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Improvement of germination & seedling vigour of maize (*Zea mays* L.) through different priming methods under salt stress

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ABSTRACT

The study was designed to evaluate the effect of different seed priming methods on growth and yield of four maize cultivars under salt stress. The present experiment was conducted in completely randomized design (CRD) having three replications. Seeds of maize were subjected to hydropriming for 12 hours while in case of osmo-priming seeds were treated with 0.5% and 1% solution of KNO_3, $ZnSO_4$ and H_3BO_3 for twelve hours, respectively. In control conditions untreated seeds were used. The seed priming significantly increased the germination rate as compared to non-primed seeds. Under salt stress germination rate decreased as compared to non-primed seeds i.e. 28% to 35% for all four cultivars at 100 Mm NaCl. Shoot length and Root length was also significantly increased for all four maize cultivars by all priming methods as compared to the to control treatment. The maximum shoot length (6.22, 5.37, 5.27 and 4.82 cm), root length (15.18, 13.18, 12.06 and 10.74 cm), leaf area (470, 465, 462 and 460) was computed for all four cultivars at 1% of KNO_3 treatment while minimum values was calculated at salinity level of 100 Mm NaCl for all cultivars. Similarly shoot fresh and root fresh weight significantly increased among all four maize cultivars treated with osmo-priming and hydro priming methods as compared to control at different salinity levels. Among all four cultivars, the maximum shoot fresh and root fresh weight was calculated for PEARLat 1% of KNO_3 priming, followed by 0.5% of KNO_3 priming as compared to control, hydro priming, $ZnSO_4$ and H_3BO_3 respectively. Whereas parameters negatively significantly affected at 75 mM NaCl and 100 mM NaCl levels.

Key words: Maize, seed priming, salt stress

Introduction

Among all the cereal crops in the world, maize (*Zea mays* L.) ranked third after wheat and rice in Pakistan. Maize (*Zea Mays L.*) is a cereal crop, belongs to family poaceae (Kynast 2012). Salinity is a Global environmental issue. Salinity among other abiotic stresses is a common factor which seriously affects crop production particularly in arid and semi-arid regions (Karmoker el al., 2008).

Akram *et al.* (2007) used solution culture technique to determine salinity tolerance in maize genotypes at three levels of salinity (0, 50 and 100 mM NaCl). The NaCl stress considerably reduces the plant shoot and root weight. Reduction in plant height and root length was also observed. Bilgin *et al.* (2008) studied the relationship between salinity and available water level at seedling stage of dent corn genotype. Root and shoot weight showed negative effect with increase in salt stress. Khayatnezhad and Gholamin, (2011) investigated the effect of different levels of salinity (0, 50, 100, 150, 200, 250 mM NaCl) on five maize cultivars at seedling stage. The traits under observation were root dry weight, shoot dry weight, root length and shoot length. They observed the decrease in root and shoot dry weight of cultivars as the level of salinity increased from 0-250 mM NaCl. Salinity negatively affected the all traits under observation and shoot weight was more affected as compared to root weight. Aydİnșakİr*et al.* (2013) studied the effect of different salinity levels on the development of fodder maize crop at initial growth stage. The experiment was conducted in Petri dishes where eight seeds of each genotype were placed. After 15 days of germination different traits including root length, shoot length, wet root and shoot weight and dry root and shoot weight were studied. The results indicated that these traits were negatively affected by salinity. High NaCl concentrations greatly reduced root length, shoot length, wet root and shoot weight and dry root and shoot weight. Salinity mostly affect the seed emergence and crop growth (Ibrahim, 2016). Seed priming is a simple, minimal effort and generally safe procedure as of late used to beat the salinity issue in farming areas (Ibrahim, 2016). A pre sowing treatment, with variety of techniques such as halo- (absorbing seeds inorganic salt arrangements), solid matrix priming (treatment of seeds with solid materials), osmo-priming (absorbing seeds arrangements of different natural osmotic) and bio priming (using bio chemicals and molecules) (Nouman *et al*., 2014). The above-mentioned priming methods can be used to 1) motivate metabolic procedures engaged with the early periods of germination, delivering greater emergence and extraordinary emergence rate 2) incite consistency and quicker rise of seedlings from prepared seeds, acquire fiery development unfriendly conditions (Imran *et al*., 2013).

Seed priming, an encouraging technique being effectively utilized to conquer the issue related with low emergence and consequent inconsistent yield remain under ordinary and saline situations (Farooq *et al*., 2009; Jafar *et al*., 2012). Hydro priming produces critical upgrades in crop vigor and growth; there is likewise proof that seeds can be treated with watery solutions such as water and nutrients to defeat inadequacies in explicit circumstances. Priming of crop seeds with little measures of supplements has been appeared to in part defeated supplement immobilization issue in soils and to build nutrient use efficacy. The present examination was led to research the emergence and seedling growth of maize under hydro and osmo-priming to reduce salinity stress.

Objectives:

- Determination of germination of maize seeds under different levels of salinity (NaCl).
- Investigation of morphological attributes under different levels of hydro priming and osmo-priming

Materials and methods

The experiment was performed to find out the effects of various seed priming techniques on maize (*Zea mays* L.) in sand filled glass under normal and controlled conditions. The experiment was performed in CRD with three replications. Four maize varieties (MALKA-2017, Pearl, MMRI yellow, Pak Afghoi were used in current study. The NaCl was used in different concentration (0 mM, 75 mM and 150 mM) for salt stress. Potassium nitrate, Zinc sulphate and Boric acid were used with various concentrations in osmo-priming while distilled water was used in hydropriming. Seeds of maize were subjected to hydropriming for 12 hours while in case of osmo-priming seeds were treated with 0.5% and 1% solution of KNO_3, $ZnSO_4$ and H_3BO_3 for twelve hours, respectively. Seeds were dipped in mentioned solutions or in water with ratio 1:5 (w/v) at 25±2°C for the period of 12 h. In control treatment untreated seeds were used. Seeds were removed from the solution after given time and washed by using distilled water three times and dried in forced air to achieved the optimum moisture contents. Seeds were used for further study after they dried. Seeds were sown in sand filled glass which was subjected to sodium chloride stress. Fifteen seeds were used in each replication. The data on the following parameters like germination rate, root fresh weight, shoot fresh weight, root length and shoot length were collected. Data was analyzed in Statistix 8.1. LSD test was used at 0.05 probability level to compare the means difference (Steel *et al*., 1997).

Results and discussion

Germination Rate

The data indicated that germination rate of all genotypes was higher in hydro and osmopriming methods as compared to control while with the increase of salt stress to 75 & 100 mM NaCl application, the germination slightly increased as compared to control but decreased at higher salinity stress (table 1).

The genotype pearl showed higher germination rate followed by Malka while Pak Afghoi showed lower germination rate. Higher germination rate was observed in seeds treated with osmo-priming as compared to the hydropriming and non-treated seeds. Under hydropriming, the maximum germination (66.67) was observed for Pearl, followed by Malka and MMRI Yellow whereas the minimum (59.66) for Pak Afghoi i.e. 28 % higher as compared to control value of Pak Afghoi. In case of osmo-priming, seed treated with 1 % of KNO_3 and $ZnSO_4$ of all four cultivars showed higher germination rates for Pearl (73.33 & 66.66) as compared to seed primed with 0.5 % of KNO_3 and $ZnSO_4$ (70 & 63.33) as compared to other maize cultivars. However, 0.5% seed treatment of H_3BO_3 of all four cultivars showed higher germination rate (60, 58, 56 & 53) respectively as compared to seed treated with 1 % of H_3BO_3 i.e. 56.66, 57.67, 52.66 and 49.66. At the salt stress concentration of 75 mM NaCl, the germination rate was slightly higher as compared to the control treatment with maximum values (67.50) for Pearl, followed by 65.50 for Malka, 63.49 for MMRI Yellow and 58 for Pak Afghoi. In case of 150 mM salinity, lower germination rate was reduced a little as compared to 75 mM NaCl with maximum values for Pearl (58.57) and the minimum values (51.75) were measured for Pak Afghoi.

Table 1: Effect of salt stress on mean germination rate of four maize cultivars treated with different priming methods.

Factor	**Germination Rate**			
Treatment (T)	Pearl	Malka	MMRI Yellow	Pak Afghoi
No priming (Control)	53.33 G	51.33 G	49.33 G	46.33 G
Hydro-priming	66.67 C	64.66 C	62.67 C	59.66 C
Priming with Potassium Nitrate (KNO_3) 0.5%	70.00 B	68.00 B	66.00 B	63.00 B
Priming with Potassium Nitrate (KNO_3) 1%	73.33 A	71.33 A	69.33 A	66.33 A
Priming with Zinc Sulphate ($ZnSO_4$) 0.5%	63.33 D	61.33 D	59.33 D	56.33 D
Priming with Zinc Sulphate ($ZnSO_4$) 1%	66.66 C	64.67 C	62.66 C	59.66 C
Priming with Boric Acid (H_3BO_3) 0.5%	60.00 E	58.00 E	56.00 E	53.00 E
Priming with Boric Acid (H_3BO_3) 1%	56.66 F	54.67 F	52.66 F	49.66 F
Salinity Level (SL)				
Control	65.00 A	63.01 A	61.00 A	60.50 A
75 mM NaCl	67.50 B	65.50 B	63.49 B	58.00 B
150 mM NaCl	58.75 C	56.74 C	54.75 C	51.75 C
LSD (T) ($p \leq 0.05$)	**	**	**	**
LSD (SL) ($p \leq 0.05$)	**	**	**	**
T × SL ($p \leq 0.05$)	**	**	**	**

Note: Means followed by different letters are significantly different from one another at $p \leq 0.05$ using LSD test.

Shoot Fresh Weight (g)

The shoot fresh weight was higher under hydro and osmo-priming methods as compared to control. The findings concerning shoot fresh weight (g) of four maize cultivars affected by different priming techniques is presented in table 2. The maximum shoot fresh weight (g) was found for Pearl followed by Malka whereas lower shoot fresh weight (g) was observed for Pak Afghoi. The seed treated with osmo-priming showed higher shoot fresh weight (g) as compared to the hydropriming and non-treated seeds. Under hydropriming, the maximum shoot fresh weight (20.30 g) was observed for Pearl, followed by Malka and MMRI Yellow whereas the minimum (13.30 g) for Pak Afghoi i.e. 27 % higher as compared to control value of Pak Afghoi. In case of osmopriming, seed treated with 0.5 % and 1 % of KNO_3 of all four cultivars showed higher shoot fresh weight for Pearl (31.08 g & 34.29 g) as compared to seed primed with 0.5 % and 1 % of $ZnSO_4$ and H_3BO_3 (27.56 g, 29.72 g & 22.63 g, 25.41 g) followed by Malka and MMRI Yellow whereas the minimum shoot fresh weight was computed for Pak Afghoi.

The shoot fresh weight was decreased as salinity increased (table 2). All genotypes showed higher fresh shoot weight in control followed by 75 mM & 150mM. At 75 mM NaCl, the pearl showed maximum values (25.10 g) for for shoot fresh weight followed by Malka (23.11 g), MMRI Yellow (21.10 g) and Pak Afghoi (18.09 g). In case of 150 mM salinity, shoot fresh weight greatly reduced as compared to control and 75 mM NaCl with maximum values for Pearl (17.10 g) and the minimum values (10.11 g) were measured for Pak Afghoi.

Table 2: Effect of salt stress on shoot fresh weight (g) of four maize cultivars treated with different priming methods.

Factor	**Shoot Fresh Weight**			
Treatment (T)	Pearl	Malka	MMRI Yellow	Pak Afghoi
No priming (Control)	17.34 H	15.34 H	13.34 H	10.34 H
Hydro-priming	20.30 G	18.30 G	16.30 G	13.30 G
Priming with Potassium Nitrate (KNO_3) 0.5%	31.08 B	29.08 B	27.08 B	24.08 B
Priming with Potassium Nitrate (KNO_3) 1%	34.29 A	32.29 A	30.29 A	27.29 A
Priming with Zinc Sulphate ($ZnSO_4$) 0.5%	27.56 D	25.56 D	23.56 D	20.56 D
Priming with Zinc Sulphate ($ZnSO_4$) 1%	29.71 C	27.71 C	25.71 C	22.71 C
Priming with Boric Acid (H_3BO_3) 0.5%	22.63 F	20.63 F	18.63 F	15.63 F
Priming with Boric Acid (H_3BO_3) 1%	25.41 E	23.41 E	21.41 E	18.41 E
Salinity Level (SL)				
Control	35.91 A	33.90 A	31.91 A	28.90 A
75 mM NaCl	25.10 B	23.11 B	21.10 B	18.09 B
150 mM NaCl	17.10 C	15.10 C	13.10 C	10.11 C
LSD (T) ($p \leq 0.05$)	**	**	**	**
LSD (SL) ($p \leq 0.05$)	**	**	**	**
T × SL ($p \leq 0.05$)	**	**	**	**

Note: Means followed by different letters are significantly different from one another at $p \leq 0.05$ using LSD test.

Root Fresh Weight (g)

The data regarding root fresh weight (g) of four maize cultivars affected by different priming techniques is showed in table 3. The results indicated that the root fresh weight markedly decreased with the increase of salinity level among all four maize cultivars as indicated in table 4.5. It was noted that root fresh weight of four cultivars was higher under hydro and osmopriming methods as compared to control while with the increase of salt stress, the root fresh weight decreased as compared to control. A significant difference of root fresh weight was computed all four maize cultivars.

Among all four cultivars, higher root fresh weight was recorded for Pearl followed by Malka whereas minimum root fresh weight was computed for Pak Afghoi. Under hydropriming, maximum root fresh weight (2.65 g) was noted for Pearl and minimum (2.24 g) for Pak Afghoi as compared to control treatment. In case of osmopriming, seeds treated with 0.5 % and 1 % KNO_3 of all four cultivars showed higher root fresh weight as compared to those treated with 0.5 % and 1 % of $ZnSO_4$ and H_3BO_3 with a maximum value of 5.84 g and 6.25 g for Pearl, followed by Malka, MMRI Yellow whereas the minimum 4.46 g and 4.87 g for Pak Afghoi. Similar trend of root fresh weight was noticed for all four maize cultivars at different levels of $ZnSO_4$ and H_3BO_3.

The root fresh weight under different salt stress concentration was in the order of control> 75 mM NaCl>150 mM NaCl as indicated in table 3. At the salt stress concentration of 75 mM NaCl, the root fresh weight decreased by 56 % for Pearl, 61 % for Malka and MMRI Yellow and maximum decrease percentage (62 %) was computed for Pak Afghoi as compared to the control. In case of 150 mM salinity, root fresh weight ranged from 1.35 g to 2.91 g with maximum values for Pearl and the minimum values for Pak Afghoi.

Table 3: Effect of salt stress on root fresh weight (g) of four maize cultivars treated with different priming methods.

Factor	**Root Fresh Weight**			
Treatment (T)	Pearl	Malka	MMRI Yellow	Pak Afghoi
No priming (Control)	2.0433 H	1.7000 H	1.6833 H	1.6533 H
Hydro-priming	2.6500 G	2.3033 G	2.2767 G	2.2467 G
Priming with Potassium Nitrate (KNO_3) 0.5%	5.8467 B	4.8467 B	4.4933 B	4.4633 B
Priming with Potassium Nitrate (KNO_3) 1%	6.2567 A	5.2567 A	4.9033 A	4.8733 A
Priming with Zinc Sulphate ($ZnSO_4$) 0.5%	5.1500 D	4.1500 D	3.7967 D	3.7667 D
Priming with Zinc Sulphate ($ZnSO_4$) 1%	5.5433 C	4.5433 C	4.1900 C	4.1600 C
Priming with Boric Acid (H_3BO_3) 0.5%	4.4467 F	3.4467 F	3.0933 F	3.0633 F
Priming with Boric Acid (H_3BO_3) 1%	4.7800 E	3.7800 E	3.4267 E	3.3967 E
Salinity Level (SL)				
Control	7.55 A	6.56 A	6.53 A	6.50 A
2. 75 mM NaCl	3.30 B	2.54 B	2.52 B	2.47 B
150 mM NaCl	2.91 C	2.15 C	1.38 C	1.35 C
LSD (T) ($p \leq 0.05$)	**	**	**	**
LSD (SL) ($p \leq 0.05$)	**	**	**	**
T × SL ($p \leq 0.05$)	**	**	**	**

Note: Means followed by different letters are significantly different from one another at $p \leq 0.05$ using LSD test.

Shoot length (cm)

The shoot length (cm) of all genotypes decreased significantly with the increase of salt stress (table 4). There was a significant interaction was noted between priming treatments and different salt concentrations for Pearl, Malka and Pak Afghoi while non-significant interaction was observed for MMRI Yellow. The maximum percentage reduction in shoot length was noticed at 150 mM NaCl followed by 75 mM NaCl as compared to control. It was observed that shoot length of all genotypes was higher under hydro and osmo-priming methods as compared to control while with the increase of salt stress, the shoot length decreased.

Among all the cultivars, the maximum shoot length was found for Pearl followed by Malka whereas minimum shoot length was observed for Pak Afghoi. A slightly higher shoot length (g) was noticed in seeds treated with osmo-priming as compared to the hydropriming and non-treated seeds. Under hydropriming, the maximum shoot length (3.59 cm) was observed for Pearl, followed by Malka and MMRI Yellow whereas the minimum (2.19 cm) for Pak Afghoi. In case of osmopriming, seed treated with 0.5 % and 1 % of KNO_3 of all four cultivars showed higher shoot length for Pearl (6.10 cm & 6.22 cm) as compared to seed primed with 0.5 % and 1 % of $ZnSO_4$ and H_3BO_3 (5.85 cm, 5.94 cm & 5.60 cm, 5.68 cm) followed by Malka (5 cm, 5.09 cm & 4.75 cm, 4.83 cm) and MMRI Yellow (4.90 cm, 4.99 cm & 4.98 cm, 4.73 cm) whereas the minimum shoot length was computed for Pak Afghoi at 0.5 % and 1 % of KNO_3, $ZnSO_4$ and H_3BO_3.

The shoot length under different salt stress concentration was in the order of control>75 mM NaCl> 150 mM NaCl and decreased significantly with the increase of salt stress (table 4). At the salt stress concentration of 75 mM NaCl, the shoot length was slightly lower as compared to the control treatment with maximum values (5.24 cm) for Pearl, followed by 4.39 cm for Malka, 4.29 cm for MMRI Yellow and 3.84 cm for Pak Afghoi. In case of 150 mM salinity, shoot length was slightly lower as compared to control and 75 mM NaCl with maximum values for Pearl (5.15 cm) and the minimum values (3.75 cm) were measured for Pak Afghoi.

Table 4.8: Effect of salt stress on shoot length (cm) of four maize cultivars treated with different priming methods.

Factor	**Shoot Length**			
Treatment (T)	Pearl	Malka	MMRI Yellow	Pak Afghoi
No priming (Control)	2.95 H	2.10 H	2.00 E	1.55 H
Hydro-priming	3.59 G	2.74 G	2.64 D	2.19 G
Priming with Potassium Nitrate (KNO_3) 0.5%	6.10 B	5.25 B	5.15 AB	4.70 B
Priming with Potassium Nitrate (KNO_3) 1%	6.22 A	5.37 A	5.27 A	4.82 A
Priming with Zinc Sulphate ($ZnSO_4$) 0.5%	5.85 D	5.00 D	4.90 BC	4.45 D
Priming with Zinc Sulphate ($ZnSO_4$) 1%	5.94 C	5.09 C	4.99 A-C	4.54 C
Priming with Boric Acid (H_3BO_3) 0.5%	5.60 F	4.75 F	4.98 A-C	4.20 F
Priming with Boric Acid (H_3BO_3) 1%	5.68 E	4.83 E	4.73 C	4.28 E
Salinity Level (SL)				
Control	5.33 A	4.48 A	4.50 A	3.93 A
2. 75 mM NaCl	5.24 B	4.39 B	4.29 B	3.84 B
150 mM NaCl	5.15 C	4.30 C	4.20 B	3.75 C
LSD (T) ($p \leq 0.05$)	**	**	**	**
LSD (SL) ($p \leq 0.05$)	**	**	*	**
T × SL ($p \leq 0.05$)	**	**	NS	**

Note: Means followed by different letters are significantly different from one another at $p \leq 0.05$ using LSD test.

Root length (cm)

The result showed that the root length markedly decreased with the increase of salinity level among all four maize cultivars. It was found that root length of four cultivars was higher in hydro and osmo-priming methods as compared to control while with the increase of salt stress, the root length decreased as compared to control. A non-significant interaction was computed between salinity levels seed priming treatments of root length as presented in table 5.

Among all four cultivars, higher root length was recorded for Pearl followed by Malka while minimum root length was found for Pak Afghoi. Under hydropriming, maximum root length (10.91 cm) was noted for Pearl and minimum (6.47 cm) for Pak Afghoi as compared to control treatment. In case of osmopriming, seeds treated with 0.5 % and 1 % KNO_3 of all four cultivars showed higher root length as compared to those treated with 0.5 % and 1 % of $ZnSO_4$ and H_3BO_3 with a maximum value of 15 cm and 15.18 cm for Pearl, followed by Malka, MMRI Yellow whereas the minimum 10.56 cm and 10.74 cm for Pak Afghoi. Similar trend of root length was noticed for all four maize cultivars at different levels of $ZnSO_4$ and H_3BO_3. At the salt stress concentration of 75 mM NaCl, the root length significantly decreased as compared to control. In case of 150 mM salinity, root length ranged from 8.72 cm to 13.16 cm with maximum values for Pearl and the minimum values for Pak Afghoi table 5.

Table 5: Effect of salt stress on shoot length (cm) of four maize cultivars treated with different priming methods.

Factor	**Root Length**

Treatment (T)	Pearl	Malka	MMRI Yellow	Pak Afghoi
No priming (Control)	9.74 H	7.74 H	6.62 H	5.30 H
Hydro-priming	10.91 G	8.91 G	7.79 G	6.47 G
Priming with Potassium Nitrate (KNO_3) 0.5%	15.00 B	13.00 B	11.88 B	10.56 B
Priming with Potassium Nitrate (KNO_3) 1%	15.18 A	13.18 A	12.06 A	10.74 A
Priming with Zinc Sulphate ($ZnSO_4$) 0.5%	13.92 D	11.92 D	10.80 D	9.48 D
Priming with Zinc Sulphate ($ZnSO_4$) 1%	14.05 C	12.05 C	10.93 C	9.61 C
Priming with Boric Acid (H_3BO_3) 0.5%	13.54 F	11.54 F	10.42 F	9.10 F
Priming with Boric Acid (H_3BO_3) 1%	13.66 E	11.66 E	10.54 E	9.22 E
Salinity Level (SL)				
Control	13.34 A	11.34 A	10.22 A	8.90 A
2. 75 mM NaCl	13.25 B	11.25 B	10.13 B	8.81 B
150 mM NaCl	13.16 C	11.16 C	10.04 C	8.72 C
LSD (T) ($p \leq 0.05$)	**	**	**	**
LSD (SL) ($p \leq 0.05$)	**	**	**	**
T × SL ($p \leq 0.05$)	**	**	**	**

Note: Means followed by different letters are significantly different from one another at $p \leq 0.05$ using LSD test.

Conclusion

From the findings of the above study, it can be concluded that seed priming can improved seed emergence rate and other growth parameters of different maize cultivars. However, osmo-priming with 1% of salt KNO_3 was the most effective method of priming which resulted in higher germination rate along with all growth parameters for all maize cultivars as compared to hydro, $ZnSO_4$ and H_3BO_3 priming techniques at various salinity levels.

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