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Alleviation of salt stress on seed germination and seedling growth in wheat by seed priming with melatonin

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ABSTRACT

The present study was conducted at the Department of Laboratory, China. To know the impact of melatonin priming in alleviating the negative impact of salt stress on seed germination and seedling establishment of winter wheat (*Triticum aestivum* L. cv Elnilein). Wheat seeds were primed with melatonin and then subjected seedlings under salt stress. Six concentrations were used: control, salt (300 mM NaCl) four concentrations of melatonin (M) 10, 100, 500, 1000 μ M with salt (300 mM NaCl) to each concentration: CT, ST, M1+ST, M2+ST, M3+ST, M4+ST. Effect of melatonin priming on seed germination under salt stress was studied and optimum melatonin concentration screened. Thereafter, three-leaf seedlings from the melatonin-primed subjected to two levels of salt stress for evaluation of the alleviation effect of melatonin on seedling establishment. Five concentrations used as follows: Control, salt stress (300 mM NaCl), salt stress (100 mM NaCl), 500 μ M of melatonin (M) +300 mM NaCl, 500 μ M of melatonin (M) + 100 mM NaCl: CK, S1, S2, M+S1, M+S2. The data obtained on different seed germination and seedling. Parameters measured under study were analyzed statistically using SPSS (SPSS Inc, Chicago, IL). Significantly different means of the measured data separated at the 0.05 probability level using Duncan's Multiple Range Test (DMRT). The results revealed that salt stress caused significant reduction in seed germination rate, germination index, lengths, dry weights of radicle and coleoptile, amylase, antioxidant enzymes activities, soluble sugar, sucrose contents, while melatonin priming alleviated these reductions. Also, salt stress decreased seedling growth attributes, water content (RWC), leaf area, and chlorophyll content, whereas melatonin priming increased these parameters. The photosynthetic rate (Pn), actual photosynthetic efficiency (Fv/Fm), maximum photochemical efficiency (Fv/Fm), and potassium content (K⁺) in stressed seedlings inhibited, where melatonin priming improved these traits. We found that salt stress plants had increased the reactive oxygen species (ROS) malondialdehyde (MDA), hydrogen peroxide (H₂O₂) contents, superoxide (O₂⁻)

production rate, and sodium (Na⁺) content, in contrast, seed primed with melatonin lowered these attributes. Then, we recommended that Melatonin well be used as priming sources to enhance salt stress present new opportunities for it is employ in agriculture.

Keywords: Melatonin priming, antioxidant enzymes, gas exchange, wheat (*Triticum aestivum* L.), salt stress

1. INTRODUCTION

Salt stress is one of the significant factors limiting crops production globally (Zhu et al., 2016). Wheat is one of the most important staple foods crop in world, and is also one of the primary cereal food supporting the increased world population in the future. The grain yield and quality are significantly affected by salt stress in wheat production (Zhang et al., 2011). Significantly the seed germination and seedling establishment stages are considered the most sensitive stages to salinity than other growth stages, and the salt stress tolerance during these stages mentioned to directly correlate to crop production (Iqbal and Ashraf, 2007). Salt causes a series adjustment in plant physiological processes, including osmotic stress, ion toxicity and oxidative injury (Sreenivasulu et al., 2000).

Under salt stress, reactive oxygen species (ROS) like superoxide (O₂^{-•}), hydrogen peroxide (H₂O₂) and hydroxyl radicals were highly induced (Radyukina et al., 2007). Meantime, the antioxidative enzymes such as catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), and ascorbate peroxidase (APX) were activated to defense against the oxidative stress (Wang et al., 2016). Enhancing the antioxidant enzyme activity in plants is essential for improving tolerance to salinity stress in plants (Sairam et al., 2002). Chemical priming to plants with certain compounds before stress events is considered an effective practice tolerance to stresses (Antonioni et al., 2016; Samota et al., 2017). For instance, molecules such as nitric oxide (NO) Zheng et al., (2009) and hydrogen sulfide (H₂S) Christou et al., (2013) recommended as effective priming agents for salt tolerance. Melatonin (N-acetyl-5-methoxytryptamine) is a low molecular-weight molecule its structure includes an indole circle (Li et al., 2012).

Previously well-known as an animal hormone, has functions in the timing rhythms, signaling environmental changes and free radicals detoxification (Zhang et al., 2014). After that, melatonin is known to function in plants as an antioxidant, growth promoter, and so on (Tan et al., 2013). Exogenous application of melatonin was found to improve tolerance to a biotic stresses such as drought effectively Ma et al., (2018), heat Zhang et al., (2017) and, also salt Zhang et al., (2014) in some plant species. However, if melatonin could applied as an effective seed priming chemical to alleviate the salt stress during the seed germination and seedling establishment stages in wheat is not clear.

Therefore, we first primed wheat seeds with different melatonin concentration and then subjected germinating seeds to salt stress to screen the optimum concentration. After that, wheat seeds primed using the optimum dose of melatonin the seedlings subjected to two levels of salt stress. The objectives of this study were: (1) To evaluate the alleviation effects of seed priming with melatonin on salt stress during both seed germination and seedling establishment stages; and (2) to elucidate the underlying physiological mechanisms in the views of antioxidation, cell membrane protection, and leaf photosynthesis in wheat.

2. MATERIALS AND METHODS

Experimental design

Two experiments were performed in the laboratory department China, using winter wheat (*Triticum aestivum* L cv Elnilein) as experimental material.

Experiment I: This experiment was to determine the optimum concentration of melatonin a priming substance for wheat seed under salt stress. Uniform seeds were sterilized with 2.5% sodium hypochlorite for 10 min and then rinsed several times with sterile distilled water. Five melatonin concentrations were designed as 0, 10, 100, 500, and 1000 µM, respectively. The seeds were pre-soaked with or without melatonin for 20 h and then arranged on a filter paper in dishes (50 sources each) for salt stress treatment, and 300 mM NaCl was applied and lasted for seven days in a growth chamber at 22 ± 0.5 °C. After that, six treatments placed as CT: No melatonin with no salt stress; ST: Salt stress only, M1+ST: 10 µM melatonin + salt stress, M2+ST: 100 µM melatonin + salt stress, M3+ST: 500 µM melatonin + salt stress; M4+ST: 1000 µM melatonin + salt stress, respectively. Germinating seeds sampled during 2, 3, 4, 5 7 after treatment for measurements of physiological traits. 7 days after salt treatment, seed germination rate determined, and the seedlings harvested for assays of dry weight, ROS production, and antioxidant enzymes activities.

Experiment II: This experiment evaluated to study the alleviation effect of melatonin priming on salt stress during the seedling establishment stage. Uniform seeds were sterilized with 2.5% sodium hypochlorite for 10 min and then washed several times with sterile distilled water, after that divided into two groups, pre-soaked with distilled water, and pre-soaking with 500 μ M melatonin for 20 h in a growth chamber at 22 ± 0.5 °C. After that, at the two-leaf stage, the plants were transplanted in the plastic containers (45 cm in length, 35 cm in width, 18 cm in height) for hydroponic cultivation, with a density of 30 plants per container. At the three-leaf stage, plants were subjected to salt stress with concentrations of 100 mM NaCl and 300 mM NaCl, and lasted for seven days, respectively. Five treatments formed: CK: Control; S1: 100 mM NaCl only; S2: 300 mM NaCl only; M+S1: melatonin+100 mM NaCl; M+S2: melatonin+300 mM NaCl. Samples harvested on Day 3 and Day 7 after the salt treatment to analyze the physiological traits.

Seed germination indexes traits and dry weight

Germination rate (GR) is the proportion of germinated seeds in each petri dish during seven days after germination. Mean germination time (MGT) was determined using the method of (Ellis and Roberts, 1980). Germination index (GI) calculated according to practice of Wang et al., (2004) as $GI = \sum (Gi / Ti)$, Gi is the germination percentage at the 7th day, and Ti is days of germination examination. Thus, GR and GI are positive with germination velocity, while MGT is negative with germination velocity; the long MGT means low germination. The radicles, coleoptile, seed residues separated and heated at 105 °C for two hours, then dried to constant weight at 80 °C to get the dry weight of each organ.

Activities of amylases in grains

The extraction and activity of α - and β -amylase measured according to the method of (Kishorekumar et al., 2007). The fresh germinating seeds collected and frozen in liquid nitrogen for starch enzyme extraction. The concentration of soluble protein in germinating seeds estimated using the method described by Bradford, (1976) using bovine serum albumin (BSA) as standard. Activities of both α - and β -amylase were calculated as unit mg⁻¹ protein, and one team protein is equivalent the release of one mg maltose from starch per minute by the amylases.

Contents of Starch and Soluble Sugars

Germinating seed powder (1 g) mixed with 10 ml 0.33 mM of HCl' and then heated at 100 °C for 10 min to remove starch. 0.5 ml of ZnSO₄ (30 %, w/v) added to the extraction to remove proteins. After that, 0.5 ml K₃[Fe (CN) 6] (30 %, w/v) added and mixed, and then the mixture volume tuned to 20 ml with distilled water. After thoroughly shaking, it filtrated, then the concentration of starch was determined like the amount of glucose produced at 20–25 °C using the polarimetric analysis through an automatic recording polarimeter (WZZ-2B, Shanghai, China) according to (Xie et al., 2003). Moreover, to extract total soluble sugars, 0.1 g oven-dried germinating seed powder sample was homogenized in (80%) ethanol at 80 °C for 10 min and centrifugation at 600g. The supernatant collected for the content of soluble sugar and sucrose measurement by anthrone method (Fales, 1951).

Shoot height and root length, fresh and dry weight of shoot and root

The shoot height and root length of five randomly selected plants from each replicate were measured using the measuring tape. Seedling shoots and roots of five randomly chosen seedlings from each experimental unit weighted using digital balance immediately. The final fresh weight calculated in (g plant⁻¹). The fresh sample of the five plants left to dry in an oven at 80 °C for 36 hours, then weighted with digital balance to estimate final dry weight.

Leaf relative water content, leaf area, and chlorophyll concentration

The leaf relative water content (RWC) determined following the method of (Ritchie et al., 1990). Leaf area (LA) measured with Li-Cor leaf area meter (LI-3100C, Lincoln, NE, USA). The pigments content in leaves extracted using 100 mg fresh leaves extracted overnight with 80% acetone solution in the dark according to the method of (Arnon, 1949). The chlorophyll a and Chlorophyll b content measured with a spectrophotometer (Unicam Helios Beta, Spectronic Unicam, Cambridge, UK) and calculated as follows (Arnon, 1949).

Leaf gas exchange and chlorophyll fluorescence parameters

After 3 and 7 days of salt stress treatment, the latest fully expanded leaf used for the gas exchange measurement following the method of (Wang et al., 2011). The LI-6400 system (LI-COR Biosciences, Lincoln, NE, USA) used to measure the photosynthesis rates (PN) at 9:00 a.m. to 11:30 a.m. 6 leaves were taken as one replicate for each treatment. The same leaves for gas exchange measurements used for chlorophyll fluorescence parameters assay with a portable pulse amplitude modulation fluorescence monitoring system (PAM chlorophyll fluorimeter, M-series, Heinz Walz, Effeltrich, Germany). Plants were firstly dark-adapted for at least 20 min before to measuring the actual PSII photochemical efficiency (Φ PSII), and maximum photochemistry efficiency (Fv/Fm).

Contents of malondialdehyde (MDA) and H₂O₂, production rate of O₂·, and activities of antioxidant enzyme

The extraction of MDA, ROS, antioxidant enzymes in germinating seed and seedling leaves conducted according to the method of (Tan et al. 2008). Fresh samples (500 mg) were homogenized into powder with 5 ml of ice-cold extraction buffer (50 mM potassium phosphate buffer, pH 7.0, 0.4 % polyvinylpyrrolidone) at four°C. The extract centrifuged at 10,000 g for 30 min, and the supernatant used as crude extract. MDA content measured following the method described by (Madhava-Rao and Sresty, 2000). The content of H₂O₂ measured by the process of Moloi and Van-der-Westhuizen, (2006), the - production rate of O₂ by following the procedure of (Sui et al., 2007). The extract from the above was further used for analysis of the activities of antioxidant enzyme analysis. The actions of superoxide dismutase (SOD), peroxidase (POD) measured following Tan et al., (2008), ascorbate peroxidase (APX) and glutathione reductase (GR) measured using the method of (Fryer et al., 1998).

Contents of K⁺ and Na⁺ in seedling plants

The extractions of K⁺ and Na⁺ in seedlings conducted according to the method of (Zheng et al., 2009). Briefly, 100 mg powder of oven-dried shoot or root sample was incubated in 5 ml concentrated sulphuric acid to fully digested and added in 200 µl 30% H₂O₂ a catalyst. K⁺ and Na⁺ contents were measured using an atomic absorption spectrometer (Beijing Purkinje General Instrument Co., Ltd., China)

Statistical analyses

In our study, all data shown as the mean values ± SD (standard deviations). At least three independent replicates conducted for each experiment. Data were analyzed using the one-way ANOVA using SPSS (SPSS Inc, Chicago, IL). Significantly different means of the measured data separated at the 0.05 probability level using Duncan's Multiple Ranges Test.

3. RESULTS

Effect of seed priming with melatonin on seed germination under salt stress

To evaluate the effects of melatonin on seed germination and to screen the optimum concentration of melatonin, we primed wheat seeds with different concentrations of melatonin and then we subjected the seeds to salt stress over the whole germination process. Salt stress (ST) significantly reduced germination rate (GR) and germination index (GI) while increasing mean germination time (MGT) as compared with the non-stress treatment (CT) (Table 1). However, pretreatment seeds with any concentrations of melatonin in the present experiment significantly alleviated the adverse effects of salt stress on seed germination in comparison with ST, with the best performance at a concentration of 500 µM. Salt stress significantly decreased the length and dry weights of radicle and coleoptile, while increases the dry weight of seed residues when compared by the non-stress treatment. While, the melatonin pretreatments improved seed germination under salt stress exemplified the less reduced length and weights of radicle and coleoptile and lowered dry weight of the seed residue. The optimum concentration observed at 500 µM (Table 1).

Effect of seed priming with melatonin on starch degradation and amylase activities in germinating seed

The starch content in seeds continuously decreased while activities of α -amylase and β -amylase increased along with seed germination (Figure 1). The starch degradation in germinating seeds was depressed under salt as indicated by the increase of starch content and lower activities of α -amylase and β -amylase as compared with the non-stressed control. Under salt stress, the melatonin priming treatments showed lower starch content than the non-priming treatment ST (Figure 1a). In addition, α -amylase and β -amylase activities

were much higher in melatonin-primed germinating seeds than ST during germination. However, movement of α -amylase was much higher in the primed seeds from 3 DAT to 7 DAT (Figure 1b), while the activity of β -amylase was from 2 DAT to 7 DAT when compared to ST (Figure 1c). In consistence, the contents of soluble sugars and sucrose in germination seeds increased from 2 DAT to 7 DAT, and they were decreased by salt stress (Figure 2a, b). However, the melatonin-primed-seed improved contents of soluble sugar and sucrose compared to non-primed seeds under salt stress. Moreover, the best alleviation performance observed at a concentration of 500 μ M.

Table 1 Effects of melatonin priming on seed germination rate (GR), germination index (GI), mean germination time (MGT), length of radicle and coleoptile, and dry weight of radicle, coleoptile, and whole seed after seven days germination under salt stress in wheat

Treatments	GR (%)	GI	MGT (d)	Length(cm)		Dry weight (mg seed-1)		
				Radicle	Coleoptile	Radicle	Coleoptile	Seed residue
CT	97.32±0.28a	93.88±0.06a	1.59±0.04d	10.28±0.06a	9.25±0.04a	14.18±0.02a	11.22±0.05a	8.19±0.02f
ST	65.89±0.26e	22.47±0.38f	5.23±0.24a	0.93±0.01f	0.89±0.01f	2.27±0.04f	2.14±0.01f	33.39±0.05a
M1+ST	76.28±0.23d	27.15±0.29e	4.33±0.17b	2.16±0.03e	2.12±0.01e	3.62±0.03e	3.07±0.02e	26.79±0.15b
M2+ST	88.47±0.41b	52.49±0.25c	1.98±0.14d	6.17±0.04c	5.56±0.24c	8.22±0.04c	6.12±0.04c	13.51±0.18d
M3+ST	94.23±0.20a	75.17±0.19b	1.87±0.02d	9.12±0.01b	8.33±0.05b	13.08±0.02b	9.36±0.24b	9.59±0.09e
M4+ST	81.63±0.46c	47.53±0.33d	2.78±0.05c	4.11±0.02d	4.31±0.03d	5.13±0.02d	4.21±0.01d	21.23±0.04c

Different small letters in the same column refer to significant differences between treatments at $P < 0.05$ level.

Effect of seed priming with melatonin on O₂- production rate, concentrations of H₂O₂ and MDA, and activities of antioxidant enzymes in germinating seeds under salt stress condition

O₂-. Production rate and contents of H₂O₂ and MDA generally increased during seed germination (Figure 3). Salt stress significantly increased O₂-. Production rate, H₂O₂ and MDA contents in germinating seeds compared to control. However, melatonin-primed seeds showed much lower O₂- production rate and contents of H₂O₂ and MDA are higher than non-primed seeds (Figure 3a-c). The activities of the antioxidant enzymes of SOD, POD, and APX continuously decreased along with seed germination, and salt stress significantly decreased activities of the antioxidant enzymes as compared with the non-salt stress treatment (Figure 4a-c). The melatonin pretreatment retarded the decreases in the activities of the antioxidant enzymes in germinating seeds under salt stress, and the best performance at a concentration of 500 μ M. Therefore, melatonin priming significantly alleviated the harmful effects of salt stress on seed germination in wheat.

Effect of seed priming with melatonin on seedling establishment under salt stress

Here, we primed wheat seeds with the optimum concentration of melatonin screened from Experiment I, and then subjected the wheat seedlings to two levels of salt stress. The plant growth was significantly inhibited by salt stress, as indicated by decreased shoot height, root length, and plant biomass, and the inhibition was much more severe under salt level (S2) than level (S1) (Table 2). Seedlings from primed seeds with melatonin (M+S1, M+S2) were less inhibited on plant growth than from non-primed seeds under both salt stress, respectively. In addition, leaf relative water content (RWC), leaf area, chlorophyll content significantly decreased by salt stress the reduction rate was much higher under S2 than under S1 (Table 3). Meanwhile, the melatonin-primed plants (M+S1, M+S2) showed higher RWC, leaf area, chlorophyll content than the corresponding salt stress treatment.

Effect of seed priming with melatonin on seedling photosynthesis, and chlorophyll fluorescence under salt stress

Plant photosynthesis (Pn) was quite sensitive to salt stress in the present study. However, Pn was significantly depressed under both salt stress treatments, especially the 7th day of salt treatment (DAT) compared with the non-salt control (Figure 5a). Consequently, seedlings from melatonin-primed seeds (M+S1, M+S2) showed much higher Pn than those from the non-primed seeds under salt stress. Further, the chlorophyll fluorescence indicators of Fv/Fm and Fv/Fm showed the same tendency of Pn in response to the salt stress and melatonin priming treatments, compared to the non-salt stress control (Figure 5b, c).

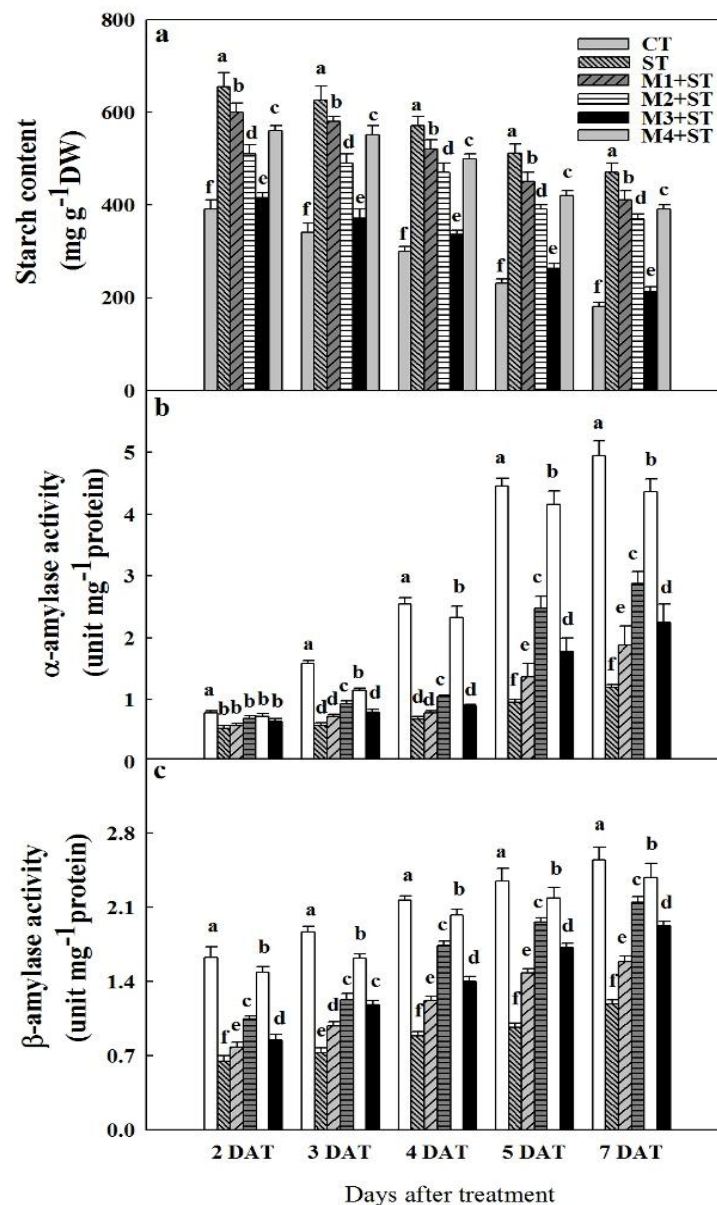


Figure 1 Effects of melatonin priming on starch content (a), activities of α - (b) and β - amylase (c) in wheat seeds during germination under salt stress. Data are mean $\pm$ SD (n=3). Different small letters in each day indicate a significant difference between treatments at $P < 0.05$

Effects of seed priming with melatonin on O₂- production rate, concentrations of H₂O₂ and MDA and activities of antioxidant enzymes in the seedlings leaves under salt stress

O₂- Production rate and H₂O₂ content were highly induced by salt stress at both 3 DAT and 7 DAT (Figure 6). While plants from melatonin- primed seeds showed significantly lower O₂- production rate and H₂O₂ content, and were higher under salt stress than non-primed plants (Figure 6a, c). The activities of the antioxidant enzymes of SOD, POD, APX, and GR in wheat seedling leaves were significantly decreased by salt stress, especially under the severe salt stress of S2 (Figure 7a-d). Melatonin pretreatment significantly improved the activities of the antioxidant enzymes to more effectively scavenge the ROS in salt stress seedlings compared to the non-

primed treatments. While, the cell membrane content of MDA was less induced in primed plants than non-primed plants under salt stress (Figure 6b).

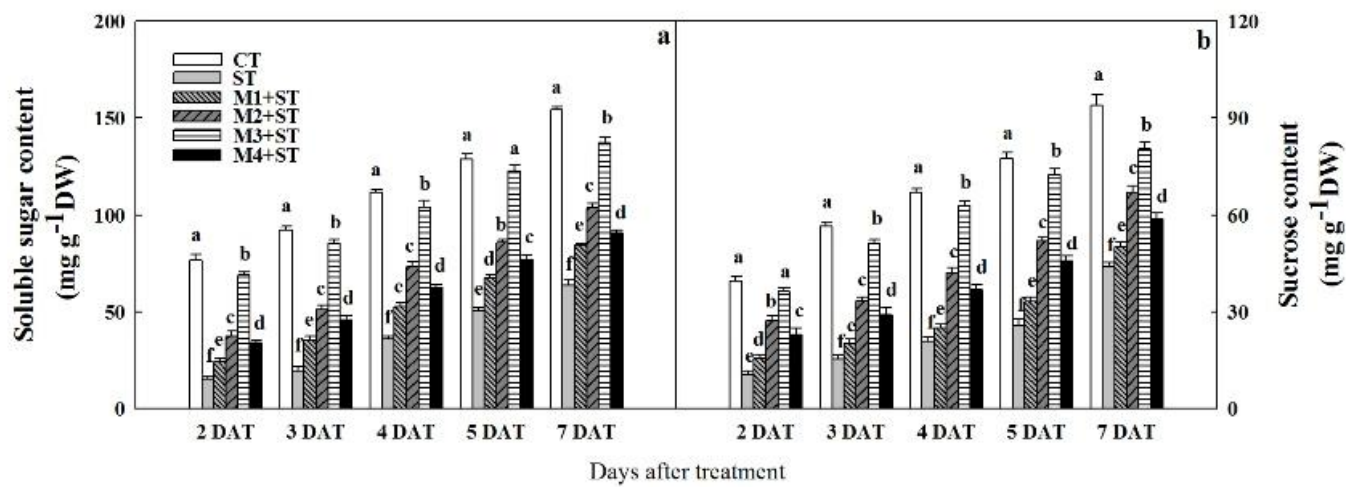


Figure 2 Effects of melatonin priming on contents of soluble sugar (a) and sucrose (b) in wheat seeds during germination under salt stress. Data are mean $\pm$ SD (n=3). Different small letters in each day indicate a significant difference between treatments at $P < 0.05$

Effect of seed priming with melatonin on contents of K⁺, Na⁺ and K⁺/Na⁺ ratio in shoot and root under salt stress

Salt stress increased the Na⁺ content while decreases K⁺ content, and then resulted in reduction of K⁺/Na⁺ ratio in seedlings (Figure 8). Melatonin pretreatment effectively prevented Na⁺ accumulation while promotes K⁺ accumulation in seedlings as compared with the non-pretreated plants under salt stress. Here, the Na⁺ content in primed seedlings was only half of the non-primed plants. Better balanced K⁺/Na⁺ was kept in the primed seedlings than in the non-primed seedlings under salt stress.

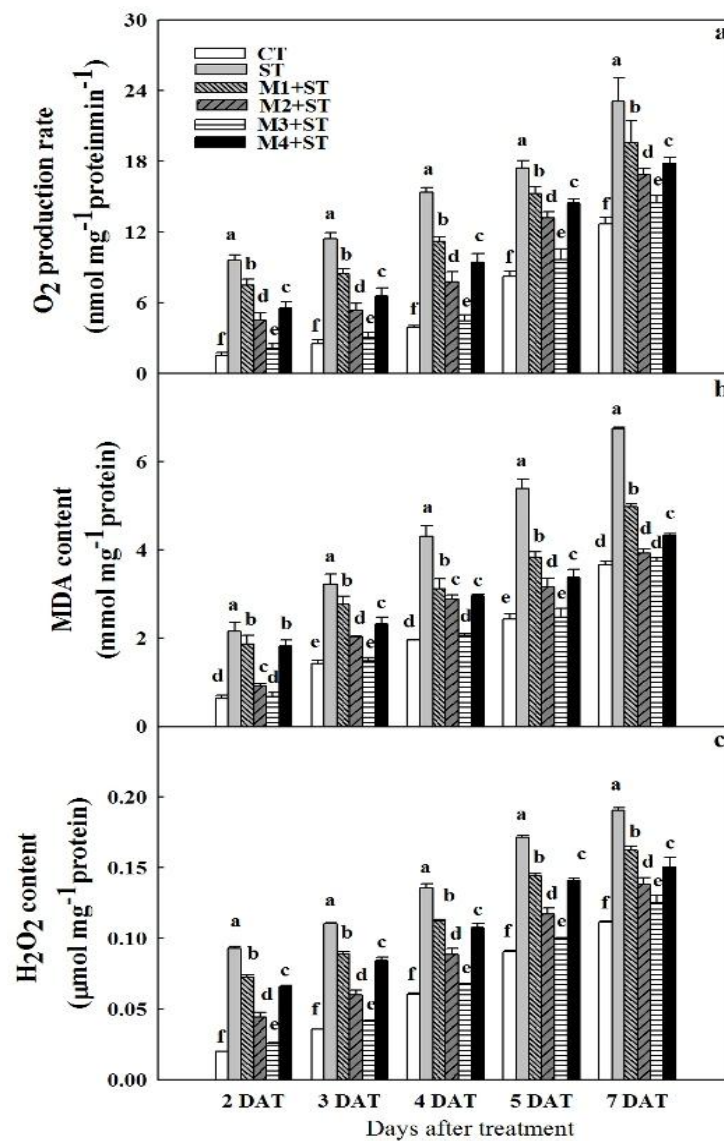


Figure 3 Effects of melatonin priming on O₂⁻ production rate (a), contents of malondialdehyde (MDA) (b), hydrogen peroxide (H₂O₂) (c) in wheat seeds during germination under salt stress. Data are mean ±SD (n=3). Different small letters in each day indicate a significant difference between treatments at P<0.05

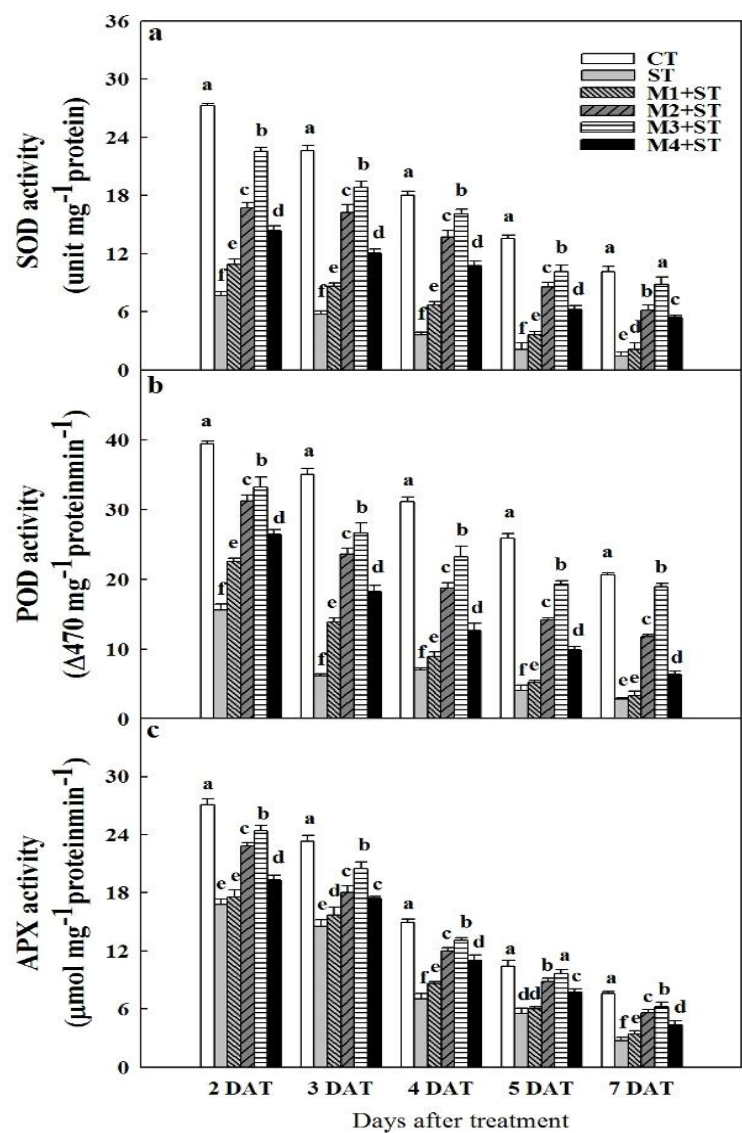


Figure 4 Effects of melatonin priming on activities of superoxide dismutase (SOD) (a), peroxidase (POD) (b), and ascorbate peroxidase (APX) (c) in wheat seeds during germination under salt stress. Data are mean ±SD (n=3). Different small letters in each day indicate a significant difference between treatments at P < 0.05

Table 2 Effects of melatonin priming on seedling morphology during wheat seedling establishment under salt stress.

Treatments	Shoot height (cm)	Root length (cm)	Fresh weight (g plant ⁻¹)		Dry weight (g plant ⁻¹)	
			Shoot	Root	Shoot	Root
CK	26.69±1.70a	29.77±1.81a	3.18±0.31a	2.68±0.31a	0.383±0.02a	0.214±0.017a
S1	15.93±1.45d	19.67±1.52d	1.36±0.30c	0.69±0.26d	0.227±0.02c	0.123±0.015d
S2	13.89±1.66e	16.81±1.75e	1.12±0.33c	0.58±0.28e	0.177±0.03d	0.083±0.012e
M+S1	23.34±0.50b	26.78±0.82b	2.97±0.09b	2.23±0.18b	0.311±0.03b	0.171±0.009b
M+S2	21.56±0.36c	23.45±0.42c	2.59±0.26b	1.48±0.13c	0.307±0.04b	0.131±0.013c

Different small letters in the same column refer to significant differences between treatments at P < 0.05 level.

Table 3 Effects of melatonin priming on relative water content, leaf area, and chlorophyll contents in the leaf of wheat seedling during wheat seedling establishment under salt stress.

Treatments	RWC (%)	Leaf area (cm2)	Chl-a (mg g-1FW)	Chl-b (mg g-1FW)	Chl (a+b) (mg g-1FW)	Chl a/b ratio
CK	90.12±1.75a	21.17±1.38a	3.04±0.029 ^a	0.956±0.020a	3.99±0.032a	3.18
S1	80.31±1.86d	13.69±1.22d	2.35±0.004c	0.891±0.031b	3.24±0.027c	2.63
S2	76.4±0.53e	10.97±1.43e	2.12±0.005c	0.854±0.011c	2.97±0.010d	2.48
M+S1	88.87±1.3b	19.26±0.53b	2.69±0.003b	0.931±0.021a	3.62±0.025b	2.88
M+S2	85.64±1.49c	17.02±0.26c	2.57±0.005b	0.904±0.036a	3.47±0.031b	2.84

Different small letters in the same column refer to significant differences between treatments at P < 0.05 level.

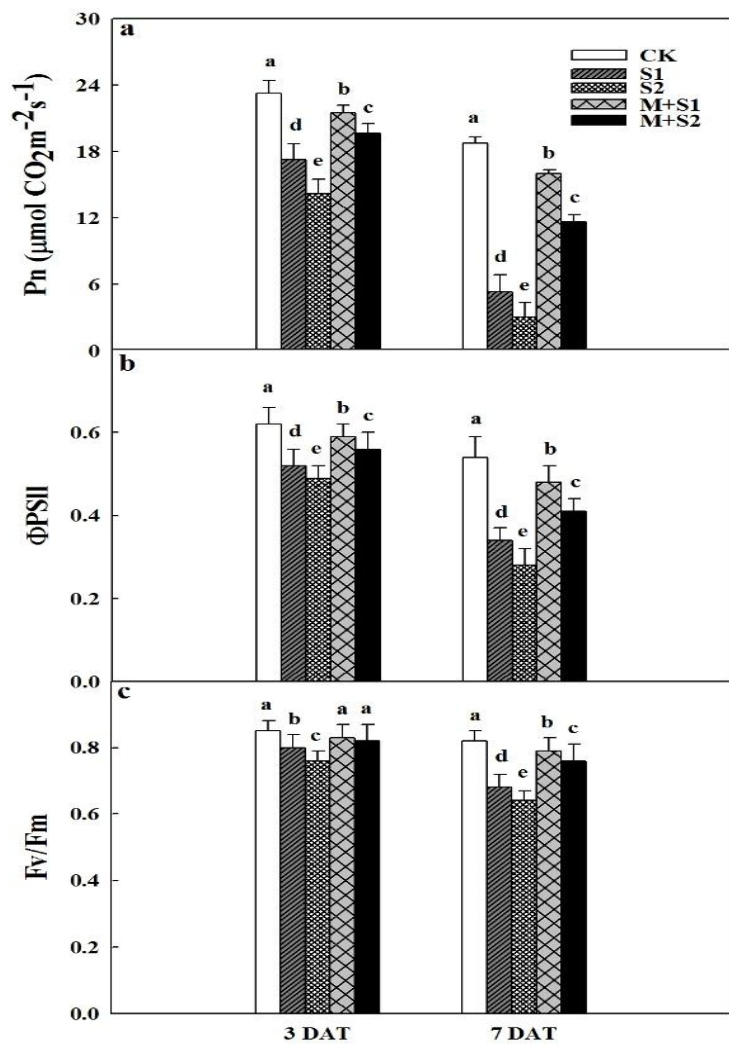


Figure 5 Effects of melatonin priming on photosynthetic rate (Pn) (a), actual photosynthetic efficiency (ΦPSII) (b), and maximum photochemical efficiency (Fv/Fm) (c) of wheat leaf during seedling establishment under salt stress. Data are mean +SD (n=3). Different small letters indicate significant differences between treatments at P < 0.05

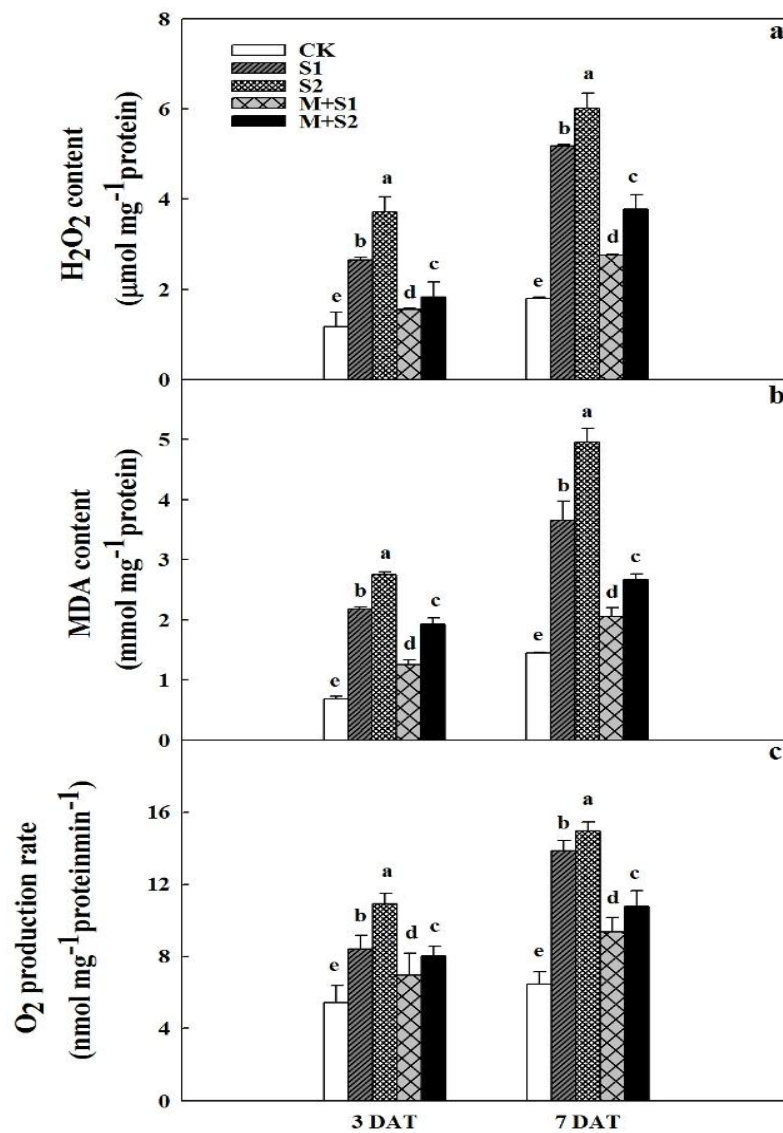


Figure 6 Effects of melatonin priming on contents of hydrogen peroxide (H₂O₂) (a), malondialdehyde (MDA) (b), and O₂ -Production rate (c) in wheat leaf during seedling establishment under salt stress. Data are mean +SD (n=3). Different small letters indicate significant differences between treatments at P < 0.05

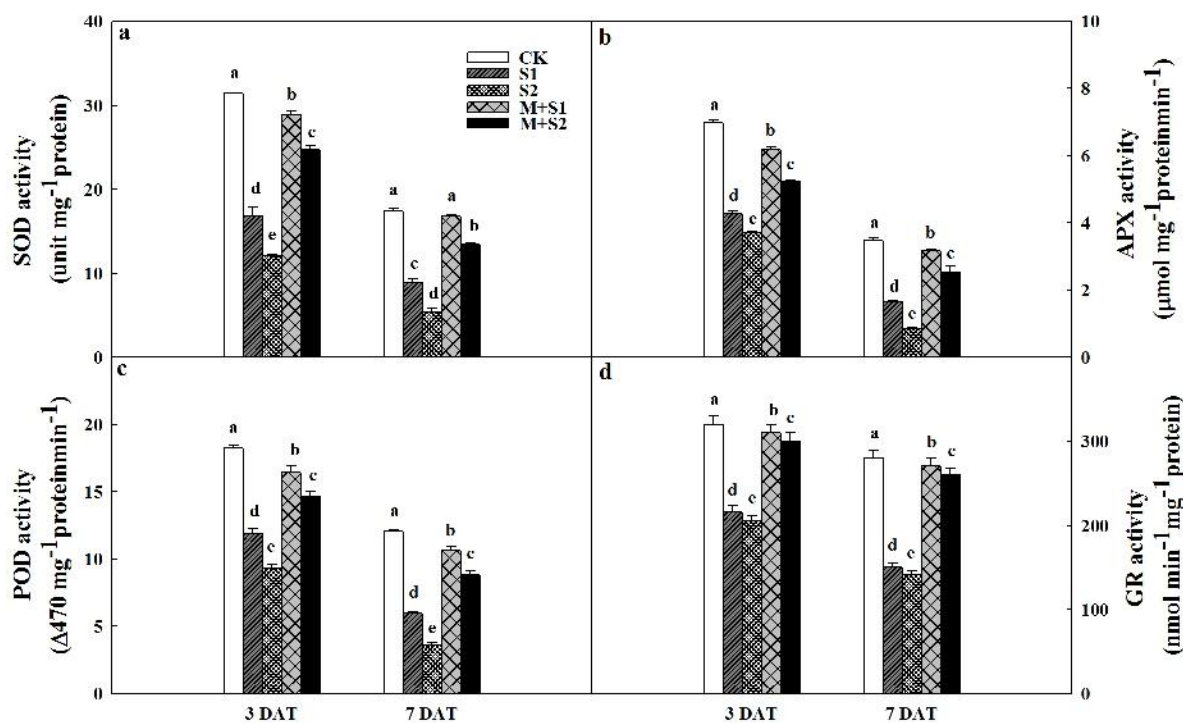


Figure 7 Effects of melatonin priming on activities of superoxide dismutase (SOD) (a), ascorbate peroxidase (APX) (b), peroxidase (POD) (c), and glutathione reductase (GR) (d) in wheat leaf during seedling establishment under salt stress. Data are mean +SD (n=3). Different small letters indicate significant differences between treatments at $P < 0.05$

4. DISCUSSION

Salt stress affects a series of critical physiological processes, such as plant photosynthesis, ion homeostasis, and oxidative reductive processes, which finally leads to the reduction of plant dry matter accumulation (Wang et al., 2016). It has found that melatonin is a potential modulator of plant growth its role is a dose-dependent way (Afreen et al., 2006; Hernández-Ruiz and Arnao, 2008). In addition, melatonin has considered a potential alleviating substance for improvement plant tolerance to salt stress in *Malus Hupehensis* (Li et al., 2012). Here, we further observed that melatonin priming on seeds could effectively alleviate the harmful effects of salt stress on seed germination and seedling establishment.

The underlying physiological explanations in terms of morphology, metabolism, photosynthesis, and antioxidation elucidated in the present study. It has studied that salt stress significantly inhibited seed germination in wheat (Zheng et al., 2009). In this study, salt stress clearly reduced seed germination rate, germination indexes, and weights of coleoptiles and radicals. However, seed priming with melatonin effectively alleviated the inhibition effects of salt stress on seed germination (Table 1). This result was consistent with the early study that melatonin promoted seed germination under salt stress in cucumbers (Zhang et al., 2014). These results indicated that melatonin priming has a positive effect on seed germination rate and morphogenesis of radicle and coleoptile under salt stress conditions this was directly related to the rapid seed starch degradation into sugar as exemplified by the improved activities of α -amylase and β -amylase (Figure 1) along with the speedy increase in contents of soluble sugars and sucrose in germinating seeds under salt stress (Figure 2), while lowered in dry weight of germinating seed residues as compared with the non-primed sources under salt stress.

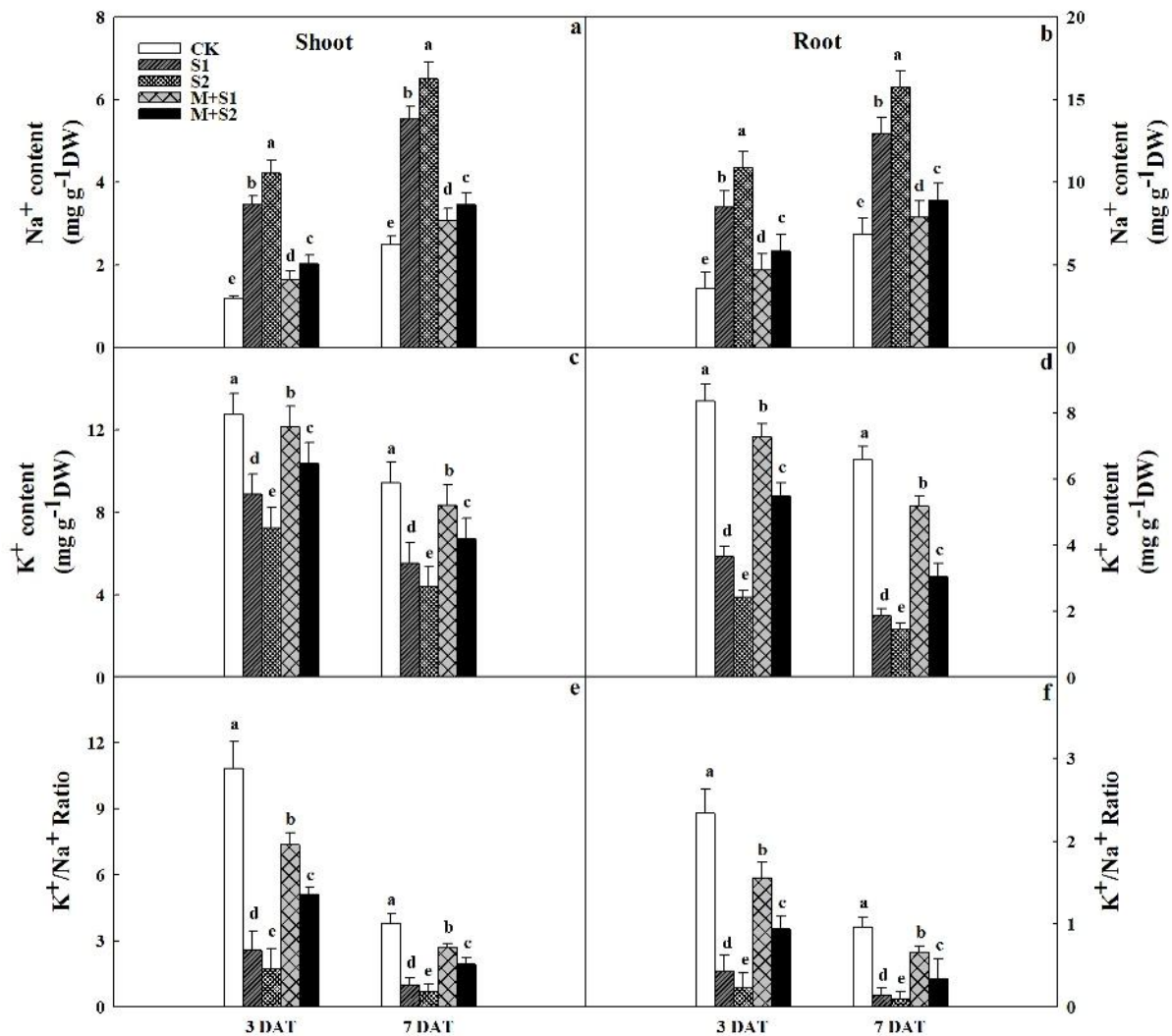


Figure 8 Effects of melatonin priming on contents of Na⁺ and K⁺ in wheat shoot and root during seedling establishment under salt stress. Data are mean +SD (n=3). Different small letters indicate significant differences between treatments at P < 0.05

In addition, our results demonstrated that the optimum melatonin dose of seed priming was 500 μ M for wheat seed germination under salt stress. The establishment of seedling is very crucial for the survival and grain yield formation of crops under salt stress. In the present study, we used the optimum dose of melatonin screened from the seed germination experiment for seed priming. We subjected the seedlings to two levels of salt stress to further evaluate the effect of melatonin pretreatment on seedling establishment in wheat. It is known that the adverse effects of salt stress on plant growth usually include the impacts of drought stress and ion imbalance. In this study, we found that values of all growth parameters measured decreased under salt stress compared to the control. Meanwhile, seed priming with melatonin alleviated those inhibitory effects (Table 2).

These results indicated priming with melatonin has a positive impact on seedling growth in wheat under salt stress conditions. This may be the early completion of pre-germination metabolic activities during seed priming. Leaf relative water content (RWC) is essential indicator of water relation for assessing tolerance to salinity stress. However, we also found that salt stress significantly decreased the

leaf RWC in wheat (Table 3). Furthermore, we observed that priming seeds with melatonin effectively maintain a better leaf water status of seedlings under salt stress, indicating that melatonin might play a significant role in wheat water relation under salt stress and help the plants to absorb more water to tolerate salt stress. Our results, also showed that melatonin-primed plants higher in leaf area compared to those grown under salt stress ones alone (Table 3).

The chlorophyll content is considered a sensitive indicator of the cellular metabolic status of plants and could further adversely affect leaf photosynthesis under salt stress. In this study, salt stress caused a significant reduction in leaf chlorophyll content and depressed leaf photosynthesis in levels of gas exchange and the photon conversion and electron transition. Furthermore, seed priming with melatonin delayed the reduction in leaf chlorophyll content and gas exchange rate of seedlings under salt stress as compared with the non-primed plants (Table 3 and Figure 5). This was in agreement with the alleviation effect of melatonin pretreatment on leaf photosynthesis in *Malus hupehensis* under salt stress (Li et al., 2012). In conformity with these conclusions, the protective effect of melatonin on chlorophyll showed that melatonin could mitigate the impact of salt stress on photosynthesis, which is the most important physiological process of the plant.

Parameters of chlorophyll fluorescence are important indicators for photon conversion and electron transition processes in Photosystem II (PSII) of leaves and are very sensitive to a biotic stress (Sayed, 2003). Moreover, the maximal photochemistry efficiency (Fv/Fm) and the actual photochemistry efficiency (ΦPSII) of PSII were clearly depressed by salt stress, at the same time; the melatonin priming effectively alleviates the negative effect of salt on Fv/Fm and ΦPSII (Figure 5). The results suggested that salt stress stimulated the inhibition on the photon conversation and blocked the electron transport from the primary acceptor plastoquinone to the secondary acceptor plastoquinone at the acceptor side of PSII (Mehta et al., 2010). While, melatonin pretreatment alleviated these harmful effects, which could modify the homeostasis of ROS in response to the salt stress and the melatonin priming.

The malfunction of PSII could result in the more accumulation of ROS, after that leading to oxidative damage to cells membrane (Flexas et al., 2004; Chaves et al., 2009). Oxidative stress is considered a major damaging factor in plants exposed to salt stress. Generation of H₂O₂ leads to lipid peroxidation, which causes membrane damage and electrolyte leakage (Sairam and Srivastava, 2002). ROS accumulation reportedly to be inhibited by exogenous melatonin (Zhang et al., 2013). However, salt stress increased the production rate of O₂⁻, and contents of H₂O₂ and MDA in both germinating seeds and leaves of wheat seedlings. Moreover, the melatonin pretreatment significantly decreased the production rate of O₂⁻, and contents of H₂O₂ and MDA (Figure 3 and 6), indicating a pronounced alleviation effect of melatonin on the oxidation damage induced by salt stress.

This alleviation effect on oxidation was further related to the up-regulation of the ROS-scavenging enzymes. It has reported that melatonin plays important roles in ROS-scavenging by enhancing actions of the antioxidant systems include the antioxidative enzymes (Tal et al., 2011; Park et al., 2013). In this study, the actions of SOD, POD, APX, and GR in the germinating seeds and leaves of wheat seedlings significantly decreased by salt stress, while the melatonin priming induced much higher activities of the antioxidative enzymes (Figure 4 and 7). Thus, the better activation of the antioxidant enzyme activities to enhance the scavenging capacity of ROS could be one of the critical explanations for the alleviation effect of melatonin priming on salt stress in wheat germinating seeds and seedlings in the present study.

In addition, ion balance and compartmentalization are critical for plants to cope with salt stress (Chen et al., 2007). Keep a balance between K⁺ and Na⁺ by promoting the uptake of K⁺ while reducing the accumulation of Na⁺ is one essential point for improving plant tolerance to salt stress (Ashraf et al., 2010; Zhu et al., 2016). Li et al., (2012) reported that salt stress induced the incensement of Na⁺ content and decrease of K⁺ contents in *Malus hupehensis* plants, and melatonin pretreatment enabled plants to maintain relatively higher K⁺ levels as compared with the non-pretreatment. In the present study, salt stress significantly increased Na⁺ accumulation while decreased K⁺ contents and K⁺/Na⁺ ratio in both shoots and roots of wheat seedlings. However, priming seeds with melatonin promoted K⁺ uptake while reducing Na⁺ uptake, and thus maintained a higher K⁺/Na⁺ ratio in wheat seedlings (Figure 8). These findings suggested that melatonin reduced the toxic effects of Na⁺. This was in accordance with the enhancement of shoot and root lengths, seedling fresh and dry weights in wheat.

5. CONCLUSIONS

The study confirmed that salt stress adversely affected seed germination and seedling establishment in wheat, while seed priming with melatonin effectively alleviated the adverse effects on seed germination with an optimum concentration at 500 μM. In addition, seed

priming with melatonin benefited wheat seedling establishment under salt stress by delaying leaf chlorophyll degradation and better maintenance of ion balance between K⁺ and Na⁺, and further enhanced leaf photosynthesis and alleviated the oxidative stress by improving activities of the antioxidant enzymes to reduce production of ROS. Melatonin then suggested as a potential substance of seed priming for improvement of salt stress during the seed germination and the seedling establishment stages in wheat.

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Author Contributions

Feisal M Ismaeil: Correspondence author, experiments implementation and manuscript writing.

Salah Eldeen E Ahmed: Manuscript tables data arrangement and analysis.

Abderhim A Jabereldar: Manuscript figures data arrangement and analysis.

Ahmed M El-Naim: Revision and correction of manuscript writing.

Entisar Elilah B Mohammed Ahmed: Technical review.

Informed consent

Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interests.

Ethical approval

In this article, as per the plant regulations followed in the Department of Crop Science, Faculty of Natural Resources and Environmental Studies, University of Kordofan, Elobeid, Sudan; the authors observed the impact of melatonin priming in alleviating the negative impact of salt stress on seed germination and seedling establishment of winter wheat (*Triticum aestivum* L. cv Elnilein). The ethical guidelines for plants & plant materials are followed in the study for observation, identification & experimentation.

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Data and materials availability

All data associated with this study are present in the paper.

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