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Effect of Seed Pretreatment on Summer Squash (*Cucurbita pepo*) Seed Germination and Seedling Characteristics under Salinity Condition

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ABSTRACT

Salinity impairs seed germination and reduces crop yield. Salt and osmotic stresses are responsible for both inhibition or delayed seed germination and seedling establishment. Seed priming is one of the physiological methods that improve seed performance under salinity. This research was carried out in order to assess the effect of seed priming on seed germination of summer squash (*Cucurbita pepo*). Germination percentage was not influenced by salt stress and priming treatment. Salt stress increased mean germination time and decreased seedling fresh weight, root and shoot length in summer squash seedlings. Results show that seed priming with PEG solution decreased seedling fresh weight and shoot length under saline conditions (at -2 MPa). Under saline conditions, seed priming with KNO_3 (-1.27 MPa at Ec = 7 ds/m) was better than other priming treatments because seedling fresh weight, root and shoot length increased.

Keywords: KNO_3, NaCl, PEG, priming, salt stress
Abbreviations: EC, electrical conductivity; **MGT**, mean germination time

INTRODUCTION

Abiotic stresses such as salt and drought are among factors most limiting plant productivity (Bohnert *et al*. 1995). High salinity in soil or irrigation water is a common environmental problem affecting seed germination and plant growth. Soil 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). Salt and osmotic stresses are responsible for both inhibition or delayed seed germination and seedling establishment (Almansouri *et al*. 2001).

Summer squash (*Cucurbita pepo*) is moderately sensitive to salinity stress (Blaylock 1994). Seed priming is one of the physiological methods which improves seed performance and provides faster and synchronized germination. In fact, 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 (Heydecker *et al*. 1975). This technique has become a common seed treatment that can increase the rate, percentage and uniformity of germination or seedling emergence, mainly under unfavorable environmental conditions (Nascimento 2003; Khajeh-Hoseni 2003). Seed priming has been successfully demonstrated to improve germination and emergence in seeds of many crops, particularly seeds of vegetables and small seeded grasses (Bradford 1985). Demir and Mavi (2004) showed that watermelon seed priming with KNO_3 solution effectively improved germination and seedling growth of the seeds of watermelon. In tomato and cucumber seeds, seed priming improves seed germination, seedling emergence and growth under saline conditions (Passam and Kakouriotis 1994; Cayuela *et al*. 1996). Farhoudi *et al.* (2007) suggested that canola seed priming with NaCl improved salinity tolerance in canola seedlings because seed priming increased the K^+/Na^+ ratio in canola seedlings. Rao *et al*. (1987) reported that primed *Brassica* seeds may reduce the risk of poor stand establishment in cold and moist soils.

The aims of the present study were to examine the possibility of overcoming salt stress in seed summer squash by seed treatments with water (hydro priming), PEG, NaCl and KNO_3 solutions and to choose the best method for summer squash seed pretreatment under salinity stress.

MATERIALS AND METHODS

This study was carried out at the Department of Agronomy, Faculty of Agriculture, University of Tehran, Iran. The experimental design had two factors factorial (2 × 4) arranged in a completely randomized design (CRD); with four replications and 25 seeds per replicate. The first factor was seed pretreatments (priming) and the second factor was salt treatment.

Seed treatments

Summer squash seeds (cultivar S-12) were primed in solution of polyethylene glycol (PEG 6000) at -2 MPa, KNO_3 at -1.27 MPa and NaCl at -1.5 MPa in a dark room (**Table 1**). 100 seeds were placed in 300 ml of each solution in a beaker of 500 ml sealed with Para film with a hole in the top to allow for air supply by little pipes (Mauromicale and Cavallaro 1996). The control treatment was seeds that were primed for 24 h in water (hydro priming). For priming, summer squash seeds were immersed in priming solution at 20°C for 24 h in the dark. Following treatments, the seeds were washed three times for 5 min each in distilled water, and then dried with blotting paper and subsequently in a flow of dry air at 30°C, until the original moisture content was approximately reached (**Table 1**).

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Table 1 Summer squash seed moisture content after priming in different osmotic solutions and after drying.

Osmotic solution	Conc. (mol/l)	Osmotic potential (MPa)	Seed moisture after priming (%)	Seed moisture after drying (%)
KNO_3	0.40	-1.27	42.4	5.1
NaCl	0.60	-1.50	44.0	6.1
PEG	0.10	-2.0	42.9	5.6
Hydro priming	---------	--------	61.2	7.3

Salt treatments

EC at 25°C of the two levels were 0.4 ds/m (control) and 7 ds/m (salinity treatment), respectively. The required amount of each solid salt for preparing 1 l of salt solution was calculated first through the following formula (Al-Ansari 2002):

$$TDS\ (mg/l) = EC \times 640$$

where TDS = total soluble solid salt amount (mg/l) and EC = given electro conductivity value (ds/m).

Germination test

25 seeds were germinated in 9-cm Petri dishes, on Whatman paper No. 1 and wet with 10 ml of salt solutions. After that seeds were placed in a growth chamber. Germination conditions were 25°C, 70% relative humidity, and 16-hr photoperiod. The traits measured were: germination percentage, Mean Germination Time (MGT), radicle length, shoot length and seedling fresh weight. Germination was considered to have occurred when the radicles were 10 mm long. Germination percentage was recorded every 24 h for 7 days. Radicle length, shoot length and seedling fresh weight were measured on the 7th day of the experiment. MGT was calculated by the method of Schilin *et al*. (2003).

Data analysis was carried out with MSTATC software, Mean comparison was performed with Duncan's multiple range test at the 5% level of significance ($P < 0.05$) and graphs were drawn using Excel 2003 software.

RESULTS AND DISCUSSION

Germination percentage

The germination percentage of summer squash was not influenced by salt stress and priming treatment (**Table 2**). In fact, none of the treatments had any effect on germination percentage (**Fig. 1**). Although Salehzade *et al*. (2009), Farhoudi *et al*. (2007) and Demir and Mavi (2004) found that seed priming increased seed germination under unfavorable conditions, in this study we did not find any effect of seed priming on summer squash seed germination under salt and non-salt conditions.

Mean Germination Time (MGT)

Both of the factors studied and their interaction significantly affected MGT (**Table 2**). Under non-saline conditions summer squash seed priming with PEG solution and NaCl solution increased MGT compared to hydro priming (control) or KNO_3 solution. Under salinity condition, salt stress increased MGT in all seeds but this increase was greater in seed primed with PEG solution (4.33 days) and control (4.17 days). Under salinity stress, KNO_3 priming had a positive effect on MGT (2.71 days) compared to other seed priming treatments (**Fig. 2**). In fact, MGT was shortened by seed priming with KNO_3. Under non-saline conditions, compared to PEG and NaCl, MGT for KNO_3 and control was shorter. This could be explained by more rapid water uptake in KNO_3 or hydro priming of seeds (Barlow and Haigh 1987). Lower MGT in KNO_3 treatments than in NaCl treatments suggests no toxicity by KNO_3 (Demir and van de Venter 1999). Demir and Mavi (2004) reported watermelon seed priming with 3% KNO3 solution increased seed germination and decreased MGT of watermelon seedling.

Sung and Chiu (1995) observed that MGT was accelerated by hydro priming without changing the amount of

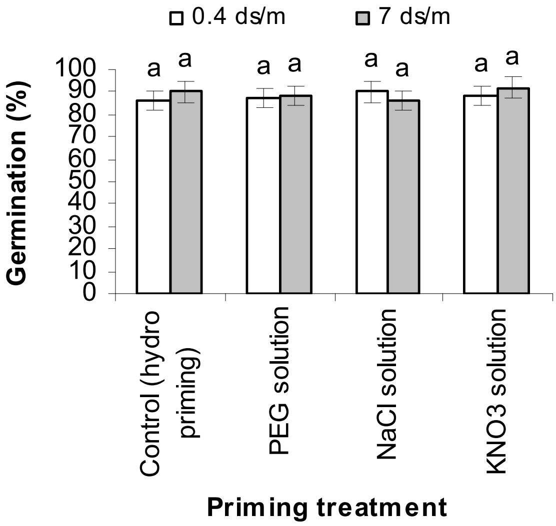

Fig. 1 Effect of priming treatment on summer squash seed germination under non-saline and saline conditions.

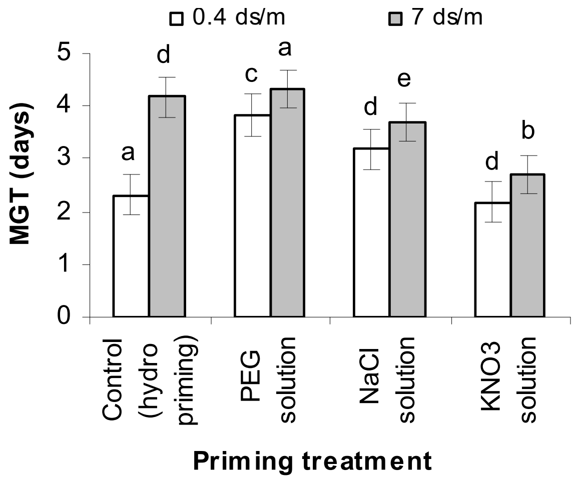

Fig. 2 Effect of priming treatment on summer squash Mean Germination Time under non-saline and saline conditions.

Table 2 Analysis of variance of the traits under study.

	Mean of square				
Source of variance	Germination (%)	MGT	Root length (mm)	Shoot length (mm)	Seedling fresh weight (g)
Rep	98.3^{ns}	9.2 ^{ns}	116.5 ^{ns}	201.3 ^{ns}	47.3 ^{ns}
Priming	184.1 ^{ns}	104.3*	898.1*	1103.9*	213.4*
Salinity	163.1 ^{ns}	76.9*	383.6*	653.3*	200.4*
Salinity*Priming	125.4 ^{ns}	40.0*	345.1*	580.4*	143.7*
Error	111.4	5.7	57.1	118.1	15.8

Ns: non significant
*: Significant at the 0.05 level of probability according to Duncan test.

water uptake in watermelon. Singh and Rao (1993) reported that 3% KNO_3 solution effectively improved germination, seedling growth and seedling vigor index of the seeds of sunflower (*Helianthus annuus*) varieties with low germination. The beneficial effects of 2% KNO_3 solution on MGT were also found by Kaya *et al.* (2006) in sunflower (*Helianthus annuus*).

Radicle and shoot length

Both of the factors studied and their interaction significantly affected radicle and shoot length (**Table 2**). Under non-saline condition summer squash radicle length was not influenced by priming treatments but under saline conditions, salinity decreased radicle length in summer squash seedlings. In salt conditions highest radicle length was observed in seed primed with KNO_3 and shortest radicle length was shown in seed primed with PEG solution (**Fig. 3**).

Under non-saline condition longest shoots formed from seeds primed with KNO_3 solution and shortest shoots in seeds primed with PEG solution. Salt stress decrease shoot length compared to non-saline conditions. Under saline conditions longest shoots formed from KNO_3 but shortest shoots formed from PEG solution (**Fig. 4**). Our results showed that under saline conditions shortest shoots formed from seeds that were primed with PEG solution. This may be due to the uptake of Na^+ and Cl^- ions by the seed, maintaining a water potential gradient and allowing water uptake during seed germination. Shorter shoots and higher MGT obtained from PEG priming compared with NaCl priming (**Figs. 2, 4**) suggest that the adverse effects of PEG on germination are due to an osmotic effect rather than specific ion accumulation (Khajeh-Hosseini *et al.* 2003; Kaya *et al.* 2006). These results agree with those of Murillo-Amador *et al.* (2002) in cowpea. They found that seedling growth was inhibited by both -2 MPa NaCl and PEG solution, but higher inhibition occurred due to PEG. They affirmed that drought may influence root and shoot growth by decreasing water uptake. Nascimento (2003) reported that muskmelon seed primed with 3% KNO_3 solution increase root and shoot length compared to other priming treatments like PEG and mannitol solution. Unlike our results, Gray *et al.* (1991) and Corbineuea *et al.* (1994) reported that -2 MPa PEG solution is the best treatment to improve seed germination and seedling growth of onion and leek (*Allium porrum*) seeds under salinity stress. Mehra *et al.* (2003) indicated that PEG molecules do not enter the seed and Khajeh-Hosseini *et al.* (2003) found that in soybean (*Glycine max*) there was no toxicity of PEG (-2MPa) but under salt stress, and if Na^+ and Cl^- are taken up by the seed a toxic effect of NaCl might appear. However, our findings at a salinity of 7 ds/m showed that the decrease in shoot and radicle length was greater in seeds primed with PEG than in other priming solutions.

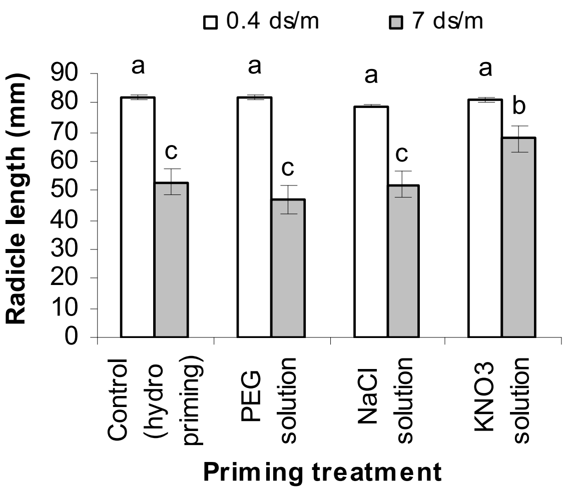

Fig. 3 Effect of priming treatment on summer squash radicle length under non-saline and saline conditions.

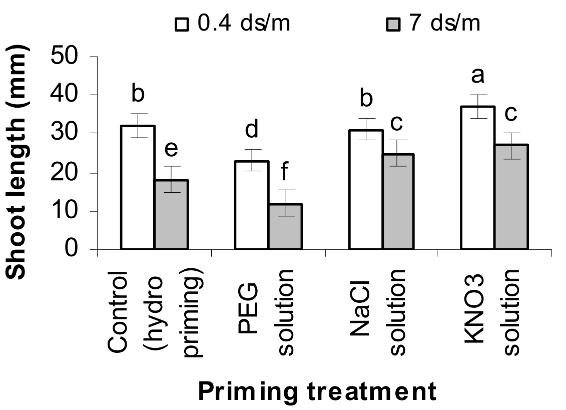

Fig. 4 Effect of priming treatment on summer squash shoot length under non-saline and saline conditions.

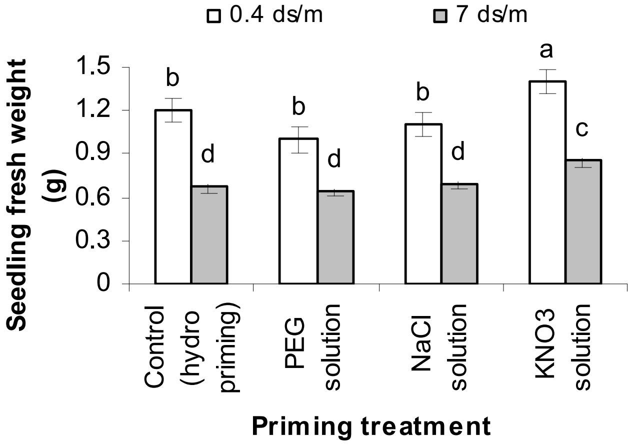

Fig. 5 Effect of priming treatment on summer squash seedling fresh weight under non-saline and saline conditions.

Seedling fresh weight

Both of the factors studied and their interaction significantly affected Seedling fresh weight (**Table 2**). Salt stress decrease seedling fresh weight (**Fig. 5**). Lowest seedling fresh weight in saline and non saline condition was obtained from seeds primed with PEG solution and highest seedling fresh weight in saline and non saline condition was obtained from seeds primed with KNO_3. In fact, summer squash seed priming with KNO_3 and hydro priming improved seedling fresh weight under non saline condition but seed priming with KNO_3 significantly was better than other treatments. Our result showed seed priming with PEG solution decrease seedling fresh weight in compared to other treatment. These result agreed by Murillo-Amador *et al.* (2002). They found that seedling growth of cowpea was inhibited by both NaCl and PEG solution, but higher inhibition occurred due to PEG. 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 KNO_3 increase muskmelon seedling fresh weight under low temperature stress in compared to non primed seeds. Our results showed that KNO_3 priming increase summer squash seedling fresh weight under saline condition. Kaya *et al.* (2006) reported that KNO_3 priming and hydro priming increase sunflower seedling fresh weight. Their research showed that KNO_3 did not have any toxic effect on sunflower seedling. Haigh and Barlow (1987) suggested tomato seed priming with solution of KNO_3 were more effective than PEG solution in promoting early germination and seedling growth of tomato.

CONCLUSION

In many seeds, germination and subsequent seedling growth can be inhibited by environmental stress such as salt stress (Sung and Chiu 1995; Cano *et al*. 1991). Priming may be helpful in reducing the risk of poor stand establishment under salt stress conditions (Kaya *et al*. 2006; Farhoudi *et al*. 2007). Salehzade *et al*. (2009) showed that osmopriming improved germination and seedling vigor than that control in wheat (*Triticum aestivum* L.) seeds. Our study showed that salt stress decrease summer squash seedling fresh weight and increase MGT but seed priming especially with KNO_3 solution demonstrated its potential in improving tolerance to salinity by increasing root and shoot length and decrease MGT of summer squash seedling under salinity condition. The beneficial effects of seed priming on seed germination at salt stress condition have been yet observed in other crop research such as sunflower (Kaya *et al*. 2006), watermelon (Demir and Mavi 2004) and muskmelon (Cayela *et al*. 1996). In this study, it was concluded that KNO_3 priming diminished inhibiting effect of salinity on seed germination and seedling growth of summer squash as has been shown in other priming treatments in muskmelon (Nascimento 2003) and sunflower (Kaya *et al*. 2006). Singh and Rao (1993) stress that KNO_3 effectively improved germination, seedling growth and seedling vigour index of the seeds of sunflower varieties with low germination.

Decreased Mean Germination Time of the seeds primed in KNO_3 as compared to the PEG and NaCl primed seeds may be explained by more advanced stage of the germination processes due to a higher water absorption rate (Mauromicale and Cavallaro 1996). Its may be related to the absorption of ion $NO3^-$ and K^+ by the seeds which reduces the internal osmotic potential of the seeds. 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 observed in many studies (Mauromicale and Cavallaro 1996). In our study KNO_3 priming decreased MGT and increase seedling fresh weight and this is suggesting non toxicity of KNO_3 due to ion accumulation in the embryo (Demir and van de Venter 1999; Kaya *et al*. 2006).

In conclusion, this study showed that KNO_3 priming of summer squash seeds was more effective than other treatment such as PEG solution and NaCl solution. Our study showed summer squash seed priming with KNO_3 decrease MGT and increase seedling fresh weight under salinity stress in compared to other priming treatments.

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