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Full Length Research Paper

The effect of NaCl seed priming on salt tolerance, antioxidant enzyme activity, proline and carbohydrate accumulation of Muskmelon (*Cucumis melo* L.) under saline condition

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Seeds of Muskmelon (*Cucumis melo* L.) cultivars' Garmsar (local cultivar in south of Iran) and Flexuosus were primed with 100 mmol NaCl solution for 36 h at 20°C. In experiment 1, after priming four replicates of 25 seeds were germinated between two rolled sheets of filter paper with 10 ml of respective salinity test solutions. Seeds were allowed to germinate at 22 ± 1°C in the 16/8 dark and light for 12 days. The experimental design was two factors factorial arranged in a completely randomized design (CRD), with four replications. The first factor was salinity stress (0, 50, 100 and 150 mmol NaCl solution) and the second factor was NaCl Primied (P) and nonprimed seeds (NP). Results indicated that salt stress decreased seed germination and seedling dry weight of muskmelon cultivars but seed priming increased seed germination and seedling growth. At 150 mmol NaCl solution, highest seedling dry weight obtained from cv. Garmsar. In experiment 2, after priming, P and NP muskmelon seeds were sown in germination boxes filled with perlit and sphangnum peat (3:1). 5 seeds sown in any pot and the germination boxes were placed in greenhouse where temperature ranges between 16/26°C, night/day for 3 weeks. The experimental design was two factors factorial, arranged in a completely randomized block design, with four replications. The factors were like experiment 1. Results revealed that salt stress decrease shoot dry weight of muskmelon cultivars, but shoot dry weight of P seeds were higher compare to NP seeds. Seed priming increased antioxidant enzyme activity, soluble carbohydrate and proline content and decrease seed membrane damage of muskmelon cultivars because MDA concentration was low in P group in contrast to NP group. Under 150 mmol salinity level, highest shoot dry weight, antioxidant activity, carbohydrate accumulation and lowest MDA concentration obtained in cv. Garmsar at P group.

Key words: Antioxidant enzyme, muskmelon, osmotic adjustment, priming.

INTRODUCTION

Salinity in soil or water is one of the major stresses and, especially in arid and semi-arid regions, can severely limit crop production. The deleterious effects of salinity on plant growth are associated with (1) low osmotic potential of soil solution (water stress), (2) nutritional imbalance, (3) specific ion effect (salt stress), or (4) a combination of these factors (Ashraf et al., 2001). Sivritepe et al. (2003) reported salt stress decrease seedling growth of melon because under salt stress, toxin ion concentration increased in melon leaf. Shahi et al. (2009) found that salt stress decrease seed germination, seedling growth and cell membrane stability of Snake Melon but increase mean germination time. Cell membrane stability has long been an indicator of stress tolerance. Farooq and Azam

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Abbreviations: CAT, Catalase; **POD,** peroxidase; **ROS,** reactive oxygen species; **MDA,** malondealdehyde; **P,** prime seed; **NP,** non prime seed.

(2006) and Munns and James (2003) suggested that the assessment of cell membrane stability is an appropriate technique to screen plants under saline condition. Bandeoglu et al. (2004) found that salt stress increase MDA production and cell membrane damage in leaf of rice seedling. Reduction in cell membrane damage and lipid distribution (MDA production) improvement with antioxidant enzyme activity.

The production of Reactive Oxygen Species (ROS) in cells increases during abiotic and biotic stresses like salt stress, as does the level of ROS-induced damage. Elevated production of ROS can seriously disrupt cellular homeostasis and normal metabolisms through oxidative damage to lipids, protein, and nucleic acid (Bandeoglu et al., 2004). Plants possess antioxidant enzymes (like catalase, peroxidase and superoxide dismutase) as well as antioxidant compounds to scavenge these ROS, and antioxidant capacity of plants is directly related to their salt tolerance. For example, while determining the role of various antioxidants in the salt tolerance of canola, Ashraf and Ali (2008) found that antioxidant enzymes activity likes catalase and peroxidase in canola leaf increase under salinity condition.

Seed priming is a pre-sowing treatment that involves exposure of seeds to a low external water potential that limits hydration. This hydration is sufficient to permit pregerminative metabolic events but insufficient to allow radicle protrusion through the seed coat. This technique has become a common seed treatment that can increase rate, percentage and uniformity of germination or seedling emergence, mainly under unfavorable environmental conditions. Nascimento (2003) reported muskmelon seed priming with PEG or KNO_3 solution improve seeds germination at low temperature condition. Demir and Mavi (2004) found watermelon seed priming with KNO_3 solution, effectively improved germination and seedling growth of the seeds under salinity compared to non-primed seeds. In tomato seeds, seed priming improves seed germination, seedling emergence and seedling growth under saline conditions (Cayuela et al., 1996). Higher salt tolerance of plants from primed seed seems to be the results of a higher capacity of osmotic adjustment (proline or carbohydrate synthesis) in leaves than plants from non primed seed. Sivritepe et al. (2003) confirmed that, NaCl seed priming increased proline concentration and salt tolerance in melon seedling, under saline condition compared to non-priming seed. Farhoudi et al. (2007) suggested that canola seed priming with NaCl improved salinity tolerance in canola seedling because seed priming decreased seedling cell membrane damage and increased seedling proline concentration. Moosavi et al. (2009) indicated that amaranth seed priming with osmotic solution increase seed germination and antioxidant enzymes activity compared to non-priming seeds. Seed priming is one of the physiological methods which improves seed performance and provides faster and synchronized germination and seedling growth. Therefore, the present study was conducted to examine the effect of NaCl priming on salt tolerance of muskmelon at the germination and seedling stage, to evaluate the physiological effects of priming like antioxidant enzymes activity and osmotic adjustment.

MATERIALS AND METHODS

This study was carried out at the Department of Agronomy, Faculty of Agriculture, Islamic Azad University, Shoushtar Branch, Iran.

Seed treatment

Seeds of muskmelon (*Cucumis melo L.*) cultivars' Garmsar (local cultivar in south of Iran) and Flexuosus obtained from vegetable research center, Ahvaz, Iran. Seeds were superficially sterilized with 0.1% $HgCl_2$ solution for 3 min. and then thoroughly washed for 5 min. Seeds were primed in 100 mmol NaCl solution in a dark room for 36 h at 20°C. On the basis of previous experiments (data not shown), the best dose of priming agent (NaCl) was selected for this experiment to observe the effects of seed priming on performance of muskmelon under different salinity levels. Following treatment, seeds were washed three times for 5 min in distill water. Following this, seeds were dried in room temperature for 24 h at 25°C.

Experiment 1

After priming four replicates of 25 seeds were germinated between two rolled sheets of filter paper with 10 ml of respective salinity test solutions. Seeds were allowed to germinate at 22 ± 1°C in the 16/8 dark and light for 12 days. A seed was considered germinated when the emerging radicle elongated to 5 mm. Seed germination was recorded everyday. The experimental design was two factors factorial arranged in a completely randomized design (CRD), with four replications. The first factor was salinity stress (0, 50, 100 and 150 mM NaCl solution) and the second factor was priming treatment (NaCl priming and nonprime seeds). In order to determine dry weight, seedling dried for 24 h at 70°C. Germination percentage was calculated using the formula:

$$\text{Germination percentage (GP)} = 100 \times \frac{\text{Number of germinated seeds}}{\text{Total of number seeds}}$$

Mean germination time (MGT) was calculated using Schelin et al. (2003) method:

$$MGT = \frac{\sum f_i n_i}{N}$$

where fi = day number during germination period, ni = number of germinated seeds per day and N = sum of germinated seeds.

Analysis of variance of all data for all parameters was carried out using MSTAT-C computer Package. Differences between mean values were determined with Duncan's Multiple Range Test (Snedecor and Cochran, 1980) and P value was $P < 0.01$.

Experiment 2

After priming (experiment 1), primed and non primed muskmelon

Table 1. Effect of seed priming on seed germination of muskmelon under saline condition.

Salinity level (mM NaCl)	Seed priming	Flexuosus			Garmsar		
		Germination (%)	MGT (day)	Seedling dry weight (g)	Germination (%)	MGT (day)	Seedling dry weight (g)
0	NP	96.2 a	2.7 e	0.31 a	97.0 a	2.6 e	0.34 a
	P	97.4 a	2.1 e	0.33 a	97.1 a	2.9 de	0.37 a
50	NP	95.2 a	3.3 d	0.27 b	97.2 a	2.8 e	0.28 ab
	P	93.7 a	3.4 d	0.3 ab	95.4 a	2.0 e	0.28 ab
100	NP	80.2 c	7.3 b	0.18 d	83.7 c	6.1 b	0.21 c
	P	89.4 b	5.4 c	0.25 b	91.4 ab	4.0 c	0.24 b
150	NP	52.2 e	9.0 a	0.11 f	68.4 d	8.4 ab	0.17 d
	P	75.6 d	7.0 b	0.14 e	84.0 c	4.9 c	0.20 c

Means followed by the same letter(s) are not significantly different at *P = 0.01* according to Duncan test.

seeds were sown in germination boxes filled with perlit and sphangnum peat (3:1). 5 seeds sown in any pot and the germination boxes were placed in a greenhouse where temperature ranges between 16°C at night and 26°C during the day for 3 weeks. The boxes were irrigated everyday with NaCl solution. Surplus water drained naturally from the bottom of boxes to avoid build up of salt in the growth media. The experimental design was two factors factorial, arranged in a completely randomized block design, with four replications. The first factor was salinity stress (0 for control, 50, 100 and 150 mmol NaCl solutions) and the second factor was priming treatment (NaCl priming and nonpriming seeds).

After 3 weeks, in order to determine dry weight, one plant (stem+leave) in each treatment, was harvested and dried for 48 h at 70°C. Proline contents of seedlings were determined according to Bates et al. (1973). Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) formation, using the thiobarbituric acid method described by Valentovic et al. (2006). Briefly, 200 mg of fresh tissue was homogenized in 2 ml of 0.1% (w/v) trichloroacetic acid (TCA) solution on ice. The homogenate was centrifuged at 14,000 rpm for 10 min, and 0.5 ml of the supernatant was added to 0.5 ml of 0.5% (w/v) TBA in 20% TCA. The mixture was incubated in boiling water for 20 min and the reaction was stopped by incubation in ice. Samples were then centrifuged for 5 min at 14,000 rpm, and the absorbance of the supernatant was measured at 532 nm, subtracting the value for nonspecific absorption at 600 nm (Valentovic et al., 2006). Catalase (CAT) and peroxidase (POD) were extracted by homogenizing frozen fresh leaf material in ice-cold solution containing 100 mM Tris (pH 7.0), 10 mM D-isoascorbic acid, 1.5 g insoluble PVP, 0.1 mM EDTA and 2ml L^{-1} Triton X-100. CAT activity was determined following Chanes (1995) by monitoring the disappearance of H_2O_2 by measuring the decrease in absorbance at 240 nm of a reaction mixture containing 1.9 ml H_2O, 1.0 ml of 5.9 mM H_2O_2 in potassium phosphate buffer (pH 7.0), and 1.0 ml extract. POD activity was determined following the protocol of Chanes (1995) using guaicol as a reactant. POD activity was measured by monitoring the H_2O_2-dependent oxidation of reduced 2, 3, 6-trichloroindophenol at 675 nm, using a UV–vis spectrophotometer (Model U-2001, Hitachi, Tokyo, Japan).

Analysis of variance of all data for all parameters was carried out using MSTAT-C computer package. Differences between mean values were determined with Duncan's Multiple Range Test (Snedecor and Cochran, 1980) and P value was $P < 0.01$.

RESULTS

Experiment 1

Results indicated that salinity reduced the final emergence percentage of both primed and non-primed seeds with more intensity as salinity levels were increased. However, priming reduced the adverse effects of salinity on muskmelon emergence as compared to non-primed seeds (Table 1). At 150 mmol NaCl solution, the highest and the lowest germination percentage obtained in P seeds of cv. Garmsar (84.0%) and NP seeds in cv. Flexuosus (52.2%) (Table 1). Salinity increased the time required for emergence of muskmelon seeds.

Results showed that, seed priming improve MGT of both muskmelon cultivars, because of P seeds emerged earlier than NP seeds under saline condition. The P seeds of cv. Garmsar had a lower MGT under 150 mmol NaCl solution compared to cv. Flexuosus, the values were 4.9 to 7 days for both cultivars respectively (Table 1). Salt stress decrease muskmelon seedling dry weight but seed priming improved it in both cultivars under saline condition (Figure 1a and b). At the highest salinity level, cv. Garmsar and cv. Flexuosus seedling, dry weight were 0.2 and 0.14 mg at P group while the values were 0.17 and 0.11 mg at NP group of cultivars respectively. Figures 3 and 4 shows that, seed priming improves muskmelon seedling growth at saline condition in both cultivars.

Experiment 2

Shoot dry weight of both cultivars examined in the present study reduced significantly with increased salinity

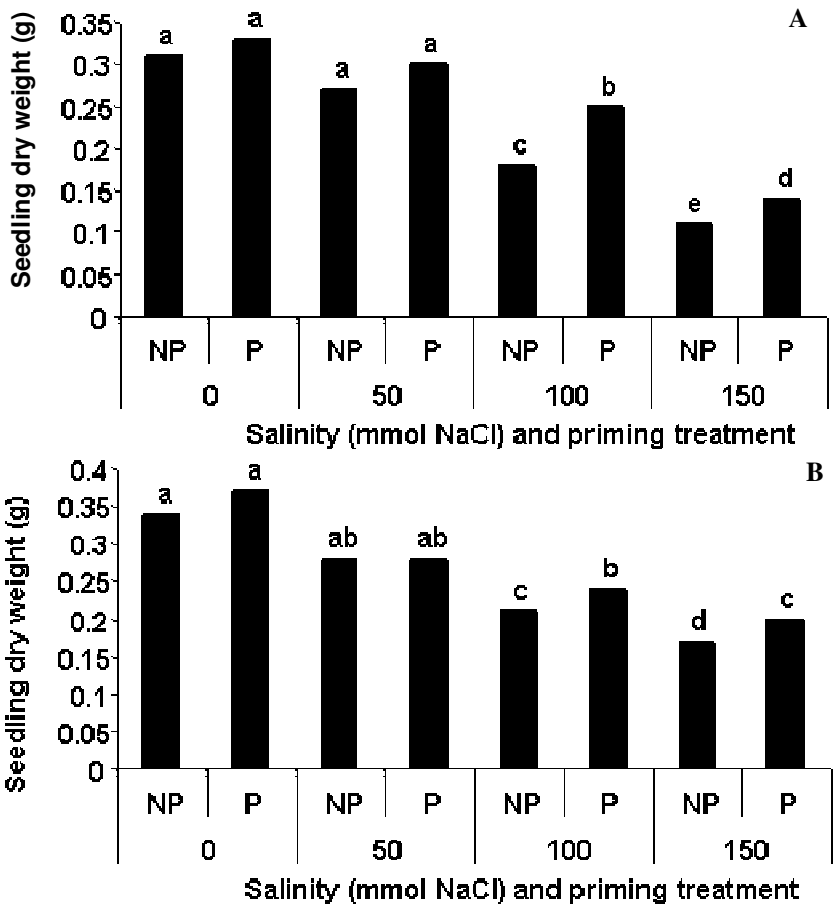

Figure 1. Effect of seed priming on muskmelon seedling dry weight under salinity stress. (a) Flexuosus cultivar and (b) Garmsar cultivar.

levels with more extent in NP compared to P seeds, (Figure 2). Salt stress increased leaf proline concentration of both cultivars (Table 2) but in cv. Garmsar, it did not obtain any significant difference between leaf proline content at P and NP seeds in each salinity level; however, in cv. Flexuosus, seed priming increased leaf proline content under 100 and 150 mmol NaCl solution compared to NP seeds (Table 2). Leaf carbohydrate content increased with increase in salt concentration in P and NP seedling of both cultivars (Table 2) but under 100 and 150 mmol NaCl solution, carbohydrate concentration was higher at P seeds compared to NP seeds.

Concurrently, MDA concentration of both cultivars of muskmelon also increased with increase in external salt concentration (Table 2). In both cultivars, seed priming improved leaf MDA content compared to NP seeds. Up to an external NaCl concentration of 150 mM, MDA concentration was 0.026 and 0.018 $nmolg^{-1}$ in NP and P seeds of cv. Flekuosus compare to 0.018 and 0.013 $nmolg^{-1}$ in cv. Garmsar, respectively (Table 2). There was considerable variation between the two cultivars with respect to CAT activity under saline condition (Table 2).

Comparison of the two cultivars shows that, cv. Flexuosus had lower CAT activity than cv. Garmsar in both P and NP seeds (Table 2). There were no significant differences found in CAT activity of P and NP seeds of Garmsar cv. Data for POD activity shows that, this variable affected in both cultivars, with more activity in P than NP seeds when exposed to increase in salt concentration of the growth medium (Table 2).

DISCUSSION

It is clear from the present results that increasing concentrations of NaCl salinity reduced germination and growth of muskmelon cultivars seedling (Tables 1 and 2). Salt stress decreased germination and seedling growth of some plants like melon (Sivritepe et al., 2003), canola (Farhoudi et al., 2007), safflower (Farhoudi and Motamedi, 2010) and pepper (Khan et al., 2009). To improve salt tolerance of the plants, especially horticultural commodities, at different stages of their lifecycle, we need more methods for improving the crops under salt stressed condition by simple, un-expensive

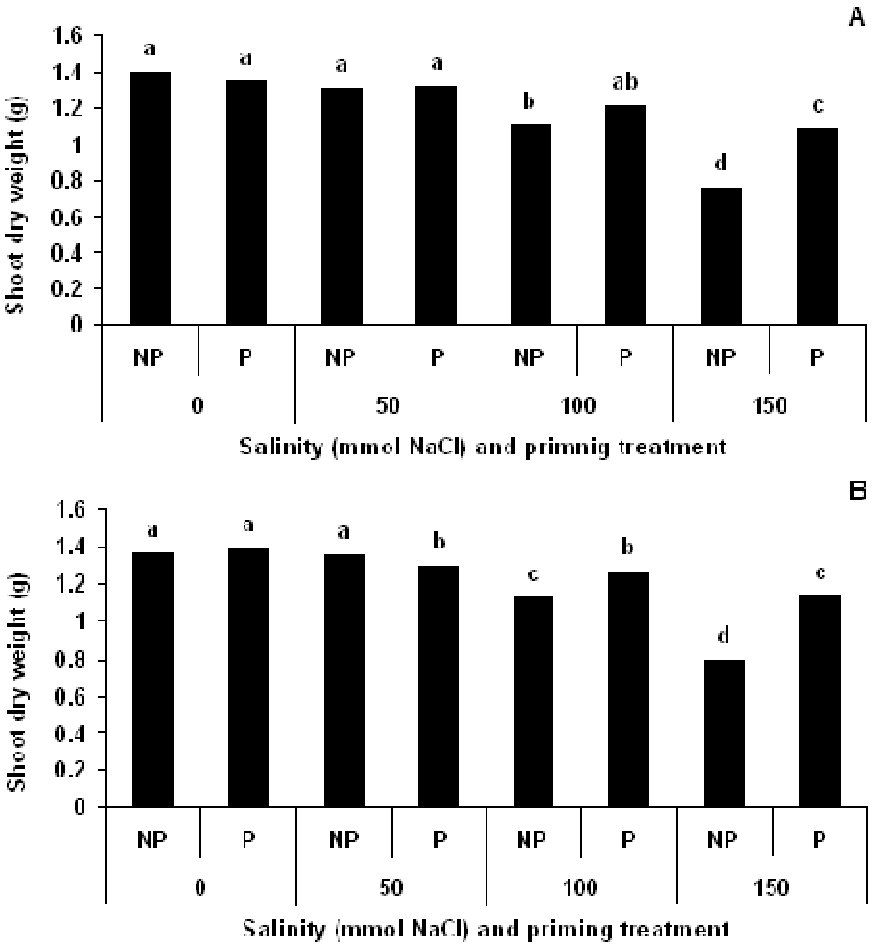

Figure 2. Effect of seed priming on muskmelon shoot dry weight under salinity stress (a) Flexuosus cultivar and (b) Garmsar cultivar.

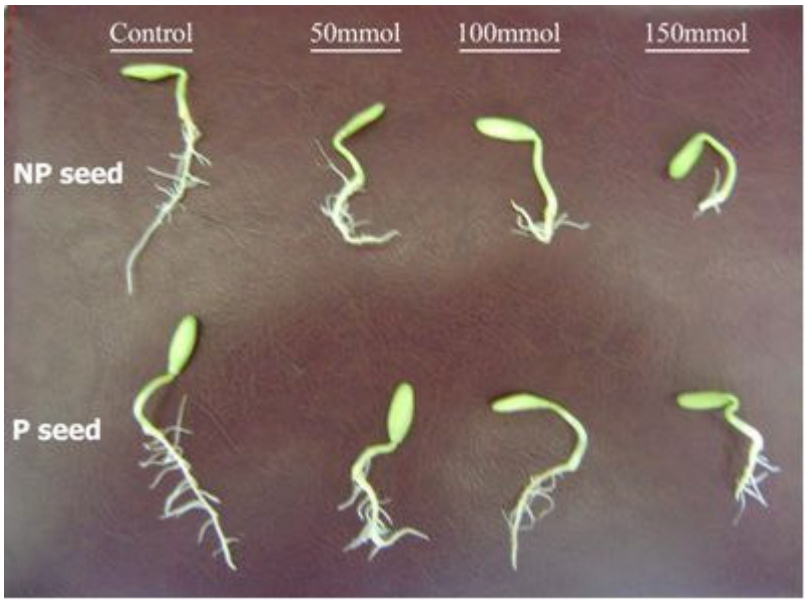

Figure 3. Effect of salt stress and seed priming on seed germination of cultivar Flexuosus.

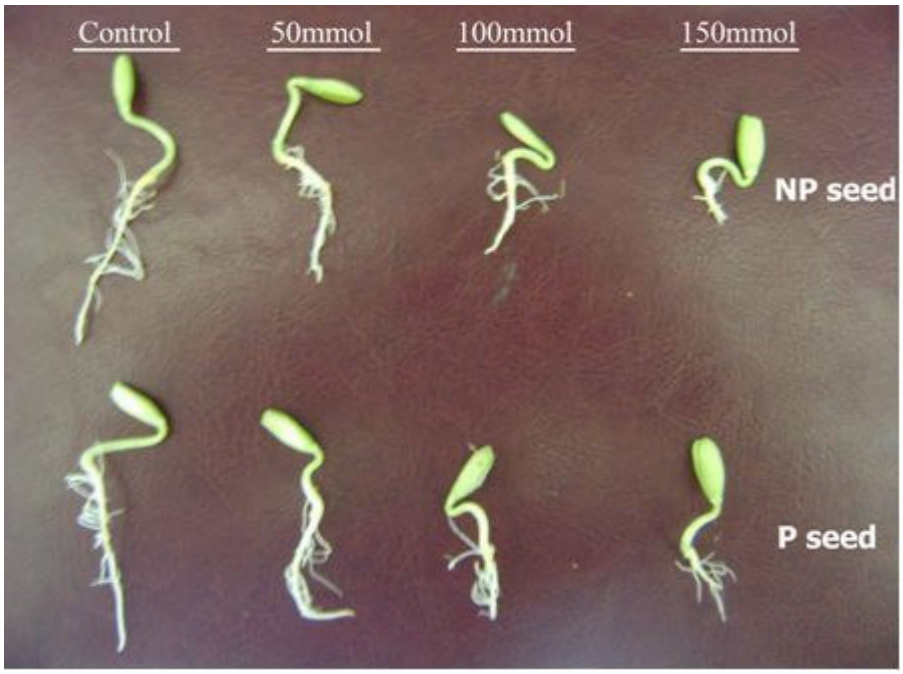

Figure 4. Effect of salt stress and seed priming on seed germination of cultivar Garmsar.

and environment friendly ways. Seed priming is one of such type of methods and NaCl priming has shown improved germination and growth of many crops under stressed conditions (Sivritepe et al., 2003; Farhoudi et al., 2007; Shahi et al., 2009; Cayuela et al., 1996). Results show that salt stress caused growth inhibition and increase in mean germination time of muskmelon cultivars. Priming decreased mean germination time and increased muskmelon seedling emergence and seedling dry weight in saline conditions compared to non priming, especially in cv. Garmsar (Table 1). These results indicated that, seed priming may be helpful in reducing the risk of poor stand establishment, under stress condition and permit more uniform seedling growth under saline condition (Figures 1 and 2). Priming with NaCl also showed improvement in growth of melon seedling (Sivritepe et al., 2003) when salt treatments were applied with seed sowing. Khan et al. (2009) reported that, NaCl priming was much efficient in improving germination and seedling growth of pepper seeds and our results also confirmed it. In earlier studies, it was observed that seedlings from NaCl primed canola seeds maintained greater mean seedling dry weights and cell membrane stability than untreated seedlings (Farhoudi et al., 2007). Salt stress decreased shoot dry weight of muskmelon cultivars and led to substantial reduction in leaf proline, sugar content and CAT activity (Table 2).

However, seed priming improves shoot dry weight, antioxidant activity and compatible solute content like sugars and proline of muskmelon under saline condition. These results indicated that in both muskmelon cultivars, seedling derived from P group have higher shoot dry weight compared to the NP group under saline condition. However, it was concluded that NaCl priming diminished inhabiting effect of salinity on seedling growth and shoot dry matter accumulation of muskmelon cultivars (Table 2). Hasegawa et al. (2000) suggested that, osmoregulation helps plants to tolerate salt stress. Osmoregulation can occur in plants by active synthesis of organic solution like proline, carbohydrate and organic acids. The present study emphasizes that seed NaCl priming increase proline and carbohydrate concentration in both muskmelon cultivars than non-priming seeds. Muskmelon seedling from P group showed highest carbohydrate concentration in both cultivars but proline concentration was significantly higher in cv. Flexuosus than cv. Garmsar at P group (Table 2). These results are supported by Cayuela et al. (1996) in tomato. Sivritepe et al. (2003) found that NaCl priming enhanced proline and carbohydrate solution in melon seedling and caused salinity tolerance in melon seedling. Recent studies suggest that proline may play as an enzyme stabilizing role (Maggio et al., 2002; Bhattacharjee and Mukherjee, 2002) and reduce lipid peroxidation (Jain et al., 2001; Farhoudi et al., 2007) under salt stress. Our results showed that cv.Garmsar seedling from P group had the highest carbohydrate concentration and shoot dry weight compared to cv.Flexuosus under the highest salinity level.

Adverse environmental conditions, such as salinity, lead to secondary stresses like oxidative stress (Munns and James, 2003; Bandeoglu et al., 2004; Farhoudi, 2010).

Table 2. Effect of seed priming on some physiological parameters of muskmelon seedling under saline condition.

Salinity level (mmol NaCl)	Seed priming	Flexuosus						Garmsar					
		Shoot dry weight (g)	CAT activity (mgH_2O_2/g.pro/min)	POD activity (mgH_2O_2/g.pro/min)	MDA concentration (µmol/g fw)	Proline concentration (mg/g fw)	Carbohydrate solute concentration (mg/g dw)	Shoot dry weight (g)	CAT activity (mgH_2O_2/g.pro/min)	POD activity (mgH_2O_2/g.pro/min)	MDA concentration (µmol/g fw)	Proline concentration (mg/g fw)	Carbohydrate solute concentration (mg/g dw)
0	NP	1.4 a	4.3 c	9.12 e	0.0016 d	0.54 ef	2.3 e	1.36 a	3.9 ed	8.6 e	0.0017 d	0.39 g	2.7 e
	P	1.35 a	4.1 c	8.9 e	0.0016 d	0.47 f	2.3 e	1.39 a	4.0 c	8.5 e	0.0016 d	0.37 g	3.0 d
50	NP	1.31 a	5.4 bc	14.3 c	0.0017 d	0.57 e	3.5 d	1.35 a	6.9 b	9.7 e	0.0019 d	0.69 d	3.7 d
	P	1.32 a	5.1 bc	18.0 b	0.0018 d	0.59 e	3.9 d	1.3 b	7.3 b	11.3 d	0.0016 d	0.61 ed	3.3 d
100	NP	1.1 d	3.1 d	13.0 c	0.020 a	0.98 d	5.1 b	1.13 d	8.3 a	13.1 c	0.017 b	1.52 b	4.8 c
	P	1.21 cd	5.4 d	20.1 ab	0.012 c	1.28 d	5.9 a	1.27 c	11.0 a	18.2 b	0.010 c	1.98 b	5.3 b
150	NP	0.76 f	2.1 e	14.1 c	0.026 a	1.52 b	4.7 c	0.80 ef	9.4 a	14.9 c	0.018 b	2.47 ab	5.4 b
	P	1.08 e	5.8 d	21.2 ab	0.018 b	2.61 a	6.2 a	1.14 d	13.1 a	26.2 a	0.013 c	2.83 a	6.7 a

Means followed by the same letter(s) are not significantly different at *P = 0.01* according to Duncan test.

MDA concentration and cell membrane damage increased under salt stress condition because of elevating of ROS production. Enzymatic anti-oxidant defense system can protect plant cells from oxidative injury. POD and CAT are the most important protective enzymes to remove reactive oxygen species. Our results demonstrated that seed priming increase CAT and POD activity of cv.Garmsar compare to cv.Flexuosus. The highest antioxidant activity help plants under environmental stress like salt stress (Munns and James, 2003). A correlation between the antioxidant enzyme activities and salinity tolerance were reported in many plants (Ali and Ashraf, 2008; Munns, 2002). The differences in the activity of antioxidant system among the two muskmelon cultivars revealed that, salinity stress induced CAT and POD activity which were significantly higher than in cv.Garmsar at P and NP group.

Thus, it could be concluded that there was a strong correlation between salt tolerance and seed priming, because P group had the highest shoot dry weight and antioxidant activity under 100 and 150 mmol salinity levels, especially in cv.Garmsar (Table 2). Ali and Ashraf (2008) reported that antioxidant enzyme activities like CAT and POD improves salt tolerance of canola seedling. Our results indicated that salt stress increases MDA production in muskmelon shoot but seed priming decreases it (Table 2). Antioxidant activity helps plant to decrease MDA concentration and cell damage under salinity stress (Munns, 2002; Bandeoglu et al., 2004). Enhanced CAT and POD activity in P group under 100 decreases it (Table 2). Antioxidant activity

helps plant to decrease MDA concentration and cell damage under salinity stress (Munns, 2002; Bandeoglu et al., 2004). Enhanced CAT and POD activity in P group under 100 and 150 mmol NaCl salinity level causes decline in MDA concentration (Table 2).

Conclusion

In conclusion, this study showed that salt stress decrease seed germination and seedling growth of muskmelon cultivars but NaCl priming helps plants to decrease salt stress injury. Seed priming has positive effects on germination, seedling growth and seedling dry weight of muskmelon cultivars. Antioxidant enzyme activities and osmo-protectants were accumulated and MDA concentration reduced in primed seeds. It has been suggested that a higher activity of antioxidant enzymes and osmotic protection could increase tolerance of primed seeds to environmental stresses such as salinity. Therefore, NaCl seed priming could be used as pre-sowing treatment in muskmelon under saline conditions.

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