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RESEARCH ARTICLE

**Lei BI, Yuxing ZHANG, Bharat Kumar POUDYAL**

# Effects of growth regulators on the respiration metabolism of pear buds during dormant period

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**Abstract** The effects of growth regulators on the respiration metabolism of pear buds during dormant period were studied in this experiment. The results showed that, during early dormant period, the respiration intensity of pear buds was infirm and increased slowly. As the weather became very cold, the respiration intensity rapidly declined, and after that it changed a little. In the later stage of dormant period, the respiration intensity rose rapidly. The maximum value appeared on January 4th, at 0.390 mol $O_2 \cdot g^{-1}$ FW$\cdot$min$^{-1}$, but thereafter declined to its original level. Under natural conditions, three respiratory pathways showed different changes. Pentose phosphate pathway might be the main reason for dormancy release in pear buds. The exogenous gibberellins were more efficient than salicylic acid in increasing the respiration rate. The exogenous SA appeared to play a more important role than exogenous $GA_3$ in phosphopentose pathway. The effect of gibberellins would be more effective than SA in tricarboxylic acid cycle (TCA). The respiration rate of glycolysis was not affected by gibberellins and salicylic acid.

**Keywords** pear, dormancy, respiration intensity, growth regulators

## 1 Introduction

The biomechanics of dormancy of deciduous fruit trees evolved during the long ages to adapt to environmental and climatic changes in winter. The physiologic and biochemical metabolisms do not stop but are merely arrested or inactivated by the dormancy process. Resting or dormant periods are common in the plant kingdom, especially in deciduous trees, and have been widely studied in organs like buds, seeds, tubers, corms, etc. Many chemicals in numerous ways increase the rate of growth of plants (Sponsel, 1983; Garcia et al., 1987). Gibberellins (GA), as one kind of phytohormones, are essential for many processes of plant development, such as seed germination, stem elongation, leaf expansion, flowering, and seed development. The germination-promoting effect of GA on mature seeds has been well documented in a number of species (Hilhorst, 1995; Finch-Savage and Leubner-Metzger, 2006). Researchers paid much attention to the studies about dormant respiration (Myking, 1997; Ögren, 2000). Chinese scientists have been studying the relationship between dormancy and respiration for many years (Li et al., 2001) in many plant species including fruit trees. For the purpose of gaining insight into the physiologic and biochemical aspects of dormancy, we carried out the study of dormancy of pear buds.

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Lei BI, Yuxing ZHANG (✉)
College of Horticulture, Agricultural University of Hebei, Baoding 071001, China
E-mail: jonsonzhyx@yahoo.com.cn

Bharat Kumar POUDYAL
Department of Agriculture, Fruit Development Directorate, Kirtipur, Kathmandu, Nepal

## 2 Materials and methods

### 2.1 Materials

Ten-year-old *Qiyuesu*'s pears were used as materials in our experiment. The samples were taken from the orchard of Agricultural University of Hebei, Baoding City, Hebei Province, China. Trees were selected in November of 2007. The branches were collected every five days from November 20th, partially treated immediately (120 mg$\cdot$L$^{-1}$ $GA_3$ or 0.2 mmol$\cdot$L$^{-1}$ SA was smeared around the cross-section of the branches) and cultured under a temperature of 25°C, photoperiod regime 12/12 light/dark, and the light intensity was 2000 lx. The branch at each experimental time contained 10 to 20 buds. The respiration rate and the proportion of respiratory channels of buds were measured when the branches collected have been cultured for 10 d.

2.2 Methods

Respiration was measured at 25°C using a Hansatech Clark oxygen electrode (Hansatech Instruments, Pentney King's Lynn, UK) in 0.4 mL respiration medium. The electrode was calibrated against air-saturated water, where $O_2$ concentration was set at 250 ppm. Experiments were conducted with dormant buds. First, a drop of electrolyte was placed on top of the dome of the sensor unit and 3 more drops were added at equal intervals in the electrode well containing the silver anode. Different compositions of electrolyte were used. However, 50% saturated solution of potassium chloride worked well in many different applications. The solubility of the anhydrous salt was 35 g per 100 g of water at 25°C. Hence, the electrolyte solution was easily made by dissolving 17.5 g of anhydrous salt in 100 mL of de-ionised water at 25°C. Once the electrolyte was added, a 1.5 cm × 1.5 cm paper spacer (cigarette paper which is manufactured to keep its tolerance working well) was placed over the electrolyte and covered by a 2.5 cm × 2.5 cm area of membrane. The paper spacer acted as a wick to continuously provide an electrolyte layer of uniform thickness above the electrode dome during operation. The membrane combination was tensioned and held in place by an O-ring which was applied over the electrode dome using the membrane applicator shaft. Initially, the O-ring was placed at the end of the applicator shaft. The applicator was then pressed vertically over the center of the dome and the applicator cone slid down the shaft, slipping the O-ring off the applicator and over the dome of the electrode disk. The resulting membrane application should be smooth with the spacer providing a uniform layer of electrolyte between the electrodes. Finally, some more drops of electrolyte might be added as necessary to the electrode well to provide a reservoir of electrolyte during operation. Potassium sodiumfluorde (NaF), malonic acid (MA), and trisodium phosphate ($Na_3PO_4$) were used to block the glycolytic pathway, tricarboxylic acid cycle (TAC) and phosphopentose pathway respectively.

# 3 Results

3.1 Changes in the intensity of respiration of *Qiyuesu* dormancy buds

Respiratory intensity was used as a measure of rate of metabolism in the work reported here. Before attaining low temperature (from November 20th to December 5th), the respiratory intensity rose continuously. When the temperature dropped (December 10th), the total respiratory intensity ranged from 0.256 mo1$O_2 \cdot g^{-1}$ FW$\cdot$min$^{-1}$ to 0.080 mo1$O_2 \cdot g^{-1}$ FW$\cdot$min$^{-1}$. Since then, natural pear developed into the dormant bud stage, and its respiratory intensity changed a little. At the end of dormancy (December 25th), the respiratory intensity began to increase rapidly. The maximum value appeared on January 4th, which was 0.390 mo1 $O_2 \cdot g^{-1}$ FW$\cdot$min$^{-1}$, but thereafter declined to its original level. In this experiment, we found that low-temperature increased the respiration intensity of pear buds during dormancy. Obviously, there was a limitation to respiration during the early rest period, and this limitation was removed as exposure to low temperature removed the rest period block. The respiration intensity of buds from chilled trees rose as a result of previous low temperature exposure which broke rest. Thus, when the data were expressed as the total respiratory intensity (Fig. 1), we found that dormant pear buds were consistent with other plants in the changes in respiration intensity.

3.2 Changes of respiratory pathway of *Qiyuesu* dormancy buds

From Fig. 2, it can be seen that the respiratory activity of glycolysis, tricarboxylic acid cycle and phosphopentose pathway changed little before December 20th. Variations between the individual enzyme's pathways were not significant. During dormancy the buds mainly relied on glycolysis and tricarboxylic acid cycle as its energy was supplied through oxidation. The respiration in phosphopentose pathway reached the maximum value on December 25th during the dormant process, which suggested that the activation of this pathway also played a vital role in breaking the dormancy of the buds. At the end of dormancy (December 25th), the respiratory activity of glycolysis and phosphopentose pathway were characterized by a rise followed by a fall. Although tricarboxylic acid pathway was also characterized by a rise followed by a fall, the peak of tricarboxylic acid appeared on January 4th. The total respiratory rate of glycolysis and phosphopentose pathway ranged from approximately 50% to 90%.

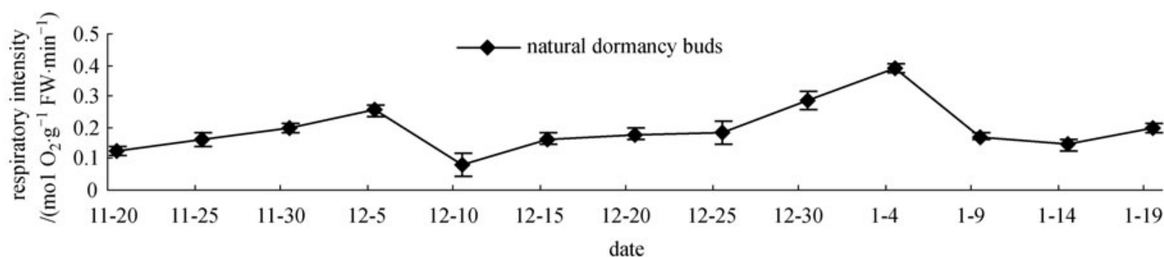


**Fig. 1** Changes in the intensity of respiration of *Qiyuesu* dormancy buds

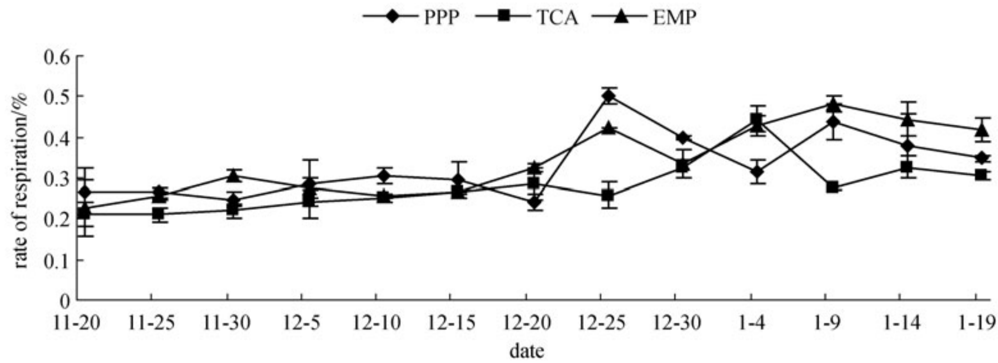


**Fig. 2** Changes of respiratory pathway of *Qiyuesu* dormancy buds

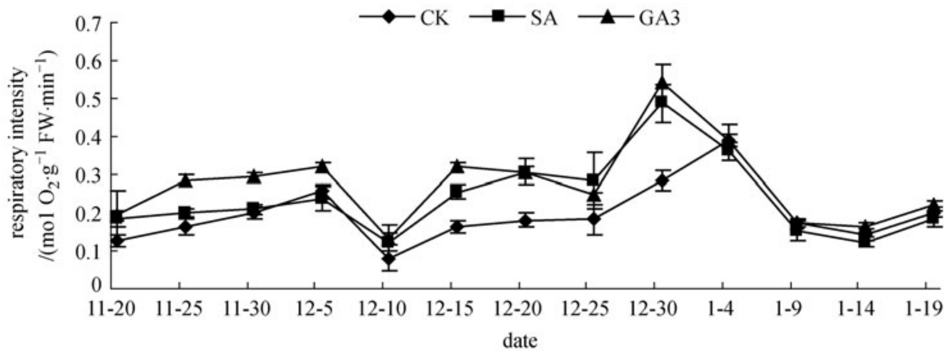


**Fig. 3** Effects of regulators on respiratory intensity of dormancy buds

The phosphopentose pathway appeared to play a more important role than the glycolytic pathway and tricarboxylic acid cycle in the dormant release. The results suggested that the dormancy was released mainly via the pentose phosphate pathway.

3.3 Effects of growth regulators on respiratory intensity of "*Qiyuesu*" pear dormancy buds

Different growth regulators affected respiratory intensity of dormant pear buds differently. It was shown (Fig. 3) that the respiration intensity of such buds increased after being treated by exogenous $GA_3$ and exogenous salicylic acid. $GA_3$ was more efficient than salicylic acid in increasing the respiration intensity. Respiration intensity was conductive to the release of dormancy. $GA_3$ and Salicylic acid would help to break the dormancy, and the effect of $GA_3$ would be more effective (Table 1).

3.4 Effects of growth regulators on respiratory pathway of "*Qiyuesu*" dormancy buds

Results showed that phosphopentose pathway might be the main reason of dormancy relief. During dormancy the buds mainly relied on glycolysis and tricarboxylic acid cycle for its energy supply through oxidation. From Fig. 4 and Table 2, we can see that the exogenous SA appeared to play a more important role than exogenous $GA_3$ on phosphopentose pathway. In SA treatment, respiration intensity in phosphopentose pathway reached the maximum on

**Table 1** Effects of integration treatment on respiratory intensity of dormancy buds

| treatment | time of treatment/d | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 11–20 | 11–30 | 12–10 | 12–20 | 12–25 | 12–30 | 1–4 | 1–9 | 1–14 | 1–19 |
| CK | 0.13<br>Ab | 0.20<br>Bb | 0.08<br>Ab | 0.18<br>Bb | 0.18<br>Ab | 0.28<br>Bb | 0.39<br>Aa | 0.17<br>Aa | 0.14<br>ABb | 0.20<br>Aab |
| SA | 0.19<br>Aab | 0.21<br>Bb | 0.12<br>Aab | 0.31<br>Aa | 0.28<br>Aa | 0.49<br>Aa | 0.36<br>Aa | 0.15<br>Aa | 0.12<br>Bc | 0.18<br>Ab |
| $GA_3$ | 0.19<br>Aa | 0.29<br>Aa | 0.13<br>Aa | 0.30<br>Aa | 0.25<br>Aab | 0.54<br>Aa | 0.39<br>Aa | 0.17<br>Aa | 0.16<br>Aa | 0.22<br>Aa |

Note: Different small and capital letters in the same column mean significantly differences at 0.05 and 0.01 probability levels, respectively.

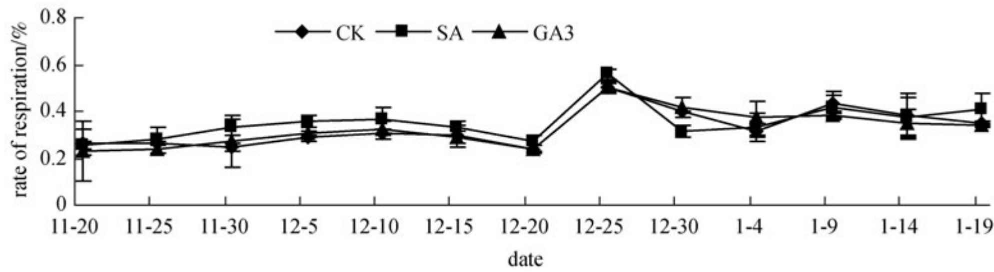


**Fig. 4** Effects of regulators on phosphopentose pathway of dormancy buds

**Table 2** Effects of integration treatment on phosphopentose pathway of dormancy buds

| treatment | time of treatment/d | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 11–20 | 11–30 | 12–10 | 12–20 | 12–25 | 12–30 | 1–4 | 1–9 | 1–14 | 1–19 |
| CK | 0.27 Aa | 0.25 Aa | 0.30 Aa | 0.24 Aa | 0.50 Ab | 0.40 ABa | 0.32 Aa | 0.44 Aa | 0.38 Aa | 0.35 Aa |
| SA | 0.26 Aa | 0.33 Aa | 0.37 Aa | 0.28 Aa | 0.56 Aa | 0.32 Bb | 0.33 Aa | 0.42 Aa | 0.38 Aa | 0.41 Aa |
| $GA_3$ | 0.23 Aa | 0.27 Aa | 0.33 Aa | 0.24 Aa | 0.50 Ab | 0.42 Aa | 0.37 Aa | 0.39 Aa | 0.35 Aa | 0.34 Aa |

Note: Different small and capital letters in the same column mean significantly differences at 0.05 and 0.01 probability levels, respectively.

December 25, which suggested that the activation of this pathway also played a vital role in breaking the dormancy of buds. Tricarboxylic acid cycle is an important way of respiration pathways. Fig. 5 and Table 3 showed that $GA_3$ would be helpful in increasing the rate of respiration gradually and the effect of $GA_3$ would be more effective than SA. We can see that the respiration rate of glycolysis was not affected by $GA_3$ and salicylic acid from Fig. 6 and Table 4.

## 4 Discussion

Dormancy is not a state of general inactivity, in fact, it is due to some specific metabolic blockages (Nir et al., 1986; Wang et al., 1999). We analyzed the increases of respiratory intensity by using phytohormones like gibberellins and salicylic acid. The results of this experiment suggested that, the initial effect of gibberellins and salicylic

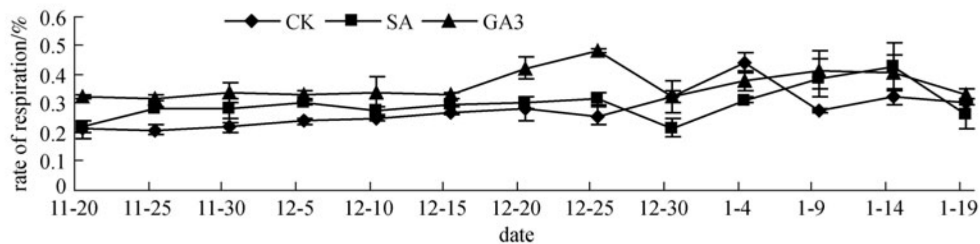


**Fig. 5** Effects of regulators on tricarboxylic acid cycle of dormancy buds

**Table 3** Effects of integration treatment on tricarboxylic acid cycle of dormancy buds

| treatment | time of treatment/d | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 11–20 | 11–30 | 12–10 | 12–20 | 12–25 | 12–30 | 1–4 | 1–9 | 1–14 | 1–19 |
| CK | 0.21 Bb | 0.22 Bb | 0.25 Ab | 0.28 Bb | 0.26 Bc | 0.32 Aa | 0.44 Aa | 0.27 Ab | 0.33 Aa | 0.30 Aab |
| SA | 0.22 Bb | 0.29 ABa | 0.28 Ab | 0.30 Bb | 0.32 Bb | 0.21 Ab | 0.31 Bc | 0.39 Aa | 0.43 Aa | 0.26 Ab |
| $GA_3$ | 0.33 Aa | 0.34 Aa | 0.34 Aa | 0.42 Aa | 0.48 Aa | 0.33 Aa | 0.38 ABb | 0.42 Aa | 0.41 Aa | 0.33 Aa |

Note: Different small and capital letters in the same column mean significantly differences at 0.05 and 0.01 probability levels, respectively.

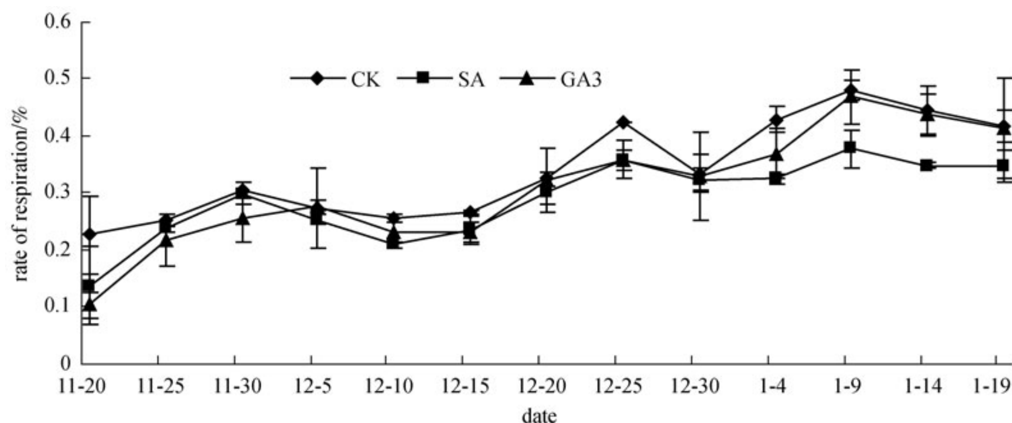


**Fig. 6** Effects of regulators on glycolytic pathway of dormancy buds

**Table 4** The effect of integration treatment on glycolytic pathway of dormancy buds

| treatment | time of treatment/d | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 11–20 | 11–30 | 12–10 | 12–20 | 12–25 | 12–30 | 1–4 | 1–9 | 1–14 | 1–19 |
| CK | 0.23<br>Aa | 0.31<br>Aa | 0.26<br>Aa | 0.32<br>Aa | 0.42<br>Aa | 0.33<br>Aa | 0.43<br>Aa | 0.48<br>Aa | 0.44<br>Aa | 0.42<br>Aa |
| SA | 0.14<br>Aab | 0.30<br>Aa | 0.21<br>Bb | 0.30<br>Aa | 0.36<br>Ab | 0.32<br>Aa | 0.33<br>Bb | 0.38<br>Ab | 0.35<br>Ab | 0.35<br>Aa |
| $GA_3$ | 0.10<br>Ab | 0.26<br>Aa | 0.23<br>ABb | 0.32<br>Aa | 0.40<br>Ab | 0.33<br>Aa | 0.37<br>ABb | 0.47<br>Aa | 0.44<br>Aa | 0.41<br>Aa |

Note: Different small and capital letters in the same column mean significantly differences at 0.05 and 0.01 probability levels, respectively.

acid was to increase the efficiency with which respiratory enzyme systems operate, thus a greater supply of available energy was provided to the buds, and complex reactions that led to active growth were triggered eventually. Possibly, gibberellins induced growth inhibited by bud inhibitors (Shulman et al., 1983) or limited by other growth substances or metabolic processes. The same explanation applied to buds in which SA stimulated bud growth to some extent. The following observations supported the concept that gibberellins are substances directly involved in the release of dormancy of these buds (Vegis, 1964). It had also been stated that gibberellins can promote bud germination by enhancing the hydrolase synthesis (Donohue et al., 2005; Gubler et al., 2005). Salicylic acid increased the respiration rate. The increased percentage was higher in phosphopentose pathway than in glycolysis and tricarboxylic acid cycle. Similarly, gibberellins also increased the respiration rate, but the increased percentage was higher in tricarboxylic acid cycle than other respiratory pathways. The phosphopentose pathway appeared to play a more important role than the glycolytic respiratory pathway and tricarboxylic acid cycle. The results suggested that breaking dormancy was metabolized mainly via the pentose phosphate pathway. During the dormancy, the viability of phosphopentose pathway was enhanced, which promoted the release of dormancy (Bogatek, 1997). This showed that phosphopentose pathway might be the main reason of dormancy release, which was previously reported in the study on seeds (Boilek, 1953; Abbott, 1955).

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