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Effects of Exogenous Melatonin on *Chrysanthemum* Physiological Characteristics and Photosynthesis under Drought Stress

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Abstract: Melatonin has been confirmed to relieve drought stress in various plant species. However, the mechanism in garden chrysanthemums remains to be elucidated. Here, we examined the putative role of exogenous melatonin application in improving drought-stress tolerance in *Chrysanthemum* seedlings. Treatment with melatonin (100 μM) significantly mitigated the negative effects of drought stress on the seedling photosynthetic parameters and physiological and biochemical indexes. The increased tolerance conferred by exogenous melatonin was attributable to increased photosynthetic activity, which alleviates drought-induced oxidative damage by reducing malondialdehyde accumulation. In other words, the combined application of MT and PEG was better than their individual applications, as it prevented heat-induced membrane damages by declining the hydrogen peroxide (28%), malondialdehyde contents (38%), and relative electrical conductivity (19%) as compared to PEG. Interestingly, the melatonin application stimulated the activity of major antioxidant enzymes such as superoxide dismutase (21%), catalase (26%) and polyphenol oxidase (35%). In addition, exogenous melatonin promoted photosynthesis. Compared with the PEG drought-stress treatment, the melatonin treatment significantly increased the net photosynthetic rate (39%), stomatal conductance (27%), and transpiration rates (18%), while decreasing the intercellular CO_2 concentration (10%). The photochemical quenching coefficient (46%), photosystem II (PSII) maximum photochemical quantum yield (22%), apparent electron transfer rate (37%), and PSII potential photochemical efficiency (18%) showed an upward trend. In summary, exogenous spraying of MT can effectively improve drought tolerance and mitigate the damage caused by drought stress in the "Xuanqiu ninghong" *Chrysanthemum*.

Keywords: exogenous melatonin; *Chrysanthemum*; PEG drought stress; physiological characteristics; photosynthesis

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1. Introduction

Chrysanthemum (*Chrysanthemum morifolium* (Ramat.) Hemsl.) is a perennial herbaceous plant belonging to the Asteraceae family; it is the second most popular cut flower after rose and is a commercially important ornamental plant across the global flower market [1]. However, this species is susceptible to various abiotic stresses during active production, among which drought is an important stress factor that seriously affects *Chrysanthemum* quality, yield, and geographical distribution [2]. Northwest China (NWC) occupies most of the drylands of East Asia, and it is one of the most frequently and severely affected drought-prone areas in China [3]. Most areas are located in arid and semiarid regions, characterized by evapotranspiration much greater than precipitation [4]. The damage caused by drought stress to plants mainly manifests as dehydration, wilting, increased membrane permeability, slowed growth, and reduced photosynthetic function, with the main cause of these injuries being oxidative damage caused by reactive oxygen species [5]. In the early stages of drought, plants are often able to cope with the stress and form

defense mechanisms of their own, but prolonged stress leads to the accumulation of large amounts of reactive oxygen species in their tissues, which then become imbalanced, leading to membrane lipid peroxidation, which results in impaired cell membrane function and decreased photosynthesis, and which will seriously affect the growth of seedlings [6–9] and, in turn, the development of the *Chrysanthemum* industry. Therefore, it is important to identify methods for improving *Chrysanthemum* resistance to drought-stress conditions [10]. The application of plant growth regulators has been considered an efficient and ecofriendly approach to solve the problem [11,12].

Melatonin (N-acetyl-5-methoxytryptamine, MT) is a pleiotropic molecule with a wide range of cellular and physiological actions in living organisms, including animals and plants, that can effectively alleviate the plant redox imbalance induced by drought stress and prevent oxidative disturbance [13]. It plays a vital role in the growth and development of plants and their resistance to stress, assisting plants in survival and thriving [14–17]. To illustrate the important role of this biomolecule in plant resilience, MT can alleviate plant damage caused by abiotic stresses such as heavy metals [1], high temperature [18,19], salinity [20], and factors that cause leaf senescence [21,22]. Many studies have investigated MT-mediated regulation of plant biology in response to drought stress, such as in apple (*Malus domestica*) [23], cucumber (*Cucumis sativus*) [24], *Malus prunifolia* [25], tomato (*Solanum lycopersicum*) [26], and maize (*Zea mays*) [27]. These findings suggest it is advantageous for horticulture crop improvements. Compared with the benefits of melatonin under various abiotic stress conditions, the underlying mechanism of melatonin in alleviating drought stress has rarely been investigated in ornamental horticulture, especially in garden chrysanthemum. To our knowledge, very few studies have systematically discussed the alleviating role of melatonin in garden chrysanthemums grown under drought-stress conditions. Specifically, little is known about whether foliar and root irrigation application of melatonin improves stress tolerance or not. In the current study, we aimed to evaluate the effects of melatonin on physiological characteristics and photosynthesis in the amelioration of drought stress in *Chrysanthemum* seedlings. Our goal was to provide a theoretical basis and valuable insights into the regulatory role of MT in the drought response mechanism of *Chrysanthemum*.

2. Materials and Methods

2.1. Plant Materials and Experimental Treatments

The experiment was carried out at the Horticultural Plant Health and Product Safety Laboratory of Ningxia University. Garden chrysanthemum cuttings were purchased from Beijing Flower and Wood Company. Melatonin (MT, N-acetyl-5-methoxytryptamine) was purchased from Solarbio. Equal-stemmed cuttings of 7 cm in length were prepared from healthy garden chrysanthemum morifolium "Xuanqiu ninghong" plants. The cuttings were placed in a perforated tray with a mixed substrate (peat: Coconut bran: perlite = 2:3:2) and allowed to root for 2 months. Thereafter, the chrysanthemums were subjected to four treatment levels: (a) well-watered without any treatment (CK); (b) 100 μM melatonin root irrigation (MT); (c) 20% polyethylene glycol (PEG) root irrigation; and (d) 100 μM melatonin foliar spray plus drought-stress root irrigation (MT+PEG). Plants were exposed to melatonin treatment using foliar spray for 3 days (once a night) before 20% PEG root irrigation to create drought stress. For the other three treatment levels, the treatments were administered as 300–350 mL of water, 100 μM melatonin, or 20% PEG every three days for 12 days. Fertilizer is applied by root irrigation once a week with the ratio of N:P:K = 20:20:20, 800 times per gram to water. It is important to note that the optimal concentrations of melatonin and PEG were chosen following preliminary experimental results. Experiments were carried out in triplicate using pooled samples. Sampling and index determination were performed after the experimental treatments.

2.2. Physiological and Biochemical Indexes and Photosynthetic Parameter Measurements

2.2.1. Physiological and Biochemical Measurements

Chlorophyll content was measured using a portable Soil Plant Analysis Development (SPAD) chlorophyll meter. The relative water concentration (RWC) was measured according to a previously described method [28]. Relative electrical conductivity (REC), superoxide dismutase (SOD), and peroxidase (POD) activity were determined according to the method of Gao Junfeng [29]. Malondialdehyde (MDA) was measured by the method of Zhao Shijie [30]. The activities of hydrogen peroxide (H_2O_2) and proline (Pro) were analyzed using a Solarbio kit (Beijing, China) following the manufacturer's instructions. Catalase (CAT) activity was determined according to Li Hesheng's method [31]. Soluble sugar and soluble protein contents were determined using anthrone colorimetry [32] and Coomassie brilliant blue G-250 [33].

2.2.2. Photosynthetic Parameter Measurements

Photosynthetic parameters: The net photosynthetic rate (Pn), transpiration rate (Tr), intercellular carbon dioxide concentration (Ci), and stomatal conductance (Gs) were measured using a Li-6800 portable photosynthesis system (LI-COR, Lincoln, NE, USA). Measurements were taken between 9:30 a.m. and 11:00 a.m. the day after treatment, and three plants were randomly selected for each treatment.

Measurement of chlorophyll fluorescence parameters: At the end of the experimental treatment, dark adaptation of seedling leaves was performed for 30 min at 20:00 on the same night. Fluorescence parameters such as the photochemical quenching coefficient (qP), nonphotochemical quenching coefficient (NPQ), apparent electron transfer rate (ETR), dark-adapted maximum fluorescence yield (Fm), maximum photochemical quantum yield (Fv/Fm) and potential photochemical efficiency of PSII (Fv/Fo) were measured using a compact multichannel continuous monitoring fluorometer (Micro-PAM, Zealquest Scientific Technology Co., Ltd., Shanghai, China). In addition, Photosynthetically Active Radiation (PAR) is 285 $\mu mol \cdot m^{-2} \cdot s^{-1}$; temperature (Temp) is 25.8 °C. The formula of some parameters is:

$$qP = \frac{F'm - F}{F'm - F'o}. \quad (1)$$

$$NPQ = \frac{Fm}{F'm} - 1 \quad (2)$$

$$Fv/Fm = \frac{Fm - Fo}{Fm} \quad (3)$$

$$ETR = PAR \cdot ETR - Factor \cdot PPS_2/PPS_{1+2} \cdot Y(II) \quad (4)$$

$$Fv/Fo = \frac{Fm - Fo}{Fo}. \quad (5)$$

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2.3. Statistical Analysis

The data were analyzed with SPSS Statistics 20.0 (SPSS Inc., Chicago, IL, USA) for one-way ANOVA and Duncan's test. The effects were considered significant at $p < 0.05$. GraphPad Prism 9.1.2 software plotting (San Diego, CA, USA).

3. Results

3.1. Effect of MT on Photosynthetic Pigments and Water Status of Chrysanthemum Seedlings under Drought Stress

As seen in Figure 1, compared with the CK control, the PEG-treated seedlings showed significant wilting; however, the wilting was alleviated after exogenous MT. Relative water content (RWC) is the primary factor indicating the water status of plants. A significant decrease in the RWC was observed in the drought-stressed *Chrysanthemum* seedlings compared to the well-watered seedlings. In contrast, there was a significant improvement in the

RWC of the melatonin-treated *Chrysanthemum* seedlings under drought-stress conditions (Figure 1C). Compared to the PEG treatment, the MT+PEG treatment increased the RWC by 27%, and compared with a normal water supply, drought stress (PEG) reduced the *Chrysanthemum* leaf Chl content. Under drought stress, exogenous melatonin (PEG+M) increased the *Chrysanthemum* leaf Chl content to a certain extent. Compared to the PEG treatment, the MT+PEG treatment increased leaf Chl content by 20. The above results indicate that the spraying exogenous MT has a protective effect on the reduction in the Chl content and the RWC in the leaves of the *Chrysanthemum* seedlings under drought stress.

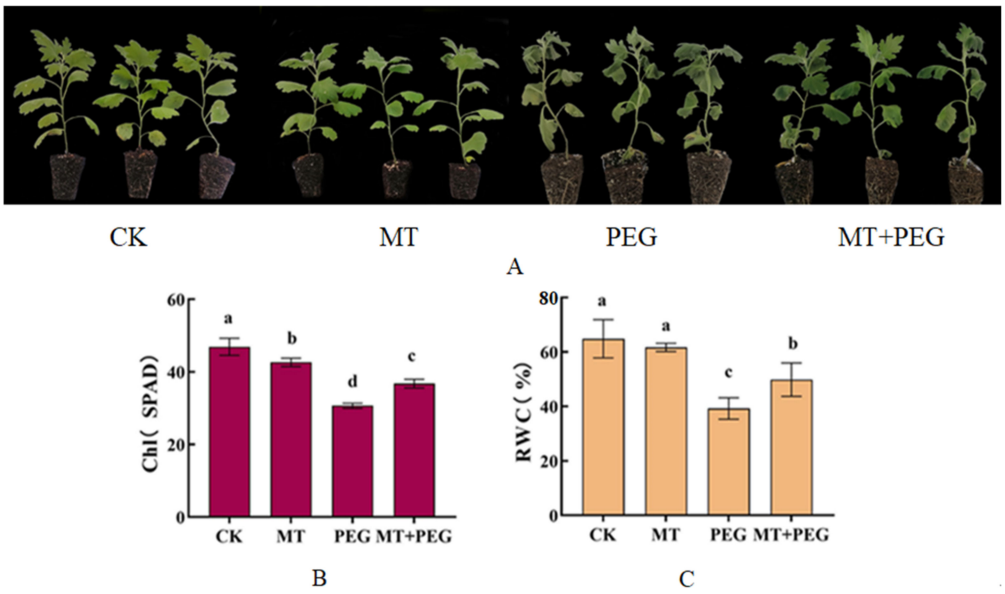

Figure 1. Effect of MT on the phenotype, photosynthetic pigments, and water status of *Chrysanthemum* seedlings under drought stress. (CK): well-watered without any treatment; (MT) 100 μM melatonin root irrigation; (PEG) 20% polyethylene glycol root irrigation; (MT+PEG) 100 μM melatonin foliar spray plus drought-stress root irrigation; (**A**): phenogram (**B**): Chl (chlorophyll content); (**C**): RWC (relative water content). Data represent means ± SEs of three replicate samples. Different letters indicate significant differences ($n = 3$ and $p < 0.05$).

3.2. Effect of MT on Reactive Oxygen Species and the Antioxidant System of Chrysanthemum Seedlings under Drought Stress

In the present study, the effect of drought stress on antioxidant enzyme activity in *chrysanthemum* plants with (MT+PEG) or without melatonin treatment (PEG) was determined (Figure 2). Among them, the standard curve of hydrogen peroxide was adopted from the study of Wang et al. [34]. The results showed significantly lower SOD, CAT and POD activity levels in plants without the melatonin treatment (PEG) than in the melatonin-treated (MT+PEG) plants under drought conditions. In terms of H_2O_2 content (Figure 2A), the trend first increased and then decreased, and the H_2O_2 content of the different treatments was significantly different from that of the MT+PEG treatment. The H_2O_2 content in the leaves of the *Chrysanthemum* seedlings under the PEG treatment reached the maximum value of 7.23 μmol/g, which was 40% higher than the H_2O_2 content of the seedling leaves under the MT+PEG treatment. SOD (Figure 2B) activity showed an increasing trend, and the SOD levels in the other treatment groups were 10% higher than that of the CK group. Compared with SOD content of the CK group, the SOD content of the other treatment groups increased by 9%, 54% and 86%. Under the MT+PEG treatment, the POD (Figure 2C) content in the seedling leaves reached the maximum value of 111.10 mg/g, which increased by 115%, 184% and 35% in the other treatment groups. CAT (Figure 2D) content also showed an increasing trend; treatment with MT+PEG resulted in a maximum CAT content of 25.23 mg/g, which was 1.26 times higher than the CAT content of the PEG treatment. Apparently, the application of MT under drought stress improves antioxidant enzyme

activity and increases the amount of antioxidant substances in the leaves of *Chrysanthemum* seedlings, thus improving their drought tolerance.

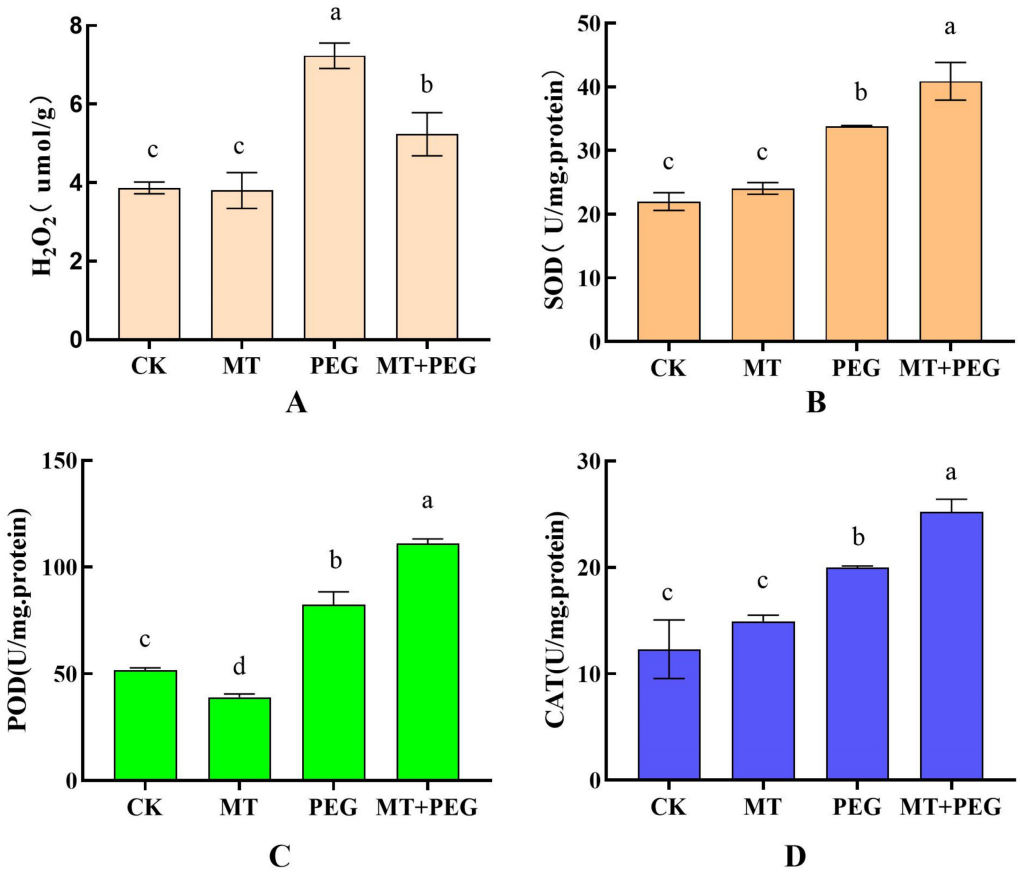

Figure 2. Effect of MT on reactive oxygen species and the antioxidant system of *Chrysanthemum* seedlings under drought stress. (CK): well-watered without any treatment; (MT) 100 μM melatonin root irrigation; (PEG) 20% polyethylene glycol root irrigation; (MT+PEG) 100 μM melatonin foliar spray plus drought-stress root irrigation; (**A**): H_2O_2 (hydrogen peroxide content); (**B**): SOD (superoxide dismutase content); (**C**): POD (peroxidase content); (**D**): CAT (catalase content). Data represent means ± SEs of three replicate samples. Different letters indicate significant differences ($n = 3$ and $p < 0.05$).

3.3. Effect of MT on Organic Osmoregulatory Substances in Seedlings under Drought Stress

As shown in Figure 3, the soluble protein (SP) and soluble sugar (SS) contents of the *Chrysanthemum* seedlings showed a decreasing trend and the proline (Pro) content showed an increasing trend under drought stress, while the MT+PEG treatment showed the opposite trend. Among them, the standard curve of proline was adopted from Feng's study [35]. The SP content (Figure 3A) in the leaves of seedlings under the MT+PEG treatment reached a maximum value of 20.28 mg/g, which was 1.10 times higher than that under the PEG treatment. After MT was sprayed, the SS content (Figure 3B) was significantly different from that under the PEG treatment, with the seedling leaf SS content being 1.44 times higher than that under the PEG treatment. The Pro content (Figure 3C) in the leaves of the seedlings under the CK and MT treatments was significantly lower than that under the other treatments, with levels that were 84% and 81% lower in the CK and MT treatments, respectively, than in the PEG treatment. This suggests that exogenous melatonin can increase the accumulation of soluble protein and soluble sugar in *Chrysanthemum*.

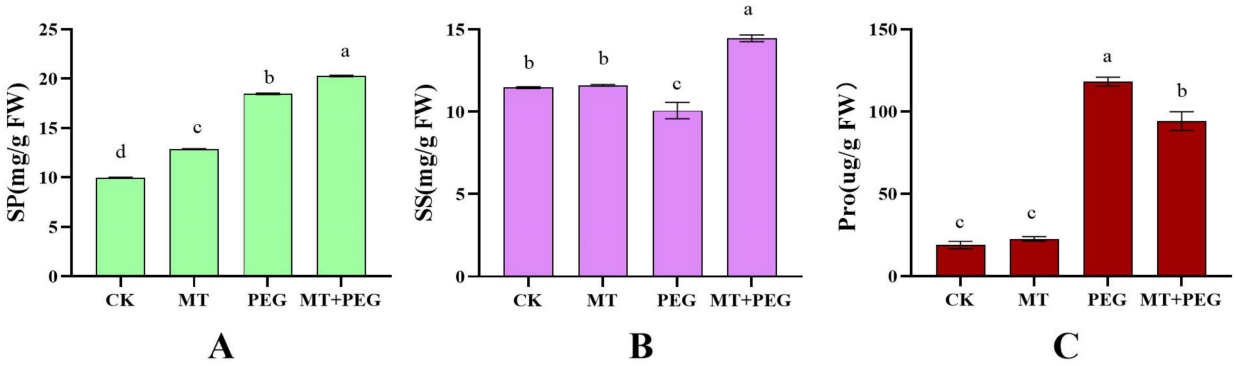

Figure 3. Effect of MT on organic osmoregulatory substances in *Chrysanthemum* seedlings under drought stress. (CK): well-watered without any treatment; (MT) 100 μM melatonin root irrigation; (PEG) 20% polyethylene glycol root irrigation; (MT+PEG) 100 μM melatonin foliar spray plus drought-stress root irrigation; (**A**): SP (soluble protein content); (**B**): SS (soluble sugar content); (**C**): Pro (proline content). Data represent means ± SEs of three replicate samples. Different letters indicate significant differences (n = 3 and $p < 0.05$).

3.4. Effect of MT on Relative Plasma Membrane Permeability and Membrane Peroxidation in Chrysanthemum Seedlings under Drought Stress

To assess the effects of drought stress and the melatonin treatment on the membrane integrity, MDA levels and electrolyte leakage were studied. As shown in Figure 4, the relative electrical conductivity (REC) and malondialdehyde content (MDA) of the leaves were significantly higher under PEG drought stress than under the other treatments. Regarding the REC of seedling leaves (Figure 4A), under the PEG treatment there was a decreasing trend after exogenous MT spraying, and under the MT+PEG treatment, the REC was 19% lower than in the PEG treatment. As for the MDA content in the seedling leaves (Figure 4B), under the MT+PEG treatment, the MDA content was 38% lower than under the PEG treatment, indicating that the exogenous MT treatment inhibited the increase in the MDA content in the plants within a short period of time. Therefore, it was confirmed that the external application of melatonin could alleviate the damage caused by membrane lipid peroxidation to the *Chrysanthemum* seedlings under drought stress.

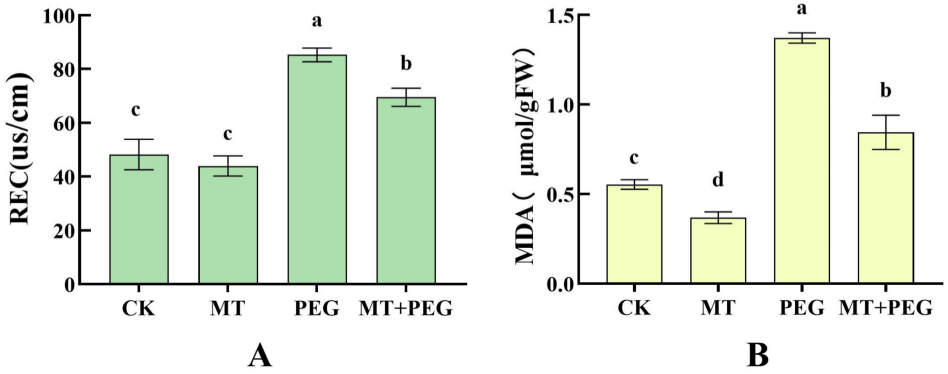

Figure 4. Effect of MT on the relative permeability of the plasma membrane and membrane peroxidation of *Chrysanthemum* seedlings under drought stress. (CK): well-watered without any treatment; (MT) 100 μM melatonin root irrigation; (PEG) 20% polyethylene glycol root irrigation; (MT+PEG) 100 μM melatonin foliar spray plus drought-stress root irrigation; (**A**): REC (relative conductivity); (**B**): MDA (malondialdehyde content). Data represent means ± SEs of three replicate samples. Different letters indicate significant differences (n = 3 and $p < 0.05$).

3.5. Effect of Exogenous MT on Chrysanthemum Seedling Leaf Photosynthesis under Drought Stress

The photosynthetic gas exchange parameters can be used to measure photosynthetic activity in the leaves of *Chrysanthemum* seedlings. As shown in Figure 5, all photosynthetic

parameters except intercellular carbon dioxide concentration (Ci) differed significantly ($p > 0.05$) between the CK and MT treatments, and under the PEG stress leaf transpiration rate (Tr), the photosynthetic rate (Pn) and stomatal conductance (Gs) were significantly reduced, while Ci was significantly increased. The Tr (Figure 5A), Pn (Figure 5B) and Gs (Figure 5D) of the seedling leaves showed an increasing trend after the spraying of exogenous MT and were 18%, 39% and 27% higher, respectively, than the values under the PEG treatment, while Ci (Figure 5C) significantly decreased by 10% and gradually returned to normal. Gs showed a reverse tendency for change versus Ci, suggesting that nonstomatal limitations on Pn existed in the plants that received 100 μM melatonin, meanwhile, the melatonin-treated plants maintained higher Tr and Pn, which were consistent with the higher Gs, suggesting that melatonin may enhance the plant roots' ability for water uptake, taking advantage of plant growth. In conclusion, the exogenous MT treatment slows the inhibitory effect of PEG on the photosynthesis of the *Chrysanthemum* seedlings under PEG stress and maintains normal photosynthesis in the *Chrysanthemum* seedlings under PEG stress.

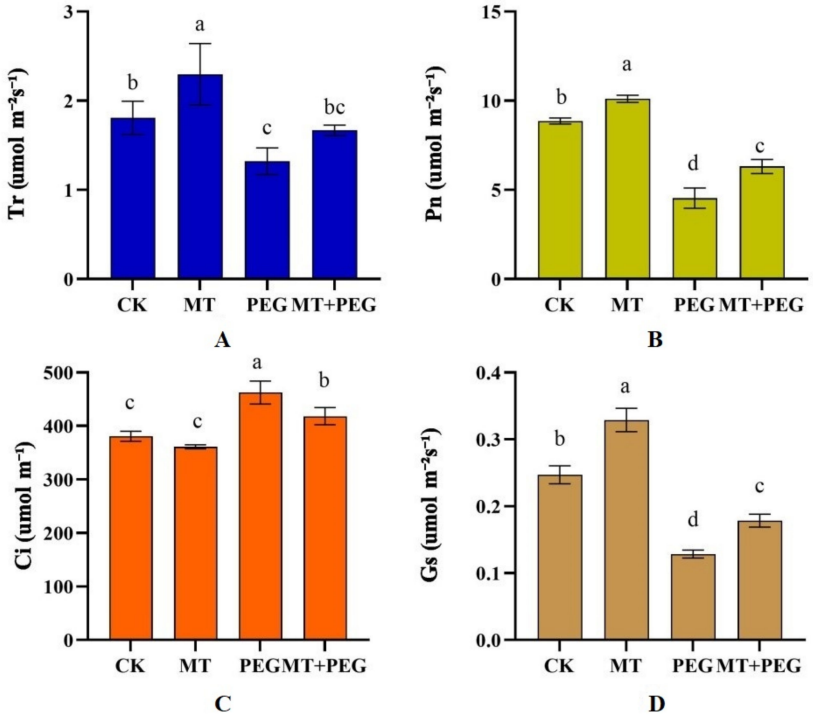

Figure 5. Effect of MT on photosynthetic parameters of *Chrysanthemum* seedlings under drought stress. (CK): well-watered without any treatment; (MT) 100 μM melatonin root irrigation; (PEG) 20% polyethylene glycol root irrigation; (MT+PEG) 100 μM melatonin foliar spray plus drought-stress root irrigation; (**A**): Tr (transpiration rate); (**B**): Pn (net photosynthetic rate); (**C**): Ci (intercellular carbon dioxide concentration); (**D**): Gs (stomatal conductance). Data represent means ± SEs of three replicate samples. Different letters indicate significant differences ($n = 3$ and $p < 0.05$).

3.6. Effect of Exogenous MT on Chrysanthemum Seedling Chlorophyll Fluorescence Parameters under Drought Stress

Among the chlorophyll fluorescence parameters, there is a competitive relationship between the photochemical quenching coefficient (qP) and the nonphotochemical quenching coefficient (NPQ). As shown in Figure 6A,B, the *Chrysanthemum* seedling leaves showed a decreasing trend for qP and an increasing trend for NPQ under the PEG drought-stress treatment, with qP decreasing by 32% and NPQ increasing by 45% when compared to the qP and NPQ values under the MT+PEG treatment. Meanwhile, the potential photochemical efficiency (Fv/Fo), maximum photochemical quantum yield (Fv/Fm), and apparent electron transfer rate (ETR) values in PSII were lowest under the PEG drought-stress treatment,

but showed an increasing trend after exogenous MT, increasing by 18%, 22% and 37%, respectively, compared with the PEG treatment alone. This suggests that water deficiency causes a shutdown or disruption of the PSII system, resulting in the weakening and inhibition of the capacity and efficiency of the light response, and that exogenous MT can effectively prevent damage to photosynthetic organs. As shown in Figure 6F, the maximum fluorescence yield (Fm) of dark adaptation tended to decrease and then increase during the stress process, and the overall seedling Fm values in the four treatment groups were in the following order: MT+PEG group > CK group > MT group > PEG group. However, there was no significant difference between the treatment groups. In summary, drought stress in *Chrysanthemum* seedlings can reduce the activity of the photosynthetic mechanism and damage it, while exogenous MT can mitigate the effect of drought stress on the photosynthetic mechanism and facilitate the growth of seedlings.

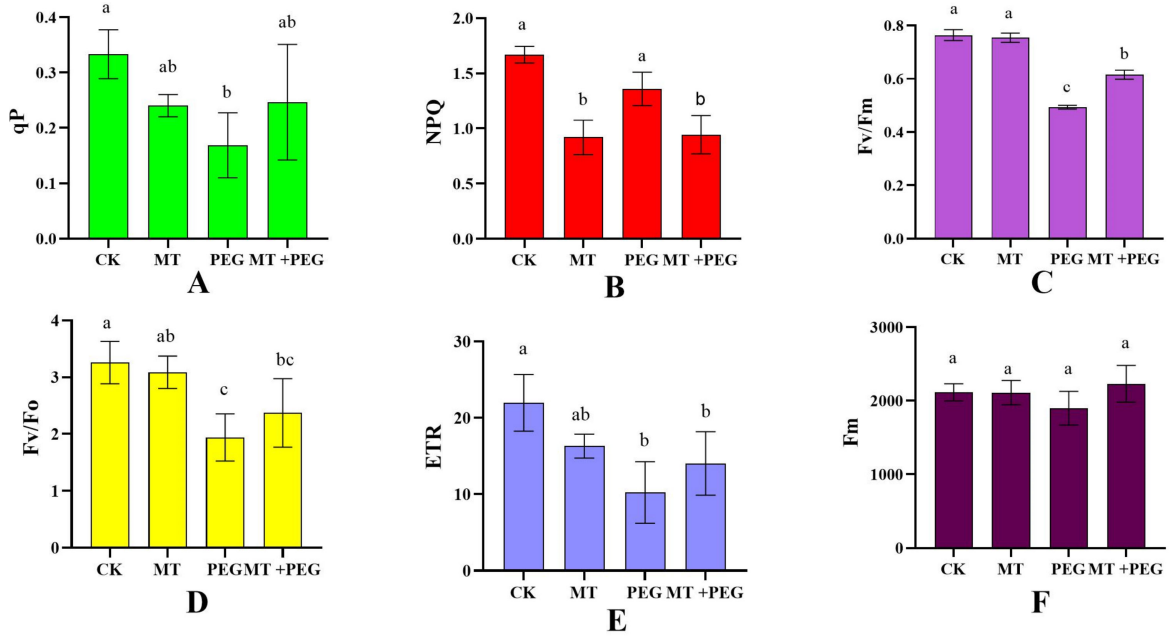

Figure 6. Effects on the fluorescence parameters of *Chrysanthemum* seedlings under drought stress. (CK): well-watered without any treatment; (MT) 100 μM melatonin root irrigation; (PEG) 20% polyethylene glycol root irrigation; (MT+PEG) 100 μM melatonin foliar spray plus drought-stress root irrigation; (**A**): qP (photochemical quenching coefficient); (**B**): NPQ (not photochemical quenching coefficient); (**C**): Fv/Fm (maximum photochemical quantum yield of PSII); (**D**): Fv/Fo (potential photochemical efficiency of PSII); (**E**): ETR (apparent electron transfer rate); (**F**): Fm (dark-adapted maximum fluorescence yield). Data represent means ± SEs of three replicate samples. Different letters indicate significant differences ($n = 3$ and $p < 0.05$).

4. Discussion

To mitigate the adverse effects of drought stress on plants, exogenous hormones have gradually become an effective alleviation method [36–38]. In a study of maize seedlings, Du Zhuo et al. [39] found that exogenous spraying of MT was effective in both reducing the loss of leaf relative water content and improving chlorophyll synthesis. Consistent with previous studies, the growth of the *Chrysanthemum* seedlings was significantly inhibited after drought stress. However, exogenous MT alleviated the wilting caused by drought stress, and the MT-treated plants had a higher relative water content and chlorophyll content than the PEG-treated plants.

Under drought conditions, both relative conductivity and the membrane lipid peroxidation product MDA showed an increasing trend [40,41], which also became a marker of the degree of membrane lipid peroxidation during plant damage. Drought stress first caused the accumulation of ABA [25]. This leads to oxidative damage of cell membranes,

chlorophyll degradation, and reduced photosynthetic performance; eventually cell turgidity is limited, causing growth retardation or even arrest [25,42]. High malondialdehyde content indicates a high degree of peroxidation and serious damage to the cell membrane. In contrast, exogenous melatonin can reduce the degree of cell membrane oxidative damage and the rate of chlorophyll degradation and enhance the occurrence of photosynthesis. Put differently, exogenous melatonin can increase the tolerance of plants under drought stress, and this tolerance is mainly attributed to the increase in photosynthetic activity, which must be achieved by reducing the malondialdehyde content. The results of the study by Wang et al. also provide evidence that melatonin can function as a general stress regulator that maintains ion homeostasis, prevents membrane lipid oxidation, and enhances photosynthetic activities [43]. In this study, foliar spraying of MT under drought stress was shown to reduce the build-up of relative conductivity and MDA, suggesting that MT may alleviate cell membrane damage under drought stress, thereby mitigating the damage caused by drought stress on *Chrysanthemum* seedlings. The same results were obtained in the study by Li, who found that exogenous MT significantly inhibited the accumulation of MDA in perilla seedlings under drought-stress conditions [44].

The adaptation of plants to the external environment and their own ability to prevent senescence can be a reflection of the level of antioxidant enzyme activity [45]. The decrease in antioxidant enzyme activity in plants under drought-stress conditions can cause the accumulation of reactive oxygen species in their tissues, which in turn can lead to toxic damage [6]. In this study, exogenous spraying of MT effectively increased the antioxidant enzyme activity in the leaves and suppressed the accumulation of the reactive oxygen species. This is consistent with the findings of Qin Bin [46] concerning melatonin on soybean seed germination, where exogenous spraying of MT under drought conditions reduced the level of membrane lipid peroxidation, increased antioxidant enzyme activity, and promoted plant growth. This study demonstrates that melatonin effectively provides a safeguard against oxidative damage in response to drought.

The accumulation of the osmotic substance content plays an important role in reducing the cellular osmotic potential and maintaining intracellular pressure, and the SP content can, to some extent, reflect the metabolic level of plant life activities [47]. Osmotic regulatory substances such as proline and soluble sugar that accumulate in plants can not only reduce the water potential but also enhance the water absorption ability of cells, thus improving their drought resistance [48,49]. Our study indicated that exogenous melatonin increased the soluble protein and soluble sugars content while decreasing the proline levels in drought-stressed *Chrysanthemum* seedlings, which suggested that exogenous melatonin improved the drought tolerance by accumulating soluble protein and soluble sugars rather than proline in *Chrysanthemum*. This is contrary to the findings of Luo et al. and may be due to different species [50]. However, these results all show that MT may regulate osmotic metabolism under drought stress and improve drought tolerance.

The important factors during drought stress that lead to the decrease in photosynthetic function in plants are stomatal restriction and nonstomatal restriction. The main factor of stomatal limitation under drought-stress conditions is the insufficient supply of CO_2—the raw material for photosynthesis—caused by the reduction in stomatal conductance, and the nonstomatal limitation factors are related to light intensity, where excessive light damages the photosynthetic organs of plants, which directly leads to the weakening of the light reaction process and a decrease in light capture and photosynthetic efficiency [51]. Bhusal demonstrated that Pn and Gs, due to heavier leaves and increased chlorophyll content, increased. This helps the leaf to capture more light, and consequently, more chlorophyll facilitates a higher Pn [52]. Chen also reported that the increased chlorophyll content also enhanced corn's net photosynthetic rate and improved salt stress tolerance [53]. In the present study, we found that exogenous spraying of melatonin under drought stress significantly increased Pn, Gs and Tr in the leaves of *Chrysanthemum* seedlings, while Ci showed a different trend. Our data showed that the Pn decreased when Gs was reduced by drought stress. Pretreatment with melatonin led to a greater Gs and a

significant rise in photosynthetic capacity because the stomata were more likely to remain open. According to a previous study [54], stomatal limitation on Pn occurs only when Gs decreases in parallel with a decline in Ci. Notably, Gs showed a reverse tendency for change versus Ci, suggesting that nonstomatal limitations on Pn existed in plants that received 100 μM melatonin, while the 100 μM MT+PEG groups showed higher efficiency of CO_2 conversion. It is notable that the melatonin-treated plants maintained higher Tr, which was consistent with higher Gs, suggesting that melatonin may enhance the plant roots' ability for water uptake. The above results suggest that exogenous spraying of MT under drought stress can reduce damage to photosynthetic organs and improve drought resistance in chrysanthemums.

Chlorophyll fluorescence emitted from the chloroplast thylakoid membrane is often used as a very sensitive intrinsic indicator of the photosynthetic reaction in photosystem II [55]. The chlorophyll fluorescence parameters can reflect the absorption, conversion, and transfer of light energy in plants, and changes in the external environment are the most important factors leading to fluctuations in the chlorophyll fluorescence parameters [56]. The occurrence of drought stress causes damage to the two photosystem reaction centers, resulting in hindered photosynthetic activity and a decreased photosynthetic rate [57]. The photochemical quenching (qP) is a measure of the proportion of energy absorbed by the PSII antenna pigments, which is used for photochemical reactions. In this context, qP also indicates the openness of the PSII reaction center [58]. ETR is a measure of the electron transfer rate in the leaves, thus reflecting PSII activities, and positively correlates to the photosynthetic rate [59]. The Fv/Fm ratio is a parameter that allows detection of any damage to PSII and possible photoinhibition [55]. The drought might not only inactivate the PSII reaction centers and reduce Fv/Fm through a rise in Fo, but they might also change the optical properties of the leaf, in which case the individual observed values of Fm and Fo may be a result of changes in leaf absorptance [60]. This will affect the estimation of NPQ but has, a priori, no effect on Fv/Fm. Additionally, a decline in Fv/Fm does not necessarily imply that the photosynthetic performance of the plant is compromised [60–62]. The results of the present study show that exogenous melatonin increases qP, Fv/Fo, Fv/Fm, ETR and Fm and decreases NPQ in the leaves of the *Chrysanthemum* seedlings under drought stress, suggesting that exogenous MT has a mitigating effect on drought-stress-induced photoinhibition. This is similar to the results of previous studies [63]. Therefore, based on our present findings and those of previous studies, it is clear that melatonin has a strong potential to alleviate drought stress in chrysanthemums.

5. Conclusions

In conclusion, exogenous melatonin under drought stress can effectively achieve the following in chrysanthemums: reduction in the loss of relative water content and chlorophyll in leaves; inhibition of the increase in malondialdehyde content and the relative permeability of plasma membranes in leaves; increase in the activity of antioxidant enzymes; reduction in reactive oxygen species production; improvement of the osmoregulatory ability; improvement of photosynthetic efficiency; acceleration of the accumulation of photosynthetic products to alleviate the damage of drought stress in seedlings; and, in general, improvement of *Chrysanthemum* drought resistance to a certain extent.

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