RESEARCH ARTICLE

Changes of endogenous hormone content in flower bud differentiation period of different *Sophora japonica* cv. "Jinhuai" in northern Guangxi

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Sophora japonica **cv. "Jinhuai", which owes its name to the golden yellow color of its dry buds, is an important representative of the *S. japonica* species and contains as much as 35% rutin, which has significant medicinal value. This tree species is extensively cultivated in the northern region of Guangxi, China with a large number of excellent cultivars being artificially bred to meet diverse production needs. However, there remains a significant gap in knowledge with respect to endogenous hormone dynamics in these cultivars. To establish a scientific foundation for regulating the flowering period of *S. japonica* cv. "Jinhuai" varieties, this study investigated the contents and dynamic changes of trans-zeatin-riboside (ZR), indole acetic acid (IAA), gibberellin (GA_3), and abscisic acid (ABA) in leaves of three *S. japonica* cv. "Jinhuai" varieties including Zihua, Qiyi, and Zaoshu *via* enzyme-linked immunosorbent assay (ELISA). The results showed that the flower bud differentiation process differed among *S. japonica* cv. "Jinhuai" varieties depending on the endogenous hormones used. In Zihua *S. japonica* cv. "Jinhuai", high levels of ABA, IAA, and ZR, as well as high ABA/ GA_3 and ZR/IAA ratios were found to be beneficial for flower bud differentiation, while GA_3 had little effect on flower bud differentiation with the rank order as ABA > ZR > IAA > GA_3. In Zaoshu *S. japonica* cv. "Jinhuai", high levels of ABA and IAA, as well as high ABA/ GA_3 and ABA/IAA ratios were observed to be beneficial for flower bud differentiation, while low levels of ZR and ZR/IAA promoted flower bud differentiation and GA_3 had also little effect on flower bud differentiation. The rank order for this variety was ABA > IAA > GA_3 > ZR. In Qiyi *S. japonica* cv. "Jinhuai", high levels of ABA, IAA, and GA_3, as well as a high ABA/IAA ratio were to be beneficial for flower bud differentiation, while the level of ZR and ratios of ZR/IAA and ABA/ GA_3 were low with the rank order as GA_3 > ABA > IAA > ZR. The results suggested that multiple endogenous hormones differentially regulated flower bud differentiation processes in these three varieties, providing a foundation for further research focusing on flowering regulation mechanisms and flower quality improvement of *S. japonica* cv. "Jinhuai".**

Keywords: Zihua *Sophora japonica* cv. "Jinhuai"; Zaoshu *Sophora japonica* cv. "Jinhuai"; Qiyi *Sophora japonica* cv. "Jinhuai"; endogenous hormone; flower bud differentiation.

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Introduction

Sophora japonica is a tall tree belonging to the *Fabaceae* family with a maximum height of 25 m. *S. japonica* grows in both northern and southern China and is most common in northern China and on the Loess Plateau [1]. The dried buds of *S. japonica* (known as Huaimi) have high medicinal value, as they contain rutin, flavin, and other active ingredients. This plant is recorded in the

"Chinese Pharmacopoeia" as having cooling, convergence, hemostasis, and blood pressure-lowering effects [2]. The *Sophora japonica* cv. "Jinhuai" is a cultivated variety of *S. japonica*, named for the golden color of its buds and flowers [3]. Compared to the other species of *S. japonica*, this cultivar has the highest rutin content up to 30%, making it extremely valuable both economically and medicinally. Hydrolyzed quercetin derived from rutin can help maintain normal capillary tension, dilate coronary vessels, and improve heart contractility and output [4]. Furthermore, quercetin exhibits anti-inflammatory, antispasmodic, anti-ulcer, anti-oxidative, anti-tumor, and other effects. Therefore, artificial technologies have been employed to select high-quality *S. japonica* cv. "Jinhuai" varieties to improve the economic benefits associated with planting this species and to meet diversified market needs. The varieties of *S. japonica* cv. "Jinhuai" include Zihua, Zaoshu, and Qiyi varieties obtained through artificial hybridization and breeding. Currently, there have been few research reports focused on these three varieties domestically or internationally.

Research focused on *S. japonica* cv. "Jinhuai" to date has primarily focused on seedling breeding [5, 6], chemical composition [7, 8], and physiological characteristics [9]. However, there is lack of research focused on endogenous hormones, which are organic substances produced by plants to regulate plant growth and development. Four key phytohormones including zeatin riboside (ZR), indole acetic acid (IAA), abscisic acid (ABA), and gibberellin (GA_3) are central regulators of plant growth and development including the process of flower bud differentiation that marks a key step in flowering plant development, corresponding to the transition between vegetative and reproductive growth. Flower bud differentiation can significantly affect the quantity and quality of flower buds produced by *S. japonica* cv. "Jinhuai", thereby impacting its yield. This study aimed to clarify the relationship between changes in endogenous hormone levels and the process of floral bud differentiation by measuring the content of different endogenous hormones in various varieties of *S. japonica* cv. "Jinhuai" during flower bud differentiation. Endogenous hormone changes in three different cultivars of *S. japonica* cv. "Jinhuai" varieties were measured using enzyme-linked immunosorbent assay (ELISA) to analyze the changes in the levels of these phytohormones during the process of flower bud differentiation. The results of this study would provide new insight into the dynamic nature of these processes, thereby providing a valuable theoretical basis for the high-yield cultivation and scientific management of *S. japonica* cv. "Jinhuai".

Materials and methods

Experimental location and sample collection

The testing site is located at the Guangxi Institute of Guangxi Botany in Guilin, Guangxi, China, which falls within a subtropical monsoon climate zone characterized by a mild climate, abundant rainfall, and sufficient light. The mean yearly sunshine, temperature, precipitation, frost-free time, and relative humidity are 1,680 hours, 23.5°C, 1,949.5 mm, 300 days, and 82%, respectively. The leaves of three *S. japonica* cv. "jinhuai" varieties, Zihua (ZH), Zaoshu (ZS), and Qiyi (QY), were collected at different stages including the vegetative stage (I), the early (II), middle (III), late (IV) stages of flower bud differentiation, the flower bud stage (V), and the initial flowering stage (VI). Healthy leaves free of insects were cut along with the branches that had flowers and marked before being sealed in a bag and stored at -80°C.

Endogenous hormone content determination

The levels of endogenous hormones including IAA, ZR, GA_3, and ABA in each stage (I-VI) for the ZH, ZS, and QY varieties were determined by using ELISA (Shanghai Hepeng Biotechnology Co., Ltd., Shanghai, China) following the manufacturer's instructions [11]. Briefly, 1.0 g of each sample was weighed and combined with 2.0 mL of 80% extraction buffer (80% methanol containing 1 mmol/L di-tert-butyl-pcresol/antioxidant (BHT)) and a small amount of quartz sand. The mixture was homogenized in an ice bath and transferred to a 10 mL centrifuge tube. The remaining sample was washed on the grinder twice using 2 mL of extract buffer and transferred to the centrifuge tube. The contents were mixed thoroughly and incubated at 4°C for 4 hrs. After centrifugation at 3,500 rpm for 8 mins, the supernatants were transferred into new centrifuge tubes. Then, 1.0 mL of each extract was added to the precipitate and shaken thoroughly. The solution was incubated again at 4°C for 1 h. After centrifugation, the two supernatants were combined, and the volume of the sample solution was recorded. The sample solution was placed in a 45°C water bath, dried under nitrogen, and diluted to a volume of 2 mL with the sample diluent. The optical density of the sample solution was measured using an ELX 808 microplate reader (BioTek, Winooski, Vermont, USA). A standard curve was used to calculate the endogenous hormone content in the sample.

Statistical analysis

SPSS 21 (IBM, Armonk, New York, USA) was employed in this study. The data were analyzed using ANOVA and Duncan's test with $P < 0.05$ as the significance difference. Origin 2022 (OriginLab, Northampton, MA, USA) was used to visualize the resultant data.

Results

Endogenous hormone dynamics in three *S. japonica* cv. "Jinhuai" varieties during flower bud differentiation

During flower bud differentiation in ZH, the highest level of ABA was observed in the late stage (IV) with a decreasing and then increasing trend (Figure 1a). Similarly, the highest level of IAA was observed in the late stage (IV) with an increasing-then-decreasing trend. At the late stage (IV), ZR content exhibited an increasing trend followed by a decrease with a distinct peak. At the flower bud stage (V), the GA_3 content level exhibited a decreasing trend followed by an increase and then a decrease. During flower bud differentiation in the ZH variety, ABA levels were significantly higher than those of other endogenous hormones with the rank order as ABA > ZR > GA_3 > IAA. Additionally, these four endogenous hormones presented with varying degrees of fluctuation over the course of floral bud differentiation. The largest fluctuation was observed for ZR, while ABA, GA_3, and IAA exhibited smaller fluctuations.

The ZS variant demonstrated the highest ABA level during the flower bud stage (V), and these levels varied significantly throughout each stage of flower bud differentiation (Figure 1b), while IAA level peaked in the early stage (II) and exhibited an initial increase followed by a decrease and then another increase. ZR level peaked during the early stage (II). At the initial flowering stage (VI), GA_3 level reached the highest and exhibited relatively little variation across different stages. ABA levels were significantly higher than those of other endogenous hormones with the following rank order of ABA > ZR > GA_3 > IAA. When comparing the different floral bud differentiation stages for this cultivar, significant changes in ABA and ZR levels were observed, whereas IAA and GA_3 levels remained relatively stable.

The highest ABA level of QY during the flower bud differentiation was observed in the flower bud stage (V) (Figure 1c). There were two distinct peaks with one in the sepal during the middle stage (III) and the other in the flower bud stage (V). ABA content exhibited an initial increase followed by a decrease, then another increase and finally a decrease. During the middle stage (III), IAA level exhibited a pattern of increasing, decreasing, and then increasing again. During the flower bud differentiation process, GA_3 content peaked during stage III, exhibiting an initial increase followed by a decrease and then another increase. In contrast, ZR level peaked during the vegetative period (I) and initial flowering period (VI), whereas they were the lowest during the late stage of bud differentiation (IV) with a decreasing-then-

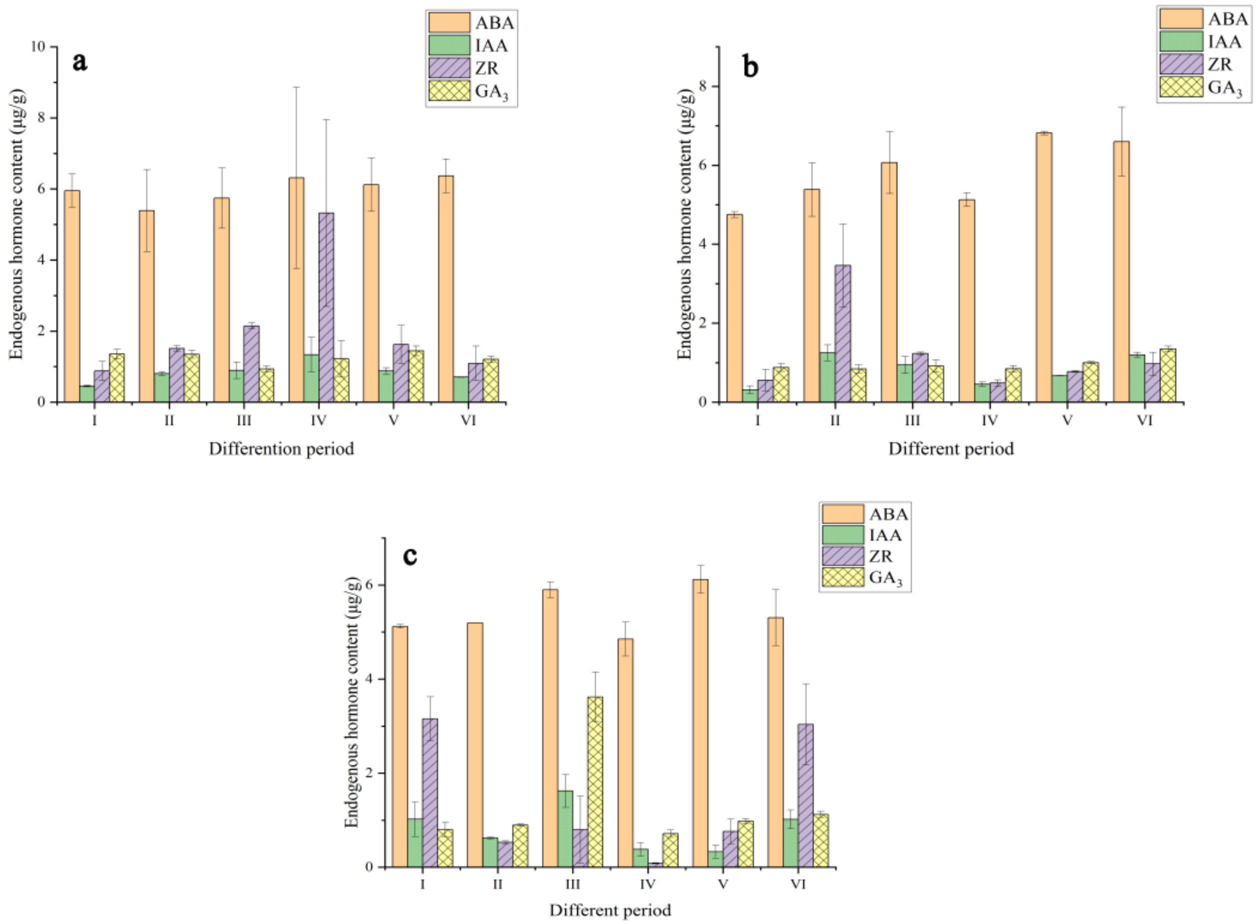

Figure 1. Changes in the contents of four endogenous hormones in each period of paraphlomis japonica. **a.** Zihua *S. japonica* cv. "Jinhuai". **b.** Zaoshu *S. japonica* cv. "Jinhuai". **c.** Qiyi *S. japonica* cv. "Jinhuai". **I:** the vegetation stage. **II:** the early stage of flower bud differentiation. **III:** the middle stage of flower bud differentiation. **IV:** the late stage of flower bud differentiation. **V:** the flower bud stage. **VI:** the initial flowering stage.

increasing trend. GA_3 level peaked during stage III with an initial increase followed by a decrease and then another increase. The content of ZR was higher during the vegetative stage (I) and the first flowering stage (VI) and the lowest during the late stage (IV) with a tendency to decrease and then increase. ABA level was significantly higher than those of other endogenous hormones with the following rank order of ABA > GA_3 > ZR > IAA. Further analyses of endogenous phytohormone levels throughout the differentiation process in QY variety revealed that, while IAA level remained relatively consistent, marked changes in ABA, GA_3, and ZR levels were noticeable with ZR showing the most significant changes.

Analysis of endogenous phytohormone ratio dynamics in *S. japonica* cv. "Jinhuai" during the process of flower bud differentiation

During floral bud differentiation, the three *S. japonica* cv. "Jinhuai" varieties exhibited different trends in terms of their ABA/IAA levels with the largest changes being observed in QY variety (Figure 2a). The ABA/IAA levels of QY initially rose, then declined, and ultimately peaked during budding. In ZH variety, this ratio initially decreased to a minimum during the late stage of bud differentiation (IV) before increasing. In contrast to the other two *S. japonica* cv. "Jinhuai" varieties, the ABA/IAA ratio for ZS variety first declined, then increased, and decreased again, reaching a minimum during the early stage of differentiation (II) followed by a

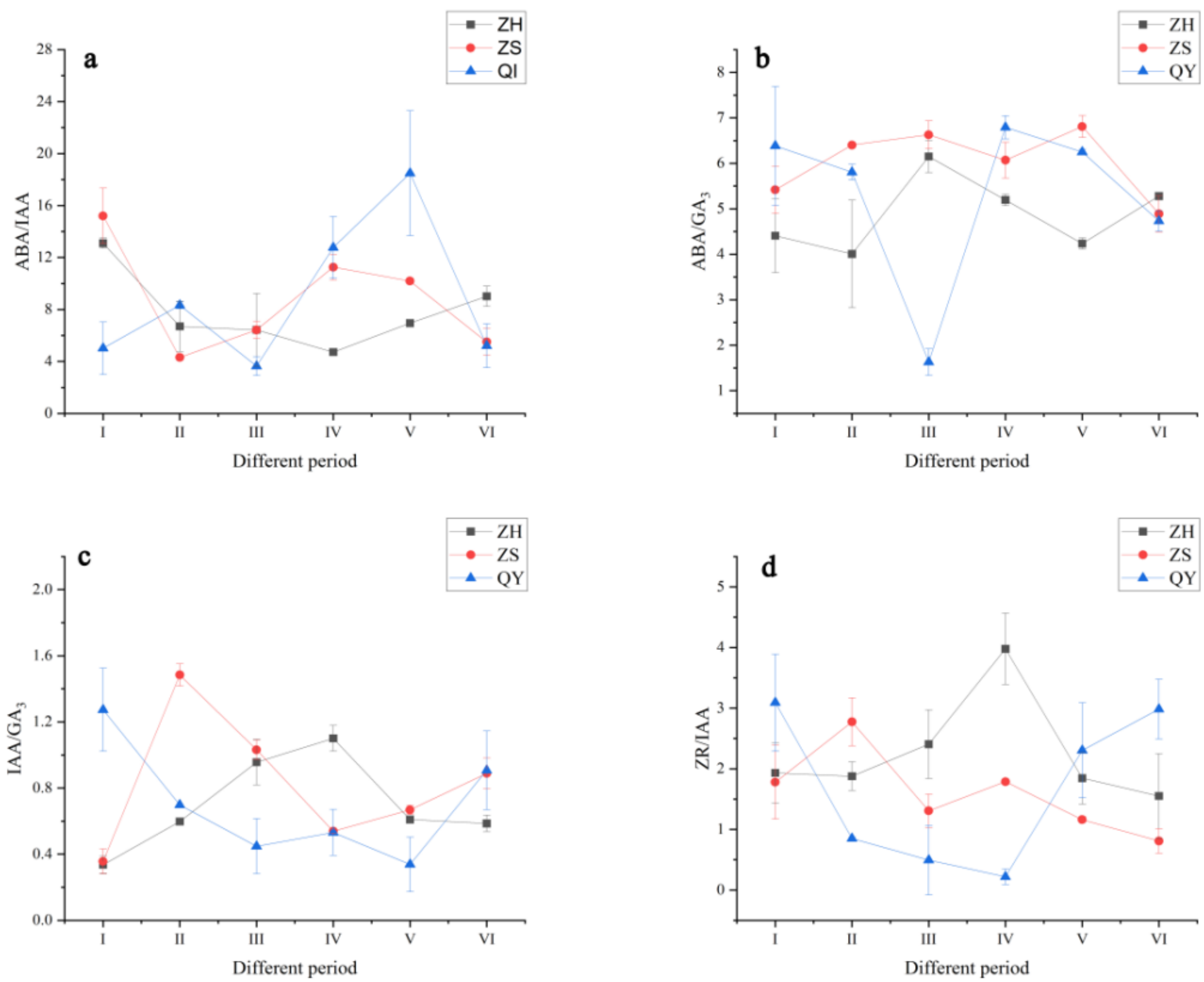

Figure 2. Changes of different endogenous hormone ratios during flower bud differentiation of three varieties of *S. japonica* cv. "Jinhuai". **a.** ABA/IAA. **b.** ABA/GA_3. **c.** IAA/GA_3. **d.** ZR/GA_3. **I:** the vegetation stage. **II:** the early stage of flower bud differentiation. **III:** the middle stage of flower bud differentiation. **IV:** the late stage of flower bud differentiation. **V:** the flower bud stage. **VI:** the initial flowering stage.

weak peak during the late stage of differentiation (IV).

The ABA/GA_3 ratios of three *S. japonica* cv. "Jinhuai" varieties exhibited different trends in floral bud differentiation (Figure 2b). The QY variety exhibited the largest change in this ratio, which initially decreased, then increased, then decreased again. The ratio remained consistently low during the flower bud differentiation stage, reaching a minimum during stage III. In the ZH variety, the ABA/GA_3 ratio varied significantly with consistently remaining high during bud differentiation with a clear peak during stage II. Conversely, the ABA/GA_3 ratio in the ZS variety exhibited minimal fluctuation, remaining high throughout the differentiation period without any noticeable peak.

The IAA/GA_3 ratio in the ZS variety changed significantly during the process of bud differentiation (Figure 2c). The ratio initially increased, then decreased, then increased again with a clear peak during the early differentiation stage (II). Similarly, the ZH variety exhibited an increasing and then decreasing trend for this ratio, which peaked at the end of flower bud differentiation but remained relatively high from stages II-IV. In contrast, IAA/GA_3 level in the QY variety initially declined and then rose without a clear peak, reaching a minimum during the flower bud stage (V) but remaining consistently low throughout bud differentiation stages II-IV.

The changes in the ZR/IAA ration in the QY variety were the most pronounced, with first decreasing and then increasing (Figure 2d). The ratio reached its minimum value during late bud differentiation (IV) and remained consistently low throughout bud differentiation. In the ZH variety, this ratio exhibited an increasing trend followed by a decrease, which contrasted with what was observed in the QY variety. The ZR/IAA ratio peaked at the end of flower bud differentiation and remained high throughout this process. No consistent fluctuations of the ZR/IAA ratio were observed in ZS variety during the flower bud differentiation process with two peaks during the early (II) and late (IV) stages, the latter of which was smaller than the former.

Discussion

The association between endogenous hormone levels and floral bud differentiation in three *S. japonica* cv. "Jinhuai" varieties

Endogenous hormones including ABA play a crucial role in regulating plant flowering and inflorescence development [12]. ABA is mainly synthesized in dormant and abscission organs where it acts as a growth inhibitor. It regulates dormancy, abscission, and the plant stress response. Additionally, ABA also plays a significant role in the flora bud differentiation process. In general, high ABA level can promote bud differentiation, which has been reported in studies of *Prunus domestica* × *armeniaca* 'Fengweimeigui' [13], *Syringa microphylla* [14], *Xanthoceras sorbifolium* Bunge [15], *Agave* hybrid No. 11648 [16], and other species. Similarly, this study found that the ABA peaks for the three species of *S. japonica* cv. "Jinhuai" occurred during the flower bud differentiation period, which indicated that high levels of ABA could promote the initiation of bud differentiation in these cultivars, supporting the transition from vegetative to reproductive growth. High levels of ABA can increase the concentration of sugars in the vacuole of flower buds, providing sufficient raw materials for differentiation. The role of IAA in flower bud differentiation process is controversial. Some studies found that IAA levels were low during bud differentiation in *Bougainvillea*, whereas others found that high IAA concentrations could promote bud differentiation in plants such as apricot [17] and longan [18]. Similarly, this study found that, in three varieties of *S. japonica* cv. "Jinhuai", high levels of IAA were associated with floral bud differentiation and opening as elevated levels of IAA facilitating the blooming of these three *S. japonica* cv. "Jinhuai" varieties. The results suggested that IAA not only played a crucial role in the flower bud differentiation process but also in the formation and blooming of flowers. ZR is an important natural cytokinin (CTK) and involved in plant xylem transport and bud differentiation. Studies showed that a high ZR concentration could promote floral bud differentiation in sweet cherry [19], *Camellia oleifera* [20], and other plants. Similar results were found in Zihua *S. japonica* cv. "Jinhuai". Throughout the period of flower bud differentiation, ZR level remained consistently high in all three varieties of *S. japonica* cv. "Jinhuai" included in this study, suggesting that elevated ZR levels played a role in the initiation of flower bud differentiation in these varieties. High levels of ZR might contribute to the accumulation of proteins and nucleic acids in the branches of *S. japonica* cv. "Jinhuai", promoting normal differentiation of flower primordia. During the early, middle, and late stages of bud differentiation in Zaoshu *S. japonica* cv. "Jinhuai" and Qiyi *S. japonica* cv. "Jinhuai", ZR levels remained consistently low, which suggested that a low level of ZR was related to this differentiation process in these varieties. Similar observations were found during floral bud differentiation in Olea europaea [21] and *Cerasus subhirtella* "Autumnalis" [22] with low ZR concentration promoting the differentiation of floral bud. Hoad, *et al*. found that GA_3 inhibited the differentiation and morphogenesis of flower buds in several plants by promoting the formation of α-amylase, which accelerated starch hydrolysis and thus inhibited flowering in fruit trees [23]. In contrast, the results of this study revealed that the levels of GA_3 in Zihua and Zaoshu *S. japonica* cv. “Jinhuai” varieties remained largely unchanged, suggesting that this phytohormone was largely unrelated to bud differentiation in these varieties, which was in line with what was described for *S. microphylla* [14]. However, the levels of GA_3 varied greatly and there was an apparent peak at the middle stage of bud differentiation in Qiyi *S. japonica* cv. “Jinhuai”, suggesting that high levels of GA_3 might contribute to bud differentiation for this variety in line with what was reported in *Hibiscus mutabilis* Linn [24] and *Crocus sativus* L. [25]. In addition, rising GA_3 levels were detected in the budding and early flowering stages, suggesting that this phytohormone might promote flowering. Similar results have been described in studies of garden flowers such as *Camellia* L., *Paeonia* × *suffruticosa* Andrews and *Gardenia jasminoides* J. Ellis [26].

The relationship between endogenous hormone levels and flower bud differentiation

The process of flower bud differentiation in plants is not only influenced and regulated by a single endogenous hormone, but instead depends on the balance between several hormones [27]. Wang, *et al*. found that lower levels of ABA/GA_3 and ABA/IAA were beneficial for the transition of *Crocus sativus* L. from vegetative to reproductive growth [25]. Similarly, Li, *et al.* found that high levels of ABA/IAA and ABA/GA_3 were beneficial for the maintenance of the nutritional status of *Hemerocallis*, while reductions in these ratios were beneficial for floral bud differentiation [28]. In Zihua *S. japonica* cv. “Jinhuai”, this phenomenon was also observed. Low levels of ABA/IAA and ABA/GA_3 could promote the transition of Zihua *S. japonica* cv. “Jinhuai” from vegetative growth to reproductive growth. Compared to Zihua *S. japonica* cv. “Jinhuai”, the higher ABA/GA_3 in Zaoshu *S. japonica* cv. “Jinhuai” was beneficial to the transformation of physiological morphology. However, in Qiyi *S. japonica* cv. “Jinhuai”, although the lower ABA/GA_3 ratio was associated with the transition from vegetative to reproductive growth, a higher ABA/IAA ratio was also found to exhibit a promoting effect in line with the results of Zhang, *et al*. [29]. Many studies showed that a high ABA/IAA ratio was conducive to the differentiation of flower buds [30, 31]. Consistently, this study also found that higher ABA/IAA ratios were associated with floral bud differentiation in Zaoshu *S. japonica* cv. “Jinhuai” and Qiyi *S. japonica* cv. “Jinhuai”, whereas the same was true for a lower ABA/IAA ratio in Zihua *S. japonica* cv. “Jinhuai”, consistent with the results published by Liu, *et al*. in the vine rose “Angela” [32]. In addition, high levels of ABA/GA_3 can also facilitate the differentiation of floral buds [33]. Similar phenomena were found in Zihua *S. japonica* cv. “Jinhuai” and Zaoshu *S. japonica* cv. “Jinhuai”. High levels of ZR/IAA also play a role in promoting floral budding [31]. Although this was only found to be true in the Zihua *S. japonica* cv. “Jinhuai” variety in this study, the low levels of promotion were observed in the Zaoshu *S. japonica* cv. “Jinhuai” and Qiyi *S. japonica* cv. “Jinhuai”.

Conclusion

Through the investigation of changes in endogenous hormone contents in leaves of three *S. japonica* cv. “Jinhuai” varieties at different floral bud differentiation stages, the results provided new insights into endogenous phytohormone dynamics during the budding process. Specifically, these endogenous hormones were found to play varied regulatory roles, differentially shaping the budding of these three varieties. The different responses of different *S. japonica* cv. “Jinhuai” varieties to different endogenous hormones were observed. Through the elucidation of the relationship between endogenous hormones and budding in *S. japonica* cv. “Jinhuai”, this study provided a theoretical reference for further studies focused on the mechanisms that regulate the flowering and flower quality improvement of this plant.

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