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# Warm winter temperature induced changes in the dormant buds of 'Dangshansuli' pear (*Pyrus bretschneideri* Rehd.)

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## ABSTRACT

**Winter dormancy is one of the crucial ways leading to the survival of deciduous fruit trees in the temperate and boreal zones. In order to study the effects of higher winter temperatures, such as caused by global warming, on bud dormancy and related hormone abundance in 'pear, a greenhouse was set up to enclose adult trees of the pear in field, the temperature inside the greenhouse was maintained about 3.0°C higher than that in the open field. Our results indicated that, greenhouse grown pear trees resulted in about 7-day delay of dormancy induction, 4-5 days ahead of dormancy breaking and much quickly into deep dormancy compared to control trees. Higher temperature led to significant changes of indole-3-acetic acid (IAA), gibberellin acid ($GA_3$), zeatin-riboside (ZR), and abscisic acid (ABA) contents as well as lower ratios of ZR/$GA_3$, ZR/IAA, ABA/$GA_3$, and ABA/IAA in flower buds but not vegetative buds, suggesting that higher winter temperature did not favour flower bud dormancy breaking. Over the dormancy period, ABA content, but not IAA, $GA_3$ and ZR, in flower bud increased alone with the decreased temperature and peaked at the deep dormancy stage, suggesting a great role of ABA in flower bud dormancy formation. The data suggested that higher winter temperature has negative effects on pear flower bud dormancy.**

**Key words:** Endogenous hormones, pear, bud dormancy, winter warming.

## INTRODUCTION

Increased winter temperature is one of the greatest challenges for deciduous fruit trees. An increase in temperature affects numerous plant physiological processes, plant growth and biomass allocation directly or indirectly (Aerts *et al*., 2). Deciduous fruit trees require a certain amount of chilling to break dormancy (Luedeling, 10). However, due to warm winter, some deciduous fruit trees can not break dormancy regularly and the majority of flower buds fail to open normally (Adnane *et al*., 1).

Dormancy initiation of deciduous fruit trees is induced by many factors, among which, phytohormones play the key roles, such as initial of dormancy and dormancy breaking, in bud dormancy (Rajeev *et al*., 13). Endogenous hormones are important regulators of many genes related to dormancy of deciduous fruit trees. The initiation and termination of dormancy process are affected by hormone biosynthesis and transportation, mainly through the hormone-regulated nutrient metabolism and modulation of dormancy-related genes (Fennell, 3). It is widely accepted that low concentration of indole-3-acetic acid (IAA) is essential for flower bud formation, while its high level inhibits the process of bud initiation (Gao *et al*., 4). Gibberellic acid ($GA_3$) is not crucial for breaking dormancy (Park *et al*., 11), while zeatin riboside (ZR) helps to induce the bursting of dormant buds (Park et al., 11). Abscisic acid (ABA) has been reported to promote bud dormancy apparently (Sonnewald, 15).

'Dangshansuli' cultivar of pear is widely grown in China, probably the most widely grown pear cultivar in the world and accounts for 1/5 of total output of pear all over the world (Zhu *et al*., 18). Production of 'Dangshansuli' may have a significant impact on the global pear market. In the past 30 years, studies on 'Dangshansuli' mainly focused on the aspects related to tree growth and productivity (Reig *et al*., 14; Vítor *et al*., 16). Studies on warm winter induced physiological changes in 'Dangshansuli' were rarely reported. In order to better understanding the effect of the warm winter on 'Dangshansuli' and to improve the cultivation management and regionalization, effects of 3°C higher winter temperature on the dormancy process, including dormancy initiation and termination, and phytohormone abundances were studied in the current study.

## MATERIALS AND METHODS

Field experiments were conducted in Dangshan County (34°30'N, 116°32'E), Anhui Province, China,

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which is the main production region and cradleland of 'Dangshansuli'. The County belongs to the warm temperate monsoon climate zone with the annual average temperature 14.1°C, minimum (-12°C) and maximum (39°C) temperatures in January and July, respectively.

In 2013, 56-year-old 'Dangshansuli' pear trees, which usually has a longevity more than 180 years old, with good cultivation management and vitality were selected. These trees uniform in height about 3 m, truck diameter, and canopy structure, with 6 m × 8 m space were selected for further examination.

Plastic arch greenhouses each with 5.0 m for top height, 4.0 m for shoulder height and 6.0 m in width were set up using iron skeleton and PEP film with 12 mm in thickness. Auto-rolling curtains, electric light sources, and dehumidification devices were also set up in the greenhouses. The temperature inside of the greenhouse was automatically set 3°C higher than that outside of the greenhouse and light intensity and humidity were set similarly as those in natural conditions. The temperature, humidity, and light intensity inside and outside of the greenhouse were automatically recorded over the period from November 20th to February 28th of the next year. The experiment was conducted with single-tree in triplicate.

For the measurement of bud dormancy stages, six current-year shoots with flower and leaf buds were randomly excised from each selected tree at a 10-day interval from November 26, 2013 to March 14, 2014. The shoots were rinsed with tap-water and placed into a container with clean water (2-3 cm in depth). The shoots were maintained in a growth chamber with 25°/21°C (day/night), a 16 h photoperiod (3000 lux), and 80% humidity. Every day, the rate of bursting buds was calculated, and number of days required for the first bud burst was recorded, and then dormancy stages were determined according to Jian *et al*. (8) and Lang *et al*. (9).

For the measurement of plant hormones (IAA, ABA, $GA_3$ and ZR), six apical flower buds and 10 apical leaf buds were excised for further testing. The amounts of IAA, ABA, $GA_3$ and ZR were determined using the kit of enzyme-linked immunosorbent assay (ELISA) (Grana *et al*., 5) and counted using the comparison of Logit model with the standards. Calculation of the Logit value was as follows: Logit (B/B0) =ln [B/( B0-B)]; B0, chromogenic value of the 0 ng/ml pot□B, chromogenic value of another pot. The sampling was conducted in triplicate.

## RESULTS AND DISCUSSION

Over the experimental period, the monthly average temperature in the greenhouse (T) was 4.12 °C while that in the control orchard was 1.16 °C (CK) (Table 1). No significant differences in humidity and light intensity was obtained in the experiment.

**Table 1.** Temperature, humidity, and light intensity between of the greenhouse (T) and open field (CK).

| Particulars | Temperature (°C) | Humidity (%) | Light (Lux) | Temp. (°C) | Humidity (%) | Light (Lux) |
|---|---|---|---|---|---|---|
| Date | | Nov 20 - Nov 30 | | | Dec1 - Dec 10 | |
| T | 8.57±1.85 | 71.94±4.31a | 5608± 728ab | 3.57±0.81 | 57.42±4.27a | 4492±707b |
| CK | 5.60±1.04 | 68.52±3.28ab | 5866±651a | 0.57±0.24 | 55.83±6.29a | 5473± 834a |
| Date | | Dec 11- Dec 20 | | | Dec 21- Dec 31 | |
| T | 1.67±0.32 | 47.1±5.30a | 4531±614a | 0.53±0.17 | 48.58±6.25a | 3832±426a |
| CK | (-)1.20±0.21 | 44.8±5.07a | 4699±525a | (-)2.37±0.50 | 44.63±3.98a | 4058±383a |
| Date | | Jan 1-Jan 10 | | | Jan11-Jan 20 | |
| T | 1.54±0.22a | 43.35±4.52a | 3014±402a | 1.22±0.24 | 43.32±5.03a | 3801±454a |
| CK | (-)1.57±0.27c | 41.42±5.02a | 3157±294a | (-)1.62±0.19 | 41.92±6.08a | 4199± 506a |
| Date | | Jan21-Jan 31 | | | Feb1-Feb 11 | |
| T | 2.01±0.28 | 43.73±4.23a | 3931±397a | 4.84±1.02 | 48.26±6.01a | 4381±375a |
| CK | (-)0.86±0.24 | 41.9±5.07a | 4199±456a | 1.96±0.3 | 47.1±5.44a | 5084±463a |
| Date | | Feb11-Feb 20 | | | Feb 21- Feb 28 | |
| T | 8.9±1.82 | 61.9±5.84a | 4928±503a | 8.3±2.03 | 62.9±5.44a | 4407±609a |
| CK | 5.9±1.57 | 60.1±6.73a | 5189±452a | 5.2±1.24 | 60.1±5.79a | 4696±583a |

Note: one-way ANOVA was used for statistical analysis, different lower-case letter indicated significant differences ($p<0.05$). (-), minus temperature.

Results showed that the flower and leaf buds in the greenhouse sampled on December 2nd began to bursting on 11th and 12th day, while open field buds sampled on November 26th required 12 d (>10) for burst, indicated that an increase of 2.96°C in temperature in winter season resulted in 7-day delay of dormancy induction. Moreover, the greenhouse buds turned into deep dormancy without experiencing dormancy deepening stage (Table 2).

Greenhouse buds taken on December 8th, December 20th, did not burst, while buds taken on December 31st and January 12th, required a minimum of 55 and 43 d, respectively, for bud bursting, which is the same with that in open field (Table 2). These results suggested that higher temperature had no significant impact on deep dormancy.

The greenhouse buds collected in early March and open field buds collected on March 14th need a minimum of about 10 d for bud bursting, indicated that dormancy ended around March 7 or March 14. These data indicated that higher temperature led to about 7 d earlier for dormancy breaking (Table 2), this may be due to the chilling treatment had been satisfied before March 7 (Luedeling, 10).

IAA abundance in the greenhouse flower buds was significantly lower than that in the open field flower buds (*p<0.05*), which consistent with previous reports that low IAA abundance promotes the initiation of buds (Gao *et al*., 4). No significant differences in $GA_3$ abundance (*p≥0.05*) was found between the greenhouse flower buds and open field buds, suggesting that the 3°C higher temperature did not significantly affected $GA_3$ abundance (Fig. 1B). ZR levels in the buds at the increased temperature were all lower than those at regular temperatures (*p<0.05*), except for the data collected on December 2nd (Fig. 1C). Our data suggested that the morphological differentiation might require high level of cytokines for cell division and differentiation, which was consistent with that cytokines can promote flower bud differentiation (Zhang *et al*., 17). ABA levels in the buds in the greenhouse were significantly lower than those in the open field (*p<0.05*). This indicates that ABA abundance was negatively correlated with winter temperature and positively correlated with dormancy degree, which was consistent with previous findings (Sonnewald, 15).

No significant differences of IAA, $GA_3$, ZR and ABA abundances between greenhouse leaf buds and open field leaf buds (p≥0.05) (Fig. 2 A-D), suggested that higher temperature did not affected leaf buds dormancy. The ratios of $ZR/GA_3$ in the greenhouse flower buds were lower than those in the open field grown buds after the date of December 10th (Fig. 3B), suggested that higher temperature led to a decrease of $ZR/GA_3$ ratio. The change of ZR/IAA ratio was found similar with the change of $ZR/GA_3$ (Fig. 3C).

The ratio of $ABA/GA_3$ changed similar with ABA/IAA, which peaked at the deep dormancy stage (Fig. 3D). These results were consistent with previous findings that both ABA and $GA_3$ are involved in the induction of bud dormancy through their balance modulation (Hamadina *et al*., 6). Higher ratios of $CTK/GA_3$, CTK/IAA, $ABA/GA_3$ and ABA/IAA favour flower bud morphological differentiation and formation (Gao *et al*., 4). Our data indicated that

**Table 2.** Influence of higher winter temperature on dormancy (mention year also).

| Treatment | Sampling date | Nov 26 | Dec 02 | Dec 08 | Dec 20 | Dec 31 | Jan 21 |
|---|---|---|---|---|---|---|---|
| | | Days for bud burst | Days for bud burst | Days for bud burst | Days for bud burst | Days for bud burst | Days for bud burst |
| T | Flower bud | 8±1* | 11±1** | No burst | No burst | 79±5 | 43±5 |
| | Leaf bud | 9±1* | 14±2** | No burst | No burst | 55±4 | 48±6 |
| CK | Flower bud | 12±2** | 21±3*** | No burst | No burst | 84±4 | 33±5 |
| | Leaf bud | 13±2** | 34±2*** | No burst | No burst | 47±3 | 42±4 |
| Treatment | Sampling date | Jan 24 | Feb 04 | Feb 16 | Feb 28 | March 07 | March 14 |
| | | Days for bud burst | Days for bud burst | Days for bud burst | Days for bud burst | Days for bud burst | Days for bud burst |
| T1 | Leaf bud | 29±4**** | 17±3**** | 17±3**** | 16±2**** | 9±2***** | 12±3***** |
| | Flower bud | 34±5**** | 24±3**** | 25±4**** | 14±5**** | 13±3***** | 15±2***** |
| CK | Leaf bud | 15±3**** | 12±2**** | 10±2**** | 11±2**** | 12±2***** | 10±3***** |
| | Leaf bud | 21±3**** | 18±4**** | 22±2**** | 19±2**** | 16±3***** | 15±2***** |

Note: Stars showed different dormancy stages: *growth stage; **dormancy initiation; ***dormancy deepening; without stars indicated deep dormancy; ****dormancy; *****dormancy breaking; greenhouse (T) and open field (CK).

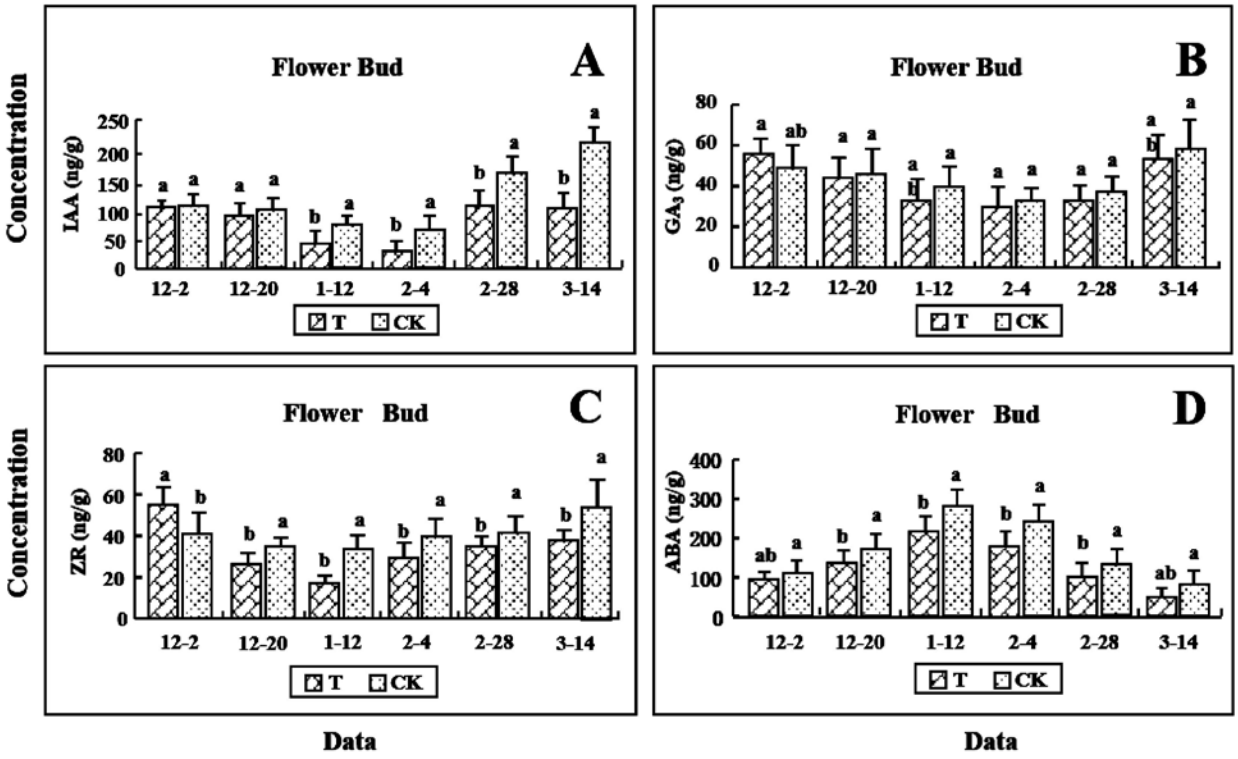


**Fig. 1.** AA (A), $GA_3$ (B), ZR (C) and ABA (D) contents in flower buds of '*Dangshansuli*' grown at open field (CK) and greenhouse (T). Statistical analysis was done using Duncan's multiple range test.

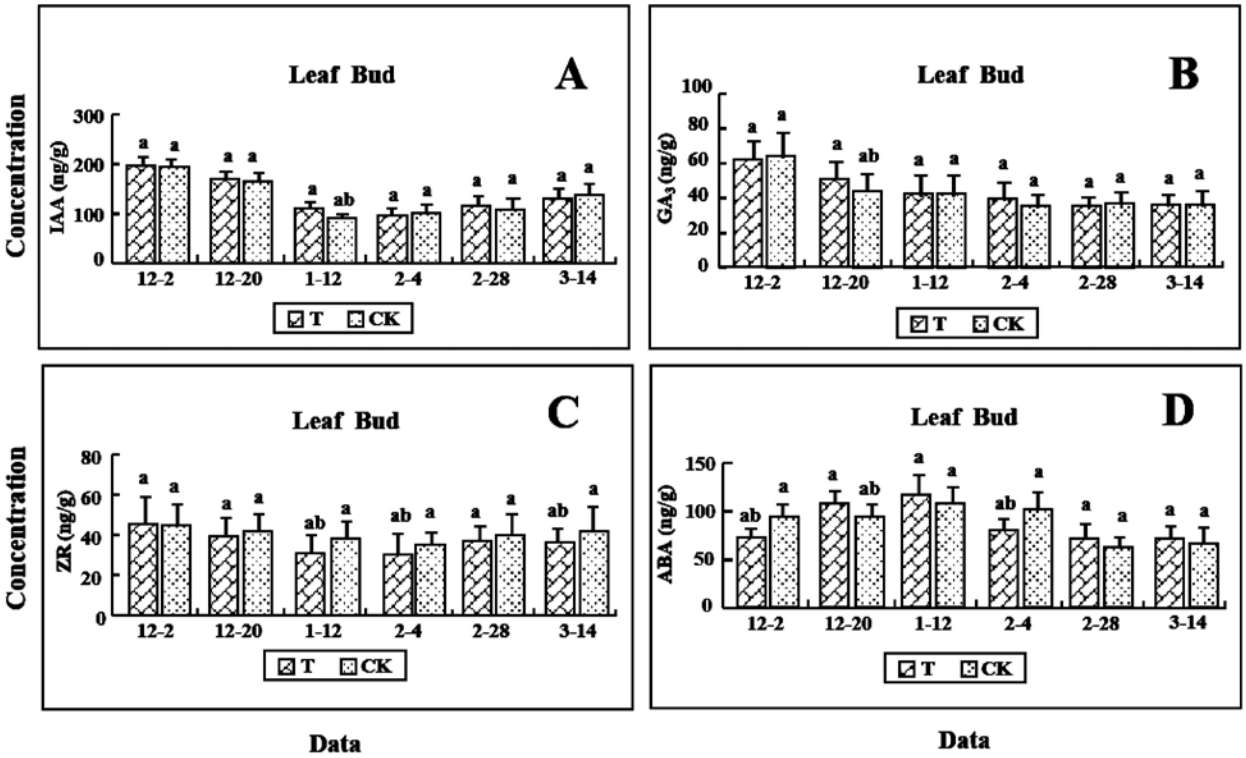


**Fig. 2.** Changes of hormone IAA (A), $GA_3$ (B), ZR (C) and ABA (D) content leaf buds of Dangshansuli pear grown in open field (CK) and greenhouse (T) over the winter days. Statistical analysis was done using Duncan's multiple range test.

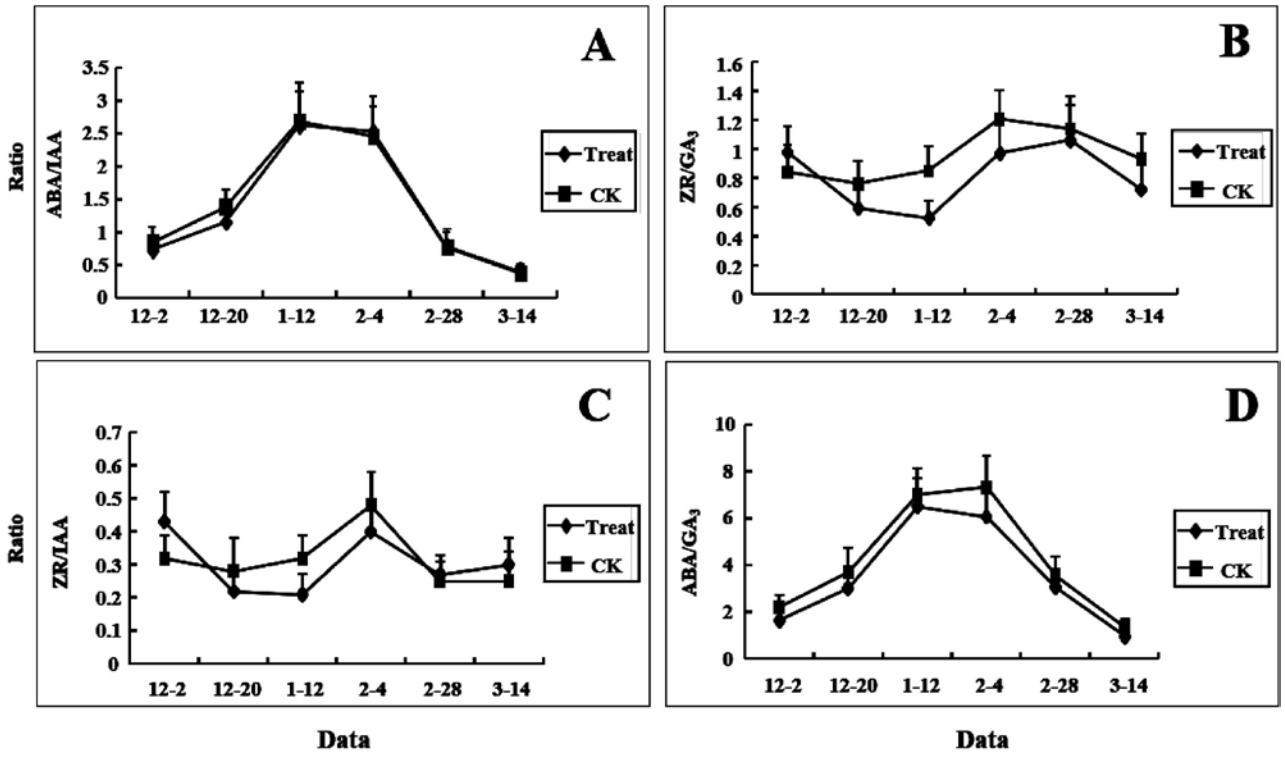


**Fig. 3.** Ratios of ABA/IAA (A), ZR/$GA_3$ (B), ZR/IAA (C) and ABA/$GA_3$ (D) in dormant flower buds. Treat, trees grown in greenhouse; CK, trees grown in open field.

the ratios of ABA/$GA_3$, and ABA/IAA, as well as ZR/$GA_3$ and ZR/IAA, in the greenhouse grown flower buds were lower than those in the open field grown buds, suggesting that higher winter temperature did not favor the flower bud dormancy breaking, which was consistent with previous findings that hormone induced responses include the binding of initiative signals with their corresponding receptors, complex signal transduction, alteration of cell morphology and metabolisms, and signal output (Pieterse *et al*., 12). Further investigations are required on the dormancy regulation by signaling molecules, related gene expression regulation.

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