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Application of melatonin-mediated modulation of drought tolerance by regulating photosynthetic efficiency, chloroplast ultrastructure, and endogenous hormones in maize

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Abstract

Background: Melatonin played an essential role in numerous vital life processes of animals and captured the interests of plant biologists because of its potent role in plants as well. As far as its possible contribution to photoperiodic processes, melatonin is believed to act as a growth regulator and a direct free radical scavenger/indirect antioxidant. The objective of this study to identify a precise melatonin concentration for a particular application method to improve plant growth requires identification and clarification.

Methods: This work establishes unique findings by optimizing melatonin concentration in alleviating the detrimental effects of drought stress in maize. Maize plants were subjected to drought stress (40–45% FC) after treatments of melatonin soil drenching at different concentrations (50, 100, and 150 μM) to consider the changes of growth attribute, chlorophyll contents, photosynthetic rate, relative water content (RWC), chloroplast ultrastructure, endogenous hormonal mechanism, and grain yield.

Results: Our results showed that the application of melatonin treatments remarkably improved the plant growth attributes, chlorophyll contents, photosynthetic rate, RWC, hormonal mechanism, and grain yield plant^{-1} under drought conditions at a variable rate.

Conclusion: Our current findings hereby confirmed the mitigating potential of melatonin application 100 μM for drought stress by maintaining plant growth, hormone content, and grain yield of maize. We conclude that the application of melatonin to maize is effective in reducing drought stress tolerance.

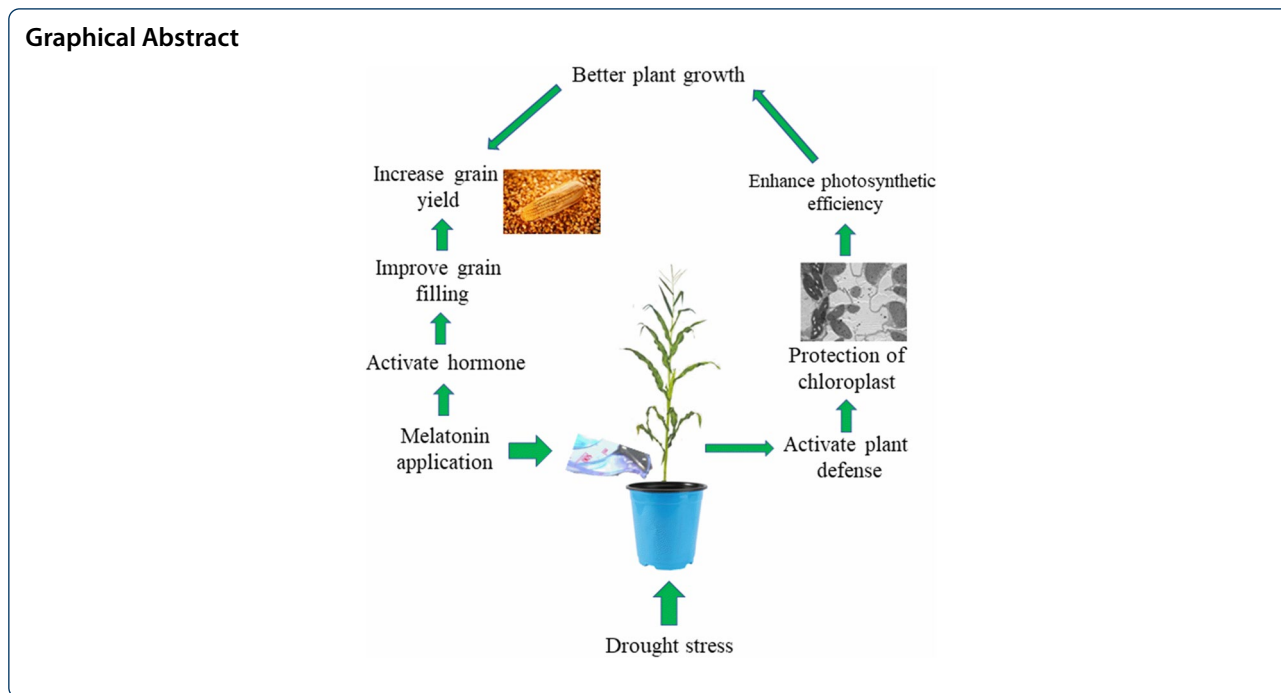
Keywords: Melatonin, Photosynthetic pigments, Chloroplast ultrastructure, Hormone contents, Maize yield, Drought stress

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Background

Plant growth and production can be severely affected by environmental stresses, such as drought, high temperatures, heavy metals, or salinity [1]. Among them, drought is a global concern due to climate change and increasingly insufficient water resources [2]. In particular, drought stress affects many morphological, physiological, and biochemical processes, such as stomatal closure, leaf ultrastructure, cellular dehydration, membrane lipid peroxidation, and reduced antioxidant ability and endogenous hormones, affecting the survival and growth of plants [3–5]. Plants have evolved extensive stress resistance systems that allow them to finish their life cycles in the presence of drought [6]. Drought is one of the most extreme environmental stress and eventually triggers crop yield reductions [7]. Maize is a significant cereal crop in many countries where it is used as a staple food, although in some countries, it is used as a raw material for the biofuel industry [8]. Therefore, numerous studies have been undertaken to enhance crop tolerance and mitigate the negative impact of various abiotic stresses, including drought stress. However, the exogenous application of plant hormones plays a crucial and even decisive role in regulating plant growth and development [9].

Plant hormones are essential signaling molecules that lead to climatic changes in plant growth and development, such as gibberellins (GA_3) and abscisic acid (ABA) are well-known phytohormone that plays crucial roles in seed germination and early seedling establishment [2]. ABA, a universal abiotic stress hormone,

responded positively to abiotic stress, while GA acts as a plant growth regulator to promote seed germination in response to stress [10]. Many physiological changes in photosynthesis, transpiration, and transport lead to structural changes in plant leaves [11]. Plants organize structure and function to adapt in most cases without causing permanent harm to different environmental stress conditions [12]. To withstand the complex physiological reactions that control drought stress, photosynthetic pigments efficiency was stimulated, and plant growth survival was promoted [13, 14]. The net photosynthetic rate and chlorophyll content decreased in drought stress conditions due to decreased stomatal conductance, as stomatal closure is one of the first responses to drought stress [15]. Drought has been shown to change the proportion and arrangement of the leaf cell thickness, palisade, spongy tissues and chloroplast structure. The chloroplast synthesis of melatonin provides one possible protection for chloroplasts from oxidative stress. As to the roles of melatonin in chloroplasts, these have not been adequately examined because research on plant melatonin is still in its early stages [16]. However, several studies have uncovered evidence that melatonin effectively prevents chlorophyll deterioration and preserves chloroplast physiology. The destruction of chlorophyll involves oxidative stress [17]. By preserving chloroplast integrity, melatonin promotes photosynthesis, which is essential for the survival of the plant. Therefore, the cell's structural and functional integrity is compromised by abiotic stress conditions [18]. Plant hormones are

essential molecules that respond to changes in the climate condition during growth and physiological response to abiotic stress [19]. Previously studies reported that cytokinin (CK), such as zeatin (Z), is strongly associated with the biogenesis of chloroplasts, particularly with their maturation [20].

On the other hand, abscisic acid (ABA) is generally associated with chloroplast-based gene expression. The ABA biosynthesis mechanism is also related to plastids, such as carotenoid formation; its precursor occurs in plastids [21]. In different crops, the plant hormones abscisic acid (ABA), indole acetic acid (IAA), gibberellic acid (GA_3), and zeatin riboside (ZR) influence the process of grain filling [22]. Also, ABA, ZR, and GA increase the chlorophyll and net photosynthetic rate in duckweed and are critical for cell division, chloroplast development, senescence, and the resistance of plants to stresses [23, 24]. This study shows that using plant bio-stimulators or phytohormone to enhance plant adaptability and defense against unfavorable environmental conditions is revolutionary.

The multi-regulatory biological hormone melatonin (*N-acetyl-5-methoxytryptamine*) plays a crucial role in plants and animals' life cycles [25]. Several studies have examined the hormone production, growth, and reaction of melatonin to biotic and abiotic stresses in plants [26]. Exogenous melatonin treatment improves crop development, improves photosynthetic pigments, ultrastructure of the leaves, regulates endogenous hormones level, and increases the crop yield under drought stress conditions [5, 27]. Melatonin treatment up-regulates the expression of critical genes involved in GA_3 biosynthesis. It down-regulates critical genes involved in ABA biosynthesis to promote the use of nutrients, synthesize new proteins, and increase *Limonium bicolor* seeds' germination under abiotic stress conditions [9, 28]. Melatonin is a plant growth hormone that is closely related to auxin [29]. Plant hormones are involved in the germination and dormancy of seeds. The exogenous melatonin treatments can mitigate seed germination under abiotic stress conditions via the regulation of GA_3 synthesis and ABA decomposition [30, 31]. Melatonin application increased endogenous melatonin and cytokinin content, decreasing the ABA range in ray grass under heat stress conditions [8, 26]. Researchers have revealed melatonin role from various aspects under stresses, including phenotypic, physiological, and molecular levels, increasing research on melatonin in plants [29].

Given the numerous hypotheses previously proposed, it is critical to comprehend melatonin regulatory effects on stressed plants. However, little is known about the regulatory impact of melatonin as a soil drenching on photosynthetic pigments, the ultrastructure of leaf chloroplast,

and the regulatory mechanism of melatonin on endogenous hormones ABA, IAA, GA_3 , and ZR, as well as maize yield under drought stress conditions. Therefore, in the current study, we analyzed exogenous melatonin role in the drought tolerance of maize by evaluating the morphological features, physiological parameters, associated endogenous hormone contents, and grain yield.

Materials and method

Plant materials, experimental design, and locations

The experiment was performed in the glass house of Guangxi University, Nanning, Guangxi, China. The most well-known maize (*Zea mays* L.) hybrid seeds (Wanchuan-1306) of the south part of China were used in the current experiment. The healthy hybrid maize seeds were selected and five seeds were planted in each pot. The pot size was (29 × 31 cm); 20 kg of soil mixture (farmland topsoil and perlite 3:1 v/v) was added to each pot. The physical–chemical properties of the collected soil are shown in Table 1. All the pots were arranged in a randomized complete block design (RCBD) and placed in a glass house under natural light conditions. At the early growth stage of maize, the soil moisture content was retained at the normal 75–80%. At the eight-leaf stage, progressive drought stress was imposed on the maize plants up to maturity. During the drought stress period, the water holding capacity was maintained (40–45%) based on daily pot weight measurements. The exogenous treatments of melatonin were applied as a soil drenching method in three growing stages: the nine-leaf stage (V-9), the Tasseling stage (R-1), and the Milk stage (R-3) to each pot for three consecutive days. The treatments designated for this study include 1) Control well-watered (CW) + 0 μ M melatonin, 2) Control drought (CD) + 0 μ M melatonin, 3) Melatonin 50 μ M + drought stress (MD1), 4) Melatonin 100 μ M + drought stress (MD2), and 5. Melatonin 150 μ M + drought stress (MD3). Flag fully open leaves sampling was collected from each treatment, and measurements for different tests, such as chloroplast ultrastructure, photosynthetic pigments, and

Table 1 Physical and chemical composition of the experimental pots soil

Parameters	Values
Soil organic matter	14.61 g/kg
Available nitrogen	0.88 g/kg
Available phosphorus	48.85 g/kg
Available potassium	96.37 mg/kg
Soil pH	6.83
Water holding capacity	30.31%

endogenous hormone level, were performed after 1 week of treatment application at each growing stage. To ensure the accuracy of determination each treatment was performed with three biological replications at each growing stage of maize and then samples were immediately processed or stored at $-80\text{ }^{\circ}\text{C}$ for further analysis.

Measurement of growth and physiological and biochemical parameter

Leaf area and plant height

Three plants from each replicate were selected for measuring leaf area plant^{-1} and plant height at each growth stage. The leaf area plant^{-1} and plant height were counted manually by using the tape meter. The leaf length and maximum leaf width were measured by a tape meter. The following formula was used to determine the leaf area plant^{-1} [32]:

$$\begin{aligned} \text{Leaf area (cm}^2\text{)} \\ &= \text{Leaf length} \times \text{Maximum leaf width} \\ &\quad \times 0.75 \text{ (correction factor).} \end{aligned}$$

Determination of chlorophyll contents and net photosynthetic rate

The chlorophyll content was determined according to Arnon et al. [33]. In each growth stage, the middle portion of fresh flag leaves (0.1 g) was cut into small pieces and grind with liquid nitrogen. The ground samples were put in 10 mL tubes and acetone, ethanol, and distilled water (4.5:4.5:1) were added to it, and then all the sample tubes were put in the dark area overnight. The absorbance was measured by UV spectrophotometer at the 663 and 646 nm wavelengths and was expressed as mg g^{-1} fresh weight. The net photosynthetic rate was measured in each pot at three growing stages of maize includes (V-9, R-1, and R-3). The LI-6800XT (LI-COR, Lincoln, Nebraska, USA) portable photosynthesis system was used on clear sunny days from 9:00 to 11:00 am, and three replications were measured for each treatment.

Relative water contents and dry biomass accumulation

The technique of Su et al. [34] was used to determine the RWC of leaves during the drought stress condition. The fresh leaf sample was collected at V-9, R-1, and R-3 growing stages of maize, and then fresh weight (FW) of each leaf sample was recorded. Then, the samples were immersed in distilled water in beakers and left for 24. Thereafter, fully turgid leaves weight (TW) at each growing stage was recorded. To obtain the dry weight (DW), the leaf samples were then oven-dried at $75\text{ }^{\circ}\text{C}$ for 72 h.

The following formula was used to determine the RWC (%):

$$\text{RWC} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

For dry biomass measurements, three plants were collected from each treatment at different growth stages, such as (V-9, R-1, and R-3). For the constant dry weight, the plant sample was put in the oven at $105\text{ }^{\circ}\text{C}$ for half-hour and then at $75\text{ }^{\circ}\text{C}$ for 72 h, a high precision (0.001 g) analytical scale was used to weight the plant samples.

Analysis of transmission electron microscope (TEM)

The three healthy and uniform leaf samples (1 mm^2) were selected for chloroplast ultrastructure from each treatment. The leaf sample was rinsed with distilled water, and measurement was observed using the transmission electron microscope (TEM). The samples obtained (6 h, $4\text{ }^{\circ}\text{C}$) were fixed using 4% glutaraldehyde and 0.2 M sodium phosphate buffer (pH 6.8), followed by a four-fold wash with 0.1 M sodium phosphate buffer (pH 6.8). Samples were post fixed in 1% osmic acid in a 0.2 M phosphate buffer (pH 6.8), dehydrated in a graded ethanol sequence, and dried using critical point drying. The ultrathin sections were finally stained with lead citrate and 2% uranyl acetate. They were examined using the HITACHI transmission electron microscope (Carl Zeiss, Göttingen, Germany) at 80 kV acceleration voltage [35].

Extraction of endogenous hormones

Plant endogenous hormone's content ABA, IAA, GA_3 , and ZR contents were determined using the method of Pan et al. [36]. Approximately 0.5 g of maize fresh leaf powder was used for the extraction and purification of phytohormone (ABA, IAA, GA_3 , and ZR). In each sample of maize leaves, approximately 25 μL d6-ABA (0.25 $\text{ng}/\mu\text{L}$), 2 μL d5-IAA (2 $\text{ng}/\mu\text{L}$), 50 μL d2- GA_3 (2 $\text{ng}/\mu\text{L}$), and 10 μL d5-ZR (0.25 $\text{ng}/\mu\text{L}$) were added as internal standards (Shanghai, Yuanye Bio-Technology Co., Ltd., Shanghai, China). Subsequently, 0.5 mL of extraction solvent (isopropanol: H_2O : concentrated HCl = 2:1:0.002, vol/vol/vol) was added and shaken for 30 min at $4\text{ }^{\circ}\text{C}$ at a speed of 100 rpm. Then 1 mL of dichloromethane was added and the mixture was shaken for 30 min. The solvent was extracted and condensed by a nitrogen evaporator after centrifugation. The residues were subsequently dissolved in 0.1 mL of aqueous methanol solvent (methanol: H_2O = 1:1, vol/vol) and centrifuged at 1200 rpm for 5 min. The supernatant was transferred to a vial for LC-MS analysis after layering.

Quantification of phytohormone by LC–MS

In the multiple reaction monitoring (MRM) mode, LC–MS was used for quantitative phytohormone measurement. An Agilent 1260 HPLC system and an AB Qtrap 5500 triple quadrupole mass spectrometer with an electrospray ionization source were integrated into the LC–MS system. Samples were injected into the Agilent SB-C18 (50 × 4.6 mm, 1.8 μm) column and separated by a mobile step with a flow rate of 0.8 mL/min as follows: distilled water with 0.1% acetic acid (A) and acetonitrile (B). The HPLC gradient program and multiple reaction monitoring (MRM) settings were applied to measure phytohormone. The injected volumes for ABA, IAA, and GA₃ were 5.0 μL and 1.0 μL for ZR. The initial HPLC running conditions were solvent A: B 90:10 (v/v). The gradient elution program was as follows: 10% solvent B for 5 min; a linear gradient from 10 to 30% solvent B in 5 min; 10 min at a constant 30% solvent B; another linear gradient from 30 to 50% solvent B in 10 min; 9 min at a constant 50% solvent B; a linear gradient from 50 to 100% solvent B in 1 min; and 5 min at a constant 100% solvent B. Before each analytical run, the column was equilibrated with the starting composition of the mobile phase for 15 min. The mass spectrometer was run in positive mode with a 3500 V capillary spray voltage and a scan speed of 22,000 (m/z)/s within 50 and 400 m/z. The nebulizer gas pressure, drying gas flow rate, and drying gas temperature were set to 30 psi, 8 L min⁻¹, and 350 °C, respectively. LC–MS (QTRAP5500, AB Sciex, Boston, MA, USA) was used to measure each phytohormone, with the conditions listed in Additional file 1: Tables S1 and S2.

Maize yield

For yield and yield components, three plants were collected from each treatment. The number of grains rows⁻¹, grains ear⁻¹, hundred grains weight (g), and grain yield plant⁻¹ (g) were calculated from each plant.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using SPSS 22.0 software (IBM SPSS Corp., Armon, NY, USA). The data obtained from every sampling unit were analyzed separately, and the means were tested by the least significant difference test (LSD test) at $P \leq 0.05$. Data were plotted by using Origin 8.6 software (Origin Lab, Northampton, MA, USA). For each treatment, three replicates were measured, and data are presented as the mean ± standard deviation (SD). Pearson's correlation was used to analyze the determination of correlation between all the indicators by using R software.

Results

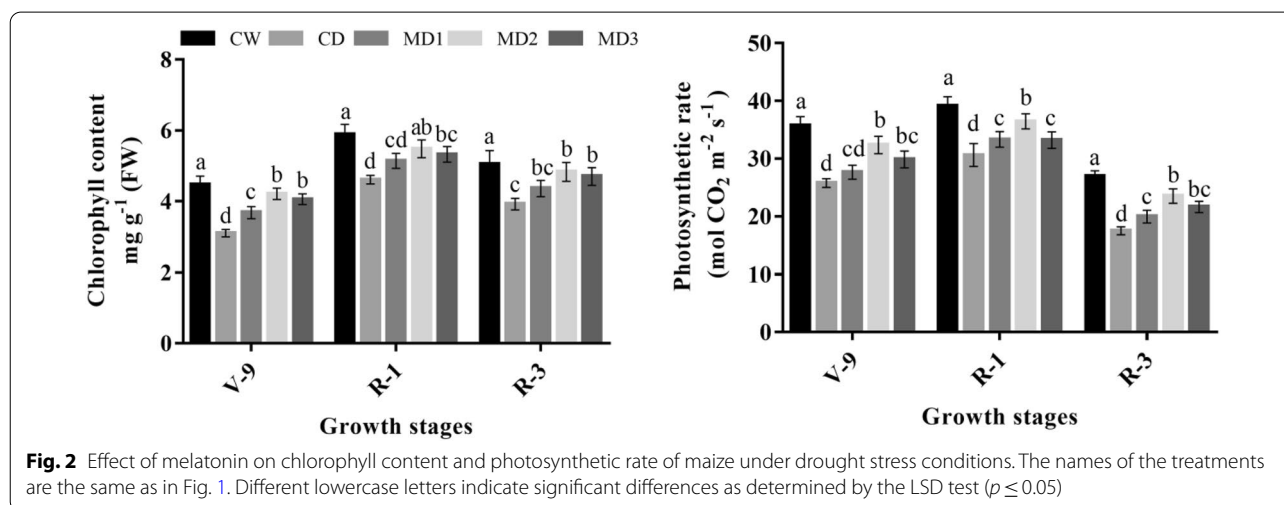
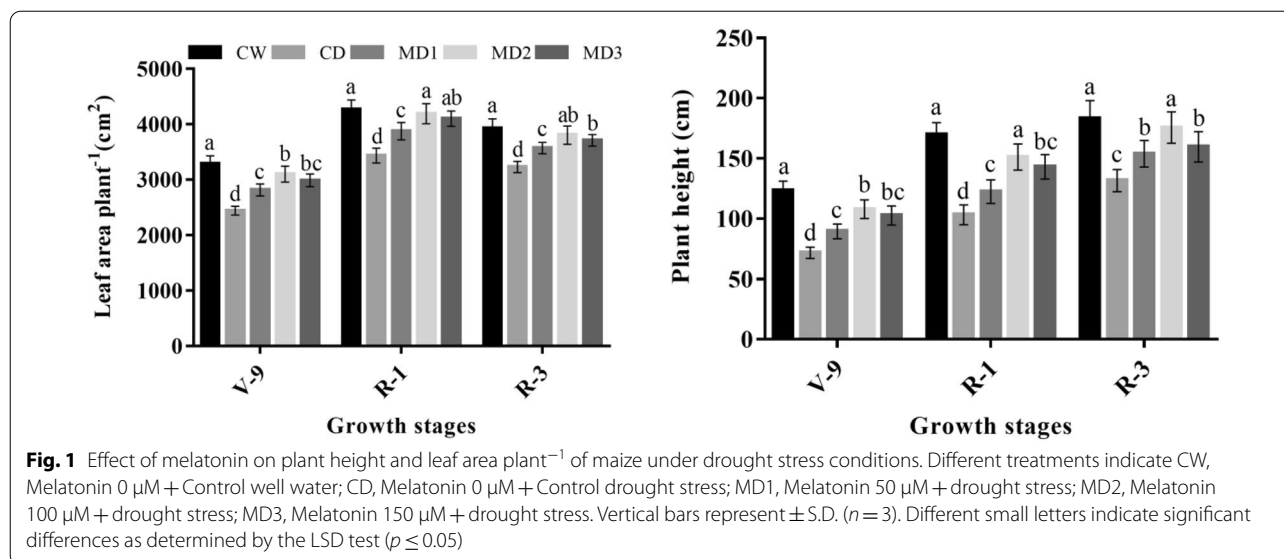
Effect of melatonin treatment on plant height and green leaf area plant⁻¹

Plant height and leaf area of maize crops were evaluated in melatonin-treated plants as compared with drought stress. Exogenous melatonin treatments significantly increase the plant height and leaf area at a variable degree in different growth stages of maize plants under drought stress conditions. Increasing melatonin concentration from low to optimum level improves the plant height, while a higher level significantly declines the plant height at all growth stages of maize. The mean results showed that treatment MD2 achieved taller plant height and was greater by 50.16% at V-9, 45.52% at R-1, and 33.45% at R-3 growing stages of maize compared to drought stress control (Fig. 1). Overall results showed that a significantly taller plant height was observed in treatment MD2 at all maize growth stages than CD and other melatonin-treated plants under drought stress conditions.

Exogenous application of melatonin increased the leaf area plant⁻¹ at various growth stages of maize under drought stress conditions. The total green leaf area plant⁻¹ increased from V-9 to R-1 growth stage and then gradually declined from R-1 to R-3 growing stages of maize in all treatments. As compared to drought stress control (CD), melatonin-treated plants have a higher leaf area plant⁻¹. Under drought stress conditions, the melatonin treatment MD2 showed a maximum leaf area of plant⁻¹ at all growing stages compared with CD. The treatment MD2 increased the leaf area plant⁻¹ by 26.94% at V-9, 21.95% at R-1, and 17.82% at the R-3 growth stage of maize under drought stress conditions (Fig. 1).

Melatonin improved the chlorophyll content and net photosynthetic rate

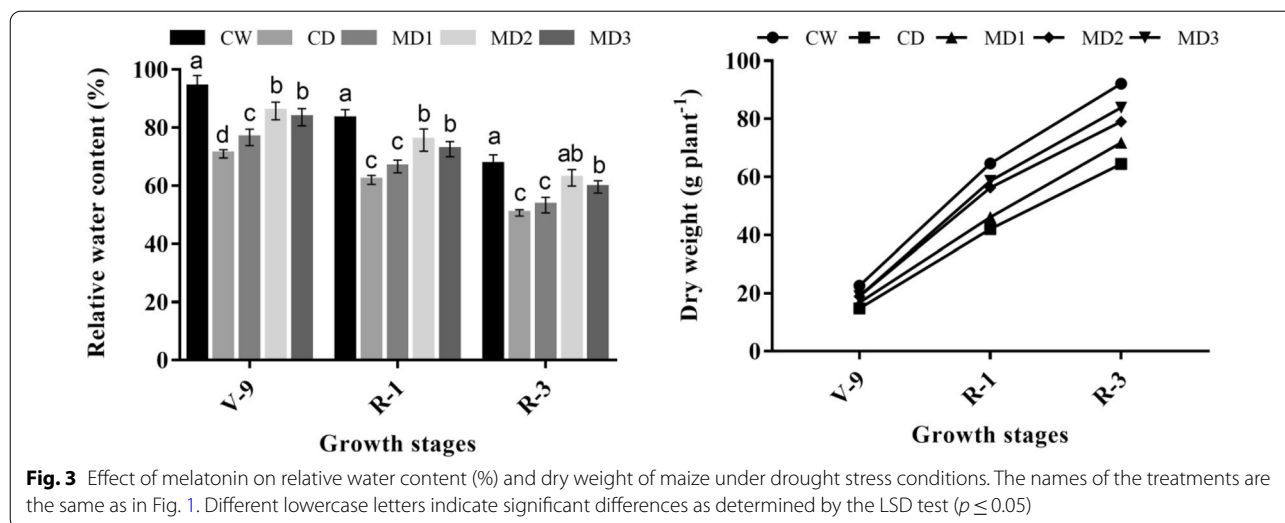
The exogenous application of melatonin increased the chlorophyll contents of maize at various growth stages. Our results revealed that chlorophyll contents increased from V-9 and reached their maximum value at the R-1 growth stage. Under well water conditions, the chlorophyll contents were higher at all growing stages than drought stress conditions. Under drought stress, the maximum chlorophyll contents were found at R-1 before decreasing until the R-3 growing stage. Compared with drought stress control (CD), melatonin treatment MD2 increased the chlorophyll contents by 35.83% at V-9, 18.90% at R-1, and 23.22% at R-3 growing stages of maize (Fig. 2). The minimum chlorophyll contents were observed at CD at all growing stages of maize compared with CW and melatonin-treated plants.



Drought stress significantly affected the net photosynthetic rate of maize. Exogenous application of melatonin dramatically increased the net photosynthetic rate at all growing stages of maize compared to drought stress control (CD). Our results showed that the net photosynthetic rate was higher at the R-1 stage and then gradually decreased from R-1 to R-3 growing stages of maize. Under drought stress conditions, the melatonin treatment MD2 showed a maximum photosynthetic rate at all growing stages compared with CD. Exogenous melatonin treatment MD2 improved the net photosynthetic rate by 19.06% at V-9, 25.58% at R-1, and 34.50% at R-3 growing stages of maize compared with drought stress control (Fig. 2).

Effect of melatonin on RWC and dry matter accumulation of maize

Leaf relative water content is the key indicator that shows the survival capability and leaf water status of the plants. Drought stress significantly reduced the RWC by 23.86% at V-9, 25.47% at R-1, and 24.96% at R-3 growing stages of maize as compared to the well water conditions. The leaf RWC was markedly greater in all of the melatonin treatments than that in the control. Under drought stress conditions, the most significant increase in RWC was observed in melatonin treatment MD2 by 21.31% at the V-9 growth stage of maize as compared to CD (Fig. 3). The total above-ground dry matter plant⁻¹ was considerably increasing by different concentrations of melatonin.



Our results demonstrated that a significant increase occurred in dry matter plant⁻¹ from the vegetative stage to physiological maturity at a variable degree. Melatonin treatments MD2 increased significantly improved the dry matter plant⁻¹ at all growing stages as compared to control. In Fig. 3 our results showed that melatonin treatment MD2 increased the dry matter plant⁻¹ by 28.85% at V-9, 39.32% at R-1, and 30.17% at R-3 growing stages of maize compared to drought stress control (CD).

Effect of melatonin on leaf ultrastructure of maize

Chloroplasts are the sites of chlorophyll existence and play an essential role in plant photosynthesis. In the normal non-stressed plants, the chloroplasts clung to the cell wall and were arranged in a single direction. They exhibited a typical structure, an ellipsoidal shape with

well-arranged and smooth thylakoid membranes in distinct grana regions. Under drought stress conditions, the chloroplasts' lengths decreased and their widths increased, rendering them round in shape. Drought stress also significantly changed the internal structure of the chloroplasts. The edges of most chloroplasts were clear and complete. Drought stress plants have intact cell wall (CW), intact cell membranes; mesophyll cells have irregular shape nucleus (n), plum protoplast, and other organelles in cytoplasm, most of them distributed close to chloroplast. However, the melatonin-treated plants have a complete cell wall and cell membrane, having a nucleus in the mesophyll cell and protoplast is full (Fig. 4). Drought stress (CD) significantly affected the structure of chloroplasts compared with melatonin treatments. Pretreatment with melatonin mitigated this

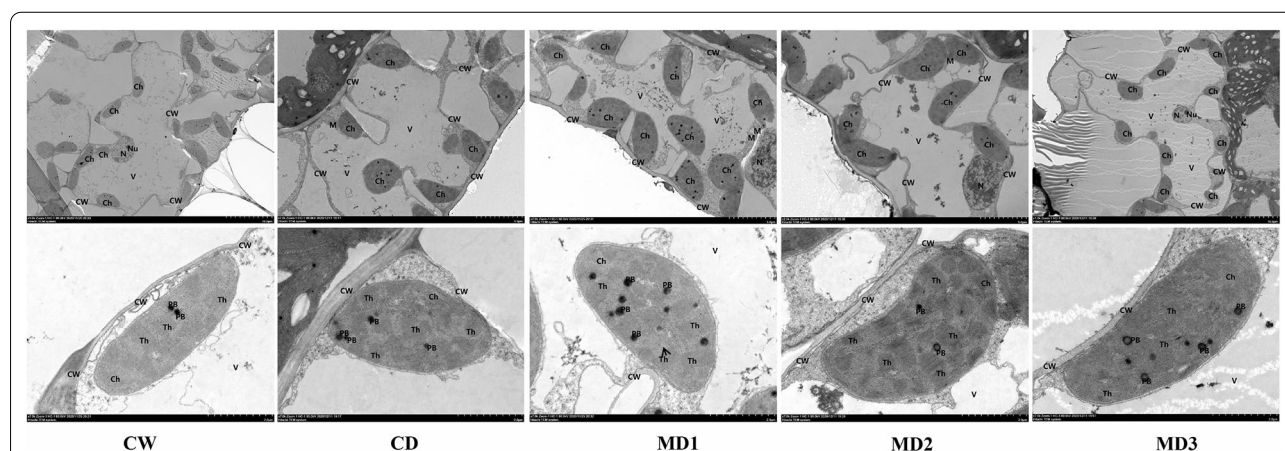


Fig. 4 Effect of melatonin on leaf chloroplast ultrastructure at V-9 growth stage of maize under drought stress conditions. In figure of transmission electron micrographs of leaf chloroplast ultrastructure, the abbreviations indicate cell wall (CW), nucleus (n), mitochondria (m), chloroplast (CH), plastid globules (PB), and vacuole (V). The names of the treatments are the same as in Fig. 1

damage to chloroplasts. In drought stress plants, vacuole (V) are large and contain flocculent metabolites, while melatonin-treated plants have large vacuole but are rich in flocculent metabolites. Among all treatments, drought stress plants chloroplast (CH) has an available quantity, in which there are a certain amount of plastid globules (PB), also known as osmiophilic granules. In melatonin-treated plants, chloroplast (CH) is abundant, in which there are abundant plastid globules (PB), also known as osmiophilic granules. Thylakoid membranes were also more compact and regular than those in the leaves of the stressed control plants. The number and volume of chloroplasts increased in all treatments, and the plastid globules and cysts gradually enriched, but some cysts are fuzzy. Chloroplasts in the plants that received melatonin pretreatment had well-preserved internal lamellar systems compared to control. There was no significant difference in other organelles (Fig. 4).

Effect of melatonin on endogenous hormones content of maize under drought stress

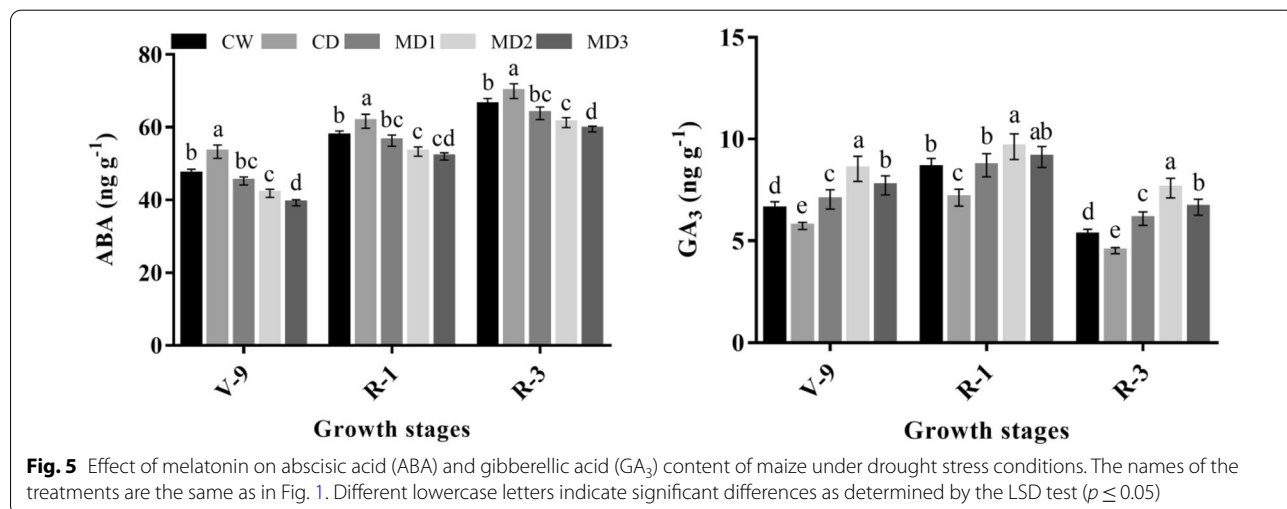
Drought stress significantly affected the endogenous levels of hormone content as compared to well water control (CW). The ABA level increased dramatically under drought stress conditions, whereas exogenous melatonin treatment lowered the ABA level by increasing the concentration. Under drought stress conditions, the ABA level increased from the early growth stage (V-9) and reached its maximum value at the (R-3) growing stage of maize. Our results showed that melatonin treatment (MD2 & MD3) decreased the ABA level by 37.71% and 31.39% at V-9, 38.40%, and 23.46% at R-1, and 34.90% and 21.77% at R-3 growing stages of maize crop (Fig. 5). Exogenous melatonin treatment increased ABA catabolism and substantially affected ABA contents under drought

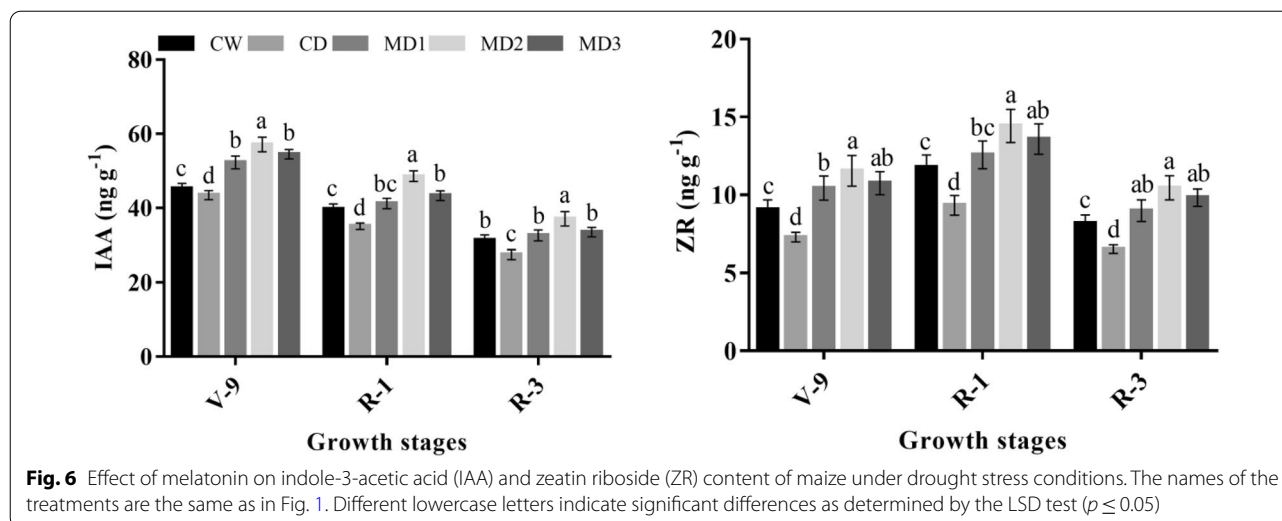
stress conditions. Under drought stress conditions, the classical plant hormones ABA and GA₃ have antagonistic effects on maize.

Exogenous treatment of melatonin had a positive effect on GA₃ content in maize leaves. The drought stress reduced the level of GA₃ contents in maize leaves, but the exogenous application of melatonin significantly increased the GA₃ level. The GA₃ content increased from V-9 to R-1 and then declined gradually from R-3 to physiologically maturity of maize under drought stress conditions (Fig. 5). Drought stress (CD) significantly decreased the GA₃ contents by 12.73% at V-9, 19.04% at R-1, and 14.37% at R-3 growing stages of maize compared to well water control (CW). However, exogenous application of melatonin significantly improved the level of GA₃ contents in maize leaf under drought stress conditions. The results showed that GA₃ content was higher in melatonin treatment MD2 by 48.95% at V-9, 35.13% at R-1, and 67.55% at R-3 growing stages of maize than drought stress control (CD).

Drought stress transiently decreased the IAA levels from V-9 to R-3 growing stages in maize leaves. The exogenous treatment of melatonin significantly improves the IAA levels in maize leaves compared to control. Melatonin treatment MD2 significantly increased the IAA content by 31.71% at V-9, 38.40% at R-1, and 34.90% at R-3 growing stages of maize compared with drought stress control (CD) (Fig. 6).

The exogenous treatment of melatonin improved the ZR contents in maize leaves as compared to control. Our results showed the same pattern of ZR as that of GA₃ contents, which increased transiently from V-9 and reached the maximum value at R-1 and then gradually declined at R-3 growing stage of maize. ZR contents were significantly higher in the melatonin-treated





plant as compared to control under drought stress conditions. Our results showed that melatonin treatment MD2 improved the level of ZR in maize leaves by 58.52% at V-9, 54.72% at R-1, and 60.31% at R-3 growing stages compared to drought stress control (CD) (Fig. 6). The mean results showed that melatonin treatment MD2 and MD3 were significantly similar at all growing stages of maize under drought stress conditions.

Effect of melatonin on yield and yield components of maize under drought stress

Melatonin application has a significant effect on the grain yield of maize under drought stress conditions. Our results showed that exogenous melatonin treatment MD2 improved the maximum number of grains row⁻¹ by 7.57%, grains ear⁻¹ by 72.57%, 100 grains weight by 31.34%, and higher grain yield plant⁻¹ by 125.61% as compared to drought stress control (CD) (Table 2).

Correlation analysis

The correlation analysis was carried out to find the relationship between physiological and biochemical indicators and grain yield of maize under drought stress conditions. Pearson’s correlation analysis results demonstrated that there was a positive correlation with each other in the leaf area plant, plant height, chlorophyll contents, photosynthesis, relative water content, dry matter, IAA, GA3, ZR, grain ear⁻¹, 100-grain weight, and grain yield plant⁻¹ (Fig. 7). Moreover, ABA content was negatively correlated with all other parameters. What is more, almost all of the relationships were significant, except for the relationships between ABA with other parameters.

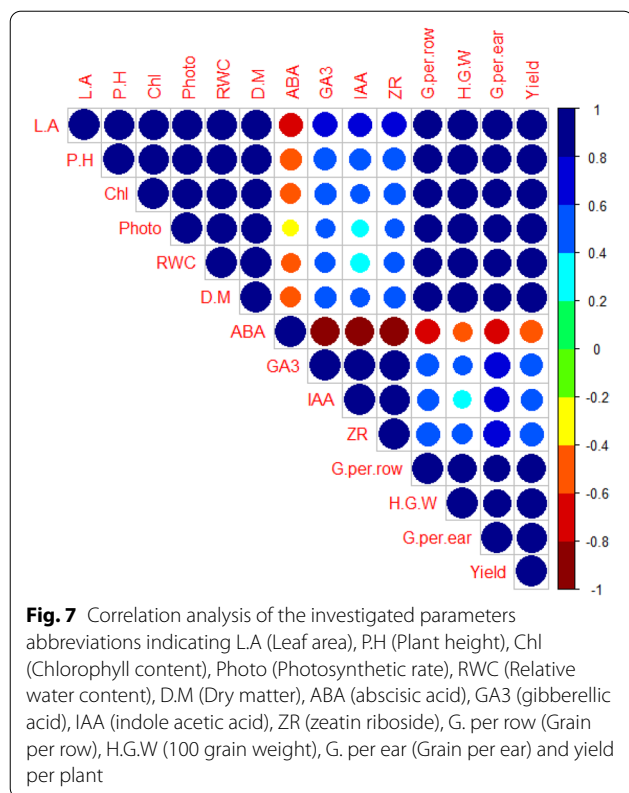
Discussion

Drought stress is one of the most severe abiotic stresses that plants face, affecting many aspects of their physiology and biochemistry (e.g., photosynthesis, protein synthesis, endogenous hormones, and leaf ultrastructure) that are critical for plant growth and development [37, 38]. Melatonin has been shown to protect plants from

Table 2 Effect of exogenous treatment of melatonin on number of grain rows⁻¹, number of grain ear⁻¹, 100 grain weight (g), and grain yield plant⁻¹ of maize under drought stress conditions

Treatments	No. of grains rows ⁻¹	No. of grains ear ⁻¹	100 grains wt. (g)	Yield plant ⁻¹ (g)
CW	23.93 ± 1.29a	410.21 ± 18.36a	25.83 ± 1.06a	105.93 ± 3.97a
CD	21.83 ± 0.52b	233.20 ± 10.60c	17.01 ± 0.83d	38.16 ± 2.59d
MD1	22.17 ± 1.14b	360.48 ± 14.38b	19.18 ± 0.92c	69.48 ± 3.56c
MD2	23.48 ± 1.16ab	385.18 ± 16.42ab	22.34 ± 1.01b	86.23 ± 3.86b
MD3	22.85 ± 1.14ab	371.72 ± 15.73c	22.02 ± 0.98b	81.69 ± 3.38b

Different treatments indicate CW, Melatonin 0 μM + Control well water; CD, Melatonin 0 μM + Control drought stress; MD1, Melatonin 50 μM + drought stress; MD2, Melatonin 100 μM + drought stress; MD3, Melatonin 150 μM + drought stress. Values are given as treatments mean ± SD (standard deviation and n = 3). The different small letters in each column indicate significant difference across the treatments at $p \leq 0.05$



both abiotic and biotic stresses; however, the mechanisms remain unclear, with some studies arguing that it acts as an antioxidant and others suggesting it acts as a growth regulator [39, 40]. In the current study, the protective effect of melatonin in maize growth and the endogenous hormonal level was explored under drought stress conditions. Drought-induced growth inhibition was minimized by exogenous application of melatonin as a soil drench, and maize resistance to drought stress was improved in terms of plant growth and production. Drought stress causes a severe reduction in leaf area and degrades the photosynthetic pigments, which undeniably impairs and reduce photosynthesis, directly affecting plant growth. Melatonin has been suggested to regulate plant growth and development [6]. This study revealed that melatonin-treated plants maintained large leaf areas, taller plant height, and high photosynthetic pigments despite drought stress, allowing for a much greater supply of assimilates to growing tissues than control. Our results are consistent with the previous study, that exogenous melatonin can alleviate drought stress and positively affected plant growth attributes [3, 41, 42]. Various other studies have also shown the stimulatory effect of melatonin on growth and development, in compliance with the current research work evaluated that increased dry biomass, relative water content,

and chlorophyll pigments composition [6]. There is evidence that melatonin can be linked to the receptor of auxin and acts directly as auxin agonists, increasing cell division and expansion, and ultimately increases plant biomass. Furthermore, we observed that the melatonin soil drenching strategy was a more effective strategy in enhancing drought tolerance. Therefore, our experiment indicated that melatonin application effectively enhances the adaptability of maize to drought stress by ameliorating the suppression of growth characteristics caused by drought stress.

In plants, chloroplast is the major source of free radical generation, which require strong protection from free radicals and associated oxidative stress [16]. Plant's melatonin biosynthesis might be taking place in chloroplasts. Previous research showed that exogenous melatonin treatment might cause endogenous melatonin production in chloroplasts [3]. In the current study, we demonstrated that exogenous melatonin might alter endogenous hormone accumulation during drought stress. Enhanced endogenous melatonin could help retain chloroplast integrity and increase the net photosynthetic rate. Photosynthesis takes place in the chloroplasts, which are also the leaf organelle most sensitive to abiotic stress. Chlorophyll plays a crucial part in improving the efficiency of photosynthesis, a principal physicochemical process that governs the synthesis of organic compounds by use of light energy and is the basis of plant growth and development. Drought stress lowers photosynthesis efficiency and damages the photosynthetic system and chlorophyll [43]. Chlorophyll is also highly susceptible to water shortages, and certain plant species have reported chlorophyll levels dropping due to drought stress [37, 38]. In the present experiment, exogenous application of melatonin significantly increased the chlorophyll content under drought stress conditions (Fig. 2). Previous research revealed that the absence of endogenous melatonin resulted in a decrease in tomato chlorophyll contents, but exogenous melatonin dramatically boosted chlorophyll concentration [44]. Chlorophyll has become an important tool for assessing plant photosynthetic rates under abiotic stress. Net photosynthesis is lowered when plants are subjected to drought conditions due to regulatory mechanisms, such as decreased stomatal conductance and chlorophyll concentrations [45]. We observed that melatonin-treated plants maintained a higher photosynthetic rate in all growing stages of maize as compared with drought stress control. This could explain why stressed plants in that treatment were able to maintain a higher photosynthetic rate, because melatonin has been proven to be a growth stimulant that also improves plant tolerance to abiotic stress. While many stressors inhibit plant growth by affecting multiple

physiological processes, photosynthesis is most strongly affected by changes in biomass production, which may be due to reduced assimilate requirements. The higher assimilation of carbon corroborates the improvement in biomass production and growth characteristics due to the increased photosynthetic capacity of melatonin-treated plants [46]. Furthermore, melatonin pretreatment improved tolerance to abiotic stress by reducing stomatal restriction and enhancing photosynthetic rate in plants seedling [47].

Plant hormones regulate growth and development and provide effective protection against biotic and abiotic stresses [48]. Some plants' stress tolerance has been linked to morphological changes that are controlled by certain hormones [44]. ABA is a plant hormone that plays a key function in giving tolerance to a wide range of environmental stresses [49]. The ABA content plays a crucial role in physiological processes, such as plant growth and development, stomatal closure and chloroplast ultrastructure, and responses to abiotic stress tolerance [50]. Our results revealed that the ABA level increased from the early growth stage (V-9) and reached its maximum value at (R-3) growing maize under drought stress conditions. Furthermore, our findings revealed that exogenous melatonin could improve maize drought tolerance by reducing its ABA concentration. Our results are consistent with previous studies, which reported that exogenously applied melatonin down-regulates genes involved in ABA biosynthesis and then up-regulates genes involved in ABA catabolism/degradation, resulting in lower ABA content and improved plant growth under stress conditions [5, 51]. The higher ABA level in the later growing stage of maize would impede the growth. Consequently, adding an appropriate amount of melatonin concentration could relieve stress tolerance, while having no inhibiting effect on plant growth and productivity of maize under drought stress conditions. Previous research confirmed that ABA had been implicated in regulating plant growth and environmental stressors [52]. Exogenous application of melatonin accelerates the ABA degradation and increases the GA biosynthesis under drought stress condition [53]. According to our findings, the content of ABA increased, whereas the content of GA₃ decreased under drought stress conditions. In fact, a negative correlation was detected between ABA contents and the various physiochemical attributes, while GA was positively correlated, indicating ABA as a major stress-related hormone. However, melatonin application inhibited the ABA and increased the GA₃ level under drought stress conditions. Melatonin also interacts with gibberellins (GAs) through GA signaling under abiotic stress conditions [54, 55]. Our

study showed that melatonin application increases the GA₃ level in leaves by increasing the melatonin concentration up to some extent, but a higher level will reduce the GA₃. Previous research showed that plant hormone GA₃ is also regulating the process of seed filling and grain development under abiotic stress conditions [5].

Stress tolerance has been associated with morphological modifications in some plants regulated by other hormones [56]. It has already been shown that IAA plays a critical function in plant growth and development [44]. Our results showed that drought stress drastically reduces the IAA contents from the early growing stage to physiologically maturity. It is conceivable that the need for IAA is higher during the seedling growth stage. Moreover, exogenous application of melatonin encourages the plants to generate more IAA that help the plant growth and yield under drought stress condition. Previous research demonstrated that the decline in IAA content in the later growth stages was due to lower demand for IAA; conversely, the melatonin in the late growth stages has dropped, and IAA elevation has been little [44, 57]. The IAA and ZR content plays a vital role in the seed filling process of cereals crops [23]. A previous study revealed that exogenous application of plant growth regulator enhanced the cytokinin content, and this increase in cytokinin resulted in a considerable improvement in chlorophyll biosynthesis [58]. Our results showed that exogenous application of melatonin increases the ZR levels under drought stress conditions. The zeatin riboside (ZR) regulates leaf senescence and chlorophyll breakdown, which reduces CK biosynthesis and transport, resulting in decreased cytokinin and quicker leaf senescence under abiotic stress conditions [59]. Exogenous application of melatonin improves photosynthetic efficiency and drought tolerance by up-regulating ZR levels and several associated signaling pathways [55, 60]. Our study found that exogenous melatonin increases GA₃, IAA, and ZR while reducing the ABA levels in leaves under drought stress conditions. Based on our results, it is clear that melatonin plays a vital role in improving photosynthetic efficiency by modulating phytohormone and increasing the production of maize crops under drought stress conditions. Plants could adapt to drought stress by increasing chlorophyll and photosynthetic rate and endogenous hormone levels, thus maintaining grain yield. Our results showed that the maize growth was inhibited by drought stress, but the application of melatonin significantly increased the growth and production. The overall results suggested that the application of melatonin treatment MD2 (100 μM) significantly increased the photosynthetic efficiency, thereby regulating hormonal levels and grain yield of maize under drought stress conditions.

Conclusion

Drought stress is a severe problem than all other abiotic stresses that significantly reduced the maize yield. This study evaluated different melatonin concentrations applied as a soil drenching at different growth stages under drought stress conditions. Taken together, our results indicated that exogenous melatonin treatment is one of the most promising approaches for increasing maize productivity under drought stress. Exogenous melatonin application alters the hormonal profile and hormonal crosstalk improved the photosynthetic efficiency and grain filling rate by enhancing the grain yield of maize under drought stress conditions. From our results, we suggested that treatment (MD2) 100 μ M melatonin application as a soil drenching to roots is an effective strategy to improve the drought tolerance of maize. The morphological, physiological, and biochemical analysis from the present study could be employed as an effective approach in many crops and will be useful in elevating drought stress conditions.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40538-021-00272-1>.

Additional file 1: Table S1. Selected reaction monitoring conditions for protonated or deprotonated plant hormones. **Table S2.** Reverse-phase HPLC gradient parameters for plant hormones.

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Authors' contributions

SA contributed to conceptualization and writing—original draft preparation. SA and GYW were involved in methodology. SA, GYW, and IM performed formal analysis. SA, SF, MZ, TJ, and SU did investigation. XBZ and JHH contributed to resources. SA and MK were involved in data curation. MK and IA were involved in writing—review and editing. XBZ supervised the study. All authors read and approved the final manuscript.

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Data availability

The data that support the finding of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

This manuscript is an original paper and has not been published in other journals. The authors agreed to keep the copyright rule.

Consent for publication

The authors agreed to the publication of the manuscript in this journal.

Competing interests

The authors declare no conflict of interests.

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