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Effects of Seed Priming on Components Germination the Sweet Corn (*Zea mays* Cv. Basin) at Different Levels of Salinity

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ABSTRACT

Priming is one of the seed enhancement methods that might be resulted in increased seed performance (germination and emergence) under stress conditions, such as salinity, temperature and drought stress. In order to study the effect of seed priming on seed germination index and some of physiological properties of super sweet corn in different levels of salinity, an experiment conducted with two factors that arranged as factorial based on completely randomize design with three replications. Factors were consisted five levels of seed priming solutions (2% potassium chloride, 3% potassium nitrate, polyethylene glycol (8000) 10%, 1% potassium dehydrogenate phosphate with control) and four NaCl concentration (0, 50, 100,150, mM). Results showed seed priming and salinity had significant effect on germination rate, germination percent and some antioxidants such as catalase and peroxidase. In general, results of this experiment revealed that seed priming improved the seed vigor and increased resistance to salinity stress at germination stage.
KEY WORDS: Corn, Enzyme, Germination, Priming, Salinity.

INTRODUCTION

Germination is the first stage and one of the important and sensitive stages of the plant life cycle. It is an important process in seedling growth (De Villiers, et al, 1996). This stage of growth is strictly influenced of environmental factors, especially, temperature and humidity (Soltani et al, 2008). Seed germination capacity under stress conditions such as water stress increases establishing chance and consequence more plant stand in farm (Baalbaki, et al, 1999). Researchers have proposed seed priming as a technique for germination rate improvement. In this method, seeds will enter in the second stage of germination i.e. imbibition, but they don't enter to the third stage of germination process. In fact, this operation is one of the most important seed treatments before seeding. After this act, primed seeds, just like the untreated seeds, will be stored (McDonald, et al, 1999). Harris, et al (1993) reported implementing preculture treatments seed of sweet corn, significant improved factors such as the time to germination (%50), radicle length, and average time of seedling emergence. Some researchers have indicated that priming increases percent, rate and uniformity of seed germination (Murungu, et al, 2003). Kaya, et al (2006) reported that priming increased percent and rate of germination and seedling dry weight, and reduce number of abnormal seedling of sunflower plants under drought stress. Khaje hosseyni, et al (2003) reported that NaCl, caused more decreasing of germination rate and percent of soybean seeds, as compared to ethylene glycol. Hosseine, et al (2008) reported that germination pattern and its rate are almost similar and the lowest percent of germination is associated with polyethylene glycol and NaCl (1/5 N). Fazliani (2000) reported that primed pea seeds with PEG (8000) effect a significant difference ($P \leq 0.05$) of germination in compared with control. During priming, DNA replication, RNA and protein synthesis will be increased which result in more level of ATP synthesis, cell division, embryo growth and cell membrane repairing and lower level of leaching of cytoplasm solutes. Basra, et al (1988) suggested that the level of sterol phospholipids increase during priming. This compound plays an important role in construction of cell membranes. They observed that the amount of this compound in primed and control seeds is 36.2 µg and 76.2 µg, respectively. Basra, et al (1988) observed level of diphosphatidyl glycerol in primed seeds was increased. This compound organizes mitochondria membranes and increase ATP synthesis which, in turn, results increased radicle growth. Chiu, et al (2002) observed during priming, amount of seed antioxidants such as ascorbate and glutathione increased and vice versa activity of peroxidase which catalyzes lipids was decreased. So, aims of this research are to study: 1) effects of priming, salinity and NaCl on germination components and some of the physiological parameters of super sweet corn, var. Basin; and 2) germination response of sweet corn, var. Basin to priming under different levels of salinity.

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MATERIALS AND METHODS

In this study investigated effect of five solutions (%2 potassium chloride, %3 potassium nitrate, polyethylene glycol(8000), potassium dehydrogenate phosphate and control) and four level of salinity (0, 50, 100, 150 μM NaCl) on germination and physiological parameters (germination rate, germination percent, catalase enzyme, peroxidase enzyme) of super sweet corn, var. Basin. The experiment was carried out in factorial as randomized completely design with 3 replication.

All instruments and tools were washed with distilled water and then sterile with autoclave. A sample of 2800 seeds was placed in a cotton bag. There was no aerating during imbibition. For one limitation of this method is lacking of ventilation which requires aerating. Beakers containing experiment solution and seeds were incubated in a germinator (20±2 C) (Akrmyan, Et al 2008) for 24 hours (Ghana, et al, 2003). After this time, seeds were brought out of germinator and with water rinsed the control for 2 minutes and air dried. Fifty seeds of each Petri dish were cultured via inter-paper method for germination tests. Six ml solution were added to each Petri dish and labeled with their specification. Once again, they were incubated in germinator for 16 hours on daylight (Temp=25 ° ℃) and 8 hours at night (Temp=16 C). Germination changes were controlled on 12 hour intervals. A seed was germinated if its radicle length was at least 2 mm. If required, a same amount of treatment solution was added to each Petri dish during experiment. In case of no germination changes after two successive days, the last day of germination was taken into account and germination features such as rate and percent were studied. Germination rate (per hour) is calculated according to the following equation (Soltani, et al, 2008):

Germination Rate = R50 = 1/D50

Protein extraction is completed according to the following way:

a) Some of the fresh material of plants (shoot parts) with liquid nitrogen are grinded by a mortar to fine particles, of which 0.5 g put in a certain quantity of solvent (5ml buffer trisglycin), and shaken vigorously to a homogenous solution.

b) Solution was poured into the special capped glass tubes, labeled by sample specification.

c) Samples centrifuging is carried out in 12000 rpm for 10 minutes (4 C).

d) Surfactant was distributed between some opendorf and froze at -20 C until testing. These were used for enzyme and protein assessments.

Catalase enzyme activity is evaluated according to Pereira, et al (2002) through evaluation of decreased level of hydrogen peroxide at 240 nm. The solvent applied in this experiment contains buffer Triss (pH= 7.5, 50 mM) and hydrogen peroxide %1(v/v). Buffer Triss(2.5 ml) was mixed with hydrogen peroxide(300µl) in ice bath and then 60µl of enzyme extract was added to it. Absorption index was recorded by using of spectrophotometer at 240nm wavelength. Enzyme activity was evaluated as absorption index (units/min^{-1}/ g FW^{-1}) of leaves. Peroxiase enzyme Activity was calculated according to Koroi, et al (1989) at wavelength 530 nm. Applied mixture contains buffer STAT (0.2 mM, pH = 4.8), hydrogen peroxide (0.1 VP) and methanol solution of benzidin (0.04 M). Two ml of buffer STAT and 200 µl of Benzidin solution as well as 200 µl hydrogen peroxide were mixed in ice bath. Then, after adding 100 µl of enzyme extract, absorption index was recorded by using of spectrophotometer at 530nm.

Enzyme activity was calculated as absorption changes (units/ min^{-1}/ g FW^{-1}) of leaves. Statistical analyzing is done by statistic software MSTAT-C. Means are compared by Duncan test ($P \leq 0.01$) and diagrams are drawn by EXCEL software.

RESULTS AND DISCUSSION

Germination Rate

Data analyzing showed that salinity has a significant effect ($P \leq 0.01$) on germination rate of super sweet corn, var. Basin (Table 1). Mean comparison showed that the highest and lowest level of germination rate is related to treated seeds with solutions containing 0 mg/l and 150 mg/l NaCl, respectively. Although 50 mg/l NaCl caused lower rate of germination, but this difference was non-significant (Fig.1).

Fazliani (2011) reported that decreased water potential reduced germination rate of wheat genotypes. Disrupted water absorption indicates that seed intrinsic reactions are done slowly and the radicle time to appearance is increased, in other words, germination rate is decreased.

Data analyzing showed that priming had a significant effect ($P \leq 0.01$) on germination rate of seeds (Table 1). Means comparison shows that the highest and lowest rate of germination was related to primed seeds with KH_2PO_4 and KNO_3, respectively. Though, there was non significant difference between primed seeds and the control (Fig.1). Analysis of data indicated that germination rate of primed seeds of corn with priming solutions under saline conditions was increased significantly ($P \leq 0.01$) (Table 1).

Najafi, et al (2011) reported that primed seeds of sunflower for 3-5 days increased germination rate and improved seedlings growth. Bradford and Dohall (1990) suggested that effect of priming is mainly through decreasing of the time required for final activation of endosperm and increasing capacity water absorption of embryo.

Soltani, et al (2008) showed that raised levels of drought stress will decrease germination rate linearly, but primed seeds as compared to the control show less decreasing rate of germination. Their results showed effect of priming on germination rate under both stress and normal conditions was positive. Although different experiments have shown that priming has a positive effect on germination rate; many others demonstrated its decreasing after preculture treatments.

Hosseine and Koucheaki (2008) has demonstrated increased rate of germination of seed sugar beet cultivars after priming treatment. These researchers mentioned seeds rinsing with water and HCl (0.1 and 0.5 Normal) as the reason for elimination of germination inhibiting compounds of seeds cuticle and increased rate of germination. They also revealed that PEG and NaCl (1.5 Normal) decreases germination rate significantly. This shows that increased level of drought and salinity has an inhibiting effect on germination. It seems this effect is due to increasing of osmotic pressure and decreasing of water intake by seeds which shows of sugar beet seedlings as compared to the next stages of development more susceptible to salinity and drought stresses (Fazliani, 2011).

Germination Percent

Data analyzing indicates significant influence of NaCl ($P \leq 0.01$) on germination percent of super sweet corn, var. Basin (Table 1). Comparison between means shows that the highest and lowest level of germination percent is related to seeds treated with 0 mg/l and 150 mg/l NaCl solutions, respectively. Although salinity levels of 50 and 100 mg/l NaCl, compared to base levels, shows lower percent of germination, but this effect is non significant (Fig. 2).

Analysis of data indicates significant effect ($P \leq 0.01$) of priming treatment on germination rate of super sweet corn seeds (Table 1). Comparison of means reveals that the highest and lowest levels of germination percent is associated with control treatment and seeds primed with KNO_3 and KCl solutions, respectively, and there was not significant difference between seeds treated with KH_2PO_4 and PEG (Fig. 2).

Concentration of salts and chemicals applied to the experiment are very important. It is reported that if salt concentration is above 4ds/l, final level of germination will be decreased (Fazliani, 2011).

Khaje Hosseine, et al (2003) stated that NaCl as compared to PEG cause greater decreasing of germination rate and percent of soybean seeds. Data analyzing showed that interaction of salinity and priming have a significant effect on germination rate of seeds (Table 1). Means comparison showed the highest level of germination percent when seeds treated with 0 mg/l NaCl and the control and lowest germination percent related to those seeds when treated with 150 mg/l NaCl and KNO_3.

Although other treated seeds show less germination percent than these two extremities, this difference is not significant (Fig. 2).

Priming improves some of the components of germination. There are many reports demonstrating positive effect of priming on germination percent. Soltani, et al (2008) showed that seed priming compared to the control increase germination percent. These findings supported Demir Kaya, et al (2006) results in sunflower plants and those of Tip, et al (2003) in melon.

Catalase (EC1.11.1.6)

Data analyzing showed that salinity has a significant effect ($P \leq 0.01$) on the level of catalase enzyme activity of super sweet corn, var. Basin (table 1).

Means comparison showed that the most and least levels of catalase enzyme activity are related to treated seeds with 0 mg/l NaCl and 150mg/l, respectively.

Although salinity levels of 50mg/l and 100mg/l NaCl as compared to other levels of salinity treatments show lower degree of catalase enzyme activity, but this difference is not significant (Fig. 3).

Data analyzing showed that priming seed has a significant influence ($P \leq 0.01$) on the level of catalase enzyme activity of super sweet corn, var. Basin (Table 1). Also, comparison of means revealed that the greatest level of catalase enzyme activity is belong to primed seeds with KH_2PO_4 and its lowest level is associated with primed seeds with KNO_3 but there is no significant difference between treated seeds with KCL and control and seeds treated with PEG solutions(Fig. 3).

Analysis of data suggested that priming solutions have a significant influence ($P \leq 0.01$) on the activity levels of catalase enzyme of super sweet corn, var. Basin under salinity conditions (Table 1). Mean comparisons revealed that the most and least amounts of catalase enzyme activity is associated with seeds treated by 0 mg/l NaCl and PEG and those treated with 150 mg/l NaCl and KNO_3, respectively.

Although, remainder seeds show lower percent of germination than these two extremities, this difference is not significant statistically (Fig. 3).

Peroxidase (EC1.11.1.7)

Data analysis indicates that NaCl has a significant influence ($P \leq 0.01$) on the level of peroxidae enzyme activity of super sweet corn plants, var. Basin (Table 1).

Mean comparisons show that the most and least levels of peroxidase enzyme activity are related to seeds treated with 150 mg/l NaCl and 0 mg/l NaCl. Although 50 mg/l NaCl and 100 mg/l NaCl show lower level of peroxidase enzyme activity than the second and third levels of salinity, but this difference is not significant statistically (Fig. 4).

Data analysis demonstrates significant influence of priming ($P \leq 0.01$) on the activity levels of peroxidase enzyme in super sweet corn (var. Basin) (Table 1). Mean comparisons indicated that the greatest level of peroxidase enzyme activity is related to primed treatments with PEG and the minimum level is associated with primed treatments with KNO_3, and that there is no statistical significant difference between treated seeds with KCL and KH_2PO_4 and control, (Fig. 4).

Hassanzadeh, et al (2011) reported that priming increase seed levels of antioxidant enzymes such as glutathione and ascorbate which, in turn, decrease activity levels of peroxidation lipid during germination and, consequently, increase germination percent

Data analysis shows that salinity and priming interaction has a significant influence ($P \leq 0.01$) on the activity levels of peroxidase enzyme in super sweet corn (Table 1). Mean comparisons indicate that the most and least levels of peroxidase enzyme activity are associated with seeds treated by 150 mg/l NaCl and KCL, and those treated with 150mg/l NaCl and KNO3, respectively. Although the remainder seeds show lower level of peroxidase enzyme activity than these two extremities, this difference is not significant statistically (Fig.e 4).

TABLE1. Analysis of variance of salinity and germination on super sweet corn studied enzyme basin

S.O.V	DF	Germination Rate	Germination Percent	Catalase	Peroxidase
Salinity	3	4.980**	2812.711**	18.757**	3.945**
Priming	4	18.533**	10682.0**	760.39**	20.955**
Salinity and Priming	12	0.848**	488.933**	6.151**	4.676**
Error	38	0.236	136.323	0.064	0.132

significant at level of 1% probability ($P \leq 0.01$)**

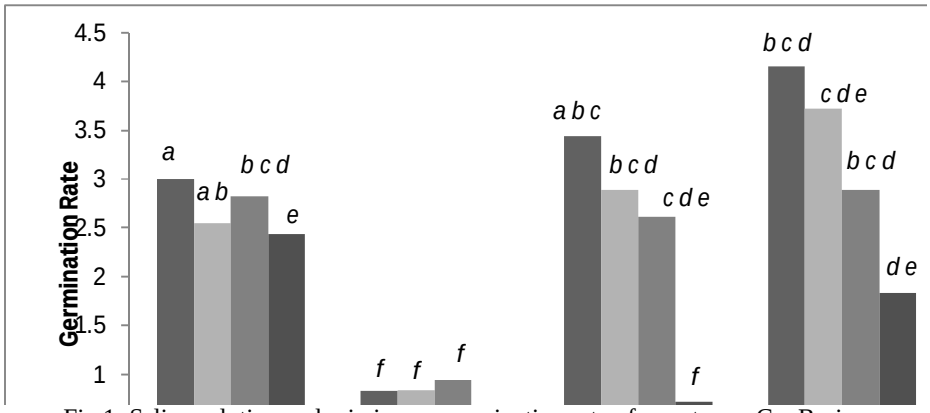

Fig 1. Saline solution and priming on germination rate of sweet corn Cv. Basin

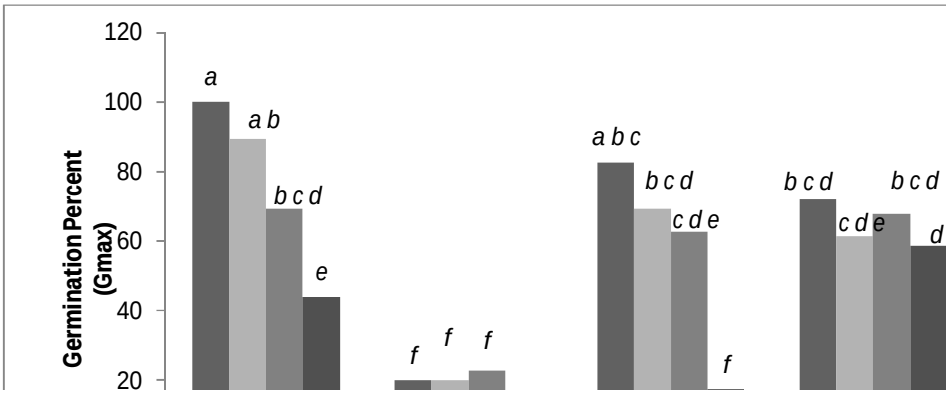

Fig 2. Saline solution and priming on germination of sweet corn Cv. Basin

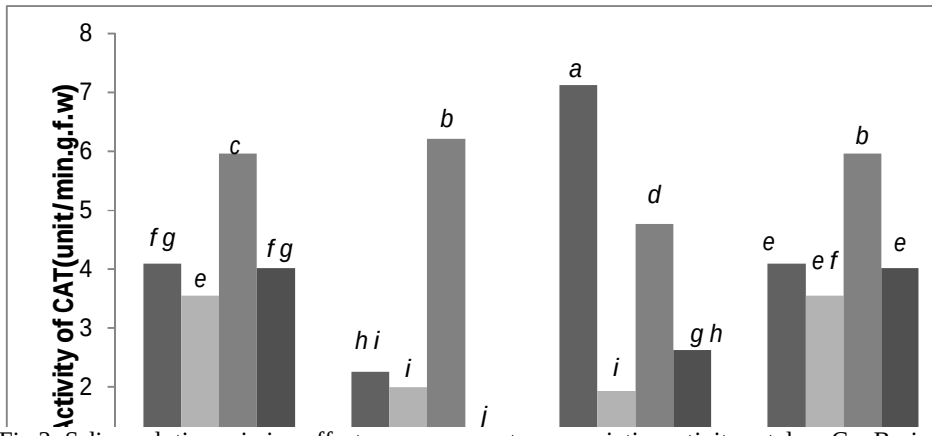

Fig 3. Saline solution priming effect on super sweet corn varieties activity catalase Cv. Basin

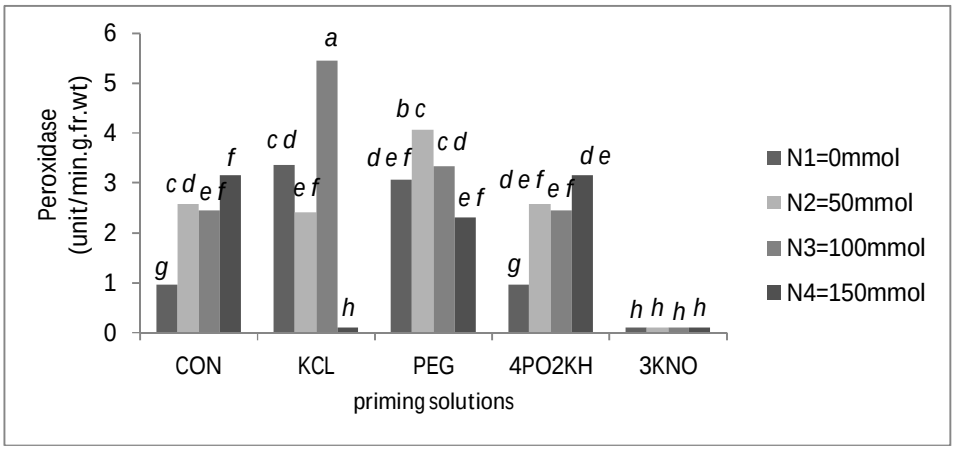

Fig4. Saline solution priming effect on super sweet corn activity Peroxidase Cv. Basin

Conclusion

The results of this experiment showed that priming improves some of the components of germination and increase growth rate of super sweet corn, var. Basin under salinity stress. Implementing of priming treatments before seedling, particularly, under adverse environmental conditions and unfavorable medium, can promote germination and growth during the early days of biological life and improves seedling establishment. This results in plants better using of medium constituents and, finally, increased quantity and quality of products. Priming is a simple and inexpensive technique which don't need chemicals material and without any toxic effect for seeds and so this method can suggest for enhancement seed vigor.

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