Biological Sciences in Space, Vol.23 No.3, 131-136, 2009

Role of the Cell Wall-Sustaining System in Gravity Resistance in Plants

Takayuki Hoson†, Kouichi Soga and Kazuyuki Wakabayashi
Department of Biology, Graduate School of Science, Osaka City University, Sumiyoshi-ku, Osaka 558-8585, Japan

Abstract

Gravity resistance is one of two principal gravity responses in plants, comparable to gravitropism. In the final step of gravity resistance, plants increase the rigidity of their cell walls via modifications to the metabolism. Various constituents of the plasma membrane and the cytoskeleton play an important role in sustaining functions of the cell wall in gravity resistance. Mechanoreceptors located on the plasma membrane are involved in the perception of gravity signal. The perceived signal may be, at least partly, transformed and transduced via membrane sterol rafts, depending on its magnitude. Cellulose synthases and proton pumps are responsible for modifications to the cell wall metabolism and the apoplastic environment, respectively. On the other hand, the reorientation of cortical microtubules contributes to modification of growth anisotropy, which is related to gravity resistance. Also, microtubule-associated proteins are important in maintenance of the structure and induction of the reorientation of cortical microtubules. Gravity resistance in plants is thus mediated by the structural continuum or physiological continuity of cortical microtubules-plasma membrane-cell wall. Article ID: 092302014

Introduction

When laid onto its side, the stem organ of plants begins to bend upward and then continues to grow in the direction opposite to the pull of gravity. The whole process all together has been called gravitropism. However, the curvature and the following straight growth are quite different in origin and mechanism. We separated the latter process from gravitropic curvature and termed it gravity resistance. We have examined the nature and mechanism of gravity resistance using hypergravity conditions, up to 300 *G*, produced by centrifugation and in space experiments. As a result, we have clarified the outline of the sequence of events leading to the final response in gravity resistance (Hoson and Soga, 2003; Hoson *et al*., 2005). These studies have also shown that gravity resistance is a principal gravity response in plants distinct from gravitropism. The development of gravity resistance appears to have played an important role in the transition of plant ancestors from an aquatic environment to a terrestrial environment and in the consequent establishment of land plants (Hoson, 2003, 2006).

Plant protoplasts are surrounded by well-developed cell walls, which is the major source of mechanical strength for plant body. Thus, the cell wall may be responsible for gravity resistance. Actually, we have obtained evidence supporting this hypothesis by hypergravity and space experiments (Hoson and Soga, 2003; Hoson *et al*., 2005; Hoson, 2006). Hypergravity has been shown to increase the cell wall rigidity in various plant materials. On the contrary, the cell wall rigidity of space-grown Arabidopsis hypocotyls and rice coleoptiles was lower than that of the controls. The cell wall rigidity is determined by the chemical nature of cell wall constituents, such as the level and the molecular mass. Hypergravity has been shown to increase cell wall thickness in various materials. Hypergravity also caused a polymerization of certain matrix polysaccharides: in dicotyledons, hypergravity increased the molecular mass of xyloglucans, whereas hypergravity increased that of 1,3,1,4-β-glucans in Gramineae plants. In addition, hypergravity decreased xyloglucan-degrading activity in dicotyledons and 1,3,1,4-β-glucanase activity in Gramineae organs. Modifications to xyloglucan metabolism under hypergravity conditions were brought about by specific down-regulation of expression of one of xyloglucan endo-transglucosylase/hydrolase (XTH) genes (Soga *et al*., 2007), which is devoted to xyloglucan breakdown (Tabuchi *et al*., 2001). On the other hand, cell wall thickness was decreased under microgravity conditions in space. The space-grown Arabidopsis hypocotyls and rice coleoptiles also contained xyloglucans and 1,3,1,4-β-glucans with lower molecular masses, respectively, resulting from the increases in xyloglucan-degrading activity and 1,3,1,4-β-glucanase activity. Thus, xyloglucans and 1,3,1,4-β-glucans appear to act as anti-gravitational cell wall polysaccharides. In addition, cellulose and lignin play a role in gravity resistance (Hoson, 2009; Karahara *et al*., 2009; Wakabayashi *et al*., 2009a, b). Taken together, these results show that plants increase the rigidity of their cell walls via modifications to the metabolism as the final step in gravity resistance (Hoson and Soga, 2003; Hoson *et al*., 2005; Hoson, 2006).

The physiological functions of the cell wall are sustained by its intimate crosstalk with the symplast (Hoson, 2002). In particular, the plasma membrane and the cytoskeleton play an important role in regulating the metabolism of cell wall constituents and therefore

Received: June 24, 2009; Accepted: August 24, 2009
†To whom correspondence should be addressed:
Tel./Fax: +81-66605-2577; E-mail: hoson@sci.osaka-cu.ac.jp

functions of the cell wall. The data obtained by hypergravity and space experiments have shown the involvement of these cellular components in perception of gravity signal as well as transformation and transduction of the perceived signal in gravity resistance. In the present article, we describe the roles of the plasma membrane and the cytoskeleton in induction of the final response of gravity resistance by the cell wall.

Role of the Plasma Membrane in Gravity Resistance

Mechanoreceptor

Perception of the gravity signal is the first step of a series of events leading to gravity responses. Mechanoreceptors (mechanosensitive ion channels) are present on the plasma membrane of plants, as of other organisms (Kanzaki *et al.*, 1999; Nakagawa *et al.*, 2007). We examined the effects of lanthanum and gadolinium ions, which are blockers of mechanoreceptors, on gravity resistance and found that hypergravity had no effects on the mechanical or chemical properties of the cell wall of shoot or root organs in the presence of such ions (Soga *et al.*, 2004, 2005). The results suggest the involvement of mechanoreceptors in the perception of gravity signal in gravity resistance (Fig. 1). Horizontal- and acropetal-hypergravity induced gravity resistance, as did basipetal-hypergravity, supporting the hypothesis. Because typical mechanoreceptors are Ca^{2+}-permeable channels (Toyota *et al.*, 2007), changes in the cytoplasmic Ca^{2+} concentration may play a role in transformation and transduction of perceived signal.

On the other hand, lanthanum and gadolinium ions at the same concentration had no effects on gravitropism of shoot or root organs. Also, *pgm* (*phosphoglucomutase*) and *sgr1* (*shoot gravitropism 1*) mutants of Arabidopsis, which have reduced or no gravitropic responses because of lack of sedimentable amyloplasts (Tasaka *et al.*, 2001), showed normal gravity resistance as wild-type (Soga *et al.*, 2004, 2005). These findings show that the gravity perception mechanism in gravity resistance is independent of that of gravitropism.

Sterol (raft)

The expression of a gene encoding 3-hydroxy-3-methylglutaryl-Coenzyme A reductase (HMGR), which catalyzes a reaction producing mevalonic acid, a key precursor of terpenoids, was greatly up-regulated under hypergravity conditions (Yoshioka *et al.*, 2003). Out of various membrane constituents of azuki bean epicotyls, the level of sterols was specifically increased by hypergravity (Koizumi *et al.*, 2007). T-DNA insertion mutants for two *HMGR* genes (*hmg*) have been isolated in Arabidopsis (Suzuki *et al.*, 2004). These loss of function mutants showed a decreased sterol level and are hypersensitive to the gravitational stimuli. Also, lovastatin, an inhibitor of HMGR, made the epicotyls hypersensitive to gravity. These results suggest that membrane sterols are involved in maintenance of capacity of plant organs to resist the gravitational force. It has been shown that sterols consist of microdomains called rafts in plant membranes, as in other organisms (Lefebvre *et al.*, 2007). The data obtained by detailed analysis of sterol composition and microarray assay of expression of related genes in plant materials grown under hypergravity conditions support the hypothesis that membrane sterols form the raft structure and thereby act in signal transformation and transduction of gravity resistance (Fig. 1).

Proton pump

The activity of cell wall enzymes, that are responsible for the metabolism of cell wall constituents, is determined not only by their protein levels but also by the cell wall environment. Out of various factors that determine the cell wall environment, the apoplastic pH is the most important one regulating the activity of cell wall enzymes *in situ* (Hoson, 2002). Hypergravity has been shown to increase the apoplastic pH of various materials (Soga *et al.*, 2000a, 2000b). Because vanadate, an inhibitor of P-type ATPase, also increased the apoplastic pH and hypergravity had no further effects on the pH in the presence of vanadate, hypergravity may increase the apoplastic pH via the reduction of the activity of the plasma membrane H^+-ATPase (proton pump). When the activities of xyloglucan and 1,3,1,4-β-glucan degradation

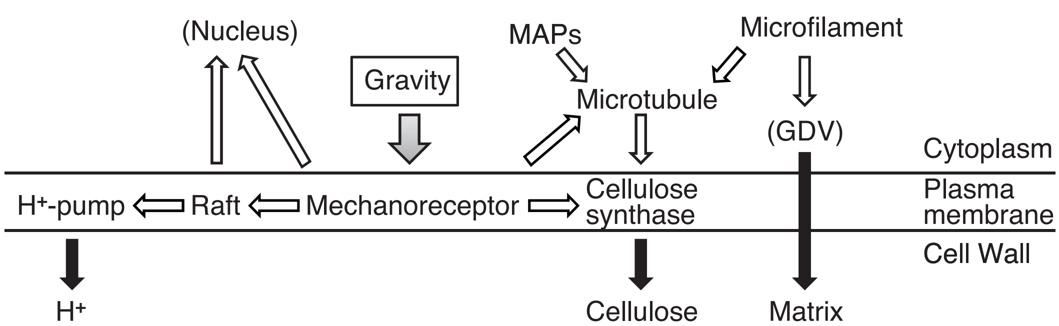

Fig. 1. Mechanism of gravity resistance in plants. The gravity signal is perceived by mechanoreceptors located on the plasma membrane, and then transformed and transduced through the plasma membrane and into the cytoplasm. The increase in cell wall rigidity is brought about as the final step in gravity resistance. Various constituents of the plasma membrane and the cytoskeleton play an important role in sustaining functions of the cell wall in gravity resistance. MAPs, microtubule-associated proteins; GDV, Golgi-derived vesicle.

were assayed at the pH corresponding to the pH value of the hypergravity-treated cell wall, a significant decrease in activity was detected (Soga *et al.*, 2000a, 2000b). Thus, the gravity signal may modify the metabolism of cell wall constituents not only directly but via regulation of the cell wall environment, when it increases the cell wall rigidity. These data suggest the involvement of proton pumps in signal transformation and transduction of gravity resistance (Fig. 1).

Cellulose synthase

The analyses of changes in mechanical and chemical properties of the cell wall in response to gravity had been limited to the growing regions of stem organs, and no data had been reported for the basal supporting regions. Effects of hypergravity on the metabolism of cell wall polysaccharides were examined along azuki bean epicotyls (Nakano *et al.*, 2007; Wakabayashi *et al.*, 2009a). The levels of matrix polysaccharides were almost constant over the regions. The matrix levels were increased by hypergravity in the upper regions, but not in the basal ones. Also, the levels of xyloglucans were increased by hypergravity only in the upper regions. On the other hand, the levels of cellulose gradually increased from the apical toward the basal regions, and hypergravity increased the levels only in the basal regions. Cellulose microfibrils in general play an important role in determining the cell wall rigidity. These findings suggest that cellulose, instead of xyloglucans, acts as anti-gravitational polysaccharide in the basal supporting regions (Hoson, 2009). Cellulose is synthesized on the plasma membrane by cellulose synthase complexes, indicating that the plasma membrane contributes to gravity resistance also via action of cellulose synthase (Fig. 1). It has been reported that the expression of cellulose synthase genes was up-regulated by hypergravity (Martzivanou and Hampp, 2003; Tamaoki *et al.*, 2009), which matches with the above-mentioned results. Cellulose is also responsible for tension wood formation in arborescent dicotyledonous angiosperms in response to the gravitational stimulus (Hoson, 2009).

Role of the Cytoskeleton in Gravity Resistance

Cortical microtubule

The cytoskeleton gives the cytoplasm structural stability and mechanical strength. Thus, cytoskeletal components may play a role in gravity resistance, in concert with the cell wall. Actually, the analysis of the changes in gene expression in Arabidopsis hypocotyls grown under hypergravity conditions has shown that the expression of most α- and β-tubulin genes is up-regulated by hypergravity, depending on the magnitude of the gravitational force (Yoshioka *et al.*, 2003; Matsumoto *et al.*, 2007). The involvement of microtubules in gravity resistance was examined with microtubule-disrupting agents and with tubulin mutants. The microtubule-disrupting agent colchicine was shown to completely prevent hypergravity-induced suppression of elongation growth as the gravity resistance (Matsumoto *et al.*, 2007). On the other hand, a number of amino acid substitution mutants in α- or β-tubulins have been isolated in Arabidopsis (Hashimoto, 2002; Ishida *et al.*, 2007). In hypocotyls of mutant of α-tubulin 6 (*tua6*), the length was shorter and the thickness was larger than those in wild-type hypocotyls at 1 *G*. Hypergravity suppressed elongation growth and stimulated lateral thickening of wild-type hypocotyls, but the degree of such changes was smaller in *tua6* mutant than in the wild-type, suggesting that tubulin mutants are hypersensitive to the gravitational force. In addition, tubulin mutants showed left-handed or right-handed helical growth, derived from disordered organization of cortical microtubules, even under 1 *G* conditions, and such a phenotype was intensified under hypergravity conditions. These results support the hypothesis that cortical microtubules play an important role in gravity resistance in plants (Fig. 1).

Hypergravity also modified the orientation of cortical microtubules. In the epidermis of azuki bean epicotyls grown at 1 *G*, cells with transverse cortical microtubules were predominant. With increasing the gravitational force, the percentage of cells with transverse microtubules was decreased, whereas that with longitudinal microtubules was increased (Soga *et al.*, 2006). The reorientation of cortical microtubules occurred promptly after transfer of seedlings from 1 *G* to hypergravity conditions. Lanthanum and gadolinium ions suppressed the reorientation of cortical microtubules. These results indicate that the reorientation of cortical microtubules is also involved in gravity resistance. The direction of plant cell growth is primarily determined by the pattern of deposition of cellulose, which, in turn, is thought to be regulated by the cytoskeleton. The co-alignment hypothesis states that the movement of cellulose synthase complexes in the plasma membrane is constrained by interactions with cortical microtubules. It is likely that cellulose microfibrils and cortical microtubules are mutually dependent in their functions such as gravity resistance.

Microtubule-associated proteins

Microtubule-associated proteins (MAPs) are proteins that bind to or interact with the microtubules and regulate their functions. Because MAPs are important for maintaining dynamics of cortical microtubules, they may also play a role in gravity resistance (Fig. 1). Elongation growth of mutant of one of MAPs, MICROTUBULE ORGANIZATION 1 (MOR1), was suppressed at 1 *G* as compared with wild-type, and was not further affected by hypergravity (Higuchi *et al.*, 2008). Also, *mor1* mutant showed helical growth due to disordered organization of cortical microtubules even at 1 *G*, and such a phenotype was intensified under hypergravity conditions. These results suggest that *mor1* mutant is hypersensitive to the gravitational force and that MOR1 plays a role in maintenance of normal growth capacity against the gravitational force probably via stabilizing the structure of cortical microtubules.

It has been proposed that the reorientation of cortical microtubules is brought about by branching of existing microtubules (Murata *et al.*, 2005). First, γ-tubulin complex may bind onto pre-existing cortical microtubules

Table 1 Roles of constituents of the plasma membrane and the cytoskeleton in gravity resistance.

Constituent	Role in gravity resistance
Plasma membrane	
Mechanoreceptor	Signal perception
Sterol (Raft)	Signal transformation and transduction
Proton pump	Regulation of the apoplastic pH
Cellulose synthase	Cellulose synthesis
Cytoskeleton	
Cortical microtubule	Regulation of growth anisotropy
Microtubule-associated protein	Maintenance of microtubule function
MOR1	Stabilization of microtubule structure
γ-Tubulin	Nucleation of new microtubule branch
Katanin	Separation of microtubule branch

and nucleate new microtubules as branch. Katanin then may sever the newly synthesized microtubule branch, and repeat of these processes may induce the reorientation. To clarify whether this model is applicable to the reorientation of cortical microtubules in gravity resistance, we examined the changes in the expression of genes encoding γ-tubulin complex and katanin in azuki bean epicotyls grown under hypergravity conditions (Soga *et al.*, 2008, 2009). Hypergravity increased transiently the expression of γ-tubulin complex genes, which was followed by a transient up-regulation of expression of katanin gene. Also, lanthanum and gadolinium ions nullified hypergravity-induced reorientation of microtubules as well as increases in expression of both genes. Furthermore, elongation growth of katanin mutant was suppressed at 1 *G* as compared with wild-type, and was not further affected by hypergravity (Higuchi *et al.*, 2008). These results suggest that γ-tubulin complex and katanin contribute to gravity resistance via induction of the reorientation of cortical microtubules.

Microfilament

Microfilaments (actin filaments) are other major constituents of the cytoskeleton. In signal perception in gravitropism, amyloplast sedimentation may induce tensional changes within the microfilament network, leading to the activation of the downstream signaling cascades responsible for final curvature (Sievers *et al.*, 1991). It has been reported that disruption of microfilaments promotes gravitropic curvature in shoot and root organs (Yamamoto and Kiss, 2002; Hou *et al.*, 2003). The results of our preliminary experiments suggest that microfilaments also play a role in gravity resistance (Fig. 1).

Conclusions and Prospects

Various constituents of the plasma membrane and the cytoskeleton are involved in perception of gravity signal as well as transformation and transduction of the perceived signal in gravity resistance (Table 1). These constituents may play a role in the structural continuum or physiological continuity of cortical microtubules-plasma membrane-cell wall, leading to the increase in cell wall rigidity as the final step in gravity resistance (Hoson *et al.*, 2005). Such a hypothesis was formulated mainly based on results of hypergravity experiments. However, it is uncertain whether the hypothesis is applicable to gravity resistance of plants to 1 *G* gravity on earth, as to the resistance to hypergravity (Hoson *et al.*, 2007). To clarify this point, we have conducted the space experiment, denoted as the Resist Wall, with Arabidopsis *hmg* and *tua6* mutants in the European Modular Cultivation System (EMCS) on the International Space Station (ISS). Unfortunately, the experiment was incomplete, because of serious anomalies of water supply system, and only limited information was obtained (Hoson *et al.*, 2009; Kamada *et al.*, 2009). Therefore, we are now preparing for the next space experiments in the Kibo Module. These experiments will greatly advance our knowledge of the mechanism of gravity resistance in plants.

References

Hashimoto, T. (2002) Molecular genetics analysis of left-right handedness in plants, *Philos. Trans. Royal Soc. London B. Biol. Sci.*, **357**, 799-808.

Higuchi, S., Kumasaki, S., Matsumoto, S., Soga, K., Wakabayashi, K., Hashimoto, T. and Hoson. T. (2008) Effects of gravity on growth phenotypes in MAPs mutants of *Arabidopsis*, *37th COSPAR Scientific Assembly*, F11-0036-08.

Hoson, T. (2002) Physiological functions of plant cell coverings, *J. Plant Res.*, **115**, 277-282.

Hoson, T. (2003) Development of the anti-gravitational system in land plants and its implication for the interaction between plants and other organisms, *Biol. Sci. Space*, **17**, 54-56.

Hoson, T. (2006) The mechanism and significance of gravity resistance in plants, *J. Gravit. Physiol.*, **13**, 97-100.

Hoson, T. (2009) Cellulose as the anti-gravitational polysaccharide. In Cellulose: Structure and Properties, Derivatives and Industrial Uses, Nova Science Publishers, New York (in press).

Hoson, T., Matsumoto, S., Soga, K., Wakabayashi, K., Hashimoto, T., Sonobe, S., Muranaka, T., Kamisaka, S., Kamada, M., Omori, K., Ishioka, N. and Shimazu, T. (2007) The outline and significance of the Resist Wall experiment: Role of microtubule-membrane-cell wall continuum in gravity resistance in plants, *Biol. Sci. Space*, **21**, 56-61.

Hoson, T., Matsumoto, S., Soga, K., Wakabayashi, K., Hashimoto, T., Sonobe, S., Muranaka, T., Kamisaka, S., Kamada, M., Omori, K., Ishioka, N. and Shimazu, T. (2009) Growth and cell wall properties in hypocotyls of Arabidopsis *tua6* mutant under microgravity conditions in space, *Biol. Sci. Space*, (in press).

Hoson, T., Saito, Y., Soga, K. and Wakabayashi, K. (2005) Signal perception, transduction, and response in gravity resistance. Another graviresponse in plants, *Adv. Space Res.*, **36**, 1196-1202.

Hoson, T. and Soga, K. (2003) New aspects of gravity responses in plant cells, *Int. Rev. Cytol.*, **229**, 209-244.

Hou, G., Mohamalawari, D. R., and Blancaflor, E. B. (2003) Enhanced gravitropism of roots with a disrupted cap actin cytoskeleton, *Plant Physiol.*, **131**, 1360-1373.

Ishida, T., Kaneko, Y., Iwano, M. and Hashimoto, T. (2007) Helical microtubule arrays in a collection of twisting tubulin mutants of *Arabidopsis thaliana*, *Proc. Natl. Acad. Sci. U.S.A.*, **104**, 8544-8549.

Kamada, M., Omori, K., Yokoyama, R., Nishitani, K., Hoson, T., Shimazu, T. and Ishioka, N. (2009) Preparation and outline of space-based studies on gravity responses and cell wall formation in plants, *Biol. Sci. Space*, (in press).

Kanzaki, M., Nagasawa, M., Kojima, I., Sato, C., Naruse, K., Sokabe, M. and Iida, H. (1999) Molecular identification of a eukaryotic, stretch-activated nonselective cation channel, *Science*, **285**, 882-886.

Karahara, I., Tamaoki, D., Nishiuchi, T., Schreiber, L. and Kamisaka, S. (2009) Effects of altered gravity conditions on lignin and secondary wall formation in herbaceous dicots and woody plants, *Biol. Sci. Space*, (in press).

Koizumi, T., Sakaki, T., Usui, S., Soga, K., Wakabayashi, K. and Hoson, T. (2007) Changes in membrane lipid composition in azuki bean epicotyls under hypergravity conditions: Possible role of membrane sterols in gravity resistance, *Adv. Space Res.*, **39**, 1198-1203.

Lefebvre, B., Furt, F., Hartmann, M.-A., Michaelson, L. V., Carde, J.-P., Sargueil-Boiron, F., Rossignol, M., Napier, J. A., Cullimore, J., Bessoule, J. J. and Mongrand, S. (2007) Characterization of lipid rafts from Medicago truncatula root plasma membranes: A proteomic study reveals the presence of a raft-associated redox system, *Plant Physiol.*, **144**, 402-418.

Martzivanou, M. and Hampp, R. (2003) Hyper-gravity effects on the Arabidopsis transcriptome, *Physiol. Plant.*, **118**, 221-231.

Matsumoto, S., Saito, Y., Kumasaki, S., Soga, K., Wakabayashi, K, and Hoson, T. (2007) Up-regulation of tubulin genes and roles of microtubules in hypergravity-induced growth modifications in *Arabidopsis* hypocotyls, *Adv. Space Res.*, **39**, 1176-1181.

Murata, T., Sonobe, S., Baskin, T.I., Hyodo, S., Hasezawa, S., Nagata, T., Horio, T. and Hasebe, M. (2005) Microtubule-dependent microtubule nucleation based on recruitment of γ-tubulin in higher plants, *Nat. Cell Biol.*, **10**, 961-968.

Nakagawa, Y., Katagiri, T., Shinozaki, K., Qi, Z., Tatsumi, H., Furuichi, T., Kishigami, A., Sokabe, M., Kojima, I., Sato, S., Kato, T., Tabata, S., Iida, K., Terashima, A., Nakano, M., Ikeda, M., Yamanaka, T. and Iida, H. (2007) *Arabidopsis* plasma membrane protein crucial for Ca^{2+} influx and touch sensing in roots, *Proc. Natl. Acad. Sci. U.S.A.*, **104**, 3639-3644.

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.

Sievers, A., Buchen, B., Volkmann, D. and Hejnowicz, Z. (1991) Role of cytoskeleton in gravity perception. In CW Lloyd, ed, The Cytoskeletal Basis of Plant Growth and Form, Academic Press, London, pp 169–182.

Soga, K., Kotake, T., Wakabayashi, K., Kamisaka, S. and Hoson, T. (2008) Transient increase in the transcript levels of γ-tubulin complex genes during reorientation of cortical microtubules by gravity in azuki bean (*Vigna angularis*) epicotyls, *J. Plant Res.*, **121**, 493-498.

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., Wakabayashi, K., Hoson, T. and Kamisaka, S. (2000a) Hypergravity-induced increase in the apoplastic pH and its possible involvement in suppression of β-glucan breakdown in maize seedlings, *Aust. J. Plant Physiol.*, **27**, 967-972.

Soga, K., Wakabayashi, K., Hoson, T. and Kamisaka, S. (2000b) Changes in the apoplastic pH are involved in regulation of xyloglucan breakdown of azuki bean epicotyls under hypergravity conditions, *Plant Cell Physiol.*, **41**, 509-514.

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. (2005) Mechanoreceptors rather than sedimentable amyloplasts perceive the gravity signal in hypergravity-induced inhibition of root growth in azuki bean, *Funct. Plant Biol.*, **32**, 175-179.

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., Wakabayashi, K., Kamisaka, S. and Hoson, T. (2007) Effects of hypergravity on expression of *XTH* genes in azuki bean epicotyls, *Physiol. Plant.*, **131**, 332-340.

Suzuki, M., Kamide, Y., Nagata, N., Seki, H., Ohyama, K., Kato, H., Masuda, K., Sato, S., Kato, T., Tabata, S., Yoshida, S. and Muranaka, T. (2004) Loss of function of *3-hydroxy-3-methylglutaryl coenzyme A reductase 1 (HMG1)* in *Arabidopsis* leads to dwarfing, early senescence and male sterility, and reduced sterol levels, *Plant J.*, **37**, 750-761.

Tabuchi, A., Mori, H., Kamisaka, S. and Hoson, T. (2001) A new type of endo-xyloglucan transferase devoted to xyloglucan hydrolysis in the cell wall of azuki bean epicotyls, *Plant Cell Physiol.*, **42**, 154-161.

Tamaoki, D., Karahara, I., Nishiuchi, T., De Oliveira, S., Schreiber, L., Wakasugi, T., Yamada, K., Yamaguchi, K. and Kamisaka, S. (2009) Transcriptome profiling in *Arabidopsis* inflorescence stems grown under hypergravity in terms of cell walls and plant hormones, *Adv. Space Res.*, **44**, 245-253.

Tasaka, M., Kato, T. and Fukaki, H. (2001) Genetic regulation of gravitropism in higher plants, *Int. Rev. Cytol.*, **206**, 135-154.

Toyota, M., Furuichi, T., Tatsumi, H. and Sokabe, M. (2007) Hypergravity stimulation induces changes in intracellular calcium concentration in *Arabidopsis* seedlings, *Adv. Space Res.*, **39**, 1190-1197.

Wakabayashi, K., Nakano, S., Soga, K. and Hoson, T. (2009a) Cell wall-bound peroxidase activity and lignin formation in azuki bean epicotyls grown under hypergravity conditions, *J. Plant Physiol.*, **166**, 947-954.

Wakabayashi, K., Soga, K. and Hoson, T. (2009b) Modification of cell wall architecture in gramineous plants under altered gravity conditions, *Biol. Sci. Space*, (in press).

Yamamoto, K. and Kiss, J. Z. (2002) Disruption of the actin cytoskeleton results in the promotion of gravitropism in inflorescence stems and hypocotyls of *Arabidopsis*, *Plant Physiol.*, **128**, 669-681.

Yoshioka, R., Soga, K., Wakabayashi, K., Takeba, G. and Hoson, T. (2003) Hypergravity- induced changes in gene expression in *Arabidopsis* hypocotyls, *Adv. Space Res.*, **31**, 2187-2193.