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The Chemical Capping Regulation Mechanism of Cotton Main Stem Growth

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Abstract: In China, due to labor shortages and increasing labor costs, manual topping is gradually being replaced by chemical capping with mepiquat chloride (DPC). External chemicals can adjust plant growth by affecting endogenous hormones. Based on the hormone changes combined with the development of the main stem of cotton plants, a comparative experiment was conducted in 2019 and 2020 to determine the regulatory mechanism of the growth of the cotton main stem after chemical capping. In the experiment, two treatment times (T1: 12 July, T2: 18 July) and two treatment agents (CA [chemical capping agent] and DPC) were set, the hormone (auxin IAA, abscisic acid ABA, Gibberellin GA_3 and Zeatin ZR) concentrations at the top of main stem (0–5 and 5–10 cm) were continuously measured and the main stem development situation was observed and recorded. The results showed that after chemical capping, the IAA concentration decreased firstly and increased later, lower than that of CK. ABA concentration increased significantly and GA_3 concentration decreased significantly compared with CK. ZR concentrations fluctuated obviously at T1 and gently at T2. In terms of main stem growth, the plant height, number of fruit branches and average length of upper internode (fifth and above) were decreased compared with CK, while the CA treatment was inhibited more strongly than the DPC treatment. To conclude, chemical capping operation affected the hormone concentration at the plant apex significantly and effectively regulated plant development. In comparison with DPC treatment, CA regulated hormones effectively, which is favorable for conducive reasonable plant shaping.

Keywords: cotton; CA (chemical capping agent); DPC (mepiquat chloride); hormone concentration; plant development

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1. Introduction

Cotton (*Gossypium hirsutum* L.) capping technology, also known as topping, refers to the artificial control of the upward growth of cotton and the adjustment of the growth center and nutrient transport direction. For a long time, cotton production in China has mainly relied on artificial removal of the growing tip of cotton plants to achieve capping, known as manual topping (MT). With the development of technology and the increase in labor costs, chemical capping has gradually replaced MT in production. Chemical capping refers to the application of plant growth regulator spray on the top of the plant to forcibly delay or inhibit the growth of the cotton apex in order to regulate vegetative growth and reproductive growth [1]. In the 1990s, the United States, Australia and other countries applied plant growth retardants such as methylonium, combined with water and fertilizer management to cotton plant growth and achieve a natural stop during the flowering and boll period, which was an early exploration of chemical capping of cotton [2,3]. Chinese scientists have also studied cotton chemical capping technology and found that the regulator products with the plant growth retarder DPC, or plant growth inhibitor flutenylamide as the active ingredient, could achieve an ideal effect; for example, reducing plant height, shortening

the length of branches or achieving a more compact plant type [4–6]. Qi et al. [7] and Xu et al. [8] believe that chemical capping treatment time and dosage would affect the regulation effect, i.e., the earlier the treatment time and the higher the dosage, the better the effect of growth control. Overall, the application methods and effects of chemical capping of cotton have been widely studied, and a relatively mature technical system has been developed.

The basic mechanism of chemical capping technology is regulating plant growth by influencing the synthesis of endogenous hormones. As highly active substances, plant hormones are ubiquitous in plants and participate in the regulation of the whole growth process of plants [9], and obvious effects can be observed even with trace amounts. Hormones do not act independently in plants, promoting and antagonistic effects have been observed among various hormones, which regulate plant growth and development in a systematic way [10,11]. It is well-established that auxin (IAA) can promote plant growth; Thimann et al. [12] and Ljing et al. [13] concluded that apical dominance—the inhibitory effect imposed by an actively growing shoot apex on axillary buds—is mediated at least in part by the synthesis and movement of auxin from young expanding leaves into the basipetal polar auxin transport stream in the main stem. Cytokinin (CTK) can stimulate the synthesis and outward transport of endogenous IAA in buds, thus promoting lateral bud growth [14]. Christine et al. [15] suggested that abscisic acid (ABA) can inhibit lateral branch growth when it is not controlled by IAA and CTK. Gibberellins (GA) and ethylene (ET) can regulate plant extension growth, with GA stimulating cell elongation along the longitudinal axis [16], while ET is known to inhibit extension growth [17–19]. Zhang et al. [20] found that after chemical capping, the IAA content in the fourth leaves from the tip of the main stem showed an obvious and rapid decline compared with no topping and manual topping; GA_3 content was close to that of no topping; CTK content increased slowly but reached the highest peak; and ABA content maintained a high level. The ability of hormone synthesis is jointly influenced by genetic basis, development stage and external conditions [21,22]; therefore, chemical capping operations at different periods and under different growth conditions will have different effects.

The change in hormone content at the tip of the main stem, which is the most active site of plant growth, is more susceptible to exogenous substances. However, existing studies about chemical capping have mainly focused on the fourth leaves from the top of the main stem, and hormone content changes in the tip of the main stem after chemical capping have not been studied. Accordingly, a comparative experiment of different cotton chemical capping methods was carried out by comparing the hormone concentration changes and development of plant apex after chemical capping, attempting to explain the regulation mechanism of chemical capping on plant apex growth to provide a theoretical basis for further popularization and application.

2. Materials and Methods

2.1. Experimental Site Overview

The experiment was conducted in the Industrial Crop Research Institute of Liaoning province (Liaoyang, Liaoning, China). The previous crop was cotton and the soil was sandy loam with organic matter content of 1.97%, total nitrogen content of 0.08%, alkali-hydrolyzed nitrogen concentration of 73.4 mg/kg, available phosphorus of 23.6 mg/kg and available potassium of 247.5 mg/kg. The experiment was conducted via film mulching culture with planting density of 90,000 plants/hm^2. Cotton was sown on 22 April, and 375 kg/hm^2 of compound fertilizer (N:P:K = 15%:15%:15%) was applied as base fertilizer. During the experiment, the cultivation management method was the same as local production practice. Topdressing was not applied, and irrigation was properly applied according to the situation.

2.2. Experiment Materials

The earlier matured and verticillium wilt resistance cotton variety "Liao Mian 31" (Liao Shen Mian 2014002), which was bred by the Industrial Crop Research Institute of Liaoning Province, was used in the experiment. The chemical capping agent (CA) used was provided by Engineering Research Center of Plant Growth Regulator, Ministry of Education, China Agricultural University. The main ingredient was 25% methylpionium water agent, and an additive naphthenate was used to extend the control time for 5–7 days. The center also supplied the pure product of DPC (97% mepiquat chloride powder).

2.3. Experiment Design and Field Management

The experiment was conducted for 2 years over 2019 and 2020. Random block arrangement was adopted, and three repetitions were employed. The plot length was 8 m long, with 8-row blocks, and the area was 32 m^2. Uniform leaf spraying was applied during treatment with an average liquid amount of 450–600 L/hm^2. The treatment area and the surrounding area were isolated with plastic film to prevent droplet drift.

Two treatment times were set in the experiment: T1—around 12 July and T2—around 18 July; the specific treatment time was slightly adjusted according to the climate and plant development of the year. Two agents were selected: the chemical-capping agents (25% mepiquat chloride water agent + promoter, CA) and acetamine (97% mepiquat chloride powder, DPC) were used in the experiment. Water treatment was taken as control (CK), i.e., on the day of treatment, the control plot was sprayed with the corresponding amount of water.

The time and amount of treatment are shown in Table 1.

Table 1. Chemical-capping treatments design.

Treatment	No-Capping	T1: 12 July		T2: 18 July	
		CA	DPC	CA	DPC
Dosage	-	1125 mL/hm^2	120 g/hm^2	1125 mL/hm^2	120 g/hm^2
No	CK	I	II	III	IV

2.4. Measurement and Recording of Items

2.4.1. Plant Development

On the day of treatment, two points were selected, and five consecutive plants with uniform growth situation were labelled in each site. Three indexes, including plant height (cm), number of fruit branches and average length of upper (fifth and above) internodes (cm) were investigated, and then leaf spray was carried out. After treatment, the corresponding indicators were investigated every 7 days three consecutive times.

2.4.2. Hormone Concentration in Plant Apex

From the treatment day (before treatment), two plants with uniform growth were selected from each plot and then 10 cm of main stem tips were taken and immediately refrigerated in the laboratory by using an incubator with an ice pack. The leaves were removed and the samples were divided from top to bottom into two parts with 5 cm intervals. Approximately 1 g of samples was obtained from each part, wrapped in tin foil and numbered, frozen in liquid nitrogen and stored in an ultra-low temperature (−80 °C) refrigerator. After treatment, samples were taken every 3 days three times, and new plants were re-selected for each sampling.

The samples were entrusted to the Engineering Research Center of Plant Growth Regulator, Ministry of Education, China Agricultural University for hormone concentration testing, including auxin (IAA), abscisic acid (ABA), Gibberellin (GA_3), zeatin and zeatin nucleoside (ZR). Enzyme-linked immunosorbent assay (ELISA) was carried out as described by Yang et al. [23] and Zhao et al. [24].

2.5. Data Analysis

The data were averaged every year, and then both years' data were averaged for analysis. Excel 2013 was used for data collation and mapping. Using DPS 9.5 data analysis and processing statistical software, Duncan's new repolarization detection method was used to analyze the difference significance, with the significance level at 0.05 and 0.01.

3. Results and Analysis

3.1. Changes of Hormone Concentration in Plant Apex after Chemical Capping Treatment

3.1.1. Changes in IAA Concentration

As shown in Figure 1, after capping operation, the concentration of IAA at plant apex first decreased and became lower than that of CK, then increased to even higher than that of CK at T1. At T1, IAA concentration among the two treatments and CK showed significant or extremely significant differences from day 6 and remained different at day 9. At T2, significant differences were observed from day 3 and expanded on day 6, and then the gap began to narrow to no significant differences at day 9. At the same time, the IAA concentration of the samples at 0–5 cm changed dramatically, and the concentration of capping agent treatment remained lower than that of DPC treatment. For the samples at 5–10 cm, IAA concentration changed gently and the curves of the two treatments crossed.

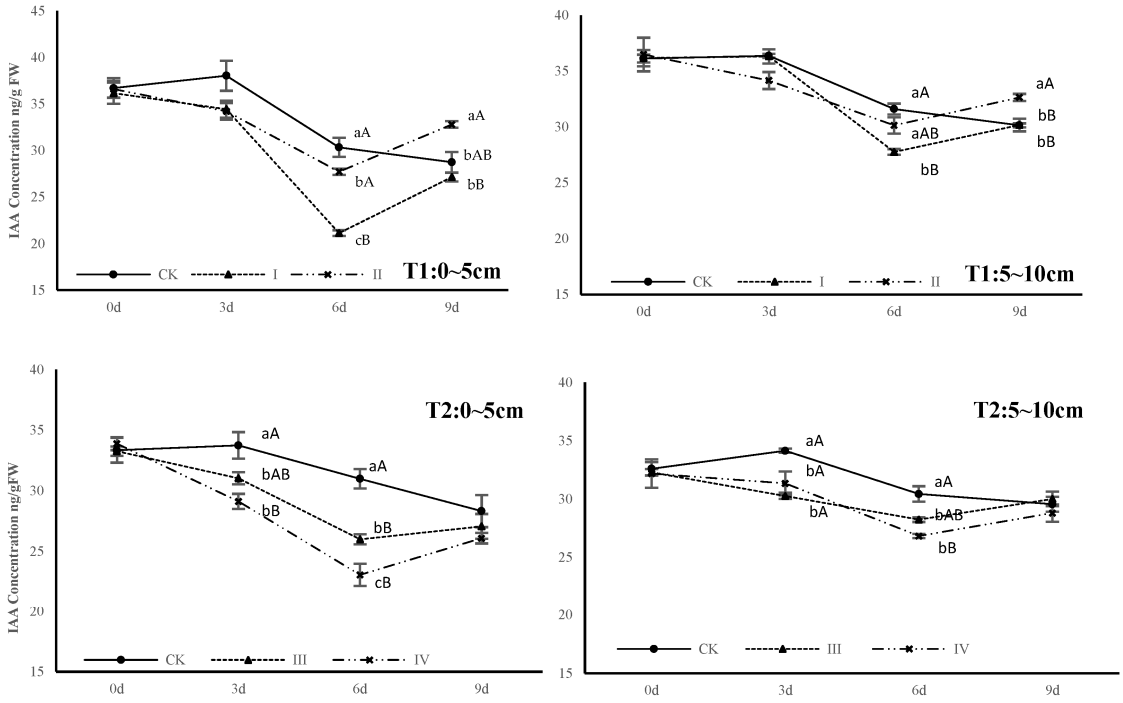


Figure 1. Changes of IAA concentration after chemical capping. Note: 1. Different letters above the bars indicate a significant difference at $p < 0.05$; different capital letters indicate an extremely significant difference at $p < 0.01$. 2. T1 refers to treatment on 12 July; T2c refers to treatment on 18 July. 3. Lines I and III represent CA treatment; lines II and IV represent DPC treatment at two treatment times, respectively. 4. The time points with significant differences in the figure are labelled with different letters; those without significance are not labelled to avoid confusion.

3.1.2. Changes of ABA Concentration

As shown in Figure 2, after capping operation, ABA concentration in 0~5 cm samples of two treatments increased from day 3, reaching a significant difference from CK on day 6. The ABA concentration of 5~10 cm samples kept lower, especially at T1, which reached significantly higher from that of CK at day 9. At the same time, except for 5~10 cm samples at T2, ABA concentration of samples treated with capping agent was higher than that treated with DPC in the other 3 groups.

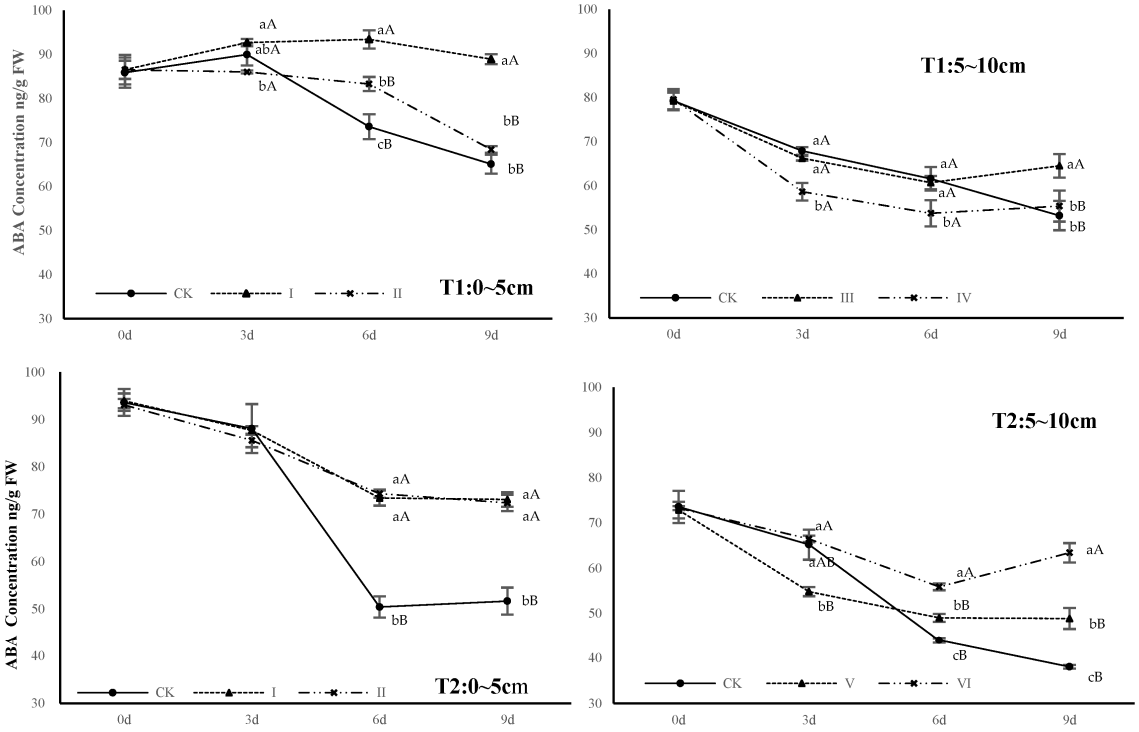


Figure 2. Changes of ABA concentration after chemical capping.

3.1.3. Changes in GA_3 Concentration

As shown in Figure 3, after capping treatment, GA_3 concentration was lower than that of CK, and that of CA treatment was lower than that of DPC treatment, and the differences were significantly or extremely significantly different from day 3. The differences of GA_3 concentration among CK and two treatments remained remarkable in T1, but narrowed in T2.

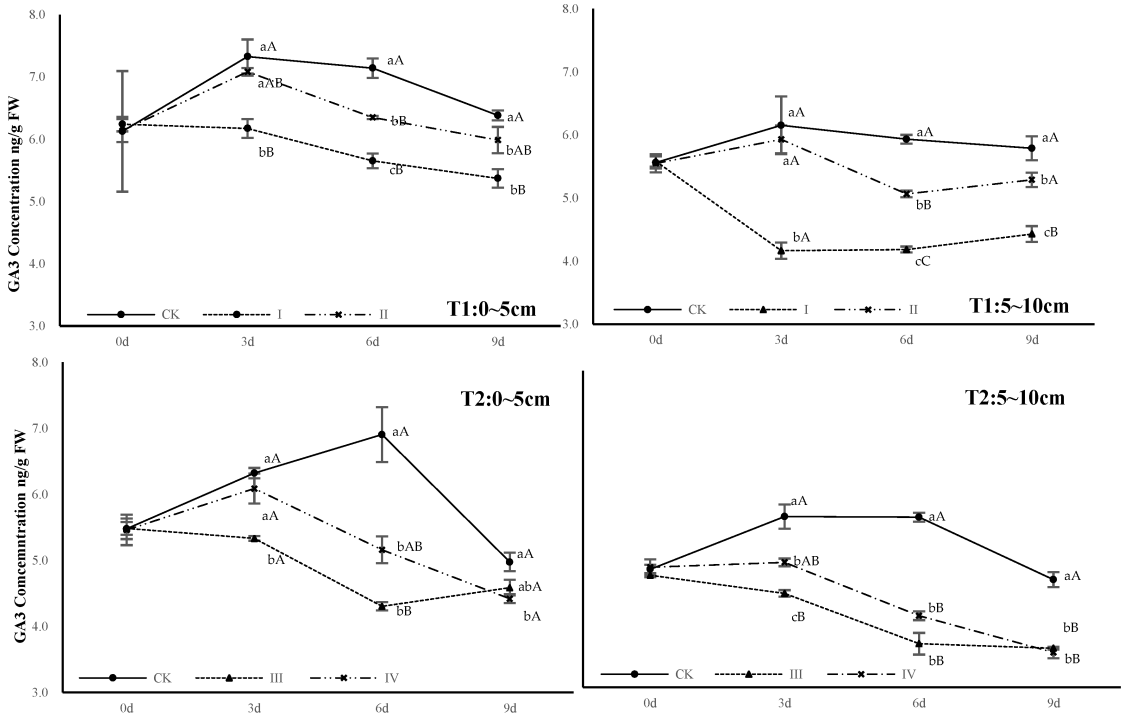


Figure 3. Changes of GA_3 concentration after chemical capping.

3.1.4. Changes in ZR and ZR Nucleoside (ZR) Concentration

As shown in Figure 4, ZR concentration changed differently in the two periods: In the T1 period, it increased rapidly and then decreased, while that of DPC treatment was significantly higher than that of CA treatment and CK. In the T2 period, the ZR concentration of CK and CA treatment fluctuated and the change range was small, while that of DPC treatment was relatively high and maintained a stable downward trend.

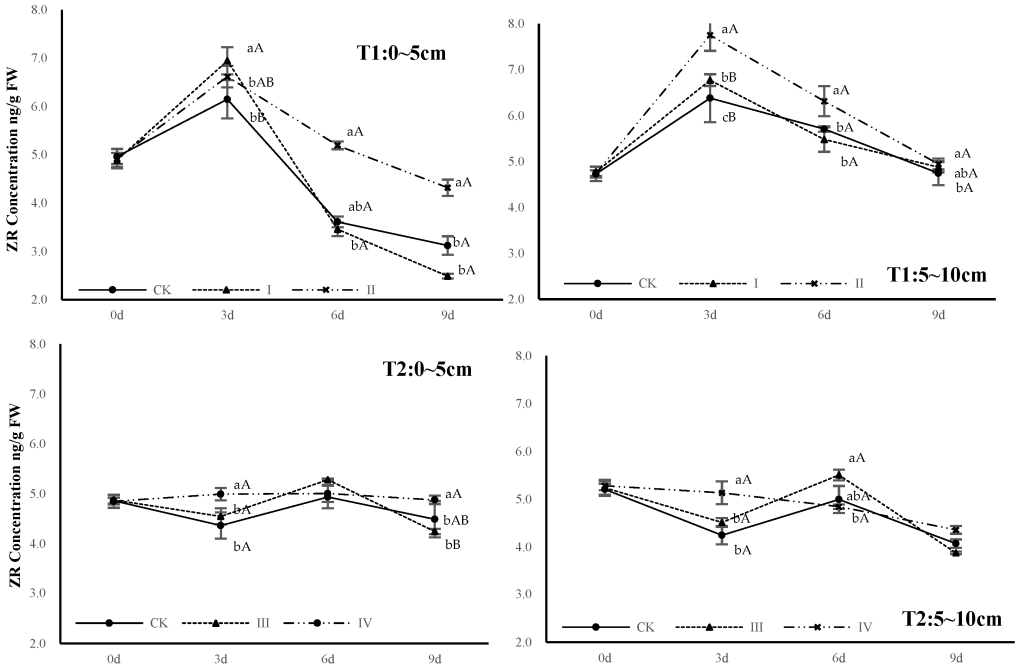


Figure 4. Changes of ZR concentration after chemical capping.

3.2. Plant Development after Capping Treatment

3.2.1. Changes in Plant Height

As shown in Figure 5, plant height still showed a stable increase after chemical capping treatment, but was obviously inhibited. The plant height was inhibited more rapidly and strongly in T1, while it showed increased lag and decreased less in T2. Compared with that of CK, plant height of CA treatments showed significance in T1 and an extremely significant difference in T2 from day 14, which continued to expand later. Between two treatments, the plant height of CA treatment was lower than that of DPC treatment, indicating that the capping agent has stronger inhibitory ability.

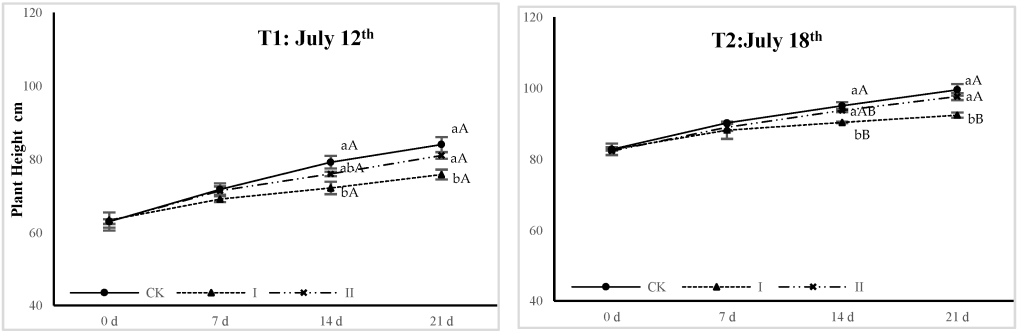


Figure 5. Changes of plant height after chemical capping.

3.2.2. Changes of Fruit Branch Number

As shown in Figure 6, after chemical capping treatment, the number of fruit branches increased steadily, while it was significantly inhibited compared with CK. The inhibitory

degree was significant at T1, and the number of fruit branches of CA treatment remained lower than that of DPC treatment. The inhibitory degree at T2 was weaker than that at T1, and the changing trend of the two treatments was very close, while the number of fruit branches of DPC treatment was slightly lower than that of CA treatment.

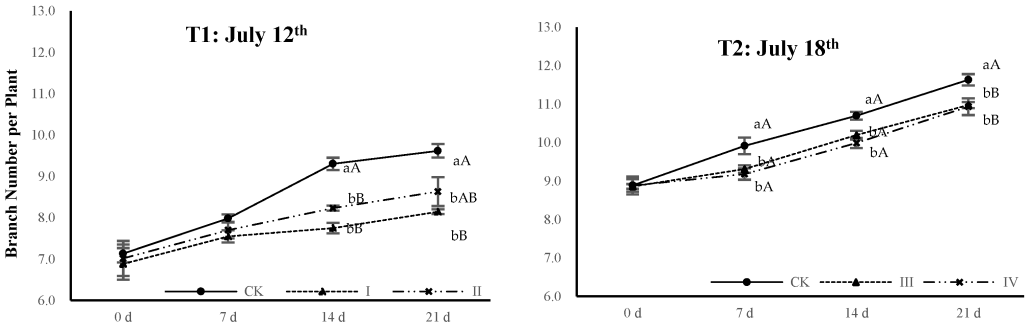


Figure 6. Changes of fruit branch number after chemical capping.

3.2.3. Change of Average Length of Upper Nodes (Fifth and Above) of the Main Stem

As shown in Figure 7, after chemical capping treatment, the growth of internodes (fifth and above) in the top of the main stem was inhibited, but the performance of the two periods varied greatly. The average length at T1 decreased first and that of CK and DPC treatments began to rise from day 7, while that of CA treatment began to rise from day 14. For T2, the changing trend of DPC treatment was still similar to that of CK, which began to rise rapidly from day 7 and slowed down after day 14, while that of CA treatment maintained a steady upward trend. The average length of upper internodes (fifth and above) of the main stem of CA treatment was significantly lower than that of DPC treatment.

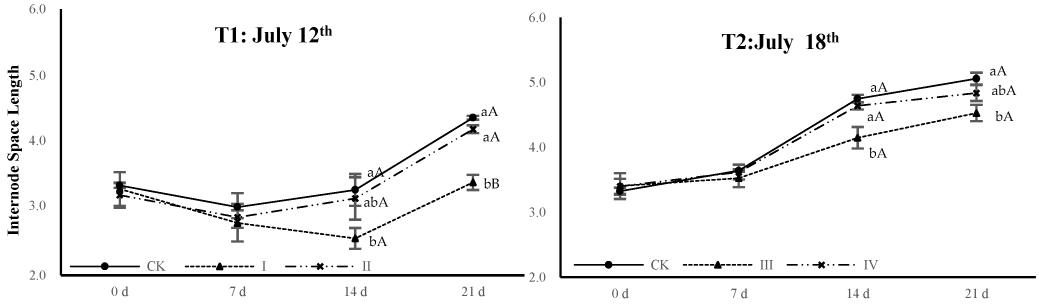


Figure 7. Changes of average length of upper nodes (fifth and above) after chemical capping.

4. Discussion

It should be noted that the starting points of the three monitored plant development indicators of T1 and T2 are differently significant, indicating that chemical control is necessary in the vigorous growth period. The results show that chemical capping operations affect hormone concentrations in the top of the main stem of cotton plants. In general, the changes in the T1 period were more obvious than that in the T2 period, and the changes of 0–5 cm samples were more obvious than that of 5–10 cm samples, hinting that the influence is more intense in the more active parts and periods of plant growth, which is consistent with the research results of Qi et al. [7] and Xu et al. [8]. At the same time, no significant difference was observed in the three monitored plant development indicators (plant height, number of fruit branches and average length of upper internode [fifth and above] of main stem) in the first 7 days, which was especially obvious in the T2 period, confirming that the effect of hormone changes on plant development requires a series of physiological processes. Notably, represented by the concentration changes of IAA, along with the growth process, hormone concentrations of each treatment gradually became close to that of CK, indicating that exogenous chemical compounds could be absorbed

and decomposed in the plant to inhibit the apical dominance at the flowering stage while ensuring normal plant growth later, beneficial to the formation of larger photosynthetic organs, which provides theoretical support for the "secondary growth" of plants [8,20].

After chemical capping treatment, compared with CK, the concentration changes of the four kinds of hormones were different. Specifically, the overall performance of IAA concentration decreased significantly in the early stage but slightly increased later, indicating that the influence of exogenous chemical compounds was gradually weakened. The concentration of IAA in the samples at 0–5 cm changed more rapidly and dramatically, reflecting the characteristics of downward polar transport of IAA. ABA concentration changed differently in two parts for the 0–5 cm samples that were significantly or extremely significantly higher than that of CK from day 3, which may be due to the ability of the leaf to synthesize ABA being enhanced after chemical capping treatment, the affection being stronger on the upper leaves and the ability to synthesize ABA being stronger, and then rapidly aggregated to the main stem. Plant height decreased significantly in response to hormone concentration changes, which was in line with expectations. The IAA concentration of the two treatments gradually became close to each other at T2; in particular, that of the samples at 5–10 cm treated with DPC was lower than that treated with CA at the later stage, but the gap of plant height still continued to widen. This could be related to the change in ABA concentration, especially the change in the samples at 5–10 cm, i.e., the difference in ABA concentration between the two treatments remained stable at T1 but continued to widen at T2, indicating that ABA also plays an important role in the differentiation and elongation of stem apical cells. Meanwhile, the elongation of stem subapical (5–10 cm samples) cells can significantly increase plant height.

After chemical capping treatment, the GA_3 concentration at the top of the main stem decreased overall and increased slightly later, so the average length of the upper internode (fifth and above) shortened; this is consistent with the result of Paciorek et al. [25], i.e., the reduction of GA levels led to the inhibition of cell elongation in internodal tissues, which reduced plant height too. The GA_3 concentration of CA treatment was lower than that of DPC treatment, and the difference in T1 was greater than that in T2, which was consistent with the variation of the average length of the upper internode. The average length of the upper internode decreased first and then increased in the T1 period due to the strong vitality of the plant and active cell differentiation, the number of fruit branches increased rapidly while the plant height was strongly inhibited and the internodes were shortened consequently. The variation in ZR concentration was different at two periods. At T1, the value increased firstly then decreased, and the number of fruit branches increased greatly; that of DPC treatment was higher and the number of fruit branches was increased too. At T2, the change curves of CA treatment and DPC treatment crossed, and consequently the curves of the number of fruit branches changing were very similar. Referring to previous studies, it can be seen that ZR can promote the growth of lateral buds [12]; according to the results of this experiment, it is concluded that ZR also has a strong effect on promoting lateral bud differentiation. Notably, although the ZR concentration of CA treatment was higher than that of CK, the number of fruit branches was still lower; it is speculated that IAA still plays a dominant role in the growth of the plant apex, and after treatment, the concentration of IAA was significantly reduced, and the differentiation of the apex cells was inhibited.

Undeniably, although the changes of four hormones (IAA, ABA, GA3 and ZR) in this study basically explained the change mechanism of three plant growth indexes (plant height, number of fruit branches and length of upper [fifth or more nodes] internode), it does not cover the complex plant endogenous hormone system, and further research is needed on other hormones such as ethylene. At the same time, the development of lateral branches directly affects the construction of reasonable shape of cotton plants; therefore, the hormone changes and development rule of lateral branches after chemical capping will also be future research projects. On the other hand, different hormones are synthesized in different parts of the plant, and it will be interesting to study how their movement patterns

inside the plant change after chemical capping. Therefore, there are many problems to be learned about chemical capping of cotton.

5. Conclusions

In this study, the effects of chemical capping with CA and DPC were examined combined with the hormone changes and plant growth. The results showed that chemical capping could significantly affect hormone concentration at the main stem apex. After chemical capping, the IAA concentration decreased firstly and increased later, lower than that of CK. ABA concentration increased significantly and GA_3 concentration decreased significantly compared with that of CK. ZR concentrations fluctuated obviously at T1 and gently at T2. By affecting the concentration of hormones at the plant apex, the differentiation and elongation of the apex cells were reduced; the plant height and the number of fruit branches were decreased; and the average length of the upper internode (fifth and above) of the main stem was shortened. In comparison with DPC treatment, considering the addition of additives, CA treatment has more powerful regulation of hormones, which is conducive to shaping reasonable plant type, and it is recommended to be applied in cotton production.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

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