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Effect of Photoperiod and Gibberellin on the Bolting and Flowering of Non-Heading Chinese Cabbage

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Abstract: Non-heading Chinese cabbage (cabbage) is an essential green leafy vegetable, and bolting and flowering are necessary for reproduction. However, further research is needed to study the effect of photoperiod on the bolting and flowering of cabbage, particularly on the development of the stem. In this study, we performed phenotypic analysis and measured endogenous gibberellin levels in the cabbage. We carried out these experiments under four different photoperiodic treatments, 12 h (light)/12 h (dark), 14 h (light)/10 h (dark), 16 h (light)/8 h (dark), and 18 h (light)/6 h (dark). The results showed that the time of bolting and flowering gradually decreased with increasing light duration. The development of stems was optimal under the 16 h (light)/8 h (dark) photoperiod treatment, and the same result was obtained via cytological observation. In addition, the changes in the endogenous gibberellin3 (GA_3) content under different photoperiodic treatments were consistent with the development of stems and peaked at 16 h (light)/8 h (dark). At the same time, qRT-PCR analysis showed that the relative expression of the key gibberellin synthase genes, *BcGA3ox2* and *BcGA20ox2*, exhibited upregulation. When treated with exogenous GA_3 and its synthesis inhibitor, paclobutrazol (PAC), exogenous gibberellins significantly promoted bolting; conversely, gibberellin inhibitors suppressed the bolting, flowering, and stem elongation of cabbage. Therefore, the photoperiod may regulate cabbage bolting by regulating endogenous GA_3.

Keywords: cabbage; photoperiodic; gibberellin; bolting; flowering; stem development

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1. Introduction

Non-heading Chinese cabbage (cabbage) (*Brassica campestris* spp. *chinensis* Makino), a vernalization-responsive, long-day (LD) plant of the Brassica genus in the Cruciferae family [1], is an important leafy vegetable cultivated worldwide [2]. The life cycle of higher plants can be divided into two stages, namely, vegetative growth and reproductive growth [3]. Completing the transition from vegetative growth to reproductive growth at the right time is essential for plant reproduction [4], which occurs in the meristem within the rosette [5]. Bolting and flowering are landmark features of cabbage entering the reproductive growth stage and critical agronomic traits in production [6]. Bolting refers to the phenomenon of the flower moss gradually elongating and growing from the rosette of leaves after the completion of floral bud differentiation [7], which is a characteristic of cabbage stem development [8]. In cruciferous vegetables, bolting is an important process in the transition to flowering, with flowering being an evolutionary component [9]. Endogenous and environmental signals jointly manipulate the timing of bolting and flowering [10–12], and these signals form a complex regulatory network to determine the transition of reproductive growth. Environmental signals, such as light and temperature, particularly through the photoperiodic pathway and the vernalization pathway, and endogenous developmental signals, including phytohormones like salicylic acid (SA), jasmonic acid (JA), gibberellin (GA), and auxin (IAA) [13–15], play critical roles in this process.

Light is an essential environmental condition for normal plant development, being an important factor in crop growth and development and quality formation [16]. Plants can sense changes in the photoperiod with their biology and regulate the time of bolting and flowering according to the duration of light exposure, as well as provide energy for plant development [17]. Plants regulate growth in response to environmental variations, which rely on the interaction between endogenous factors and environmental signals, such as hormone and light signaling pathways [18]. On the one hand, plants absorb different light levels through a series of photoreceptors, inducing light signaling to regulate plant flowering via sensing the presence or absence, direction, and intensity of light [19]. On the other hand, related studies have demonstrated that light signaling synergistically with gibberellin signaling mediates plant flowering [20]. Wang found that GA induced the expression of the *CO* (*CONSTANS*) gene and thus facilitated the expression of *FT* (*FLOWERING LOCUS T*) under LD conditions [21]. However, other studies have also shown that flowering is associated with the apical bioactivity of gibberellins under short-day light (SD) conditions [22]. In addition, bolting can affect the structure of the plant, and a longer light duration can favorably induce the initial elongation of flowering stems [23]. The stem is not only one of the important production organs but also a storage organ for supplying nutrients to the plant [24]. The photoperiodic response to flowering is a hot research topic today. At the same time, the study of light and stem development has also attracted increasing attention. Current research has focused on onion species [25], and there is still a demand to deepen the study in *Brassica*.

Active gibberellins (GAs) are involved in various processes of plant development, including seed germination, stem and leaf development, and flowering time; they also play a pivotal role in plant cell expansion and elongation, and they can respond to endogenous and environmental signals in plants [26–29]. Secondly, promoting branch tip elongation and plant height is also a more prominent feature of GAs [30]. Wang found in cabbage that the knockout of the *BraRGL1* (DELLA protein) gene using the CRISPR/Cas9 gene editing system resulted in a delay in the process of bolting initiation and flowering in the mutant material [31]. It has been shown that in the gibberellin synthesis pathway, three classes of oxidases, *GA3ox*, *GA20ox*, and *GA2ox*, positively regulate the synthesis and inactivation of active GAs, and the overexpression of the *GA20ox* gene in Arabidopsis was found to be effective in promoting branch elongation [32]. Although many studies have shown that GAs are involved in the flowering process [11], the changes and functions of endogenous GAs in the photoperiodic regulation of cabbage bolting need to be further investigated, especially under extended light duration conditions.

In this study, we investigated the effects of photoperiod on the time of bolting and flowering, stem development, changes in endogenous GA_3 content, and the expression of related genes using morphology, cytology, and molecular biology to explore the regulation of photoperiod on the bolting and flowering of cabbage and the mechanism of the endogenous gibberellin response.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

The cabbage cultivar 'Qingtai No. 4' was obtained from Fujian Jinpin Agricultural Technology Co. (Fujian, China). The cabbage seeds were sown into the substrate in a 25 °C growth chamber until they grew to 4–5 true leaves. Afterward, the plants were placed in a climate chamber (Jiangnan Instrument Factory, Ningbo, China) for ten days of low-temperature vernalization at 8 °C/6 °C (day/night), 14 h/10 h photoperiod, photosynthetic photon flux density (PPFD) of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and relative humidity of 75%.

2.2. Photoperiod Processing

Uniform and healthy plants were selected for photoperiod treatment after vernalization. Four different photoperiod treatment groups were set up as 12 h (light)/12 h (dark) (recorded as Ph12), 14 h (light)/10 h (dark) (Ph14), 16 h (light)/8 h (dark) (Ph16), and

18 h (light)/6 h (dark) (Ph18) in a special growth chamber. The plants were grown at a temperature of 25 °C and white light (LED with a wavelength of 400–700 nm) (Fujian Jiupu Biotechnology Co. Fuzhou, China) with PPFD of 200 μmol·m^{-2}·s^{-1}.

2.3. Treatment with Exogenous GA$_3$ and Inhibitors

The cabbage plants with the same growth after vernalization were selected for treatment. The exogenous GA_3 (300 mg/L) and PAC (20 mg/L) treatments were sprayed once every two days individually under the 18 h (light)/6 h (dark) photoperiod. Each treatment application was sprayed only twice during the entire growth period, with water spray as the control. The experiment was over when the plants bloomed.

2.4. Plant Phenotyping and Morphological Characterization

The beginning of sowing was recorded as the first day, and we recorded the time taken for the emergence of green flower buds as the time of the squaring stage. The bolting stage was the time of the elongation of the flower moss and rosette leaf flush. The time that elapsed until the first flower fully opened was recorded as the flowering stage. The growth indices of the different treatments were determined at different stages. Plant height and stem height were measured using a ruler. We took the length from the cotyledon to the top as the plant height. Stem height was defined as the distance from above the rosette to the stem tip. A Vernier caliper was used to determine the stem diameter, and the diameter of the stem with the first internodal distance greater than 1cm was the thickness of the stem. Fresh weight and dry weight were determined using an electronic balance. Chlorophyll content was determined using the ethanol extraction colorimetric method [3] from the young leaf to the outer third mature functional leaf.

2.5. Measurement of Endogenous GA Content

The endogenous GA_3 content was determined at the apical of the cabbage stems, including the stem tips and young leaves, at the bolting and flowering stages under different photoperiod treatments. The endogenous gibberellin content was determined using an enzyme-linked immunosorbent assay with a gibberellin (GA_3) ELISA kit (Enzyme-linked Biotechnology, Shanghai, China).

2.6. Cytologic Observations

Stems from the same nodulation locus (where the internode distance on the rosette first appeared to be greater than 1 cm nodulation) of each treatment at the flowering stage were immersed in FAA fixative (70% ethanol: acetic acid: formaldehyde = 90:5:5) for 24 h. Afterward, paraffin section preparations were embedded in paraffin wax and stained with Senna red and solid green. Finally, the cells were observed and photographed using a fluorescence inverted microscope (Leica, Wetzlar, Germany). Data measurements were performed using ImageJ 1.8.0 software.

2.7. Gene Expression Analysis

Total RNA was extracted from plants of different treatments at the bolting and flowering stages using the Plant Total RNA Isolation Kit (Vazyme, Nanjing, China). First-strand cDNA was synthesized with a FastKing gDNA Dispelling RT SuperMix Kit (Tiangen, Beijing, China). The real-time PCR analysis was performed using the 2× RealStar Green Fast Mixture reagent (Genstar, Beijing, China) in a LightCycler® 96 Real-Time PCR instrument (Roche, Basel, Switzerland). The β-actin gene was used as an internal control, and relative expression was calculated using the $2^{-\Delta\Delta Ct}$ calculation method. Gene-specific primers are listed in Table S1.

2.8. Statistical Analysis

Three replicates were set up for each treatment, and each replicate consisted of twenty plants. Statistical analysis was performed using SPSS 26.0 software, and the significance of

differences between treatments was compared using a one-way ANOVA ($p = 0.05$). Plotting was performed using Origin 2022 software.

3. Results

3.1. Bolting and Flowering Time of Cabbage under Different Photoperiod Treatments

The development of buds, bolting, and flowering of cabbage are closely related to light duration. The experimental results show (Figure 1) that photoperiodic treatments caused positive effects on the bolting and flowering stages of the cabbage. And the bud appearance, bolting, and flowering stages were gradually shortened with increasing light duration. Among them, the Ph18 treatment took the shortest time in the flowering process, followed by the Ph16 treatment, but the difference between the two treatments was slight. In contrast, the Ph12 treatment delayed bolting and flowering time the longest compared with the Ph18 treatment. It could be seen that prolonging the light duration had a promoting effect on bolting and flowering, but the promotion effect was weakened beyond the Ph16 treatment. Flowering was advanced by 8.6%, 12.1%, and 13.8% (median as a reference) under Ph14, Ph16, and Ph18 treatments, respectively, compared with the Ph12 treatment.

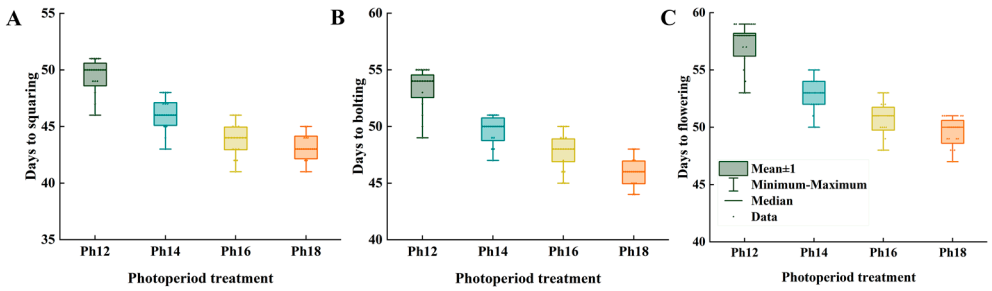

Figure 1. Effect of photoperiod on flowering of non-heading Chinese cabbage (cabbage). (**A**) Analysis of the squaring time under different photoperiod treatments. (**B**) Analysis of the bolting time. (**C**) Analysis of the flowering time. The horizontal line in the figure indicates the median ($n > 20$).

Photoperiod affected the accumulation of photosynthetic pigments in cabbage. This study determined changes in the photosynthetic pigments content of the third mature functional leaf from the young leaf outward. As shown in Figure 2A,B, the total chlorophyll and carotenoid content showed the same trend of change under the same light duration treatment as the growth process advanced. The photosynthetic pigments all showed a gradual decrease under the Ph12 treatment. In contrast, they showed a gradual increase in the Ph14 and Ph16 treatments. Beyond that, they all increased before bolting and decreased after bolting under the Ph18 treatment. In terms of the developmental period, there was no marked difference in the pigment content between the different light treatment groups at the squaring stage. At the bolting stage, the Ph18 treatment showed a distinct advantage, and the carotenoid content and total chlorophyll content were 1.5 and 1.3 times higher than those under the Ph12 treatment, respectively. During the flowering stage, the pigment content significantly increased under the Ph14 and Ph16 treatments, whereas it was downregulated under the Ph12 treatment, and its content decreased by 29.3% and 12.6%, respectively, compared with that at the squaring stage.

Plant dry and fresh weights are also indicators for evaluating plant quality and yield. The growth morphology and weight changed after the cabbage entered the carex stem development stage. In this study, the dry and fresh weights of the aboveground parts of the cabbage with different light duration treatments were measured at the bolting and flowering stages. There were significant differences in fresh weight but not in dry weight among the treatments, while both dry and fresh weights peaked under the Ph16 treatment, as shown in Figure 3.

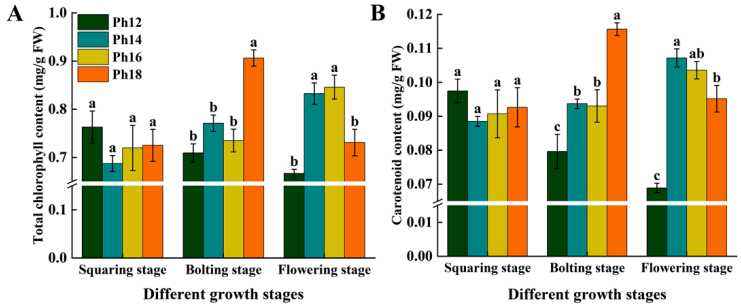

Figure 2. Effect of photoperiod on the photosynthetic pigments of cabbage. (**A**,**B**) Total chlorophyll content and carotenoid content of the third mature functional leaf of cabbage at squaring, bolting, and flowering stages. Data shown are means and error lines indicate SE (n = 3). All data were evaluated statistically using one-way ANOVA ($p \leq 0.05$). Different lowercase letters indicate a significant difference.

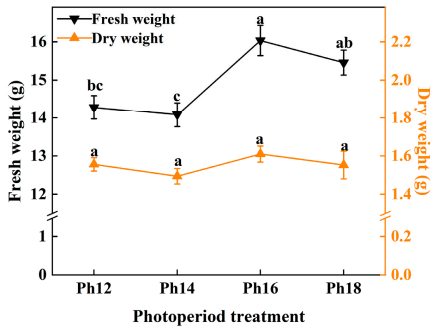

Figure 3. Effect of photoperiod on the aboveground fresh and dry weights of cabbage at flowering. The data shown are means and error lines indicate SE (n > 15). All data were evaluated statistically using one-way ANOVA ($p \leq 0.05$). Different lowercase letters indicate a significant difference.

3.2. Analysis of Stem Phenotypes and Endogenous GA_3

In the experiment, it was found that photoperiod not only affected the time of bolting and flowering but also had a significant effect on plant height. According to the measured plant height data (Figure 4A), at the squaring stage, the plant height showed a decreasing and then increasing trend with increasing light duration. However, the aboveground height at flowering showed a trend of increasing and then decreasing, peaking under the Ph16 treatment, which was 16.5%, 11.2%, and 4.3% higher than that under the Ph12, Ph14, and Ph18 treatments, respectively. The flowering phenotype is shown in Figure 4B.

Photoperiod affects the development of stems in cabbage. In the present study, we observed the phenotypes of carex stems in the upper part of the rosette at the bolting (Figure 5A) and flowering stages (Figure 5B). As shown in Figure 5C, the results of stem length indicated that the maximum was achieved under the Ph16 treatment, which was 5.4% higher than that of the Ph12 treatment at the bolting stage. At the flowering stage, it showed a unimodal trend, peaking under the Ph16 treatment and being 18.5% higher than that under the Ph12 treatment. Stem diameter is also one of the indicators of carex stem development. Moreover, the stem diameter in the upper part of the rosette (Figure 5D) showed a tendency to increase and then decrease at both the bolting and flowering stages and peaked under the Ph16 treatment. This indicates that carex stems develop both horizontally and vertically under the influence of the photoperiod.

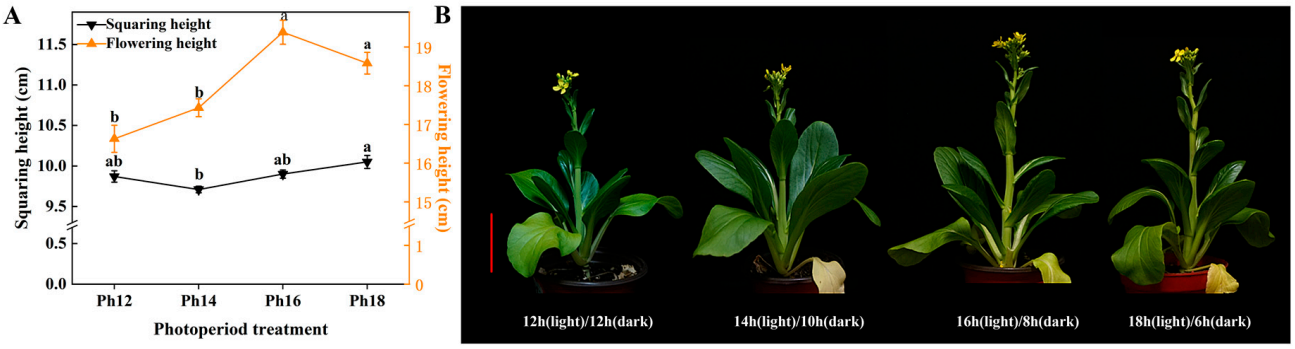

Figure 4. Effect of photoperiod on the phenotype of cabbage. (**A**) Aboveground plant heights at squaring stage and flowering stage, data shown are means and error lines indicate SE ($n > 20$). (**B**) Phenotypes at the time of 1–3 flowers in each photoperiod treatment, scale bar = 5 cm. All data were evaluated statistically using one-way ANOVA ($p \leq 0.05$). Different lowercase letters indicate a significant difference.

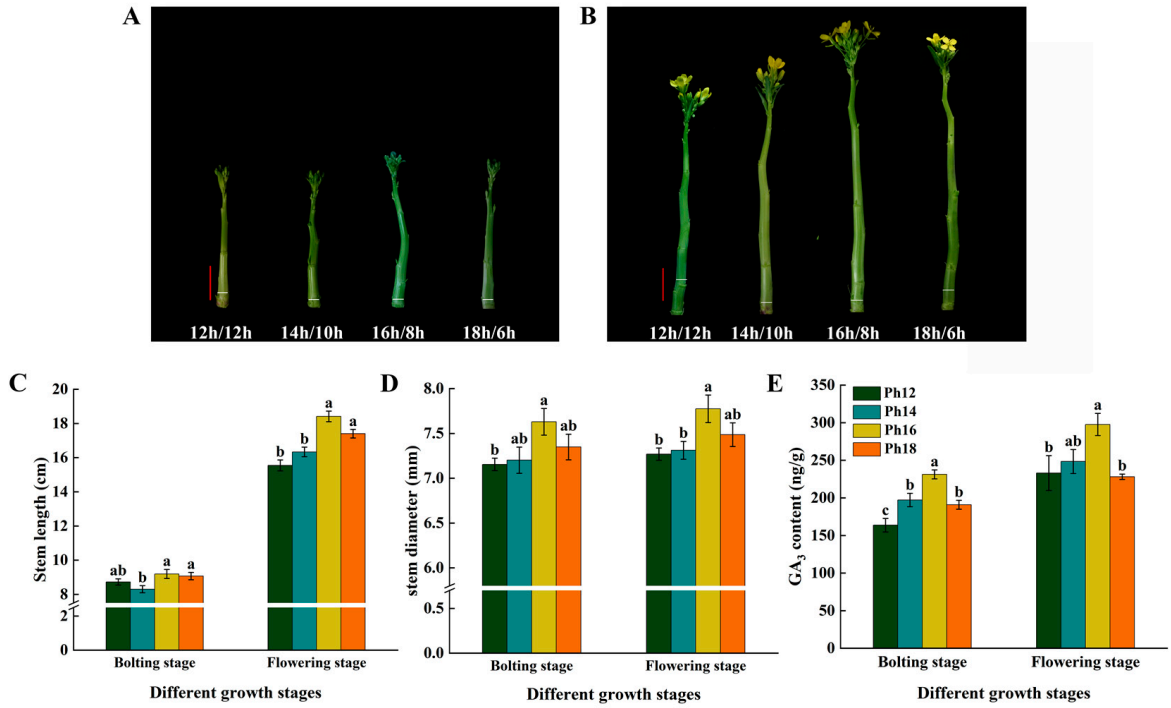

Figure 5. Effect of photoperiod on the development of stems during the bolting and flowering stages of cabbage. (**A**,**B**) Stems on the rosette at the bolting and flowering stages, scale bar = 2 cm. (**C**) Stem length on the rosette at bolting stage and flowering stage, data shown are means, and error lines indicate SE ($n > 20$). (**D**) Stem diameter at the bolting stage and flowering stage, data shown are means and error lines indicate SE ($n > 20$). (**E**) Changes in endogenous GA_3 content in cabbage at the bolting and flowering stages under different photoperiod treatments, the data shown are means, and error lines indicate SE ($n = 3$). All data were evaluated statistically using one-way ANOVA ($p \leq 0.05$). Different lowercase letters indicate a significant difference.

To investigate the role of gibberellin in development under different photoperiodic treatments, we measured the levels of endogenous GA_3 content at both the bolting and flowering stages of the cabbage. The data showed that the GA_3 content was at its maximum under the Ph16 treatment, both at the bolting and flowering stages. However, further extension of light duration caused the gibberellin content to decrease under the Ph18 treatment (Figure 5E). At the bolting stage, compared to the Ph16 treatment, endogenous GA_3 was

29.2%, 14.7%, and 17.4% lower in the Ph12, Ph14, and Ph18 treatments, respectively. At the flowering stage, endogenous GA_3 under the Ph16 treatment was 27.7%, 19.8%, and 30.5% higher than under the Ph14, Ph16, and Ph18 treatments.

3.3. Cellular Observation

Morphological indicators showed that different photoperiod treatments affected the development of the cabbage stems. For this reason, we further observed the rosette stem nodes at the flowering stage from a cytological point of view in paraffin sections. The morphology of the transverse cut cells is shown in Figure 6A, and the longitudinal cut cells are shown in Figure 6B. By observing the cells in the pith of the stems between different treatments, we found that both the area of the cells in the transverse section (Figure 6C) and the length of the cells in the longitudinal section (Figure 6D) were higher under the Ph16 treatment than under the other three treatments. In addition, the cell area and length under the Ph16 treatment reached 1.2 times that of the minimum value under the Ph12 treatment. It is evident that the photoperiod induces the development of stems by affecting the elongation and division of stem cells.

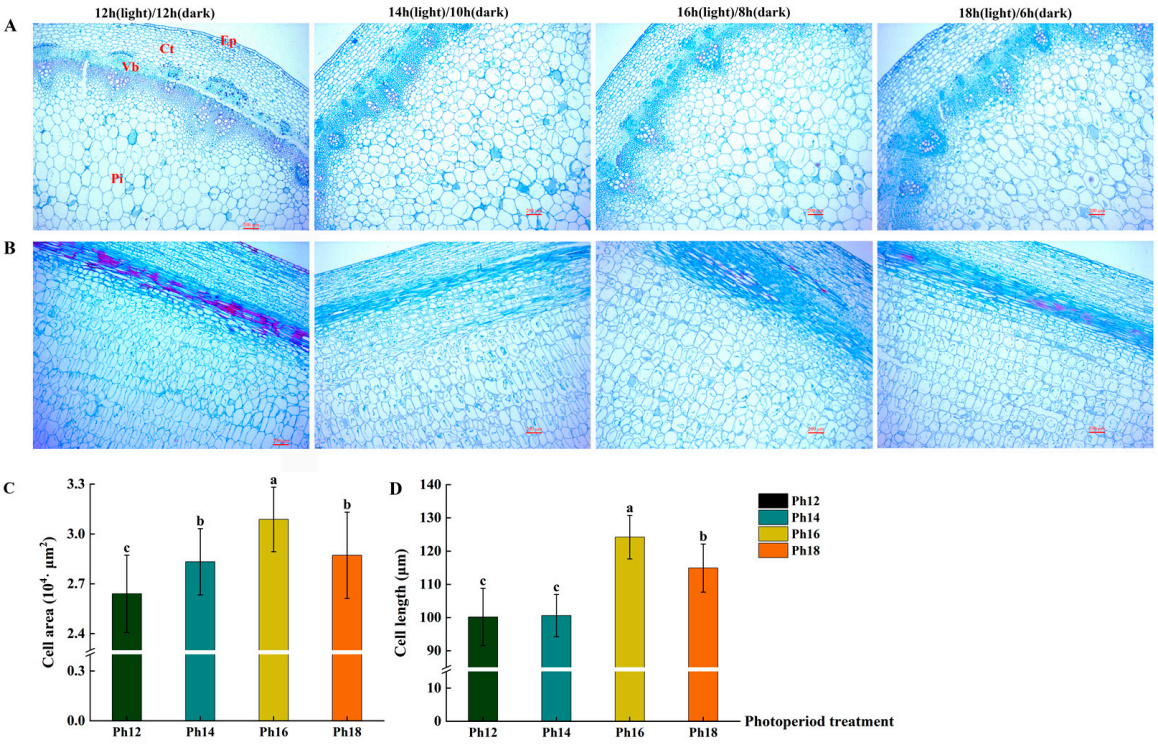

Figure 6. Photoperiodic effects on carex stem cells at the flowering stage of cabbage. (**A**) Anatomical drawings of the cross-section of the stem of cabbage at the flowering stage, EP, epidermis; Ct, cortex; Vb, vascular bundle; Pi: pith. Scale bar = 200 μm. (**B**) Anatomy of the longitudinal interface of carex stems at the flowering stage. Scale bar = 200 μm. (**C**,**D**) Cell area and cell length of cabbage stems at the flowering stage. Three medullary regions were photographed in each tissue section, no less than 50 cells were randomly selected from each region for counting, and the data shown are means and error lines indicate SE ($n > 150$). All data were evaluated statistically using one-way ANOVA ($p \leq 0.05$). Different lowercase letters indicate a significant difference.

3.4. Molecular Characterization of Related Genes in the Process of Bolting and Flowering in Cabbage

Photoperiod affected the expression levels of related genes. To further investigate the influence of photoperiod on stem development, according to previous research [33] and prior laboratory study [34], we analyzed the expression of the key enzyme-encoding genes for GA synthesis (*BcGA20ox2* and *BcGA3ox2*), the flowering-associated genes (*BcCO*,

BcSOC1, and *BcFT*), and the cell elongation genes (expansion protein (*BcEXPA10*) and xyloglucan endotransferase (*BcXTH4*)) at both the bolting (Figure 7) and flowering (Figure 8) stages under different photoperiodic treatments. The results showed that the gibberellin synthase genes *BcGA3ox2* and *BcGA20ox2* exhibited distinct expression patterns in photoperiodic regulation. In contrast, the flowering-related genes were gradually upregulated with the prolongation of light duration at both the bolting and flowering stages. The experimental results show that the photoperiod also affected the relative expression of the cell expansion-related genes *BcEXPA10* and *BcXTH4*.

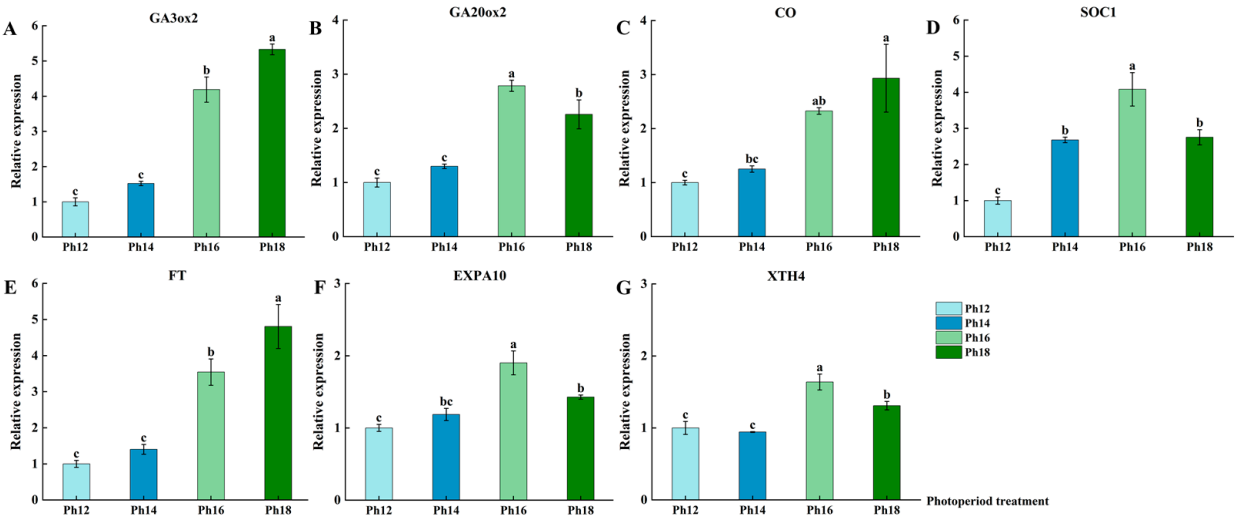

Figure 7. During the bolting stage, (**A**,**B**) relative expression levels of photoperiod-regulated gibberellin synthesis genes, (**C**–**E**) flowering-related genes, (**F**,**G**) cell elongation-related genes in the cabbage. The data shown are means and error lines indicate SE (n = 3). All data were evaluated statistically using one-way ANOVA ($p \leq 0.05$). Different lowercase letters indicate a significant difference.

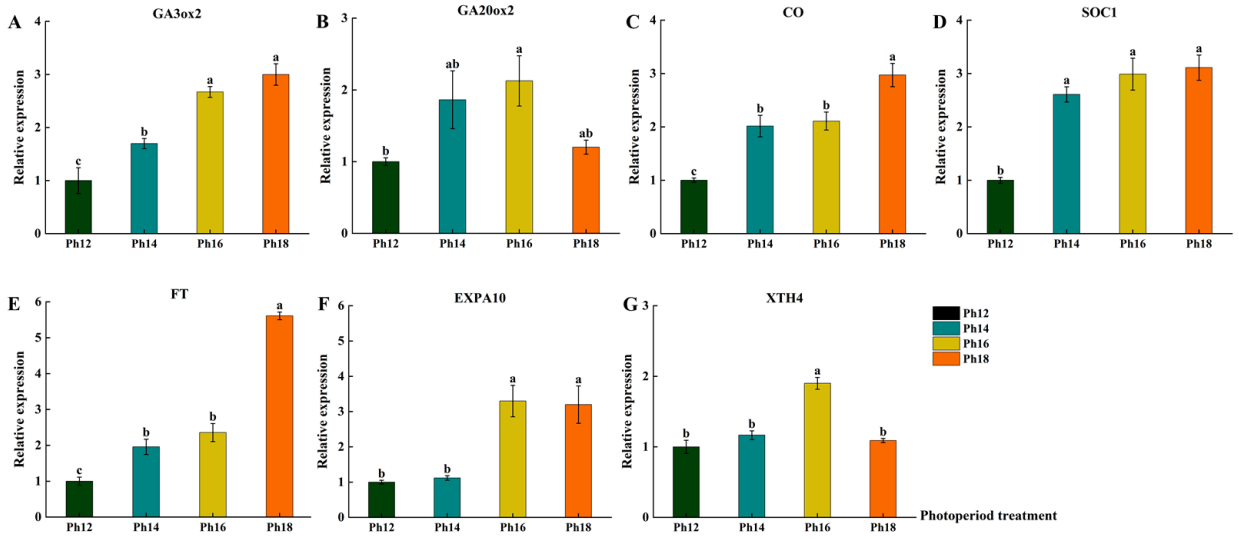

Figure 8. During the flowering stage, (**A**,**B**) relative expression levels of photoperiod-regulated gibberellin synthesis genes, (**C**–**E**) flowering-related genes, (**F**,**G**) cell elongation-related genes in the cabbage. The data shown are means and error lines indicate SE (n = 3). All data were evaluated statistically using one-way ANOVA ($p \leq 0.05$). Different lowercase letters indicate a significant difference.

3.5. Effect of Exogenous GA_3 on Bolting, and Flowering, and Stem Development in Cabbage

To further evaluate the role of GA in bolting and flowering, we exogenously sprayed GA_3 and PAC on the vernalized cabbage. As shown in Figure 9A, the spraying of GA_3 promoted bolting initiation and flowering under the 18 h (light)/6 h (dark) light condition. However, the PAC treatment significantly delayed carex stem development and flowering time. The externally applied GA_3 treatment significantly affected the morphology of the carex stems in the cabbage (Figure 9B). In addition, the differences in carex stem development were also significant. Compared with the control, the GA_3 treatment significantly increased the stem diameter by 9.6% (Figure 9C) and the elongation by 24.5% (Figure 9D). In contrast, the sample under the PAC treatment until flowering did not reach the flush with the rosette of the cabbage and became significantly thinner and weaker. Moreover, internode was not obvious, but the number of leaves increased. The data showed a 20.3% reduction in stem diameter and a 61.4% reduction in stem length compared to the control. The above results indicate that GA_3 is more prominent in the role of carex stem development.

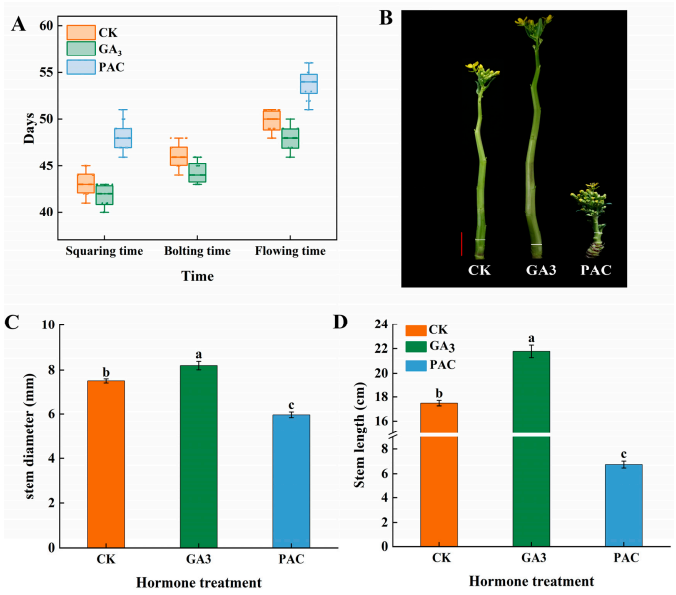

Figure 9. Effects of externally applied GA_3 and PAC on the time of bolting and flowering and the development of carex stem in cabbage. (**A**) Quantitative analysis of the time of squaring, bolting, and flowering in GA_3 treatment, PAC treatment and control, and the horizontal line in the figure indicates the median ($n > 20$). (**B**) Morphological phenotypes of stems at flowering stage after external application of treatments, scale bar = 2 cm. (**C**,**D**) Stem diameter and length of stems at flowering stage, the data shown are means and error lines indicate SE ($n > 20$). All data were evaluated statistically using one-way ANOVA ($p \leq 0.05$). Different lowercase letters indicate a significant difference.

We further observed the alterations in the paraffin sections of stem cells. The transverse cell morphology of the stems under different treatments is shown in Figure 10A, and the longitudinal cell morphology is shown in Figure 10B. By comparing the area (Figure 10C) and length (Figure 10D) of pith cells, the results show that the area and length of the pith cells in the GA_3 treatment increased by 31.2% and 33.3%, respectively, compared with those in the CK. However, the area and length of the pith cells in the PAC treatment decreased by 69% and 53%, respectively, compared with those in the CK group, and the cells were more tightly arranged. Therefore, it follows that exogenous GA_3 can influence the bolting and stem development of cabbage.

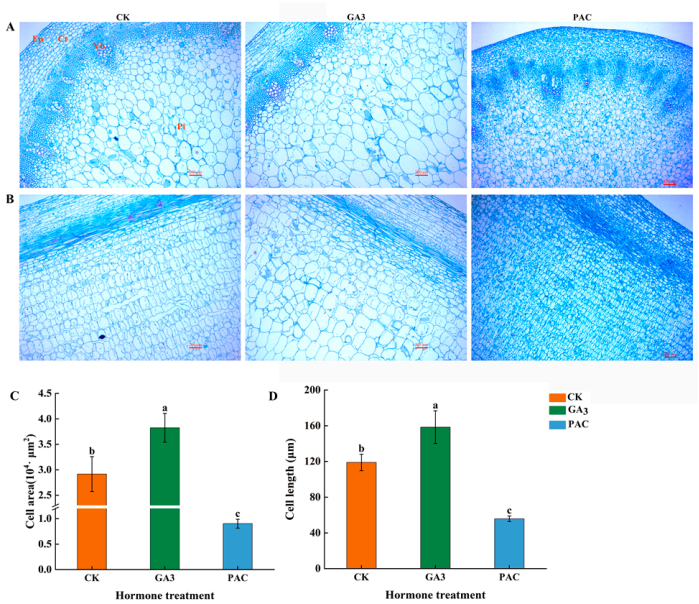

Figure 10. Effects of externally applied GA_3 and PAC on the cells of stems during the flowering period of cabbage. (**A**) Anatomical drawings of stems in cross-section at the flowering stage, EP: epidermis, Ct: cortex, Vb: vascular bundles, Pi: pith. Scale bar = 200 μm. (**B**) Anatomical drawings of longitudinal sections of stems at flowering stage. (**C**,**D**) Stem cell area and cell length of cabbage under different exogenous treatments, data shown are means and error lines indicate SE ($n > 150$). All data were evaluated statistically using one-way ANOVA ($p \leq 0.05$). Different lowercase letters indicate a significant difference.

4. Discussion

In this study, we demonstrated that photoperiod affects the growth of bolting, flowering, and stem development in cabbage and that gibberellin plays an important role in this process. Non-heading Chinese cabbage is an LD plant. However, the length of the bolting and flowering time varied under different LD conditions. Moreover, the time of bolting and flowering not only directly affects the yield and quality of cabbage [35] but also features critical impacts on the breeding of floral regulation among different varieties. Therefore, proper bolting and flowering under suitable light conditions can ensure the quality of cabbage and reduce energy consumption. In this study, we found that prolonging the light duration could accelerate the bolting and flowering of cabbage under LD. Santos [36] also found that the flowering time of cassava was significantly advanced when the light duration was extended via artificial supplementation. The photoperiodic change also affected the photosynthesis of leaves. Relatively high chlorophyll could be more favorable for photosynthesis, providing a material basis for the next stage of the flowering process [37]. It was also found in rapeseed that prolonging photoperiod could promote plant growth [38]. The findings of this study revealed that the pigment content under the Ph18 treatment, which caused a short flowering time, peaked at the bolting stage, possibly indicating a reserve of nutrition for the early flowering stage, thus facilitating early flowering. However, the pigment content under the Ph14 and Ph16 treatments increased gradually with the advancement of the bolting and flowering time, and thus, under these two treatments, blossoming successively occurred following the Ph18 treatment. The pigment content under the Ph12 treatment gradually decreased with the advancement of the floral transition. Reduced pigmentation may not be favorable for photosynthesis, so the time of bolting and flowering was also longer.

The process of the bolting and flowering of cabbage is accompanied by the development of carex stems. Stems are also considered as nutrient storage organs of plants [24]. Therefore, the quality of carex stems can be used as an index to evaluate the quality of bolting. In this study, we found from the morphological observation that the stem development

was optimum under the Ph16 treatment at the bolting and flowering stages. This indicates that photoperiod affects stem development in the process of flower formation. When the light duration was sufficient, incomplete stem growth may have been due to premature flowering and nutrient bias in preparing for the flowering of cabbage. In contrast, the time of bolting and flowering was delayed under shorter photoperiod conditions, while the development of the carex stems was also hindered. The proliferation and expansion of the internodal meristematic tissue cells significantly affected stem elongation and development [39]. The results of paraffin sections of the stems showed consistency with the phenotype. We can hypothesize that photoperiod affects the elongation and growth of the cells inside carex stems inside the cells, which causes the differences in stem length and stem diameter phenotypes.

In previous studies, GAs have been found to respond positively to the photoperiodic regulation of flowering, especially under SD [40,41]. However, significant effects of GAs have also been demonstrated in Viola philippica [42], garlic [25], spinach [43], and sugar beet [44]. They also proved the actions of GAs to be remarkable under LD, while the GAs requirement was reduced by CO and FT relative to under SD [45]. Temperature and light have also jointly affected plant elongation and growth in Brassica juncea [46]. However, using shade and PAC to treat Arabidopsis, Alabadi found that endogenous GAs repress photomorphogenesis in the dark [47]. Therefore, the functional role of GA varies with light conditions. The present study, however, focused on LD to investigate the function of GAs in the photoperiodic regulation of bolting and flowering. GAs are essential hormones that harmonize plant development, with the most critical functions including flowering, stem development, and cell elongation [48]. GAs have been reported to be involved in the photoperiodic induction of the flowering pathway [21,49]. GAs and SLs have been shown to cooperate in the regulation of stem development in cucumber [50]. Measurements of endogenous GA_3 showed that within a specific range of light duration, a higher GA_3 content promoted bolting and flowering. Beyond this range, the GA_3 content declined, while flowering still advanced. This may be attributable to the synergistic action of GA_3 with other species of active gibberellins in inducing flowering under more prolonged photoperiodic conditions [51,52]. It is also assumed that feedback regulation by endogenous GAs resulted in a lower endogenous GA_3 content in the cabbage under longer light durations. In addition, we found that changes in the endogenous GA_3 content coincided with the development of carex stems, and we hypothesized that endogenous GA_3 is involved in photoperiod induction of stem growth in cabbage.

The feedback regulation of *GA20ox*, *GA3ox*, and *GA2ox* can mediate GAs level homeostasis [53]. In this experiment, data from the corresponding relative quantitative analysis of genes showed that the expression tendency of *BcGA3ox2* was the same as that of *BcFT*, which may be involved in the flowering process of cabbage. Osnato showed that, in Arabidopsis, *GA3ox2* affected flowering by regulating *FT* with the TEM1 transcription factor [41]. We hypothesize that a conserved function exists since cabbage belongs to the same cruciferous family as Arabidopsis to some extent. *BcGA20ox2* prefers to regulate carex stem development, while the different gibberellin synthase oxidase genes are expressed in separate patterns during the bolting and flowering process. It is hypothesized that *BcGA20ox2* may promote active GAs synthesis to accelerate the rapid growth of the stems of cabbage and bolting. *EXPAs* and *XTHs* performed cell wall expansion by decreasing the viscosity of the polysaccharides between cell walls and cleaving xyloglucan chains to regulate cell wall relaxation, respectively [54]. Alabadi [55] proposed that GAs regulate cell expansion by integrating light signals. Related studies have also shown that GAs can promote cell wall relaxation and elongation by stimulating the expression of *EXPAs* and *XTHs* [56]. The data from this experiment showed that the relative expression of *BcEXPA10* and *BcXTH4* responded prominently to photoperiod. Therefore, the photoperiod may further affect the expression of cell expansion genes by regulating endogenous GA_3 content, leading to stem elongation and stem thickness.

To verify the above inference, we subjected cabbage to exogenous GA_3 and PAC treatments. The results confirmed that GA_3 significantly affected the elongation and development of inflorescence stems. Exogenous GA_3 promoted stem elongation and thickness, while PAC significantly inhibited stem growth. It was also confirmed in lettuce, showing a pronounced stem elongation after 12 days of exogenous spray treatment with 25 mg/L GA_3 [57]. Wang also demonstrated that exogenous GA_3 could increase the height of dwarfed plants [58]. Therefore, we speculate that the photoperiod affects the bolting and flowering of cabbage with the regulation of endogenous GA_3, which may be a key target for intervention in stem development. This was also shown in a recent study of gibberellin involvement in the photoperiodic regulation of chrysanthemum flowering [59].

5. Conclusions

In summary, the photoperiodic regulation of the bolting and flowering of cabbage showed that the longer the light duration, the shorter the bolting and flowering time. Cabbage stems developed preferably under 16 h (light)/8 h (dark) photoperiodic conditions. Meanwhile, endogenous GA_3 responded positively to the bolting process. Exogenous GA_3 induced a more prominent development of stems in the cabbage under LD conditions. Our results provide a scientific basis for the rational use of light facilities to regulate the breeding process and the mechanism of stem development in cabbage.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9121349/s1. Table S1: Primer sequences used for qRT-PCR amplification of relevant genes.

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