Gravimorphogenesis of Cucurbitaceae plants: Development of peg cells and graviperception mechanism in cucumber seedlings

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Abstract We examined the effect of microgravity on the peg formation of cucumber seedlings for clarifying the mechanism of gravimorphogenesis in cucurbitaceous plants. The spaceflight experiments verified that gravity controls the formation of peg, hypocotyl hook and growth orientation of cucumber seedlings. Space-grown cucumber developed a peg on each side of the transition zone of the hypocotyl and root, indicating that on the ground peg formation is regulated negatively by gravity (Takahashi et al. 2000). It was found that the auxin-regulated gene, CS-IAA1, was strongly expressed in the transition zone where peg develops (Fujii et al. 2000). In the seedlings grown horizontally on the ground, CS-IAA1 transcripts were much abundant on the lower side of the transition zone, but no such differential expression of CS-IAA1 was observed in the space-grown cucumber (Kamada et al. 2000). These results imply that gravity plays a role in peg formation through auxin redistribution. By the negative control, peg formation on the upper side of the transition zone in the horizontally growing seedlings might be suppressed due to a reduction in auxin concentration. The threshold theory of auxin concentration accounted for the new concept, negative control of morphogenesis by gravity (Kamada et al. 2000). Anatomical studies have shown that there exists the target cells destined to be a peg and distinguishable at the early stage of the growth. Ultra-structural analysis suggested that endoplasmic reticulum develops well in the cells of the future peg. Furthermore, it was found that reorganization of cortical microtubules is required for the change in cell growth polarity in the process of peg formation. The spaceflight experiment with cucumber seedlings also suggested that in microgravity positive hydrotropic response of roots occurred without interference by gravitropic response (Takahashi et al. 1999b). Thus, this spaceflight experiment together with the ground-based studies has shown that cucumber seedling is an ideal for the study of gravimorphogenesis, hydrotropism and their interaction. Although peg formation is seen specifically in cucurbitaceous seedlings, it involves graviperception, auxin transport and redistribution and cytoskeletal modification for controlling cell growth polarity. This system could be a useful model for studying important current issues in plant biology.

Key words: auxin, cucumber (Cucumis sativus L.), gravimorphogenesis (peg formation), hydrotropism, space-experiment (STS-95)

Objective and procedure

Objective

Most members of cucurbitaceous plants develop a protuberance called peg on the transition zone between the hypocotyl and root soon after germination (Fig. 1). The peg holds the lower seed coat during the emergence of the seedling from the seed coat (Fig. 2). Peg usually develops on the lower side of the transition zone when seeds were placed in a horizontal position for germination (Takahashi 1997a). When cucumber seeds were placed up side down at a certain time after imbibition, the seedlings ultimately develop a peg on each side of the transition zone (Wizzum and Gersani 1975, Takahashi 1997a). When seedlings were grown on a clinostat or vertically with the root tip down, they developed a peg on each side of the transition zone or became pegless (Takahashi 1997a). Its lateral positioning of a peg appeared to depend on gravity, although we did not know whether gravity was required for peg formation itself.

Thus, peg formation of cucurbitaceous seedlings is probably a species-specific gravimorphogenesis (Darwin and Darwin 1880, Wizzum and Gersani 1975, Takahashi and Suge 1988, Takahashi et al. 1993, Takahashi and Scott 1994, Takahashi 1997a). Following hypothesis for the gravity-regulated formation of peg in cucumber seedlings has been proposed. 1) Graviperception for peg formation

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by cucumber seedlings takes place in the bundle sheath cells that contain sedimentable amyloplasts. 2) The gravitropism causes an asymmetric distribution of auxin in the transition zone of the seedlings. 3) Cortical cells change their growth direction to become a peg tissue in response to the higher concentration of auxin on the bottom side of the horizontally growing seedlings. This process may involve alteration of microtubule arrangement in cortical cells (Kobayashi et al. 1999, Takahashi et al. 1999a). We conducted a spaceflight experiment in order to verify the hypothesis of the gravity-regulated formation of cucumber peg and to study the structural and molecular mechanisms of peg formation.

Preparation by ground experiments

Hypothesis: From the studies with clinostat, we hypothesized three possibilities: under microgravity conditions in space, cucumber seedlings 1) become pegless, 2) develop two pegs and 3) develop one peg on the concave side if the seedlings curve. If the seedlings become pegless, it implies that gravity is necessary for peg formation. If two or more pegs develop, gravity is not required for peg formation but controls the positioning of the peg. If one peg develops on the concave side in the curved seedling, then gravity may not directly regulate peg formation (Takahashi 1997a).

Cloning the auxin-regulated genes from cucumber and establishing their in situ hybridization: We have cloned several auxin-regulated genes from cucumber and determined the full sequences (Fuji et al. 2000). Then, the expression pattern of the cDNA was examined (Fuji et al. 2000). As a result, CS-IAA1 was found to reflect auxin concentration in the expression pattern. Transcript accumulation of CS-IAA1 was also examined by in situ hybridization and found to be greater in the transition zone between the hypocotyl and root where peg developed. In a horizontally placed seedling, CS-IAA1 transcript was much abundant in the lower side than the upper side of the transition zone. Since in situ hybridization with CS-IAA1 was likely useful for evaluating endogenous auxin concentration, we examined various factors such as fixation period and concentration of proteinase K important for the incorporation of CS-IAA1 probe and hybridization. From the results of those studies, it was suggested that in situ hybridization could be adopted for spaceflight experiment.

Orientation and re-arrangement of cortical microtubules (MTs): We could successfully observed MTs in cucumber seedlings (Kobayashi et al. 1999, Takahashi et al. 1999a). It was found that reorganization of MTs is required for peg formation (Kobayashi et al. 1999, Takahashi et al. 1999a). That is, MTs are originally oriented perpendicular to the elongating axis of the hypocotyl and root, but it must be reorganized just before outgrowth of the cortical cells. To examine MTs in the space-grown seedlings, we tested fixative and fixation period simulating the conditions of space experiment (Takahashi et al. 1999a).

We also prepared and tested the growth containers for BRIC, seed-holding materials and methods, water reservoir, watering system, seedling growth and peg development at different temperature and humidity conditions, photography and sampling methods for fixation and so forth.

Onboard procedure

Cucumber seeds were placed in two Petri-dishes enclosed in a half-BRIC-60, one Petri-dish contains rock wool and the other contains plastic foam (Fig. 3). Twenty-four cucumber seeds were placed in the rock wool medium and 36 in the plastic foam. Three BRICs (A, B, C), each contains those two Petri-dishes, were stored in a refrigerator
of the space shuttle before the launch. At 22:16 (completion time) of MET 0, three BRICs were transferred from the refrigerator to the cabin temperature. Seeds in the BRICs A, B, and C were watered in orbit at 0:51 of MET 1, 20:31 of MET 4, and 3:52 (all completion time) of MET 3, respectively. Those seedlings grown in the plastic foam container of BRIC A, B and C were photographed and chemically fixed with glutaraldehyde or formaldehyde fixatives 29, 30 and 70 h after imbibition. The chemically fixed samples together with the seedlings of the rock wool containers were refrigerated for return to the earth. Using the cabin temperature of SpaceHab, we conducted ground control experiment with 24 h delay from the operations in orbit.

Recovered samples
We made morphological observation of all seedlings grown in orbit and photographed them by still camera and under stereomicroscope (Takahashi et al. 1999b, 2000, Kamada et al. 2000). Germination and growth of the seedlings differed among individuals, but we did obtain seedlings at different stages of growth from BRIC A, B and C as we expected. Seedlings grown in BRIC C for 70 h developed two pegs as in the ground control seedlings grown vertically. In space, seedlings did not grow straight but curved or nutated during the growth due to a factor other than gravitropism. But, they still developed two pegs regardless of the seedling curvature. Roots of the seedlings grown in plastic foam elongated longer than those in rockwool, but the developed pegs were smaller than those in rock wool. Seedlings in rock wool showed much greater radial growth and developed larger pegs. Hypocotyl hook of the space-grown seedlings was less developed than that of the ground control. Primary roots of the space-grown seedlings in plastic foam container curved, and their lateral roots grew toward the side of wet plastic foam. Seedlings grew faster in orbit compared with those of the ground control. This difference in growth was probably due to the difference in temperature. That is, HOBO thermo-recorder in BRIC showed much higher temperature than cabin temperature used for the ground control experiment. Nine plants grown in rock wool and recovered in refrigerated
(three plants each from BRIC A, B and C) were brought to our laboratory of Tohoku University. Remaniders of the rock-wool-grown seedlings were chemically fixed with glutaraldehyde and shipped to our laboratories in Japan. Some of the fixed samples with formaldehyde were also shipped to Japan, and the remainder was dehydrated and embedded in paraffin blocks before shipping. During the shipment, samples in fixatives or paraffin were kept refrigerated. We also conducted the ground control experiments in our laboratory of Tohoku University using the temperature recorded in the BRICs during spaceflight.

**Morphogenesis controlled negatively by gravity**

When cucumber seeds were germinated in rock wool, root lengths (mean ± SD) of the space-grown seedlings at stages A, B and C were 2.9±1.2, 3.3±1.7 and 17.9±9.0 mm, respectively. Hypocotyl length at stage C was 6.1±9.2 mm in average, but seedlings at stages A and B did not show significant elongation of hypocotyl (Takahashi et al. 2000). In an aeroponic culture with wet plastic foam, root lengths of the space-grown seedlings at stages A, B and C were 0.3±0.3, 1.3±0.8 and 20.9±15.4 mm, respectively. These seedlings under aeroponic conditions did not show significant elongation of hypocotyls (Kamada et al. 2000). Seedling growth of the space-grown cucumber was similar to that of the ground control. The rate of seed germination under both space and ground conditions were greater than 96%.

We did obtain seedlings at three different stages of growth; 1) seedlings at stage A did not show the initiation of peg, 2) at stage B seedlings had initiated their peg that could be seen under stereomicroscope, and 3) seedlings at stage C had completed peg formation. When seeds were germinated in a horizontal position, a peg developed on the concave side of the bending transition zone (Fig. 4). Seedlings grown in a vertical position with the root tips down developed a peg on each side of the transition zone (Fig. 4). Morphological feature of space-grown seedlings of cucumber was similar to that of the vertically grown seedlings on the ground (Fig. 4). That is, 92% of the seedlings in rock wool and 81% of the seedlings in plastic foam developed a peg on each side of the transition zone (Takahashi et al. 2000, Kamada et al. 2000). These results demonstrate that gravity is not necessarily required for peg formation in cucumber seedlings and that cucumber seedlings potentially develop a peg on each plane of the cotyledons. On the ground, however, cucumber seedlings develop a peg on the concave side of the bending transition zone because peg formation on the convex side is inhibited in response to gravity. This inhibition of peg formation due to gravity is considered to be a morphogenesis controlled negatively by gravity (Takahashi et al. 2000, Kamada et al. 2000).

Primary roots and hypocotyl grew straight downward and upward, respectively, on the ground. On the other hand, number of seedlings showed bending growth of primary roots and hypocotyl, which could occur due to nutritional...
movement, hydrotropism or physical contact in the absence of gravitropic response (Takahashi et al. 2000, Kamada et al. 2000). Nevertheless, those seedlings still developed two pegs symmetrically. These results suggest that bending growth is not the cause for the lateral positioning of the peg although it usually develop on the concave side of the gravitropically bending transition zone. Because bending growth of the space-grown seedlings was observed on photographs taken by the crew 30 h or 70 h after imbibition, however, we could not conclude whether the bending occurred during or after peg initiation.

**Hydrotropic response of roots in microgravity**

On the ground, primary roots showed positive orthogravitropism and grew downward when grown aeroponically. Lateral roots of the ground control grew perpendicular to the primary roots growing down (Fig. 5). Primary roots of the space-grown seedlings were not straight but grew away from the wet plastic foam in humid air (Takahashi et al. 1999b, Kamada et al. 2000). Surprisingly, the lateral roots of cucumber seedlings grew toward the wet plastic foam in microgravity (Fig. 5). The deviated angle of the lateral root from the primary root was much smaller than 90 degrees. Namely, the deviated...
angles (mean±SD) for the space-grown seedlings and the ground control were 49.3±15.4 and 94.0±16.2 degrees, respectively. The directional growth of the lateral roots on the ground is considered to result from the diagnostropic response.

It has been known that seedling roots show positive hydrotropism in response to a moisture gradient (Jaffe et al. 1985, Takahashi, 1994, 1997b, Takahashi et al. 1996). Because there could be a moisture gradient in the container, we examined whether the hydrotropic response was attributable to the directional growth of the lateral roots in microgravity. For that purpose, we prepared containers having cucumber seeds in the crack of the wet plastic foam and placed them on a three-dimensional clinostat. In the stationary control, the angle between primary root and lateral root was approximately 90-100 degrees, whereas in the clinorotated seedlings it was approximately 40-50 degrees (Figs 5, 6). Lateral roots developed on the proximal region of the primary root, closer to the wet plastic foam, showed greater

Fig. 5 Cucumber seedlings grown aeroponically in microgravity and on the ground. For aeroponical culture of cucumber seedlings, we glued three blocks of water-absorbable plastic foam (45 mm x 10 mm x 10 mm) onto the inner surface of the cap of the plastic container (60 mm x 60 mm). Thirty-six (12 onto each block) seeds were inserted into cracks of the plastic foam, and the cap with the seed-holding plastic foam sealed the container. In this aeroponic culture, it was expected that roots would grow into an aerial space in the container. In orbit, cucumber seeds were imbibed by watering 10 mL distilled H2O to a block of water-absorbable plastic foam. Ground control experiments were conducted by using temperatures recorded by the HOBO every hour during the experiments in orbit. When seedlings were clinorotated on the ground, the plastic containers containing cucumber seeds in the water-absorbable plastic foam were placed on the three-dimensional clinostat and rotated at 1 rpm. A, seedlings grown for 96 h in a stationary condition on the ground. B, clinorotated seedlings for 96 h on the ground. C, seedlings grown for 70 h in microgravity. Arrowhead indicates the lateral root. g, direction of gravitational force. (J. Plant Res. 112: 497-505, 1999)

Fig. 6 Directional growth of the lateral roots developed on different position on the primary roots of cucumber seedlings. Cucumber seedlings were aeroponically grown. The y axis indicates the deviated angle of the lateral root from the axis of the primary root. The x axis indicates the position of the lateral root numbered from the proximal of the primary root. (J. Plant Res. 112: 497-505, 1999)
curvature toward the wet plastic foam with smaller angle between primary root and lateral root (Fig. 6). Thus, clinorotation of cucumber seedlings successfully mimicked the directional growth of the lateral roots in microgravity. We also confirmed a moisture gradient between wet plastic foam glued to the ceiling of the container and the bottom. We assume that such a moisture gradient might be maintained in microgravity because of the lack of convection. When cucumber seedlings were entirely grown in wet rock wool, orientation of lateral root growth on clinostat was virtually the same as that of the stationary control (Fig. 6). When the intensity of moisture gradient was decreased, by placing wet filter paper or wet rock wool onto the inner surface of the container, the angle between primary root and lateral root of the clinorotated seedlings became much greater (Fig. 7).

Taken together, these data strongly implied that in microgravity the lateral roots of cucumber seedlings positively responded to a moisture gradient and showed hydrotropism. Our spaceflight experiment demonstrated that on the ground hydrotropic response of roots is always interfered by gravitropic response. Hydrotropic response of primary roots of space-grown seedlings was not evident in our spaceflight experiments. However, it was found that the primary roots also became sensitive to a moisture gradient showing positive hydrotropism on clinostat when roots were placed parallel to the wet plastic foam in the container. Because primary roots initiated to grow parallel to the direction of a moisture gradient in microgravity, the root cap possessing a sensory apparatus in the primary root might be unable to perceive the moisture gradient. However, lateral roots on the primary root initiated to grow transversely to the direction of the moisture gradient so that the root cap was hydrotropically stimulated. Microgravity in space provides us with a suitable experimental environment for the study of hydrotropism in roots.

Expression pattern of an auxin-inducible gene, CS-IAA1

To estimate the localization of endogenous auxin in the transition zone between hypocotyl and root, we isolated the auxin-inducible genes from cucumber seedlings (Fujii et al. 2000). Characterization and expression analysis of these genes revealed that the cDNA of CS-IAA1 could be used as a maker gene for the distribution of endogenous auxin, indole-3-acetic acid (Fujii et al. 2000). Its expression depended on the level of auxin concentration (Fig. 8). We
also established the *in situ* hybridization method to analyze the expression pattern of *CS-IAA1* in cucumber seedlings (Fujii *et al.* 2000), which led us to successful achievement of spaceflight experiment on the studies of auxin distribution in microgravity (Kamada *et al.* 2000). Without *in situ* hybridization, we would not be able to know the distribution pattern of endogenous auxin in cucumber seedlings under microgravity conditions. That was because crew time available for the experiment was strictly limited, and because the crew was not allowed to excise the tissues for collecting a number of materials from different parts of the seedlings enough for the extraction of endogenous auxin in orbit. Instead, the crew fixed whole seedlings chemically in orbit for the *in situ* hybridization of *CS-IAA1*.

The results of the expression analysis of *CS-IAA1* showed that its mRNA accumulated around the transition zone just prior to or during the initiation of peg formation (Kamada *et al.* 2000). When cucumber seedlings completed peg formation, we hardly detected the accumulation of *CS-IAA1* mRNA around the transition zone, and its accumulation was abundant in the elongating regions of the hypocotyl and roots (Fujii *et al.* 2000, Kamada *et al.* 2000).

Space-grown cucumber seedlings at stages A and B showed accumulation of *CS-IAA* mRNA all around the transition zone (Kamada *et al.* 2000). The signal was much stronger in the epidermal region. This accumulation pattern of *CS-IAA1* was similar to that of the vertical control on the ground. In the horizontal control, accumulation of *CS-IAA1* mRNA on the lower epidermis of the transition zone appeared to be the same as that of the space-grown seedlings or greater. However, its accumulation in the upper epidermal region was much less compared to that of space-grown seedlings or the vertical control. There was an asymmetric accumulation of *CS-IAA1* mRNA between the lower and the upper cortical regions of the horizontally placed transition zone. It was evident that the expression of auxin-inducible gene was much less in the upper half than that of the lower half. This differential accumulation of *CS-IAA1* mRNA in the transition zone was also confirmed in the analysis by Northern blotting (Kamada *et al.* 2000). Compared with the accumulation in the vertical control, *CS-IAA1* mRNA accumulation increased to approximately 7 fold in the lower side of the horizontal transition zone and decreased to approximately 0.7 fold in the upper side. The difference of the mRNA accumulation between the upper and lower sides of the horizontal transition zone was therefore approximately 10 times. Our spaceflight experiment verified for the first time that auxin did not redistribute in microgravity.

The results of the gene expression analysis of cucumber seedlings grown in space and on the ground suggested an

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**Fig. 9** Threshold model for auxin level in inducing peg formation. In the horizontally placed seedlings, auxin concentration on the upper region of the transition zone decreases below the threshold value for peg formation. On the other hand, the lower region of the transition zone is engaged to maintain auxin level above the threshold. Numbers in parentheses indicate the rate of *CS-IAA1* mRNA accumulation. Numbers in square indicate the hypothesized percentage of auxin distribution between the upper and lower sides of the transition zone (Modified from Kamada *et al.* 2000).
existence of a threshold level of auxin required for peg formation (Kamada et al. 2000). This hypothesis (Fig. 9) was supported by the following result. Application of exogenous auxin to the seedlings in a horizontal position caused the development of a peg on each side (the lower and the upper) of the transition zone, while the control seedlings developed a peg only on the lower side of the transition zone (Kamada et al. 2000). As the concentration of auxin was increased, a collar-like protuberance developed around the transition zone, but a larger peg still developed on each plane of the cotyledons (Kamada et al. 2000). From the accumulation pattern of CS-IAA1 mRNA in cucumber seedlings grown in space, we could speculate that auxin enough for peg formation distributed all around the transition zone. However, space-grown seedlings developed a peg on each plane of the cotyledons. This result implies that tissue on the cotyledonary plane of the transition zone is much sensitive to auxin than the others in inducing peg formation.

Thus, cucumber seedlings potentially develop a peg on each side of the transition zone. On the ground, however, the development of one peg on the upper side of the transition zone is inhibited in response to gravity because the graviresponse leads to a reduction of auxin level below the threshold for peg formation. On the other hand, the lower side of the horizontally placed transition zone is engaged to develop a peg because of more accumulation of auxin. This negative control of peg formation by gravity is therefore involved with the gravity-induced redistribution of auxin in the transition zone. As has been discussed by Link and Cosgrove (1999), however, whether gravity plays positively or negatively for peg formation may be determined by a total amount of auxin available in the transition zone. If the auxin concentration is less than the threshold level, gravity plays only positively by increasing the auxin level on the lower side of the horizontal transition zone. If auxin concentration is above the threshold level as in our spaceflight experiment, the negative control by gravity plays a role in the inhibition of peg formation on the upper side, while peg formation on the lower side occurs due to the positive control by gravity.

Changes in cytological and cytoskeletal structure for gravimorphogenesis

It was found that there exists specific structure of epidermal cells where peg develops eventually; that is, more development of endoplasmic reticulum (ER) in the region of peg initiation. It was also found that cell division takes place both in epidermis and cortex at the early stage of peg development but not in those of non-peg side of the transition zone. The region of the future peg was distinguishable by observing the epidermal cells at the early stage of growth under a scanning electron microscope. Those structural features were retained in both space-grown

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Fig. 10 A new concept for the gravity-regulated formation of peg in cucumber seedlings. Morphogenesis is negatively controlled by gravity.
and ground control seedlings.

Cortical microtubules are considered to regulate the direction of cell expansion via oriented position of cellulose microfibrils in the cell wall. In the present study, therefore, orientation of cortical microtubules was examined during peg development using cucumber seedlings grown in microgravity and on the ground. The results showed that in cucumber seedlings grown in a horizontal position, cortical microtubules of the lower transition zone re-oriented perpendicular to protrusion of the developing peg. Cortical microtubules of the upper transition zone persisted transversely to the axis of a seedling. In the seedlings grown vertically on the ground or the space-grown seedlings, cortical microtubules in many cells re-oriented random or parallel to the axis of a seedling in both sides of the transition zone. These results support the idea that re-orientation of microtubules is involved in signal transduction pathway of gravimorphogenesis in cucumber seedlings.

A model for the mechanism of gravimorphogenesis in cucumber seedlings

Based on the results of the spaceflight and ground experiments, we hypothesized the mechanism for the gravimorphogenesis (peg formation) in cucumber seedlings as follows (Fig. 10).

1) Cells of vascular bundle sheath in the transition zone perceive gravity by sedimenting amyloplasts.

2) The gravistimulation causes redistribution of auxin on the transition zone. On the lower region of the transition zone in the horizontally growing seedlings, auxin level increases promising the development of a peg.

3) Auxin at the concentration above the threshold causes an alteration of microtubule arrangement in the transition zone. This re-organization of microtubules is required for the change in cell growth polarity for peg formation. Cell division and development of ER in the epidermis or cortical cells may somehow play important roles in peg formation.

4) In contrast, gravistimulation causes a decrease in auxin level on the upper region of the transition zone in the horizontally growing seedlings. Then, the upper region of the transition zone cannot maintain auxin level above the threshold for peg formation.

5) Thus, cucumber seedlings potentially develop two pegs and do not require gravity for peg formation itself, but on the ground the development of one peg is suppressed in response to gravity. This may be considered as negative control of morphogenesis by gravity. The decrease in auxin level is possibly a key factor responsible for the gravity's negative control of morphogenesis.

6) The gravity-regulated auxin transport/auxin redistribution and the auxin-regulated re-orientation of microtubules as well as graviperception mechanism need to be clarified in future studies.

Conclusion

Our spaceflight experiment with cucumber revealed the morphogenesis that is negatively controlled by gravity. The gravity's negative control of morphogenesis involves with the level of auxin whose distribution is obviously regulated by gravity. This concept, negative control by gravity, may account for other aspects of plant growth and development such as gravitropism (Kamada et al. 2000). It is noteworthy that isolation and in situ hybridization of an auxin-inducible gene brought about the successful analysis and results of the spaceflight experiment, which provides us with a hint for studying the gravity's effect on auxin transport, the auxin-regulated growth polarity and future spaceflight experiments.

A surprising finding, positive hydrotropism of roots in microgravity, verified on that the ground gravitropic response strongly interferes with hydrotropism. The results suggest that space environment is useful for the studies involving with hydrotropic sensitivity and its interaction with gravitropism. As we have demonstrated, gravitropic mutants and clinostat are certainly useful for simulating plant growth in microgravity although we must pay careful attention to the different aspects and effects brought by clinostat and microgravity conditions.

Gravimorphogenesis, peg formation, is seen specifically in the seedlings of cucurbitaceous plants. However, this unique system may provide a clue to answer important questions in plant physiology and space biology.

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