Proteomic analysis provides insights into changes in the central metabolism of the cambium during dormancy release in poplar

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ABSTRACT

Seasonal cycling of growth and dormancy is an important feature for the woody plants growing in temperate zone, and dormancy is an effective strategy for surviving the winter stress. But the mechanisms of dormancy maintenance and its release are still not clear, especially little information is available with regard to the changes of proteome during the process. A better understanding in the function of proteins and their related metabolic pathways would expand our knowledge of the mechanisms of dormancy maintenance and its release in trees. In this study, we employed the isobaric tags for relative and absolute quantification (iTRAQ) approach with LC–MS/MS analysis to investigate the protein profile changes during dormancy release in poplar. In addition, the change of lipid, total insoluble carbohydrates and starch granules in the cambium was investigated by histochemical methods. A total of 3789 proteins were identified in poplar cambial tissues, 1996 of them were significantly altered during the dormancy release. Most of the altered proteins involved in signaling, phytohormone, energy metabolism, stress and secondary metabolism by functional analysis. Our data shows that the lipid metabolism proteins changed significantly both in the release stage of eco- and endodormancy, while the changes of carbohydrate metabolism proteins were mainly in endo-dormancy release stage. Moreover, histochemical results were consistent with the proteomic data. Our results reveal diverse stage-specific metabolism changes during the dormancy-release process induced by chilling in poplar, which provided new information regarding the regulation mechanisms of dormancy maintenance and its release in trees.

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1. Introduction

Trees growing in temperate zones show the cycle of active growth and dormancy to adapt to seasonal changes. There are five processes in the cycle: growth arrest, dormancy establishment, maintenance, release, and growth restoration (Little and Bonga, 1974; Rohde and Bhalerao, 2007). Growth arrest is the first step of plant dormancy, which can be induced by many environmental factors, such as photoperiod, temperature, and drought, and these environmental factors trigger induced dormancy establishment after the growth arrest (Rinne and van der Schoot, 1998). Photoperiod plays an important role in the regulation of trees' growth arrest and dormancy establishment (Junttila and Jensen, 1988; Little and Bonga, 1974). In poplar, trees growing in short

day (SD) for about 3 weeks begin to arrest growing and form dormancy apical buds. Trees' growth is completely arrested in SD lasting over 4 weeks, while the trees can restore active growth upon exposure to growth promoting conditions at this time, showing its eco-dormancy or quiescence stage. Growing in SD for 8 weeks renders trees into a physiological dormant state, and the trees can't restore active growth upon exposure to growth promoting conditions, showing its endo-dormancy or rest stage (Ding et al., 2014; Little and Bonga, 1974; Ruttink et al., 2007). Another environmental factor to control the growth cycle is temperature. Low temperature also plays roles in inducing growth cessation and dormancy (Heide and Prestrud, 2005). Sustained chilling must be achieved to release endo-dormancy or eco-dormancy, and then warm temperature could reinitiate growth subsequently (Heide, 2008).

Much progress has been made in the study of hormonal and transcriptional regulation of bud and cambium dormancy establishment in recent years. It is known that environmental factors regulate the growth cycle through modulating phytohormone levels or altering the sensitivity of the cells to phytohormones. The

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trees perceive the change of photoperiod through phytochrome A (Olsen et al., 1997) and FT/CO are the key mediators for growth cessation induced by SD photoperiod (Böhlenius et al., 2006). In addition, phytochrome A can regulate gene FT by controlling gene *CO*. Recently, *Like-AP1* (*LAP1*), a poplar ortholog of *Arabidopsis* gene *APETALA1* (*AP*1), was found to mediate photoperiodic control of seasonal growth cessation and dormancy establishment downstream of CO/FT (Azeez et al., 2014). Callose deposited at plasmodesmata between cells of the shoot apical meristem prior to dormancy establishment (Rinne and van der Schoot, 1998). A comprehensive genome-wide analysis of small RNAs revealed diverse stage-specific microRNAs in cambium during dormancy-release and suggested a role of miRNAs in the regulation of this biological process (Ding et al., 2014). However, the mechanisms of dormancy maintenance and its release are still not clear. Switching between dormancy and active growth is a very complex biological phenomenon involving a large number of genes, proteins and metabolic processes. It has been reported that poplar xylem proteins showed seasonal variation (Vander Mijnsbrugge et al., 2000), post-transcriptional modification on cell cycle proteins was observed (Schrader et al., 2004), and proteins involved in auxin signaling pathway were regulated in the physiological dormant period (Baba et al., 2011). However, limited information is available with regard to the changes of proteome during dormancy maintenance and its release in trees. Thus, interrogation of changes in the protein profile in cambium during dormancy-release could help form a controlling network which will facilitate the understanding of the regulation mechanisms of dormancy maintenance and its release in trees.

Proteome can be defined as the total set of protein species present in a biological unit at any developmental stage under specific environmental conditions. Using proteomics, one can expect to know the thousands of individual protein species produced in a living organism, their functions and interaction with each other to fit in the programmed growth and to respond to abiotic environment (Abril et al., 2011). The isobaric tags for relative and absolute quantification (iTRAQ) technique employed in proteomic workflows allows the simultaneous identification and quantification of proteins obtained on the MS/MS level (Evans et al., 2012). It has been successfully used to quantify protein changes in plant biological processes, such as grape berry ripening (Lücker et al., 2009; Martínez-Esteso et al., 2013), pathogenic process of sweet orange (Fan et al., 2011), iron (Fe) deficiency (Lan and Schmidt, 2011), and rice grain chalkiness (Lin et al., 2014). Here, we present an iTRAQ quantitative proteomic exploration of the cambial meristem during dormancy-active growth transition in poplar.

A total of 3789 proteins were identified and quantified in poplar cambial meristem, 1996 proteins showed significantly differential expression during dormancy-active growth transition and 195 proteins were specifically induced by chilling treatment from endo-dormancy to eco-dormancy. The results revealed diverse stage-specific metabolic changes in poplar cambium during dormancy-release induced by chilling, which provided new information regarding the regulation mechanisms of dormancy maintenance and its release in trees.

2. Materials and methods

2.1. Plant materials and tissue collections

Clonal poplar seedlings (*Populus alba* × *P. glandulosa* cv. "84 k") were transplanted to sterilized soil and grown in greenhouse for 4 months under long day photoperiod (16 h light/8 h dark, 25 °C, 55%-65% ambient humidity) until stems reached 100 in height centimeter. The induction of dormancy and resumption of growth in

poplar were performed according to Espinosa-Ruiz et al. (2004) and Ding et al. (2014) with some modifications. Sixty trees in active growth were transferred to a short day photoperiod (8 h light/16 h dark, 25 °C, 55%–65% ambient humidity) for 8 weeks (SD8) to induced dormancy. One quarter of the trees were collected after SD8 treatment as samples, while the rest were placed under consistent chilling treatment (4 °C, darkness) for 3 weeks (CT3) and 5 weeks (CT5), respectively, to break the endo-dormancy, and then the trees were transferred to the long day photoperiod (16 h light/8 h dark, 25 °C, 55%–65% ambient humidity) conditions for 3 weeks (LD3) to restore the growth.

Cambial tissues in stems using the girding method were collected from at least 12 trees each stage at SD8, CT3, CT5, and LD3, then immediately frozen in small aliquots in liquid nitrogen and stored at −80 °C for protein extraction and quantification. In order to accurately verify the iTRAQ data, cambial tissues in stems using the frozen section method were collected for qRT-PCR analysis. The 10th internodes of the trees at different stages were fixed in FAA (90 mL of 70% ethanol, 5 mL of formaldehyde and 5 mL of glacial acetic acid) for anatomical observation.

2.2. Anatomical and histochemical observation

The FAA-fixed samples were dehydrated in a gradient of ethanol solutions (70%, 85%, 95%, and 100%), embedded into Spurr's resin and sectioned using a Leica microtome. The sections were stained with 0.1% (w/v) toluidine blue O (Sigma, St. Louis, MO, USA) and observed under a Zeiss Axioskop2 Plus microscope equipped with a computer-assisted digital camera. The FAA-fixed samples were also used for preparation of hand-cut transverse sections with a razor blade. The histochemical examination involved staining for total insoluble carbohydrates (periodic acid-Schiff's reaction, PAS), starch (iodine-potassium iodide, I_2-KI) and lipids (Sudan III) according to Riding and Little (1984).

2.3. Protein extraction and quantification

Total protein was extracted from frozen cambium tissue collected and stored at −80 °C using a phenol extraction procedure as described previously (Hurkman and Tanaka, 1986; Wang et al., 2009) with minor modifications. The cambial tissues of 6 plants were collected as one pool to give sufficient sample quantity for analysis and two biological replicates with 12 plants were collected. 200 mg powder of each sample was extracted with 400 μL cooled Tris-phenol (pH 8.8) and 400 μL cooled extraction buffer with 0.1 M Tris-HCl, 10 mM EDTA, 0.4% 2-mercaptoethanol, 0.9 M sucrose, proteinase inhibitor, and phosphatase inhibitor. After vortexing for 30 min at 4 °C and centrifugation for 20 min at 20,000g at 4 °C, the phenol phase of the supernatant was swiftly collected into a new centrifuge tube and placed on ice immediately. Subsequently, after adding additional 400 μL cooled Tris-phenol (pH 8.8) into the aqueous phase and vortexing for 2 min, the mixture was centrifuged for 20 min at 20,000g and 4 °C. The phenol phase of the supernatant was combined with the former and centrifuged for 15 min at 5,000g and 4 °C, after which the whole supernatant was transferred into a new centrifuge tube. The whole supernatant was then precipitated overnight in five volumes of 0.1 M ammonium acetate at −20 °C. The protein was eventually collected by centrifugation at 20,000g and washed twice with cooled 0.1 M ammonium acetate, twice with 80% acetone, and once with 70% methanol. The protein was dried for 20 min at room temperature and added in 50 μL dissolvant buffer, which consists of 100 mM HEPES (pH 8.5), 7 M urea, 2 M thiourea, 40 mM DTT, 1 x protease inhibitor mixtures, and phosphatase inhibitor, dissolved at room temperature for 30 min. The solution was again centrifuged prior to the addition of 1 μL of freshly prepared iodoacetamide solution (to prepare