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Change of Endogenous Hormones Inside *Paeonia lactiflora* Buds during Winter Dormancy

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ABSTRACT

Variation in and the ratios of endogenous hormones in the terminal buds of 4-year old traditional Chinese peony cv. 'Da Fu Gui' throughout the dormant period were studied by HPLC to attempt to unravel the mechanism of winter bud dormancy in this woody ornamental. Abscisic acid (ABA) content began to increase on November 20 and peaked on December 10 while gibberellic acid (GA_3) content started to increase on December 10 and dropped off by December 30 while the trend for cytokinin (CK) was similar to that of GA_3. The level of indole-3-acetic acid changed little throughout the period of dormancy. GA_3/ABA and CK/ABA values dropped as dormancy progressed and rose as dormancy was released.

Keywords: ABA, GA_3, HPLC, plant hormones

INTRODUCTION

Herbaceous peony (*Paeonia lactiflora*) is a traditional flower in China that has a long history of cultivation. Herbaceous peonies generally bloom from late April until early May, which is too short to fulfill the requirement for all-year round cut flower production for international markets. The main method of extending the period of supply is through forcing culture. However, through evolution, herbaceous peonies have developed characteristics of dormancy in which dormancy of seeds and buds needs to be broken under low temperature in winter in order to sprout in spring (Qin 2004). After plants grow roots in autumn, epicotyls and terminal buds break dormancy under 0-4°C. To induce blooming of herbaceous peonies in winter, they must first be chilled for a minimum of 4 weeks at 0-5.6°C to break dormancy (Byrne 1986; Evans 1990; Aoki 1991a, 1993b; Fulton 2001; Fei 2008; Cheng 2009).

Dormancy, which includes seed dormancy and winter bud domancy, is an ecological adaptation of perennial plants to dry and cold climates in winter. Bud dormancy is a phenomenon in which the growth or development of a plant is temporarily suspended. Traditionally, all phases of dormancy are controlled by endogenous hormones (Seeley 2002). Hormones play an important regulative effect during dormancy release in tree peony (Liu 2003), asparagus (Miao 2011), peach bud (Wang 2006), poplar (*Populus deltoides* Bartr. cv. Lux (ex.i-69/55)) buds (Zhu 1990), and pear stock (*Pyrus betulaefolia* Bge.) and *Pyrus calleryana* Dcne.) seeds (Bao 2011). The process of release in tree peony is well reflected in changes of both activators and inhibitors, among which the ratio of gibberellic acid (GA_3) to abscisic acid (ABA) was directly related to the progress of dormancy release (Zheng *et al*. 2009). At present, most attention has focused on the relationship of plant hormones and breaking of dormancy in fruit trees and forest species rather than herbaceous peonies.

The content of endogenous hormones (GA_3, indole-3-acetic acid (IAA), cytokinin (CK), ABA) and variations in the ratio of IAA/ABA, GA_3/ABA and CK/ABA were determined by high performance liquid chromatography (HPLC) in order to investigate the function of hormones in the process of breaking dormancy at low temperature. By doing so would assist in elucidating the mechanism of forcing in herbaceous peony. Forcing culture is a cultivation method that allows flowers to grow and develop in protected facilities in the cold season, shortening thus the cultivation period.

MATERIALS AND METHODS

Plant materials

The experiment was conducted in 2009 on 4-year-old herbaceous peony cv. 'Da Fu Gui' at Beijing Forestry University, China. At least 10 apical buds were collected as sample materials over 10-day intervals. Sterilized secateurs were used to harvest the samples, which were then rinsed in distilled water and sterilized in 70% alcohol for 50-60 sec. The collected bud samples were immediately placed in an ice box, transported to the laboratory, dipped in liquid nitrogen and stored at -80°C until analyses were conducted.

Hormone extraction and analysis

The extraction of endogenous hormones (GA_3, IAA, ABA and CK) was conducted as described by Chen et al. (1991), with slight modifications. Axillary bud samples (0.5 g) were ground in 10 ml of 80% cold methanol extraction medium containing 1 mmol.L^{-1} butylated hydroxytoluence (BHT, Sigma Chemical Co.) as antioxidant until completely homogenous. The homogenate was then transferred into a test tube and 30 mg of polyvinylpolypyrrolidone (PVP, China Chem Source Co., Ltd.) was added, then thoroughly mixed on a shaker for 10 min and incubated at 4°C overnight. The next morning, the supernatant was put into a 10-ml tube and centrifuged at 6,000 rpm for 20 min. The residue was washed and re-extracted in 2 ml cold methanol for another 12 h, then centrifuged under the same conditions as above before discarding the residue. The combined extract, after adding 1-2 drops of ammonia (NH_3), was condensed (35-40°C) to an aqueous phase using a rotary evaporator. The aqueous phase was then dissolved using distilled

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water and the mixture was separated into two equal parts.

For the determination of GA_3, IAA and ABA, the pH of one part of the mixture was adjusted to 2.5-3.0 with 1 N HCl and then extracted three times in an equal volume of ethyl acetate (HAc). The pH of the other part of the mixture was adjusted to 7.5-8.0 with 1 N NH_3 for the determination of CK and also partitioned three times into equal volumes of butanol in phosphate buffer (pH 8.0). Both the ethyl acetate and butanol fractions were evaporated to dryness at 40 and 60°C, respectively. Hormone purification was performed with 80% aqueous methanol and passed through a C18 Sep-Pak cartridge (Waters Corp., Milford, MA). The residues were collected, dissolved in methanol and dried under N_2 gas. The purified extracts were dissolved in 50% methanol, filtered through a 45-µl membrane and subjected to HPLC analysis. Hormonal analysis was done on a computer-assisted Agilent 1100 HPLC unit (Agilent Technologies, CA, USA) equipped with a vacuum degasser, an auto-sampler, a quandary pump, thermostated column compartment and a diode array detector. HPLC conditions were as follows: ZORBAX RX-C8 column (250 × 4.6 mm); mobile phase consisted of 3% methanol and 97% 0.1 M acetic acid for IAA, GA_3 and ABA determination, and 3% ammonia and 97% pure water (pH 7.0) for CTK determination (after filtration through a 0.45-µm filter membrane); detection wavelength of different hormones (IAA = 280 nm, ABA = 260 nm, GA_3 = 210 nm, CK = 260 nm); a sample quantity of 10 µl was automatically injected at a flow rate of 1 ml min^{-1}. Hormones were quantified by comparing the peak area of the samples with those of standard samples (Sigma Chemical Co. USA).

Statistical analyses

Statistical analyses were conducted using SPSS v. 17.0. The level of each hormone was measured three times from different buds from one tree. The mean values of the targeted hormones were measured and one-way ANOVA analysis performed to determine the significance level between treatments at $P \leq 0.05$ using the LSD test.

RESULTS AND DISCUSSION

Endogenous hormones in *Prunus persica* buds affect endodormancy by regulating nutrient metabolism and activating dormancy-related genes (Marquat 1999). The natural changes in endogenous hormones in dormant buds of peony are shown in **Fig. 1**. ABA peaked in early December and when its level began to decrease in late December, both GA_3 and CK peaked. IAA showed no major peaks or troughs from between October and March.

Interestingly, the peak of the GA/ABA and CK/ABA ratios were in a trough in early winter when ABA peaked and these ratios peaked when all three hormones (excluding the effect of IAA) reached a plateaued trough in early January (**Fig. 2**).

Breaking dormancy is critical to forcing culture of herbaceous peonies and can be effectively achieved with GA_3 and chilling (Qin 2004; Cheng *et al*. 2009). A high level of ABA promoted dormancy while a high level of GA and CK promoted the breaking of dormancy. The balance ratio or opposing effects among several hormones is crucial in the progress of dormancy rather than the level of certain single hormone (Liu 2003).

A high GA/CK ratio promotes the breaking of dormancy, ABA inhibits dormancy and IAA has different effects in different species, including the dormancy of seeds (Huang *et al*. 1998). ABA and GA are endogenous signaling molecules regulating seed dormancy and germination. ABA induces the end of the division of embryo cells and initiates and keeps seed in a dormant state while GA_3 generates an opposite activity, initiating and promoting seed germination (Jiang and Wan 2007). ABA and GA_3 regulated the induction of endo-dormancy of peach flower buds by changing the balance of ABA and GA_3 by spraying the two hormones at the dormant stage. There was an important critical point determining the transition to endo-dormancy, which was possible when the ratio of GA_3 and ABA was higher than that critical point, otherwise endo-dormancy would not be induced (Wang *et al*. 2006). Exogenous hormones, particularly GA_3, break dormancy, accelerate flower bud differentiation and promote flowering in flower production.

Although IAA increases obviously during the release from dormancy, it does not directly participate in the regulation of dormancy (Zhu and Wu 1990). When the total IAA content increased to a certain level, new shoots of apple stopped growing and dormancy began, although the content of free IAA decreased at the beginning of dormancy and increased at the end of dormancy (Eggert 1953). IAA has some effect on promoting hawthorn seed germination, which does not depend on a certain level of content to promote or inhibit, but is the result of various factors (Zhang 1999).

ABA is considered to be the signal substance of dormancy. A series of studies on seed found that inhibiting the increase of ABA promoted the conversion of seed reserves in the embryo into substances which can be utilized for

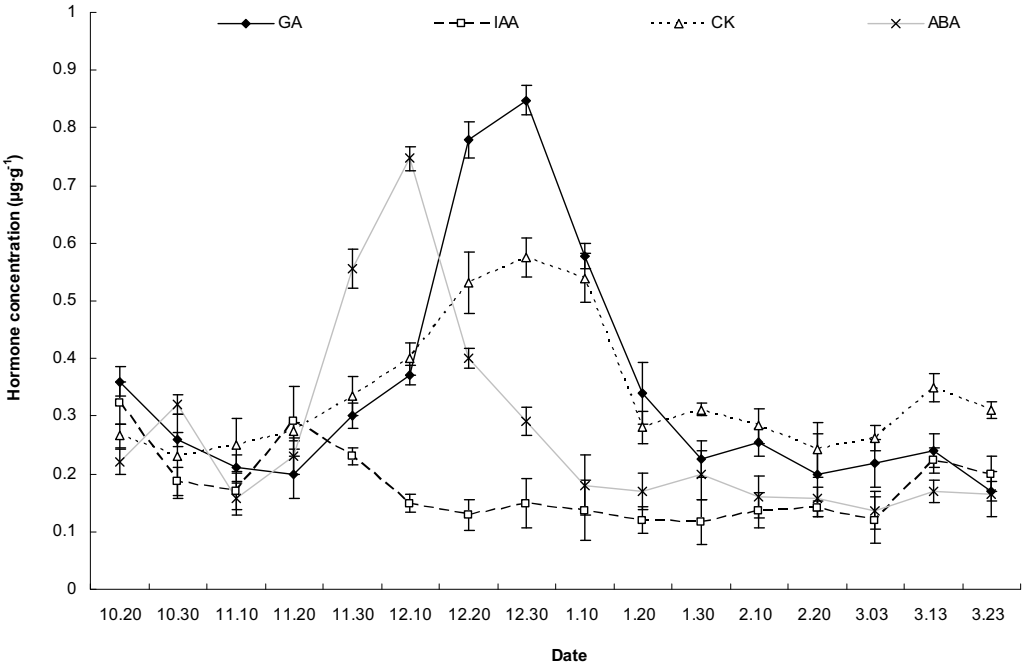

Fig. 1 Change in hormones inside dormant *Paeonia lactiflora* buds over time.

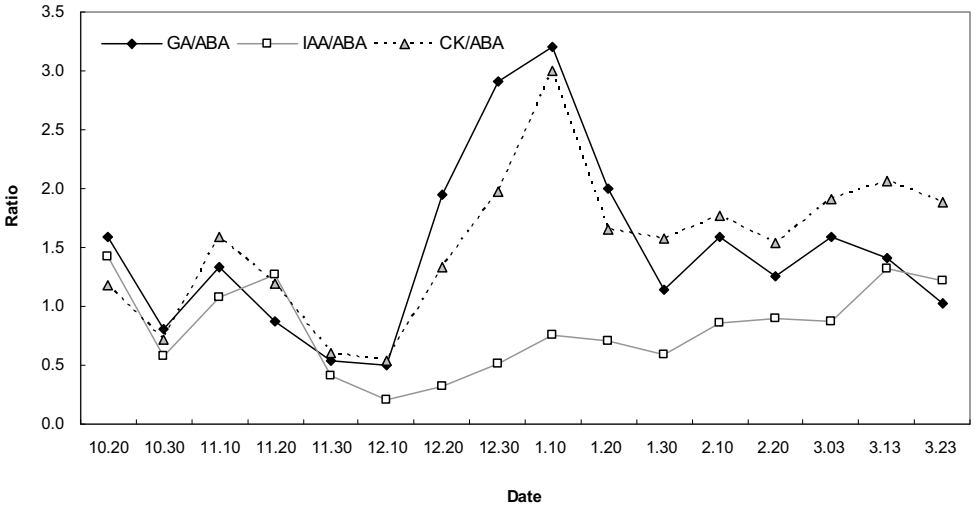

Fig. 2 Change in GA/ABA, CK/ABA, IAA/ABA ratios in dormant *Paeonia lactiflora* buds over time.

cell division and growth (Fu *et al*. 1998). The endogenous ABA content in peach, which was treated with ABA, induced a marked increase in photoperiod as the treatment progressed, finally resulting in terminated growth and initiation of dormancy (Wang *et al*. 2006).

CK can resist apical dominance and break dormancy of axillary buds, but only of those buds to which CK was directly applied (Li 1996). 6-Benzyladenine (BA) and ABA display an opposite effect: the former promotes degradation of ABA and breaks dormancy while BA has a lower concentration and a shorter treatment period than GA (Huang 1987).

Endogenous hormones affect the whole process of natural dormancy by regulating nutrient metabolism and activating dormant genes, which lays the foundation for regulating dormancy by using exogenous growth regulators in production (Marquat 1999). Breaking dormancy is key to the success of forcing culture. Using GA_3 with chilling is an effective means to break dormancy in peony. Moreover, dormancy in peony depends on the balance between the levels of several hormones, rather than that of a single one. IAA provides energy to the plant before entering dormancy and promotes flower bud growth and differentiation after the end of dormancy, but is not involved in the dormancy process. Therefore, a future study should apply exogenous hormones, including GA_3, to break dormancy.

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REFERENCES

Aoki N (1991) Effects of chilling period on the growth and cut-flower quality of forced herbaceous peony. *Bulletin of the Faculty of Life and Environmental Science, Shimane University* **25**, 149-154

Aoki N (1993) Effect of pre-chilling on the growth and development of flower bud and the flowering of forced herbaceous peony. *Bulletin of the Faculty of Life and Environmental Science, Shimane University* **27**, 73-78

Bao JP, Zhang SL (2011) Changes in endogenous hormone contents of pear stock (*Pyrus betulaefolia* Bge. and *Pyrus calleryana* Dcne.) seeds during cold stratification. *African Journal of Biotechnology* **10**, 16884-16886

Byrne TG, Halevy AH (1986) Forcing herbaceous peony. *Journal of the American Society for Horticultural Science* **111**, 379-383

Cheng FY, Zhong Y, Long F, Yu XN, Kamenetsky R (2009) Chinese herbaceous peonies: Cultivar selection for forcing culture and effects of chilling and GA_3 on plant development. *Israel Journal of Plant Sciences* **57**, 357-367

Eggert FP (1953) The auxin content of spur buds of apple as related to the rest period. *Proceedings of the American Society for Horticultural Science* **62**, 191-200

Evans AH, Anderson NO, Wilkins HF (1990) Temperature and GA_3 effect emergence and flowering of potted *Paeonia lactiflora*. *Hortscience* **25**, 923-924

Fei F (2008) Study on the technology of multi-seasonal production of herbaceous peony cut flowers. MS thesis, Beijing Forestry University, Beijing, China, 21 pp (in Chinese, English abstract)

Fu J, Qi WQ, Gu ZH, You RL (1998) Seed dormancy and germination in *Cimicifuga nanchuanensis*. *Acta Botanica Sinica* **40**, 303-308

Fulton TA, Hall AJ, Catley JL (2001) Chilling requirements of *Paeonia* cultivars. *Scientia Horticulturae* **89**, 237-248

Huang BQ, Zhang WJ, Zhang ZH (1998) Pretreatment techniques for eggplant seeds to break dormancy. *Journal of Fujian Agriculture University* **27**, 166-171

Huang H (1987) Effects of 6-benzyladenine on abscisic acid degradation in wheat leaves. *Acta Phytophysiologica Sinica* **13**, 325-329

Jiang L, Wan JM (2007) Advances in seed dormancy and germination regulated by plant hormones ABA and GA. *Jiangsu Journal of Agriculture Science* **23**, 360-365

Li XD (1996) Effect of endogenous hormones on the dormancy of garlic (*Allium sativum* L.). *Acta Horticulturae Sinica* **23**, 150-154

Liu B (2003) Study on the chilling requirement and some physiological and biochemical changes of tree peony during dormancy release. MS thesis, Shandong Agriculture University, Shandong, China, pp 43-44 (in Chinese, English abstract)

Marquat C, Vandamme M, Gendraud M (1999) Dormancy in vegetative buds of peach: Relation between carbohyrate absorption potentials and carbohydrate concentration in the bud during dormancy and its release. *Scientia Horticulturae* **79**, 151-162

Miao JL, Nie LC, Shi LZ (2011) Effect of low temperature bud break and changes endogenous hormones of asparagus. *Acta Horticulturae Sinica* **38**, 165-170

Qin KJ (2004) *Herbaceous Peony*, China Forestry Press, Beijing, pp 18-42

Seeley SD, Damavandy H, Anderson JL (1992) Autumn applied growth regulators influence leaf retention, bud hardiness, bud and flower size, and endodormancy in peach and cherry. *Journal of the American Society for Horticultural Science* **117**, 203-208

Wang HB, Gao DS, Wang XD, Li J (2006) Role of gibberellin and abscisic acid in peach bud endodormancy induction. *Journal of Fruit Science* **23**, 599-601

Zhang PY, Yang XL, Xiang DF, Qi YS, Guo MJ, Yang YD (1999) Variation of endogenous hormones in hawthorn seeds during the period of dormancy and germination. *Journal of Hebei Agrotech Teachers College* **13**, 8-10

Zheng GS, Gai SP, Gai WL (2009) Changes of endogenous hormones during dormancy release by chilling in tree peony. *Scientia Silvae Sinicae* **45**, 48-52

Zhu YL, Wu GM (1990) Analysis for endogenous hormones in poplar (*Populus deltoides* Bartr. cv. Lux (ex.i-69/55)) bud during dormancy and its release. *Journal of Nanjing Forestry University* **14**, 7-15