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Effects of Seed Priming on Seed Germination and Seedling Emergence of Cotton Under Salinity Stress

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Abstract: To determine effects of hydro-priming and priming with potassium nitrate on seed germination and seedling emergence of cotton (Sahel cv.) under salinity stress, we set up two laboratory and greenhouse studies using completely randomized factorial design with 4 replications in Agricultural Faculty of Bu-Ali Sina University, Iran. Treatments were hydro-priming (priming with distilled water) and priming with potassium nitrate at two concentrations of 3 and 6 g l^{-1}, all under three levels of salinity stress (induced by sodium chloride) i.e. 0, 4 and 8 ds m^{-1}. Results revealed that under introduced salinity stress, hydro-primed seeds and those primed with potassium nitrate showed increased properties such as: seed germination and seedling emergence, length of radicle and plumule, weight of dried seedling and plant, average length of plant and per plant leaf area. Average germination and emergence time were also reduced by priming. Meanwhile, priming with 6 g $KNO_3\,l^{-1}$ had the most positive effects on measured traits and at the highest level of salinity stress.

Key words: Hydro-Priming · Potassium nitrate · Cotton · Germination · Stress

INTRODUCTION

The process of seed germination is a tri-phase phenomenon which includes an initial phase of rapid water uptake, followed by a plateau phase with little change in water content; and subsequently, the last phase which coincides with radicle emergence and resumption of growth [1]. In stressed environments, germination and early seedling growth are especially crucial and important stages of plant's life-cycle [2, 3].

Sometimes, due to increasing temperature and decreasing rainfall, emerged salinity of soil elements as a secondary source of stress, could halt the germination process [2]. High salinity stress could interrupt homeostasis in plant water potential and ion distribution, at both cellular and whole plant levels and consequently reduce yield quality and quantity [3]. Seeds that succeed germinating under stress, it may increase their chance of continuing growth and development, so as to overcome other environmental stresses as well [2].

There are many strategies on how to overcome negative impact of salinity stress. Bradford [4] introduced seed priming as a method to overcome negative effects induced by salinity. Apart from inducing early resistance and therefore preparing the plant for any future environmental stress, seed priming is also a pre-germination physiological method that improves seed performance leading to faster and more synchronized seed germination [3].

Positive effects of seed priming with various priming agents have been reported in some crops, like chickpea [5], wheat [6], Sugarcane [3], sunflower [7] and Oregano [8]. Sung and Chiu [4] observed that mean germination time of hydro-primed seeds accelerated in watermelon without changing the water uptake. Effects of hydro-priming in sunflower [10], cotton [11] and Soybean [12] have also been encouraging. Demir and Van De Venter [13] researching on watermelon reported that hydro-priming and priming with KNO3 reduced mean germination time while increased overall germination. Another experiment revealed that priming with KNO3

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increased seed germination of sunflower in drought stress whereas hydro-priming showed its maximum positive effect on radicle and plumule growth [10]. Meanwhile, Afkari [14] discovered that when salinity stress increased, final emergence of primed seeds with KNO3 overwhelmed un-primed seeds. Hopper *et al.* [15] expressed that in primed seeds, radicle and plumule appeared faster because of more water uptake efficiency and metabolic activity during germination. The present study was conducted to investigate the effects of seed priming by water or potassium nitrate on seed germination traits of cotton (cv. Sahel) in the condition of salinity stress.

MATERIALS AND METHODS

To evaluate the effects of hydro-priming and priming with potassium nitrate on germination and emergence traits of cotton (Sahel cv.) in salinity stress, authors managed two laboratorial and greenhouse studies utilizing factorial experiment. It was based on a completely randomized design with 4 replications in the Agricultural Faculty of Bu-Ali Sina University, Iran. Treatments were hydro-priming (with distilled water) and priming with potassium nitrate at 3 and 6 g l^{-1} concentrations under three levels of salinity namely 0, 4 and 8 ds m^{-1} induced by sodium chloride (S1=0 (control) S2=4, S3=8 (ds m^{-1}).

Seed Treatments: Cotton seeds were collected from Gorgan University of Agricultural Sciences. After sterilizing with 1% sodium hypochlorite for 3 minutes, seeds were washed twice in distilled water. Then, based on so-called priming treatments, seeds were placed on filter paper (Whatman No.1) impregnated with distilled water or equal amount of KNO3 solutions for 12 hours. Finally, seeds were left for two days to dry at 25°C until their moisture reached the initial content.

Germination Test: Twenty five seeds were placed on filter paper (Whatman No.1) at each 9 cm diameter Petri dish. Then, 20 ml of designed saline solution was added to the correspondent Petri dishes. Later Petri dishes were transferred into a dark germinator with 20±2°C in temperature. Seed germination was recorded daily at a certain time. Of course, seeds were considered normally-germinated when their radicle had emerged by about 2 mm in length [12]. After the 7^{th} day, seeds' radicle and plumule length were measured. Then, seedlings were dried in the oven for 48 hours at 72°C and their weights measured subsequently. The seedlings with thin, spiral-formed hypocotyls, or stunted primary root were considered to be abnormally germinated [16].

Greenhouse Experiment: Twenty five seeds were planted 2 cm deep in each 35 cm (in diameter) pot which contained loamy soil with 0.3 ds m^{-1} electrical conductivity. Each pot was irrigated on two day intervals with 200 ml of salinity solution induced by sodium chloride based on designed treatment. Seed germination was recorded daily. After the 7^{th} day, when seedling emergence remained unchanged, plants were thinned so that four plants remained per pot. After the 50^{th} day, plants' heights were measured. They were harvested and their dry weights were determined similar to those coming from laboratorial experiment. Final Germination or Emergence Percentage (FGP or FEP) and Mean Germination or Emergence Time (MGT or MET) were assessed using the following equations. The second equation was introduced by Ellis and Roberts [17]:

$$FGP \text{ or } FEP = (\frac{S}{E}) \times 100 \qquad \text{(eq.1)}$$

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

where S, T, n and d stood for: number of germinated seeds on final day, number of seeds, number of germinated seeds per day and number of days passed from the beginning of the experiment; respectively. Means were compared using Duncan test (P<0.05) in SAS medium.

RESULTS AND DISCUSSION

Priming and salinity stress showed to have significant effects on evaluated traits of the study in both laboratorial and greenhouse experiments (Table 1 and 2). In the no salinity treatment (S0), unlike other priming treatments that revealed no significant difference, FGP and MGT of primed seeds (with 3 g KNO3 l^{-1}) were significantly different from those of unprimed (Table 3). When salinity stress increased to 4 ds m^{-1}, FGP decreased –i.e. FGP in unprimed, hydro-primed, primed with 3 and 6 g KNO3 l^{-1} reported to be less than control by 28, 24, 14 and 10 percents; respectively. At both 0 and 4 ds m^{-1}salinity stresses, FGP of primed seeds with 3 compared to 6 g KNO3 l^{-1} did not show any significant difference (Table 3). At the highest level of salinity stress (8 ds m^{-1}), priming with 6 g KNO3 l^{-1} increased FGP of seeds by 39 percent (Table 3).

Yagmur and Kaydan [18] found that seed priming of triticale with KH_2PO_4 was more effective on germination percentage than other priming treatments. Ghana and Schillinger [19] observed that primed seeds of wheat with

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

		Mean of square				
S.O.V.	df	FGP	MGT	Length shoots hoot	Root length root	Seedling dry weight
Prim (P)	3	1229**	5.196**	6.6**	2.92**	0.0191**
Salinity (S)	2	9247**	15.362**	21.9**	23.26**	0.0809**
S×P	6	225**	0.538**	0.7**	0.45**	0.0022**
Error	36	24.5	0.126	0.1	0.13	0.0003
CV (%)	-	6.7	10.9	8.1	8.7	7.8

(FGP) Final Germination Percentage; (MGT) Mean Germination Time.
**: significant at 99 % confidence interval

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

		Mean of square				
S.O.V.	df	FEP	MET	LA	Height	Plant weight
Prim (P)	3	1507**	5.038**	7449**	96.2**	5.040**
Salinity (S)	2	10009**	10.429**	109176**	4481.9**	33.791**
S×P	6	218**	0.478**	1044**	97.9**	1.096**
Error	36	43	0.132	223	16.4	0.087
CV (%)	-	9.8	9.4	9	10.5	5.7

(FEP) Final Emergence Percentage; (MET) Mean Emergence Time; (LA) leaf area per plant
**: significant at 99 % confidence interval

Table 3: Effects of priming treatments on cotton seed germination and its vigor under salinity conditions in laboratory experiment (means comparision)

Priming	Salinity (dsm^{-1})	FGP (%)	MGT (day)	Plumule length (cm)	Radicle length (cm)	Seedling dry weight (g)
Control	0	91^{bc}	2.6^{de}	4.4^{b}	5.1^{a}	0.277^{b}
Hydro	0	98^{ab}	2.4^{def}	5.1^{a}	5.4^{a}	0.290^{ab}
Kno$_3$ 3gl^{-1}	0	99^{a}	2.0^{f}	5.2^{a}	5.5^{a}	0.292^{ab}
KNO3 6gl^{-1}	0	96^{ab}	2.1^{ef}	5.0^{a}	5.5^{a}	0.312^{a}
Control	4	63^{ef}	4.4^{b}	2.3^{e}	3.1^{f}	0.161^{e}
Hydro	4	74^{d}	3.2^{c}	3.3^{cd}	3.9^{cd}	0.206^{d}
KNO3 3gl^{-1}	4	85^{c}	2.6^{def}	4.2^{b}	4.6^{b}	0.249^{c}
KNO3 6gl^{-1}	4	86^{c}	2.7^{d}	4.2^{b}	4.3^{bc}	0.274^{b}
Control	8	27^{h}	5.3^{a}	1.3^{f}	2.1^{g}	0.085^{g}
Hydro	8	43^{g}	4.7^{b}	2.5^{e}	2.8^{f}	0.125^{f}
KNO3 3gl^{-1}	8	57^{f}	3.6^{c}	3.2^{d}	3.3^{ef}	0.178^{e}
KNO3 6gl^{-1}	8	66^{e}	3.4^{c}	3.6^{c}	3.8^{de}	0.214^{d}

Abbreviations: FGP, Final Germination Percentage; MGT, Mean Germination Time. Similar letters at each column indicate the non-significant difference at 95 % confidence interval

KH_2PO_4 and water enhanced germination compared to un-primed seeds. They believed that higher water-uptake ability in primed seeds than un-primed ones led to a positive effect on this trait. Positive effects of hydro-priming and priming with KNO3 were reported by Kaya *et al*. [11] as well. Bocian and Holubowicz [20] found that priming with KNO3 enhanced seed germination of tomato. Also, studies of Afkari [14] showed that germination percentage of primed with KNO3 were more than un-primed seeds.

At the non-stress condition, priming with 3 g KNO3 l^{-1} reduced MGT of cotton seeds significantly compared to unprimed seeds. By increasing salinity stress, this trait increased at all priming treatments. However at all treatments of salinity stress, MGP of hydro-primed seeds were significantly higher than primed seeds with KNO3, while the differences between 3 and 6 g KNO3 l^{-1} priming treatments were not significant (Table 3).

Similar research also reported reduction of MGT in primed seeds [20, 11, 9]. The accelerated germination of primed seeds might be due to increased rate of cell division [21] and stimulation of metabolic activities during early phases of seed germination [4, 22].

Positive effects of priming on plumule length appeared in non-stress condition, while no significant difference was seen between primed seeds. At the 4 ds m^{-1} salinity, plumule length of primed seeds (with 3 and

6 g KNO3 l^{-1}) enhanced compared to unprimed by 82.6 percent. Maximum plumule length (3.8 cm) was obtained at the 8 ds m^{-1} salinity and by primed seeds with 6 g KNO3 l^{-1} (Table 3).

There was a statistically significant difference in radicle length between priming treatments at the salinity levels of 4 and 8 ds m^{-1} i.e primed seeds showed a significant superiority over un-primed ones. Kaya *et al*. [11] found that seed priming in sunflower enhanced root length at both osmotic and salinity stress. Similar results were reported by Afkari [14].

In non-stress condition, only dry weight of seedlings coming out of primed seeds with 6g KNO3l^{-1} increased significantly. At the salinity levels of 4 and 8 ds m^{-1}, seedling dry weight of primed seeds were higher than un-primed ones and priming with 6 g KNO3 l^{-1} had the highest positive effect on this trait. At the highest level of salinity stress, seedlings dry weight of hydro-primed and primed seeds with 3 and 6 g KNO3 l^{-1} were 1.5, 2.1 and 2.5 times higher than control; respectively (Table 3).

Soltani *et al*. [23] explained reduction in seedling dry weight of wheat cultivars in drought and salinity stress to be a consequence of decrease in mobilized seed reserve due to low water uptake by the germinating seeds. Results of Yagmur and Kaydan [18] showed that priming with water and salt in addition to improved seed germination in salinity stress, increased seedling growth of primed seeds. Research of Afkari [14] indicated positive effects of priming on seedling dry weight.

There was no significant difference between FEP of primed seeds at the non-stress condition. At the 4 ds m^{-1} salinity, priming increased this trait significantly and there were significant differences between hydro-primed and primed seeds with 3g KNO3 l^{-1} in this trait. At the highest level of salinity stress, differences of FEP between un-primed, hydro-primed and primed with 3 and 6g KNO3 l^{-1} were significant (Table 4).

Mean Emergence Time (MET) of primed seeds in non-stress conditions and 4 ds m^{-1} salinity showed no significant differences, while a significant decrease was observed comparing primed seeds with control. At the highest level of salinity stress, MGT in both priming treatments with KNO3 were significantly less than un-primed and hydro-primed seeds (Table 4). Leaf area per plant (LA) obtained from primed seeds at non-stress showed a significant increase compared to non-primed seeds. At the 4 ds m^{-1}, LA from primed seeds with KNO3 was significantly higher than un-primed and hydro primed seeds (Table 4).

Mean plant height of cotton in priming treatment with 6 g KNO3 l^{-1} at 4 ds m^{-1}, in comparison with other treatments was significantly superior. At 8 ds m^{-1}, mean plant height between two priming treatments with KNO3 was not significant (Table 4).

Mohammadi [18] indicated that osmotic priming with KNO3 in comparison with control had maximum positive effect on leaf area and plant height. Also, Afkari [14] reported that priming with KNO3 enhanced height and number of leaves per plant than un-primed seeds of sunflower.

In non-stress, plant dry weight from hydro-primed seeds showed a significant difference compared with un-primed. But at the 4 and 8 ds m^{-1}, priming with KNO3 showed the most positive effect on this trait; meanwhile, priming with 6 g KNO3 l^{-1} was seen to be the best treatment. For instance, at the highest level of salinity

Table 4: Effects of priming treatments on cotton seed emergence and growth under salinity conditions in greenhouse (means comparision)

Priming	Salinity (dsm^{-1})	FEP (%)	MET (day)	LA (cm^{-2})	Plant height (cm)	Plant dry weight (g)
Control	0	83^{abc}	3.8^{cde}	232^{b}	56.3^{ab}	6.42^{b}
Hydro	0	91^{ab}	2.7^{h}	270^{a}	59.0^{a}	7.08^{a}
KNO3 3gl^{-1}	0	93^{a}	2.8^{gh}	263^{a}	52.3^{bc}	6.78^{ab}
KNO3 6gl^{-1}	0	92^{ab}	3.1^{fgh}	256^{a}	51.5^{bc}	6.73^{ab}
Control	4	55^{fg}	4.8^{b}	114^{ef}	34.0^{e}	4.00^{f}
Hydro	4	70^{de}	3.6^{def}	138^{d}	36.5^{de}	5.00^{d}
KNO3 3gl^{-1}	4	78^{cd}	3.3^{efg}	166^{c}	40.3^{d}	5.48^{c}
KNO3 6gl^{-1}	4	82^{bc}	3.5^{def}	182^{c}	46.8^{c}	5.60^{c}
Control	8	20^{i}	5.9^{a}	52^{h}	15.3^{h}	2.50^{h}
Hydro	8	32^{h}	4.9^{b}	81^{g}	19.0^{gh}	3.48^{g}
KNO3 3gl^{-1}	8	48^{g}	4.2^{c}	105^{f}	23.0^{fg}	4.53^{e}
KNO3 6gl^{-1}	8	61^{ef}	3.9^{cd}	133^{de}	28.0^{f}	4.95^{d}

FEP, final emergence percentage; MET, mean emergence time. LA, leaf area per plant. Similar letters at each column indicate the non-significant difference at 95 % confidence interval stress, priming with 6 g KNO3 l^{-1} in comparison with control increased plant dry weight by 98 %; while hydro-priming and priming with 3 g KNO3 l^{-1} increased this trait by only 39.2 and 81.2 %; respectively (Table 4).

CONCLUSION

Priming with salinity stress showed to have significant effects on evaluated traits of the study in both laboratorial and greenhouse experiments. Under the salinity stress, priming treatments in all traits showed significant superiority over non-primed seeds. At the 4 ds m^{-1} in greenhouse experiment, in traits of FEP, MET and plant height there was no significant difference between hydro-priming and priming with 3 g KNO3l^{-1}. But the difference between other traits in both experiments and also at the highest level of salinity stress were significantly lower than KNO3 treatments. Despite no significant deference between concentrations of KNO3 in most traits in stress levels of 0 and 4 ds m^{-1}; at the highest level of salinity stress, seed priming with 6 g KNO3 l^{-1}, FGP and FEP, plumule length, seedling and plant dry weight and LA had significant superiority over hydro-priming and priming with 3 g KNO3l^{-1}. Overall, the results showed that in sodium chloride induced salinity stress priming with KNO3 could lead to positive effects on seedling and enzymes production meaning more resistance to salinity. Moreover, it seems to us that at high salinity stress, priming with higher concentration of salt (less than seed toxicity, of course) could be an appropriate strategy to induce stress resistance in seeds and seedlings. Cakmak [24] suggested that the improvement of K-nutritional status of plants might be of great importance and crucial for the survival of crop plants under environmental stress conditions, such as drought, chilling and high light intensity. Afkari [14] reported that potassium content of primed seeds was relatively more than un-primed ones.

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