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A Study on Petal Morphological and Physiological Characteristics of *Styrax japonicus* during the Flowering Period

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Abstract: *Styrax japonicus* is a small ornamental tree with medicinal values, although its flowering period is short. To date, information about the morphological and physiological characteristics of the petals during the flowering period is limited. In this study, we observed the structure of the petals at the full flowering stage with a scanning electron microscope and detected the contents of nutrients, minerals, and endogenous hormones and the activities of enzymes at different flowering stages. The results showed that the content of soluble sugar exhibited an 'increase-decrease' trend, whereas the contents of soluble protein, nitrogen (N), phosphorus (P), and abscisic acid (ABA) showed a 'decrease-increase' pattern. The content of starch descended continuously, but the contents of potassium (K), gibberellic acid (GA_3), indoleacetic acid (IAA), and malondialdehyde (MDA) ascended continuously. The activities of peroxidase (POD) and superoxide dismutase (SOD) first rose and then declined during the flowering period. Higher contents of soluble sugar, N, K, and IAA promoted *S. japonicus* flowering; meanwhile, lower contents of starch, soluble protein, P, and GA_3 in addition to the lower activity of SOD might be some of the causes of the short flowering period. This work will serve as the foundation for a scientific technique to utilize the flowers and extend the flowering period in *S. japonicus*.

Keywords: *Styrax japonicus*; enzymes; hormones; minerals; nutrients; senescence

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1. Introduction

Controlling the flowering time and understanding the physiological mechanism of the induction of flowers are essential to the tourism, research, and flower industries [1]. In recent years, many researchers have studied the flowering process, including flower morphological structure, nutrients, mineral elements, enzymes, and relevant genes in flowers, so as to reveal the biological characteristics and regulate the flowering period of different plants [2–5]. Based on the study of flower morphology in the genus *Capsella*, Neuffer and Paetsch [6] specifically disclosed the petal size, pollen number, stigma length, and so on in *Capsella grandiflora*, *C. rubella*, and *C. bursa-pastoris*. Experiments were conducted to determine whether changing nutrient concentrations helped to induce *Eichhornia crassipes* flowering, and the result demonstrated that very low nutrient concentration conditions induced inflorescence formation [7], which reconfirmed the conclusion of Richards [8]. High soluble sugar content promoted *S. tonkinensis* flowering [9], while it was the opposite for olive [10]. Starch is one of the nutrients that may be converted into soluble sugar to meet the need for metabolic energy during the process of flower bud differentiation [9]. Moreover, soluble protein is one of the most important energy sources in plant bud initiation and the basis for flower organ morphogenesis [11]. Shahri et al. [12] pointed out that the soluble protein content in the *Helleborus orientalis* flower was similar from the tight bud stage to the half-open stage, but its content increased at the fully open stage and then decreased during senescence. Research on the changes of endogenous hormones is of great significance for the regulation of plant flowering. It was suggested that IAA content was

directly related to flowering [13], ABA affected flowering through the regulation of the vegetative growth of plants [9], and GA controlled various development processes, such as flower development [14,15]. IAA may be an inhibitor of flower bud formation because the photoperiod induction of leaves was interfered with by IAA [16]. GA is also considered to inhibit flower bud growth, although it could contribute to the flowering of *Juglans regia* [17]. SOD, POD, and catalase (CAT) belong to the protective enzyme system and are considered to be key enzymes in removing reactive oxygen species (ROS). To investigate the activity of antioxidant enzymes in *Dahlia pinnata* petals, Kan et al. [18] divided its flowering period into six stages. They concluded that an 'increase-decrease' trend of CAT and SOD activity appeared with the development of flowers, but POD activity was the opposite. Li et al. [19] used RNA-seq technology to examine the expression of flowering-related genes during *S. japonicus* flower development. The experimental data showed that 31,471 differentially expressed unigenes were generated, and these genes were associated with four pathways, including phytohormone signaling, transcription factor, protein kinase, and circadian rhythms.

Numerous researchers have carried out studies about the regulation of the flowering period. Severe nutrient stress delayed *Arabidopsis thaliana* from flowering; however, flowering was accelerated when plants were grown with alternating 'high-low' nutrient levels [3]. Insufficient nutrients and cell membrane damage were the main causes of petal senescence and a short flowering period, which was proved by Liu et al. [20] in the research of *Nelumbo nucifera* flowering regulation. On the basis of mastering the law of plant growth and development, some technical measures could be adopted to regulate the flowering time or prolong the flowering period [21]. Exogenous GA_3 application could replace low temperature to enable some plants that need to undergo vernalization to bloom at room temperature and promote the flowering of wild *A. thaliana* under short-day conditions [22]. It was found that the exogenous application of KNO_3 advanced the flowering time of *Mangifera indica* and enhanced its flowering rate [23].

Styrax japonicus, a low-branched landscape tree species with slight fragrance [24], is widely distributed in Korea, Japan, and southern China [25]. Although *S. japonicus* is an ornamental species with a huge number of flowers, the flowering period is relatively short, which limits its production and ornamental value to some extent. Hence, artificial methods to prolong the flowering period and improve flowering quality are in demand. According to our observation of phenology, a single flower bloomed for 4–5 days then withered, and the flowering period for a group of flowers was about two weeks. Until now, little information has been available regarding the morphological and physiological characteristics of *S. japonicus* petals during the flowering period.

Therefore, we conducted experiments to investigate the structure of petals at the full flowering stage and the contents of nutrients, mineral elements, hormones, and the activities of antioxidant enzymes in petals at different flowering stages. We attempted to answer the following questions: (1) Which physiological indexes have the greatest effects on *S. japonicus* flowering? (2) What is the relationship between these indexes and flowering? We hope our work may contribute to a better understanding of the flowering physiological mechanism of *S. japonicus* and consequently facilitate effective methods to regulate its flowering period.

2. Materials and Methods

2.1. Plant Material

Plant material was collected and processed at the Jiangsu Guoxing Biotechnology Co. Ltd., located in Luhe District, Nanjing, China (32°54′ N, 118°50′ E) in 2019. The average temperature, length of the day, and rainfall per year were 15.3 °C, 2200 h, and 970 mm, respectively. *S. japonicus* blooms in late April and quickly enters the blooming stage with white flowers (Figure 1). Nine-year-old *S. japonicus* trees originating from Yichang, Hubei Province, were used in our experiment. These plants grew in natural conditions, and no fertilizer was used. Ten trees with similar height, growth, and development conditions

were selected and tagged. Hence, each tree was regarded as one biological repetition. *S. japonicus* bloomed with stamens and pistil exposed at first, and then stigma exerted with bright anthers. Stigma, anthers, and petals were dry when flowers began to fall. Its flowering period was divided into three stages, including initial flowering (5% of the flowers on a flowering tree blossomed), full flowering (50% of the flowers on a flowering tree blossomed), and end flowering (95% of the flowers on a flowering tree blossomed).

Figure 1. General look of a flowering tree.

2.2. Sample Collection

To avoid the influence caused by different water, temperature, and light conditions, we harvested all the samples between 9:00–9:30 a.m. on a single day [26]. Thirty flowers were collected from the middle part of each tree at three stages. Then samples were mixed and taken back to the laboratory. Ten flowers from the full flowering stage were observed and photographed by scanning electron microscope (SEM) observation. Thirty flowers were chosen to measure the length and width of petals, calyx, and pedicel using a vernier caliper. The rest of the flowers at three flowering stages were used to determine several physiological indexes (including soluble sugar, starch, soluble protein, N, P, K, IAA, GA_3, ABA, SOD, POD, and MDA). Each index measurement contained three technical repetitions, then the average of the three values was calculated.

2.3. Determination Methods

SEM: Fresh petals were fixed in 4% glutaraldehyde in 0.2 M phosphate buffer and rinsed with 0.1 M phosphate buffer solution. Then these petals were fixed in 1% osmium tetroxide and rinsed with 0.1 M phosphate buffer again. After being dehydrated in ethanol, replaced with anhydrous alcohol and isoamyl ester, dried in a Hitachi HCP-2 critical point dryer (Hitachi, Tokyo, Japan) and coated with a mixture of gold/palladium in a Hitachi E-1010 sputter coater (Hitachi, Tokyo, Japan), fixed petals were observed and photographed by an FEI Quanta-200 SEM (FEI Company, Hillsboro, OR, USA) [27].

Soluble sugar and starch: A 0.2 g of sample in each replicate was ground and diluted to 10 mL. After two times of 30-min extraction with boiling water, they were diluted to 25 mL. Then 0.2 mL of extracting solution, 1.8 mL of distilled water, 0.5 mL of anthrone ethyl acetate, and 5 mL of 98% H_2SO_4 were added, respectively. Calculation of soluble sugar content was accomplished according to the method described by Li [28] and Wu et al. [29], and the OD value (at 630 nm) was read by a Beckman DU 800 UV-visible spectrophotometer

(Beckman Coulter, Inc., Brea, CA, USA; the same hereafter). The residues from extracting soluble sugar were transferred into test tubes and diluted to 10 mL. The tubes were placed in boiling water for full extraction for 15 min, then 2 mL of 9.2 mol/L perchloric acid were added, then the residues were extracted for another 15 min. The measurement steps were the same as above [9].

Soluble protein: A 0.2 g sample was ground and diluted to 5 mL. After centrifugation at 8000 r/min for 15 min at 4 °C (Allegra X-22R, F1010 Rotor, Beckman Coulter, Inc., USA), 1 mL of extraction and 5 mL of Coomassie brilliant blue G-250 were added to measure soluble protein content according to Li [28] and Bradford [30]. The OD value at 560 nm wavelength was read by a spectrophotometer.

Hormones: A fresh sample (0.3 g) was extracted with 80% cold methanol at 4 °C for 4 h. After purifying, vacuuming, and drying, the filtering samples were solved with PBS. GA_3, IAA, and ABA contents were measured using enzyme-linked immunosorbent assay (ELISA) according to Koshita et al. [31] and Weiler et al. [32].

N, P, and K: Petals were dried at 65 °C for 72 h, and then 0.1 g of petals were transferred into a Kjeldahl flask. A total of 9 mL of H_2SO_4 and 1 mL of $HClO_4$ were added, boiled, and filtered into a glass bottle for determination. Indigo colorimetry was adopted to estimate N using a spectrophotometer at 625 nm, molybdenum-antimony colorimetry to estimate P using a spectrophotometer at 700 nm, and flame atomic absorption spectrophotometry (AA900T, PerkinElmer, Waltham, MA, USA) to estimate K at 776 nm according to National Standards of the People's Republic of China.

POD, SOD, and MDA: A total of 0.2 g of petals were ground in 5 mL of phosphate buffer (pH = 7.8) using a pre-chilled mortar and pestle. The homogeneous liquid was made to determine POD, SOD, and MDA. The activities of POD and SOD were estimated based on the methods described by Beauchamp and Fridovich [33]. Specifically, 2.9 mL of the reaction solution (containing 28 μL of guaiacol and 19 μL of hydrogen peroxide) and 0.1 mL of the supernatant were mixed. POD activity was estimated with a spectrophotometer at 470 nm.

The homogenate was transferred to a centrifuge tube and centrifuged at 10,000 rpm for 20 min. The reaction system, including 1.5 mL of 50 mmol/L phosphate buffer, 0.3 mL of 130 mmol/L methionine, 0.3 mL of 750 μmol/L nitroblue tetrazolium, 0.3 mL of 100 μmol/L ethylene diamine tetraacetic acid, 0.3 mL of 20 μmol/L riboflavin, and 50 μL of phosphate buffer or supernatant, was made to estimate SOD activity using a spectrophotometer at 560 nm.

The homogenate was centrifuged at 3000 rpm for 20 min, 2 mL of supernatant and 2 mL of 0.6% thiobarbituric acid were added to the tube and placed in boiling water for 20 min. The OD values at 450 nm, 532 nm, and 600 nm were read, and the content of MDA was estimated according to the methods described by Hodges et al. [34] and Ennajeh et al. [35].

2.4. Data Analysis

Statistics were processed by Excel (Office 2013 Pro Plus, Microsoft Corporation, Redmond, WA, USA), and values were expressed as mean ± standard deviation for three replicates. One-way analysis of variance of assessments in *S. japonicus* petals at different flowering stages was performed by SPSS 22.0 (IBM, Armonk, NY, USA). The homogeneity of variance test was conducted and verified. The differences in Duncan's multiple comparisons at the significance level of 0.05 were established. p values less than 0.05 were considered to indicate significance within groups.

3. Results

3.1. Structure Observation of S. japonicus Flowers

S. japonicus flowers were comprised of 5–6 white petals and looked like pendulous bells. Flowers at the full flowering stage were used as an example to exhibit the structural characteristics. Unfolded petals had a length of 18.504 ± 0.302 mm and a width of

8.184 ± 1.621 mm. Many stellate hairs were present on both sides of the petals, and the hairs were bigger and in greater number on the upper surface of the petals (Figure 2a). Hairs on the lower surface of the petals were sparser and darker (Figure 2b). The pistil was covered by several stamens, which consisted of yellow-narrow anthers (Figure 2c). Pollens were dense and oval-shaped (Figure 2d). The length of the calyx and pedicel was 5.02 ± 0.211 mm and 24.251 ± 2.472 mm, respectively.

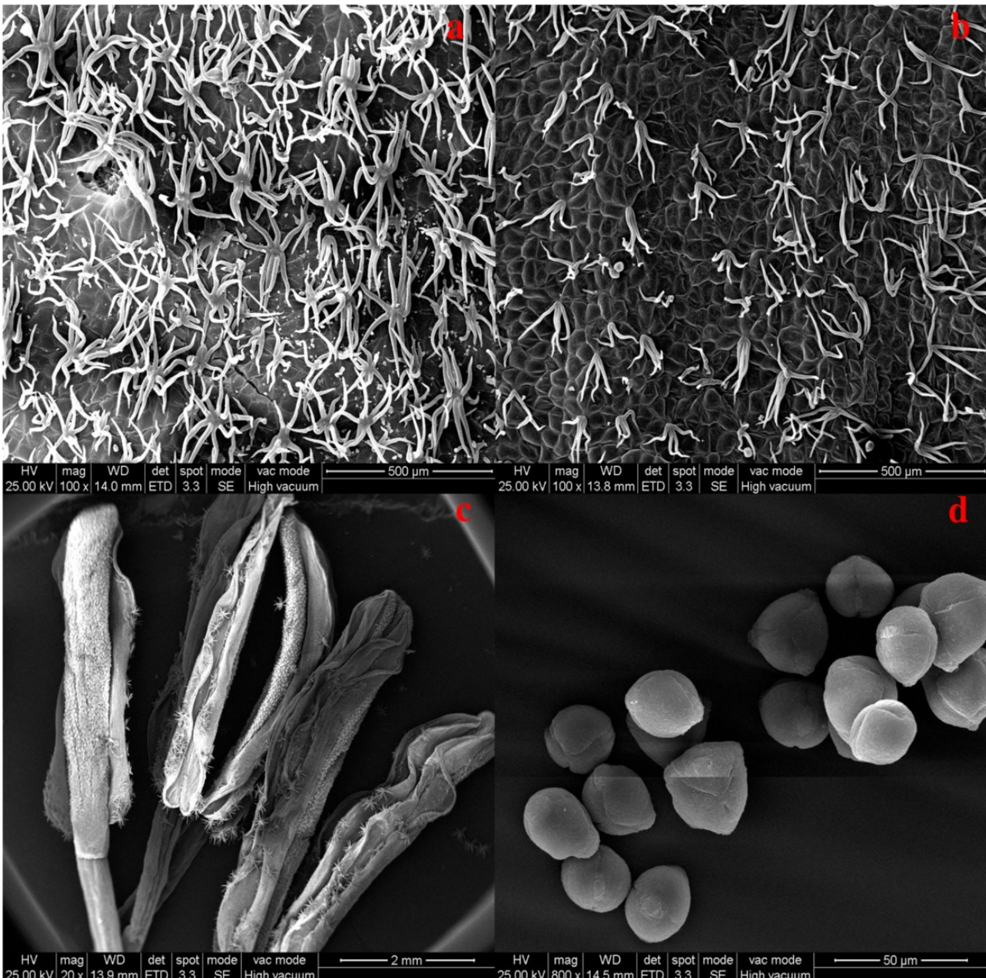

Figure 2. Structure of *S. japonicus* flowers at full flowering stage. (**a**) upper surface of petals; (**b**) lower surface of petals; (**c**) anthers; (**d**) pollens.

3.2. Comparison of Soluble Sugar, Starch, and Soluble Protein at Different Flowering Stages

To analyze the contents of soluble sugar, starch, and soluble protein in *S. japonicus* petals at different flowering stages, we applied one-way analysis of variance and found an 'A' pattern in the change of soluble sugar content (Figure 3). The content at the full flowering stage (36.91 mg/g FW) was significantly higher than at the initial flowering and end flowering stages; however, the contents at these two stages were very close (30.77 mg/g FW and 29.54 mg/g FW, respectively). Starch content displayed a continuous decreasing trend from the initial flowering to end flowering stages (Figure 3). As shown in Figure 3, the content of soluble protein at the initial flowering stage was 2.33 mg/g FW and then decreased to 2.22 mg/g FW at the full flowering stage. Soluble protein content exhibited a 'decrease-increase' trend and reached the maximum at the end flowering stage (2.37 mg/g FW). No significant differences were found among the three stages.

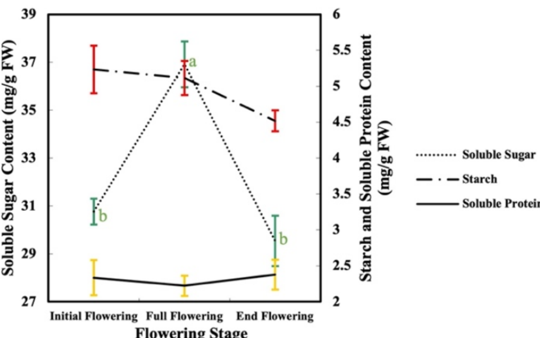

Figure 3. Changes of soluble sugar, starch, and soluble protein in *S. japonicus* petals during the flowering period. Data were shown as the average of three repetitions ± SD; different lowercase letters indicated significant differences at different stages.

3.3. Relationship between Mineral Elements and Flowering

The content of N at the initial flowering stage was significantly higher than at the full flowering and end flowering stages (Figure 4). With regard to P, the highest contents appeared at the initial flowering stage, and the lowest contents appeared at the full flowering stage, so the change trends of the content were 'decrease-increase' patterns (Figure 4). The contents of K at the initial flowering and full flowering stages were 0.30% DW and 0.31% DW, respectively, with no significant difference. Whereas the content at the end flowering stage was 0.39% DW, which was a significant difference from those at the other two stages (Figure 4).

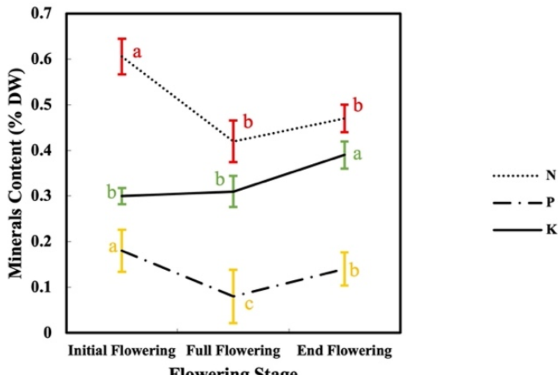

Figure 4. Changes of N, P, and K in *S. japonicus* petals during the flowering period. Data were shown as the average of three repetitions ± SD; different lowercase letters indicated significant differences at different stages.

3.4. Differences of the Contents of Hormones in S. japonicus Petals

GA_3 content increased continuously during the flowering period (Figure 5), and the difference between the full flowering and end flowering stages was significant (3.49 ng/g FW and 3.76 ng/g FW, respectively). The content of GA_3 increased slightly at the early stage and increased sharply at the late stage. The content of IAA increased continuously, and the difference among the three flowering stages was significant (Figure 5). The contents of ABA were more abundant at the initial flowering (54.50 ng/g FW) and end flowering (55.40 ng/g FW) stages than at the full flowering (51.90 ng/g FW) stage; however, no significant difference was observed among the three stages (Figure 5).

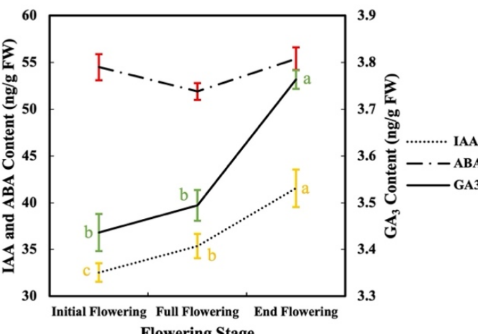

Figure 5. Changes of IAA, ABA, and GA_3 in *S. japonicus* petals during the flowering period. Data were shown as the average of three repetitions ± SD; different lowercase letters indicated significant differences at different stages.

3.5. Changes of Antioxidant Enzymes and MDA in S. japonicus Petals

The activity of POD ascended significantly from the initial flowering (472.17 U/g FW) to the full flowering (930.99 U/g FW) stage and thereafter descended significantly at the end flowering (666.16 U/g FW) stage (Figure 6a). The activity of POD at the full flowering stage was 97.2% higher than at the initial flowering stage. Figure 6a shows an 'increase-decrease' trend of the activity of SOD. Although there were no significant differences between the initial flowering and full flowering stages (23.43 U/g FW and 29.08 U/g FW, respectively), SOD activity at the end flowering (11.52 U/g FW) stage was significantly lower than those at the former two stages. MDA contents at the full flowering (2.61 µmol/mg FW) and end flowering (2.86 µmol/mg FW) stages were very close with no significant differences; however, they had significant differences with MDA content at the initial flowering (1.81 µmol/mg FW) stage. The changing pattern of the content of MDA rose continuously (Figure 6b).

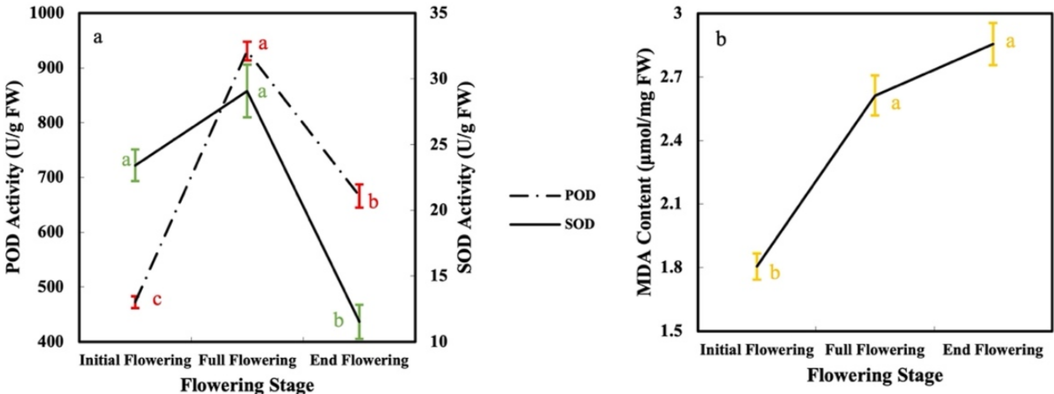

Figure 6. Changes of (**a**) POD, SOD, and (**b**) MDA in *S. japonicus* petals during the flowering period. Data were shown as the average of three repetitions ± SD; different lowercase letters indicated significant differences at different stages.

4. Discussion

4.1. Abundant Nutrients Promoted S. japonicus Flowering

Flowers are important reproductive organs of angiosperms, and the formation process of flowers consumes a large amount of energy. The dynamic changes of nutrients, for instance, soluble sugar, starch, and soluble protein, could reflect the growth and development of plants. Soluble sugar is a carrier of plant energy, as well as the main form of the transportation of carbohydrates, which could be directly utilized by plants, and its content change connects to the quality of flowers [36,37]. The present study indicated that soluble sugar content in petals at the initial flowering stage was 30.77 mg/g FW and then reached

36.91 mg/g FW at the full flowering stage; however, content declined to 29.54 mg/g FW at the end flowering stage. The possible reason behind this was the transition of the source-sink relationship caused by the energy demand during flowering. At the initial flowering stage, the flower organ was an important sink, which contained sufficient soluble sugar. The growth rate of *S. japonicus* flowers slowed down at the full flowering stage, resulting in the accumulation of soluble sugar [38]. Towards the end flowering stage, a decrease in soluble sugar content was presented because the flower as a sink was reduced. It was reported that soluble sugar was the main energy for flowers to sustain lifespan, and the decrease in soluble sugar in the late stage caused flower resistance to decline and accelerated the aging process [39]. Therefore, it was possible to enhance soluble sugar content so that the *S. japonicus* flowering period might be extended. Girdling would be an appropriate approach to accumulate soluble sugar content, thus facilitating bud induction and flowering [40].

Starch is a carbohydrate that has been reported as a resource for respiration and metabolic intermediates and structural components [41]. Numerous evidence confirmed that starch had great effects on plant flowering [42–44]. Wang et al. [44] explored starch mobilization in Oncidium 'Gower Ramsey' during the flowering process, and they indicated that little relationship was observed between the transition mechanism from vegetative to reproductive growth and the onset of starch mobilization. Obviously, their experimental result was not consistent with our common understanding that starch mobilization was helpful for floral induction [45]. Starch accumulation emerged as an 'S'-curve during sorghum flowering [46], whereas in the current experiment, starch content was much lower and decreased during the flowering period. We assumed that the starch in *S. japonicus* petals might be converted into soluble sugar to meet the need for metabolic energy during the flowering process.

Yu et al. [47] reported that soluble protein was one of the factors that impacted plant flowering, and our research proved their viewpoint. At the early stage, soluble protein was maintained at a relatively high level. Due to the flower's development, *S. japonicus* consumed many nutrients; thus, soluble protein content declined, then the petals absorbed and stored soluble protein from vegetative organs, leading to an increase in content.

4.2. Mineral Elements Affected S. japonicus Flowering Variously

In the present study, the average contents of N, P, and K were 0.5% DW, 0.13% DW, and 0.33% DW, respectively, and the change trends of these elements during the flowering stages were different. Yang et al. [48] analyzed the content of several minerals in *Rosa hybrida* petals and found that K was the most abundant mineral, followed by P, which was consistent with our results. Li et al. [49] discussed the link between the amount of minerals and the color of the petals in *P. lactiflora* and stated that red or purple petals had a higher amount of minerals than those that were white. K was the highest element in *P. lactiflora* petals (1.26%), which was much higher than in *S. japonicus* petals. In light of the above evidence, we might adopt some artificial measures to enhance K content in petals in order to attempt to change flower color. Fertilization was a common mineral supplement that directly affected plant growth. A profound understanding of the appropriate time and type of fertilization could effectively alleviate the increase of membrane permeability of petals, improve the activity of SOD, and significantly increase the longevity of flowers [50]. Low phosphorus content in *Matricaria chamomilla* petals promoted the synthesis of anthocyanin, which played a crucial role in free radical scavenging [51]. Similarly, P content in our study was lower when compared to N and K, so it could be considered to apply P fertilization to investigate its effect on the flowering of *S. japonicus*.

4.3. Hormones Closely Correlated with S. japonicus Flowering Period

Flowering is influenced by a complex network of genes that integrate multiple environmental factors and endogenous signals, thus ensuring that flowering occurs at the right time. Hormone regulation is vital in this process, and its signal transduction pathways are

involved in the formation of flowers [52,53]. A study conducted by Halevy and Mayak [54] revealed that ABA content correlated with flower longevity negatively. Muller et al. [55] reached the same conclusion. Although no significant difference existed in ABA content at the three flowering stages, the average ABA content (53.93 ng/g FW) was much higher when compared to the other two hormones. Hence, we assumed that high ABA content could shorten the flowering period in *S. japonicus*, and lowering ABA content was favored for flowering regulation [56].

GAs are crucial plant growth regulators that play an important role in flowering pathways and affect floral transition [14,57]. Chrispeels and Varner [58] expounded that the action of GAs and ABA was antagonistic in most physiological processes. As we mentioned above, high ABA content probably shortened the *S. japonicus* flowering period, so we speculated that more GA_3 was used in other physiological activities or transferred to other organs, leading to the low content of GA_3 when compared to ABA. With the development of *S. tonkinensis*, GA_3 content in petals displayed an increasing trend [39], which was identical to our experimental result. A high concentration of GA_3, especially 25 mg/kg, obviously postponed *M. indica* flowering, but the fruiting quality was improved [59]. Therefore, the impacts of GA_3 on flowering depended on the plant species and varieties [60].

Keeping IAA at a suitable level was beneficial for plant growth and development [61]. Additionally, high IAA content correlated with intense cell division [62]. Distribution or gradient of auxin within plant tissues was different because of the polar movement of IAA, and these changes were dynamic during the plant development process, such as flowering [62,63]. Xu et al. [39] determined IAA content in petals during *S. tonkinensis* flowering, and the result showed that IAA content first increased and then decreased; however, IAA content increased continuously in *S. japonicus* petals. It was concluded that IAA acted dissimilarly on flower development in different tree species, even in the same genus. Compared to CK, IAA extended the *Brassica napus* flowering period for five days, and Zhu et al. [64] attributed this phenomenon to the capacity of IAA in increasing antioxidants. We wondered whether exogenous IAA treatment could regulate the *S. japonicus* flowering time, and if yes, reasonable IAA concentration required further research.

4.4. Antioxidant Enzyme Activity and MDA Content Significantly Impacted S. japonicus Flowering

MDA is produced by membrane lipid peroxidation of unsaturated fatty acids in membrane lipids and could be inactivated by binding with proteins and enzymes. Normally, MDA is recognized to evaluate the degree of membrane damage [34]. The content of MDA is extremely low in growing plants, but it increases during flowering. Generally, MDA content equals the degree of petal senescence. As described in our study, MDA content increased gradually, indicating that the degradation of cell inclusions was intensified. The function of the enzymatic defense system decreased, and the degree of membrane lipid peroxidation was enhanced, revealing that flowering is an aging process.

POD is a biochemical marker, which could characterize the induction phase of the flowering process [65,66]. In addition, POD is involved in the production of ethylene and the oxygenolysis of IAA. McCord and Fridovich [67] found that SOD was capable of removing various free radicals and ROS produced by aerobionts. Both POD and SOD can convert superoxide anions or hydrogen peroxide into less active substances, reducing or eliminating their attacks on membrane lipids, so those membrane lipids are protected. The activities of POD and SOD in *S. japonicus* petals had the same trend of rising first and then declining. We supposed that *S. japonicus* could eliminate excessive ROS by enhancing the activities of POD and SOD to maintain the balance of metabolism at the early flowering stage; nonetheless, due to the reduction of protein degradation or synthesis, the activities of these two enzymes and scavenging capacity of ROS decreased at the late flowering stage. Our result was in agreement with Bartoli et al. [68] that the activities of SOD and POD increased first and then decreased. To conclude, *S. japonicus* inevitably produced MDA and other aging-promoting substances during the flowering period, but at the same time, SOD,

POD, and other anti-aging substances were generated, illustrating that these substances were antagonistic.

5. Conclusions

The current study elaborated the change trends of nutrients, minerals, hormones, and antioxidant enzymes and established the relationship between them and *S. japonicus* flowering. These physiological indexes were closely related to plant flowering, and they could be regulated by using appropriate flowering control techniques to achieve early, delayed, or even prolonged plant flowering periods. In general, light, temperature, and hormone regulation could be manipulated to affect the plant's flowering period. In recent years, with the deepening of flowering mechanism study, genetic engineering technology has also been applied to regulate plant flowering. Combined with high-throughput techniques, such as genomics, transcriptomics, and proteomics, genes associated with flowering were isolated, and the mechanism of plant flowering was illuminated, thereby realizing accurate regulation of the flowering period and improvement of flowering quality.

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