were subcultured on MS medium containing 7 g. L^{-1} agar, 30 g L^{-1} sucrose, 1 mg L^{-1} kin and different concentrations of NaCl (50, 100 or 150 mM). Explants were incubated in the growth chamber under 16 hr light (80-100 $\mu E.m^{-2}.s^{-1}$ fluorescent light)/8 hr dark at 24 ± 2°C. Number of micoshoots, number of leaves, microshoots fresh weight and microshoots length were reported after six weeks.

Proline Content Assay:

Briefly, 0.5 g of fresh plant material of each species was homogenized in 10 ml of 3% aqueous 5-sulfosalicylic acids. The homogenate was filtrated. Then, 2 ml of supernatant, 2 ml of acidic ninhydrin and 2 ml of acetic acid were mixed in a test tube and heated in water bath at 90°C for 60 minutes. The reaction was terminated in an ice bath. To extract the chromophor, 4 ml of toluene was added to the tube and mixed vigorously for 15-20 sec, and the extract was aspirated from aqueous phase. Absorbance of the solution was read at 520 nm. Proline concentration was determined by using a standard curve and was calculated on fresh weight bases according to Bates et al. (1973):

[(µg proline / ml toluene) / 115.5 µg / µmole] / [(g sample) / 5] = µmole

Proline / g fresh weight material.

Lipid Peroxidation Content:

Lipid peroxidation level was measured as malondialdehyde (MDA) content according to Carmak and Horst (1991). Fresh leaves (0.5 g) were homogenised in 10 ml 0.1% trichloro acetic acid (TCA). Then, the homogenate was centrifuged at 15.000 g for 10 minutes. Then, 2 ml supernatant were mixed with 4

ml 0.5% thiobarbituric acid (TBA) in 20% TCA. The mixture was heated in a water bath at 95°C for 30 minutes, and then cooled in an ice bath. The mixture was centrifuged at 10.000 g for 10 minutes. The absorbance of the supernatant was read at 532 nm and 600 nm. The blank was 0.5% TBA in 20% TCA solution. MDA content was determined using the extinction coefficient of 155/mM/cm.

Total Nitrogen:

Microshoots were oven dried overnight at 70 °C and milled to pass 1.0 mm sieve size. A one hundred mg sample was used to determine total nitrogen content by using Micro-Kjeldahl digestion procedure (Ma and Zuazaga, 1942). Crude protein content was determined by multiplying total nitrogen by a factor of 6.25 (Bulman and Smith, 1993).

Na and K Analysis:

Microshoots were dried overnight at 70 °C and milled to pass 1.0 mm sieve size. Then, 0.2 g sample was ignited in Muffle Furnace at 550 °C for 14 hours. Five ml 0.2% HCl were added (mixed well until dissolved). The mixture was filtrated through using a Whatman filter paper No. 42. The filtrate was diluted to 50 ml with distilled water. The diluted sample was stored in bottles to determine the concentration of sodium and potassium through using flame photometer (Corning, M410).

Statistical analysis

All experiments were repeated three times, for each experiment at least five independent replicates were used. SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for analysis of variance (ANOVA). Means were separated by using Tukey t-test at 0.05 level of probability.

RESULTS

In vitro Propagation:

To examine the possibility of *in vitro* propagation of *C. prophetarum*, different cytokinin types and levels were used (Kin and BA; 1.0, 2.0 and 3.0 mg L^{-1}) to determine the best type and level of cytokinin on shoot proliferation. Results showed the possibility of obtaining multiple shoots with high proliferation rate from *C. prophetarum* that was grown in MS medium supplemented with Kin (Figure 2A). The optimum Kin level for microshoot multiplication (7 microshoots) of *C. prophetarum* was 1.0 mg L^{-1} (Figure 2A). Similar response was observed for medium supplemented with 2.0 mg L^{-1} Kin (6.5 microshoots per explants). Maximum number of leaves for *C. prophetarum* was obtained in MS medium supplemented with 1.0 mg L^{-1} Kin (31 leaves/explant) (Figure 2B). Microshoots length of *C. prophetarum* was affected by type and concentration of the growth regulator ($P \leq 0.05$). In general, microshoot grown in free MS medium was taller than those grown in medium supplemented with BA or Kin (Figure 2C). However, the Kin supplementation produced taller microshoots than those obtained from BA supplementation (Figure 2C).

For roots formation, the basal medium was supplemented with several concentrations of IBA or NAA (0.5, 1.0 and 2.0 mg L^{-1}). Root induction, number of roots and root length were measured after 6 weeks. All MS media supplemented with IBA or NAA showed similar root formation percentage (100%) except the media supplemented with 2.0 mg L^{-1} NAA which showed 66.7% (Figure 3A). Maximum number of roots of *C. prophetarum* was obtained in MS medium supplemented with 0.5 mg L^{-1} NAA (26.3 roots) (Figure 3B). *C. prophetarum* grown in media supplanted with 1.0 mg L^{-1} IBA produced the maximum number of roots. In general, results show that root length of *C. prophetarum* plants grown in MS medium supplemented with IBA was longer than those grown in MS medium supplemented with NAA (Figure 3C). The longest roots were obtained from plants grown in media

supplemented with 1 mg L^{-1} IBA (18.5 cm) and 0.5 mg L^{-1} IBA (17.4 cm).

Salinity Stress:

In the present study, different salinity levels (0, 50, 100, 150 and 200 mM NaCl) were used. However, data were recorded only up to 150 mM NaCl, as none of the species survived at 200 mM. Analysis of variance revealed significant effect for salt level and species for all the physiological and biochemical parameters. Results show that the increases in salinity decreased number of shoots, number of leaves, shoot length and shoot fresh weight of *C. prophetarum* and *C. sativus*. *C. prophetarum* showed higher salt tolerance than *C. sativus*.

Growth rate of *C. prophetarum* microshoots grown in media containing NaCl was slower than those grown in control medium. Microshoots number of *C. prophetarum* grown in MS medium supplemented with 50 mM NaCl was 43.8% of those produced from the control (Figure 4A). Significant reduction was observed in microshoots number of *C. prophetarum* at 100 and 150 mM NaCl (25% of the control). Growth of *C. sativus* microshoots was also negatively influenced by salt level. Moreover, NaCl at high concentrations (100 and 150 mM) were lethal for *C. sativus* microshoots. Microshoots grown at 50 mM NaCl showed only 25% of the number of shoots produced from control (Figure 4A).

Number of microshoots of both species was adversely affected by increasing salt level and the severity was higher in *C. sativus* than *C. prophetarum*. *C. prophetarum* microshoot survived and grown at 100 and 150 mM NaCl levels whereas *C. sativus* was killed. This indicates the ability of the crop wild relative *C. prophetarum* to tolerate salinity.

Salinity decreased leaves production (Figure 4B). Leaves production of Growing C. *prophetarum* and *C. sativus* plants grown in 50 mM NaCl was 44% and 60%, of the control, respectively.

Increasing NaCl concentration decreased microshoots length of *C. prophetarum* (Figure 4C). Microshoots length of *C. prophetarum* grown at 50 mM NaCl was 84.34% of control. Similar microshoots length was obtained at 100 mM NaCl. Severe reduction in microshoot (59.04% of the control) was obtained from medium supplemented with 150 mM NaCl. For *C. sativus,* increased salinity also decreased microshoot length. Microshoot length of *C. sativus* exposed to 50 mM NaCl was 74.8% of the control.

Fresh weight of *C. prophetarum* was gradually decreased by increasing NaCl concentration. Fresh weight of *C. prophetarum* in medium contained 50 mM NaCl was similar to medium supplemented with 100 mM NaCl (Figure 4D). Fresh weight of *C. prophetarum* exposed to 150 mM NaCl decreased to minimum and reached 60% of the control weight. Furthermore, *C. sativus* plants exhibited significant reduction (48.15% of the control) in microshoots fresh weight when grown in medium containing 50 mM NaCl.

Microshoots proline content was estimated after 6 weeks of stress under *in vitro* conditions. NaCl treated *C. prophetarum* microshoots showed higher level of proline compared levels (Figure 5A). The maximum proline accumulation was found in microshoots grown in media supplemented with 150 mM NaCl. Proline content decreased with the decreases of salt concentration. Similarly, proline content of *C. sativus* microshoot was increased with increasing salt contents of the media. At 50 mM NaCl, proline content increased to 181.2%of the control. A higher proline accumulation rate in *C. prophetarum* than in *C. sativus* microshoots was observed, this indicates that *C. prophetarum* is more tolerant to salinity stress than *C. sativus*.

The influence of salt stress on microshoots lipid peroxidation rate was estimated by measuring MDA content, which is the product of lipid peroxidation.

Increased salinity level decreased lipid peroxidation content in *C. prophetarum* (Figure 5B). MDA content of *C. prophetarum* at 50 mM NaCl was similar to the control (99.22%). In contrast, lipid peroxidation rate in *C. sativus* increased to 143.2% relative to control at 50 mM NaCl.

Salinity reduced *C. prophetarum* microshoots content of crude protein. In contrast, protein content of *C. sativus* that was grown in 50 mM NaCl was 139.66% of the control content (Figure 5C). The biochemical consequences of salt stress on *C. prophetarum* and *C. sativus* were assessed in relation to sodium and potassium content (Figure 6). Na contents of both species increased with increasing salt contents of the media. Na accumulation in microshoots of *C. prophetarum* that was grown in media contained 100 and 150 mM NaCl was 265% of the control, whereas, the Na accumulation in microshoots grown in medium contained 50 mM NaCl was 246% of the control. Na content of *C. sativus* microshoots grown in medium contained 50 mM NaCl was 420% of the control (Figure 6A). Potassium content of *C. prophetarum* and *C. sativus* decreased with the increases in salinity levels (Figure 6B). Maximum K content was observed in control plants of *C. prophetarum* and the lowest was less than 150 mM NaCl with only 40% of the control.

The K/Na ratio in the microshoots of both species decreased as salinity level increased in the MS medium (Figure 6C). At 50 mM NaCl, K/Na ratio in *C. prophetarum* was 23.7% of the control; whereas K/Na ratio in *C. sativus* was only 13.4% of the control. At 100 mM NaCl, K/Na decreased to 17.65% of the control. Furthermore, K/Na ratio decreased to 15.2% of control at 150 mM NaCl in *C. prophetrum*. The K/Na ratios of *C. prophetrum* were than those of *C. sativus*.

DISCUSSION

In vitro propagation of C. prophetarum

In vitro propagation of *C. prophetarum* is necessary to obtain plantlets with uniform growth characteristics of the mother plant. A successful micropropagation protocol was established for *C. prophetarum*. Previous studies on *in vitro* propagation of *C. sativus* and *C. melo* addressed the importance of supplementing the media with BA in shoot multiplication (Ahmad and Anis, 2005; Mohammadi and Sivritepe, 2007; Shalaby et al., 2008). However, in the present study, the best shoot multiplication rate was achieved in media supplemented with Kin. The maximum microshoots formation of *C. sativus* was obtained from MS media containing Kin (Abed Alrahman et al., 2005). Cytokinins stimulate multiple shoot formation and shoot elongation (Chawla, 2009; Gomes et al. 2010; Hashemabadi and Kaviani, 2010; Van Staden, 2008; Tornero et al., 2009). Cytokinins organize cell division and morphogenesis, regulate flower and seed development, seed germination, and the uptake of nutrients (Zalabák et al., 2013).

The optimum cytokinin concentrations vary from species to another species; therefore determination the exact amount is necessary to achieve the efficient rates of proliferation. It has been shown that adding Kin in low concentrations to MS medium resulted in the best rate of shoot multiplication for many plant species. For example, adding low concentration of Kin (1.9 mg L^{-1}) to MS medium was the best for microshoot multiplication rate of *Arbutus unedo* (Gomes et al., 2010). Callus formation of *C. sativus* from plant grown in low BA concentration was higher those planted in high concentration (Ahmad and Anis, 2005). Maximum shoot differentiation rate was obtained inr *C. melo* at low concentrations of BA and 2iP (Kathal et al. 1988). Also, Singh et al (1996) found that using lower levels of cytokinin was better for multiple shoot formation than higher levels in *Cucumis melo*.

For *in vitro* root formation, different types and

concentrations of auxin were used. In general; results show that NAA was better than IBA for roots induction. Similar result was reported by Selvaraj et al (2007) on *in vitro* rooting of wild and cultivated *Cucumis* species. In addition, our results agree with the findings of Ahmad and Anis (2005), Mohammadi and Sivritepe (2007), Sultana et al (2004). They found that NAA was more suitable for root formation than IBA. Anand and Jayachandran (2004) found that NAA was an ideal growth regulator for root elongation of *Zehneria scabra*. In contrast, Sarowar et al. (2003) found that *Cucurbita* hybrid variety, Shintoza rooted most successfully in IBA supplemented MS medium. Selvaraj et al. (2007) found that IBA was better than NAA for root formation and root elongation in *C. sativus*.

Salinity Stress

To the best of our knowledge, this is the first study that investigates the possibility of using *C. prophetarum* as a crop wild relative for *C. sativus* and assesses their salinity tolerance. One of the present study objectives was to investigate the effect of salinity on growth and physiological responses of *in vitro* grown *C. prophetarum*. Also, we compared *C. prophetarum* vs. *C. sativus* response to salinity stress. Salinity reduced microshoots number, leaves number, microshoots length and microshoots fresh weight of *C. prophetarum* and *C. sativus* ($P \leq 0.05$). *C. prophetarum* tolerate salinity more than *C. sativus*.

Salinity lowered the production of microshoots of both species. It has been shown previously that salinity reduced number of microshoots for different species including *C. sativus* (Malik et al., 2010), rice (Prakash and Prathapasenan, 1988), *Solanum pimpinellifolium* (Sun et al., 2010). Number of leaves of *C. prophetarum* was also decreased by increasing salinity level. Abd-Alla et al. (1992) found that salinity decreased leaves production in *C. sativus*. Sun et al. (2010) reported that salinity stress causes leave wilting for both Tomato and its wild relative *Solanum pimpinellifolium*. However, the crop wild relative *S. pimpinellifolium* recovered faster than the cultivated tomato cultivar 'Moneymaker'.

Salinity reduced microshoots length of both species. Many previous studies agree with our result. Similar results reported by Ztaimeh et al. (2007) and Carvajal et al. (1998) on muskmelon (*Cucumis melo* L.). Chelli-Chaabouni et al. (2010) found that the wild relative *Pistacia atlantica* Desf is more salt tolerant than the cultivated *Pistacia vera*. In another study, shoot length of the crop wild relative *Solanum pimpinellifolium* and cultivated tomato (*Solanum lycopersicum)* cultivar 'Moneymaker' was adversely affected by salinity stress, but *Solanum pimpinellifolium* showed a relatively taller plants than *Solanum lycopersicum* 'Moneymaker' (Sun et al., 2010).

Proline is an osmoticum, a preserving agent of enzyme and cellular structure and a storage compound of decreasing nitrogen for rapid re-growth after stress is removed (Debnath, 2008). In this study, proline content increased in *C. prophetarum* microshoots with elevated salt level ($P \leq 0.05$). In addition, proline content in *C. prophetarum* was higher than *C. sativus*. Many salt-tolerant plants have higher concentration of proline than salt sensitive plants (Ashraf and McNeilly, 2004). Katz and Tal (1980) demonstrated that proline content under salinity stress increased in both cultivated tomato *Lycopersicon esculentum* and its wild relative *L. peruvianum*, and the proline accumulation was higher in the wild relative *L. peruvianum*.

Salinity may cause accumulation of ROS in plant tissues and oxidative damage to the membranes (Blokhina et al., 2003). Peroxidations of the cell membrane produce lipid hydroperoxides and aldehydes which react with thiobarbituric acid (TBA) to produce MDA (Ayala-Astorga and Alcaraz-Meléndez, 2010).

MDA content has often been used as a tool to assess the degree of plant sensitivity to oxidative damage (Blokhina et al., 2003). The amount of lipid peroxidation in *C. prophetarum* was decreased by increasing salt level (Figure 5B). Whereas, it was increased in *C. sativus* leaves as salinity level increased. Shalata and Tal (1998) showed that lipid peroxidation level decreased in the leaves of salt tolerant tomato exposed to salinity stress in contrast to salt sensitive cultivars. Many found that low levels of lipid peroxidation under high level of salinity could be an indicator of salt tolerance (Malik et al., 2010). Shalata et al. (2001) agree with present findings, they reported that lipid peroxidation rate decreased in salt-tolerant wild species, *Solanum pennellii*, in comparison with the cultivated tomato.

Salinity also affected nutrient content in both species. Results show that Sodium content increased with the increases in salinity level ($P \leq 0.05$) Higher Na accumulation rate in plants disturbs ionic balance, has specific ion effects, induces nutrient shortage symptoms, osmotic regulation and disrupted metabolism by the toxic effects of accumulated ions (Gunes et al., 1996; Tiwari et al., 2010). Sodium is not an essential element for plant growth; plants accumulate Na^+ more than K^+ and Ca^{+2} under salinity stress (Cramer et al., 1985). The present study demonstrates that Na^+ content increased as salinity level increased in *C. prophetarum* and *C. sativus*, whereas, K^+ content decreased. Earlier studies are in agreement with this result (Abed Alrahman et al., 2005; Al-Harbi, 1995; Furtana and Tipirdamaz, 2010; Tiwari et al., 2010; Ztaimeh et al., 2007) in *C. sativus* and *C. melo* shoots. On the other hand, Lo-Piero et al. (2000) found that K^+ content is not affected in pepper by salinity stress. Also, Al-Harbi (1995) reported a similar result in tomato. The competition between Na^+ and K^+ on the absorptive sites of the plant roots may be responsible for the decreases in K^+ content under salinity stress (Abed Alrahman et al., 2005; Ali et al., 1993; Cordovilla et al., 1995; Essa, 2002; Shibli, 1993). The reduction in K^+ concentration lowered the capacity of osmotic adjustment and turgor maintenance and/or harmfully affects metabolic functions (Greenway and Munns, 1980). The potassium/sodium ratio of *C. prophetarum* microshoots was decreased due to the high accumulation of sodium under high saline condition. The rate of Na^+ in *C. sativus* was higher than *C. prophetarum*; therefore, K^+ level and K/Na ratio were higher in *C. prophetarum* than *C. sativus*. Salt tolerant species have the ability to exclude Na^+ and preserve higher K/Na ratio (Abed Alrahman et al., 2005; Creda et al., 1995; Perez-Alfocea et al., 1996). For example, *Pistacia vera* L. (cultivated crop) accumulated Na^+ more than *P. atlantica* Desf (CWR) under salinity stress (Chelli-Chaabouni, 2010). Similarly, *Solanum lycopersicum* shoots accumulate higher levels of Na^+ than its crop wild relative *Solanum pimpinellifolium* L. (Sun et al., 2010). Whereas, K^+ accumulation in *S. Pimpinellifolium* (crop wild relative) was higher than *S. Lycopersicum* (Fernández-Ruiz et al., 2011).

In conclusion, an efficient micropropagation protocol for *C. prophetarum* was achieved using MS medium supplemented with Kin and NAA for microshoot induction and root formation, respectively. Furthermore, *C. prophetarum* plants showed the ability to tolerate salt stress and could be used as a crop wild relative to improve salt tolerance in *C. sativus*.

ACKNOWLEDGMENT

This research was supported by the Deanship of Research at Yarmouk University/Jordan (Project # 12/2011) as M.Sc. thesis project for May Abu Serdane.

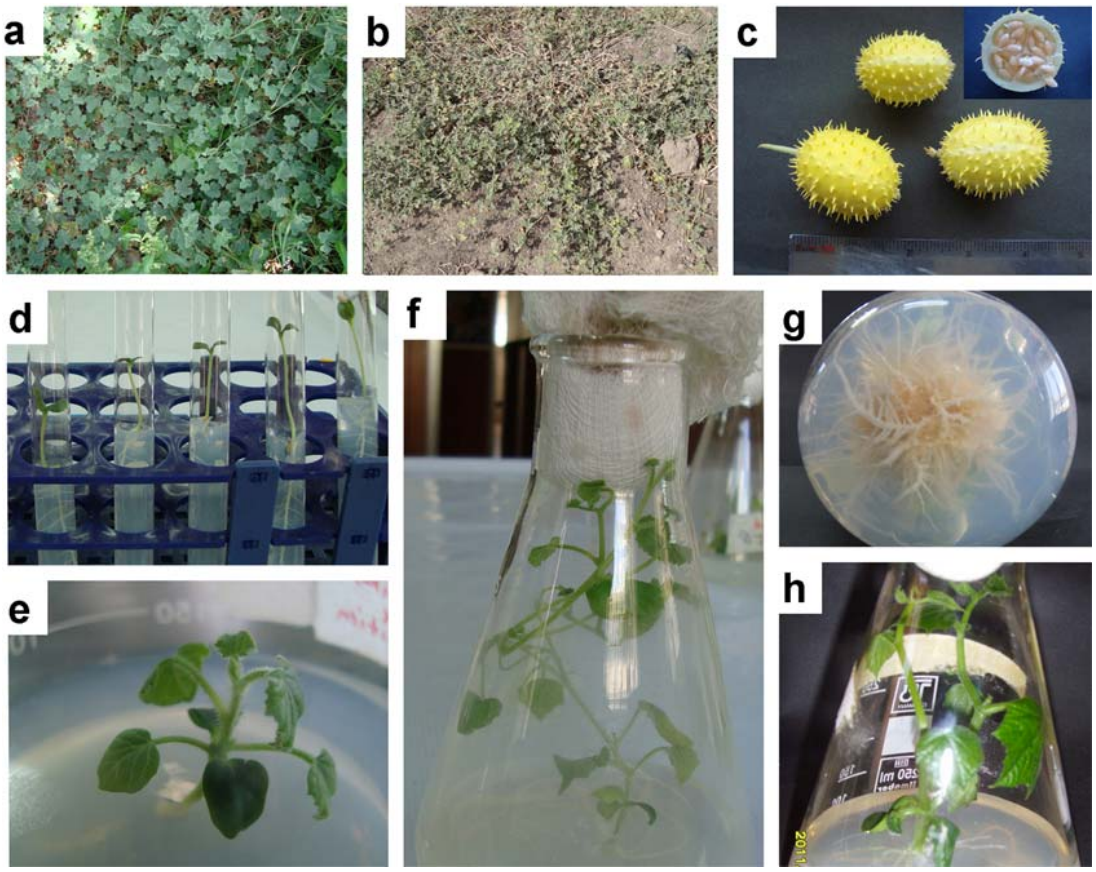

Figure 1: A & B: Wild grown *Cucumis prophetarum* L., near the Dead Sea/Jordan C: Mature fruits of *Cucumis prophetarum* L. D: *in vitro* germinated seedlings on 1/2 MS medium, E & F: microshoots after 20 or 40 days respectively of inoculation on MS medium, G: root induction, H: *in vitro* grown *Cucumis sativus* L.

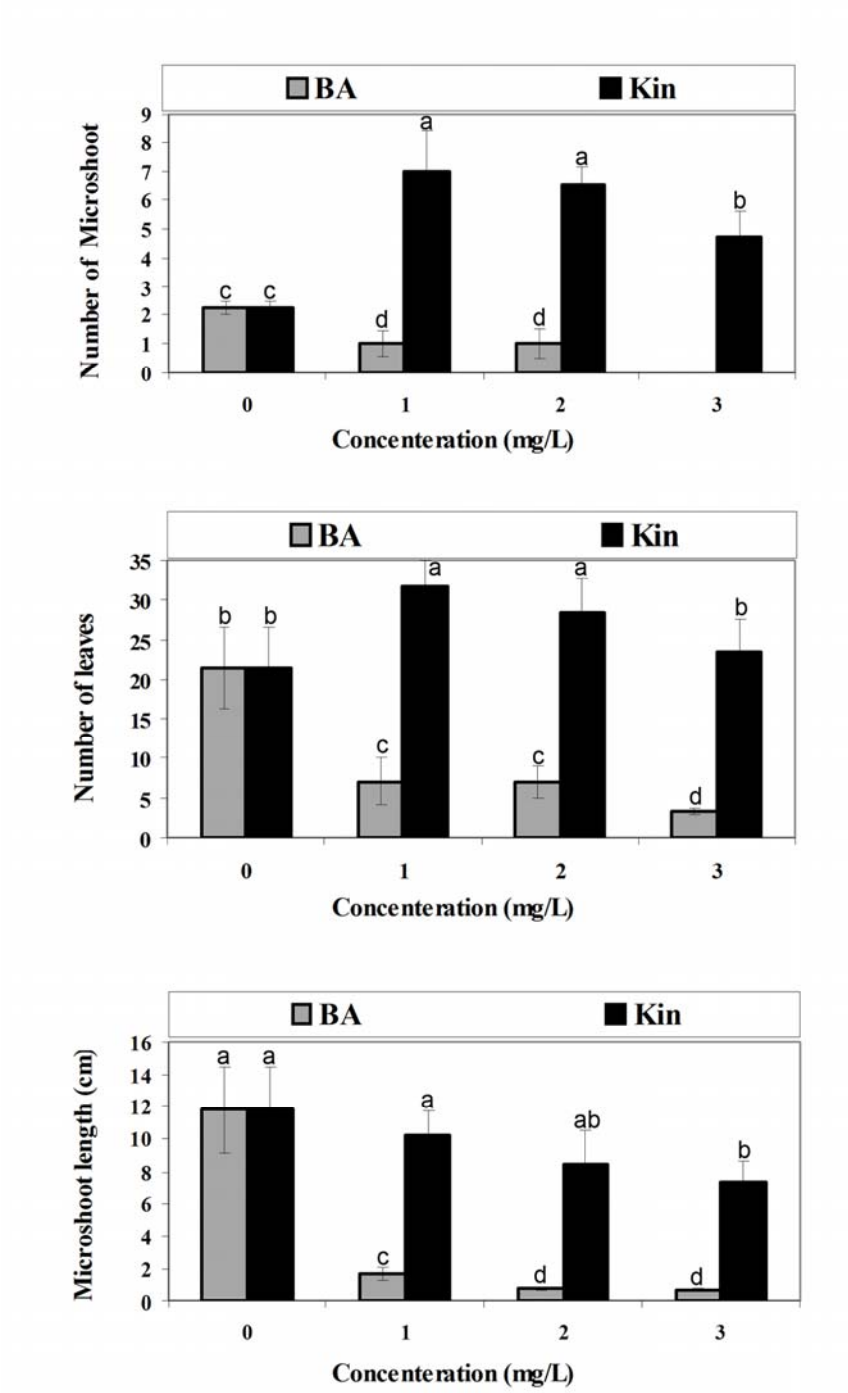

Figure 2: Effects of BA and Kin on *Cucumis prophetarum* shoot proliferation. (A) Number of microshoots, (B) Number of leaves and (C) microshoot length (cm). Data are shown as means ±SE of 8 replicates. Bars with different letters are significantly different at $p \leq 0.05$.

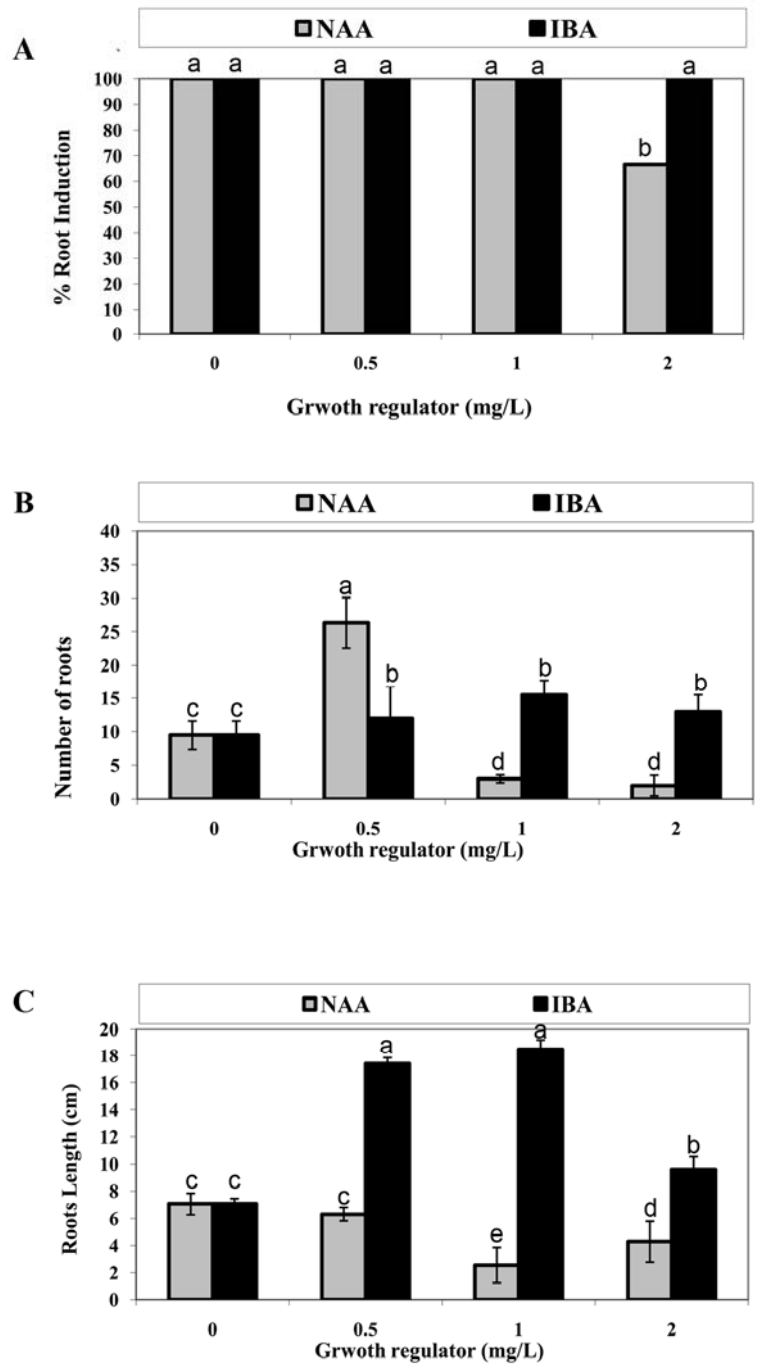

Figure 3: Effect of NAA and IBA on *in vitro* rooting of *Cucumis prophetarum*. A: root induction rate, B: Number of roots, C: Root length. Data are shown as means ±SE of 8 replicates. Bars with different letters are significantly different at $p \leq 0.05$.

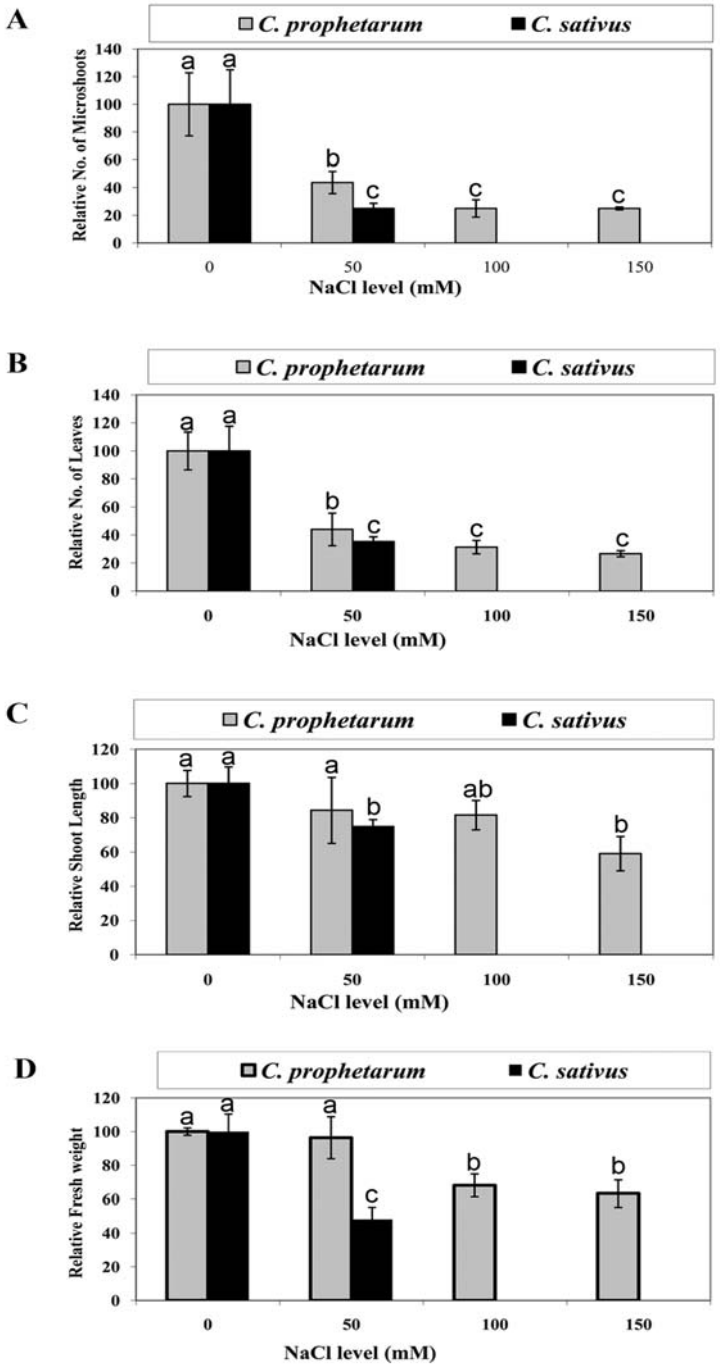

Figure 4: The effect of NaCl stress on shoot growth of *Cucumis sativus* L. and *Cucumis prophetarum*. A: Relative number of microshoots, B: Relative number of leaves C: Relative microshoots length, D: Relative fresh weight of *Cucumis sativus* L. and *Cucumis prophetarum* plants grown under 0, 50, 100 and 150 mM NaCl. Data is expressed as relative to control of the same species. Error bars indicate ± SE (n = 8). Bars with different letters are significantly different at $p \leq 0.05$.

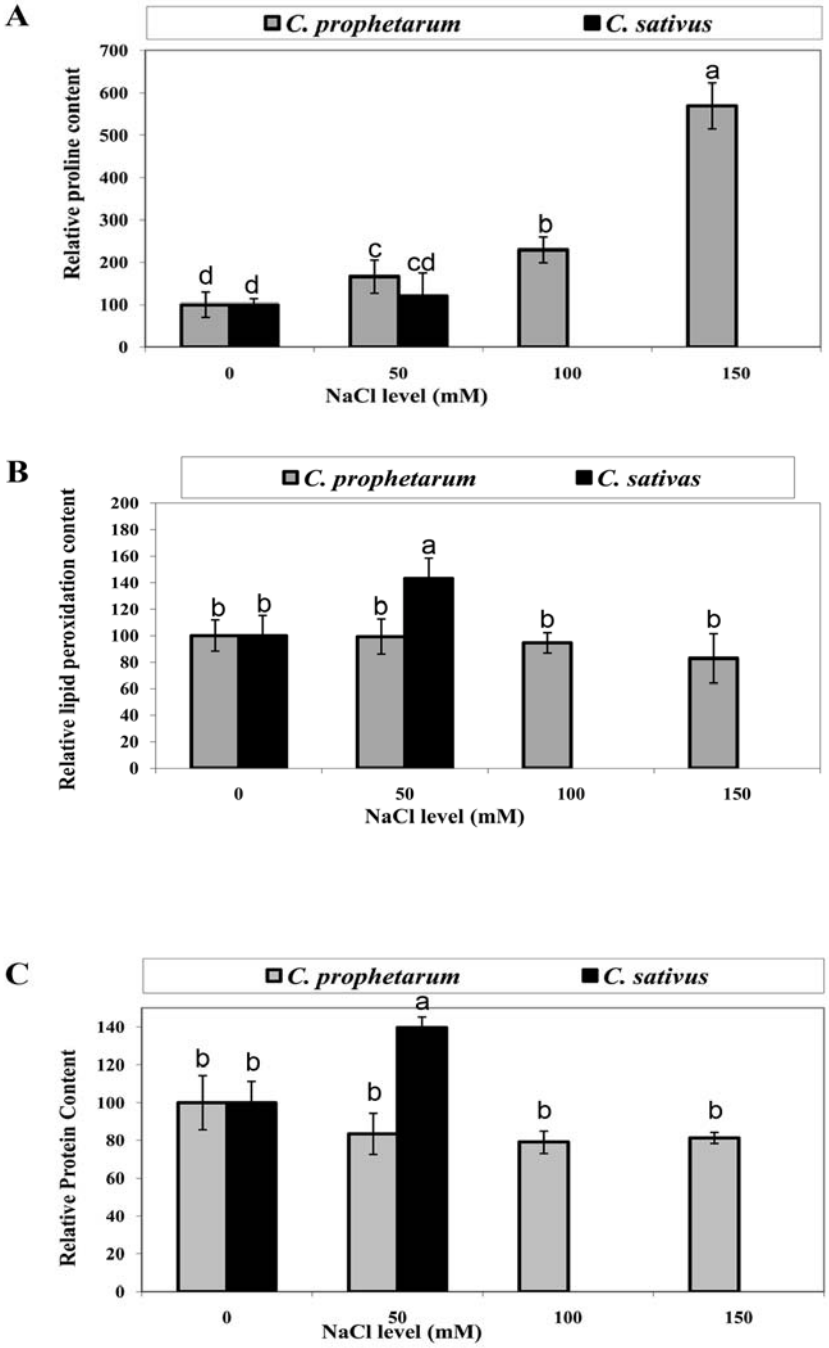

Figure 5: The effect of NaCl stress on *Cucumis sativus* L. and *Cucumis prophetarum* A: proline content, B: lipid peroxidation, C: protein content of plants grown under 0, 50, 100 and 150 mM NaCl. Data is expressed as relative to control of the same species. Error bars indicate ± SE (n = 8). Bars with different letters are significantly different at $p \leq 0.05$.

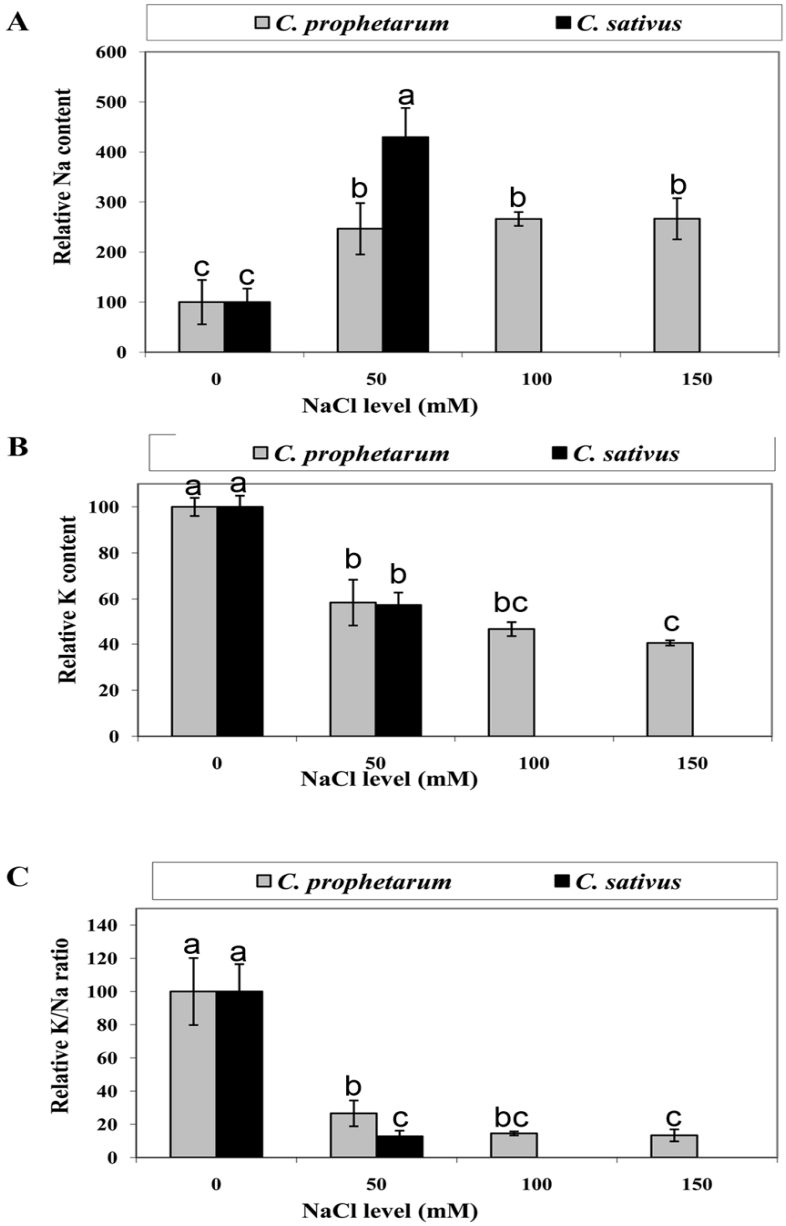

Figure 6: The effect of NaCl stress on *Cucumis sativus* L. and *Cucumis prophetarum* A: Na content, B: K content, C: K/Na ratio of plants grown under 0, 50, 100 and 150 mM NaCl. Data is expressed as relative to control of the same species. Error bars indicate ± SE (n = 8). Bars with different letters are significantly different at $p \leq 0.05$.

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تقييم تحمل نبات .*Cucumis prophetarum* L المزروع في أنابيب الاختبار للملوحة

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ملخص

تعد الملوحة من أهم العوامل التي تحد من نمو وإنتاجية النباتات. تم فحص امكانية اكثار (*Cucumis prophetarum* L.) في المختبر داخل الانابيب والذي يعد أحد النباتات البرية القريبة للخيار. استخدمت أنواع وتراكيز مختلفة من الهرمونات (BA او Kin)لاكثار السويقات و(NAA او IBA) لانتاج الجذور، وتم دراسة تأثيرها في المختبر على نمو الخيار البري. أظهرت النتائج أن الوسط المزود بهرمون النمو 1ملغم/ليتر Kin هو أفضل وسط لنمو وتكاثر الخيار البري وبينت النتائج إعطاء معدل 7 سويقات و31 ورقة والطول وصل الى 10.27سم. وكانت أفضل نتائج للجذور هي الوسط الذي يحتوي 0.5 ملغم/ليتر وكانت النتائج بمعدل 26.3 جذر وطول الجذر 6.3 سم. تم أيضا تم المقارنة من الناحية البيوكيميائية والفسيولوجية بين الخيار البري والخيار المزروع تحت تأثير الاجهاد الملحي. أظهرت النتائج أن الملوحة لها تأثير سلبي على الخيار البري والخيار المزروع ولكن كان الخيار البري أكثر تحملاً من الخيار المزروع حيث وجد أن الخيار المزروع لم يتحمل تركيز. ارتفاع نسبة الملوحة في الوسط أدى الى تناقص كمية الدهن البيروكسيدي وزيادة كمية البرولين في الخيار البري، أما في الخيار أظهر نتائج عكسية. تركيز الصوديوم في الخيار البري والخيار المزروع تزايد تحت تأثير الملوحة وكان تركيز الصوديوم أقل في الخيار البري من الخيار المزروع،أما تركيز البوتاسيوم ومعدل البوتاسيوم/الصوديوم تناقص مع ازدياد نسبة الملوحة ولكنه كان أعلى في الخيار البري من الخيار المزروع. بناء على ما سبق فإن هذه الدراسة دلت على إمكانية استخدام الخيار البري كمصدر للجينات لتحسين أداء محصول الخيار المزروع.

الكلمات الدالة: الأصول البرية، الخيار، إكثار داخل الأنابيب، الملوحة.

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تاريخ استلام البحث 2015/12/2 وتاريخ قبوله 2016/2/16.