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Effect of salt stress on *in vitro* grown *Solanum nigrum* L.

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Abstract

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Biotic and abiotic stresses such as salinity, drought and heavy metals affect biochemical, morphological, physiological and molecular processes in plants. The yield and productivity of major crops including *Solanum lycopersicum* are reduced as a result of these stresses worldwide. Plant tissue culture has been used to study the effect of various environmental factors on plants in a small space and short time. The aim of the present study is to investigate the effect of salt stress on germination, growth and biochemical parameters of the crop wild relative (CWR) *Solanum nigrum* compared to tomato (*S. lycopersicum*). Results showed that presence of NaCl in MS medium reduced significantly most of the studied germination and growth characteristics (plant fresh weight, shoot length, number of leaves and roots) for both *S. nigrum* and *S. lycopersicum*. Proline accumulation rate increased in *S. nigrum* and *S. lycopersicum* under salinity stress. However, *S. nigrum* showed higher proline accumulation rate than tomato. Also, chlorophyll content was higher in tomato plants compared to *S. nigrum* under salt stress. Results of this study indicate that the crop wild relative (*S. nigrum*) is more tolerant to salt than tomato plants.

Keywords: Solanum nigrum L.; *Solanum lycopersicum* L.; crop wild relative; salinity

Introduction

The response of a biological system to extreme environmental factors that lead to functional changes in plant life such as growth inhibition, decrease in bioproduction and adaptation or combination of these changes is called stress and it is divided into two types; biotic and abiotic stress (Mandre, 2002). Salinity, drought, flooding, high and low temperatures, ultraviolet (UV) radiation, heavy metals, wind, nutrient deficiency, shade, air pollution and photoinhibition are examples of abiotic stresses which are based on the interactions between the physical environment and organisms. On the other hand, biotic stress involves the interaction of plants with other living organisms such as herbivores or microorganisms (Schulze et al., 2005; Ashraf and Harris, 2005). Biotic and abiotic stresses lead to biochemical, morphological, physiological and molecular changes in plants (Al-Qwasemeh**,** 2013). It has been shown that the average yield of major crops reduced for more than 50% of the normal yield under stress conditions. More than 50% of agricultural land is threatened by salinization (Alam et al., 2015).

Salinity affects plants in two ways. In the first one, salinity decreases the ability of roots to extract water (Munns and Mark, 2008). This way happened when the irrigated water has high salt content, with time water will evaporate and salts will accumulate and remain in the soil (Zhu, 2007). The second way happened when salt concentrations become high within the plant itself, which is toxic to plant cells (Munns and Mark, 2008). Plants are divided into two types according to their ability to adapt to salt stress, glycophytes which includes species whose growth is inhibited under salt stress as *Zea mays*. Another type is halophytes, including those species that can

grow and survive in nature at high salt concentration, so they tolerate salt stress, as *Atriplex vesicaria* growing in the presence of 700 mM NaCl (Zhu, 2007). Currently, many biotechnological techniques are used to assess the effect of abiotic factors on plants but plant tissue culture remains one of the most widespread methods (Wang et al., 2003). It is used for *in vitro* growing of explants (shoot, root, etc.) aseptically on suitable medium that allow the division and regeneration into callus or particular plant organs (Rai et al., 1990). Also, it is used to study the effect of various environmental factors on plants in small space and short time (Hassanein and Soltan, 2000) as well as to enhance different defensive mechanisms to stress. Stress tolerance in plants may be induced *in vitro* by adding selective agents such as NaCl or mannitol (for salt and drought stresses, respectively) to the medium, plants that survive are considered as tolerant plants.

Solanaceae is a large family with more than 95 genera and about 2800 species are distributed throughout tropical to temperate regions with centralization in central and south America and Australia (Edmonds and Chweya, 1997). Also, it has varied morphology and growth habits from trees to small annual herbs (Foolad, 2007). *Solanum* is considered as the largest and most important and complex genus within the *Solanaceae* family. *Solanum* species show variation in phenotypic appearance, such as plant habit, leaf size and forms. Its predominantly self pollinating with natural inter and intra-specific hybridization and out and cross-breeding are also occurring (Edmonds and Chweya, 1997). Most of *Solanum* species have a basic chromosome number (x = 12) (Lou et al., 2010). *Solanum nigrum* L. is considered as an annual herb with different ploidy level: diploid (2n = 2x = 24), tetraploid (2n = 4x = 64) and hexaploid (2n = 6x = 72). *Solanum nigrum* L. or black nightshade is an annual plant growing up to one-meter tall, native to north-western Africa, Europe, western and central Asia (Edmonds and Chweya, 1997; Sridhar and Naidu, 2011).

Crop wild relatives (CWR) defined as wild plant species that have a close genetic relationship to cultivated crops or may be the progenitors of crops or other species related to them. CWR have beneficial traits that could be transferred to cultivated crops (Hajjar and Hodgkin, 2007). The relevance between wild plants and cultivated crops could be determined according to gene pool concept. The primary gene pool includes wild and cultivated plants with close relatives between them, secondary gene pool includes more distance between them and it contains the whole genus, and finally tertiary gene pool which contain the most distance one (Maxted et al., 2007).

The objective of this study was to study the effect of salinity on growth, biochemical and physiological responses of *in vitro* grown *Solanum nigrum* L. and tomato (*Solanum lycopersicum* L.).

Materials and Methods

Plant materials

Solanum nigrum L. fruits were collected from Jordan valley during the summer of 2016 and stored in the lab for drying. Tomato (*Solanum lycopersicum* L.) seeds (cv. Sunqueen) were purchased from a seed store.

Germination experiments

S. nigrum and *S. lycopersicum* seeds were surface sterilized by washing in 10% sodium hypochlorite containing 0.3% (v/v) tween 20 for 15 minutes, then rinsed twice with sterile distilled water. Seeds were plated in Petri dishes over 9 cm filter paper, then distilled water (as control) or different amounts of NaCl were added. Petri dishes were incubated in the growth chamber at 24(±1)°C with a 16/8 h light/dark photoperiod. Germination percentage was recorded after 4 days in which seed showing radical extrusion were considered as germinated seed.

Growth experiments

In vitro culture of *S. nigrum* and *S. lycipersicum* were established according to Al Khateeb and Al-Qwasemeh (2014). *S. lycopersicum* and *S. nigrum* seedlings were subcultured into MS medium (full strength) (Murashige and Skoog, 1962), supplemented with 7 g/L plant agar, 30 g/L sucrose and different concentrations of NaCl (0, 50, 100, 150 or 200 mM). Explants were incubated in growth chamber at 24(±1)°C under 16/8 hour light/dark photoperiod. Data were taken after 5 weeks for shoot length, number of roots and leaves and fresh weight.

Proline content

Proline content was performed according to Bates et al. (1973) protocol with few modifications. Proline (Sigma-Aldrich, Germany) was used as standard. One gram of plant tissues was grinded in 10 ml of 3% sulphosalicylic acids, and then filtered through filter paper. After addition of 2 ml glacial acetic acid and 2 ml acid ninhydrin to 2 ml of the filtrate, the mixture was incubated at 100°C for one hour at 100°C. The reaction then was stopped using ice bath. Then, the chromophore was extracted with toluene and mixed thoroughly for 30 seconds, after separation by centrifugation, the chromophore layer was moved to another tube. The absorbance at 520 nm was assessed. Finally, standard curve was used to determine proline content on fresh weight bases (Bates et al., 1973).

Chlorophyll content

Fifty mg of leaf samples were soaked in 1 ml of 80% acetone, the soaked samples were incubated overnight in the dark under shaking, then the soaked samples were centrifuged at 14000 rpm for 4 minutes. The absorbance of the supernatant was taken at 645 and 663 nm and 80% acetone was used as blank (Arnon, 1949).

Statistical analysis

All data were analyzed using ANOVA. Means, standard deviation and standard error were calculated using SPSS. At least six replicates were used for each treatment. To test the significance of treatments, statistical significance was accepted at $P \leq 0.05$ using Tukey's test.

Results

In this study, effect of different NaCl concentrations (0, 50, 100, 150 and 200 mM) on germination, growth parameters (number of roots and leaves, shoot length and fresh weight) and biochemical indices (proline and chlorophyll) were assessed for 5 weeks of *in vitro* grown *S. nigrum* and *S. lycopersicum*. Analysis of variance (ANOVA) revealed significant effect of NaCl amounts and the species for all growth and biochemical studies.

Effect of salinity stress on germination parameters

Germination percentage of *S. nigrum* and *S. lycopersicum* seeds were measured 3 days after planting under different concentrations of NaCl. Results showed that germination percentage of both species significantly ($P \leq 0.05$) reduced as NaCl content increased (Fig. 1A). Similar relative germination percentage was observed between *S. nigrum* and *S. lycopersicum* under control and 50 mM NaCl. In contrast, significant difference between the two species was observed under 100, 150 and 200 mM NaCl. *S. nigrum* showed significantly higher relative germination percentage than *S. lycopersicum* under these NaCl concentrations.

S. nigrum and *S. lycopersicum* hypocotyl length was measured 7 days after planting under different concentrations of NaCl. Hypocotyl lengths of both species were affected significantly ($P \leq 0.01$) when the NaCl is increased (Fig. 1B). Similar relative hypocotyl length was observed for *S. nigrum* and *S. lycopersicum* under control and 50 mM NaCl. Enhancement of NaCl (100, 150 and 200 mM) led to a huge decrease in relative hypocotyl length in *S. lycopersicum* seedlings compared to *S. nigrum*. *S. nigrum* seedlings showed relatively long hypocotyls under higher NaCl amount.

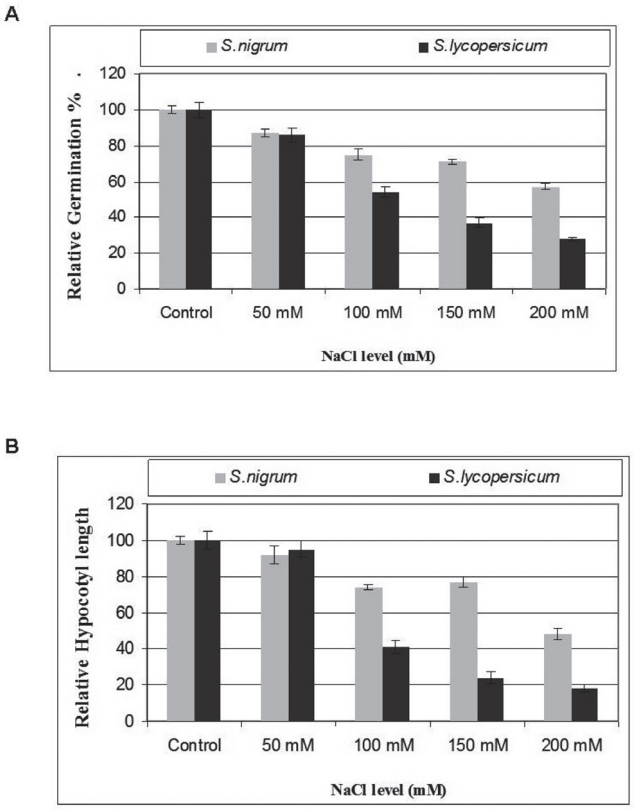

Fig. 1. Relative germination percentage (a) and hypocotyl length (b) of *S. lycopersicum* and *S. nigrum* grown *in vitro* under different NaCl concentrations

*Error bars represent standard error

Effect of salinity stress on growth parameters

Fresh weight of *in vitro* grown *S. nigrum* and *S. lycopersicum* were affected negatively with increased NaCl concentration in the growing medium (Fig. 2A). For both species, sharp reduction of shoot fresh weight was obtained at medium supplemented with 200 mM NaCl (6.68% relative to control for *S. nigrum* and 9.18% for *S. lycopersicum*). The lowest reduction in fresh weight of both species was achieved at medium supplemented with 50 mM NaCl (93.85% of control for *S. nigrum* and 75.87% for *S. lycopersicum*) (Fig. 2A). Results showed that *S. lycopersicum* have higher fresh weight than *S. nigrun* under 150 and 200 mM NaCl. Significant differences ($P \leq 0.05$) were observed between the two species under all NaCl concentrations used except 200 mM.

Leaves number of both species were reduced as NaCl content increased (Fig. 2B). The maximum number of *S. nigrum* leaves was observed in medium supplemented with 100 mM NaCl (93.41% relative to control). Higher concen-

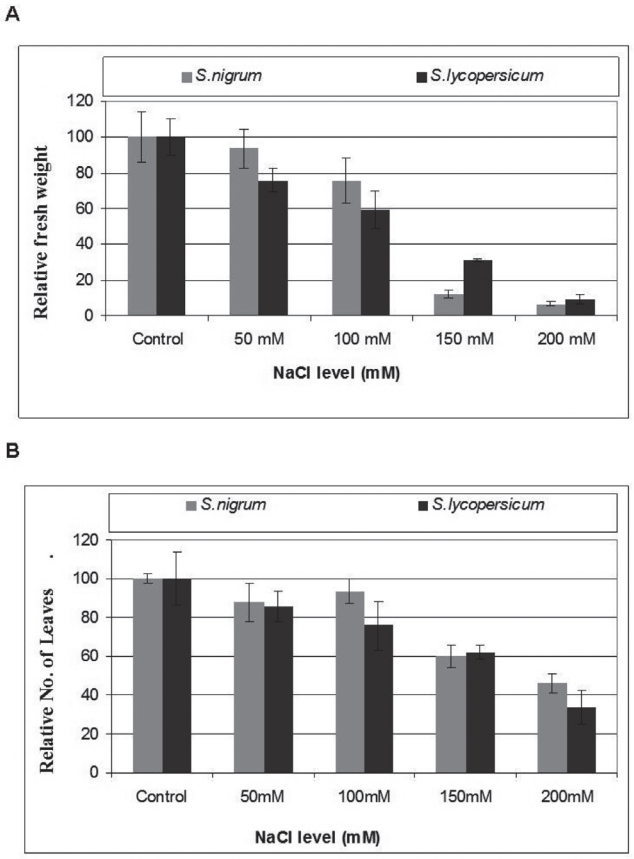

Fig. 2. Relative fresh weight (a) and leaves number (b) of *S. lycopersicum* and *S. nigrum* grown *in vitro* under different levels of NaCl stress

*Error bars represent standard error

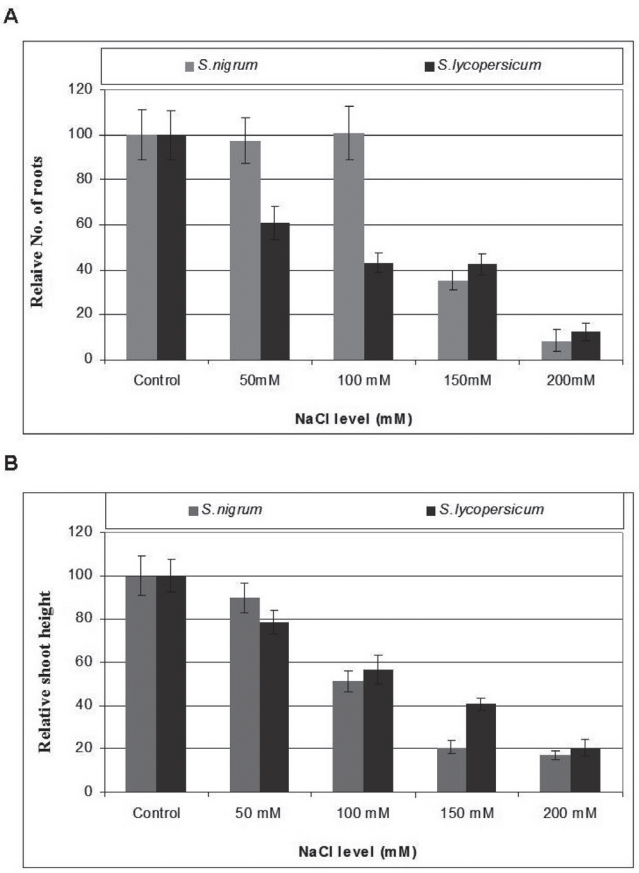

Fig. 3. Relative roots number (a) and shoot length (b) of *S. lycopersicum* and *S. nigrum* grown *in vitro* under different levels of NaCl stress

*Error bars represent standard error

tration of NaCl (150 and 200 mM) reduced leaves number to 60% and 46.15% relative to control, respectively ($P \leq 0.05$). On the other hand, leaves number of *S. lycopersicum* decreased gradually with the increase of NaCl, from 85.71% leaves relative to the control in 50 mM NaCl to reach the minimum leaves number in 200 mM NaCl (33.73% relative to control). No significant differences on leaves number were observed between *S. lycopersicum* and *S. nigrum* at 50 and 150 mM NaCl, while at 100 and 200 mM NaCl, significant differences between the two species were observed. A slightly higher leaves number produced in *S. lycopersicum* than *S. nigrum* when grown at 150 mM NaCl.

As shown in Fig. 3A, roots number of both *S. nigrum* and *S. lycopersicum* were affected adversely by NaCl concentration. The highest roots number was observed in *S. nigrum* plants grown in medium supplemented with 100 mM NaCl (100.8% relative to control). Minimum number of roots was observed in medium supplemented with the highest quantity of NaCl (8.4% compared to control). Furthermore, the lowest roots number of *S. lycopersicum* was observed in medium containing 200 mM NaCl (12.28% relative to control). At 100 and 150 mM NaCl, no difference was observed in roots number with less than 43.13% roots compared to the control. At 50 mM NaCl, roots number reduced to only 60.78% relative to control. Results showed that roots number in *S. nigrum* was higher than *S. lycopersicum* at all NaCl concentrations used except in 200 mM NaCl. A significant difference between the two species was observed at lower NaCl amounts (50 and 100 mM) ($P \leq 0.05$) while at higher salt content (150 and 200 mM) no significant differences were observed.

Shoot length of both *S. nigrum* and *S. lycopersicum* decreased as NaCl concentration increased (Fig. 3B). The shortest shoot in *S. nigrum* was observed in medium sup-

plemented with 200 mM NaCl (15.76% relative to control). At lower quantity of NaCl (50 and 100 mM), shoot length was 81.86% and 46.64% relative to control, respectively. Similarly, shoot length of *S. lycopersicum* decreased only to 75.12% compared to control when grown in medium supplemented with 50 mM NaCl then the length decreased to half of control when grown in medium supplemented with 100 mM NaCl ($P \leq 0.05$). Growing *S. lycopersicum* microshoots in medium supplemented with 200 mM NaCl resulted in decreased shoot length to the minimum (19.79% relative to control). Shoot of *S. lycopersicum* was longer than *S. nigrum* at all NaCl concentrations except at 50 mM NaCl. No significant differences were observed between the two species for shoot length at all tested NaCl concentrations except at 150 mM NaCl.

Effect of salinity stress on proline content

Proline content of both species was examined after 5 weeks of *in vitro* growth under different concentrations of NaCl. Proline content increased gradually in both tested species as the NaCl concentration increased ($P \leq 0.05$) (Fig. 4A). The highest proline content in *S. nigrum* was obtained from shoot grown on medium supplemented with 200 mM NaCl which resulted in 593.75% relative to control. Similar proline content was observed in *S. nigrum* plants grown on 100 and 150 mM NaCl (381.58% and 376.64% compared to control). Minimum proline content was observed at 50 mM NaCl (240.79% compared to the control). Similarly, proline accumulation reached the highest amount in *S. lycopersicum* when grown in medium supplemented with 200 mM NaCl (510.84% relative to control). Also growing *S. lycopersicum* microshoots in medium supplemented with 50 mM NaCl resulted in the lowest amount of proline (112.9% compared to control). Significant differences between *S. nigrum* and *S. lycopersicum* for proline accumulation rate were observed at all NaCl concentrations ($p \leq 0.05$). *S. nigrum* showed a significantly higher proline content than *S. lycopersicum* under all tested NaCl concentrations. This indicates that *S. nigrum* is more tolerant than *S. lycopersicum* to salinity stress.

Effect of salinity stress on chlorophyll content

Chlorophyll content for both *S. nigrum* and *S. lycopersicum* was affected differently by different NaCl concentrations in the growing medium. In terms of *S. nigrum*, higher concentrations (150 and 200 mM NaCl) decreased chlorophyll content to 76.14% and 82.78% relative to control, respectively. Growing microshoots on medium supplemented with 50 and 100 mM NaCl resulted in approximately the same values and it was found the highest chlorophyll content at 100 mM NaCl (94.41% relative to control) (Fig. 4B). A

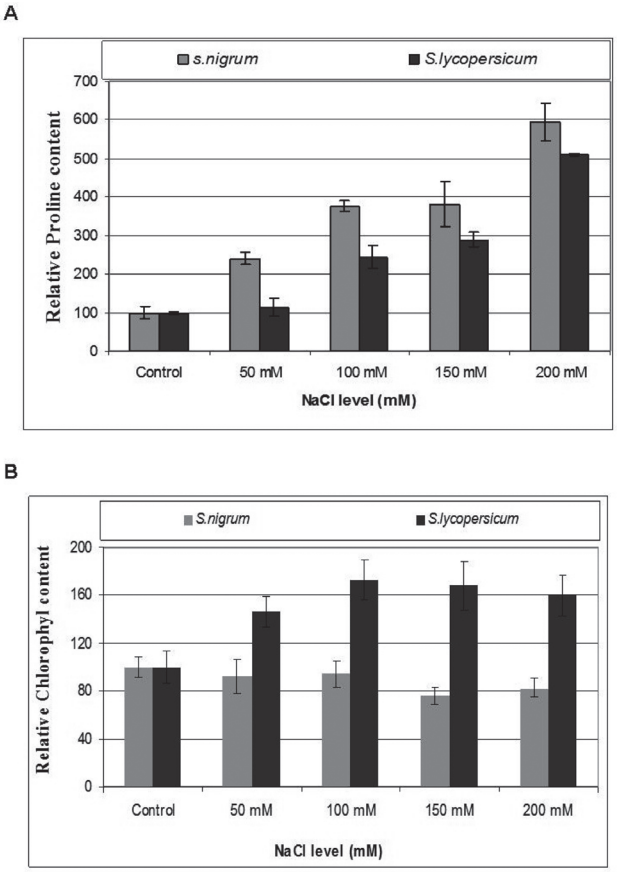

Fig. 4. Effect of different amounts of NaCl on proline content (µg/ml) (a) and chlorophyll content (µg chlorophyll/mg fresh weight) (b) of *in vitro* grown *S. lycopersicum* and *S. nigrum*

*Error bars represent standard error

different trend was observed in *S. lycopersicum*. Growing *S. lycopersicum* microshoots in medium supplemented with various amounts of NaCl (100, 150 and 200 mM) resulted in nearly the same chlorophyll content (173%, 168.3% and 160.1% relative to control, respectively). At 50 mM NaCl, chlorophyll content decreased to reach 146.7% relative to control. Results showed a significantly higher chlorophyll content ($P \leq 0.05$) in *S. lycopersicum* leaves than in *S. nigrum* under all NaCl levels used.

Discussion

As the problem of food supply is in progress and the risk of climate change and environmental stress threats most crops worldwide, an emergence crop improvement strategies are in need (Ford-Lloyd et al., 2011). Crop wild rela-

tives (CWR) are wild plants with a close genetic relationship to cultivated crops; they can be used as gene donor for crop improvement since they have more adaptation to stress conditions. CWR have more genetic diversity than cultivated crops which undergo several domestication processes resulted in loss of genetic variation (Heywood et al., 2007; Jarvis et al., 2008; Ford-Lloyd et al., 2011).

As far as we know, this is the first study which examined the probability of using *S. nigrum* as a crop wild relative to *S. lycopersicum* and evaluates their tolerance to some abiotic stresses. Salinity as one of the abiotic stresses affects plants at morphological, physiological, biochemical and molecular levels and subsequently reduced their growth and productivity.

The effect of salinity stress on germination, growth and biochemical responses of *in vitro* grown *S. nigrum* and *S. lycopersicum* was studied in present study. Our results show that salt stress affects germination and growth adversely and leads to an increase of proline content of the both tested species. It has been shown previously that salinity reduces growth and yield of more than 5% of arable land. Salt stress affects plant growth adversely at various levels: physiological, biochemical and molecular level (Molazem et al., 2010). Results show that some species are more tolerant to salt stress than others (Ashraf and Harris, 2005). In this study, assessment of stress tolerance for the crop wild relative (*S. nigrum)* and the cultivated *S. lycopersicum* is presented.

Seed germination is one of the most crucial phases of plant development. In this study, results showed significant reduction in germination % of both the cultivated and wild relative plants under salt stress. Similarly, Singh et al. (2012) found that high salt concentrations negatively affected germination and shoots growth of tomato seedlings. Also, they observed a delay in germination under salt stress compared to normal conditions. Our results showed that NaCl adversely affected growth parameters of both species. However, the crop wild relative (*S. nigrum*) showed higher degree of salinity tolerance than *S. lycopersicum*. Inhibition of growth under salt stress may be due to the excess accumulation of ions such as Na^+ and Cl^-, these are toxic to plant or reduce K^+ levels inside the cell (De Lacerda, 2003). The inhibition in growth start when the concentration of salt in growing medium increased to a threshold levels which increased the external osmotic pressure (Munns and Mark, 2008). The higher adaptation capability of wild plants to NaCl stress than cultivated one could be attributed to the consequences of the living organisms under harsh environmental conditions which may led to presence of tolerance mechanisms as changes in plant cell which appear to counteract the osmotic stress including decrease cell expansion in leaves and root tips, production of smaller and thicker leaves and reduction in stomatal aperture (Munns and Mark, 2008). Also, might be due to production of antioxidant enzymes such as SOD, CAT and APX as a result of oxidative stress which is caused by salinity that decrease the damage caused by reactive oxygen species (ROS). Sun et al. (2010) reported that the wild *Solanum pimpinellifolium* and the cultivated 'Moneymaker' both showed reduced growth under salt stress but the relative shoot elongation decrease severely in 'Moneymaker' cultivar compared to a little reduction in the wild one. Also, Chaabouni et al. (2010) studied the effect of different NaCl concentrations on three *Pistacia atlantica* rootstocks that showed differences in salt tolerance. They found that the treatment with 100 and 200 mM NaCl leads to a reduced number of leaves in two rootstocks of *P. atlantica* L. more than the third one. In this study, we found higher proline content under salt stress than in control of both species. The highest accumulation rate was observed in *S. nigrum* compared to *S. lycopersicum*. Proline has a major rule in adaptation against harsh condition as salt stress (Jaleel et al., 2007), it is considered as important osmoregulatory solute under saline conditions (Delauney and Verma, 1993). Hassanein (2004) found that the stress tolerant *Alhagi graecorum* accumulate higher proline content than stress sensitive *Lycopersicon esculentum* L. when both grown under NaCl. Similarly, Koca et al. (2007) showed that *Sesamum indicum* cultivar Cumhuriyet which assumed to be stress tolerant accumulated more proline than Orhangazi which is sensitive cultivar. The higher proline accumulation rate in addition to higher growth rates of most of the studied growth parameters seen in the crop wild relative (*S. nigrum*) indicates that *S. nigrum* is more tolerant than *S. lycopersicum* to salt stress. One of the suggested reasons behind this is the presence of certain genes responsible for salt tolerance expressed only or in higher rates in the wild species (*S. nigrum*) compared to the cultivated crops (*S. lycopersicum*). Further studies are in need to identify these genes and to transfer them to cultivated crops in order to improve their tolerance ability to environmental stresses.

In conclusion, salinity reduces most of the growth parameters for both *S. nigrum* and *S. lycopersicum*. The presence of NaCl, in MS medium cause an increase in proline contents for both *S. nigrum* and *S. lycopersicum* species with a significant higher amount in *S. nigrum*. Based on biochemical and growth parameters*, S. nigrum* showed higher degree of tolerance. Therefore, *S. nigrum* could be considered as a crop wild relative for its close relative *S. lycopersicum*, to improve their stress tolerance capability.

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References

Alam, M., Juraimi, A. S., Rafii, M. Y., & Abdul Hamid, A. (2015). Effect of salinity on biomass yield and physiological and stem-root anatomical characteristics of Purslane (Portulaca oleracea L.) accessions. *BioMed Research International*, *2015*.

Al-Qwasemeh, H. A. (2013). *Effect of Various Abiotic Stresses on in Vitro Grown Solanum Nigrum L*. M.Sc. thesis, Department of Biological Sciences, Yarmouk University, Jordan.

Al Khateeb, W., & Al-Qwasemeh, H. (2014). Cadmium, copper and zinc toxicity effects on growth, proline content and genetic stability of Solanum nigrum L., a crop wild relative for tomato; comparative study. *Physiology and Molecular Biology of Plants*, *20*(1), 31-39.

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. *Plant Physiology*, *24*(1), 1.

Ashraf, M., & Harris, P. (Eds.). (2005). *Abiotic stresses: plant resistance through breeding and molecular approaches*. CRC Press, New York.

Bates, L. S., Waldren, R. P., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil*, *39*(1), 205-207.

Chaabouni, A. C., Hammami, M., Gouia, M., Gargouri, K., Gargouri, R., & Drira, N. (2010). Effect of salt stress on Pistacia atlantica rootstock seedlings in nursery conditions. *Options Méditerranéennes. Série A, Séminaires Méditerranéens*, *94*, 135-140.

Delauney, A. J., & Verma, D. P. S. (1993). Proline biosynthesis and osmoregulation in plants. *The plant journal*, *4*(2), 215-223.

Edmonds, J. M., & Chweya, J. A. (1997). *Black nightshades: Solanum nigrum L. and related species* (Vol. 15). Bioversity International.

Foolad, M. R. (2007). Genome mapping and molecular breeding of tomato. *International Journal of Plant Genomics*, *2007*, 1-52.

Ford-Lloyd, B. V., Schmidt, M., Armstrong, S. J., Barazani, O., Engels, J., Hadas, R., ... & Li, Y. (2011). Crop wild relatives—undervalued, underutilized and under threat? *BioScience*, *61*(7), 559-565.

Hajjar, R., & Hodgkin, T. (2007). The use of wild relatives in crop improvement: a survey of developments over the last 20 years. *Euphytica*, *156*(1-2), 1-13.

Hassanein, A. M. (2004). Effect of relatively high concentrations of mannitol and sodium chloride on regeneration and gene expression of stress tolerant (*Alhagi graecorum*) and stress sensitive (*Lycopersicon esculentum* L.) plant species. *Bulg. J. Plant Physiol*, *30*(3-4), 19-36.

Hassanein, A. M., & Soltan, D. M. (2000). Solanum nigrum is a model system in plant tissue and protoplast cultures. *Biologia Plantarum*, *43*(4), 501-509.

Heywood, V., Casas, A., Ford-Lloyd, B., Kell, S., & Maxted, N. (2007). Conservation and sustainable use of crop wild relatives. *Agriculture, Ecosystems & Environment*, *121*(3), 245-255.

Jaleel, C. A., Manivannan, P., Lakshmanan, G. M. A., Sridharan, R., & Panneerselvam, R. (2007). NaCl as a physiological modulator of proline metabolism and antioxidant potential in Phyllanthus amarus. *Comptes Rendus Biologies*, *330*(11), 806-813.

Jarvis, A., Lane, A., & Hijmans, R. J. (2008). The effect of climate change on crop wild relatives. *Agriculture, Ecosystems & Environment*, *126*(1-2), 13-23.

Koca, H., Bor, M., Özdemir**, F., & Türkan, I.** (2007). The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. *Environmental and Experimental Botany*, *60*(3), 344-351.

Lou, Q., Iovene, M., Spooner, D. M., Buell, C. R., & Jiang, J. (2010). Evolution of chromosome 6 of Solanum species revealed by comparative fluorescence in situ hybridization mapping. *Chromosoma*, *119*(4), 435-442.

de Lacerda, C. F., Cambraia, J., Oliva, M. A., Ruiz, H. A., & Prisco, J. T. (2003). Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress. *Environmental and Experimental Botany*, *49*(2), 107-120.

Mandre, M. (2002). Stress concepts and plants. *Forestry Studies*, *36*, 9-17.

Maxted, N., Scholten, M., Codd, R., & Ford-Lloyd, B. (2007). Creation and use of a national inventory of crop wild relatives. *Biological Conservation*, *140*(1-2), 142-159.

Molazem, D., Qurbanov, E. M., & Dunyamaliyev, S. A. (2010). Role of proline, Na and chlorophyll content in salt tolerance of corn (Zea mays L.). *American-Eurasian J. Agric. & Environ. Sci*, *9*(3), 319-324.

Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.*, *59*, 651-681.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, *15*(3), 473-497.

Rai, R., Campus, P., Culture, A., & Conservation G. (1990). Genetics and Plant Breeding: Introduction to Plant Biotechnology. *Google Scholar,* 1-42.

Schulze, E. D., Beck, E., & Hohenstein, K. M. (2005). Environment as stress factor: stress physiology of plants. *Plant Ecology*, *702*, 7-11.

Singh, J., Sastry, E. D., & Singh, V. (2012). Effect of salinity on tomato (Lycopersicon esculentum Mill.) during seed germination stage. *Physiology and Molecular Biology of Plants*, *18*(1), 45-50.

Sridhar, T. M., & Naidu, C. V. (2011). An efficient callus induction and plant regeneration of Solanum nigrum (L.)-An important antiulcer medicinal plant. *Journal of Phytology*, 3(5): 23-28.

Sun, W., Xu, X., Zhu, H., Liu, A., Liu, L., Li, J., & Hua, X. (2010). Comparative transcriptomic profiling of a salt-tolerant wild tomato species and a salt-sensitive tomato cultivar. *Plant and Cell Physiology*, *51*(6), 997-1006.

Wang, W., Vinocur, B., & Altman, A. (2003). Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta*, *218*(1), 1-14.

Zhu, J. K. (2007). Plant Salt Stress. Encyclopedia of Life Sciences. *CrossRef Google Scholar*, 1-3.

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