# **4.CULTIVATION SYSTEM**

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Because spatial volume and energy consumption are limited in plant cultivation in space, it is necessary to increase plant density while simultaneously increasing lighting efficiency. Light-emitting diodes (LEDs), which have excellent luminous efficiency, durability, and safety, and are capable of accurate wavelength control over a wide range, are used as light sources. The environment, such as temperature and humidity, is controlled to the general optimum value on earth, and  $CO_2$  concentration is controlled to 0.7 to 1 mmol mol<sup>-1</sup>.

To establish environmental control technology for efficient plant production using space plant cultivation equipment, physical environmental factors for gas exchange and plant growth (light intensity, light-dark period, light wavelength, temperature, humidity,  $CO_2$  concentration, airflow, medium moisture, and medium aeration), as well as the effects of space-specific environmental conditions, such as microgravity and a low-pressure environment, must be considered.

An environmental control that maximizes the photosynthetic rate is important in plant production. Here, we first examined the control of environmental factors common to plant production on earth, the effects of major physical environmental factors on photosynthesis, and environmental control issues under the microgravity of the universe.

## 4.1 Basic points to remember regarding the control of the crop cultivation environment on the lunar farm

#### 4.1.1 Light environment

Because the spatial volume and energy consumption are limited in plant cultivation in space, it is necessary to increase plant density while increasing lighting efficiency. The light source uses an LED with excellent luminous efficiency, durability, and safety and is capable of accurate wavelength control over a wide range. The effective photosynthetic photon flux density (PPFD) for leaf vegetables, fruit vegetables, grains, and potatoes is 100-300, 200-500, 400-800, and  $200-400 \mu$ mol m<sup>-2</sup> s<sup>-1</sup>, respectively. It is necessary to change the conditions depending on the plant species and varieties and the purpose of cultivation regarding light quality.

#### 4.1.2 Temperature environment

Plant body temperature (or leaf temperature) directly affects biochemical reactions and physiological functions in plants. For example, the photosynthetic reaction has an optimum temperature, and in general, the leaf temperature at which the net photosynthetic rate is maximized is in the range of 20 to 30°C. Leaf temperature increases with increasing radiant energy absorbed by the leaves and decreases with increasing sensible and latent heat energy transported from the leaves to the surrounding air. Therefore, environmental factors (light intensity, temperature, humidity, and air velocity) involved in these energy balances affect leaf temperature, which subsequently affects photosynthesis and growth. Therefore, the concept of combined environmental control is important.

# 4.1.3 Moisture environment

The relationship between relative humidity and net photosynthetic rate varies with other environmental conditions (e.g., air velocity), but generally, the net photosynthetic rate is maximal at 75%–85% relative humidity. When the relative humidity reaches 90%, the net photosynthetic rate decreases, which means that under high relative humidity (low water vapor saturation), water loss because of transpiration is suppressed, and simultaneously, water absorption caused by root pressure is maintained. Therefore, it is speculated that under high relative humidity, the water vapor in the leaves becomes excessive, and the stomatal opening becomes small because of the pressure.

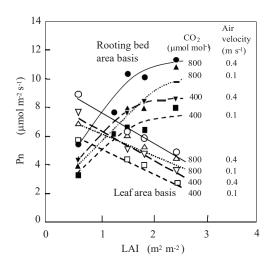
# 4.1.4 CO<sub>2</sub> environment

Generally, the net photosynthetic rate increases as the  $CO_2$  concentration increases, but the net photosynthetic rate does not increase when the  $CO_2$  saturation point is exceeded. The effect of  $CO_2$  concentration on plant photosynthesis varies greatly depending on other environmental factors, such as light intensity and temperature. The  $CO_2$  saturation point rises as light intensity increases. The  $CO_2$  compensation point ( $CO_2$  concentration when the net photosynthetic rate becomes zero) decreases as light intensity increases. As the  $CO_2$  concentration increases,

the effect of leaf temperature on the net photosynthetic rate becomes significant, and the leaf temperature that maximizes the net photosynthetic rate shifts to a higher temperature. Under low  $CO_2$  concentration, even if the leaf temperature is increased to stimulate photosynthetic enzyme activity, the amount of  $CO_2$ , a required material for photosynthesis, is a limiting factor. Consequently, the effect of increasing the leaf temperature on the increase in the rate of net photosynthesis is minimal.

### 4.1.5 Airflow environment

It is necessary to circulate the air in the cultivation space to create uniform environmental conditions in the cultivation space and increase the net photosynthetic and transpiration rates to promote growth. When the air velocity decreases, leaf boundary layer resistance increases, photosynthesis (Fig. 4.1) and transpiration are suppressed, and consequently, growth is suppressed. When the air velocity around the plant is  $0.5 \text{ m s}^{-1}$  or less, growth suppression is remarkable as the air velocity decreases. The air velocity around plants that are growing close together is often 0.1 m s<sup>-1</sup> or less. Additionally, control of the airflow environment is important because many environmental factors affect the photosynthesis and transpiration of crops through the foliar boundary layer.<sup>1)</sup>



## Fig. 4.1.

Effects of the leaf area index (LAI) on net photosynthetic rates (Pn) based on the rooting bed area (solid symbols) and the leaf area (open symbols) of tomato seedling canopies under different air velocities above the canopies and atmospheric carbon dioxide (CO<sub>2</sub>) concentrations (from Kitaya et al., 2004 <sup>2</sup>). Photosynthetic photon flux density: 250  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>; air temperature: 23 °C; relative humidity: 55%; plant height: 0.05–0.2 m.

#### 4.1.6 Rhizosphere environment

Generally, nutrient composition, pH, and liquid temperature are controlled in a nutrient solution tank. However, the environmental gas regulation is not sufficient in the nutrient solution tank alone. Especially in hydroponics, wherein the roots are always in the nutrient solution, the concentration of  $O_2$  dissolved in the solution is often a limiting growth factor. For example, the maximum amount of  $O_2$  that can be dissolved in 1 L of water at 20°C is 9.3 mg, consumed in a short time by root respiration. Therefore, to maintain normal root function, it is necessary to supplement  $O_2$  in the nutrient solution continually. In particular, when the liquid temperature rises, the roots' respiration rate increases,  $O_2$  consumption increases, and the saturated  $O_2$  concentration in the liquid decreases, such that  $O_2$  deficiency is likely to occur. To increase the amount of  $O_2$  supplied to the roots, various measures have been used in the liquid feeding method and the cultivation bed structure. Because the  $O_2$  diffusion coefficient in still water is approximately 1/10,000 in still air, the concentration immediately decreases when  $O_2$  is absorbed in the vicinity of the roots in hydroponics. In hydroponics, where the roots are always in the culture medium, the  $O_2$  concentration around the roots tends to decrease; therefore, it is important to forcibly increase the nutrient solution flow to transport  $O_2$  to the root surface quickly. In spray tillage, the supply of  $O_2$  to the roots is not restricted.

#### 4.2 Plant cultivation under microgravity in space

# 4.2.1 Problems in controlling the environment for plant cultivation under space microgravity

The microgravity of space is simulated in many pseudo-microgravity experiments using a device (clinostat) that rotates a plant placed horizontally on the ground, which disturbs the direction of gravity. Plant physiology research on gravitropism has been conducted using space shuttles, and research has shown that plants can grow even under microgravity. An experiment was conducted in which plants were hung upside down such that the direction of gravity was the opposite of that on earth (gravity of -1 g) at a light intensity of 200 µmol m<sup>-2</sup> s<sup>-1</sup>. The results showed that even with gravity in the opposite direction to normal, the plant's growth direction could be controlled by phototropism (Fig. 4.2) and the plant could be grown almost normally <sup>3</sup>. Long-term cultivation tests at the

International Space Station have recently demonstrated that plants can be cultivated even under the microgravity of space, blooming and bearing fruit.

To date, the reproductive processes of wheat and *Arabidopsis thaliana* analyzed in space experiments showed that although flowering and fruiting occurred, the formation rate of fertile seeds with germination ability was significantly reduced. For mustard seeds, the number of seeds was almost equal to that on the ground. Although they could be developed, there were reports that mutations occurred in seed traits and during the maturation process <sup>4), 5), 6))</sup>. There are many unclear aspects regarding the causes of these abnormalities.

The cultivation in space of plants like cereals and beans for human consumption is essential, and to produce these crops, it is necessary for normal reproductive growth, such as flowering and fertilization, to occur in space. To support long-term manned activities, the life cycle of seed germination, vegetative growth, reproductive growth, and seed formation must be repeated for generations. However, from the results of space experiments to date, it is expected that there might be problems with the reproductive growth and seed formation of plants, which do not function normally, as described above.

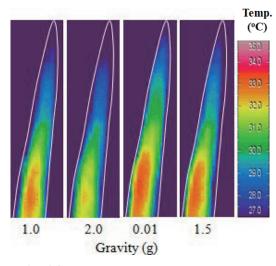


Fig. 4.2. Upside down cultivation of lettuce in the ground based experiment (Kitaya et al., 1992)

### 4.2.2 Effects of space microgravity on heat and gas exchange in plant leaves

Heat convection does not occur because of temperature differences in the microgravitational field of space, therefore it is thought that the exchange rate of heat and gas is significantly suppressed, resulting in increased temperature in plant organs and suppression of photosynthesis in leaves. Here, to investigate the effects of microgravity on leaf heat and gas exchange, we introduce results of an investigation on leaf temperature changes and the net photosynthetic rate of leaves caused by changes in gravity (0.01 to 2 G) during parabolic flight of an aircraft.

In one parabolic flight, from level flight at gravity 1 G, through ascending acceleration flight (2 G) for approximately 20 s, and microgravity flight (0.01 G) for approximately 20 s, the leaves received 1.5 G of gravity for approximately 30 s when returning to level flight. Fig. 4.3 shows the leaf temperature distribution of fresh barley leaves during the horizontal flight (gravity 1 G), ascending acceleration flight (2 G), microgravity flight (0.01 G), and 1.5 G before returning to horizontal flight <sup>7</sup>). The leaf temperature distribution was acquired with an infrared thermal image camera. When gravity increased from 1 to 2 G, the high-temperature region in the leaf center decreased. At 0.01 G, the high-temperature region increased; and at 1.5 G, it further decreased. The average leaf temperature was approximately 30°C during level flight but decreased by approximately 0.3°C during ascending acceleration flight. After the start of microgravity flight, the leaf temperature increased linearly, and after 20 s, it had increased by approximately 1°C from the leaf temperature at 1 G. As gravity increased when returning to level flight, leaf temperature decreased again. Under microgravity, the leaf temperature in the thin part at the tip of the leaf increased by 2 to 3°C, and the increase in leaf temperature was remarkable compared to that in the wide part of the leaf  $^{8)}$ . Convection occurred under 1 G gravity, and the leaf surface boundary layer at the narrow part of the tip of the leaf was thinner than that at the wide part of the leaf, thus the resistance to heat (latent heat + sensible heat) transport was small. Leaf temperature in thin areas was lower than that in large areas. However, under microgravity without convection, the thickness of the leaf surface boundary layer was almost constant regardless of the leaf width, such that the leaf temperature approached the same value regardless of the leaf width. Therefore, when the gravity dropped from 1 to 0.01 G, the increase in temperature in the thin parts became very high, and the temperature distribution on the leaf surface became uniform.





Thermal images of the barley leaf at different gravity levels (from Kitaya et al., 2003 7)). Ambient air temperature: 28°C, Irradiance: 260 W m–2, Air velocity: 0.2 m s–1.

Fluctuations in the average surface temperature of the fresh barley leaves, a simulated copper plate leaf, and a simulated wet filter paper leaf with the change in gravity were compared by the same method as in the above experiment. The emissivity of each simulated leaf (the ratio of the energy of the radiation emitted from an object at a specific temperature to the energy of the radiation emitted from a blackbody at the same temperature, which is the same value as the emissivity), and heat capacity was almost the same as that of the fresh leaves. The evaporation or transpiration rates were higher in ascending order: copper plate simulated leaves < fresh barley leaves < wet filter paper simulated leaves. The temperature increase of each sample caused by the decrease in gravity from 1 to 0.01 G was 0.1°C for the copper plate simulated leaves, 0.8°C for fresh leaves, and 1.1°C for the filter paper simulated leaves, and the order of the temperature increase was consistent with the evapotranspiration rate of the materials. From this, it was concluded that in the heat exchange process of each sample in the microgravitational field, the temperature increase was greater with decreased evapotranspiration rate, and the latent heat transport was suppressed because of the suppression of convection.

The effects of gravity on the net photosynthetic rate of barley leaves and the transpiration rate of strawberry leaves

are shown in Fig. 4.4 <sup>7), 8), 9), 10). The net photosynthetic rate of barley leaves, which was approximately 4  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> during level flight, decreased at the beginning of microgravity flight (gravity 0.01 G), and after 20 s, decreased by 20% relative to that of level flight (gravity 1 G). The net photosynthetic rate sharply increased as gravity increased in return to level flight. The transpiration rate of strawberry leaves during microgravity flight was reduced by approximately 40% when compared with that during level flight.</sup>

Because heat convection does not occur because of the temperature difference in the microgravitational field, heat and gas exchange between the leaves and the surrounding air is suppressed, leaf temperature increases, and the net photosynthetic rate decreases with a decrease in transpiration rate. In this experiment, the microgravity period was as short as 20 s, and heat and gas exchange were not in a steady state. When a plant is placed under microgravity for an extended time, such as in a plant experiment in space, it is predicted from the plant's thermal characteristics that the increase in leaf temperature will be approximately twice that in this experiment where microgravity was only maintained for 20 s. Additionally, the decrease in net photosynthesis and transpiration rates will be even more remarkable under the continuous microgravity of space.

Increased plant body temperature and decreased net photosynthetic rate under microgravity are important issues to consider when producing plants in space. How are photosynthesis, transpiration, and leaf temperature affected in plants subject to the universe's microgravity for long periods? For example, pollen formation of rice, wheat, and tomato is impaired by a high temperature of approximately 32 to 34°C.

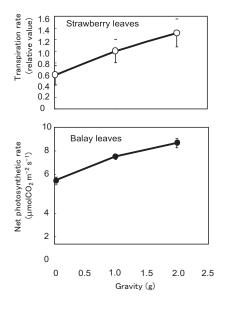


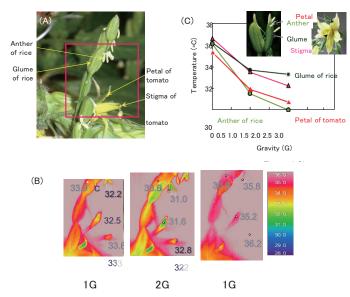
Fig. 4.4

Effects of gravity levels on photosynthetic rates (Pn) of barley leaves 20 s after exposure to each gravity level in airplane parabolic flight experiments (from Kitaya et al., 2001<sup>9)</sup>; Hirai & Kitaya, 2009<sup>10)</sup>). Each value is the mean of four parabolic flights. Vertical bars represent standard deviations.

Therefore, we

investigated the temperature dynamics of the reproductive organ microtissue when heat convection does not occur under microgravity using an aircraft parabolic flight experiment. This experiment was used to predict the effects of microgravity on pollen formation and development with the temperature increase of the reproductive organs of plants. Thus, we examined the effects of the space environment on the reproductive growth of plants. The temperatures of rice stamens and stamen anthers, and tomato petals and pistil stigmas decreased when gravity increased from 1 to 2 G and increased when gravity decreased to 0.01 G (Fig. 4.5). As the gravity decreased from 1 to 0.01 G, the temperature of rice stalks increased by 2°C, that of stamen anthers increased by 3.6°C, that of tomato petals increased by 2.7°C, and that of pistil stigma increased by 2.4°C. The increase of the surface temperature of each part of the plant caused by the decrease in gravity became particularly remarkable in the delicate parts, such as the stamen anthers of rice. This experiment suggests that elevated temperatures might cause reproductive organ abnormalities in the absence of thermal convection under microgravity. From the above, it was concluded that microgravity suppresses gas exchange, such as in photosynthesis and transpiration, causing suppression of vegetative growth. Furthermore, the suppression of substance production and an increase in the temperature of reproductive organs caused by the suppression of photosynthesis are thought to cause abnormal reproductive growth, such as sterility (Fig. 4.6). Appropriate airflow environmental control is indispensable to prevent such growth suppression and reproductive abnormalities.

As described above, heat convection (density convection) is unlikely to occur under microgravity. Therefore, in the absence of forced airflow, even for 20 s of microgravity (0.01 G) generated during parabolic flight, heat exchange between plant leaves and ambient air was suppressed by decreased heat convection, and a leaf temperature increase of approximately 2 to  $3^{\circ}$ C can occur. Especially under microgravity, the suppression of latent heat exchange by suppressing transpiration is intimately involved in increasing leaf temperature. The suppression of convection under microgravity also reduces the CO<sub>2</sub> absorption rate (net photosynthetic rate) of the leaves. Therefore, from the viewpoint of heat and gas exchange, convection promotion by airflow control is indispensable in plant cultivation in space. When attempting to fertilize seeds in space for sustainable plant production, it is necessary to force air flow around the plant to promote heat and gas exchange in the reproductive organs to prevent excessive temperature increases of the reproductive organs that cause reproductive abnormalities. Additionally, because the air velocity is low in a high-density plant community, airflow control is indispensable for promoting gas diffusion, photosynthesis, and transpiration.



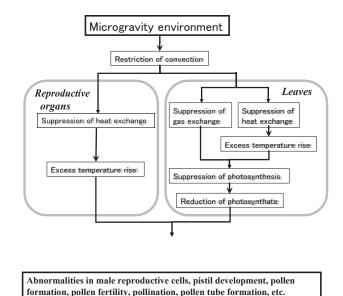


Fig. 4.5 Effects of gravity on the surface temperature of plants (Kitaya and Hirai, 2013). (A) Photograph of the measurement site; (B) Thermal images under different levels of gravity (1, 2, and 0.01 G) during aircraft parabolic flight; (C) Effects of gravity on the surface temperature of plant reproductive organs.

Fig. 4.6 Possible effects of microgravity on plant reproductive growth from the perspective of gas and heat exchanges.

## 4.3 Other issues regarding plant cultivation in the space environment

Because the lunar surface is almost a vacuum at 0 atm, and the surface of Mars is 0.06 to 0.09 atm, it is easy to reduce the pressure inside the space station partially. Under such low-pressure conditions, the structure of the facility's outer wall can be simplified by reducing the pressure inside the facility. Therefore, during plant cultivation in space, the establishment of plant cultivation technology in a low-pressure environment is also an important issue. It has been reported that spinach and similar plants grow normally in cultivation under a low pressure of 1/4 of the atmospheric pressure <sup>11</sup>. In addition to low pressure, microgravity is also advantageous in reducing the weight of the facility's load-bearing structure.

In a closed environment, various trace levels of harmful gases can be generated and accumulated. In particular, ethylene is an important plant growth regulator, and the plant itself is also a source. In general, when the ethylene concentration in the air rises above  $0.1 \mu mol mol^{-1}$ , the plants are damaged. In a closed environment, it is necessary to monitor the behavior of various trace gas components, and a trace gas removal system is indispensable when problems occur because of excessive accumulation.

Water supply to plant roots under microgravity is also an important issue. On earth, the waterfall phenomenon because of gravity is used in plant cultivation methods in the water supply system. However, in the microgravity environment of space, water falling is restricted. Therefore, it may be necessary to utilize pressure gradients or capillary forces to supply water to the roots.

## 4.4 Function and structure of the cultivation system

Assuming a vacuum environment on the lunar surface, the building structure in which the cultivation equipment is installed is preferably a pressure-resistant container having a cylindrical shape that can withstand the difference in air pressure between the inside and outside, or a half-cylindrical shape obtained by cutting the cylinder in half. It is conceivable to introduce a hydroponic cultivation system having a multi-layer structure with a pressure-resistant structure container. Multi-layer hydroponic cultivation equipment that uses LEDs as a light source has already been developed in Japan and overseas, and cultivation results have been attained. For example, Photo 4.7 shows the LED multi-layer hydroponic cultivation system developed at Tamagawa University (Watanabe, 2011). It has a structure where many cultivation systems are stacked on a multi-stage cultivation frame by combining a water-cooled LED panel light source with improved durability and a hydroponic system of a thin hydroponic solution method (NFT hydroponic method). By pouring the hydroponic solution into shallow gutters and letting the roots crawl there, it is possible to produce hydroponic method), and which has a multi-layer structure. In this case, it is possible to build many cultivation system in a limited space, advantageous in terms of space efficiency. Fig. 4.8 shows a vegetable production system in which the cultivation system is stacked in 12 stages (Hagiya & Watanabe, 2015).



Fig. 4.7 Multi-layer nutrient film technique hydroponics system with light emitting diodes (FST laboratory, Tamagawa University).



Fig. 4.8 Twelve-layer leaf lettuce production facility (LED Farm, Tamagawa University)

#### 4.5 Cultivation method, conditions, and management

The cultivation method, conditions, management, and calendar were summarized for the eight crops that are to be cultivated on the lunar farm. The main points are below.

### (1) Leaf vegetables with a short cultivation period: leaf lettuce.

The cultivation period is short and can be harvested in approximately 5 weeks. They have a good track record in LED hydroponics.

### (2) Cereals with a long cultivation period: rice, soybeans.

It takes approximately 16 weeks to harvest rice and soybean, and long-term cultivation is required. It is appropriate to deploy the cultivation equipment in a plane and replant the entire crop at each harvest.

### (3) Root vegetables: sweet potatoes, potatoes.

For root vegetables, the use of mist hydroponics is effective. Currently, both sweet potatoes and potatoes require a cultivation period of approximately 25 weeks, and shortening the cultivation period is an issue. By adopting the hydroponic method, it is possible to harvest from tubers and rhizomes that have reached the harvest time.

#### (4) Fruit vegetables: strawberries, tomatoes, cucumbers.

For strawberries, research examples have been reported using the LED multi-layer cultivation method. There are few research examples of LED cultivation for tomatoes and cucumbers, but it is possible to adopt a multi-layer cultivation system by arranging the harvested products horizontally on the cultivation panel. Tomatoes are harvested 16 weeks after sowing, and cucumbers are harvested 10 weeks after sowing.

These features, further detailed growth processes, notable monitoring items in each growth stage, and cultivation work according to each crop species are summarized in Japanese version of this report. If you need the details, see the attachment data "Growth characteristics and cultivation calendars by crop species" in Chapter 4 of the Japanese version.

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