

## Effects of Gravity on Stem Sap Flow and Water and Heat Exchange in the Leaves of Sweetpotato

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### Abstract

Plants play an important role in bio-regenerative life support systems (BLSSs) for long-term manned space missions. During parabolic airplane flights, we investigated stem sap flow without forced air movement and water vapor conductance with forced air movement using sweetpotato plants. Stem sap flow was promoted under microgravity, but only when forced air movement was applied to the plants. The water vapor conductance of the plant leaves increased under microgravity at an air velocity of 0.25 m·s<sup>-1</sup>. Leaf temperatures also increased under microgravity at an air velocity of 0.02 m·s<sup>-1</sup>. This suggests that forced air movement is important in maintaining long-term, healthy plant growth in BLSSs.

**Keyword(s):** Bio-regenerative life support systems, Leaf conductance, Parabolic airplane flight, Sweetpotato, Transpiration

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### 1. Introduction

Manned space exploration has become feasible in recent years, the establishment of environments for making long-term manned space missions possible has become an urgent requirement<sup>1, 2)</sup>. Plants will play an important role in food production, CO<sub>2</sub>/O<sub>2</sub> conversion and water purification in the bio-regenerative life support systems (BLSSs) that will be needed in long-term missions, and thus, the ability to cultivate plants stably and efficiently will be critical to their success<sup>3-8)</sup>. It is necessary, therefore, to investigate the physiological and ecological responses of plants to low gravity during both the vegetative and reproductive growth stages. In the International Space Station (ISS), the effects of long-term microgravity on the entire plant life cycle, from the germination of seeds and including vegetative and reproductive growth and seed formation, have been actively studied in recent years<sup>9, 10)</sup>.

Water transport from roots to stems, petiole and leaves mainly with transpiration demand as a driving force; this also delivers nutrients to the plants' organs. We previously reported that air movement has a significant effect on transpiration<sup>11-14)</sup>, and that forced air movement promotes stem sap flow<sup>14)</sup> under microgravity conditions in parabolic airplane flights. Information on the effect of gravity on the stem sap flow in the absence of forced air movement has been lacking in the literature. In this study, therefore, we investigated the effect of gravity on water movement in stems in the absence of forced air movement. In addition, we investigated the effects of gravity on leaf temperatures and on water vapor conductance between leaves and the atmosphere.

### 2. Materials and Methods

#### 2.1 Low Gravity Conditions and Materials

Sweetpotato [*Ipomoea batatas* (L.) Lam.] plants were used in this study. The plants had been cultivated for 2–3 months in a rockwool substrate in a growth chamber. During cultivation, nutrient solution was constantly supplied from the bottom of the substrate. Root zones of plants, including the substrate, were sealed in a plastic case during the experiment. We carefully avoided water stress during both cultivation and the experiment by controlling the proportion of air and water volumes in the substrate appropriately.

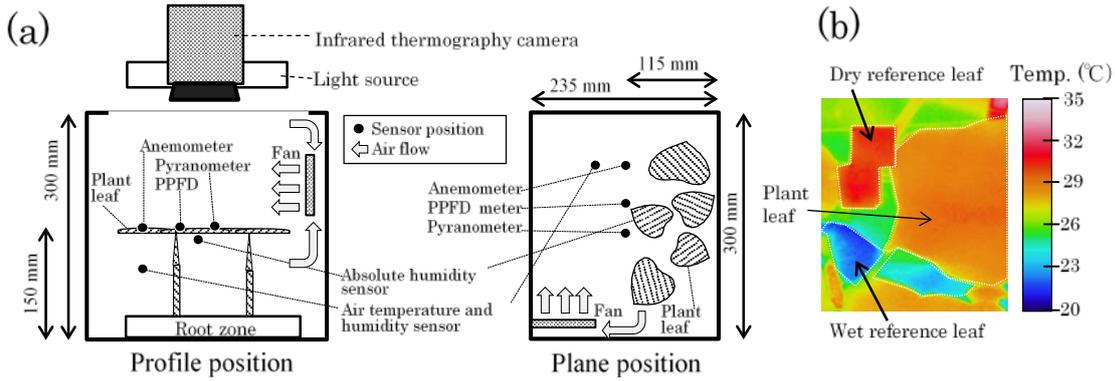
Parabolic airplane flight experiments were conducted for approximately 10–20 seconds at 0.01 G (equivalent to microgravity conditions in the ISS), 0.16 G (similar to the gravity on the Moon), and 0.38 G (equivalent to the gravity on Mars). Gravity was approximately 1.0 G when the airplane was flying horizontally before and after the parabolic flights and increased to 1.3–2.0 G just before and after each low-gravity period.

All components of the experimental system, including plants, were placed in a frame structure and fixed in the airplane (**Fig. 1a**). We controlled forced air convection using a fan unit that was installed in the cultivation chamber. Air velocities during the fan operation and without it were 0.25 and 0.02 m·s<sup>-1</sup>, respectively, which was measured using an anemometer with non-directional needle sensor (Model 6071; Kanomax Japan, Inc., Japan) set near measured leaves as shown in **Fig. 1a**. An ambient air near the plants was moved with a circulation fan

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**Fig. 1** (a) Diagram of the experimental system, and (b) Sample thermal images of a sweetpotato leaf, the dry reference leaf and the wet reference leaf at an air velocity of  $0.25 \text{ m}\cdot\text{s}^{-1}$  and gravity of  $1.0 \text{ G}$ .

unit. The maximum air velocity was  $0.25 \text{ m}\cdot\text{s}^{-1}$  near the plants, so that there were no swaying plants and leaves and accompanying G-jitters.

The light source was a mixed system with light-emitting diodes and tungsten lamps to promote transpiration and to provide appropriate heat loads to leaves. The air temperature was  $26 \pm 2 \text{ }^\circ\text{C}$ , the relative humidity was  $30 \pm 5\%$ , the radiation flux density from light sources and surrounded materials was  $380 \pm 10 \text{ W}\cdot\text{m}^{-2}$ , and the photosynthetic photon flux density (PPFD) was  $25 \text{ }\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . The air pressure was  $892 \pm 3 \text{ hPa}$ . The horizontal gravities of  $G(x)$  and  $G(y)$  ranged between approximately  $0.02 \pm 0.09 \text{ G}$  and  $0.02 \pm 0.03 \text{ G}$ , respectively.

## 2.2 Measurement of Stem Sap Flow

The index of the stem sap flow (ISSF) was calculated from the stem surface temperature based on Tokuda et al.<sup>14)</sup> and is briefly explained in this section. We heated the inside of the stem by inserting a tiny heater made from a nickel-chrome alloy wire  $0.2 \text{ mm}$  in diameter, which connected with a dry-cell for continuously supplying electricity at  $1.5 \text{ V}$ . The input heat energy was transferred upside and downside from the heater through heat conduction and stem sap flow in the vascular tissue. We compared the difference in stem surface temperature above and below the heater under  $1.0 \text{ G}$  and low gravity and evaluated the increase and decrease of stem sap flow using ISSF calculated from equations (1) and (2).

$$T_R = T_{s(\text{low } G)} - T_{s(1.0 \text{ G})} \quad (1)$$

$$\text{ISSF} = T_{R(\text{Max})} - T_{R(\text{Min})} \quad (2)$$

In equation (1), we calculated the relative stem surface temperature ( $T_R$ ) by subtracting the stem surface temperature under reference gravity [ $1.0 \text{ G}$ ;  $T_{s(1.0 \text{ G})}$ ] from that under low

gravity [ $T_{s(\text{low } G)}$ ] in order to remove effects of peripheral radiation and G-jitter. The heat supplied from the heater is transported to the upside from the downside due to the sap flow in the stem. The ISSF was assumed to be the difference in stem surface temperature between  $5 \text{ mm}$  above [ $T_{R(\text{Max})}$ ] and below [ $T_{R(\text{Min})}$ ] the heater [equation (2)], and to increase with increasing stem sap flow.

The experiment was conducted without forced air movement, and a white fluorescent lamp was used as a light source to maintain leaf transpiration. The air temperature was  $27 \pm 2 \text{ }^\circ\text{C}$ , the relative humidity was  $30 \pm 10\%$  and the PPFD was  $350 \pm 50 \text{ }\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . The temperature of the stem surface was measured using an infrared thermography camera (TH9100PMV; Nippon Avionics Co., Ltd.) every  $0.2$  seconds. The temperatures were determined from the intensity of infrared radiation ( $8\text{--}14 \text{ }\mu\text{m}$  in wavelength) from the surfaces according to the Stefan-Boltzmann law. The accuracy of temperature measurement was  $0.06 \text{ }^\circ\text{C}$ , and the minimum spatial resolution was  $95 \text{ }\mu\text{m} \times 95 \text{ }\mu\text{m}$ . We set the emissivity value at  $0.93$  based on the work of Jones<sup>15)</sup>.

## 2.3 Calculation of Water Vapor Conductance in Plant Leaf ( $g_{vL}$ ) and Wet Reference Leaf ( $g_{vW}$ ) under Forced Air Movement

Water vapor conductance was calculated using the surface temperatures of a sweetpotato leaf, a dry reference leaf and a wet reference leaf, and environmental factors including air temperature, relative humidity and atmospheric pressure. We measured the surface temperatures of all materials at the same time in order to assess their heat balance (Fig. 1b). The dry reference leaf and the wet reference leaf were used for investigating the physical variation of sensible heat and latent heat, respectively, excluding the biological factors. The dry reference leaf and the wet reference leaf were made to resemble black bodies. The wet leaf was made from a paper that was

**Table 1** List of symbols

$E$	Water vapor flux ( $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )
$H$	Sensible heat flux ( $\text{W}\cdot\text{m}^{-2}$ )
$L_a$	Long-wave radiation from atmosphere ( $\text{W}\cdot\text{m}^{-2}$ )
$L_{out}$	Long-wave radiation at T °C ( $\text{W}\cdot\text{m}^{-2}$ )
$R_M$	Radiation of light sources and surrounded materials ( $\text{W}\cdot\text{m}^{-2}$ )
$R(T)$	Net radiation energy absorbed at T °C ( $\text{W}\cdot\text{m}^{-2}$ )
$T_a$	Air temperature (°C)
$T_D$	Dry reference leaf temperature (°C)
$T_L$	Plant leaf temperature (°C)
$T_R$	Relative stem surface temperature
$T_{s(XG)}$	Stem surface temperature at X gravity (°C)
$T_W$	Wet reference leaf temperature (°C)
$c_p$	Specific heat capacity of air ( $\text{J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$ )
$e_a$	Air water vapor pressure (kPa)
$e_s(T)$	Saturation water vapor pressure at T °C (kPa)
$g_{Ha}$	Boundary layer conductance for sensible heat on leaves ( $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )
$g_{vL}$	Water vapor conductance in plant leaf ( $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )
$g_{vW}$	Water vapor conductance in wet reference leaf ( $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )
$p_a$	Atmospheric pressure (kPa)
$\epsilon$	Overall infrared emissivity
$\sigma$	Stefan-Boltzmann constant ( $\text{W}\cdot\text{m}^{-2}\cdot\text{K}^{-4}$ )
$\lambda$	Water evaporative latent heat ( $\text{J}\cdot\text{mol}^{-1}$ )

uniformly dyed with a black dye and constantly supplied with distilled water from a water absorbing yarn attached to a water reservoir. The dry leaf was made from an aluminum plate painted with a black body paint (TA410KS, Ichinen TASCO Co.,Ltd.).

When the leaf temperature variation is within a small range, the surface temperature in the plant leaf and the wet reference leaf are expressed by equations (5) and (6), which are derived from the heat balance equation (3), and the surface temperature in the dry reference leaf is expressed by equation (7), which is derived from the heat balance equation (4).

$$\begin{aligned} R(T) - H - \lambda E \\ = R(T_L) - c_p g_{Ha} (T_L - T_a) - \frac{\lambda g_{vL} (e_s(T_L) - e_a)}{p_a} \\ = 0 \end{aligned} \quad (3)$$

$$R(T) - H = R(T_D) - c_p g_{Ha} (T_D - T_a) \quad (4)$$

$$T_L = T_a + \frac{R(T_L) - \lambda g_{vL} \frac{e_s(T_L) - e_a}{p_a}}{c_p g_{Ha}} \quad (5)$$

$$T_W = T_a + \frac{R(T_W) - \lambda g_{vW} \frac{e_s(T_W) - e_a}{p_a}}{c_p g_{Ha}} \quad (6)$$

$$T_D = T_a + \frac{R(T_D)}{c_p g_{Ha}} \quad (7)$$

Under forced air movement, the boundary layer conductance ( $g_{Ha}$ ) for sensible heat on these leaves is considered to be mostly constant due to only small variations of surface temperatures, and the water vapor conductance in the plant leaf ( $g_{vL}$ ) and the wet reference leaf ( $g_{vW}$ ) are described by equations (8) and (9) which are derived from (5), (6) and (7).

$$g_{vL} = \frac{p_a \left( R(T_L) - R(T_D) \left( \frac{T_L - T_a}{T_D - T_a} \right) \right)}{\lambda (e_s(T_L) - e_a)} \quad (8)$$

$$g_{vW} = \frac{p_a \left( R(T_W) - R(T_D) \left( \frac{T_W - T_a}{T_D - T_a} \right) \right)}{\lambda (e_s(T_W) - e_a)} \quad (9)$$

The net absorbed radiation flux densities at T °C [ $R(T)$ ] were calculated using the following equation (10):

$$\begin{aligned} R(T) &= R_M + L_a - L_{out} \\ &= R_M + \sigma (273.15 + T_a)^4 - \epsilon \sigma (273.15 + T)^4 \end{aligned} \quad (10)$$

The radiation from the lighting system and surrounding materials ( $R_M$ ) was regarded as the sum of the short-wave and long-wave radiation measured with a pyranometer (LI-200R, Li-Cor Inc.). In the experiment, we also measured the PPF in the range of short-wave radiation (LI-190R, Li-Cor Inc.), and it was approximately  $25 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . We assumed that short-wave radiation was negligibly small when considering the reflectance of short-wave radiation on material surfaces. Long-wave radiation from the atmosphere ( $L_a$ ) was derived from the Stefan-Boltzmann constant ( $\sigma$ ), and air temperature ( $T_a$ ) and long-wave radiation from leaves ( $L_{out}$ ) were derived from the overall infrared emissivity ( $\epsilon$ ) and the temperature of the object ( $T_L$ ,  $T_D$ , or  $T_W$ ). The  $\epsilon$  for  $T_D$  and  $T_W$  was 0.99, which is close to the value for a black body, and the  $\epsilon$  for  $T_L$  was 0.95, which is the mean value reported for plants (0.90 to 0.99)<sup>16)</sup>.

Atmospheric pressure ( $p_a$ ) was measured in the airplane cabin. The saturated water vapor pressure of the plant leaf [ $e_s(T_L)$ ] and the wet reference leaf [ $e_s(T_W)$ ] were determined at the surface

temperatures. Water evaporative latent heat ( $\lambda$ ) was corrected based on  $T_a$  variations. The  $R(T)$ ,  $T_a$  and atmospheric water vapor pressure were recorded every 0.5 seconds and the other parameters every 0.2 seconds.

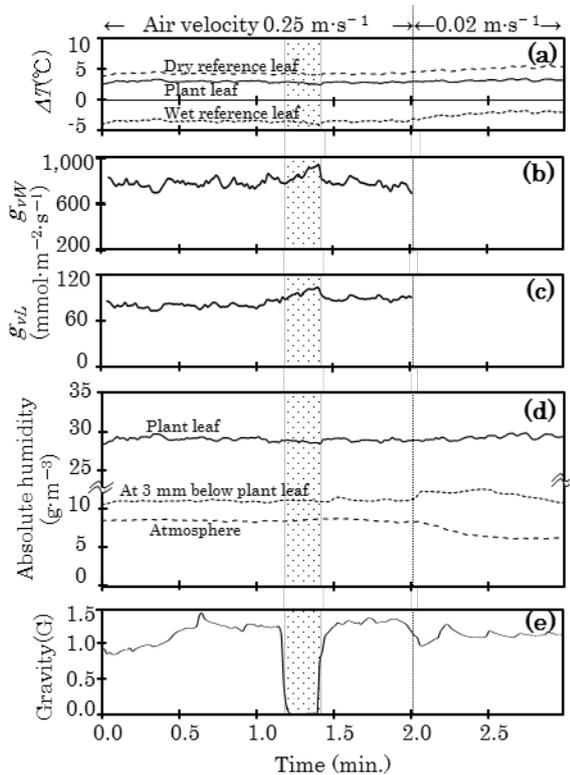
The  $T_L$ ,  $T_D$  and  $T_W$  were measured with an additional infrared thermography camera (R300SR, Nippon Avionics Co., Ltd., Japan) and thermal images were recorded every 0.2 seconds.

In addition, absolute humidity was measured below the lower surface of the leaf every 0.5 seconds. The sensor of a high-speed absolute humidity meter (RHM-1000s, Ricoh Elemex Co., Ltd.) was fixed at approximately 3 mm below the leaf. Measurement accuracy was  $\pm 1 \text{ g}\cdot\text{m}^{-3}$ .

Measured leaves were fixed using thin nylon threads to avoid movement due to gravity variation.

### 3. Results and Discussion

The experiments were repeated three times and we confirmed the same trends repeatedly. Representative trends of measured and calculated parameters at air velocities of  $0.25 \text{ m}\cdot\text{s}^{-1}$  and



**Fig. 2** Variations in: (a) Temperature ( $\Delta T$ ) obtained by subtracting the air temperature from the surface temperatures of the sweetpotato leaf, the dry reference leaf and the wet reference leaf; (b) Water vapor conductance in the wet reference leaf ( $g_{vW}$ ); (c) Water vapor conductance in the sweetpotato leaf ( $g_{vL}$ ); (d) Absolute humidity inside the plant leaf, at 3 mm below the leaf, and of the atmosphere; and (e) Gravity.

$0.02 \text{ m}\cdot\text{s}^{-1}$  are shown in **Fig. 2** and **Fig. 3**, respectively.

There were no significant changes in the surface temperature of any leaf with gravity changes at 0.01 G at an air velocity of  $0.25 \text{ m}\cdot\text{s}^{-1}$  (**Fig. 2a**). The  $g_{vL}$  and the  $g_{vW}$  increased under microgravity (**Fig. 2b, 2c** and **Table 2**).

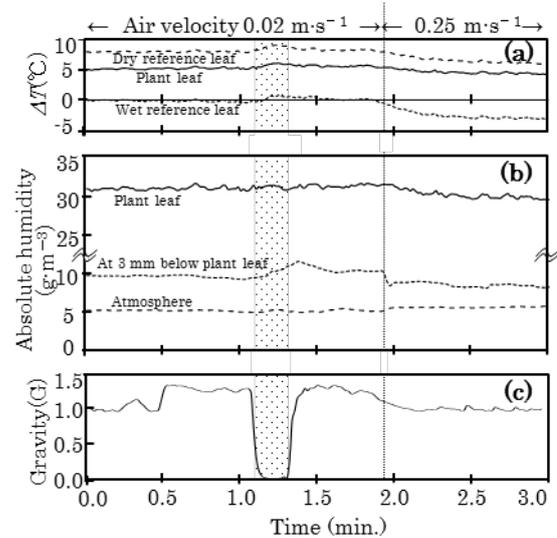
Absolute humidity inside the leaf (the saturated value calculated from the leaf surface temperature), the measured absolute humidity below the leaf, and the atmospheric absolute humidity did not change significantly with gravity changes at an air velocity  $0.25 \text{ m}\cdot\text{s}^{-1}$  (**Fig. 2d**).

The surface temperature of each leaf increased at 0.01 G at an air velocity of  $0.02 \text{ m}\cdot\text{s}^{-1}$  (**Fig. 3a**), thus the  $g_{vL}$  and the  $g_{vW}$

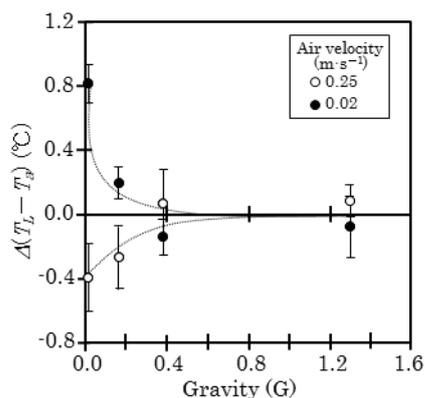
**Table 2** Water vapor conductance in the sweetpotato leaf ( $g_{vL}$ ) and in the wet reference leaf ( $g_{vW}$ ) under 1.0 G or 0.01 G.

	$g_{vL}$ ( $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	$g_{vW}$ ( $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )
1.0 G	$86.4 \pm 4.8$	$813.4 \pm 36.1$
0.01 G	$115.5 \pm 8.7$	$1029.0 \pm 82.0$
<i>P-Value</i>	0.043	0.074

The values are averages of the 5 seconds just before the change in gravity (Means  $\pm$  SE, unpaired two-tailed Student's  $t$  test,  $n = 3$ ).



**Fig. 3** Variations in: (a) Temperature ( $\Delta T$ ) obtained by subtracting the air temperature from the surface temperatures of the sweetpotato leaf, the dry reference leaf and the wet reference leaf; (b) Absolute humidity inside the plant leaf, at 3 mm below the leaf, and of the atmosphere; and (c) Gravity. The air velocity was switched from  $0.02 \text{ m}\cdot\text{s}^{-1}$  to  $0.25 \text{ m}\cdot\text{s}^{-1}$  during the gravity variation. The variation of the water vapor conductance in the wet reference leaf ( $g_{vW}$ ) and the sweetpotato leaf ( $g_{vL}$ ) were not determined because they did not satisfy the precondition that those leaves' temperatures and boundary layer conductance for sensible heat were constant.



**Fig. 4** Effects of gravity on variation in the difference between the surface temperature of the sweetpotato leaf and the air temperature [ $\Delta(T_L - T_a)$ ] at air velocities of  $0.02 \text{ m}\cdot\text{s}^{-1}$  and  $0.25 \text{ m}\cdot\text{s}^{-1}$ , compared to those at  $1.0 \text{ G}$ . (Means  $\pm$  SE,  $n = 2-9$ )

could not be calculated at this velocity because the boundary layer conductance ( $g_{Ha}$ ) for sensible heat on leaves was not constant due to the variations in surface temperatures, which did not satisfy the precondition that those leaves temperatures and boundary layer conductance for sensible heat must be constant.

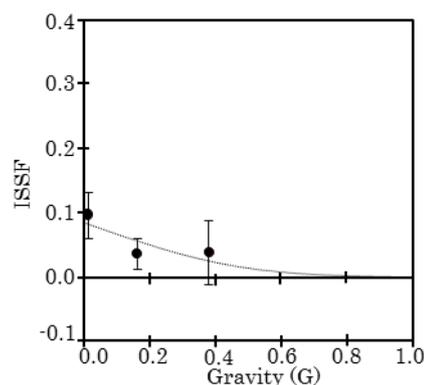
Absolute humidity at 3 mm below the leaf increased significantly at  $0.01 \text{ G}$  and at an air velocity  $0.02 \text{ m}\cdot\text{s}^{-1}$  (**Fig. 3b**).

The surface temperatures of the plant leaf under low gravity relative to the temperatures at  $1.0 \text{ G}$  are shown in **Fig. 4**.

The plant leaf temperatures at an air velocity of  $0.02 \text{ m}\cdot\text{s}^{-1}$  (**Fig. 3a** and **Fig. 4**) were consistent with the results of previous studies<sup>11, 12)</sup> conducted without force air movement. At an air velocity of  $0.02 \text{ m}\cdot\text{s}^{-1}$ , suppression of air convection under low gravity decreased the conductance in the boundary layer on the leaf and transpiration was suppressed, resulting an increase in leaf temperature. However, the increase in leaf temperature was not significant at an air velocity of  $0.25 \text{ m}\cdot\text{s}^{-1}$  (**Fig. 2d** and **Fig. 4**).

The absolute humidity just below the leaf at an air velocity of  $0.02 \text{ m}\cdot\text{s}^{-1}$  (**Fig. 3b**) was also consistent with the results of a previous study<sup>13)</sup> conducted without forced air movement. At an air velocity of  $0.02 \text{ m}\cdot\text{s}^{-1}$ , suppression of air convection under low gravity made the absolute humidity near the leaf increase and resulted in suppression of the transpiration. However, there was no change in the absolute humidity just below the leaf at an air velocity of  $0.25 \text{ m}\cdot\text{s}^{-1}$  (**Fig. 2d**), which indicates continuing transpiration.

In a previous study, we reported that an air velocity of  $0.25 \text{ m}\cdot\text{s}^{-1}$  promoted the stem sap flow of sweetpotato and that ISSF increased to  $0.37$  under microgravity<sup>14)</sup>. In this study,



**Fig. 5** Effect of gravity on the index of stem sap flow (ISSF) of sweetpotato. (Means  $\pm$  SE,  $n = 4-8$ )

although ISSF at an air velocity of  $0.02 \text{ m}\cdot\text{s}^{-1}$  increased slightly to  $0.07$  under microgravity (**Fig. 5**), it was significantly lower than the previous result at an air velocity of  $0.25 \text{ m}\cdot\text{s}^{-1}$ .

It has been reported that photosynthesis, evapotranspiration and water use efficiency do not change under microgravity in the ISS using a canopy of wheat plants grown in a moderate light environment<sup>17)</sup>. There was, however, no mention of the air velocity near the plants. In this study, promotion of water transport in plants under microgravity was observed in a forced air movement, although the periods of low gravity lasted only 10–20 seconds. We need to confirm this phenomenon in a precisely controlled environment for a long duration in a future experiment on the ISS.

#### 4. Conclusion

Stem sap flow of sweetpotato was promoted under microgravity, but only when forced air movement was applied to the plants. The water vapor conductance of the plant leaves increased under microgravity at an air velocity of  $0.25 \text{ m}\cdot\text{s}^{-1}$ . Leaf temperatures also increased under microgravity at an air velocity of  $0.02 \text{ m}\cdot\text{s}^{-1}$ . These results suggest that transpiration of plants would be promoted under microgravity with a forced air movement and control of air movement is important in plant growth facilities in space.

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