Scientia Horticulturae 239 (2018) 114–122

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Protein changes in response to photoperiod during dormancy induction in peach leaves and flower buds

Shaoxuan Li[a,b], Qiuping Tan[a,b], Mingyue Sun[a,b], Gongxun Xu[a,b], Chen Li[a,b], Xiling Fu[a,b], Ling Li[a,b], Dongsheng Gao[a,b], Dongmei Li[a,b,*]

[a] College of Horticulture Science and Engineering, Shandong Agricultural University, Taian, 271018, PR China
[b] State Key Laboratory of Crop Biology, Shandong Agricultural University, Taian, 271018, PR China

ARTICLE INFO

Keywords:
Dormancy induction
Temperature
Photoperiod
Peach

ABSTRACT

Bud dormancy in deciduous fruit trees is a key feature for the survival of winter chill but is a limiting factor for forcing cultivation in the greenhouse. Photoperiod is an important signal in dormancy induction; the quality and quantity of many genes and proteins can change and undergo a transition from active growth to dormancy. The aim of this work was to provide new insights into these changes during the early stage of dormancy. We analyzed protein changes in response to photoperiod during dormancy induction in peach (*Prunus persica* cv. Chunjie) leaves and flower buds. The results showed that short photoperiod could get the buds into dormant-induction period under non-low temperature conditions. However, the dormancy induction factor for long photoperiod treatment was the decreasing of the temperature. Sixty-five differentially expressed proteins were revealed, 42 of which were identified. The critical expression period of the differentially expressed proteins was mid-September in the leaves and mid-October or slightly earlier in the flower buds. Compared with that of the other identified proteins, the expression of proteins associated with stimulus responses and stress defense was higher and occurred earlier in short-photoperiod dormancy induction, whereas the expression of proteins associated with photosynthesis and growth was lower. This study is the first to identify the name, expression pattern, functional category, and biological function of various proteins in peach leaves and flower buds during photoperiod dormancy induction.

1. Introduction

Most fruit trees require bud dormancy during the winter in northern China. Buds that do not undergo the dormancy stage cannot successfully enter the next reproductive cycle, even under warm conditions. Therefore, dormancy is one of key factors limiting the protected cultivation of fruit. Greenhouse cultivation can advance the mature period of peach to late February, resulting in improve economic benefits. Therefore, it is important to study the dormancy mechanism of fruit trees. This study identified 42 dormancy-related proteins and outlined the differential proteomic profiles of peach leaves and flower buds in response to photoperiod-induced dormancy. These findings are highly important for in-depth investigations of dormancy-induced gene regulation and protein function.

Bud dormancy in woody perennial plants in temperate regions constitutes an important strategy to withstand low winter temperatures. It is essential that dormancy preparations occur within the plant well in advance of the cold season; buds maintain very low metabolic activity, thereby preserving their nutrient and carbohydrate reserves for new growth (Adamec, 2010). Bud dormancy of deciduous fruit trees is a complex process that is induced by several factors (Cook et al., 2005; Rinne and van der Schoot, 1998), including temperature, water deficit, photoperiod, and the combination of these factors (Tanino et al., 2010; Kozlowski and Pallardy, 2002; Heide and Sønsteby, 2015; Renaut et al., 2008; Olsen, 2010). With respect to dormancy-related research, low temperature and short photoperiod are timely topics, but these stimuli differ. Heide indicated that low temperature, not photoperiod, controlled dormancy induction and that near-freezing nighttime conditions (21/4 °C, 14/10 h) can induce growth cessation, even under continuous light (Heide and Prestrud, 2005). Heide (2008, 2011) further studied more species in the Rosaceae family and verified this conclusion. However, for some species, short days (SDs) are considered as the major prerequisite, since SDs can induce a high level of dormancy comparable to that need for natural development (Jian et al., 1997; Rohde et al., 2002, 2007; Victor et al., 2010). Furthermore, peach trees may be very sensitive to SD and/or low-temperature conditions, and the

* Corresponding author at: 61 Daizong Street, Taian City, 271000, PR China.
E-mail address: dmli2002@163.com (D. Li).

https://doi.org/10.1016/j.scienta.2018.05.010
Received 13 December 2017; Received in revised form 3 May 2018; Accepted 7 May 2018

combination of the two factors plays either a synergistic or additive role in dormancy induction (Renaut et al., 2008; Wang et al., 2008).

When the photoperiod induces growth cessation in woody species, terminal buds develop, and the plants progress from a dormant to a more freezing-tolerant state (Rinne et al., 2001; Ruonala et al., 2006). Metabolic changes, including physiological and molecular changes (Keskitalo et al., 2005; Rinne et al., 2010; Lee et al., 2014), can occur. Examples include changes in nutrients (Penfold and Buchanan-Wollaston, 2014), photosynthesis (Oláh et al., 2017), carbohydrates, amino acids, enzyme activities, hormones and gene expression (Wang et al., 2016; Galindo González et al., 2012). Specifically, bark storage proteins (BSPs), which may play a role in nitrogen storage during overwintering, is controlled by photoperiod. BSPs can accumulate during dormancy, after which their presence decreases during spring shoot growth (Zhu and Coleman, 2001). Photoperiod-induced dormancy studies have been reported in many species such as in pears and apple (Heide and Prestrud, 2005). Furthermore, proteomics analysis was performed in many crops, e.g. poplar (Liu et al., 2011), grape (Victor et al., 2010) etc. Quantitative proteomic analyses of short-photoperiod and low-temperature responses in peach bark (Renaut et al., 2008) and poplar cambium (Jin et al., 2017) have revealed that differentially expressed proteins are involved in carbohydrate metabolism (e.g., enolase and malate dehydrogenase), defense or protective mechanisms (e.g., dehydrin, heat-shock proteins (HSPs), and pathogenesis-related (PR) proteins), energy production and electron transport (e.g., adenosine triphosphate synthase and lyase), and cytoskeleton organization (e.g., tubulin and actin).

We previously reported that many physiological metabolic processes such as photosynthesis (Zhang et al., 2015; Li et al., 2014), respiration (Li et al., 2011), and stress resistance as well as some hormones and genes (Wang et al., 2016; Chen et al., 2016) respond to photoperiod during dormancy, but how leaf and bud metabolism respond to the photoperiod remains unclear. In this study, proteins in leaves and flower buds were analyzed to investigate the responses under different photoperiods. Our results about the molecular mechanisms underlying the biological functions of differentially expressed proteins in the leaves and buds are discussed in depth.

2. Materials and methods

2.1. Plant material and experimental design

The experimental treatments were applied during peach dormancy induction (from August to November) at Shandong Agricultural University in Tai'an. Special 6-year-old protected peach trees (*Prunus persica* cv. Chunjie) that exhibited similar growth vigor were used for treatment; their chilling requirement is 200 chilling units (CU). The experiment consisted of two treatments: long days (LDs) and SDs. Trees under natural conditions constituted the control (CK).

LD treatment: 16-h light/8-h dark photoperiod. The day time was prolonged with artificial lighting at a luminous flux density of 350 μmol $m^{-2} s^{-1}$.

SD treatment: 8-h light/16-h dark photoperiod. The day time was shortened by shading.

For each treatment, every 3 trees composed a plot; the plots were replicated 3 times. Functional leaves and flower buds were frozen in liquid nitrogen and stored at −80 °C for further analyses.

2.2. Definition of bud dormancy status

Ten 1-year-old branches (same orientation and height from beginning to end) were collected randomly at 7-day intervals beginning on the treatment date. The clean-water method was used to determine dormancy status (Jian et al., 1997; Wang et al., 2008). The leaves and terminal buds were removed and placed in a growth chamber, whose environmental conditions were as follows: temperature of 25 °C/21 °C (day/night), light intensity 40 μmol $m^{-2} s^{-1}$, photoperiod of 14 h/10 h (day/night), and relative humidity of 80–90%. The first bud underwent dormancy induction when it required more than 10 d to germinate. If shoot still had not sprouted under these conditions after 6 weeks, those buds were transported to the natural dormancy period conditions.

2.3. Protein extraction

The total soluble proteins were extracted from the peach leaves and flower buds by the trichloroacetic acid (TCA)/acetone precipitation method as described by Renaut et al. (2008), with modifications: 1000 mg of tissue was ground to a fine power in liquid nitrogen by using a prechilled mortar and pestle (1% polyvinylpyrrolidone (PVP) could be applied to the tissue if the quantity of proteins from the peach buds was perhaps insufficient because of high amounts of phenolic compounds). The powder was resuspended in −20 °C prechilled acetone containing both 10% (w/v) TCA and 0.07% (w/v) dithiothreitol (DTT), after which the resuspended powder was incubated overnight at −20 °C. The material was then centrifuged at 20,000 × *g* for 20 min at 4 °C. Afterward, the supernatant was discarded, after which the pellet was washed with −20 °C prechilled acetone containing 0.07% (w/v) DTT for approximately 2 h at −20 °C and then centrifuged at 18,000 × *g* for 20 min; the previous step was repeated until the supernatant became nearly colorless. The pellet was dried overnight under vacuum at room temperature, after which it was stored in a dry, clear Eppendorf centrifuge tube at −80 °C until further analysis.

2.4. Protein determination

The proteins from the acetone/TCA insoluble dry powder (DP) were extracted with 2% (v/v) pH 4–7 immobilized pH gradient (IPG) buffer containing 7 M urea, 2 M thiourea, 4% (w/v) 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), 1% Triton-X 100 and 40 mM DTT (GE Healthcare). One microgram of leaf DP was suspended in 15 μl of IPG buffer, and 1 mg of flower buds was suspended in 8 μl of IPG buffer; more flower bud material was needed because of its lower protein content. The samples were thoroughly vortexed and then incubated at room temperature for 30 min, after which the samples were vortexed again and subsequently centrifuged at 40,000 × *g* for 30 min at room temperature to remove any insoluble matter. The protein content in the supernatant was determined by the modified Bradford method, and bovine serum albumin (BSA, Sigma) was used a standard.

2.5. Two-dimensional gel electrophoresis (2D-GE)

The first dimension involved isoelectric focusing (IEF), which was carried out on an Ettan IPGphor Manifold (GE Healthcare). Hydration buffer (8 M urea, 4% [w/v] CHAPS, 0.5% [v/v] IPG buffer, 0.28% [w/v] DTT, 0.002% [v/v] bromophenol blue) and sample solutions were mixed together and then centrifuged at 40,000 × *g* for 10 min at room temperature; the supernatant was transferred to an IPGphor IEF unit via Immobiline DryStrips (GE Healthcare, pH 4–7, 24 cm). The system settings were as follows: 30 V for 12 h, 500 V for 1 h, 1000 V for 1 h and 8000 V for 9 h at 20 °C at 50 μA $strip^{-1}$.

After the IEF, the IPG strips were equilibrated for 15 min in 15 ml of equilibration buffer (6 M urea, 75 mM Tris−HCl (pH 8.8), 30% (v/v) glycerol, 2% (w/v) SDS) supplemented with 1% (w/v) DTT. Afterward, a second 15-min equilibration step involving the same equilibration buffer and the same volume (but containing 2.5% (w/v) iodoacetamide) was carried out. The IPG strips were then sealed with 0.5% agarose in SDS running buffer at the top of slab gels (280 × 210 × 1 mm) polymerized from 12.5% (w/v) acrylamide and 0.1% *N*, *N*′-methylenebis acrylamide. The gels were poured between low-fluorescent and bind-silane-treated glass plates. SDS-PAGE was performed at 15 °C using an Ettan Dalt II tank (GE Healthcare) at 5 W gel^{-1} for 45 min followed by 17 W gel^{-1} for 8 h until the