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Improvement in tolerance to salt stress during tomato cultivation

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Abstract: This study was conducted to evaluate the physiological impact of seed priming on improving the salt tolerance of tomato cultivars H-2274 and Rio Grande. Tomato seeds were primed with 5 M NaCl solution at 20 °C for 3 days in a dark environment. The seeds of the control treatments were handled in a similar way, using tap water instead of NaCl solution. After the priming procedure, seeds were washed and sown in standard germination trays. From each treatment group, 21 homogeneous seedlings were transplanted into 10-L pots full of turf for subsequent salinity experiments. In order to avoid osmotic shock, the volume of the applied NaCl solutions (0 mM, 100 mM, and 200 mM NaCl) was increased from 30 mL/day to 50 mL/day after 5 days. As a major physiological parameter, the chlorophyll content was evaluated 3 times during the experimental phase. Furthermore, the mineral compositions (Na, K, Ca, and Mg) of the leaves as well as the content of soluble sugars (glucose, fructose, and sucrose) were evaluated at the end of the experiment. Consequently, it was found that seed priming with NaCl may provide a good alternative to reduce salinity-induced stress in tomato plants.

Key words: *Lycopersicon esculentum*, plant physiology, salinity, seed priming

1. Introduction

Tomatoes are one of the most frequently consumed vegetables in many countries, being the main source of several phytonutrients and providing important nutritional value to the human diet (Willcox et al., 2003). During their lifespan, plants are frequently exposed to various negative conditions. Such conditions affect or inhibit growth, development, and metabolism due to stress. Mahajan and Tuteja (2005) classified stress factors, based on their origins, into 2 groups: abiotic and biotic stress factors. Abiotic stress factors include cold and hot temperatures, drought, salinity, excessive water, radiation, various chemicals, oxidative stress, wind, and lack of soil nutrients. Biotic stress factors consist of pathogens like viruses, bacteria and fungi, insects, and herbivores. Salinity is one of the most severe problems in agricultural production worldwide. Soil and irrigation water salinity reduces soil water availability and inhibits germination and growth, consequently decreasing yield (Tanji, 1990). In arid or semiarid regions, in particular, salinity is the major and most common abiotic stress factor in plants (Umezawa et al., 2000). Different researchers have reported this problem in different fashions. Serrano and Gaxiola (1994) reported that about 40% of agricultural lands worldwide were under threat of salinity. Today, almost 1 million hectares, corresponding to 7% of the earth's surface area, are under threat of salinity (Metternicht and Zinck, 2003). Yaylacı and Alikamanoğlu (2012) indicated that such an area may reach up to 50% by the year 2050 unless measures are taken.

Plants have different responses to salinity. While some have relative resistance, others are sensitive to even low levels of salinity (Afzal et al., 2005). In particular, salinity increases in the soils of arid and semiarid climates. It is generally a major problem in catchments without a discharge outlet to rivers and is mostly observed due to spoiled soil texture, stiff soil layers, improper irrigation practices, excessive fertilization, insufficient drainage systems, and excessive evaporation (Rabie and Almadini, 2005). Most studies on the physiological responses of plants to stress are based on the assumption that the only possible way a plant can survive under stress conditions is to express preexisting genetic attributes that counteract the effect of stress (Cayuela, 1996). Bolarín et al. (1993) observed higher salt tolerance in tomato plants that were treated at the germination stage than in plants treated after emergence. In this study, a 5 M NaCl solution was used for seed trimming of Rio Grande and H-2274 tomato cultivars, and 0 mM, 100 mM, and 200 mM NaCl solutions were used in salinity experiments to evaluate the variations in element, carbohydrate, and chlorophyll contents of the tomato cultivars.

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2. Materials and methods

Rio Grande and H-2274 tomato varieties supplied by May Seed Inc. were used in this study. Rio Grande is a standard industrial tomato cultivar with a strong plant structure and is available for field culture. H-2274 is a table cultivar and available for field culture. The seeds of Rio Grande and H-2274 tomato cultivars were primed with 5 M NaCl solution and tap water for 3 days at 20 °C in dark conditions. Control seeds were primed with distilled water instead of NaCl. Evaporation was prevented by taping the petri dishes. A total of 50 seeds were primed for each treatment. After the priming procedure, seeds were washed and sown in standard germination trays; germinated seeds were counted daily. In each treatment group, the germination rate was determined and the mean germination time was calculated according to the procedures given by Ellis and Roberts (1981). A total of 21 homogeneous seedlings were transplanted into 10-L pots on 5 August 2010 for the salinity treatments. In order to avoid osmotic shock, the volume of the applied NaCl solutions (0 mM, 100 mM, and 200 mM NaCl) was increased from 30 mL/day to 50 mL/day after 5 days. Weekly chlorophyll content measurements were started 1 week after salt treatment from the fully formed initial leaf with a SPAD device as a rational unit. A portable chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc., Japan) was used to measure the leaf greenness of the plants. The SPAD-502 chlorophyll meter can estimate total chlorophyll amounts in the leaves of a variety of species with a high degree of accuracy and is a nondestructive method (Neufeld et al., 2006). At the end of salt treatments, mineral (Na, K, Ca, and Mg) contents were studied in 3 leaflets taken from fully formed initial leaves on 20 August 2010 (45 days after priming, DAP) and in 3 leaflets taken from fully formed fourth leaves on 3 September 2010 (60 DAP). Furthermore, at the end of the salt treatments, the soluble sugar (glucose, fructose, and sucrose) contents were studied in 3 leaflets taken from fully formed fourth leaves on 3 September 2010 (60 DAP). Elemental analyses were performed using an atomic absorption spectrometer (AAS) device as follows: initially, 10 mL of 65% nitric acid (HNO_3) and 4 mL of 35% hydrogen peroxide (H_2O_2) were added to 0.2-g mashed samples and then placed in a microwave oven (MARS Xpress, CEM Corporation) at 180 °C for 45 min. The mixture was then transferred to a 50-mL flask and 50 mL of demineralized water was added. Elements were calculated by AAS using a PerkinElmer analyzer. Sugars (glucose, fructose, and sucrose) were analyzed with a high-performance liquid chromatography method as follows: 0.2 g of mashed sample was placed into a test tube, to which 5 mL of demineralized water was added, and the sample was then placed into a 60 °C water bath for 60 min. Next, the sample was centrifuged at 4000 rpm for 20 min, pipetted, and passed through a membrane filter. Measurements were taken by using a diffracted refractometer produced by the Knauer Company. Experiments were performed using a randomized-plots experimental design with 7 replications. The data were subjected to variance analysis by SPSS 13.0 for Windows. Least significant difference tests were also used for mean separation.

3. Results

The germination rates in the control treatments of Rio Grande and H-2274 were 98% and 86%, respectively. Under the same conditions, the rates in salt-primed seeds were 83% and 73%, respectively. While the mean emergence time in water-primed seeds was 5.3 days for Rio Grande and 4.2 days for H-2274, the values in salt-primed seeds were 7.5 and 6.7 days, respectively, for Rio Grande and H-2274. Results revealed lower germination rates and longer mean emergence times in salt-primed seeds than in control water-primed seeds (Table 1). Variation in K^+ contents and relevant statistical data are presented in Table 2. Significant differences were observed among the treatments with regard to potassium retention and accumulation. The potassium contents of the leaves of both salt-primed Rio Grande and H-2274 seedlings at 45 DAP were found to be significantly higher than those of the leaves of control seedlings treated with water priming (Table 2).

However, a significant decrease was observed in the K^+ content of 200 mM NaCl-treated H-2274 seedlings, which yielded similar results to the controls, and a decrease was observed in K^+ contents in 200 mM NaCl-treated H-2274 leaves. Significant differences were also observed among treatments with regard to K^+ retention and accumulations in the fourth leaf at 60 DAP. The potassium contents of the leaves of both salt-primed Rio Grande and H-2274 seedlings at 60 DAP were found to be significantly lower than those of the control seedlings treated with water priming (Table 2). The leaves used in these analyses were picked 15 days after the initial samples; therefore, they were exposed to higher salt solutions than the first samples. Since the leaf samples were taken from the fully formed fourth leaf, they were older than the leaves of the first samples. Contrary to the first analyses, control-treatment seeds had higher potassium contents in both cultivars than the salt-primed ones (Table 2). Researchers generally reported decreasing K^+ levels with increasing salinities in various plants (Ashraf, 1994; Parida and Das, 2005). With regard to K^+ uptake, salt-primed seedlings yielded similar results to the control seedlings. Therefore, salt priming can be considered as an effective practice to improve salt tolerance in plants.

Variation in the Ca^{+2} contents of Rio Grande and H-2274 tomato cultivars and relevant statistical data are

Table 1. Germination rates and mean emergence times for 5-M NaCl-primed and water-primed seeds.

Treatments	Rate of germination (%)	Mean emergence time (day)
H_2O-primed Rio Grande	98.00	5.31
NaCl-primed Rio Grande	83.33	7.48
H_2O-primed H–2274	86.00	4.19
NaCl-primed H-2274	73.33	6.70

Table 2. Effects of 0, 100, and 200 mM NaCl treatments on potassium and calcium uptake of the leaves of NaCl- and water-primed Rio Grande and H-2274 tomato cultivars.

Cultivars	Seed treatment	NaCl treatment dose (mM)	K (mg g^{-1}) DW, 45 DAP, first leaf	K (mg g^{-1}) DW, 60 DAP, fourth leaf	Ca (mg g^{-1}) DW, 45 DAP, first leaf	Ca (mg g^{-1}) DW, 60 DAP, fourth leaf
Rio Grande	NaCl-priming	0	36.35 a*	34.85 bc	45.60	27.85
		100	36.29 a	35.21 bc	46.95	29.39
		200	36.49 a	31.92 c	47.19	29.22
	Water-priming	0	34.62 ab	40.87 a	44.90	30.79
		100	33.12 bc	40.27 a	46.03	31.70
		200	31.72 c	37.43 ab	44.85	28.56
H-2274	NaCl-priming	0	38.66 a	31.34 b	43.82 c	23.93
		100	36.96 ab	31.58 b	46.71 bc	25.27
		200	32.70 cd	30.16 b	46.23 c	26.15
	Water-priming	0	34.52 bc	37.32 a	45.50 c	24.23
		100	30.23 de	37.21 a	50.66 ab	27.71
		200	28.60 e	36.12 a	52.50 a	25.40

*: Values indicated with different letters are significantly different ($P < 0.05$). DW: Dry weight.

also presented in Table 2. Statistical differences were not observed in Ca^{+2} retention and accumulation in Rio Grande at either 45 or 60 DAP. However, significant differences were observed in Ca^{+2} retention and accumulation in H-2274 at 45 DAP. Water-primed H-2274 seeds subjected to 100 mM and 200 mM NaCl treatments yielded higher Ca^{+2} contents than the other treatments (Table 2). With regard to Ca^{+2} uptake, statistical differences were not observed between the seedling leaves of salt-primed and water-primed seeds of H-2274 at 60 DAP (Table 2).

Younger leaves had higher Ca^{+2} contents than older ones in both cultivars. These findings are parallel to the findings reported in the literature. Yeo et al. (1991) reported the special impacts of salt stress on leaf aging as accumulation of toxic ions (Na$^+$ and Cl$^-$) or depletion of K$^+$ and Ca^{+2} ions. Ashraf (1994) found that plant nutrient balance was negatively affected by high salinity around the root region of the plants and reported decreases in K$^+$ and Ca^{+2} uptake and transport with increasing salinity levels. While the leaves of salt-primed plants had higher K$^+$ contents at 45 DAP, the K$^+$ contents of the leaves of water-primed plants (control treatments) were higher at 60 DAP. The ion balance of 60-DAP control plants was spoiled; cell membranes lost their osmosis and took up the K$^+$ ions at high levels in an uncontrolled fashion. Likewise, Ca^{+2} uptakes at 45 DAP in control-treatment plants of the less salt-tolerant H-2274 cultivar were higher than in salt-primed plants. With regard to Ca^{+2} uptake of the fourth leaf of H-2274 at 60 DAP, similar results to those in control-treatment plants may be related to a decrease in Ca^{+2} uptake due to salt stress and the transfer of stress conditions in older leaves to younger ones, because 60-DAP analyses were performed 15 days later than those conducted at 45 DAP. In several plants, salinity increases Na$^+$ and Cl$^-$ contents and decreases Ca^{+2}, K$^+$, and Mg^{+2} contents (Parida and Das, 2005). In the present study, while all treatments yielded similar results to the control treatment with regard to the Mg^{+2} uptake of the Rio Grande cultivar at 45 DAP, the Mg^{+2} contents of plants subjected to 200 mM NaCl treatment decreased at 60 DAP. Statistical differences were not observed in Ca^{+2} uptakes in salt-primed and water-primed 45-DAP– and 60-DAP–old plants. However, the Mg^{+2} contents of the leaves at 60 DAP were lower than those of leaves at 45 DAP in both cultivars. These findings are in agreement with Parida and Das (2005) (Table 3). Certain factors causing a decrease in Mg^{+2} uptakes and playing a role in protein synthesis may also cause a decrease in chlorophyll content. Therefore, the highest Mg^{+2} content (5.33 mg g^{-1}) in the Rio Grande cultivar was observed in the leaves of plants from water-primed seeds, and the highest chlorophyll content (50.55%) was also observed in the leaves of the same plants without salinity treatment (Table 3). Variation in the Na^+ contents of the Rio Grande tomato cultivar and relevant statistical data are presented in Table 3. Na accumulations in younger leaves at 45 DAP revealed the highest value (20.42 mg g^{-1}) in plants from salt-primed seeds and with 200 mM NaCl treatment; this was followed by plants from water-primed seeds and with 200 mM NaCl treatment (18.97 mg g^{-1}) (Table 3).

The highest Na accumulation at 60 DAP was observed in salt-primed and water-primed control plants without NaCl treatment, and the other plant groups were placed in the same statistical group. In the H-2274 cultivar, significant differences were not observed among treatments with regard to Na retention and accumulations in young leaves at 45 DAP. However, the Na^+ contents of older leaves at 60 DAP increased in the plants of salt-primed seeds with increasing NaCl doses. Lower Na^+ accumulation levels were observed in plants from water-primed seeds. In this study, the findings of increasing Na^+ contents in younger leaves of Rio Grande and H-2274 at 45 DAP with increasing NaCl doses are in agreement with the findings of Parida and Das (2005). Researchers reported that plants under stress conditions with high salinity levels in their cytoplasm stored excessive Na^+ in their vacuoles to maintain metabolic functions (Parida and Das, 2005). In H-2274, similar results to the control treatments with regard to Na^+ retention and accumulation of younger leaves at 45 DAP are also in agreement with the findings of Cayuela (1996) in tomato cultivars. Significant differences were observed among treatments with regard to the chlorophyll contents of Rio Grande and H-2274 cultivars, and decreasing chlorophyll contents were observed with increasing salinity stress. The highest chlorophyll contents were observed in control groups in all 3 measurements of both salt-primed and water-primed seeds, and chlorophyll contents increased with increasing NaCl doses (Table 4).

Salinity increase in photosynthetic tissues causes accumulation in adjacent grana membranes, shrinking of the thylakoid membranes, and break-off of chlorophyll (Franco et al., 1993; Ashraf, 2004). Low salinity levels increase chlorophyll content and high levels spoil the structure of chlorophyll (Ashraf, 2004). The current study yielded similar findings, and increasing chlorophyll contents were observed with increasing salt stress. The results of the current study are parallel to the findings of Sivritepe et al. (2005) on melon (cv. Kirkagac) plants. Several plants exposed to salt stress tend to accumulate various organic materials with low molecular weights in their cells to sustain osmotic balance. The amount of

Table 3. Effects of 0, 100, and 200 mM NaCl treatments on sodium and magnesium uptake of the leaves of NaCl- and water-primed Rio Grande and H-2274 tomato cultivars.

Cultivars	Seed treatment	NaCl treatment dose (mM)	Mg (mg g^{-1}) DW, 45 DAP first leaf	Mg (mg g^{-1}) DW, 60 DAP fourth leaf	Na (mg g^{-1}) DW, 45 DAP first leaf	Na (mg g^{-1}) DW, 60 DAP fourth leaf
Rio Grande	NaCl-priming	0	5.49	4.70 ab*	18.62 ab	17.55 b
		100	5.70	4.69 ab	18.89 ab	16.99 bc
		200	5.81	4.61 b	20.42 a	16.63 bc
	Water-priming	0	5.64	5.33 a	18.32 ab	19.49 a
		100	5.95	4.91 ab	17.44 b	17.15 bc
		200	5.98	4.58 b	18.97 ab	15.19 c
H-2274	NaCl-priming	0	6.12	4.45	17.91	17.85 b
		100	6.01	4.49	19.69	18.53 ab
		200	6.16	4.79	19.34	20.05 a
	Water-priming	0	5.97	4.40	18.84	15.34 c
		100	6.76	4.57	17.97	15.55 c
		200	6.59	4.56	19.71	14.67 c

*: Values indicated with different letters are significantly different ($P < 0.05$).

Table 4. Effects of 0, 100, and 200 mM NaCl treatments on chlorophyll contents (rational unit) of NaCl- and water-primed Rio Grande and H-2274 tomato cultivars.

Cultivars	Seed treatment	NaCl treatment dose (mM)	13.08.2010 Measurement 1	19.08.2010 Measurement 2	26.08.2010 Measurement 3
Rio Grande	NaCl-priming	0	38.82 b*	40.10 b	48.80 ab
		100	37.94 b	37.55 c	48.31 ab
		200	36.22 c	36.84 c	47.77 b
	Water-priming	0	39.15 a	42.01 a	50.55 a
		100	35.31 c	36.98 c	43.08 c
		200	34.97 c	37.24 c	42.44 c
H-2274	NaCl-priming	0	41.18 a	42.90 a	51.02 a
		100	37.60 bc	39.27 b	47.90 bc
		200	36.10 cd	38.12 bc	46.35 c
	Water-priming	0	40.87 a	41.62 a	49.17 ab
		100	37.8 b	39.38 b	45.47 c
		200	35.82 d	36.97 c	45.64 c

*: Values indicated with different letters are significantly different ($P < 0.05$).

sugars with low molecular weight generally increases under saline conditions depending on plant type, variety, and parts of the plant (Ashraf and Harris, 2004). Sugars (sucrose, glucose, fructose, fructans) and polysaccharides are accumulated under saline conditions to clean out different radicals, and to provide and maintain osmotic balance (Parvaiz and Satyawati, 2008). In this study, the leaves of seedlings from the salt-primed seeds of the Rio Grande cultivar had lower glucose, fructose, sucrose, and total sugar contents than those of controls. Seedlings subjected to salt-priming and 200-mM NaCl treatment had the maximum sugar accumulation levels (Table 5). In the H-2274 cultivar, the glucose, fructose, and total sugar contents of all groups were found to be similar to the contents of the control-treatment plants; a significant difference was observed only in sucrose content. The

Table 5. Effects of 0, 100, and 200 mM NaCl treatments on chlorophyll sucrose, glucose, and fructose contents of NaCl- and water-primed Rio Grande and H-2274 tomato cultivars.

Cultivars	Seed treatment	NaCl treatment dose (mM)	Sucrose (mg g^{-1}) DW, 60 DAP, fourth leaf	Glucose (mg g^{-1}) DW, 60 DAP, fourth leaf	Fructose (mg g^{-1}) DW, 60 DAP, fourth leaf
Rio Grande	NaCl-priming	0	5.99 d*	23.55 bc	31.03 c
		100	6.32 cd	22.72 c	31.12 c
		200	7.49 bc	21.15 c	30.76 c
	Water-priming	0	8.24 ab	30.46 a	37.43 b
		100	9.54 a	26.83 b	37.81 b
		200	8.41 ab	33.54 a	42.69 a
H-2274	NaCl-priming	0	12.14 abc	25.80	34.97
		100	10.56 c	24.81	36.65
		200	11.86 abc	27.76	37.30
	Water-priming	0	12.87 ab	27.65	35.97
		100	11.02 bc	26.45	36.13
		200	13.56 a	26.89	35.57

*: Values indicated with different letters are significantly different ($P < 0.05$).

accumulation of sugars against salt stress in H-2274, which is less tolerant to salinity, and sugar accumulation in water-primed control-treatment plants indicate that the salt-priming practice in Rio Grande might be effective in improving salt tolerance. Cayuela (1996) observed higher sucrose content in the roots and lower values in the leaves at 60 DAP, and also observed higher glucose and fructose accumulation in leaves. The findings of the present study are in agreement with these findings.

4. Discussion

Plants under salinity stress uptake lower levels of K^+ with increasing uptake levels of Na^+ (Yeo et al., 1991; Parida and Das, 2005; Mugdal et al., 2010), and they tend to accumulate simple sugars under stress conditions (Ashraf and Harris, 2004). Low salinity levels increase chlorophyll content (Franco et al., 1993; Ashraf, 2004). In the present study, the control-treatment seeds and salt-primed seeds of Rio Grande were placed in the same statistical group in analyses of the first real leaves carried out at 45 DAP; however, a decrease was observed in the K^+ contents of the water-primed plant leaves. Ion balance in the control plants of Rio Grande and H-2274 cultivars at 60 DAP was negatively affected; they lost cell wall osmosis capability and absorbed K^+ ions in an uncontrolled fashion. In the low-salt–tolerant H-2274 cultivar, the Ca^{+2} uptake of control-treatment plants at 45 DAP was higher than that of salt-primed plants. The glucose, fructose, and sucrose contents of fourth real leaves at 60 DAP revealed that the salt-primed plant leaves had higher rates of sugar accumulation than control-treatment plants. The chlorophyll contents in control-treated plant leaves were lower than in the leaves of salt-primed plants (Table 4). In the present study, it might have been interesting to investigate the K^+ contents in the roots of Rio Grande and H-2274 tomato cultivars, since 45 DAP K^+ uptake in the leaves of control-treatment plants was lower than in the leaves of salt-primed plants, while 60 DAP K^+ uptake and accumulation were higher.

Considering all these findings, the practice of salt-priming may be considered as an effective alternative method to improve the salt tolerance of tomato cultivars, especially of Rio Grande.

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