RESEARCH PAPER

Changes in gravitational forces induce the modification of *Arabidopsis thaliana* silique pedicel positioning

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Abstract

The laterals of both shoots and roots often maintain a particular angle with respect to the gravity vector, and this angle can change during organ development and in response to environmental stimuli. However, the cellular and molecular mechanisms of the lateral organ gravitropic response are still poorly understood. Here it is demonstrated that the young siliques of *Arabidopsis thaliana* plants subjected to 3-D clinostat rotation exhibited automorphogenesis with increased growth angles between pedicels and the main stem. In addition, the 3-D clinostat rotation treatment significantly influenced the development of vascular bundles in the pedicel and caused an enlargement of gap cells at the branch point site together with a decrease in *KNAT1* expression. Comparisons performed between normal and empty siliques revealed that only the pedicels of siliques with normally developing seeds could change their growth angle under the 3-D clinostat rotational condition, while the pedicels of the empty siliques lost the ability to respond to the altered gravity environment. These results indicate that the response of siliques to altered gravity depends on the normal development of seeds, and may be mediated by vascular bundle cells in the pedicel and gap cells at branch point sites.

Key words: *Arabidopsis*, 3-D clinostat, gravitropism, *KNAT1*, pedicel.

Introduction

The ability of plant organs to change their orientation with respect to gravity is termed gravitropism (Myers *et al.*, 1994). Different organs exhibit different responses to gravitational force; for example, stems show negative gravitropism, whereas roots show positive gravitropism, lateral branches and lateral roots demonstrate plagiotropism, and stolons reveal diageotropism (Stockus, 1994; Mano *et al.*, 2006). Young seedlings typically show a vertical upward growth movement of the shoot (negative orthogravitropism) and a downward growth movement of the root (positive orthogravitropism). However, most plant organs, especially in mature plants, remain at a particular angle with respect to the vertical growth axis and are not parallel to the gravity vector. Experiments in the microgravity environment of space showed that the angle of lateral organ growth with respect to the main stem was altered, and the plants exhibited automorpho-

genesis (Halstead and Dutcher, 1987; Brown *et al.*, 1990; Musgrave *et al.*, 1997, 2000; Kiss *et al.*, 1998; Hoson *et al.*, 1999; Takahashi *et al.*, 1999; Kiss 2000; Hoshino *et al.*, 2007; Zheng *et al.*, 2008). In addition, plants grown on a 3-D clinostat, which can be used to simulate certain aspects of the microgravity environment (Hoson *et al.*, 1996; Stankovic *et al.*, 2001), also displayed an architecture similar to that of space-grown plants (Hoson *et al.*, 1996; Hoson and Soga, 2003; Driss-Ecole *et al.*, 2008). In recent years, studies on the model plant *Arabidopsis thaliana* have accumulated a lot of knowledge on the mechanisms of gravitropism of stems and roots (Okada and Shimura, 1992; reviewed by Fukaki *et al.*, 1996a; Tasaka *et al.* 1999; Terao-Morita and Tasaka, 2004), but information on the response of lateral organs to altered gravitropic growth conditions is quite limited (Digby and Firt, 1995).

The laterals of both shoots and roots are often maintained at particular angles with respect to the gravity vector during different stages of growth. By changing the gravity direction against the axis of the plant, Mano *et al.* (2006) found that the orientation of *Arabidopsis* rosette leaves was due to negative gravitropism and the nastic movement. Studies on the gravitropic behaviour of trailing plant organs suggested that the developing organs changed their orientation with respect to gravity (Digby and Firn, 1995). As the position of lateral organs, such as siliques or fruits, is not always 'vertical', it is difficult to build a model because lateral organs sense their displacement with respect to the root-shoot axis during changes in the gravitropic environment (Feldman, 1985; Chen *et al.*, 1999). It has been proposed that the direction of lateral organ growth might reflect a balance between the forces of negative and positive gravitropism or a balance between negative gravitropism and epinasty (Digby and Firn, 1995). Still missing is information on the cellular and molecular mechanisms that mediate the response of lateral organs to altered gravitropic growth conditions.

The angle between the pedicel and the inflorescence stem is an important morphological characteristic of inflorescences, and influences both the beauty and yield potential of plants (Wang and Li, 2008). Several genes that play key roles in regulating pedicel development in *Arabidopsis* have been identified. For example, *POLTERGEIST* (*POL*) and *PLL1* (*POL*-like gene) regulate pedicel length by interacting with *ERECTA* (Song and Clark, 2005), *Arabidopsis* expansin-10 (*AtEXP10*) modulates pedicel abscission (Cho and Cosgrove, 2000), and ectopic expression of *Arabidopsis MYB-13* (*AtMYB13*) leads to peculiar hook structures at pedicel branching points (Kirik *et al.*, 1998). However, very little is known about the genes or the pathways that regulate pedicel angle. One *KNAT1* null mutant, *brevipedicellus* (*bp*), displaying downward-pointing siliques (or flowers) (Venglat *et al.*, 2002), is valuable for understanding whether gravity is involved in controlling pedicel angle. *KNAT1* is one of the class I *KNOX* genes which play key roles in defining the architecture of the inflorescence, including the pedicel development of *Arabidopsis* (Douglas *et al.*, 2002).

Silique (or fruit), a lateral organ derived from the shoot apical meristem like leaves (Douglas and Riggs, 2005), might have fundamentally the same gravitropism as stem (Fukaki *et al.*, 1996b; Mano *et al.*, 2006). However, the detailed analysis of silique gravitropism has not been reported yet. In this study, the effects of 3-D clinostat rotation (clinorotation) on pedicel architecture, and the relationship between *KNAT1* expression and pedicel gravity response have been characterized

Materials and methods

Plant materials and the 3-D clinostat treatment

Arabidopsis thaliana (L.) Heynh. (ecotype Columbia), *pro-KNAT1::GUS* transformants (Ori *et al.*, 2000), and *pro35S::*

KNAT1 transformants (Lincoln *et al.*, 1994) were planted in a medium (vermiculite:perlite 3:1) with Plant-Soul special water-soluble fertilizer 20-20-20+TE (Wintong Chemicals Co., Ltd) and watered with distilled water in a test tube (15 mm in diameter, 80 mm in length) or in a plastic bottle (60 mm in diameter, 80 mm in length). Plants usually grew up to the first flower opening stage with a photoperiod of 16 h light and 8 h dark at $22 \pm 2^\circ\text{C}$ in a greenhouse. Afterwards, all plants were moved to an air-conditioned room at $22 \pm 2^\circ\text{C}$ and grown under constant white light provided with a bank of fluorescent tubes (TD-L 36W/7-426 made by Koninklijke Philips Electronics NV, The Netherlands), with an intensity of $120 \mu\text{mol m}^{-2} \text{s}^{-1}$ at plant level. *Arabidopsis* plants with inflorescence stems of 8–10 cm in length (*Arabidopsis* growth stage number 6; Boyes *et al.*, 2001) were selected and then exposed to a 3-D clinostat (SM-31 made by the Center of Beijing Space Science CAS, China) rotated condition as described by Zheng *et al.* (2008). For a 1 g stationary control (i.e. normal gravity), plants with 8–10 cm inflorescence stems at the same developmental stage were grown in the same culture condition as described above without clinorotation.

Tissue preparation for light microscopy

Light microscopy was performed as described by Kiss and Sack (1989). Briefly, the node region containing 4–6 mm of the inflorescent stem and ~2 mm of the basal part of the pedicels of the 3-D clinorotation-treated (clinorotated) plants and the controls were cut and fixed with glutaraldehyde and paraformaldehyde (PFA). Serial sections were taken at 2 μm and stained with toluidine blue. The sections were observed under a Leica DMLB microscope; images were captured using a JVC TK-1381EG digital microscope camera.

Scanning electron microscopy (SEM)

Plant materials for SEM were fixed in 3% glutaraldehyde overnight. The materials were mounted on aluminium stubs and coated with gold in JEOL JFC-1600 after graded dehydration and replacement. The samples were viewed in a JEOL JSM-6360LV scanning electron microscope at 6 kV.

Detection of β -glucuronidase (*GUS*) activity

Histochemical detection of *GUS* activity was carried out as described by Shao *et al.* (2004). Briefly, 4 cm of the apical part on the inflorescence stem with siliques of the 3-D clinorotated plant and its control were cut and placed in the *GUS* staining solution containing 100 mM Na_2PO_4 (pH 7.0), 10 mM EDTA, 1 mM $\text{K}_3\text{Fe}(\text{CN})_6$, 0.1% (v/v) Triton X-100, and 160 $\mu\text{g ml}^{-1}$ X-gluc (5-bromo-4-chloro-3-indolyl- β -D-glucuronic acid cyclohexylammonium salt) (Bio Basic Inc., Canada) under vacuum for 10 min at room temperature, then incubated at 37°C in the dark overnight. The hand-cut sections through the node regions of inflorescence stems were stained with the same procedure without vacuum treatment as described above. The *GUS* stained tissues or sections were rinsed with 75% ethanol, viewed, and photographed in 50% glycerol.

Quantitative real-time PCR (RT-PCR)

Total RNA was extracted from the node region containing ~2 mm of main stem and ~2 mm of pedicel using an RNAiso plus Kit (TaKaRa Biotechnology Co., Ltd, China) according to the manufacturer's instructions. cDNA was synthesized using 2 μg of total RNA and 100 U of ReverTra Ace reverse transcriptase (Toyobo Co., Ltd, Japan) according to the manufacturer's instructions.

RT-PCR was performed on a Rotor-Gene 3000 (Corbett Research, Australia) using the SYBR Green Realtime PCR Master Mix (Toyobo Co., Ltd, Japan) with *KNAT1*-specific primers

(forward primer, 5'-TCCTGATGGGAAGAGTGACAAT-3'; reverse primer: 5'-CCACTTGTAATGCAACTCCCAC-3'). Amplification was carried out at 95 °C for 5 min and 41 cycles at 95 °C for 10 s, 61 °C for 15 s, 72 °C for 30 s, followed by 1 °C s⁻¹ ramping up to 95 °C for fusion curve characterization. The data were normalized with respect to *UBQ10* (forward primer, 5'-GTCCTCAGGCTCCGTGGTG-3'; reverse primer, 5'-TGCCATCCTCCAAGTCTTTC-3'; Perry *et al.*, 2005). Three replicates were performed.

Fluorescence microscopy for xylem analysis

The hand-cut sections of pedicels at the basal 1–2 mm part were soaked in 1 mg ml⁻¹ berberine (Sigma Chemical Co., St Louis, MO, USA) for 30 min, washed with distilled water three times, and then transferred to a staining solution containing 5 mg ml⁻¹ aniline blue (Sigma) for 15 min. The sections were mounted in 50% (w/w) glycerol. The xylem area was examined by fluorescence microscopy (Leica DMLB with UV filter A, excitation filter BP 340–380 nm/Suppression Filter LP 425). The autofluorescence of xylem vessels was blue under UV light, but yellow after staining with berberine.

Results

Positioning of silique pedicels of *Arabidopsis* was influenced by the 3-D clinorotation

Arabidopsis plants grew up to the flowering stage (corresponding to *Arabidopsis* growth stage number 6; Boyes *et al.*, 2001) in the greenhouse. Afterwards the plants with two siliques grew for 5 d under the normal gravity (1 g) stationary control condition or the simulated microgravity condition on a 3-D clinostat. Under the 1 g control condition, *Arabidopsis* siliques formed upward angles on the inflorescence (Fig. 1A). When *Arabidopsis* plants were exposed to the 3-D clinorotation, an altered architecture characterized by orthogonal or downward-oriented siliques was observed (Fig. 1B). The angles between the inflorescence stem and the pedicels of the clinorotated plants ranged from 40 ° to 100 °, with nearly 40% of siliques oriented approximately perpendicular to the inflorescence axis (90 °) (Fig. 2). In contrast, the stem–pedicel angles of

the 1 g control plants ranged from 10 ° to 80 °, and >70% of them possessed an angle of 20–40 ° under the 1 g control condition (Fig. 2). These results indicate that the angle between the inflorescent stem and pedicels significantly increased under the 3-D clinorotated growth condition.

Developing seeds in the silique are essential for the pedicel response to the 3-D clinorotation

Silique growth and development were characterized by observations of the branching sites of seedlings (Fig. 3). Figure 3A shows that the angles of pedicels from P4 to P14 were significantly increased by the 3-D clinorotation. This was not the case for the older silique pedicels (e.g. from P1 to P3) and for the most recently formed silique pedicels at the shoot apex (e.g. P15), neither type of which demonstrated a response to the 3-D clinorotation. To determine whether the ability of the silique responding to the clinorotation depended on the development of seeds in the silique, the correlation between the presence of developing seeds and the gravity response of the siliques was studied. As seen in Fig. 3C the siliques (e.g. P15) at the apex of the shoot in which the embryos had just started to develop (2 days after flowering; DAF) exhibited no response to the 3-D clinorotation treatment. However, as the embryos developed to the globular stage (Fig. 3D, E), the siliques (e.g. P14) became responsive to the altered gravity. The most pronounced response of siliques to the 3-D clinorotation (e.g. P5 and P6) was observed when the embryos reached the heart stage of development at ~5 DAF (Fig. 3F). As the embryos in the siliques completed their development (Fig. 3G) after ~6 DAF, the siliques (e.g. P1–3 siliques in Fig. 3A) lost the ability to respond to the altered gravity created by the 3-D clinostat.

To confirm further the influence of seed development on the response of siliques to the 3-D clinorotation, empty siliques (without seeds) were produced experimentally by

Fig. 1. Phenotypes of *Arabidopsis thaliana* plants (A) grown under normal (1 g) gravity conditions (control) and (B) after growth on a 3-D clinostat for 5 days. Scale bars: 10 mm.

Fig. 2. Distribution of angles between *Arabidopsis* inflorescence stems and silique pedicels. *Arabidopsis* plants were grown either under normal gravity (1 g) conditions (Control) or under the 3-D clinorotated conditions (3-D) for 5 d. The angle between the orientation of the transition zone connecting the main inflorescence stem and the pedicel was read in a clockwise direction and called the pedicel angle (α). The pedicel angle with respect to the inflorescence axes upwards is 0 ° and downwards is 180 °. The data represent the mean of five replications, and each replication contains ~50 siliques of four plants. Bars designate the SD.

Fig. 3. The ability of *Arabidopsis* pedicels to respond to the 3-D clinorotation changed during the development of seeds in the siliques. (A) Phenotype of an inflorescence stem of an *Arabidopsis* plant grown under 3-D clinorotated conditions for 5 d. To establish a baseline that facilitated identification of the response of pedicels to the altered gravity, the pedicels (P1 is the pedicel of the first formed silique, P2 is that of the second silique, P15 is that of the 15th silique) were marked according to the position of the siliques on the inflorescence stem. (B) Examples of siliques at different developmental stages. (C–G) Developmental stages of embryos in the siliques of B. Scale bars: 6 mm in (A), 3 mm in (B), 20 μm in (C), 50 μm in (D–F), 200 μm in (G).

removing anthers from the flowers before the pollen matured. In such plants, no difference between the angles of normal siliques (with developing seeds) and empty siliques was observed under the 1 g control growth condition (Fig. 4C, D). However, on the 3-D clinostat, the angles of normal siliques increased (Fig. 4A, B) and the average angle was $\sim 70^\circ$ (Fig. 4E), while the average angle of the empty siliques (Fig. 4B) was only $\sim 40^\circ$ (Fig. 4E), which did not change significantly in comparison with those of both normal and empty siliques under the 1 g control condition (Fig. 4D). These results indicate that the presence of developing seeds in the siliques is essential for the pedicel response to altered gravity.

Effects of 3-D clinorotation on pedicel vascular patterning and branch point cell elongation

The vascular patterns of *Arabidopsis* pedicels exhibit dorsoventral asymmetry with respect to vascular positioning (Douglas and Riggs, 2005). To assess the influence of the 3-D clinorotation treatment on pedicel vascular patterning, serial transverse sections through the proximal regions of pedicels were made. Sections of pedicels of young siliques showed that the vasculature of pedicels consists of two lateral bundles and one abaxial bundle during early development (e.g. P15 in Fig. 3A, Supplementary Fig. S2A–C available at JXB online). Pedicels at this stage of development did not respond to 3-D clinorotation. The adaxial bundles arise during the middle phase of pedicel development (e.g. from P9 to P14 in Fig. 3A, Supplementary Fig. S2D, E, Fig. 5A, B), but the abaxial vascular bundles at this stage (e.g. P9 in Fig. 3A) were larger than the adaxial bundles in the 1 g control samples (Fig. 5A, C). This difference between the abaxial and adaxial bundles disappeared in the 3-D clinorotated samples during the middle stage of pedicel development (Fig. 5B, D). The amount of vessel cells in the two lateral and the abaxial

bundles was similar at the middle developmental stage and was not influenced by the 3-D clinorotation treatment, while the vessel cells in the adaxial bundle were significantly increased in the 3-D clinorotated samples in comparison with those in the control pedicels. The number of vessel cells in the adaxial bundles was only about one-fifth of that in the abaxial bundles in the controls, but the numbers of vessel cells in adaxial and abaxial bundles were almost the same in the 3-D clinorotated plants (Fig. 5E, F). These results suggested that the adaxial vascular bundle was more strongly influenced by the 3-D clinorotation compared with the other three bundles during the middle stage of pedicel development (e.g. P9–P14 in Fig. 3A).

Longitudinal sections through the node region of *Arabidopsis* plants grown on the 3-D clinostat and under 1 g control conditions demonstrated that the overall patterns of the tissue types were similar (Fig. 6A, B). The cells in the adaxial region of the pedicel and main stem junction constitute the branch point site. As shown in Fig. 6C and D, the branch point cells become elongated under the 3-D clinorotation conditions compared with those in the 1 g control plants (Fig. 6C, D; Supplementary Fig. S4 at JXB online). Interestingly, the SEM results showed that the epidermal cells at the abaxial pedicel–stem junction of the 3-D clinorotated plants were shorter than those of the control (Fig. 6E, F), but there was no statistical difference in the average size of epidermal cells in the pedicel tissues between the 3-D clinorotated plants and the control (Supplementary Fig. S4C). Thus it is assumed that the elongation of the branch point cells might be one of the primary reasons for the enlargement of the angle between the pedicel and the stem under the 3-D clinorotation condition.

Expression of KNAT1 in the node region

Previous studies reported that a *KNAT1* null mutant, *brevipedicellus* (*bp*), showed downward-oriented siliques

Fig. 4. Responses of pedicels with empty or normal siliques to the 3-D clinorotation growth condition. (A) Phenotype of pedicels of normal siliques with developing seeds subjected to 3-D clinorotation conditions for 5 d. The growth directions of the pedicels with empty siliques (arrowheads) on the inflorescence stems of 3-D clinorotated (B) or the normal gravity (1 g) grown plants (C). The distribution of pedicel angles of normal and empty siliques under the 1 g control condition (D) and the 3-D clinorotation condition (E) is shown in the form of a histogram. The data of D and E were based on 150–200 pedicels of 20 plants from five biological repeats, and the angles of pedicels with empty and normal siliques were collected from the same plant. Scale bars: 6 mm in (A–C). Bars in (D) and (E) indicate the SD.

(Douglas *et al.*, 2002), which is similar to the morphology of wild-type plants grown on the clinostat in this study (Fig. 1B). *KNAT1* is expressed in the node region at the base of the pedicels (e.g. the branch point region) of *proKNAT1::GUS* transformants. Such transformants were used to study *KNAT1* expression under 3-D clinorotated and 1 g stationary control growth conditions. The GUS activity in branch point cells of the 3-D clinorotated samples (Fig. 7D–F) showed a drastic reduction compared with the control samples (Fig. 7A–C). Serial cross-sections through the node region showed that in the cortical tissues

Fig. 5. Influence of the 3-D clinorotation treatment on the development of the pedicel vascular bundles. Fresh hand-cut transverse sections (A and B) and half thickness glutaraldehyde-fixed sections (C and D) through the basal region of pedicels at 5 days after flowering (e.g. P9 in Fig. 3A) were viewed using fluorescence and light microscope. Two lateral bundles (a left and a right bundle) and two middle bundles (an abaxial and an adaxial bundle) were observed in pedicels at this stage, but the adaxial bundle is obviously smaller than the abaxial bundle in the 1 g control condition (A and C). The abaxial and adaxial bundle appeared the same size in the 3-D clinorotation condition (B and D). The ratios of the number of adaxial/abaxial (ad/ab) xylem vessel cells in the middle bundles (E) and left/right (L/R) xylem vessel cells in the lateral bundles (F) were calculated from the data based on 48 pedicels of 12 different plants from three repeats. ad, adaxial; ab, abaxial; L, left; R, right xylem vessel cells of vascular bundles. Scale bars: 50 μ m in (A–D). Bars in (E) and (F) are the SD, ***P* < 0.01; ****P* < 0.001.

there was no difference in GUS expression between the 3-D rotated samples and the controls. In contrast, GUS activity decreased in the branch point cells of the 3-D clinorotated samples (Fig. 7K–N) compared with the controls (Fig. 7G–J).

The relative transcript level of *KNAT1* in the pedicel node region was determined using real-time PCR (Fig. 8A). This analysis demonstrates that the expression of *KNAT1* in the node of the 3-D clinorotated plants was only about one-third of that of the control (Fig. 8A). The *KNAT1*-overexpressing plants, *pro35S::KNAT1* transformants, were also rotated on the 3-D clinostat. The result showed that the

Fig. 6. Influence of 3-D clinorotation on the development of branch point cells. (A–D) Light micrographs of sections through the basal region of pedicel stem nodes of glutaraldehyde-fixed plants. (A) and (C) Longitudinal section of a control pedicel. (B) and (D) Longitudinal section of a pedicel of a clinorotated plant. Note the expansion of the branch point cells (bpc) of the 3-D clinorotated plants compared with the bpcs of the control plants. Scanning electron micrograph of the pedicel nodes of control (E) and 3-D clinorotated plants (F) showing the files of cells on the pedicel–stem junction surface (arrowheads point). Scale bars: 200 μm in (A) and (B), 50 μm in (C) and (D), 100 μm in (E) and (F).

pedicels of *pro35S::KNAT1* plants were insensitive to the 3-D clinorotation (Fig. 8B, C) and consistent with the hypothesis that *KNAT1* might be involved in the response of pedicels to 3-D clinorotation.

Discussion

In this study, the response of pedicels of plants grown on a rotating 3-D clinostat was analysed. The findings were as follows. First, pedicel positioning was affected by altered gravity, and the ability of the pedicels to respond to the 3-D clinorotation was dependent on the presence of developing seeds in the silique. Secondly, the development of vascular bundles of pedicels was also influenced by the 3-D clinorotation. Thirdly, the elongated branch point cells, in which expression of *KNAT1* was drastically reduced in the 3-D clinorotated plants, might contribute to the pedicel response to altered gravity.

The growth response of plants to altered gravity conditions has been shown to be plant specific, yielding different architectural phenotypes, such as ‘trailing plants’ (Digby and Firn, 1995), ‘hooked flower stalks’ (Kohji *et al.*, 1981, 1995), and altered apical hooks of dicotyledonous seedlings (Myers *et al.* 1994). Common to all of these growth responses is that they involve the main shoot–root axis. In contrast, little is known about how altered gravity forces affect lateral branch and silique formation. The present study provides new evidence to support the hypothesis that the gravitropic response of siliques is also subject to developmental regulation. In particular, the data indicate that the ability of a pedicel to respond to 3-D clinorotation depends on the presence of developing seeds in the silique. In *A. thaliana*, the pedicel response to altered gravity can be divided into three phases related to the development stage of the seeds in the siliques. The first phase coincides with the initial elongation of the siliques and the earliest stages of embryo development. Such siliques demonstrated plagiogravitropism, but the pedicel growth angle did not change in response to 3-D clinorotation growth conditions. During the second phase, the siliques elongated to maximum length as their seeds expanded and differentiated, and during this growth period the pedicels exhibited a characteristic growth response to 3-D clinorotation. During the last phase, which coincided with the embryo becoming metabolically quiescent and tolerant to desiccation, the pedicel lost its ability to respond to altered gravity.

To determine whether the weight of the seeds influenced the pedicel response, the orientation of similar weight siliques of 3-D clinorotated and control plants was compared. This experiment demonstrated that under 1 g control growth conditions the average silique exhibited an upward growth direction, whereas the siliques of plants grown on the 3-D clinostat grew in a downward direction. In contrast, when plants were grown in a 2-D vertical clinorotation condition (Wang *et al.*, 2006) the angles of pedicels with siliques at the same developmental stage and weight were not statistically different from those of the stationary control (Supplementary Fig. S1 at *JXB* online). However, the growth angles of pedicels under the 2-D horizontal rotation condition were apparently larger than those in both the vertical and stationary condition (Supplementary Fig. S1). This result was consistent with the larger growth angles of pedicels in the 3-D clinorotated condition (Figs 1B, 2). Thus, the enlargement of the pedicel growth angle under the 2-D or the 3-D clinorotation conditions was a gravitational response.

In addition, the gravitational response of pedicels with a silique containing unfertilized, aborted seeds produced by the removal of the anthers during flower development was studied. The pedicels of these empty siliques did not respond to the 3-D clinorotation. It was concluded that the aborted seeds not only led to a reduction in silique weight but also blocked some putative pathway involved in controlling the direction of silique growth. Young developing organs, such as young leaves and young fruits, are

Fig. 7. Influence of 3-D clinorotation on the expression pattern of *KNAT1* in the node region. Histochemical localization of GUS activity in *proKNAT1::GUS Arabidopsis* transformants grown under the 1 *g* control (A–C and G–J) and 3-D clinorotated condition (D–F and K–N). C and F are detailed images of the boxed areas of A, B, D, and E, respectively. (G–J) Serial hand-cut cross-sections through the node region of a control inflorescence stem. (K–N) Serial hand-cut cross-sections through the node region of a 3-D clinostat-treated inflorescence stem. Scale bars: 6 mm in (A) and (D), 400 μ m in (B) and (E), 50 μ m in (C) and (F), 200 μ m in (G–N).

regarded as the primary sites of auxin synthesis, which influences xylem generation in the stem internodes and the pedicels (Jacobs, 1952; Dražeta *et al.*, 2004). In this study, the number of xylem vessel cells of the two lateral vascular bundles in the basal region of the pedicel was not significantly affected by the 3-D clinorotation, whereas the xylem vessel cells in the middle vascular bundles, especially in the adaxial vascular bundle of the 3-D clinorotated plants, appeared earlier than those of the 1 *g* control plants. This result is in agreement with the effect of the 3-D clinorotation on vessel development in woody stems of *Prunus jamasakura* (Yoneyama *et al.*, 2004). Pedicel ontogeny in control plants revealed that the abaxial side vessel element was developmentally more advanced than the corresponding elements on the adaxial side of 3–5 DAF stage pedicels, and at this stage became sensitive to both 3-D and 2-D clinorotation. At maturity, the loss of pedicel response to altered gravity coincided with the loss of visible adaxial–abaxial asymmetry. The asymmetry of the adaxial and abaxial vascular bundles of middle developmental stage pedicels might be one of the primary determinants for the upward-pointing pedicels of *Arabidopsis* plants grown under normal gravity conditions. If this were the case, the clinostat-induced perturbation of the asymmetrical develop-

ment of adaxial and abaxial vascular bundles might be responsible for the observed alterations in pedicel angles.

KNAT1 was reported to have a function in controlling pedicel growth angle, and loss of *KNAT1* produces downward-pointing pedicels (Douglas *et al.*, 2002; Venglat *et al.*, 2002; Douglas and Riggs, 2005). In this study, it was found that reduced expression of *KNAT1* in the branching point cells of the 3-D clinorotated inflorescence stem was correlated with an increase in size of these cells. This change in size of the branching point cells might be important for controlling the angle of pedicel growth. Interestingly, the angles of *pro35S::KNAT1* transformants did not show significant changes when the plants were exposed to the 3-D clinorotation condition. This result suggests that the expression of *KNAT1* in branch point cells might control the developmental state of these cells, and *KNAT1* expression could be modified by the simulated microgravity.

Because the other class I *KNOX* genes have a similar expression pattern to *KNAT1* (Lincol *et al.*, 1994; Pautot *et al.*, 2001; Lenhard *et al.*, 2002; Dean *et al.*, 2004), the expression of the other *Arabidopsis* class I *KNOX* genes, such as *STM*, *KNAT2*, and *KNAT6*, was also examined in the node region. The data showed that the expression of these genes was not influenced by the 3-D clinorotation

Fig. 8. *KNAT1* expression in the node region of the main inflorescence stems of both *Arabidopsis* wild-type (WT) and *KNAT1*-overexpressing (*pro35S::KNAT1* transformants) plants grown under normal gravity (1 *g*) or 3-D clinorotation conditions. The relative expression of *KNAT1* in node regions of the WT (A) and the *pro35S::KNAT1* transformant (B) inflorescence stems were detected using real-time PCR. (C) Distribution of pedicel angles of *pro35S::KNAT1* transformants under both the 1 *g* control and the 3-D clinorotated conditions. The pedicel angles were read as described in Fig. 2. Data were obtained from three repeats; each repeat contains ~40 pedicels from four plants. Bars indicate the SD. ****P*<0.001.

treatment (Supplemental Fig. S3 at *JXB* online). Thus, these other class I *KNOX* genes might not take part in regulating the gravity-dependent growth angle of the pedicels.

Supplementary data

Supplementary data are available at *JXB* online.

Figure S1. Distribution of pedicel angles of *Arabidopsis* plants grown on a 2-D clinostat. To investigate whether the enlargement of the pedicel growth angle under clinorotation conditions is a mechanical stimulation response or a gravitational response, a 2-D clinorotation treatment in this study consisted of a clinostat orientated horizontally (H, simulated weightlessness), and a vertically oriented clinostat (V, clinostat control) was used. The distribution of pedicel angles of vertically rotated plants was significantly different from the distribution of the horizontally rotated plants,

which were similar to that of the stationary control. The pedicel angles of stationary and vertically rotated control plants varied from 30 ° to 50 °, whereas those of the horizontally rotated plants were closer to 90 °.

Figure S2. Vascular development in the basal region of pedicels. Sections through the basal region of pedicels showed that the vascular pattern changed as the pedicel developed. Vascular bundles in younger pedicels (e.g. from P11 to P15) consisted of two lateral bundles and one abaxial bundle. The adaxial bundle appeared and was smaller than the abaxial bundle in the pedicel until 4–5 DAF (e.g. P8 and P5). Sections through the basal region of a mature pedicel (e.g. P3) revealed a typical radial tissue pattern of stems with two lateral bundles, one abaxial bundle and one adaxial bundle.

Figure S3. Other class I *KNOX* genes expressed in the nodal region of *Arabidopsis* plants grown under 3-D clinostat rotational conditions. Reverse transcription-PCR characterization of class I *KNOX* gene expression in nodal region of pedicels. The results demonstrate that the expression of *STM*, *KNAT2*, and *KNAT6* in the basal region of the pedicels of control and of siliques devoid of seeds was similar to their expression in 3-D clinorotated normal and seedless pedicels. The PCR primers are presented in Supplementary Table S1.

Figure S4. Quantitative characterization of cells in tissues of the pedicel basal region of *Arabidopsis* plant grown under 3-D clinostat rotational conditions. Light micrographs of longitudinal sections through the basal region of pedicels of the control samples and the 3-D clinorotated plants were selected. The cell walls of the tissues in these micrographs were traced to highlight the cell size, and the cross-sectional area of epidermal, cortical, and branch point cells in the sections was measured using Image J 1.42q software (<http://rsbweb.nih.gov/ij>). The results show that the 3-D clinorotation does not influence the size of cells in tissues of the pedicel basal region except that of branch point cells (bpcs). To confirm this result, the average volume of bpcs was measured and calculated using another method described by Steer (1981). The results also indicate that the volume of the bpcs apparently increased under the 3-D clinostat rotation condition in comparison with the controls.

Table S1. RT-PCR primers.

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