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Morphological and anatomical responses of two Palestinian tomato (*Solanum lycopersicon* L.) cultivars to salinity during seed germination and early growth stages

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The effect of salinity (50, 100 and 150 mM NaCl) on seed germination and early growth of two Palestinian tomato (*Solanum lycopersicon*) cultivars (J1 and Ram) was investigated. Salinity delayed seed germination of both cultivars, and none of them reached 50% germination at 150 mM NaCl even after 21 days of incubation. The seedling height increased with time but decreased with increasing salinity level in both cultivars. Ram cultivar showed higher relative seedling height and higher relative root length than the J1 cultivar. The development of root system and leaves was reduced as salinity increased. Salt stress caused reduction in the vascular system in both root and stem. Meanwhile, salinity stress resulted in an increase in the thickness of the cortex region of the stem and a reduction in that of the root. The reduced severity of morphological and anatomical deformations observed in the Ram line is more tolerant to salinity stress than the J1 cultivar, during the early growth stage.

Keywords: Anatomy, morphology, salinity stress, *Solanum lycopersicon*, tomato.

INTRODUCTION

Severe abiotic stresses, such as salinity and drought, are more harmful to crop plants than the biotic, where the later causes less than 10% loss of crop yields, while the former can reduce yields up to 65%. Salinity is considered as the major abiotic stress that affects negatively the yield of crops in arid and semiarid regions (Greenway and Munns, 1980; Albino et al., 2001). The major problems caused by salt stress are osmotic stress and ion toxicity (Serrano et al., 1999). Osmotic stress results from the excessive ions, especially Na^+ and Cl^- accumulating around the roots. On the other hand, ion toxicity occurs when the sodium ions accumulate to a detrimental level in plant leaves.

High salt concentrations impose adverse effects on plant growth and development throughout the ontogeny of the plant. Salinity stress delays the onset, reduces the rate, and increases the dispersion of germination events, reduces the plant leaf number (Oztekin and Tuzel, 2011), reduces the number of fruits, number of flowers variability in crop maturation thereby, reducing the yield (Raja et al., 2012). It may also cause reduction in Adenosine-5'-triphosphate (ATP) and growth regulators in plants (Allen et al., 1994). This would lead to a sequence of events such as a lower growth rate, a decrease in leaflet growth and in the number of leaflets per leaf (Najla et al., 2009). In addition, salinity stress slows the rate of cell expan-

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sion, which limits the size of the leaves leading to their death and an eventual death of the plant (Volkmar et al., 1998).

Alterations in plant morphology, for example significant reductions in shoot weight, plant height, root length and number of lateral roots, are also evident due to salt stress (Hajer et al., 2006; Maggio et al., 2001). The roots play a crucial role in the amount of salt taken into the plant (Maggio et al., 2001). The salt stress is firstly perceived in the roots. As a consequence, the root sends the signal hormone abscisic acid to the leaf causing the closure and/or decreasing the density of stomata in developing leaves in order to reduce the transpiration rate. This will inhibit the photosynthesis because of the reduction in the CO_2 supply. As a result, the net photosynthesis will decrease significantly whereby prohibiting leaf expansion (Lerner et al., 1994; Rausch et al., 1996). On the other hand, most of the salt ions are translocated from the roots to the leaves via the xylem stream, where they are compartmentalized into vacuoles as an adaptive strategy (Robinson et al., 1997). The compartmentalization consumes energy from a cell and this hinders its growth (Volkmar et al., 1998). Another energy consuming adaptive mechanism at a cellular level involves extrusion of ions via active transport processes (Taiz and Zeiger, 2010).

Tomato (*Solanum lycopersicon* L.) is one of the most economically important crop in the world (Amini and Ehsanpour, 2005; Estan, et al., 2005). In Palestine, it is considered as one of the major vegetable crops. Tomato is sensitive to high levels of salt in the soil (Turhan et al., 2009). Development of salt tolerant cultivars would allow exploitation of larger areas for the cultivation of this crop. Screening for salt-tolerant cultivars is a prerequisite step to achieve this target. The aim of this study was to identify Palestinian tomato cultivar(s) showing salt tolerance during the seed germination and early growth stages. The study focus on exploring anatomical and morphological features related to salt tolerance during the early development stage of the seedling.

MATERIALS AND METHODS

Plant seed germination

Two Palestinian tomato cultivars, J1 and Ram were investigated. The seeds of the cultivars were kindly provided by the Palestinian Ministry of Environmental Affair, the Botanical Garden, Jericho. Seeds were surface sterilized by soaking in 1% (v/v) sodium hypochlorite solution for 20 min and washed with sterile distilled water three times. *In vitro* germination was done in 9 cm Petri dishes containing water agar medium (0.8%) supplemented with 0, 50, 100 and 150 mM NaCl for 21 days. The pH of the medium was adjusted to 5.8 with NaOH. The medium was autoclaved for 20 min at 121°C and 1 atmosphere. Under aseptic condition, ten seeds of each cultivar were placed in each Petri dish in a triplicate form for each concentration and were incubated in plant growth room under fluorescent cool light with 16 h photoperiod at 24 ± 2°C.

During the germination stage, the number of germinated seeds (the emergence of radicle) was recorded daily for 21 days. The G_{50} is defined as time (in days) required for the germination of 50% of the seeds.

Early vegetative growth

For the evaluation of salt stress effect on the early vegetative growth stage, 21-days old seedlings were transferred to 0.5 strength MS medium (Murashige and Skoog, 1962) and 0.8% agar in Magenta boxes supplemented with the same salt concentrations as applied in the germination stage. The seedlings were then maintained under the same conditions of seed germination for additional 10 days after which, the growth parameters, for example seedling height, relative root length, relative stem length and stem diameter, were determined.

Light microscopy (LM) and statistical analysis

The 31 days old seedlings were used for anatomical investigation. Segments of roots and stems (hypocotyls) were fixed in 5% formaldehyde overnight at 4°C (refrigerator). After dehydration in graded solution series combined of tertiary butanol, ethanol and distilled water, and infiltrated in Histoclear-Paraplast plus, the samples were embedded in freshly prepared resin “Paraplast plus”. Semi-thin sections (8 µm thick) were cut on a Shandon Finesse E+ AND ME+ microtome (Thermo Electron Corporation, series A77510300GB, United Kingdom) and mounted on glass slides over a 40°C hot plate for an overnight.

The sections were cleared in double bath of histoclear and rehydrated in decreased graded ethanol series. They were then stained with 0.5% toluidine blue O in 5% borax solutions (Pickett-Heaps and Northcote, 1969; Ge et al., 2005). Another set of sections were stained with 1% safranin O and 0.5% fast green (Johansen, 1940). Micrographs of the sections were taken using a microscope OLYMPUS CK40 with photographic attachment (Dino-Lite Handheld Digital Microscope Device, AM-311S/AM311-ST).

All statistical tests were performed using the statistical software package SPSS for Windows (11.5.1, SPSS Inc., USA). Differences in means of anatomical and morphological variables were assessed by means of the analysis of variance (ANOVA). Correlation analysis was used to examine relationships among plant tissue variables.

RESULTS

Effect of salinity on seed germination

Salinity stress delayed seed germination and reduced germination percentage of both cultivars (Figures 1 to 3). Ram cultivar showed lower values of G_{50} than the J1 when germinated in 50 and 100 mM NaCl. A significant difference in G_{50} was recorded for Ram (6.3 days) over J1 (9.1 days) when they germinated in 100 mM NaCl. On the other hand, when salinity increased to 150 mM, none of the two cultivars was able to reach 50% germination. The seed germination percentage for Ram and J1 cultivars at this elevated level of salinity reached only 25 and 13.33%, respectively after 21 day of incubation. This

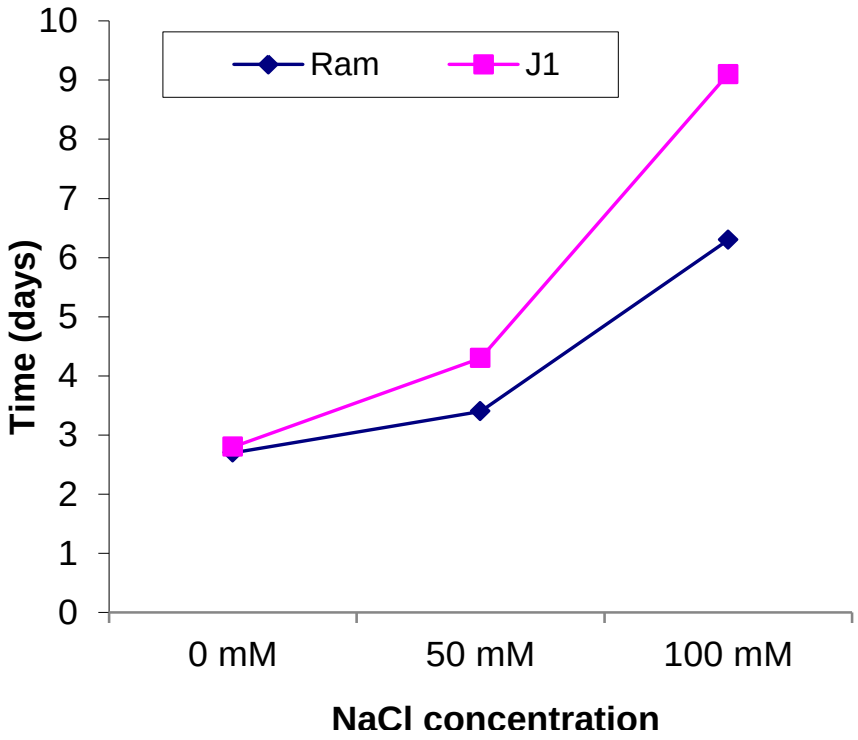

Figure 1. G_{50} values for Ram and J1 tomato cultivars germination in 50 and 100 mM NaCl; none of the cultivars reached G_{50} in 150 mM NaCl.

indicates that Ram cultivar is more tolerant to salinity stress during the seed germination stage, than the J1 cultivar.

Regarding the morphological data collected at day 21 from sowing, the total seedlings height of both cultivars increased with time but decreased with increasing salinity. Increasing salinity caused reductions in plant height, stem length, root length and repressed the emergence of lateral roots (Figures 2 to 3). The cotyledons and hypocotyls of the 50 mM NaCl treated plants were greater in size than the corresponding ones of the plants grown in other salinity levels including the control. The cotyledons were highly reduced in size when plants were grown in 150 mM NaCl. In addition, the cultivars showed a swelling and/or succulence in the transition zone between the root system and the shoot system (Figures 2 to 3).

Effect of salinity on early growth stage

The 31 days-old seedlings of both cultivars showed a reduction in seedling height, stem length and root length at 100 and 150 mM NaCl (Figures 4 to 5). Nevertheless, in 100 mM NaCl, the Ram stems possessed greater length relative to the control than the J1 (88 vs. 70%). Stem average thickness was significantly correlated with increasing salinity levels in both cultivars. However, Ram cultivar (r = 0.83) was less correlated than the J1 (r = 0.99). This indicates that Ram cultivar shows fewer changes in stem diameter as a consequence of salt stress than the J1 cultivar, particularly when grown at salinity levels of 50 and 100 mM NaCl (Table 1).

Morphologically, Figures 4 to 5 show that the control seedlings of both cultivars succeed to develop the first and second leaves. At 50 mM NaCl, however, the seedlings of both cultivars developed only the first leaf. Moreover, the higher level of salinity (100 mM NaCl) hindered the development of leaves in both cultivars. However, the highest level of salinity (150 mM NaCl) prevented development of leaves in the J1 cultivar, while the Ram line succeeded to develop the first leaf.

Transverse sections from the stem of J1 cultivar revealed an increase in the thickness of the stem cortex (Table 1; Figures 6 to 8). The cortex region (starting from the outer side of the vascular cylinder) was 5 to 7 cell layers in the control, while it was 8 to 10 and 11 to 13 cell layers in the 50 mM and 100 mM NaCl treated seedlings, respectively (Figures 6 to 8). Similar increase in cortex thickness was observed in Ram cultivar at 50 mM NaCl. However, no further increase in cortex thickness was observed at levels beyond this salinity level (Table 1;

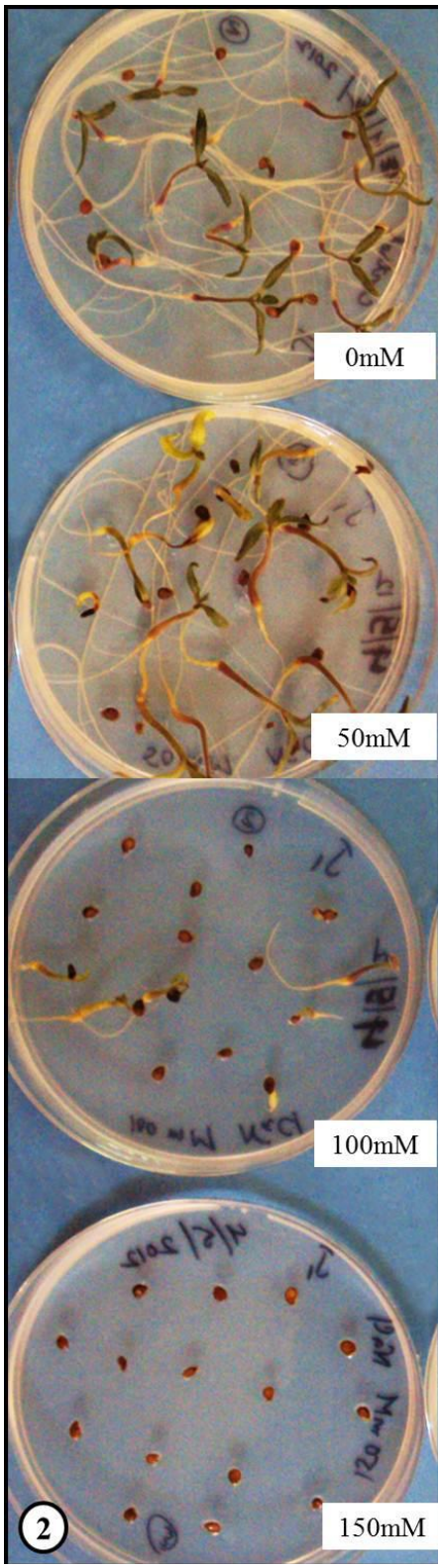

Figure 2. J1 cultivar: Photos of 21 days germination of tomato seedlings illustrate the morphological changes induced by 0, 50, 100 and 150mM salt.

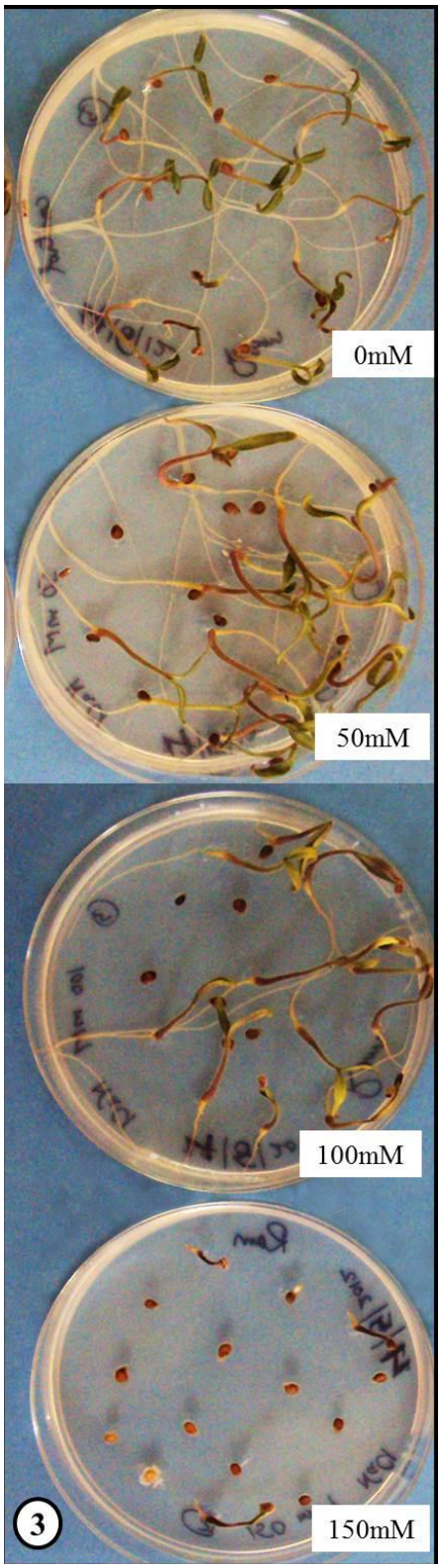

Figure 3. Ram cultivar: Photos of 21 days germination of tomato seedlings illustrate the morphological changes induced by 0, 50, 100 and 150mM salt.

Figure 4. J1 cultivar: Photos of 31 days-old tomato seedlings illustrate the morphological changes induced by 0, 50, 100 and 150mM salt. Scale bar = 10 mm.

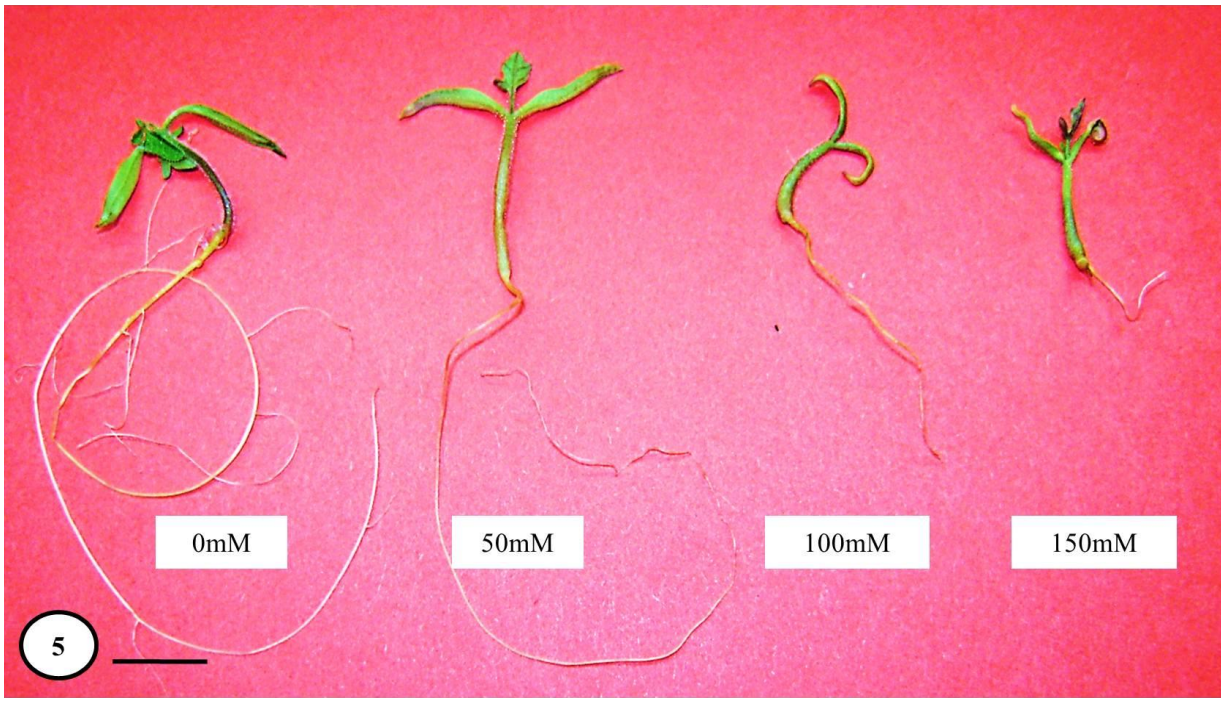

Figure 5. Ram cultivar: Photos of 31 days-old tomato seedlings illustrate the morphological changes induced by 0, 50, 100 and 150 mM salt. Scale bar = 10 mm

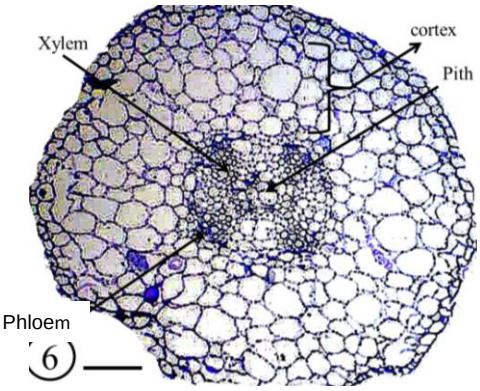

Figure 6. Stem transverse section of J1 cultivar grown with no salt treatment (control). Scale bar = 100 µm

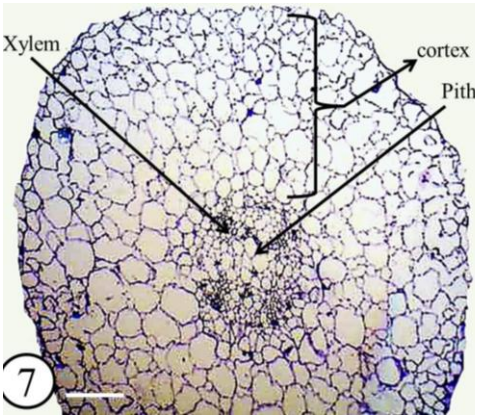

Figure 7. Stem transverse section of J1 cultivar grown in 50 mM NaCl containing media reveals a slight increase in the cortical cell layers. Scale bar = 100 µm

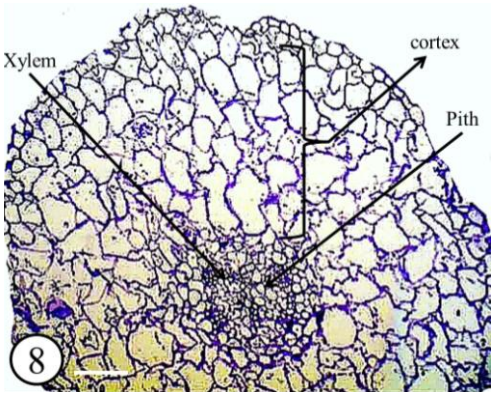

Figure 8. Stem transverse section of J1 cultivar grown in 100 mM NaCl containing media shows an increase in cortical cell layers, increase in cortical cell volume and a reduction in vascular system. Scale bar = 100 µm

Figures 9 to 11).

Pertaining to the effect of salinity on root morphology, the Ram cultivar showed less changes in root diameter and length than the J1 (Figures 4 to 5; Table 2). The relative root length of Ram seedlings grown in 50 and 100 mM NaCl to the control were 92 and 45%, respectively. On contrast, the relative root length of J1 seedlings in the corresponding concentrations was 86 and 33%, respectively.

The anatomical investigations on the region just below the hypocotyl-root transition zone, revealed a reduction in the vascular system of the roots of salt treated seedlings (Figures 12 to 17). The vascular cylinder was dimensioned in the treated plants of both cultivars particularly those grown in 100 mM NaCl. The Ram cultivar showed a reduction of 1 to 4 cell layers in the cortex thickness with increased salinity from control to 50 and 100 mM NaCl (Figures 15 to 17; Table 2, r = - 0.56). In addition, the salt stressed tomato seedlings showed few and small metaxylem vessels, while large prominent metaxylem vessels were present in the root of the controls. Moreover, the parenchyma and phloem profiles were reduced in the salt stressed plants (Figures 12 to 17).

DISCUSSION

Seed germination is initiated by imbibition of water followed by activation of hydrolytic enzymes catalyzing the breakdown of the food reserve material stored in the seed. The degradation products are then used for the building up of new tissues through a wide range of biosynthetic pathways involving various enzymes (Taiz and Zeiger, 2010). All of these processes are coordinated and controlled by several hormones like brassinosteroids, gibberellins, abscisic acid and other factors (Clouse and Sasse, 1998). In another study, we found that tomato seedlings grown under saline conditions accumulates sodium ions, in roots and leaves, several folds greater than amounts accumulated in the control (unpublished data). The harmful effects of salinity on seed germination observed in both cultivars might be attributed to the toxic effect of the accumulated ions and/or the impairment of the water absorption process (Cuartero and Fernandez-Munoz, 1999; Neamatollahi et al., 2009).

The different G_{50} values (germination rate) under salt stress conditions exhibited by the two cultivars (Figure 1) could be a consequence of difference in ion plasma lamella extrusion pumps, where the Ram cultivar harbors a larger number of such transporters than the J1 line. Also, the Ram cultivar may have more efficient mechanisms for salt compartmentalization into the vacuoles. The reduced amount of toxic ions due to the action of these mechanisms of ion translocation, would exert less toxic effect on the metabolic enzymes, whereby allowing

Table 1. Effect of salinity (0, 50 and 100 mM NaCl) on the morphology and anatomy of stems of the Palestinian tomato cultivars, J1 and Ram, of 31 days-old seedlings. [n=10 for each concentration, average (ave) ± Standard Deviation (SD)].

NaCl concentration (mM)	Tomato cultivar					
	J1 Stem			Ram stem		
	Length (mm)	Diameter (mm)	Cortex cell layers	Length (mm)	Diameter (mm)	Cortex cell layers
0	18.56 ± 3.09	1.13 ± 0.10	5-7	17.00 ± 2.59	1.06 ± 0.12	5-7
50	20.00 ± 2.35	1.48 ± 0.27	8-10	18.55 ± 2.43	1.55 ± 0.37	8-9
100	13.14 ± 3.58	1.71 ± 0.45	11-13	15.00 ± 3.92	1.52 ± 0.41	8-10
Correlation coefficient (r)	- 0.75	0.99	0.99	- 0.56	0.83	0.93

The values for length and diameter are mean ± standard deviation.

Table 2. Effect of salinity (0, 50 and 100 mM NaCl) on the morphology and anatomy of roots of the Palestinian tomato cultivars, J1 and Ram, of 31 days-old seedlings. [n=10, ± SD for each concentration].

NaCl concentration (mM)	Tomato cultivar			
	J1 roots		Ram root	
	Length (mm)	Cortex cell Layers	Length (mm)	Cortex cell layers
0	122.56 ± 26.83	8-9	128.40 ± 30.87	8-12
50	105.44 ± 35.16	6-8	118.40 ± 31.61	7-8
100	40.86 ± 20.24	8-9	58.63 ± 30.43	8-9
Correlation coefficient (r)	- 0.95	0.006	- 0.92	- 0.59

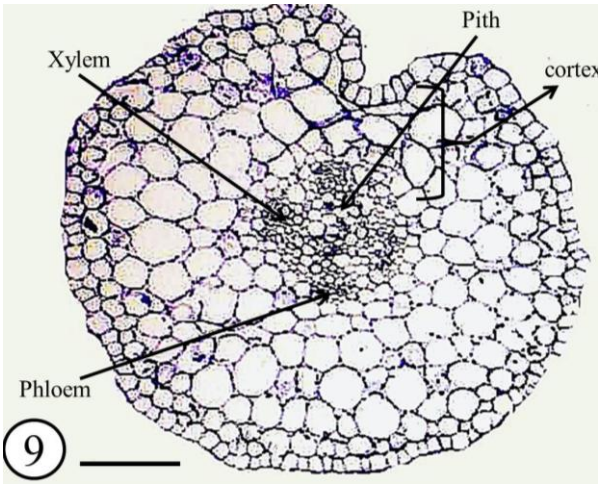

Figure 9. Stem transverse section of Ram cultivar grown with no salt treatment (control). Scale bar = 100 µm

seed germination processes to occur (Kaveh et al., 2011).

The salt treatment of 100 and 150 mM NaCl caused a substantial reduction in size of cotyledons and hypocotyls of both cultivars (Table 1, Figures 4 to 5). The decrease in cell size upon exposure to salt stress is a well-documented phenomenon in plants. It brings important advantages to the cell while coping with the salt stress. For example, a smaller cell size would require synthesis of smaller amount of organic solutes to counter balance the osmotic effect of the external excessive salt ions (Berger et al., 1998; Babu et al., 2012).

Our results indicate that the Ram cultivar showed a less reduction in root and shoot sizes compared to J1 in 50 and 100 mM NaCl (Tables 1 and 2). This is probably due to less toxic effect imposed by ions in the Ram cultivar due to their extrusion by active transport in the roots. The reduced root volume and/or root-to-shoot ratio may improve salinity tolerance by restricting the flux of toxic ions to the shoot leading to a delay in the onset of the tolerance threshold (Maggio et al., 2001). The inhibition of lateral root development in all salt treated samples (Figures 4 to 5) could be explained as an adaptive change aimed at decreasing the absorption surface area to reduce uptake of toxic ions (Dalton et al., 2000). The increased size in root and shoot of 50 mM NaCl treated seedlings compared to the other treatment cannot be explained.

The anatomical studies on the root of the salt treated seedlings revealed a decrease in the cortex thickness of the Ram cultivar in relation to its control. However, in the J1 cultivar, the root cortex thickness did not change significantly (Table 2). The plasmalemma of cortical cells

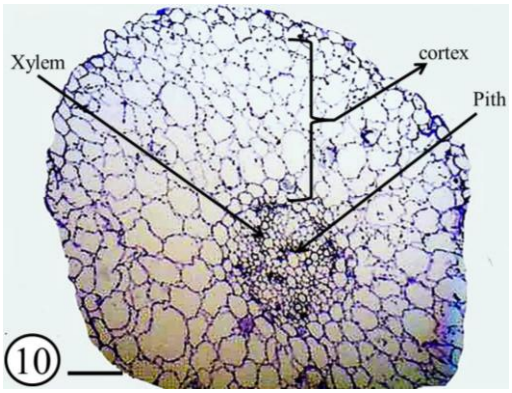

Figure 10. Stem transverse section of Ram cultivar grown in 50 mM NaCl containing media reveals a slight increase in the cortical cell layers. Scale bar = 100 µm

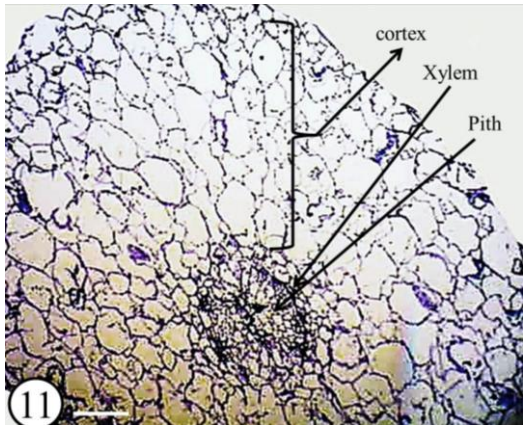

Figure 11. Stem transverse section of Ram cultivar grown in 100 mM NaCl containing media shows the same increase in cortical cell layers as in the previous salt level (50 mM NaCl). Scale bar = 100 µm

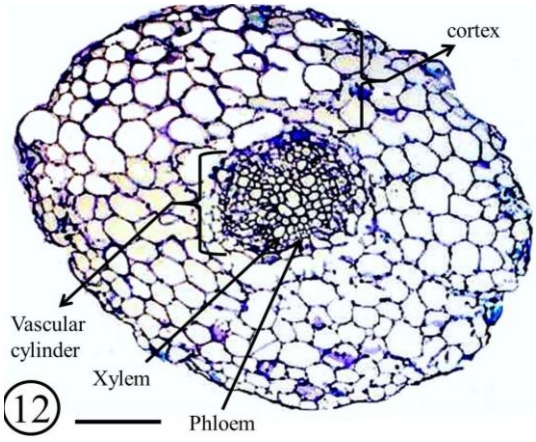

Figure 12. Root transverse section of J1 cultivar grown with no salt treatment (control).

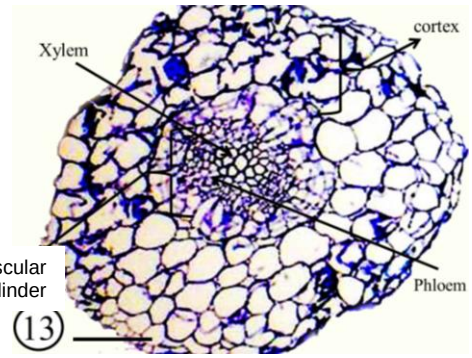

Figure 13. Root transverse section of J1 cultivar grown in 50 mM NaCl containing media reveals an ignored increase in the cortical cell layers. Scale bar = 100 µm

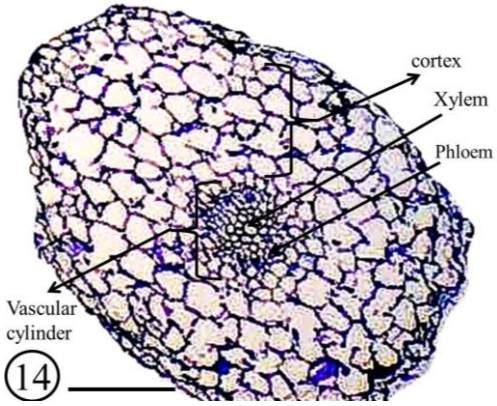

Figure 14. Root transverse section of J1 cultivar grown in 100 mM NaCl containing media reveals an ignored increase in the cortical cell layers. Scale bar = 100 µm

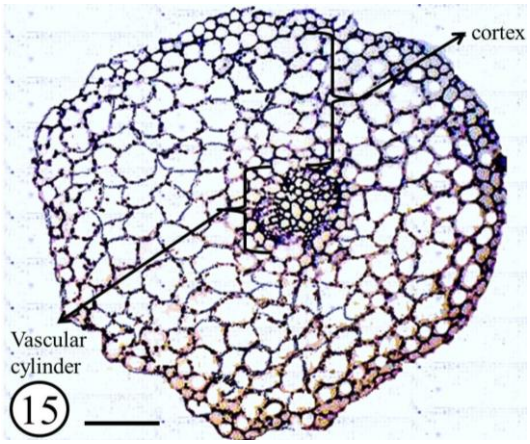

Figure 15. Root transverse section of Ram cultivar grown with no salt treatment (control). Scale bar = 100 µm

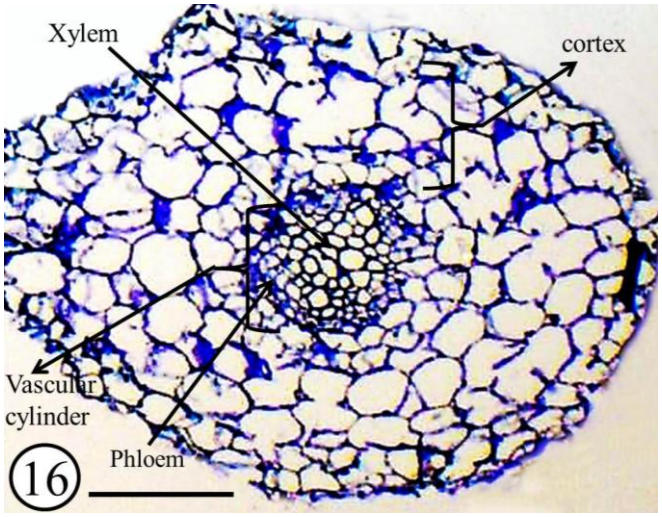

Figure 16. Root transverse section of Ram cultivar grown in 50 mM NaCl containing media reveals increase in the cortical cell layers. Scale bar = 100 µm

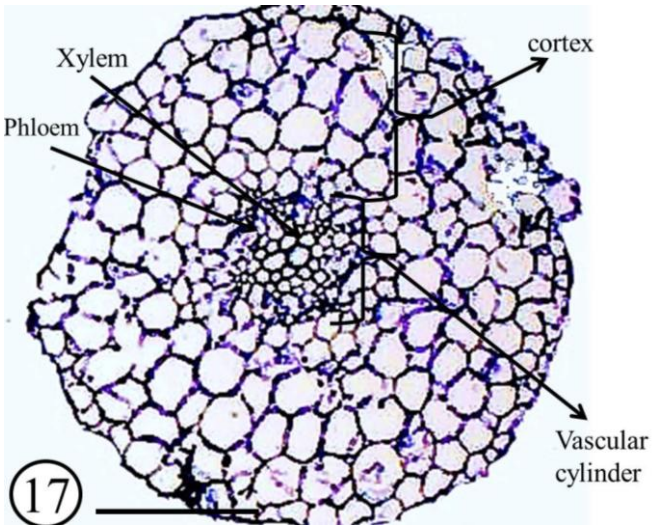

Figure 17. Root transverse section of Ram cultivar grown in 100 mM NaCl containing media reveals the same increase in the cortical cell layers as in the previous salt level (50 mM NaCl).

in young roots actively engaged in absorption, constitute a surface for absorption of water and salt from the apoplast (Weier et al., 1982). A reduction in the density of the cortical cells would result in a reduction in total salt absorption. The reduction in membrane surface would be an adaptive change that may increase salt tolerance. Accordingly, the Ram cultivar could be considered as salt tolerant compared to the J1.

The vascular bundles in roots of salt treated (all salinity levels) seedlings showed a decrease in size in both cultivars compared to the control (Figures 12 to 17).

Similar effect of salinity on root vascular system was observed in a study on mungbean (*Phaseolus radiatus* L. cv. BARI-3) seedlings. In this study, a relatively low salt concentration (50 mM NaCl) inhibited the growth of root vascular system (Rashid et al., 2004).The decrease in the xylem size is probably a result of the decrease in amounts of water translocation (Reinhardt and Rost, 1995; Sánchez-Aguayo et al., 2004). In the present study, it was found that salinity stress reduces the lateral root formation as well as the root cortical thickness. These changes would most likely lead to a decrease in water absorption and translocation in the xylem.

The stem cortex thickness of both cultivars was increased significantly at 100 mM NaCl compared to the control. However, the thickness of the J1 cortex was significantly higher than that of the Ram cultivar (Table 1). Stem cortical cells ordinarily function in storage. In the salt treated seedlings, these cells may function in compartmentalization of toxic ions. When the salinity level reaching the stem cells becomes higher, there will be a need for larger number of cortical cells for the aforementioned compartmentalization (Cunhua et al., 2012). On the other hand, when the amount of salt reaching the stem, becomes lower, one would expect that less cortical cells will be needed for salt compartmentalization. This assumption is consistent with our findings where the Ram cultivar contains less root cortical thickness, that is, less water and salt absorption. These assumptions could be assessed by conducting anatomical studies on cortical cells of root and stems of salt sensitive and/or tolerant cultivars.

In conclusion, Ram cultivar, when compared to J1 line, shows more salt tolerance adaptations at both morphological and anatomical levels. Further research is required to assess the capacity of this cultivar to extrude toxic ions in the root and an improved potassium/sodium balance in the roots and shoots which constitutes another indication for salt tolerance.

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