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Effect of Seed Priming with Potassium Nitrate on Germination and Emergence Traits of Two Soybean Cultivars under Salinity Stress Conditions

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Abstract: To evaluate the effect of seed priming with potassium nitrate on germination and emergence traits of two soybean cultivars under salinity stress, two laboratorial and greenhouse experiments were carried out as a factorial experiment based on a completely randomized design with 4 replications in the Seed Physiology Research Department of Agricultural Faculty of Bu-Ali Sina University, Iran. Treatments were included of two soybean cultivars (Sahar and Gorgan-3), two levels of non-priming and priming with potassium nitrate at 6 gl^{-1} concentration and three levels of salinity (0, 4 and 8 dS m^{-1} induced by sodium chloride). The results showed that Sahar cv. was superior to Gorgan-3 cv. in all traits and in both experiments. Seed priming with KNO_3 significantly increased germination and emergence percentages, radicle and plumule length, seedling dry weight, plant height, plant leaf area and plant dry weight. Also at the salinity stress, mean germination and emergence time of primed seeds were less than non-primed seeds, significantly. At the salinity stress, specific highest salinity level, final germination percentage, radicle and plumule length, plant height, plant leaf area and plant dry weight of primed seeds were more than non-primed seeds, significantly. Positive effects of priming with potassium nitrate on seeds of Gorgan-3 cv. were greater than those of Sahar cv.

Key words: Priming · Potassium nitrate · Soybean · Germination · Stress

INTRODUCTION

Soybean (*Glycine max* L.) is one of the most important oil seed crops, which is planted in many areas of Iran [1], such as Khorasan, Golestan and Mazandaran stats. Salinity stress is considered as the most important problems in Iran that led to reduced crops yield.

Soil salinity may affect the germination by creating osmotic potential to preventing water uptake or through toxic effects of Na^+ and Cl^- ions [3-5]. Almansouri *et al*., [6] expressed that salinity stress is an important factor in preventing or delaying seed germination and seedling establishment. Some researchers have reported negative effects of salinity stress on seed germination and crop production [7-9].

Seed priming is beneficial technique to improvement seed germination and growth in stress condition. Nowadays, various seed priming techniques have been developed, including hydro priming (soaking in water), halo priming (soaking in inorganic salt solutions), osmo priming (soaking in solutions of different organic osmotic), thermo priming (treatment of seed with low or high temperatures), solid matrix priming (treatment of seed with solid matrices) and bio priming (hydration using biological compounds) [10].

Harris *et al*., [11-13] reported that seed priming led to better establishment and growth, earlier flowering and greater yield. Kattimani *et al*., [14] indicated that primed seeds with nitrate solutions produced vigorous seedlings, more dry matter accumulation and root length in compared with non-primed seeds. Mohammadi [1] found that among the priming treatments, primed seeds with potassium nitrate showed the highest values for all traits and priming with KNO_3 than control, increased germination percentage, germination rate and seedling dry weight by 28.3, 129.4 and 58.1 percentage, respectively. Kaya *et al*., [15] indicated that priming of sunflower seeds with KNO_3 led to increasing of germination percentage in drought and salinity stresses. Several studies confirmed positive effects of seed priming on germination [5, 8, 16-18].

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Therefore, it is probably that in unfavorable condition, seedlings from primed seeds less affected by stress because of better germination and growth.

This study was conducted to evaluate the effect of seed priming by nitrate potassium solutions on two soybean cultivars under salinity stress conditions.

MATERIALS AND METHODS

To evaluate the effect of seed priming with potassium nitrate (KNO_3) on germination and emergence traits of two soybean cultivars in salinity stress, in the Seed Physiology Research Department of Agricultural Faculty of Bu-Ali sina University, Iran, two laboratorial and greenhouse experiments were carried out as a factorial experiment based on a completely randomized design with 4 replications. Treatments were included of two soybean cultivars (Sahar And Gorgan-3), two levels of non-priming and priming with Kno3 at 6 g l^{-1} concentration and three levels of salinity (0, 4 and 8 ds m^{-1} induced by sodium chloride).

Seed treatments: Seeds of soybean cultivars were obtained from the seed bank of Bu-Ali Sina University and after sterilize with 1% sodium hypochloride for 1 minute were washed twice with distilled water. Then seeds were placed on filter paper (Whatman No.1) impregnated with equal amount of solution 6 g KNO_3 l^{-1}, for 6 hours in dark condition. Finally, seeds for two days were dried at 25°C until moisture of seeds reach to its initial content (%4).

Germination Test

Laboratorial Experiment: Twenty five seeds were placed on filter paper (Whatman No.1) in per glass Petri dish with 9 cm diameter and added 20ml salinity solution of desired treatment. Finally, Petri dishes were transferred into the dark germinator with 25±2°C and darkness. Seed germination was recorded daily in a certain time. A seed was considered as germinated when its radicle emerged by about 2 mm in length [1]. After the 7^{th} day, radicle and plumule length was measured. Then seedlings were dried in oven for 48 hours at 72°C and their weight was measured by a Scale with precision 0.001. The seedlings with think and spiral formed hypocotyls and stunted primary root were considered as anormally germinated [19].

Greenhouse Experiment: Pots with 35cm diameter and contain loamy soil with 0.3 dS m^{-1} electrical conductivity were prepared. In each pot twenty five seeds were planted 2 cm in depth. Each pot was irrigated with a 200 ml of salinity solution induced by sodium chloride of desired treatment, two days interval. Seed germination was recorded daily. After 8^{th} day that emergence was fixed, plants were thined and maintained four plants per pot. After 20^{th} days of start of the experiment, height of plants were measured and then plants were harvested from soil surface and their dry weight was determined similar to laboratorial experiment. Final germination and emergence percentage by the equation 1 and mean germination and emergence time were calculated according to the equation of Ellis and Roberts [20]. (equation 2):

$$FGP \text{ or } FEP = (\frac{S}{T}) \times 100 \quad (1)$$

$$MGT \text{ or } MET = \frac{\sum n.d}{\sum n} \quad (2)$$

Where FGP and FEP is the final germination percentage and final emergence percentage, S is the number of germinated seeds on final day, T is the number of seeds, MGT and MET is mean germination and emergence time, n is the number of seeds germinated on day and d is the number of days from the start of each experiment.

Data analysis was carried out using SAS. The differences between the means were compared using Duncan test ($P<0.05$).

RESULTS AND DISCUSSION

In laboratory experiment, characters of FGP, MGT, Root length, Shoot length and Seedling dry weight were affected significantly by cultivar, priming and salinity (Table 1). Also, in the greenhouse experiment, the effect of cultivar, seed priming and salinity on FEP, MET, plant height, plant LA and plant dry weight were significant (Table 2).

Increasing of salinity stress led to significant reduction in FGP in both non-primed and primed seeds. Minimum value of this trait was obtained at levels of 8 dS m^{-1} and non-priming, so that at the mentioned salinity level, germination of primed seeds with KNO_3 was 21.5 percent more than non-primed seeds (Table 5). Also, Guzman and Olave [21] reported that seed priming with nitrate solutions led to improved germination rate and germination index.

Table 1: Analysis of variance of the evaluated traits in the laboratory.

		Mean of square				
Source Of Variance	df	FGP	MGT	Radicle Length (cm)	Plumule Length (cm)	Seedling Dry Weigh (g)
Cultivar (C)	1	1260**	5.280**	10.6**	19.6**	0.0161**
Prim (P)	1	2852**	16.10**	13.8**	9.62**	0.0657**
Salinity(S)	2	11805**	46.42**	16.8**	36.1**	0.0396**
P× C	1	36.7ns	0.258*	1.81**	0.54*	0.0015*
S×C	2	12.2ns	0.006ns	0.65*	0.71**	0.0014**
S×P	2	226**	3.136**	2.57**	1.06**	0.0002ns
S×P×C	2	2.25ns	0.080ns	0.75*	0.13ns	0.0002ns
Error	36	36.5	0.058	0.19	0.103	0.0002
CV (%)	-	11	6.4	12.9	8.1	12.2

Abbreviations: FGP, final germination percentage; MGT, mean germination time. ns, * and **: non-significant and significant at the 0.05 and 0.01 level of probability, respectively.

Table 2: Analysis of variance of the evaluated traits in the greenhouse.

		Mean of square				
Source Of Variance	df	FEP	MET	Height (cm)	LA (cm)	Plant dry weight (g)
Cultivar (C)	1	1045**	7.908**	138.7**	1010**	7.249**
Prim (P)	1	901**	4.445**	205.8**	3945.8**	13.23**
Salinity(S)	2	15474**	73.06**	846.8**	21013.4**	41.06**
P×C	1	21.3ns	0.259*	0.270ns	52.08*	0.116ns
S×C	2	42.2ns	0.118ns	1.173ns	3.967ns	0.230*
S×P	2	86.3ns	1.102**	16.83**	42.85*	0.561**
S×P×C	2	10.4ns	0.013ns	0.910ns	23.39ns	0.078ns
Error	36	32.9	0.047	4.9	9.9	0.062
CV (%)	-	12.8	4.3	12.7	4.7	6

Abbreviations: FEP, final emergence percentage; MET, mean emergence time. LA, leaf area per plant. ns, * and **: non-significant and significant at the 0.05 and 0.01 level of probability, respectively.

Table 3: The effect of priming on seed germination, seedling emergence and growth of two soybean cultivars at laboratory and greenhouse conditions.

		Laboratory				Green house	
Cultivar	priming	MGT (day)	RL (cm)	PL (cm)	SDW (g)	MET (day)	LA (cm^{-2})
Sahar	Control	4.0b	3.1b	4.0b	0.105c	4.9c	62.7c
	Kno3 6gl^{-1}	2.9d	4.6a	5.1a	0.168a	4.4d	78.7a
Gorgan-3	Control	4.8a	2.6c	2.9d	0.057d	5.8a	51.4d
	Kno3 6gl^{-1}	3.5c	3.3b	3.6c	0.143b	5.1b	71.6b

Abbreviations: MGT, mean germination time; RL, radicle length; PL, pulumule length; SW, seedling dry weight; MET, mean emergence time; LA, leaf area per plant. Similar letters at each column indicate the non-significant difference at the 0.05 level of probability.

Table 4: Effect of salinity levels on seed germination, seedling emergence and growth of two soybean cultivars at laboratory and green house conditions

		Laboratory		Green house	
Cultivar	Salinity (dsm^{-1})	Radicle length (cm)	Plumule length (cm)	Seedling dry weight (g)	Plant dry weight (g)
Sahar	0	4.8a	6.5a	0.202a	5.86a
	4	3.6c	4.1c	0.121c	4.89c
	8	3.1d	3.1d	0.086d	2.85e
Gorgan-3	0	4.1b	4.7b	0.145b	5.35b
	4	2.8d	3.0d	0.089d	3.91d
	8	1.7e	2.2e	0.066e	2.01f

Similar letters at each column indicate the non-significant difference at the 0.05 level of probability.

Table 5: Effect of priming on seed germination and seedling emergence and growth at salinity levels on laboratory and greenhouse conditions

		Laboratory				Green house			
Priming	Salinity (dsm^{-1})	FGP (%)	MGT (day)	RL (cm)	PL (cm)	MET (day)	Height (cm)	LA (cm^{-2})	PDW (g)
Control	0	81.0b	2.0e	4.3a	5.4b	2.8e	23.6a	90.5b	5.19b
	4	38.0d	5.0b	2.8c	3.2d	5.9c	14.3c	61.0d	3.66c
	8	21.0e	6.2a	1.5d	1.9e	7.4a	8.0d	19.6f	2.02e
Kno_3 6gl^{-1}	0	88.0a	1.8e	4.6a	5.8a	2.7e	25.7a	107.7a	6.03a
	4	55.7c	3.5d	3.7b	3.9c	4.9d	20.5b	82.7c	5.14b
	8	42.5d	4.3c	3.4b	3.4d	6.6b	12.2c	35.0e	2.85d

Abbreviations: FGP, final germination percentage, MGT, mean germination time; RL, radicle length; PL, plumule length; MET, mean emergence time; LA, leaf area per plant; PDW, plant dry weight. Similar letters at each column indicate the non-significant difference at the 0.05 level of probability.

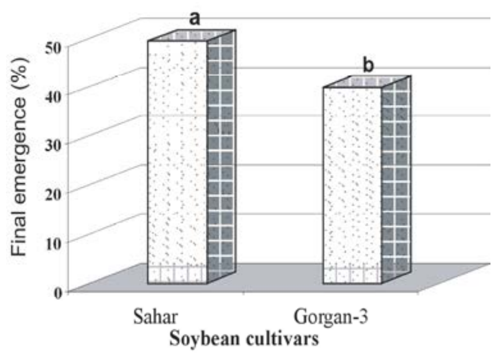

Fig. 1: Mean comparison of final emergence percentage in two soybean cultivars (Duncan 0.05).

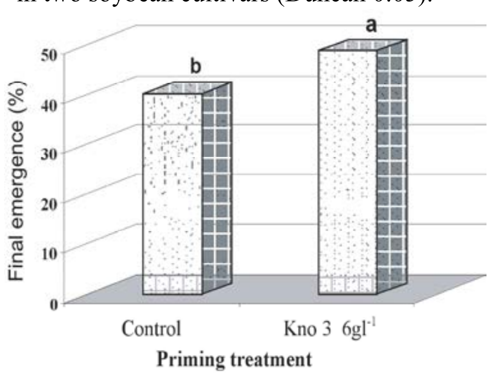

Fig. 2: Mean comparison of final emergence percentage in priming treatment (Duncan 0.05).

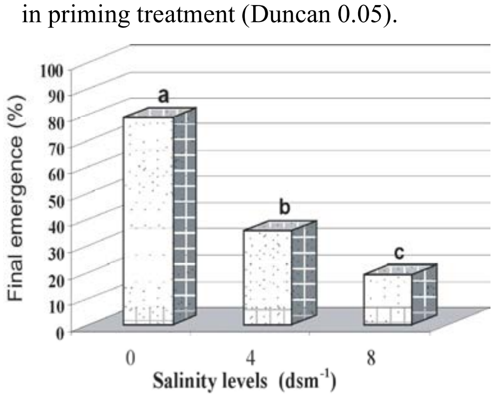

Fig. 3: Mean comparison of final emergence percentage in salinity levels (Duncan 0.05).

In the greenhouse test only the simple effects of priming, cultivar and salinity on FEP was significant (Table 2). Final percentage emergence of Sahar cv. was more than Gorgan-3 cv. (Figure 1). FEP of primed seeds were more than non-primed, significantly (Figure 2).

By increasing of salinity stress, FEP reduced, so that at the 4 and 8 ds m^{-1} than control, this trait reduced by 43.5 and 60.25 percentages, respectively and a significant difference was observed in all levels of salinity stress (Figure 3). Cultivar × prim and prim × salinity stress interaction in traits of mean germination and emergence time were significant (Tables 1 and 2). MGT of Sahar cv. in non-priming and priming was less than that of Gorgan-3 cv., significantly. Seed priming with KNO_3 caused to reduction of MGT of Gorgan-3 cv. by 37%, while this reduction in Sahar cv. was 27.5%. Also similar results were obtained for MGT, but reduction effect of seed priming on MGT of Sahar and Gorgan cv. were 10 and 12%, respectively (Table 3).

At 4 and 8 ds m^{-1} salinity stress treatments, there was significant difference between MGT in priming and non-priming (Table 5). However increasing of salinity led to significant increase of MGT of primed and non-primed seeds, but salinity effect on primed seeds was less than un-primed seeds, so that at the 8 ds m^{-1} MGT of non-primed seeds than own control was 3.1 times, but in primed seeds it was 2.4 times. Variation of MET in non-primed and primed seeds at the levels of salinity stress was similar to MGT. At the highest level of salinity stress, MGT of non-primed seeds than own control was 2.6 and this value was reduced to 2.4 in primed seeds. Afkari [9] reported that by increasing of salinity stress levels, final emergence reduced in both non-primed and primed seeds, but, negative effects of salinity stress on primed seeds were less than non-primed seeds. Bocian and Holubowicz [22] showed that seed priming with Kno3 for 6 and 12 hours, improved seed germination of tomato and reduced mean germination time. In other studies the positive effects of priming on seed germination has been confirmed [23, 24].

Simple effects and interaction of all treatments on radicle length were significant and in plumule length only interaction of cultivar × prim × salinity stress was not significant (Table 1). In both cultivars radicle and plumule length of primed seeds significantly was more than non-primed seeds, also non-primed and primed seeds of Sahar cv. in both traits had significant priority than Gorgan-3 cv. (Table 3). With increasing of salinity stress, radicle and plumule length of both soybean cultivars showed significant reduction and Sahar cv. than Gorgan-3 cv at the each level of salinity stress was superior (Table 4).

At the non-stress, radicle length of both primed and non-primed seeds were the same statistically (Table 5). Also, with increasing of salinity from 4 to 8 ds m^{-1}, significant reduction in radicle length of primed seeds wasn't observed. At the highest level of salinity stress than control, radicle length of non-primed seeds was reduced by 65 %, so that in primed seeds, this decline was 26 %. At the whole salinity levels, plumule length of primed seeds was significantly more than non-primed seeds, while at the levels of 0 and 8 dS m^{-1}, plumule length of primed seeds was more than non-primed seeds by 6.9 and 44.1 %, respectively (Table 5).

Only, the effect of prim and salinity on height of plant was significant (Table 2). At the non saline treatments, there was significant difference in height between non-primed and primed seeds, but with increasing salinity stress in each level, primed seeds than non-primed showed significant superiority (Table 5). Hopper *et al*., [2] indicated that in primed seeds because of more water uptake efficiency and faster metabolic activity in term of germination, radicle and plumule appeared faster. The similar results were reported by other researchers [9, 14, 21]. Except of cultivar × salinity stress and cultivar × prime × salinity stress interaction on leaf area per plant, other simple and interaction effects were significant (Table 2).

Leaf area per plant of Sahar cv. in both non-primed and primed seeds was more than Gorgan-3 cv., significantly. In both Sahar and Gorgan-3 cv. leaf area per plant from primed seeds than non- primed seeds showed significant increase by 25.5 and 39 percent, respectively (Table 3). Seed priming led to significant increase of leaf area per plant at each salinity level. At the 8 dS m^{-1} than 0 dS m^{-1}, leaf area of non-primed seeds was decreased by 78%, but this reduction in primed seeds was 67 percent (Table 5). In a field experiment, soybean plants from the primed seeds with Kno_3 showed the highest LAI [1].

In trait of seedling dry weight, further simple effect of treatments and interaction of cultivar × prim and cultivar × salinity stress were significant (Table 1). Also, interaction of cultivar × salinity stress and prim × salinity stress was significant (Table 2). Seedling dry weight of Sahar cv. obtained of non-primed and primed seeds were more than Gorgan-3 cv. Seed priming led to more positive effects on seedling dry weight of Gorgan-3 cv. (Table 3). At the each salinity level, seedling dry weight and plant dry weight of Sahar cv. was more than that of Gorgan-3 cv., significantly (Table 4). Seedling dry weight obtained of primed seeds significantly was more than non-primed seeds (Table 5). Afkari (2010) found that seedlings dry weight of primed seeds were more than non-primed seeds at the all salinity levels.

CONCLUSION

Seed priming led to increase all traits of germination and emergence and also dry weight, plant height and leaf area of both soybean cultivar in laboratorial and greenhouse. However, Gorgan-3 cv. than Sahar cv. was weaker in all traits, but seed priming caused to more positive effects on traits of this cultivar. Singh and Rao [25] reported that in stress condition, potassium nitrate effectively improved germination, seedling growth and seedling vigor index of the seeds of sunflower (*Helianthus annuus*) varieties with low germination. Tzortzakis [24] expressed that priming with KNO_3 improved rate of germination and seedling emergence and stated that priming is a practical method with economic benefit for producers.

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