Original Research Article

Effects of melatonin priming on seed germination of wheat under salt stress

ABSTRACT: Melatonin (N-acetyl-5-methoxytryptamine) has been identified as a new growth regulator in plants due to its beneficial effect in abiotic stress reduction. The experiment was conducted at the Wheat Cultivation Department Laboratory, Gezira Scheme, Sudan. To investigate the effects of melatonin priming in alleviating the negative effect of salt stress on seed germination of winter wheat (*Triticum aestivum* L. cv Elnilein and Imam). Six concentrations were used: control, salt (300 mM NaCl) and four concentrations of melatonin (M) 10, 100, 500 and 1000 µM with salt (300 mM NaCl) add to each concentration: CT, ST, M1+ST, M2+ST, M3+ST, M4+ST. The treatments were arranged in a Randomized Complete Block Design with three replications. The parameters studied included: seed germination rate (%), germination index, mean germination time (d), lengths (cm) and dry weight (mg) of coleoptile and radicle, starch, soluble sugar, and sucrose concentrations, amylase activities, activities of the antioxidant enzymes including superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX) and catalase (CAT), reactive oxygen species (ROS) including malondialdehyde (MDA), Hydrogen peroxide (H_2O_2) contents and superoxide (O_2^-) production rate, abscisic acid (ABA). The parameters of seedling establishment were: Shoot height, root length, fresh and dry weight of shoot and root. The treatment M3+ST (500 µM Melatonin + 300 mM NaCl) had the highest seed germination rate, germination index lengths and dry weights of coleoptile and radicle, soluble sugar, sucrose

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concentrations, amylase and antioxidant enzymes activities, and ABA concentration, seedling shoot and root length, and fresh and dry weight of shoot and root in Imam and Elnilein cultivars, respectively.

The study revealed that melatonin priming effectively alleviates salt stress during seed germination and seedling establishment in winter wheat.

Keywords: Salt stress, Melatonin, Germination, Antioxidant system, Winter wheat (*Triticum aestivum* L.)

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1. Introduction

" Amongst the various abiotic stresses that plants convene, heat and salt stress are mainly imperative in agriculture since both stresses can lead to a considerable reduction in crop yields and are predictable to raise in incidence in the near future because of climate change" [1]. "Presently about 6% of total land area of 20% agricultural total lands are influenced by salt in Sudan" [2]. "Seed germination and seedling establishment are the two critical stages for the crop establishment" [3], and "these were to be harmfully affected by salt stress" [4]. "Salinity causes reduced emergence and impaired seedling growth presumably due to salt-induced osmotic effects, specific ion toxicity, nutrient imbalance, oxidative damage, and alterations in endogenous levels of hormones" [5]. In this admiration [6] confirmed that "increases in salt content reduce the germination percentage and delays germination time".

"Salinity can influence the seeds germination by the toxic effect of sodium and chloride ions on the viability of embryo" [7] and [8]. "The toxic effects include disruption to the structure of enzymes and other macromolecules, damage to cell organelles and the plasma membrane, the disruption of respiration, photosynthesis, and protein synthesis" [9], [8], and [10]. "accumulation of mono di-Aldehyde (MDA) in different

crops" [11]. "MDA content is a signal of membrane injury at the cellular levels in salt stress. Hence, the concentration of MDA can serve as an essential oxidative stress sign" [12]. "Oxidative stress is the term utilized to define the imbalance between the production of ROS and scavenging or detoxification" [13]. "Plants have developed the antioxidant defense system to reduce the harmful impact of ROS including antioxidant enzymes i.e., superoxide dismutase (SOD), peroxidase (POD) catalase (CAT), glutathione reductase (GR), and ascorbate peroxidase (APX) but salt stimulate ROS production exceeds capacity of antioxidant in the cells, oxidative stress arises" [14] and [15].

"Melatonin (*N*-acetyl-5-methoxytryptamine) has comprehensively be known as an important hormone of animal occupied in many biological processes" [16]. However, "its role in animals the same like a hormone has been well-characterized, it's information in plants kingdom is still bitty" (Arnao and Hernández-Ruiz 2014). "Melatonin is considered as a growth regulator of plant, and has role has as that of growth regulation of the shoots, root and explants, germination of seed, rhizogenesis and remain senescence in leaf" [17]. "The melatonin mechanisms mediated tolerance of stress occupy the antioxidant support biosynthesis, the start of connected enzymes and the directly ROS scavenging subsequent the plants exposure to harm environments" [18] and [19]. "Previous study has reported that while applied exogenously melatonin alleviates oxidative injury through ROS scavenging and stimulating antioxidant system that are in different environmental stresses" [20] and [21].

In view of the above information, this study focused on determining the effect of melatonin pretreatment on seed germination, physiological, and biochemical changes happening in winter wheat geminating seeds exposed to salt stress.

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2. Materials and methods

2.1 Experimental design

The study was performed in the wheat cultivation department Laboratory, Gezira scheme, Gezira state, Sudan. Uniform seeds of two cultivars of winter wheat (*Triticum aestivum* L. cv Elnilein and Imam) were chosen and surface-sterilized with 2.5% sodium hypochlorite for 10 min and washed seven times with sterile distilled water [22]. Two treatments were placed with soaking the seeds in distilled water as control and 10, 100, 500, and 1000 µM of melatonin solution for 20h, respectively. The concentrations of melatonin used here were established to be optimal in the preliminary experiments. The soaked 50 seeds of each cultivar were arranged on a filter paper in Petri dishes with a diameter of 9 cm, and 300 mM of NaCl was then added to each dish. The dishes were incubated at 22± 0.5 ^{0}C for 7 days in Chamber. At the two-leaf stage (ca. 20 days after germination), wheat plants were transplanted to plastic containers (45 cm in length, 35 cm in width and 18 cm in height). The density was 30 plants in each container. The Hoagland nutrient solution was used for hydroponic culture. The growth temperatures were 25 ^{0}C/20 ^{0}C (day/night), six treatments were used: Control (CT), 300 mM of salt NaCl (ST), 10 µM of melatonin+300 mM NaCl (M1+ST), 100 µM of melatonin+300 mM NaCl (M2+ST), 500 µM of melatonin+300 mM NaCl (M3+ST), 1000 µM of melatonin+300 µM NaCl (M4+ST). The experiment was a Randomized Complete Block Design with three replications. 50 seed were used in each treatment for the germination test for each cultivar and were sampled at days 2, 3, 4, 5 and 7 after treatment for biochemical and physiological measurements, and another batch was sampled at day 7 after treatment for determinations of dry weight, seed germination rate, and ABA content. Wheat seedlings were harvested at

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the four-leaf stage in order to measure the seedling shoot height, root length, and fresh and dry weights of the shoot and root.

2.2 Germination rate (GR), mean germination time (MGT), germination index (GI), dry weight of germinating seeds

GR is the proportion of germinated seeds in each Petri-dish during 7 days after germination. MGT was determined fallowing the method of (Ellis and Roberts 1978), i.e. MGT = $\sum$ (f x i)$/\sum$f. where, f is the number of recently germinated seeds on germinating day i. Subsequently, long MGT indicates slow germination. Germinated seeds percentage on each day was measured. "The method of [23] was used to calculate the GI as GI= $\sum(G_i/T_i)$, where Gi is the germination percentage per day, and Ti is day of germination test. High GI demonstrates high quality of seed and performance" [23].

Radicles, coleoptile and the seed residues of germinating seeds of two cultivars were selected and dried at 105 ^{0}C for 2 h, after that kept at 80 ^{0}C to obtain stable weight.

2.3 Contents of starch and soluble sugars

"Germinating seed oven- dried powder (1 g) was mixed by 10 ml 0.33 mM of HCl` and then heated at 100 ^{0}C for 10 min to remove starch. Half ml of ZnSO4 (30 %, w/v) was added to the extraction to remove proteins. After that, 0.5 ml K3 [Fe (CN) $_6$] (30 %, w/v) was added and mixed, and then the mixture volume was attuned to 20 ml with distilled water. After completely shaking it was filtrated, then the concentration of starch was determined like the amount of glucose produced at 20–25 ^{0}C using the polarimetric analysis through an automatic recording polarimeter (WZZ-2B, Shanghai, China)" according to [24]. "Moreover, to extract total soluble sugars, 0.1 g oven-dried germinating seed powder sample was homogenized in (80 %) ethanol at 80 ^{0}C for 10 min and centrifugation at

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600g. The supernatant was collected for content of soluble sugar and sucrose measurement using the method of anthrone" [25].

2.4 Amylase extraction and activity assess

The activity of amylase was estimated after [26]. "The concentration of soluble protein in germinating seeds was estimated following the method of [27] by using bovine serum albumin (BSA) like standard. Activities of both α- and β-amylase were articulated as unit mg^{-1} protein, and one unit is equivalent to release of one mg maltose from starch per minute by the amylases" [27].

2.5 Activities of antioxidant enzymes and O_2- production rate, H_2O_2, MDA concentrations

The MDA and H_2O_2 concentration measurements and antioxidant enzymes activities in germinating seed were measured suggested by [28]. "In brief, 0.5 g samples of germinating seed were sliced and homogenized in a mortar and pestle with 5 ml of ice-cold extraction buffer (50 mM potassium phosphate buffer, pH 7.0, 0.4 % polyvinylpoly pyrrolidone (PVP)). The homogenate was centrifuged at 10,000xg for 30 min and the supernatant was used like crude extract. All procedures were done at 4 ^{0}C. The MDA concentration was estimated after" [29]. "In brief, 20 % trichloroacetic acid containing 0.5 % 2-thiobarturic acid was added to 0.2 ml of the suspension and heated at 95^{0}C for 25 min after that cooled down in a cool water bath and centrifuged at 3,000xg for 5 min. Concentration of MDA was calculated at 532 nm and corrected by subtracting the absorbance at 600 nm. The concentrations of H_2O_2 were carried out by the method" given by [30]. "Briefly, 1 ml suspension was added to 0.1 ml 20% titanium tetrachloride ($TiCl_4$) and mixed with shaking. 0.2 ml of 25% ammonia was added to the mixture. The reaction solution was after that centrifuged at 5,930xg for 5 min, and the

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precipitate was washed three times with cold acetone to eradicate the color. Lastly, the precipitate was dissolved in 3 ml 1 M H_2SO_4, and filtered prior to measurement of the absorbance at 415 nm. The O^-_2 production rate was measured by using the method" of [22]. The activities of SOD, and CAT were calculated according to [28]. "For SOD activity measurement, 3 ml reaction mixture contained 130 mM methionine, 750 mM NBT, 100 mM EDTA and 50 ml of enzyme extract in 50 mM phosphate buffer (pH 7.8). The reaction was started with 20 mM riboflavin through exposing the cuvette to a 15W circular white light tube for 10 min. One unit of SOD activity was identified as the amount of enzyme per mg of protein sample causing 50% inhibition of the rate of NBT reduction at 560 nm" [31]. CAT activity was estimated as described by [28]. The activity of POD was determined according to the changes in absorbance at 470 nm due to guaiacol oxidation. Ascorbate peroxidase (APX) was measured using the method of [32].

2.6 Determination of endogenous hormone concentration

"The concentration of abscisic acid (ABA) was analyzed by an indirect ELISA technique. Briefly, the samples of 0.1 g dry seeds were homogenized in liquid nitrogen and removed in cold 80% (v/v) methanol by butylated hydroxytoluene (1 mM) overnight at 4 ^{0}C. The extracts were then collected after centrifugation at 10,000 x g (4 ^{0}C) for 20 min, and after that the extracts were passed throughout a C_{18} Sep-Pak catridge (Waters, Milford, MA) and dried in N_2. Then the residues were melted in PBS (0.01 M, pH 7.4). To estimate the content of ABA using the method" described by [33].

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2.7 Statistical analysis

All data were subjected to the one-way analysis of variance (ANOVA) to determine the significant differences between the treatments using the software of SPSS (Ver. 10.0 SPSS, Chicago, IL, USA).

3. Results

3.1 Seed germination parameters

Seed germination rate was significantly reduced under salt stress. During 7 days of germination, seed GR and GI were considerably enhanced whereas MGT was reduced by melatonin priming treatment when compared with salt stressed-seed in both cultivars, with values being higher in Imam than that in the Elnilein (Table 1). Also, lengths and dry weights of coleoptile and radicle were clearly increased in both cultivars while seed residue dry weight was significantly ($P < 0.05$) reduced with application of melatonin in both cultivars, as compared with salt stress alone. Therefore, this increase was more prominent in Imam than that in the Elnilein (Table 1). However, seed pretreatment with melatonin enhanced seed germination in wheat under salt stress. However, the control treatments performed better in all the germination parameters studied indicating that there is no problem of both the wheat cultivars.

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Table 1 Effect 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 7 days of germination under salt stress in wheat.

					Length (cm)		Dry weight (mg seed^{-1})		
Cultivar	Treatments	GR(%)	GI	MGT(d)	Radicle	Coleoptile	Radicle	Coleoptile	Seed

									residue
Elnilein	CT	95.36±0.28a	92.78±0.05a	1.54±0.03d	9.38±0.05a	8.27±0.03a	13.18±0.02a	10.26±0.05a	7.13±0.02f
	ST	64.39±0.26e	21.49±0.36f	4.62±0.22a	0.9±0.01f	0.82±0.01f	1.39±0.05f	1.12±0.01f	32.36±0.04a
	M1+ST	76.28±0.23d	26.13±0.27e	3.59±0.13b	1.59±0.02e	1.21±0.02e	2.84±0.02e	2.03±0.01e	25.71±0.15b
	M2+ST	88.47±0.41b	51.47±0.23c	1.87±0.13d	5.23±0.02c	4.78±0.14c	6.35±0.04c	5.01±0.04c	12.45±0.16d
	M3+ST	94.23±0.20a	74.19±0.18b	1.73±0.01d	8.04±0.01b	7.14±0.04b	12.03±0.02b	8.63±0.28b	8.49±0.09e
	M4+ST	81.63±0.46c	46.68±0.33d	2.42±0.04c	3.12±0.01d	3.29±0.03d	4.15±0.01d	3.23±0.01d	20.18±0.04c
Imam	CT	97.83±0.40a	95.32±0.17a	1.47±0.02c	10.89±0.09a	9.43±0.23a	15.72±0.06a	11.79±0.09a	9.27±0.05f
	ST	67.82±0.37f	25.46±0.30f	3.48±0.08a	0.95±0.01f	0.91±0.01f	1.98±0.01f	1.35±0.01f	36.55±0.27a
	M1+ST	78.53±0.31e	29.38±0.49e	2.92±0.06b	1.85±0.02e	1.98±0.05e	3.94±0.02e	3.28±0.03e	29.18±0.36b
	M2+ST	91.72±0.35c	69.37±0.90c	1.69±0.02c	7.33±0.03c	5.83±0.05c	9.47±0.18c	7.37±0.19c	13.95±0.03d
	M3+ST	95.57±0.27b	78.67±0.65b	1.58±0.02c	8.63±0.22b	8.65±0.27b	13.69±0.14b	9.72±0.29b	10.87±0.31e
	M4+ST	82.68±0.67d	55.03±0.63d	2.12±0.07b	5.63±0.08d	4.61±0.04d	7.44±0.04d	5.04±0.01d	23.59±0.04c

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

3.2 Starch, soluble sugar, and sucrose contents

During seed germination progress starch level decreased, but total soluble sugar and sucrose concentrations were increased in germinated seeds of wheat in both cultivars (Fig. 1). Nevertheless, in both cultivars, starch concentration of seed was lower under melatonin priming compared to salt stress during 7 days of germination (Fig. 1). In comparison, the concentration of total soluble sugar and sucrose were higher in the control and melatonin than that under salt stress in both cultivars. However, total soluble sugar and sucrose concentrations were significantly ($P < 0.05$) higher in Elnilein than those in Imam and oppositely in case of starch concentration (Fig. 1). These clearly indicated that melatonin priming improved the resistance of wheat seed to salt stress.

3.3 Activities of α- and β- amylase

In control the α-amylase activity in both wheat cultivars was improved in seeds germinating than that in the level of salt stress during 7 days of germination (Fig. 2). Nevertheless, melatonin priming significantly ($P<0.05$) enhanced α-amylase activity; when contrasted with salt stress alone. Furthermore, in both cultivars β-amylase activity decreased under salt stress. On other hand, melatonin pretreatment significantly ($P<0.05$)

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increased the activity of β-amylase compared to salt stress alone (Fig. 2). Conversely, under control and melatonin mutually activities of α- and β- in germinated seeds in both cultivars were higher than those of under salt stress levels, and they increased more in Elnilein than that in Imam.

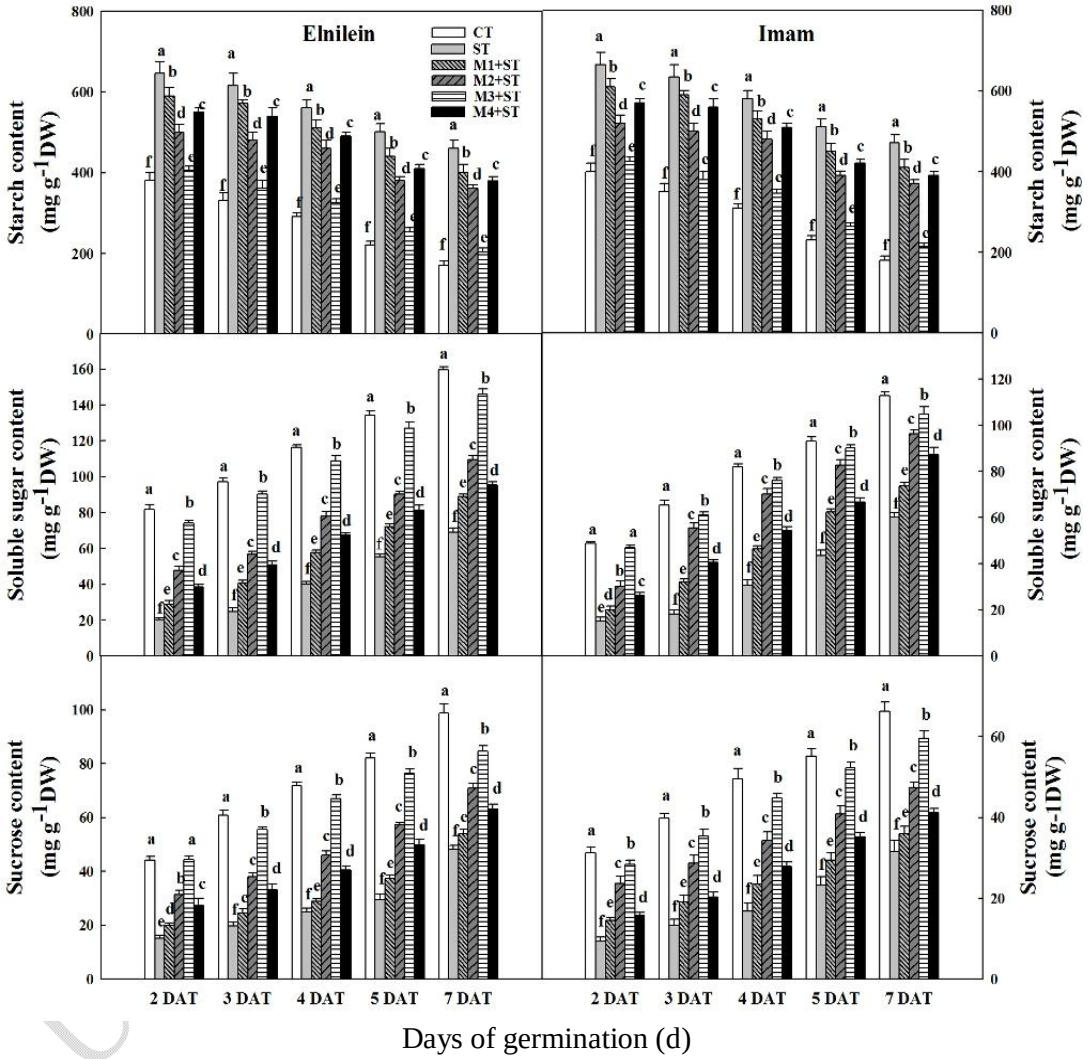

Fig. 1 Effect of melatonin priming on changes in contents of starch, soluble sugar, and sucrose in wheat seeds during 7 days germination under salt stress. Data are means ±SD (n=3). Different small letters at each day indicate significant difference between treatments at $P < 0.05$.

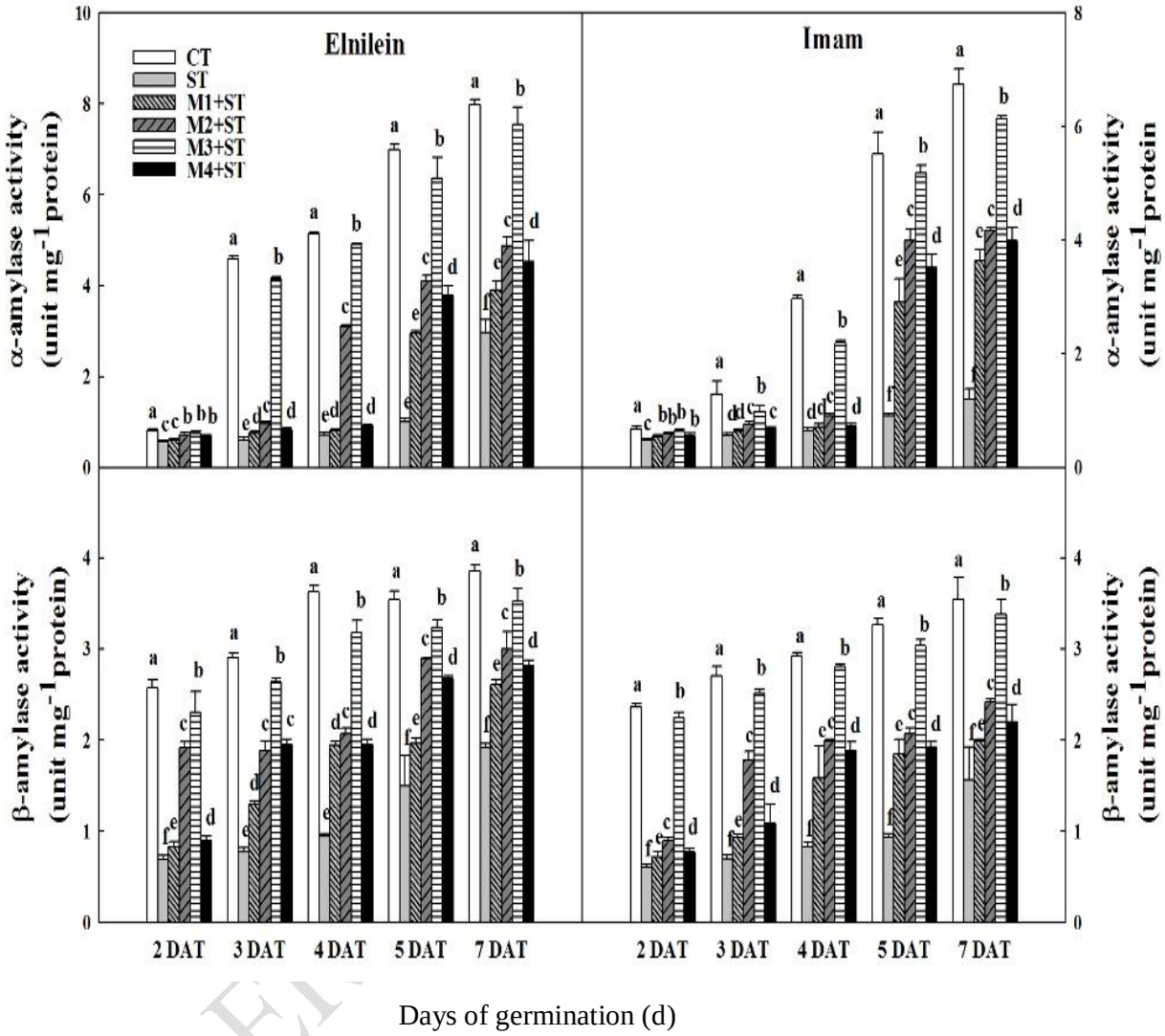

Fig. 2 Effect of melatonin priming on changes in activities of α- and β- amylase in wheat seeds during7 day's germination under salt stress. Data are means ±SD (n=3). Different small letters at each day indicate significant difference between treatments at $P< 0.05$.

3.4 O^{-}_{2} production rate, concentrations of H_2O_2 and MDA, and activities of antioxidant enzymes

Compared to the control, salt stress significantly ($P < 0.05$) increased the concentration of MDA and H_2O_2 in wheat germinating seed in both cultivars (Fig.3). In contrast, exogenous application of melatonin significantly ($P < 0.05$) decreased the concentration of MDA and H_2O_2 in both cultivars compared to salt stress alone. In both cultivars the production rate of O^{-}_{2} demonstrated the same pattern as similar to H_2O_2 and MDA accumulation during the germinating seeds (Fig. 3). Reversely, salt stress alone significantly ($P < 0.05$) reduced the activities of SOD, POD, CAT, and APX compared to control in both cultivars (Fig.4). In contrast, pretreatment of melatonin remarkably enhanced ($P < 0.05$) SOD, CAT, POD, and APX activities in both cultivars and they increased more in Elnilein than that in Imam. These results confirmed that priming of melatonin was responsible for the improvement of adaptive responses of wheat seed germination against salt stress.

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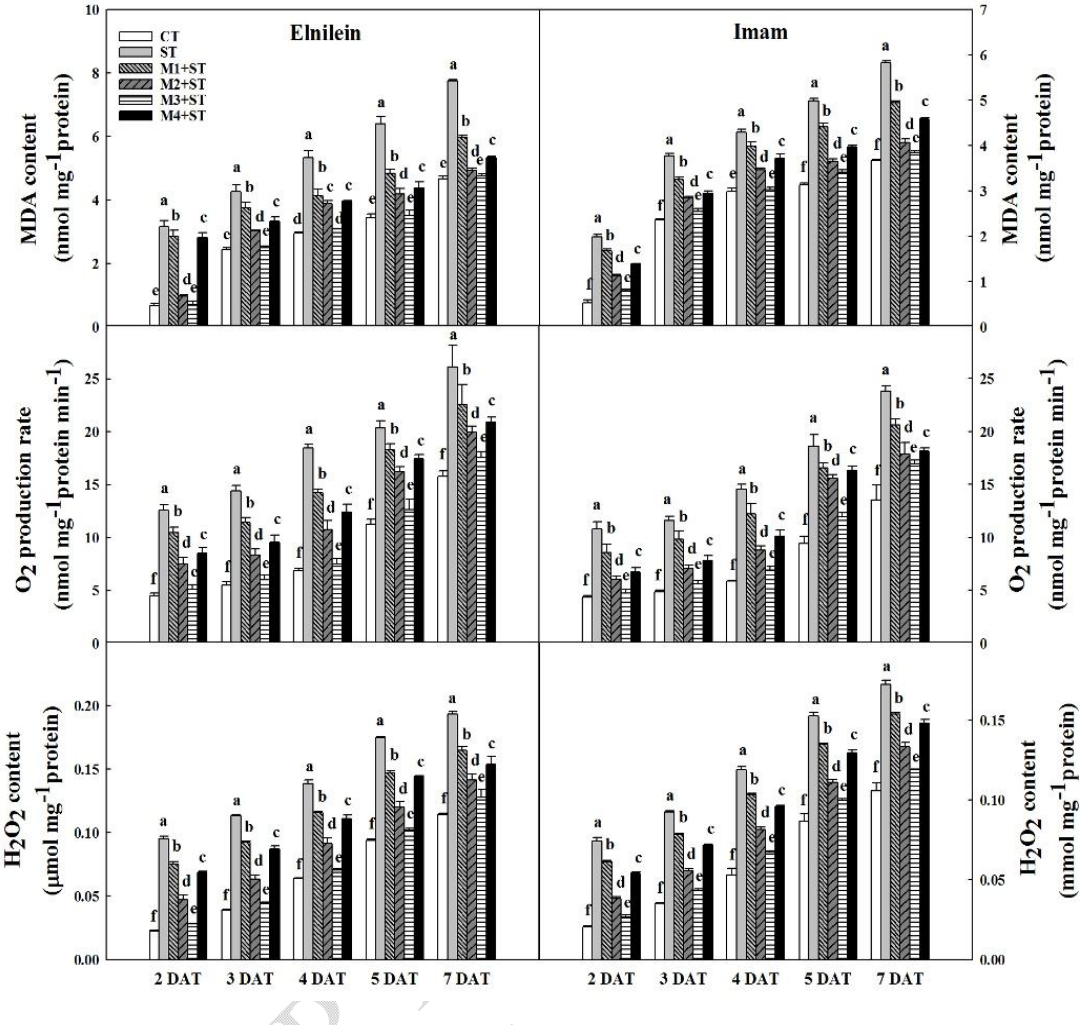

Fig.3 Effect of melatonin priming on changes in contents of malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and $O2^-$ production rate in wheat seeds during 7 days germination under salt stress. Data are means ±SD (n=3). Different small letters at each day indicate significant difference between treatments at $P < 0.05$

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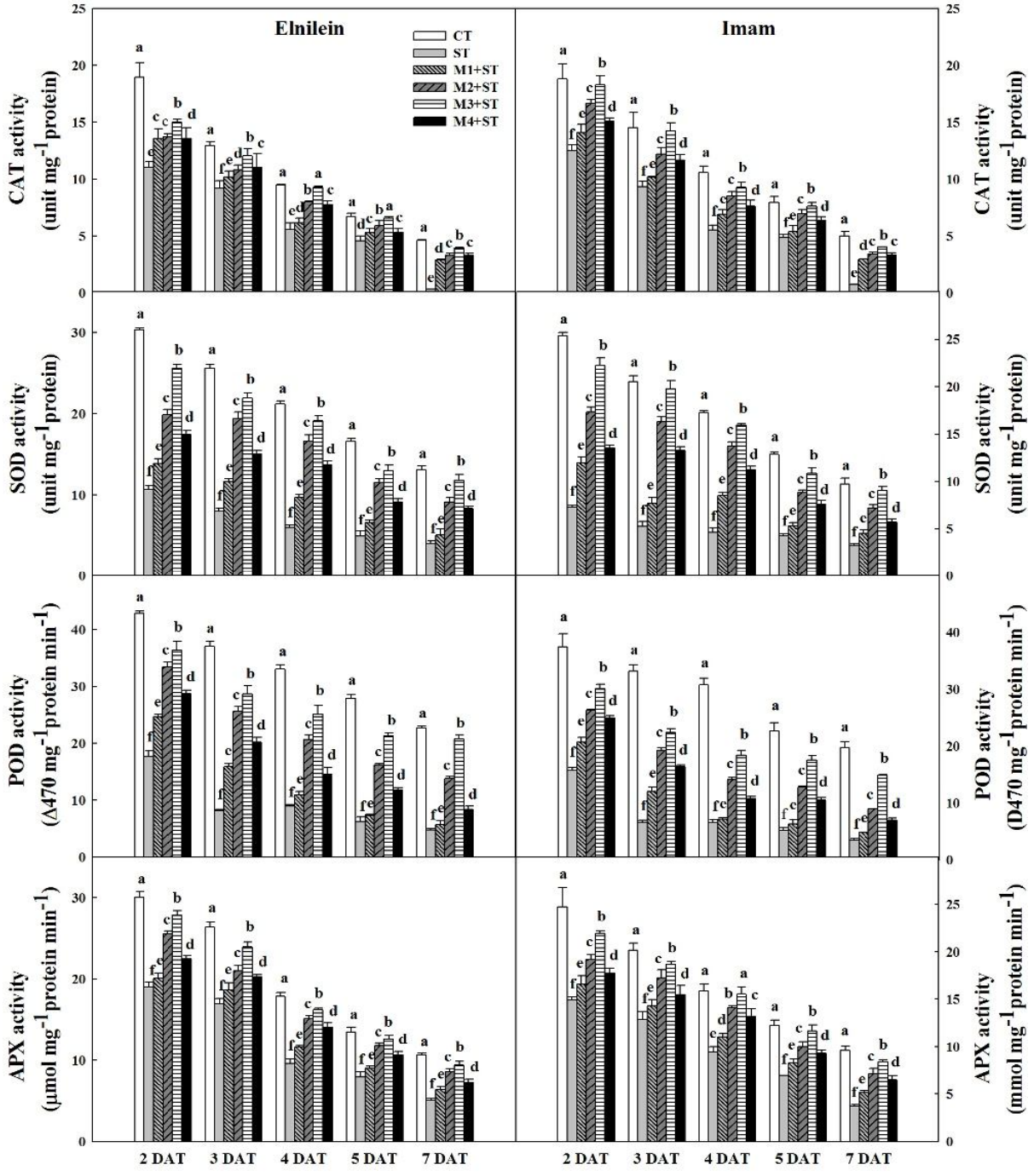

Fig. 4 Effect of melatonin priming on changes in activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) in wheat seeds during 7 days germination under salt stress. Data are means ±SD (n=3). Different small letters at each day indicate significant difference between treatments at $P < 0.05$

3.5 ABA concentration

After set of treatments the concentration of ABA in germinating seeds of wheat was much higher under salt stress when compared to control in both cultivars (Fig. 5). On the other hand, under salinity stress melatonin priming significantly ($P < 0.05$) enhanced germinated seeds ABA concentration in both cultivars (Fig. 5). In general, melatonin priming had higher levels of ABA in germinated seeds as compared to both control and salt stress alone. The protective effect of melatonin on ABA concentration was greater in Imam than in Elnilein ($P < 0.05$).

3.6 Seedling growth parameters

After germination, wheat plants were allowed to growing at the same temperature conditions till the 4-leaf stage, for further investigation (Table 2). Salt stress induced by 300 mM NaCl significantly inhibited the growth of wheat seedlings. This attribute was demonstrated by comparing the decrease in the shoot height, root length, fresh and dry weight of shoot and root, in both cultivars. However, melatonins priming significantly ($P < 0.05$) alleviate this response. The positive effects of melatonin priming had more profound in Imam than that in the Elnilein.

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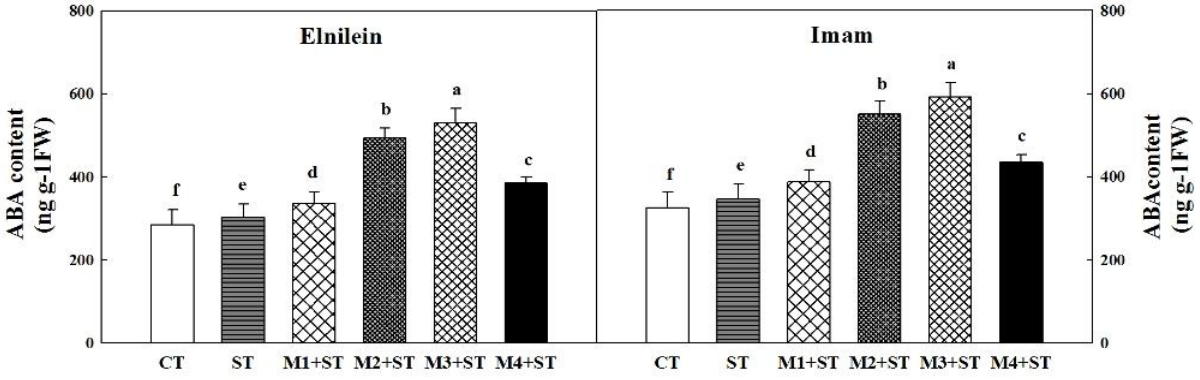

Fig. 5 Effect of melatonin priming on changes in content of ABA in wheat seeds during 7 days germination under salt stress. Data are means ±SD (n=3). Different small letters indicate significant difference between treatments at $P < 0.05$

Table 2 Effect of melatonin priming on seedling shoot height and root length, fresh and dry weight of shoot and root of four- leaf-stage seedling grown under salt stress in wheat.

Variety	Treatments	Shoot height (cm)	Root length (cm)	Fresh weight (mg plant^{-1})		Dry weight (mg plant^{-1})	
				Shoot	Root	Shoot	Root
Elnilein							
	CT	23.56±1.70a	26.82±1.84a	23.97±0.54a	16.85±0.33a	6.97±0.44a	3.96±0.37a
	ST	11.98±0.66f	13.27±0.68f	9.81±0.41f	6.19±0.41f	2.38±0.42f	1.03±0.42d
	M1+ST	15.11.±1.45e	17.93±1.54e	13.21±0.39e	8.88±0.32e	3.92±0.40e	1.97±0.42d
	M2+ST	19.67±0.50c	20.19±0.28c	17.66±0.36c	13.44±0.63c	4.98±0.57c	2.89±0.53b
	M3+ST	22.44±0.36b	23.74±0.84b	20.13±0.31b	15.67±0.23b	5.11±0.31b	3.58±0.13b
	M4+ST	17.22±0.36d	18.87±0.84d	15.23±0.31d	10.16±0.45d	4.09±0.38d	2.01±0.35c
Imam							
	CT	25.61±1.70a	28.87±1.81a	26.96±0.49a	19.79±0.28a	8.96±0.49a	5.89±0.48a
	ST	13.18±1.66f	15.59±0.81f	12.14±0.19f	7.92±0.45f	3.56±0.49f	1.98±0.46e
	M1+ST	16.96±0.78e	18.24±1.52e	16.15±0.56e	10.53±0.16e	4.32±0.57e	2.67±0.46e
	M2+ST	20.73±0.50c	22.43±0.42c	22.22±0.19c	15.91±0.62c	6.12±0.43c	4.78±0.63c
	M3+ST	23.47±0.36b	25.91±0.82b	24.42±0.12b	17.19±0.49b	7.43±0.18b	5.17±0.19b
	M4+ST	18.23±0.36d	20.34±0.82d	19.21±0.27d	13.39±0.39d	5.83±0.47d	3.32±0.39d

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

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4. Discussion

Seed germination and plant growth inhibition in saline surroundings were caused by the reduce in the supply of available energy of biochemical essential for synthesis reactions, most important to an inhibition growth of root and coleoptiles [34]. It has been recommended that melatonin acting a significant function in responses of plant to environmental stimulus, including biotic and a biotic stresses [19] and [35]. In this study, salt stress clearly reduced seed germination, growth of root and coleoptiles in both wheat cultivars (Table. 1). But the melatonin priming improved these parameters even under salt stress. These results are in agreement with the results obtained, in barley, bean, and maize [36] . Because melatonin pretreatment was effectual to defend plant cell from oxidative harm [37]. In agreement with this, our results revealed that

melatonin pretreatment significantly enhance germination rate and morphogenesis of radicle and coleoptile under salt stress. This was directly related with the rapid seed starch humiliation during with speedy increase in concentrations of soluble sugars and sucrose in germinating seeds under salt stress (Fig.1).

Throughout seed germination, different hydrolytic enzymes are created and implicated in reserve mobilization [38]. In harmony with this, our results discovered that salt stress caused extremely inhibition on amylase activity while melatonin use considerably alleviated this inhibition improved the activities of α-amylase and β-amylase in both cultivars (Fig. 2). Hence, melatonin priming assists the starch change into sugars (Fig. 1), which enhancement wheat seed germination under salt stress.

Salt stress stimulates more ROS production, which create injure to macromolecules like, proteins, lipids and nucleic acids [39] and [40], mentioned that salt stress increased the concentration of H_2O_2 and MDA in the tomato mitochondria. This might be caused by a salt-induced raise in the O_2 production rate [41]. In agreement with the previous researches mentioned by [36]. Our study confirmed that salt stress quickly increased the concentration of MDA, H_2O_2, and O_2, in wheat germinating seed. But, there was a significant reduces in these factors upon the exogenous of melatonin as contrasted by salt-stressed wheat germinating seeds in both cultivars (Fig. 3). A similarly stress role of anti-oxidative of melatonin priming was also seeing in salt-stressed barley plants [42].It is strongly documented that the antioxidant enzymes like SOD, CAT, POD, and APX cooperate an important function in ROS scavenging in salt stressed plants [43]. Previous researches showed that exogenous melatonin defends contents against oxidative stress in reed under heat stress [44] and in wheat under drought. This associated with increased the activities of SOD, CAT, POD and APX. In agreement with these results, we

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experiential that activities of these enzymes in the mitochondria of wheat seed were significantly improved by melatonin pretreatment application in the current study (Fig. 4), and which may have provided to the alleviated oxidative stress in the mitochondria of wheat germinating seeds and thus enhanced germination rate under salt stress.

Abscisic acid (ABA)) is essential in the rule of a-amylase through germination [45] and [46] mentioned that ABA plays a role in alleviating oxidative damage under a biotic stress. “Abscisic acid (ABA) has been proposed to act as a mediator in plant responses to a range of stresses, including drought and salt stress” [47] “Among different phytohormones, abscisic acid (ABA), is the central controller of a biotic stress resistance in plants and organizes group of functions [48], allowing plants to cope with various stresses”. ABA content considerably increases in plants under stress conditions like drought, extreme temperature, and high salinity, stimulating stress-tolerance effects that help plants adapt and survive under these stressful conditions [49], and is necessary in the plants for stress tolerance [50]and [51], stated that, the level of ABA in soybean seed germination increased significantly under salt stress. It is thus feasible that ABA improves salt tolerance in soybean. Our results also demonstrated that the salt stress increased the ABA content of both cultivars when compared to the control germinating seed. Furthermore, melatonin pretreatment significantly increased seed ABA contents as compared to the salt stress and control during germination under salt stress (Fig.5). Therefore, we can deduce that increasing ABA concentration due to seeds priming with melatonin may be one of the mechanisms that enabled wheat seeds to tolerate salt stress [52] mentioned that, during the onset and development of salt stress all seedlings growth parameter are affected and reduced when compared with control plants. In this study, we found that values for almost all of

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the parameters of seedling growth measured were significantly ($P < 0.05$) decreased in plants under salt stress as contrasted with the control. Nevertheless, melatonin priming application considerably alleviated those inhibitory causes (Table 2). Various studies have established that melatonin can perform as a potential modulator of plant growth and improvement in a dose-dependent way [53]. The increased shoot and root heights and weights of seedlings growing under salt stress may serve as a good illustration of this. This was initially attributed to the significance of melatonin pre-treatment improving seed germination.

Conclusions

The study clearly demonstrated that the deleterious effects of salt stress on seed germination and early seedling growth of wheat cultivars were considerably alleviated by melatonin priming. On one hand, the exogenous application of melatonin improved soluble sugar, sucrose contents, α- and β activities under salt stress. Also enhanced ABA content in germinated seeds. Conversely, melatonin could effectively scavenge ROS and improved antioxidant enzymes activities. These findings provide a novel use of melatonin in wheat during seed germination and seedling establishment under salt stress. We expect that the positive effect of melatonin in mitigation salt stress present new opportunities for its employ in agriculture. Highest values for most parameters measured were obtained at 500 µM of melatonin level and it is used for our next experiment of wheat seedling performance under salt stress.

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