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Effects of Different Regulating Measures on the Floral and Nutritional Physiology of Lemon

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Abstract: Lemon is an important economic crop in the world and can bloom several times each year. The Dehong prefecture of Yunnan province is one of the main lemon-producing areas in China, and is a suitable area for planting high-quality lemons in China. However, the hot and rainy summers in Yunnan are not conducive to flower bud differentiation, which leads to low lemon yield. Therefore, normal flower bud differentiation is important to guarantee lemon production and quality. In this study, we selected some lemon trees for a pot experiment, and we sprayed the lemon leaves with gibberellin and paclobutrazol to regulate lemon flower formation. We set four separate concentration gradients for each regulator: 50, 100, 150, and 200 mg/L. The PBZ concentration gradients were 200, 400, 600, and 1200 mg/L. After the experiment, we determined and analyzed the morphological index and fruit quality of the lemon trees. The results showed that under the same cultivation and management conditions, spraying paclobutrazol substantially inhibited the growth of lemon shoots, increased the fruit setting rate, and improved the fruit yield. However, gibberellin considerably reduced the number of lemon flowering branches and promoted the vegetative growth of the lemons. When the concentration of paclobutrazol was 600 mg/L, the amount of lemon fruits reached the maximum, which remarkably increased the titratable acid and soluble solids contents of the fruit and ultimately increased the fruit quality compared with the control. Altogether, selecting the appropriate concentration of regulators to control the flowering and fruit setting of fruit trees is important and has value in guiding actual production.

Keywords: lemon; paclobutrazol; gibberellin; flowering; fruit quality

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1. Introduction

Lemons (*Citrus limon* (L.) Burm. F.), a fruit tree that can bloom several times a year, is an important economic crop in the world. The fruit ripening time of lemons is in the fruit-offseason—spring—so is suitable for the domestic market when spring fruits are scarce. The Dehong prefecture of Yunnan province is one of the main lemon-producing areas and is suitable for planting high-quality lemons in China [1] due to its early, high-quality, off-season production, and annual output [2]. However, the high temperatures and rainy summers in Yunnan hinder lemon flowering, which considerably reduce the fruit yield. Therefore, normal flower bud differentiation is crucial for the quality and production of lemons.

In recent years, plant growth regulators have been widely used in agricultural production. They have a remarkable effect on promoting flowers and controlling branches of fruit trees [3,4]. Plant growth regulators, such as gibberellin (GA), play an important role in the transportation and distribution of nutrients from the source to the reservoir in fruit trees, and inhibit the flowering effect of fruit loads [5]. This inhibiting effect of gibberellin has been confirmed in fruit trees, such as peach [6], plum [7], pear [8], and apple [9]. The application of gibberellin during the stage of citrus flower bud differentiation can reduce the flowering intensity [10], increase the fruit setting rate of non-pollinated ovaries [11], delay chlorophyll decomposition, inhibit carotenoid accumulation, and promote the vegetative growth of plants. Spraying GA_3 can deploy enough nutrients to the growing branches in time, which promotes vegetative growth but is not conductive to the reproductive growth (flowering) of branches [12,13]. Gibberellin treatment resulted in a decreased expression of many MADS-box genes in shoots, some of which are involved in the development of floral meristems and floral organs [14]. When performing foliar spraying with GA_3 (20–50 mg/L), the flowering of citrus was reduced by 25–60%. In the process of the flower bud differentiation of citrus, GA_3 inhibited the production of flower buds, resulting in leafy branches in the apex as well as a higher percentage of flowers, which promoted fruit development [10]. Pollination induced GA_1 synthesis in the ovary, and when pollination was presented by removing the male flowers, the levels of this hormone reduced in the ovary, and young fruit abscission increased. However, these effects can be counteracted by the administration of GA_3 [11]. Paclobutrazol (PBZ) is an inhibitor of GA biosynthesis. The use of paclobutrazol has considerable potential for controlling excessive branch growth in citrus. The effect of PBZ on the stem is reflected in its control of the longitudinal expansion of cells, thereby shortening the length of the internodes and dwarfing the plant. At the same time, the number of vascular tissues in the stem increases, the thickness of the stem wall increases, and the basal stem thickens [15]. The application of PBZ can increase the flowering and fruiting of plants [16,17]. In a previous study, the application of PBZ to mangoes changed the contents of gibberellin, abscisic acid, and cytokinin, and induced early flowering [18]. Moreover, PBZ promotes flowering in many citrus species, such as *C. aurantifolia* and *C. sinensis*. Limes planted in Nepal even had their flowering date advanced by 70 days through PBZ application [19]. The effect of PBZ on citrus flowering depends on the fruit load, and PBZ cannot promote flowering beyond its threshold. Flower bud differentiation is associated with changes in hormones and sugars in plants [20,21]. In citrus, PBZ can inhibit growth by influencing the biosynthesis of gibberellin and inducing flowering [22]. High and low levels of endogenous GA in leaves were inversely or positively proportionally correlated with the number of flowers, respectively [23]. In citrus varieties with a low parthenocarpic ability, the application of the exogenous hormone GA_3 can substantially reduce the ABA content in the ovary after flowering, thereby reducing the shedding of small fruits. In contrast, in parthenocarpic cultivars such as mandarin oranges, the inhibition of GAs synthesis by PBZ increases ABA levels and fruitlet abscission [24].

However, few detection methods for the positive and negative regulation of lemon flower promotion are available either at home or abroad. As such, in this study, we used different concentrations of PBZ and gibberellin to spray lemons to investigate the effects of the two regulators on flower formation and nutritional physiology, to provide some theoretical basis for high-quality, high volumes, and stable yields of lemons.

2. Materials and Methods

2.1. Plant Materials

We obtained the plant materials from three-year-old grafted Eureka lemons, which were located at the scientific research base of the Lemon Experimental Station of the Institute of Tropical and Subtropical Economic Crops of the Yunnan Academy of Agricultural Sciences, Ruili city, Dehong prefecture (97°52′12″ E, 24°1′9″ N, elevation 750.4 m).

2.2. Experiment Design and Methods

We conducted the experiment under potting conditions. The growth medium was a 3:1 mixture of latosolic red soil and perlite, using 65 kg of the mixture per pot. We selected lemon trees with the same growth status for exogenous hormone treatment. In the experiment, we used gibberellin (GA_3) and paclobutrazol (PBZ) as plant growth regulators, and we set four concentration gradients to each regulator. The GA_3 concentration gradient was 50 (GA 50), 100 (GA 100), 150 (GA 150), and 200 mg/L (GA 200). The PBZ concentration gradient was 200 (PBZ 200), 400 (PBZ 400), 600 (PBZ 600), and 1200 mg/L (PBZ 1200). We sprayed the plant growth regulators on the leaves once every 10 days for 30 days, for a total of 3 times. According to the experimental design, we set 9 treatments (1 control) and 9 replicates, with a total of 81 lemon trees. The experiment duration was 72 days.

2.3. Measurement Indexes and Methods

2.3.1. Investigation of Plant Growth Index

At the end of the experiment, we observed the morphology of the plants and leaves that we had treated with different hormones. We measured the length of the topmost branches, number of leaves, leaf longitudinal and transverse diameter, petiole length, length of the internode, and diameter of the stem were measured with Vernier calipers and rulers. We manually counted the total, flowering, and new branches on the 52nd and 72nd days of the experiment (when the lemons began to blossom). We divided the flowering branches into four types: the top flowering branch with leaves, the top flowering branch without leaves, the branch with inflorescences and leaves, and the non-leaf branch with inflorescences. We separately recorded the number of these four types of flowering branches on the 52nd and 72nd days.

2.3.2. Fruit Load and Fruit Quality of Lemons Regulated by Different Hormones

We counted the number of fruits per tree for each treatment, then, we fit the data of the hormone concentration-fruit number curve. For each treatment, we randomly selected nine fruits on the basis of health, size consistency, color uniformity, and features of commercial maturity. We brought these fruits back to the laboratory to determine the fruit quality. We measured the longitudinal and transverse diameters and skin thickness of the lemons with a Vernier caliper. The fruit shape index is the ratio of the transverse and longitudinal diameters of the fruit. We also weighed the single fruits. We used a citrus juice squeezer, extract the juice. Juice yield was calculated by using the weight of juice divided by the weight of lemons. We determined the soluble solids (TSS) and titratable acid (TA) of the lemon fruits with a hand-held refractometer (PAL-BX | ACID1, Atago Co., Ltd., Tokyo, Japan). We titrated vitamin C (VC) with 2, 6-dichlorindophenol [25].

2.3.3. Dynamic Changes C/N Ratio in Leaves

We sampled the leaves every seven days from the beginning of the experiment. We collected 15 leaves from each treatment, and we sampled 3 replicates to obtain the total soluble sugar, starch, and nitrogen contents. We determined the total soluble sugar contents in the leaves with the modified anthrone method [26], and the starch content in the leaves using the anthrone-sulfuric acid method [27]. We determined the nitrogen content using the Kjeldahl method [28]. Our calculations of the leaf carbon-to-nitrogen (C:N) ratio were based on the total content of both carbon (the total soluble sugar and starch) and nitrogen.

2.4. Data Processing and Analysis

We used SPSS 17.0 statistical software (SPSS Inc., Chicago, IL, USA) for variance analysis and the least significant difference (LSD) test. We used Origin 8.5 software (Origin lab cooperation, Hampton, MA, USA) for drawing.

3. Results

3.1. Effects of Hormone Treatment on Lemons

Different hormone types and levels had different effects on the lemon shoot characteristics (Figure 1). Compared with the control, we found a substantial difference in the length of the topmost shoots according to the PBZ spraying concentration. The length of the topmost shoots increased under PBZ600 and PBZ1200, but decreased under PBZ200 and PBZ400 treatments (Figure 1A). The length of the topmost shoots varied from 27.34 (GA50) to 35.06 (GA200) cm after spraying GA_3. The length of the topmost shoots increased with the increase in the gibberellin concentration. For the number of leaves, PBZ600 (22.60) and PBZ1200 (33.06) plants had more leaves than the control plants (Figure 1B). GA100 treatment decreased the number of leaves, but other gibberellin treatments increased the number of leaves. The leaf longitudinal diameter of the lemons was no different in any PBZ or GA treatments (Figure 1C). Moreover, spraying PBZ notably increased the leaf transverse diameter (Figure 1D), and shortened the petiole and internode lengths (Figure 1E,F), but we noted no significant association with the spraying concentration. The GA_3 treatment increased the leaf longitudinal diameter of the lemon compared with that in the control (Figure 1C), and we found no significant difference among the spraying concentration treatments. With the increase in the GA_3 concentration, GA_3 substantially shortened the transverse diameter of the leaves (Figure 1D), but had little effect on the petiole and internode lengthes or stem diameter (Figure 1E–G).

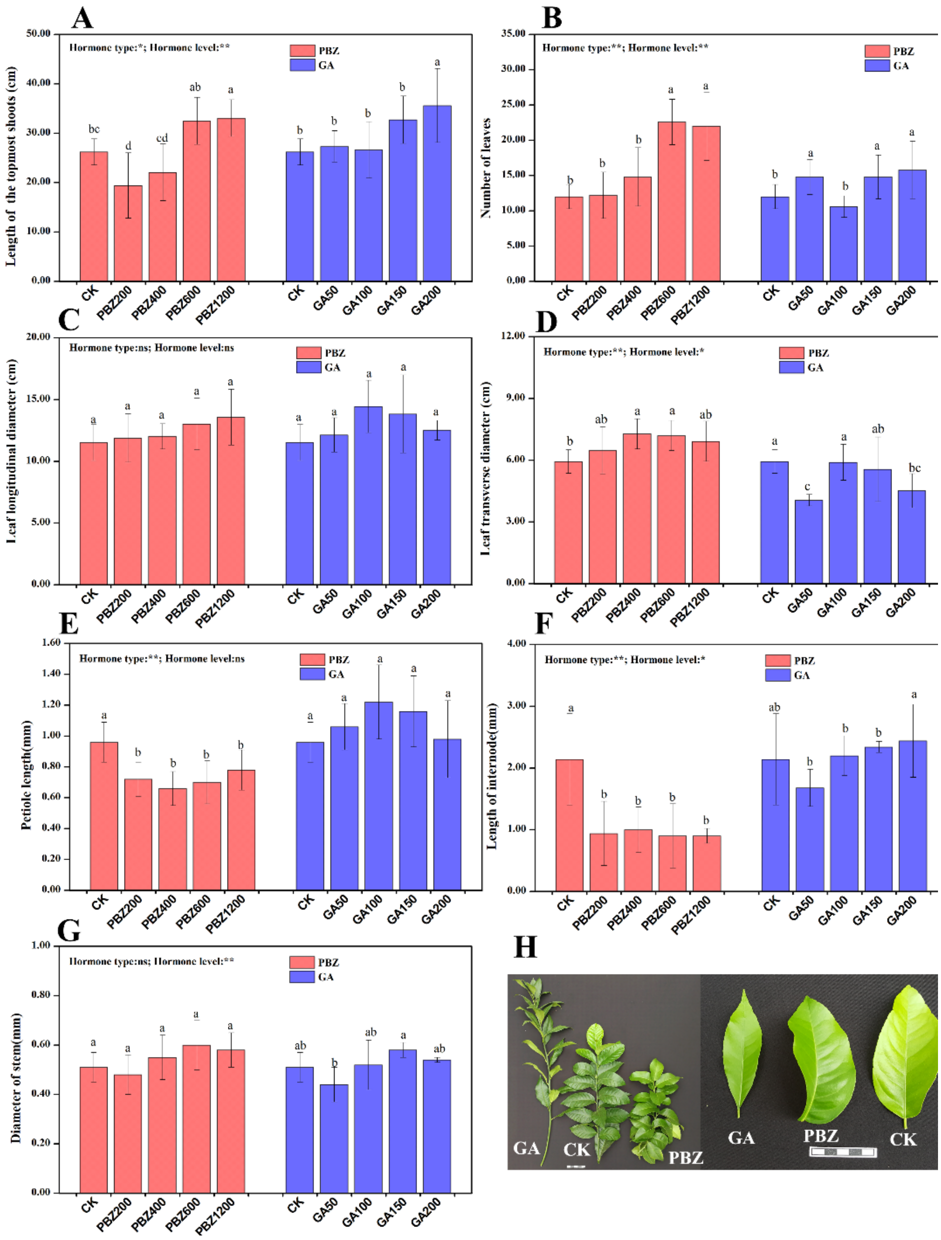

Figure 1. Effects of hormone regulation on the length of the topmost shoots (**A**), number of leaves (**B**), leaf longitudinal diameter (**C**), leaf transverse diameter (**D**), petiole length (**E**), length of internode (**F**), diameter of stem (**G**). (**H**) Plant and leaf morphology after treatment with different hormones. The

red and blue bars represent PBZ and GA, respectively. Different letters (a, b, c and d) denote significant differences based on a p value < 0.05. * denotes significant difference (p < 0.05), and ** denotes extremely significant difference (p < 0.01).

3.2. Types of Lemon Branches Regulated by Different Hormones

We found no significant difference in the total branches of the trees treated with different hormones (Figure 2A), but the effects on the number of flowers and new branches were different (Figure 2). On the 52nd day of the experiment, the application of PBZ, which we applied in the same way as GA_3, considerably reduced the number of flowering branches. At the end of the experiment (72nd day), the application of PBZ increased the number of branches for all types of lemons. However, the number of new branches increased, and the number of flowering branches was reduced under the GA treatment (Figure 2B,C). When the concentration of PBZ was 600 mg/L, the number of flowers and inflorescence branches reached the maximum 211.00 (Figure 2B).

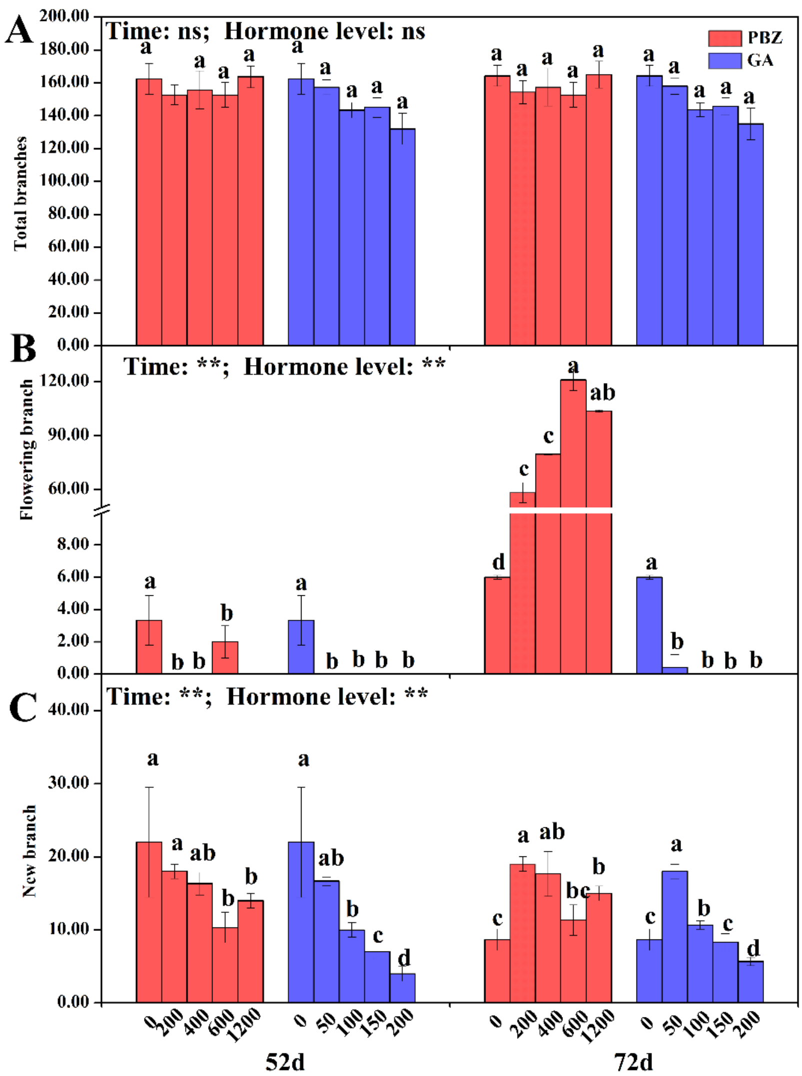

Figure 2. Effects of hormone regulation on types of lemon total (**A**), flowering (**B**), and new branches (**C**). Different letters (a, b, c and d) denote significant differences based on a p value < 0.05. ** denotes extremely significant difference (p < 0.01).

We mainly observed the effects of hormone regulation on the number of lemon flower branches on the 72nd day of the treatment (Figure 3). The number of top flowering branches with leaves first increased and then decreased with the increase in the PBZ concentration on the 72nd day (Figure 3A). The number of top flowering branches without the leaves of the lemons increased with the increase in the PBZ concentration on the 72nd day (Figure 3B). The number of top flowering branches without leaves was 13.15- to 43.45-fold higher than that of the control, indicating the promoting effect of PBZ on flowering branches. The numbers of branches with inflorescences and leaves were also higher than in the control, especially for plants in the PBZ600 treatment (Figure 3C). The branches with inflorescences and leaves were the largest in number of the other three types of flowering branches. The PBZ application also increased the number of non-leaf branches with inflorescences, especially for plants under the PBZ200 and PBZ1200 treatments (Figure 3D). The application of gibberellin substantially reduced the number of flowering branches of all types (Figure 3), which all decreased with the increase in the gibberellin concentration. Thus, GA treatments would not be favorable for the four types of flower branches.

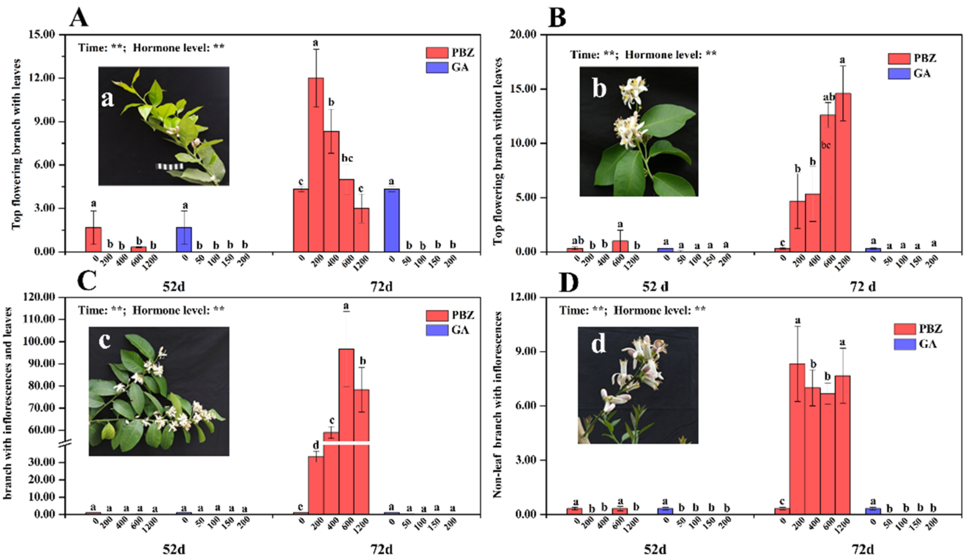

Figure 3. Effects of hormone regulation on the types and number of lemon flower branches: top flowering branch with leaves (**A**), top flowering branch without leaves (**B**), branch with inflorescences and leaves (**C**), and non-leaf branch with inflorescences (**D**).Different letters (a, b, c and d) denote significant differences based on a p value < 0.05. ** denotes extremely significant difference (p < 0.01).

3.3. Fruit Load, Fruit Morphology, and Fruit Quality of Lemons Regulated by Different Hormones

The effects of different types of hormones and hormone levels on the fruit load of lemon were remarkably different (Figure 4A). PBZ substantially increased the fruit load of the lemons, and the fruit load of the lemons first increased and then decreased with the increase in the PBZ concentration, which reached the maximum when the PBZ concentration was 600 mg/L. However, gibberellin application was not conducive to increasing the fruit load. We observed only a small amount of fruit in the GA50 treatment. The curve fitting between PBZ concentration and fruit load showed that when the PBZ spraying concentration was 850 mg/L (Figure 4B), the fruit load reached a maximum of 48 per plant, which was 5.2 times higher than that in the control. Different types of hormone treatments affected the lemon fruit quality and morphology (Figure 4D and Table 1). The PBZ treatment considerably reduced the fruit weight, fruit longitudinal diameter, fruit transverse diameter, and skin thickness of the lemons, but notably increased the juice yield. The GA50 treatment plants had higher values than the control plants in terms of fruit weight, fruit thickness, and juice yield. The fruit shape index values ranged from 0.70 (PBZ200) to 0.92 (PBZ600). A high concentration of PBZ (600 and 1200 mg/L) improved the fruit shape index, but GA had no noticeable effect. With PBZ1200 concentration, the juice yield of the lemon fruits was 1.4 larger than that of the control. The application of gibberellin decreased the VC content and increased the TA and TSS contents (Figure 4C).

The VC content also decreased in the PBZ treatment group, and the TA and TSS varied with the PBZ concentration.

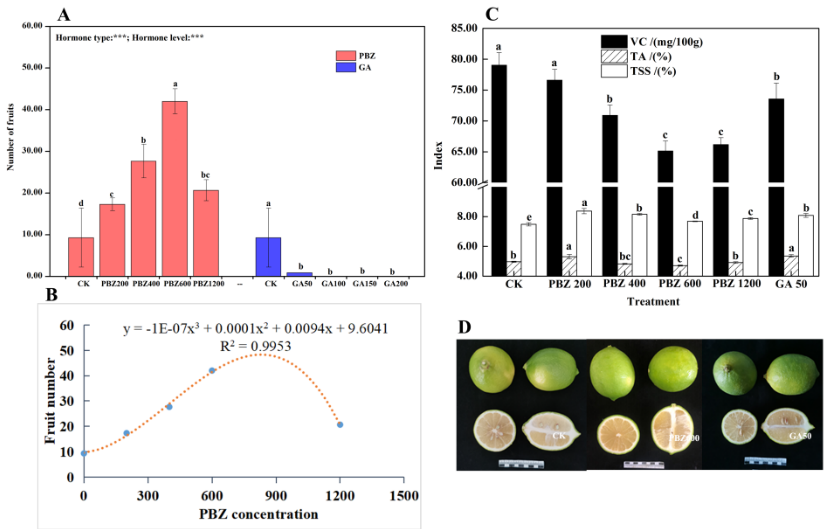

Figure 4. Effects of hormone regulation on fruit load and quality of lemons. (**A**) Effects of hormone regulation on number of fruits; (**B**) PBZ concentration–fruit number curves fit to pooled data calculated by an equation; (**C**) effects of hormone regulation on TSS, TA, and VC contents; (**D**) fruit morphologies under different treatments. Different letters (a, b, c and d) denote significant differences based on a p value < 0.05. *** denotes extremely significant difference (p < 0.001).

Table 1. Effects of hormone regulation on quality of lemon fruit. Different letters (a, b, c and d) denote significant differences based on a p value < 0.05.

Treatment	Fruit Weight (g)	Longitudinal Diameter (cm)	Transverse Diameter (cm)	Fruit Shape Index	Fruit Thick (cm)	Juice Yeild (%)
CK	133.87 ± 3.40 a	7.91 ± 0.23 a	5.69 ± 0.13 a	0.72 ± 0.00 c	0.61 ± 0.03 ab	39.83 ± 1.23 e
PBZ200	132.90 ± 2.02 a	8.18 ± 0.03 a	5.71 ± 0.07 a	0.70 ± 0.01 c	0.57 ± 0.01 cd	45.11 ± 1.22 d
PBZ400	128.90 ± 1.37 b	7.48 ± 0.23 b	5.47 ± 0.12 b	0.73 ± 0.04 c	0.59 ± 0.02 bc	48.02 ± 0.32 c
PBZ600	123.10 ± 1.47 c	6.23 ± 0.16 d	5.75 ± 0.09 a	0.92 ± 0.04 a	0.64 ± 0.02 a	52.35 ± 1.02 b
PBZ1200	127.83 ± 1.65 b	7.05 ± 0.14 c	5.45 ± 0.07 b	0.77 ± 0.01 b	0.56 ± 0.02 d	55.81 ± 1.48 a
GA50	134.6 ± 4.21 a	7.84 ± 0.42 a	5.56 ± 0.11 a	0.71 ± 0.02 c	0.64 ± 0.04 ab	42.70 ± 3.11 d

3.4. Contents of Soluble Sugar, Starch, and C/N Ratio Regulated by Different Hormones

With the growth of lemons from July to August in 2017, the soluble sugar contents in the lemon leaves increased under the PBZ treatment, but decreased under the GA_3 treatment (Figure 5A,B). When the concentration of PBZ was 600 mg/L, the soluble sugar content of the lemon leaves reached the maximum during the prophase of sampling. Most of the plants in the treatments (except for PBZ1200) had a lower TSS content than those in the control on 12 August 2017. The starch content in the lemon leaves decreased with the increase in both the PBZ and GA_3 hormone levels (Figure 5C,D). The results also revealed that the starch content of the plants in the PBZ and GA_3 treatments decreased as the experiment continued. Carbohydrates are the main product of plant photosynthesis and are an important substance involved in plant life metabolism, the C/N ratio of the lemon leaves showed a trend of first increasing and then decreasing with the increase in PBZ concentrations (Figure 5E). Among the different PBZ concentrations treatments, when the concentration of PBZ was 1200 mg/L, the C/N of the leaves was the highest. For the GA_3 treatment, the C/N ratio of the lemon leaves was lower compared to those in the control as the experiment continued (Figure 5F).

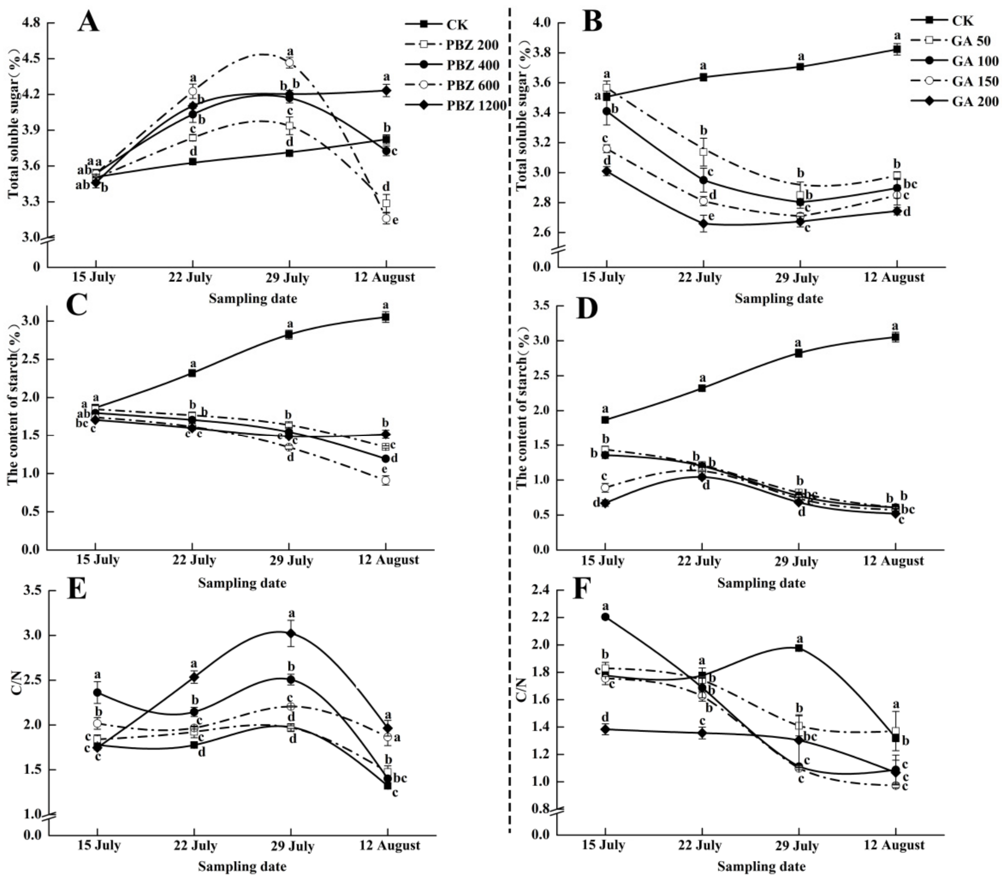

Figure 5. Effects of hormone regulation on total soluble sugar (**A**,**B**), and the starch contents (**C**,**D**), and C/N ratio (**E**,**F**) of lemon leaves. The left-hand plants were treated with paclobutrazol, and the right-hand plants were treated with gibberellin. Different letters (a, b, c and d) denote significant differences based on a p value < 0.05.

4. Discussion

4.1. Lemon Growth and Development under the Regulation of Different Hormones

Spraying PBZ on fruit trees can inhibit the growth of new shoots, promote fruit setting, and increase the fruit yield [29]. The application of PBZ can not only increase the number and ratio of fruit flower buds, but can also improve the quantity and quality of flowers, and promote the flowering process and early flowering [30]. In this experiment, the lengths of the petiole and interleaf of the lemons were shortened by spraying PBZ. Liu [31] also reached the same conclusion when studying the regulation effect of different flower promotion measures on lemons. Figure 2B shows that the application of PBZ increased the number of all flowering branches of lemons. Our results showed that the application of gibberellin reduced the numbers of lemon flower, terminal flower, and inflorescence branches: all decreased with the increase in gibberellin concentration, which is consistent with the finding reported by Du [32], who found that the application of gibberellin inhibited the flowering of lemons and reduced the number of lemon flower buds. Sun [33] also reached the same conclusion in *Vernicia fordii*.

The physiological effect of PBZ is mainly directly or indirectly regulating the distribution of photosynthetic products by blocking the synthesis of gibberellin in plants. Thus, the flowering, fruit setting rate, and fruit yield of fruit trees increase [34,35]. In this study, the application of PBZ increased the fruit load of the lemons, and with the increase in the PBZ concentration, the fruit load of the lemons first increased and then decreased, which reached a maximum when the PBZ concentration was 600 mg/L. Gibberellin reduced the fruit load of the lemons, which was consistent with the findings of Du [32]. PBZ application increased the titratable acid and soluble solids contents, but reduced the VC content of the lemon fruits, which is consistent with the results reported by Yan [36] and Yang [37].

4.2. Effects of Hormone Regulation on the Nutritional Physiology of Lemon Leaves

Spraying PBZ can regulate the nutritional growth of fruit trees, improve the soluble sugar and starch contents in plants, and increase the C/N ratio, which promotes flower bud differentiation, improves the flowering rate, and increases the yield [38,39]. The application of gibberellin inhibits the formation of flower buds by altering the distribution of carbohydrates in plants [40]. In this study, the soluble sugar content in the lemon leaves treated with PBZ first increased and then decreased with the increase in hormone levels, and the soluble sugar content in the lemon leaves reached a maximum when the PBZ concentration was 600 mg/L, but the starch content in the lemon leaves was reduced for different PBZ concentrations. This is consistent with the results of Yang [37]. Gibberellin treatment reduced the soluble sugar and starch contents in lemon leaves, and both of them decreased with the increase in hormone level. Zhang [41] also reached the same conclusion using litchi.

The C/N ratio of lemon leaves was increased by the application of PBZ. Yang [42] studied the effects of PBZ application, and drought stress on seedling vigor, photosynthetic capacity, and non-structural carbohydrates of *Phyllostachys edulis*, and concluded that drought stress increases the carbon–nitrogen ratio in the leaves *of Phyllostachys edulis*. However, spraying PBZ at the same time of drought stress may reduce the carbon–nitrogen ratio in the leaves. Gibberellin also reduced the C/N ratio of the lemon leaves.

4.3. Effects of Hormone Regulation on the Nutritional Physiology of Lemon Leaves

Spraying PBZ reduced the single fruit weight, fruit longitudinal diameter, fruit transverse diameter, skin thickness, and VC content of the lemons, but increased the juice yield and TSS content of the lemons, which is similar to the research results obtained by Fu [43] and Cao [44]. After spraying GA, the content of VC decreased, but the TSS of the fruit increased. However, Wu [45] found that spraying GA resulted in a substantial increase in the acid content of the pulp and a notable decrease in the sugar content of the pulp, which may have related to the spraying time and concentration. From the field experiment in this study, we found that the regulation technical measures used for autumn-flowering and fruiting can considerably improve the yield of lemon autumn flowers and fruits, but has little effect on the quality of autumn lemon flowers and fruits.

5. Conclusions

By regulating the distribution of photosynthetic products in plants, PBZ applications can promote the flowering process of fruit trees and make them bloom earlier. In this study, we found that the optimum PBZ concentration to promote lemon flowering was 600 mg/L, and the fruit quality increased by spraying PBZ on fruit trees. The physiological effect of gibberellin was the opposite to that of PBZ. Therefore, hormones should be reasonably applied to control the flowering and setting of fruit trees, and the concentration of hormones should be controlled.

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