Evalution effect of KNO3 seed priming on seedling growth and cell membrane damage of sunflower (*Heliantus annus*) under salt stress

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Abstract: This research was carried out in order to evolution effect of seed priming on seed germination and seedling growth of sunflower (*Heliantus annus* var. *Azargol*) in Islamic Azad University, shoushtar Branch, Iran, in 2011. The experimental design was two factors factorial (3×4) arranged in a completely randomized design; with six replications. The first factor was salt treatments (0, 40 and 80 mmol NaCl solution) and the second factor was seed priming (0, 0.3, 0.6 and 0.9 MPa KNO3 solution). Results showed sunflower GP, seedling fresh weight and seedling growth decreas under salinity condition but MGT and MDA concentration was increased. Results showed under saline condition seed priming with KNO3 improved sunflower seed germination, POX activity and seedling growth compared non priming seeds.
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Key words: salt stress, sunflower, peroxidase activity, priming, KNO3

Abbreviations: GP, Germination percentage; MGT, Mean Germination Time; MDA, Malondealdehyde; POX, Peroxidase activity

1. Introduction

Generally, it is known that salt stress depresses a number of morphological, physiological, biochemical and anatomical characteristics, which enable the plants to grow in the presence of high concentrations of toxic ions. Seed germination and seedling growth is one characteristic severely affected by salt stress (Ashraf, 2001). Okcu et al. (2005) reported that salt stress decrease seedling growth and increase mean germination time of pea seeds. Kaya and Day (2008) reported that salt stress decrease seed germination and seedling growth of sunflower due to salt stress. Farhoudi et al. (2007) founded that Na^+ increase in seed germination environment due to decrease seedling growth of canola.

One of the biochemical changes possibly occurring when plants are subjected to harmful stress conditions in the production of activity oxygen species. The increasing evidences also suggest that high salinity induces oxidative stress which is a key underlying component of most abiotic stresses. Reactive oxygen species (ROS) are generated as by-products of plant cellular metabolism and are also important as signaling molecules (Mittler 2002). Elevated production of ROS can seriously disrupt cellular homeostasis and normal metabolisms through oxidative damage to lipids, protein, and nucleic acid (Charles et al. 2007). Membrane injury induced by salt stress in different plant species is also related to an enhanced production of ROS (Sairam et al., 2005). Plants possess antioxidant enzymes as well as antioxidant compounds to scavenge these ROS, and antioxidant capacity of plants is directly related to their salt tolerance. In order to avoid the production of these reactive molecules plants have evolved an effective scavenging system involving antioxidant molecules like carotenoids, ascorbate, glutathione and tocopherols as well as antioxidant enzymes such as super oxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase (Sairam et al., 2005). For example, while determining the role of various antioxidants in the salt tolerance of tomato, Mittova et al. (2004) found that higher salt tolerance of wild tomato (*Lycopersicon pennelli*) as compared to cultivated tomato was correlated with increased activities of antioxidant enzymes. Similarly, enhanced activities of these antioxidant enzymes under salt stress have been reported in salt-tolerant cultivars of wheat (Sairam et al., 2005) and seedlings of millet *(Setaria italica)* (Sreenivasasulu et al., 2000).

Seed priming is one of the physiological methods, which improves seed performance and provides faster and synchronized germination. This technique has become a common seed treatment that can increase percentage and uniformity of germination or seedling emergence, mainly under unfavorable environmental conditions (Farhoudi et al.,2007) . Demir and Mavi (2004) showed that watermelon seed priming with KNO3 solution

effectively improved germination and seedling growth of the seeds of watermelon under salinity condition in compared to non primed seeds. In tomato and cucumber seeds, seed priming improves seed germination, seedling emergence and growth under saline conditions (Cayuela et al. 1996; Passam and Kakouriotis, 1994). Shahi-Gharahlar et al (2009) suggested that Summer Squash (*Cucurbita pepo*) seed priming with KNO3 improved salinity tolerance in Summer Squash.

The aims of the present study were study examined the possibilities to overcome salt stress in sunflower (*Heliantus annus*) seed by seed pretreatments and effect of seed priming on seedling growth of sunflower under salinity stress.

2. Material and Methods

This study was carried out at the Department of Agronomy, Faculty of Agriculture, Islamic Azad University, Shoushtar Branch, Iran. The experimental design was two factors factorial arranged in a completely randomized design (CRD); with six replications. The first factor was salinity stress levels and the second factor was KNO3 priming treatments.

Seed treatments

Sunflower (*Heliantus annus* var *Azargol*) seeds were primed in -0.3, 0.6 and 0.9 MPa of KNO3 solutions in dark room. For priming, seeds were placed in 500 ml of each solution at 24 ◦C for 12 h and following treatments, the seeds were washed for 5 minutes in distilled water. Following this, seeds were dried between two filter papers and allowed to dry for 24 hours in 25◦C. Control treatment was the non priming seeds. Salt stress treatments were 0(control), 50 and 100 mmol NaCl solution.

Germination test

Seeds were superficially sterilized with 0.1%$HgCl_2$ solution for 3min., and then thoroughly washed for 5 minutes. Six replicates of 15 seeds were evaluated under light (16 h) and dark (8h) conditions using 15×100 mm Petri dishes on top of one sheet of moistened filter. A seed was considered germinated when the emerging radicle elongated to 3 mm. Seed germination was recorded every 24 h for 10 days. Radicle length, shoot length and seedling fresh weights were measured on the 10th day of experiment.

Germination percentage was calculated using formula below (Siddiqi et al, 2007):

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

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

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

fi: Day number during germination period
ni: Number of germinated seeds per day
N: Sum of germinated seeds

Peroxidase (POX) activity was determined following the protocol of Chanes (1995) using guaicol as a reactant. POX activity was measured by monitoring the H2O2-dependent oxidation of reduced 2,3,6-trichloroindophenol at 675 nm using a UV–vis spectrophotometer (Model U-2001, Hitachi, Tokyo, Japan). Lipid peroxidation was determined by measurement the amount of malondialdehyde (MDA) formation using the tiobarbituric acid method (Dionisio-Sese and Tobita, 1998).

Data was analyzed using MSTATC software. Mean comparison was performed with Duncan's test, and graphs were drawn using Excel 2000 software.

3. Results and Discussions

Sunflower GP and MGT were significantly influenced by salt stress, priming and interaction of salt stress and priming . Results showed salt stress decrease sunflower seed germination compared control but MGT increased under salt stress. Lowest sunflower seed germination obtained from 80 mmol NaCl and no-priming treatment (21%). Seed priming improved seed germination under salinity stress condition. In fact, the primed seeds emerged earlier and maintained a higher level of emergence throughout the emergence period. At 40 and 80 mmol NaCl salinity level, highest seed germination obtained when sunflower seed treatment with 0.6 and 0.9 MPa KNO3 solutions (Fig.1).

At both salinity levels, seed priming improved MGT of sunflower because MGT of non-priming seed at 40 and 80 mmol NaCl solution were 3.4 and 5.4 day but priming treatment shortened the time to seed germination(Table1). Under salinity stress, KNO3 priming had a positive effect on snake melon mean germination time (Shahi-Gharahlar et al.,2009). The beneficial effects of KNO3 on Mean Germination Time were found in Kaya et al (2006) study, too. Salinity may affect the germination of seeds either by creating an osmotic potential external to the seed preventing water uptake, or through the toxic effects of Na+ and Cl− ions on the germinating seed (Khajeh-Hosseini et al. 2003). Farhoudi et al (2007) reported seed priming increased canola seed germination under salinity stress because seed priming decrease harmful ion absorption and cell

damage of canola seedling. Mauromicale and Cavallaro (1996) found herbage grasses seed priming with KNO3 solution decrease mean germination time in these seeds compared to PEG solution because KNO3 did not have toxicity or prevent water uptake compared other material like PEG or NaCl (Demir and Van De Venter 1999).

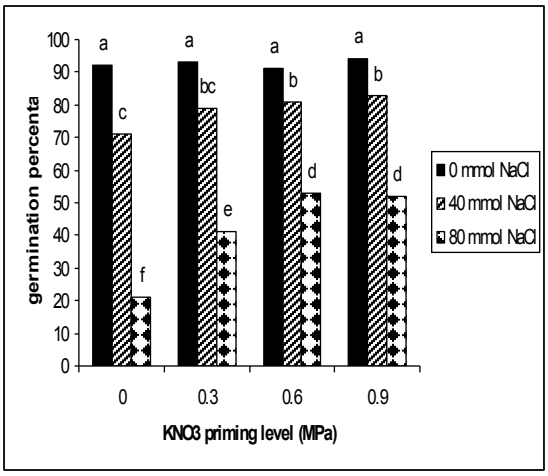

Figure1. Effect of salt stress and seed priming on sunflower seed germination

Table 1. Effect of salt stress and seed priming on seed germination and seedling characteristics of sunflower

Salt stress (mmol NaCl)	Seed priming	Mean Germination Time (day)	Shoot length (cm)	Root length (cm)	MDA concentration (µmol/gr fw)	POX activity mgH2O2/g.pro/min
0	0	1.4 e	2.4 a	3.2 a	0.0011 d	13.2 cd
	0.3	1.3 e	2.7 a	2.9 a	0.0013 d	11.7 d
	0.6	1.3 e	2.3 a	3.1 a	0.0011 d	14.0 cd
	0.9	1.4 e	2.3 a	3.0 a	0.0012 d	12.3 d
40	0	3.4 c	1.3 c	2.3 b	0.035 b	18.2 c
	0.3	2.8 cd	1.9 b	2.4 b	0.033 b	18.3 c
	0.6	2.1 d	1.9 b	2.9 a	0.021 c	19.8 c
	0.9	2.2 d	1.8 b	3.0 a	0.022 c	24.3 ab
80	0	5.4 a	0.9 d	1.2 d	0.049 a	23.6 b
	0.3	4.1 b	1.3 c	2.1 c	0.034 b	31.2 a
	0.6	3.2 c	1.5 bc	2.1 c	0.023c	30.1 a
	0.9	3.6 c	1.3 c	1.9 c	0.025 c	30.0 a

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

Sunflower seedling fresh weight, radicle length and shoot length were significantly influenced by salt stress, priming and interaction of salt stress and priming. Greater reduction in shoot and root length due to 80 mmol NaCl solution (9 mm for shoot lenght and 15 mm for radicle lenght). In each salinity leavel, seed priming enhanced shoot and radicle growth compared non priming seeds. Under 40 mmol NaCl solution did not any significant different between radicle length of seed priming at 0.3 MPa KNO3 solution and control but 0.6 and 0.9 KNO3 solution increased root length compared

control(Table1). Results indicated seed priming increase radicle length at highest salinity level cpmpared control but did not show any significant difference between priming level (Table1). Shoot length was higher in each priming treatment in 40 and 80 mmol NaCl solution compared control (Table 1). Depending on decrease in shoot and root length, seedling fresh weight gradually declined with the decreasing salt stress level but in two salt stress level seed priming improved seedling fresh weight compared non-priming seeds (Fig.2). Nascimento (2003) reported that muskmelon seed priming with KNO3 increase root and shoot length in compared to other priming treatment like PEG and Manitol solution. These results are supported by Okcu et al. (2005). They stress that pea seed priming with solution of KNO3 were more effective than PEG solution in promoting early germination and seedling growth of Pea seedling. Kaya et al. (2003) reported salt stress decrease safflower seedling weight. They suggested that osmotic stress and ionic stress of salt stress decrease growth of safflower seedling. Nascimento (2003) reported that muskmelon seed priming with KNO3 increase muskmelon seedling fresh weight under low temperature stress in compared to non primed seeds.

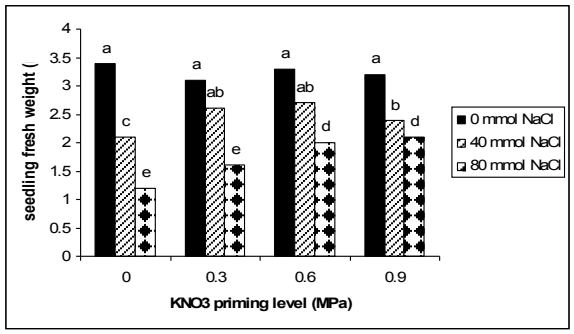

Figure2. Effect of salt stress and seed priming on sunflower seedling fresh weight

Sunflower seedling MDA concentration and POX activity significantly influenced by salt stress, priming and interaction of salt stress and priming . Under non saline condition, did not show any significant different between seedling MDA concentration but salt stress increase cell memberane damage and MDA concentration. Results in Table 1 showed at 40 and 80 mmol NaCl levels, seed priming with 0.6 and 0.9 MPa KNO3 solution decrease MDA concentration compared control. Results showed salt stress and priming increase sunflower seedling POX activity. At 40 mmol NaCl level, the 0.9 MPa KNO3 solution increased POX activity in sunflower seedling compared 0.3 and 0.6 MPa KNO3 solution but all priming treatment increased POX activity of sunflower seedling at 80 mmol salinity level (Table1). Farooq and Azam (2006) and Meloni et al. (2003) founded cell membrane stability of wheat seedling decreased under salinity condition, too. Results indicated seed priming improve cell memberane stability and decrease MDA production under salt stress. Farhoudi et al (2007) stress that canola seed priming by NaCl solution increased canola seedling cell membrane stability and decreased seedling damage under salinity condition. Salinity has a pronounced effect on plasma membrane and lipid peroxidation, there by affecting its permeability which in turn modulates the pattern of ion leakage (Sairam et al., 2002).

In many plants, germination and subsequent seedling growth can be inhibited by environmental stress such as salt stress (Farooq and Azam, 2006; Kaya et al., 2006; Okcu et al., 2005). Priming may be helpful in reducing the risk of poor stand establishment under salt stress conditions (Kaya et al. 2006; Nascimento, 2003). The results of this study clearly showed that salinity increased MDA concentration of seedlings but the seeds, that priming with KNO3 had loweer cell memberane damage and highest seedling fresh weight compared non-priming seedlings. Farhoudi et al (2007) founded that canola seed priming by NaCl solution under salinity stress, decrease canola seedling cell membrane damage because increased seedling K^+ and proline content. Jain et al. (2001) suggested that the higher adaptation capacity of seedlings in priming groups to salinity could be due to osmoregulation induced by organic solutes like proline, antioxidant activity and cell membranes stability. In this study, sunflower seed priming by KNO3 under salinity condition increase seedling fresh weight and seedling growth and decrease seedling MDA concentration compared non priming seeds. In fact, results showed sunflower seed priming by KNO3 solution demonstrated its potential in improving tolerance to salinity by increasing seedling fresh weight and POX enzyme activity. The beneficial effects of seed priming by KNO3 solution on seed germination at salt stress condition have been yet observed in other crop research such as safflower (Kaya et al. 2003), Pea (Okcu et al., 2005) and muskmelon (Nascimento,2003). Singh and Rao (1993) stress that KNO3 effectively improved germination, seedling growth and seedling vigour index of the seeds of sunflower varieties with low germination. Improving seedling sunflower growth by KNO3 priming under salinity condition can suggesting nontoxity of KNO3 due to ion accumulation in the embryo (Kaya et al. 2006; Demir and Van De Venter 1999). Khajeh-Hosseini et al. (2003) found that under salt stress, Na^+ and Cl^- may be taken up by the seed and toxic effect of NaCl

might appear but higher embryo K^+ and seed water content following priming in potassium salts as compared to untreated seed was obsverd in many studies (Mauromicale and Cavallaro, 1996).

In conclusion, this study showed KNO3 priming of sunflower seeds, especially 0.6 and 0.9 MPa KNO3 solution, was more effective than non priming seeds under salinity condition because decrease seedling fresh weight and seedling growth of sunflower under salinity stress in compared to non priming seeds.

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