

Effects of regulators on the respiratory enzyme of pear branches during dormant period

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Abstract The effects of regulators on the respiratory enzymes of Qiyuesu's pear branches during dormant period were studied. The results showed that enzyme activities of SDH, G-6-PDH and 6-PGDH were increased by 0.02 mmol/L SA and 120 mg/L GA₃. The enzyme activities of phosphohexoisomerase were reduced by 0.02 mmol/L SA and 120 mg/L GA₃. Compared with the control, the PGI activity was reduced by 20.5% and 13.6% using 0.02 mmol/L SA and 120 mg/L GA₃. Similarly, the SDH activity was increased by 6.1% and 29.2%, respectively. Likewise, the activity of G-6-PDH and 6-PGDH was increased by 93.9% and 24.8%. Changes of respiratory enzymes were consistent with respiratory pathway of regulators, which indicated that SA and GA₃ were helpful to break the dormancy by enhancing activities of SDH, G-6-PDH and 6-PGDH.

Keywords pear, dormancy, respiratory enzyme

Introduction

Respiration is one of the physiologic processes frequently measured in relation to the dormancy period. In general, respiration increases slowly with the shift from the dormant to the potentially growing condition, and then very rapidly with the beginning of active growth. Obviously there is a limitation to respiration during early dormancy. This limitation is removed by exposure to low temperature. Plants grown under low temperatures characteristically possess increased carbohydrate concentrations (Neales and Incoll, 1968) that may attain levels inhibitory to photosynthesis and stimulatory to respiration. Phosphohexoisomerase (PGI), succinic dehydrogenase (SDH) and glucose-6-phosphate dehydrogenase (G-6-PDH) and 6-phosphogluconate dehydrogenase (6-PGDH) are the key enzymes for respiratory metabolic pathway (Bewley and Black, 1994). The control of dormancy in perennial branch is not yet clear, although many changes in growth regulators have been reported during its onset and release (Li et al., 2001). Gibberellins (GA), one kind of phytohormones,

are essential for many processes of plant development, such as seed germination, stem elongation, leaf expansion, flowering, seed development, etc. The germination-promoting effect of GA on mature seeds has been well documented in a number of species including fruit trees (Hilhorst, 1995; Finch-Savage and Leubner-Metzger, 2006). Salicylic acid (SA) has profound effects on many developmental events and environmental responses of plants (Erez, 1971; Faust et al., 1997; Fuchigami and Wisniewski, 1997). Thus, it is of interest to study the mechanism of Qiyuesu's action by regulators on dormant branches. In particular, we have tried to determine the involvement of the respiratory enzyme in the development of and release from dormancy in pear branches with the help of SA and GA₃. In this article, the physiologic and biochemical changes of branches were determined during defoliating period, dormancy initial period, deep dormancy period and finally the dormancy breaking period.

Materials and methods

Materials

Ten-year-old Qiyuesu's pears were used in this experiment. Samples were taken from the pear orchard of Agricultural University of Hebei, Baoding, Hebei Province, China. In

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November 2007, the branches were collected and stored in an artificial climate chamber (RXZ280A) at 25°C and a light intensity of 2000 lx for 12 h daily. For each five-day interval, the respiration rate of the branches and the proportion of respiratory channels were measured.

Methods

The activity of PGI, G-6-PDH and 6-PGDH was assayed with the help of Brown and Wary’s method (1968), and the activities of SDH were done by Hiatt (1961). The enzyme activity of PGI was determined by the content of F-6-P. Likewise, the activities of G-6-PDH and 6-PGDH were determined by the content of NADPH. Similarly, the activity of SDH was determined by the content of 2,6-dichlorophenolindophenol (DCPIP).

Results

Effects of regulators on PGI of dormancy branches

The content of F-6-P determined the activity of PGI, which was the key enzyme for glycolytic pathway (EMP). From Fig. 1, the activity of PGI of dormancy branches shows a trend of increasing first, then decreasing with the temperature reducing gradually, increasing again after dormancy released (December 25), and decreasing finally. It also can be seen that the change of PGI activity of dormancy branches treated with salicylic acid is similar to that treated with gibberellin in early dormancy and deep dormancy stages. Spraying regulators of salicylic acid and gibberellin can obviously reduce the PGI

content of dormant branches. The change of PGI activity is similar to that of glycolytic pathway.

From Fig. 2, compared to control, addition of salicylic acid and gibberellin obviously reduced the F-6-P content of dormant branches. At the end of dormancy (December 25), compared with the control, the content of F-6-P was reduced by 20.5% and 13.6% using 0.02 mmol/L SA and 120 mg/L GA₃, respectively. According to this result, we can infer that the activity of PGI can be also reduced by 0.02 mmol/L SA and 120 mg/L GA₃.

Effects of regulators on SDH of dormancy branches

The content of DCPIP stands for the activity of SDH, which is the key enzyme for tricarboxylic acid cycle (TCA). From Fig. 3, the SDH activity of dormant branches showed a trend of increasing, then decreasing with the passage of date, increasing again after rest released (December 25), and then decreasing. The change of SDH activity of dormancy branches treated with salicylic acid was similar to that treated with gibberellin in early dormancy and deep dormancy stage. Spraying regulators of salicylic acid and gibberellin obviously increased the SDH activity of dormant branches. At the same time, it can be seen that the SA appeared to play a lesser role than GA₃. The change of SDH activity was similar to the change of TCA.

From Fig. 4, the content of DCPIP was increased by 0.02 mmol/L SA and 120 mg/L GA₃. At the end of dormancy (December 25), compared with the control, the content of DCPIP was increased by 6.1% and 29.2% using 0.02 mmol/L SA and 120 mg/L GA₃ respectively. Therefore, we come to a

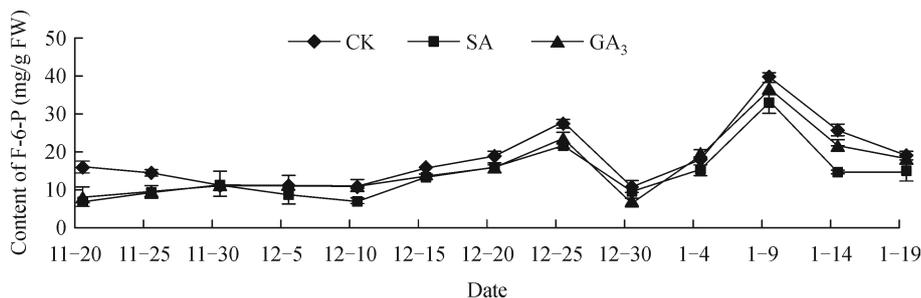


Figure 1 Effects of regulators on content of F-6-P of Qiyuesu dormancy branches.

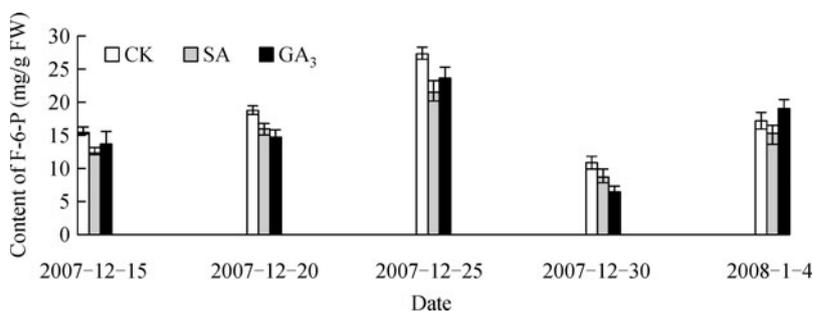


Figure 2 Effects of regulators on content of F-6-P of Qiyuesu dormancy branches during respiratory climacteric period.

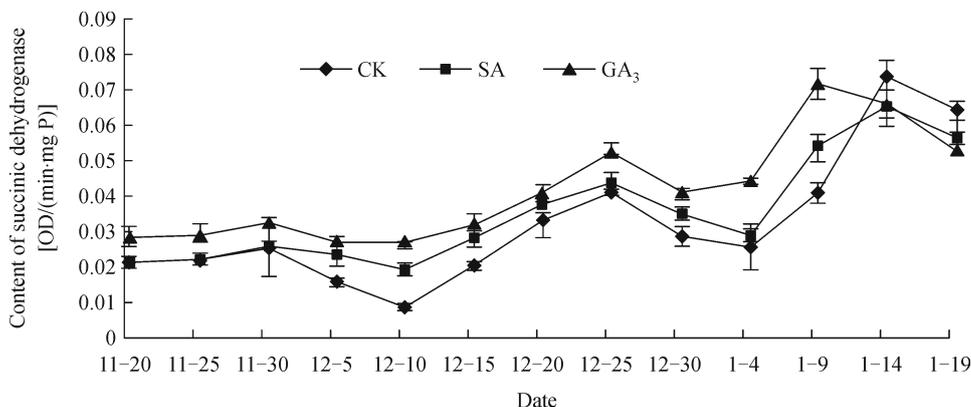


Figure 3 Effects of regulators on succinic dehydrogenase of Qiyuesu dormancy branches.

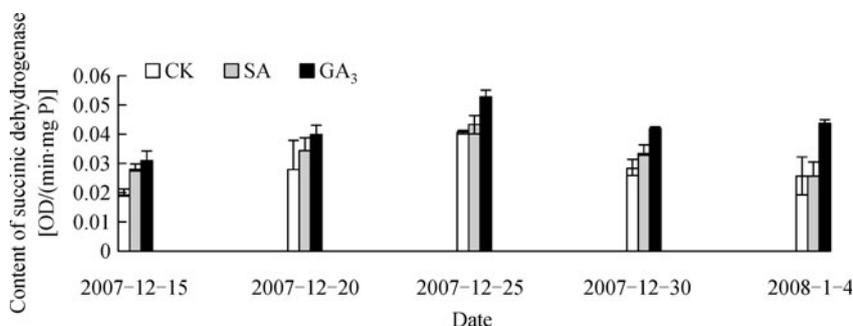


Figure 4 Effects of regulators on succinic dehydrogenase of Qiyuesu dormancy branches during respiratory climacteric period.

conclusion that addition of SA and GA₃ can obviously increase the SDH activity of dormant branches.

Effects of regulators on G-6-PDH and 6-PGDH of dormancy branches

The content of NADPH reflected the activities of G-6-PDH and 6-PGDH, which are the key enzymes for phosphopentase pathway (Leslie, 1986; Li and Wu, 2004). From Fig. 5, the activities of G-6-PDH and 6-PGDH of dormant branches showed a trend of increasing and then decreasing before dormancy released (December 25). The change of enzymes from dormancy branches treated with SA was similar to one

treated with GA₃ in early dormancy and deep dormancy stage. Spraying regulators of SA and GA₃ obviously increased the G-6-PDH and 6-PGDH activities of dormant branches. The GA₃ played a lesser role than SA in early dormancy and deep dormancy stage. The change of G-6-PDH and 6-PGDH activity was similar to that of phosphopentase pathway (PPP).

From Fig. 6, it is clear that the content of NADPH was increased by 0.02 mmol/L SA and 120 mg/L GA₃. In December 20 (which was about to arrive the time of rest released), compared with the control, the contents of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were increased by 93.9% and 24.8% using

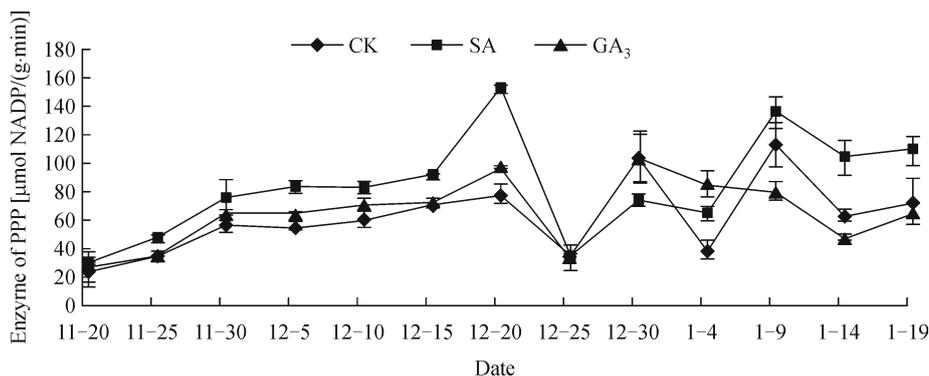


Figure 5 Effects of regulators on G-6-PDH and 6-PGDH of Qiyuesu dormancy branches.

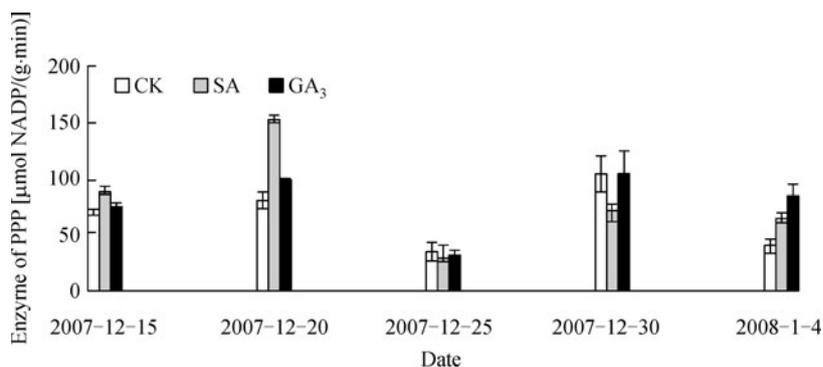


Figure 6 Effects of regulators on G-6-PDH and 6-PGDH of Qiyuesu dormancy branches during respiratory climacteric period.

0.02 mmol/L SA and 120 mg/L GA₃ respectively. In comparison with control, the addition of salicylic acid and Gibberellins obviously increased the glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase content of dormant branches.

Discussion

PGI, SDH, G-6-PDH and 6-PGDH are the key enzymes for respiratory pathway of EMP, TCA and PPP, respectively. The activities of enzymes participating in the respiratory pathway were determined in situ during shifts of carbon sources.

The data indicated the close interrelationship between respiratory enzymes and respiratory pathway. For example, we can see the enzyme activity of PGI from the content of F-6-P. The results showed that enzyme activities of SDH, combined with G-6-PDH and 6-PGDH, were increased by 0.02 mmol/L SA and 120 mg/L GA₃. The activity of PGI was reduced by 0.02 mmol/L SA and 120 mg/L GA₃. Compared with the control, PGI activity was reduced by 20.5% and 13.6% when treated with 0.02 mmol/L SA and 120 mg/L GA₃, respectively, SDH activity was increased by 6.1% and 29.2%, and the activity of G-6-PDH and 6-PGDH was increased by 93.9% and 24.8%. Li (2004) discovered that changes of respiratory enzymes were consistent with the respiratory pathway of regulators in cherry buds. The results were similar with ours. Changes of respiratory enzymes consistent with respiratory pathway of regulators indicated that salicylic acid and gibberellins were helpful to dormancy release by enhancing activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase.

Bogatek and Rychter (1984) observed that the breaking dormancy was metabolized mainly via the phosphopentase pathway. The increased level of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase might cause the activation of the phosphopentase pathway, thus leading to dormancy termination, bud burst and rapid growth. The glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are the third enzymes of the oxidative branch of the pentose phosphate pathway (Kurodaet et al., 1991; Konget et al., 1999). This pathway

serves two major functions: the production of ribulose 5-phosphate required for the nucleotide synthesis, and the generation of NADPH providing a major reducing power essential for protecting the cell against oxidative stress and a variety of reductive biosynthetic reactions, particularly lipid production. Both thioredoxin reductase and glutathione reductase require NADPH as the electron donor to reduce oxidized thioredoxin or glutathione (GSSG) (Jamieson, 1998). Nir and Lavee (1993) investigated respiratory changes in grapevine buds and concluded that the increased level of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase might cause the activation of the phosphopentase pathway. Wang (1991) observed that the relative content of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in apple decreased quickly at dormancy breaking stage.

New protein bands appeared when sprout occurred. These polypeptides may be associated with the dormancy release of tissues (Lang, 1994; Li and Foley, 1995). Recent progress in high-throughput biotechnology (Fiehn et al., 2000) has made advances in understanding of cell level (Karssen et al., 1989). Although it is known that genetic variability for sprouting resistance exists, the underlying physiologic mechanisms are poorly understood.

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