Growth Regulation Mechanisms in Higher Plants under Microgravity Conditions - Changes in Cell Wall Metabolism

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Abstract During Space Shuttle STS-95 mission, we cultivated seedlings of rice (*Oryza sativa* L. cv. Koshihikari and cv. Tan-ginbozu) and Arabidopsis (*Arabidopsis thaliana* L. cv. Columbia and cv. *etr1-1*) for 68.5, 91.5, and 136 hr on board, and then analyzed changes in the nature of their cell walls, growth, and morphogenesis under microgravity conditions. In space, elongation growth of both rice coleoptiles and Arabidopsis hypocotyls was stimulated. Also, the increase in the cell wall extensibility, especially that in the irreversible extensibility, was observed for such materials. The analyses of the amounts, the structure, and the physicochemical properties of the cell wall constituents indicated that the decreases in levels and molecular masses of cell wall polysaccharides were induced under microgravity conditions, which appeared to contribute to the increase in the wall extensibility. The activity of certain wall enzymes responsible for the metabolic turnover of the wall polysaccharides was increased in space. By the space flight, we also confirmed the occurrence of automorphogenesis of both seedlings under microgravity conditions; rice coleoptiles showed an adaxial bending, whereas Arabidopsis hypocotyls elongated in random directions. Furthermore, it was shown that spontaneous curvatures of rice coleoptiles in space were brought about uneven modifications of cell wall properties between the convex and the concave sides.

1)Introduction

Plants developed the cell wall with a complicated but well-organized structure during their evolution for more than 400 million years after they first went ashore. The plant cell wall plays an important role in supporting its body under 1 g on earth. At the same time, the cell wall encloses each cell and determines its size and shape. Growth and development of plant body are thus most directly controlled by the nature of the cell wall. Therefore, microgravity in space would greatly modify the properties of the cell wall, thereby influencing growth regulation mechanisms.

The results obtained by our ground-based experiments with the water immersion method and the hypergravity conditions produced by centrifugation support such an idea[1)-3)]. For instance, rice coleoptiles grow faster under water than in air. Air-bubbling partially suppresses

coleoptile growth under water, suggesting that the stimulation of growth under water is caused by both gaseous factors and microgravity effect due to buoyancy. The rheological analysis of the cell wall properties of rice coleoptiles showed that the extensibility of the cell wall of water-grown coleoptiles was higher than that of air-grown ones. Submergence also causes diverse changes in the levels and the structure of the cell wall constituents, such as the decrease in the levels of cellulose, the matrix polysaccharides, the structural glycoproteins, and phenolics, that are supposed to be responsible for the increase in the cell wall extensibility of rice coleoptiles. On the contrary, hypergravity caused a suppression of elongation growth and a decrease in the capacity of the cell wall to extend in various plant stems. Hypergravity also induces various types of changes in cell wall constituents such as the increase in the levels of cellulose, the matrix polysaccharides, and lignin. From these results it is expected that microgravity in space causes an increase in the mechanical extensibility of the cell wall through modifying the metabolism of the wall constituents, thereby stimulating elongation growth of various stem organs.

When above-mentioned wall modifications occur uniformly about stems, only the changes in growth rate

are brought about. However, if such modifications occur unevenly, they should induce curvatures. Our ground-based experiments with a 3-D clinostat indicated that such spontaneous curvatures occurred under simulated microgravity conditions[4)5)]. In general, shoots showed a curvature either toward the seed (adaxial direction) or away from the seed (abaxial direction), whereas roots grew in the direction of the tip of primordia in the early stage of growth and later in a random fashion with characteristic curvatures in a couple of portions on the clinostat. These spontaneous curvatures due to differential modifications of the cell wall nature are principal components of the morphogenesis of plants in a stimulus-free environment, termed automorphogenesis. It is another exciting theme whether such an automorphogenesis occurs under true microgravity conditions in space.

The present study was planned and carried out according to the theoretical background and the results of ground-based experiments mentioned above. The objective of this experiment was to understand the role of gravity in regulation of growth and morphogenesis of plants by examining the properties of the cell wall of seedlings grown on board.

2) Materials and Procedures

A) Ground-based Preliminary Experiments

Because there are various differences in the cell wall structure between monocotyledonous Gramineae and dicotyledons, it is required to select plant materials for space experiments from each of these two groups. We finally decided to use rice (*Oryza sativa* L.) and Arabidopsis (*Arabidopsis thaliana* L.) by considering growth characteristics, the fitness to flight procedures, the amount of background information, and scientific and social impact of various plant species. Both species are also popular for molecular biological analyses, because they have genomes with a small size. Of these species, rice was flown to space for the first time as an official experimental material. A gibberellin-deficient dwarf mutant Tan-ginbozu of rice and an ethylene-resistant mutant *etr1-1* of Arabidopsis were used in addition to tall or wild strains, Koshihikari (rice) and Columbia (Arabidopsis), in order to analyze the involvement of phytohormones in gravity-dependent regulation of the cell wall properties.

There are two methods to hydrate seeds or caryopses for space experiments. One is to launch dry seeds and hydrate them on orbit. The other is that imbibed seeds are launched at cool temperature and growth is triggered by their transfer to cultural temperature. We employed the latter, because it was essential to obtain uniform samples for the analyses of the cell wall properties. This selection favors the determination of germination procedures of Arabidopsis: the seeds need light exposure for germination, which can be fulfilled by imbibing them in the light before launching. We performed various preliminary experiments for elevating germination rate of rice caryopses and keeping

their ability to germinate for longer period in a refrigerator.

The culture dish was newly designed for the present experiment. Testing its capacity, we decided the type of culture medium (agar), its concentration (1%), cultural temperature (23°C), cultural periods (72, 96, and 120 hr), as well as the method of seed sterilization and imbibition. Cultural temperature was a kind of compromise between the culture of rice which prefers higher temperature, and that of Arabidopsis which lives usually in cooler climate. In the present experiment we could not use an incubator on orbit. Therefore, we had made a temperature compensating table in advance to adjust the cultural periods flexibly according as the cabin temperature.

Various methods exist for collecting plant materials to the ground, such as the freezing, chemical fixation, and drying. We selected the freezing, because it provides us with maximal information on the nature of the cell wall. We also compared different methods of freezing, but such a consideration became unnecessary because we can use a GN_2 freezer on orbit.

Finally, we checked the fitness of the selected materials and procedures to space experiment by using a vibration apparatus in TKSC. We confirmed their reliability.

B) Flight Experiments
Plant materials:
   Rice (*Oryza sativa* L.) cv. Koshihikari and cv. Tan-ginbozu
   50 caryopses/dish, one dish each for three different cultural periods
   Arabidopsis (*Arabidopsis thaliana* L.) cv. Columbia and cv. *etr1-1*
   1000 seeds/dish, one dish each for three different cultural periods

Experiment procedures:
  i)(Pre-launch operation)
   Set rice caryopses of two cultivars on agar in three dishes each.
   Set Arabidopsis seeds of two cultivars on agar in three dishes each.
   Store all dishes at 4°C in three half-BRIC-60s.
  ii) Launch three BRICs at 4°C in OSRF.
  iii) Remove three BRICs from refrigerator and set them in cabin at day 3, 4, and 5, respectively. The seedlings will be cultivated in the dark.
  iv) At day 8 (TBD upon cabin temperature), open the BRICs and take pictures of the dishes (5 snaps each).
  v) Put two BRICs in GN_2 freezer and one BRIC in OSRF. Return at -80 or 4°C condition.

Onbord procedures:
   Seeds and caryopses for space experiments were soaked on agar in a culture dish on Oct. 27, 1998 at Hangar L in KSC. Four culture dishes each were stored in three BRICs and kept in the OSRF during launching. Samples for 120 hr culture (BRIC-A), 96 hr culture (BRIC-B), and 72 hr culture (BRIC-C) were transferred to the cabin at MET 2/6:18, 4/2:53, 5/1:59, respectivey. Cultural periods were modified so as to adjust the fluctuation of cabin

temperature. Actual cultural periods on board were 136, 91.5, and 68.5 hr for BRICs A, B, and C. At MET 7/20:52 Astronaut Chiaki Mukai took out all samples from the BRIC, took pictures, and then froze them with a GN_2 freezer (completed at 7/22:31). The materials were kept frozen during the flight, landing, and transportation to our laboratory in Osaka City Univ., and stored at -80˚C until the analysis. The ground control experiments were carried out at Hangar L one and three days after the space experiments. A supplementary control experiment was performed in our laboratory in Osaka City Univ. by reproducing the data on temperature within BRIC on orbit exactly.

3) Results

A) Growth and Cell Wall Properties

i) Growth

When we compared the length of rice coleoptiles and Arabidopsis hypocotyls between samples frozen on orbit and those at Hanger L, elongation growth appeared to be suppressed under microgravity conditions. However, it was turned out later that the information on cabin temperature down-linked from orbit during the flight did not reflect enough the temperature within the BRIC and the temperature set at Hanger L was significantly higher than in space, which cause such a result. Therefore, we carried out a supplementary control experiment in our laboratory by reproducing the temperature within BRIC on orbit exactly. By comparison between space-grown seedlings and the true control, it was shown that elongation growth of rice coleoptiles and Arabidopsis hypocotyls was stimulated under microgravity conditions (Figs. 1, 2). In rice seedlings grown in space, the stimulation of coleoptile growth was less than 20% in length and clearer in Tan-ginbozu than in Koshihikari (Fig. 1). The degree of growth stimulation decreased with aging. On the other hand, the hypocotyl length of Arabidopsis was greater by about 50% in space-grown seedlings of both cultivars (Fig. 2). All the differences in length between seedlings grown on the ground and in space were significant, indicating that elongation growth of rice coleoptiles and Arabidopsis hypocotyls was stimulated under microgravity conditions in space.

ii) Cell Wall Extensibility

We analyzed the mechanical properties of the cell wall of rice coleoptiles and Arabidopsis hypocotyls grown in space and on the ground by the stress-relaxation method and the strain/stress method in our laboratory using a tensile tester. It has been shown that two stress-relaxation parameters, the minimum stress-relaxation time and the stress-relaxation rate, correlate with the capacity of the cell wall to expand. However, no clear differences were detected in these parameters between rice coleoptiles grown in space and those on the ground (Figs. 3, 4). On the other hand, prominent differences existed in the cell wall extensibility calculated from the slope of load-extension curve. The total cell wall extensibility, that tended to decrease with the age, was always higher in space-grown rice coleoptiles (Fig. 5). By two successive extensions of segments, the total extensibility was separated into the reversible and the irreversible components. No differences were found in the reversible extensibility between space-grown coleoptiles and the controls (Fig. 6), whereas space-grown coleoptiles showed a clearly higher irreversible extensibility (Fig. 7). Such a difference in the irreversible extensibility between coleoptiles grown in space and on the ground decreased with aging.

Similar results on the cell wall extensibility were obtained for Arabidopsis hypocotyls. Space flight did not influence the stress-relaxation parameters also in this material irrespective of strains or the age (Figs. 8, 9). On the other hand, the total cell wall extensibility of space-
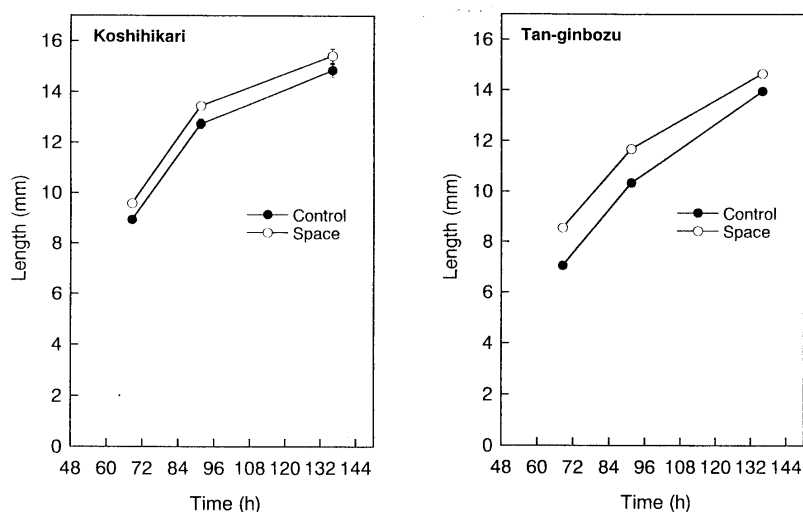

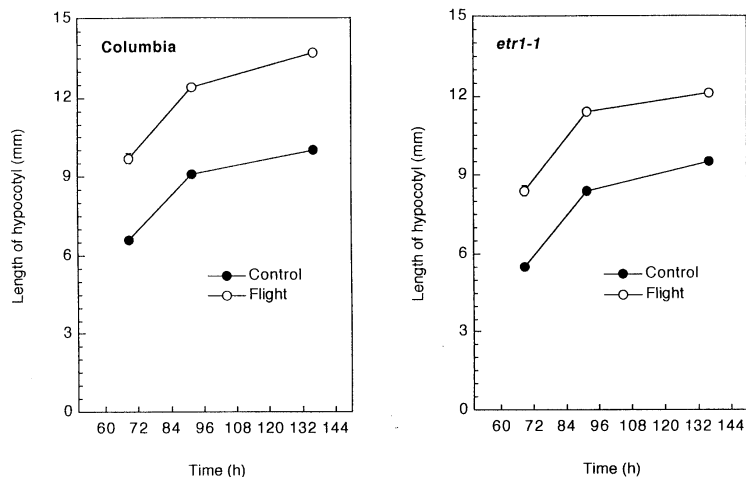**Fig.1** Effect of microgravity on elongation of rice coleoptiles.

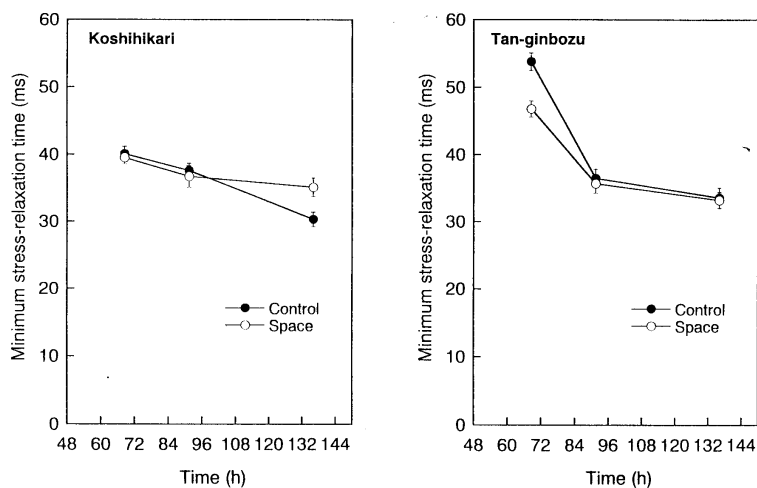**Fig.2** Effect of microgravity on elongation of Arabidopsis hypocotyls.

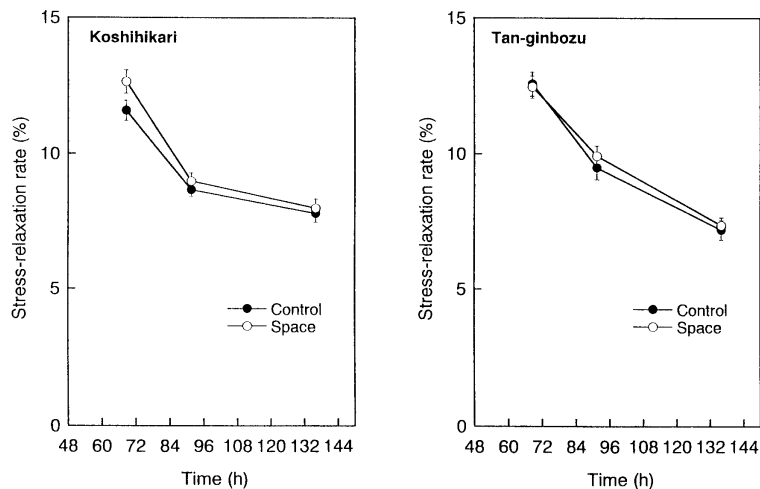**Fig.3** Effect of microgravity on the minimum stress-relaxation time of rice coleoptiles.

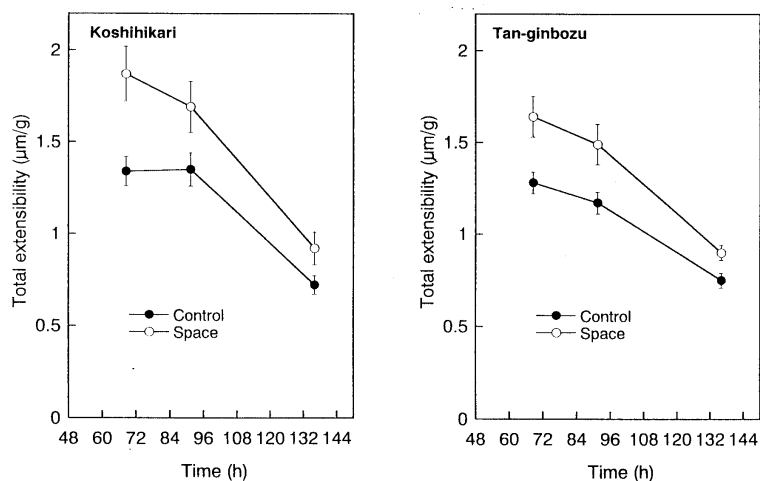**Fig.4** Effect of microgravity on the stress-relaxation rate of rice coleoptiles.

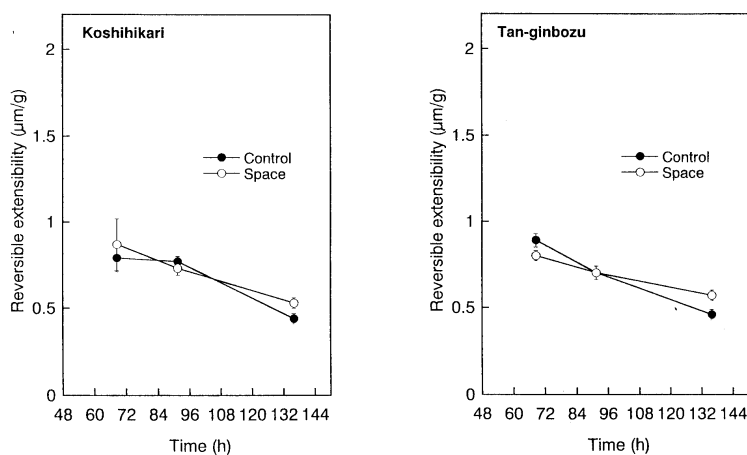**Fig.5** Effect of microgravity on the total cell wall extensibility of rice coleoptiles.

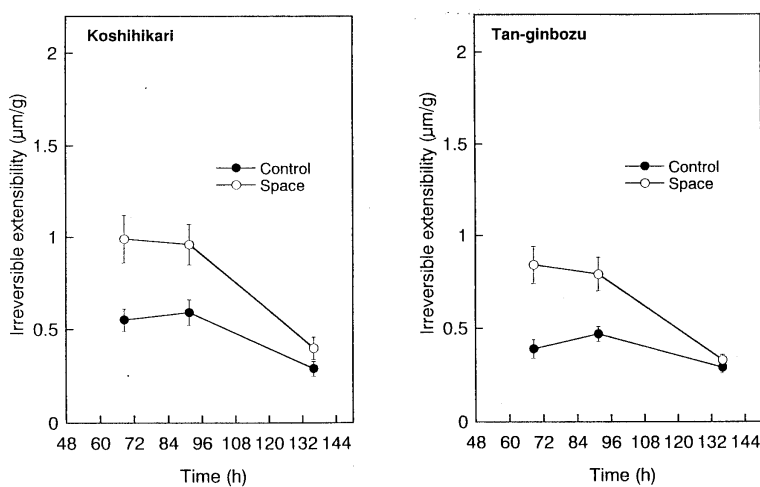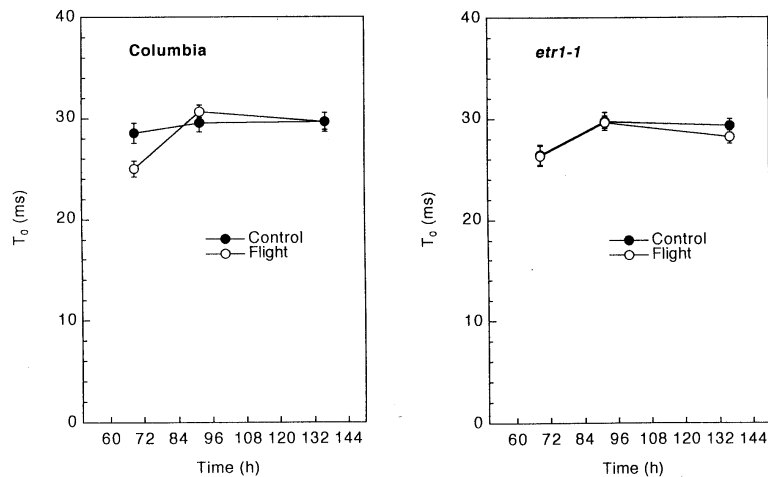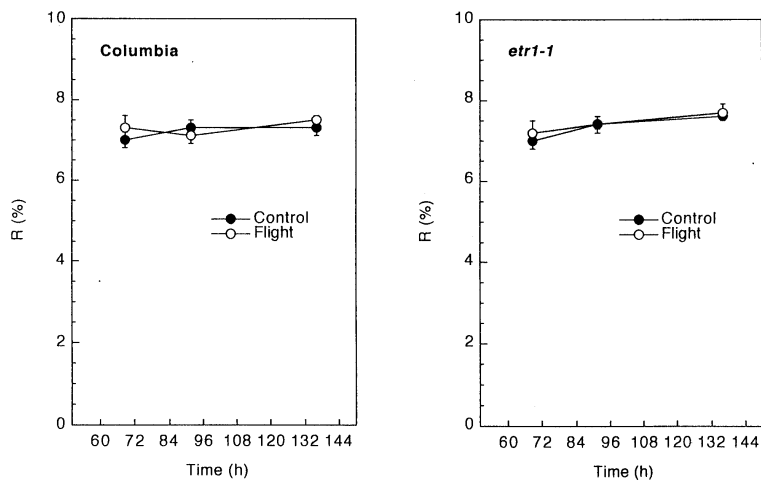**Fig.6** Effect of microgravity on the reversible cell wall extensibility of rice coleoptiles.

Fig.7 Effect of microgravity on the irreversible cell wall extensibility of rice coleoptiles.

Fig.8 Effect of microgravity on the minimum stress-relaxation time of Arabidopsis hypocotyls.

Fig.9 Effect of microgravity on the stress-relaxation rate of Arabidopsis hypocotyls.
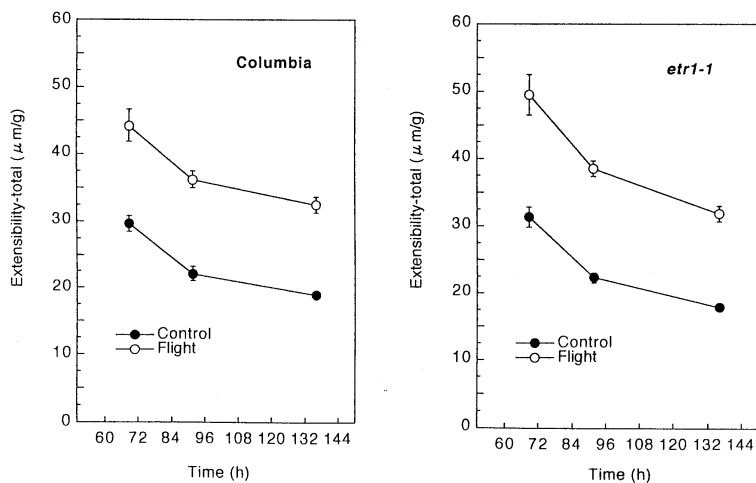
Fig.10 Effect of microgravity on the total cell wall extensibility of Arabidopsis hypocotyls.

grown hypocotyls was clearly higher than the controls (Fig. 10). As in rice coleoptiles, the difference in the total extensibility of Arabidopsis hypocotyls between in space and on the ground was almost completely due to the difference in the irreversible extensibility (Fig. 12). The cell wall extensibility of Arabidopsis hypocotyls decreased with the age and no clear differences were detected between cultivars. Thus, it was clearly shown that the cell walls of rice coleoptiles and Arabidopsis hypocotyls grown in space have a higher capacity to expand than the controls.

iii) Level and Molecular Mass of Cell Wall Polysaccharides

In order to understand the mechanisms by which the cell wall extensibility is increased under microgravity conditions in space, we analyzed the levels and the physico-chemical properties such as the molecular mass of cell wall polysaccharides. Rice coleoptile cell walls were

fractionated into the pectic, the hemicellulose, and the cellulose fractions, and their amounts were measured. During cultivation from 68.5 to 136 hr after the start of incubation the levels of the cell wall polysaccharides per coleoptile increased several times. Nevertheless, microgravity did not influence the amounts, irrespective of strains or the age (Figs. 13, 14). On the other hand, the amounts of the cell wall polysaccharides per unit length were significantly smaller in space-grown coleoptiles (Figs. 15, 16). Namely, microgravity appears to have the capacity to decrease the thickness of the cell wall, and thus increase the capacity of the cell wall to expand. No influences of space flight were detected in the proportions among three cell wall polysaccharide fractions.

On of the major parameters that determine the viscosity regulating the irreversible cell wall extensibility is the molecular size of constituting polysaccharides. Therefore, we measured the molecular mass of the hemicellulose, the
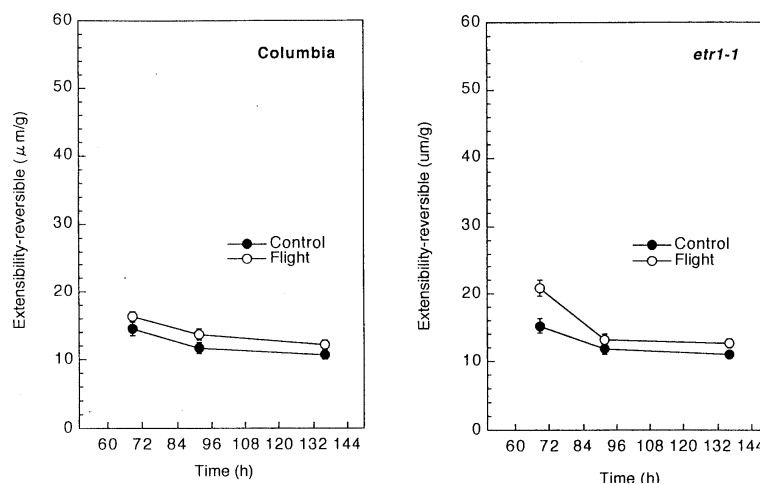

Fig.11 Effect of microgravity on the reversible cell wall extensibility of Arabidopsis hypocotyls.
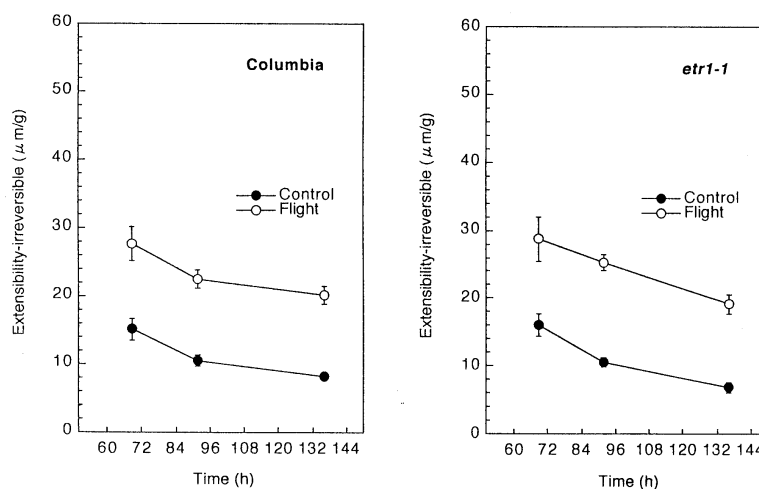

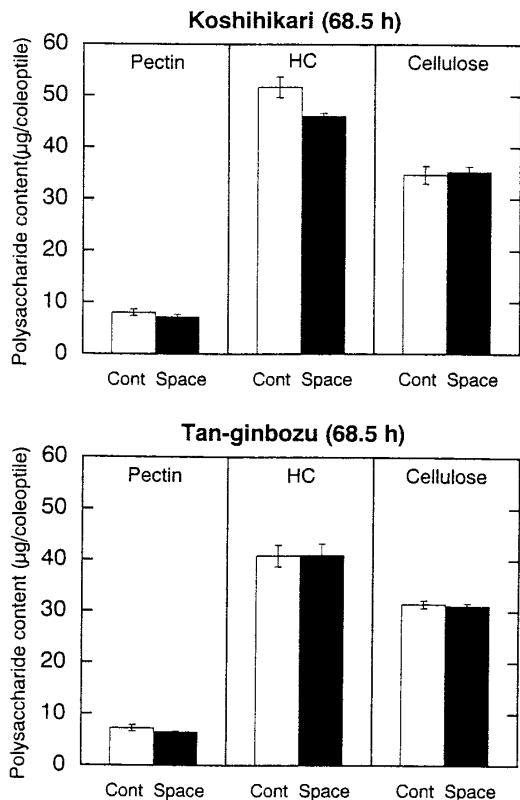**Fig.12** Effect of microgravity on the irreversible cell wall extensibility of Arabidopsis hypocotyls.

Fig.13 Effect of microgravity on the levels of the cell wall polysaccharides of 68.5 hr-old rice coleoptiles.
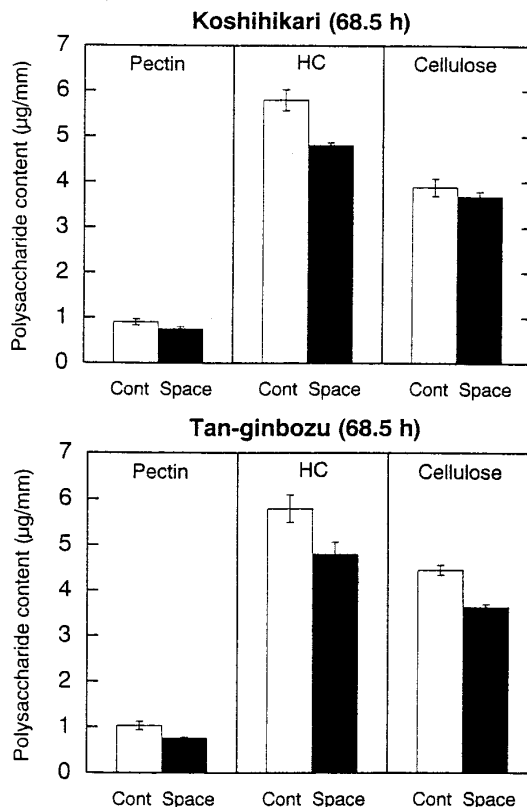

Fig.14 Effect of microgravity on the levels of the cell wall polysaccharides of 136 hr-old rice coleoptiles.

Fig.15 Effect of microgravity on the levels of the cell wall polysaccharides per unit length of 68.5 hr-old rice coleoptiles.
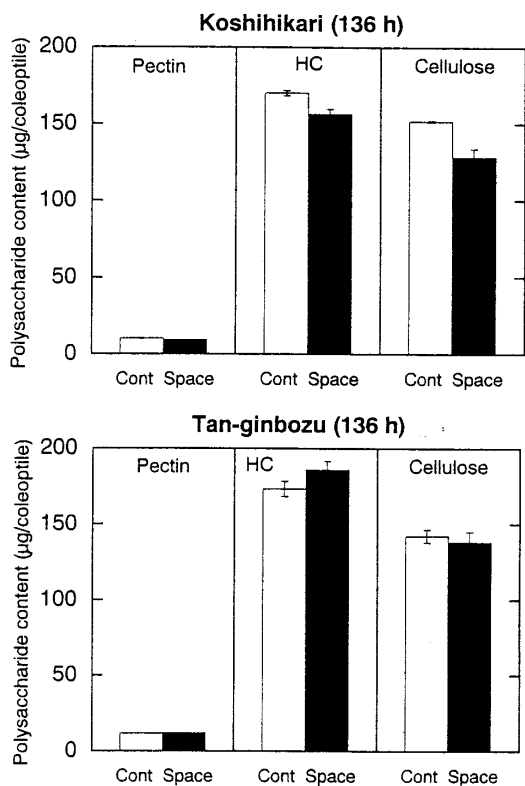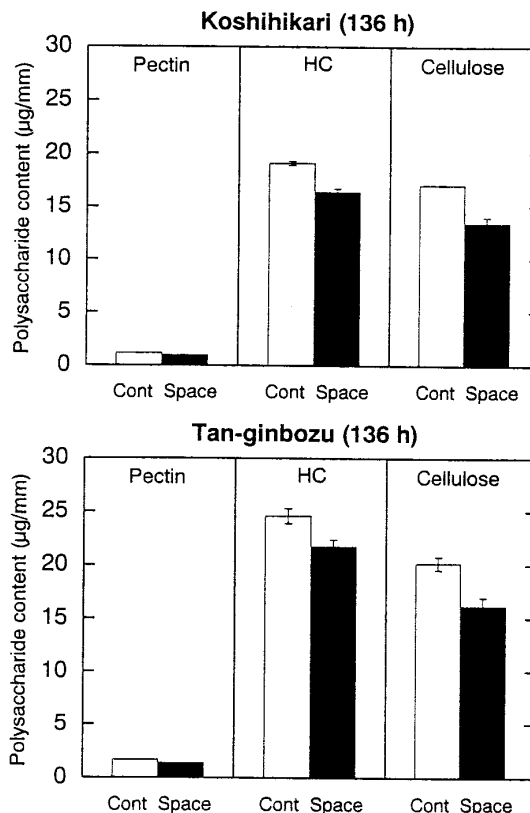

Fig.16 Effect of microgravity on the levels of the cell wall polysaccharides per unit length of 136 hr-old rice coleoptiles.

Koshihikari (68.5 h)
Hemicellulose
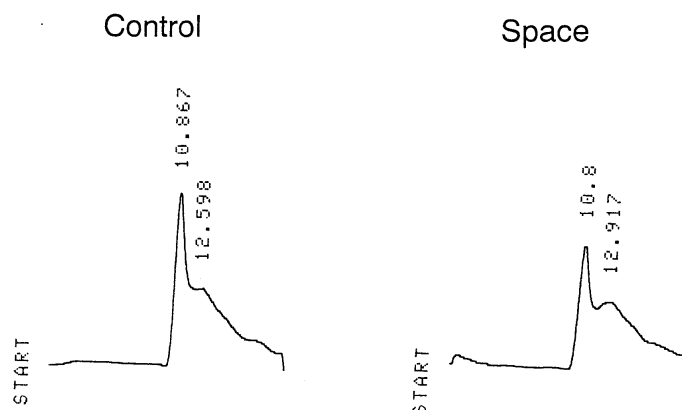
Control   Space

Fig.17 Molecular mass distributions of the hemicellulose polysaccharides from rice coleoptiles grown under 1 *g* and microgravity conditions.

major component of matrix, of rice coleoptiles with an HPLC equipped with a gel filtration column. Figure 17 shows the elution profile of the hemicellulose of Koshihikari on such a column. The general profile looked similar between space-grown and control coleoptiles. However, in the hemicellulose of rice coleoptiles grown in space, the relative area of high molecular mass region decreased: the ratio of this area to the area of the next major peak was 1.0 for space samples, whereas that was 1.2 for ground samples. Similar trend was detected also in the hemicellulose from Tan-ginbozu coleoptiles (data not shown). Because this high molecular mass region consists of $(1 \rightarrow 3),(1 \rightarrow 4)$-ß-glucans, that in general play a major role in determining the viscosity of the cell wall in Gramineae, it is likely that the breakdown of these glucans was stimulated on board. Further analysis of the influence of microgravity on the metabolic turnover of the glucans could not be carried out, because the amount of samples obtained in the present experiment was very low.

  The cell walls of Arabidopsis hypocotyls grown either in space or on the ground were fractionated into the pectic, the hemicellulose I, the hemicellulose II, and the cellulose fractions, and their levels were examined, as for rice coleoptiles. During cultivation of Arabidopsis seedlings the levels of the cell wall polysaccharides per hypocotyl increased but were not influenced by microgravity, irrespective of strains or the age (Figs. 18, 19). No influences of space flight were also detected in the proportions among the cell wall polysaccharide fractions. However, the amounts of the cell wall polysaccharides per unit length were significantly smaller in space-grown hypocotyls (Figs. 20, 21). These results indicate that microgravity causes a decrease in the thickness of the cell wall, thereby increasing the capacity of the cell wall to
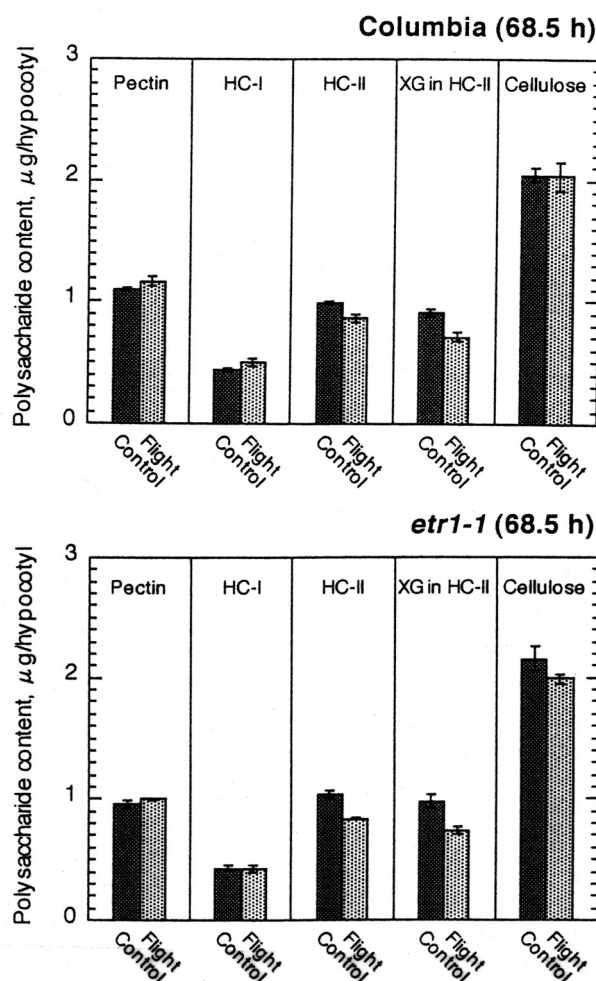

Fig.18 Effect of microgravity on the levels of the cell wall polysaccharides of 68.5 hr-old Arabidopsis hypocotyls.
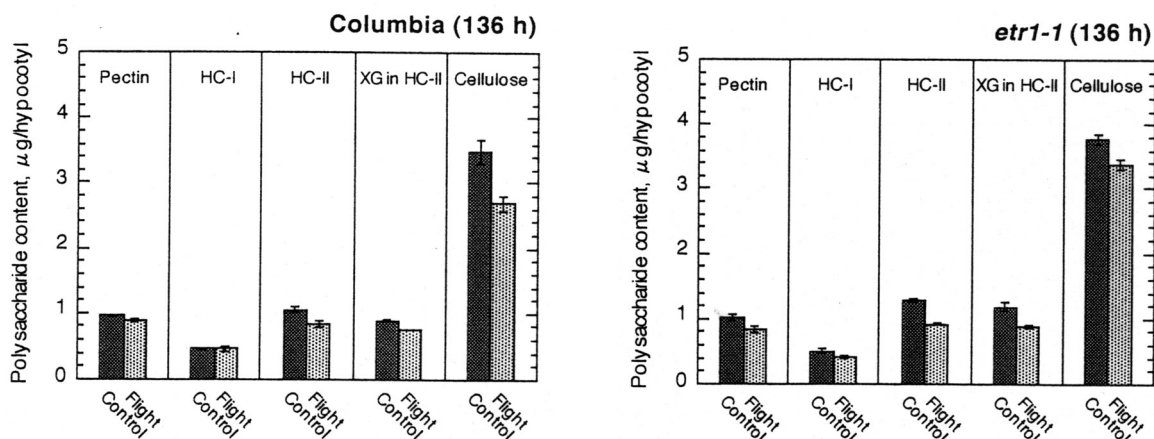
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Fig.19 Effect of microgravity on the levels of the cell wall polysaccharides of 136 hr-old Arabidopsis hypocotyls
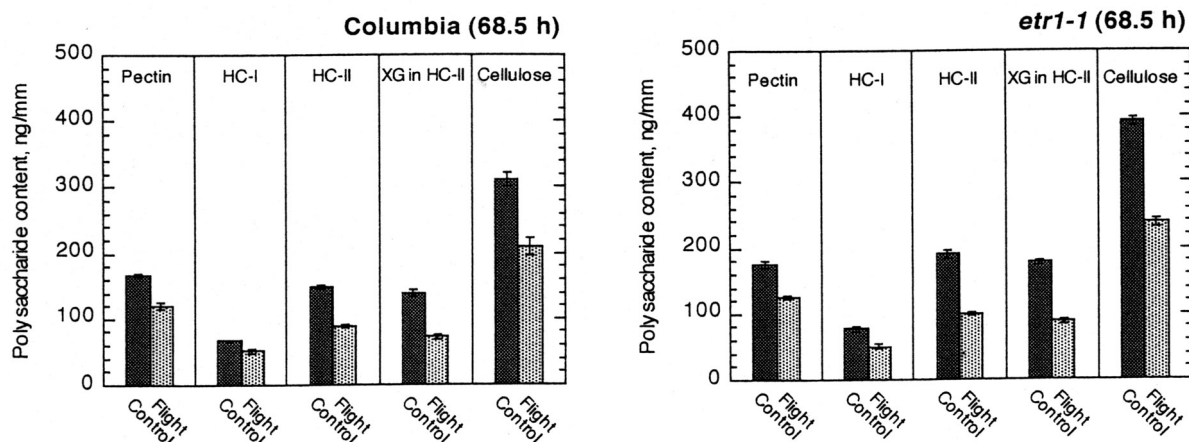

Fig.20 Effect of microgravity on the levels of the cell wall polysaccharides per unit length of 68.5 hr-old Arabidopsis hypocotyls
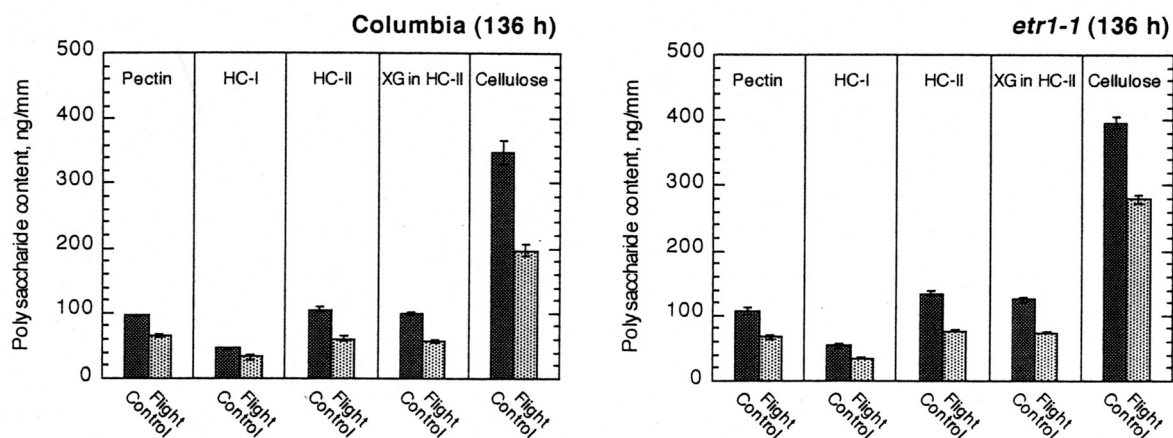

Fig.21 Effect of microgravity on the levels of the cell wall polysaccharides per unit length of 136 hr-old Arabidopsis hypocotyls.

expand in Arabidopsis hypocotyls, as in rice coleoptiles.

The molecular masses of the pectic, the hemicellulose I, and the hemicellulose II polysaccharides of Arabidopsis hypocotyls were analyzed with an HPLC equipped with a gel filtration column. The profiles of these polysaccharides changed during growth. Microgravity did not influence the elution proflies of the pectic or the hemicellulose I polysaccharides of Columbia hypocotyls (Figs 22, 23). On the contrary, the molecular mass distributions of the
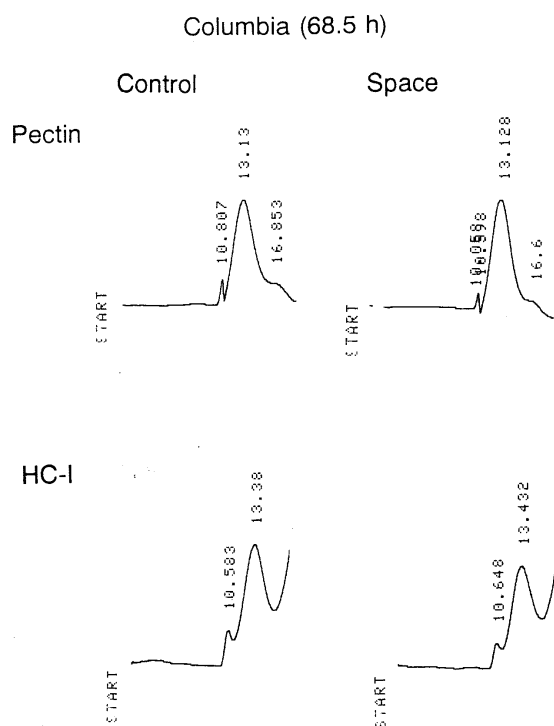
Columbia (68.5 h)

Fig.22 Molecular mass distributions of the pectic and the hemicellulose I polysaccharides from 68.5 hr-old Arabidopsis hypocotyls grown under 1 *g* and microgravity conditions.
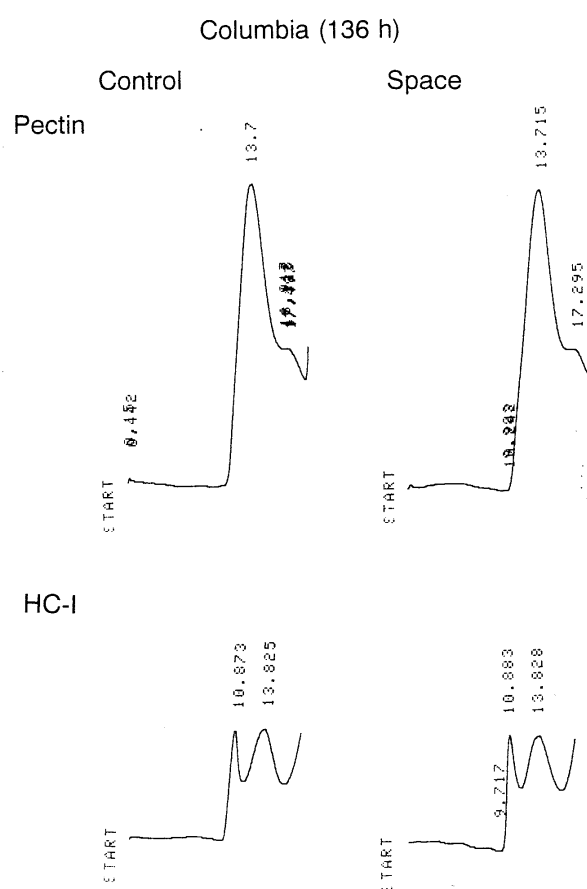
Columbia (136 h)

Fig.23 Molecular mass distributions of the pectic and the hemicellulose I polysaccharides from 136 hr-old Arabidopsis hypocotyls grown under 1 *g* and microgravity conditions.

hemicellulose II polysaccharides from space-grown hypocotyls shifted to the lower molecular mass region as compared with the controls (data not shown). Similar results were obtained for *etr1-1* (data not shown). The major component of the hemicellulose II fraction is xyloglucans that play a principal role in determining the viscosity of the cell wall in dicotyledons. Therefore, we examined the molecular mass distribution of xyloglucans contained in this fraction with the iodine method. It was shown that microgravity caused a decrease in the molecular size of xyloglucans, irrespective of cultivars and the age (Figs. 24, 25). Such an effect of microgravity was prominent in Tan-ginbozu cultivar as compared with Koshihikari.

iv)Activity of Cell Wall Enzymes

One of the mechanisms inducing the changes in the levels and the molecular masses of cell wall polysaccharides under microgravity conditions appears to be modifications of activities of the cell wall enzymes. Therefore, the cell wall proteins were extracted with 1 M NaCl from the cell wall preparations of rice coleoptiles and Arabidopsis hypocotyls grown either on orbit or on ground, and the cell wall polysaccharide-hydrolyzing activities were measured. In rice coleoptiles, the extracted cell wall protein fractions showed the activities to degrade $(1 \rightarrow 3),(1 \rightarrow 4)$-ß-glucans and $(1 \rightarrow 3)$-ß-glucans

(laminarin), but did not show the activities to hydrolyze cellulose derivatives or xylans (Fig. 26). So, the effect of microgravity was analyzed for these glucanase activities. $(1 \rightarrow 3),(1 \rightarrow 4)$-ß-Glucan-hydrolyzing activity per coleoptile (Fig. 27) and protein (Fig. 28) increased almost linearly from 68.5 hr to 136 hr. The activity was somewhat higher in space-grown coleoptiles. However, the difference in the activity was not so prominent.

When the protein fraction from Arabidopsis hypocotyls was incubated with carboxymethylcellulose or arabinogalactans, the activities were low (Fig. 29) and not different between space and ground samples. However, a high xyloglucan-hydrolyzing activity was present in this fraction (Fig. 29) and the activity was clearly higher in space-grown Arabidopsis hypocotyl cell wall than that in control cell wall, irrespective of cultivars and the age (Figs. 30, 31). The increase in xyloglucan-degrading activity in space was clearly detected, when the activity was expressed on the basis of hypocotyl, proteins, and cell wall polysaccharides.
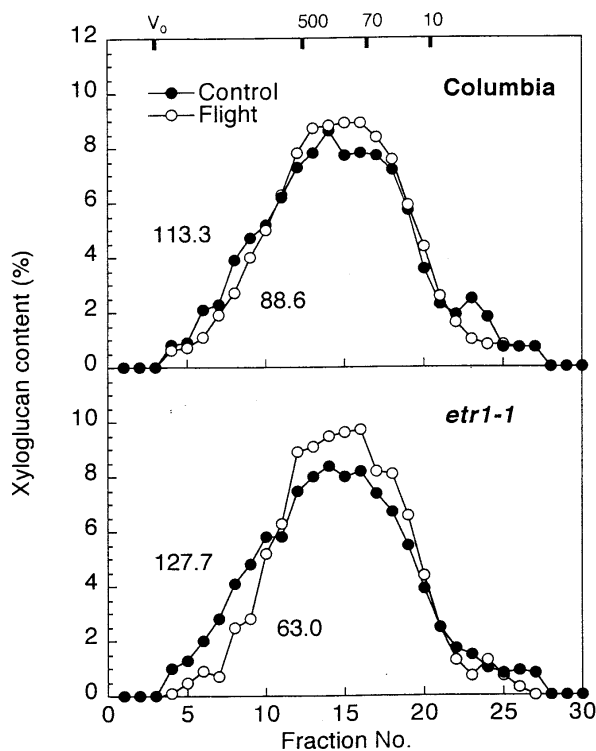
B) Morphogenesis
i) Automorphogenesis

Fig.24 Molecular mass distributions of xyloglucans from 68.5 hr-old Arabidopsis hypocotyls grown under 1 *g* and microgravity conditions.

Fig.25 Molecular mass distributions of xyloglucans from 136 hr-old Arabidopsis hypocotyls grown under 1 *g* and microgravity conditions.

By comparison between the pictures taken on orbit by Astronaut Chiaki Mukai and those of the controls, we analyzed the effects of microgravity on morphogenesis of both rice and Arabidopsis seedlings. On the ground, coleoptiles of rice grew upward and their roots downward along gravity vector, although some deviations from the vertical exist (Figs. 32, 33). The morphology of rice seedlings changed greatly in space. Coleoptiles were not straight but bent in the direction of the picture shown in Figs. 32 and 33 on orbit. Because rice caryopses were planted along a groove on agar with the embryos located to the right, the direction of bending was toward the caryopses (adaxial), as observed on the 3-D clinostat. Such curvatures were more prominent in Koshihikari than Tanginobozu. On the other hand, rice roots grew in various directions on orbit. However, the direction was not completely random and majority group formed a constant angle with the orientation of caryopses.

Arabidopsis seeds planted on 1% agar germinated and their hypocotyls elongated upward on the ground (Figs. 34, 35). In space Arabidopsis hypocotyls elongated in various directions, including into the agar medium. However, no clear curvatures were observed in their elongating regions. On the other hand, Arabidopsis roots grew along the surface of the agar plate and never into the medium. Microgravity did not influence such a growth
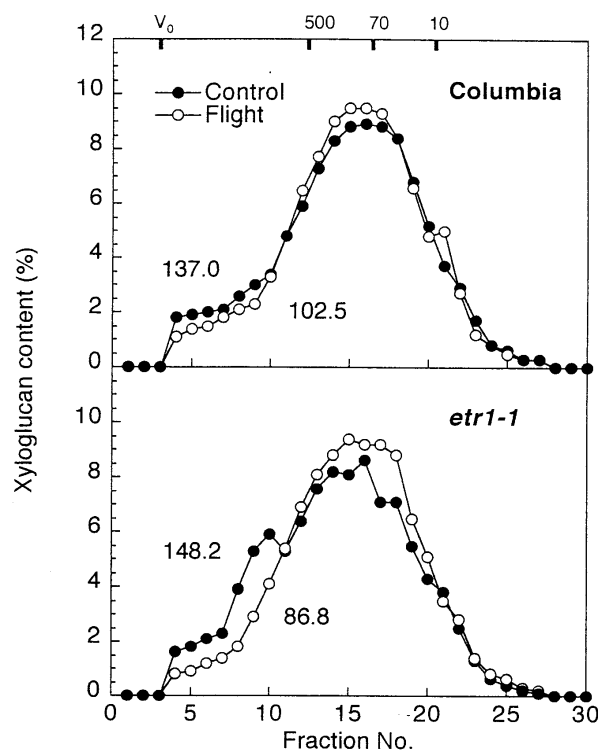
behavior of Arabidopsis roots.

ii) Growth Direction and Spontaneous Curvatures

The bending of rice coleoptiles observed in space appeared to consist of the inclination in the basal region and the curvature in the elongating region. The bending in these two regions was estimated separately by measuring the angle between the base line and the orientations of the basal and tip regions. To analyze the inclination in the basal
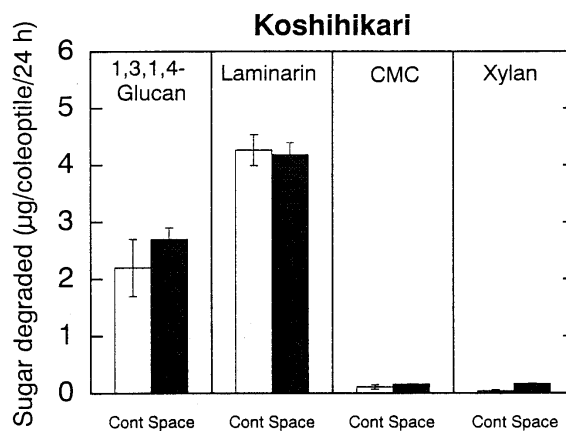

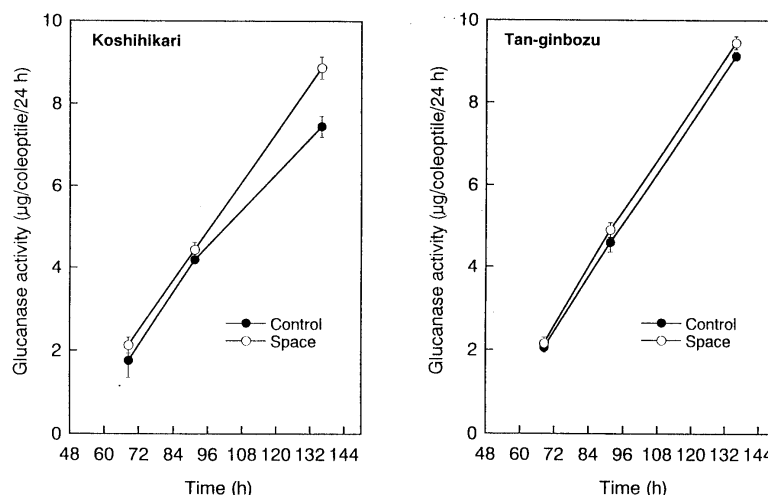**Fig.26** Effect of microgravity on activities of the cell wall enzymes of Koshihikari coleoptiles.

Fig.27 Changes in total activity of $(1 \rightarrow 3),(1 \rightarrow 4)$-ß-glucanase in rice coleoptile cell walls
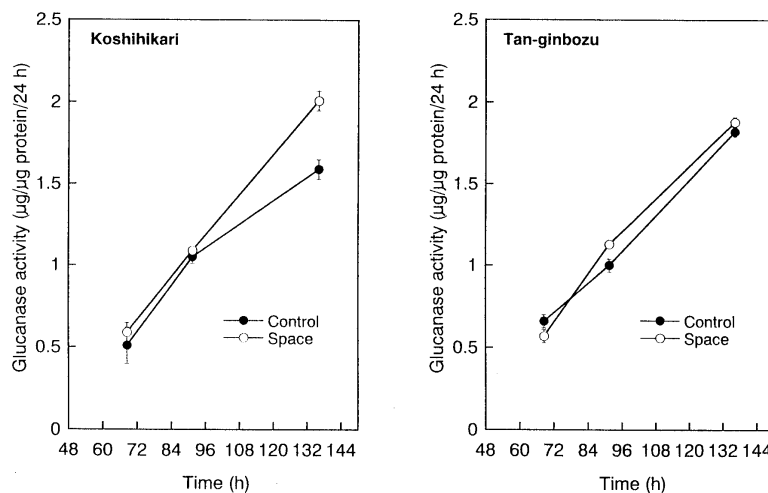
Fig.28 Changes in specific activity of $(1 \rightarrow 3),(1 \rightarrow 4)$-ß-glucanase in rice coleoptile cell walls.
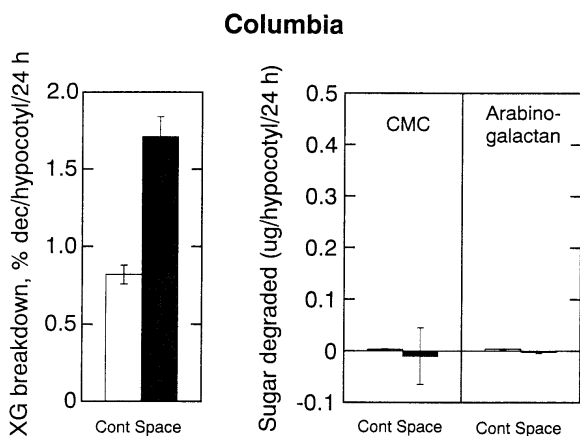
Columbia

Fig.29 Effect of microgravity on activities of the cell wall enzymes of Columbia hypocotyls.

regions of coleoptiles, the angle between the base line perpendicular to the long axis of caryopses and the orientation of the basal regions was read in the clockwise direction. In control coleoptiles of Koshihikari cultivar, the angle was almost 0° (Fig. 36). The angle in space-grown coleoptiles distributed from 10 to 50°, with the average angle of 28° (Fig. 36). Koshihikari coleoptiles grown on the 3-D clinostat also showed the inclination toward the caryopsis, although the angle was slightly smaller than in space. The bending in the subapical, elongating region was expressed as the angle between the orientations of the basal and the tip regions, read in the clockwise direction (Fig. 37). Control coleoptiles of Koshihikari elongated almost straightly, whereas those grown in space showed an adaxial curvature with the average angle of 32° (Fig. 37). The bending angle in coleoptiles grown on the 3-D clinostat was smaller than those grown in space, although the direction was the same. Similar analyses were applied also to a gibberellin-deficient dwarf cultivar, Tan-ginbozu (Figs.
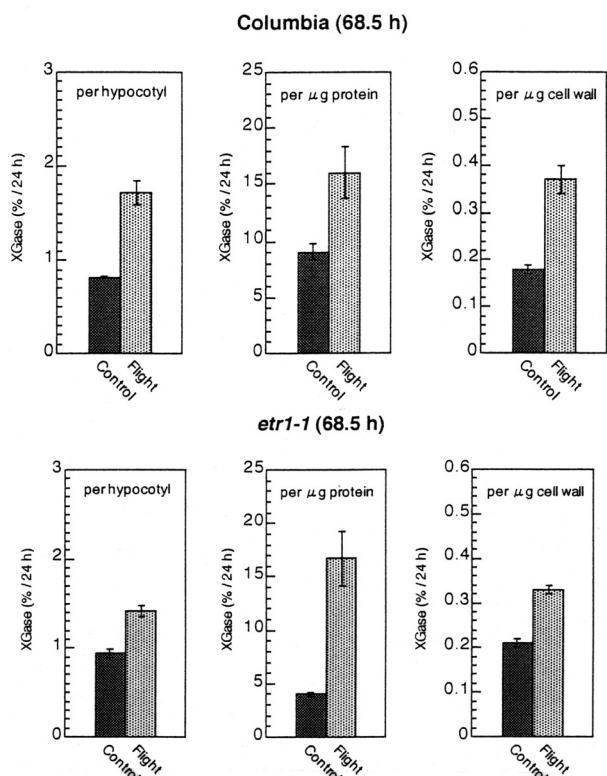
87

Fig.30 Effect of microgravity on activities of xyloglucan-degrading enzymes in the cell walls of 68.5 hr-old Arabidopsis hypocotyls.
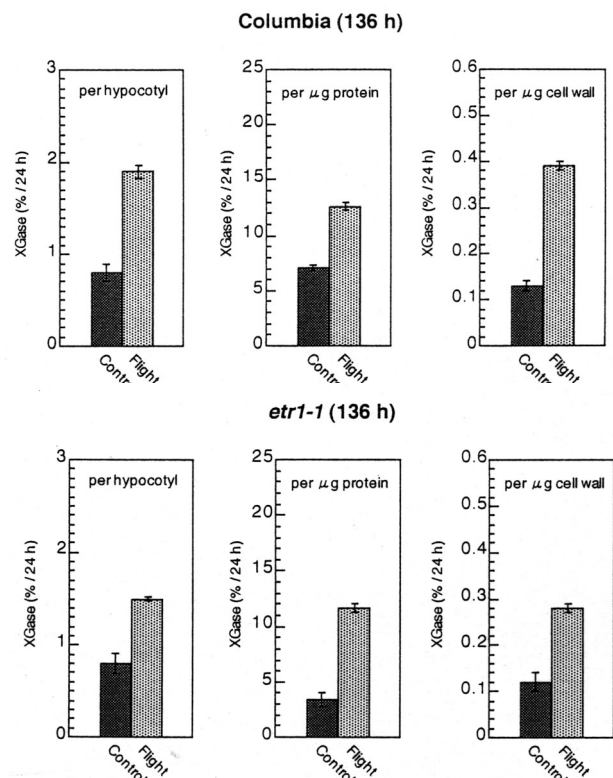

Fig.31 Effect of microgravity on activities of xyloglucan-degrading enzymes in the cell walls of 136 hr-old Arabidopsis hypocotyls.
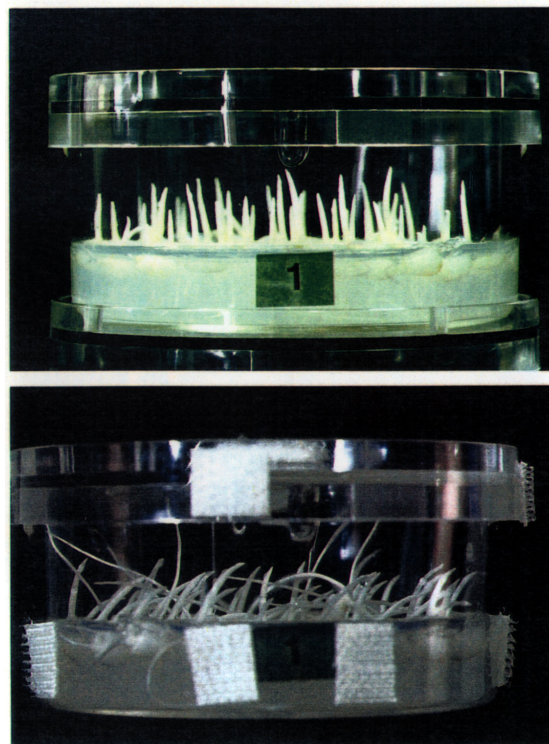

Fig.32 The morphology of 68.5 hr-old Koshihikari seedlings grown under 1 *g* (upper) and microgravity (lower) conditions.
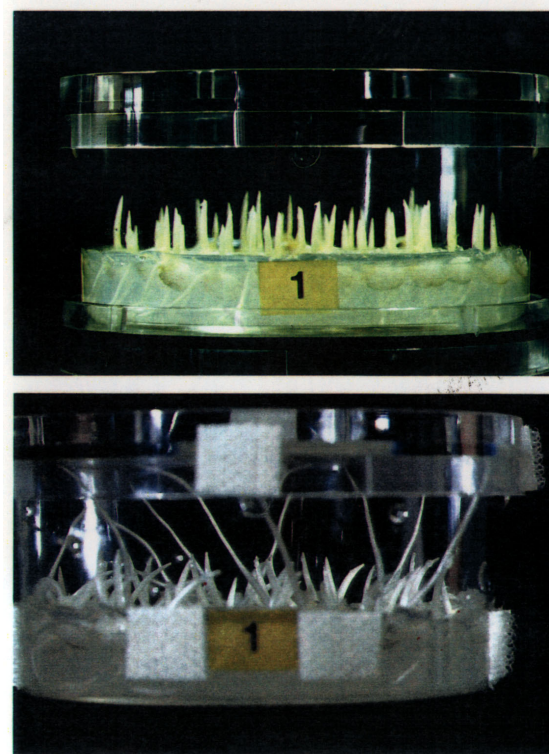

Fig.33 The morphology of 68.5 hr-old Tan-ginbozu seedlings grown under 1 *g* (upper) and microgravity (lower) conditions.

Fig.34 The morphology of 68.5 hr-old Columbia seedlings grown under 1 *g* (upper) and microgravity (lower) conditions.

Fig.35 The morphology of 68.5 hr-old *etr1-1* seedlings grown under 1 *g* (upper) and microgravity (lower)conditions.
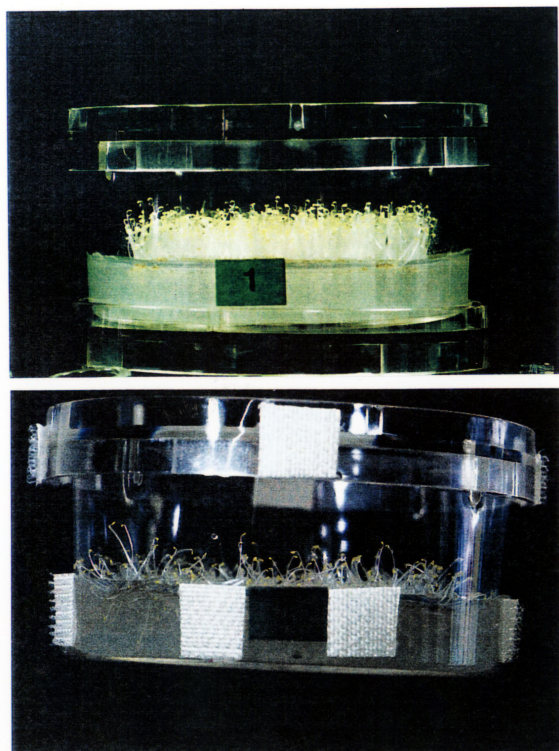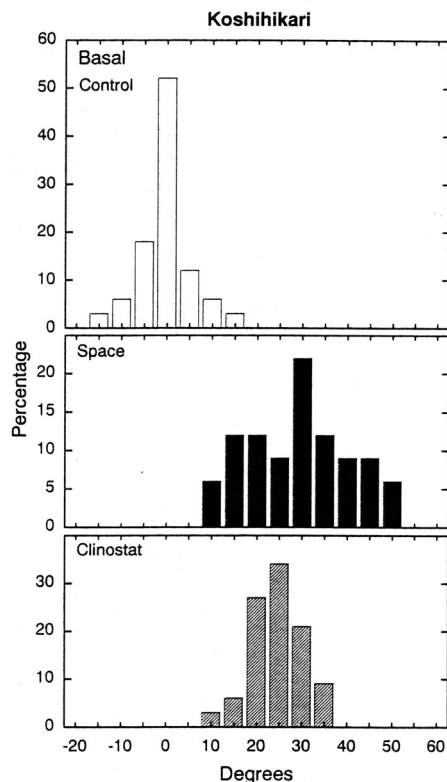

Fig.36 Distribution of the angle in the basal region of Koshihikari coleoptiles.
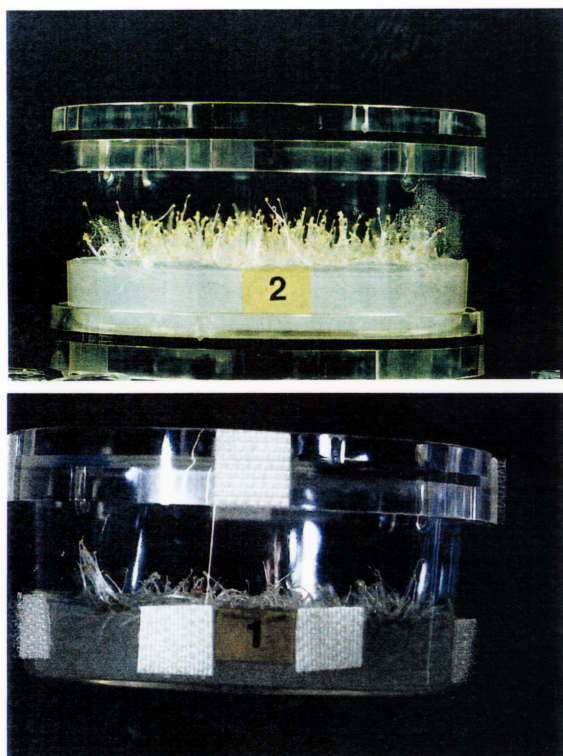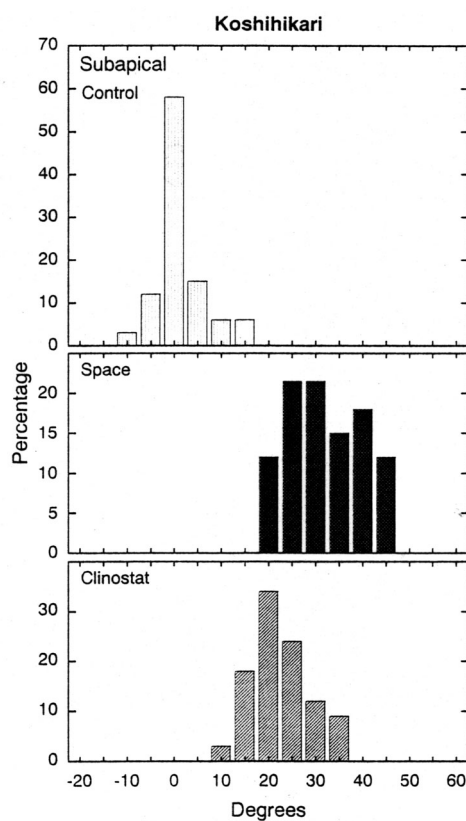

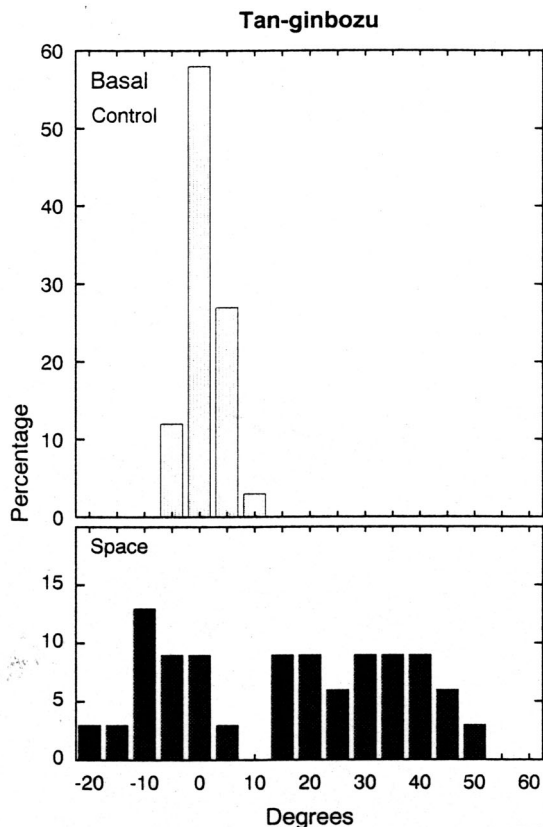**Fig.37** Distribution of the angle in the subapical region of Koshihikari coleoptiles.

Fig.38 Distribution of the angle in the basal region of Tan-ginbozu coleoptiles.
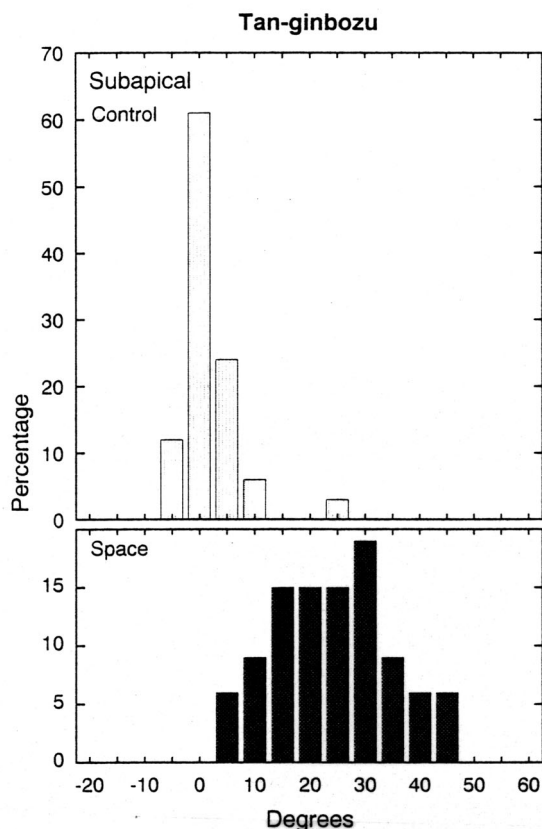

Fig.39 Distribution of the angle in the subapical region of Tan-ginbozu coleoptiles.

38, 39). Control coleoptiles also exhibited straight elongation as in Koshihikari. On the other hand, space-grown Tan-ginbozu coleoptiles also showed an inclination toward the caryopsis in the basal region and also a spontaneous curvature in the same direction in the elongating region. However, the angles in both regions were smaller than Koshihikari. Especially, in the basal region, some Tan-ginbozu coleoptiles showed an inclination in the opposite direction and so the average angle in this cultivar was almost a half of that in Koshihikari. The inclination in the basal regions of rice coleoptiles was constant, whereas the bending in the subapical regions decreased with aging (Fig. 40).

Morphology of rice roots was estimated by measuring the angle between the base line perpendicular to the long axis of caryopses and the direction of the basal regions of roots in the clockwise direction. Control roots of Koshihikari cultivar elongated mostly downward and showed the angle around 0° (Fig. 41). Koshihikari roots elongated in various directions under microgravity conditions in space, more than 20% of which emerged into the air. However, the direction was not completely random and two thirds of the roots formed a constant angle of about 55° with the perpendicular base line. Similar results were obtained for Koshihikari roots grown on the 3-D clinostat, although the angle of majority group was slightly smaller than that in space. Roots of Tan-ginbozu cultivar grown on

the ground and in space also showed similar patterns of distribution of orientation (Fig. 42), excepting that control roots also showed some inclination in the direction as the majority of space-grown roots elongated.

For estimating the orientation of Arabidopsis hypocotyls, the angle between the base line perpendicular to the long axis of seeds and the direction of hypocotyls was read in the clockwise direction. Because Arabidopsis seeds are very small and it was difficult to align them on the agar plate, they were planted in a random fashion, and then the original orientation of seeds was presumed from the direction of hooks in seedlings later. Control hypocotyls of Columbia cultivar elongated around the angle 0° (Fig. 43). In space Columbia hypocotyls elongated in a variety of directions; about 10% of which grew even into the agar medium (Fig. 43). On the 3-D clinostat, Columbia hypocotyls showed a similar distribution of the orientation, although the angle was slightly dispersed. The orientation of hypocotyls of an ethylene-resistant mutant *etr1-1* grown on the ground and in space was fundamentally similar to that in Columbia (Fig. 44). However, in this cultivar control hypocotyls showed slightly dispersed distribution of the angle as compared with Columbia.

iii) Mechanisms Inducing Spontaneous Curvatures

In order to understand the mechanisms by which spontaneous curvatures of rice coleoptiles are induced
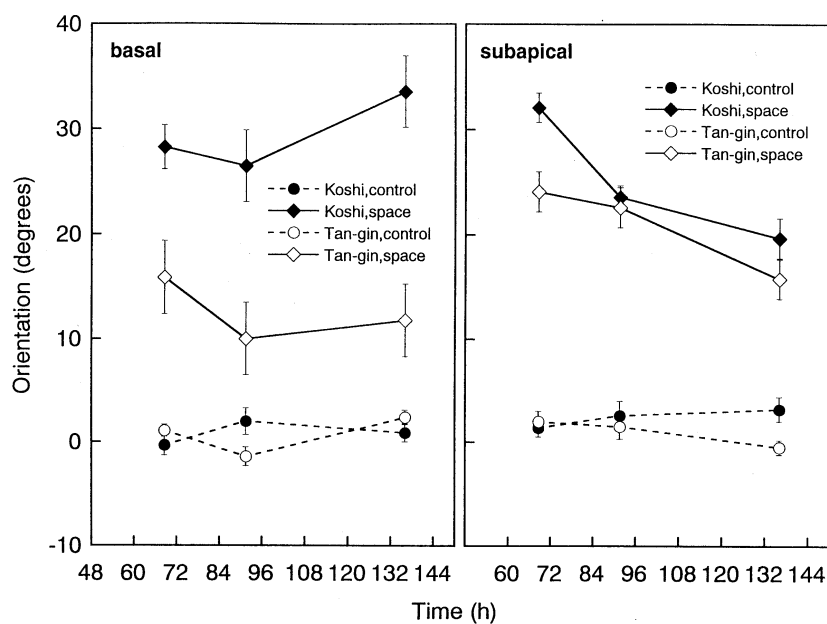
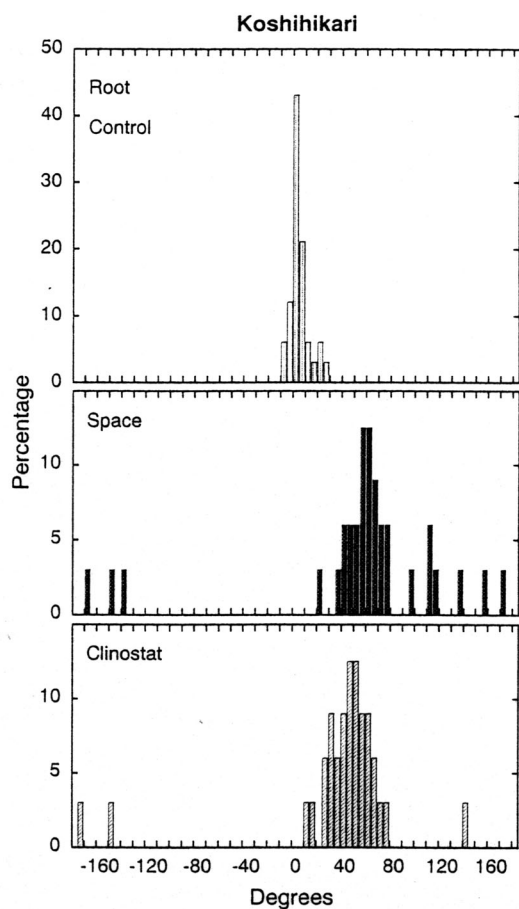**Fig.40** Changes in the orientations of the basal and subapical regions of rice coleoptiles.

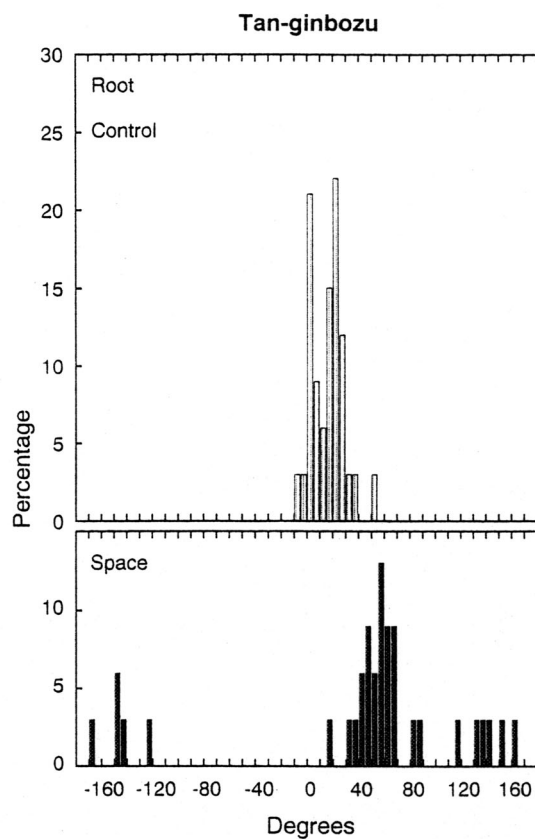**Fig.41** Distribution of the angle in the basal region of Koshihikari roots.

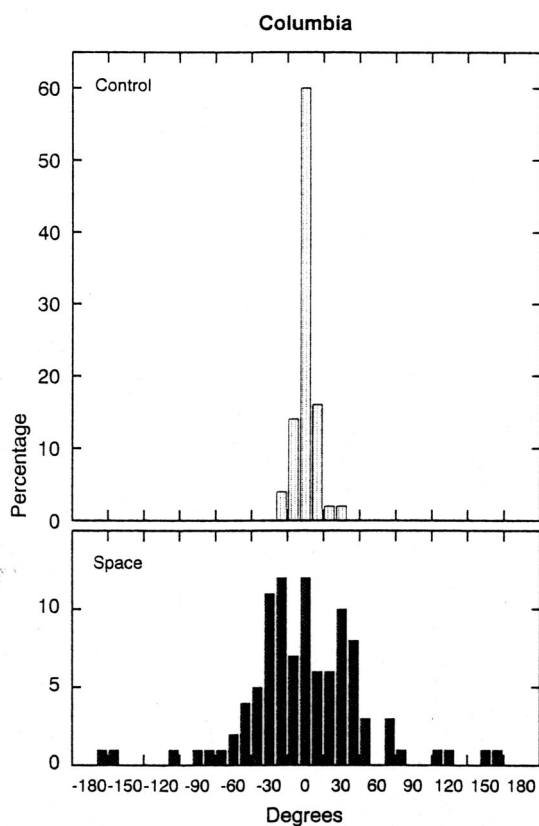**Fig.42** Distribution of the angle in the basal region of Tan-ginbozu roots.

Fig.43 Distribution of the angle in the basal region of Columbia hypocotyls.
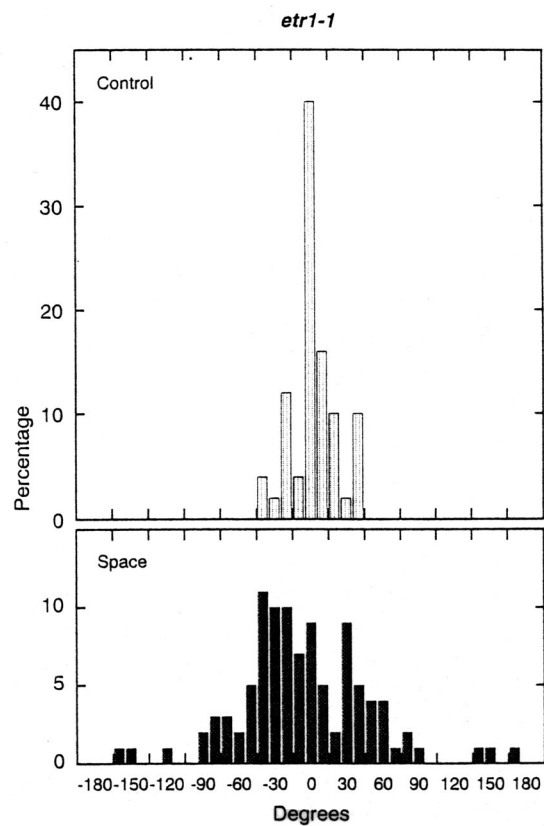

Fig.44 Distribution of the angle in the basal region of *etr1-1* hypocotyls.

under microgravity conditions, segments cut from the curvature portions of rice coleoptiles were separated into the ventral and the dorsal halves, and then the mechanical properties of their cell walls were measured with a tensile tester. No differences were detected in the stress-relaxation parameters between both sides (data not shown). However, the dorsal, convex sides of rice grown in space showed a higher mechanical extensibility of the cell wall than the opposite sides (Fig. 45). There were no clear differences in the extensibility between the ventral and the dorsal sides of control coleoptiles. The difference in the extensibility between the convex and the concave sides was prominent in Koshihikari and decreased with aging (Fig. 45). Such a difference in the cell wall extensibility was not due to the reversible extensibility(data not shown) but due to the irreversible one (Fig. 46). There was a high correlation between such a difference in the cell wall extensibility and the angle of curvatures of rice coleoptiles (Fig. 47).

The chemical basis for the difference in the cell wall extensibility was examined by comparing the levels and the molecular masses of the wall polysaccharides and their turnover between the convex and the concave sides of curved regions of rice coleoptiles. One clear result obtained was that the levels of the wall polysaccharides per unit length were lower in the convex sides than the opposite sides (Fig. 48). The results indicate that the decrease in the

thickness of the cell wall in the convex sides due to uneven modifications of the wall polysaccharide metabolism caused the automorphic curvatures in rice coleoptiles under microgravity conditions. The data obtained also suggest that the decrease in the molecular masses of the hemicellulose polysaccharides (Fig. 49) and the activation of $(1 \rightarrow 3),(1 \rightarrow 4)$-ß-glucanases (Fig. 50) in the convex sides are also involved in the curvatures. However, the further analyses on such changes were impossible in the present study, because of limitations of the amount of samples obtained.

4) Discussion

Under microgravity conditions in space, elongation growth of rice coleoptiles and Arabidopsis hypocotyls was stimulated significantly (Figs. 1, 2). Microgravity also caused an increase in the cell wall extensibility in both materials (Figs. 5, 7, 10, 12). In these organs the levels of cellulose and the matrix polysaccharides per unit length decreased as compared with controls (Figs. 15, 16, 20, 21). The decrease in the molecular mass of xyloglucans was brought about in Arabidopsis hypocotyls grown on orbit (Figs. 24, 25). The changes in activities of cell wall enzymes which appears to be involved in the modifications of the cell wall metabolism were also detected in space-grown
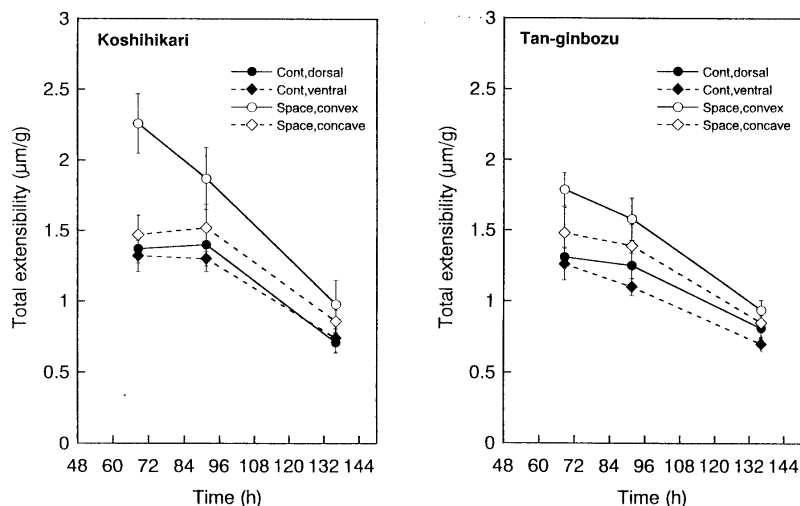
Fig.45 The total cell wall extensibility of the curvature portions of rice coleoptiles grown under microgravity conditions.
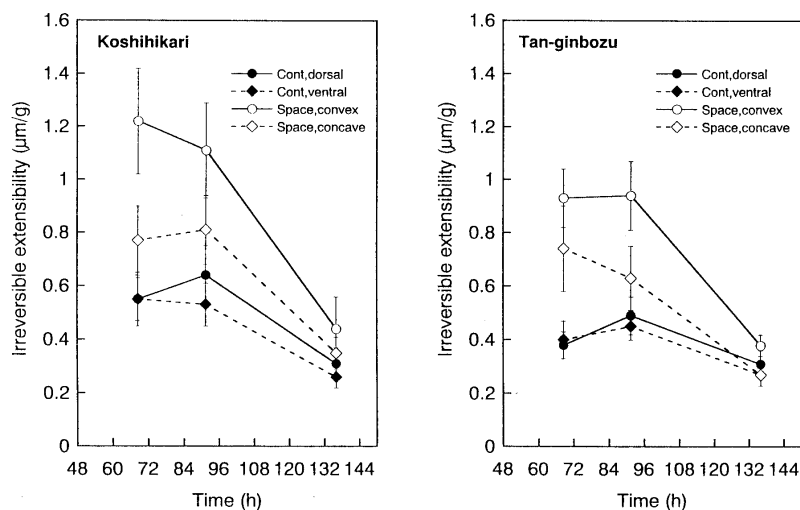

Fig.46 The irreversible cell wall extensibility of the curvature portions of rice coleoptiles grown under microgravity conditions.
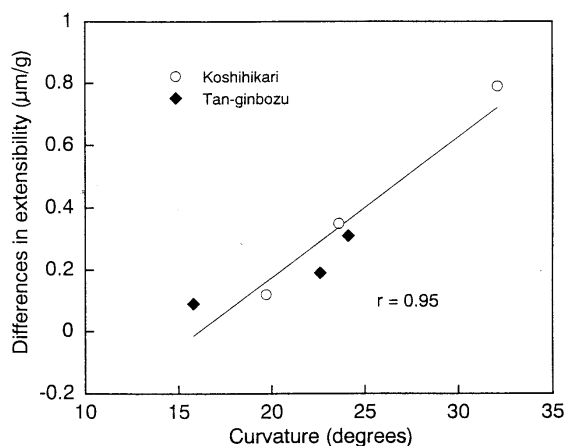
Fig.47 Relationship between the angle of automorphic curvatures and the difference in the cell wall extensibility between the convex and the concave sides of rice coleoptiles grown under microgravity conditions.

seedlings (Figs. 30, 31). Such changes in the cell wall properties are similar to those observed in rice coleoptiles grown under conditions of submergence[1)3)], but opposite to those induced by hypergravity[2)3)]. The results support the hypothesis that under microgravity in space the cell wall metabolism of plant seedlings is modified, which leads to an increase in the cell wall extensibility, thereby stimulating elongation growth.

The effects of microgravity in space on elongation growth of plant seedlings so far reported were controversial. In some experiments, not the stimulation but the suppression of growth has been reported[6)]. We, therefore, first considered the possibility that the stimulation of elongation might be brought about by the difference in the experimental conditions other than gravity between on orbit and on the ground in the present study. One of the factors that influence the growth rate most would be temperature. However, the temperature for cultivating control seedlings
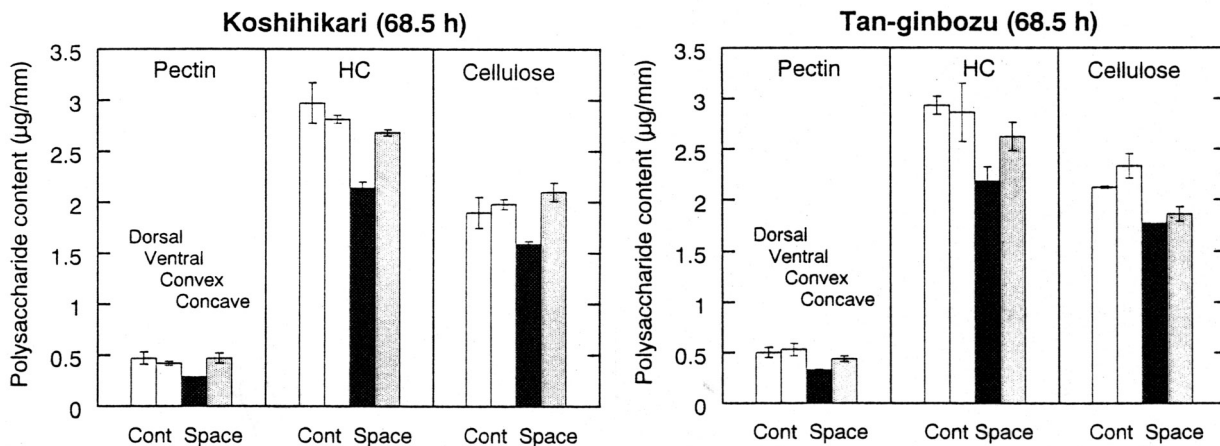
Fig.48 The levels of the cell wall polysaccharides per unit length in the curvature portions of rice coleoptiles grown under microgravity conditions.

Fig.49 Molecular mass distributions of the hemicellulose polysaccharides in the curvature portions of rice coleoptiles grown under microgravity conditions.
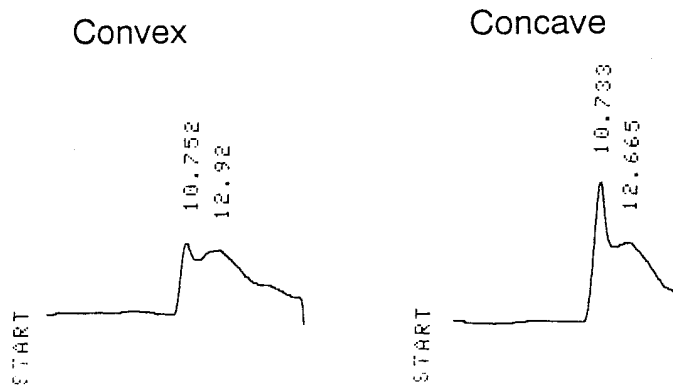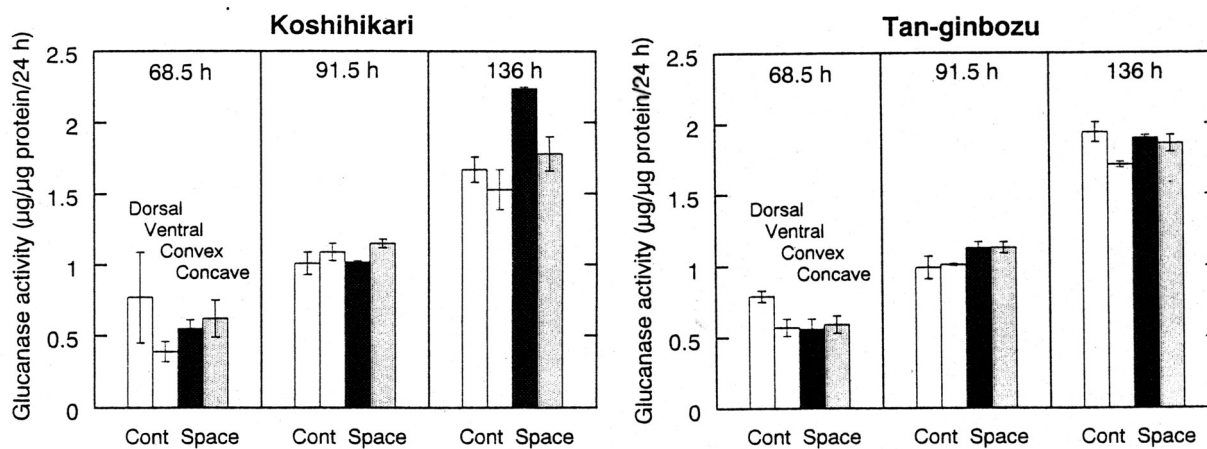

Fig.50 Specific activities of $(1\rightarrow3),(1\rightarrow4)$-ß-glucanases in the curvature portions of rice coleoptiles grown under microgravity conditions.

were controlled very precisely in the present experiment, based on the data stored in the data logger-type thermo-recorder on orbit, and the difference in temperature between in space and on the control experiment was estimated as less than 0.2ºC. Also, the degree of growth stimulation was quite different between rice coleoptiles and Arabidopsis hypocotyls (Figs. 1, 2), although they were cultivated simultaneously, suggesting that the difference in culture temperature is not the cause of growth difference between in space and on the ground. Furthermore, if the temperature of control cultivation was too low, control seedlings should be kept younger and so should show a higher cell wall extensibility than space-grown seedlings. However, the actual results were quite opposite (Figs. 5, 7, 10, 12). These results suggest the difference in temperature is not the primary cause of growth stimulation observed in the present study.

The analyses of experimental procedures in the past space experiments indicate that there were various problems in conditions for cultivation of plant materials in these experiments. For instance, in most of the past experiments, plant seedlings were exposed to light continuously or temporally during cultivation. However, light is another factor that strongly influences the cell wall metabolism, thereby affecting elongation growth of seedlings[3]. Actually, light is recognized as one of gravity-substituting factors and so the effect of microgravity on growth may be diminished in the light. Therefore, rice and Arabidopsis seedlings were grown in complete darkness in the present space experiment. Also, it is required to use independent culture dishes to obtain seedlings with difference ages, as in our study. Another problem in the past space experiments is that seeds had been imbibed on orbit and the amount of growth, that was measured after some days, reflected not only the effect of microgravity on elongation growth but the effects on imbibition and germination. In general, the imbibition process tends to be delayed under microgravity conditions. For avoiding such effects, imbibed seeds and caryopses were launched at cool temperature, and then the cultivation was started by elevation of temperature in the present study. Such a protocol is also advantageous for obtaining uniform seedlings.

In some of the past space experiments, the amounts of certain cell wall constituents have been measured, although the data are fragmentary and not conclusive. Most famous result reported so far is that the amounts of cellulose decrease in space[3][6]. The decrease in the levels of cellulose per unit length of rice coleoptiles (Figs. 15, 16) and Arabidopsis hypocotyls (Figs. 20, 21) was observed also in the present study. However, the levels of other cell wall polysaccharides also decreased in space, of which the decrease in the hemicellulose II fractions was most prominent. Moreover, the increase in cellulase activity in the cell wall was detected in neither rice coleoptiles (Fig. 26) nor Arabidopsis hypocotyls (Fig. 29). Thus, the results obtained in the present study did not support the above view.

The molecular mass of xyloglucans was smaller (Figs. 24, 25) and xyloglucan-hydrolyzing activity was higher (Figs. 30, 31) in space-grown Arabidopsis hypocotyl cell walls than that in control cell walls, irrespective of cultivars and the age. These changes are just opposite to those observed in seedlings of dicotyledons grown under hypergravity conditions[3][7]. Microgravity did not influence the elution profiles of the pectic or the hemicellulose I polysaccharides (Figs 22, 23) or enzyme activities to degrade these polysaccharides (Fig. 29). Namely, the changes are xyloglucan specific. These results suggest that xyloglucans rather than cellulose serve as anti-gravitational polysaccharides. Xyloglucans, the principal matrix polysaccharides, are hydrogen bounded to cellulose and play an important role in growth regulation in dicotyledons[8][9]. It is thus acceptable that xyloglucans have an anti-gravitational function. On the other hand, certain matrix polysaccharides other than xyloglucans appear to play such a role in Gramineae, because their cell walls contain only a small amount of xyloglucans[8][9]. One of candidates would be $(1 \rightarrow 3),(1 \rightarrow 4)$-ß-glucans. Actually, in the present study, we obtained some data suggesting that the turnover of the glucans might be stimulated under microgravity conditions. Unfortunately, the detailed analyses on this point were impossible because of the limited amount of samples obtained.

In order to analyze the involvement of phytohormones in gravity-dependent regulation of the cell wall properties, a gibberellin-deficient dwarf mutant Tan-ginbozu of rice and an ethylene-resistant mutant *etr1-1* of Arabidopsis were used in addition to tall or wild strains, Koshihikari (rice) and Columbia (Arabidopsis) in the present study. The stimulation of rice coleoptile growth under microgravity conditions was clearer in Tan-ginbozu than in Koshihikari (Fig. 1). Because gibberellin plays a principal role in cell wall formation, it is likely that elongation growth of Tan-ginbozu is suppressed at 1 g but not influenced under microgravity conditions. Also, the angles of spontaneous inclinations and curvatures under microgravity conditions were smaller in Tan-ginbozu than in Koshihikari (Figs. 36-40), suggesting that gibberellin is involved in induction of automorphic curavatures. On the other hand, there were no clear differences in growth or morphogenetic parameters between Columbia and *etr1-1*. The development of the anomalous hook in space-grown Arabidopsis hypocotyls due to ethylene had been reported and this was one of reasons we used *etr1-1* mutant in addition to Columbia for the present experiment. However, from the results obtained, it was turned out that such a consideration on the action of ethylene was unnecessary.

The amounts of plant materials obtained in the present study were very small as one cultural dish per treatment, which is about one tenth of the amounts required for the ordinary analyses. Based on the experience accumulated through preliminary experiments, we were successful to use the materials very efficiently and to get a full set of data on the cell wall properties. Especially, for Arabidopsis seedlings information on the nature of their cell wall have

been lacking, although the plant is very popular for the molecular biological analysis. In the present study we could clarify for the first time the physical, chemical, and biochemical properties of the cell wall of Arabidopsis hypocotyls. At the same time, the limitation of the amount of samples made it difficult to clarify various points, such as the chemical basis for the increase in the cell wall extensibility of rice coleoptiles in space, the involvement of modifications of $(1 \rightarrow 3),(1 \rightarrow 4)$-ß-glucan metabolism in automorphic curvatures of rice coleoptiles, and the effect of microgravity on expression of genes coding cell wall enzymes. These points should be clarified by future space experiments in International Space Station.

From the results obtained in the present experiment, we could confirm the occurrence of automorphogenesis under microgravity conditions in space, which should be another major fruit of our experiment. In space, rice coleoptiles showed an inclination toward the caryopsis in the basal region and also a spontaneous curvature in the same direction in the elongating region (Figs. 32, 33, 36-39). Rice roots elongated in various directions including into the air on orbit, but two thirds of the roots formed a constant angle with the axis of the caryopsis (Figs. 41, 42). Arabidopsis hypocotyls in space also elongated in a variety of directions without obvious curvatures in the elongating region (Figs. 43, 44). In the past space experiments, the morphology of various plant seedlings have been recorded. However, the results were variable, because of differences in cultural conditions by experiments[6]. The results on automorphogenesis obtained in the present study provide us with a basis for further analyses on the mechanism of morphogenesis in higher plants.

The direction of elongation and the pattern of spontaneous curvatures of rice coleoptiles and Arabidopsis hypocotyls in space were fundamentally the same as those formed on the 3-D clinostat (Figs. 36, 37, 41). The results support the effectiveness of this clinostat in the study of plant morphogenesis under microgravity conditions, as suggested[5]. Furthermore, in rice coleoptiles the faster-expanding convex side exhibited a higher extensibility of the cell wall than the opposite side (Figs. 45-47), as observed in maize coleoptiles grown on the 3-D clinostat[4]. Thus, not only the occurrence of automorphogenesis was confirmed in space but also the mechanism inducing such a morphogenesis, via the differential modifications of their cell walls, was clarified in the present space experiment. As to the chemical basis for the difference in the cell wall extensibility between the convex and the concave sides in bending rice coleoptiles, what clearly shown in the present study is only the decrease in the thickness of the cell wall in the convex sides (Fig. 48). The contribution of other metabolic changes in the cell wall constituents to the curvature should be in the future experiments.

We carried out the ground control experiments at Hangar L one and three days after the space experiments. However, it was turned out later that the temperature we set was significantly higher than in space. One reason of such a discrepancy is that the information on cabin temperature down-linked did not reflect the temperature around the place where BRIC was placed. The other reason would be that the adjustment of temperature within BRIC was delayed than outer environment due to lack of convection under microgravity conditions. The cabin temperature fluctuated greatly during the present flight, which may have made the situation worse. Because the nature of the cell wall deeply depends on growth rate of plants, the controls stricter than other experiments are required for analyzing the effect of microgravity on the cell wall. The improvement on this point is indispensable for the future space experiments.

5) Literature cited

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