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Original Paper

Comparisons of the Effects of Vibration of Two Centrifugal Systems on the Growth and Morphological Parameters of the Moss *Physcomitrella patens*

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Abstract

As pioneer plants mosses are adaptable to extreme environments and are the first to colonize previously uncolonized land. Recently, we designed two centrifuges, MIJ-17 and MK-3, to investigate the effects of long-term cultivation under moderate hypergravity conditions on the growth of mosses. We examined the effects of the vibration generated by these centrifuges on the growth and morphological parameters of the moss *Physcomitrella patens*, because plants are generally sensitive to vibration. In MIJ-17, random vibration was detected by a micro three-axis acceleration sensor during centrifugation at 2, 5, and 10 *G*, whereas vibration was negligible in MK-3. Therefore, we compared the growth and morphological parameters of *P. patens* gametophores cultivated with MIJ-17 and MK-3 at 10 *G*. The vibration generated by MIJ-17 did not significantly affect the growth and morphological parameters of *P. patens* gametophores. Thus, we conclude that the vibration of MIJ-17 has a negligible impact on the growth of *P. patens* gametophores. doi: 10.2187/bss.31.9

Introduction

Mosses, which are tiny plants without roots and a vascular system, are pioneer plants that are the first to colonize previously uncolonized land, showing characteristic resistance against extreme environmental stresses. Together with bacteria and other microbes, mosses contribute to soil formation, which enables larger vascular plants to grow on the substrate. Thus, mosses are key organisms for space development and terraforming of the Moon or Mars where gravity is different from that of Earth's, so that their growth responses to different gravity conditions (microgravity or hypergravity) will be an important subject to investigate. Nevertheless, few experiments have examined the effects of gravitational change on the growth of mosses (Kern *et al.*, 2005; Takemura *et al.*, 2017).

Recently, we designed two new centrifuges, named MIJ-17 and MK-3, equipped with a lighting system, in order to investigate the response of the moss *Physcomitrella patens* to long-term moderate hypergravity (< 10 *G*). In the use of these centrifuges, however, it is necessary to examine whether vibration generated from the centrifuges affects the growth and morphological parameters of *P. patens*, because previous studies have shown that plants may be sensitive to vibration (Appel and Cocroft, 2014; Gagliano *et al.*, 2012; Niklas, 1998; Takahashi *et al.*, 1991; Uchida and Yamamoto, 2002). Furthermore, in a hypergravity experiment with a large-diameter centrifuge (LDC; ESA-ESTEC, Noordwijk, The Netherlands), significant differences in morphological parameters of *Arabidopsis thaliana* seedlings were observed between the 1-*G* external control experiment and the 1-*G* rotational control experiment (Manzano *et al.*, 2012). These findings imply that vibration during centrifugal hypergravity experiments can affect growth and morphological parameters of plants.

The MIJ-17 and MK-3 centrifuges are so small compared with the LDC that it is difficult to do 1-*G* rotational control experiments as with the LDC, in which the central gondola is used for the control experiments. Therefore, the objectives of the present work were to describe the vibration properties of MIJ-17 and MK-3, and to investigate whether the vibrations affect the growth and morphological parameters of *P. patens* gametophores.

Materials and Methods

Plant material

Gametophores of *Physcomitrella patens* Bruch & Schimp subsp. *patens* (Ashton and Cove, 1977) were pre-cultivated on BCD medium (Nishiyama *et al.*, 2000) solidified with 0.8% (w/v) Difco Bacto Agar for 1–2 months in a growth chamber at 25°C. Newly generated gametophores cut into 3-mm lengths from the shoot apex, which were equivalent to approximately 0.08 mg of dry mass in a gametophore, were placed on BCD agar medium in 5-cm Petri dishes (six gametophores per dish) and incubated for 5 days under continuous white fluorescent light to immobilize the gametophore explants. Before centrifugal cultivation, the Petri dishes were sealed with breathable surgical tape (type 21N, NICHIBAN Co., Ltd., Tokyo, Japan) to prevent rapid desiccation. We confirmed that CO_2 concentrations in the Petri dish were

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Table 1. Fluctuation of gravity values during centrifugation with the MIJ-17 and MK-3 centrifuges.

	MIJ-17			MK-3		
	68 rpm*	108 rpm	154 rpm	90 rpm	140 rpm	200 rpm
Mean gravity (*G*)	2.0	5.0	10.0	2.1	5.0	10.0
Peak to peak value** (*G*)	0.3 (0.15)	0.6 (0.12)	0.8 (0.08)	0.0 (0)	0.0 (0)	0.1 (0.01)
RMS*** (*G*)	0.027	0.098	0.120	0.000	0.000	0.001

Values are obtained from 500 recorded gravity values at 10 ms interval (see Fig. 2A, B). The values in parentheses are relative peak to peak values, in which the mean gravity values were normalized to unity. * rpm, revolutions per minute, **Peak to peak value, difference between the maximum and the minimum, *** RMS, root mean square from mean gravity.

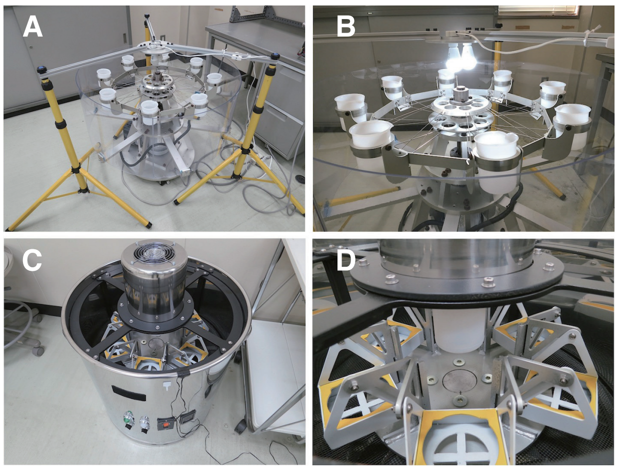

Fig. 1. Photographs of the two centrifuges, MIJ-17 (A, B) and MK-3 (C, D).

enough for photosynthesis of the moss, because CO_2 conductance of the sealed Petri dish (10.0 μmol m^{-2} s^{-1}) was much higher than the photosynthetic rate of the 8-week-cultivated gametophores (0.47 μmol m^{-2} s^{-1}).

Centrifugal cultivation

In the hypergravity experiments, we used two different custom-built centrifuges, namely MIJ-17 (Environmental Measurement Japan, Co., Ltd, Fukuoka, Japan) and MK-3 (Matsukura Co., Kurobe, Japan) (Fig. 1). In MIJ-17, a direct driving system with a Mitsubishi Super Line motor, SF-HR type (Mitsubishi Electric Co., Tokyo, Japan) was used, whereas MK-3 adopted a belt drive system with a MLH8085M three-phase induction motor (Fuji Electric Motor Co., Ltd., Tokyo, Japan). These centrifuges were designed to cultivate small plants at 2–10 *G*, and incorporated a LED-lighting system to allow long-term cultivation of plants. The photosynthetic photon flux density was adjusted to 18 μmol m^{-2} s^{-1} at the surface of the Petri dishes in both MIJ-17 and MK-3. After pre-incubation for 5 days, the *P. patens* gametophore explants were centrifuged at 10 *G* for 4 weeks with both centrifuges. In order to prevent desiccation, 0.8 ml sterile ultra-pure water were added to the medium once a week.

Measurement of vibration

In the present work, we defined vibration as change of acceleration, and measured it with a three-axis micro acceleration sensor (G-MEN DR20, SRIC Co., Nagano, Japan). The G-MEN was placed into a bucket and centrifuged at 2, 5, and 10 *G* in the MIJ-17 and MK-3, with gravity values recorded every 10 ms at the resolution capability of 0.1 *G*. The resultant three-axis forces were analyzed to evaluate the vibration properties.

Analysis of plant growth and morphogenesis

P. patens gametophores grown at 10 *G* for 4 weeks were used to measure shoot length, shoot diameter, rhizoid length, and number of leaves for each gametophore (*n* = 34–36), and dry mass and number of gametophores in the canopy (*n* = 12). The gametophore size was analyzed with ImageJ software (National Institutes of Health, Bethesda, MD, USA) after the gametophores were photographed with a SMZ-1000 microscope (Nikon, Tokyo, Japan) or PowerShot S100 digital camera (Canon, Tokyo, Japan).

Results

Vibration properties in MIJ-17 and MK-3

The waveforms of vibration generated by MIJ-17 and MK-3 are shown in Figure 2. For MIJ-17, random vibration was clearly observed and the gravitational change was more distinct with increasing rotation speed; the peak-to-peak value, which indicates the maximum excursion of the wave, and a useful quantity to evaluate the maximum stress considerations, increased from 0.3 *G* at 68 rpm to 0.8 *G* at 154 rpm (Table 1). In addition, the RMS (root mean square from mean gravity), which takes into account both the time history of the wave and the amplitude value that is directly related to the energy content of the vibration, also increased from 0.027 *G* at 68 rpm to 0.120 *G* at 154 rpm (Table 1). However, when the mean gravity values were normalized to unity, the peak-to-peak values decreased as the rotation speed increased, from 0.15 at 68rpm to 0.08 at 154 rpm (Table 1). This result indicated that the relative vibration in MIJ-17 stabilized as rotation speed increased.

On the other hand, the vibration generated by MK-3 was almost negligible even at 10 *G* (Fig. 2B). Indeed, the both peak-to-peak value and RMS were very small even at 10 *G* and undetectable at 5 *G* and 2.1 *G* (Table 1). Thus, these data indicated that MK-3 could be considered to be a vibrationless centrifugal culture system.

Table 2. Gametophore and canopy features of *Physcomitrella patens* cultivated with MIJ-17 and MK-3 at 10 *G* for 4 weeks.

Trait	MIJ-17	MK-3	*t*-test
Gametophore features			
Rhizoid length (mm)	2.9 ± 0.2	2.9 ± 0.1	n.s.
Shoot length (mm)	3.6 ± 0.1	3.8 ± 0.1	n.s.
Stem (caulid) diameter (mm)	0.25 ± 0.00	0.25 ± 0.00	n.s.
Number of leaves	20.0 ± 0.5	21.0 ± 0.5	n.s.
Canopy features			
Dry mass (mg)	1.5 ± 0.1	1.5 ± 0.1	n.s.
Number of plants	31.0 ± 3.6	35.0 ± 3.2	n.s.

The mean ± SE values of rhizoid length, shoot length, stem diameter, number of leaves for each gametophore cultivated in MIJ-17 (n = 36) and in MK-3 (n = 34) are shown. The mean ± SE values of dry mass and number of gametophores for each canopy (n = 12) are shown. The significance of differences between means was analyzed using Student's t-test ($P < 0.05$). n.s., non-significant.

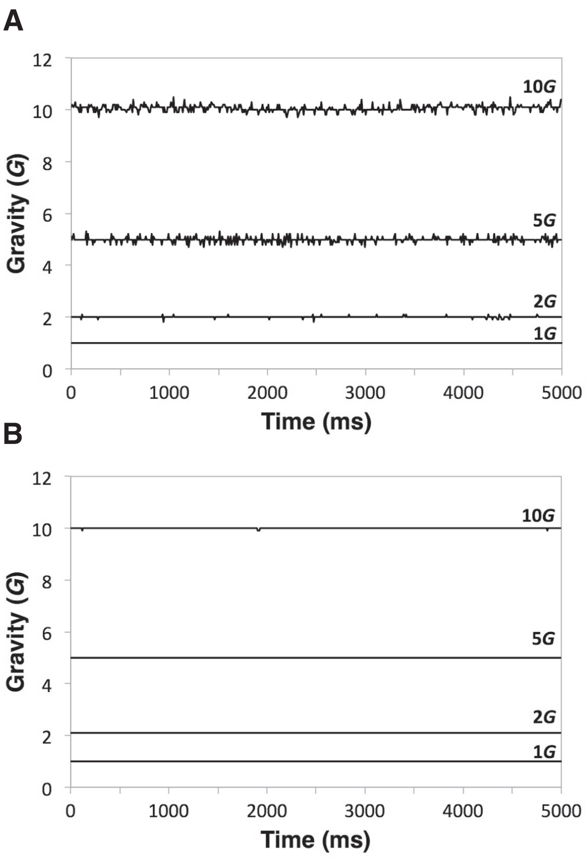

Fig. 2. Vibration waveforms generated by MIJ-17 (A) and MK-3 (B) during centrifugation at 2, 5, and 10 *G*, and the stationary condition (1 *G*). For MIJ-17, the rotation speeds to obtain 2, 5, and 10 *G* were 68, 108, and 154 rpm, respectively, whereas those for MK-3 were 90, 140, and 200 rpm, respectively. The waveforms were obtained with a three-axis micro acceleration sensor (G-MEN DR-20) at 10 ms interval.

Effects of centrifugal vibration on the growth of gametophores

We examined how the centrifugal vibration in MIJ-17 affects the growth and morphological parameters of *P. patens* gametophores in a long-term centrifugal environment. Given that MK-3 generated little vibration, we used it as a vibrational control experiment. In this work, we adopted the 10 *G* condition for the experiments because growth of *P. patens* was significantly influenced at 10 *G*: increases in canopy-based biomass and plant numbers, rhizoid length and stem (caulid) diameter, and decreases in shoot length and leaf cell wall thickness (Takemura *et al.*, 2017). After cultivation for 4 weeks at 10 *G*, the gametophore explants properly proliferated, showing similar features of the canopies between the gametophores cultivated with MIJ-17 and MK-3 (Fig. 3). This finding implied that the vibration generated by MIJ-17 may not notably affect the growth of *P. patens* gametophores.

In order to determine the effects of the vibration more precisely, rhizoid length, shoot length, stem (caulid) diameter, number of leaves per gametophore, and dry mass and number of gametophores in canopies were measured (Table 2). No significant differences were detected (Student's *t*-test, $P < 0.05$) among all traits examined. These results indicated that the effects of the vibration of MIJ-17 were negligible on the growth of *P. patens* gametophores.

Discussion

A centrifugal hypergravity experiment is useful to investigate gravitational effects on plant growth on Earth (Herranz *et al.*, 2013; Manzano *et al.*, 2012; Nava *et al.*, 2015; Soga *et al.*, 2006; Takemura *et al.*, 2017; Tamaoki *et al.*, 2014). However, a rigorous 1-*G* control experiment is difficult to perform, because several factors (vibration, pressure, gas circulation, humidity and temperature) are presumed to change during centrifugation in addition to gravity. The most difficult factor to control is vibration; there is no information available on the vibration generated during centrifugation, and reproducing the vibrating condition in a 1-*G* control experiment is problematic. In the present work, vibration was distinctly

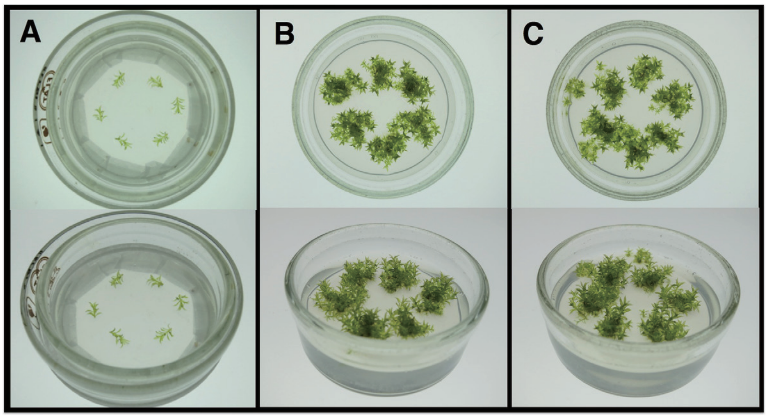

Fig. 3. *Physcomitrella patens* gametophores pre-cultivated on BCD agar medium (A), and cultivated at 10 *G* for 4 weeks with MIJ-17 (B) and MK-3 (C).

detected in MIJ-17, whereas little vibration was generated by MK-3. For MIJ-17, the peak-to-peak value was 0.3 *G* when the mean gravity was 2.0 *G* (68 rpm). This means that the effective radius rotation ranged from 35 to 41 cm. Thus, the radius rotation changed at most 6 cm during centrifugation. However, such a large change is unlikely to occur in our experimental system. Alternatively, because MIJ-17 is driven by a direct drive system, an irregular rotation may be caused by the lack of motor torque, especially at a low rotation speed. As the rotation speed increased, the difference in estimated effective radius rotation became small; at 5.0 *G* (108 rpm) and 10.0 *G* (154 rpm), the differences in the estimated effective radius rotation were 5 and 3 cm, respectively. Thus, this may be the reason why the relative peak-to-peak value declined with increasing rotation speed from 0.15 to 0.08 (Table 1). Thus, we conclude that the vibration in MIJ-17 may be caused by the irregular rotation of the motor, especially at a low rotation speed. Conversely, the vibrationless properties in MK-3 may be attributable to its belt drive system.

We showed that the vibration generated by MIJ-17 at 10 *G* did not affect the growth and morphological parameters of *P. patens* gametophores, because no significant differences in the measured parameters were observed between the gametophores cultivated with MIJ-17 and vibrationless MK-3 (Table 2). However, previous studies show that vascular plants are sensitive to vibration, which stimulates seed germination and root elongation in rice and cucumber (Takahashi *et al*., 1991) and also seed germination in *Arabidopsis thaliana* (Uchida and Yamamoto, 2002), affects mechanical properties and biomass allocation in *Capsella bursa-pastoris* (Niklas, 1998), and elicits glucosinolate and anthocyanin production as chemical defenses in *A. thaliana* (Appel and Cocroft, 2014). The present results imply that anatomy and growth of mosses may be less sensitive to vibration compared with vascular plants. However, the vibration properties generated by the MIJ-17 centrifuge are different from those of previous studies, in which a high frequency of vibration (50—250 Hz) was generated. Therefore, it is essential to compare sensitivities under the same vibration properties. Indeed, different vibration properties cause different biological effects in the anthocyanin response in *A. thaliana* (Appel and Cocroft, 2014).

In conclusion, our results confirmed that the vibration generated by MIJ-17 did not significantly affect the growth and morphological parameters of *P. patens* gametophores. Therefore, the influence of the vibration in MIJ-17 may be negligible in experiments to investigate the effects of long-term hypergravity conditions (< 10 *G*) on the growth of *P. patens* gametophores.

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