

## **The effect of seed priming by KNO<sub>3</sub> on bioactive compounds of germinated Rye (*secale cereale*) under salinity stress**

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**ABSTRACT:** Seed priming is one of the ways to reduce negative effects of salt which is used for increasing germination percentage and seed resistance increase in salty zones. In order to study the effect of pre treatment of rye seed (*secale cereale* L.) by KNO<sub>3</sub> on germination an experiment was conducted in triplet in factorial form and based on fully randomized plan in vitro. Factors include KNO<sub>3</sub> with density 2% and osmosis potential produced using NaCl 15 and 30 molar were composed the second factor. Germinated seeds were counted daily for eight days. Then the percent of germination, germination speed, the average daily germination, the length of the shoot, root and the weight of dry plant were measured. Regarding mean data resulted from the experiment the results showed that KNO<sub>3</sub> affects on the percent of germination, germination speed, the average daily germination, the weight of dry plant, the length of the shoot and the root on the level of one percent ( $p>0/01$ ) significantly. In sum the results showed that pre treatment seed by the KNO<sub>3</sub> can help the farmers in setting and producing suitable rye plant.

**Keywords:** KNO<sub>3</sub>, NaCl, seed priming, germination indices, rye

### **INTRODUCTION**

Germination is one of the most critical and sensitive stages of canola growth in facing salt stress affecting the settlement of germ and plant survival in saltiness soils. Hence, numerous attempts are made to develop policies for reducing the adverse effects of salt stress. Today, priming technique or seed preparation is one of the physiological methods to increase the germination percentage, seed efficiency, and preparation of speed and consistency of germination and deployment under saltiness conditions. Accordingly, rye seed preparation to detect its effects on traits including germination and salt stress tolerance increase in germination stage is of particular significance. Mansouri *et al* (1999) concluded that salt resistance is a complicated trait resulted from mutual effect of several physiological and morphological processes and leads to the production of strong cellular line while maintaining renewal trait finally transforming to reformed plant. Exterior use of ascorbic acid in *Nicotiana tabacum* in saltiness condition will significantly affect poisoning resulted from H<sub>2</sub>O<sub>2</sub> by enhancing the activity of catalase and peroxide (Khan & Gul, 2006). Studies by Moradshahi *et al* (2004) regarding the effect of salt stress on nine floral plants demonstrated that as salt stress increases, growth of the roots of all cultivars increases but aerial member of almost all cultivars decreases. Rye is one of the most important agricultural plants and increase in germination percent of the rye seed is an important factor in improving the plant (Kafi *et al* 2005). Although among the plants, rye is one of the best adjusted agricultural categories but amount of production and performance of this plant as other agricultural plants is hardly affected by environmental factors and constantly there is a concern that whether produced rye can be enough for growing population in the world (Satorr and Slafar 2001). Harris *et al* 2001 reported that priming the seed causes the powerful development, more rye branch fills the rye better increase the products and length of the branches of the rye. In India the effect of priming in decreasing the development duration caused that farmers can have 3 products in a year (Harris *et al* 2001). Karaki (1998) reported the increase

of the wet weight and the length of the shoot and the root of the rye and barley along with priming. Determining a suitable time of priming prevents a negative effect of it. Penalosa and Eira 1993 reported that the suitable time of priming prevents a negative effect of priming on the germination seed of tomato. Seed germination play an considerable role in determining the final density of the plant in the square unit so that the enough density of the plant in square unit will be achieved that cultivated seeds erupt completely with enough speed (Baalbaki et al 1990). The advantages of priming the seed is reported so that include increasing the resistance of the plant in the salty areas, Asada 1992, and under dry condition (Adams et al 1999), seed cultivation, (Benson et al 1998), increasing the performance of the seeds with low naming power, (Afzal et al 2004) and also increase the products (Dumet and Benson 2000). According to the studies, it has been specified that the role of increasing of  $KNO_3$  among the root attract cause appearing the root that it is affected by  $KNO_3$  and nitrogen fertilizer of the develop indicator and the amount of the leaf chlorophyll will be changed (Khan et al). Using  $KNO_3$  cause increase the nutrients from the soil and in result increase the performance in agricultural plant (Lone et al 1999). Treating the seeds with  $KNO_3$  was so easy. In addition increase the cropping indicator and the tank capacity (Khan et al 2001). Corn seed was prepared with  $KNO_3$  solution. The results showed that treating the seed with  $KNO_3$  before cultivating it, increase the characteristics of germination and final grows of the corn plant (Eradatmand Asli and Houshmand Far 2001). Ashrafi and Ramzjoo (2010) prepared three categories of Safflower in an experiment under hydro priming and asmo priming condition. Hydro priming improved germination, the amount of germination, balance of germination and amount of shoot to the root significantly but decreased the duration of attending to 50% germination. Ansari et al 1990 in an experiment of mash seed declared that  $KNO_3$  application increase access to high nutrients. By studying the effect of  $KNO_3$  on the plant, the critical role  $KNO_3$  was confirmed on the plants development and their resistance to the tension. In order to study the effect of  $KNO_3$  on rye rate an experiment was done and according to its results it was determined that its proficiency can be improved by drenching the seed in  $KNO_3$  solution so that  $KNO_3$  attend the seed on the suitable level of germination (Samiullah et al 1991). The end of this research is studying the effect of preparing the rye seeds by  $KNO_3$  on the germination power increase germination seed, the average of daily germination, the length of the root and the shoot, the weight of the dry plant.

## MATERIALS AND METHODS

To examine the effect on  $KNO_3$  on germination indices of rye seed in salt stress conditions it was studied in terms of full randomized blocks factorial plan in Arable Plants Physiology Laboratory, faculty of Agriculture of Saveh Azad University. For this reason, rye's cultivar *Secale montanum* was used, namely, first, 50 seeds were separated and disinfected for each Petri dish and in order to disinfect, the seeds were drenched in sodium hydro chloride 5% for 5 minutes then they were washed with water. Related seeds were put in the  $KNO_3$  solution for 8 hours in  $20^{\circ}$  in density of 2%. After this time the seeds were transferred to the sterilized pottery dish in which bottom there was a paper filter. The diagonal of all pottery dishes was 9 cm. Then, 10ml distilled water or chlorine and sodium solutions in concentration 15 and 30 molar and after that all of them were transferred to a germinator with  $25 \pm 1$  and duration of day light 16 hours and darkness was 8 hours. Light intensity was 1500 lux. Counting the germinated seeds was done daily in a specific time. In the time of counting, the seeds were considered germinated that the length of their roots was 2mm or more. Counting will be continued till the increase in the number of germinated seed won't be observed and the number of the seed in pottery dish will be fixed. According to the data, in order to calculate the percent and speed of germination, the following equation is used.

$$\text{Germination Percentage} = S/T \times 100$$

$$\text{Germination Speed} = N1/D1 + N2/D2 + \dots + Ni/Di$$

Where s is the number of germinated seeds, T is the total seeds and Ni the number of germinated seed in day Di.

$$\text{The average of daily germination (MDG)} = \sum Cpsgt/T$$

Where Cpsgt is the percent of the germinated seed during the period and T is the total germination period. In order to achieve the length of the shoot and the root, 1ml ruler was used then the dry plant was measured. All samples were dried in an oven at  $70^{\circ}C$  for 72 h, and then weighed for dry matter.

## RESULTS AND DISCUSSION

Results showed that  $KNO_3$  has significant effect on germination percent, germination speed, the average of daily germination, dry weight of plant, the length of the shoot and the root on the one percent probable level ( $p > 0/01$ ). Also NaCl has significant effect on germination speed, dry weight of plant, the length of the shoot and the root, germination percent and the average of daily germination on the one percent probable level ( $p > 0/01$ ).

Interaction of KNO<sub>3</sub> to NaCl has significant effect on germination percent, germination speed, the average of daily germination, dry weight of plant, the length of the shoot and the root on the one percent probable level (p>0/01). The results of the average of comparison effect of different level of KNO<sub>3</sub> (table 1) showed that increase of the KNO<sub>3</sub> improve the situation of germination components related to witness. The most germination indicator is related to the KNO<sub>3</sub>.

Table 1. comparing the average effect of different level of KNO<sub>3</sub> on the eruptive and biochemical indicators of the rye.

KNO <sub>3</sub>	Germination percent	Germination speed	Average daily germination	Dry weight of the plant	Shoot length	Root length
0	98.5 <sup>b</sup>	27.71 <sup>d</sup>	19.7 <sup>b</sup>	0.315 <sup>c</sup>	4.93 <sup>c</sup>	3.14 <sup>d</sup>
2%	99.3 <sup>ab</sup>	35.19 <sup>b</sup>	19.86 <sup>ab</sup>	0.513 <sup>b</sup>	6.89 <sup>b</sup>	5.06 <sup>b</sup>

The same letters in every column don't have significant statistical difference.

The results of the averages of the KNO<sub>3</sub> (table 1) showed that the increase of KNO<sub>3</sub> improves the situation of germination components related to the witness. KNO<sub>3</sub> doesn't have much effect on the germination percent and the average of daily germination. The most germination speed, dry weight, length of the shoot and the root is related to KNO<sub>3</sub> 2% treatment.

Table 2. Comparison the average effect of different level of KNO<sub>3</sub> on the eruptive and biochemical indicators of the rye.

NaCl	Germination percent	Germination speed	Average daily germination	Dry weight of plant	Shoot length	Root length
0	97.17 <sup>a</sup>	29.34 <sup>d</sup>	19.43 <sup>a</sup>	0.21 <sup>d</sup>	3.98 <sup>d</sup>	2.7 <sup>d</sup>
15	98 <sup>a</sup>	34.74 <sup>c</sup>	19.6 <sup>a</sup>	0.37 <sup>c</sup>	5.58 <sup>c</sup>	4.21 <sup>c</sup>
30	98.17 <sup>a</sup>	36.16 <sup>b</sup>	19.63 <sup>a</sup>	0.58 <sup>b</sup>	7.98 <sup>b</sup>	5.48 <sup>b</sup>

The same letters in every column don't have significant statistical difference

Studying the effect of NaCl on germination percentage shows that as salt level increases, germination percentage decreases resulted from the disruption in ionic effect, osmosis effect and also nutritional imbalance emanated from the presence of chlorine and sodium ions; namely, the plant consumes more energy to gain a certain amount of water. As a result, a part of energy the plant requires to grow is consumed to gain water. Another possibility for proving the matter can be mentioned so that the increase of the concentrations of chlorine and sodium in external environments can decrease ionic activity and bring about the reduction of large Na/Ca, Na/K, and Na/Mg ratios, but for primed seeds (cause of the presence of ascorbic acid as a small water soluble antioxidant and strong reducer in disinfecting oxygen reactive species and in particular H<sub>2</sub>O<sub>2</sub>) itself is oxidized and reduces superoxide compounds; for instance, in these seeds, some proteins and carbohydrates are decomposed as a result of hydrolyzing enzymes and reactions and ready for taking part in the germination process. The issue can be an explanation for acceleration of germination and reduction of mean germination time which the results are in accordance with the results of Bikort et al (2005), Neuman and Shalta (2001), and Dowlatabadian et al (2008). Results from the mean comparisons of different levels of ascorbic acid showed that the acid increase leads to the increase of seed resistance against salt stress and improvement in the status of germination components' growth

Table 3. comparing the average interaction of the KNO<sub>3</sub> to NaCl on the studied adjectives.

Treatment		Adjective average					
NaCl	KNO <sub>3</sub>	Germination percent	Germination speed	Average daily germination	Dry weight of the plant	Shoot length	Root length
0	0	95.6 <sup>d</sup>	25.44 <sup>k</sup>	19.13 <sup>d</sup>	0.07 <sup>o</sup>	2.53 <sup>k</sup>	1.86 <sup>i</sup>
0	2%	98 <sup>bc</sup>	27.03 <sup>j</sup>	19.6 <sup>bc</sup>	0.17 <sup>n</sup>	4.71 <sup>l</sup>	2.4 <sup>k</sup>
15	0	100 <sup>a</sup>	36.66 <sup>e</sup>	20 <sup>a</sup>	0.61 <sup>c</sup>	8.34 <sup>d</sup>	6 <sup>d</sup>
15	2%	100 <sup>a</sup>	43.16 <sup>b</sup>	20 <sup>a</sup>	0.8 <sup>b</sup>	9.99 <sup>b</sup>	7.05 <sup>b</sup>
30	0	100 <sup>a</sup>	44.22 <sup>a</sup>	20 <sup>a</sup>	0.89 <sup>a</sup>	10.96 <sup>a</sup>	8.38 <sup>a</sup>
30	2%	89.3 <sup>f</sup>	33.77 <sup>f</sup>	17.87 <sup>f</sup>	0.76 <sup>c</sup>	9.12 <sup>c</sup>	6.42 <sup>e</sup>

The average in which a column at least has one common letter with Donken test on the level of 5% is in the similar statistical group.

The results of comparing the interaction effects of different levels of KNO<sub>3</sub> (table 3) showed that level of KNO<sub>3</sub> 2% with NaCl 15 molar has the most germination speed, dry weight of the plant, the length of the shoot and the root. Also the most germination percent and the amount of daily germination related to the lack of KNO<sub>3</sub> existence

and NaCl 30 molar.  $\text{KNO}_3$  can act as a new type of plant antioxidants and also are involved in a wide range of biochemical reactions, including the metabolism of glycogen and amino acid synthesis and nucleic and the synthesis and metabolism of hemoglobin, also this material in synthesis is involved, sphingomilin and other sphene lipids neurotransmitters. 5-phosphate  $\text{KNO}_3$  is involved in acid metabolism of gamma - amino butyric. The reason of the increasing the germination in  $\text{KNO}_3$  application is for stimulating the respiratory inhibitor. The similar results were reported (Khan et al 1995). The probably, the reason of increasing the length of the root and the shoot is  $\text{KNO}_3$  application because of root and shoot system development by using this material that increase the nutrients attraction of in result increase the performance in agricultural plants. The similar results were reported by (Farokhi, Eradatmand, 2007), (Khan et al, 1995), (Lone et al, 1999) and (Samiullah, 1991). Also (Chen and Xiong, 2005) by studying the effect of  $\text{KNO}_3$  on plants, confirmed the vital role of  $\text{KNO}_3$  on the development of the plants. Based on the research done by (Khan et al 1995) and the increasing role of  $\text{KNO}_3$  in the amount of root drawing, cause to appear the leaf soon. It changes the ability of photosynthesis and natural attraction rate NAR. Based on the research of treating the seed with  $\text{KNO}_3$  will have nitrogen attraction increase and phosphor in Safflower plan, vetch and lentil (Smiullah *et al*, 1992), rye (Khan *et al*, 1996) and canola (Khan *et al*, 1995) and (Smiullah *et al* 1991) (Chojnowski *et al* 1997) reported that priming the seed of the sunflower for 3 to 5 days increase the germination speed and improve the plant development. They also declare that the reason of this reaction in respiratory activity is producing ATP, stimulating the activity of RNA and making protein in the primed seeds. Probably the reason of decreasing the germination percent because of eliminates the free radicals directly, which reduces the damage caused by reactive species, so membrane lipid per oxidation will be decreased. Similar results on the other materials have been reported by (Yasar *et al*, 2008), (Dolatabadian *et al*, 2009) and (Burguieres *et al*, 2006).

## CONCLUSION

According to the results in this experiment and the different level of treating the chemical  $\text{KNO}_3$  and NaCl of this matter we can conclude that probably,  $\text{KNO}_3$  by increasing the root development and raising the ability of nutrient attraction by the plant represents this possibility in order to use the potential of the water and nutrient in the soil. The results of this research showed that seed treatment with  $\text{KNO}_3$  can be as an economic simple way and also be effective on increasing the plant output.

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