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INFLUENCE OF SEED PRIMING AND NITROGEN APPLICATION ON THE GROWTH AND DEVELOPMENT OF MAIZE SEEDLINGS IN SALINE CONDITIONS

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Abstract

Seed priming and nitrogen application can promote plant tolerance and resistance to salt stress. To explore the combined effects of these two factors on the growth of salt-stressed seedlings, four treatments (priming + nitrogen application, PN; priming + no nitrogen application, P; unprimed + nitrogen application, UPN; and control treatment unprimed + no nitrogen application, UP) were applied to evaluate the responses of plant morphology, antioxidase systems, physiological and biochemical parameters of the maize seedlings under different concentrations of salt stress (0, 100, 200, and 300 m*M*). The results indicated that under salt stress, the priming treatment facilitated the growth of seedlings of root and stems, increased the amount of osmoregulatory substances, and enhanced the antioxidase activity and resistance of the maize seedlings. After nitrogen application during the maize growth stage, the growth of young leaves was greatly promoted along with an increase in the soluble protein and chlorophyll content. The combination of seed priming and nitrogen application significantly improved the plant growth, antioxidase activities and physiological and biochemical parameters.

Key words: Seed priming, Nitrogen, Salt stress, Maize seedlings.

Introduction

Soil salinization refers to the process of salt accumulation in the soil surface due to the evaporation of salt-laden water moving upwards from bottom soil or groundwater via capillary action (Metternicht & Zinck, 2003). In recent years, the area of saline soil has been increasing on a global scale due to population growth and the intensification of human activities and climate change. In inland China, the area of saline soil has reached 34.3 million hectares and there are ~17.3 million hectares of potentially salinized soil that is prone to secondary salinization due to improper soil development and utilization (Wang, 1993). This phenomenon will result in the deterioration of soil quality and a reduction in available soil area, thus causing a large adverse impact on crop yield.

Maize is one of the major food crops whose yield is restricted by soil salinization. The growth of maize can be affected to a varying extent by salt-alkali stress, leading to crop failure. Therefore, it is urgent to promote the salt tolerance of maize seedlings.

Seed priming (or osmotic adjustment), proposed in 1973, is a seed preprocessing method (Heydecker et al., 1973). Seeds are first soaked in a solution with a low water potential so that the seeds can absorb water slowly and fully. Then the seeds are dried at room temperature to their original moisture content, dehydrated to their original state. This method can promote the germination ability of seeds under stress conditions (Kulkarni & Shanna., 1998; Ulfat *et al*., 2017). Nitrogen is an indispensable macroelement during seedlings development and growth, and maize is very sensitive to nitrogen deficiency during growth. If nitrogen deficiency occurs, maize plants cannot develop normally and their yield will decline (Kitchen *et al*., 2010). It has been reported that the appropriate use of nitrogen fertilizer during crop growth is conducive to improvements in photosynthetic efficiency and the activity of antioxidant enzyme systems (Ramalho *et al*., 2000; Misra & Gupta., 2006). However, very few studies have investigated the effects of seed priming in conjunction with nitrogen applications on salt-stressed seedlings. This article focuses on the combined effects of seed priming and nitrogen treatments on the growth and development of maize seedlings under salt stress. The results provide a theoretical basis for the use of priming technology to improve crop production in saline soils.

Materials and methods

Experimental materials: The maize seed *Zea mays* L.'Xianyu 335' used in the experiment were provided by Jilin Agricultural University.

Seed priming: Seeds were surface sterilized and then primed for 24 hours by soaking in 100 m*M* KCl at 15 °C then rinsed with distilled deionized water (dd H_2O) for 2 min to wash off the KCl solution from the surface of the seeds. The primed seeds were slowly dried at room temperature for 2 days to their original (non-primed) moisture content (9- 10%); the control seeds received no priming treatment.

Salt stress and nitrogen application: Farmland soil was sieved and placed in a plastic pot with a diameter of 25 cm and a height of 28 cm. Then, NaCl solutions of different concentrations (0, 100, 200, and 300 m*M*) were used to imitate salt stress, with six replicates for treatment of each salt concentration. Before sowing, each pot was flood-irrigated with one of the NaCl solutions. Fifteen primed or control seeds were sown in each pot. After entering the seedling stage, 1L of a 3% urea solution was

added to the nitrogen application group. For each salt concentration, three pots received the nitrogen treatment and three pots were irrigated with an equal amount of tap water (control).

In total, four treatments (priming + nitrogen application, PN; priming + no nitrogen application, P; unprimed + nitrogen application, UPN; and unprimed + no nitrogen application, UP) were applied in each of the salt concentration treatments.

Measurement indicators: Biomass: After 30 days of treatment, the stem length (the length from the base of the plant to the base of the uppermost blade), root length, seedling height (absolute height above ground), number of leaves, fresh weight, and dry weight were determined.
Leaf area: The area of all the leaves of each seedling was measured using a laser leaf area meter (YMJ-C, TOP Instrument, Zhejiang).

Physiological and biochemical parameters: The malonaldehyde (MDA) and soluble protein contents were determined with a protein quantification kit manufactured by the Nanjing Jiancheng Bioengineering Institute. The proline and chlorophyll contents were determined according to established methods reported in literature (Troll & Lindsley., 1955; Zhang & Qu, 2006).

Antioxidase system: Superoxide dismutase (SOD) and peroxidase (POD) were determined using the enzyme assay kit manufactured by the Nanjing Jiancheng Bioengineering Institute.

Statistical analyses

SPSS16.0 was used for data processing. A one-way analysis of variance was performed to determine the differences between all the treatments and a *P*-value of < 0.05 was used to indicate significant differences. Two-way ANOVA was used to test the effects of the main factors (priming, nitrogen and salinity) and their interactions on the content of biochemical parameters (soluble protein, proline, Chlorophyll, MDA) and enzyme activities (SOD, POD).

Results

Influence of different treatments on maize seedling growth: As seen in Table 1, the stem lengths of the UP group were the shortest, whereas those of the PN group were the longest under the same salt concentrations. When compared with other salt concentrations, the stem lengths of seedlings from each treatment in 100 m*M* salt solution reached or approximately reached the maximum. The descending order of stem length was: PN > P > UPN > UP. The priming treatment group had significant longer stem lengths compared with the non-priming treatment group ($p<0.05$). For the 200 m*M* salt solution, the seedlings receiving the nitrogen application had longer stem lengths than those with no nitrogen. The priming treatment had a positive effect on root growth. For the seedlings stressed with a salt concentration of 100 m*M*, the root lengths of the P group were higher than the other groups ($p<0.05$). The priming and nitrogen treatments had the greatest impacts on seedlings height, and the values of the PN group were higher than other three groups under all tested concentrations of salt stresses ($p<0.05$). In the PN group, the seedlings height decreased significantly with increasing salt concentration. Obvious changes were found in other treatment groups with the salt concentrations.

Table 1. Effects of different treatments on seedling growth.

	Stress concentration	Treatments			
	(m*M*)	PN	UPN	P	UP
Stem length (cm)	0	9.23 ± 1.19^{Aa}	7.41 ± 1.93^{Bb}	8.33 ± 0.96^{ABb}	7.43 ± 0.59^{Ba}
	100	9.76 ± 1.11^{Aa}	8.57 ± 1.08^{BCa}	9.20 ± 1.39^{ABa}	7.77 ± 1.12^{Ca}
	200	9.07 ± 1.67^{Aa}	8.61 ± 1.02^{ABa}	7.43 ± 1.27^{Bc}	6.90 ± 0.83^{Ba}
	300	7.62 ± 0.57^{Ab}	7.20 ± 0.56^{Ab}	7.22 ± 0.89^{Ac}	5.87 ± 1.36^{Bb}
Root length (cm)	0	73.67 ± 7.51^{Aa}	54.47 ± 8.57^{ABa}	62.83 ± 14.15^{ABa}	47.73 ± 3.89^{Ba}
	100	59.67 ± 6.03^{Ab}	59.00 ± 19.31^{Aa}	75.33 ± 7.23^{Aa}	69.33 ± 22.94^{Aa}
	200	55.00 ± 6.08^{Bb}	50.00 ± 7.00^{Ba}	68.67 ± 10.60^{Aa}	47.00 ± 3.46^{Ba}
	300	49.33 ± 7.02^{Ab}	43.83 ± 3.55^{Aa}	43.00 ± 2.65^{Ab}	36.07 ± 11.33^{Aa}
Seedling height (cm)	0	65.10 ± 5.96^{Aa}	57.30 ± 7.78^{Ba}	48.77 ± 6.84^{Ca}	49.83 ± 4.17^{Ca}
	100	59.23 ± 4.46^{Ab}	44.80 ± 3.19^{Bb}	46.83 ± 4.72^{Ba}	37.97 ± 6.82^{Cb}
	200	48.03 ± 7.61^{Ac}	40.20 ± 3.32^{Bb}	35.10 ± 3.89^{Cb}	35.83 ± 4.09^{Cb}
	300	36.83 ± 3.16^{Ad}	34.20 ± 2.13^{Bc}	32.93 ± 2.87^{Bb}	28.50 ± 4.28^{Cc}

priming + nitrogen application, PN; unpriming+ nitrogen application, UPN; priming + no nitrogen application, P; and unpriming + no nitrogen application, UP. Capital letters represent significant differences among treatment groups within the same salt stress ($p<0.05$). Small letters represent significant differences among salt stress within the same treatment groups ($p<0.05$).

Table 2. Effects of different treatments on seedling dry weight (g) and water content (%)

	Stress concentration	Treatments			
	(m*M*)	PN	UPN	P	UP
Aboveground dry weight (g plant^{-1})	0	3.98 ± 1.73^{Aa}	0.81 ± 0.44^{Ba}	2.28 ± 0.95^{Ba}	0.97 ± 0.26^{Ba}
	100	3.62 ± 1.80^{Aa}	0.76 ± 0.38^{Ba}	1.79 ± 0.75^{Bab}	0.74 ± 0.48^{Ba}
	200	2.93 ± 1.7 3^{Aab}	1.08 ± 0.49^{Ba}	1.22 ± 0.76^{Bbc}	0.59 ± 0.22^{Ba}
	300	1.29 ± 0.60^{Ab}	0.63 ± 0.36^{Aa}	0.65 ± 0.28^{Ac}	0.85 ± 0.66^{Aa}
Belowground dry weight (g plant^{-1})	0	1.77 ± 0.12^{Aa}	0.66 ± 0.08^{Ca}	2.03 ± 0.20^{Aa}	1.30 ± 0.16^{Ba}
	100	1.06 ± 0.24^{Ab}	0.60 ± 0.09^{Ba}	0.68 ± 0.05^{Bc}	0.89 ± 0.15^{Ab}
	200	0.76 ± 0.08^{Bb}	0.84 ± 0.13^{Ba}	1.36 ± 0.29^{Ab}	0.86 ± 0.14^{Bb}
	300	0.74 ± 0.10^{Ab}	0.71 ± 0.18^{Aa}	0.55 ± 0.07^{Ac}	0.64 ± 0.06^{Ab}
Aboveground water content (%)	0	78.52 ± 8.82^{Ac}	82.39 ± 4.12^{Aa}	79.50 ± 6.07^{Aa}	82.91 ± 3.26^{Aa}
	100	85.75 ± 6.03^{Ab}	82.11 ± 4.56^{Aa}	83.42 ± 4.36^{Aa}	80.67 ± 4.82^{Aa}
	200	87.57 ± 5.18^{Aa}	80.71 ± 3.34^{Ba}	85.53 ± 4.72^{Ba}	78.79 ± 6.45^{Ba}
	300	78.77 ± 3.59^{Ac}	81.04 ± 5.27^{Aa}	81.42 ± 6.61^{Aa}	71.67 ± 6.48^{Bb}
Belowground water content (%)	0	81.65 ± 0.67^{Bc}	84.41 ± 0.20^{Aa}	76.20 ± 2.63^{Cb}	80.62 ± 0.34^{Ba}
	100	85.63 ± 1.37^{Ab}	82.62 ± 1.42^{Ba}	86.98 ± 1.12^{Aa}	78.87 ± 2.67^{Ca}
	200	86.96 ± 1.35^{Aab}	84.36 ± 2.01^{Aa}	87.06 ± 0.82^{Aa}	82.00 ± 6.30^{Aa}
	300	88.62 ± 0.47^{Aa}	70.00 ± 8.38^{Aa}	85.28 ± 3.32^{Aa}	81.33 ± 3.94^{Aa}

priming + nitrogen application, PN; unpriming+ nitrogen application, UPN; priming + no nitrogen application, P; and unpriming + no nitrogen application, UP. Capital letters represent significant differences among treatment groups within the same salt stress ($p<0.05$). Small letters represent significant differences among salt stress within the same treatment groups ($p<0.05$).

Table 3. Effects of different treatments on seedling leaf number and leaf area

	Stress concentration	Treatments			
	（m*M*）	PN	UPN	P	UP
Leaf numbers	0	5.40 ± 0.74^{Aa}	5.80 ± 0.68^{Aa}	4.33 ± 0.62^{Bab}	4.53 ± 0.52^{Ba}
	100	5.47 ± 0.64^{Aa}	4.53 ± 0.52^{BCb}	4.80 ± 0.41^{Ba}	4.20 ± 0.68^{Ca}
	200	4.80 ± 0.41^{Ab}	4.33 ± 0.82^{ABb}	4.07 ± 0.70^{Bb}	4.20 ± 0.56^{Ba}
	300	4.33 ± 0.49^{Ac}	4.20 ± 0.56^{Ab}	4.40 ± 0.63^{Aab}	4.07 ± 0.96^{Aa}
Leaf area (cm^2 plant^{-1})	0	567.25 ± 98.19^{Aa}	132.90 ± 55.36^{Ca}	258.36 ± 109.73^{Ba}	183.86 ± 42.75^{BCa}
	100	435.46 ± 80.17^{Ab}	59.24 ± 26.42^{Cb}	281.39 ± 100.23^{Ba}	96.29 ± 28.38^{Cb}
	200	239.23 ± 51.27^{Ac}	134.26 ± 34.01^{Ba}	162.60 ± 33.34^{Bb}	73.57 ± 18.54^{Cb}
	300	163.28 ± 24.85^{Ad}	63.67 ± 29.09^{Bb}	68.62 ± 11.10^{Bc}	39.52 ± 1.71^{Cc}

priming + nitrogen application, PN; unpriming+ nitrogen application, UPN; priming + no nitrogen application, P; and unpriming + no nitrogen application, UP. Capital letters represent significant differences among treatment groups within the same salt stress ($p<0.05$). Small letters represent significant differences among salt stress within the same treatment groups ($p<0.05$).

Influence of different treatments on dry weight and water content of maize seedlings: Table 2 shows the influence of the different treatments on the dry weight and water content of aboveground and belowground sections of the maize seedlings. The PN treatment significantly increased the dry weight of the aboveground section under the 0, 100, and 200 m*M* salt concentrations. The dry weight of belowground seedling sections varied with salt concentration among the different treatment groups. Under the 200 m*M* salt concentrations, the P treatment significantly increased the belowground dry weights compared with the other treatments ($p<0.05$). Under moderate salt stress (100 and 200 m*M*), the priming groups had higher water contents than those of the non-priming groups, the nitrogen treatments increased water contents compared with non-nitrogen treatments,. Under the 200 m*M* salt stress, the water content of the aboveground section of PN treatment was the highest. Moreover, compared with no salt stress groups, the priming treatments significantly increased the water content of belowground seedling sections under salt stress (100, 200 and 300 m*M*) ($p<0.05$).

Influence of different treatments on number of leaves and leaf area of maize seedlings: The influence of different treatments on the number of leaves is shown in Table 3. Compared with the groups without the nitrogen application, those with the nitrogen application had higher leaf numbers at low and moderate salt stress (0, 100, and 200 m*M*). The PN treatment significantly increased the leaf number of seedlings at a salt concentration of 100 m*M* ($p<0.05$). With the increase of salt stress, the leaf area decreased significantly, except the UPN group at 100 m*M* salt concentration. Interestingly, the priming treatments had a significant impact on the leaf area of the maize seedlings ($p<0.05$). No matter with or without nitrogen treatment, the leaf area with priming treatment was higher than without priming treatment.

Influence of different treatments on biochemical parameters: As seen from Fig. 1A, the content of soluble protein, proline and chlorophyll was significantly affected by salinity and priming or nitrogen treatment ($p<0.001$).

The average soluble protein content in the nitrogen application groups was significantly higher than those with no nitrogen application under the same salinity stress level ($p<0.05$). At salt concentrations of 200 and 300 m*M*, priming treatment significantly increased soluble protein content. The PN group had a higher soluble protein content than the other treatment groups at salt concentrations of 300 m*M*.

As shown in Fig. 1B, No significant differences were observed between different treatments at a salt concentrations of 0, 100, 200 m*M*. Higher salt stress (300 m*M)* significantly increased the proline content ($p<0.05$). Under high salt stress (300 m*M*), maize seedlings in the nitrogen application groups had significantly decreased proline content when compared with those without nitrogen application.

It can be seen from Fig. 1C that the chlorophyll content was higher in the nitrogen treatment groups under the same salt-stress conditions except in P group under 200 m*M* salt concentration. Within the nitrogen application groups, chlorophyll content of the priming groups was significantly higher than unprimed groups under some salt stress conditions (0, 100 and 300 m*M*). The chlorophyll content was lowest at UP groups under 200 and 300 m*M* salt concentrations.

Influence of different treatments on SOD, POD, and MDA contents: The enzyme activity was significantly affected by the treatment (priming or nitrogen), and the interaction with salinity ($p<0.01$) (Table 4).

The SOD activity of the PN group was higher than the UPN group, with significant differences under 0 and 100 m*M* salt concentrations ($p<0.05$), respectively (Fig. 2A). The SOD activity was lower in the P group than in the UP group in 100 m*M* salt concentration, and no significant differences were observed in other salt concentrations. When compared with different salt treatments in the priming treatment, the SOD activity was significantly higher under 300 m*M* than other salinity concentrations ($p<0.05$).

It can be concluded from Figure 2B that under salt stress, the priming and nitrogen treatments reduced the POD activity of the maize seedlings to some extent. Under each salt concentration, the POD activity was significantly lower in the P group than in the UP group ($p<0.05$). Moreover, the POD activity was higher without nitrogen treatment than in the nitrogen application groups in unprimed groups under the same salt concentration ($p<0.05$). However, when the salt stress was higher than 100 m*M*, no significant differences was observed between nitrogen and no nitrogen treatment in priming groups. When Compared with the same treatment of UPN, the POD activity was significantly higher under high salt concentration (200 m*M* , 300 m*M*) than lower salt concentrations ($p<0.05$).

The content of MDA was significantly affected by salinity, treatment (priming or nitrogen) and their interaction ($p<0.001$) (Table 4). The priming treatments significantly reduced the MDA content of the maize seedlings under salt stress when there was no nitrogen application (Fig. 2C). No significant differences among different treatments were observed at 0 m*M* salt concentration. Under the UP treatment, the content of MDA was significantly higher under high salt concentrations (200 and 300 m*M*) than under lower concentrations ($p<0.05$).

Discussion

Influence of different treatments on growth of maize seedlings: It is clear from the results that increase in salt stress reduced the germination and growth of the maize seedlings (Table 1). The mechanisms by which salt affects germination are not clear, but osmotic and/or toxic effects may be responsible for salt injury (Essa, 2002). However, we know that seed priming has resulted in improved germination and growth of many crops under salt-stressed conditions (Zhang & Hu., 2007). Our experiments indicated that under salt stress, the indices of the different plant seedling sections (stem length, seedlings height, root length, dry weight, and water content), number of leaves, and chlorophyll contents of the primed maize seedlings declined at a slower rate and were superior to those of the other treatment groups under the same salt concentrations. Similar results were also obtained by previous study, and the mechanism was proved that priming allowed the hydration of membranes and proteins and the initiation of various metabolic systems, and these processes were arrested when seeds were dried or moisture was withheld (Ashraf & Rauf., 2001).

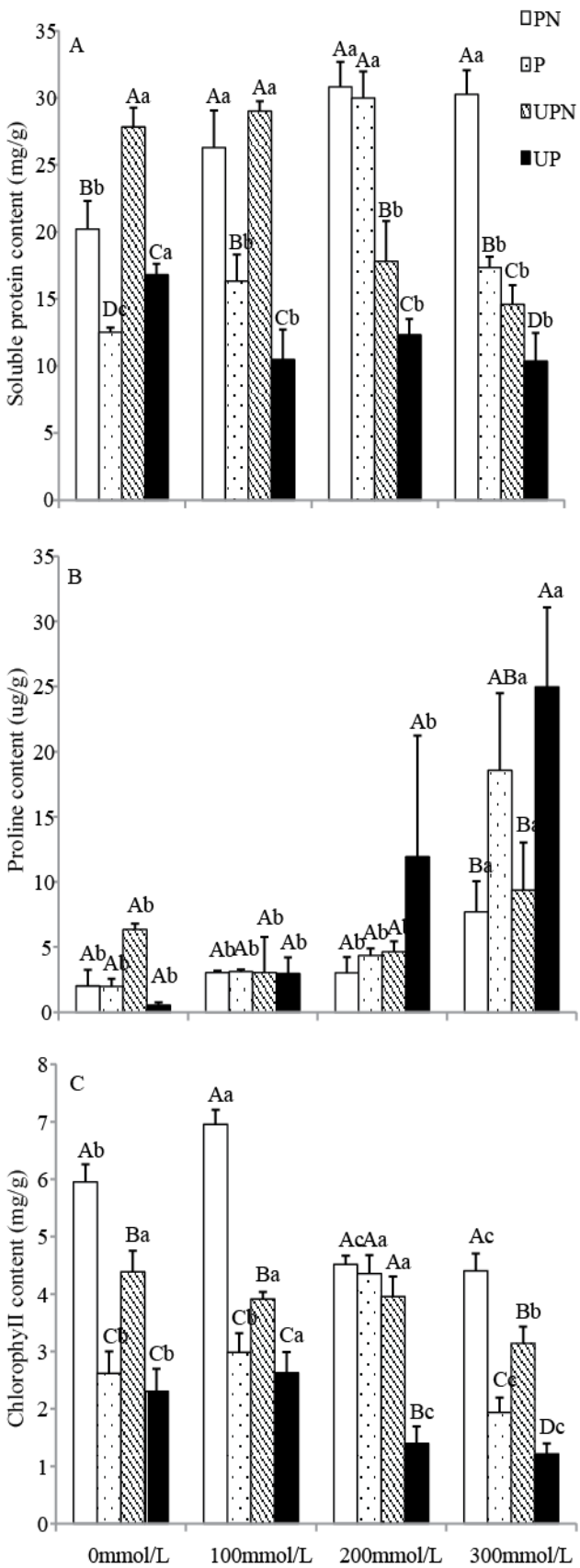

Fig. 1. Effects of different treatments on seedling biochemical indexes (A: soluble protein content, B: proline content, and C: chlorophyll content). Bars represent ±S.D. (n=3) Capital letters represent significant differences among treatment groups within the same salt stress (p <0.05). Small letters represent significant differences among salt stress within the same treatment groups (p<0.05).

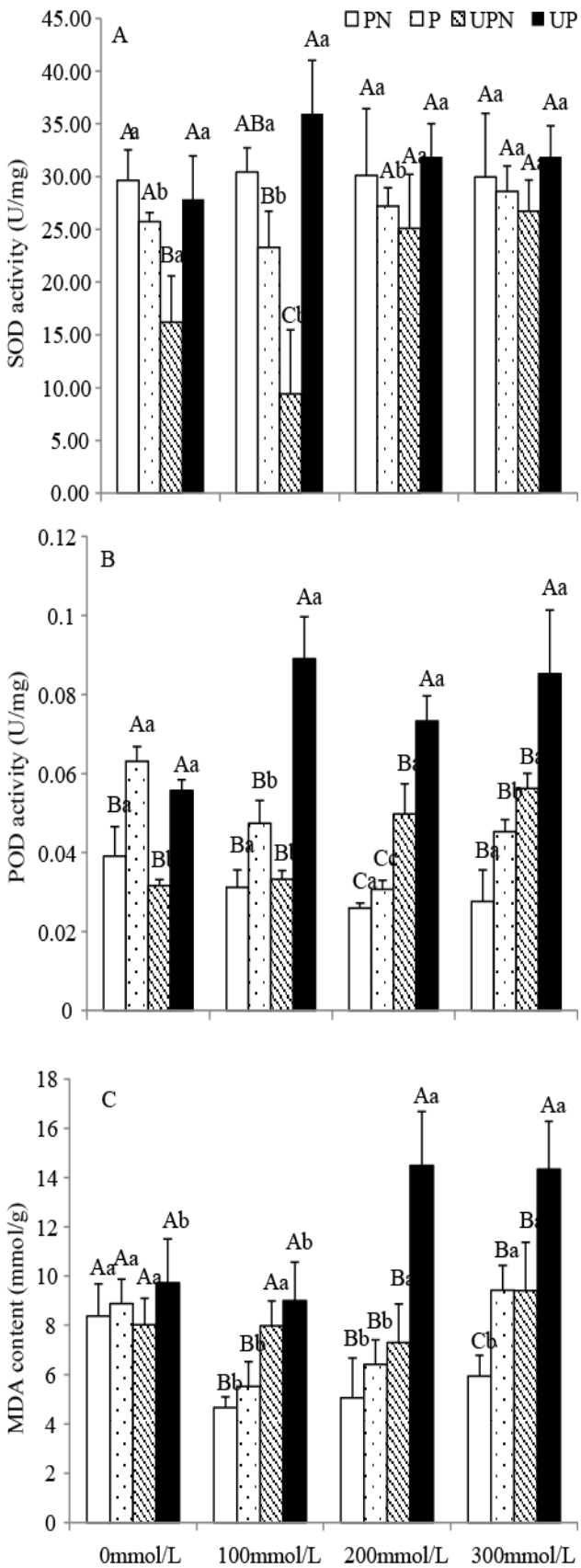

Fig. 2. Effects of different treatments on POD (A) and SOD (B) activity and MDA content (C). Bars represent ± S.D. (n=3). Capital letters represent significant differences among treatment groups within the same salt stress (p<0.05). Small letters represent significant differences among salt stress within the same treatment groups (p<0.05).

Table 4. Two way ANOVA for salinity, nitrogen or priming treatments and their interactions

Independent variable	Soluble protein content				Proline content				Chlorophy Ⅱ content			
	df	Mean-square	F-ratio	Sig	df	Mean-square	F-ratio	Sig	df	Mean-square	F-ratio	Sig.
Salinity	3	45.95	14.83	0.000	3	471.53	39.50	0.000	3	5.05	57.55	0.000
Priming or Nitrogen treatment	3	439.36	141.81	0.000	3	94.40	7.91	0.000	3	26.23	299.22	0.000
Salinity x Priming or Nitrogen treatment	9	126.80	40.93	0.000	9	52.11	4.37	0.001	9	1.78	20.27	0.000
Independent variable	**SOD**				**POD**				**MDA content**			
	df	Mean-square	F-ratio	Sig	df	Mean-square	F-ratio	Sig	df	Mean-square	F-ratio	Sig.
Salinity	3	112.16	5.72	0.003	3	0.00	2.07	0.124	3	18.41	11.96	0.000
Priming or Nitrogen treatment	3	369.10	18.84	0.000	3	0.004	55.34	0.000	3	74.56	48.43	0.000
Salinity x Priming or Nitrogen treatment	9	67.70	3.46	0.004	9	0.001	6.76	0.000	9	9.54	6.20	0.000

Nitrogen is an essential element for seedlings growth and development, and nitrogen application can positively influence the morphological characteristics, such as seedlings type, leaf shape, and stem and leaf weight (Bloomfield *et al*., 2014). It was found in our study that the combination of seed priming and nitrogen treatments facilitate the growth of the aboveground seedling sections and the accumulation of biomass of the salt-stressed seedlings. Under a salt concentration of 100 m*M*, the indices of the aboveground seedling sections of the PN group were superior to those of the other groups (P, UPN, UP). It is evident that the combination of seed priming and nitrogen application improved the adaptability of seedling's roots to salt stress and enabled the redistribution of nutrients and energy within the seedlings. Thus, the nitrogen or other nutrients absorbed by the roots were preferentially supplied to the aboveground seedling sections to promote their growth. The P treatment was superior to the PN treatment under high salt stress. Moreover, all the root indices in the P group were significantly higher than those in the other groups at a salt concentration of 200 m*M*. This may be because the nitrogen application at high salt stress increased the solute content in the soil, reduced the water potential, and inhibited the water absorption by roots, thus making it possible for cells to expand and divide normally. Nitrogen is a critical component of chlorophyll (Sandhu *et al*., 1986), and the nitrogen application in our study increased the number of leaves, leaf area, and chlorophyll content of the salt-stressed maize seedlings. Of the four treatments, PN caused the most evident increases in the leaf area and chlorophyll content of the maize seedlings under salt stress. Seed priming in conjunction with nitrogen application resulted in a greater increase in the growth of young maize leaves compared with either treatment alone

Influence of different treatments on physiological and biochemical indices: Osmoregulation can occur in plants by the active uptake of organic solutes (e.g., sugar, proline, and soluble protein), which varies among species (Jan *et al*., 2017; Guan *et al*., 2017). The results of this study clearly showed that priming enhanced the proline and soluble protein accumulation in the maize seedlings (Fig. 1A, 1B). Recent studies have suggested that various impacts of salt stress on seedlings can be detected using indicators such as proline. However, similar impacts have not yet been discovered in seedlings tissues under unfavorable conditions such as drought, salinity stress, extreme temperature, and inadequate light intensity (Abdelkader & Esawy., 2011). The higher capacity of the primed seedlings to adapt to salt stress could be due to osmoregulation induced by organic solutes by *Sivritepe* et al. (Sivritepe *et al*., 2003). Our results also indicated that the application of nitrogen could increase the soluble protein content of maize seedlings.

Seedlings antioxidase activity was greatly increased under high salt stress. These antioxidases can protect cells against excess oxygen free radicals by synergistically scavenging them (Dionisio & Tobita., 1998). SOD can rapidly catalyze oxygen free radicals and water molecules into hydrogen peroxide and oxygen molecules (Dou *et al*., 2010). In this experiment, the SOD activity was lower in the priming treatments than in the non-primed treatments at each salt concentration, which differed from the conclusion reported by Amooaghaie (2011). Priming may selectively enhance other antioxidation mechanisms while inhibiting SOD activity according to the oxidative status of the plasma membrane (Chen & Arora., 2011). Under salt stress, the groups receiving the nitrogen application had lower SOD activity compared with those without nitrogen application. Wang et al.(2010) argued that nitrogen application alone will not necessarily promote SOD activity and may inhibit antioxidase activity in plants. However, the SOD activity increased after the PN treatment. This suggests that priming or nitrogen treatments cannot promote SOD activity alone, but the combination of the two treatments can achieve a promoting effect to some extent.

POD not only participates in the degradation of chlorophyll and the production of reactive oxygen species, but it is also a component of the enzyme system that can protect cells against damage by reactive oxygen species (Yan

et al., 1996). Under salt stress, the groups with the nitrogen application had lower POD activity than those without the nitrogen treatment. It has been reported that nitrogen application alone will not necessarily enhance POD activity (Guo *et al*., 2010). Here, POD activity declined in the PN treatment, which may be explained by the fact that POD activity is likely more efficient under lower hydrogen peroxide concentrations. As seed priming and nitrogen application enhance seedling's reactive oxygen species scavenging system, the activity of POD declines under low hydrogen peroxide concentrations, whereas that of other antioxidases increases (Mitler, 2002).

Meloni et al. (2003) showed that MDA was produced when polyunsaturated fatty acids in cell membranes underwent peroxidation, and lower degrees of membrane damage correlated with low MDA concentrations. We arrived at the same conclusion as *Polesskaya* et al. (2006), that is, seed priming and nitrogen treatments applied together can reduce the MDA content of seedlings. In this work, the MDA content decreased after the PN treatment, indicating that the combination of priming and nitrogen treatments can reduce the damage caused by salt stress to plasma membranes and enhance seedlings tolerance.

Taken together, this study showed for the first time that KCl priming and nitrogen application could be used to increase the salt tolerance of maize seedlings. We are conducting other studies to investigate whether the beneficial effects of KCl priming and nitrogen persist in the later growth and development stages of maize seedlings.

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References

Abdelkader, A.F. and M.A. Esawy. 2011. Case study of a biological control: Geobacilluscaldoxylosilyticus (IRD) contributes to alleviate salt stress in maize (*Zea mays* L.) plants. *Acta. Physiol. Plant*., 33(6): 2289-2299.

Amooaghaie, R. 2011. The effect of hydro and osmopriming on alfalfa seed germination and antioxidant defenses under salt stress. *Afr. J. Biotechnol*., 10(33): 6269-6275.

Ashraf, M. and H. Rauf. 2001. Inducing salt tolerance in maize (*Zea mays* L.) through seed priming with chloride salts: Growth and ion transport at early growth stages. *Acta. Physiol. Plant*., 23(4):407-414.

Bloomfield, K.J., G.D. Farquhar and J. Lloyd. 2014. Photosynthesis–nitrogen relationships in tropical forest tree species as affected by soil phosphorus availability: a controlled environment study. *Funct. Plant. Biol*., 41(8): 820-832.

Chen, K. and R. Arora. 2011. Dynamics of the antioxidant system during seed osmopriming, post-priming germination, and seedling establishment in Spinach (Spinaciaoleracea). *Plant. Sci*., 180(2): 212-220.

Dionisio-Sese, M.L. and S. Tobita. 1998. Antioxidant responses of rice seedlings to salinity stress. *Plant. Sci*., 135(1): 1-9.

Dou, J.H., S.X. Yu, S.L. Fan, C.Y. Pang and M.Z. Song. 2010. SOD and Plant Stress Resistance. *Mol. Plant. Breed*., 8(2): 359-364.

Essa, T.A. 2002. Effect of salinity stress on growth and nutrient composition of three soybean (*Glycine max* L. Merrill) cultivars. *J. Agron. Crop. Sci*., 188(2): 86-93.

Guan, B., J.B. Yu, A.X. Hou, G.X. Han, G.M. Wang, F.Z Qu, J.B. Xia and X.H. Wang. 2017. The Ecological adaptability of *Phragmites australis* to interactive effects of water level and salt stress in the yellow river delta. *Aquat. Ecol*., 51: 107-116.

Guo, W.Q., B.L. Chen, R.X. Liu and Z.G. Zhou. 2010. Effects of nitrogen application rate on cotton leaf antioxidant enzyme activities and endogenous hormone contents under short-term waterlogging at flowering and boll-forming stage. *Chinese J. App. Ecol.*, 21(1): 53-60.

Heydecker, W.J., J. Higgins and K. Gulliver. 1973. Accelerated germination by osmotic seed treatment. *Nature*, 246: 42-46.

Jan, S.U.M. Jamil, H. Alipour, M.F. Bhatti and A. Gul. 2017. Analysis of salinity tolerance potential in synthetic hexaploid wheat. *Pak. J. Bot*., 49(4): 1269-1278.

Kitchen, N.R., K.A. Sudduth, S.T. Drummond, P.C. Scharf and H.L. Palm. 2010. Ground-based canopy reflectance sensing for variable-rate nitrogen corn fertilization. *Agron. J*., 102(1): 71-84.

Kulkarni, G.N. and M.R. Shanna. 1998. Effect of pre-soaking of corn seed on seed quality. *Seed. Res.*, 16: 37-46.

Meloni, T., D.A. Oliva, M.A. Martinez and C.A. Cambraia. 2003. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environ. Exp. Bot.*, 49(1): 69-76.

Metternicht, G.I. and J.A. Zinck. 2003. Remote sensing of soil salinity: potentials and constraints. *Remote. Sens. Environ*., 85(1): 1-20.

Misra, N. and A.K. Gupta. 2006. Effect of salinity and different nitrogen sources on the activity of antioxidant enzymes and indole alkaloid content in Catharanthusroseus seedlings. *J. Plant. Physiol.*, 163(1): 11-18.

Mitler, R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends. Plant. Sci*., 7: 405-410.

Polesskaya, O.G., E.I. Kashirina and N.D. Alekhina. 2006. Effect of salt stress on antioxidant system of plants as related to nitrogen nutrition. *Russ. J. Plant. Physl.*, 58(2): 186-192.

Ramalho, J.C., T.L. Pons, H.W. Groeneveld, H.G. Azinheira and M.A. Nunes. 2000. Photosynthetic acclimation to high light conditions in mature leaves of *Coffeaarabica* L.: Role of xanthophylls, quenching mechanisms and nitrogen nutrition. *Aust. J. Plant. Physiol.*, 27(1): 43-51.

Sandhu, B.S., B. Singh, B. Singh and K.L. Khera. 1986. Maize response to intermittent submergence, straw mulching and supplemental N-fertilization in subtropical region. *Plant. Soil*, l96:45-56.

Sivritepe, N., H.O. Sivritepe and A. Eris. 2003. The effects of NaCl priming on salt tolerance in melon seedlings grown under saline conditions. *Sci. Hortic-Amsterdam*., 97(3): 229-237.

Troll, W. and J. Lindsley. 1955. A photometric methods for the determination of proline. *J. Biol. Chem*., 215(2): 655-660.

Ulfat, A., S.A. Majid and A. Hameed. 2017. Hormonal seed priming improves wheat (*Triticum aestivum* L.) field performance under drought and non-stress condition. *Pak. J. Bot*., 49(4): 1239-1253.

Wang, Y.B., L. Wang, S.G. Shan and Y.L. Bai. 2010. Effects of amounts of N application on seedlings growth of maize and the activity of major antioxidant enzymes. The Chinese society of plant nutrition and fertilizer 2010 academic essays, Beijing.

Wang, Z. 1993. China Saline Soil. *Chinese Science.*, Beijing, China p. 1-2.

Yan, B., Q.J. Dai, X.Z. Liu, Huang, S.B. and Z.X. Wang. 1996. Flooding induced membrane damage, lipid oxidation and activated oxygen generation in corn leaves. *Plant. Soil*, 179: 261-268.

Zhang, S. and J. Hu. 2007. Seed priming with brassinolide improves lucerne (*Medicago sativa* L.) seed germination and seedling growth in relation to physiological changes under salinity stress. *Aust. J. Agr. Res*., 58: 811-815.

Zhang, Z.L. and W.J. Qu. 2006. Experimental guide of physiology plant. Higher Education, Beijing, China p.74-76.

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