

RESEARCH ARTICLE

Comparative study of endogenous hormone dynamics during the first and second growth cycles in *Sophora japonica* cv. *jinhuai* harvested twice a year

Yishan Yang, Zongyou Chen, Rong Zou, Jianmin Tang*, Yunsheng Jiang

Guangxi Key Laboratory of Plant Functional Phytochemicals and Sustainable Utilization, Guangxi Institute of Botany, Chinese Academy of Sciences, Guilin, Guangxi, China

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Sophora japonica cv. *jinhuai* is the best Chinese Flos Sophorae Immaturus variety with high medicinal and economic values. Generally, *Sophora japonica* harvests once a year. However, *Sophora japonica* cv. *jinhuai* harvests twice a year. This study targeted the flowering mechanisms of *Sophora japonica* cv. *jinhuai* by investigating the changes of endogenous hormones in the flower bud differentiation process to explore the important scientific foundation in the production of *Sophora japonica* cv. *jinhuai* that harvests twice a year. The contents of indole acetic acid (IAA), gibberellin (GA3), zeatin riboside (ZR), and abscisic acid (ABA) in vegetative period, flower bud stage, budding period, full flowering stage, and fruit stage of flower bud differentiation of *Sophora japonica* cv. *jinhuai* were determined by using enzyme-linked immunosorbent assay (ELISA). The hormones' actions of the above different stages were analyzed and compared to each other for further exploring the process of flower bud development (FBD) of *Sophora japonica* cv. *jinhuai*. The results showed that, in the process of flower bud differentiation period, from low to high, the endogenous hormones' contents in the first and second harvest times were: ABA > IAA > ZR > GA3. The content of ABA was the highest one among the four endogenous hormones at each stage, which could promote flowering. Low content of IAA might promote flower bud initiation, while GA3 inhibited flower formation. Low level of IAA/ABA ratio (0.374), GA3/ABA ratio (0.073), (IAA+GA3)/ZR ratio (1.887) could promote the transformation of *Sophora japonica* cv. *jinhuai* from vegetative growth to reproduction development during flower bud differentiation period. The results provided a solid theoretical basis and technical guarantee for the development of *Sophora japonica* cv. *jinhuai* planting industry in northern Guangxi area.

Keywords: *Sophora japonica* cv. *jinhuai*; flower bud differentiation; endogenous hormone; flower formation.

*Corresponding authors: Jianmin Tang, Guangxi Key Laboratory of Plant Functional Phytochemicals and Sustainable Utilization, Guangxi Institute of Botany, Chinese Academy of Sciences, Guilin, Guangxi 541006, China. E-mail: 18877384841@163.com.

Introduction

Sophora japonica L, whose unopened buds are called Flos Sophorae Immaturus, is a perennial deciduous tree of *Papilionoideae*, *Leguminosae*. It can be used not only as medicine, but also as tea. In addition, it can be used as building materials and has ornamental value [1]. Flos

Sophorae Immaturus is a characteristic Chinese herbal medicine enriched of Rutin, Flavine, and other chemical components [2]. China's most high-quality Flos Sophorae Immaturus is produced in northern Guangxi. The *Sophora japonica* cv. *jinhuai* in northern Guangxi area is an excellent tree species bred from *P. japonica*. Most Flos Sophorae Immaturus in China is

harvested only once a year. With the development of industrialization, farmers pay more and more attention to the growth state of *Sophora japonica cv. jinhuai* and the situation of flowers. During the planting, some farmers found that the Flos Sophorae Immaturus appeared the second harvest period after branches of *Sophora japonica cv. jinhuai* were broken in the first harvest, which made the farmers considering cultivating two harvest cycles a year. However, at present, the flowers of the secondary sophora rice are sparse or even unable to be hung. It is difficult to form a yield, and the maturity seasons of the secondary flos sophorae are inconsistent. The basic reason is the lack of knowledge about the flower bud differentiation and the formation mechanism of secondary sophora flower bud. Flower bud differentiation is the basis of flowering, fruits, and yield. The first growth cycle is from April to July, while the second growth cycle is from July to October. In order to fully germinate the flower buds for the second growth, 3-4 leaves at the top of the branches need to be cut off after the first growth cycle of harvest. However, the production of the second cycle has been low [3]. A previous study showed that pruning in winter could promote growth, while pruning in summer could promote flower bud formation [4]. Endogenous hormones are important factors affecting flower bud differentiation and morphogenesis [5], which play the important roles in plant growth and development [6]. The levels of endogenous hormones and their ratios in plants also play important physiological roles in flower bud differentiation. Williams reported that inhibiting vegetative growth before flower bud formation was beneficial to the formation of flower buds, which was the premise of flower bud formation [7]. Vegetative growth is the basis of reproductive growth, which is contradictory and interdependent to reproduction development [8]. At present, most studies on *Sophora japonica cv. jinhuai* focused on genetic diversity, cultivation techniques, selection of fine varieties, germplasm resources investigation, extraction of active ingredients, nutritional ingredients, and content determination [9-13]. There is no report

on the comparison of endogenous hormones' changes in the first and second growth cycles of *Sophora japonica cv. jinhuai*. The synthesis and function of plant endogenous hormones are closely related to plant physiological growth. Wang, *et al.* demonstrated that reasonable fertilization could keep the balance of endogenous hormones in plants [14]. Nitrogen has great influence on root growth and zeatin riboside (ZR) synthesis. Wang, *et al.* also reported the seasonal variation in rooting of the cuttings from tetraploid Locust in relation to nutrients and endogenous plant hormones of the shoot, indicating that the reproductive growth of *sophora japonica* was closely related to the changes of endogenous hormones [15]. This study compared the dynamic changes of endogenous hormones in each period of flower bud differentiation in the first and second growth cycles of *Sophora japonica cv. jinhuai* to explore the causes for the low yield of Flos Sophorae Immaturus and provide a scientific theoretical basis for improving the quality of Flos Sophorae Immaturus in the first and second growth cycles, species conservation, and artificial regulation of flowering.

Materials and methods

Collection of plant leaves

The sampled *Sophora japonica cv. jinhuai* were from Guangxi Institute of Botany (Guiin, Guangxi, China) (110°18'E, 25°04'N, altitude 175 meters). The plants were growing in a mild subtropical monsoon climate with the annual average temperature of 23°C, rainfall of 1,949.5 mm, frost-free period of 300 days, and sunshine of 1,680 hours. Healthy plants with the same age and similar growth cycle in the plantation (three-year-old tree) were selected. 20 grams of fresh and pest-free *Sophora japonica cv. jinhuai* leaves were collected at vegetative stage, flower bud stage, budding stage, full flowering stage, and fruit stage during the two growth cycles of the year, respectively. Three groups of samples were collected for each growth stage. All the samples were stored at -20°C.

Extraction and determination of endogenous hormones

1.0 g of the leaf samples was homogenized with 2 mL of 80% methanol in a mortar under the condition of ice bath. The homogenate was rinsed with 2 mL of extracting solution, and then, was placed at 4°C for 4 hours before centrifugation at 3,500 rpm at 4°C for 8 mins. 1 mL of supernatant was re-added into the precipitate, stirring well, sitting at 4°C for 1 h, followed by centrifugation at 3,500 rpm at 4°C for 8 mins. The supernatants of both centrifugations were combined, and blow dried with nitrogen in a 45°C thermostatic water bath. After passing the supernatant through a C-18 solid phase extraction column, 2 mL of sample diluent was used for endogenous hormones determination by using enzyme linked immunosorbent assay (ELISA) kits (Shanghai Hepeng Biotechnology company, Shanghai, China) for abscisic acid (ABA), indole acetic acid (IAA), zeatin riboside (ZR), and gibberellin (GA3). The OD₄₅₀ values of the standard and each sample were measured by using Biotek ELX808 microplate reader (Agilent, Santa Clara, CA, USA). The content of each endogenous hormone was calculated according to the standard curve.

Statistical analysis

The data were statistical analyzed by using Microsoft Excel 2007 (Microsoft, Redmond, WA, USA). The data were expressed as the mean ± SD. The graphs were developed by using Origin 2015 software (Guangzhou Origin Software Co., LTD, Guangzhou, Guangdong, China).

Results

Dynamic changes of endogenous hormone contents in leaves of *Sophora japonica* cv. *jinhuai* in the first and second growth cycles

The endogenous hormone contents in the first and second growth cycles were the same sequence as ABA > IAA > ZR > GA3. The content of ABA was the highest among the four endogenous hormones at each stage in both cycles. However, in the first growth cycle, ABA

demonstrated a trend of increasing first and then decreasing with a peak of 5.928 µg/g of leaves at the budding stage, while the content of IAA showed a trend of decreasing first, then increasing, and then decreasing with the highest peak of 5.835 µg/g of leaves at the budding stage too. The content of ZR showed a fluctuant pattern with increasing first and decreasing at the end, and the peak of 1.966 µg/g of leaves at full flowering stage. The content of GA3 displayed a trend of increasing first, then decreasing and then increasing with the peak of 0.513 µg/g of leaves at the budding stage (Figure 1). In the second growth cycle, ABA content firstly increased and then decreased, then increased slightly, and finally decreased again with peak of 6.455 µg/g of leaves at the flower bud stage, while IAA content showed a trend of increasing first, then decreasing, and increasing again with the peak of 4.915 µg/g of leaves at the flower bud stage too. The content of ZR demonstrated a rather great fluctuation with a trend of decreasing first, then increasing, and then decreasing with the peak of 4.489 µg/g of leaves at the budding stage. The content of GA3 decreased first and then increased with the peak of 0.897 µg/g of leaves at the vegetative stage (Figure 1).

Comparison of each endogenous hormone in the first and second growth cycles

(1) Abscisic acid (ABA):

The changing trends of ABA contents in the first and second growth cycles were different. Before the budding stage, the content of ABA in the first growth cycle was increasing, while it showed a trend of increasing first and then decreasing before the budding stage in the second growth cycle. After the budding stage, the changing trends of ABA contents in the two growth cycles were basically the same in a downward trend. In general, the content of ABA in the second growth cycle was higher than that in the first growth cycle. The peaks of ABA in the two growth cycles were different. The ABA contents reached the peaks at the budding stage and flower bud stage in the first and second growth cycles, respectively, with the significant changes in the contents.

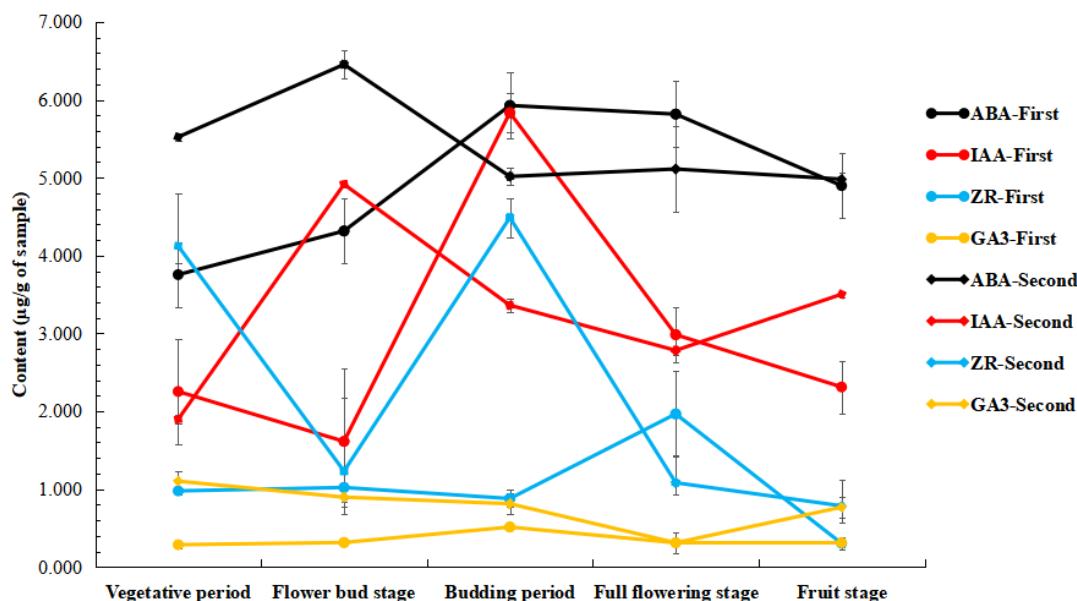


Figure 1. Changes of four endogenous hormone contents in the first and second growth cycles of *Sophora japonica* cv. *jinhuai*.

(2) Indole acetic acid (IAA):

In the first growth cycle, the content of IAA decreased slowly at first, then increased sharply, and reached the peak at the budding stage, and finally decreased again. The content of IAA was the highest at the budding and the lowest at the flower bud stage. In contrast, in the second growth cycle, the content of IAA began to increase sharply and reached peak at the flower bud stage. After that, its content decreased slowly, and there was a slight increase after the full flowering stage. During the first growth cycle, the content of IAA was the lowest at flower bud stage and the highest at budding stage. In the second growth cycle, the content of IAA was the lowest in vegetative stage and the highest in flower bud stage. The content of IAA in the flower bud stage of the second growth cycle was higher than that in other stages of second growth cycle, which indicated that low content of IAA could promote flower bud formation, while high content of IAA could inhibit flower bud formation during flower bud differentiation (Figure 1).

(3) Zeatin riboside (ZR):

During the first growth cycle, the content of ZR in the first three periods did not change much. After the budding stage, the content of ZR showed an

increasing trend and reached the peak at the full flowering stage. After that, the content of ZR decreased and reached a minimum at the fruit stage. In the second growth cycle, the content of ZR decreased significantly at first, then increased significantly after the flower bud stage, and reached the peak at the budding stage. After that, the content of ZR decreased sharply and then slowly, and reached the lowest value at the fruit stage. In contrast, although the peak time of ZR content was different in the two growth cycles, which were full flowering stage and budding stage, the lowest content was the same in fruit stage. In addition, the content of ZR at flower bud stage and fruit stage in the two growth cycles were similar. In summary, the content of ZR in the second growth cycle was higher than that in the first growth cycle, which indicated that the increase of ZR content would promote flower bud development (FBD) (Figure 1).

(4) Gibberellin (GA3):

During the first growth cycle, the content of GA3 increased slowly and reached the peak at the budding stage. After that, it fell slowly and finally rose slowly. During the second growth cycle, the content of GA3 through the first four stages showed different degrees of decline, among

which the degree of decline from flower bud stage to budding stage was the smallest, while from flower bud stage to full flowering stage was the largest. The content of GA3 was the lowest at full flowering stage and the highest at vegetative stage. The content of GA3 in the second growth cycle was higher than that in the first growth cycle, which indicated that GA3 inhibited flower formation (Figure 1).

Endogenous hormone ratio changes

(1) IAA/ABA ratio

The changing trend of IAA/ABA ratio was different in the two growth cycles. The result showed that the ratio of vegetative stage : flower bud stage : budding stage : full flowering stage : fruit stage in the first and second growth cycles were 0.600 : 0.374 : 0.984 : 0.513 : 0.472 and 0.343 : 0.761 : 0.670 : 0.544 : 0.703, respectively. During the first growth cycle, the ratio of IAA to ABA decreased first, then increased, and finally decreased again. The ratio was the highest at budding stage and the lowest at flower bud stage. After reaching the highest point at the budding stage, the next two stages were in a downward trend. From the budding stage to the full flowering stage, the ratio decreased significantly, and the downward trend slowed down after the full flowering stage. During the second growth cycle, the ratio of IAA to ABA increased first, then decreased, and finally increased slowly. The ratio was the lowest at vegetative stage and reached the peak rapidly at flower bud stage. After the flower bud stage, the ratio decreased slowly. After the full flowering stage, the ratio raised slowly again. The results indicated that lower IAA/ABA ratio was beneficial to flower bud differentiation (Figure 2).

(2) ZR/ABA ratio

The changing trend of ZR/ABA ratio was different in the two growth cycles. The result showed that the ratios of vegetative stage : flower bud stage : budding stage : full flowering stage : fruit stage were 0.260 : 0.237 : 0.148 : 0.338 : 0.062 and 0.747 : 0.191 : 0.895 : 0.212 : 0.158 in the in the first and second growth cycles, respectively. In each growth cycle, the ratio changed more

frequently. In the first growth cycle, the ratio of ZR/ABA slowly increased from vegetative stage to flower bud stage, then slowly decreased to budding stage, then increased to full flowering stage and peaked, and finally decreased to fruit stage. In the second growth cycle, the ratio decreased sharply from vegetative stage to flower bud stage, then increased sharply to budding stage and peaked, and finally kept declining. The ratio dropped to the lowest value at the fruit stage (Figure 3).

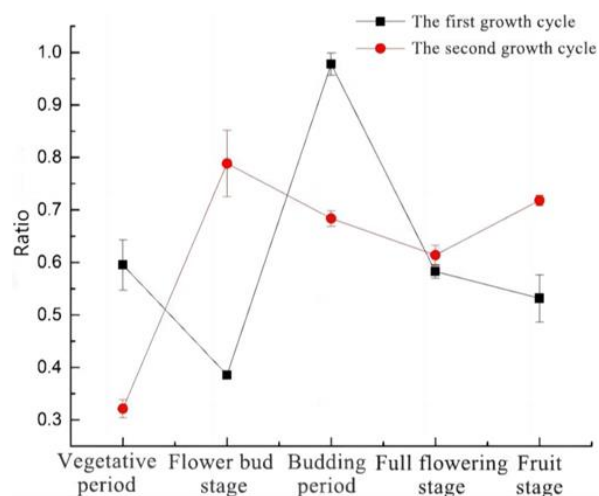


Figure 2. The change of IAA/ABA ratio at different stages of first and second growth cycles.

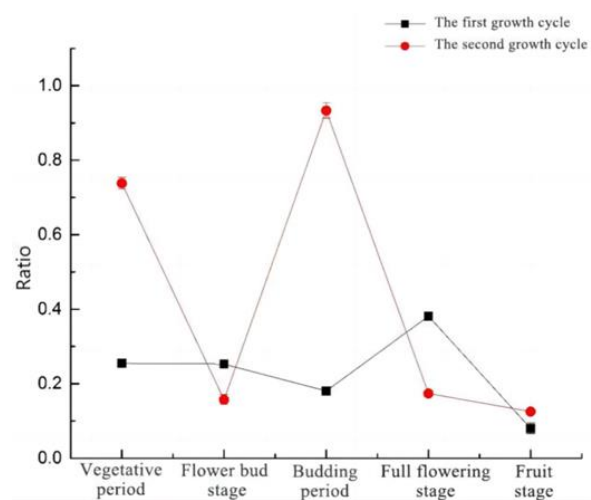


Figure 3. The change of ZR/ABA ratio at different stages of first and second growth cycles.

(3) GA3/ABA ratio

The result showed that the ratios of vegetative stage : flower bud stage : budding stage : full flowering stage : fruit stage in the first and second growth cycles were 0.076 : 0.073 : 0.087 : 0.053 : 0.062 and 0.200 : 0.139 : 0.162 : 0.061 : 0.154, respectively. During the first growth cycle, the ratio of GA3/ABA increased slowly and reached the peak at the budding stage, then the ratio decreased slowly to the full flowering stage, and finally increased slowly. The ratio was at the lowest in vegetative stage and at the highest in budding stage. During the second growth cycle, the ratio of GA3/ABA decreased rapidly at first, and then increased slowly. After the budding stage, it decreased rapidly to the full flowering stage, and reached the lowest point before rising rapidly. During this growth cycle, the change of GA3/ABA ratio was greater than that in the first growth cycle. The ratio was the highest in vegetative period and the lowest in full flowering period (Figure 4).

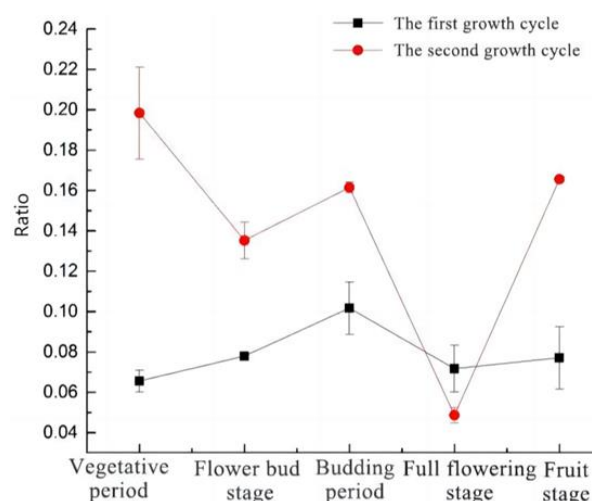


Figure 4. The change of GA3/ABA ratio at different stages in the first and second growth cycles.

(4) (IAA+GA3)/ZR ratio

The result showed the ratios of vegetative stage : flower bud stage : budding stage : full flowering stage : fruit stage in the first and second growth cycles were 2.600 : 1.887 : 7.222 : 1.676 : 8.630 and 0.727 : 4.721 : 0.930 : 2.859 : 5.441,

respectively. During the first growth cycle, the ratio of (IAA+GA3)/ZR changed frequently, showing a “W” shape change. The ratio decreased from vegetative stage to flower bud stage. After that, it increased first and then decreased during the flowering stage. Finally the ratio rose again after the fruit matured and reached the peak at the fruit stage. In the second growth cycle, the ratio of (IAA+GA3)/ZR showed a trend of “up-down-up”, which increased from vegetative stage to flower bud stage, then decreased to budding stage. After that, the ratio increased rapidly and peaked at the fruit stage. In contrast, in the first growth cycle, the lowest value of (IAA+ GA3)/ZR ratio appeared in the flowering stage, while the second growth cycle appeared in the vegetative stage. In both growth cycles, the ratio peaked at the fruit stage (Figure 5).

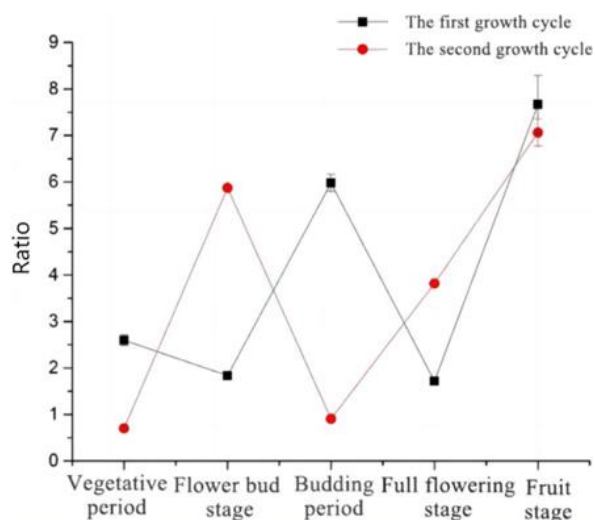


Figure 5. The change of (IAA+GA3)/ZR ratio at different stages in the first and second growth cycles.

Discussion

Flower bud differentiation is a physiological and morphological marker for the transition from vegetative growth to reproductive growth [16]. The content and balance of endogenous hormones in plants are closely related to the formation of flowers [17-19]. Studies have shown

that plant flower bud differentiation was the result of a combination of nutrients and endogenous hormones, which were controlled by internal genetic genes [20]. In this study, the content of IAA in the second growth cycle was higher than that in the first growth cycle. In the first growth cycle, the content of IAA decreased from the vegetative stage to the flower bud stage, and then increased with the flowering, showing a trend of rising-declining. During the second growth cycle, the content of IAA peaked at the flower bud stage. The results showed that the low content of IAA promoted the development of flower buds, and the high content of IAA inhibited the inoculation of flower buds. Studies have shown that low levels of IAA were also required during the floral initiation phase of jujube [21]. Wang, *et al.* found that high level of ZRs, low level of GA1/3 and IAA were needed in the flower bud differentiation period of cherry by studying the content of endogenous hormones [22].

ABA has both promoting and inhibiting effects on flower formation. ABA inhibits flower formation by inducing dormancy of growth points, while, on the other hand, ABA and GA have antagonistic effects, so that the branches stop growing, which eventually allows starch and sugar to accumulate and promotes flower formation [23]. In addition, ABA is considered to be an important hormone for promoting flowering [24] and may indirectly affect flower-bud induction [25]. This study showed that the increase of ABA content could promote flowering. Zeng, *et al.* believed that the reasons for ABA promoting flowering were as follows: (1) after the branches stopped growing, ABA could accumulate cytokinin at the growth point, and (2) it could promote the accumulation of starch [26].

In the first growth cycle, the content of GA3 was the highest at the bud stage and increased significantly in the flower bud differentiation. However, the GA3 content was decreased at the developmental stage. In contrast, during the second growth cycle, the content of GA3 reached the highest level in the vegetative stage, and

decreased sharply in the full flowering stage. It can be concluded that GA3 has inhibitory effect on flower formation. The study of Wu, *et al.* showed that during the litchi FBD the content of GA3 lowered constantly [27]. In addition, Cao, *et al.* found that, in the process of apple FBD, the content of GA3 in the terminal bud of spurs which could blossom decreased over the time [28].

The formation of flower is the result of interaction of various endogenous hormones. The ratios of IAA/ABA, GA3/ABA, and (IAA+GA3)/ZR decreased during flower bud differentiation of *Sophora japonica cv. jinhuai*, which were beneficial to the transformation from vegetative growth to reproductive growth and the formation of flower buds. Most studies suggested that, under the action of hormones, floral genes were out of the repressed state. So that, the genetic information was expressed, and the flower bud differentiation was started [26]. The formation of flowers is a very complex process, which is affected by various factors. The flowering process can be summarized as nutrient accumulation, the activation of flowering genes, the formation of flower morphology, and the synthesis of new proteins [29]. Flower bud differentiation is a series of complex processes involving multiple factors and multi-step regulation. This process is an accumulation process from quantitative change to qualitative change [30]. By analyzing and comparing the endogenous hormones of flower bud differentiation in two growth cycles, the changes of endogenous hormones in each stage were clarified, which was important to improve the yield and quality of *Sophora japonica cv. jinhuai*.

Acknowledgments

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References

- Liu JG, He GH, Li LY, Song XH, Liao SQ. 2020. Effects of grafting and pruning on the growth of *Sophora japonica* 'Shuangjiminhuai' in hilly area of Chongqing. *J Chin Med Mater*. 2020(04):797-801.
- Yang N, Zhu KM, Gu SJ. 2010. The research progress on extraction methods of rutin from *Sophora japonica*. *Lishizhen Med and Mate Med Res*. 21(12):3340-3341.
- Tang JM, Zhu CH, Gu R, Zou R, Shi YC, Xiong ZC, et al. 2020. Comparative and analysis of nutrients in different periods of *Sophora japonica* cv. *jinhuai* harvested twice a year. *J Guangxi Acad Sci*. 27(04):387-393.
- Magness JR. 1920. The influence of summer pruning upon bud development in the apple. *Oregon Agri Expt Sta Bull*. 317:3-34.
- Pan RC. 2004. *Plant physiology*. Beijing: Higher Educ. Press.
- Ting Y, Li JG, Shi R, Zhang J, Wang N, Cao RJ. 2016. Relationship between endogenous hormone content in Jun jujube and physiological fruit drop. *Nonwood Forest Res*. 34(02):45-49.
- Williams MW. 1989. Control of flowering, fruit set, and seed development in apple (*Malus domestica borkh.*) with chemical growth regulators. *Acta Hort*. 240:221-228.
- Cao SY. 2000. Study on the differentiation course and the fluctuations of endogenous phytohormones of flower buds in apple. Nanjing: Nanjing Agriculture University.
- Tang JM, Zou R, Shi YC, Shu WJ, Zhu SY, Jiang YS, et al. 2017. Optimization of ISSR-PCR reaction system and screening of primers in *Sophora japonica* cv. *jinhuai*. *Lishizhen Med and Mate Med Res*. 28(11):2747-2749.
- Jiang YS, Wei X, Zou R, et al. 2016. Breeding and cultivation techniques promotion of Jinhuai good variety. Guangxi Zhuang autonomous region, Guangxi Institute of Botany, Chin Acad Sci, Guangxi Zhuang Autonomous Region.
- Shu WJ, Shi YC, Jiang YS, Zou R, Tang JM, Wei X. 2017. *Sophora japonica* germplasm resources in Guangxi. *Chin Exp Traditional Med Formulae*. 23(15):53-59.
- Yao XL, Shu WJ, Li H, Zhang DD, Zhu KM, Gu SJ. 2019. Optimization of ultrasonic-assisted extraction of Rutin from *Sophora japonica* in northern Guangxi by response surface methodology. *Jiangsu Agri Sci*. 47(02):197-200.
- Tang JM, Zou R, Shi YC, et al. 2017. Effects of different fertilization treatments on yield and quality of flos sophorae immaturus. *J Guangxi Acad Sci*. 33(04):280-284.
- Wang XJ, Jiang YS, Qi XX, Zou R. 2011. Effects of nitrogen fertilizer on growth and endogenous hormones of Locust tree. *Hunan Agri Sci*. 2011(01):113-116.
- Wang XL, Zhao Z. 2012. Seasonal variation in rooting of the cuttings from Tetraploid Locust in relation to nutrients and endogenous plant hormones of the shoot. *Turkish Agri Forestry*. 36(2):257-266.
- Guo C, Yang X, Yang GQ, Wu RH, Pan ZP, Wei X. 2016. Dynamic changes of exogenous hormones in blossom and fruit period of camellia *tunghinensis* Chang. *Guangxi Sci*. 23(03):278-285.
- Yang S, Bai MD, Gao P, Guo HP. 2017. Research progress on flower bud differentiation mechanism of fruit trees. *Deciduous Fruits*. 49(06):22-25.
- Anton JM, Will G, Gerard WM, George JW. 1991. *In vitro* flower bud formation in tobacco: Interaction of hormones. *Plant Physiol*. 97:402-408.
- Shalit A, Rozman A, Goldshmidt A, Alvarez JP, Bowman JL, Eshed Y, et al. 2009. The flowering hormone florigen functions as a general systemic regulator of growth and termination. *Proc Natl Acad Sci USA*. 106(20):8392-8397.
- Wang LP, Li ZG, Tan LH, Song YH, Lian FS, Yang FC, et al. 2011. Research progress of plant endogenous hormone. *J Anhui Agri Sci*. 39(04):1912-1914.
- Niu HL, Zhang HW, Bian Y, Li XG. 2015. Flower formation and endogenous hormones dynamic in Chinese Jujube. *Acta Horticulturae Sinica*. 42(04):655-664.
- Wang YH, Fan CH, Shen X, Qu GM, Shi JD. 2002. Changes in endogenous hormones during the flower bud differentiation of sweet cherry. *Acta Agri Boreali-occidentalis Sinica*. 2002(01):64-67.
- Zeng X. *Fruit tree physiology*. Beijing: Beijing Agricultural University Press, 1992.
- Luckwill LC. 1974. In proceedings of the XLX. *Int Hort Congr*. 11:169-177.
- Rakngan J, Gemma H, Iwahori S. 1995. Flower bud formation in Japanese pear trees under adverse conditions and effects of some growth regulators. *Jpn Trop Agr*. 39(1):1-6.
- Zeng X. 1985. Flower bud differentiation of fruit trees-internal factors affecting flower bud differentiation. *Life World*. 1985(02):25-27.
- Wu ZX, Zhou ZD, Tao ZL, Wang LX. 2005. Changes of endogenous hormones in Feizixiao and Edan litchi during flower bud differentiation. *Chin Trop Crop*. 2005(04):42-45.
- Cao SY, Zhang JC, Wei LH. 2000. Studies on the changes of endogenous hormones in the differentiation period of flower bud in apple trees. *J Fruit Sci*. 2000(04):244-248.
- Cao SY, Tang YZ, Zhang JC. 2001. Effects of GA3 and PP333 on the apple flower bud differentiation course and content of endogenous hormones. *J Fruit Sci*. 2001(06):313-316.
- Hou JX, Wang M, Gao YJ. 2010. Research progress on regulation mechanism of flower bud differentiation in fruit trees. *Northern Fruits*. 2010(03):1-3.