Biological Resource

Development of an LED-Attached Box for Phytochrome Response Experiments on Lettuce Seed Germination in Senior High School Biology

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The germination of lettuce seed is known to be a phytochrome-mediating phenomenon: red light promotes it, while far-red light inhibits it. In Japan, this topic has been included in biology textbooks for senior high schools, but the phytochrome response experiments have not yet been practiced widely. In order to enable senior high school biology teachers to conduct these experiments, the authors developed an apparatus for the experiments, LED-attached box. This article attempts to explain how to set up the LED-attached box. By means of the LED-attached box, the experimental procedures written in Japanese biology textbooks and in some research articles were followed. The phytochrome responses in photoblastic lettuce seed germination mentioned in Japanese biology textbooks and in other articles were successfully confirmed by using this box. This experimental apparatus was piloted in biology laboratory classes for senior high school students in Japan and pre-service high school teacher trainees in Cambodia. The participants could obtain good results and they were interested in using this LED-attached box.

Keywords: *experimental apparatus, LEDs, photoblastic seed germination, phytochrome, senior high school biology*

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INTRODUCTION

Phytochrome is a photoreceptor sensitive to red light (R) and far-red light (FR). Light absorbed by phytochromes, which consist of two forms, the R-absorbing form (Pr) and the FR-absorbing form (Pfr), has an effect on gene regulation that influences plant growth and development (Casal *et al.*, 1998; Park and Song, 2003). In lettuce (*Lactuca sativa* L.), seed germination is known to be under phytochrome control: R promotes and FR inhibits the seed germination, and the effects of R and FR are reversible (Borthwick *et al.*, 1952; Kendrick and Russell, 1975; Choi and Takahashi, 1979; Toyomasu *et al.*, 1998; Sawada *et al.,* 2008). The response of photoblastic lettuce seed germination to light conditions can be explained as illustrated in Figure 1. This topic is included in recent biology textbooks for senior high schools in Japan (Akasaka *et al.*, 2014; Agata *et al.*, 2015; Baba *et al.*, 2015; Asashima *et al.*, 2018).

In a wide range of plant species, seed germination is also regulated by two plant hormones: gibberellin (GA) promotes seed germination whereas abscisic acid (ABA) inhibits seed germi-

Dark				Do not germinate
R	Dark			Germinate
FR	Dark			Do not germinate
R	FR	Dark		Do not germinate
FR	R	Dark		Germinate
R	FR	R	Dark	Germinate
FR	R	FR	Dark	Do not germinate

Figure 1: Germination responses of photoblastic lettuce seed to light treatment with red light (R) and far-red light (FR)

nation (Piskurewicz *et al.*, 2009). The treatment of R on a photoblastic lettuce seed causes the conversion of Pr to Pfr in the seed, which up-regulates the gene expression of GA to induce seed germination (Toyomasu *et al.*, 1998) and, in contrast, when FR is irradiated, Pfr is converted to Pr which results in producing ABA to inhibit seed germination (Piskurewicz *et al.*, 2009).

Even though many articles have described the effects of R and FR on lettuce seed germination, the equipment used by the researchers as the light sources of R and FR might not have been applied for high school laboratory classes. Traditionally, the light sources for the experiment were contrived by using incandescent or fluorescent lamps together with colored cellophane or gelatin filters. For example, Shanklin *et al.* (1987) used a slide projector in conjunction with either an R interference filter or an FR cut-off filter for their experiment. Jackson *et al.* (1985) proposed the use of LEDs (light-emitting diodes) as light sources in plant physiology. Researchers might have used an industrial plant growth chamber with attached LEDs to conduct their researches on the effects of R and FR on lettuce seed germination. As this kind of experimental apparatus might be too expensive for ordinary high schools even in Japan, phytochrome experiments have not yet been practiced extensively in biology education at schools. Jomori, a senior high school biology teacher in Japan, got results mostly similar to those of phytochrome experiments reported by Borthwick *et al.* (1952) by using commercial panels with many LEDs (Jomori, 2010). Nowadays, LEDs are readily available in the market in Japan. Even senior high school students could set up LED-installed apparatuses for their experiments on seed germination (Website 1) and seedling growth (Website 2), though their apparatuses could not be used for experiments on phytochrome responses. So, in the present study, the authors developed a simple LED-attached apparatus specified for phytochrome response experiments for high schools. This article introduces the methods of setting up the apparatus and reports its usefulness for the experiments.

DEVELOPMENT OF LED-ATTACHED BOX AND ITS APPLICATION TO CLASSROOMS

Development of LED-attached Box

Materials

The materials needed for setting up the apparatus can all be easily purchased. Five bulbs of R-LED or FR-LED of 5 mm diameter (Figure 2a), five sets of LED bulb holders (Figure 2b), resistors of 2.2 Ω and 51 Ω (Figure 2c), an on-off switch (Figure 2d), an electric current meter (Figure 2e), and a dial with a variable resistor from zero to 2 KΩ (“variable resistor dial” Figure 2f) are needed to set up one LED circuit. A 4-battery case (Figure 2g) and four 1.5 V batteries are needed as a power source. Some of these electrical parts were purchased at electrical shops and the others were ordered online from companies in Japan. Other materials such as electrical wires and batteries were purchased at markets. A cubical plastic kitchen canister with a side length of 8 cm which was bought from a 100-yen shop was used as the container box.

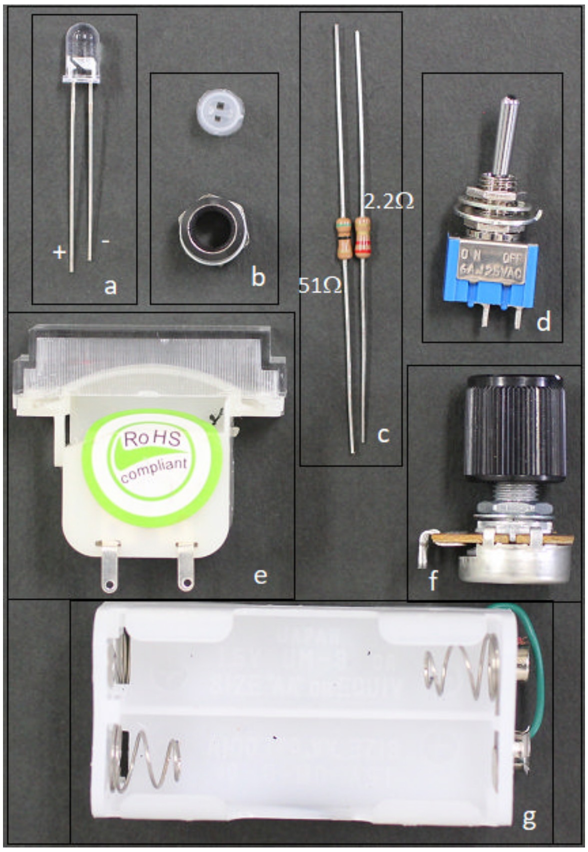

Figure 2: Essential parts for setting up an LED-attached box

Setting up an LED-attached box

A black paint was sprayed on the outside wall of the plastic kitchen canister in order to prevent the light from penetrating through the box. Aluminum foil was attached on the inside wall of the box to make the light reflect internally as well as to block the light from outside completely. Holes were drilled on the top-cover of the plastic box for inserting LED bulb holders. Five R-LEDs and the other five FR-LEDs were attached inward to the top-cover of the box as shown in Figure 3a. The wiring diagram of one LED circuit, which includes five bulbs of R-LED or FR-LED is shown in Figure 3b. Using electrical wire, a handmade LED-circuit to connect one LED to another LED following the wiring diagram in Figure 3b is shown in Figure 4a. Black paper board was folded to make the outer cover of the LED circuit. A completed LED-attached box is shown in Figure 4b.

Analysis of light spectrum

To ensure the correct light spectrum emitted from the light sources, a light analyzer LA-105 (NK-system Co. Ltd., Japan) was used to measure the light features in the LED-attached box. The parameters of the light features indicated by the analyzer include illuminance (LUX), dominant wavelength (Lambda D), and photon flux density (PFD). Lambda D of the light from both R-LED bulbs and FR-LED bulbs was almost constant: 623 or 624 nm for the former and 690 nm for the latter. In the light of R-LED, PFD-R was prominently higher than PFD-FR (Table 1), and in the light of FR-LED, PFD-FR was prominently higher than PFD-R (Table 2).

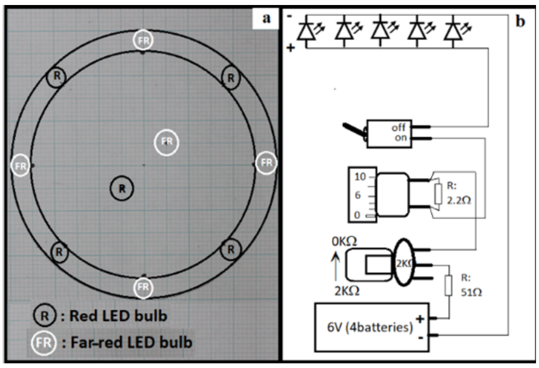

Figure 3: a: Distribution of LED bulbs on the box cover, b: The diagram of each LED circuit.

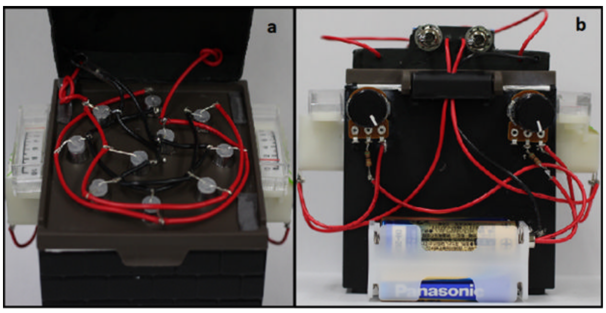

Figure 4: a: Top view of an LED-attached box, b: Side view of an LED-attached box.

Table 1: Features of light from red-LED bulbs in an LED-attached box for each marked value on its electric current meter measured by LA-105

Light features	Marked values on the electric current meter in the LED-attached box									
	1	2	3	4	5	6	7	8	9	10
Lux (lx)	35.9	95.4	143.0	190.0	259.0	306.0	376.0	476.0	585.0	694.0
PFD-R (600-700 nm) ($\mu mol/m^2/s$)	0.80	2.29	3.48	4.69	6.41	7.63	9.46	12.10	14.90	17.80
PFD-FR (700-780 nm) ($\mu mol/m^2/s$)	0.06	0.08	0.10	0.11	0.14	0.16	0.18	0.22	0.26	0.30

Table 2: Features of light from far-red-LED bulbs in an LED-attached box for each marked value on its electric current meter measured by LA-105

Light features	Marked values on the electric current meter in the LED-attached box									
	1	2	3	4	5	6	7	8	9	10
Lux (lx)	7.3	10.0	12.0	15.2	17.4	21.2	26.5	30.3	35.4	41.2
PFD-R (600-700 nm) ($\mu mol/m^2/s$)	0.10	0.21	0.29	0.40	0.49	0.64	0.81	0.97	1.14	1.35
PFD-FR (700-780 nm) ($\mu mol/m^2/s$)	1.06	4.11	5.98	8.95	11.30	15.30	19.70	24.20	29.00	35.00

The operation of the LED-attached box

The LED-attached box developed can be used mainly to conduct an experiment on phytochrome-mediating seed germination under the irradiation of R and FR. The box has two on-off switches that can allow users to switch on one type or both types of light at one time. The combination of the variable resistor dial and the electric current meter allows users to determine the intensity of LED light as shown in Table 1 and Table 2. Turning the dial clockwise results in reducing the resistance which would then generates a higher intensity of electricity current. However, if the batteries are low, the indicator of the electric current meter cannot reach the maximum marked value, 10, even though the dial is turned to maximum.

Application of LED-attached Box to Phytochrome Response Experiments

Materials

The seeds of the lettuce cultivar being used in this study must not germinate in the dark. In our preliminary experiments, we selected one lettuce cultivar, "Furiru (frill)" lettuce (Sakata Seed Co. Ltd., Japan), out of 25 cultivars commercialized in Japan.

Gibberellic acid (GA_3) and abscisic acid (Sigma-Aldrich Co. Ltd., USA) were used for GA and ABA treatment, respectively.

A Petri dish of 5.5 cm in diameter, which suited to the LED-attached box, was used with four layers of kitchen paper towel at the bottom.

Methods

A total of 20 Furiru lettuce seeds were put on each prepared Petri dish, then the preparation was placed into the LED-attached box. Two milliliters of tap water were added to the Petri dish in the box while the acclimation light, R or FR, was irradiating, and the box was closed immediately. The acclimation light continuously irradiated for 10 minutes. In the case that only one kind of light was used, the acclimation light was switched off to keep the seeds in the dark in the box. In the case of alternative light treatment, the treatment light, R or FR, was irradiated immediately after the acclimation light or the previous light was switched off, and the treatment light was irradiated continuously for 10 minutes before being switched off to keep the seeds in the dark in the box. In practice, there were seven different light treatments for one experiment which were dark (D), R-D, FR-D, R-FR-D, FR-R-D, R-FR-R-D, and FR-R-FR-D (Figure 1). The experimental settings were kept in a room of the temperature around 24°C for 3 days with the box cover being closed completely. The same experiments were repeated five times.

Experiments which observe the effects of plant hormones on lettuce seed germination were also conducted for showing the results to students in the classroom. Instead of tap water, 2 ml of 10 ppm ABA or GA was added to each Petri dish containing 20 Furiru lettuce seeds, and the seeds were treated with R, FR, or room light, or kept in the dark.

RESULTS AND DISCUSSION

The germination of Furiru lettuce seeds was inhibited completely within 3 days in the dark, or when the imbibed seeds received the last irradiation of FR before being kept in the dark. In contrast, seeds germinated whenever they received R before being kept in the dark (Figure 5 and Figure 6). In some previous studies, lettuce seed germination was not completely inhibited in the dark or by the final exposure to FR, *i.e.*, the germination rate was 8.5% in the dark and 43 to 54% by the exposure to FR (Borthwick *et al.*, 1952), 26% in the dark and up to 34% by the final exposure to FR (Jackson *et al.*, 1985), and 29% in the dark and up to about 30% by the exposure to FR (Jomori, 2010). The results of Japanese students' experiments on the effect of light on seed germination (Website 1) also indicated that lettuce seed germination was inhibited in their dark box. However, their box was not developed for phytochrome response experiments because it was equipped with neither R-LED nor FR-LED. Although it is not possible simply to compare our results with those of the previous studies because the lettuce cultivars they used and their experimental conditions are different from our experiments, the Furiru lettuce seeds and the LED-attached box used in this study were shown to be good materials for conducting an experiment to confirm photoblastic seed germina-

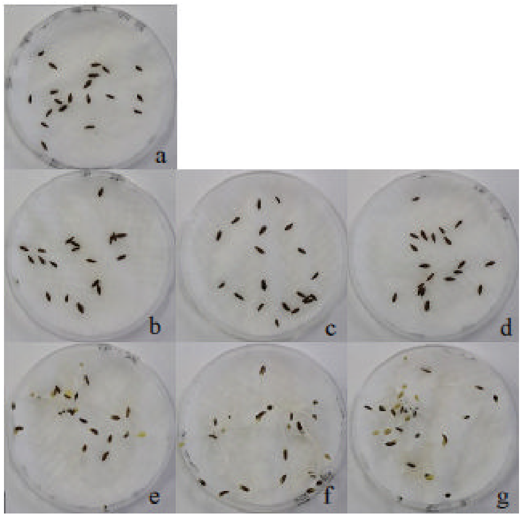

Figure 5: Germination responses of Furiru lettuce seeds to different light treatments 3 days after experiment started
a: dark (D), **b**: far-red (FR)-D, **c**: red (R)-FR-D, **d**: FR-R-FR-D, **e**: R-D, **f**: FR-R-D, **g**: R-FR-R-D.

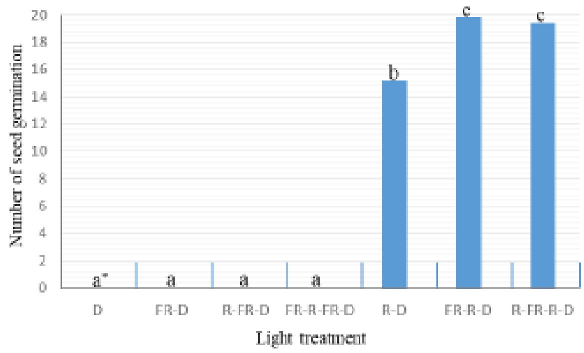

Figure 6: Germination of Furiru lettuce seeds on the 3rd day after different light treatments
D: dark, **FR**: far-red, **R**: red
*Different letters on the bars indicate significant differences among treatments by Real Statistics Using Excel (Charles Zaiontz) one factor Anova follow-up Turkey HSD, *p*-value <0.05.

tion phenomena which were described in Japanese biology textbooks and other articles. While Jomori (2010) used a black curtain or a windowless incubator to make dark conditions and the seeds were sown on an agar medium, we used simple materials and methods, such as a small dark box and the seeds were sown on wet paper, which are more suitable to apply to student laboratories.

The mode of action of phytochrome, which responses either to R irradiation to produce GA or to FR irradiation to produce ABA, can be explained by the results of the experiment using the respective plant hormones (Figure 7). The lettuce seeds treated with GA could germinate in dark conditions as well as they were irradiated with R before being kept in the dark. The seeds, however, did not germinate when they were treated with ABA in the dark or under the room light as well as they were irradiated with FR before being kept in the dark (Figures 5, 6 and 7).

At around 24°C, the Furiru lettuce seed germination generally started within 2 days after being imbibed. However, the results, whether the seeds have germinated, can be checked one week after the experiment was started. Therefore, the

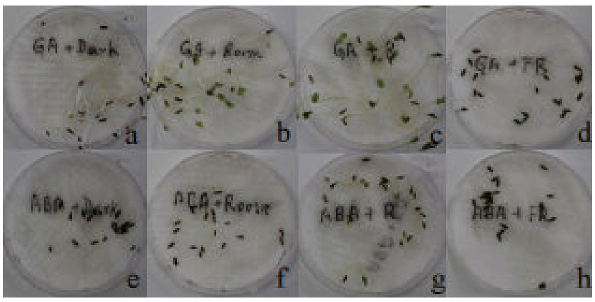

Figure 7: Germination of Furiru lettuce seeds on the 4th day after different light treatments with GA or ABA.
a to **d**: 10 ppm of GA was applied to the seeds, and the seeds were kept in the dark (**a**), under the room light (**b**), under red light (**c**), and under far-red light (**d**). **e** to **h**: 10 ppm of ABA was applied to the seeds, and the seeds were kept in the dark (**e**), under the room light (**f**), under red light (**g**), and under far-red light (**h**).

experiment can be adapted to the curriculum of some countries including Cambodia, where biology lessons are scheduled once a week. But temperature must be one of the concerning factors in this experiment. The effect of light on seed germination of some photoblastic lettuce cultivars depends on temperature (Hannay, 1967). According to Ikuma (1964), the optimum temperature to observe the phytochrome responses to R and FR is 25°C, and the seed germination of some photoblastic lettuce cultivars such as Grand Rapids is inhibited at 35°C if the seeds are maintained at the same temperature throughout. Therefore, to introduce this experiment into biology laboratories of ordinary secondary schools in the tropics where any air conditioner is not equipped (with), further examinations of the effects of temperature on the phytochrome-mediating germination of Furiru lettuce seeds should be needed.

APPLICATION TO CLASSROOMS

The LED-attached box developed was piloted with senior high school students in biology laboratory classes at the Senior High School Affiliated to Aichi University of Education in Japan and with teacher trainees in a pre-service teacher training course at the National Institute of Education in Cambodia. Furiru lettuce seeds were used for examining the seed germination. In the experiment, in order to make the PFD values of R and FR almost similar, the intensities of electric current for R-LED and for FR-LED were adjusted to 8 (PFD-R = 12.10 μmol/m^2/s) and to 6 (PFD-FR = 15.30 μmol/m^2/s), respectively.

Situation in Japan

The first trial was carried out in a biology laboratory class having 24 students of 2nd-year senior high school (11th grade) in 2018. An experiment was designed to confirm the theory about the phytochrome-mediating lettuce seed germination which was explained in the biology textbook that students used. In the first lesson on this topic, students carried out the experiment. Seeds were irradiated with R or FR for 5 minutes immediately after being soaked, and then they were irradiated with FR or R, respectively, for 5 minutes before being kept in the dark. The results were checked 3 days later in the second lesson. The results obtained were consistent with the theory written in the textbooks as well as the results of previous studies shown in Figure 1, despite the failure of some seeds to germinate after receiving R.

In 2019, the second trial was carried out in another biology laboratory class with the same students when they had become 3rd-year students (12th grade). An experiment was designed with a more advanced question of scientific inquiry. The imbibed seeds were irradiated with acclimation light, R or FR, for 10 minutes and then irradiated with treatment light, FR or R, of different durations from 1 to 10 minutes before being kept in the dark. The results were checked 4 days later. The lettuce seeds which received the final irradiation of FR did not germinate whereas the seeds which received the final irradiation of R germinated depending on the duration of light irradiation from 1 minute (16 seeds out of 20 germinated) to 10 minutes (all = 20 seeds germinated). The students were asked to fill in pre-lesson and post-lesson questionnaires (Appendixes 1 and 2).

In comparing the results of the pre-lesson questionnaire with those of the post-lesson questionnaire, students' comprehension did not change considerably after carrying out the experiment (Table 3). The high average scores of pre-lesson indicate that students still remembered the phenomena that R promotes and FR suppresses seed germination which they had learnt in the biology laboratory class in the previous year (2018). However, as the average score for Question 1 rose from 2.25±0.60 (pre-lesson) to 2.74±0.61 (post-lesson), students' understanding of the relation between wavelength and light was improved by the lesson. Although students understood well about the effects of R and FR on seed germination (pre-lesson average score was 3.08±0.76 and post-lesson average score was 3.43±0.58 for Question 2), their understanding of the mode of action of light, that causes the change in phytochrome structure which results in the promotion or suppression of seed germination, seemed to be insufficient even after carrying out the experiment (pre-lesson average

Table 3: The results of multiple-choice questions given to the Japanese students (n = 24)

Questions	Average scores	
	Pre-lesson	Post-lesson
Q-1: How well do you understand the relation between wavelength and light that blue light has a shorter wavelength and red light has a longer wavelength?	2.25±0.60	2.74±0.61
Q-2: How well do you understand the promotion and suppression of seed germination by the irradiation of red light (R) and far-red light (FR)?	3.08±0.76	3.43±0.58
Q-3: How well do you understand the mode of action of light in Question 2 on seed germination caused by the change in the structure of a substance called phytochrome?	2.71±0.79	3.04±0.62
Q-4: How well do you understand the change of phytochrome structure by R and FR irradiation affecting the contents of plant hormones to promote and suppress seed germination?	2.42±0.86	2.74±0.85
Q-5: Totally, to what extent did you understand the contents of this class?		2.96±0.62
Q-6: Was this class interesting for you?		2.78±0.72
Q-11: How useful is this experimental apparatus for you to understand the phytochrome response?		3.24±0.53

score was 2.71±0.79 and post-lesson average score was 3.04±0.62 for Question 3). The lesson also improved students' understanding that plant hormones promote or suppress seed germination since the average score for Question 4 rose from 2.42±0.86 (pre-lesson) to 2.74±0.85 (post-lesson). In general, students expressed that they could understand the contents of the lesson (the average score for Question 5 was 2.96 ± 0.62). However, a few students could not understand some aspects of this class, for example, why the indicator of electric current meter should be adjusted to marked value of 8 for R and 5 for FR, and why the experiment similar to that in the last year had to be conducted. Other two students could not understand about phytochrome or light-wavelength relationship. Most of the students also answered that the experiment was interesting for them (the average score for Question 6 was 2.78±0.72). Only three students were not interested in this class because the contents were difficult, the experiment was similar to that in the previous year, or the results of experiment were not the same as predicted ones.

Pieces of knowledge which students obtained from this class were the promotion of seed germination by R and the suppression of seed germination by FR, the effect of different duration of R and FR irradiation on seed germination, and the relation between light and wavelength (Table 4).

Twenty students mentioned further experimental activities which they wanted to do. As shown in Table 5, nearly two-thirds of the students wanted to conduct an experiment with shorter periods of light irradiation.

Some students gave comments on this class or wrote their impressions: five students gave comments that they are happier to learn with conducting experiments than reading textbooks, six students were surprised or disappointed that the results of the experiment were different from their expected ones, and three students stated that they could not understand clearly the mode of action of phytochrome. Students highly evaluated that the LED-attached box was helpful for them to understand the phytochrome response phenomenon (as shown in Table 3, the average score for Question 11 was 3.24±0.53).

Situation in Cambodia

The LED-attached box was applied to a laboratory class with 23 trainees in the biology teacher

Table 4: Pieces of knowledge which students obtained from the class (n = 24)

Pieces of knowledge	Number of students
Red light promotes seed germination and far-red light suppresses seed germination	16
The number of seeds germinated is affected by the duration of red and far-red light irradiation	13
The structure of phytochrome can be changed by red and far-red light irradiation	8
The relation between light and wavelength	4

Table 5: Further experimental activities which students wanted to do (n = 20)

Further activities	Number of students
To examine how lettuce seed germination is affected by shorter periods of light irradiation (in seconds)	15
To examine how lettuce seed germination is affected by increasing light intensity	3
To conduct the same experiment using the seeds of other lettuce cultivars	1
To conduct the experiment on the effects of plant hormones	1

training course at the National Institute of Education, Cambodia. The teacher trainees will be high school teachers after they finish their studies at this teacher training institution. The concept of the phytochrome-mediating phenomenon was not adopted in this lesson because the trainees had not learned about this biological phenomenon before. Therefore, the LED-attached box was piloted in relation to the topic "the Effects of Light on Seed Germination and Seedling Growth." The soaked seeds in one box were irradiated with FR for 30 minutes, then irradiated with R for 30 minutes, before being kept in the dark. Those in the other box were irradiated with R for 30 minutes, then irradiated with FR for 30 minutes, before being kept in the dark. The results were checked one week later. The seeds that received the last irradiation of FR did not germinate, but the seeds that received the last irradiation of R germinated well. After the class, the trainees evaluated the lesson and the LED-attached box by answering the questionnaire which is shown in Appendix 3.

The results of the questionnaire are shown in Table 6. The attendants could understand the contents of the lesson well (the average score was 2.96±0.46), and this experimental class was remarkably interesting for them (the average score was 3.61±0.49). The trainees expressed that this LED-attached box is useful for biology education in Cambodia (the average score for Question 4 was 2.91±0.50), but they were not sure whether they can set up the apparatus by themselves (the average score for Question 5 was 2.26±0.44). The view that "This equipment is not dangerous for students" was shared by all the trainees (the score 4.00±00 for Question 6).

To the Question 7, the trainees replied that this LED-attached box is appropriate for biology education at university (3 trainees), senior high school (12 trainees), and junior high school level (12 trainees) in Cambodia corresponding to the chapters of plant growth and response (15 trainees), and photosynthesis (13 trainees). No trainees mentioned the use of this apparatus to check the phytochrome response on seed germination. Trainees noticed that the difficult points in this pilot lesson are the setting up of the apparatus by themselves (15 trainees), the availability of materials for setting up the apparatus (6 trainees), the explanation of the effects of different light wavelengths on seed germination (4 trainees), and the identification of the difference between R and FR (1 trainee). They suggested us to explain more in detail how to set up this LED-attached box (10 trainees) and why the lettuce seed germination is affected by different light wavelengths (13 trainees).

CONCLUSION

The LED-attached box developed in this study is suitable to biological education at the high school level. By means of this apparatus, students can study the effects of R or FR on phytochrome-

Table 6: The results of multiple-choice questions given to the Cambodian teacher trainees (n = 23)

Questions	Average scores
Q-1: Can you understand this science lesson?	2.96±0.46
Q-2: Is this science class interesting for you?	3.61±0.49
Q-3: Did you get new knowledge or new ideas from this lecture?	2.83±0.38
Q-4: Do you think that this LED-attached box is useful for biology education?	2.91±0.50
Q-5: Do you think that you can set up this apparatus by yourself if there are enough materials available?	2.29±0.44
Q-6: Do you think that this apparatus is dangerous for students?	4.00±0.00

mediating lettuce seed germination. Teachers can set up this experimental box by themselves if enough materials are available. There is no danger even if they have made a wrong circuit. Using small batteries can provide a stable electric current and they are appropriate for any school setting such as schools with unreliable electrical supply in a developing country. This LED-attached box can provide different intensities of R and FR independently or simultaneously so that students can design further experiments to examine the effects of R and/or FR of different intensities on seed germination.

REFERENCES

Borthwick, H. A., Hendricks, S. B., Parker, M. W., Toole, E. H. and Toole, V. K. (1952) A reversible photoreaction controlling seed germination. *Proc. Natl. Acad. Sci. USA* **38**: 662-666.

Casal, J. J., Sánchez, R. A. and Botto, J. F. (1998) Modes of action of phytochromes. *Journal of Experimental Botany* **49**: 127-138.

Choi, K. S. and Takahashi, N. (1979) Studies on the lettuce seed germination with special reference to light responses. *Japanese Journal of Breeding* **29**(3): 197-204.

Hannay, J. W. (1967) Light and seed germination - An experimental approach to photobiology. *Journal of Biological Education* **1**: 65-73.

Ikuma, H. (1964) The effects of temperature on photosensitive lettuce seed germination. *Plant Cell Physiology* **5**(4): 429-439.

Jackson, D. L., Walker, J. R. L. and McWha, J. A. (1985) The use of light-emitting diodes (LEDs) as green and red/far-red light sources in plant physiology. *Journal of Biological Education* **19**: 79-82.

Jomori, H. (2010) Experiment on germination of photoblastic-seeds – Using lettuce seeds soled at stores. *Resources for Science* **68**: 14-15 (in Japanese)

Kendrick, R. E. and Russell, J. H. (1975) Photomanipulation of phytochrome in lettuce seeds. *Plant Physiology* **56**: 332-334.

Park, C. M. and Song, P. S. (2003) Structure and function of the phytochrome: Light regulation of plant growth and development. *Journal of Photoscience* **10**(1): 157-164.

Piskurewicz, U., Tureckova, V., Lacombe, E. and Lopez-Molina, L. (2009) Far-red light inhibits germination through DELLA-dependent stimulation of ABA synthesis and ABI3 activity. *The EMBO Journal* **28**: 2259-2271.

Sawada, Y., Aoki, M., Nakaminami, K., Mitsuhashi, W., Tatematsu, K., Kushiro, T., Koshiba, T., Kamiya, Y., Inoue,Y., Nambara E. and Toyomasu, T. (2008) Phytochrome-and gibberellin-mediated regulation of abscisic acid metabolism during germination of photoblastic lettuce seeds. *Plant Physiology* **146**: 1386-1396.

Shanklin J., Jabben, M. and Vierstra, R. D. (1987) Red light-induced formation of ubiquitin-phytochrome conjugates: Identification of possible intermediates of phytochrome degradation. *Proc. Natl. Acad. Sci. USA*, **84**: 359-363.

Toyomasu, T., Kawaide, H., Mitsuhashi, W. Inoue, Y. and Kamiya, Y. (1998) Phytochrome regulates gibberellin biosynthesis during germination of photoblastic lettuce seeds. *Plant Physiology* **118**: 1517-1523.

BIOLOGY TEXTBOOKS FOR JAPANESE SENIOR HIGH SCHOOLS (in Japanese)

Agata, K., Fujimoto, H., Furumoto, H., *et al*. (2015) *Biology* (Certified by MEXT, No. 304) Daiichi Gakushusha Co. Ltd. pp. 260-262.

Akasaka, K., Hirata, K., Iijima, K., *et al*. (2014)

Biology (Certified by MEXT, No. 302) Shin-koshuppansha Keirinkan Co. Ltd. pp. 273-275.

Asashima, M., Fujiwara, H., Fukagawa, O. *et al.* (2018) *Revised Biology* (Certified by MEXT, No. 306) Tokyo Shoseki Co., Ltd. pp. 264-265.

Baba, S., Iguchi, I., Katsumata, S., *et al.* (2015) *Biology* (Certified by MEXT, No. 305) Jik-kyo-Shuppan Co. Ltd, pp. 240-241.

WEBSITES

1. Experiments on the effects of light on seed germination (in Japanese)
   https://www.nagano-c.ed.jp/omc-shin/gakka/risuuka/2015/se-2.pdf
2. Experiments on the effects of light on seedling growth (in Japanese)
   https://school.gifu-net.ed.jp/ena-hs/ssh/H24ssh/sc3/31216.pdf

APPENDIXES

Appendix 1: Pre-lesson Questionnaire for Senior High School Students in Japan

Note: A Japanese version was prepared for the students.

<Answer the following questions by ticking the number of the applicable item>

Question 1: How well did you understand the relation between wavelength and light that blue light has a shorter wavelength and red light has a longer wavelength?

Choices: Understand well (4), Understand (3), Did not understand so well (2), Did not understand at all (1)

Question 2: How well did you understand the promotion and suppression of seed germination by the irradiation of red light (R) and far-red light (FR)?

Choices: Understand well (4), Understand (3), Did not understand so well (2), Did not understand at all (1)

Question 3: How well did you understand the mode of action of light in Question 2 on seed germination caused by the change in the structure of a substance called phytochrome?

Choices: Understand well (4), Understand (3), Did not understand so well (2), Did not understand at all (1)

Question 4: How well did you understand the change of phytochrome structure by R and FR irradiation affecting the contents of plant hormones to promote and suppress seed germination?

Choices: Understand well (4), Understand (3), Did not understand so well (2), Did not understand at all (1)

Appendix 2: Post-lesson Questionnaire for Senior High School Students in Japan

Note: A Japanese version was prepared for the students.

<Answer the following questions by ticking or writing>

Question 1: How well did you understand the relation between wavelength and light that blue light has a shorter wavelength and red light has a longer wavelength?

Choices: Understood well (4), Understood (3), Did not understand so well (2), Did not understand at all (1)

Question 2: How well did you understand the promotion and suppression of germination by the irradiation of FR and R?

Choices: Understood well (4), Understood (3), Did not understand so well (2), Did not understand at all (1)

Question 3: How well did you understand the change of the substance called phytochrome and its effect on seed germination from taking the previous class and this class?

Choices: Understood well (4), Understood (3), Did not understand so well (2), Did not understand at all (1)

Question 4: How well did you understand the change of phytochrome structure by R and FR irradiation affecting the contents of plant hormones (GA and ABA); the former promotes and the later suppresses seed germination?

Choices: Understood well (4), Understood (3), Did not understand so well (2), Did not understand at all (1)

Question 5: Totally, to what extent did you understand the contents of this class?

Choices: Understood well (4), Understood (3), Did not understand so well (2), Did not understand at all (1)

Question 6: Was this class interesting for you?

Choices: Remarkably interesting (4), Interesting (3), Not so interesting (2), No, not at all (1)

Question 7: Please write three kinds of knowledge you have obtained from this class. It does not matter that you confirm them.

Question 8: After taking this class, what do you want to do and what do you want to know for further activities?

Question 9: Please write what you could not understand in this class.

Question 10: Please write reasons if you answered "Not so interesting" or "No, not at all" for this science class in Question 6.

Question 11: How useful is this experimental apparatus for you to understand the phytochrome response?

Choices: Especially useful (4), Useful (3), Not especially useful (2), Not at all, studying with textbook is enough (1)

Question 12: Comments and impressions (if any)

Appendix 3: Questionnaire for teacher trainees of the National Institute of Education in Cambodia

Note: An English version was prepared for the trainees.

<Tick and answer the following questions>

Question 1: Can you understand this science lesson?

Choices: Very well (4), Well (3), Some extent (2), Not at all (1)

Question 2: Is this science class interesting for you?

Choices: Remarkably interesting (4), Interesting (3), A little interesting (2), Not interesting at all

Question3: Do you get new knowledge or new ideas from this lecture?

Choices: A lot (4), Some (3), A little or a few (2), Not at all (1)

Question 4: Do you think that this LED-attached box is useful for biology education?

Choices: Especially useful (4), Useful (3), Not so useful (2), Not at all (1)

Question 5: Do you think that you can set up this apparatus by yourself if there are enough materials available?

Choices: Very sure (4), Sure (3), Not sure (2), Not at all (1)

Question 6: Do you think that this apparatus is dangerous for students?

Choices: Not at all (4), Some attentions should be required (3), Dangerous (2), Extremely dangerous (1)

Question 7: If you think that this equipment is useful for biology education in Cambodia, for which level and which chapter can this equipment be used?

Question 8: What kinds of experiments do you want to do by using this equipment?

Question 9: What are difficult points in this science class?

Question 10: Comments and Suggestions (if any)