

## Growth and Morphogenesis of Azuki Bean Seedlings in Space during SSAF2013 Program

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### Abstract

**Seedlings of azuki bean were cultivated under microgravity conditions in space during SSAF2013 program, and then growth, morphology, and the cell wall rigidity of their etiolated epicotyls were analyzed. Epicotyls grown on the ground oriented vertical direction, whereas epicotyls grown in space oriented oblique upward direction away from the cotyledons. The length of epicotyls grown in space was varied, but the proportion of seedlings with larger epicotyls was higher than that of the controls. These results indicate that growth and morphology of epicotyls are modified under microgravity conditions in space. On the other hand, the breaking load of epicotyls was analyzed using a spring balance for determination of the cell wall rigidity of epicotyls. The breaking load of epicotyls grown in space tended to be smaller than that of controls. Also, epicotyls grown in space had resistance to bending than the controls. Thus, microgravity affected the cell wall rigidity of epicotyls. Taken together, azuki bean seedlings performed an automorphogenesis and cell wall modification under microgravity conditions. Under microgravity conditions, where plants need not to resist the gravitational force, the body shape and the cell wall rigidity of stems may be modified.**©2014 Jpn. Soc. Biol. Sci. Space; doi: 10.2187/bss.28.6

### Introduction

Gravity is always present on the Earth's surface in a constant direction and magnitude. Plants have utilized gravity as the most reliable directional environmental cues for growth and morphogenesis. In general, plant shoots grow in the direction opposite to the pull of gravity, whereas roots grow in the direction to the pull of gravity. This response to gravity is called gravitropism and enables plants to orient their leaves to sunlight and roots to water and minerals. It has been shown that the relative directional change of gravity is detected by specialized cells called statocytes in gravity sensing for gravitropism (Sack, 1991). In a stimulus-free environment, such as space, plants show spontaneous morphogenesis, termed automorphosis or automorphogenesis (Hoson *et al.*, 1999). For example, pea epicotyls oriented oblique upward direction away from the cotyledons, and roots oriented upward asymmetric to the epicotyls in space (Ueda *et al.*, 1999).

Plants also show the other gravity response that is to resist the gravitational force by developing a rigid body. We have termed this phenomenon "gravity resistance" (Hoson and Soga, 2003) and have analyzed the nature and mechanisms of gravity resistance using hypergravity conditions produced by centrifugation and microgravity conditions in space (Soga, 2010, 2013). Our results indicate that the gravity signal is perceived by mechanoreceptors (mechanosensitive ion channels) on the plasma membrane of not only statocytes but also other types of cells (Soga *et al.*, 2004). As the final step of gravity resistance, plants developed a short and thick body and increased cell wall rigidity to resist the gravitational force. The modification of body shape was brought about by the rapid reorientation of cortical microtubules that was caused by the action of microtubule-associated proteins in response to the magnitude of the gravitational force (Soga *et al.*, 2006, 2009). The regulation of the cell wall rigidity was brought about by modification of the metabolisms of anti-gravitational cell wall polysaccharides (Hoson and Soga, 2003; Nakano *et al.*, 2007).

The "Space Seeds for Asian Future (SSAF)" program is one of the activities of the "Asian Beneficial Collaboration through Kibo Utilization (Kibo-ABC)" initiative under the "Asia-Pacific Regional Space Agency Forum (APRSAF)" (Takaoki *et al.*, 2014). One of the objectives of the program is to provide young people in the Asia-Pacific region with opportunities to learn about leading scientific discoveries through their participations in space experiments. In the SSAF2010-2011 program, seeds native to the Asia-Pacific region were flown to the International Space Station (ISS) and kept in the Kibo Module. Both the seeds returned to the Earth and control seeds not flown to space were germinated on the ground, and then growth and morphology of their seedlings were compared. In the SSAF2013 program, seeds of azuki bean were germinated and their seedlings were cultivated by astronauts in the Kibo. Participants of the program also cultivated azuki bean seedlings on

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the Earth. Participants observed changes in growth and morphology of azuki bean seedlings grown in space using downlinked images of space grown seedlings. Also, the physical strength (cell wall rigidity) of epicotyls was measured in this program. In the present paper, we report the modifications to growth and morphogenesis in azuki bean seedlings under microgravity conditions in space during SSAF2013 program. We also show the changes in the cell wall rigidity of space-grown epicotyls.

## Materials and Methods

### *Plant materials and preflight procedures*

Azuki bean (*Vigna angularis* (Willd.) Ohwi et Ohashi cv. Erimowase) was used in the present experiment. The frequency of seeds with 110-120 mg weight was the highest, when we examined the distribution of the dry weight of the seeds. Thus, we selected seeds in this range to reduce variation in size of seedlings. The selected seeds were sterilized in 5% (v/v) sodium hypochlorite solution for 3 min, and then sterilized in 99.5% (v/v) ethanol for 2 min. The sterilized seeds were dried in a clean bench. Strophiole of the dried seeds was cut off using file to enhance initial water absorption for germination. On July 5, 2013, the seeds were embedded into the slits of rock wool block (Yasai-hana block 30, Nippon Rockwool Co., Tokyo, Japan) with the embryos located to the right at Tsukuba Space Center. The rock wool block containing 18 seeds was placed into plastic container (Tight box 25020 1, Cho Pla Co., Aichi, Japan; Inside dimension: 180 × 120 × 40 mm) with watering and ventilation ports.

### *Inflight procedures*

Plastic container containing dry seeds of azuki bean was launched on the fourth H-II Transfer Vehicle (HTV4) on August 4, 2013, and stored in the Kibo at room temperature until watering. On August 30, 2013, the seeds were provided with water for germination. Plastic container which was in the lightproof bag (Lamizip AL-J, Seisannipponsha, Tokyo, Japan; 340 × 240 mm) was incubated inside the storage rack of the Kibo module of the ISS. The average temperature during incubation was 23.1°C. After incubation for 93.0 h, seedlings were observed by shooting video images. The seedlings were continuously incubated after shooting video images. On September 6, 2013, video and still images of seedlings were obtained after incubation for 147.5 h. Then, some seedlings were removed from plastic container and used for measurement of the breaking load of epicotyls. The middle region of epicotyls were hooked to a spring balance, and pulled until they were broken. Also, another epicotyls were bended by hand until they were broken. These operations in the Kibo were shot in video. Ground control experiments were carried out at the laboratory of Osaka City University, by reproducing materials, hardware, and procedures during flight experiment, as much as possible. The difference in temperature between space and control experiment was less than 0.1°C.

### *Postflight analysis*

The orientation, the length, and the breaking load of epicotyls were measured from video or still images taken in the Kibo or on the ground. In the measurements of epicotyl orientation, the angle between the surface line of rock wool block and the orientation of the basal region of epicotyls was read in the counterclockwise direction. The breaking load was measured by reading the value of a spring balance when epicotyls were broken.

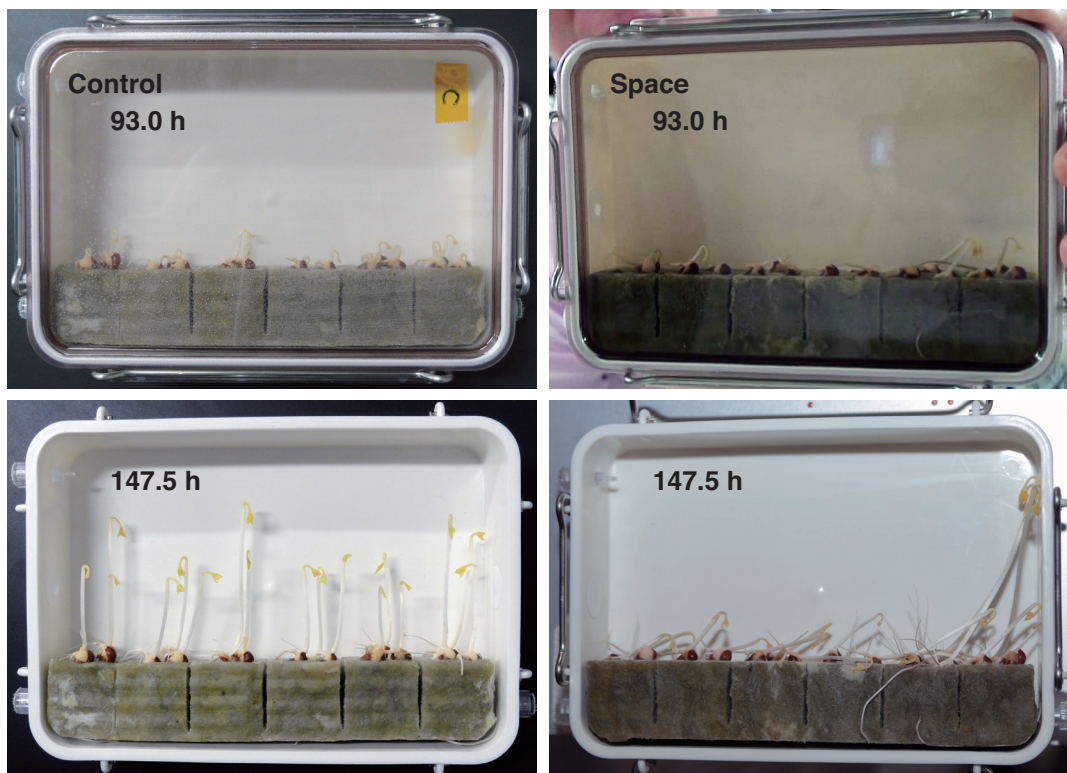
## Results and Discussion

All seeds of azuki bean were germinated irrespective of the gravitational conditions. However, microgravity strongly affected the morphology of seedlings (Fig. 1). On the ground, epicotyls grew upward along gravity vector, although they were slightly inclined (Fig. 1, Table 1). Under microgravity conditions in space, epicotyls oriented oblique upward. Because seeds were embedded into the slits of rock wool block with the embryos located to the right, direction of bending was away from the cotyledons (abaxial). The average angle was not changed during incubation on the ground or in space (Table 1), although the variation was slightly reduced (Fig. 2). Hoson *et al.* (1992) reported that azuki bean epicotyls grown on the three-dimensional (3-D) clinostat exhibit an abaxial curvature. These results indicate that azuki bean epicotyls perform an automorphogenesis under not only omnilateral but also true microgravity conditions. The present results also confirm the effectiveness of the 3-D clinostat in the analysis of morphogenetic processes in microgravity.

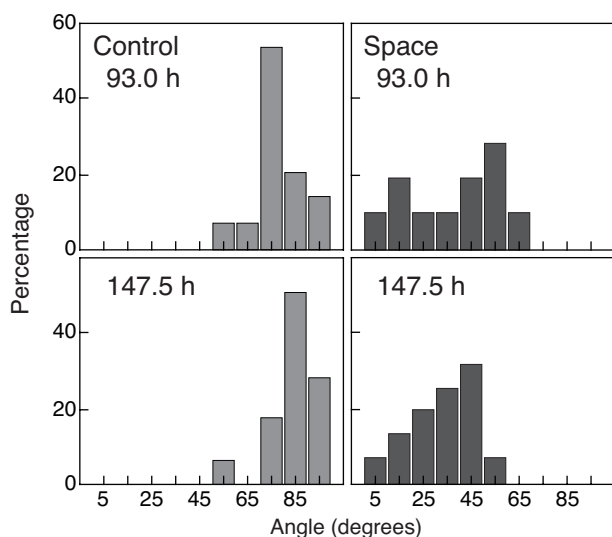
The morphology of shoots has been examined in various space experiments. Heathcote *et al.* (1995) showed that wheat coleoptiles oriented away from the caryopsis (abaxial). Pea epicotyls also oriented abaxial direction (Ueda *et al.*, 1999). On the other hand, rice coleoptiles bended adaxial direction toward the caryopsis (Hoson *et al.*, 1999). Arabidopsis hypocotyls elongated in various directions (Hoson *et al.*, 1999), and cucumber hypocotyls elongated straight in predetermined direction (Takahashi *et al.*, 1999). In the present study, we showed that azuki bean epicotyls bended abaxial direction (Fig. 1). These results indicate that growth pattern of shoots in space is different by plant species and by the type of organs. Epicotyls of both pea (Ueda *et al.*, 1999) and azuki bean (Fig. 1) elongated abaxially in space. These results suggest that epicotyls in general may elongate in abaxial direction under microgravity conditions in space.

**Table 1.** The angle in the basal region and the length of azuki bean epicotyls. Seedlings were grown as shown in Fig. 1. The angle was read as shown in Fig. 2. Values are means ± SE (n=16). Asterisk denote significant difference between control and space seedlings at 5% probability level.

	Angle (degrees)		Length (mm)	
	93.0 h	147.5 h	93.0 h	147.5 h
Control	76.7±2.7	83.4±2.2	6.4±0.6	38.9±2.9
Space	37.1±5.8*	33.7±3.6*	10.5±1.6*	49.1±5.8

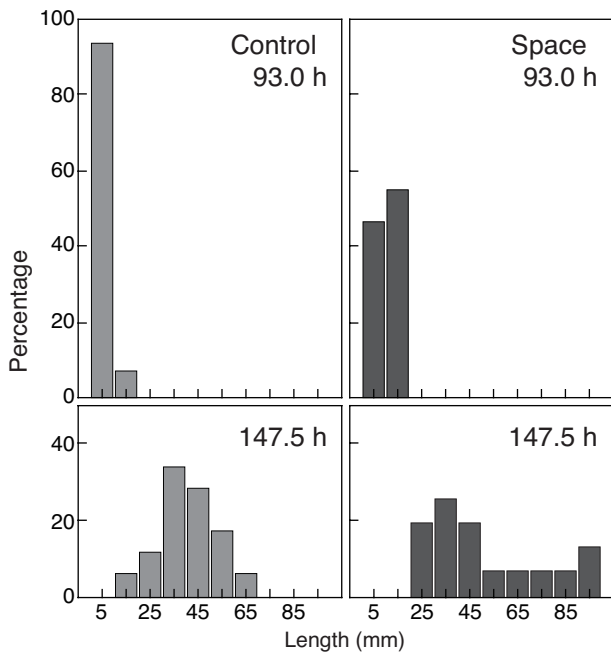


**Fig. 1.** Azuki bean seedlings grown on the ground or in space. Seedlings were cultivated for 93.0 h (upper panel) and 147.5 h (lower panel) on the ground (left panel) and in the Kibo (right panel). Seeds were embedded into the slits of rock wool block with the embryos located to the right.



**Fig. 2.** Distribution of the angle in the basal region of azuki bean epicotyls. Seedlings were grown as shown in Fig. 1. The angle between the surface line of rock wool block and the orientation of the basal region of epicotyls was read in the counterclockwise direction. The values were divided into categories by 10 degrees, and the numbers of each category were shown as percentages.

Variation in the length of epicotyls grown in space was larger than that on the ground (Figs. 1 and 3). In space, the length of the epicotyls in the right side of plastic container was longer than that in the left side. The roots of the seedlings in the left side grew in the air and did not grow into the rock wool medium (Fig. 4). Thus, water supply may be suppressed in the seedlings grown in the left side. Also, lateral expansion of epicotyls in the upper growing region was observed in the seedlings in the left side (Fig. 4). The present cultivation container has not active ventilation system, although it has ventilation ports sealed with a membrane filter. Also, the left side of the container was placed in the bottom of the lightproof bag, where air exchange is lower than the entrance of the bag. Because of the limited ventilation and the lack of buoyancy convection under microgravity (Lappa 2004), the gas composition in the container may be different between the right and the left sides. Ethylene, a plant hormone, stimulates lateral expansion and inhibits elongation growth in shoot organs (Neljubow, 1911). Thus, the accumulation of ethylene in the left side of the container may induce stimulation of lateral expansion and inhibition of elongation growth. The temperature condition may also be different between the right and left sides of the container, because the container was incubated in the storage rack of the Kibo, but not in an incubator in the present study. The difference in the temperature may induce a difference in the moisture conditions in the gas between the right and left sides of the container.

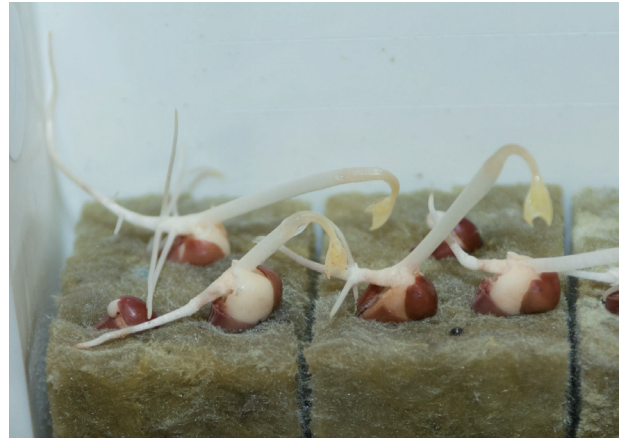


**Fig. 3.** Distribution of the length of azuki bean epicotyls. Seedlings were grown as shown in Fig. 1. The lengths of epicotyls were divided into categories by 10 mm, and the numbers of each category were shown as percentages.

Taken together, the high variation in the length of space-grown epicotyls may be induced by the difference in the environmental conditions, such as water and atmospheric composition including ethylene and temperature.

The distribution of the length of epicotyls grown in space was shifted to longer region (Fig. 3). Therefore, the average value of the length of space-grown epicotyls was higher than that of control epicotyls (Table 1). The length of epicotyls grown in space for 93.0 h was significantly longer at 5% level by Student's *t*-test. In cultivation for 147.5 h, there was no statistically significant difference, although the average value in space was higher than that of control. Under hypergravity conditions, elongation growth of azuki bean epicotyls was suppressed with increasing gravity (Soga *et al.*, 2006). In the *Arabidopsis* hypocotyls, elongation growth was suppressed by hypergravity, whereas that was stimulated by microgravity (Soga *et al.*, 2001, 2002). The hypocotyl length of *Arabidopsis* varied in proportion to the logarithm of the magnitude of gravity in the range from microgravity to hypergravity. These results indicate that growth of azuki bean is also changed in response to the magnitude of gravity in the range from microgravity to hypergravity. These results also suggest that development of a short and thick body may be regarded as a part of the response that enables plants to grow against the gravitational force.

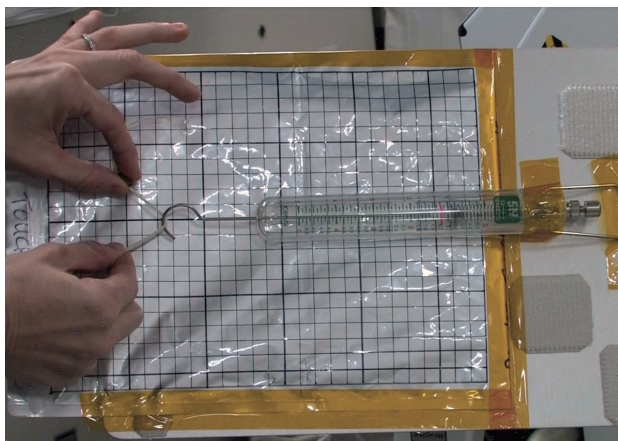
We have reported that hypergravity increased the cell wall rigidity in shoots of various plants, such as garden cress, azuki bean, maize and *Arabidopsis* (Hoson *et al.*,



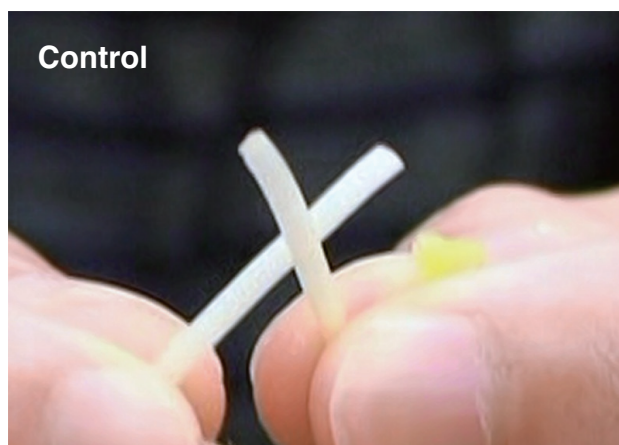
**Fig. 4.** Azuki bean seedlings grown in space. Seedlings were cultivated in left side of the container for 147.5 h under microgravity conditions.

1996; Soga *et al.*, 1999a, 1999b, 2001; Nakabayashi *et al.*, 2006). On the other hand, the cell wall rigidity of both *Arabidopsis* hypocotyls and rice coleoptiles decreased under microgravity conditions (Hoson *et al.*, 2002; Soga *et al.*, 2001, 2002). The cell wall rigidity of *Arabidopsis* hypocotyls varied in proportion to the logarithm of the magnitude of gravity in the range from microgravity to hypergravity (Soga *et al.*, 2001). In the present study, to measure the cell wall rigidity of azuki bean epicotyls in the ISS, we searched for instruments available in the ISS. As a result, we found a spring balance as a candidate (Fig. 5). To check whether a spring balance could be utilized for the measurement of cell wall rigidity, we compared the breaking load of azuki bean epicotyls grown under hypergravity conditions with that of the controls. Cultivation at 300 G clearly increased the breaking load of epicotyls, which is comparable to the result obtained with a tensile tester. Thus, we measured the breaking load of epicotyls as the measure of the cell wall rigidity in the present study. The breaking load of epicotyls grown in space tended to be smaller than that of controls (Table 2). We also performed the bending test; epicotyls were bended by hand until they were broken (Fig. 6). Epicotyls grown in space had resistance to bending, as compared with those grown on the ground. These results indicate that microgravity decreased the cell wall rigidity of azuki bean epicotyls. Thus, the increase in the cell wall rigidity may be regarded as a part of the response that enables plants to grow against the gravitational force.

We have mainly analyzed the responses to hypergravity of azuki bean seedlings to clarify the nature and mechanisms of gravity resistance (Soga, 2010, 2013). Thus, we need to analyze the responses to microgravity in azuki bean seedlings. In the SSAF2013 program, azuki bean seeds were shown to germinate and grow under microgravity conditions, as at 1 G on the ground. Also, the present results showed that the body shape and the cell wall rigidity were modified in space. The details of gravity resistance remain to be clarified in



**Fig. 5.** Measurement of the breaking load of azuki bean epicotyls. The middle region of epicotyls were hooked to a spring balance, and pulled until they were broken.



**Fig. 6.** Bending test in azuki bean epicotyls. Seedlings were cultivated for 147.5 h on the ground and in the Kibo. Epicotyls were bended by hand until they were broken.

**Table 2.** The breaking load of azuki bean epicotyls. Seedlings were cultivated for 147.5 h on the ground and in the Kibo. The breaking load was measured as shown in Fig. 5. Values are means  $\pm$  SE (n=8).

	Breaking load (g)
Control	140.3 $\pm$ 8.9
Space	122.5 $\pm$ 14.1

further space experiments using azuki bean seedlings. In the present study, high variation in the growth of epicotyls was observed (Figs. 1 and 3). The present observation suggests that the plants responded to the heterogeneous distribution of atmospheric components under microgravity in which buoyancy convection was suppressed. Gas components around plant body diffuse slowly under microgravity due to lack of buoyancy convection and that condition could be utilized to study plant response to gaseous substances. On the contrary, lack of buoyancy convection may suppress normal growth of plants. Therefore, in future space experiments, gas conditions in the cultivation vessels should be controlled by forced convection system to reduce the variation in the growth of epicotyls. Also, primordia of root should be embedded into the medium to increase the seedlings whose roots grow into the medium, although the changes in growth direction of epicotyls by microgravity may be suppressed.

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#### References

- Heathcote, D.G., Chapman, D.K. and Brown, A.H. (1995) Nastic curvatures of wheat coleoptiles that develop in true microgravity. *Plant Cell Environ.*, **18**, 818-822.
- Hoson, T., Kamisaka, S., Masuda, Y. and Yamashita, M. (1992) Changes in plant growth processes under microgravity conditions simulated by a three-dimensional clinostat. *Bot. Mag. Tokyo*, **105**, 53-70.
- Hoson, T., Nishitani, K., Miyamoto, K., Ueda, J., Kamisaka, S., Yamamoto, R. and Masuda, Y. (1996) Effects of hypergravity on growth and cell wall properties of cress hypocotyls. *J. Exp. Bot.*, **47**, 513-517.
- Hoson, T., Soga, K., Mori, R., Saiki, M., Wakabayashi, K., Kamisaka, S., Kamigaichi, S., Aizawa, S., Yoshizaki,

- I., Mukai, C., Shimazu, T., Fukui, K. and Yamashita, M. (1999) Morphogenesis of rice and *Arabidopsis* seedlings in space. *J. Plant Res.*, **112**, 477-486.
- Hoson, T., Soga, K., Mori, R., Saiki, M., Nakamura, Y., Wakabayashi, K. and Kamisaka, S. (2002) Stimulation of elongation growth and cell wall loosening in rice coleoptiles under microgravity conditions in space. *Plant Cell Physiol.*, **43**, 1067-1071.
- Hoson, T. and Soga, K. (2003) New aspects of gravity responses in plant cells. *Int. Rev. Cytol.*, **229**, 209-244.
- Lappa, M. (2004) Fluids, Materials and Microgravity: Numerical Techniques and Insights into Physics. Oxford, England, Elsevier Science.
- Nakabayashi, I., Karahara, I., Tamaoki, D., Masuda, K., Wakasugi, T., Yamada, K., Soga, K., Hoson, T. and Kamisaka, S. (2006) Hypergravity stimulus enhances primary xylem development and decreases mechanical properties of secondary cell walls in inflorescence stems of *Arabidopsis thaliana*. *Ann. Bot.*, **97**, 1083-1090.
- Nakano, S., Soga, K., Wakabayashi, K. and Hoson, T. (2007) Different cell wall polysaccharides are responsible for gravity resistance in the upper and the basal regions of azuki bean epicotyls. *Biol. Sci. Space*, **21**, 113-116.
- Neljubow, D. (1911) Geotropismus in der Laboratoriumsluft. *Ber. Dtsch. Bot. Ges.*, **29**, 97-112.
- Sack, F. D. (1991) Plant gravity sensing. *Int. Rev. Cytol.*, **127**, 193-252.
- Soga, K., Wakabayashi, K., Hoson, T. and Kamisaka, S. (1999a) Hypergravity increases the molecular size of xyloglucans by decreasing xyloglucan-degrading activity in azuki bean epicotyls. *Plant Cell Physiol.*, **40**, 581-585.
- Soga, K., Harada, K., Wakabayashi, K., Hoson, T. and Kamisaka, S. (1999b) Increased molecular mass of hemicellulosic polysaccharides is involved in growth inhibition of maize coleoptiles and mesocotyls under hypergravity conditions. *J. Plant Res.*, **112**, 273-278.
- Soga, K., Wakabayashi, K., Hoson, T. and Kamisaka, S. (2001) Gravitational force regulates elongation growth of *Arabidopsis* hypocotyls by modifying xyloglucan metabolism. *Adv. Space Res.*, **27**, 1011-1016.
- Soga, K., Wakabayashi, K., Kamisaka, S. and Hoson, T. (2002) Stimulation of elongation growth and xyloglucan breakdown in *Arabidopsis* hypocotyls under microgravity conditions in space. *Planta*, **215**, 1040-1046.
- Soga, K., Wakabayashi, K., Kamisaka, S. and Hoson, T. (2004) Graviperception in growth inhibition of plant shoots under hypergravity conditions produced by centrifugation is independent of that in gravitropism and may involve mechanoreceptors. *Planta*, **218**, 1054-1061.
- Soga, K., Wakabayashi, K., Kamisaka, S. and Hoson, T. (2006) Hypergravity induces reorientation of cortical microtubules and modifies growth anisotropy in azuki bean epicotyls. *Planta*, **224**, 1485-1494.
- Soga, K., Kotake, T., Wakabayashi, K., Kamisaka, S. and Hoson, T. (2009) The transcript level of katanin gene is increased transiently in response to changes in gravitational conditions in azuki bean epicotyls. *Biol. Sci. Space*, **23**, 23-28.
- Soga, K. (2010) Gravity resistance in plants. *Biol. Sci. Space*, **24**, 129-134.
- Soga, K. (2013) Resistance of plants to gravitational force. *J. Plant Res.*, **126**, 589-596.
- Takahashi, H., Mizuno, H., Kamada, M., Fujii, N., Higashitani, A., Kamigaichi, S., Aizawa, S., Mukai, C., Shimazu, T., Fukui, K., Yamashita, M. (1999) A spaceflight experiment for the study of gravimorphogenesis and hydrotropism in cucumber seedlings. *J. Plant Res.*, **112**, 497-505.
- Takaoki, M., Yano, S., Mathers, N., Dwivany, F., Esyanti, R., Djamaluddin, T., Asillam, M. F., Kamarudin, F., Madom, M. S., Tantiphanwadi, S., Giang, N. V. and Kibo-ABC (2014) Space seeds for Asian future. *Trans. Japan Soc. Aeronautical and Space Sci., Aerospace Technol. Japan*, in press.
- Ueda, J., Miyamoto, K., Yuda, T., Hoshino, T., Fujii, S., Mukai, C., Kamigaichi, S., Aizawa, S., Yoshizaki, I., Shimazu, T. and Fukui, K. (1999) Growth and development, and auxin polar transport in higher plants under microgravity conditions in space: BRIC-AUX on STS-95 space experiment. *J. Plant Res.*, **112**, 487-492.