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Effect of Endogenous Hormone Concentrations on Wizened Bud in Pear

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Additional index words: Pear, Wizened bud, Endogenous hormone, Flower bud differentiation

Abstract

To investigate the relationships between changes in endogenous hormone concentrations and occurrence of wizened bud in pear, we sampled buds of 'Yuluxiang' pear from a pear orchard with occurrence of wizened bud (abnormal) and orchards with normal bud development in Weixian, Hebei, China, and in Taigu, Shanxi, China. Vegetative shoots on trees in the abnormal orchard grew later in the season and were longer than shoots on trees in the normal orchards. Wizened buds became obvious during the sepal differentiation phase in early to mid-July. We used ELISA to analyze changes in bud endogenous hormone concentrations at the flower bud differentiation stage. The indole acetic acid (IAA) concentration in abnormal buds was higher than that in normal buds, at the occurrence of wizened bud stage. The abscisic acid (ABA) concentration in normal pear buds was significantly higher than that in abnormal buds from mid-June to early Aug., whereas the gibberellic acid (GA_3) concentration in normal pear buds was lower than that in abnormal buds. Concentrations of zeatin riboside (ZR) in normal buds were lower than those in abnormal buds from early June to mid-June (initial stage of flower bud differentiation). Ratios of ZR/GA_3, ABA/GA_3, ABA/IAA, ABA/ZR, ABA/(ZR+GA_3), ABA/(IAA+GA_3), and ABA/(ZR+GA_3+IAA) in normal buds were significantly higher than those in abnormal buds in Weixian. We hypothesize that orchard practices that suppress vegetative growth, regulate plant hormone metabolism, and create balance between vegetative growth and reproductive growth, may decrease the incidence of wizened bud.

Wizened buds are derived from normal flower buds but during development bud scales are loose, and the pith and base of scales become dry and black (Liu et al., 2016). Occurrence of wizened bud in some pear cultivars, such as 'Xuehua' (*Pyrus bretschneideri* Rehd.) and 'Korla' (*Pyrus sinkiangensis* Yü.), has been observed in some regions of China for many years, with climatic conditions such as temperature in winter being recognized as the causal agent (Yang et al., 2016). During the spring season, pear flower buds develop rapidly and cold resistance declines. Pear buds lose cold resistance completely, causing wizened buds during cold spells in later spring (Zhang and Guo, 1998). Recently, in some regions of China, wizened bud was also observed in new pear cultivars such as 'Yuluxiang' and 'Hongxiangsu' (Yang et al., 2016). To solve this issue as soon as possible, we investigated the occurrence of wizened bud in pear in regions such as Shenze, Shenzhou, Zhaoxian, and Weixian of Hebei, and Daxing of Beijing in China, where the incidence of wizened bud is severe, and over 90% of the buds were affected in some areas. We observed that in Aug. the bud became soft, ruptured, and

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turned brown, and anatomically the inflorescence was smaller than the normal bud with thorough browning.

In the current study, we confirmed that the occurrence of wizened bud in pear during flower bud differentiation is not caused by freeze injury, but rather is related to endogenous hormones. Endogenous hormones have significant regulatory function in flower bud differentiation and development. Low indole acetic acid (IAA) concentration is necessary for flower bud formation whereas high concentration inhibits flower bud formation, suggesting it may have an indirect function on flower bud formation (Kinet, 1993). High concentrations of gibberellic acid (GA$_3$) can inhibit flower bud formation in fruit trees (Irene et al., 2001). Low abscisic acid (ABA) concentration positively affects flower development (Li et al., 2017; Wu et al., 2011); however, this positive effect is based on its ability to enhance the sugar concentration of the vacuole, which then enhances the ability of nutrient absorption in the cell (Kojima, 1982). Tanaka et al. (2006) focused on relationships between hormonal balance and flower bud differentiation, indicating that high values of ABA/IAA, ABA/GA$_3$, and zeatin riboside (ZR)/GA$_3$ may be favorable for flower bud differentiation.

In the present study, buds from a 'Yuluxiang' orchard in Weixian, Hebei, China, with severe wizened bud were compared to normal buds from orchards in Weixian, Hebei and Taigu, Shanxi, China. We analyzed changes in endogenous hormone concentrations during flower bud formation and their association with nutrition and the occurrence of wizened bud. The relationships between hormonal metabolism and flower bud differentiation, which can provide the foundation for control measures of wizened bud in pear, were also studied.

Materials and Methods

Test materials and design. 'Yuluxiang' flower buds were sampled from three pear orchards. The first orchard exhibited serious of wizened bud: trees were cultivated according to current regulations for integrated fruit production (Irrigate 4 times a year: before germination, after flowering, fruit expansion period and before winter. Nitrogen fertilizer is applied once a year in March, the amount of fertilizer is 10kg·ha^{-2},and trimmed once between July and August. but produced no fruit throughout the whole orchard. The second orchard had normal bud development and was adjacent to the abovementioned orchard: yield was 28,500–31,500 kg·ha^{-2} and received routine management combined with cultivation measures such as nitrogen control (No nitrogen fertilizer), water control (Watered only before winter and during fruit expansion, the amount of water are 80,000 and 60,000 L·ha^{-2}, respectively), branch pulling and ring cutting between May and June, and spraying growth retardant "Chlormequat chloride" in Weixian, Hebei, China. The third orchard with normal flower bud formation had trees cultivated according to current regulations for integrated fruit production and yield was 34,500–37,500 kg·ha^{-2}) in Taigu, Shanxi, China. The study was performed from 2011 to 2013 and started on 1 June 2011. During the stage of flower bud morphological differentiation (from early June to mid-Aug.), 6 'Yuluxiang' pear trees were randomly selected in each of the three pear orchards. The experimental design was considered completely randomized. Eight buds were collected from the east, south, west, and north sides of trees randomly according to growth and development and buds from the different trees were analyzed separately.

Measure of annual shoots. Shoot length (m), shoot diameter (cm), and shoot growth cessation were measured for 30 annual shoots per tree from six trees in 2013 from June to Aug.

Observation of flower bud morphological differentiation. Thirty short buds (less than 2.5 cm long with a rosette of leaves at the terminal end) were randomly selected every 5 days and stored in FAA fixative (Formaldehyde: Acetic Acid: 75% Ethanol=1:1:18) in

2013. The paraffin section method was used to observe the flower bud differentiation process by Microscope BH50.

Determination of endogenous hormone concentrations. Buds were collected six times (1 and 15 June, July and Aug.), brought to the laboratory, quickly frozen under liquid nitrogen, and preserved in the freezer at -70°C for future analysis. To prepare samples, 2 mL of 80% cold methanol was added to 0.5 g of frozen sample and ground in an ice bath to produce a homogenate. The homogenate was then transferred to a 10 mL test tube. The tube was refrigerated at 4°C and was shaken for 1 h. The tube was then centrifuged (1000×g, 15min) and the volume of the supernatant was recorded. The residue was removed. Solid liquid purified by C-18 solid phase extraction column. Concentrations of IAA, GA_3, ZR, and ABA were detected by ELISA kit provided by College of Agriculture and Biotechnology, China Agricultural University (He, 1993). ELISA kits were provided by the College of Agriculture and Biotechnology of China Agricultural University.

Data analysis. Excel and SAS System 8.6 were used to analyze the data. Differences between replications were evaluated by one-way analysis of variance (ANOVA). Duncan's multiple range test was used to determine significant differences among results. Differences with $P < 0.05$ were considered statistically significant. Correlation analysis was used to evaluate the linear relationships between hormones.

Results

Vegetative growth. Length and diameter of shoots were greatest in orchards with wizened buds, where shoots grew later in the summer (Table 1).

Bud morphological differentiation. Upon comparing the microstructure, wizened buds were less developed (Fig. 1A, made in Oct 2013), than normal flower buds (Fig. 1B). In the process of normal flower bud differentiation, before early June, (Fig. 1C), the growing point was narrow and smooth with no herniation, and the longitudinal section showed a circular conoid. In the differentiation phase, in early- to mid-June, the growing point increased and rolled, and the apex gradually flattened, indicating that morphological differentiation had began in the flower bud (Fig. 1 D). In the flower bud differentiation phase, mid-June to early-July, the hypertrophied point did not become round and this processes continued all around, the growth cone extended upward, and the apex gradually formed a floral primordium (Fig. 1E); at this time, each floral primordium appeared and extended upward at the bract primordium axillary, which formed the center flower bud and the original body of the adnation bud. In the sepal differentiation phase, (early July to mid-July, the apex of the floral primordium gradually flattened, and the intermediate part became relatively invaginated; this process appeared around the calyx original body (Fig. 1F). In the petal differentiation phase, mid- to late-July,) the processes

Table 1. Characteristics of current season shoots on 'Yuluxiang' pear trees growing in three orchards.

Shoot growth characteristics	Orchard with wizened buds (abnormal; Weixian)	Orchard with normal flower buds (Weixian)	Orchard with normal flower buds (Taigu)
Shoot length （m）	2.11A[z]	1.53B	1.13C
Shoot diameter （cm）	1.12A	0.83B	0.81B
Shoot growth cessation	Early Sep.	End of Aug.	Late July

[z]Means in rows followed by common letters do not differ at the 1% level by Duncan's multiple range test.

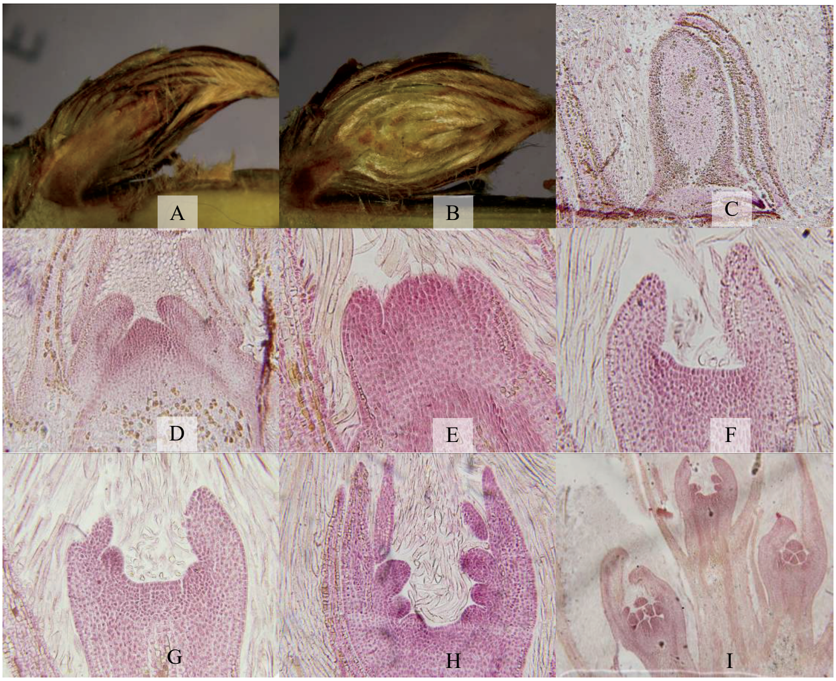

Fig. 1. Bud morphological differentiation of 'Yuluxiang' pear. A: Wizened bud in late winter ×10. B: Normal bud in late winter ×10. C: Undifferentiating phase, ×200. D: Initial differentiation phase, ×100. E: Flower bud differentiation phase, ×200. F: Sepal differentiation phase, ×200. G: Petal differentiation phase, ×200. H: Stamen differentiation phase, ×100. I: Pistil differentiation phase, ×40.

began in the base of the sepal primordium, which was named the petal original body (Fig. 1G). During the stamen differentiation phase, late June to early Aug., the stamen primordial developed in the basal interior of the petal primordium, which was named the stamen original body (Fig. 1H). During the pistil differentiation phase, mid-Aug. to early Sept., after the stamen primordium appeared, the processes then appeared in the bottom of flower original body center, which was the pistil original body (Fig. 1I). In addition, histological slices showed that the undifferentiated phase, initial differentiation phase, and flower bud differentiation phase in wizened buds were normal. Normal histological slices were not obtained during the sepal differentiation phase because part of the organization was blighted. It was inferred that wizened buds began at the sepal differentiation phase. Wizened buds became obvious during the sepal differentiation phase, (early- to mid-July), and increased from 23.7% to 49.3% during the petal differentiation phase, (mid- to late-July), to 88.5% during the stamen differentiation phase (late July to early Aug.), to 97.6% during the pistil differentiation phase (mid-Aug. to early Sept.).

Changes in the concentrations of endogenous hormones during bud formation. IAA concentrations in buds from the Weixian abnormal orchard were significantly lower than those in buds from the other orchards in June but increased rapidly in July before declining rapidly after mid-July (Fig. 2). IAA concentrations from the Weixian normal orchard were lower than buds from the normal Taigu orchard on all dates except for 1 July.

Bud ABA concentrations in all orchards generally declined through the season (Fig. 2). ABA concentration in buds from the Weixian and Taigu normal orchards was significantly higher than that in buds from the Weixian abnormal orchard from mid-June to mid-Aug. During bud morphological differentiation, the ABA concentration in buds from the Weixian normal pear orchard was 37.9, 44.4, 48.2, and 36.3% higher than that in buds from the Weixian abnormal orchard on the July 1, July 15, Aug. 1, and Aug. 15, respectively (Fig. 2).

GA_3 concentrations in buds from the Weixian normal orchard peaked in mid-June and were similar to those in the Taigu normal orchard during July (Fig. 2). GA_3 was highest in buds from the Weixian abnormal orchard on all sampling dates.

Differences in bud ZR concentrations were inconsistent through most of the season (Fig. 2). ZR concentration was highest for buds from the Weixian abnormal orchard in early June, then declined in early July before increasing in late July. Bud ZR concentrations were lowest for buds from the Weixian normal orchard on all but one sampling date.

Hormone ratios during bud morphological differentiation. The ZR/GA_3 ratio from early June to mid-Aug in buds from the Weixian abnormal pear orchard was dramatically lower than that in buds from the normal pear orchards in Weixian and Taigu (Fig. 3). The ABA/GA_3 ratio in buds from the abnormal pear orchard in Weixian was also lower than that in buds from the normal orchards in Weixian and Taigu. The ABA/IAA ratio in buds of the different 'Yuluxiang' pear or-

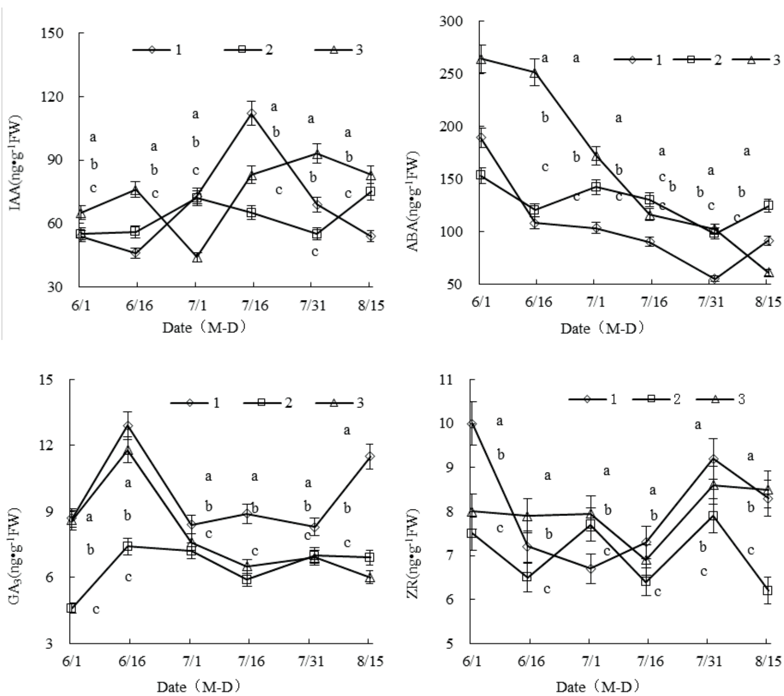

Fig. 2. IAA, ABA, GA_3, and ZR concentration changes in bud of 'Yuluxiang' pear during the growing season at three orchards. 1 = abnormal orchard in Weixian of Hebei Province; 2= normal orchard in Weixian of Hebei Province; and 3 = normal orchard in Taigu of Shanxi Province. Means within dates followed by common letters do not differ at the 0.1% level, by Duncan's multiple range test.

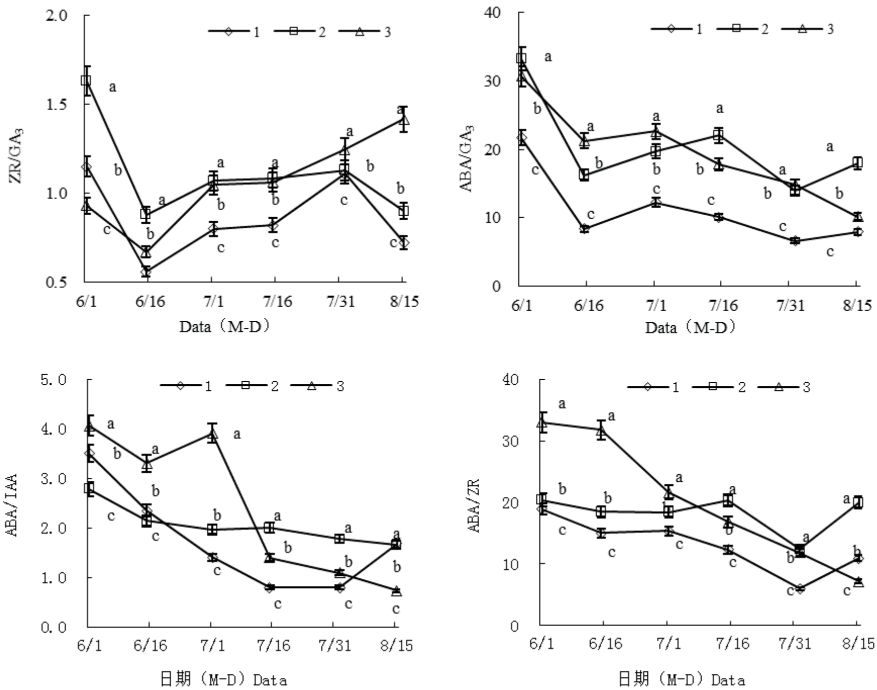

Fig. 3. Changes in ZR/GA_3, ABA/GA_3, ABA/ IAA, and ABA/ZR ratios during the bud differentiation of 'Yuluxiang' pear. 1 = abnormal pear orchard in Weixian of Hebei Province; 2 = normal pear orchard in Weixian of Hebei Province; 3 = normal pear orchard in Taigu of Shanxi Province. Means within dates followed by common letters do not differ at the 0.1% level, by Duncan's multiple range test.

chards generally declined over time, being significantly lower in buds from the abnormal pear orchard in Weixian from late June to early Aug. than that in buds from the normal pear orchards in Weixian and Taigu. The ABA/ZR ratio in buds from the abnormal Weixian orchard from early June to late Aug. was significantly lower than that in buds from the normal orchards in Weixian and Taigu.

Changes in ABA/(ZR+GA_3), ABA/(IAA+GA_3), and ABA/(ZR+GA_3+IAA) ratios during bud morphological differentiation. ABA/(ZR+GA_3) and ABA/(IAA+GA_3) ratios in buds declined during the season. The ABA/(ZR+GA_3) ratio in buds from the abnormal Weixian orchard was significantly lower than that in buds from the two normal orchards from early June to mid-Aug. The ABA/(IAA+GA_3) ratio in buds from the abnormal orchard was significantly lower than in buds from the two normal orchards from mid-June to early Aug. The downward trend of the ABA/(ZR+GA_3+IAA) ratio was similar to that of the ABA/(IAA+GA_3) ratio in the three orchards. The ABA/(ZR+GA_3+IAA) ratio in buds from the abnormal orchard was significantly lower than that in buds from the two normal orchards from mid-June to early Aug. (Fig. 4).

Relationship between hormone concentratins during bud differentiation. The correlation matrix in Table 2 shows that during bud differentiation of 'Yuluxiang' pear, ZR was positively correlated with IAA and GA3, but ABA was negatively correlated with GA3.

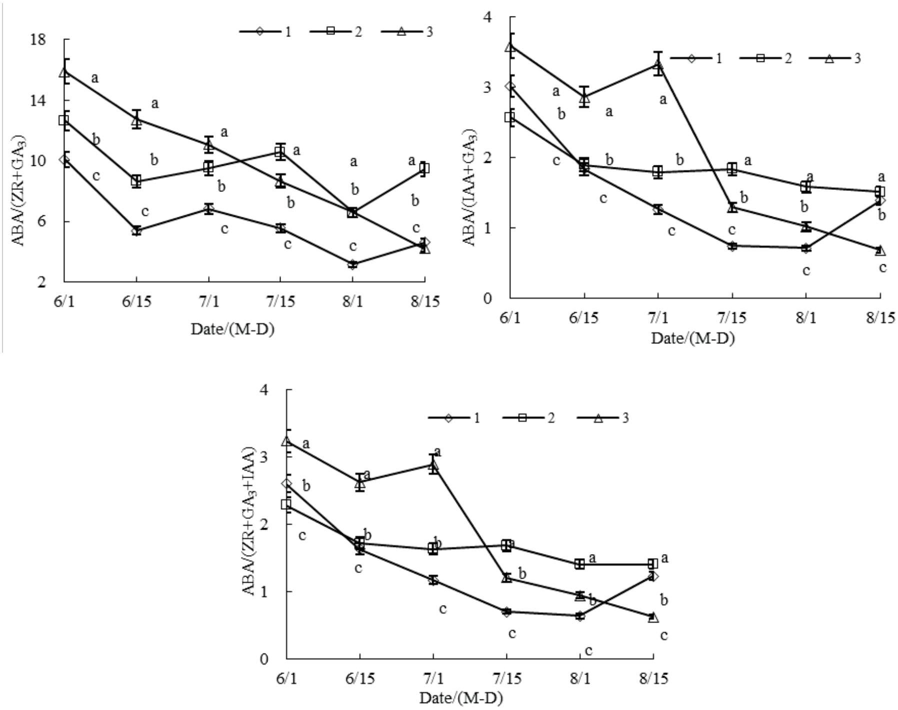

Fig. 4. Changes in ABA/(ZR+GA_3), ABA/(IAA+GA_3), and ABA/ (ZR +GA_3+IAA) ratios during bud differentiation of 'Yuluxiang' pear. 1 = abnormal pear orchard in Weixian of Hebei Province; 2 = normal pear orchard in Weixian of Hebei Province; 3 = normal pear orchard in Taigu of Shanxi Province. Means within dates followed by common letters do not differ at the 0.1% level, by Duncan's multiple range test.

Discussion

Reasonable cultivation measures can promote the transition of vegetative growth to reproductive growth (Wan et al., 2018). Judicious fertilizer application, irrigation, branch bending, branch girdling, etc. are used in the Weixian orchards, and these practices can suppress shoot growth and induce normal formation of flower buds. There was a close relationship between shoot growth cessation and flower bud differentiation. Some studies have reported that growth and development of branches are the premises of normal flower bud differentiation (Yang et al., 2016). Cultural practices that suppress vegetative growth of pear trees and encourage normal development of flower buds, may inhibit the occurrence of wizened bud.

The concentrations and ratios of endogenous hormone in buds were closely related to flower bud differentiation. We discovered that the wizened bud phenomenon of 'Yuluxiang' appeared from early July to mid-July, during which IAA, GA_3 and ZR concentration in buds was significantly higher than that in buds that grew normally in Weixian and Taigu. Studies of flower bud differentiation in 'apple-pear' showed that IAA is an inhibitor of flower bud formation (Li et al., 1999). Our current study agreed with the findings related to 'apple-pear' trees performed by Li et al. (1999). High concentration of GA3 can also inhibit the transition of vegetative growth to reproductive growth in 'Yuluxiang' pear, similar to findings described by Cao et al. (2001). We inferred that IAA and

Table 2. Correlation matrix for endogenous hormone concentrations in 'Yuluxiang' pear buds during bud differentiation stage(n=144).

	IAA	ABA	GA_3	ZR
IAA	1.000			
ABA	0.2811	1.0000		
GA_3	0.6683	-0.4742	1.000	
ZR	0.8610*	0.0623	0.9730**	1.000

GA_3 concentration was high in buds, which hindered the transition of vegetative growth to reproductive growth, and was an important factor determining the development of wizened bud. Low concentration of GA_3 promoted the conversion of vegetative growth to reproductive growth (Sha et al. 2007). Proper cultivation practices decreased IAA and GA_3 concentration in buds of Weixian pear orchards significantly, and as a result flower buds developed normally. We speculated that the relatively low concentration of ZR in buds from the normal orchard maintained slow cell division at the prophase of differentiation, thereby allowing buds to grow more plump; the higher concentration of ZR in buds from the abnormal orchard may have excelerated cell division, causing decreased resistance toward stress. The results agree with the findings of Wu et al. (2013). High concentrations of ABA can halt growth of branches and promote flower bud differentiation (Sha et al., 2007). In this study, the ABA concentration in buds from the Weixian normal pear orchard (treated by cultivation measures) was higher than that in buds from the Weixian pear orchard with the occurrence of wizened bud. ABA itself can induce the enzyme that is essential for biosynthesis of ABA, and accordingly promote further biosynthesis of ABA (Cao et al., 2014). This study showed that cultivation methods can increase ABA concentration, which may further activate the vast biosynthesis of ABA through a positive feedback mechanism, enhancing the signal transduction pathway of ABA, and thereby enabling plants to adapt much better to environmental stress and reducing the occurrence of wizened bud.

The effects of individual plant hormones have complicated relationships. The balance of plant hormones has a very important effect on flower bud differentiation in pear, controlling different types of metabolism and regulating flower bud differentiation and adaptability to stress (Wu et al., 2011). In this study, the ratios of ZR/GA_3, ABA/GA_3, ABA/IAA, and ABA/ZR in buds from the Weixian normal pear orchard were significantly higher than those in buds from the abnormal Weixian pear orchard. These results support the hypothesis of Luckwill et al. (1979) based on plant hormone balance of bud differentiation. The ratios of $ABA/(ZR+GA_3)$, $ABA/(IAA+GA_3)$, and $ABA/(ZR+GA_3+IAA)$ in buds from the normal orchard were significantly higher than those in buds from the abnormal orchard, in accordance with the findings of Cao et al. (2001).

In conclusion, the wizend bud of 'Yuluxiang' is a complex biological process induced by environmental factors. It can be effectively controlled by nitrogen control, water control, branch opening angle, and ring cutting.

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About the Cover:

Jackfruit (*Artocarpus heterophyllus*) is native to the humid tropics of Asia and is cultivated throughout the tropics and subtropics of Asia, Africa and the Americas. Fruits may weigh up to 55 kg and a mature tree can produce up to 500 fruits per year. The fruit and tree are staples in much of the world and there is increasing commercial export trade, particularly with the advent of value add, fresh cut and frozen products. In the Americas, Mexico is the largest producer (Nayarit, Jalisco and Sinaloa states), with over 5,000 ha for domestic consumption and export. Superior cultivars were introduced from Asia, improved and have been clonally propagated for the last 2 decades and account for the majority of the acreage grown in the Americas for commercial trade. Photo by Richard Campbell.