Scientia Horticulturae 242 (2018) 36–43

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Dynamic of carbohydrate metabolism and the related genes highlights PPP pathway activation during chilling induced bud dormancy release in tree peony (*Paeonia suffruticosa*)

Zhang Yuxi[a], Yu Dan[a,b,1], Liu Chunying[a], Gai Shupeng[a,*]

[a] College of Life Science, Qingdao Agricultural University, Key Lab of Plant Biotechnology in Universities of Shandong Province, Qingdao, China
[b] College of Food Science & Nutritional Engineering, China Agricultural University, Beijing, China

ARTICLE INFO

Keywords:
Tree peony
Dormancy release
Chilling
Respiratory metabolism
Enzyme activity
Expression pattern

ABSTRACT

Tree peony is a well-known ornamental plant, and its dormancy is a biological characteristic and a necessary process before bud sprouting and flowering. Carbohydrate metabolism plays an important role in regulating many physiological processes of plants. With the extension of chilling treatment, morphology changes indicated that the percentages of sprouting and blossom increased, and nearly 100 percentages of the apical buds sprouted and then blossomed after 21 d 0–4 °C artificial chilling duration. Sufficient chilling fulfillment significantly increased the total respiration rate. Ten putative respiratory metabolism and starch degradation-related genes including *PsAMY*, *PsBMY*, *PsHK*, *PsPFK*, *PsPK*, *PsPDH*, *PsIDH*, *PsOGDH*, *Ps6PGDH* and *PsG6PDH* were isolated, and the qPCR results indicated that chilling duration induced their expressions and most of them reached the maximum value after 21 d chilling exposure. The activities of the three key enzymes of EMP, TCA and PPP pathway, HK, IDH and G6PDH, were consistent with the expression levels of the corresponding genes (*PsHK*, *PsIDH* and *PsG6PDH*). Although the related genes of EMP were up-regulated and the activities of key enzymes increased, but the contribution of EMP gradually decreased during dormancy release in tree peony, and the increasing contribution of PPP pathway was higher than that of TCA pathway. Our results suggested that the activation of PPP pathway might play an important role in chilling-induced dormancy release of tree peony.

1. Introduction

Tree peony (*Paeonia suffruticosa* Andrews), originated from China, has been spread to many countries and become well known as a horticultural and medicinal plant in the world. Forcing culture accounts for a great part in the production of tree peony. Dormancy is a biological characteristic of tree peony and also is a necessary process before bud sprouting and flowering (Gai et al., 2012). Bud dormancy is a protective mechanism of plant to survive at unfavorable environmental conditions, which was regulated by various environmental and internal factors. The dormancy of tree peony belongs to endo-dormancy (Lang et al., 1987), as well as peach grape, and kiwifruit (Wu et al., 2012; Takemura et al., 2013; Khalil-Ur-Rehman et al., 2017). The buds require sufficient chilling to break endo-dormancy and turn into eco-dormant state subsequently (Rohde and Bhalerao, 2007), and they will sprout with the comfortable environment coming (Horvath, 2009).The growth of bud almost ceased at the stage of dormancy, but the physiological and biochemical activities including respiration metabolism during dormancy maintenance and release were still going on (Malyshev et al., 2016). As known, respiration pathways play pivotal roles in the regulation of growth and development. Researches on biophysiological and expression of related genes indicated that respiratory metabolism participated in dormancy process. The activities of glycolysis and Tricarboxylic Acid (TCA) cycle increased and Pentose Phosphate Pathway (PPP) activity gradually declined during budbreak in apple (Wang et al., 1991). The activities of G6PDH and 6PGDH increased during dry storage after-ripening in dormant groundnut seeds (Swamy and Sandhya Rani, 1986). Low temperature promoted the PPP activation, which might shorten the natural dormant process of *Corylus avellana* (Gosling and Ross, 1980). In grape, dormancy release by

Abbreviations: EMP, Embden-Meyerhof-Parnas; TCA, tricarboxylic Acid; PPP, pentose phosphate pathway; HK, hexokinase; PFK, phosphofructokinase; PK, pyruvate kinase; PDH, pyruvatedehydrogenase; IDH, isocitrate dehydrogenase; OGDH, 2-oxoglutarate dehydrogenase; G6PDH, glucose-6-phosphate dehydrogenase; 6PGDH, 6-phosphogluconate dehydrogenase; RACE, rapid amplification of cDNA ends; qPCR, real-time quantitative RT-PCR; ORF, open reading frame

* Corresponding author.
E-mail address: spgai613@163.com (G. Shupeng).
[1] This author contributed equally to the work.

https://doi.org/10.1016/j.scienta.2018.07.022
Received 14 November 2017; Received in revised form 21 June 2018; Accepted 17 July 2018
0304-4238/

chemical methods was related to the activation of PPP (Halaly et al., 2008; Vergara and Pérez, 2010). Whereas during chilling-induced bud dormancy release in grape, the contributions of Embden-Meyerhof-Parnas (EMP) pathway and TCA cycle increased continuously (Cong et al., 2013). As mentioned above, the performance of respiratory pathways varied during dormancy release in different plants.

Molecular evidences also indicated that carbohydrates metabolism and respiration metabolism involved in dormancy release. Starch is hydrolyzed to soluble sugar by amylase, which provides plenty of carbohydrates for plants. The grape alpha-amylase genes (*VvAMYs*) and leafy spurge beta-amylase gene (*EeBAM1*) were induced in dormant buds (Rubio et al., 2014; Chao and Serpe, 2010). In potato tuber bud dormancy, alpha- and beta-amylase genes (*StAMY1*, *StBAM2*) were up-regulated during the gibberellin-mediated dormancy release (Rentzsch et al., 2012). The hexokinase (HK), glucose sensor, plays a role as signal to regulate plant growth and development and exhibits high activity before breakage of dormancy in potato tuber buds (Rolland et al., 2006; Akoumianakis et al., 2008). The bud dormancy release of grape, which was induced by hydrogen cyanamide and heat shock, was accompanied by the transient expression of pyruvate decarboxylase and alcohol dehydrogenase (Wang et al., 1991; Or et al., 2000). These suggested that the changes of respiratory metabolic pathways might play a vital role in dormancy-breaking process, but there was difference among plants. How the respiratory metabolic pathway and the key genes change during the process of dormancy release in tree peony has not been well characterized.

In this study, we intended to detect the dynamic changes of respiratory metabolic pathways by measuring total respiration rate and the contributions of EMP, TCA and PPP pathway. At the same time, based on the results of 454 sequencing (Gai et al., 2012), full cDNA sequences of 10 putative respiratory metabolism-related key enzyme genes were obtained by rapid amplification of cDNA ends (RACE)-PCR, and then their expression patterns were analyzed during chilling induced dormancy release by Real-time quantitative RT-PCR (qPCR), which including *AMY* and *BMY* (alpha and beta amylase genes, encoding key enzymes of starch hydrolysis), *HK*, *PK* and *PFK* (hexokinase gene, pyruvate kinase gene and phospho-fructokinase gene, encoding critically rate-limiting enzymes of EMP pathway), *PDH*, *IDH* and *OGDH* (genes encoding pyruvate dehydrogenase, isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase, the rate-limiting enzymes in TCA cycle), *G6PDH* and *6PGDH*, (glucose-6-phosphate 1-dehydrogenase gene and 6-phosphogluconate dehydrogenase gene, encoding the marker enzymes of oxidative and not oxidative PPP pathway). Our results aimed to provide useful information about the potential role of respiratory metabolic pathways during dormancy release of tree peony buds.

2. Materials and methods

2.1. Plant materials

In November 2015, four-year-old tree peonies (*P. suffruticosa* 'Luhehong') were transferred to a dark refrigeration house (0–4 °C) when the daily average temperature was below 10 °C. After 1-week intervals until 4-weeks (0, 7, 14, 21 and 28 d), ten pots per replicate were transferred to a greenhouse (18–22 °C, 8-h-light 16-h-dark cycle) for observation of bud burst rate, respectively. The apical buds of 3 plants per replicate were harvested, of which twelve apical buds were used to measure respiration rates, and the others were immediately frozen in liquid nitrogen and stored at −80 °C refrigerator until use. Three replicates were performed for all treatments.

2.2. Morphological observation

Sprouting rate and blossom rate of buds were monitored based on the number of unfolding leaves and flowers during the observation period. Sprouting rates were checked after being transferred into greenhouse for continuous 30 d, and blossom rates were counted for continuous 60 d after that.

2.3. Bud respiration rate assessment

All oxygen consumption measurements were performed using a Clarke-type oxygen electrode (Hansatech, United Kingdom). Equal-sized buds were freshly harvested, divided with a scalpel and weighed. Then they were immediately transferred to the temperature-controlled chamber containing 2 mL of deionized water at 25 °C. Total respiration was calculated by measuring oxygen consumption rates after the sequential addition of specific inhibitor. According to the method of Tan et al., respiration rates of EMP pathway, TCA cycle and PPP pathway were measured by addition of competitive inhibitors of EMP, TCA and PPP pathways, NaF (10 mmol/L), Malonic Acid (50 mmol/L) and Na_3PO_4 (10 mmol/L), respectively (Tan et al., 2010). Specifically, respiratory rate of EMP pathway was total respiratory rate minus respiratory rate in the presence of NaF, and the contribution of EMP pathway was respiratory rate of EMP pathway accounting for a proportion of total respiratory rate. The measurements of respiratory rates and contributions of TCA and PPP pathways were similar.

2.4. Determination of key enzymes activities of EMP, TCA and PPP

2.4.1. HK activity

The activity of HK was determined using a procedure with minor modifications (Wiese et al., 1999). 0.2 g frozen buds were homogenized with 5 mL cold extraction medium containing 50 mmol/L Tris-HCl (pH 8.5), 5 mmol/L $MgCl_2$, 1 mmol/L EDTA, 5 mmol/L DTT and 1% Triton X-100. The slurry was centrifuged at 13,000 × *g* for 15 min at 4 °C.

Enzyme activity was measured with the absorbance at 340 nm using UV spectrophotometer (Kyoto, Japan). The reaction was initiated by adding the supernatant into the buffer containing 100 mmol/L imidazole-HCl (pH 8.5), 1.5 mmol/L $MgCl_2$, 0.5 mmol/L NADP, 1.1 mmol/L ATP, 2 U glucose-6-phosphate-dehydrogenase, and 2.5 mmol/L glucose.

2.4.2. IDH activity

According to the method of Chen et al. (1988), bud samples (0.2 g) were ground in liquid nitrogen and suspended in 3 mL buffer A (50 mmol/L HEPES-KOH, pH 7.5, 5 mmol/L $MgCl_2$, 1 mmol/L EDTA, 1 mmol/L EGTA, 5 mmol/L DTT and 0.1% (v/v) glycerol). The mixture was centrifuged at 4000 × *g* for 5 min. The supernatant was re-centrifuged at 13,000 × *g* for 15 min. 0.2 mL crude extract was added into 5 mL buffer B (100 mmol/L potassium phosphate buffer (pH 7.5), 5 mmol/L $MgCl_2$ 2 mmol/L isocitrate and 0.1 mmol/L $NADP^+$). Enzyme activity was measured with the reduction of $NADP^+$ at 340 nm.

2.4.3. G6PDH activity

According to the method of Meillon et al. (1990), buds (0.2 g) were homogenized in 5 mL cold enzyme extracting solution (100 mol/L potassium phosphate buffer, pH 7.5, 0.5 mmol/L DTT, 2 mmol/L EDTA, 2 mmol/L cysteine, β-mercaptoethanol and 1% w/v insoluble PVPP), then centrifuged at 12,000 × *g* for 15 min at 4 °C. Enzyme extract supernatant was pipetted into enzyme reaction mixture (50 mmol/L Tris HCl, pH 8.0, 0.5 mmol/L NADP, 5 mmol/L $MgCl_2$ and 0.5 mmol/L glucose-6-phosphate). The absorbance at 340 nm was recorded.

2.5. Total RNA extraction

Total RNA was extracted from homogenized buds of tree peony using RNA isolation kit (TIANGEN, China) according to the manufacturer's protocol. RNA samples were photometrically quantified, verified on an agarose gel and stored at −80 °C until use.