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Dwarfing Effect of Plant Growth Retarders on *Melaleuca alternifolia*

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Abstract: Rapid growth and scattered plant types are urgent issues for potted *Melaleuca alternifolia*. An effective strategy for dwarfing cultivation is the exogenous application of plant growth regulators (PGRs) to plants. However, for many non-wood forest species, there is currently limited understanding of the regulatory mechanism of dwarfing effects of PGRs, which greatly limits the application of PGRs. In this study, three PGRs, paclobutrazol (PP333), uniconazole (S3307), and chlormequat chloride (CCC) were applied to dwarf terpinen-4-ol *M. alternifolia*. By observing the morphological characteristics and leaf anatomy of *M. alternifolia* after dwarfing and measuring its photosynthetic characteristics and physiological and biochemical indexes, the dwarfing effect of the three PGRs and the underlying mechanisms were investigated to provide a reference for the cultivation of *M. alternifolia* by dwarfing. The results show that the PP333 (P3) treatment at 2000 mg·L^{-1} had the best dwarfing effect on *M. alternifolia*, with a compact plant shape, thicker stems, and green leaf color. At the same time, it increased the chlorophyll contents, changed the blade structure, increased the content of soluble substances and the activity of antioxidant enzymes, increased the endogenous hormones indole-3-acetic acid (IAA), gibberellin A3 (GA3), and trans-zeatin-riboside (ZR), and decreased abscisic acid (ABA) levels.

Keywords: chlormequat chloride; dwarf; endogenous hormones; *Melaleuca alternifolia*

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1. Introduction

Melaleuca alternifolia is an evergreen shrub or small tree of the genus *Melaleuca* in the Myrtle family (Myrtaceae) that grows to a height of 2–14 m. The tree has a neat shape with a dense, fluffy crown and a laminar, papery, grayish-white bark. The cylindrical branchlets have alternating bright green, lance-shaped leaves with a length of 1–6 cm; the leaves are glandular, with a pleasant scent of terpenes and nutmeg. The flowers appear from summer to autumn [1] and have a particularly sweet scent, and it is a useful landscape plant [2]. For many years, the development and application of this species have been focused on extracting the essential oils contained in its branches and leaves [3,4]. With an increasing demand for healthful landscape plants, *M. alternifolia* is being used in more forms. Ornamental potted plants are often considered a passive way to improve indoor air quality (IAQ). *M. alternifolia* is rich in volatile terpinen-4-ol [5,6] and 1,8-cineole [7], which have insect-repellent [8], mosquito-repellent [9], fly-repellent, and air-purifying properties [10]; thus, potted plants have great development potential [11]. At present, *M. alternifolia* grows too fast, too tall, and in a scattered way, which is a problem that needs to be solved in potted plants. This is also an important direction for *M. alternifolia* breeding and a hot spot for research.

Plant growth retardants (PGRs) are a class of synthetic compounds that can control the synthesis of gibberellin A3 (GA3) in plants, causing the plant body to exhibit biochemical characteristics, such as physiological dwarfing, thickened stalks, the greening of the leaf

color, thickened leaves, the development of root systems, and improved resistance [12,13] and have been systematically used since as early as the 1930s [14]. Compared with traditional dwarfing methods, the use of PGRs to dwarf plants is more convenient and efficient, as well as cost-effective, and has become an important method in modern agriculture as it can maintain the ornamental characteristics of plants to a great extent while conforming to the scale of plant production. Currently, PGRs have been widely used on horticultural crops [15,16], fruit trees [17], and flowers [18], and they have different dwarfing effects on different plants [19]. PP333 can inhibit the height of potted sour pulp plants and increase the number of fruits per plant at a concentration of 60 mg·L^{-1}, resulting in more compact and delicate plants with better aesthetic appearance [20]. In addition, the application of PGRs induces various morphological and physiological responses in plant leaves, increases antioxidant levels [21], and enhances resistance to abiotic stresses [22,23]. Spraying low concentrations of CCC has been shown to increase the superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) activity, reduce the malonaldehyde (MDA) content and the relative permeability of the plasma membrane, and increase the total chlorophyll (C(a + b)) and carotenoid (Car) content in poplar leaves, resulting in a significant increase in the net photosynthetic rate, thus increasing the accumulation of soluble sugar (SS) and protein in the pre-bud differentiation stage, improving the flowering rate and fruit set on branches and the resistance of leaves to acid rain [24]. Foliar sprays of PP333 at 1500 mg·L^{-1}, CCC at 450 mg·L^{-1} CCC, and sodium nitrophenolate, a sodium compound, at 2000 mg·L^{-1} reduced the IAA and GA3 content, elevated the ZR content, and increased the ratio of ABA/IAA, ABA/GA3, ZR/IAA, and ZR/GA3, leading to a significant increase in the number of macrosporophylls in southern redbuds [25].

At present, the problems of fast growth rate, tall plant height, and scattered plant shape under natural growth conditions need to be solved for potted *M. alternifolia*. In this study, we investigated the effects of three PGRs on the growth indexes, leaf anatomy, chlorophyll content, osmoregulatory substances, and antioxidant enzyme and endogenous hormone content of *M. alternifolia* and correlated the indexes to study the mechanism of these PGRs. The purpose was to understand the response of *M. alternifolia* to different PGRs and determine the most suitable retarder types and concentrations for dwarfing the plant in pots in order to improve the ornamental effect and provide a reference basis for the dwarfing cultivation of *M. alternifolia*.

2. Materials and Methods

2.1. Study Area

The experiment was conducted at the outdoor testing nursery (28°10′ N; 113°23′ E), College of Forestry, Central South University of Forestry and Technology (Changsha, China) from August to early November 2020. This area belongs to a subtropical monsoon climate, with four distinct seasons and abundant rainfall. The annual average temperature is 17.2 °C, and the annual average precipitation is 1361.6 mm.

2.2. Materials

The test materials were provided by Hunan Xingtai Biotech Co., Ltd.(Yongzhou, China), and consisted of 9-month tissue culture seedlings of *M. alternifolia* in good growth condition, free from pests and diseases and with consistent morphological growth potential. The treatment agent was 15% PP333 wet powder (Sichuan Runer Technology Co., Ltd., Chengdu, China), 95% S3307, and 98% CCC (Beijing Solarbio Technology Co., Ltd., Beijing, China).

2.3. Experiment Design

For the pot experiment, the test materials were colonized in plastic pots with a mouth diameter of 27.5 cm and a height of 28 cm. They were first placed in an environment with full sunlight and air permeability outside for 14 d to recover their normal growth and heal the possible damage during transportation. The pot culture medium was a uniform

mixture of peat, vermiculite, perlite, and red soil at a ratio of 1:1:1:1, with 1 plant per pot. The plants were approximately 26 cm in height after 2 weeks of colonization, at which point dwarf treatment was initiated.

The trial was a completely randomized block design and a single-factor controlled trial. Gradients were set for each agent as follows: PP333: 500, 1000, 2000, and 3000 mg·L^{-1} (P1, P2, P3, and P4, respectively); S3307: 50, 100, 200, and 300 mg·L^{-1} (S1, S2, S3, and S4, respectively); and CCC: 1000, 2000, 3000, and 4000 mg·L^{-1} (C1, C2, C3, and C4, respectively). Clear water treatment was used as control (CK), and 12 test trees were selected for each treatment of each variety; a total of 156 trees were selected, and each treatment was repeated 3 times. The test was carried out in August 2020 using the foliage spraying method; each mass concentration was treated with foliar spray using a pressure spray, and the amount of spraying liquid was appropriate to the rigid dripping on the blade surface. The spraying time was 9:00~11:00 am. Each treatment maintained a distance of 1 m × 1 m; treatment was once every 7 d continuously for 5 weeks, with clean water treatment (CK) as control. Samples were taken at 7, 14, 21, 28, and 35 days after the first treatment, and the next treatment was carried out after each sampling. The leaves were stored in an ultra-low-temperature refrigerator at −80 °C for determination of physiological and biochemical parameters and endogenous hormones.

2.4. Growth and Biomass Measurement

Three seedlings were randomly selected from each treatment for index and biomass measurement, and each treatment was repeated 3 times. The height from the horizontal surface of the pot soil to the highest point of the plant measured by a tape was considered the plant height, and the thickness of the plant base about 2 cm from the horizontal surface of the pot soil measured by cross method using vernier calipers was considered the stem thickness. On the 35th day of the experiment, after the new branches stopped growing, 9 new branches were measured randomly in each experimental group with a tape measure, and the average value was taken as the new branch length.

2.5. Chlorophyll Analysis

Chlorophyll was determined using Lichtenthaler's modified Arnon method [26]. After 35 days of medication treatment, the upper, middle, and lower layers of random external function leaves of each treated plant in different directions were cut and mixed, then 0.2 g samples were weighed and placed in a mortar. Small amounts of quartz sand and calcium carbonate powder were added, and 2–3 mL 95% ethanol was added to grind the samples into a homogenate, followed by 10 mL ethanol to grind until the tissue whitened. Then, chlorophyll a (C*a*), chlorophyll b (C*b*), and carotenoid (Car) were determined using an ultraviolet-visible spectrophotometer (SPECORD 210 PLUS, Shanghai, China) at 665, 649, and 470 nm. The formula for calculating chlorophyll content is as follows [26]:

$$\mathrm{C}a\ (\mathrm{mg/g}) = 13.95\mathrm{A}665 - 6.88\mathrm{A}649$$

$$\mathrm{C}b\ (\mathrm{mg/g}) = 24.96\mathrm{A}649 - 7.32\mathrm{A}665$$

$$\text{Carotenoid}\ (\mathrm{mg/g}) = (1000\mathrm{A}470 - 2.05\mathrm{C}a - 114.8\mathrm{C}b)/245$$

$$\mathrm{C}\ (a + b)\ (\mathrm{mg/g}) = \mathrm{C}a + \mathrm{C}b$$

$$\mathrm{C}a/b\ (\mathrm{mg/g}) = \mathrm{C}a/\mathrm{C}b$$

Note: C*a* is the content of chlorophyll a, C*b* is the content of chlorophyll b, C (*a* + *b*) is the total content of chlorophyll, and carotenoid is the concentration of carotenoids; A470, A649, and A665 are the absorbance of the sample tube.

2.6. Anatomical Features of Leaves

Three seedlings were selected for each treatment, and on day 35 of the test treatment, test samples were taken from fully expanded leaves at the same position, and the fresh tissues were fixed in 50% fixative FAA (formaldehyde alcohol acetic acid) for 48 h. The tissues were removed from the fixative, and tissues at the site of interest were leveled off with a scalpel inside a fume hood. The trimmed tissues and corresponding wax-impregnated labels were then placed inside a dehydration box, which was put into a hanging basket in a dehydrator (model JJ-12J, China) and sequentially dehydrated with different gradients of alcohol (75, 85, 90, 95%). The wax-impregnated tissues were embedded in the embedding machine (model JB-P5, China). The wax blocks were removed from the embedding frame after the wax solidified and were trimmed. The tissue was flattened by floating on the 40 °C water of the spreading machine. After the wax was dried, it was taken out and stored at normal temperature for later use.

Paraffin sections were dyed with a safranin and Fast Green double-dyeing method [27,28]. After the paraffin sections were dewaxed in water, they were dyed with 1% saffron dye solution for 1–2 h. After the excess dye was slightly washed with tap water, 50, 70, and 80% alcohol were, respectively, added to decolorize for 1 min. Then, the sections were successively added to 0.5% solid green dye solution for 30–60 s, then decolorized in absolute alcohol I for 30 s and absolute alcohol II for 1 min. Sections were cleared with xylene for 5 min after drying in a 60° oven and were mounted with neutral gum. Next, microscopy was performed using a Nikon ECLIPSE Ci positive optical microscope (Nikon, Japan), and image acquisition and analysis were performed using a Nikon DS-U3 imaging system (Nikon, Japan). The thickness of leaves, fence tissue, sponge tissue, and mesophyll was measured using cross-section analysis. Three sections were measured per treatment, and the mean of 5 fields was taken per section.

2.7. Determination of Physiological and Biochemical Indicators

SOD activity was determined using the NBT photoreduction method [29], POD activity was determined using the guaiacol chromogenic method [30], CAT activity was determined using the ammonium molybdate method [31], MDA content was determined using the thiobarbituric acid (TBA) method [32], soluble sugar content was determined using anthrone colorimetry [33], and the soluble protein of *M. alternifolia* was determined using the Coomassie brilliant blue method [34].

2.8. Determination of Endogenous Hormones

An enzyme-linked immunosorbent assay (ELISA) kit [35] (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) was used to determine the content of four endogenous hormones: IAA, GA, ABA, and ZT.

2.9. Statistical Analysis

One-way analysis of variance (ANOVA) was performed on the trial data using SPSS 23.0 (IBM, New York, NY, USA), and Pearson correlation analysis (IBM, New York, NY, USA) was used to evaluate the correlations between indicators (the data used for correlation analysis were samples from day 35 of the trial). Microsoft Excel 2016 software (Microsoft, Washington, DC, USA) was used to sort and tabulate the experimental data.

3. Results

3.1. Growth Characteristics

In this paper, the spraying concentrations P3, S2, C3, and CK with the most obvious treatment effects in PGRs were selected for comparative study.

Plant height is the key to measuring the success of dwarfing. One-way ANOVA of the growth indicators of *M. alternifolia* (Figure 1a) showed that the effect of foliar PGRs on plant height was highly significant (Table S1). At 35 d, the P3, S2, and C3 treatments showed the most significant dwarfing effect, with 13.04, 7.69, and 4.29% reduction compared to CK,

respectively (Figure 2); P3 and S2 were highly significantly different from CK ($p < 0.01$), and the plants were not damaged by the drug. As can be seen in Figure 1b, the P3, S2, and C3 treatments all increased the stem thickness of *M. alternifolia* in pots, but there were no significant differences (Table S2). At 35 d, stem thickness under the P3 treatment was 0.3457 cm, an increase of 15.35% compared to CK; in comparison, S2 and C3 induced increases of 14.68 and 13.78%, respectively. Regarding the effect on new branch length (Figure 1c), overall, PP333 was better than the S3307 treatment, with P3 and S2 causing branch shortening by 39.91 and 28.06%, respectively, compared to CK, showing a highly significant difference ($p < 0.01$). The effects of the three PGRs on plant leaf length and width were not significant, and no treatments showed significant differences with CK (Table S3).

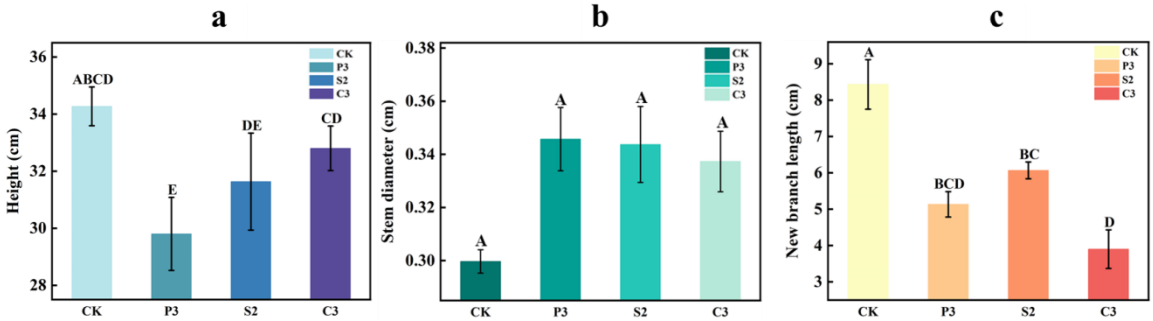

Figure 1. Effects of PGRs on growth characteristics of *M. alternifolia*. (**a**) Plant height, (**b**) stem diameter, (**c**) new branch length. Note: different letters followed the mean indicate significance at $p < 0.01$ according to Duncan's multiple range test.

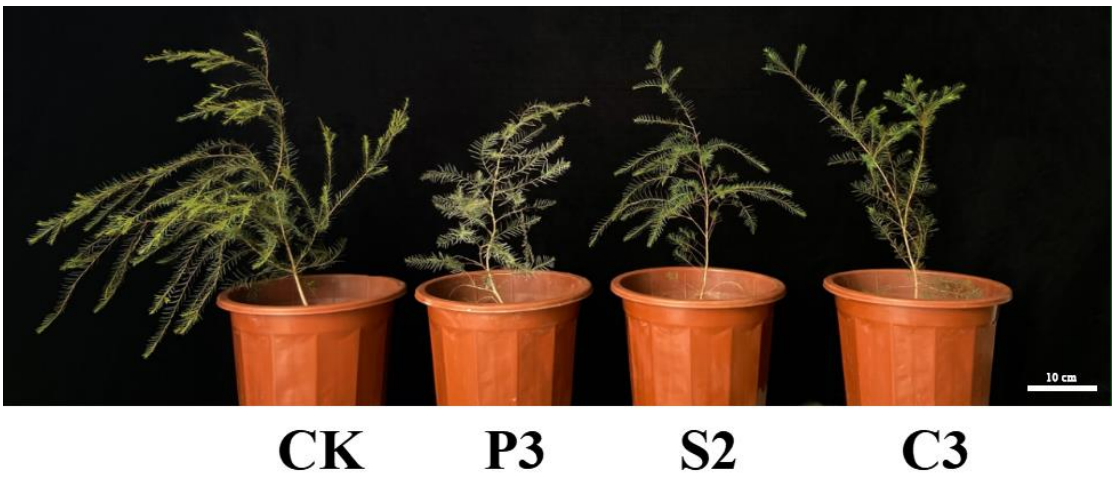

Figure 2. Comparison between CK and the treatment with the most obvious influence on plant height of each PGRs.CK: treated with water, P3: treated with 2000 mg·L^{-1} PP333, S2: treated with 100 mg·L^{-1} S3307, C3: treated with 3000 mg·L^{-1} CCC, the same below.

3.2. Chlorophyll Content

Chloroplasts are the main sites of photosynthesis in plants, and chlorophyll is the main pigment involved in the photosynthetic pathway. The higher the chlorophyll content, the stronger the photosynthesis of the plant and the better the growth and development. As shown in Figure 3, C*a*, C*b*, total chlorophyll, and carotenoids in *M. alternifolia* leaves were increased by the P3, S2, and C3 treatments at 35 d. After the S2 treatment, the contents of C*a*, C*b*, Chl, and Car increased by 22.12, 25.72, 23.12, and 22.12%, respectively, showing highly significant differences compared to CK ($p < 0.01$). After the P3 treatment, the content increased by 17.27, 14.92, 16.72, and 17.27% and after the C3 treatment by 8.25, 4.00, 7.38, and 8.25%, respectively (Table S4).

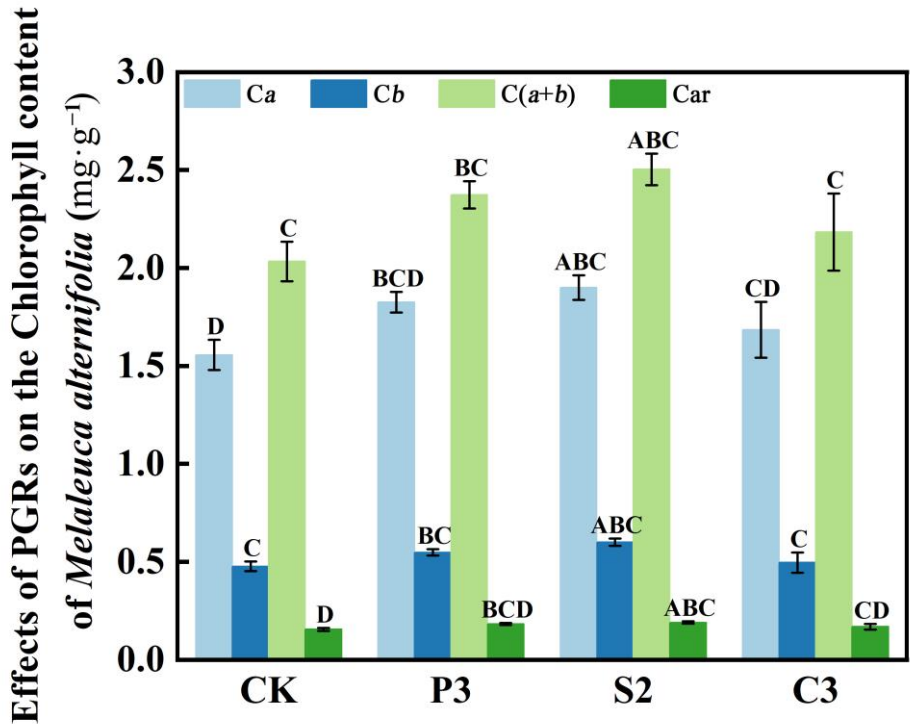

Figure 3. Effects of three PGRs on the chlorophyll content of *M. alternifolia.* Note: different letters followed the mean indicate significance at $p < 0.01$ according to Duncan's multiple range test.

3.3. Effect on Leaf Anatomy of M. alternifolia

From the cross-sectional structure diagram of an *M. alternifolia* leaf (Figure 4), it can be seen that it is an isopetalous leaf, consisting of veins, mesophyll, and epidermis. The spongy and palisade tissues are differentiated from the mesophyll cells, the upper and lower epidermis are closely arranged single-layer cells, and the palisade tissues are differentiated on the inner side of both the upper and lower epidermis, with the thickness of the upper palisade tissues greater than that of the lower palisade tissues (Table S5). The palisade tissue is mostly composed of 1–2 layers of narrow columnar thin-walled cells, with small and neatly arranged dense cell gaps, containing abundant chloroplasts. The spongy tissue is differentiated between the upper and lower palisade tissues and consists of loosely arranged irregularly shaped or sub-rounded thin-walled cells. Some irregularly shaped crystal cells are scattered in the leaf flesh. On day 35 of experimental treatment (Figure 5a,b), the P3, S2, and C3 treatments increased the thickness, upper epidermis, lower epidermis, upper palisade tissue, spongy tissue, and mesophyll of *M. alternifolia* leaves, and the thickness, spongy tissue thickness, and mesophyll thickness were highly significantly different from those of CK ($p < 0.01$). The most significant increases in leaf thickness, upper and lower epidermal thickness, palisade tissue, spongy tissue, and mesophyll thickness compared to CK were observed after the P3 treatment, with increases of 26.38, 22.27, 10.03, 13.16, 75.91, and 22.75%, respectively. After the S2 and C3 treatments, stem thickness was 91.45 and 88.98 μm, an increase of 64.63 and 60.18%, respectively, compared to CK; mesophyll thickness was 171.46 and 167.95 μm, an increase of 19.00 and 16.57%, respectively; and leaf thickness was 186.19 and 191.00 μm, an increase of 14.99 and 17.96%.

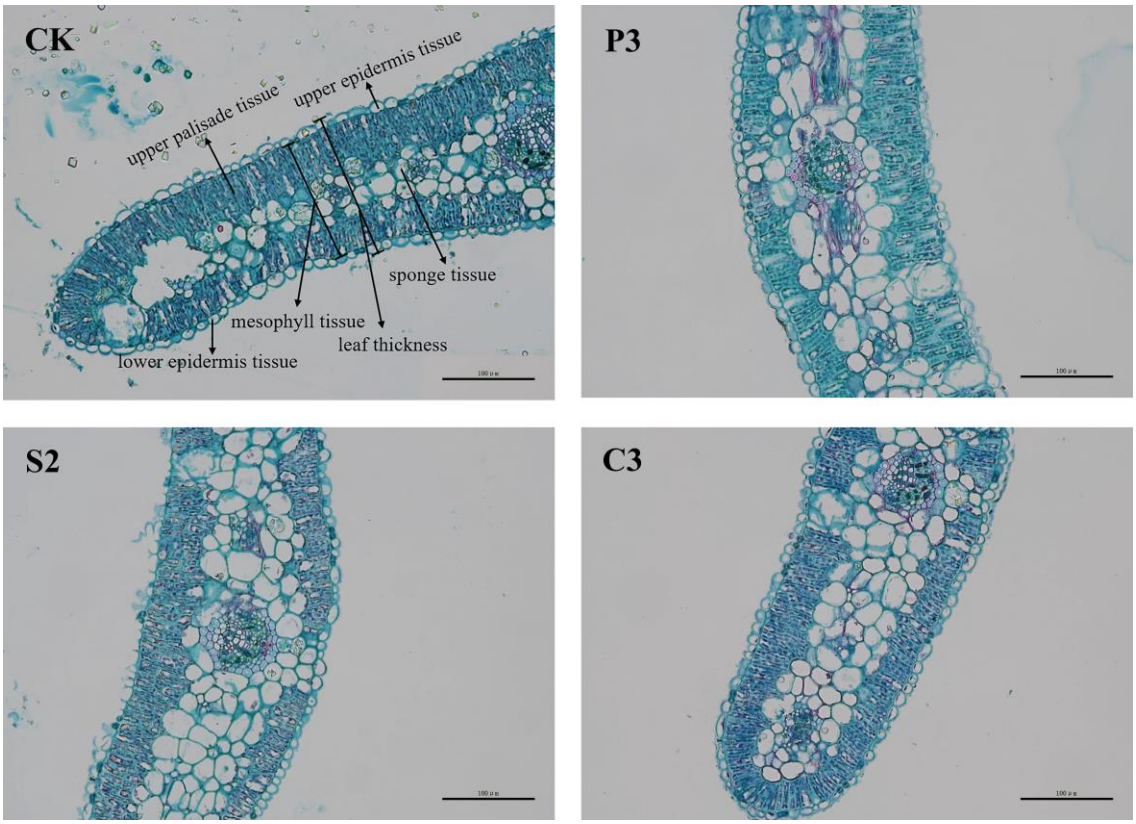

Figure 4. Anatomical structure of *M. alternifolia* leaves under different PGRs (× 20).

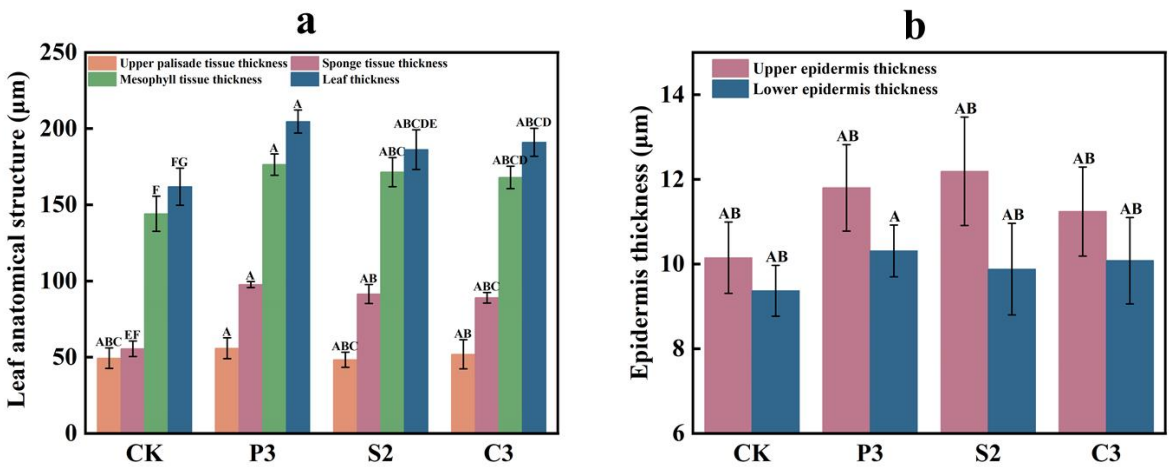

Figure 5. Effect of PGRs on anatomical structure of leaves of *M. alternifolia***.** (**a**) Upper palisade tissue thickness, sponge tissue thickness, mesophyll tissue thickness, and leaf thickness, (**b**) upper epidermis thickness and lower epidermis thickness. Note: different letters followed the mean indicate significance at $p < 0.01$ according to Duncan's multiple range test.

3.4. Physiological and Biochemical Characteristics

Effect of PGRs on the Content of Osmotic Regulating Substances

PGRs at different concentrations increased the soluble sugar content (SS) in *M. alternifolia* leaves (Table S6). It can be seen in Figure 6a that the trend of S2, C3, and CK was the same; over time, the SS content showed a trend of increasing, then decreasing, then increasing, peaking at 14 d. Compared with CK, after the S2 treatment, the SS content increased by 18.71, 24.41, 20.51, 54.78, and 41.39% and after the C3 treatment by 13.23, 13.52, 11.67, 14.86, and 15.18%, all showing highly significant differences from CK ($p < 0.01$). After

the P3 treatment, the SS content first decreased and then increased with the prolongation of the test time. The SS content was the highest on day 7 of treatment, reaching 59.05 mg·g^{-1} (43.12% higher than CK), showing a very significant difference ($p < 0.01$). On day 35 of the test, the SS content was in the order of S2 > C3 > P3 > CK.

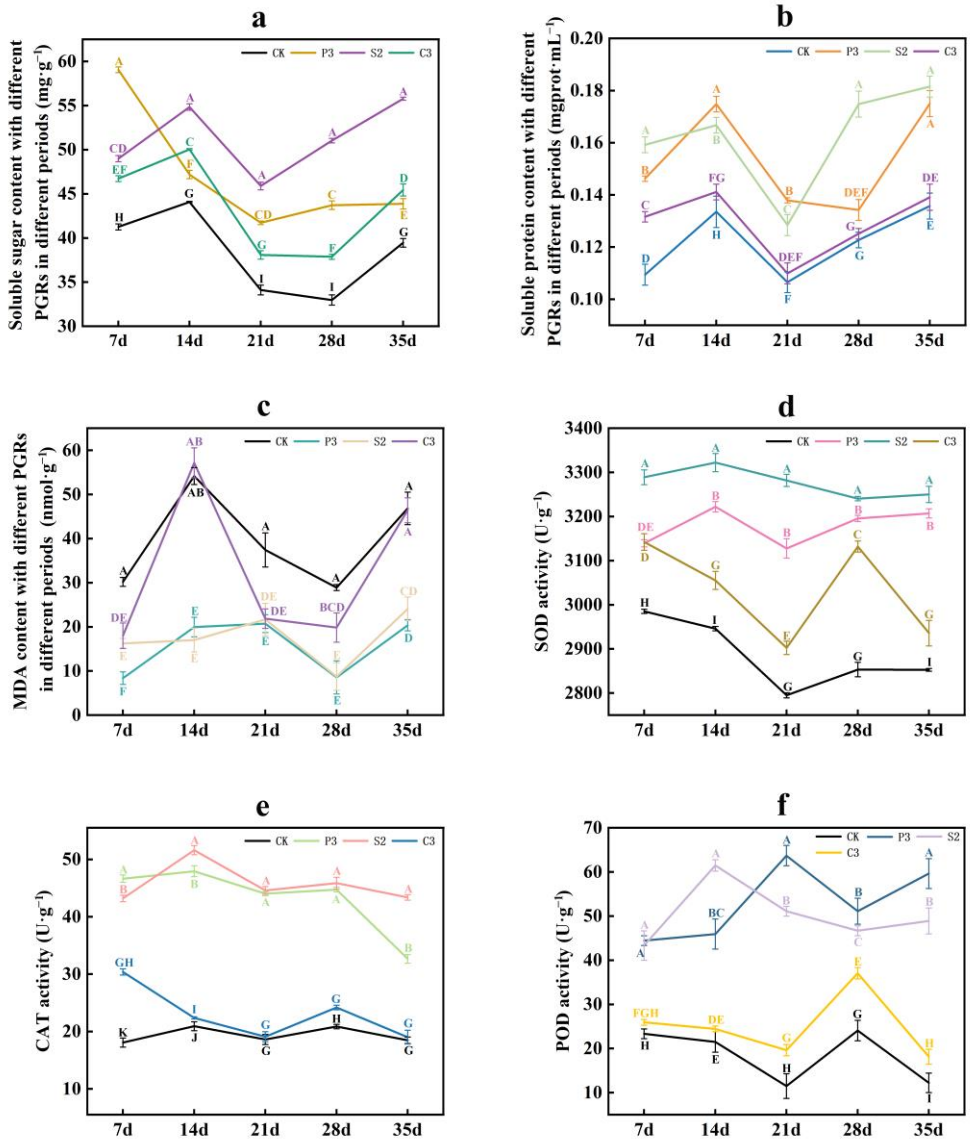

Figure 6. Effects of PGRs on physiological and biochemical indexes of *M. alternifolia* Leaves. (**a**) Soluble sugar content, (**b**) soluble protein content, (**c**) MDA content, (**d**) SOD activity, (**e**) CAT activity, (**f**) POD activity. Note: different letters followed the mean indicate significance at $p < 0.01$ according to Duncan's multiple range test.

The soluble protein (SP) concentration in *M. alternifolia* was increased by different concentrations of PGRs (Table S7). As shown in Figure 6b, with increasing treatment time in P3, S2, and C3, the SP concentration was in line with that of CK, showing a trend of increasing, then decreasing and then increasing. Both the S2 and P3 treatments reached the peak value on day 35, with an SP concentration of 0.1815 and 0.1750 mgprot·mL^{-1}, respectively (33.75 and 28.96% higher than CK). The C3 treatment reached the peak value on day 14 when the SP concentration was 0.1411 mgprot·mL^{-1} (5.69% higher than CK), and each treatment was significantly different from CK ($p < 0.01$). The lowest SP concentration in both the S2 and C3 treatments occurred on day 21, with increases of 20.56 and 3.19%, respectively, over CK. The lowest concentration of SP in the P3 treatment was 0.1342 mgprot·mL^{-1} on

day 28 (9.37% higher than CK), with the S2 and P3 treatments showing highly significant differences from CK ($p < 0.01$). In summary, the S3307 and PP333 treatments were able to retard the degradation of SP in *M. alternifolia* leaves for a longer period of time, while the CCC treatments inhibited SP degradation for a short period of time, but the inhibitory effect diminished over time. Taking all the data together, S2 was the most effective at increasing SS and SP in *M. alternifolia*, and CCC was less effective overall.

MDA is the final breakdown product of cell membrane lipid peroxidation, and changes in MDA content can reflect the degree of damage and resistance of plants under stress. The MDA levels after P3 and S2 were the lowest among the PP333 and S3307 treatments; both showed a trend of increasing, then decreasing and then increasing with treatment time (Table S8). As shown in Figure 6c, P3 reduced MDA levels by 72.28, 63.18, 44.63, 70.47, and 56.52% and S2 by 46.27, 68.59, 42.12, 69.75, and 48.60% at each period compared with CK, with all highly significantly different from CK ($p < 0.01$). The MDA content was at its lowest point on days 7 and 28 under the P3 and S2 treatments, respectively, and reached a peak on days 21 and 35. The trend of MDA content after the C3 treatment was consistent with that of CK, with both showing an increase, then a decrease, and then an increase. MDA reached the lowest point under the C3 treatment on day 7 at 18.00 $nmol \cdot g^{-1}$ (40.38% lower than CK), which was highly significantly different from CK ($p < 0.01$) and reached a peak on day 14 at 57.14 $nmol \cdot g^{-1}$ (5.50% higher than CK). On day 35 of treatment, the difference between the C3 treatment and CK was not significant. It can be seen that foliar spraying of CCC can reduce the MDA content in *M. alternifolia* leaves in a short period of time, but with time and additional applications, CCC can damage the plant's membrane lipid system and reduce its resistance.

3.5. Effect of PGRs on Antioxidant Enzyme Activity

SOD is the first line of defense for the scavenging of reactive oxygen species (ROS) in plants, acts mainly in the antioxidant enzyme system to scavenge reactive oxygen radicals and catalyze superoxide dismutase reactions, and is an indicator of plant stress resistance. The SOD, CAT, and POD activity of *M. alternifolia* leaves was significantly higher under the three PGR treatments than in CK (Table S9). As shown in Figure 6d, the SOD activity after the S2 treatment was the highest among the S3307 treatments, increasing by 10.17, 12.77, 17.42, 13.57, and 13.93% compared to CK, and after the P3 treatment, it increased by 5.16, 9.37, 11.90, 12.00, and 12.42%, with all highly significantly different from CK ($p < 0.01$). The S3307 and PP333 treatments showed the same trend with time, with the SOD activity in *M. alternifolia* leaves first increasing, then decreasing, and then increasing, and both reached a peak on day 14. The overall trend under the C3 treatment was consistent with that of CK, with the SOD activity first decreasing, then increasing, and then decreasing, reaching a peak on day 7 and a minimum on day 21. The SOD activity increased by 5.26, 3.71, 3.85, 9.77, and 2.91% after the C3 treatment compared with CK, showing highly significant differences from CK ($p < 0.01$). Comprehensive analysis showed that the S2 treatment had the most significant effect on the SOD activity in potted *M. alternifolia*, with an overall order of S3307 > PP333 > CCC.

CAT, the main antioxidant enzyme in the reactive oxygen species scavenging system, is produced in cells when plants are subjected to abiotic stress. Its synergistic action with SOD can defend plants against oxygen toxicity, effectively protect plant cells and the organism itself, and enhance the tolerance of plants under adversity stress. As shown in Figure 6e, the activity of CAT after both the P3 and S2 treatments was the highest among the PGR treatments, and the change pattern of CAT activity over the observation time was consistent with CK in both cases, showing a rising–lowering–rising–lowering trend, while the C3 treatment was the opposite, showing a falling–lowering–lowering trend. With increasing treatment days, the CAT activity increased by 158.05, 129.11, 136.49, 114.43, and 76.92% after the P3 treatment and 139.01, 146.70, 139.55, 119.85, and 134.94% after the S2 treatment compared to CK. The CAT activity under both the P3 and S2 treatments reached a peak on day 14 and was significantly different from that of CK ($p < 0.01$) (Table S10). The CAT

activity increased by 68.07, 6.93, 2.63, 15.96, and 2.98% after the C3 treatment compared with CK and peaked on day 7, showing a highly significant difference from CK ($p < 0.01$). Collectively, the P3 and C3 treatments induced the lowest CAT activity on day 35. This indicates that the ability of PGRs to increase CAT activity in *M. alternifolia* leaves diminishes with more frequent and longer applications. The S2 treatment had the most significant effect on the CAT activity of *M. alternifolia* in pots, followed by the P3 treatment.

POD is a class of oxidoreductase enzymes commonly found in plants that can convert hydrogen peroxide into water and are also somewhat closely related to the photosynthesis, respiration, and oxidation of growth hormones. The POD activity was higher in leaves of potted *M. alternifolia* than in CK under all three PGR treatments (Table S11). As shown in Figure 6f, the POD activity after the P3 and S2 treatments was the highest among the PGR treatments, and the pattern of change was consistent with that of CK over the observation time, showing a trend of increasing, then decreasing, and then increasing. Under the P3 treatment, the POD activity increased by 90.48, 113.83, 454.88, 112.34, and 387.97% compared with CK, showing a highly significant difference from CK ($p < 0.01$). The POD activity reached a peak on day 21 and its lowest value on day 7 under the P3 treatment. After the S2 treatment, the POD activity increased by 85.73, 186.22, 345.21, 93.89, and 300.08% compared to CK, which was highly significantly different from CK ($p < 0.01$); it peaked on day 14 and was at its lowest value on day 7. The POD activity showed the same trend under the CCC treatment as in CK, with both decreasing, then increasing, then decreasing. After the C3 treatment, the POD activity reached a peak on day 28 and was at its lowest value on day 35 (53.88 and 48.53% higher than CK, respectively) and was highly significantly different from CK ($p < 0.01$).

Overall, foliar sprays of PP333, S3307, and CCC all increased the SOD, CAT, and POD activity of *M. alternifolia* in pots, with the S2 treatment having the most significant effect on antioxidant enzyme activity, followed by P3. Under the PP333 treatment, SS was significantly and negatively correlated with MDA (Table S12), and under the S3307 treatment, MDA was significantly and negatively correlated with SS and CAT (Table S13). Among them, MDA was negatively correlated with every other physiological index. Under the CCC treatment, SP was highly significantly positively correlated with POD, and SS was significantly and positively correlated with SOD, while SOD, CAT, and POD were positively correlated with each other (Table S14). In conclusion, MDA in *M. alternifolia* leaves was found to be regulated by both osmoregulatory substances and antioxidant enzymes; there was a positive correlation between the antioxidant enzymes SOD, CAT, and POD, and all of them were negatively correlated with MDA content. Under the condition that the physiological indicators of the plant were acting in harmony, the stress resistance of *M. alternifolia* was improved.

3.6. Effects of PGRs on Endogenous Hormones

Leaf spraying of the three PGRs had a significant effect on the endogenous hormones of *M. alternifolia* in pots, generally decreasing the IAA, GA, and ZT contents and increasing the ABA content in the leaves, with the largest change observed in the GA content (Table S15). IAA, GA, and ZT were all positively correlated with each other and negatively correlated with ABA (Tables S16–S18). As shown in Figure 7a, the IAA, GA, and ZT contents after the P3 treatment were 4.54, 44.82, and 35.31 $ng \cdot mL^{-1}$ (38.40, 51.27, and 35.81% lower than CK, respectively), and the ABA content was 28.92 $ng \cdot mL^{-1}$ (19.80% higher than CK), with all showing highly significant differences ($p < 0.01$). The IAA, GA, and ZT contents after the S2 treatment were 4.69, 46.34, and 40.24 $ng \cdot mL^{-1}$ (36.36, 49.61, and 26.85% lower than CK, respectively), and the ABA content was 27.58 $ng \cdot mL^{-1}$ (14.25% higher than CK), with all showing highly significant differences from CK ($p < 0.01$). The IAA, GA, and ZT contents after the C3 treatment were 5.36, 57.08, and 42.73 $ng \cdot mL^{-1}$ (27.27, 37.94, and 22.32% lower than CK, respectively), and the ABA content was 26.58 $ng \cdot mL^{-1}$ (10.11% higher than CK), with all showing highly significant differences from CK ($p < 0.01$).

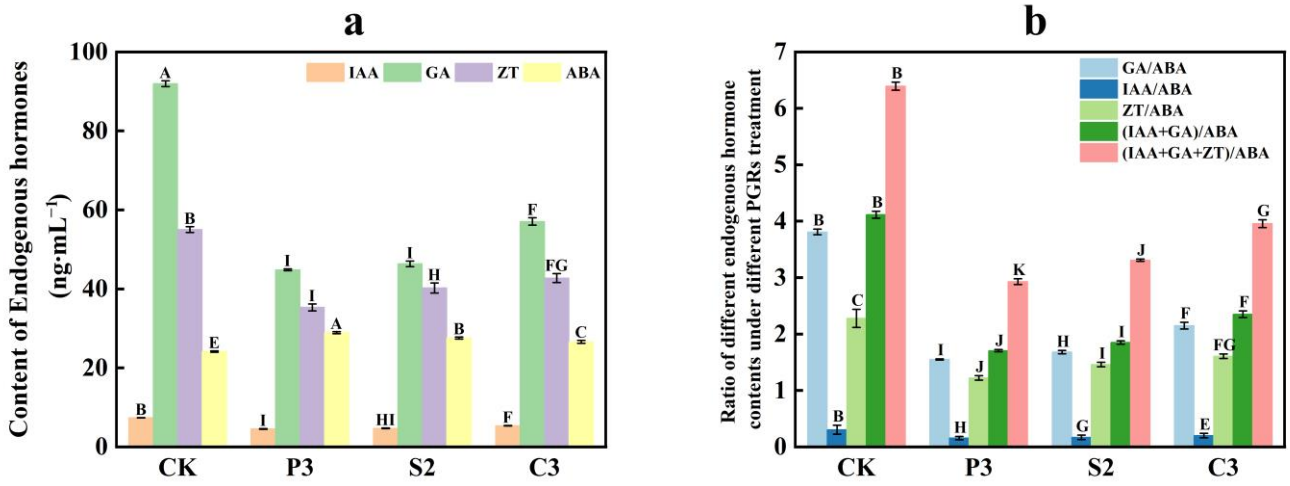

Figure 7. Effects of PGRs on endogenous hormones of *M. alternifolia.* (**a**) Effect of PGRs on endogenous hormone content of *M. alternifolia*, (**b**) ratio of different endogenous hormone contents in *M. alternifolia* leaves treated with PGRs. Note: different letters followed the mean indicate significance at $p < 0.01$ according to Duncan's multiple range test.

The GA/ABA, IAA/ABA, ZT/ABA, (IAA + GA)/ABA, and (IAA + GA + ZT)/ABA ratios in *M. alternifolia* leaves decreased significantly after the P3, S2, and C3 treatments, and their change patterns with the applied concentrations were basically the same as those of the IAA, GA, and ZT contents (Figure 7b), with the most pronounced changes in GA/ABA (Table S19). The P3 treatment had the most significant effect on the endogenous hormones of *M. alternifolia*, reducing the GA/ABA, IAA/ABA, ZT/ABA, (IAA + GA)/ABA, and (IAA + GA + ZT)/ABA ratios by 59.53, 48.39, 47.81, 58.25, and 54.39%, respectively.

3.7. Correlation Analysis of Various Indexes under PGR Treatment

As shown in Figure 8a, the plant height of *M. alternifolia* under the PP333 treatment was positively correlated with SS and CAT and negatively correlated with SP, MDA, SOD, and POD. Stem thickness was positively correlated with SP, SOD, CAT, and POD and negatively correlated with SS and MDA. New branch length was negatively correlated with SP and POD and positively correlated with SS, MDA, SOD, and CAT (Table S20-1). As shown in Figure 8b, under the S3307 treatment, plant height was highly significantly positively correlated with SOD and POD and significantly positively correlated with MDA; stem thickness was positively correlated with SP, MDA, SOD, and POD and negatively correlated with SS and CAT; and new branch length was negatively correlated with SS and positively correlated with all other indicators (Table S20-2). As can be seen from Figure 8c, under the CCC treatment, new branch length showed a highly significant positive correlation with SOD, a significant positive correlation with MDA, CAT, and POD, and a significant negative correlation with SP; stem thickness was significantly and positively correlated with POD; plant height was negatively correlated with SS and SP and positively correlated with MDA, SOD, CAT, and POD; and blade thickness was negatively correlated with SP and positively correlated with all other indexes (Table S20-3).

As can be seen from Figure 8, under the three PGR treatments, the plant height and new branch length of *M. alternifolia* were positively correlated with IAA, GA, and ZT and negatively correlated with ABA; in contrast, stem thickness was negatively correlated with IAA, GA, and ZT and positively correlated with ABA. Under the PP333 treatment, plant

height was significantly positively correlated with IAA, highly significantly positively correlated with ZT, and significantly negatively correlated with ABA; stem thickness was significantly negatively correlated with IAA, highly negatively correlated with GA, and significantly positively correlated with ABA (Figure 8a). Under the S3307 treatment, plant height was significantly positively correlated with IAA and GA and significantly negatively correlated with ABA; in contrast, stem thickness was significantly negatively correlated with IAA and GA and highly positively correlated with ABA (Figure 8b). Under the CCC treatment, stem thickness showed a highly significant negative correlation with IAA and a significant negative correlation with ZT, and new branch length showed a highly significant positive correlation with IAA and a significant positive correlation with ZT (Figure 8c) (Table S20-3).

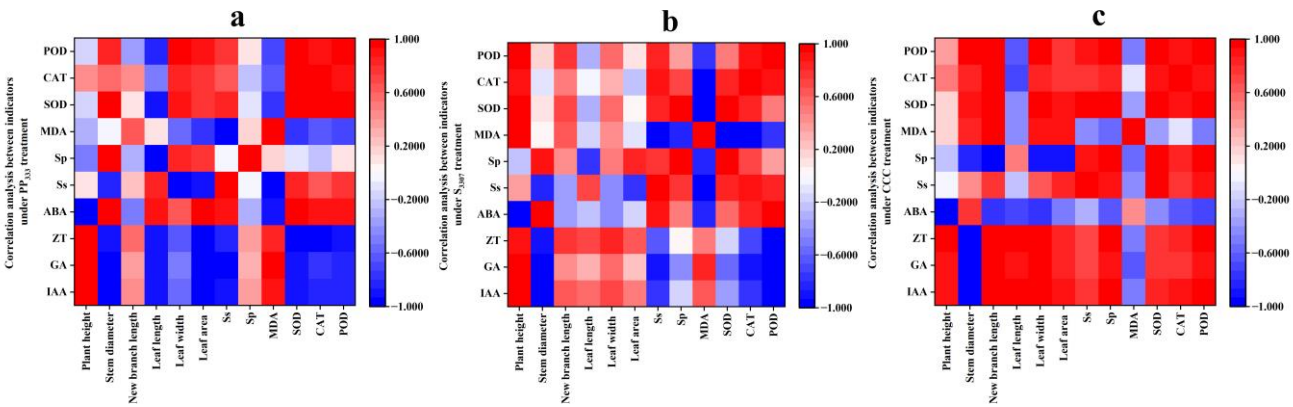

Figure 8. Correlation analysis of indexes of *M. alternifolia* under PGR treatments. (**a**) Correlation analysis of indexes of *M. alternifolia* under PP_{333} treatment, (**b**) correlation analysis of indexes of *M. alternifolia* under S_{3307} treatment, (**c**) correlation analysis of indexes of *M. alternifolia* under CCC treatment.

4. Discussion

4.1. Effect of PGRs on Morphological Indexes of M. alternifolia

The dwarfing of trees into potted plants is an important part of the commercial production of ornamentals. The technique of dwarfing plants using PGRs is an integrated and complex system involving multiple factors. Plant dwarfism is related not only to the genetic characteristics of the plant itself but also to the type and concentration of PGRs. The present study shows that the type and concentration of PGRs had significant effects on the plant height and stem thickness of *M. alternifolia*, which is consistent with the results of scholarly studies on *Epidendrum radicans* orchid [36], pomegranate (*Punica granatum* L.) [37], and apple [38].

PGRs achieve dwarfism mainly by reducing plant height and shortening internode length. The inhibition of new shoot elongation is one of the important aspects of plant growth regulation, and different concentrations of PGRs showed significant inhibition of new shoot elongation in *M. alternifolia*, which is consistent with previous studies on nursery citrus trees [39], *Osmanthus fragrans* 'Rixiang Gui' [40], and *Chrysanthemum indicum* L. [41], as well as other studies. Applying PGRs to *M. alternifolia* inhibited the synthesis of GA, which, in turn, inhibited the cell division and elongation of the subapical meristem of the plant, thereby temporarily retarding nutritional growth, resulting in reduced plant height and stalk thickening and the inhibition of new growth. Overall, the effects of the three PGRs on the morphological indexes of *M. alternifolia* were stronger for PP333 than S3307 and stronger for S3307 than CCC, which may be related to the different periods of inhibition of the GA activity. CCC acts in the early stage of GA synthesis and affects ent-kaurene synthase (KS) and copalyl pyrophosphate synthase (CPS), while PP333 and S3307 act in the middle stage of GA synthesis and affects ent-kaurene oxidase (KO), and the intensity of the inhibitory effect on GA is greater in the middle stage than the early stage [42].

4.2. Effect of PGRs on M. alternifolia Leaf Anatomy

The leaf is the organ that has the largest contact area with the environment and is also the most sensitive part of the plant to changes in external living conditions. Leaves can adapt to the living environment and conditions by changing their morphological structure [43], so the morphological anatomical structure of plant leaves can reflect the physiological adaptation of plants to a certain extent [44]. The test results show that PGRs increased the thickness of leaves, epidermis, palisade tissue, spongy tissue, and mesophyll of *M. alternifolia*, which was similar to the results of previous studies on walnut (*Juglans regia* L.) [45] and potato [46]. The spongy tissue contains more chloroplasts, which is where the main sites of plant photosynthesis are; when the chloroplasts increase, the leaves become greener, and the photosynthetic properties are enhanced [47], and at the same time, the thickened epidermal and mesophyll tissues of leaves can make the plants resistant to drought, cold, pests, and diseases [48,49].

4.3. Effects of PGRs on Physiological and Biochemical Indexes of M. alternifolia

PGRs can improve plant resistance and disease resistance by increasing the content of osmoregulatory substances, enhancing the activity of antioxidant enzymes, delaying membrane lipid peroxidation, and delaying protein breakdown [50]. As important osmoregulatory substances and nutrients in plants, SS and SP can maintain cell expansion pressure to reduce water loss to maintain water balance and normal cell function, which is crucial in alleviating damage and is a critical part of plant resilience. In the present study, the PGR treatment of *M. alternifolia* resulted in a significant increase in SS and SP, indicating that foliar spraying of the three PGRs could improve the plant's osmoregulatory ability to some extent by increasing the cytosol concentration in the plant and reducing the osmotic potential of the cells, thus improving the plant and maintaining normal growth. This is consistent with scholarly studies concluding that when plants experience stress, they can adapt by increasing their SP content, thus improving their resistance to stress [51].

Under the action of PGRs, the metabolic balance between reactive oxygen species (ROS) and free radicals in *M. alternifolia* is disrupted, and excessive ROS promotes the peroxidation of unsaturated fatty acids in membrane lipids to produce MDA, which can polymerize with enzymatic proteins in a chain reaction and degenerate the membrane system, leading to membrane lipid peroxidation. A high MDA level indicates a high degree of damage to the membrane lipid system as MDA is toxic to cells and affects the normal metabolic activities of organisms. In the present study, the MDA content of *M. alternifolia* leaves decreased significantly under PGR treatment. The MDA content in leaves was relatively the lowest after the P3 and S2 treatments, indicating that PGRs can inhibit the peroxidation of cell membrane lipids, reduce the damage to the membrane lipid system, and improve the plant. This is in general agreement with previous studies on *Zelkova serrata* [52] and *Magnolia wufengensis* [53]. In plants, SOD plays an important role as an active enzyme for ROS scavenging and cooperates with other antioxidant enzymes, such as CAT and POD, to alleviate the damage caused by ROS [54] and stabilize the biofilm structure and function to maintain the normal physiological metabolic balance and improve the plant's resistance to stress. When the plant is under adverse stress, the balance between ROS accumulation and the scavenging system is maintained by enhancing the activity of the oxidative enzymes SOD, POD, and CAT to mitigate the toxic effects of membrane lipid peroxidation on the cell membrane structure and intracellular substances. In the present study, all three PGRs increased the activity of oxidative enzymes in leaves, which is consistent with the results of studies on *Primulina yungfuensis* [55] and *Lolium perenne* [56]; among the three, the POD activity increased most significantly and was up to 4–5 times

higher than that of CK, followed by CAT, while the SOD activity increased the least. This suggests that POD and CAT, which are the second line of defense against ROS in *M. alternifolia* leaves, play an important role in breaking down the hydrogen peroxide produced by the disproportionated SOD reaction into H_2O and O_2 and reducing the formation of hydroxyl radicals, thereby adapting the plant to the dwarfing mechanism and enhancing its resistance to stress. POD mainly affects plant growth by controlling the structure of the cell wall. Increased POD activity can reduce the content of IAA in the plant and inhibit the elongation of cell walls, thus retarding the growth of the plant [57].

Pearson correlation analysis showed that SS was positively correlated with POD, SOD, and CAT under the three PGR treatments, and the antioxidant enzyme activity was negatively correlated with MDA content, indicating that the PGRs mainly increased the correlation between *M. alternifolia* antioxidant enzymes and osmoregulatory substances to affect the plant's resistance to stress.

4.4. Effects of PGRs on Endogenous Hormones of M. alternifolia

The application of PGRs had a significant effect on the endogenous hormones of *M. alternifolia* in pots, generally causing a significant decrease in the contents of IAA, GA, and ZT in the leaves and a significant increase in the content of ABA compared to CK. Further, all three were positively correlated with IAA, GA, and ZT and negatively correlated with ABA, which is consistent with previous findings on cabbage (*Brassica oleracea*) [58], *M. wufengensis* [59], and *Cyperus esculentus* [60]. Both the plant height and new branch length of *M. alternifolia* were positively correlated with IAA, GA, and ZT and negatively correlated with ABA; in contrast, stem thickness was negatively correlated with IAA, GA, and ZT and positively correlated with ABA. PGRs reduce GA levels in plants by decreasing the activity of enzymes involved in GA synthesis, thereby inhibiting cell elongation in the interstem elongation zone, shortening the internode length, and achieving dwarfism. Some precursors of GA are closely related to IAA, ABA, CAT, and ethylene (ETH), so changes in GA levels will affect the overall endogenous hormone levels in plants [61]. In addition, the physiological effects among endogenous hormones show characteristics of interaction and coupling, which, in turn, makes the relationship between endogenous hormones and physiological processes both close and complex [62]. The GA response to stress is similar to that of IAA, showing reduced levels and disruption of normal plant growth and development, as has been demonstrated in studies on many crops [63,64]. GA has the same synthetic precursor as ABA–farnesyl diphosphate (FPP), and when GA synthesis is blocked, the ABA content is increased, while the main physiological effect of plant growth retardants is to block GA synthesis [65]. ZT can promote cell division, shorten node spacing, and prevent the breakdown of SP in plants, thus delaying plant senescence.

In this experiment, the GA/ABA, IAA/ABA, ZT/ABA, (IAA + GA)/ABA, and (IAA + GA + ZT)/ABA ratios of dwarfed *M. alternifolia* were significantly reduced, which is consistent with the results of previous studies on *M. wufengensis* [66] and Phalaenopsis [67], and the reason is the synergistic effect of endogenous hormones in the plant. The ratios reflect the growth potential of the plant: when the ratios are higher, the plant has more vigorous nutritional growth; when the ratios are lower, the tendency of rapid growth is suppressed and the reproductive growth of the plant is promoted [68].

5. Conclusions

The results of this study demonstrate that foliar spraying of PGRs had a significant inhibitory effect on the growth of *M. alternifolia* and improved its ornamental value in various ways. On balance, PP333 (P3) at 2000 $mg \cdot L^{-1}$ had the best dwarfing effect, and the resulting potted *M. alternifolia* plants showed ideal dwarfing effects, with a compact plant shape and green leaf color, which greatly improved their resistance and ornamental value. On the other hand, the S2 treatment at 100 $mg \cdot L^{-1}$ S3307 was the most effective at enhancing the activity of the antioxidant enzymes of potted *M. alternifolia* plants and was relatively good at improving stress resistance.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14040732/s1, Table S1: Effects of three plant growth retardants on the height of *M. alternifolia*; Table S2: Effects of three plant growth retardants on the roughness of *M. alternifolia*; Table S3 Effects of different plant growth retardants on leaf morphology of *M. alternifolia*; Table S4: Effects of three plant growth retardants on the Chlorophyll content of *M. alternifolia*; Table S5: Leaf anatomical structure of *M. alternifolia* with different plant growth retardants; Table S6. Soluble sugar content of *M. alternifolia* treated with different PGRs in different periods ($mg \cdot g^{-1}$); Table S7. Soluble protein content of *M. alternifolia* treated with different PGRs in different periods ($mgprot \cdot mL^{-1}$); Table S8. MDA content of M. treated with different PGRs in different periods ($nmol \cdot g^{-1}$); Table S9. SOD activity of *M. alternifolia* treated with different PGRs in different periods ($U \cdot g^{-1}$); Table S10. CAT activity of *M. alternifolia* treated with different PGRs in different periods ($U \cdot g^{-1}$); Table S11. POD activity of *M. alternifolia* treated with different PGRs in different periods ($U \cdot g^{-1}$); Table S12. Correlation analysis of physiological indexes of *M. alternifolia* under PP333 treatment; Table S13. Correlation analysis of physiological indexes of *M. alternifolia* under S3307 treatment; Table S14. Correlation analysis of physiological indexes of *M. alternifolia* under CCC treatment; Table S15. Content of endogenous hormones in leaves of *M. alternifolia* treated with PGRs (35d); Table S16. Correlation analysis of endogenous hormones in *M. alternifolia* under PP3333 treatment; Table S17. Correlation analysis of endogenous hormones in *M. alternifolia* under S3307 treatment; Table S18. Correlation analysis of endogenous hormones in *M. alternifolia* under CCC treatment; Table S19. Ratio of different endogenous hormone contents in *M. alternifolia* leaves treated with PGRs; Table S20-1. Correlation analysis of physiological indexes and morphological indexes of *M. alternifolia* under PP333 treatment; Table S20-2. Correlation analysis of physiological indexes and morphological indexes of *M. alternifolia* under S3307 treatment; Table S20-3. Correlation analysis of physiological indexes and morphological indexes of *M. alternifolia* under CCC treatment; Table S20-4. Correlation analysis between endogenous hormones and morphological indexes of *M. alternifolia* under PP3333 treatment; Table S20-5. Correlation analysis between endogenous hormones and morphological indexes of *M. alternifolia* under S3307 treatment; Table S20-6. Correlation analysis between endogenous hormones and morphological indexes of *M. alternifolia* under CCC treatment.

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