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RICE SEED PRIMING TO OVERCOME SALT STRESS CONDITIONS

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ABSTRACT

This study investigated the potential of seed priming for induction of salt tolerance in rice. Two lots of rice seeds (high and low vigor) cv Sakha 106 were primed in aerated solutions of KNO_3 2%, $CaCl_2$ 2%, PEG 2%, ascorbic acid 100 ppm and control (distill water) each for 24 h. Two experiments were conducted in laboratory and pots. The primed seeds were planted in (0, 3000, 4500, 6000 and7500 ppm NaCl) at Seed Technology Research Unit, Mansoura. Seed Technology Research Department, ARC. The results revealed that priming with KNO_3 2% followed by control, $CaCl_2$ 2% , ascorbic acid 100 ppm, and PEG 2% were more effective in inducing salt tolerance owing to improved seed germination, seedling vigor index, speed of germination, rate of germination and seedling emergence in pots experiment in 0 and 3000 ppm NaCl. Freshly harvested seed recorded higher germination percentage (80%). It is therefore recommended that KNO_3 could be adopted for priming rice seed under 3000 ppm as it maintained seed viability and seedling vigor optimally among other treatments. Reduced germination parameters due to increasing salinity levels up to 7500 ppm. The results showed that application of KNO_3 or $CaCl_2$ at 2% improved all studied seed quality traits under salinity stress conditions. Thus, results signify the role of priming substances and high vigor in regulating salinity response of pre-treated seed could be used as a potential growth regulator, for improving common seedlings growth under salinity stress conditions.

Keywords: Rice (*Oryza sativa* L.), seed priming, seedling growth, salinity and seed storage.

INTRODUCTION

At the current growth rate of the world population, rice requirement dramatically and many nations are facing second generation challenge of producing more rice at less cost in a deteriorating environment (Tiwari *et al*., 2011). Egypt is the largest rice producer in the region and rice yield is one of the highest in the world. On the other side, (Ghassemi *et al.,*1995) reported that, of the 130 million hectares of land where rice is grown, about 30 percent contain levels of salt too high to allow normal rice yield. About 7% of the world's total land area is affected by salt. The salinity threshold level of rice is 3.0 dS m^{-1} with 12% yield reduction per unit increase in EC. (dS m^{-1}) above this level (Maas and Hoffman, 1977). Certified rice (Sakha 106) seeds not always able to market during the following planting season so, these seeds (carry over) are store for marketing during the second season after harvest. Prolonging storage period can lead to a gradual loss of vigor and finally a loss of viability (Moon *et al.,* 2011). Sowing carry over seed under soil salinity conditions have harmful effects in seedling vigor while, salt and osmotic stress are responsible for both inhibition or delayed seed germination and seedlings establishment (Almansouri *et al*., 2001). Also salinity stress affected many physiological processes including photosynthesis, respiration, translocation, ion uptake, nutrient metabolisms, biosynthesis of proteins, carbohydrates and growth promoters (Sadeghipour and Aghaei., 2012). Also, Yeo *et al.,* (1990) reported that, rice is sensitive to salinity at the seedlings stage and becomes tolerant at the vegetative phase and very susceptible at reproductive phase in terms of grain yield. The successful establishment of crop mainly depends upon good quality seed. Highly vigorous seeds germinate rapidly, uniformly and are able to withstand environmental adversity after sowing (Del Gi dice., 1996). Delays in germination and low seed viability are among the serious problems limiting the production of rice. However, the use of rice seeds of low physiological quality, leading to inadequate plant population in the field. To provide higher quality seeds, scientists have developed new technologies called "Seed Enhancement Techniques". The main objective of these techniques is to optimize the application of seed treatment products by improving the technical quality of seeds. The two important enhancement technologies are seed coating and seed priming that have been employed successfully for many crops. This enhancement technology is the key interface between the two highly specialized industries viz., crop protection and the seed industry.

Seed priming is widely used nowadays for betterment of seed performance in terms of higher rate of germination and uniformity of establishment (Farooq *et al*., 2009). Also a reduction in emergence time, accomplishment of uniform emergence and betterment of crop stand in many horticultural crops are known to be achieved by seed invigoration techniques like seed priming Ashraf and Foolad (2005). Osmopriming and hormonal priming are terminologies used in describing soaking of seeds in aerated low-water potential solutions to control water uptake and prevent radical protrusion (Bray 1995).When these treatments are followed by dehydration, they become more effective in improving germination in different types of vegetable seeds most especially when the environmental conditions are at sub-optimal levels (Bradford and Haigh 1994). Furthermore, the use of calcium chloride, potassium nitrate, sodium chloride and polyethylene glycol 8000 have been proved to lessen mean germination time (Ruan *et al.,* 2002). A rice farmer or producer should be able to know the best priming solution that is equally cost effective to meet his target of early seedling establishment and high profit through yield increase at the end of production period. Therefore, the primary objectives to assess whether seed priming treatment can alleviate the adverse effects of salinity of rice lots (High and Low vigor). Also study the effects of different priming treatments on seed germination and subsequent seedling growth under normal and stress conditions.

MATERIALS AND METHODS

This experiment was conducted at Laboratories of Seed Technology Research Unit, Mansoura, Seed Technology Research Department, Field Crops Research Institute, Agricultural Research Center. To study the role of seed priming treatments in alleviate the adverse effects of soil salinity on rice seed germination, seed and seedling vigor. Improvement seed and seedling vigor of carry over rice seed. Seed samples of rice lots (c.v Sakha 106) were obtained after harvest from Central Administer of Seed (CAS) in June, 2013 for old seed (carry over seed) and 2014 for fresh seed obtained seed was cleaned from remains, dust, damaged seed and any inert materials then immersed in 5% NaOCl (sodium hypochlorite solution) for 5 minutes to avoid fungal invasion. The initial seed germination was determined. Rice lots were subjected to seed priming in distilled water, potassium nitrate 2%, calcium chloride 2%, polyethylene glycol 6000 2% and ascorbic acid 100 ppm for 24 hours and dried back to the same weight and planting under salt stress conditions 3000, 4500, 6000 and 7500 ppm NaCl in addition to control (without salt stress).

Seed lots: 1- High vigor seed 2- Low vigor seed

Priming treatments: KNO_3 2%, $CaCl_2$ 2%, PEG 6000 2%, Ascorbic acid 100 ppm and control).

Salinity treatments: Solutions of NaCl were prepared at concentrations of (0 control, 3000, 4500, 6000 and 7500 ppm).

Germination tests:

Sand that had passed through the screen of 2mm diameter mesh was washed and dried at 130 ^{o}C for 4 h. Fifty seeds were placed in plastic germination box (13X19X12 cm) containing sand, each 100 g sand was mixed with 15.6 mL corresponding salinity solutions. Then the seeds were incubated in a cultivation cabinet at 25 ^{o}C under alternating cycle of 12 h light and 12 h darkness for 14 days according to ISTA., 1999. Three replicates for each treatment were used.

Germination parameters:

The number of germinated seeds, based on which germination parameters were calculated, was recorded every day for 14 days. Germination energy and germination percentage were calculated as follows:

1-Germination percentage (%): It was defined as the total number of normal seedlings at the end of the test after 14 days according to (ISTA. 1999).

2-Germination energy (GE): It was recorded on the fifth day after planting. It is the percentage of germinated seeds 5 days after planting relative to the total number of seeds tested (Ruan *et al., 2002*).

3-Speed of germination index (SGI): It was calculated as described in the Association of Official Seed Analysis (AOSA., 1992) by the following formula:

$$\text{SGI} = \frac{\textit{No of germinated seed}}{\textit{Days of first count}} + \frac{\dots\dots\dots}{\dots\dots..} + \frac{\text{No of germinated seed}}{\textit{Days of final count}}$$

4-Germination rate (GR): It was defined according to the following formula of (Bartllet., 1937).

$$GR = \frac{a + (a + b) + (a + b + c) + \cdots \ldots \ldots \ldots \ldots (a + b + c + m)}{n(a + b + c + m)}$$

Where a, b, c are number of seedlings in the first count, second count and third count, n is the number of counts.

5-Mean germination time (MGT): It was determined according to the following equation which is described by Alvardo and Bradford (1987):

$$MGT = \sum dn / \sum n$$

Where (n) is the number of seeds which is germinated on day (d), and (d) is the number of days counted from the beginning of germination.

6-Seedling length (cm): It was recorded by the average of random ten seedlings at the end of germination test.

7-Seedling dry weight (gm): Ten normal seedlings 14 days after planting, the seedlings were dried in hot-air oven at 85^{o}C for 12 hours to obtain the seedlings dry weight according to Krishnasamy and Seshu (1990).

8-Seedling vigor index (SVI): It was calculated according to the equation described by Abdul-Baki *et al.*, (1973):

$$SVI_1 = G\% \text{ X Seedling length.}$$
$$SVI_2 = G\% \text{ X SDW (seedling dry weight).}$$

9-Pots experiment: This experiment was carried out in pots under natural conditions. Seeds were sown in rows at the depth of 3 cm and replicated three times. Irrigation was applied whenever required. The data regarding emergence, were recorded up to the 14th day of sowing.

Statistical analysis:

The observed data were statistically analyzed as the technique of analysis of variance (ANOVA) of the randomized complete block design of three factors in three replicates as mentioned by (Gomez and Gomez., 1984). The means were compared using the least significant differences (L.S.D). Statistical analysis was performed using analysis of variance technique (ANOVA) by means of "MSTAT-C"computer software package.

RESULTS AND DISCUSSION

1-Seed lot effects:

The results in Tables (1and 2) showed the main effect of the two lots (high and low vigor) tested and the salinity levels of NaCl and overcome substances on seed and seedling growth parameters of rice seeds in twice times during 2014season.

Table 1: Effect of seed lot, priming substances and salinity levels on germination percentage, root length, shoot length, seedling length, seedling dry weight and seedling vigor index.

Treatments	Germination %	Germination energy	Speed of germination	Germination rate	Mean germination time
A.Seed lot					
High vigor	80.0	71	6.0	0.61	4.5
Low vigor	50.0	37	2.0	0.56	4.7
F. test	**	**	**	**	**
B.Priming substances					
Control	68.0	54	3.8	0.54	4.9
KNO3 2%	67.5	59	4.2	0.59	4.5
$CaCl_2$	59.5	55	3.9	0.57	4.6
PEG 2%	57.0	54	3.8	0.55	4.7
Ascorbic acid 100 ppm	46.5	54	3.8	0.54	4.7
F. test	**	NS	NS	NS	NS
L.S.D at 5 %	13.4	-	-	-	-
C.Salt levels					
Control	60.0	59	4.2	0.60	4.6
3000 ppm	62.5	58	4.1	0.59	4.6
4500 ppm	58.5	54	4.0	0.58	4.6
6000 ppm	58.0	52	3.7	0.58	4.6
7500 ppm	59.5	47	3.3	0.58	4.7
F. test	NS	**	**	NS	NS
L.S.D at 5 %	-	0.052	0.394	-	-
D. Interactions					
A X BX C	NS	NS	NS	NS	NS

The results exhibited significant differences between the two lots. The freshly harvested seed recorded the highest mean value of germination percentage, germination energy, speed of germination, germination rate, mean germination time, seedling length, seedling dry weight, seedling vigor index, and pot experiment of rice seeds in comparison to old harvested seed. This reduction in low vigor might be due to lost viability through a sequence of physiological manifestation, such as reduced enzymatic activities, increased permeability of cellular membranes, slower rates of cellular repair slower cell division and radical protrusion reduced respiration rates and altered pathways denaturation of macromolecules etc. These results are in the same line with those recorded by Hong Fashui (2002), Hanegave (2009) and Shi-yang Zhu *et al* (2010).

Table 2: Effect of seed lot, priming substances and salinity levels on seedling vigor index, germination energy, speed of germination, germination rate, mean germination time and pot experiment.

Treatments	Seedling Length (cm)	Seedling dry Weight (gm.)	Seedling vigor index1	Seedling vigor index 2	Pot experiment
A.Seed lot					
High vigor	6.60	0.216	538	13.1	61
Low vigor	4.50	0.167	180	10.0	40
F. test	**	**	**	**	**
B.Priming substances					
Control	5.81	0.201	381	12.3	51
KNO3 2%	5.71	0.178	388	12.4	55
$CaCl_2$	5.54	0.205	361	11.9	53
PEG 2%	5.23	0.201	333	10.5	51
Ascorbic acid 100 ppm	5.59	0.173	331	10.0	50
F. test	NS	**	NS	NS	NS
L.S.D at 5 %	-	0.019	-	-	-
C.Salt levels					
Control	7.49	0.212	558	12.3	58
3000 ppm	5.57	0.202	405	12.4	56
4500 ppm	4.92	0.193	324	11.9	54
6000 ppm	4.91	0.175	283	10.0	45
7500 ppm	4.99	0.176	225	10.5	38
F. test	**	**	**	NS	**
L.S.D at 5 %	0.98	0.019	61.33	-	6.7
D.Interactions					
A X BX C	NS	NS	NS	NS	NS

2-Priming substances effects:

Highly significant differences were detected among the priming substances regarding to germination percentage, germination energy, speed of germination, germination rate, mean germination time, seedling length, seedling dry weight, seedling vigor index, and pot experiment Tables (1 and 2). KNO3 gave the highest values in all growth parameters; however ascorbic acid gave the lowest values in all growth parameters in both times. The superiority of KNO3 may be due to its role in influencing the permeability of the membranes which ultimately leads to activation of enzymes involved in protein synthesis and carbohydrate metabolism. Similar data were obtained by Faroq *et al* (2007) and Shehzad *et al* (2012).

3-Salinity levels effect:

With respect to the main effect of the different salinity levels the 3000 ppm NaCl recorded the high value of seedling growth and pot experiment on growth parameters, in relation to the control treatment (distill water).The present results are in accordance with observation of Basra *et al.,* 2005 who suggested that seed germination and vigor in rice increased with osmoconditioning. However, osmopriming has been shown to activate processes related to germination, for instance, by affecting the oxidative metabolic such as increasing superoxide dismutase and peroxidase.

4-Interactions effects:

Data presented in Table 3 show that, the interaction between rice seed lot and salinity levels had highly significant effect on germination percentage. The highest germination percentage was produced by high vigor seed lot when primed with water before planting at soil salinity level 3000 ppm NaCl. On the other hand, the lowest values of germination percentage produced from low vigor seed lot when planted at salinity level, 7500 ppm NaCl. At the increasing salinity levels high vigor seed lot gave the lowest reduction in germination percentage 34% and for low vigor seed lot 59%, these results indicated that high vigor seed lot more tolerant than low vigor seed lot for increasing salinity levels. The reduction of rice seed growth parameters as a result of increasing salinity levels up to 7500 ppm may be due to that salinity is one of the major abiotic stresses which adversely affect seed growth and development. Generally, rice seeds are highly sensitive to salinity stress. These data are in accordance with those reported by Labidi *et al.,* 2009, Irfan *et al* (2012) and Yousef (2013).

Table 3: The interaction between seed lots and salinity levels treatments on germination percentage.

Salinity levels / Seed lot	Control	3000 ppm NaCl	4500 ppm NaCl	6000 ppm NaCl	7500 ppmNaCl
High vigor	93	90	81	71	59
Low vigor	43	45	38	43	34
F test	**				
LSD at 0.05	10.17				

Also, rice seed germination percentage (Fig 1) show affected by the interaction and increased with 3000 ppm NaCl and reached its highest percentage (80%) comparing to control (70%).

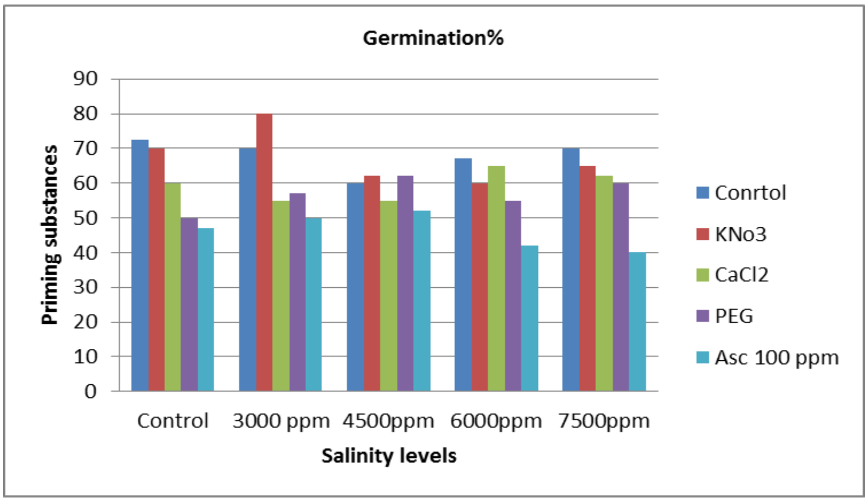

Fig 1: Effects of salinity levels and priming substances on germination percentage

The interaction between salinity levels and priming substances treatments (Fig 2) reflected significant differences on pots experiment. The zero NaCl with KNO3 gave the highest mean values followed by 4500 ppm NaCl with PEG. This positive effect may due to primed seed had better efficiency for water absorption from growing media, that is why metabolic activities in seed during germination process commence much earlier than radicle and plumule appearance. These data are in accordance with those reported by Yousef (2013).

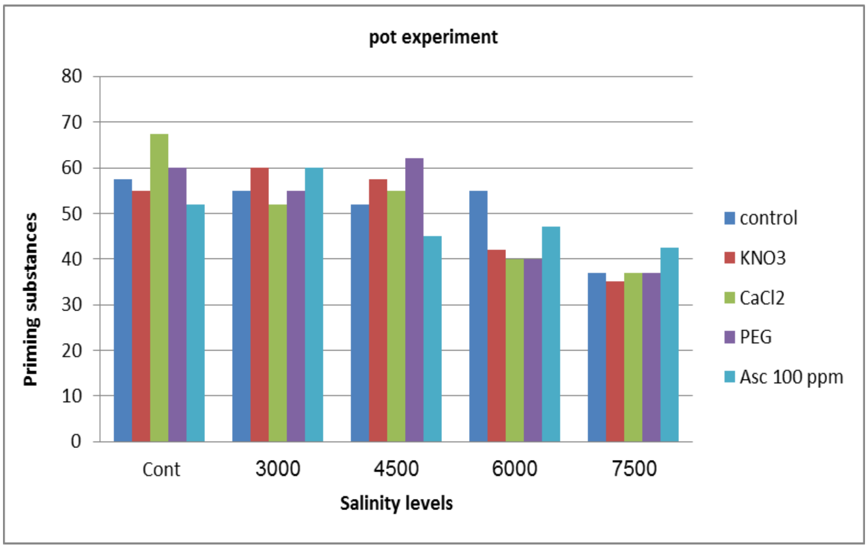

Fig 2: Effects of salinity levels and priming substances on pots experiment.

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تهيئة تقاوى الأرز للتغلب على ظروف الإجهاد الملحى

عبدالمجيد محمد سعد كشك و محمد رضا عبدالسميع الموافى
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أجريت هذه الدراسة خلال عام 2014 فى معامل بحوث تكنولوجيا البذور- معهد بحوث المحاصيل الحقلية - مركز البحوث الزراعية حيث تم تنفيذ التجارب فى تصميم التام العشوائية فى ثلاثة مكررات لتقييم تهيئة البذور للإنبات تحت ظروف الملوحة وعلاقتها بنسبة الانبات والتكشف الحقلى للوقوف على مشاكل إنخفاض حيوية تقاوى الأرز ووضع حلول مناسبة لها وذلك في لوطيين تقاوي مختلفين في الحيوية (بذور حديثة الحصاد وبذور معرضة للإجهاد الطبيعي) من تقاوى صنف سخا 106 ويمكن تلخيص اهم النتائج المتحصل عليها كالتالى:

١-أوضحت النتائج وجود فروق معنوية بين لوطات التقاوى الحديثة الحصاد (عالية الحيوية) والتقاوى المخزنة (منخفضة الحيوية) . سجلت التقاوى حديثة الحصاد اعلى نسبة انبات (80 %).

٢-أظهرت النتائج وجود فروق معنوية بين التهيئة بنترات البوتاسيوم 2% تلاها المعاملة بالماء تلاها المعاملة بكلوريد الكالسيوم 2% تلاها حمض الاسكوربيك 100 جزء فى المليون تلاها المعاملة بالبولى ايثلين جليكول 2% فى مساعدة التقاوى على تحمل الملوحة وتحسين نسب الانبات ومعدا الانبات وسرعة الانبات والانبات الحقلى فى المعاملة بالماء و 3000 جزء فى المليون كلوريد صوديوم .

٣- أشارت النتائج الى ان التقاوى حديثة الحصاد اعلى نسبة انبات (80 %) والمعاملة بنترات ةالبوتاسيوم (76%) بينما لمستويات الملوحة كانت اعلى نسبة انبات لمستو 3000 جزء فى المليون بعد الكنترول.

توصى الدراسة باستخدام التقاوى حديثة الحصاد واستخدام التهيئة الاسموزية لتقاوى الارز صنف سخا ١٠٦ بواسطة نترات البوتاسيوم بتركيز ٢% لمدة ٢٤ ساعة عند الزراعة فى ظروف اجهاد ملحى,لما له من تحفيز مقامة التقاوى ومن ثم حيوية البادرة لتخفيف الاثار الضارة للملوحة.