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RESEARCH PAPER

Involvement of auxin dynamics in hypergravity-induced promotion of lignin-related gene expression in *Arabidopsis* inflorescence stems

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Abstract

Recent studies have shown that hypergravity enhances lignification through up-regulation of the expression of lignin biosynthesis-related genes, although its hormonal signalling mechanism is unknown. The effects of hypergravity on auxin dynamics were examined using *Arabidopsis* plants that were transformed with the auxin reporter gene construct *DR5::GUS*. Hypergravity treatment at 300 *g* significantly increased β-glucuronidase activity in inflorescence stems of *DR5::GUS* plants, indicating that endogenous auxin accumulation was enhanced by hypergravity treatment. The hypergravity-related increased expression levels of both *DR5::GUS* and lignin biosynthesis-related genes in inflorescence stems were suppressed after disbudding, indicating that the increased expression of lignin biosynthesis-related genes is dependent on an increase in auxin influx from the shoot apex.

Key words: *Arabidopsis*, auxin, disbudding, *DR5::GUS*, hypergravity, lignin.

Introduction

Land plants evolutionarily developed rigid cell walls to support their weight, and to grow and extend upward in the 1 *g* environment of the earth. Previous studies have demonstrated that prolonged hypergravity treatment significantly increased the content of acetylbromide-extractable lignins in *Arabidopsis* inflorescence stems (Tamaoki *et al.*, 2006), promoted metaxylem development, and decreased extensibility of the secondary cell walls in *Arabidopsis* inflorescence stems (Nakabayashi *et al.*, 2006). In addition, microarray analysis showed that hypergravity up-regulated genes responsible for the biosynthesis or modification of secondary cell wall components such as lignin (Tamaoki *et al.*, 2009). These facts indicate that regulation of lignin formation in response to hypergravity is mediated through the transcriptional regulation of genes related to lignin formation (Karahara et al., 2009).

Mechanosensitive ion channels were thought to be involved in graviperception in plants (Soga *et al.*, 2004; Nakabayashi *et al.*, 2006; Tamaoki *et al.*, 2006). Moreover, auxin was demonstrated to be involved in signalling of gravitropism (Salisbury *et al.*, 1988; Young *et al.*, 1990), peg formation in cucumber seedlings (Witztum and Gersani, 1975; Kamada *et al.*, 2000), and the negative gravitropic response of pea epicotyls (Hoshino *et al.*, 2006). It was reported previously that application of artificial weight induced secondary cell wall growth, as well as auxin transport in *Arabidopsis* inflorescence stem (Ko *et al.*, 2004). Although little is known about the signal transduction events involved in these responses to hypergravity, microarray analysis in *Arabidopsis* callus culture demonstrated that a hypergravity stimulus (7 *g*) up-regulates the expression of genes related to plant hormones (Martzivanou and Hampp, 2003). In addition, microarray analysis showed that

hypergravity significantly altered the expression of genes related to auxin biosynthesis and signalling (Tamaoki *et al.*, 2009).

The purpose of this study is to test whether auxin is involved in the hypergravity-related induction of expression of genes related to lignin in *Arabidopsis* inflorescence stems, using plants transformed with the auxin reporter gene construct *DR5::GUS*. The synthetic promoter *DR5*, which consists of seven tandem repeats of 11 bp containing the auxin-responsive TGTCTC element (Ulmasov *et al.*, 1997), increases auxin responsiveness and is widely used to monitor auxin responses in plants (Guilfoyle, 1999).

Materials and methods

Plant material and hypergravity treatment

Wild-type and *DR5::GUS* transgenic plants (Ulmasov *et al.*, 1997) of *Arabidopsis thaliana* (L.) Heynh. ecotype Columbia-0 were used throughout the study. Seeds were treated with 99% (v/v) ethanol for 10 s for the purpose of sterilization, and planted on 4 ml of Murashige and Skoog medium (Wako Pure Chemical Industries Ltd, Tokyo, Japan) containing 2% (w/v) sucrose and 1.0% (w/v) agar in test tubes (15 mm×105 mm). After 3 d at 4 °C in the dark, plants were grown at 22 °C for 20–26 d under continuous white light with an intensity of 130 μmol m^{-2} s^{-1} at the plant level from fluorescent tubes (FL20SS·N/18; Toshiba Corp., Tokyo, Japan). *Arabidopsis* plants with 5 mm inflorescence stems (i.e. growth stage number 5; Boyes *et al.*, 2001), were exposed to 300 *g* hypergravity 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 disbudding experiments, apical buds as well as lateral buds were removed 3 h before the hypergravity treatment. For the 1 *g* control, test tubes containing plants with 5 mm inflorescence stems were placed in the dark without centrifugation.

Morphometric analysis of the cross-sectional area of inflorescence stems

Wild-type *Arabidopsis* plants were grown at 22 °C for another 3 d in continuous light after the 24 h 300 *g* or 1 *g* (hyper)gravity treatment, which took place in the dark. Cross-sections of 50 μm thickness were cut at 1 mm above the base of the inflorescence stems using a vibrating microtome (Linear slicer Pro. 7; DSK, Kyoto, Japan), and observed under a light microscope (BX-50; Olympus, Tokyo, Japan). Photographs were taken with a digital camera (Coolsnap cf, Nippon Roper, Tokyo, Japan) fitted to the light microscope. Measurements of the cross-sections were carried out on the digital micrographs using Openlab Darkroom 3.0.2 (Improvision, Coventry, UK).

Histochemical staining of β-glucuronidase activity

For whole-mount histochemical observation of β-glucuronidase (GUS) activities in *DR5::GUS* plants, seedlings were incubated with a staining solution consisting of 100 mM Na_2PO_4 (pH 7.0), 10 mM ethylenediaminetetraacetic acid (EDTA), 3 mM $K_3Fe(CN)_6$, 0.1% (v/v) Triton X-100, and 40 mg ml^{-1} 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (Wako Pure Chemical Industries Ltd) at 37 °C, fixed in a mixed solution of ethanol and acetic acid, and cleared with a mixture of chloral hydrate, glycerol, and water (8:1:2, w/v/v). The cleared seedlings were mounted on slide glasses and observed under a dissecting microscope (SZH-10; Olympus, Tokyo, Japan). Photographs were taken with a digital camera (Digital sight DS-Fi1; Nikon, Tokyo, Japan) fitted to the dissecting microscope.

Because the inflorescence stem segments of 10 mm in length were cut from the base for the fluorometric assay of GUS activity, cross-sections of 50 μm thickness were taken at 5 mm above the base of the inflorescence stems (half-way up the 10 mm segments). Cross-sections were cut using a Linear Slicer Pro. 7 (DSK), and observed under a light microscope (BX-50; Olympus). Photographs were taken with a digital camera (Coolsnap cf; Nippon Roper) fitted to the light microscope.

Fluorometric assay of GUS activity

For quantitative measurements of GUS activity, the fluorometric GUS assay was performed using 4-methylumbelliferyl-β-d-glucuronide (4-MUG) as the substrate. This compound is converted by GUS into the fluorescent product 4-methyl umbelliferone (4-MU) (Jefferson *et al.*, 1987). Ten millimetre inflorescence stem segments (as above) of *DR5::GUS* plants were homogenized with GUS extraction buffer [50 mM Na_2PO_4, pH 7.0, 10 mM EDTA, 0.1% (v/v) Triton X-100, and 0.1% sarcosyl] immediately after hypergravity treatment at 300 *g*, and centrifuged at 12 000 *g* for 5 min at 4 °C. A portion of the supernatant fraction was mixed with the GUS extraction buffer containing 1 mM 4-MUG (Wako Pure Chemical Industries Ltd). This reaction mixture was incubated at 37 °C. Aliquots of the reaction mixture were removed at 0 min and 60 min. The reaction was terminated by adding 0.2 M Na_2CO_3. Production of 4-MU was quantified by measuring the 4-MU fluorescence (365 nm excitation, 455 nm emission) using a Nanodrop-ND-3300 spectrometer (Nanodrop Technologies Inc., Wilmington, DE, USA). The amount of 4-MU was deduced from a standard curve. GUS activity was expressed as nmol 4-MU min^{-1} mg^{-1} protein. The protein concentration was determined using the Bradford method (Bradford, 1976), using the Quick Start protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA), with bovine serum albumin as standard.

Quantitative RT-PCR analysis

Basal 10 mm inflorescence stem segments were excised from plants that had been incubated at 300 *g* and 1 *g* for 24 h. Total RNA was isolated from the basal region using the Agilent Plant RNA Isolation Mini kit (Agilent Technologies Inc., Palo Alto, CA, USA). First-strand cDNA was synthesized from 500 ng of total RNA, in a 10 μl reaction mixture, using a PrimeScript RT reagent kit (Takara Bio Inc., Shiga, Japan). Quantitative RT-PCR amplifications and measurements were performed using an ABI PRISM 7000 sequence detection system (Applied Biosystems Japan Ltd, Tokyo, Japan) using SYBR Premix Ex Taq (Takara Bio Inc.). The PCR amplification had an initial denaturing step of 30 s at 95 °C, followed by 40 cycles of 95 °C for 5 s and 60 °C for 31 s. All data were normalized using *18S rRNA* as an internal standard. The sequences of the gene-specific primers were as follows: *C4H*, 5′-CGTTATGAAACCAAGGAACTGTTAAA-3′ and 5′-GCAATCGTAGAACGAACCATTTAAA-3′ (Sibout *et al.*, 2005); *C3H1*, 5′-AAGTCTAGTGGAGCGAAACAGCATT-3′ and 5′-TCCTCACTAAGATCATACTGATCCTTCA-3’ (Abdulrazzak *et al.*, 2006); *ATPA2*, 5’-CCTTGACTGGGAGTAATGGAGA-3′ and 5′-TCAACCAAAGCTTGCCAATG-3′ (Tamaoki *et al.*, 2009); and *18S rRNA*, 5′-CGGCTACCACATCCAAGGAA-3′ and 5′-GCTGGAATTACCGCGGCT-3′ (Tamaoki *et al.*, 2009).

Results

Hypergravity induces DR5::GUS *expression in inflorescence stems*

It was demonstrated previously that hypergravity up-regulated the expression of auxin signalling-related genes

[auxin-inducible genes and auxin response factor (*ARF*) genes] in *Arabidopsis* inflorescence stems (Tamaoki *et al.*, 2009), indicating that the endogenous auxin level increased upon exposure to hypergravity. To test this, the effects of hypergravity on expression of a synthetic auxin response element, *DR5*, were examined using *DR5::GUS* transgenic *Arabidopsis* plants. Histochemical data showed that GUS expression was up-regulated in inflorescence stems of *DR5::GUS* transgenic plants sampled at 12 h or 24 h after the start of the hypergravity treatment (Fig. 1A–C). This result indicates that the endogenous auxin level increased in the inflorescence stems upon hypergravity treatment. It also indicates that endogenous auxin already begins to accumulate before 12 h after the start of the hypergravity treatment, and that the area in which endogenous auxin accumulates expands. Quantitative fluorometric analysis of GUS activity showed that the GUS expression was significantly higher at both 12 h and 24 h after the start of hypergravity treatment in inflorescence stems of the *DR5::GUS* transgenic plants (Fig. 2). These results indicate that endogenous auxin levels increase in response to hypergravity treatment in *Arabidopsis* inflorescence stems.

Cross-sections were taken from inflorescence stems of the *DR5::GUS* plants, and the tissue localization of the GUS activity was determined. High GUS activity was found in both the epidermis and the cortex, whereas the activity was weak in the stele (Fig. 3).

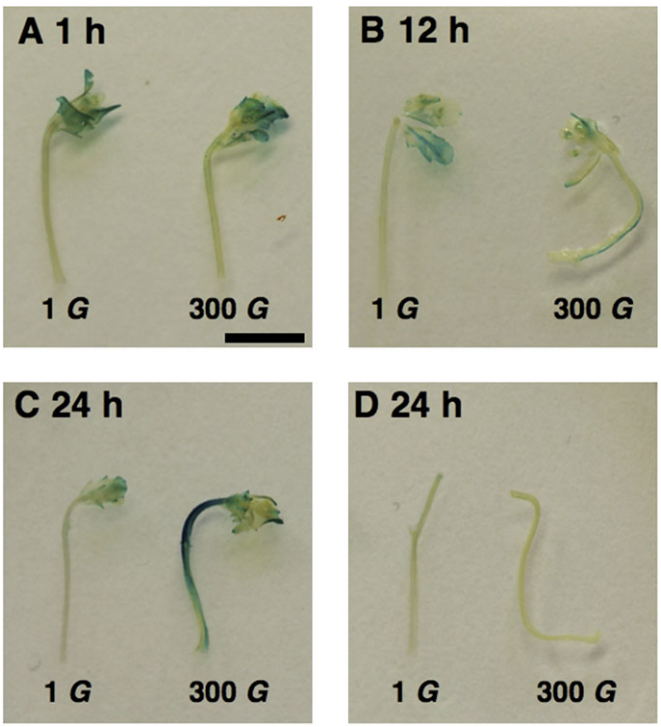

Fig. 1. Whole-mount, GUS-stained inflorescence stems of *DR5::GUS* transgenic *Arabidopsis* exposed to hypergravity for 1 h (A), 12 h (B), or 24 h (C, D). (A–C) Intact plants. (D) Transgenic plants disbudded before the hypergravity treatment. Bar=5 mm.

Disbudding treatment suppresses the hypergravity-induced increase of DR5::GUS *expression in inflorescence stems*

Microarray analysis showed that the expression of auxin biosynthesis-related genes was up-regulated by hypergravity in *Arabidopsis* inflorescence stems (Tamaoki *et al.*, 2009). Auxin is produced predominantly in shoot apical regions (Woodward and Bartel, 2005) and transported to the stem by polar transport (Lomax *et al.*, 1995). To test whether the hypergravity-induced increase in endogenous auxin in the inflorescence stems also originated from the buds, the effect of disbudding on the hypergravity-induced increase of *DR5::GUS* expression in inflorescence stems was examined. It was observed that hypergravity did not result in increased GUS activity in inflorescence stems after the plants were

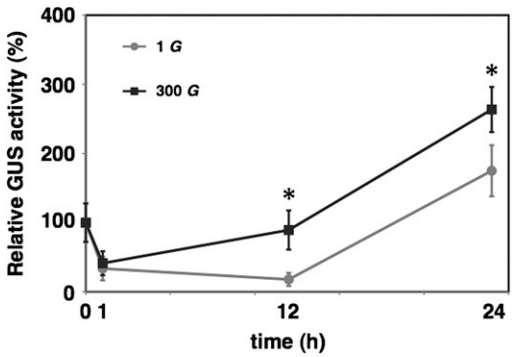

Fig. 2. Fluorometric analysis of GUS activities in the inflorescence stems of hypergravity-treated *DR5::GUS* transgenic *Arabidopsis* plants. Values are the mean ±SE (n=5–12). The values were normalized to the mean value of samples at 0 h. *P <0.05 Mann-Whitney U-test, two-tailed.

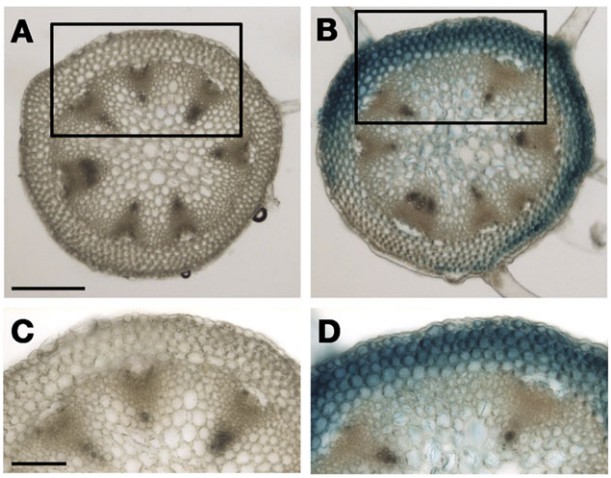

Fig. 3. Histochemical analysis of GUS expression in cross-sections of inflorescence stems of *DR5::GUS* transgenic *Arabidopsis* in response to hypergravity. GUS staining was performed just after the hypergravity treatment. (A) The 1 *g* control. (B) 300 *g*. (C and D) Magnifications of areas shown in A and B, respectively. Bars: (A) 200 μm, (C) 100 μm.

disbudded (Fig. 1D). The effect of disbudding on the GUS activity after hypergravity treatment was quantified using fluorometric analysis of inflorescence stems of *DR5::GUS* plants. The GUS activity values of hypergravity-treated plants were normalized to those of the 1 *g* samples. The GUS activity in the disbudded, hypergravity-treated samples was 98.7±27.3% (n=8) of the 1 *g* samples, while that in the control (non-disbudded, hypergravity treated) was 150.6±18.6% (n=12) that of the 1 *g* samples. These results indicate that the hypergravity-induced increase in endogenous auxin levels in inflorescence stems resulted from either enhanced production of auxin in buds or enhanced auxin transport from the buds.

Disbudding suppresses hypergravity-related induction of lignin biosynthesis genes in inflorescence stems

Previously, it was demonstrated that hypergravity treatment up-regulated the expression of lignin biosynthesis or lignin-related genes (Tamaoki *et al.*, 2009). It also increased the content of lignin in the secondary cell wall (Tamaoki *et al.*, 2006). Auxin was reported to be involved in vascular bundle formation (Mattsson *et al.*, 1999, 2003) and tracheary element differentiation (Yoshida *et al.*, 2005). To test whether hypergravity-induced endogenous auxin accumulation plays a role in the induction of lignin-related genes, the effects of disbudding on the expression of the lignin-related genes, *ATPA2 PEROXIDASE* (*ATPA2*), *CINNAMATE 4-HYDROXYLASE* (*C4H*), and *COUMARATE 3-HYDROXYLASE1* (*C3H1*), were examined in inflorescence stems of hypergravity-treated and of 1 *g* control wild-type *Arabidopsis* plants. The expression levels of these three genes in inflorescence stems of plants with buds were enhanced significantly by hypergravity, while they remained unchanged in inflorescence stems of disbudded plants (Fig. 4). This raises the possibility that the hypergravity-induced auxin accumulation causes the expression levels of lignin biosynthesis-related genes to increase.

Disbudding does not suppress the hypergravity-related increase in the cross-sectional area of inflorescence stems

Thickening of the stem, as well as lignin deposition, is important in supporting the plant shoots during growth against the gravitational force. It has been demonstrated that hypergravity increased the cross-sectional area of *Arabidopsis* inflorescence stems (Nakabayashi *et al.*, 2006). Therefore, the effect of disbudding on the hypergravity-induced increase in the cross-sectional area of inflorescence stems was examined. In disbudded and intact plants, equally, increases in the cross-sectional area of inflorescence stems were observed in response to hypergravity [Mann–Whitney *U*–test, two-tailed, z= –2.562, P=0.0104 (disbudded plants); z= –2.397, P=0.0165 (intact plants)] (Fig. 5). This indicates that the increase in endogenous auxin does not play a role in the increase in the cross-sectional area of inflorescence stems upon hypergravity treatment.

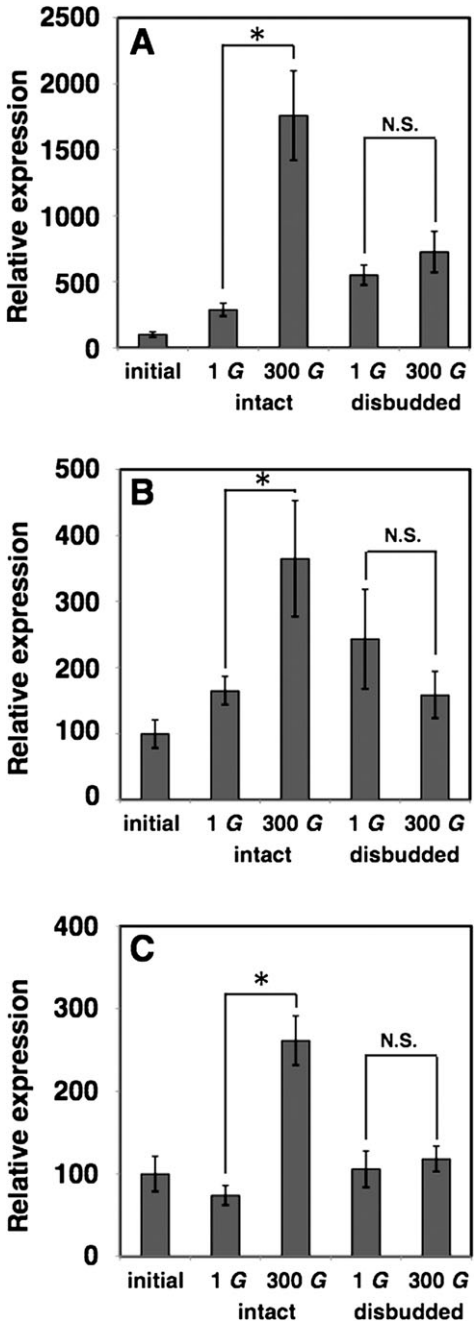

Fig. 4. Effects of disbudding on the enhanced expression levels of lignin biosynthesis-related genes in *Arabidopsis* inflorescence stems under hypergravity. The expression of (A) *ATPA2*, (B) *C4H*, and (C) *C3H1* genes was determined by quantitative RT-PCR. The values of gene expression were normalized to the mean value of those before the treatment (initial). Values are mean ±SE (n=3). *P <0.05 Mann–Whitney *U*-test, two-tailed. N.S., not significant

Discussion

Growth parameters such as elongation of *Arabidopsis* hypocotyls varied in proportion to the logarithm of the magnitude of gravity in the range from microgravity to hypergravity (Soga *et al.*, 2001). Such a linear relationship also existed between the logarithm of the magnitude of gravitational acceleration and the inhibition of root

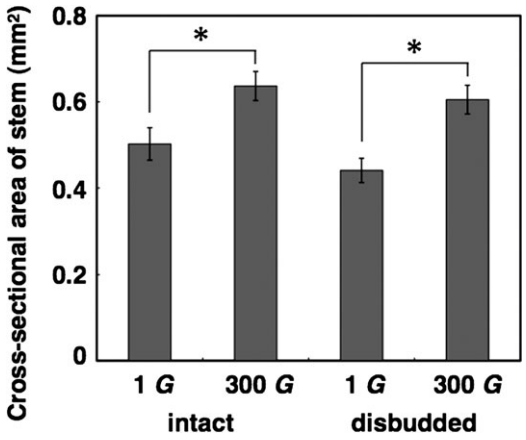

Fig. 5. Effects of disbudding on the increase in cross-sectional area of wild-type *Arabidopsis* inflorescence stems at the base by hypergravity treatment. Values are mean ±SE (n=7–8). *P <0.05, Mann–Whitney U-test, two-tailed.

elongation, at least up to 300 *g* in azuki bean roots (Soga *et al.*, 2005). In addition, the change in cell wall extensibility as a result of hypergravity at 300 *g* was shown to be reversible within a few hours in azuki bean epicotyls, and in maize coleoptiles and mesocotyls (Soga *et al.*, 2003). These results clearly indicate that such hypergravity treatment at 300 *g* is not excessive and does not cause irreversible damage. Further, it was demonstrated that hypergravity treatment at 300 *g* for 24 h increased the lignin content, as well as the expression of genes related to lignin synthesis in *Arabidopsis* inflorescence stems (Tamaoki *et al.*, 2006, 2009). The aim of the present study was to test whether auxin is involved in hypergravity-related induction of expression of genes related to lignin synthesis. Therefore, it was decided to use gravitational acceleration at 300 *g* as the most suitable hypergravity treatment.

Involvement of auxin in signal transductions of gravitropism has been well documented in plants (Salisbury *et al.*, 1988; Young *et al.*, 1990). On the other hand, only a few studies have indicated an involvement of auxin signalling in responses to changes in gravitational acceleration. Microarray analysis indicated a possible involvement of auxin signalling in the response of *Arabidopsis* callus culture to hypergravity (Marzivanou and Hampp, 2003). In addition, microarray analysis showed that hypergravity up-regulated the expression of auxin signalling-related genes in *Arabidopsis* inflorescence stems (Tamaoki *et al.*, 2009). More specifically, up-regulation of auxin-responsive genes, such as *IAA28*, by hypergravity implies that hypergravity increases the endogenous level of auxin in *Arabidopsis* inflorescence stems (Tamaoki *et al.*, 2009). In addition, the present study showed that *DR5::GUS* expression significantly increased under hypergravity in the *Arabidopsis* inflorescence stems (Figs 1, 2), indicating that hypergravity increased the level of endogenous auxin in *Arabidopsis* inflorescence stems.

Microarray analysis showed that the expression of several auxin biosynthesis-related genes was up-regulated >2-fold upon hypergravity in *Arabidopsis* inflorescence stems, raising the possibility that hypergravity enhanced auxin biosynthesis in the stems (Tamaoki *et al.*, 2009). The results obtained in the disbudding experiment (Fig. 1) clearly indicated that the accumulated endogenous auxin in inflorescence stems upon hypergravity treatment, which was important for the hypergravity-enhanced expression of genes related to lignin biosynthesis, mainly originated from the buds. However, disbudding treatment may partially affect the auxin biosynthesis in inflorescence stems under hypergravity.

Enhanced expression of *DR5::GUS* was observed in the epidermis, cortex, and stele cells, but not in the xylem region of the inflorescence stem (Fig. 3). Polar auxin transport (Lomax *et al.*, 1995) is mediated by auxin efflux carriers, such as PIN-FORMED protein (PIN) and P-glycoprotein (PGP), and auxin influx carriers, such as AUXIN/LIKE-AUXIN (AUX/LAX) families. PINs and PGPs control the direction of auxin transport because of their subcellular localization (Blakeslee *et al.*, 2007). The localization of auxin uptake and efflux proteins was investigated in detail in *Arabidopsis* roots and embryos, thereby providing a model of auxin dynamics (Petrášek and Friml, 2009). Although auxin dynamics in *Arabidopsis* hypocotyls and inflorescence stems was poorly understood, several reports showed localization and expression of auxin uptake and efflux proteins in the inflorescence stem. PIN1 localized basally in xylem parenchyma cells (Galweiler *et al*, 1998). In dark-grown seedlings, PIN1 localized in the vascular parenchyma, in the epidermis of the shoot apical hook, in the vascular parenchyma, and in adjacent cortical cells in the mid-hypocotyl (Blakeslee *et al.*, 2007). A change in the localization and/or expression of such proteins might affect auxin transport in the inflorescence stem upon hypergravity treatment.

Disbudding suppressed the hypergravity-induced increases in both *DR5::GUS* expression and the expression of lignin biosynthesis genes, such as *C4H*, *C3H1*, and *ATPA2* (Fig. 4), in inflorescence stems. This indicates that the hypergravity-related induction of the expression of lignin biosynthesis-related genes is mediated by the hypergravity-related increase in endogenous auxin. *C4H* and *C3H1* are involved in monolignol synthesis (Ruegger and Chapple, 2001; Goujon *et al.*, 2003). *C4H* encodes cinnamate 4-hydoxylase, which is the first cytochrome P450-dependent monooxygenase of the phenylpropanoid pathway (Bell-Lelong *et al.*, 1997), and controls the conversion of cinnamate into *p*-coumarate (Raes *et al.*, 2003). *C3H1* encodes *p*-coumarate 3-hydroxylase, which converts the shikimate and quinate esters of *p*-coumaric acid into the corresponding caffeic acid conjugates (Raes *et al.*, 2003). *ATPA2* encodes an extracellular anionic peroxidase, which has the ability to oxidize monolignols and is involved in lignin polymerization (Østergaard *et al.*, 2000; Nielsen *et al.*, 2001). The increase in *ATPA2* expression by hypergravity is more prominent than the *C4H* and *C3H1* expression, which is consistent with a previous report (Tamaoki *et al.*, 2009). This indicates that hypergravity enhances lignin polymerization more than monolignol

synthesis. Disbudding suppressed the hypergravity-related increases in expression of all these genes, indicating that the processes of lignin biosynthesis are regulated in response to a change in gravity and are dependent on the level of auxin. A basipetal gradient of lignification exists in *Arabidopsis* inflorescence stems (Roger and Campbell, 2004). This gradient has been suggested to be necessary to support the weight of the plant. It was reported that development of the secondary cell wall was promoted by application of artificial weight on the shoot (Ko *et al.*, 2004). In addition, the level of lignin, a characteristic component of the secondary wall, increased upon hypergravity in *Arabidopsis* inflorescence stems (Tamaoki *et al.*, 2006). Therefore, body weight of *Arabidopsis* plants is thought to be an important factor in the regulation of secondary cell wall formation in inflorescence stems. Ko *et al.* (2004) also reported that auxin transport and signalling were promoted by artificial application of a weight on *Arabidopsis* inflorescence stems. In this study, it was demonstrated that disbudding suppressed the hypergravity-enhanced expression of genes related to lignin biosynthesis (Fig. 4). These facts indicate that auxin signalling is involved in the up-regulation of lignin biosynthesis under hypergravity, thereby contributing to supporting the inflorescence stem mechanically.

A previous study demonstrated that auxin transport inhibitors prevented tracheary element differentiation in a *Zinnia elegans* cell culture system (Yoshida *et al.*, 2005), indicating that auxin signalling plays an important role in vascular formation. It is likely that hypergravity-related up-regulation of auxin signalling in the inflorescence stem also promoted xylem formation. This idea is supported by a previous study demonstrating that the number of meta-xylem elements in the basal region of inflorescence stems increased in response to hypergravity (Nakabayashi *et al.*, 2006).

On the other hand, disbudding did not affect the increase in cross-sectional area of inflorescence stems by hypergravity (Fig. 5), indicating that the hypergravity-induced lateral growth of the inflorescence stem is not mediated by auxin signalling. Microarray analysis showed that hypergravity increased the expression of genes that are related to plant hormones such as ethylene and cytokinin, as well as auxin, in *Arabidopsis* inflorescence stems (Tamaoki *et al.*, 2009). Therefore, it is possible that plant hormones other than auxin might be involved in the hypergravity-induced lateral growth of the inflorescence stem.

The findings of this study indicated for the first time that auxin signalling plays a key role in the responses of plants to hypergravity, particularly in the enhancement of expression of genes related to lignin synthesis. As a next step, these findings will be tested using *Arabidopsis* mutants with defects in auxin transport.

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