Summary report of the ISS-Kibo utilization mission,
"Mechanisms of Gravity Resistance in Plants
- From Signal Transformation and Transduction to Response (Resist Tubule)"
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Background and Objectives

Mechanical resistance to the gravitational force, termed gravity resistance, is a principal graviresponse in plants, comparable to gravitropism, and has sustained the evolution of land plants. Nevertheless, only limited information was available on the mechanism of gravity resistance. We have shown, with ground-based experiments using centrifugal hypergravity conditions, that cortical microtubules and membrane sterol rafts play an important role in signal transformation and transduction, leading to increased cell wall rigidity as the final response of resistance to hypergravity. However, it is uncertain whether this hypothesis is as applicable to resistance to 1 *g* gravity as to the resistance to hypergravity. The present experiment aims to confirm this by using true microgravity conditions in space as the control, thereby elucidating the universal mechanisms of gravity resistance, in particular the processes from the signal transformation and transduction to the response.

Research Operations

We set two specific aims for the present experiment:
1. to clarify microscopically the modifications in dynamics of cortical microtubules and membrane rafts, under microgravity conditions, and
2. to confirm and quantify the restoration of growth phenotypes of Arabidopsis tubulin and raft mutants, under microgravity conditions.

We conducted three runs for these objectives:
Run #1 On-site analysis of dynamics of cellular components
Four green fluorescent protein (GFP)-expressing Arabidopsis lines were cultivated for 3 days in the Cell Biology Experiment Facility (CBEF) incubator with a cultivation/observation chamber and video measurement unit (V-MEU) canister and then the structure and distribution of cortical microtubules and membrane rafts were examined on orbit with the CB fluorescence microscope.
Run #2 Analysis of cellular components on the ground
Wild-type Arabidopsis seedlings were cultivated for 3 days in the CBEF incubator with chamber B/V-MEU canister and fixed with an aldehyde mixture in KSC Fixation Tubes. The fixed materials were then recovered to Earth at low temperature and observed microscopically on the ground.
Run #3 Analysis of growth phenotypes of mutants
Arabidopsis tubulin and raft mutants and wild-type plants were cultivated for 39 days in the CBEF incubator with a Plant Experiment Unit (PEU) and their images were taken daily. The plants were then fixed with RNAlater® solution in Chemical Fixation Bags and recovered to Earth in frozen condition. Gene expression levels and cell wall properties of the recovered materials were analyzed.

Results

1. Role of cortical microtubules

The expression of tubulin genes was suppressed under microgravity. In the epidermis of Arabidopsis hypocotyls, reorientation of cortical microtubules from transverse to longitudinal directions occurred in regions where elongation growth had almost ceased at 1 *g*. Under microgravity, such reorientation was suppressed and the growing region was extended toward the base. α-Tubulin mutants *tua4* and *tua6* showed strong and slight dwarfism, respectively, at 1 *g*. Growth of both mutants was stimulated up to the level of wild-type growth under microgravity. These results suggest that under microgravity, the expression of tubulin genes was downregulated, which caused the suppression of cortical microtubule reorientation and thereby, stimulated growth.

2. Role of membrane rafts

The expression of membrane raft-related genes was downregulated under microgravity; in addition, the raft-derived fluorescence on the plasma membrane decreased. The raft-related *hmg1* mutant did not grow inflorescence stems at all, suggesting strong suppression of reproductive growth under microgravity conditions.

3. Role of the cell wall

The cell wall extensibility was higher under microgravity than at 1 *g*, particularly in the basal region of inflorescence stems. In the basal region, the cellulose and matrix polysaccharide levels decreased and showed a significant negative correlation with cell wall extensibility, under microgravity. In addition, the expression of cellulose synthase genes (*CES4*, *CES*7, and *CES8*) and xylan synthase genes was suppressed under microgravity. These results suggest that under microgravity, the expression of genes involved in secondary wall synthesis was downregulated, which caused a decrease in cell wall polysaccharide levels and thereby, maintained the cell wall soft and extensile.

Significance and Application

The present experiment effectively utilized microgravity conditions in the Kibo and elucidated the universal mechanisms of signal transformation and transduction underlying the response involved in gravity resistance. The results may also provide cues for understanding the evolution of land plants and the mechanism of other environmental responses in plants. Information on the mechanism of gravity resistance obtained in the present study would enable efficient plant production, which is indispensable for human life not only in space but also on Earth.

Conclusions

The results of the present experiment support the hypothesis that cortical microtubules, membrane rafts, and the cell wall play an essential role in resistance of plants to gravity in the range from 1 *g* to hypergravity. The experiment also shows that gravity resistance consists of two spatially and temporally separated processes: first, the stimulation of lateral expansion of organs, caused by the cortical microtubule reorientation from transverse to longitudinal directions in regions where elongation growth has almost ceased, and then, cell wall strengthening due to the activation of secondary wall synthesis in the basal region.