



Research article

Melatonin promotes plant growth by increasing nitrogen uptake and assimilation under nitrogen deficient condition in winter wheat

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ABSTRACT

Melatonin (MEL) has been widely reported to be beneficial to plant growth and development, but few studies have combined investigations of the performance and function of MEL with detailed physiologically based analyses of nitrogen (N) uptake and metabolism in staple crops. In this study, the effect of MEL application on winter wheat seedling growth and grain yield were investigated in hydroponic and pot experiments at different N levels. The result showed that application of 1 μM MEL in hydroponic solution significantly improved the wheat seedling growth under both N sufficient and deficient conditions, but the effect of MEL on promoting seedling growth was prominent under N deficient condition. Meanwhile, MEL-treated plants maintained higher N contents and nitrate nitrogen levels in shoot under N deficient condition, and also maintained higher nitrate nitrogen levels in root. Further investigation showed that nitrate reductase (NR) and glutamine synthetase (GS) activities were higher in MEL-treated plants than that of MEL-untreated plants under N deficiency. The N absorption calculated based on N contents and biomass showed that MEL could promote the N absorption under N deficient condition. In pot experiment, pre-soaking of seeds with 100 μM MEL enhanced per-plant yield by 16% under N sufficient condition and 23% under N deficient condition. Taken together, the results of this study indicate that MEL is involved in promoting N uptake and assimilation through up-regulating the activities of N uptake and metabolism related enzymes and, ultimately, promotes the plant growth and yield, especially under N deficient condition.

1. Introduction

Nitrogen (N) is an essential nutrient that plants require in a large amount for growth and development. Consequently, the supply of N fertilizer has significant consequences for crop yield and quality (Mäder et al., 2011). In the last 50 years, the application of synthetic N fertilizer has increased dramatically to meet the agricultural demands of growing population (FAO, 2015; Conant et al., 2013). In China, the use of chemical fertilizer has also reached a fairly high level (Gu et al., 2015). However, by the same time of increasing crop productivity, the increased input of N fertilizer also reduced plant N use efficiency and the excessive N may lead to negative effects on environment and human health by causing surface water eutrophication, excessive nitrate nitrogen content in groundwater and increasing greenhouse gas emissions (e.g. nitrous oxide) (Zhang et al., 2008). Oppositely, the insufficiency of

N fertilizer significantly inhibits plant growth and results in low crop productivity and, ultimately, in reduced food supply (Greenwood, 1982; Liu et al., 2015; Perchlik and Tegeder, 2017). Therefore, reducing the input of N fertilizer without decreasing crop growth and yield is an important challenge for sustainable development of agriculture.

Melatonin (N-acetyl-5-methoxytryptamine, MEL) is a natural pleiotropic biomolecule that can be widely found in plants and animals. It is involved in multiple plant physiological actions, such as growth and development, seed germination, rooting, flowering, branching, photosynthesis and ripening (Arnao and Hernández-Ruiz, 2014, 2017; 2018; Nawaz et al., 2016). The most prominent function of MEL in plants is considered as a protective agent against various biotic and abiotic stresses, including cold, drought, PEG-induced osmotic stress, heat, salinity, chemical pollutants, herbicides and UV irradiation etc. (Chan and Shi, 2015; Reiter et al., 2015; Arnao and Hernández-Ruiz,

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2018). Exogenous MEL can be absorbed by plant roots, cotyledons, leaves and seeds in various plant species and plays the same function with endogenous MEL (Posmyk and Janas, 2009; Nawaz et al., 2016). Meanwhile, MEL is naturally existed in both animals and plants and is also beneficial to human health (Nawaz et al., 2016; Paredes et al., 2009; Tan et al., 2015; Bajwa et al., 2014). Accordingly, if MEL was used as a plant growth regulator in crops production, it would not promote any risks to environment and human health. These special characteristics attract people to use it as a natural biostimulator in crops production in recent years (Arnao and Hernández-Ruiz, 2014; Janas and Posmyk, 2013; Wei et al., 2015; Reiter et al., 2015; Zuo et al., 2017).

In addition to protect plants against numerous biotic and abiotic stresses, MEL promotes plant nutrient uptake has also been found in recent years. A study in *Malus* showed that MEL could regulate the ROS signal and activate the CBL1-CIPK23 pathway to regulate the expression of a potassium channel protein gene, which then promoted potassium absorption under salinity or nutrient deficient conditions (Li et al., 2016). Melatonin could reverse the toxic effect of high boron in pepper plants (Sarafi et al., 2017). Rhizosphere application of MEL could promote N metabolism and proline homeostasis in drought-stressed alfalfa (Antoniu et al., 2017). In addition to those direct evidences, some indirect studies also suggest that MEL application could affect plant nutrient uptake and metabolism. First, MEL has been found to regulate the lateral root development and enhance the root/shoot ratio (Arnao and Hernández-Ruiz, 2007; Zhang et al., 2014; Chen et al., 2018), and the strong root system would increase the nutrient uptake. Second, drought and salt stresses often cause difficulties for plants to take up nutrient, and MEL alleviates these stresses partly due to its function in increasing nutrient uptake and metabolism. Third, in some field experiment, MEL is also reported to enhance the yield, partly because it could increase nutrient uptake and utilization.

As one of the most important nutrients for plant growth, N metabolism has also been proved to be regulated by MEL in some plant species. Zhang et al. (2017) found that exogenous MEL application improved the tolerance of cucumber seedlings to high nitrate stress through enhancing the activities of enzymes involved in N metabolism. In sub-low temperature suffered melon seedlings, exogenous MEL significantly decreased the leaf NH_4^+ -N content by improving the activities of N metabolism related enzymes, such as glutamine synthetase (GS) and glutamic acid synthase (GOGAT) (Gao et al., 2016). Antoniu et al. (2016) found that MEL could maintain nitro-oxidative homeostasis through the regulation of N species at the enzymatic and/or transcript level in drought-stressed *Medicago sativa*. Furthermore, MEL could protect organisms from oxidative stress by interactions with reactive oxygen species/reactive nitrogen species through a highly efficient free radical scavenging cascade (Tan et al., 2007). However, the mechanisms of MEL on plant N uptake and metabolism remain largely unclear, especially in crop plants. In addition, considering developing the MEL as a plant growth regulator that can be practically used in crop production, the performance and the underlying mechanisms of MEL on crop growth still need further research.

Wheat is an extremely important food crop that affects the world's food security. The previous study showed that exogenous application of MEL increased wheat drought tolerance by alleviating photosynthetic inhibition and oxidative damage induced by drought (Ye et al., 2016). MEL was also identified to improve the photosynthetic carbon assimilation and antioxidant enzymes activities when exposed to Nano-ZnO stress in wheat (Zuo et al., 2017). Undoubtedly, N deficiency significantly reduces wheat growth and yield (Dupont and Altenbach, 2003; Good et al., 2005; Barneix, 2007). Up to date, few studies have combined investigations of the performance and function of MEL with detailed physiologically based analyses of N uptake and metabolism in wheat. The objective of this study was to investigate the effects of exogenous application of MEL on wheat growth and yield under different N levels. The N contents, the activities of key enzymes of N

uptake and metabolism and protein contents were also investigated. The results of this study would contribute to the practically using of MEL in crop production, especially in the area where the N efficacy needs to be improved.

2. Materials and methods

2.1. Experiment 1: effect of MEL on wheat seedling growth, N uptake and metabolism in the hydroponic culture under different N levels

2.1.1. Plant materials, growth conditions and MEL treatments

Seeds of winter wheat (*Triticum aestivum* L., cv. Changhan58) were sterilized and germinated in an incubator at 24 °C under dark conditions for 4 days. After germination, uniform seedlings were selected and transplanted into a plastic container (40 × 28 × 14 cm) with 5 L of half-strength Hoagland solution (Macro-nutrition: 1 mM KH_2PO_4 , 1 mM $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$, 4 mM CaCl_2 , 14 mM KNO_3 , and 1 mM NH_4Cl ; Micro-nutrition and Fe was consistent with normal Hoagland solution), and continued to grow in a growth chamber set to a temperature of 28/23 °C and light cycle of 14/10 h (day/night), a relative humidity of 40–50%, and the amount of photosynthetically active radiation (PAR) to the upper plant was 600 $\mu\text{mol m}^{-2}\text{s}^{-1}$. Five days after transplanting, the seedlings were selected and divided into four groups for N and MEL treatments. The four treatments are: N sufficiency (NS, normal N level), N deficiency (ND, low N level), NS with MEL treatment (NS + MEL), and ND with MEL treatment (ND + MEL). The N concentration was 1.875 mM (KNO_3 1.75 mM and NH_4Cl 0.125 mM; Equivalent to the amount of N in 1/8 Hoagland solution) in ND treatment and was 7.5 mM (KNO_3 7 mM and NH_4Cl 0.5 mM; Equivalent to the amount of N in 1/2 Hoagland solution) in NS treatment. For MEL treatment, 1 μM MEL was added to the culture solution. In the pre-experiment, different concentrations of MEL (0, 0.1, 0.5, 1.0 and 10 μM) were applied. The result showed that the effects of MEL on promoting the wheat growth was increased with increasing of MEL concentration when the concentration was below 1.0 μM . However, MEL showed inhibition effect when the concentration was 10 μM (Supplemental data Fig. 1a). During the experiment, the culture solution was continuously aerated, and the pH was adjusted to 5.7 with 0.1 M HCl or 1 M KOH every day. All plants were sampled after 15 and 28 days of treatments.

2.1.2. Biomass determination

After 15 and 28 days of N and MEL treatment, the whole plants were harvested and roots were washed with distilled water for three times. The sampled plants were separated into shoot and root, and weighed after drying at 75 °C for 72 h. The dry sample was used to determine the tissue N content. Each treatment includes three replicates and each replicate includes six plants gathered from the same container.

2.1.3. Nitrate N content

After treated for 15 and 28 days, the fresh samples (shoot or root) (0.5 g) were ground in water (10 mL) and held in a boiling water bath for 30 min. Then, 0.1 mL of the supernatant was mixed with 0.4 mL of 5% salicylic- H_2SO_4 and react for 20 min. The absorbance was read at 410 nm after 9.5 mL of 8% NaOH was added, and the NO_3^- -N content was calculated. Each treatment includes at least three replicates.

2.1.4. Plant tissue N content and total N uptake per plant

The N contents in plant tissues were measured according to the method of Nelson and Sommers (1973). Finely ground dried plant samples (approximately 0.1 g) were digested in sulfuric acid under the action of catalysts (0.23 g K_2SO_4 and 0.07 g CuSO_4). The N content was determined using a Kjeltac 2300 Analyzer Unit (Foss Tecator AB, Hoganas, Sweden). Each treatment includes at least three replicates. Total N absorption per plant was calculated based on the tissue N content and dry weight.

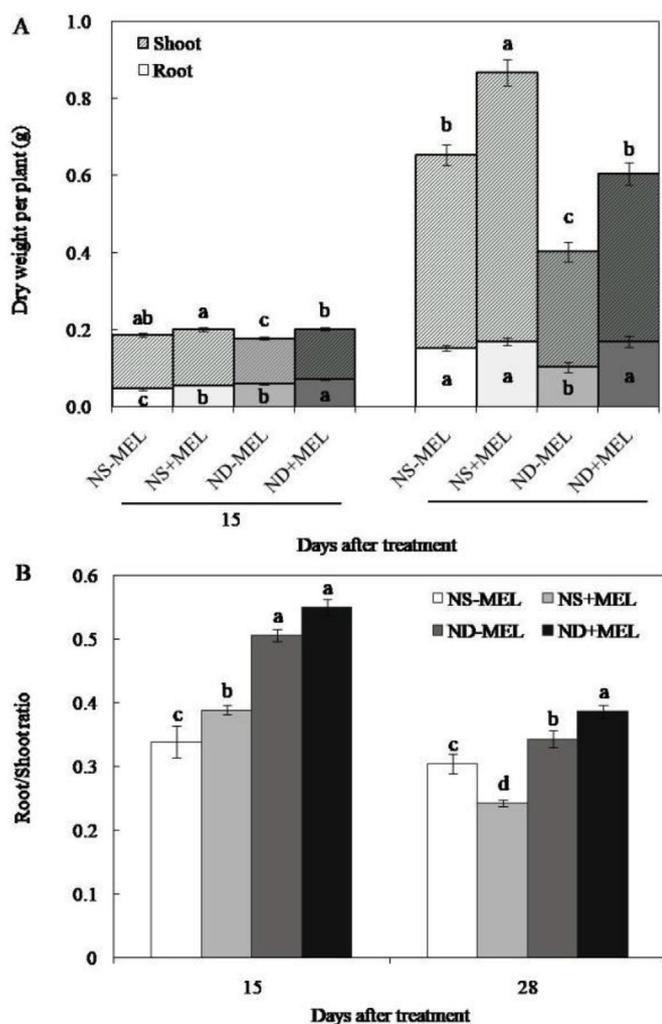


Fig. 1. Effects of melatonin (MEL, 1 μ M) on plant dry weight (A) and root/shoot ratio (B) of wheat seedlings grown under nitrogen-sufficient (NS, 7.5 mM nitrogen) and nitrogen-deficient (ND, 1.875 mM nitrogen) conditions in hydroponic experiment. All parameters were measured after 15 and 28 days of treatment. Values are presented as the means \pm SE ($n = 3$). Different letters in one measuring group indicate statistically significant differences at $p < 0.05$.

2.1.5. Enzyme activity and soluble protein content

Two grams of plant materials (shoot or root) that stored in liquid nitrogen were mixed with 2 mL of pre-cooled 20 mM HEPES-NaOH buffer (pH 7.5 with 0.5 mM β -mercaptoethanol, 5 mM EDTA, 2 mM MgCl_2 , 0.5 mM MSF, 2.5 mM DTT, 0.05% (v/v) Triton-100 and 2% (m/v) PVP). The mixture was homogenized in an ice bath, and centrifuged at 10,000 g for 15 min at 4 $^{\circ}\text{C}$. The supernatant was collected for measuring the activities of nitrate reductase (NR) and glutamine synthetase (GS). The NR activity was monitored according to the method of Kaiser and Lewis (1984), with 0.1 mL of the supernatant was added to 0.1 mL of 0.1 mL L^{-1} phosphate buffer (pH 7.5), 0.1 mL of 1 mg mL^{-1} NADH and 0.2 mL of 0.1 M KNO_3 and constant volume to 2 mL. After pre-incubation at 28 $^{\circ}\text{C}$ for 15 min, the reaction was terminated by adding 1 mL of 1% (m/v) aminobenzenesulfonamide and 1 mL of 0.02% (m/v) naphthylethylenediamine aqueous solution. The mixture was centrifuged at 5,000 g for 5 min to remove suspended solids, and the absorbance was measured at 540 nm. For the blank control, no NADH was added.

The GS activity was measured according to the method of Claussen and Lenz (1999) with minor modification. The 1.2 mL of the supernatant was added to 0.6 mL of 0.25 M imidazole-HCl buffer (pH 7.0), 0.4 mL of 0.3 M sodium glutamate solution (pH 7.0), 0.4 mL of 30 mM

ATP-Na Solution (pH 7.0), 0.2 mL of 0.6 M hydroxylamine, and 0.2 mL of 0.5 M MgSO_4 . The reaction solution was incubated in a 37 $^{\circ}\text{C}$ water bath for 15 min, and 0.8 mL of FeCl_3 reagent was added to terminate the reaction. The mixture was centrifuged at 4,000 g for 15 min, and the supernatant was measured for absorbance at 540 nm. Enzyme activity was calculated as: GS activity ($\text{A mg}^{-1} \text{ protein h}^{-1}$) = $A / (W \times t)$, where A is the absorbance at 540 nm, W is the sample mass, and t is the reaction time.

Soluble protein content was measured according to the method of Bradford (1976). Leaf (0.5 g) or root samples (1.5 g) were frozen by liquid nitrogen and then homogenized with 1.2 mL of 30 mM Tris-HCl buffer (pH 8.7), containing 0.7 M sucrose, 1 mM EDTA-2Na, 1 mM DTT, 1 mM PMSF, 1 mMAsA, and 1 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$. The homogenate was centrifuged at 12,000 g for 15 min at 4 $^{\circ}\text{C}$ after which the supernatant was centrifuged again. Bovine serum albumin was used as the standard to quantify the protein in the final supernatant. Each treatment includes at least three replicates.

2.2. Experiment 2: effect of seed pre-soaking with MEL on wheat grain yield under different N levels in pot experiment

The pot experiment was conducted during 2016–2017 year at the Institute of Soil and Water Conservation, Chinese Academy of Sciences (34 $^{\circ}$ 16'56.24"N, 108 $^{\circ}$ 4'27.95"E; 460 m above sea level), Shaanxi Province, China. Seeds of wheat cultivar 'Changhan58' were sown in pots (30 cm in diameter and 33 cm in depth) containing 15 kg air-dried brown soil. As base fertilizers, P (P_2O_5)/K (K_2O) were presented at concentrations of 0.26 g and 0.17 g kg^{-1} dried soil, respectively. The N levels were 0.23 g pure N per kilogram soil (low N level,) or 0.34 g pure N per kilogram soil (high N level) with urea. Before sowing, wheat seeds were soaked in 100 μM MEL solution or water (as control) for 24 h under dark condition. In addition, a pre-experiment with four concentrations of MEL (0, 10, 100 and 500 μM) was used for seed soaking. The result showed that the effect of MEL on wheat growth promoting was increased with increasing of MEL concentration when the concentration was below 100 μM . While the effect of 500 μM MEL was less prominent than 100 μM MEL (Supplemental data Fig. 1b). The four treatments are: high N level (HN), low N level (LN), HN with MEL treatment (HN + MEL), and LN with MEL treatment (LN + MEL). The seeds were sown on 21 October and harvested on the 5 June. Fifteen seeds were sown at a depth of 3 cm and 12 uniform plants were remained in each pot when the fourth leaf was appeared. All pots were placed under a rain shed in the field. During the experiment, the soil water contents were maintained between 75 and 95% of maximum pot capacity based on daily measurement of pot weight (Chen et al., 2016). The grain number and thousand-grain weight were investigated at the end of experiment.

2.3. Statistical analysis

All results are presented as means \pm SE. Statistical analysis was performed using SPSS Statistics software (Version 21.0, SPSS, Chicago, IL, USA). Differences between the means were compared by means of the Duncan test ($p < 0.05$).

3. Results

3.1. Effects of exogenous MEL on wheat seedling growth

After 15 days of growth, MEL application showed no significant effect on increasing plant dry weight under N sufficiency, but increased both shoot and root dry weight under N deficiency. After treated for 28 days, the dry weights of MEL-treated plants were increased by 32.7% and 50.6% under N sufficient and deficient conditions, respectively, compared with that of MEL-untreated ones (Fig. 1A). The root/shoot ratio of wheat was also affected by MEL application. After 15 days of

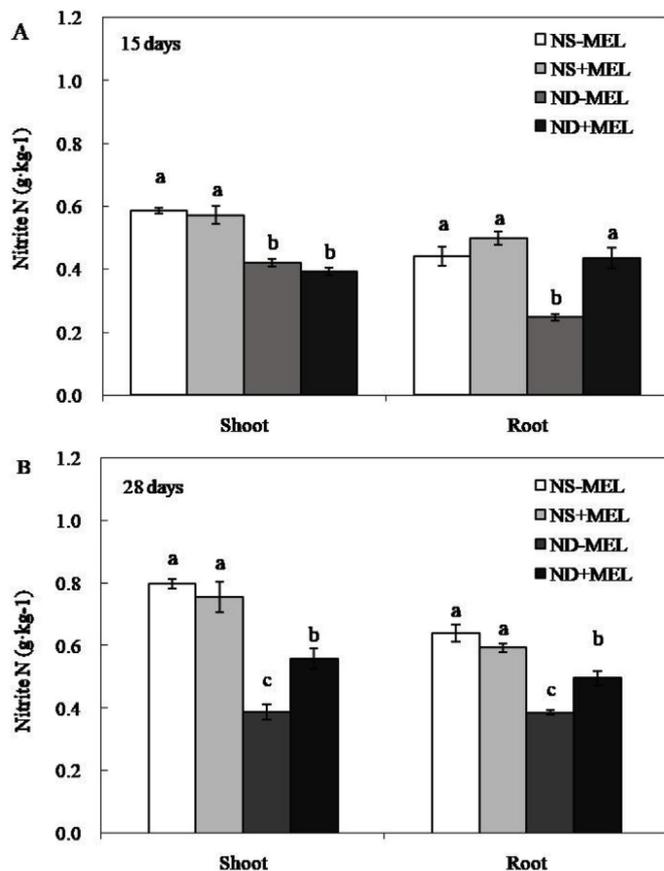


Fig. 2. Effects of melatonin (MEL, 1 μ M) on NO_3^- -N content of wheat seedlings grown under nitrogen-sufficient (NS, 7.5 mM nitrogen) and nitrogen-deficient (ND, 1.875 mM nitrogen) conditions in hydroponic experiment. All parameters were measured after 15 days (A) and 28 days (B) of treatment. Values are presented as the means \pm SE (n = 3). Different letters in one measuring group indicate statistically significant differences at $p < 0.05$.

treatment, the root/shoot ratio was significantly enhanced in MEL-treated plants under both N sufficiency and deficiency. The different results were shown after 28 days of treatment, where the root/shoot ratio was decreased by 20.3% in MEL-treated plants under N sufficient condition, while it was increased by 12.6% under N deficient condition, compared with the plants that did not applied with MEL (Fig. 1B).

3.2. Effects of exogenous MEL on NO_3^- -N content

Without MEL application, the shoot and root NO_3^- -N contents were significantly lower in plants grown under N deficiency than that grown under N sufficiency both after 15 and 28 days of treatment (Fig. 2). MEL application significantly increased NO_3^- -N contents in roots under N deficient condition, which was 75.9% higher than that without MEL treatment, but had no effect on shoot NO_3^- -N content under the same condition after 15 days of treatment. However, after treated for 28 days, MEL application enhanced both shoot and root NO_3^- -N contents under N deficiency markedly. In addition, MEL had no significant effect on shoot or root NO_3^- -N contents under N sufficient condition neither 15 nor 28 days after treatment.

3.3. Effects of exogenous MEL on N content and absorption

The N contents in shoots and roots were significantly reduced in plants grown under N deficient condition both after 15 and 28 days of treatment. In shoot, N content was not altered by MEL application after 15 days of N deficient treatment, but it was increased by 50% after 28

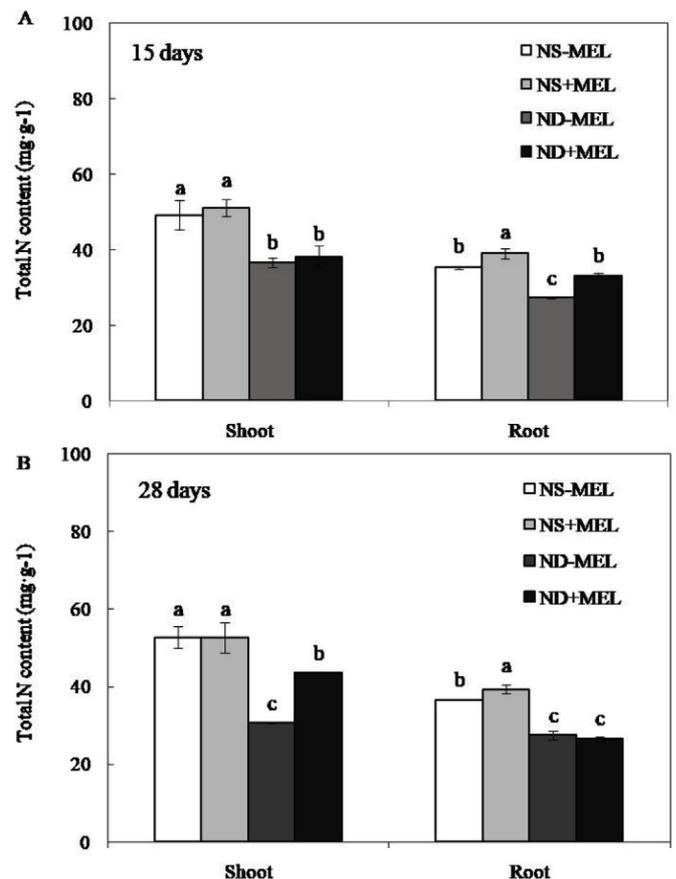


Fig. 3. Effects of melatonin (MEL, 1 μ M) on total nitrogen content of wheat seedlings grown under nitrogen-sufficient (NS, 7.5 mM nitrogen) and nitrogen-deficient (ND, 1.875 mM nitrogen) conditions in hydroponic experiment. All parameters were measured after 15 days (A) and 28 days (B) of treatment. Values are presented as the means \pm SE (n = 3). Different letters in one measuring group indicate statistically significant differences at $p < 0.05$.

days of the same treatment (Fig. 3). In root, MEL application enhanced N contents under N sufficient condition after 15 and 28 days of treatment, but such an increase effect was only found after 15 days of treatment under N deficient condition.

MEL application showed no effect on plant N absorption under N sufficient condition, however, it significantly increased N absorption under N deficiency (Fig. 4). For example, after 28 days of treatment, the total N uptake was 49.8% higher in MEL-treated plants than that of MEL-untreated plants under N deficient condition.

3.4. Effects of exogenous MEL on NR and GS activities, and soluble protein contents

The application of exogenous MEL improved the NR activity only in root of plants grown under N deficiency after 15 days of treatment (Fig. 5). After 28 days of treatment, the NR activity in shoot and root was largely increased by MEL application under N deficiency. In addition, exogenous MEL application had no effect on improving the NR activity under N sufficient condition, neither in shoot nor root and neither treated for 15 nor 28 days.

The GS activity was higher in plants grown under N deficiency than that grown under N sufficiency, regardless of MEL application (Fig. 6). After 15 days of treatment, exogenous MEL application increased the GS activity by 76.8% in root under N deficiency, but exhibited no effect in shoot. After 28 days of treatment, both the shoot and root GS activities were increased in MEL-treated plants, which showed 43.7% and 29% higher than that of MEL-untreated plants under N deficient

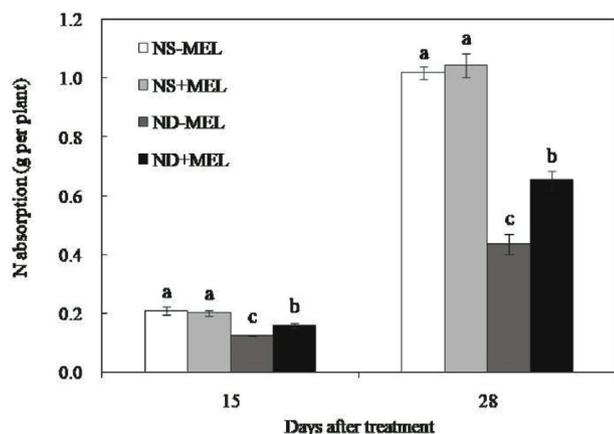


Fig. 4. Effects of melatonin (MEL, 1 μ M) on nitrogen absorption of wheat seedlings grown under nitrogen-sufficient (NS, 7.5 mM nitrogen) and nitrogen-deficient (ND, 1.875 mM nitrogen) conditions in hydroponic experiment. The total N uptake per plant was calculated based on plant N content and biomass after 15 and 28 days of treatment. Values are presented as the means \pm SE (n = 3). Different letters in one measuring group indicate statistically significant differences at $p < 0.05$.

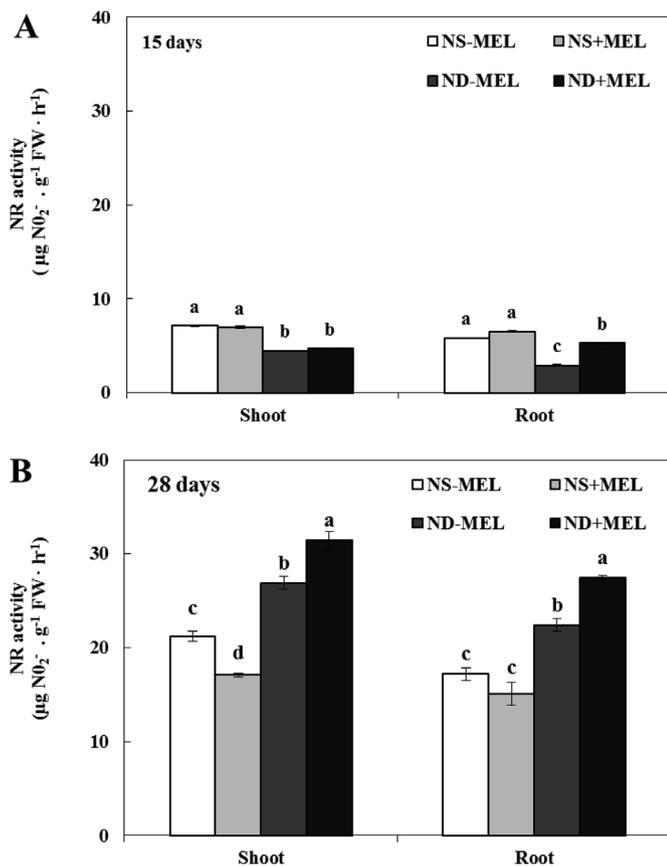


Fig. 5. Effects of melatonin (MEL, 1 μ M) on nitrate reductase (NR) activity of wheat seedlings grown under nitrogen-sufficient (NS, 7.5 mM nitrogen) and nitrogen-deficient (ND, 1.875 mM nitrogen) conditions in hydroponic experiment. All parameters were measured after 15 (A) and 28 days (B) of treatment. Values are presented as the means \pm SE (n = 3). Different letters in one measuring group indicate statistically significant differences at $p < 0.05$.

condition. In addition, MEL application did not affect the soluble protein content in shoot and root under both N sufficient and deficient conditions, except an increase in shoot after 15 days of N-sufficient treatment (Fig. 7).

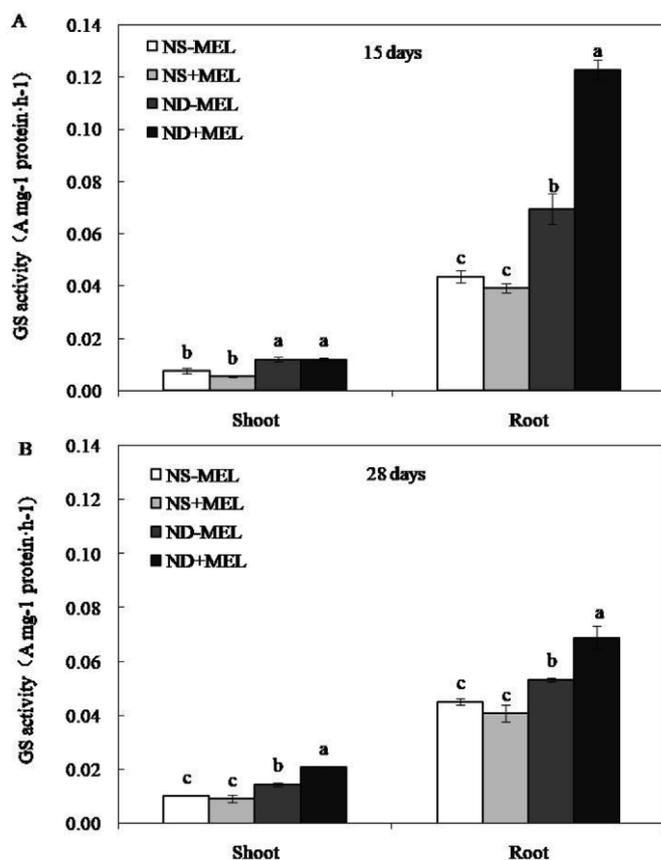


Fig. 6. Effects of melatonin (MEL, 1 μ M) on glutamine synthetase (GS) activity of wheat seedlings grown under nitrogen-sufficient (NS, 7.5 mM nitrogen) and nitrogen-deficient (ND, 1.875 mM nitrogen) conditions in hydroponic experiment. All parameters were measured after 15 (A) and 28 days (B) of treatment. Values are presented as the means \pm SE (n = 3). Different letters in one measuring group indicate statistically significant differences at $p < 0.05$.

3.5. Effects of seed pre-soaking with MEL on wheat grain yield

As shown in Table 1, without MEL treatment, the per-plant yield was 18% higher in the plants grown under N sufficient condition than that grown under N deficient condition. With MEL application, the grain yield increased by 16% and 23% under N sufficiency and deficiency, respectively. Moreover, both the gain number and thousand-grain weight were increased by MEL application under N deficient condition.

4. Discussion

Melatonin is considered as a plant master regulator which is widely involved in regulating plant growth, development and stress responses (Arnao and Hernández-Ruiz, 2018). In this study, MEL application showed promoted function on wheat seedling growth both under N sufficient and deficient conditions, but this promoted function on plant growth was more prominent under N deficient condition than that under N sufficient condition (Fig. 1). In pot experiment, the pre-soaking of seeds with MEL enhanced the wheat yield, and the greater extent in yield increasing was found under low N level, compared with that under high N level (Table 1). Generally, N deficiency significantly inhibited the plant growth and yield (Greenwood, 1982). Therefore, MEL application effectively improved plant growth and yield under low N level, suggesting that MEL could be used as an effective plant growth regulator in enhancing plant N use efficiency.

The assimilation of N is closely related to crop biomass and grain yield (Fenilli et al., 2007). Basically, plants absorb N source from the

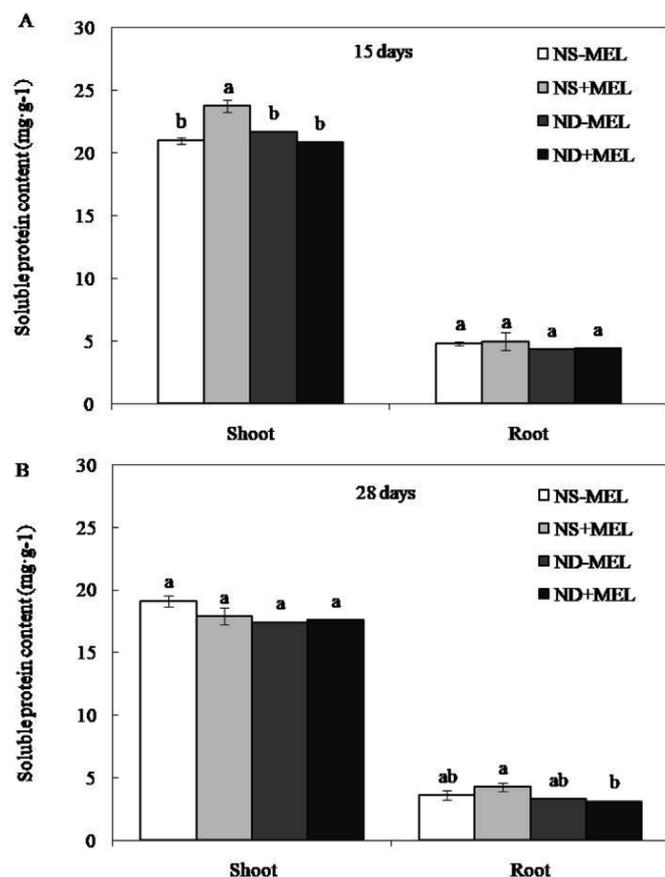


Fig. 7. Effects of melatonin (MEL, 1 μM) on soluble protein content of wheat seedlings grown under nitrogen-sufficient (NS, 7.5 mM nitrogen) and nitrogen-deficient (ND, 1.875 mM nitrogen) conditions in hydroponic experiment. All parameters were measured after 15 (A) and 28 days (B) of treatment. Values are presented as the means \pm SE ($n = 3$). Different letters in one measuring group indicate statistically significant differences at $p < 0.05$.

Table 1

Effect of seed pre-soaking with melatonin (MEL, 100 μM) on yield of wheat grown under high nitrogen (HN, 0.23 g pure N per kilogram dried soil) and low nitrogen (LN, 0.34 g pure N per kilogram dried soil) conditions in pot experiment. Values are presented as the means \pm SE ($n = 30$). Different letters in the same group indicate statistically significant differences at $p < 0.05$.

Treatment	Grain number	Thousand-grain weight (g)	Theoretical yield per plant (g)
HN-MEL	43.51 \pm 15.02a	50.47 \pm 0.31 b	4.32
HN + MEL	44.30 \pm 15.20 ab	51.35 \pm 0.14a	5.03
LN-MEL	35.91 \pm 14.09 b	48.19 \pm 0.08c	3.67
LN + MEL	43.25 \pm 12.64a	50.95 \pm 0.21 ab	4.50

soil in two forms: nitrate nitrogen (NO_3^-) and ammonium nitrogen (NH_4^+). For wheat, NO_3^- is the dominant nitrogen source (Li et al., 2013). In plants, the total N includes all forms of N, such as NO_3^- , NH_4^+ , amino acids, etc., which reflects the overall N accumulation in plant tissues and can be used as the valid physiological markers for evaluation of N absorption, assimilation and transport in relation to N supply (Kichey et al., 2006). In this study, after 28 days of growth, MEL-treated plants maintained the high NO_3^- -N content both in shoot and root under N deficient condition, suggesting that MEL could promote N uptake under this condition (Fig. 2). Meanwhile, total N content in shoot of MEL-treated plants was higher than that of MEL-untreated plants, suggesting that MEL application could also promote N assimilation and remobilization under N deficient condition (Fig. 3). These

results indicate that MEL could promote N uptake and metabolism, leading to maintaining the higher N level in shoot, which finally benefits the plant growth under N deficient condition.

Various enzymes are involved in N uptake and metabolism, including nitrate reductase (NR), nitrite reductase (NiR), nitrogenase (Nase), glutamate synthase (GS), glutamine synthetase (GOGAT), and glutamate dehydrogenase (GDH) (Andrews et al., 2013). Among these enzymes, NR and GS are the key enzymes of N metabolism and assimilation and play important roles in plant response to N deficiency (Kaur et al., 2015; Kichey et al., 2006, 2007). Nitrate reductase catalyzes the conversion of NO_3^- to NO_2^- and is the first enzyme in the NO_3^- assimilation process, in addition, the activity of nitrate reductase determines the rate of nitrate assimilating into organic N compounds (Lillo, 2008; Heidari et al., 2011). The assimilation site of NO_3^- depends on the genotype and soil NO_3^- concentrations. For temperate cereals and legumes, root is the main site of NO_3^- assimilation when the soil NO_3^- concentration is less than 1 mol m^{-3} , however, shoot NO_3^- assimilation increases when the soil NO_3^- concentration is between 1 and 20 mol m^{-3} (Andrews et al., 1992, 2004). In the present study, NR activity was increased significantly after 28 days of N deficiency treatment. Moreover, the NR activity in both shoot and root of wheat seedlings with MEL treatment was significantly higher than that without MEL application under N deficiency (Fig. 5). Meanwhile, the NO_3^- -N content was maintained high in MEL-treated plants (Fig. 2). Taken together, these results indicate that MEL is involved in promoting N assimilation through regulating NR activity under N deficiency.

Glutamine synthetase catalyzes the first step reaction of N assimilation, and the GS/GOGAT cycle accounts for more than 95% of ammonia assimilation (Céline et al., 2006). Leaf GS activity was found to be positively correlated with leaf soluble protein and N content as well as grain yield (Kichey et al., 2006, 2007). Two isozymes of glutamine synthetase exist: GS1 and GS2. The regulation of relative activity or amount of GS1/GS2 could enhance N use efficiency by a complex C-N metabolic mechanism, improving the transfer of N nutrition between source and sink, thereby accelerating the transport of nutrients to the grain (Zhang et al., 2017). Transgenic expression of plastidic GS gene increases N uptake and yield in wheat under low N condition (Hu et al., 2018). In this study, the GS activity was found to be greatly higher in MEL-treated plants than in MEL-untreated ones under N deficiency (Fig. 6), demonstrating that MEL application participates in enhancing the N assimilation. The high NR and GS activities observed in MEL-treated wheat plants suggest that MEL application could promote N uptake and assimilation and, ultimately, lead to the maintenance of high N contents in plants.

In addition to enhancing the activities of N metabolism and assimilation enzymes, a stronger root system also contributes to plant N uptake (Gavito et al., 2001). It has been confirmed that MEL can regulate the root development, particularly the adventitious root formation (Murch and Saxena, 2001; Nawaz et al., 2016). Recently, more evidences showed that auxin and H_2O_2 are participated in MEL-modulated root growth (Hernández-Ruiz et al., 2004; Nawaz et al., 2016; Liang et al., 2017; Wang et al., 2016; Chen et al., 2018; Mukherjee, 2018). In the current study, MEL-treated plants also maintained high root/shoot ratio (Fig. 1), the high root/shoot ratio could contribute to improved nutrient uptake, which could be involved in MEL-regulated wheat response to N deficiency as well.

Nitrogen uptake and assimilation by plants is a process that requires the consumption of photosynthetic assimilates (Turpin et al., 1988; Lawlor, 2002). MEL has been widely reported to improve photosynthesis of crops. Especially under stresses, MEL can alleviate the damage of photosynthetic system and delay leaf senescence by directly acting as an antioxidant or indirectly increasing the activity of antioxidant enzymes (Wang et al., 2013; Arnao and Hernández-Ruiz, 2014; Liang et al., 2015). In this study, although we did not investigate photosynthetic rate and chlorophyll content, the application of MEL promoted wheat growth and increased yield, indicating that MEL could

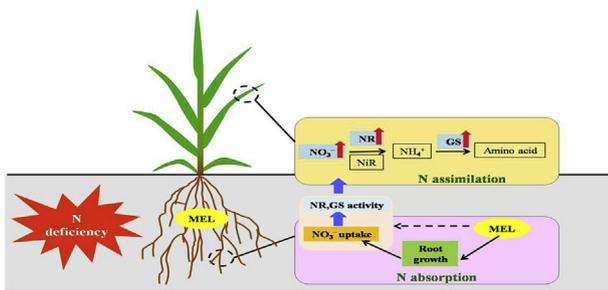


Fig. 8. Schematic model displaying the role of exogenous MEL on wheat seedling growth in exposure to N deficiency. Exogenous application of MEL enhanced the activities of NR and GS enzymes, and the root/shoot ratio under N deficient condition, which promoted the N absorption, metabolism and assimilation, meanwhile, increased the N content and N efficiency under N deficiency.

also improve photosynthesis. Meanwhile, high photosynthetic rate and sufficient photosynthetic assimilates also contribute to nitrogen uptake and assimilation, which might be an important reason for MEL to improve plant tolerance to N deficiency.

5. Conclusion

In general, N deficiency severely inhibits the growth of wheat seedlings and reduces the grain yield. In this study, the performance and function of MEL on N uptake and metabolism in winter wheat seedlings were investigated and the possible mechanism is proposed (Fig. 8). Exogenous application of MEL enhanced the activities of NR and GS enzymes, and the root/shoot ratio under N deficient condition, which promoted the N absorption, metabolism and assimilation. Meanwhile, the N content and N efficiency were also increased under N deficiency. The results of this study suggest that MEL has big potential values in reducing N fertilizer application and enhancing N efficacy in practical crop production and the field study need conducting in the future.

Author contributions

Shiwen Wang and Yujie Qiao conceived and designed the experiment, analyzed the data and prepared the draft. Yujie Qiao and Bomei Wang conducted the experiment and collected data. Lina Yin, Qingbo Ke and Xiping Deng contributed to data analysis and interpretation of the results.

Conflicts of interest

All authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.plaphy.2019.03.037>.

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