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STS-95 space experiment for plant growth and development, and auxin polar transport

Junichi Ueda[1], Kensuke Miyamoto[1], Tomokazu Yuda[1], Tomoki Hoshino[1], Keiko Sato[1], Shuhei Fujii[2], Shigeki Kamigaichi[3], Ryutaro Izumi[3], Noriaki Ishioka[3], Sachiko Aizawa[3], Izumi Yoshizaki[3], Toru Shimazu[4] and Keiji Fukui[4]

[1] College of Integrated Arts and Sciences, Osaka Prefecture University, 1-1 Gakuen-cho, Sakai, Osaka 599-8531, Japan
[2] Tezukayama College, 3-1-3 Gakuenminami, Nara 631-8585, Japan
[3] National Space Development Agency of Japan, 2-1-1 Sengen, Tsukuba 305-8505, Japan
[4] Japan Space Forum, 1-29-6 Hamamatsu-cho, Tokyo 105-0013, Japan

Abstract The principal objective of the space experiment, BRIC-AUX on STS-95, was the integrated analysis of the growth and development of etiolated pea and maize seedlings in space, and the effect of microgravity conditions in space on auxin polar transport in the segments. Microgravity conditions in space strongly affected the growth and development of etiolated pea and maize seedlings. Etiolated pea and maize seedlings were leaned and curved during space flight, respectively. Finally the growth inhibition of these seedlings was also observed. Roots of some pea seedlings grew toward the aerial space of Plant Growth Chamber. Extensibilities of cell walls of the third internode of etiolated pea epicotyls and the top region of etiolated maize coleoptiles which were germinated and grown under microgravity conditions in space were significantly low. Activities of auxin polar transport in the second internode segments of etiolated pea seedlings and coleoptile segments of etiolated maize seedlings were significantly inhibited and extremely promoted, respectively, under microgravity conditions in space. These results strongly suggest that auxin polar transport as well as the growth and development of plants is controlled under gravity on the earth.

Key words: Auxin polar transport, Cell wall extensibility, Growth and development, Maize, Microgravity, Pea, *Pisum sativum*, Space, STS-95, *Zea mays*

Introduction (objective and background)

Plants have various kinds of mechanisms for the adaptation of environmental stimuli on the earth. Gravitropism of plants is considered to be one of the most important responses to adapt to the ground conditions on the earth. The cultivation of plants as a resource of foods and oxygen, and for psychological reasons will be one of the essential and fundamental factors for human being to be alive in space conditions in near future. Therefore, to make the effects of microgravity conditions in space on the growth and development of plants much clear is considered to be the most important subject. Though several studies concerning with the effect of microgravity conditions in space on some physiological responses of plants have already been done using spacecrafts (Brown *et al.* 1990, Musgrave *et al.* 1997, Volkmann *et al.* 1986), informations obtained from these space experiments seem not to be sufficient to make clear the growth and development of plants in space.

On the other hand, many studies using a clinostat have been made to achieve the objective of this kind of research on the earth. Results of these studies showed that simulated microgravity on a clinostat strongly affected the orientation and the quantity of the growth and development (Brown *et al.* 1976, Hoson *et al.* 1992, 1995, 1996, 1997). The growth and development of inflorescence axes of *Arabidopsis thaliana* observed under simulated microgravity conditions on a clinostat was concerned with the changes in their activities of auxin polar transport (Miyamoto *et al.* 1999, Oka *et al.* 1995, Ueda *et al.* 1996), in which auxin moves basipetally in plants. Moreover, auxin polar transport in etiolated pea epicotyls, and maize coleoptiles and mesocotyls was substantially influenced under simulated microgravity conditions on a 3-D clinostat (Shimazu *et al.* 2000, Ueda *et al.* 1999a). Etiolated pea and maize seedlings have also been shown to be suitable plant materials to investigate the effect of microgravity conditions in space on auxin polar transport (Ueda *et al.* 1997). These facts together with the results described above led us to plan the space experiment using a spacecraft to confirm that the auxin polar transport as well as the growth and development of plants might be accounted for one of physiological phenomena as a gravity-dependent response. The experiments were performed during the 25th flight of Space Shuttle, Discovery, of the National Aeronautics and Space Administration in October 29 to November 7, 1998, resulted in the successful achievement of the objective. In this paper, we report that microgravity conditions in space substantially affect auxin polar transport of etiolated pea

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Address for correspondence: Junichi Ueda
ueda@el.cias.osakafu-u.ac.jp

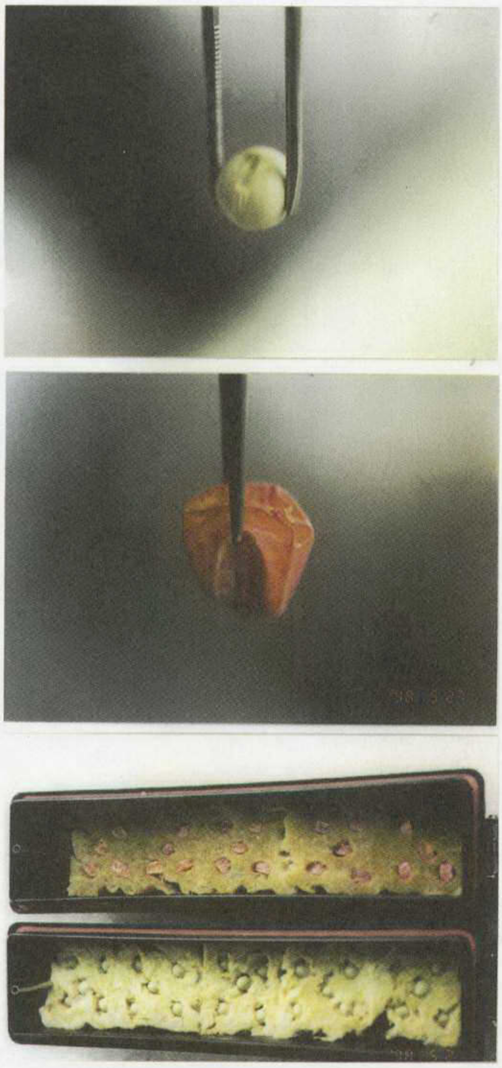

Fig. 1 Plant materials for space experiment (dry seeds of pea and maize).

and maize seedlings as well as their growth and development. We have also reported brief results of STS-95 space experiment (Ueda *et al*, 1999b).

The ground experiment

Growth and development, and auxin polar transport in higher plants under simulated microgravity conditions were studied using a 3-D clinostat. Pea (*Pisum sativum L.* cv. Alaska) and maize (*Zea mays* L. cv. Golden Cross Bantam) seedlings were grown in PGC (Plant Growth Chamber) actually used in space experiment for 6.5 days at 25 °C in the dark (Fig. 1). As shown in Fig. 2, the measurement of auxin polar transport was performed according to the method reported previously with minor modifications. Segments of the 2nd internode of etiolated pea seedlings or coleoptile of etiolated maize seedlings were prepared. Agar medium (0.9 %, 50 μl) containing radiolabeled auxin (1 μCi/ml, [^{14}C] indole-3-acetic acid) was prepared in 1.5 ml Eppendorf tubes. Radiolabeled auxin was incorporated into the apical or the basal side of the segment in the tube. After 22-h incubation, radioactivity of the opposite side (5 mm) of the donor side of the segment was directly determined using a liquid scintillation counter. This method has been considered to be suitable for the determination of auxin polar transport.

Growth of etiolated pea and maize seedlings was strongly affected by simulated microgravity conditions on a 3-D clinostat. Leaning and curvature of the seedlings on a 3-D clinostat were found in etiolated pea and maize seedlings. The growth of the seedling was finally promoted by simulated microgravity conditions on a 3-D clinostat due to the early emergence of the 3rd internode in pea seedlings and leaf in maize seedlings, respectively. In addition, almost no ethylene production was found in the seedlings grown under simulated microgravity conditions on a 3-D clinostat (data not shown). The activities of auxin polar transport in the segments of pea epicotyl (2nd

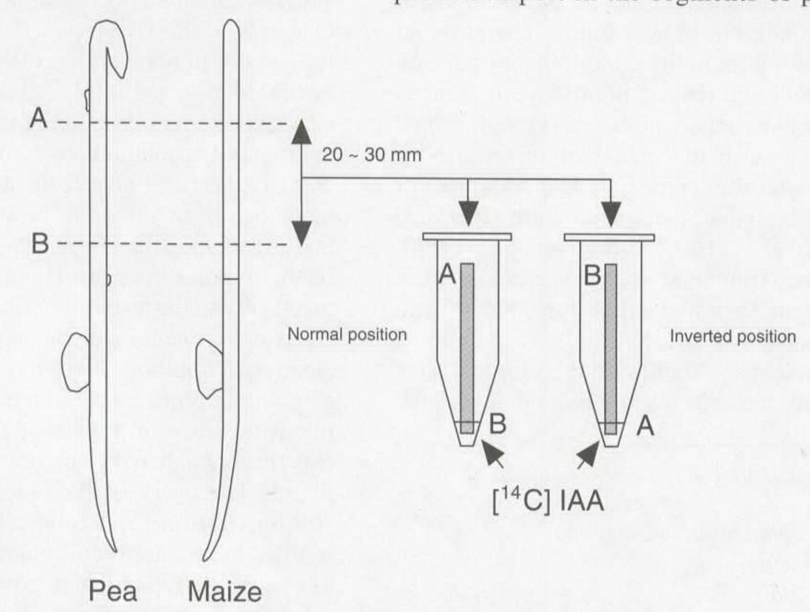

Fig. 2 Method for the determination of auxin polar transport

internode), and maize coleoptile and mesocotyl were significantly promoted by simulated microgravity conditions on a 3-D clinostat nevertheless seedlings have been grown either on 1g or under simulated microgravity. These results suggest that the responses of higher plants for microgravity conditions are different not only among the species of plants but also in their organs and/or tissues as well as auxin polar transport. We would like to confirm these results on the space experiment.

Materials and Methods

Outline of the space experiment

In order to achieve the objective of the space experiment as described in Introduction, following experimental design was planned as shown in Fig. 3.

(1) Auxin polar transport of the segments of etiolated pea epicotyls and maize coleoptiles excised from these seedlings grown on the ground of the earth (1 g) is determined on the ground (ground control).

(2) Auxin polar transport of these segments excised from the seedlings grown on the ground of the earth is determined under microgravity conditions in space (ground-flight operation).

(3) Auxin polar transport of these segments excised from the seedlings grown under microgravity conditions in space is determined under microgravity conditions in space. The growth and development of the seedlings is photographed during flight (flight-flight operation).

(4) Auxin polar transport of these segments excised from the seedlings grown under microgravity conditions in space is determined on the ground of the earth after landing of the space shuttle. Mechanical properties of cell walls of these seedlings were also determined (postflight operation).

Plant materials

Four PGCs (Plant Growth Chambers, Krikorian and Levine, 1991) were used in the space experiment. Thirty two dry seeds of pea (*Pisum sativum* cv. Alaska) and maize (*Zea mays* cv. Golden Cross Bantam) were set in dry rockwool (Minipot, Nittobo, Tokyo, Japan) and allowed to germinate after irrigating 180 ml of Distilled Water (GibcoBRL, Life Technology, N.Y.). PGC was kept in Ziploc Bag and the seedlings were grown in PGC at 25 °C in the dark for 6 days before launch of the space shuttle (PGC A for pea, PGC B for maize). Thirty two dry seeds of pea and maize were also set in dry rockwool in PGC C and D, respectively.

On-board procedures

Watering (180 ml of Distilled Water, GibcoBRL, Life Technology, N.Y.) to dry seeds of pea and maize in PGC C and D, respectively, was made in space conditions in orbit at MET (Mission Elapsed Time) O day. In order to determine the growth and development of the seedlings in PGC C and D, pictures were taken at MET 4 to 6 days. NASDA Stem Cutter, the special cutter developed by NASDA for safety of astronauts, was used for the preparation of epicotyl and coleoptile segments of etiolated pea and maize seedlings, respectively. Experiments in space were made on time according to the procedures described in BRIC EXPERIMENT OPERATION CHECKLIST. Detail schedule of the space experiment on-board has already been reported as STS-95 BRIC Experiments Quick Report (Ueda *et al.* 1999c).

(1) MET 0

6:47 - 7:15

Water (180 ml) was added to pea (PGC C) and maize (PGC D) seeds, respectively.

22:20 - 23:47

After taking pictures of etiolated pea (PGC A) and maize (PGC B) seedlings grown on the earth, the segments (3 cm) of the 2nd internode of pea seedlings and coleoptile of maize seedlings were excised. Radiolabeled auxin was applied to the apical or basal side of the segments in Eppendorf tubes. These tubes containing the segments were

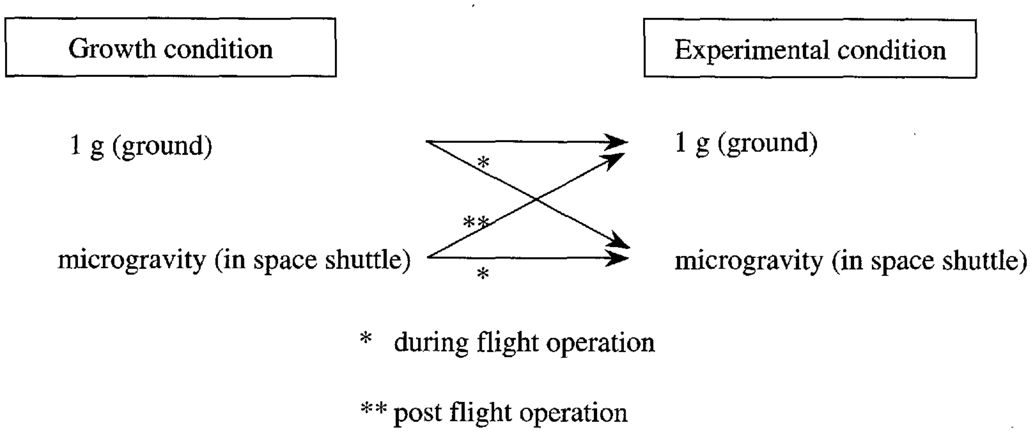

Fig. 3 Design of space experiments for IAA polar transport in etiolated pea and maize seedings

incubated in the locker of the Space Hab.

(2) MET 1

22:02 (completed)

Microtube holder containing the segments in Eppendorf tubes was frozen using liquid nitrogen in GN_2 freezer.

(3) MET 4 to 6

Photographs of the seedlings of etiolated pea and maize in PGC C and D were taken at 22:32 - 23:15 Met 4 day, 20:49 -21:26 Met 5 day and 22:20 - 22:50 Met 6 day, respectively.

(4) MET 7

After taking pictures of the seedlings at 1:45 - 3:01, feeding experiments of radiolabeled auxin were carried out using the segments of the seedlings. Experimental procedures were almost same as previous one using the seedlings in PGC A and B at Met 0 day.

(5) MET 8

00:31 (completed)

Microtube holder containing segments in Eppendorf tubes was transferred to GN_2 freezer containing liquid nitrogen.

(6) MET 10

02:50 (started)

After landing of the space shuttle, pictures of the seedlings were taken and then feeding experiments were made immediately using the segments of pea and maize seedlings grown under microgravity conditions in space (PGC C and D).

Control experiments on the earth

Control experiments were also carried out on time of the space flight and on 24-h delay using OES (Orbiter Environment Simulator) system.

Measurements of auxin polar transport

The measurement of auxin polar transport was performed according to the method reported previously with minor modifications (Oka *et al.* 1995, Ueda *et al.* 1996). The segments (30 mm length) of the 2nd internode of etiolated pea seedlings or coleoptile of etiolated maize seedlings were prepared using NASDA stem cutter (Fig. 4). Agar medium (0.9 %, 50 µl) containing radiolabeled auxin (1 µCi/ml, [^{14}C] indole-3-acetic acid, American Radiolabeled Chemicals Inc., St. Louis, Mo.) was prepared in 1.5 ml Eppendorf tubes. Radiolabeled auxin was incorporated into the apical or the basal side of the segment in the tube. After 22-h incubation, the tubes were directly frozen by liquid nitrogen in GN_2 Freezer. After landing of the space shuttle, radioactivities of the opposite side (5 mm) of the donor side of the segment was directly determined using a liquid scintillation counter. This method has been considered to be suitable for the determination of auxin polar transport (Oka *et al.* 1995, 1998, 1999, Okada *et al.* 1991, Ueda *et al.* 1996).

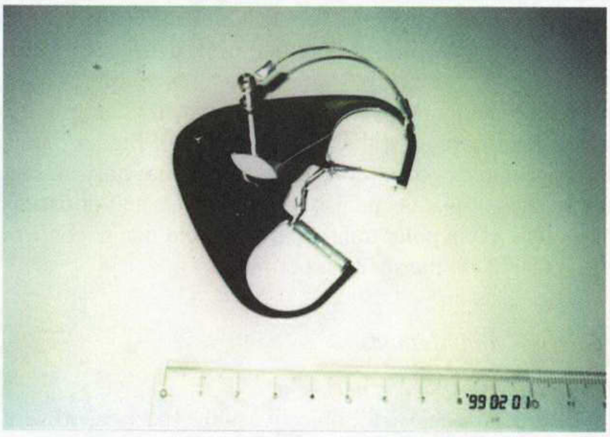

Fig. 4 NASDA stem cutter.

Determination of mechanical properties of cell walls

Just after landing of the space shuttle, pea and maize seedlings in PGC C and D as well as each ground control seedlings were immediately fixed using 80% ethanol on the ground until the determination of extensibilities of cell walls of pea epicotyls and maize coleoptiles, respectively. Extensibilities of cell walls were determined according to the method using a tensile tester (Hoson and Masuda 1991).

Results and Discussion

Middeck temperature during flight

As described above, there was no incubator in the shuttle. Plants in PGC and microtubes during the experiment of auxin polar transport were kept in middeck of the shuttle. Figure 5 shows the temperature of middeck of the shuttle. Just after launch, the temperature was a little bit low and gradually increased during flight. The difference in between the maximum and the minimum temperature was about 3 to 4 °C.

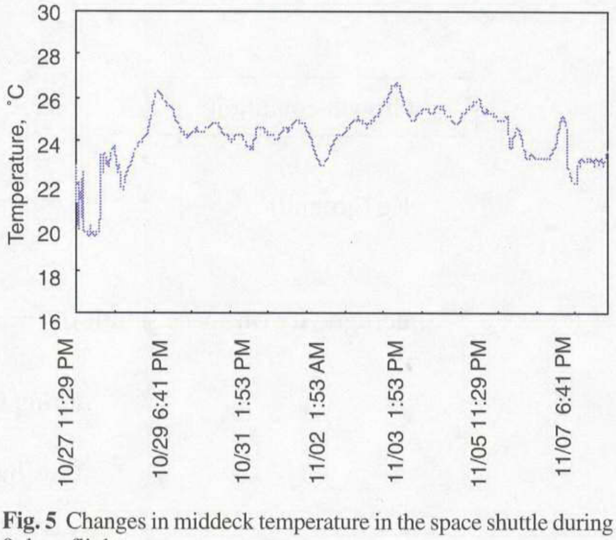

Fig. 5 Changes in middeck temperature in the space shuttle during 9 days flight.

Fig. 6 Etiolated pea seedlings grown on 1 g coditions (left) and under microgravity conditions in space (right) for 6 days.

Growth and development of plants under microgravity conditions in space

Microgravity conditions in space did not affect seed germination of pea and maize (data not shown), but strongly affected the orientation of stem elongation, and their growth and development. Judging from the pictures taken by the astronaut in orbit, seedlings leaned and/or curved were observed in PGC C and D during space flight (Figs. 6 to 9). Roots of some pea seedlings were also observed in the aerial space of PGC. Observation after landing of the space shuttle revealed that epicotyls of some pea seedlings grew toward into the rockwool in PGC. Coleoptiles of maize seedlings grew straight but mesocotyls curved at random in PGC.

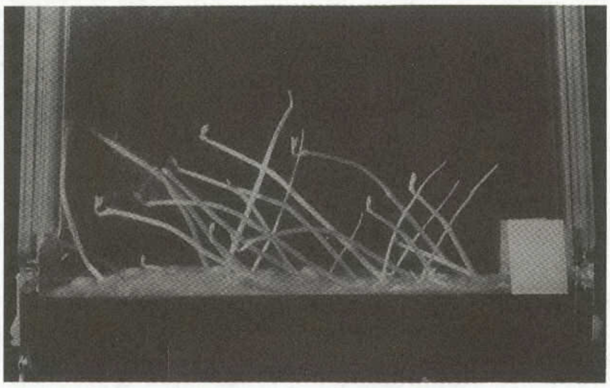

Fig. 7 Etiolated pea seedlings grown under microgravity conditions in space for 6 days.

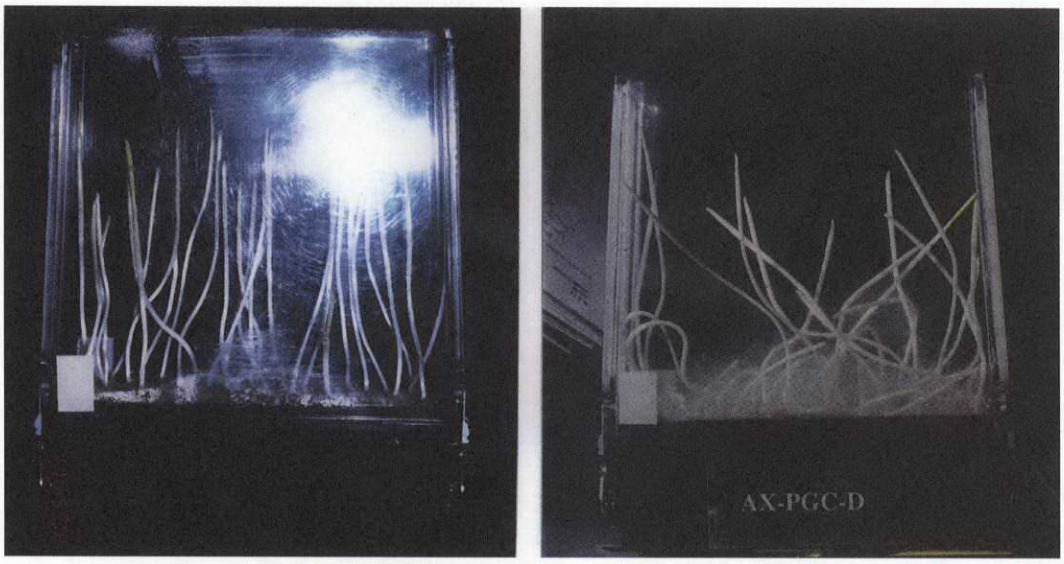

Fig. 8 Etiolated maize seedlings grown on 1 g conditions (left) and under microgravity conditions in space (right) for 6 days.

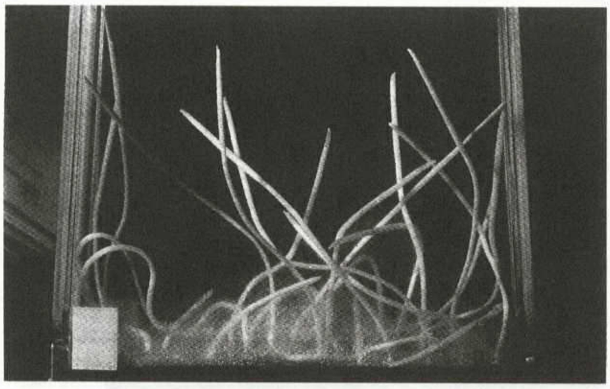

Fig. 9 Etiolated maize seedlings grown under microgravity conditions in space for 6 days.

The direction of the epicotyls of almost all pea seedlings was about 40 to 60 degrees from the vertical line, it being far from cotyledons (Fig. 10). This seems to be automorphosis of plants, which has already been found under simulated microgravity conditions on a 3-D clinostat (Hoson *et al.* 1992, 1995, 1996). Moreover, pea seedlings grown under microgravity conditions in space showed an interesting 2nd curvature in epicotyls. As shown in Fig. 11, the 2nd curvature in epicotyls increased together with hook opening in space conditions. Another differences were not found in the seedlings grown between under space and 1 g conditions.

The growth and development of etiolated pea and maize seedlings was strongly affected by simulated microgravity conditions on a 3-D clinostat (Ueda *et al.* 1999a). Leaning and curvature of the seedlings on a 3-D clinostat were also found, being similar to those in the space experiment. Moreover, the growth stage of the seedling was finally promoted by simulated microgravity conditions on a 3-D clinostat due to the early emergence of the 3rd internode in

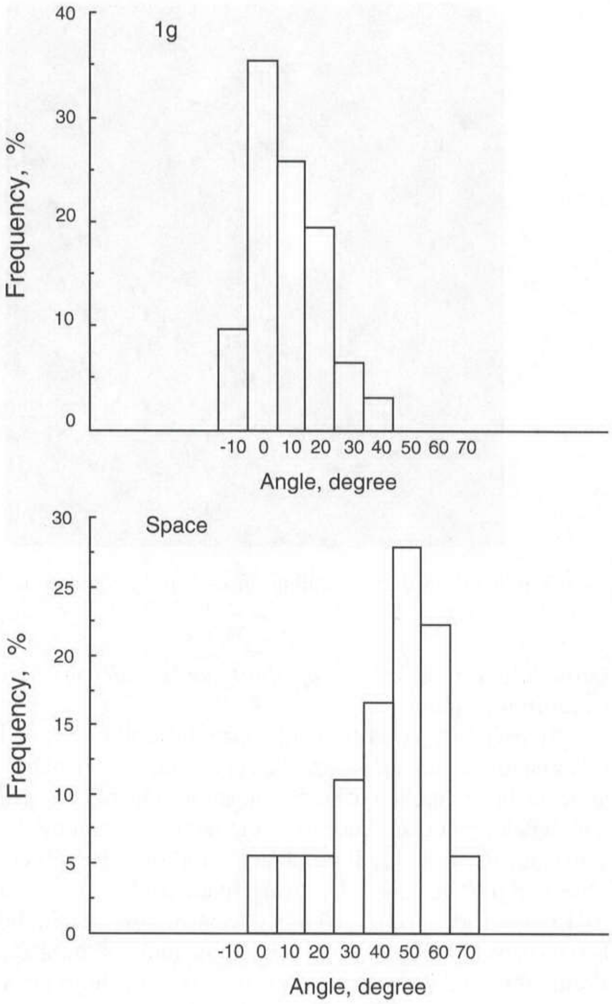

Fig. 10 Effect of microgravity conditions in space on the direction of growth of etiolated pea epicotyls. Seedlings were grown under microgravity conditions in space for 6 days at about 23 °C in the dark. Results were expressed as the direction of the vertical line is zero degree.

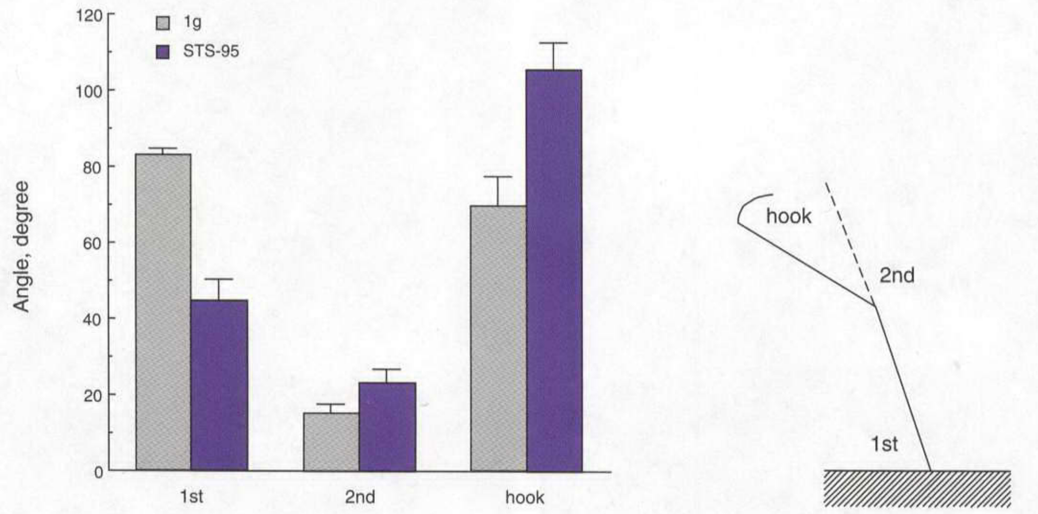

Fig. 11 Angles of etiolated pea epicotyls grown under space conditions for 6 days.

etiolated pea seedlings and leaf in etiolated maize seedlings, respectively. Microgravity conditions in space, however, did not affect the growth stage of etiolated pea and maize seedlings, but the elongation of epicotyls and mesocotyls during space flight, resulted in significant inhibition of growth (Figs. 12 to 19). These results indicate that the responses of higher plants to microgravity conditions in space are different not only among the species of plants but also in their organs and/or tissues.

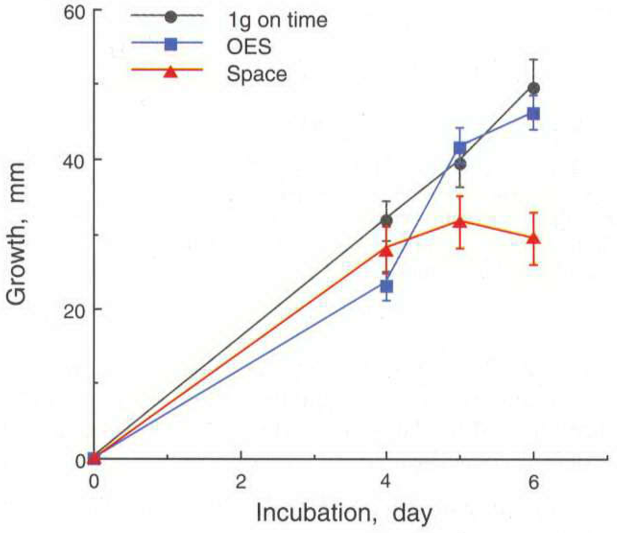

Fig. 12 Effect of microgravity conditions in space on the 1st internode growth of etiolated pea seedlings. Seedlings were grown under microgravity conditions in space for 6 days at about 23 °C in the dark. Results were expressed as the average with the standard error of the mean.

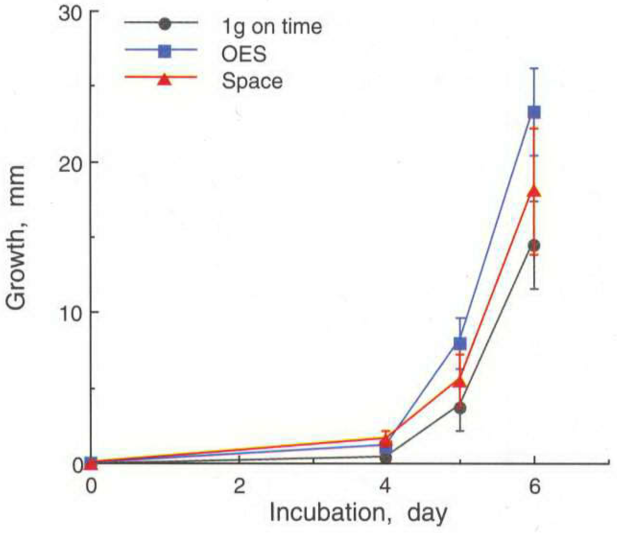

Fig. 13 Effect of microgravity conditions in space on the 2nd internode growth of etilated pea seedlings. Seedlings were grown under microgravity conditions in space for 6 days at about 23 °C in the dark. Results were expressed as the average with the standard error of the mean.

Mechanical properties of cell walls of pea epicotyls and maize coleoptiles

Pea and maize seedlings grown under microgravity conditions in space were fixed using 80% aqueous ethanol just after landing of the space shuttle. Pea epicotyls and maize coleoptiles were prepared for the determination of the mechanical properties of cell walls using a tensile tester. As shown in Table 1, the values of extensibility of the 1st and 2nd internodes of pea epicotyls and of the basal and the middle regions of maize coleoptiles were extremely low. There was no difference in these values between the

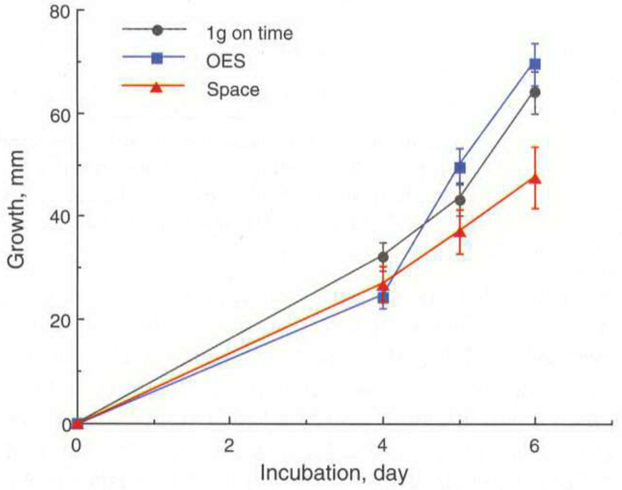

Fig. 14 Effect of microgravity conditions in space on the growth of etiolated pea seedlings. Seedlings were grown under microgravity conditions in space for 6 days at about 23 °C in the dark. Results were expressed as the average with the standard error of the mean.

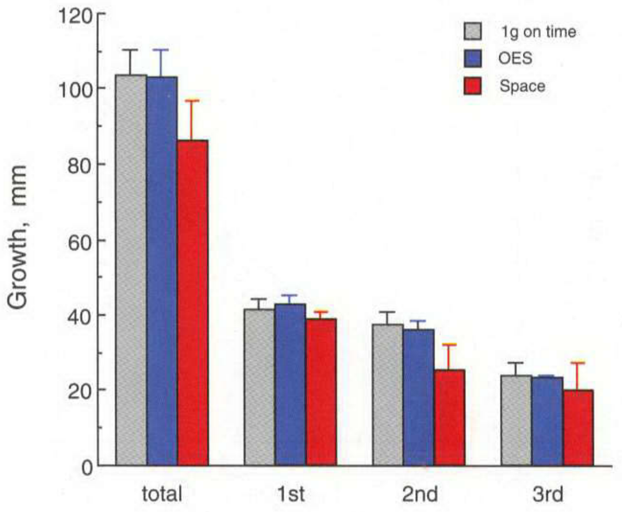

Fig. 15 Effect of microgravity conditions in space on the growth of etiolated pea seedlings. Seedlings were grown under microgravity conditions in space for 9 days at about 23 °C in the dark. Results were expressed as the average with the standard error of the mean.

Table 1 Extensibilities of cell walls of etiolated pea and maize seedlings

	Extensibility (10^{-6} m/g)		
	On time	OES	Space
pea epicotyl			
1 st internode	0.53 ± 0.06	0.59 ± 0.04	0.67 ± 0.04
2nd internode	0.75 ± 0.08	0.82 ± 0.11	0.90 ± 0.16
3rd internode	1.32 ± 0.13	1.11 ± 0.10	1.00 ± 0.08*
maize coleoptile			
basal region	0.67 ± 0.05	0.86 ± 0.10	0.69 ± 0.03
middle region	0.85 ± 0.03	0.96 ± 0.03	0.75 ± 0.05
top region	1.28 ± 0.16	1.19 ± 0.05	0.92 ± 0.10*

Extensibilities were measured on etiolated pea and maize seedlings grown under microgravity conditions in space after landing of the space shuttle of 9 days flight. Each segment was prepared for 10 mm length. Results were expressed as the average with standard error of the mean (n=15).
*Significant at the 0.05 level of confidence.

control on the ground and the microgravity conditions in space, cell walls being already hard. On the other hand, these values of the 3rd internode and the top region of maize coleoptiles were relatively high. Microgravity conditions in space significantly decreased the extensibility of the growing zone of pea seedlings and of younger region of maize coleoptiles, being a good correlation with the growth and development of the seedlings though coleoptile growth was not affected. Since the age of the seedlings were 9-day old, the values of cell wall extensibility seemed to be consequence of the growth and development of the seedlings. These results, however, strongly suggest that gravity on the earth substantially affects the growth and development of plants due to the changes of mechanical properties of cell walls.

Auxin polar transport under microgravity conditions in space

The activities of auxin polar transport in the segments of pea epicotyl (2nd internode), and maize coleoptile and mesocotyl, which were grown on 1 g conditions or under simulated microgravity conditions on a 3-D clinostat, have

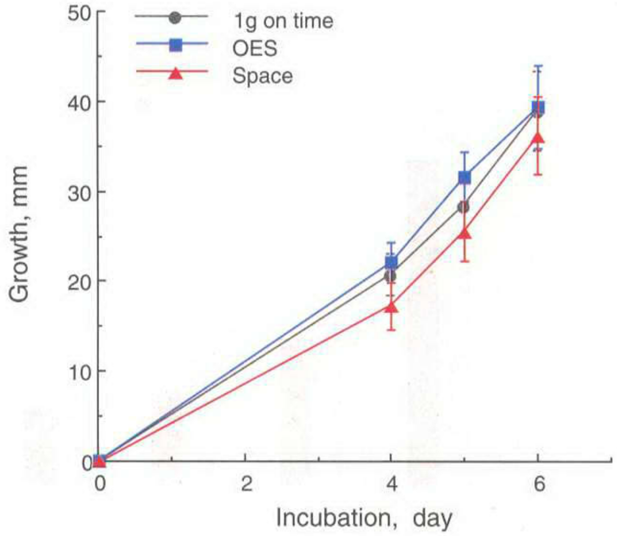

Fig. 16 Effect of microgravity conditions in space on coleoptile growth of etiolated maize seedlings. Seedlings were grown under microgravity conditions in space for 6 days at about 23 °C in the dark. Results were expressed as the average with the standard error of the mean.

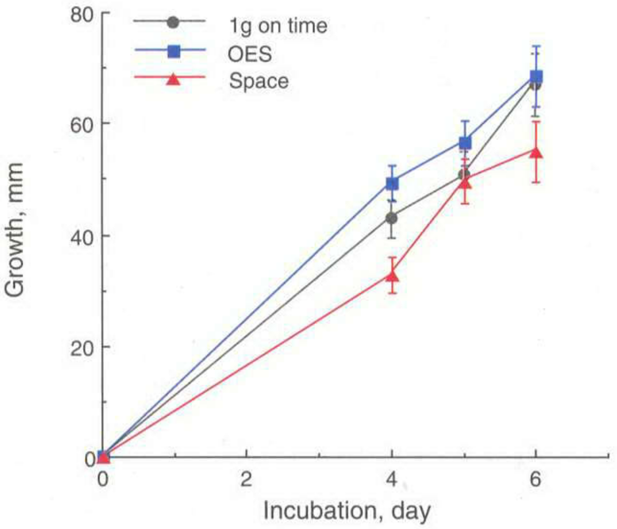

Fig. 17 Effect of microgravity conditions in space on mesocotyl growth of etiolated maize seedlings. Seedlings were grown under microgravity conditions in space for 6 days at about 23 °C in the dark. Results were expressed as the average with the standard error of the mean.

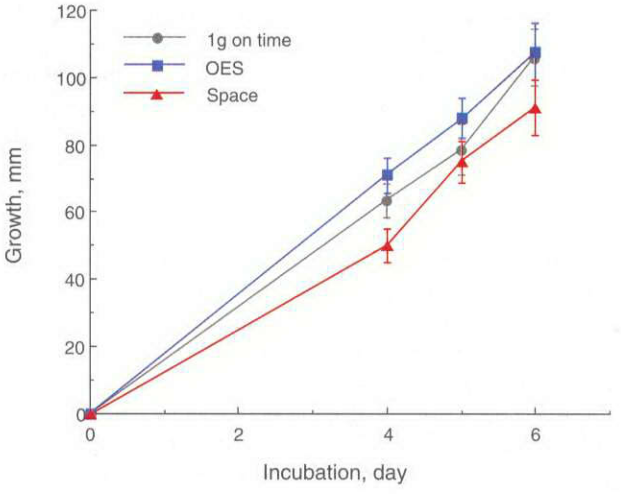

Fig. 18 Effect of microgravity conditions in space on the growth of etiolated maize seedlings. Seedlings were grown under microgravity conditions in space for 6 days at about 23 °C in the dark. Results were expressed as the average with the standard error of the mean.

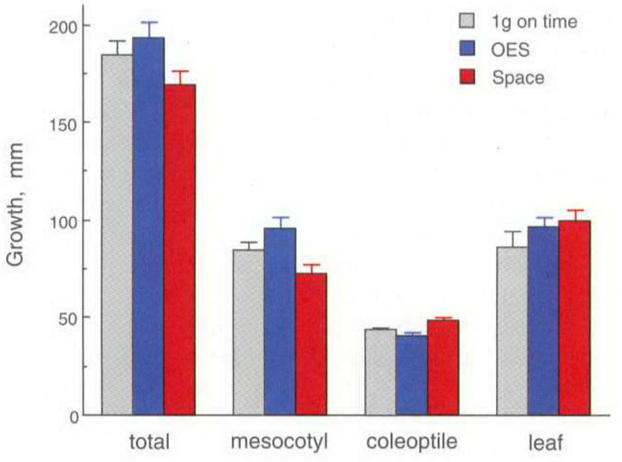

Fig. 19 Effect of microgravity conditions in space on the growth of etiolated maize seedlings. Seedlings were grown under microgravity conditions in space for 9 days at about 23 °C in the dark. Results were expressed as the average with the standard error of the mean.

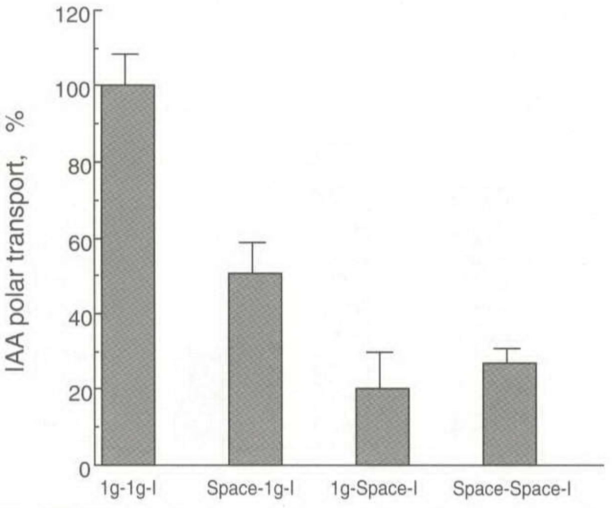

Fig. 20 Effects of microgravity conditions in space on auxin (IAA) polar transport in the segments of etiolated pea 2nd internodes. Auxin polar transport in the inverted position of the segment prepared from the seedlings grown on 1g conditions was determined under 1g conditions (1g-1g-I) or under microgravity conditions in space (1g-Space-I). Auxin polar transport in the inverted position of the segment prepared from the seedlings grown under microgravity conditions in space was also determined under 1g conditions (Space-1g-I) or under microgravity conditions in space (Space-Space-I). Results were expressed as percentages of the value of 1g-1g-I.

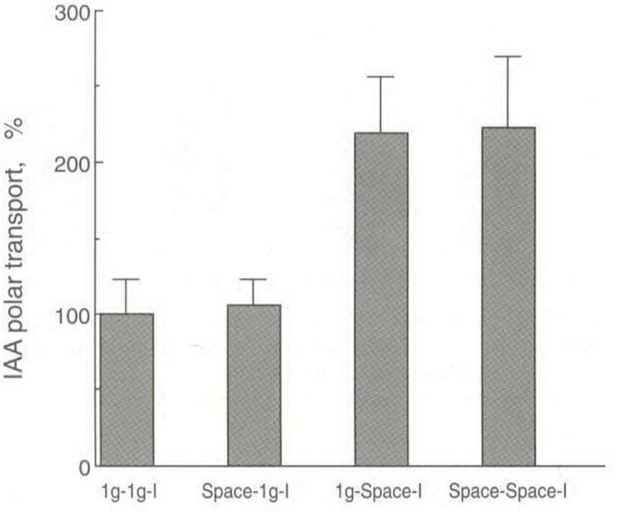

Fig. 21 Effects of microgravity conditions in space on auxin (IAA) polar transport in the segments of etiolated maize coleoptiles. Symbols in horizontal axis are the same as those in Fig. 20. Results were expressed as percentage of the value of 1g-1g-I.

already been reported to be slightly or significantly promoted by simulated microgravity conditions on a 3-D clinostat (Ueda *et al.* 1999a).

As shown in Figs. 20 and 21, microgravity conditions in space substantially affected auxin polar transport of the segments of etiolated pea epicotyl and etiolated maize coleoptile. Auxin polar transport of pea 2nd internodes grown under microgravity conditions in space was lesser than that of the seedlings grown on the ground conditions in spite of gravitational conditions in the measurement of auxin polar transport. These results were different from those in simulated microgravity conditions on a 3-D clinostat as describe above (Ueda *et al.* 1999a). The reason of this difference has not been clear yet, but it might be depend on the growth of the seedlings. The growth of the etiolated pea epicotyls was substantially inhibited in microgravity conditions in space but did not in simulated microgravity conditions on a 3-D clinostat (Ueda *et al.* 1999a).

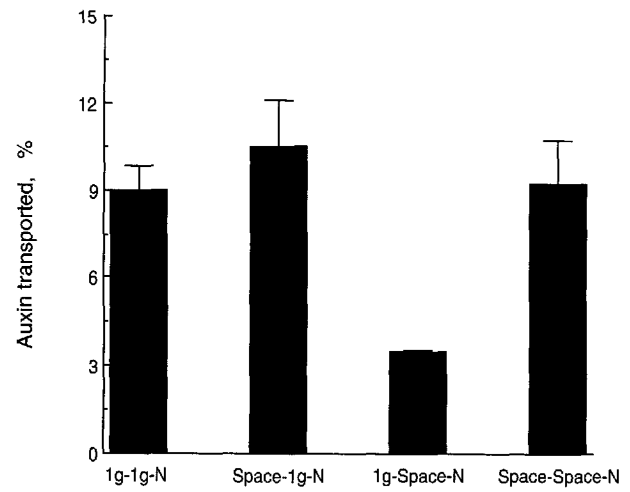

Fig. 22 Effects of microgravity conditions in space on auxin (IAA) acropetal movement in the segments of etiolated pea epicotyls. Symbols in horizontal axis are the same as those in Fig. 20. Results were expressed as percentages of the value of 1g-1g-I.

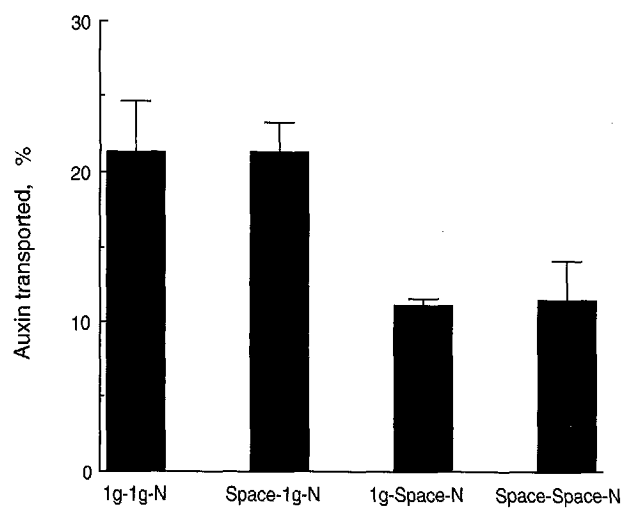

Fig. 23 Effects of microgravity conditions in space on auxin (IAA) acropetal movement in the segments of etiolated maize coleoptiles. Symbols in horizontal axis are the same as those in Fig. 20. Results were expressed as percentages of the value of 1g-1g-I.

On the other hand, the effects of microgravity conditions in space on auxin polar transport were quite different in etiolated maize coleoptile. As similar to the results obtained from the experiment using simulated microgravity conditions on a 3-D clinostat, the activities of auxin polar transport in etiolated maize coleoptile segments were promoted by microgravity conditions in space nevertheless whether etiolated maize seedlings grew under microgravity conditions in space or on the ground conditions.

Acropetal movement of auxin was also affected by microgravity conditions in space. Microgravity conditions had almost no effect on the acropetal movement of auxin in the seedlings grown under space conditions, but reduced it in the seedlings grown on 1 g conditions (Fig. 22). In etiolated maize seedlings, the effect of microgravity conditions in space was different from that in etiolated pea seedlings. Acropetal movement of auxin measured under microgravity conditions in space decreased in the segments of etiolated maize coleoptiles grown either under space conditions or on 1 g conditions (Fig. 23).

The difference of auxin polar transport in microgravity conditions in space in between pea and maize seedlings has not been clear yet. It might depend on the difference in species of plants and/or organs and tissues, and in the system of auxin polar transport between these seedlings. Jones (1990) has found that auxin moved basipetally through epidermis of maize mesocotyl using photolytically fixed [^{3}H] 5-N_3IAA. Gälweiler et al. (1998) have also reported that auxin polar transport is regulated by AtPIN1 gene expressed strongly in vascular tissues of *Arabidopsis thaliana* as an efflux carrier protein, suggesting that auxin moves basipetally through cells consisting of vascular bundle. These reports are supporting the consideration described above. Judging from the results in this study together with those in a 3-D clinostat, the activity of auxin polar transport or the construction of the system of auxin polar transport is considered to be under the control of gravity.

Gravity on the earth really influences the activities of auxin polar transport as well as the growth and development of plants. The responses of higher plants to microgravity conditions seem to be different not only among the species of plants but also in their organs and/or tissues. Moreover, as reported previously (Hoson *et al.* 1997), a 3-D clinostat is considered to be very important and valuable instrument for simulating microgravity conditions on the earth, though there are some differences in the results between the experiments using spacecrafts and a 3-D clinostat.

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