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SEED PRIMING WITH MELATONIN EFFECTS ON SEED GERMINATION AND SEEDLING GROWTH IN MAIZE UNDER SALINITY STRESS

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Abstract

The effects on seed germination and seedling growth in maize under salinity stress by seed priming with melatonin were investigated. Seeds of maize cultivar Nonghua101 were soaked in 0.4, 0.8 and 1.6 mM aerated solution of melatonin for 24 h, and primed seeds were germinated under the condition of 150 mM NaCl with paper media. The results showed seed priming with 0.8 mM melatonin was the best performance of all the treatments to seed germination and seedling growth in maize under salinity stress. Then primed with 0.8 mM melatonin or water for 24 h and unprimed seeds were germination under the condition of 150 mM NaCl with sand media. The results showed seed priming with 0.8 mM melatonin significantly improved germination energy, germination percentage, seedling vigor index, shoot and root lengths, seedling fresh and dry weights, K^+ content, relative water content, proline and total phenolic contents, superoxide dismutase, catalase and phenylalanin ammonia lyase activities; and significantly decreased mean emergence time, Na^+ content, electrolyte leakage and malondialdehyde content compared with untreated seeds under salinity stress. These results suggest that seed priming with melatonin alleviates the salinity damage to maize and seed priming with melatonin may be an important alternative approach to decrease the impact of salinity stress in maize.

Keywords: Seed priming, Salinity stress, Melatonin, Maize

Introduction

Salinity, one of the major abiotic stresses, which limits growth and development of crop plants (Rameeh, 2012). More than 800 million hectares of land is badly affected by salinity worldwide, which is a critical agricultural problem leading to low production (Munns, 2005). It is largely due to the ionic and osmotic stress that salinity adversely affects growth and metabolic processes in a wide variety of crops, some basic metabolic processes in plants including carbohydrate metabolism, lipid metabolism and protein synthesis (Parida & Das, 2005). Ion toxicity is due to high concentration of Na^+ in plant tissues comes into being membrane dysfunction and inhibition of cellular metabolism (Niu *et al*., 1995), which leads to adverse effects on plant growth and development. Osmotic stress is due to high concentration of Na^+ causing water deficiency of plant cell results in a reduction in the water potential (Munns &Tester, 2008). To alleviate the adverse effects of salinity stress on plants, a variety of approaches have been adopted to regulate the osmotic homeostasis and ion balance and to prevent damage (Ghosh *et al*., 2011; Abbasi *et al*., 2015; Ibrahim, 2015; Kurotani *et al*. 2015; Liu *et al*., 2015). Among various measures, seed priming is believed as an effective and simple way to improve the performance of plants growth under stressful conditions compared to plants grown from unprimed seed (Iqbal & Ashraf, 2007). Seed priming as a seed pre-sowing treatment, which exposes seeds to a certain solution that allows partial hydration but not germination, and redried to original moisture content. During priming, the germination process is not completed, but metabolic activities for radicle protrusion may be initiated (Heydecker *et al*., 1973). Usually, seed priming techniques include hydropriming (soaking seed in water), osmopriming (soaking seed in osmotic solutions such as polyethylene glycol), halopriming (soaking seed in salt solutions such as potassium chloride), matripriming (soaking seed in matrix such as sand) and priming with plant growth hormones such as thiamin (Afzal *et al*., 2013; Farooq *et al*., 2015).

Melatonin is a well-known regulator of circadian rhythm and sleep in animals (Mishima, 2012). It is also a natural occurring compound which has been detected in seeds, roots, fruits, and leaves in plants (Reiter *et al*., 2001; Zohar *et al*., 2011). Many evidences have shown melatonin could alleviate biotic and abiotic stresses such as copper, senescence, temperature, salinity, light, drought and pathogen (Zhang *et al*., 2014). Moreover, melatonin also could regulate the expression of a large number of genes involved in plant stress defense (Weeda *et al*., 2014; Zhang *et al*., 2014). However, little information is available pertaining to the improvement of the performance of plants growth under stress by seed priming with melatonin.

Maize (*Zea mays* L.) as one of the most important crops, is widely used for food and feed all over the world and is considered as a salt sensitive species (Farooq *et al*., 2015). Poor seed germination and early seedling growth in maize farms are often occured due to high level of salinity of soil (Zhang *et al*., 2007). Previous studies have found that seed priming with chloride salts, silicon, gibberellic acid (GA3) and etc. improve the performance of plants growth under stress in maize (Ashraf & Rauf, 2001; Zhang *et al*., 2007; Ghodrat & Rousta, 2012; Abdel & Tran, 2016). However, no research on seed priming with

melatonin alleviates salt stress in maize. In view of the role of melatonin in stress tolerance, we put forward the hypothesis seed priming with melatonin can improve the salinity tolerance in maize. Therefore, the primary objective of this study was to examine the effects of seed priming with melatonin on seed germination and early seedling growth stages in maize under salinity stress, and the optimal concentration of melatonin for seed priming.

Materials and Methods

Plant materials and treatments: Seeds of maize cultivar Nonghua101, used in this study, were harvested in 2013 and obtained from Beijing Golden Agricultural Seed Technology Company (Beijing, China). Initial moisture content is 11.06 % and initial germination percentage was 93.33 %. The following experiments were carried out at Qingdao Agricultural University, Qingdao.

Experiment 1: Seeds after surface-sterilized with 0.5% sodium hypochlorite for 10 min were soaked in 0.4, 0.8 and 1.6 mM melatonin solution (melatoninpriming) for 24 h at 20°C, and were recorded as T1, T2 and T3, respectively; seeds soaked in aerated water (hydropriming) for 24 h were used for CK1 (normal) and CK2 (salinity stress). Keeping seed to solution ratio of 1:5 (w:v) (Farooq *et al*., 2013). Seeds were then removed, rinsed thoroughly with sterile distilled water and re-dried near to their original weight with forced air at 25°C under shade. Thirty seeds for each replication were sown between double layered rolled seed germination papers (10*15 cm, Anchor Paper Co., USA) for 7 days at 25°C in a growth chamber (60% RH, 16 h day length, and light intensity of 250 µmol $m^{-2}s^{-1}$). The rolled papers with seeds were put into plastic bags (20*14 cm) to avoid moisture loss. The seed germination papers for CK2, T1, T2 and T3 were moistened with about 30 ml of Hoagland's solution with 150 mM NaCl, and the seed germination papers for CK1 were moistened with about 30 ml of Hoagland's solution, ensuring that the seeds were immerged with solution, and the papers were renewed every 2 days. These experiments were performed with five replicates per treatment, each treatment for one plastic bag.

Experiment 2: After passed through a 2 mm sieve, sand was sterilized and put in plastic pots (volume 150 ml), 100g of sterilized sand per pot. Hoagland nutrient solution content in sterilized sand was 10% (V:W). Salt treatment was performed by supplementing the Hoagland's solution with final concentrations of 150 mM NaCl. Before planted, seeds after surface-sterilized were primed with 0.8 mM melatonin solution for 24 h at 20°C; for comparison, seeds were also soaked in aerated water (hydropriming) for 24 h; seeds not soaked were used as control. There were three replications for each treatment and 10 seedlings per replication. After 12 days, the seedlings were harvested, washed carefully in tap water and surface dried with filter paper. Then plants were separated into two parts: one part was at 105°C for 30min then oven at 80°C up to constant weight for dry weight and K^+ and Na^+ content, another part was used for determination of biochemical index.

Measurement techniques

Evaluation of seed germination and seedling vigor: Seed germination was recorded daily up to the 7th day after the beginning of the experiment. A seed was considered germinated when radical emerged for 2 mm. Germination energy (GE, %) was determined on the 4th day after seed planted, it is the number of emerged seedlings expressed in percentage on the 4th day after sowing (Farooq *et al*., 2006). On the 7th day, plants were harvested to assay for shoot and root lengths (cm) and seedling fresh and dry weights (mg $plant^{-1}$). In each replication, five seedlings were randomly selected, and their averages were considered as sample data. Germination percentage (GP, %), seedling vigor index (VI) and mean emergence time (MET, days) were determined at the end of the experiment, based on the following formula: GP=n/N×100%; MET= ∑Dn/∑N (Ellis & Roberts, 1981), VI = (mean root length + mean shoot length) × Germination percentage (%) (Abdul-Baki & Anderson, 1973). Where n is total number of seeds germinated during the experiment, N is total number of seeds planted, and D is the number of days counted from the beginning of emergence.

Evaluation of physiological characteristics of seedlings: The leaves of maize as samples, relative water content (RWC) was estimated by the method of Abbasi *et al*. (2015) and expressed in percentage, relative electrolyte leakages (EL, %) were determined by measuring the conductivity of leachates owing to damaged plasma membrane as described by Voigt (2009), K^+ and Na^+ content was determinate according to Ghosh *et al*. (2011) and expressed in percentage, proline contents were determined following Bates *et al*. (1973) method, superoxide dismutase (SOD, $Ug^{-1}FW^{-1}$) activities and malonaldehyde (MDA, µ$molg^{-1}FW^{-1}$) contents were measured based on the method of Voigt (2009), catalase (CAT, $Ug^{-1}FW^{-1}min^{-1}$) activities were determined according to the method of Zhang *et al*., (2013), total phenolic contents were determined using Folin-Ciocalteu reagent method as described by Nadernejad *et al*., (2012), phenylalanin ammonia-lyase (PAL, $Ug^{-1}FW^{-1}h^{-1}$) activities were assayed using the method given by Nadernejad *et al*., (2012).

Statistical analyses: In all the experiments, analysis of variance (ANOVA) was conducted using Statistics SPSS 17.0 software. The means of each treatment were compared using Duncan´s multiple range test at 0.05 level. The data are shown as the means.

Results

Effects of seed priming on seed germination: Compared with CK1, CK2 significantly (P<0.05) decreased GE, GP, VI and significantly (P<0.05) increased MET. Compared with CK2, seed priming with

melatonin improved GE, GP, VI and decreased MET. 0.8 mM melatonin priming (melatoninpriming) was the best performance of all the treatments to significantly (P<0.05) increase GE, GP, VI and to significantly (P<0.05) decrease MET (Table 1).

Effects of seed priming on seedling growth: Shoot and root lengths, seedling fresh and dry weights were significantly (P<0.05) decreased by salinity stress (Table 2). However, under normal and salinity stress conditions, melatoninpriming and hydropriming improved shoot and root lengths, seedling fresh and dry weights (Table 2), there was a significant difference for shoot and root lengths, seedling fresh and dry weights between melatoninpriming and control. Melatoninpriming substantially improved shoot and root lengths, seedling fresh and dry weights than hydropriming under normal and salinity stress conditions. Although, there was no difference for shoot and root lengths between melatoninpriming and hydropriming at normal condition, there was a significant difference for seedling fresh and dry weights between melatoninpriming and hydropriming at normal condition, and there was a significant difference for shoot and root lengths, seedling fresh and dry weights between melatoninpriming and hydropriming under salinity stress.

Effects of seed priming on membrane EL and MDA content: Salinity stress significantly (P<0.05) increased membrane EL of leaves of maize cultivar Nonghua101 (Table 3). Under normal and salinity stress conditions, melatoninpriming and hydropriming decreased membrane EL, and there was a significant difference between melatoninpriming and control, and there was also a significant difference between melatoninpriming and hydropriming, and melatoninpriming showed greater decline in membrane EL than hydropriming (Table 3). Likewise, salinity stress also significantly increased MDA contents of leaves (Table 3). Melatoninpriming and hydropriming also decreased MDA contents under normal and salinity stress conditions, and this influence was similar with that of membrane EL.

Table 1. Influence of seed priming with melatonin on the GE, GP, MET and VI in maize under saline stress.

Treatments	GE(%)	GP(%)	MET(days)	VI
CK1	87.00a	96.67a	4.34c	15.11a
CK2	58.89d	83.33c	4.73a	5.47e
	(-32.31%)	(-13.80%)	(+8.99%)	(-63.80%)
T1	70.00c	88.89bc	4.56b	7.39d
	(-19.54%)	(-8.05%)	(+5.07%)	(-51.09%)
T2	81.11ab	94.44ab	4.38c	9.56b
	(-6.77%)	(-2.31%)	(+0.91%)	(-36.73%)
T3	76.67b	90.00b	4.51b	8.65c
	(-11.87%)	(-6.90%)	(+3.92%)	(-42.75%)

Different letters in the same column indicate a significant difference among treatments according to Duncan´s multiple range test at 0.05 level. The values of the brackets are the reduction or promotion over CK1. CK1=nomal; CK2=salt stress; T1=seed priming with 0.4 mM melatonin solution; T2=seed priming with 0.8 mM melatonin solution; T3=seed priming with 1.6 mM melatonin solution; GE= germination energy; GP= germination percentage; MET= mean emergence time; VI= seedling vigor index.

Table 2. Influence of seed priming with melatonin on the shoot and root lengths, seedling fresh and dry weights in maize under saline and normal conditions.

Treatments		SL(cm)	RL(cm)	FW($mg^{-1}plant^{-1}$)	DW($mg^{-1}plant^{-1}$)
	Control	6.85b	9.02b	405.6b	39.7b
	Hydropriming	7.04ab	9.53ab	436.2b	43.9db
Normal		(+2.77%)	(+5.65%)	(+7.5%)	(+10.6%)
	Melatoninpriming	7.31a	10.04a	466.7a	48.0a
		(+6.72%)	(+11.31%)	(+15.1%)	(+20.9%)
Salinity	Control	1.66e	4.90e	144.9e	14.9e
	Hydropriming	2.01d	6.30d	192.9d	20.2d
		(+21.08%)	(+28.57%)	(+33.1%)	(+35.6%)
	Melatoninpriming	2.48c	7.64c	241.80c	25.1c

		(+49.4%)	(+55.92%)	(+66.9%)	(+68.5%)

Different letters in the same column indicate a significant difference among treatments according to Duncan´s multiple range test at 0.05 level. The values of the brackets are the promotion over control. Control=seed unprimed; Hydropriming=seed priming with water; Melatoninpriming= seed priming with 0.8 mM melatonin solution; SL=shoot length; RL=root length; FW=fresh weight; DW=dry weight.

Table 3. Influence of seed priming with melatonin on the RWC, EL, MDA, proline and total phenolic contents in maize under saline and normal conditions.

	Treatments	RWC(%)	EL(%)	MDA(μmolg^{-1}FW^{-1})	Proline(mg^{-1}FW^{-1})	TP(mg^{-1}FW^{-1})
	Control	89.20b	13.50d	7.97d	54.90e	43.57e
	Hydropriming	90.35ab	12.70d	7.39d	59.62de	48.22de
Normal		(+1.29%)	(-5.93%)	(-7.28%)	(+8.60%)	(+10.67%)
	Melatoninpriming	91.76a	10.83e	6.61e	64.76d	53.09d
		(+2.87%)	(-19.78%)	(-17.06%)	(+17.96%)	(+21.85%)
	Control	70.35e	28.51a	15.11a	87.97c	72.97c
	Hydropriming	73.83d	20.82b	11.80b	105.25b	86.58b
Salinity		(+4.95%)	(-26.97%)	(-21.91%)	(+19.64%)	(+18.65%)
	Melatoninpriming	76.09c	17.27c	8.95c	119.58a	100.58a
		(+8.16%)	(-39.42%)	(-40.77%)	(+35.93%)	(+37.84%)

Different letters in the same column indicate a significant difference among treatments according to Duncan´s multiple range test at 0.05 level. The values of the brackets are the reduction or promotion over control. Control=seed unprimed; Hydropriming=seed priming with water; Melatoninpriming= seed priming with 0.8 mM melatonin solution; RWC=relative water content; EL= electrolyte leakage; MDA= malondialdehyde; TP=total phenolic.

Effect of seed priming on relative water content, proline content and total phenolic content: Salinity stress significantly (P<0.05) decreased leaf relative water content of maize cultivar Nonghua101 (Table 3). Under salinity stress conditions, melatoninpriming and hydropriming increased relative water content, and there were significant differences between melatoninpriming and control, there were also significant differences between melatoninpriming and hydropriming, and melatoninpriming showed greater improvement in relative water content than hydropriming (Table 3). Salinity stress significantly (P<0.05) increased proline and total phenolic contents in leaves of maize cultivar Nonghua101 (Table 3). Under salinity stress conditions, melatoninpriming and hydropriming increased proline and total phenolic contents, and there were significant differences between melatoninpriming and control, and there were significant differences between melatoninpriming and hydropriming, and melatoninpriming showed greater improvement in proline and total phenolic contents than hydropriming (Table 3).

Effect of seed priming on Na^+ and K^+ content: Salinity stress significantly (P<0.05) decreased K^+ content in leaves of maize cultivar Nonghua101 (Table 4). Under normal and salinity stress conditions, melatoninpriming and hydropriming increased K^+ content, and there were significant differences between melatoninpriming and control, and there were significant differences between melatoninpriming and hydropriming, and melatoninpriming showed greater increase in K^+ content than hydropriming (Table 4). However, salinity stress significantly (P<0.05) increased Na^+ content in leaves of maize cultivar Nonghua101 (Table 4). Under normal and salinity stress conditions, melatoninpriming and hydropriming decreased Na^+ content, and there were significant differences between melatoninpriming and control, and there were significant differences between melatoninpriming and hydropriming, and melatoninpriming showed greater decrease in Na^+ content than hydropriming (Table 4). Meanwhile, salinity stress significantly (P<0.05) decreased K^+/Na^+ ratio, melatoninpriming significantly (P<0.05) increased K^+/Na^+ ratio.

Effect of seed priming on SOD, CAT and PAL activities: Salinity stress significantly (P<0.05) increased SOD activities of leaves of maize cultivar Nonghua101. Under normal and salinity stress conditions, melatoninpriming and hydropriming increased SOD activities, and there was a significant difference between melatoninpriming and control, and there was also a significant difference between melatoninpriming and hydropriming, and melatoninpriming showed greater improvement in SOD activities than hydropriming (Table

5). Likewise, salinity stress also significantly increased CAT activities of leaves. Under normal and salinity stress conditions, melatoninpriming and hydropriming also increased CAT activities, and melatoninpriming showed greater improvement in CAT activities than hydropriming. There was a significant difference between melatoninpriming and hydropriming under salinity stress (Table 5). Salinity stress also significantly increased PAL activities of leaves. Under normal and salinity stress conditions, melatoninpriming and hydropriming also increased PAL activities, and this influence was similar with that of CAT activities (Table 5).

Table 4. Influence of seed priming with melatonin on the K^+ and Na^+ in maize under saline and normal conditions.

	Treatments	K^+ (%)	Na^+ (%)	K+/Na+ ratio
Normal	Control	1.76c	0.59d	2.98b
	Hydropriming	1.88b	0.62d	3.06ab
	Melatoninpriming	2.24a	0.69c	3.24a
Salinity	Control	0.76f	0.96a	0.79e
	Hydropriming	0.90e	0.78b	1.14d
	Melatoninpriming	1.31d	0.71c	1.84c

Different letters in the same column indicate a significant difference among treatments according to Duncan´s multiple range test at 0.05 level. Control=seed unprimed; Hydropriming=seed priming with water; Melatoninpriming= seed priming with 0.8 mM melatonin solution.

Table 5. Influence of seed priming with melatonin on the SOD, CAT and PAL activities in maize under saline and normal conditions.

	Treatments	SOD($Ug^{-1}FW^{-1}$)	CAT($Ug^{-1}FW^{-1}min^{-1}$)	PAL($Ug^{-1}FW^{-1}h^{-1}$)
Normal	Control	21.29e	11.79e	3.31e
	Hydropriming	22.38e	12.55de	3.66de
		(+5.12%)	(+6.45%)	(+10.57%)
	Melatoninpriming	23.71d	13.65d	4.02d
		(+11.37%)	(+15.78%)	(+21.45%)
Salinity	Control	29.19c	17.88c	5.45c
	Hydropriming	33.74b	21.02b	6.57b
		(+15.59%)	(+17.56%)	(+20.55%)
	Melatoninpriming	37.26a	23.51a	7.55a
		(+27.65%)	(+31.49%)	(+38.53%)

Different letters in the same column indicate a significant difference among treatments according to Duncan´s multiple range test at 0.05 level. The values of the brackets are the promotion over control. Control=seed unprimed; Hydropriming=seed priming with water; Melatoninpriming=seed priming with 0.8 mM melatonin solution; SOD= superoxide dismutase; CAT= catalase; PAL= phenylalanin ammonia lyase.

Discussion

Seed germination and early seedling growth are critical stages for plant establishment and production. Due to osmotic and ion toxicity, seed germination and seedling growth were adversely affect by salinity (Parida & Das, 2005). This study indicated GE, GP and VI were significantly (P<0.05) decreased and MET was significantly (P<0.05) increased by salinity stress (Table 1). These results clearly indicated that salinity caused significant reduction in shoot and root lengths, seedling fresh and dry weights (Table 2). These results are in line with those of (Hajer *et al*., 2006; Abbasi *et al*., 2014a; Abbasi *et al*., 2015; Liu *et al*., 2015) who reported that salinity caused reduction in plant growth. This reduction in plant growth might be due to ions toxicity or a reduction in osmotic potential (Farooq *et al*., 2015). However, priming with melatonin can improve GE, GP and VI in maize, and can decrease MET, the performance was best when melatonin was 0.8 mM, and there was a significant difference between melatoninpriming and salinity stress. Meanwhile, priming with melatonin can improve shoot and root lengths, seedling fresh and dry weights. These results indicated priming with melatonin has a positive effect on seed germination and seedling growth in maize under salinity stress condition. This may be early completion of pre-germination metabolic activities during priming (Farooq *et al*., 2013), this superiority of primed seeds resulted in improvement in seed germination and seedling growth.

Relative water content has been used as an efficient method of water relation for evaluating plants tolerance to salt stress (Suriya-arunroj *et al*., 2004). In the present study, salinity significantly decreased relative water content in maize. This confirmed the previous results that a decrease in relative water content of maize under salt stress (Saleh,

2012; Abbasi *et al*., 2014b). Priming with melatonin can improve relative water content, which showed melatonin may play an important role in maize water relation under salinity stress and help the plants to absorb more water to resist salt stress.

Salt stress causes the replacement of K^+ by Na^+ bringing about a reduction in K^+/Na^+ ratios, which lead to the disorder of biochemical reactions in plants. The maintenance of higher K^+/Na^+ ratios would be suitable for the metabolic processes occurring within the plants (Ashraf & Khanum, 1997). Therefore, a higher K^+/Na^+ ratio is considered to be one of the important biochemical mechanism of salt tolerance in plants (Maathuis & Amtmann, 1999). Priming with melatonin can improve K^+/Na^+ ratio (Table 4), which suggested melatonin reduced the toxic effects of Na^+, this was accordance with the improvement of shoot and root lengths, seedling fresh and dry weights in maize.

The accumulation of reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\cdot-}$), and hydroxyl radical (•OH) in plants is one of the most important physiological manifestations of abiotic stress (Ahmad *et al*., 2009). It is harmful to plant cell structure and function for ROS at high concentrations. Relative electrolyte leakage enables cell membrane injury to be assessed when plants are subjected to salinity stress, which was significantly increased under salinity stress (Demidchik *et al*., 2014). Maintaining integrity of the cellular membranes under salinity stress is considered a mechanism of salinity tolerance (Stevens *et al*., 2006). Seed priming with melatonin significantly decreased the membrane relative electrolyte leakages in maize (Table 3). This confirmed the protective effect of melatonin in membrane damage induced by salinity stress. This result is concordant with previous reports that melatonin facilitated the maintenance membrane functions (Li *et al*., 2012; Zhang *et al*., 2014). Moreover, MDA as a lipid peroxidation product, which is accumulated in tissues when plants are exposed to salinity stress (Parida & Das, 2005.), and has often been used as indicator of salinity induced oxidative damage in membranes. The improvement in MDA content in maize showed the increase of lipid peroxidation leading to membrane damage induced by salinity stress (Table 3). This was in agreement with the increase in membrane relative electrolyte leakages. Seed priming with melatonin decreased MDA content, which may be related to the induction of antioxidant response and protective role of membranes that increase the tolerance of plant to damage (Posmyk *et al*., 2009).

The negative effects of ROS are counteracted by enzymatic and non-enzymatic antioxidative system (Ahmad *et al*., 2009). Enzymes of SOD and CAT play an important role in scavenging ROS, SOD catalyses the dismutation of O_2^- to H_2O_2 and O_2, while CAT scavenges H_2O_2 (Foyer *et al*., 1994). Non-enzymatic antioxidative system can inhibit ROS production, including proline and phenolic compounds etc (Foyer *et al*., 1994; Michalak, 2006; Ahmad *et al*., 2009; Mehr *et al*, 2012; Kishor & Sreenivasulu, 2014; Król *et al*., 2014). In this study, the enzyme activities of SOD and CAT in maize shoots were improved under salinity stress compared with that of maize shoots grown at normal condition (Table 5). This suggests that maize activates its antioxidant system under salinity stress condition. Seed priming with melatonin increased the SOD and CAT activities under normal and salinity conditions. This indicated the ability to scavenge ROS in maize plant system was increased and the negative effects of salinity stress were alleviated. These results were further confirmed by the decrease of MDA content and the relative electrolyte leakage level. Many studies demonstrate that melatonin scavenges ROS and stimulates the antioxidant system in plant system to alleviate oxidative damage (Li *et al*., 2012; Zhang *et al*., 2013; Meng *et al*., 2014; Zhang *et al*., 2014), which are in contrast to our work.

Proline is considered as a multi-functional molecule, including osmotic pressure regulation, protection of membrane integrity, scavenger of free radicals, accumulating in high concentrations as an adaptive response to a variety of abiotic stresses including salinity stress (Kishor & Sreenivasulu, 2014). In this study, the accumulation of proline in maize is higher under salinity stress, which is in line with previous reports. Seed priming with melatonin can improve proline content (Table 3), this showed that the inhibitory effect of salinity stress on the tested maize seedling was alleviated, this also showed the possibility of melatonin inducing the increasing proline synthesis and the decreasing proline degradation (Mehr *et al*., 2012; Kishor & Sreenivasulu, 2014). The decrease of relative electrolyte leakage level and MDA content is the proof that proline has the functions of osmotic pressure regulation, protection of membrane integrity and scavenger of free radicals.

Phenolic compounds which play a part in antioxidant and free radical scavenging properties are induced in plants by environmental stresses (Michalak, 2006; Mehr *et al*., 2012; Król *et al*., 2014). In this research, the level of total phenolics in maize shoots significantly increased under salinity stress (Table 3), which is similar to that of *Anethum graveoles* reported by Mehr *et al*., (2012) and wheat reported by Mahboob *et al*., (2016). However, compared to the maize plant under salinity stress, seed priming with melatonin significantly increased the total phenolic content (Table. 3), this result is in agreement with previous results that exogenous melatonin increased the levels of phenolic compounds in *Vigna radiate* (Szafrańska *et al*., 2012). PAL plays an important role in the synthesis of phenolic compounds, which are responsible for successfully resisting adversity environment (Riov *et al*., 1969; Farkas & Szirmai, 1969). PAL is a particularly sensitive indicator of stress conditions and it is commonly considered as a biochemical marker indicating the synthesis of both structural and protective compounds (Nadernejad *et al*., 2012). In this study, the PAL activities in maize shoots significantly increased under salinity stress (Table 5), seed priming with melatonin significantly increased the PAL activities (Table 5). These results indicated the synthesis of protective compounds was stimulated in maize, which were in agreement with the total phenolic contents. Therefore, we consider that the improvement of PAL activity may be

one of the important physiological reasons for the resistance of maize to the salinity stress.

Conclusions

In conclusion, salinity stress has adverse effects on seed germination and seedling growth in maize. Seed priming with melatonin improved the salinity resistance to maize, it also improved the performance of maize at normal condition. Seed priming with melatonin alleviates the salinity damage to maize by improving SOD, CAT and PAL activities, relative water content, proline and total phenolic contents, decreasing membrane relative electrolyte leakage and lipid peroxidation product, and the performance of seed priming with 0.8 mM melatonin is most effective. Therefore, seed priming with melatonin may be an important alternative approach to decrease the impact of salinity stress in maize.

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