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宇宙環境で生育するオオムギの遺伝子発現

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Gene Expression of Barley Grown in Space

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Abstract: Barley seeds were germinated and cultured for 26 days in LADA plant growth chamber onboard Russian segment of the international space station (ISS). The seeds were germinated after 3 days of irrigation and grown to have a flag leaf as same as the ground control barley. Expression levels of defense/stress response genes in space-grown barley were compared to those in ground-grown barley by semiquantitative RT-PCR. In 17 defense/stress response genes that are up-regulated by oxidative stress or other abiotic stress, only catalase, pathogenesis-related protein 13, and phenylalanine ammonia-lyase genes were increased in space-grown barley, suggesting that the barley germinated and grown in LADA onboard ISS is not damaged by space environment, especially oxidative stress, which are suspected to be induced by space radiation and microgravity.
Key words; Barley, Gene expression, Oxidative stress, International Space Station, LADA plant growth chamber

Plant cultivation in space should be necessary to self-supply foods when number of astronauts and cosmonauts would stay and investigate in orbital and in the bases of the moon and Mars. Establishment of the culture conditions certifying plant productivity and multiple generations in space is critical to future advanced space life support systems. To date, number of plants could be cultivated under space flight conditions (Musgrave et al. 1997; Ferl et al. 2002; Berkovich et al. 2004; Levinskikh et al. 2005; Gostimskii et al. 2007), but molecular and genetic changes in plants caused by long-term spaceflight environment are not to be analyzed yet. In space, plants are exposed to the extreme environment, especially space radiation is suspected to induce oxidative stress by generating high-energy free radicals and microgravity would enhance the effect of space radiation (Horneck 1992), however, current understandings of plant growth and responses on this synergistic effect of radiation and microgravity is limited to a few experiments.

Cereals are the most important crop for not only human diet but also animal feeding. Barley, the 4th most produced cereal crops in the world, is more tolerant to environmental stress such as dry and salinity than wheat and rice, and would be a suitable crop to culture in space, where the water is limited to use. In this study, barley seeds were germinated and cultured in plant growth chamber, LADA, onboard Russian segment of ISS. Gene expression grown in space was analyzed by RT-PCR to understand plant responses and adaptation to space environment and to develop the space stress-tolerant plants.

Materials and methods

Plant cultivation and spaceflight

Seeds of barley, *Hordeum vulgare* L. cv. Haruna nijo, were transported to ISS by Progress M-56 launched on April 24, 2006, and kept in the Zvezda service module. The seeds were set in the root module of LADA plant growth chamber onboard Zvezda on August 31, 2006, and allowed to germinate after irrigating water. The seedlings were grown in LADA under a light/dark cycle of 20 hrs/4 hrs at 25 °C. After 26 days of cultivation, the plants were harvested, brought to the ground on September 29, 2006, by Soyuz TMA-8, and stored at -80 °C. Ground control cultivation was carried out under the similar temperature and light/dark cycle to the space experiment. The culture experiment was achieved by Pavel Vinogradov, the commander for Expedition 13.

RNA preparation

Leaf samples of space and ground barleys frozen at -80 °C were ground to a fine powder by mortar and pestle in liquid nitrogen. Total RNA was isolated from the powder using the RNeasy Plant mini kit (Qiagen) according to the manufacture's instructions. The quality of the isolated total RNA was examined by Agilent 2100 Bioanalyzer (Agilent Technologies).

Semiquantitative RT-PCR

The 1st-strand cDNA was synthesized from 1 mg of total RNA with Oligo(dT) using the PrimeScript 1st strand cDNA synthesis kit (Takara Bio). The cDNA was subjected to PCR with Ex Taq HT (Takara Bio) and primers designed on the basis of each barley gene sequence on a database under the following thermal cycle profile: 94 °C for 15 sec, 60 °C for 15 sec, 68 °C for 1 min. PCR cycle number for each gene is shown in Table 1. The PCR products were resolved by ethidium bromide-stained agarose gel electrophoresis. The mRNA levels of each gene were normalized with that of α-tubulin gene.

Table 1. PCR cycle number and amplified fragment size of target gene.

Target gene	Fragment size (bp)	PCR cycle No
PR1a	502	35
PR1b	494	30
PR2	500	35
PR3	749	35
PR4	442	35
PR5	713	35
PR10	496	35
PR13	395	28
LTP	350	33
CHS	490	30
α-TB	457	28
GST	502	28
SOD	459	28
CAT	504	28
APX	502	28
PAL	285	33
AOR	1020	33
PHGPX	331	35

Results and discussion

Growth of barley in space

Barley was cultured in LADA plant growth chamber, which has successfully produced a harvest of fresh vegetables in space. The seeds of barley kept in Zvezda over 4 months were germinated after 3 days of irrigation in LADA and the final germination ratio was over 90 %. The height of plants was about 50 to 60 cm and flag leaf has been opened after 26 days of irrigation. These results of barley growth in space were similar to those of the ground control barley, indicating that barley growth is not affected by microgravity and radiation of space environment in ISS.

Gene expression of barley in space

Plants respond to environmental stress in several ways and have evolved mechanisms by which to increase their tolerance to the stress through interactive molecular and cellular changes (Nelson et al. 1998; Zhang et al. 1999; Knight and Knight 2001). The components of these changes are responses of integrated signaling networks triggered under stressful conditions, and they are assigned as a potential marker. Therefore, these marker genes and proteins are important for understanding plant response, adaptation, and tolerant mechanism.

In orbital and in the bases of the moon and Mars, conditions of plant cultivation such as temperature, light, air, and water could be controlled, however, it would be hard to eliminate space radiation and microgravity, which are suspected to induce oxidative stress. To understanding the barley response to space environment, expression levels of defense/stress response genes in space- and ground-grown barleys were determined by RT-PCR (Fig. 1). Oxidative stress generates reactive oxygen species (ROS) such as superoxide anione, hydroxyperoxide, and hydroxyl radical, and plants have developed enzymatic systems for scavenging these ROS, therefore, we examined the gene expression levels of ROS-reducing proteins in space-grown barley. The gene levels of catalase (CAT) was increased but that of ascorbate peroxidase (APX) was decreased, and those of glutathione *S*-transferase (GST) and superoxide dismutase (SOD) were not changed in space-grown barley, when compared to those in ground-grown barley. Ascorbate oxidoreductase (AOR) and phospholipid hydroperoxide glutathione peroxidase (PHGPX) genes

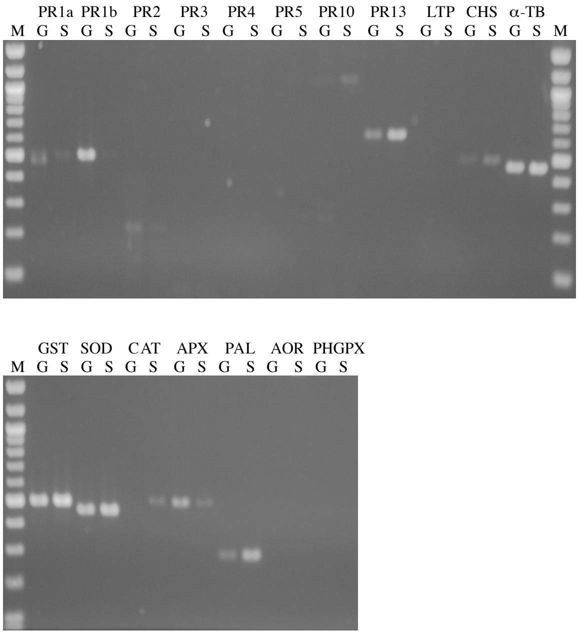

Fig. 1. Expression of defense/stress response genes in space-grown barley (S) and ground-grown barley (G). Two μl of each PCR reaction mixture was loaded on agarose gel and stained by ethidium bromide. M, 100 bp-ladder marker.

were not detected in both space- and ground-grown barleys. In the expression of pathogenesis-related (PR) proteins, which are up-regulated by not only the attack of pathogenic microorganisms but also abiotic stress, the gene level of PR13 was increased in space-grown barley, but those of PR1a and PR1b were decreased in space-grown barley, when compared to those in ground-grown barley, and PR2, PR3, PR4, PR5, and PR10 genes were not detected in both space- and ground-grown barleys. Furthermore, lipid transfer protein (LTP) gene, which is responsible to environmental changes, was not detected, and gene levels of phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS), which is up-regulated by abiotic stress, were increased and decreased in space-grown barley, respectively. Gene level of α-tubulin (α-TB) in space-grown barley was almost same as that in ground-grown barley.

The response of the genes, which only CAT, PR13, and PAL genes in 17 defense/stress response genes were induced in space-grown barley, suggests that the barley germinated and grown in LADA onboard ISS is not damaged by oxidative stress, which are suspected to be induced by space radiation and microgravity.

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