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Transcriptome profiling in *Arabidopsis* inflorescence stems grown under hypergravity in terms of cell walls and plant hormones

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Abstract

Land plants rely on lignified secondary cell walls in supporting their body weight on the Earth. Although gravity influences the formation of the secondary cell walls, the regulatory mechanism of their formation by gravity is not yet understood. We carried out a comprehensive analysis of gene expression in inflorescence stems of *Arabidopsis thaliana* L. using microarray (22 K) to identify genes whose expression is modulated under hypergravity condition (300 *g*). Total RNA was isolated from the basal region of inflorescence stems of plants grown for 24 h at 300 *g* or 1 *g*. Microarray analysis showed that hypergravity up-regulated the expression of 403 genes to more than 2-fold. Hypergravity up-regulated the genes responsible for the biosynthesis or modification of cell wall components such as lignin, xyloglucan, pectin and structural proteins. In addition, hypergravity altered the expression of genes related to the biosynthesis of plant hormones such as auxin and ethylene and that of genes encoding hormone-responsive proteins. Our transcriptome profiling indicates that hypergravity influences the formation of secondary cell walls by modulating the pattern of gene expression, and that auxin and/or ethylene play an important role in signaling hypergravity stimulus.

Keywords: *Arabidopsis*; Auxin; Cell wall; Hypergravity; Lignin; Microarray

1. Introduction

Land plants have to support their weight on the Earth. Cell walls play a crucial role in this self-supporting function of land plants. Content of cell walls in shoots has been shown to change under altered gravity conditions. Under hypergravity conditions, the content of matrix and cellulosic polysaccharides in unit length increases in shoots (Waldron and Brett, 1990; Kasahara et al., 1995; Hoson et al., 1996; Soga et al., 1999). Under microgravity conditions in space, on the other hand, the content of cell wall components such as cellulose (Cowles et al., 1984; Nedukha, 1996) and matrix polysaccharides (Hoson et al., 2002) decreases.

In addition to the polysaccharides in cell walls mentioned above, lignin, which is deposited in secondary cell walls, provides rigidity and provides mechanical support for land plants. Lignification was suppressed under microgravity condition in space (Cowles et al., 1984; Nedukha, 1996). Three-dimensional clinorotation, which is often used

Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; ACO, 1-aminocyclopropane-1-carboxylate oxidase; AGP, arabinogalactan-protein; *ATPA2*, *Arabidopsis thaliana* peroxidase A2; *C3H*, coumarate 3-hydoxylase; *C4H*, cinnamate 4-hydroxylase; Cy, Cyanine; *EXT*, extensin; *FAH*, ferulate-5-hydroxylase; GO, Gene Ontology; GRP, glycine-rich protein; GUS, beta-glucuronidase; *HEL*, Hevein-like protein; IAA, indole-3-acetic acid; IAOx, indole-3-acetaldoxime; JA, jasmonic acid; *LRP*, lateral root primordium; *PBP*, PID-binding protein; *PID*, PINOID protein kinase; RT-PCR, reverse transcription-polymerase chain reaction; *TCH*, touch-induced; Trp, tryptophan; *XTH*, xyloglucan endotransglucosylase/hydrolases.

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as a simulation of microgravity condition and, therefore, called "pseudo-microgravity", has been demonstrated to suppress secondary xylem formation (Nakamura et al., 1999; Yoneyama et al., 2004). Microgravity, or pseudo-microgravity, suppressed the formation of lignified secondary cell walls. In addition, our recent studies demonstrated that prolonged hypergravity treatment significantly increased the content of the secondary cell wall and acetylbromide-extractable lignins in *Arabidopsis* inflorescence stems (Tamaoki et al., 2006) promoted metaxylem development and decreased extensibility of secondary cell walls (Nakabayashi et al., 2006). These experimental data suggest that hypergravity promotes the formation of lignified secondary cell walls by enhancing the expression of genes involved in the formation of cell wall components including lignin. This hypothesis is supported by a recent study using a custom microarray showing that 27 of 765 genes related to secondary cell wall formation, such as genes encoding glucanase, laccase, cellulose synthase and peroxidase, are significantly changed in *Arabidopsis* inflorescence stems when plants are placed horizontally (Yokoyama and Nishitani, 2006). A recent proteomic study revealed that the formation of reversibly glycosylated cell wall proteins, which may be related to cell wall biosynthesis, was down-regulated by clinostat rotation (Wang et al., 2006). This finding also supports hypergravity-induced lignification. In order to verify our hypothesis, the effect of hypergravity on the expression of genes involved in the formation of cell wall components including lignin should be examined using a genome-scale microarray.

Mechanosensitive ion channels are suggested to be involved in the perception of gravity stimulus in plants, because La or Gd ions, which are known inhibitors of mechanosensors, inhibit the effect of hypergravity on shoot growth and cell wall properties (Soga et al., 2004; Tamaoki et al., 2006; Nakabayashi et al., 2006). In addition, a recent study demonstrated that a transient increase in the concentration of cytoplasmic free calcium ions was induced by hypergravity in *Arabidopsis* seedlings (Toyota et al., 2007). However, little is known about the signal transduction pathway after the perception of gravity stimulus.

Participation of plant hormones in signal transduction mechanism has been reported for various responses of plants to altered gravity. Auxin has been shown to play a crucial role in gravitropism (Salisbury et al., 1988; Young et al., 1990). Polar transport of auxin is reported to be involved in negative gravitropic response of pea epicotyls (Hoshino et al., 2006) and in the positive gravitropism of roots (Friml et al., 2002). In addition, auxin has been shown to be a key factor regulating peg formation in cucumber seedlings (Wiztum and Gersani, 1975; Kamada et al., 2000). A microarray analysis demonstrated that hypergravity (7 *g*) up-regulates the expression of genes related to plant hormones in *Arabidopsis* callus culture (Martzivanou and Hampp, 2003). These studies suggest a possible involvement of plant hormones in signal transduction after perception of the gravity stimulus.

The aim of this study is to verify our hypothesis that hypergravity promotes the formation of lignified secondary cell walls in *Arabidopsis* inflorescence stems through the up-regulation of the expression of genes involved in the formation of cell wall components including lignin. In addition, we aimed to get an insight into a possible involvement of plant hormones in the signal transduction in gravireponse. Therefore, we carried out a comprehensive analysis of gene expression using *Arabidopsis* microarray (22 K).

2. Materials and methods

2.1. Plant material and hypergravity treatment

Arabidopsis thaliana (L.) Heynh. ecotype Columbia was used for the experiments (Tamaoki et al., 2006). Seeds were surface sterilized with 95% (v/v) ethanol for 10 s and were planted on 1.0% (w/v) agar consisting of 4 ml Murashige and Skoog medium (Wako Pure Chemical Industries Ltd., Tokyo, Japan) with 2% (w/v) sucrose in a test tube (15 × 105 mm). After 3 days at 4 °C in the dark, plants were allowed to grow at 22 °C for 20–26 days under continuous white light provided by fluorescent tubes (FL20SS·N/18; Toshiba Corp., Tokyo, Japan), giving an intensity of 130 μmol m^{-2} s^{-1} at plant level. Plants with primary inflorescence stems of 5–10 mm in length, i.e., at *Arabidopsis* growth stage number 5 (according to Boyes et al., 2001), were selected and exposed to hypergravity at 300 *g* in the shoot to root direction for 1–24 h at 25 °C in the dark using a centrifuge (SL-05A; Sakuma Seisakusho Ltd., Tokyo, Japan). For the 1 *g* control test tubes containing plants with 5–10 mm primary inflorescence stems were placed in the dark without centrifugation.

2.2. Microarray analysis

The basal region (10 mm in length) of primary inflorescence stems was excised from plants grown for 24 h at 300 *g* or 1 *g*. Total RNA was isolated from the basal 10 mm region of primary inflorescence stems of 17–21 individual plants per biological replicate, using the Agilent Plant RNA Isolation Mini kit (Agilent Technologies Inc., Palo Alto, USA). The quality of RNA samples was assessed with the RNA 6000 Nano LabChip Kit (Bioanalyser 2100; Agilent Technologies Inc.). Samples were subjected to microarray analysis using the Agilent Arabidopsis 2 Oligo Microarray (22 K) (Agilent Technologies Inc.). Microarray analyses were performed according to the Agilent 60-mer Oligo Microarray Processing Protocol (Agilent Technologies Inc.). Total RNAs (500 ng) from hypergravity-treated or control plants (1 *g*) were labeled with Cyanine 3 (Cy3)-CTP or Cyanine 5 (Cy5)-CTP, using the Low RNA Input Fluorescent Linear Amplification Kit (Agilent Technologies Inc.). RNeasy mini spin columns (Qiagen, Valencia, CA) were used for purification of labeled cRNA probes. Labeled cRNA was hybridized to microarrays.