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Seed Priming Effect on Germination, Seedling Growth and Salt Tolerance of Loquat (*Eriobotrya japonica* Lindl.)

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Abstract

The improvement of loquat seed germination, seedling growth was investigated using seed priming technique. The experiment was conducted in completely random design with 3 replications and 24 seeds in each replication. Treatments were different solutions of sodium chloride and potassium nitrate with electrical conductivity of 0, 4, 8, 12 and dS m^{-1}. The results showed that the highest germination percentage (83%) gained in 8 dS m^{-1} NaCl solution. The NaCl primed seeds showed higher stem length, root and shoot dry weight than control. The primid seedlings were transferred into a closed hydro culture system containing different level of salinity to assisset their salt tolerance. The activities of superoxide dismutase, peroxidase, and ascorbite peroxidase were enhanced in salt-stressed condition. Pre-treated seedlings had also higher proline content than control.

Keywords: Antioxidant enzyme, germination, loquat, salinity, seed priming.

1. Introduction

Salinity is a major factor limiting crop productivity in arid and semi-arid areas of the world (6). High salt stress disturbs water potential balance and ion distribution in plants grown under saline environment, and also causes drought stress and ion toxicity (5). In such condition, reactive oxygen species (ROS) for instance superoxide, hydrogen peroxide and hydroxyl radicals, build up in plants (27). In order to avoid the oxidative damage caused by ROS compounds, plants evolve molecular defense systems that both limit the formation of ROS and promote their removal (43). The plant enzymatic defenses include antioxidant enzymes such as superoxide dismutase (SOD), peroxidase **(**POX), ascorbite peroxidase (APX), catalase (CAT) and Glutathione reductase (GR). It has been reported that the activities of the antioxidant enzymes increase under salt stress in higher plants (37, 47).

Seed germination is the most critical phase in plant life confronting saline conditions which affects plants establishment and subsequent growth (4). It was in 1964 that Strogonov (44) proposed that salt tolerance of plants could be enhanced by treatment of seed with salt solution prior to sowing. Seed priming is a controlled hydration treatment at low water potential that allows pre-germinative metabolism to proceed, but prevents radicle emergence (9). Besides, it has been reported that seedlings from primed seeds emerge faster, grow more vigorously and perform better under sub-optimal conditions such as water stress (40). Osmopriming is the most widely used type of seed priming in which seeds are soaked in aerated low water potential solutions. Examples of such osmotica used include Polyethylene Glycol (PEG), KNO3, K3PO4, KH2PO4, MgSO4, NaCl and manitol (26, 19). Oliveira et al. (35) indicated beneficial effects of osmo-conditioning associated with repair and synthesis of nucleic acids, increased synthesis of proteins and repair of membranes. The activities of detoxifying enzymes (e.g. SOD, CAT, and APX) increased with the priming duration or the concentration of priming agent (11, 33). Therefore, the improvement of seed quality by seed priming has principally been attributed to the reduction lipid peroxidation.

Loquat (*Eriobotrya japonica* Lindl.) trees belong to the family Rosaceae and are commercially produced in China and Japan. Loquat leaves and fruits traditionally have been considered to have high medicinal value and there is strong evidence of pharmaceutically active compounds (16). Loquat fruit is becoming an important industry in China as well as Spain, Japan, India, Pakistan and Turkey (22). In Iran, it is mostly consumed in the local or short distant

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markets. Loquat is commonly propagated by seeds, therefore it exhibits a wide genetic heterogeneity and the fruits show variability in size and quality. At present, the seedlings are used as a rootstock for cultivars with high fruit quality. However, the germination of loquat seed, in general, faces certain problems such as the low germination percentage and velocity as well as the slow growth of the subsequent seedlings. Having the same seedlings with improved growth is the main step in producing grafted sapling in nursery. In addition, it has been reported that the process of priming or hardening involves prior exposure to abiotic stress factor making a plant more resistant to the future stress imposition (36).

The aim of this study was to elucidate the effects of osmo-priming on seed emergence and early seedling growth of loquat and the response of obtained seedlings to salinity in relation to antioxidant enzyme activity.

2. Material and Methods

2.1.Plant material and treatments

Loquat uniform seeds were extracted from mature fruits and immediately washed with tap water and placed at 4˚C for 2 weeks (16). Then the seeds were primed with NaCl and KNO_3 solutions at 5 levels (0, 4, 8, 12, 16 dS m^{-1}) for 12 h at 25±3˚C. The treated seeds were sown in perforated polyethylene pots contained a mixture of peat-moss, sand and clay (1:1:1, v/v/v). The experiment was conducted in completely random design with 3 replications and 24 seeds in each replication. After sowing, the pots were watered regularly and shaded in a greenhouse with average temperature of 25±3˚C and relative humidity of 40%. After 3 weeks from sowing, the germination percentage was calculated weekly for seven weeks. Seed germination percentage (%) and the germination rate were determined according to Maguire (29).

Germination rate (GR) = Σ Si/Di

Where Si is the number of germinated seeds in every counting and Di is the day of counting.

Three month-old seedlings were randomly collected from each treatment (3seedlings/ replicate) and used for the measurements of seedling length (cm), leaf area (cm^2), shoot and root dry weight (mg).

Total chlorophyll content (indicated as SPAD- value) was measured by a chlorophyll meter, SPAD-502 (Minolta, Japan) (39). The data represented were the means of 3 readings from each plant of each replicate.

For assessment of salt tolerance of seedlings originated from primed seeds, from above experiment, 27 seedlings (3-months-old) of uniform size grown from primed seeds with NaCl, KNO3 (8 dSm^{-1}) and control were selected (81 seedlings totally). They were transferred into a closed hydro culture system aerated with air pomp and nourished with half-strength Hoagland solution (21). Plants were grown in a glasshouse under natural light with a mean day/night temperature of 25±3ºC. Two weeks after, when the plant were established, salt (NaCl) were added to nutrient solutions. Treatments were 0 g L^{-1} (0 dS m^{-1}), 5.12 g L^{-1} (8 dS m^{-1}) and 7.68 g L^{-1} (12 dS m^{-1}) NaCl. To avoid salinity shock, salts were added in three times. Each pot (consist of three plants) was considered as one replicate with three pots per treatment. After 30 days of salt treatment, SOD, APX, CAT and POX activities and proline contents were measured.

Free proline was determined according to Bates et al. (7). A 0.5 g sample of frozen powder was mixed with 5.0 ml aliquot of 3% (w/v) sulfosalicylic acid in glass tubes covered at the top and heated in a water bath at 100˚C. The mixture was centrifuged at 2000 g for 5 min at 25ºC. A 200 µl aliquot of the extract was mixed with 400 µl distilled water and 20 ml of the reagent mixture (30 ml glacial acetic acid, 20 ml distilled water and 0.5 g of ninhydrin) and boiled at 100ºC for 1 h. After cooling the mixture, 6.0 ml of toluene was added. The chromophore containing toluene was separated and absorbance was read at 520 nm using toluene as a blank. Proline concentration was calculated using L-proline for the standard curve.

2.2. Enzyme assay

Frozen leaves (0.5 g) were first homogenized in 50 mM potassium phosphate buffer (pH 7.8) containing 1mM EDTA, 3mM 2-mercaptoethanol, and 2% (w/v) polyvinyl polypyrrolidone (PVPP) in a chilled mortar. The homogenate was then centrifuged at 16000×g for 30 min at 4°C and the supernatant was used for enzyme assays.

SOD (EC 1.15.1.1.) activity was assayed by the nitroblue tetrazolium (NBT) method (Dhindsa et al. 1981). The reaction mixture (3 ml) contained 50 mM Na-phosphate buffer (pH 7.3), 13 mM methionine, 75 mM NBT, 0.1 mM EDTA, 4mM riboflavin, and 0.2 ml of enzyme extract. The reaction was started by the addition of riboflavin, and the glass test tubes were shaken and placed under fluorescent lamps (160 µmol m^{-2} s^{-1}). After proceeding for 5 min, the reaction was

stopped by switching off the light. Absorbance was measured at 560 nm. Blanks or controls were run in the same manner but without illumination or enzyme, respectively. One unit of SOD was defined as the amount of enzyme that produced 50% inhibition of NBT reduction under assay conditions.

APX (EC 1.11.1.11) activity was measured according to the methods of Nakano and Asada (30). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM hydrogen peroxide, and 100 µl of enzyme extract in a total volume of 1 ml. The concentration of oxidized ascorbate was calculated by the decrease in absorbance at 290 nm. The absorption coefficient was 2.8 mM^{-1} cm^{-1} One unit of APX was defined as 1 mmol ml^{-1} ascorbate oxidized per min.

Catalase (CAT, EC 1.11.1.6) activity was measured by following the reduction of H_2O_2 (ε = 39.4 mM^{-1} cm^{-1}) at 240 nm according to the method of Dhindsa et al. (14). The assay solution contained 50 mM potassium phosphate buffer (pH 7.0) and 15 mM H2O2. The reaction was started by the addition of 100 µl enzyme extract to the reaction mixture and the change in absorbance was followed 1 min after the start of the reaction. One unit of activity was considered as the amount of enzyme which decomposes 1 mM of H_2O_2 in one minute.

Peroxidase (POX, EC 1.11.1.7) activity was determined according to the method of Chance and Maehly (1955). The tetraguaiacol formed in the reaction has a maximum absorption at 470 nm and thus the reaction can be readily followed spectrophotometrically. The enzyme was assayed in a solution containing 50 mM phosphate buffer (pH 7.0), 5 mM H_2O_2 and 13 mM guaiacol. The reaction was initiated by adding of 33 µl enzyme extract at 25°C. One unit of enzyme was calculated on the basis of the formation of guaiacol to tetraguaiacol for 1 min.

2.3. Statistical design

Statistical analysis was performed for each studied parameter based on a randomized complete design model with three replications (3 seedling in each replication) using SAS 9.1 software. Means were compared by LSD test (Least Significant Difference) at $P < 0.05$

3. Results and Discussion

The germination percentage was significantly affected by types and concentrations of priming agents. The highest germination percentage was achieved in 8 dS m^{-1} NaCl (83%) which was significantly higher than control (Table 1). The seeds pre-treated in 4 and 8 dS m^{-1} NaCl solutions had higher germination rate than those pre-treated in the same concentration of KNO_3 solutions. This may be due to the uptake of Na^+ and Cl^- ions by the seed, maintaining a water potential gradient allowing water uptake during seed germination (24). It has been reported that loquat seeds display an endogenous dormancy that seems to be released by seed priming (16).

Other researchers demonstrated the same results on some vegetable seeds such as hot pepper (38), carrot and onion (49) and cowpea (41). Similarly, Ghassemi-Golezani and Esmaeilpour (18) reported that KNO_3 and NaCl priming significantly increased the germination percentage of cucumber seeds.

Table 1. Effects of seed priming on seed germination rate and percentage and also seedling behavior of loquat

OP agents	EC (dS m^{-1})	G %	GR	shoot length (cm)	Root D.W (mg)	Shoot D.W (mg)	Leaf area (cm^2)	Total chlorophyll (SPAD)
Control	0	47.2 d	1.03 b	10.76a	76.67bcd	160 bc	15.84c	47.70a
NaCl	4	63.61 b	1.12 b	10.61ab	103.33abc	216.67ab	23.24a	39.99bcd
	8	83.00 a	1.75 a	11.15a	116.67a	296.33 a	20.59ab	43.68abc
	12	61.00 bc	0.82bcd	12.5a	110.00ab	73.67 c	17.39bc	41.25abc
	16	36.08 ef	0.55d	8.32bc	73.33cd	120.00bc	9.39d	37.11cd
KNO_3	4	51.5 cd	0.93bc	11.00a	80.00bcd	152.67bc	20.04ab	41.80abc
	8	58.32bc	1.003b	8.03c	90.00abcd	195.33abc	20.33ab	44.57ab
	12	44.23 de	0.77bcd	7.706c	63.33d	166.67cb	18.63bc	41.74abc
	16	31.92 f	0.57dc	6.20c	56.67d	87.33c	17.52bc	33.76d

In each column, values with different letter are significantly different ($P < 0.05$). OP: Osmopriming agent, G: germination, GR: germination rate

The seeds pretreated in NaCl solution with EC of 8 dS m^{-1} showed best germination rate (1.75). In both priming agent (NaCl and KNO_3) solutions, 16 dS m^{-1} had adverse effect on the germination rate with comparison to control. It has been well documented that seed priming can homogenize seed germination in a short period of time (25, 37, 50).

Osmopriming involves exposure to relatively high-concentrated solutions (low water potentials) to allow only partial seed hydration. Na^+ and Cl^- can pass through cell membranes, and thus may influence the cellular mechanisms independent of the osmotic stress (11). The accelerated germination rate after priming may be explained by an increased rate of cell division in the primed seed (8, 42) and stimulation of many metabolic activities involved in the early phases of seed germination (9, 46). In this regard, it seems that, NaCl treatment achieved better than KNO_3.

Seeds pre-treated with solutions of NaCl 12 dS m^{-1} exhibited the greatest shoot length which was not significantly different with comparison to control, 4 and 8 dS m^{-1} NaCl. In both priming agents, EC 16 dS m^{-1} decreased significantly the length of shoots. Nascimento and Aragao (31) also, reported that the pre-treatment of melon seeds with different solutions increased plumule length resulted from the longer period of time for seedlings growth as a result of a faster germination.

Seed priming enhanced root dry weight using appropriate concentration of priming agents. In both priming agents, solution with 8 dS m^{-1} improved significantly root dry weight and the highest root dry weight recorded in 8 dS m^{-1} NaCl. In both priming agents (NaCl and KNO3) also, 16 dS m^{-1} had destructive effects on root dry weight. These results are in agreement with the results obtained by Takhti and Shekafandeh (45) that indicated seed priming with different priming agents and proper concentration resulted in an increase in shoot and root dry weight of *Ziziphus spina-christi* seedlings.

The greatest shoot dry weight achieved in 8 dS m^{-1} NaCl which was significantly higher than other treatments except for 4 dS m^{-1} NaCl and 8 dS m^{-1} KNO_3. Seed priming with solution of 8 dS m^{-1} NaCl increased shoot dry weight by 46% with comparison to control and was not significantly different with 8 dS m^{-1} KNO3.

Nascimento (32) reported that muskmelon seed priming with KNO_3 increase shoot length in compared to other priming treatment like PEG and Manitol solution.

Leaf area was markedly enlarged in low concentration of priming agents (4 and 8 dS m^{-1}). High concentration of priming agents 12 and 16 dS m^{-1} significantly decreased leaf areas (Table 1). The enlargement of leaf area using proper concentration of priming agents has been also reported by others (2, 48).

Total chlorophyll was decreased with seed priming treatments, but this reduction was significant with 16 dS $m^{-}1$ of both NaCl and KNO_3. Poor germination and less chlorophyll contents in loquat seedlings raised from seeds primed with higher concentration of salts (NaCl or KNO_3) might be due to increased uptake of toxic mineral elements such as Na^+, Cl^- or NO3 (1).

3.1. Proline content

In relation to leaf proline changes of seedlings originated from primed seeds in salt stress conditions, the result showed that, in control condition (without salt) the leaves of primed seedlings showed significantly higher proline content than those of unprimed seedlings. With increasing salt concentration in the medium culture, proline content of both primed and unprimed seedlings increased. NaCl primed seedlings showed significantly higher leaf proline content than those primed with KNO3 and unprimed seedlings (Figure 1).

Osmoregulation can occur in plants by active uptake of inorganic ions (such as Na, K and Cl) or synthesis of organic solutes (such as sugars, organic acids, free amino acids and proline) depend on species (28, 20). The results of this study clearly showed that NaCl priming augmented proline accumulation in loquat seedlings (Figure 1). Sivritepe et al.(42) also found that NaCl priming enhanced proline content in melon seedlings which had positive relation with their salinity tolerance. On the other hand, it has been suggested that proline may play a role as an enzyme-stabilizing agent under NaCl salinity and reduces peroxidative damage to the lipid membranes due to salt dependent oxidative stress (31).

3.2. Antioxidant enzyme activities

In relation to antioxidant enzyme activities under saline conditions, an enhancement in SOD activity was observed in primed and unprimed seedlings in saline condition (Figure 2). The maximum SOD

(72.23 U /mg F.W) was detected in NaCl pre-treatments plants supplemented with 12 dS m^{-1} NaCl, which was not significantly different in comparison to control and KNO_3 primed seedlings.

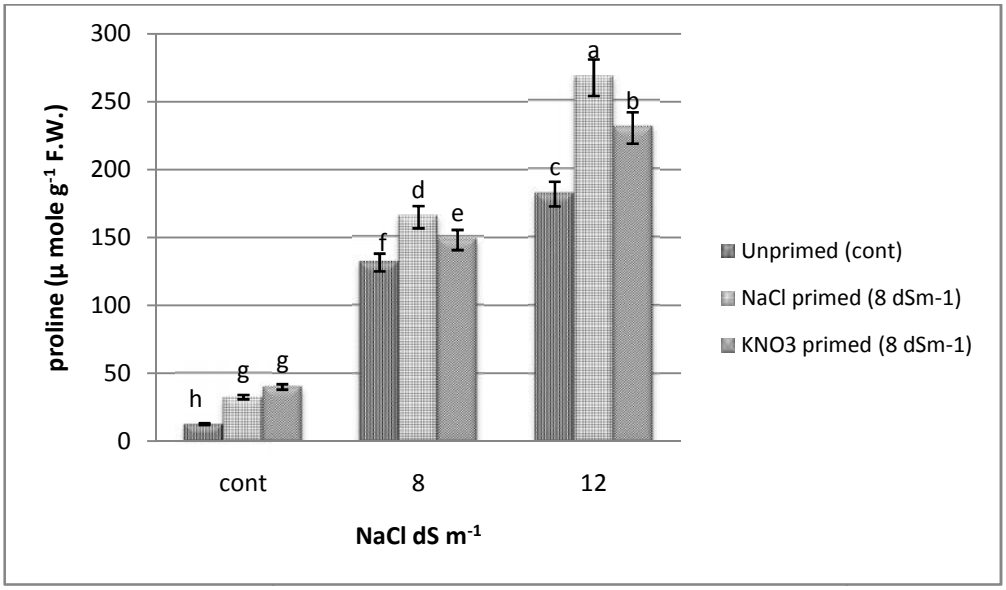

Figure1. Effect of seed osmo-priming (8 dSm^{-1} NaCl and KNO3) on proline content of seedlings in salt stress condition
Column with the same letters are not different at 5% probability using LSD test.

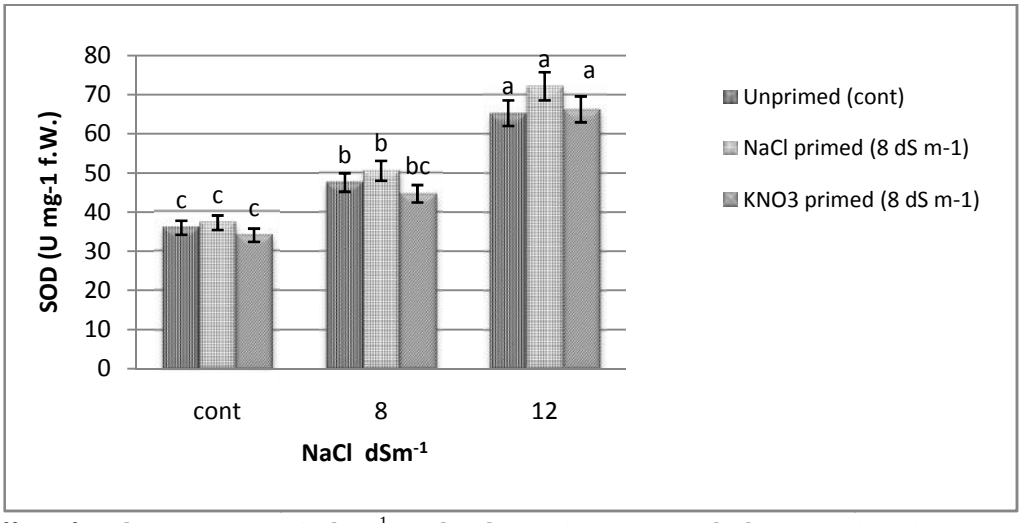

Figure 2. Effect of seed osmo-priming (8 dSm^{-1} NaCl and KNO_3) on superoxide dismutase (SOD) activity in salt stress condition
Column with the same letters are not different at 5% probability using LSD test.

In control (without NaCl) and 8 dS m^{-1} NaCl, there was no significant difference between the leaf CAT activity of the primed and unprimed seedlings. In 12 dS m^{-1} NaCl, the CAT activity decreased but this reduction for NaCl primed seedling was less pronounced than KNO_3 primed and unprimed seedlings (Figure 3). NaCl primed seedlings under saline condition kept the CAT activity high with compare to control suggests the contribution of this enzyme to minimize harmful effects of salinity on plant growth.

The results showed that POX activity in leaves of primed and unprimed seedlings increased with increasing salt concentrations to 12 dS m^{-1} (Figure 4). However, NaCl-pre-treatment seedlings showed significantly higher POX activity than those primed with KNO_3 and control.

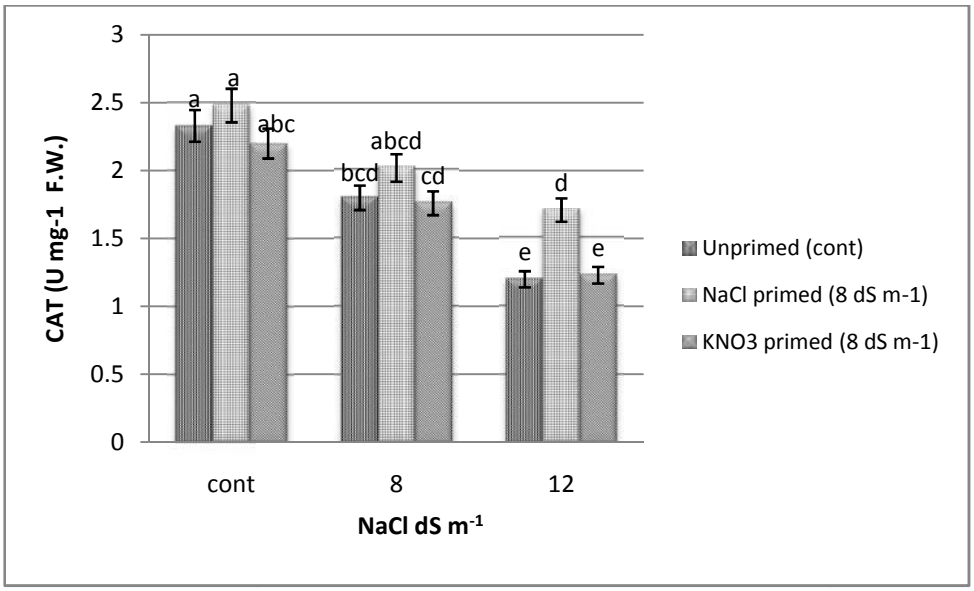

Figure 3. Effect of seed osmo-priming (8 dSm^{-1} NaCl and KNO3) on leaf catalase (CAT) activity in salt stress condition
Column with the same letters are not different at 5% probability using LSD test.

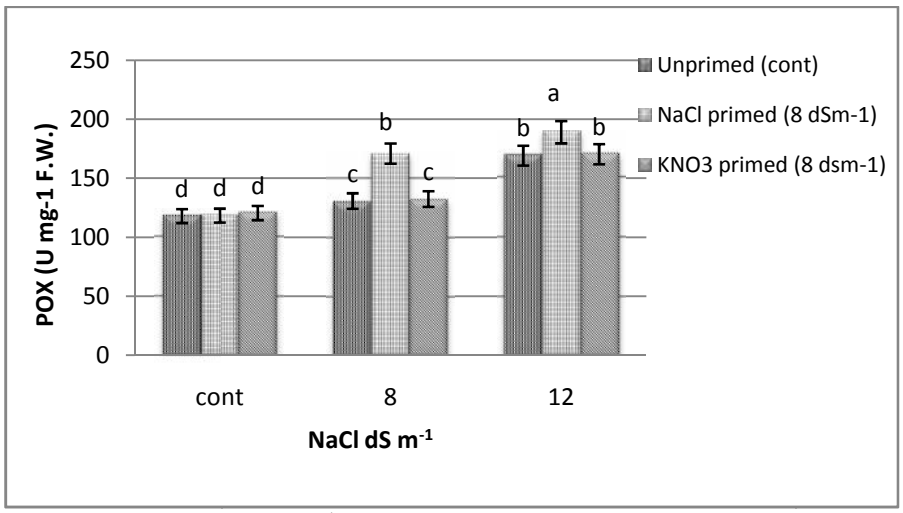

Figure 4. Effect of seed osmo-priming (8 dSm^{-1} NaCl and KNO_3) on peroxidase (POX) activity in salt stress condition
Column with the same letters are not different at 5% probability using LSD test.

Pre-treated and untreated seedlings (primed and un-primed) showed higher APX activities in saline condition than non-saline condition (Figure 5). In all conditions, seedlings from NaCl primed seeds had APX activity greater than those from control and KNO_3 primed seeds.

In the present study, high activity of antioxidant enzymes such as POX and APX in leaves of loquat seedlings pre-treated with NaCl and KNO_3 solution indicated that seed priming greatly activate plant defence system in order to alleviate oxidative damage induced by salt stress. According to our results, although with increasing salt in culture media, SOD increased (Figure 2) as a first step in scavenging free radicals but, pre-treated seedlings had no effect on the enhancement of SOD in the leaves of loquat seedlings, which is in accordance with the results obtained by Oliveira et al. (32) on NaCl- stressed sorghum seedlings. APX and POX probably had more important role in H_2O_2 detoxifying than CAT, although CAT together with APX and POX play detoxifying role in plant. On the other hand, reduction in CAT activity in loquat plants may be due to the prevention of new enzyme synthesis or catalase photo–activation (15).

Pre-treated loquat seedlings showed more resistance to the oxidative damage as compared with un-treated seedlings. Induction in antioxidant enzyme activities

in Pre-treated seedlings was reported by Farhoudi (17), Oliveira et al. (35) and Chiu et al. (12) using different species. Sivritepe et al. (43) suggested that the NaCl pre-treatments may act to alleviate salt stress in melon seedlings by decreasing the permeability of plasma membranes and maintenance of cell form and structure due to the increase of antioxidative enzymes such as CAT, POX. Jowkar et al. (23) found that priming enhanced the activities of POX and APX in *S. marianum* seeds than non-primed seeds. Thus, it could be concluded that there was a strong correlation between salt tolerance and seed priming, because pre-treatments plants had the highest antioxidant activity under salinity condition, especially in NaCl pre-treatments plants. The higher activity of antioxidant enzymes in the primed seedlings suggests, that priming probably prepares the cell to meet and overcome stress by stabilizing membranes and forming a potential of higher antioxidant capacity (3).

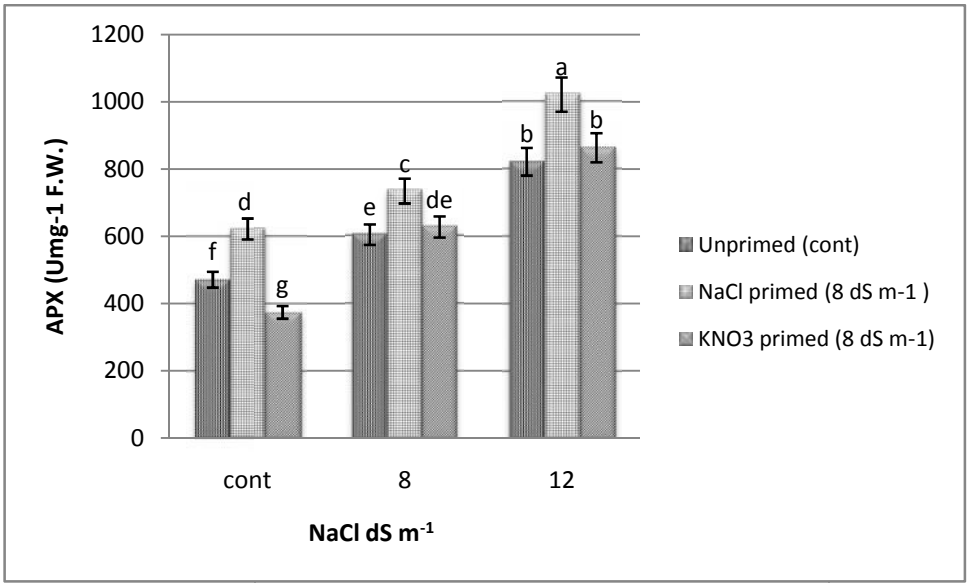

Figure 5. Effect of seed osmo-priming (8 dSm^{-1} NaCl and KNO_3) on ascorbate peroxides (APX) activity in salt stress condition.

Column with the same letters are not different at 5% probability using LSD test.

4. Conclusions

The results of experiment showed that priming with NaCl improves germination indices and seedlings growth in loquat. Priming treatments also increased the contents of proline and improved the activities of POD, APX and CAT in saline condition. The higher adaptation capacity of loquat plants induced by NaCl pre-treatments was explained by biochemical and physiological changes, which were maintained throughout the life cycle of the plants. Therefore, NaCl seed priming could be used as pre sowing treatment in loquat under saline conditions.

6. References

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