ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 104, No. 3 (2017), p. 219–228
DOI 10.13080/z-a.2017.104.028

Physiological response of plants to polyethylene glycol (PEG-6000) by exogenous melatonin application in wheat

Dongxiao LI, Di ZHANG, Hongguang WANG, Yanming LI, Ruiqi LI

Agricultural University of Hebei,
Key Laboratory of Crop Growth Regulation of Hebei Province
Baoding 071001, P. R. China
E-mail: lidongxiao.xiao@163.com

Abstract

The regulation effect of melatonin on water use efficiency of leaf and potential mechanisms related to phytohormone of leaf/root were investigated using two wheat (*Triticum aestivum* L.) cultivars with contrasting drought tolerance: drought-tolerant 'Hengguan35' (HG35) and growing in irrigated fields 'Jimai22' (JM22). Four treatments, including normal water treatment (N), 20% polyethylene glycol (PEG) (P), P + 1 μM melatonin and P + 10 μM melatonin were conducted. Results indicated that exogenous melatonin could significantly improve net photosynthetic rate (Pn), instantaneous water use efficiency (WUE_{inst}) and intrinsic water use efficiency (WUE_{intr}) of cultivar 'Jimai22'. This was possibly related to increasing root auxin (IAA), zeatin riboside (ZR) content and inhibiting abscisic acid (ABA), hydrogen peroxide (H_2O_2) and aminocyclopropane-1-carboxylic acid (ACC) production in leaf. However, for cultivar 'Hengguan35', 1 μM and 10 μM melatonin did not improve photosynthetic rate and chlorophyll content even took negative effect on that. This could be caused by high vapour pressure deficit (VPD) and high H_2O_2 contents in leaf. Also, as a drought-tolerant cultivar, 'Hengguan35' possesses some physiological regulation itself such as increasing IAA and ZR content in root and leaf, inhibiting H_2O_2 production in root. But ABA and ACC content in root was not inhibited, even increased with melatonin under drought treatment. ACC was a potential central player in the hormone cross-talks that regulated leaf and root growth and physiological function. These results suggest that the effect of exogenous melatonin application on drought-resistance in seedling was distinct owing to wheat cultivars with different drought sensitivity, and this was also involved in complicated mechanism of physiological regulation to keep water status. Suitable application of melatonin thus can be an effective way in improving plant drought tolerance in wheat.

Key words: drought, melatonin, phytohormone, *Triticum aestivum*, water use efficiency.

Introduction

Drought stress represents one of the major limitations to winter wheat productivity by alleviating both leaf growth and photosynthetic capacity, which further limit the harvestable biomass and productivity. In the North China Plain, the rainfall during the entire growth stage in wheat is far below the actual water demand level (Cao et al., 2003), which results in large decline of the groundwater resource and reduction of the irrigation area of winter wheat during past 20 years (Jia, Liu, 2002). Therefore, it is critical to improve the water use efficiency (WUE) in winter wheat production on which to promote the sustainable agriculture in North China (Guan et al., 2015).

Melatonin, a growth regulator identified in higher plants in 1995, has been reported successively on regulating various biological processes, including root formation (Zhang et al., 2014), flowering and fruit ripening (Shi et al., 2016), and leaf senescence (Wang et al., 2012 a). In particular, melatonin can trigger the plant defense responses against abiotic stress, playing an important role in improving the tolerance of plants to adverse environment (Li et al., 2012; Turk et al., 2014; Meng et al., 2015; Bałabusta et al., 2016). This has been proposed to be related to scavenging active oxygen, increment of root to stem ratio, delaying induced leaf senescence, up-regulating of anti-stress genes and so on (Wang et al., 2012 b; Weeda et al., 2014; Jiang, Zu, 2015; Li et al., 2015; Ye et al., 2015). Currently, melatonin has been shown to act as an interesting natural biostimulator in regulating field crop production (Arnao, Hernández-Ruiz, 2014; Ye et al., 2016). The same concentration of melatonin may exert different effect on different organs of crops. Exogenous application of 10 μM melatonin was efficient and induced more and longer roots in *Lupinus* (Arnao, Hernández-Ruiz, 2007); however, the same concentration of melatonin was inhibitory for adventitious rooting in the commercial sweet cherry (Sarropoulou et al., 2012). In addition, seeds treated by melatonin could promote the seedling growth and seed production of maize, soybean, and cucumber (Janas et al., 2009; Zhang et al., 2013; Wei et al., 2015). Meng (2016) suggests that wheat seed priming with melatonin

Please use the following format when citing the article:
Li D., Zhang D., Wang H., Li Y., Li R. 2017. Physiological response of plants to polyethylene glycol (PEG-6000) by exogenous melatonin application in wheat. Zemdirbyste-Agriculture, 104 (3): 219–228 DOI 10.13080/z-a.2017.104.028

could enhance the biomass and yield once plants suffered water stress during filling stage.

Thus far, although the melatonin function in regulating plant growth and abiotic stress responses has been elucidated, the effect of melatonin on water utilization and phytohormone metabolism under drought stress is still largely uncharacterized in wheat. In this study, through exogenous application of melatonin in two wheat cultivars with contrasting drought tolerance (sensitive JM22 and tolerant HG35 cultivars), we investigated the potential role of melatonin in regulation leaf WUE, phytohormone and H_2O_2 content in leaves and roots of wheat under drought treatment. Our results would elucidate the melatonin effects in mediating plant drought tolerance and provide a new strategy in improving drought resistance and water use in wheat production.

Material and methods

Study site and experiment description. The experiment was carried out in a phytotron, Agricultural University of Hebei, Baoding city, China in 2015–2016. The environmental data will be documented and controlled by the computer automatically. Growth temperature of day/night was set 22/8°C; the length of day/night was 12/12 h. Relative humility was 60%.

Plant material and experimental practices. Two wheat (*Triticum aestivum* L.) cultivars including the drought-tolerant cultivar 'Hengguan35' (HG35) and the irrigated cultivar 'Jimai22' (JM22) were used in this study. The HG35 seeds were provided by Dry Land Farming Research Institute of Hebei Academy of Agricultural and Forestry Sciences, whereas the cultivar JM22 seeds were donated by Crop Research Institute, Shandong Academy of Agricultural Sciences. At first, wheat seeds were germinated in a holed tray fitted by moist vermiculite at 22°C. After about three days, at the first leaf expansion stage, the seedlings were transferred into a plastic box (length × width × height = 19 × 13.5 × 7.5 cm) that was filled with Hogland solution (Table 1). One-leaf seedlings were moved through holes and fixed with small flexible polyfoam in each hole drilled on a flat polyurethane foam. There were 6 wheat seedlings could be fixed on each foam, covering the top of plastic box. All sides of the containers were wrapped by blank and light-proof film. And, more remarkable, all roots of wheat seedlings should be submerged into growth solution. Fresh culture solution was replaced every two days to avoid root anaerobic respiration. Fourteen days later, the plants were transferred into new culture solution to initiate four experimental treatments, including normal water treatment (N), 20% polyethylene glycol PEG-6000 (P), 20% PEG-6000 + 1 μM melatonin (P + 1 μM melatonin), 20% PEG-6000 + 10 μM melatonin (P + 10 μM melatonin). Three replications were performed for each treatment, and five measuring replications were performed on leaves with the same location from five different plants under each treatment. Four treatments were measured successively as 20% PEG-6000 (P), 20% P + 1 μM melatonin, 20% P + 10 μM melatonin and N treatment. Both polyethylene glycol (PEG-6000) and melatonin were added into the culture solution. After processing for 12 hours, plants were measured and sampled under different treatments.

Table 1. Concentration of Hogland solution constituent

Constituent		Concentration g L^{-1}
Macroelement	KH_2PO_4	0.1360
	KNO_3	0.5050
	$MgSO_4 \times 7H_2O$	0.4930
Microelement (1000 ×)	H_3BO_3	2.8600
	$MnCL_2 \times 4H_2O$	1.8100
	$ZnSO_4 \times 7H_2O$	0.2200
	$CuSO_4 \times 5H_2O$	0.0800
	H_2MoO_4	0.0180
Ca^{2+}	$Ca(NO_3)_2 \times 4H_2O$	1.1800
Fe^{2+}	$FeSO_4 \times 7H_2O$	0.0278
	$Na_2EDTA \times 2H_2O$	0.0373

Measuring items and methods. Fully unfolded leaves were selected to measure the net photosynthetic rate (Pn), stomatal conductance (gs), transpiration rate (Tr) and high vapour pressure deficit (VPD) using the portable photosynthesis system Li-6400 (LI-COR, USA). During measurements, the environmental conditions were as follows: 25 ± 0.3°C, 400 ± 5 cm^3 m^{-3} CO_2 concentration and 1500 mol m^{-2} s^{-1} radiation intensity (Fenta et al., 2012).

SPAD (Soil and Plant Analyzer Development) value. This means the relative content of chlorophyll. Fully unfolded leaf (avoiding leaf vein) was selected and measured with a chlorophyll meter model SPAD-502 (Japan).

Water use efficiency (WUE) on leaf level was expressed by instantaneous WUE ($WUE_{inst.}$) and intrinsic WUE ($WUE_{intr.}$), which were calculated from the ratio of Pn to Tr and the ratio of Pn to gs, respectively (Qiao et al., 2010).

Water potential. Fully unfolded leaf (avoiding leaf vein) was selected during midday conditions, and on each leaf we punched leaf discs with a hole puncher. Then, leaf discs were put into the closed leaf chamber of a potential meter WP4 Dewpoint (Decagon Devices Inc., USA) to measure water potential.

Phytohormone. Young leaves and roots (0.5 g) were sampled and then quickly frozen at −80°C. Then the samples were ground to homogenate with 5 mL 80% methyl alcohol (containing 1 mmol L^{-1} butylated hydroxytoluene) at 4°C. After 4 hours' standing, the solution was centrifuged at 1000 g for 15 min (LDZ5-2, China). About 1 mL supernatant was transferred into centrifuge tube to concentrate and dry for 0.5 h with vacuum chamber (DZF-6020, China). Then the concentrated solution was centrifuged at 10000 r min^{-1} for 10 min. Then the solution was leached by solid phase extraction C18 cartridges. The leached solution was transferred into 5 mL centrifuge tube and was blow-dried by N_2 to evaporate methyl alcohol. Then the dry sample was diluted with phosphate buffer (containing 8.0 g NaCl, 0.2 g KH_2PO_4, 2.96 g $Na_2HPO_4 \times 12H_2O$, 1000 mL distilled water, pH 7.5) to 2 mL. (1) Prepared four 96-well plates (8 × 12) with four phytohormone antigens including auxin (IAA), zeatin riboside (ZR) content, gibberellin (GA_3) and abscisic acid (ABA). The first three lines were added 1000, 500, 250, 125, 62.5, 31.25, 15.625 and 0 ng mL^{-1} phytohormone standard samples 50 μL with three replicates poles of each concentration. Other poles were

added sample solution 50 µL with three replicate poles. (2) Then, added 50 µL antibody into each pole for 0.5 h at 37°C. (3) Washed the plates with phosphate buffer (including 1 mL Tween-20 per 1000 mL) three times and added second antibody for 0.5 h at 37°C. (4) Washed the plates again and added 100 µL colour reagent (15 mg O-phenylenediamine; 10 mL substrate buffer solution: 5.10 g $C_6H_8O_7 \times H_2O$, 18.43 g $Na_2HPO_4 \times 12H_2O$, 1000 mL distilled water and 1 mL Tween-20; pH 5.0; 3µL 30% H_2O_2). When the differential optical density (OD) value between 1000 and 0 ng mL^{-1} holes reached 1, terminated the enzymatic reaction with 50 µL 2 mol L^{-1} H_2SO_4 in each hole. Then absorbance was read at 492 nm using an indirect enzyme-linked immune absorption (ELISA) reader (BIO-RAD680, USA). The kit was provided by China Agricultural University. Logit curve tracing was adopted to calculate hormone content. The abscissa of logit curve was indicated by natural logarithm of standard phytohormone sample concentrations (ng mL^{-1}); and the ordinate was indicated by the logit value of each colorimetric value at 492 nm.

Aminocyclopropane-1-carboxylic acid (ACC) content. ACC content was determined based on the description of Yang (2014). Fresh leaves or root sampling (0.1 g) was ground to homogenate with 2 mL 95% ethyl alcohol and placed in a hot water bath at 80°C for 15 min. The solution was centrifuged twice at 8000 × g for 15 min ("Sigma centrifuges", UK). The supernatant was blow-dried by N_2, then diluted by 2 mL H_2O. Then the diluents were added 2 mL trichloromethane to elute pigments. Finally 0.8 mL of the extracting solution was transferred into volume-known glass bottle. Then 0.2 mL, 3.3 mM HgCl was injected into sealed 2 mL bottle, and mixture of 0.1 mL 5.5% NaOCl and NaOH (v:v = 2:1) was added by injection syringe. After 5 min vibration the bottle on ice, 1 mL of gas was collected and subjected to assay of the ethylene contents using a gas chromatography (Agilent 7890B, USA).

Hydrogen peroxide (H_2O_2) content. Fresh leaf and root samples (0.5 g) were measured based on the description of Prochazkova et al. (2001). Samples were homogenised in precool acetone (1:1) with a little quartz sand. The homogenate was centrifuged at 3000 r min^{-1} for 10 min. The supernatant (1 mL) was mixed with 5% titanium sulphate and stronger ammonia water. The mixture was centrifuged at 3000 r min^{-1} for 10 min again. The precipitation was washed 3–5 times with acetone until pigment removed completely. Then the dissolved precipitation was mixed with 5 mL sulphuric acid (2 mol) and then diluted with water to 10 mL. Absorbance was measured at 415 nm in an UV-visible spectrophotometer M 36 ("Beckman", USA).

Statistical analysis. All data were run using analysis of variance (*ANOVA*) with three replicates according to *Excel 2003* and *SPSS 17.0*. The Duncan's new multiple range (DMR) test at 5% probability level was used to test the differences among the mean values. Significant differences were labelled based on DMR.

Results

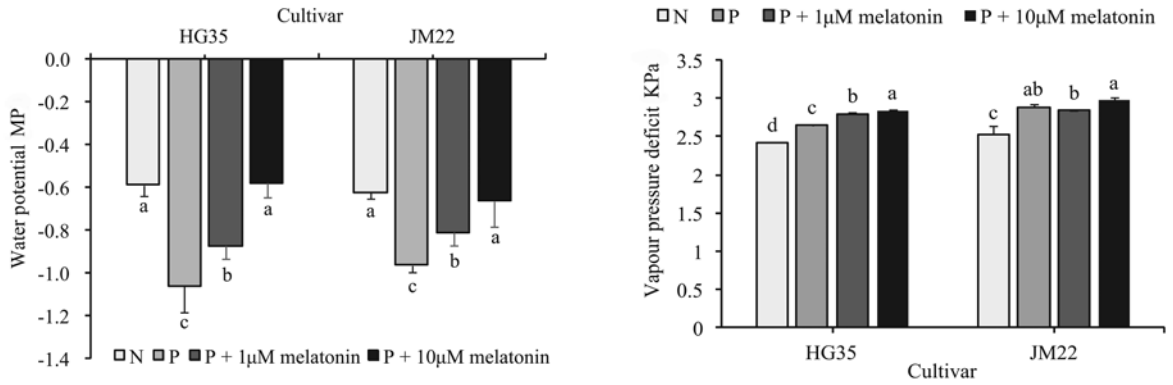
Photosynthetic indexes and water use efficiency on leaf level. The results of the experiment showed that for HG35, P treatment decreased significantly Pn, Tr and gs by 49.69, 91.71 and 94.44 % with respect to N, respectively. But it increased significantly the $WUE_{inst.}$ and $WUE_{intr.}$ by 4.99 and 6.29 times compared with N. P + 1 µM melatonin and P + 10 µM melatonin treatments reduced further the SPAD, Pn, $WUE_{inst.}$ and $WUE_{intr.}$ compared with P treatment. And the reduction of P + 1 µM melatonin was higher than P + 10 µM melatonin. This suggested that melatonin took no alleviation effect on photosynthesis of HG35 seedlings under drought stress. For JM22, P treatment decreased significantly Pn, Tr and gs by 89.03, 92.23 and 90 % compared with N, respectively. $WUE_{inst.}$ and $WUE_{intr.}$ did not change significantly. However, compared with P, P + 1 µM melatonin significantly increased Pn, $WUE_{inst.}$ and $WUE_{intr.}$ by 1.81, 1.84 and 2.40 times, respectively. Meanwhile, P + 10 µM melatonin significantly increased SPAD, Pn, $WUE_{inst.}$ and $WUE_{intr.}$ increased by 12.42%, 2.49 times, 66.13% and 1.21 times, respectively. This suggested that melatonin took obvious alleviation effect on photosynthesis and water utilization of JM22 seedlings under drought stress.

Water potential and vapour pressure deficit (VPD). The results of the experiment showed that water potential decreased significantly under drought condition compared with N for HG35 and JM22 (Fig. 1). Both P + 1 µM and P + 10 µM melatonin treatments significantly improved this value under drought treatment. However, there were no significant differences between N and P + 10 µM melatonin treatments. This suggested that 10 µM melatonin could improve water potential upon plant exposure to drought treatment. VPD of HG35 showed

Table 2. Physiological effect of melatonin on SPAD, Pn (µmol CO_2 m^{-2} s^{-1}), Tr (mmol H_2O m^{-2} s^{-1}), gs (mol H_2O m^{-2} s^{-1}), $WUE_{inst.}$ (µmol CO_2 mmol H_2O^{-1}), $WUE_{intr.}$ (µmol CO_2 mol H_2O^{-1}) of wheat leaf under drought treatment

Cultivar	Treatment	SPAD	Pn	Tr	gs	$WUE_{inst.}$	$WUE_{intr.}$
'Hengguan35' (HG35)	Normal water treatment (N)	36.92 a	9.54 a	3.62 a	0.18 a	2.73 c	54.48 c
	20% PEG (P)	37.1 a	4.74 b	0.30 b	0.01 c	16.35 a	397.04 a
	P + 1µM melatonin	30.6 b	2.24 c	0.28 b	0.02 bc	8.04 b	97.79 b
	P + 10µM melatonin	32.67 b	1.01 d	0.39 b	0.02 b	2.59 c	41.72 c
'Jimai22' (JM22)	Normal water treatment (N)	41.25 ab	16.86 a	5.02 a	0.20 a	3.45 c	83.94 c
	20% PEG (P)	38.98 b	1.85 d	0.39 b	0.02 b	4.96 c	110.48 c
	P + 1µM melatonin	38.92 b	5.19 c	0.38 b	0.01 b	14.10 a	375.50 a
	P + 10µM melatonin	43.82 a	6.45 b	0.79 b	0.03 b	8.24 b	244.66 b

Note. Pn – net photosynthetic rate, Tr – transpiration rate, gs – stomatal conductance, $WUE_{inst.}$ – instantaneous, $WUE_{intr.}$ – intrinsic water use efficiency; values followed by the same letter within each genotype are not significantly different at $P = 0.05$ as determined by the Duncan's means comparison test.

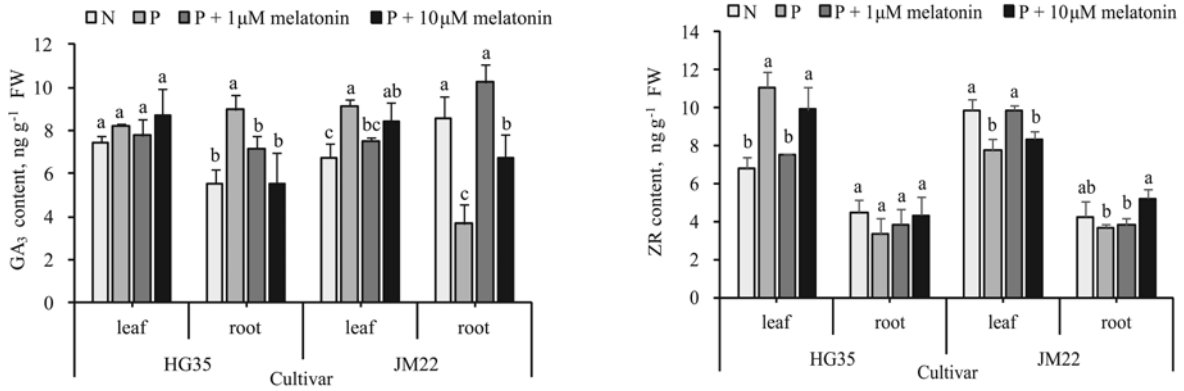


Note. Different letters indicate significant difference among treatments ($P < 0.05$).

Figure 1. Water potential and vapour pressure deficit of wheat leaves under normal water treatment (N), 20% PEG (P), drought with 1 μM (P + 1 μM melatonin) and 10 μM (P + 10 μM melatonin) melatonin treatments

a significant increase under P, P + 1 μM and P + 10 μM melatonin treatments compared with that under N. This value of P + 10 μM melatonin was the highest. Likewise, VPD increased significantly in JM22 plants under P, P + 1 μM and P + 10 μM melatonin treatments compared with N, but there were no significant differences between P and two concentrations of melatonin treatments. VPD of P + 10 μM was higher than that of P + 1 μM melatonin. It may be related to stomatal conductance changing under drought stress.

Gibberellin (GA_3) and zeatin riboside (ZR) content of leaf and root. For HG35, no obvious differences in GA_3 content of leaf were seen among four treatments (Fig. 2). GA_3 content in root under P increased significantly by 61.64% compared with N; both P + 1 μM and P + 10 μM melatonin treatments did not change significantly. For JM 22, compared with N, GA_3 content of leaf under P and P + 10 μM melatonin treatments increased significantly by 35.54% and 25.04%, respectively. However, the leaf GA_3 content under P + 1μM did not change obviously



Note. Different letters indicate significant difference among treatments ($P < 0.05$).

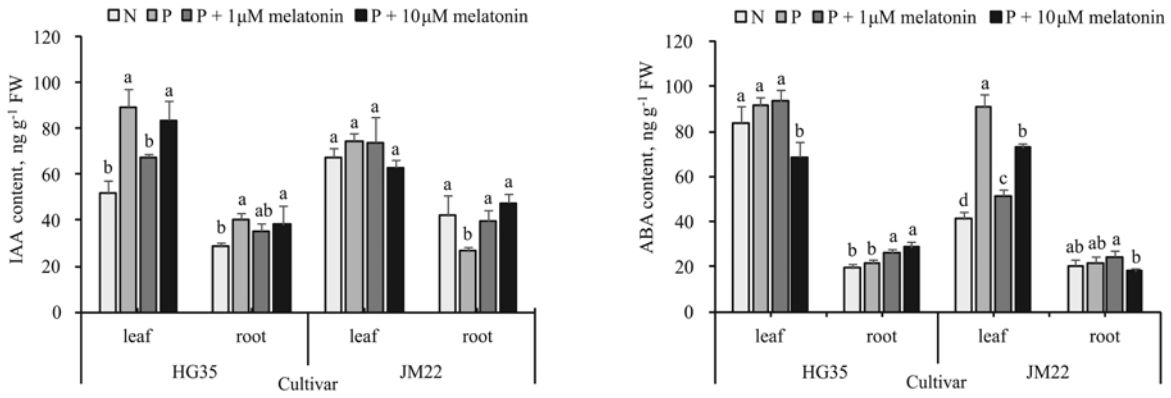
Figure 2. Content of gibberellin (GA_3) and zeatin riboside (ZR) in wheat leaf and root under normal water treatment (N), 20% PEG (P), 20% PEG with 1 μM (P + 1 μM melatonin) and 10 μM (P + 10 μM melatonin) melatonin treatments

compared with N. Conversely, GA_3 contents of root under P and P + 1 μM melatonin treatments were decreased significantly by 57.33% and 21.82% relative to that of N, respectively, whereas this value was similar under P + 10 μM melatonin and N treatments.

Analysis on leaf ZR content revealed that it was higher than root ZR content. For HG35, compared with N treatment, ZR content of leaf under P and P + 10 μM melatonin treatments increased by 63.18% and 46.31%, respectively; this value of root was changed little. Conversely, leaf ZR content in JM22 was significantly decreased under P and P + 10 μM melatonin treatments compared with that under N, with decrease of 21.57% and 15.51%, respectively. ZR content of root did not change significantly compared with N; but this value under P + 10 μM melatonin treatment was significantly higher than P and P + 1 μM melatonin treatments. The results showed that ZR content was different in different organ and should take different effect.

Auxin (IAA) and abscisic acid (ABA) content of leaf and root. The data of the experiment showed that IAA content in leaf was higher than that in root (Fig. 3). For HG35, IAA content of leaf under P and P + 10 μM melatonin treatments were significantly higher than that under N, with increase of 73.25% and 61.82%, respectively. IAA content of root under P and P + 10 μM melatonin treatments increased significantly by 41.26% and 33.75%. For JM22, no differences on leaf IAA content were seen among four treatments. Compared with N, P significantly decreased root IAA content by 36.86%; both P + 1 μM and P + 10 μM melatonin treatments did not change it evidently.

Obviously, ABA content in leaf was higher than that in root. For HG35, ABA content of leaf under P + 10 μM melatonin treatment decreased significantly by 18.01% compared with that under N. In contrast, ABA contents of root under P + 1 μM and P + 10 μM

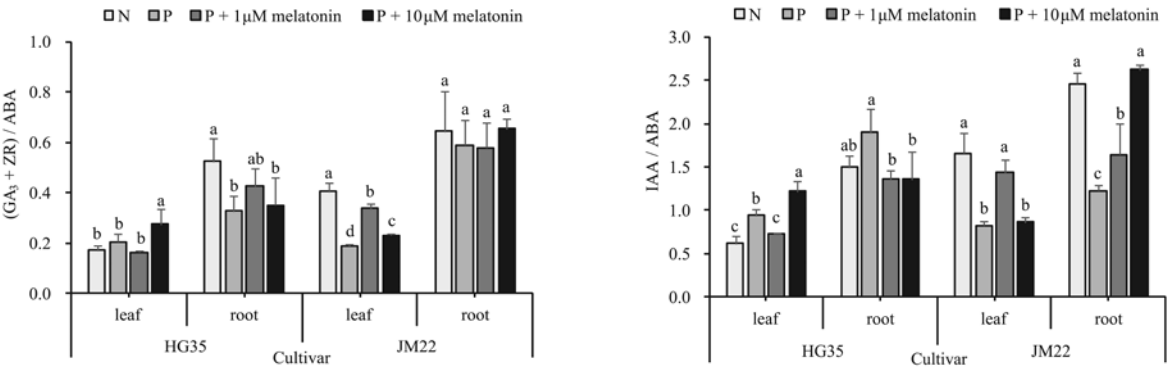


Note. Different letters indicate significant difference among treatments ($P < 0.05$).

Figure 3. Content of auxin (IAA) and abscisic acid (ABA) in wheat leaf and root under normal water treatment (N), 20% PEG (P), 20% PEG with 1 μM (P + 1 μM melatonin) and 10 μM melatonin (P + 10 μM melatonin) melatonin treatments

melatonin treatments increased significantly by 34.80% and 47.47%, respectively. For JM22, compared with N, ABA content of leaf under P, P + 1 μM melatonin and P + 10 μM melatonin treatments increased significantly by 1.21 times, 25.61% and 77.56%, respectively. Root ABA content under P + 10 μM melatonin was significantly lower than that under P + 1 μM melatonin. No obvious differences were seen among other treatments.

Hormonal balance ratio. Hormone proportion could reflect action centre on different wheat organ under different treatments. For HG35, (GA_3 + ZR) / ABA of leaf under P + 10 μM melatonin treatment was significantly increased by 60.60% compared with N (Fig. 4). Conversely, (GA_3 + ZR) / ABA of root was decreased under P and P + 10 μM melatonin treatments relative to that under N, with reduction of 37.66% and 33.30%, respectively (Fig. 4). This suggested that shoot growth had been inhibited in some extent. For JM22, compared with N, (GA_3 + ZR) / ABA of leaf under P, P + 1 μM and P + 10 μM melatonin treatments were all decreased by 53.91, 16.53 and 43.25 %, which suggested more ABA accumulation inducing stomatal closure.



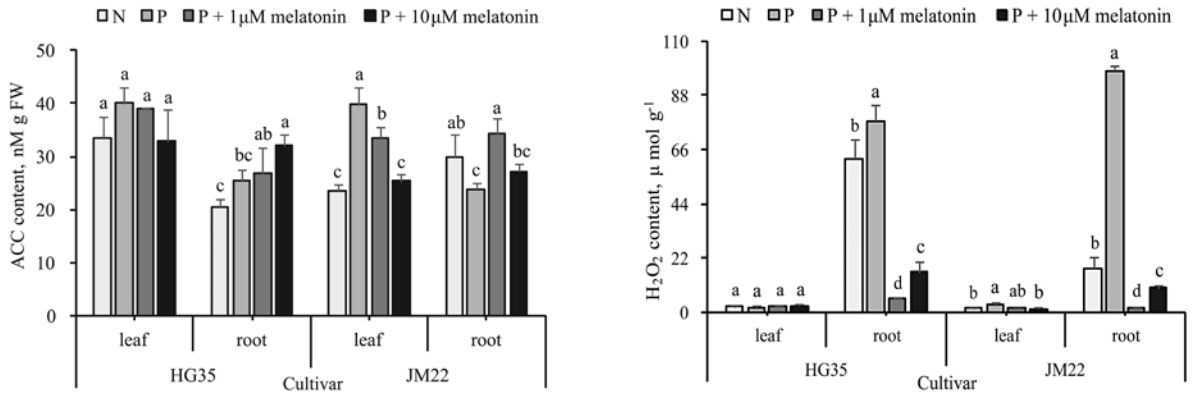
Note. Different letters indicate significant difference among treatments ($P < 0.05$).

Figure 4. Phytohormones proportion (GA_3 + ZR) / ABA and IAA / ABA in wheat leaf and roots under normal water treatment (N), 20% PEG (P), 20% PEG with 1 μM (P + 1 μM melatonin) and 10 μM (P + 10 μM melatonin) melatonin treatments

Compared with N, IAA / ABA of leaf for HG35 under P and P + 10 μM melatonin treatments increased significantly by 51.70% and 96.96%, respectively. This value of root under P + 1 μM and P + 10 μM melatonin treatments was significantly lower than that under P. For JM22, which was contrary to HG35, IAA / ABA of leaf under P and P + 10 μM melatonin treatments decreased significantly relative to N by 50.14% and 47.84%, respectively. IAA / ABA of root was decreased significantly under P and P + 1 μM melatonin treatments compared with that under N, with reduction of 50.10% and 66.70%, respectively. This suggested central hormone was different due to different drought tolerance of two wheat cultivars and different concentrations of melatonin.

Aminocyclopropane-1-carboxylic acid (ACC) and hydrogen peroxide (H_2O_2) contents. The data of the experiment showed that there were no significant differences in leaf ACC content of HG35 among four treatments (Fig. 5). Root ACC content of HG35 under P was higher significantly than that of N. However, this value under P + 1 μM and P + 10 μM melatonin treatments was both decreased significantly compared with N. For JM22, leaf ACC content under P was significantly increased compared with N. No obvious changing was observed under both P + 1 μM and P + 10 μM melatonin treatments. The changing of root ACC contents in JM22 under four treatments was similar to that in HG35, namely, P increased this value significantly, but P + 1 μM and P + 10 μM melatonin treatments both decreased it significantly.

Drought stress significantly increased the H_2O_2 contents in roots of HG35 plants and in both leaves and roots of JM22 plants. Root H_2O_2 content of two cultivars



Note. Different letters indicate significant difference among treatments ($P < 0.05$).

Figure 5. Contents of aminocyclopropane-1-carboxylic acid (ACC) and hydrogen peroxide (H_2O_2) in wheat leaves and roots under normal water treatment (N), 20% PEG (P), 20% PEG with 1 μM (P + 1 μM melatonin) and 10 μM (P + 10 μM melatonin) melatonin treatments

decreased significantly under both P + 1μM and P + 10 μM melatonin treatments compared with that under N. And there were no significant differences in leaf H_2O_2 content of HG35 among four treatments.

Correlation analysis for phytohormones. The data of the experiment showed that ABA content was very significantly positively correlated with the ACC content (n = 16, r = 0.715**). Also, IAA content was very significantly positively correlated with ACC content (n = 16, r = 0.632**). GA_3 content was significantly positively correlated with ACC content (n = 16, r = 0.503*). However, there was no significant correlation between ACC and ZR content (Fig. 6).

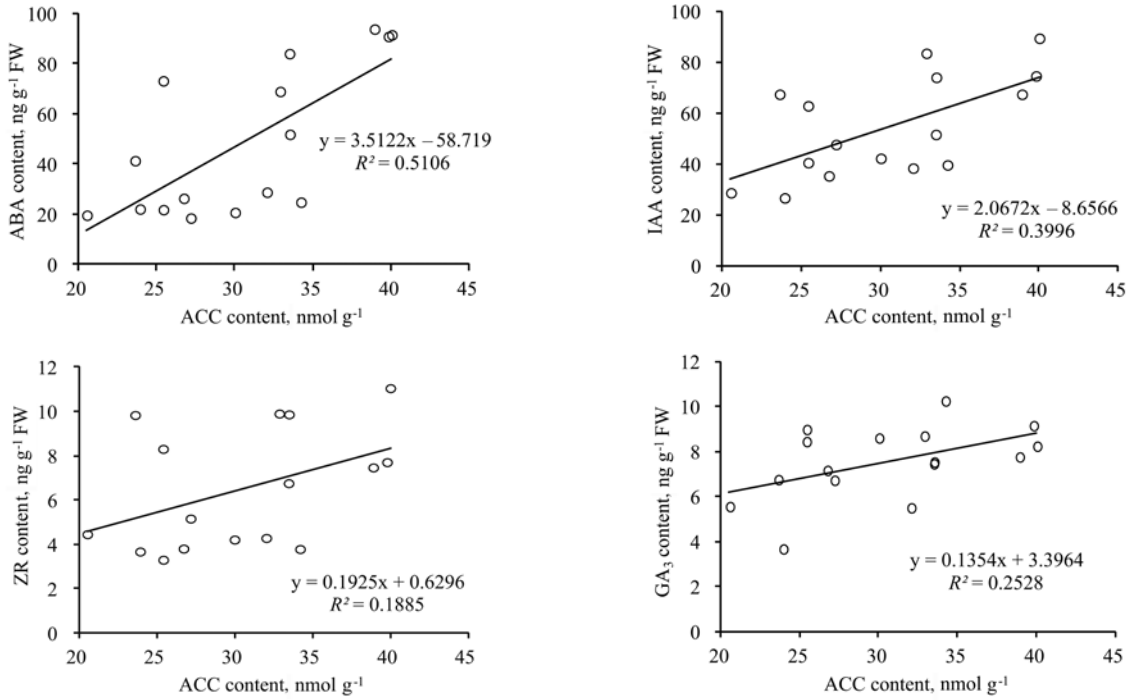


Figure 6. The correlation analysis of variance between aminocyclopropane-1-carboxylic (ACC) content and phytohormones-abscisic (ABA) acids, auxin (IAA), zeatin riboside (ZR), gibberellin (GA_3) contents

These results indicated that ACC was possible central player in the hormone cross-talks that regulated leaf and root growth and physiological function.

Discussion

Plants can alleviate the injury of drought stress by maximizing water uptake and/or minimizing water loss. Our study found that the leaf stomatal closure was induced by drought, given that the Tr and gs were drastically decreased under 20% PEG (P) treatment. Meanwhile, Pn was also significantly decreased due to CO_2 deficiency caused by lowered Tr and gs. However, $WUE_{inst.}$ and $WUE_{intr.}$, reflecting the leaf water status of plants, both showed an increasing trend under PEG condition. This result is consistent with that reported by Guo et al. (2010). Previous studies indicated that melatonin can improve chlorophyll content and photosynthetic character (Ye et al., 2016). In this research, we also found that melatonin could improve Pn, $WUE_{inst.}$ and $WUE_{intr.}$ of the JM22 plants, suggesting its function in regulating the

photosynthesis of wheat under drought. In addition, we also observed that the leaf water potential was decreased under drought significantly in two wheat cultivars, which could be recovered by both 1 μM and 10 μM melatonin. This finding may be attributed to stomatal closure and increasing VPD that reflect the evapotranspiration capacity of plant. However, exogenous application of melatonin did not exert positive roles on Pn and chlorophyll content in HG35 plants, which was possibly due to melatonin-mediated VPD elevation to lead to stomatal closure and photosynthesis decreasing (Xi et al., 2012). A large body of evidence have demonstrated that drought-induced growth limitation of crop was from hydraulic control in response to both soil water deficit and high VPD (Voisin et al., 2006; Tardieu et al., 2010). In this study, our results also indicated that drought increased VPD in two cultivars, in which HG35 showed much higher VPD under exogenous melatonin condition. The leaf and root H_2O_2 content are stimulated under drought and other abiotic stresses, which can damage cytomembrane and DNA and reduce plant growth and productivity. In our research, the leaf H_2O_2 contents of HG35 under both P + 1 μM and P + 10 μM melatonin treatments did not decrease obviously; however, the root H_2O_2 contents of HG35 decreased significantly under the two melatonin treatments. Previous researches have also reported that melatonin could eliminate H_2O_2 content directly, protecting chlorophyll damage from the induced oxidative stress injury (Wang et al., 2012 a; Turk et al., 2014). For JM22, leaf and root H_2O_2 contents both decreased with 1 μM and 10 μM melatonin under drought treatment, which was consistent with the former conclusion. These findings together indicate that melatonin can improve plant growth and photosynthesis under drought conditions through effective alleviation of oxidative stress damage initiated by water stress.

Early studies reported that ABA can induce stomatal closure in various species regulated by H_2O_2 (Desikan, Neill, 2004; Bright et al., 2006; Miao et al., 2006; Seki, Urano, 2007). Exogenous melatonin application resulted in higher ABA concentration in drought-primed plants, suggesting that the interplay between melatonin and ABA improves water status under drought (Li et al., 2016). In this study, leaf ABA content in JM22 under P increased significantly, and decreased under P condition supplemented with 1 μM melatonin and 10 μM melatonin; root ABA content in JM22 under P + 10 μM melatonin was significantly decreased. In accordance with these findings, recent research has documented that melatonin can increase drought tolerance by enhancing ABA degradation and suppressing its synthesis. Consequently, less H_2O_2 is accumulated in the guard cells (Li et al., 2015). Similar results have been reported that application of ABA to guard cells was shown to induce a burst of H_2O_2 that resulted in stomatal closure (Desikan, Neill, 2004). In this study, HG35 showed significantly decreased leaf ABA content under P + 10 μM melatonin treatment, but unreduced leaf H_2O_2 content; decreased root H_2O_2 content significantly, but significantly increased root ABA contents under P + 1 μM and P + 10 μM melatonin treatments. This can be interpreted as drought tolerant cultivar HG35, cope with drought stress under melatonin treatments possibly through an ABA-dependent pathway. The detailed mechanism underlying this pathway regulated by melatonin needs to be further studied.

As known, melatonin and IAA have the same biosynthesis precursor, i.e. tryptophan, and melatonin could stimulate IAA biosynthesis by which to stimulate root growth (Chen et al., 2009). In this study, the leaf and root IAA content in HG35 plants were increased significantly under drought condition. Application of 1 μM melatonin did not change it obviously comparing with N treatment, but P + 10 μM melatonin treatment was significantly higher than N treatment, which has no obvious differences with P treatment. This suggested that HG35 could regulate IAA content not by melatonin under drought condition. For JM22, root IAA content were decreased significantly under drought condition, but the reduced IAA contents could be recovered by application of 1μM and 10μM melatonin. This suggested that melatonin improved root drought-resistant. There were no differences on leaf IAA content were seen among four treatments. This was partly consistent with the result of exogenous application of 0.1 mM melatonin also raised the endogenous levels of free IAA in roots, while higher concentrations had no significant effect (Chen et al., 2009). Therefore, the melatonin effects on regulating IAA accumulation needs to be further characterized.

Cytokinin ZR and the ethylene precursor ACC are both root-derived hormones. Soil drying can reduce the transport of cytokinins from root to shoot (Davies et al., 2005). Also, drought can increase the generation of ethylene in shoots by promoting root ACC synthesis and xylem transporting ACC to shoots (Sobeih et al., 2004). In this study, we found that ZR content in HG35 leaf was significantly higher under P and P + 10 μM melatonin treatments than those under N treatment. ACC content in leaf did not increase obviously under above treatments. This was attributed to higher drought-tolerance. Conversely, the leaf ZR content in JM22 were significantly lower under P and P + 10 μM melatonin treatments than those under N treatment; meanwhile, the ACC content was increased under P and P + 1 μM melatonin treatments. ZR content in root was significantly increased, and ACC content decreased significantly under 10 μM melatonin treatment. These results showed that drought-inhibited ZR biosynthesis in wheat cultivars, especially in the drought sensitive ones, can be alleviated by exogenous melatonin. Correlation analysis showed that ACC possibly acted as the central player in regulating the hormone cross-talks under drought with melatonin application conditions.

All above have also indicated the effect of exogenous melatonin was tissue-selective in some extent. Cross-talk among different hormones from root to shoot would be influenced by external environment. In this study, melatonin had taken obvious inhibiting effect on root H_2O_2 content in HG35. The same concentration melatonin would promote growth in coleoptiles, but inhibit growth in roots (Hernández-Ruiz, Arnao, 2008 a; b). Drought also influenced dynamic equilibrium in endogenous hormone. In our study, IAA would take central effect for HG35, but ABA would take central effect for JM22 during drought regulation process due to different drought tolerances. Melatonin concentrations applied exert contrasting roles such as stimulatory effect or inhibitory one in different organs and wheat cultivars (Chen et al., 2009; Posmyk et al., 2009). In the present experiment, our results on different melatonin concentrations were also shown to be consistent with previous reports. The two wheat cultivars with contrasting

drought tolerance under 10 μM melatonin-exhibited improved growth under drought stress, suggesting that this melatonin concentration can be a useful index in improving plant growth under drought in wheat.

Conclusion

Our results indicated that exogenous melatonin can effectively improve the tolerance of wheat cultivar 'Jimai22' (JM22) to drought stress through its function in increasing net photosynthetic rate, water use efficiency on leaf by stomatal closure and water potential. 'Hengguan35' (HG35), as a drought-tolerant cultivar, exhibited relatively decreased net photosynthetic rate and transpiration rate under drought with 1 μM and 10 μM melatonin treatments compared with those under sole drought treatment. But this cultivar also showed some physiological regulation on increasing zeatin riboside (ZR), auxin (IAA) and inhibiting hydrogen peroxide (H_2O_2) for the drought-primed plants. Application of suitable concentration of melatonin in wheat can be an effective strategy for improving plant drought stress tolerance and productivity. There is still lack of information to explain clearly the role of melatonin in physiology regulation for drought-tolerant wheat cultivar. More attention should be paid on increasing/decreasing melatonin concentration and respiratory pathway in root considering obvious inhibiting effect on hydrogen peroxide production.

Acknowledgements

This work was supported by the Chinese National Programs of Science and Technology for High Yielding Crop Production (2013BAD07B05). The Fund of Research Group Construction for Crop Science in Hebei Agricultural University (TD2016C317).

Received 16 02 2017
Accepted 16 06 2017

References

1. Arnao M. B., Hernández-Ruiz J. 2007. Melatonin promotes adventitious- and lateral root regeneration in etiolated hypocotyls of *Lupinus albus* L. Journal of Pineal Research, 42 (2): 147–152. https://doi.org/10.1111/j.1600-079X.2006.00396.x
2. Arnao M. B., Hernández-Ruiz J. 2014. Melatonin: plant growth regulator and/or biostimulator during stress? Trends in Plant Science, 19 (12): 789–797. https://doi.org/10.1016/j.tplants.2014.07.006
3. Bałabusta M., Szafrańska K., Posmyk M. M. 2016. Exogenous melatonin improves antioxidant defense in cucumber seeds (*Cucumis sativus* L.) germinated under chilling stress. Frontiers in Plant Science, 7: 575.
4. Bright J., Desikan R., Hancock J. T., Weir I. S., Neill S. J. 2006. ABA-induced NO generation and stomatal closure in *Arabidopsis* are dependent on H_2O_2 synthesis. Plant Journal, 45 (1): 113–122. https://doi.org/10.1111/j.1365-313X.2005.02615.x
5. Cao L., Dou Y. X., Zhang D.Y. 2003. Effect of climate change on ecological environment of heihe field. Arid Meteorology, 21 (4): 45–49.
6. Chen Q., Qi W. B., Reiter R. J., Wei W., Wang B. M. 2009. Exogenously applied melatonin stimulates root growth and raises endogenous indoleacetic acid in roots of etiolated seedlings of *Brassica juncea*. Journal of Plant Physiology, 166 (3): 324–328. https://doi.org/10.1016/j.jplph.2008.06.002
7. Davies W. J., Kudoyarova G., Hartung W. 2005. Long-distance ABA signaling and its relation to other signaling pathways in the detection of soil drying and the mediation of the plant's response to drought. Journal of Plant Growth Regulation, 24 (4): 285–295. https://doi.org/10.1007/s00344-005-0103-1
8. Desikan R., Neill S. J. 2004. ABA, hydrogen peroxide and nitric oxide signalling in stomatal guard cells. Journal of Experimental Botany, 55 (395): 205–212. https://doi.org/10.1093/jxb/erh033
9. Fenta B. A., Driscoll S. P., Kunert K. J., Foyer C. H. 2012. Characterization of droughttolerance traits in nodulated soya beans: the importance of maintaining photosynthesis and shoot biomass under drought-induced limitations on nitrogen metabolism. Journal of Agronomy and Crop Science, 198 (2): 92–103. https://doi.org/10.1111/j.1439-037X.2011.00491.x
10. Guan D., Zhang Y., Al-Kaisi M. M., Wang Q., Zhang M., Li Z. 2015. Tillage practices effect on root distribution and water use efficiency of winter wheat under rain-fed condition in the North China Plain. Soil and Tillage Research, 146: 286–295. https://doi.org/10.1016/j.still.2014.09.016
11. Guo X. Y., Zhang X. S., Huang Z. Y. 2010. Drought tolerance in three hybrid poplar clones submitted to different watering regimes. Journal of Plant Ecology, 3 (2): 79–87. https://doi.org/10.1093/jpe/rtq007
12. Hernández-Ruiz J., Arnao M. B. 2008 (a). Distribution of melatonin in different zones of lupin and barley plants at different ages in the presence and absence of light. Journal of Agricultural and Food Chemistry, 56 (22): 10567–10573. https://doi.org/10.1021/jf8022063
13. Hernández-Ruiz J., Arnao M. B. 2008 (b). Melatonin stimulates the expansion of etiolated lupin cotyledons. Plant Growth Regulation, 55 (1): 29–34. https://doi.org/10.1007/s10725-008-9254-y
14. Janas K. M., Ciupińska E., Posmyk M. M. 2009. Melatonin applied by hydropriming, as phytobiostimulator improving corn (*Zea mays* L.) seedlings growth at abiotic stresses conditions. Progress in Environmental Science and Technology, 2: 383–388.
15. Jia J. S., Liu C. M. 2002. Groundwater dynamic drift and response to different exploitation in the North China plain: a case study of Luancheng County, Hebei Province. Acta Geographica Sinica, 57: 201–209.
16. Jiang C. Q., Zu C. L. 2015. Advances in melatonin and its roles in abiotic stress resistance in plants. Biotechnology Bulletin: 31 (4): 47–55.
17. Li C., Wang P., Wei Z., Liang D., Liu C., Yin L., Jia D., Fu M., Ma F. 2012. The mitigation effects of exogenous melatonin on salinity-induced stress in *Malus hupehensis*. Journal of Pineal Research, 53 (3): 298–306. https://doi.org/10.1111/j.1600-079X.2012.00999.x
18. Li C., Tan D. X., Liang D., Chang C., Jia D., Ma F. 2015. Melatonin mediates the regulation of ABA metabolism, free-radical scavenging, and stomatal behaviour in two *Malus* species under drought stress. Journal of Experimental Botany, 66 (3): 669. https://doi.org/10.1093/jxb/eru476

19. Li X., Tan D. X., Jiang D., Liu F. 2016. Melatonin enhances cold tolerance in drought-primed wild-type and abscisic acid-deficient mutant barley. Journal of Pineal Research, 61 (3): 328–339. https://doi.org/10.1111/jpi.12350
20. Meng X. P. 2016. Effects of melatonin priming seedlings treatment on growth and physiology of wheat under water stress. Northwest A&F University, Shaanxi, China (in Chinese) http://cdmd.cnki.com.cn/Article/CDMD-10712-1016157079.htm.
21. Meng J. F., Xu T. F., Wang Z. Z., Fang Y. L., Xi Z. M., Zhang Z. W. 2015. The ameliorative effects of exogenous melatonin on grape cuttings under water-deficient stress: antioxidant metabolites, leaf anatomy, and chloroplast morphology. Journal of Pineal Research, 57 (2): 200–212. https://doi.org/10.1111/jpi.12159
22. Miao Y., Dong L., Wang P., Wang X. C., Jia C., Chen M., Song C. P. 2006. An arabidopsis alutathione peroxidase functions as both a redox transducer and a scavenger in abscisic acid and drought stress responses. The Plant Cell, 18 (10): 2749–2766. https://doi.org/10.1105/tpc.106.044230
23. Posmyk M. M., Bałabusta M., Wieczorek M., Sliwinska E., Janas K. M. 2009. Melatonin applied to cucumber (*Cucumis sativus* L.) seeds improves germination during chilling stress. Journal of Pineal Research, 46 (2): 214–223. https://doi.org/10.1111/j.1600-079X.2008.00652.x
24. Prochazkova D., Sairam R. K., Srivastava G. C., Singh D. V. 2001. Oxidative stress and antioxidant activity as the basis of senescence in maize leaves. Plant Science, 161 (4): 765–771. https://doi.org/10.1016/S0168-9452(01)00462-9
25. Qiao Y. Z., Zhang H. Z., Dong B. D., Shi C. H., Li Y. X., Zhai H. M., Liu M. Y. 2010. Effects of elevated CO_2 concentration on growth and water use efficiency of winter wheat under two soil water regimes. Agricultural Water Management, 97 (11): 1742–1748. https://doi.org/10.1016/j.agwat.2010.06.007
26. Sarropoulou V. N., Therios I. N., Dimassitheriou K. N. 2012. Melatonin promotes adventitious root regeneration in in vitro shoot tip explants of the commercial sweet cherry rootstocks CAB-6P (*Prunus cerasus* L.), Gisela 6 (*P. cerasus* × *P. canescens*), and M×M 60 (*P. avium* × *P. mahaleb*). Journal of Pineal Research, 52 (1): 38–46. https://doi.org/10.1111/j.1600-079X.2011.00914.x
27. Seki M., Urano K. 2007. Regulatory metabolic networks in drought stress responses. Current Opinion in Plant Biology, 10 (3): 296–302. https://doi.org/10.1016/j.pbi.2007.04.014
28. Shi H., Wei Y., Wang Q., Reiter R. J., He C. 2016. Melatonin mediates the stabilization of DELLA proteins to repress the floral transition in Arabidopsis. Journal of Pineal Research, 60 (3): 373–379. https://doi.org/10.1111/jpi.12320
29. Sobeih W. Y., Dodd I. C., Bacon M. A., Grierson D., Davies W. J. 2004. Long-distance signals regulating stomatal conductance and leaf growth in tomato (*Lycopersicon esculentum*) plants subjected to partial root-zone drying. Journal of Experimental Botany, 55 (407): 2353–2363. https://doi.org/10.1093/jxb/erh204
30. Tardieu F., Parent B., Simonneau T. 2010. Control of leaf growth by abscisic acid: hydraulic or non-hydraulic processes? Plant, Cell and Environment, 33 (4): 636–647. https://doi.org/10.1111/j.1365-3040.2009.02091.x
31. Turk H., Erdal S., Genisel M., Atici O. 2014. The regulatory effect of melatonin on physiological, biochemical and molecular parameters in cold-stressed wheat seedlings. Plant Growth Regulation, 74 (2): 139–152. https://doi.org/10.1007/s10725-014-9905-0
32. Voisin A. S., Reidy B., Parent B., Rolland G., Redondo E., Gerentes D., Tardieu F., Muller B. 2006. Are ABA, ethylene or their interaction involved in the response of leaf growth to soil water deficit? An analysis using naturally occurring variation or genetic transformation of ABA production in maize. Plant, Cell and Environment, 29 (9): 1829–1840. https://doi.org/10.1111/j.1365-3040.2006.01560.x
33. Wang P., Sun X., Li C., Wei Z., Liang D., Ma F. 2012 (a). Long-term exogenous application of melatonin delays drought-induced leaf senescence in apple. Journal of Pineal Research, 54 (3): 292–302. https://doi.org/10.1111/jpi.12017
34. Wang P., Yin L., Liang D., Li C., Ma F., Yue Z. 2012 (b). Delayed senescence of apple leaves by exogenous melatonin treatment: toward regulating the ascorbate-glutathione cycle. Journal of Pineal Research, 53 (1): 11–20. https://doi.org/10.1111/j.1600-079X.2011.00966.x
35. Weeda S., Zhang N., Zhao X., Ndip G., Guo Y., Buck G. A., Fu C., Ren S. 2014. Arabidopsis transcriptome analysis reveals key roles of melatonin in plant defense systems. PLoS ONE, 9 (3): 1–18. https://doi.org/10.1371/journal.pone.0093462
36. Wei W., Li Q.T., Chu Y. N., Reiter R. J., Yu X. M., Zhu D. H., Zhang W. K., Ma B., Lin Q., Zhang J. S. 2015. Melatonin enhances plant growth and abiotic stress tolerance in soybean plants. Journal of Experimental Botany, 66 (3): 695–707. https://doi.org/10.1093/jxb/eru392
37. Xi X. X., Tian Y. L., He Y. H. 2012. Photosynthetic characteristics of *Ostryopsis davidiana* Decaisne and relationship with influencing factor. Journal of Inner Monggolia Agricultural University, 33 (4): 81–86.
38. Yang W.B. 2014. Polyamines and ethylene biosynthesis in response to water deficit in wheat and its relations to grain filling. Shandong Agricultural University. http://cdmd.cnki.com.cn/Article/CDMD-10434-1014341837.htm
39. Ye J., Deng X. P., Wang S. Y., Yin L. N., Xiong B. L., Wang X. Y. 2015. Effects of melatonin on growth, photosynthetic characteristics and antioystem in seedling of wheat under droug ht stress. Journal of Triticeae Crops, 35 (9): 1275–1283.
40. Ye J., Wang S., Deng X., Yin L., Xiong B., Wang X. 2016. Melatonin increased maize (*Zea mays* L.) seedling drought tolerance by alleviating drought-induced photosynthetic inhibition and oxidative damage. Acta Physiologiae Plantarum, 38 (2): 48. https://doi.org/10.1007/s11738-015-2045-y
41. Zhang N., Zhao B., Zhang H. J., Weeda S., Yang C., Yang Z. C., Ren S., Guo Y. D. 2013. Melatonin promotes water-stress tolerance, lateral root formation, and seed germination in cucumber (*Cucumis sativus* L.). Journal of Pineal Research, 54 (1): 15–23. https://doi.org/10.1111/j.1600-079X.2012.01015.x
42. Zhang N., Zhang H. J., Zhao B., Sun Q. Q., Cao Y. Y., Li R., Wu X. X., Weeda S., Li L., Ren S. 2014. The RNA-seq approach to discriminate gene expression profiles in response to melatonin on cucumber lateral root formation. Journal of Pineal Research, 56 (1): 39–50. https://doi.org/10.1111/jpi.12095

ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 104, No. 3 (2017), p. 219–228
DOI 10.13080/z-a.2017.104.028

Kviečių fiziologinė reakcija į polietilenglikolį (PEG-6000) egzogeniškai taikant melatoniną

D. Li, D. Zhang, H. Wang, Y. Li, R. Li

Hebei žemės ūkio universitetas
Hebei provincijos Pagrindinė augalų augimo reguliavimo laboratorija, Kinija

Santrauka

Siekiant nustatyti melatonino ir hormonų potencialų poveikį lapų vandens naudojimo efektyvumui, buvo pasirinktos dvi kviečių veislės, nevienodai atsparios sausrai: atspari ‘Hengguan35’ (HG35) ir auginama drėkinamuose laukuose ‘Jimai22’ (JM22). Tirti keturi daigų auginimo terpėje būdai: 1) mitybinėje terpėje (N), 2) N + 20 % polietilenglikolio (PEG) (P), 3) P + 1 μM melatonino ir 4) P + 10 μM melatonino. Egzogeninis melatonimas esmingai padidino veislės JM22 grynosios fotosintezės intensyvumą (Pn), momentinį ($WUE_{inst.}$) ir įprastinį (WUEintr.) vandens naudojimo efektyvumą. Tai galima sieti su šaknų didesnės koncentracijos auksinu (IAA) bei zeatino ribozidu (ZR) ir padidėjusia lapų abscisinės rūgšties (ABA), vandenilio peroksido (H_2O_2) bei aminociklopropan-1-karboksirūgšties (ACC) gamyba. Tačiau veislės HG35 fotosintezės greitis dėl 1 μM ir 10 μM melatonino nepadidėjo, o chlorofilo koncentracija buvo netgi nepalanki. Taip galėjo būti dėl reikšmingai sumažėjusio vandens garų slėgio ir didelės vandenilio peroksido (H_2O_2) koncentracijos lapuose. Be to, pasireiškė veislės HG35 fiziologinė savireguliacija, pvz., didėjo šios veislės kviečių šaknų bei lapų IAA ir ZR koncentracijos, buvo slopinama šaknų H_2O_2 gamyba. Naudojant melatoniną šaknų ABA ir ACC koncentracijos nesumažėjo, o sausros sąlygomis net padidėjo. Sąveikaujant hormonams didžiausią reikšmę turi ACC, kuri valdo lapų bei šaknų augimą ir kitus fiziologinius reiškinius.
Tyrimo duomenimis, išlaikant įprastinę vandens būklę išorinio melatonino poveikis daigams priklauso nuo kviečių veislės atsparumo sausrai ir sudėtingos hormonų sąveikos. Taigi, melatoninas gali būti veiksminga priemonė siekiant pagerinti kviečių atsparumą sausrai.

Reikšminiai žodžiai: drėgmės naudojimo efektyvumas, fitohormonas, melatoninas, sausra, *Triticum aestivum*.