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Influence of Snake Melon (*Cucumis melo* var. *flexuosus*) Seed Priming on Seedling Emergence and Seedling Electrolyte Leakage under Salinity

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ABSTRACT

This research was carried out in order to study the effect of seed priming on seedling emergence and seedling electrolyte leakage of snake melon (*Cucumis melo* var. *flexuosus*). Germination percentage, seedling dry weight (DW) and seedling growth decreased under salinity but mean germination time and electrolyte leakage increased. Under saline conditions seed priming by PEG solution decreased seedling DW but seed priming with KNO_3 was better than other priming treatments because seedling cell membrane stability, seedling DW, root and shoot length increased more than in other seed priming treatments.

Keywords: KNO_3, PEG, priming, NaCl, salt stress
Abbreviations: **EC**, electrical conductivity; **EL**, electrolyte leakage; **GP**, germination percentage; **MGT**, mean germination time

INTRODUCTION

Soil salinity is a major constraint to food production because it limits crop yield and restricts use of land previously uncultivated. 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). Okcu *et al*. (2005) reported that salt stress decrease seedling growth and increase mean germination time of pea (*Pisum sativum* L.) seeds. Kaya and Day (2008) reported that salt stress decreased seed germination and seedling growth of sunflower (*Helianthus annuus* L.). Meloni *et al.* (2003) and Farooq and Azam (2006) found that salt stress damaged seedling cell membrane and increased cellular membrane leakage in salt-sensitive plants (wheat varieties).

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 the percentage and uniformity of germination or seedling emergence, mainly under unfavorable environmental conditions (Rao *et al*. 1987; Farhoudi *et al*. 2007). Demir and Mavi (2004) showed that watermelon (*Citrullus lanatus*) seed priming with KNO_3 solution effectively improved germination and seedling growth of the seeds under salinity compared to non-primed seeds. 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). Under saline conditions, seed priming with KNO_3 was better than other priming treatments because summer squash (*Cucurbita pepo*) seedling fresh weight, root and shoot length increased (Shahi-Gharahlar *et al*. 2009). 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.

Snake melon (*Cucumis melo* var. *flexuosus*) is very long, grooved and consumed as a cucumber in some Asiatic and African countries. The centre of origin of snake melon is accepted as South East Anatolia, Azerbaijan, Iraq, Palestine and Central Asia (Besirli and Yanmaz 1999). In this area, snake melon is used fresh like cucumber or cooked or pickled.

The aims of the present study were to examine the possibility of overcoming salt stress in snake melon seed by seed pretreatments and to choose the best method for this plant's seed pretreatment under salinity stress.

MATERIALS 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 four replications. The first factor was salinity stress and the second factor was priming treatment.

Seed treatments

Snake melon (*Cucumis melo* var. *flexuosus*) seeds were obtained from the Seed and Plant Research Institute, Karaj, Iran. Seeds were primed in -1 MPa solution of polyethylene glycol (PEG 6000), KNO_3 and NaCl in a dark room. All chemicals were obtained from Sigma Co. The control treatment was seeds that were primed in water (hydro-priming). Seeds were placed in 500 ml of each solution at 24°C for 6 h in the dark. Following treatments, seeds were washed three times for 5 min in distilled water. Following this, seeds were dried between two sheets of filter paper and allowed to dry for 24 h at 25°C.

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Research Note

Table 1 Analysis of variance of the traits under study.

Source of variance	Germination percentage (%)	MGT	Root length (mm)	Shoot length (mm)	Seedling dry weight (g)	Seedling electrolyte leakage (%)
Priming	498.1**	53.3**	438.2**	1345.0**	71.0**	3.2**
Salinity	476.1***	67.1**	287.6**	612.3*	69.4**	2.4**
Salinity*Priming	134.4 **	33.0**	311.1**	549.6*	43.1**	1.7**
Error	21.1	3.2	35.1	135.5	8.0	0.04

* and**: Significant at the 0.05 and 0.01 level of probability respectively

Salt treatments

The EC at 25°C of the two levels were 0 (control) and 8 ds/m (salinity treatment), respectively. The required amount of each solid salt for preparing one liter salt solution was calculated by the following formula first (Al-Ansari 2002):

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

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

Germination test

Seeds were superficially sterilized with 0.1% $HgCl_2$ solution for 3 min. and then thoroughly washed for 5 min. Four replicates of 25 seeds were germinated between two rolled sheets of filter paper with 10 ml of respective test solutions. The sheets of paper were replaced daily to prevent the accumulation of salts. Seeds were allowed to germinate at 22 ± 1°C in the dark for 8 days. A seed was considered germinated when the emerging radicle elongated to 5 mm. Seed germination was recorded every 24 h for 8 days. Radicle length, shoot length and seedling dry weights (DWs) were measured on the 8^{th} day of the experiment.

Germination percentage was calculated using the formula:

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)'s 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.

Electrolyte leakage was measured as described by Valentovic *et al*. (2006). 0.5 g of fresh seedlings were washed with deionized water and placed in tubes with 10 ml of deionized water and incubated for 2 h at 25°C. Electrical conductivity (EC) was then determined (L1). Samples were autoclaved at 120°C for 20 min and the final EC was measured following equilibration at 25°C. Electrolyte leakage was defined as Valentovic *et al*. (2006):

$$\text{EL (\%)} = 100 \times \frac{L1}{L2}$$

Data was analyzed using MSTATC software. Mean comparison was performed with Duncan's test (0.01 level of probability respectively), and graphs were drawn using Excel 2003 software.

RESULTS AND DISCUSSION

Germination percentage (GP) and Mean Germination Time (MGT)

Snake melon (*Cucumis melo* var. *flexuosus*) GP and MGT was significantly influenced by salt stress, priming and interaction of salt stress and priming (**Table 1**). Our results showed salt stress decrease snake melon seed germination but seed primed by NaCl and KNO_3 solution had higher seed germination compared other treatments (**Fig. 1**). Farhoudi *et al*. (2007) and Demir and Mavi (2004) found that seed priming increase seed germination percentage under unfavorable condition.

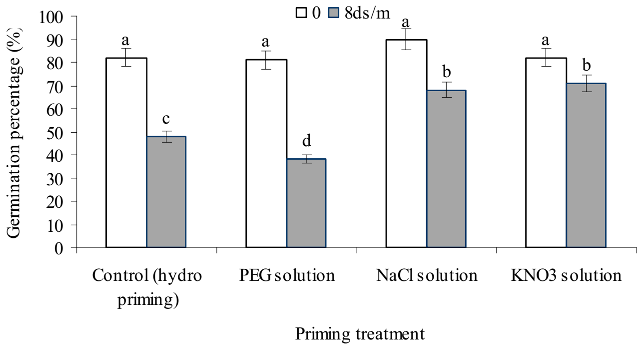

Fig. 1 Effect of salt stress and seed priming on snake melon germination percentage.

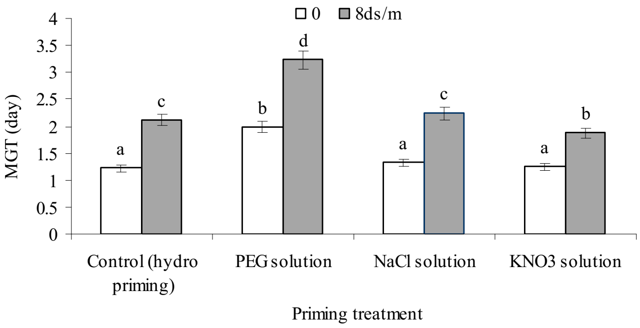

Fig. 2 Effect of salt stress and seed priming on snake melon mean germination time (MGT).

Under non-saline conditions seed priming with PEG increase MGT compared to other treatments (**Fig. 2**). Salt stress increased MGT but this increase were slower in seed priming with KNO_3 solution (**Fig. 2**). In fact, under salinity stress, KNO_3 priming had a positive effect on snake melon MGT. The beneficial effects of KNO_3 on MGT were found by Kaya *et al.* (2006) study, too. In our study, lower MGT from KNO_3 in compared to NaCl and PEG, suggesting no toxicity or prevent water uptake of KNO_3 (Demir and Van De Venter 1999). Mauromicale and Cavallaro (1996) found herbage grasses seed primed with KNO_3 decrease MGT in these seeds compared to PEG solution.

Radicle and shoot length

Radicle and shoot length was significantly influenced by salt stress, priming and interaction of salt stress and priming (**Table 1**). Results showed that under non-saline conditions radicle length increased significantly when seeds were pretreated with KNO_3 or NaCl (**Fig. 3**). Salinity stress decreased radicle length in seedlings. Longest radicles formed in seed primed with KNO_3 and shortest in seed primed with PEG solution and water (**Fig. 3**). Nascimento (2003) reported that muskmelon seed priming with KNO_3 increase root and shoot length compared to other priming treatments like PEG and mannitol.

Fig. 4 shows under non-saline conditions no significant differences in shoot length between seed priming. Salt stress decrease shoot length in compared to the non-saline treatment. **Fig. 4** shows that under saline condition tallest shoots formed when seeds were primed with KNO_3 and

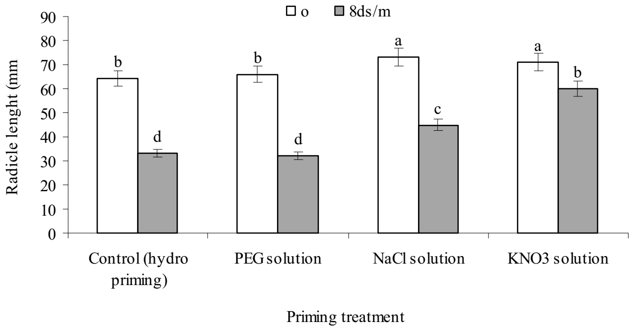

Fig. 3 Effect of salt stress and seed priming on snake melon radicle length.

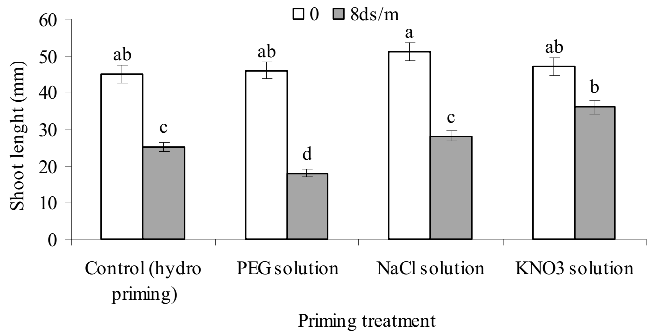

Fig. 4 Effect of salt stress and seed priming on snake melon shoot length.

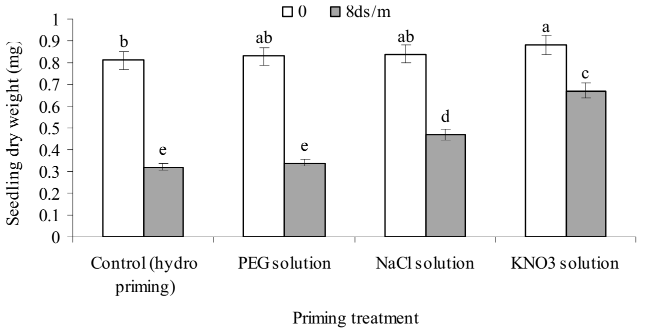

Fig. 5 Effect of salt stress and seed priming on snake melon seedling dry weight.

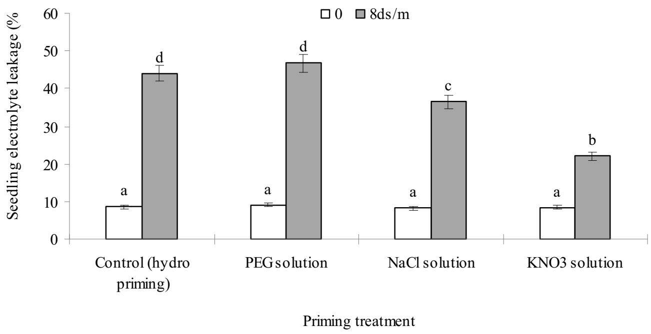

Fig. 6 Effect of salt stress and seed priming on snake melon seedling electrolyte leakage.

shortest with PEG (**Fig. 4**). Unlike our results, Gray *et al.* (1991) reported that PEG is the best treatment to improve seed germination and seedling growth of onion seeds under salinity stress. However, our findings at salinity of 8 ds/m showed that a decrease in shoot and radicle length was higher in seeds primed with PEG compared with the salt priming treatment.

Seedling dry weight

DW was significantly influenced by salt stress, priming and interaction of salt stress and priming (**Table 1**). Under non-saline conditions heaviest seedlings were obtained from seed primed with KNO_3, NaCl and PEG (**Fig. 5**). Salt stress decreased seedling DW (**Fig. 5**). Under salinity seedling DW was lowest showed in seeds primed with PEG solution but highest seedling DW was obtained from seeds primed with KNO_3 (**Fig. 5**). Seed priming with PEG decreased seedling DW compared to other treatments. These results are supported by those of Okcu *et al.* (2005) who showed that priming with KNO_3 was more effective than PEG in promoting early germination and seedling growth of pea (*Pisum sativum* L.) seedlings. Kaya *et al.* (2003) reported salt stress to decrease safflower (*Carthamus tinctorius* L.) seedling weight. They suggested that osmotic stress and ionic stress of salt stress decreased the growth of safflower seedlings. Nascimento (2003) reported that muskmelon seed primed with KNO_3 increased seedling fresh weight under low temperature stress compared to non-primed seeds.

Seedling electrolyte leakage

Seedling electrolyte leakage was significantly influenced by salt stress, priming and the interaction between salt stress and priming (**Table 1**). Under non-saline conditions, no significant differents in electrolyte leakage were shown (**Fig. 6**). Under salinity seeds treated by KNO_3 solution had lowest seedling electrolyte leakage compared other seed priming treatments (**Fig. 6**). Farooq and Azam (2006) and Meloni *et al.* (2003) found that cell membrane stability of wheat seedlings decreased under salinity but Farhoudi *et al.* (2007) stress that canola seed primed by NaCl solution increased canola seedling cell membrane stability and decreased seedling damage under salinity.

CONCLUSIONS

In many plants, germination and subsequent seedling growth can be inhibited by environmental stress such as salt stress (Okcu *et al.* 2005; Farooq and Azam 2006; Kaya *et al.* 2006; Shahi-Gharahlar *et al.* 2009). Priming may be helpful in reducing the risk of poor stand establishment under salt stress conditions (Nascimento 2003; Kaya *et al.* 2006). The results of this study clearly show that salinity increased electrolyte leakage in snake melon seedlings (**Fig. 6**) but the seeds primed by KNO_3 had lowest electrolyte leakage and highest seedling DW compared to seedlings from other treatments. Farhoudi *et al.* (2007) found that canola seed priming by NaCl under salinity stress decreased seedling electrolyte leakage because of increased seedling K^+ and proline content. Jain *et al.* (2001) suggested that the higher adaptation of seedlings to salinity could be due to osmoregulation induced by organic solutes like proline and cell membrane stability. In this study, seed priming by KNO_3 under salinity increase DW and seedling growth (**Figs. 3-5**) and decreased seedling electrolyte leakage (**Fig. 6**) compared to other seed priming treatments. In fact, our results showed snake melon seed primed by KNO_3 improve tolerance to salinity by increasing seedling DW, root and shoot length and decreasing electrolyte leakage under salinity. The beneficial effects of seed priming by KNO_3 solution on seed germination under salt stress has been observed in other crops: safflower (Kaya *et al.* 2003), pea (Okcu *et al.* 2005) and muskmelon (Nascimento 2003). Singh and Rao (1993) stress that KNO_3 effectively improved germination, seedling growth and seedling vigor index of sunflower seeds.

Improving seedling snake melon growth by KNO_3 priming under salinity suggests the non-toxity of KNO_3 due to ion accumulation in the embryo (Demir and Van De Venter 1999; Kaya *et al.* 2006). Khajeh-Hosseini *et al.* (2003) found that under salt stress, Na^+ and Cl^- may be taken up by

Fig. 7 Snake melon seeds used in this study.

the seed and the toxic effect of NaCl might appear but higher embryo K^+ and seed water content following priming in potassium salts as compared to untreated seed is observed (Mauromicale and Cavallaro 1996).

In conclusion, this study showed that KNO_3 priming of snake melon seeds (**Fig. 7**) was more effective than other treatments such as PEG or NaCl solution. Our study showed snake melon seed priming with KNO_3 decrease MGT and increase seedling DW under salinity stress compared to other priming treatments.

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