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EFFECT OF RICE SEED PRIMING WITH CALCIUM CHLORIDE ($CaCl_2$) ON GERMINATION AND SEEDLINGS VIGOR UNDER SALINITY STRESS

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ABSTRACT

The successful establishment of crop mainly depends on good quality seed which germinate rapidly, uniformly and able to withstand under environmental adversity after sowing. Laboratory experiment was conducted at Seed Technology Unit, Mansoura, Egypt to evaluate the effect of rice seed priming (c.v. Giza 177) with $CaCl_2$ at osmotic potentials (- 0.75, -1.00 , -1.25 , -1.50 MPa and distilled water) for 6 , 12, 24 , 36 and 48 hours on seed germination, seed and seedling vigor under normal and salinity stress. The obtained results of this study indicated that salinity levels (9 dS/m) delayed germination, seed and seedling vigor characters compared with normal salinity (0.3 dS/m). Rice seed priming with $CaCl_2$ at (-1.00 MPa) followed by (-1.25 MPa) gave the highest values of germination percentage, speed of germination index, germination rate, germination co-efficient, germination energy %,seedlings length, seedlings dry weight, water uptake % and decreasing mean germination time as well as time to 50 % germination compared with control (distilled water) under salinity stress. Whereas, priming seed with distilled water (control) resulted in the lowest germination characters under salinity stress. Priming duration 24 h showed its superiority in improving germination characters comparing with other priming durations. It could be concluded that, priming rice seed (c.v. Giza 177) with osmotic potential (-1.00 MPa) for 24 h can reduce the injurious effects of salinity stress.

INTRODUCTION

Rice (*Oryza sativa*, L) is one of the most important cereal crops of the world. 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 (*Oryza sativa* L.) 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).Salt and osmotic stresses are responsible for both inhibition or delayed seed germination and seedlings establishment (Almansouri *et al.*, 2001) . 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 the reproductive phase in terms of grain yield.

Delay in germination and germination rate consequently seedlings establishment in the field are the main problems in direct seeded method especially at the adverse environmental conditions like salinity stress. (Du and Toung, 2002) reported that, poor seedling establishment is one of the major yield limiting constraints both in transplanted and direct seeded rice especially under stressful conditions.

So, using high quality seed resulted in good establishment seedlings which have rapid emergence and tolerate the adverse conditions, (Harris *et al.*, 1999) reported that, seed priming is one of the most important developments to help rapid and uniform germination and emergence of seeds and to increase seed tolerance to adverse environmental conditions. Seed priming is a technique in which seeds are partially hydrated until the germination process begins, but radical emergence does not occur. Priming allows the metabolic processes necessary for germination to occur without actual germination. In priming, seeds are soaked in different solutions of sugars, polyethylene glycol (PEG), glycerol and $CaCl_2$ with high osmotic potentials. These prevent the seeds from absorbing enough water for radical protrusion, thus suspending the seeds in the lag phase (Taylor *et al.*, 1998). The low water potential of the treatment solution allows partial seed hydration so that pre germination metabolic processes begin but germination is inhibited. When the primed seed are planted in the field, they usually exhibit rapid and uniform germination. Osmohardening of rice seed with $CaCl_2$ at $\psi s = -1.25$ MPa for 24 hrs gave the highest germination percentage and lowest days to 50 % germinated compared with control (Farooq *et al.*, 2007). Low Ca concentration in the soil as well as in the seed result in greatly reduced germination and seedlings survival (Adams & Hartzog, 1991). Poor germination and seedlings survival were observed in soils with Ca levels below 21 mg kg-1 in the upper 15 cm of the soil profile (Gascho and Davis ,1994). Osmohardening of rice seed with $CaCl_2$ at 22.2 g/L (-1.25 MPa) at 27 °C for 24 h recorded the highest final emergence percentage, root/shoot, seedlings dry weight and seedlings fresh weight compared with control (Basra *et al.*, 2004). Yari, *et al.*,(2012) reported that seed primed duration of 24h with $CaCl_2$ was suitable for all rice cultivars.

The purpose of this investigation was to study the effect of using seed priming technique with $CaCl_2$ for enhancement rice seed quality, also study the role of priming rice seed with $CaCl_2$ in increasing seed tolerance to saline stress.

MATERIALS AND METHODS

This Study was carried out at the Laboratory of Seed Technology Research Unit, Mansoura, Egypt, to study the effect of priming rice seed with Calcium Chloride solution for different periods under salinity stress. Rice seed (c.v. Giza 177) were obtained from Central Administer of Seed (CAS) and immersed in 5 % NaOCl (sodium hypochloride solution) for 5 min. to avoid fungal invasion. The studied factors were:-

1-Priming treatments:- Rice seeds were soaked in aerated $CaCl_2$ solutions with a osmotic potentials of (distilled water , -0.75 , -1.00 , -1.25 and -1.5 MPa) according to Michel and Kaufmann (1973).

2-Priming time:- Rice seed were soaked in $CaCl_2$ solution for (6, 12, 24, 36 and 48 hrs), after that the primed seed were dried in open air and its moisture contents were determined and it arranged between 14 ±2 .

3-Salinity levels:- The primed seeds were planted at salinity level (0.3 dS/ m) from tab water (control) and salinity stress (9 dS/m) , by dissolving Rashid salt in water, dS/m is deci-siemens per meter .

Germination tests: Germination percentage was performed according to ISTA, 1999, while 400 seeds of rice were sown in four replicates at 25° C±2 in sterilized Perti dishes (15 × 1.5 cm) covered at the bottom with two sheets of Whatman filter paper that had been autoclaved and germination was performed daily to study the following characters:-

1- Water uptake percentage: water uptake percent was calculated by the formula (Rahman *et al*., 2008).

$$\%\ \text{water uptake} = \frac{W2-W1}{W1}$$

W1 = Initial weight of seed.
W2 = Weight of seed after absorbing water in a particular time.

2- Germination percentage defined as the total number of normal seedlings at the end of the test after fourteen days.

3- Speed germination index (SGI): It was calculated as described in the Association of Official Seed Analysis (AOSA, 1983) by following formula:

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

Seeds were considered germinated when the radicle was at least 2 mm. long.

4- Germination energy (GE): It was recorded on the 4th day after planting. It is the percentage of germinated seeds 4 days after planting relative to the total number of seeds tested (Ruan *et al*., 2002).

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

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

Where a, b, c are No. of seedlings in the first, second and third count, n is the number of counts.

6- Co-efficient of germination (CG): It was calculated using the following formula (Copeland 1976).

$$\text{Co-efficient of germination} = \frac{100(A1 + A2 + \ldots\ldots An)}{A1T1 + A2T2 + \ldots\ldots An\,Tn}$$

Where ,

A = Number of seed germinated .
T = Time (days) corresponding to A.
n = No. of days to final count.

7- Mean germination time (MGT): It was calculated based on the following equation of Ellis and Roberts (1981).

$$MGT = \frac{\Sigma Dn}{\Sigma n}$$

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

8- The time to get 50 % germination: (T 50 %) was calculated according to the following formula of Coolbear *et al.*,(1984).

$$T\ 50\ \% = ti + \frac{(N/2 - ni)(tj - ti)}{nj - ni}$$

Where N is the final number of germination and ni, nj cumulative number of seeds germinated by adjacent counts at times ti and ti when ni < N/2 < nj.

9- Seedlings length (cm) : It was measured of ten normal seedling 14 days after planting.

10- Seedlings dry weight (gm): Ten normal seedlings 14 days after planting, the seedlings were dried in hot-air oven at 85° C for 12 hours to obtain the seedlings dry weight (g),according to Krishnasamy and Seshu (1990).

All obtained data of characters were subjected to the statistical analysis according to the technique of analysis of variance (ANOVA) of completely randomized design, as described by Gomez and Gomez (1984).

RESULTS

Presented data in (Fig. 1 a, b and c), show the effect of interaction between osmotic potentials and salinity conditions on rice seed germination, speed of germination and germination energy % was significant. Under normal salinity level, germination percentage of the primed rice seed did not affected significantly and ranged between 90 and 92%. Whereas, with sowing unprimed rice seed under salinity level (9.0 dS/m) germination percentage decreased to 72 % and it enhanced to 82% with priming at osmotic potential (-1.00 MPa). Speed of germination index and germination energy under normal saline reached maximum values with priming by $CaCl_2$ at osmotic potential (-1.00 MPa). On contrast sowing rice seed under salinity level (9dS/m) resulted in decreasing both of speed of germination index and germination energy but these traits were enhanced with priming by $CaCl_2$ at osmotic potential (-1.00 MPa) comparing unprimed rice seed.

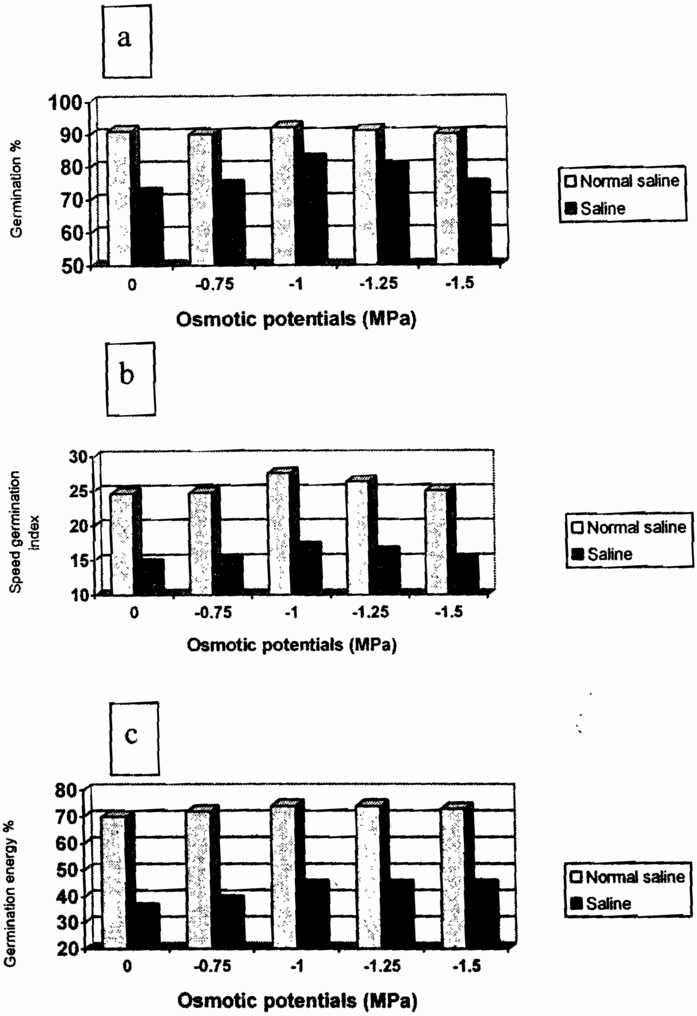

Fig 1 : Effect of ($CaCl_2$) osmotic potentials solution on (a) germination percentage, (b) speed germination index (SGI) and (c) germination energy % (GE) of rice seed under normal and saline stress.

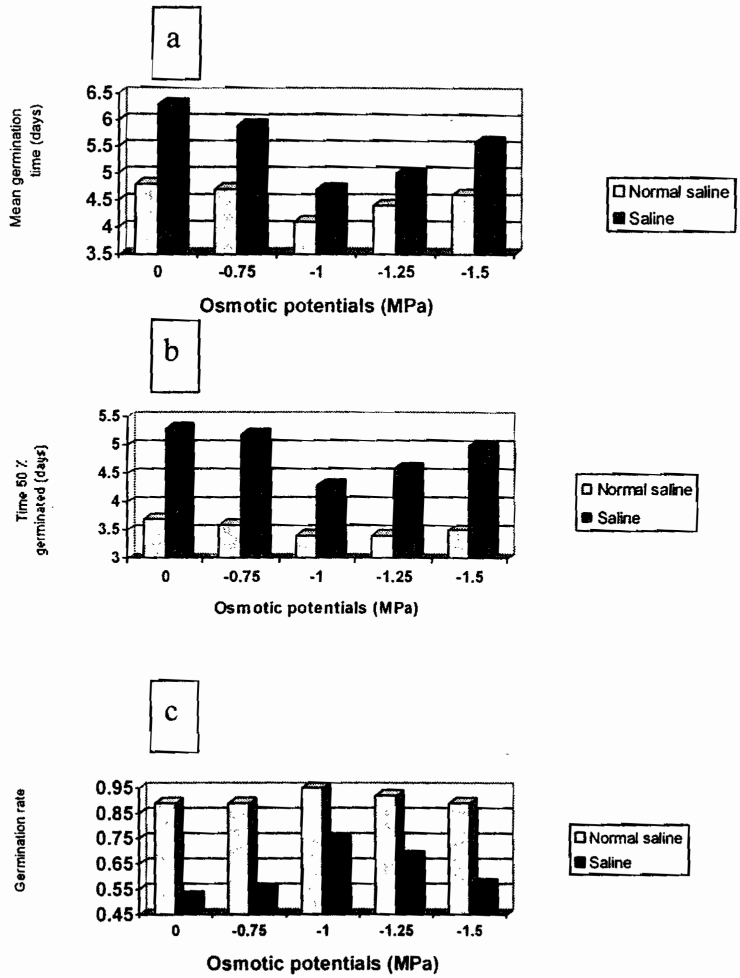

Fig 2: Effect of ($CaCl_2$) osmotic potentials solution on (a) mean germination time (MGT), (b) time 50 % germinated (T 50 %) and (c) germination rate (GR) of rice seed under normal and saline stress.

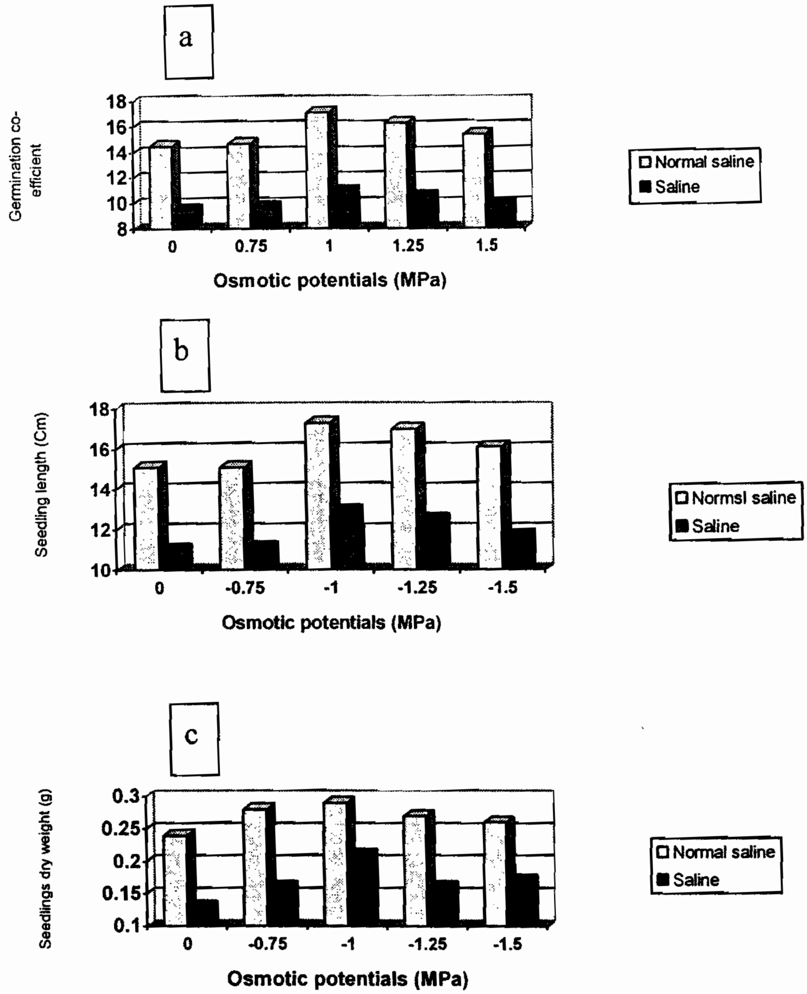

Fig 3: Effect of ($CaCl_2$) osmotic potentials solution on (a) co-efficient of germination (CG), (b) seedlings length (cm) and (c) seedlings dry weight (g) of rice seed under normal and saline stress.

Germination rate and germination co-efficient significantly affected by the interaction between salinity levels and osmotic potentials in Fig. (2c) and Fig (3a),respectively, germination rate of planted rice seed in normal salinity significantly increased with priming by $CaCl_2$ at osmotic potential (-1.00 MPa). In general, under salinity level (9.0 dS/m), germination rate significantly decreased but it improved significantly with priming and reached its highest rate 0.74, at osmotic potential (-1.00 MPa), the same trends were obtained from co-efficient germination.

With respect to the effect of interaction between osmotic potentials and salinity conditions on mean germination time and time to get 50%

germination, significantly effects were noticed Fig. (2 a and b), the minimum mean germination time and time to get 50% germination were obtained from sowing primed rice seed with $CaCl_2$ at (-1.00 MPa) in normal salinity level (0.3 ds/m)comparing unprimed seed. Meanwhile, mean germination time and time to get 50% germination were increased by sowing in salinity level (9.0 dS/m) and decreased with priming especially at osmotic potential (-1.00 MPa).

Seedlings vigor characters as measured by seedlings length and seedlings dry weight significantly affected by interaction between osmotic potentials and salinity conditions (Fig.3 a and b). Planted seed in normal salinity condition produced the tallest and heaviest seedlings especially from pre-sowing priming by $CaCl_2$ (-1.00 MPa) comparing untreated seeds.

On the other hand, seedlings vigor as measured by (seedlings length and seedlings dry weight) was decreased under salinity conditions and improved by priming before sowing especially by $CaCl_2$ at (-1.00 MPa). The major effect of seed priming on seedling growth was observed due o earlier germination, which gave the seedling a longer time to develop.

With respect to the effect of interaction between osmotic potentials of $CaCl_2$ solution and priming time on water uptake percentage after priming (Fig.4a), significant effect was noticed while water uptake was increased with increasing priming time for 24 hours at all osmotic potentials and reached its highest value at osmotic potential (-1.00 MPa). On contrast, with increasing priming time more for 36 hours and osmotic potential to (-1.25 MPa) water uptake decreased.

Also, rice seed germination percentage (Fig.4b) significantly affected by this interaction and increased with increasing priming time for 24 hours and reached its highest percentage (92%) at osmotic potential (-1.00 MPa) comparing to primed seed in distilled water. Seed vigor as measured by speed germination index (Fig 4c), also was improved by priming with $CaCl_2$ at osmotic potential (-1.00 MPa) for 24 hours.

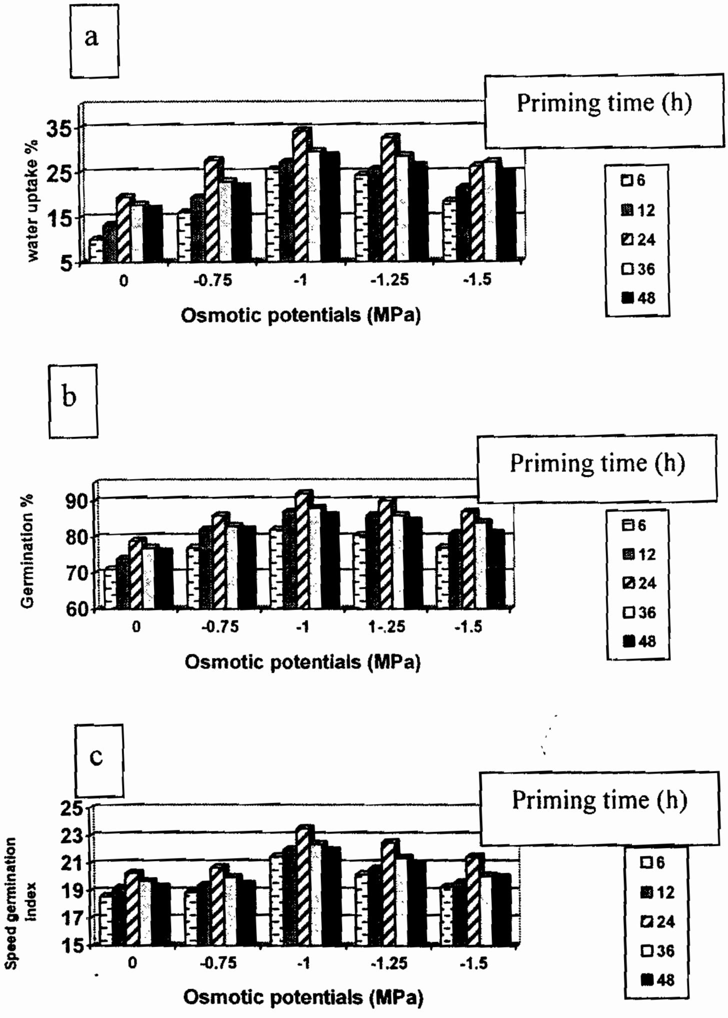

Fig 4: Effect of ($CaCl_2$) osmotic potentials solution and priming time (h) on (a) water uptake %, (b) germination percentage and (c) speed germination index of rice seed under normal and saline stress.

DISCUSSION

Seed germination is a critical stage in the history of plants and salt tolerance during germination is crucial for the establishment of plants that grow in saline soils. It is well documented that salinity reduces the germination as well as seedlings growth in crop plants and seed priming ameliorates salinity affects during early seedlings growth. In present study, salt stress (9 dS/m) gave the lowest values of traits under study and the highest mean germination time , time 50 % germinated. These results agreed with (Dubey, 1982) , who indicated that increased salinity decreased germination percentage, seedlings growth, these is due to decrease in water uptake by endosperm which cause decrease in protease , amylase activity and RNA content,.

The three early phases in seed germination are (i) imbibition, (ii) lag phase and (iii) protrusion of the radicle through the testa (Simon, 1984). Priming affects the lag phase and causes early DNA replication (Bray *et al.*, 1989), increased RNA and protein synthesis , greater ATP availability (Mazor *et al.*, 1984), faster embryo growth and repair of deteriorated seed parts (Saha *et al.*, 1990). However, osmopriming has been shown to activate processes related to germination, through affecting the oxidative metabolism such as increasing SOD and POD enzymes or through activating ATP ase , acid phosphatase and RNA synthase (Fu *et al.*, 1988).

Rice seed osmopriming with $CaCl_2$ (-1.00 MPa) achieved a good results in mitigating the adverse effects of salinity and promotion germination characters. Seeds primed with $CaCl_2$ had an advantage in maintaining germination under saline conditions perhaps due to the influence of Ca^{+2} on membranes (Shannon and Francois, 1977) and enhanced antioxidant proteins like SOD enzyme . (Hameed *et al.*, 2010). Calcium thus protects plants from adverse affects of salt stress and improves the growth of plants under saline conditions, greater efficiency of osmohardening with $CaCl_2$ and KCl is possibly related to the osmotic advantage that both k+ and Ca^{+2} have an improving cell water statues, and also they acts as cofactors in the activities of numerous enzymes (Taiz and Zeiger,2002).The beneficial influence of Ca^{+2} ion on root growth of rice seedlingss was most likely due to competition between Ca^{+2} and Na^{+1} leading to a reduced level of internal Na^{+1} (Lin and Kao,1995).

With respect to, priming durations effect, Rice seed osmopriming with $CaCl_2$ for 24 h showed its superiority compared with other priming durations. These results were consistent with (Yari *et al.*, 2012). Probably, increased duration priming make uncontrolled water uptake which caused seed coat weakening and vital electrolyte may leak which results in low colloids content in seed, low water uptake for germination and germination interference (Jet *et al.,* 1996).

This study decided that ($CaCl_2$) Calcium chloride plays an important role in the germination process, especially under saline conditions and therefore, it can be concluded that seed priming of Giza 177 cultivar seed which sensitive for salinity at osmotic potential (-1.00 MPa) for 24 h can reduce the harmful effects of salinity.

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تأثير التهيئة الأسموزية بكلوريد الكالسيوم على إنبات و حيوية بادرات تقاوي الأرز تحت الإجهاد الملحي

فيصل إبراهيم يوسف
قسم بحوث تكنولوجيا البذور– معهد بحوث المحاصيل الحقلية– مركز البحوث الزراعية– مصر .

أجريت تجربة معملية بمعمل قسم بحوث تكنولوجيا البذور بالمنصورة في تصميم التام العشوائية ذات أربع مكررات لدراسة تأثير التهيئة الأسموزية بواسطة محلول كلوريد كالسيوم بجهود أسموزية مختلفة (صفر، -٠.٧٥، -١.٠٠ و- ١.٥ميجا باسكال) على إنبات وقوه بادرات تقاوي الأرز صنف جيزة ١٧٧(حساس للملوحة) وذلك تحت إجهاد ملحي قدره (٠.٣ و ٩ ديسيسيمنز/م).

ويمكن تلخيص أهم النتائج فيما يلي :-

أدت زيادة مستويات الملوحة من ٠.٣ إلى ٩ ديسيسيمنز /م إلى انخفاض النسبة المئوية للإنبات و تدهورصفات حيوية البذور والتي تم قياسها بواسطة (معدل الإنبات ، متوسط زمن الإنبات ، الزمن اللازم لإنبات ٥٠% من البذور ، دليل سرعه الإنبات ، طاقه الإنبات ، نسبة الماء الممتص ومعامل الانبات) وقوة البادرات والتي تم قياسها بواسطة (طول البادرة ، الوزن الجاف للبادرة). سجلت التهيئة الأسموزية (-١.٠٠ ميجا باسكال) أفضل القراءات تحت ظروف الإجهاد الملحي (٩ ديسيسيمنز / م) لصفات نسبة الإنبات، حيوية التقاوي(معدل الإنبات ، متوسط زمن الإنبات ، الزمن اللازم لإنبات ٥٠% من البذور ، دليل سرعة الإنبات،معامل الإنبات وطاقه الإنبات و نسبة الماء الممتص)، حيوية البادرات(الوزن الجاف للبادرة).

سجلت التهيئة الأسموزية لمدة ٢٤ ساعة أعلى القراءات لصفات نسبة امتصاص الماء خلال اختبار الإنبات، نسبة الانبات و دليل سرعة الإنبات.

لذلك توصي هذه الدراسة بإجراء التهيئة الأسموزية لتقاوي الأرز صنف جيزة ١٧٧ بواسطة كلوريد الكالسيوم عند جهد أسموزي (-١.٠ ميجا باسكال) لمدة ٢٤ ساعة عند الزراعة في ظروف الإجهاد الملحي ، لما له من تأثير فعال في تحسين حيوية التقاوي وقوة البادرات و تخفيف الآثار الضارة المتسببة عن الإجهاد الملحي .

قام بتحكيم البحث

أ.د / محب طه صقر	**كلية الزراعة – جامعة المنصورة**
أ.د / ابراهيم فتحى مرسال	**مركز البحوث الزراعية**