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Revealing critical mechanisms of BR-mediated apple nursery tree growth using iTRAQ-based proteomic analysis

Liwei Zheng, Juanjuan Ma, Lizhi Zhang, Cai Gao, Dong Zhang, Caiping Zhao, Mingyu Han*

College of Horticulture, Northwest A & F University, Yangling, Shaanxi 712100, China

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ABSTRACT

Brassinosteroid is identified as an important hormone. However, information about brassinosteroid has not been fully elucidated, and few studies concerned its role in apple. The aim of this work was to study the role of brassinosteroid for apple tree growth. In our study, the effect of brassinosteroid on apple nursery tree was analyzed. The biomass, cell size and xylem content of apple nursery tree were obviously evaluated by brassinosteroid treatment; mineral elements contents, photosynthesis indexes, carbohydrate level and hormone contents were significantly high in brassinosteroid treated trees. To explore the molecular mechanisms of these phenotypic differences, iTRAQ-based quantitative proteomics were used to identify the expression profiles of proteins in apple nursery tree shoot tips in response to brassinosteroid at a key period (14 days after brassinosteroid treatment). A total of 175 differentially expressed proteins were identified. They were mainly involved in chlorophyII biosynthesis, photosynthesis, carbohydrate metabolism, glycolysis, citric acid cycle, respiratory action, hormone signal, cell growth and ligin metabolism. The findings in this study indicate that brassinosteroid mediating apple nursery tree growth may be mainly through energy metabolism. Important biological processes identified here can be useful theoretical basis and provide new insights into the molecular mechanisms of brassinosteroid.

Biological significance: Brassinosteroid is very important for plant growth and development. However, the molecular mechanism of brassinosteroid mediating growth process is not perfectly clear in plant, especially in apple nursery tree. We used a combination of physiological and bioinformatics analysis to investigate the effects of brassinosteroid on apple nursery tree growth and development. The data reported here demonstrated that brassinosteroid regulates apple nursery tree growth mainly through energy metabolism. Therefore it can provide a theoretical basis from energy points for developing dwarfed or compact apple trees. This will benefit for low orchard management cost as well as early bearing, and high fruit yield as well as quality.

1. Introduction

The sixth major plant hormone brassinosteroid (BR) is closely related with plant growth. Once isolated and identified from the pollen of rape, BR has been identified as a new class of growth-promoting phytohormone in regulating many processes, including seed germination, vegetative and reproductive development, and stress responses [1,2]. In *Arabidopsis*, BR synthetic and BR-insensitive mutants show multiple defects in growth and development. BR-deficient mutants (such as *cpd*, *dwf4* and det*2*) exhibit dark green dwarfs with epinastic leaves, reduced or abolished fertility and delayed development [3,4,5]. BR exerts its biological functions mainly through signal transduction. BR-perceptional mutants can't be recovered by exogenous BR treatment and display dramatically dwarfed phenotypes which are mainly caused by the reduced cell size and cell number [4,5]. The complicated regulatory functions of BR are also identified in other plants. *MicroRNA1848* (*miR1848*) and its target gene *cytochrome P450 subfamily 51G3* (*CYP51G3*) mediate BR biosynthesis and have potential applications to modulate leaf angle, and the size and quality of seeds in rice [6]. Cotton BR synthetic mutant *pagoda1* (*pag1*) exhibits dwarfism and reduces fiber length via controlling the level of endogenous bioactive BRs [7]. RNA interference (RNAi) *maize brassinosteroid insensitive1* (*ZmBRI1*), BR signal receptor, compromises BR signaling and results in dwarf plant with shortened internodes and small leaves [8]. Overexpression of *PtCYP85A3*, a BR synthetic gene, increases endogenous BR levels, plant height, shoot fresh weight, xylem formation, stem diameter and leaf area [9].

BR can interact with other hormones in plant growth and stress responses. Previous researches have focused on the crosstalk in BR-auxin, BR-gibberellin (GA), and BR- abscisic acid (ABA) in several

* Corresponding author.
E-mail address: hanmy@nwsuaf.edu.cn (M. Han).

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aspects of plant growth and development. BR and auxin commonly regulate numerous physiological processes, and many genes which are involved in their biosynthesis and signal transduction are both auxin and BR inducible [10,11]. Direct interactions between auxin and BR were identified through integration of *brassinosteroid-insensitive 2* (*BIN2*) and A*uxin Response Factor 2* (*AFR2*) in photomorphogenesis [12]; and BR can enhance polar auxin transport and distribution [13]. The interaction between BR-activated *brassinazole-resistant 1* (*BZR1*) and GA-inactivated *DELLA* defines a core transcription module which can mediate cell growth [14]; BR regulates GA biosynthesis by inducing the expression of the key GA biosynthesis genes, and this function is important for the growth and development of vascular plants [15]. Interactions between BR and ABA are identified in seed germination, stomatal closure and responses to environmental stresses [16,17].

In addition, previous studies have proved that BR plays positively roles in photosynthesis during plant life cycle. BR spray application significantly increases the light-saturated net CO_2 assimilation, carboxylation rate and quantum yield of photosystem II (PSII) electron transport [18]. In cucumber, BR-induced photosynthesis involves in increasing activity of enzymes in the calvin cycle and affecting CO_2 assimilation [19]. Transgenic experiment indicates that overexpressing *SlDWARF* (a BR synthetic gene in tomato) promotes net photosynthetic rate (P_n), whereas knockdown *SlDWARF* leads to a significant inhibition in photosynthesis [20]. Under cold stress, BR can accelerate recovery of photosynthetic apparatus by balancing the electron partitioning, carboxylation and redox homeostasis [21]. BR can also combine with carbohydrates through photosynthesis. BR has a positive role in increasing sucrose, soluble sugars, and starch contents by activing sucrose phosphate synthase (SPS), sucrose synthase (SS), and acid invertase (AI) activities, which may be caused by increasing photosynthesis [18]. In Cucumber, BR treatment increases soluble sugar, sucrose and starch content, while Brz (BR inhibitor) application decreases their content which is associated with light-saturated rate of CO_2 assimilation [22]. After downregulating *constitutive photomorphogenic dwarf* (*CPD*), a BR synthetic gene, plant shows a clear reduction in starch content which may be partly due to reduced photosynthesis [23]. In addition, two β-amylases genes (*BAM7* and *BAM8*) possess BZR1-type DNA binding domains and are reported to link starch metabolism to BR signaling [24].

Previous studies have provided important clues and basis for revealing the biological function of BR. However, complex mechanisms underlying BR on plant growth and development are still not fully elucidated. Proteomics can provide new insights into the biological function of genes at post-transcriptional level. It can be able to analyze key biological process and provide direct insights into metabolic processes by global protein expression patterns. Spectrometry-based iTRAQ is a new quantitative proteomics technology which can simultaneously perform quantitative protein analysis of eight samples with high quantitative quality and repeatability. It has been used to identify many kinds of biological processes. However, it has not been used to investigate the apple nursery tree responses to BR, and there is little information regard to BR in apple nursery trees which are perennial woody fruit trees and differ from annual model plants. Apple is one of the most important fruit which is widely cultivated in the temperate region all over the world, and it occupies a very important position in the world fruit market. Proper vegetative growth and architecture of apple nursery trees are very important for apple tree early bearing, high fruit yield and quality. Therefore, we researched the function of BR on apple tree growth and development using iTRAQ-based quantitative proteomics approach.

In our study, the morphological indexes, anatomical observation, photosynthetic indexes, energy substance and endogenous hormones content in apple nursery tree were checked after BR treatment. Moreover, the molecular mechanism of BR regulating apple nursery trees growth was identified by iTRAQ. We found the differentially expressed proteins (DEPs) between BR treatment and control group were mainly involved in energy metabolism. Physiological and bioinformatics analyses were combined to explore the biological functions of BR in apple. The findings in this study indicated various pathways (especially energy metabolisms) may be response to BR and take part in controlling apple nursery trees growth. These data obtained in this study provide new points for studying mechanism of BR on plant growth and will lay a foundation for better understanding the roles of BR in future. The study will also contribute to develop apple lines with proper tree size.

2. Materials and methods

2.1. Plant material and BR treatment

In this study, uniform one-year-old Malling 9-T337 (M.9-T337) nursery trees (rooting tissue culture seedlings), an apple rootstock wildly used in the world, were planted in plastic pots containing culture medium (garden soil, peat, and vermiculite at a 3:1:1 ratio) and normally irrigated in greenhouse. The growth conditions were 16-h day (24 °C)/8-h night (18 °C), and light intensity was 600 μmol m^{-2} s^{-1} in the day. When the nursery trees were about 7 cm height and 2.4 mm thickness of stem, the whole tree (including, whole stem, shoot tips, and leaves) was sprayed with 3.0 mg/L BR (Sigma Chemical Co., Deisenhofen, Germany) using a low-pressure hand-wand sprayer. The spray treatment was performed according to previous method [19,25]. The proper BR treatment concern was determined according to pilot experiment and previous studies in apple [26,27,28]. The BR was dissolved in ethanol and stored at − 20 °C. A total of 24 times sprays were performed with once every 2 days. The phenotypic of trees were analyzed at 0, 14, 28, 42, 56 and 70 days after BR treatment (DABT), and they were photographed at 70 DABT (Fig. 1) and the obvious phenotypic differences appeared at 28 DABT (Fig. 3). The nursery trees which were sprayed with water adding corresponding ethanol were used as controls. Main shoot tips (2–3 cm below and including the apical portions) which are important part for tree architecture were sampled at 0, 14, 28, 42, 56 and 70 DABT. Because distinct phenotypic differences of trees in control and treatment groups appeared at 28 DABT, the samples

Fig. 1. Morphology of control and treated apple trees at 70 days after BR treatment (DABT).
(A) BR treated trees. (B) Control trees. Red ovals indicate sampling tissues (shoot tips) for iTRAQ analysis, Scale bars = 1 cm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)