



# The Asian Journal of Biology Education

ISSN 1447-0209

Volume 12: September 2020

## Editorial Board

### Editor-in-Chief

Dr. Nobuyasu Katayama (*Tokyo Institute of Biology Education, Japan*)

### Associate Editors

Professor Shigeki Mayama (*Tokyo Gakugei University, Japan*)

Dr. Kiyoyuki Ohshika (*Aichi University of Education, Japan*)

### Editorial Committee

Dr. Edona Amparado (*University of the Philippines, Diliman, Philippines*)

Dr. Chi Chiu Cheang (*The Education University of Hong Kong, China*)

Professor Ka Hou Chu (*The Chinese University of Hong Kong, China*)

Dr. Narendra D. Deshmukh (*HBCSE, TIFR, India*)

Dr. C. H. Diong (*National Institute of Education, Singapore*)

Professor Juneuy Hong (*Seowon University, South Korea*)

Dr. Nirankush V. Khubalkar (*L. A. D. College for Women, India*)

Dr. John Donnie A. Ramos (*University of Santo Tomas, Philippines*)

Professor Kew-Cheol Shim (*Kongju National University, South Korea*)

Professor Morakot Sukchotiratana (*Chiang Mai University, Thailand*)

Dr. Anne M. Wallis (*Deakin University, Australia*)

Dr. Chen Zhong (*National Institute of Education, Singapore*)

Professor Cheng Zhong (*Tianjin University of Science and Technology, China*)

**The Asian Journal of Biology Education (AJBE)** is published electronically by the AJBE Editing and Publishing Office for the Asian Association for Biology Education (AABE). The Journal is on the AABE website: <http://www.aabe.sakura.ne.jp/Journal.htm>

Copyright ©2004 by the AJBE Editing and Publishing Office. All rights reserved.

# The Asian Journal of Biology Education

Volume 12: September 2020

## Biological Resource

### Wisconsin Fast Plants, Ideal Plant Materials for Student Laboratories on Flower Initiation at the Secondary Level

*Hideto Tojo and Nobuyasu Katayama* ..... 2

### Inquiry into the Onion

*Teiko Nakamichi* ..... 11

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

*Chansean Mam, Youhei Noda, Hiroyoshi Funai, Tsutomu Iwayama, Juntaro Kato* ..... 17

**Publications** ..... 29

**From the Editor-in-Chief** ..... 29

---

**Biological Resource**

---

**Wisconsin Fast Plants, Ideal Plant Materials for Student Laboratories on Flower Initiation at the Secondary Level****Hideto Tojo<sup>1)</sup>, Nobuyasu Katayama<sup>2)</sup>\***<sup>1)</sup> *Shiraume High School, Japan*<sup>2)</sup> *Tokyo Institute of Biology Education, Japan*

(Received: 01 April 2018; Accepted for publication: 16 April 2019;  
Communicating editor: S. Mayama)

For developing a student laboratory exercise on flower initiation (floral development) in senior high school biology, we selected the Wisconsin Fast Plants (WFPs) which are the new varieties of rape (*Brassica rapa*, syn. *campestris*) as our experimental material. We examined the effects of temperature, day length, light intensity and the plant hormone gibberellic acid (GA<sub>3</sub>) on the flower initiation of WFPs. Under continuous light, the days required for detecting the first flower bud were dependent on the growth temperature being between 15°C and 35°C; the higher the temperature was, the faster the flower bud formation was. In the temperature range examined, the first flower bud could be detected within at least nine days (at 15°C) after sowing the seeds. Flower initiation was dependent on the day length at 15°C, but not at 25°C. At 15°C, flower buds formed nine days after sowing the seeds under continuous light, while no flower bud was detected nearly two weeks after the seeds were sown under a short-day condition (L:D = 8:16). The intensity of light in a long-day treatment (L:D = 16:8), from about 10 μmol·m<sup>-2</sup>·s<sup>-1</sup> (ca 1,500 lux) to 100 μmol·m<sup>-2</sup>·s<sup>-1</sup> (ca 13,000 lux) at 15°C, did not affect flower bud formation. The flower initiation in the plants treated with 0.01 μg·mL<sup>-1</sup> GA<sub>3</sub> was faster than in the untreated plants. The results obtained in the present study reveal that by using the WFPs students can get clear results on the effects of photoperiod, temperature, and the plant hormone gibberellin on the flower initiation of long-day plants within a shorter period than by using any other plant. Biology teachers possibly can organize a student laboratory on the photoperiodic response of plants referring to the present report.

**Keywords:** *Brassica rapa* (syn. *campestris*), flower initiation, laboratory exercise, photoperiodism, secondary biology, Wisconsin Fast Plants

\* **Author for correspondence:** E-mail: katayama@u-gakugei.ac.jp

**INTRODUCTION**

Flower initiation (floral development) is the point at which vegetative growth turns to the reproductive stage. Therefore, it is one of the important biological phenomena to be learned at the secondary level (Lockard and Shortess, 1960). Students might be interested in studying this topic in the chapter “Plant Growth and Its Regu-

lation” in secondary biology because they can learn the relationships between plant morphogenesis and environmental factors, such as light, photoperiod and temperature.

In current biology textbooks for Japanese senior high school students, there are many plant names (e.g., *Ipomoea nil*, *Lemna* sp., *Xanthium occidentale* and *Kalanchoe* sp.) and terms (e.g.,

short-day and long-day treatments, short-day plants, long-day plants, indeterminate plants, and flowering hormone) in relation to flower initiation and photoperiodism. Some examples of experiments, such as leaf removal, grafting and stem girdling techniques, also appear in these textbooks. However, in practice, such experiments are rarely carried out as a student laboratory because the teaching of this topic through observation and experimentation is time-consuming. Therefore, learning of this topic has been mainly through lectures and deskwork. As a result, understanding of this topic has largely come by obtaining enumerated fragmentary knowledge of the phenomenon, and students mostly might be bored in studying this topic.

Among many plants, the Japanese morning glory (*Ipomoea nil*), a short-day plant, has been used as an experimental material for flower initiation and the flowering itself in research on plant physiology in Japan (Imamura, 1967; Takimoto and Kaihara, 1984). There have been a considerable number of trials to introduce the results of this serial research work to high school biology classes so far (e.g., Madrazo, Jr. and Hounshell, 1978). However, only a small number of teachers may carry out this experiment for student laboratories, because it takes at least a few weeks to get the experimental results.

Some experiments using other short-day plants, such as duckweed, *Lemna* sp., (Rhodes, 1968; Fujioka, 1986) and *Bryophyllum* sp. (Hibbs and Yokum, 1976; Hinata, 1988, 1989) were developed for high school student laboratories. Regarding duckweed, it seems difficult for students to detect its flower buds, because they are too small. The latter species are familiar as flower-pot plants and are very useful as teaching materials for vegetative reproduction. Unfortunately, these plants also require several weeks for

detecting the flower buds after a short-day treatment. Therefore, there has been no report on such trials in student laboratories.

Regarding long-day plants, Hollis and Miller (1968) introduced *Silene pendula* into the laboratory exercise for university students. However, as it takes more than one month until flowering, the plant does not seem to be suitable for secondary school biology laboratories. Gotoh (1998) proposed the use of *Arabidopsis thaliana*, an ideal plant for research, for the secondary school student laboratory on flower initiation. This plant is suitable for this kind of experiment because of its shorter life cycle. Flowering in this plant is well-known to be induced by long-day photoperiods (Hayama and Coupland, 2003) and by plant hormones such as gibberellin (Boss *et al.*, 2004). But, there is a difficulty in detecting a flower bud at its early stages since the color of its petals is white, and it seems very hard for students to distinguish the juvenile flower bud from leaf primordia.

Friend and Helson (1966) indicated that the floral induction in rape (*Brassica rapa*, syn. *campestris*) occurs by one long-day treatment. Wisconsin Fast Plants (WFPs, see details in Appendices) which are the new varieties of rape have a short life cycle, as does *A. thaliana*. As the color of their petals is yellow, detecting their flower buds may be easier than with *A. thaliana*. However, the use of WFPs in students' laboratories on flower initiation has not been reported, yet. Therefore, in the present study, we examine the experimental conditions on the flower initiation of the WFPs for the sake of secondary biology teachers to introduce this plant material into their teaching of photoperiodism.

## MATERIALS AND METHODS

### Seeds

The seeds of the standard type of WFPs were purchased from Carolina Biological Supply Company, USA, through Nakamura Rika Kogyo Co. Ltd., Tokyo, Japan, or from *InTheWoods*: Kobayashi Hard Ware Co. Ltd., Aomori, Japan.

### **Plant Growth**

Fifty seeds were sown on a wet vermiculite bed in a tray (planter). Although the plants are generally grown with the addition of some fertilizer to assist good growth, only water was given to the plants in the present study to restrict their height. The trays were placed in a temperature-controlled growth cabinet. The light was shone from above by four fluorescent light tubes (Toshiba FL15N, 100V, 15W). The light intensity was determined by a photometer, Memory Sensor MES-101 which can be equipped with the Quantum Sensor IKS-27/101 or the Illuminance Sensor IKS-17/101 (Koito Industries, Ltd., Japan). Each experiment was repeated four times.

### **Gibberellin Treatment**

We used gibberellic acid (GA<sub>3</sub>, Tokyo Chemical Industry Co. Ltd., Japan) for gibberellin treatment. GA<sub>3</sub> was given to the seedlings throughout. Twenty seeds were placed on 0.8% water-agar plates containing 0, 0.01, 1, 10 or 100 µg·mL<sup>-1</sup> GA<sub>3</sub>, each in a 9 cm Petri dish or a Plant Box (BC-PB851, Bio Medical Science Co. Ltd., Tokyo). When a Petri dish was used, a plastic cover was placed on each Petri dish to prevent water loss from the agar plate. Then the Petri dishes and Plant Boxes were placed in the growth cabinet mentioned above. The experiment was repeated four times.

In our experiments, we did not conduct sterilization treatment because we did not notice any microbial infections on the seeds or seedlings.

### **Detection of Flower Buds**

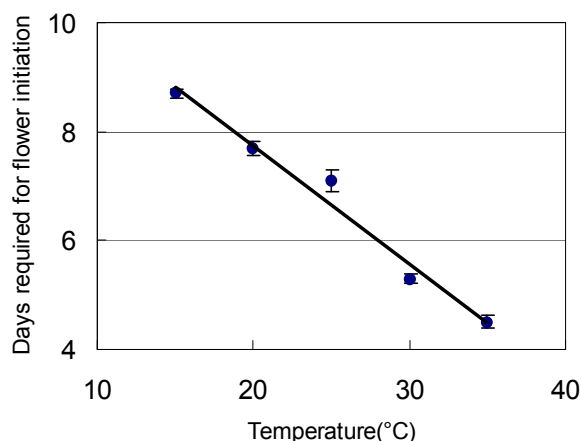
The presence of the first flower bud, whose color was pale yellow, of each plant was exam-

ined with the naked eye by opening juvenile leaves at the shoot apex of each plant with a pair of tweezers. The number of days required for detecting the first flower bud after sowing the seeds was recorded. Data were analyzed statistically: the mean value and significant difference (SD) of the number of days required for detecting the first flower bud were calculated for four replications.

## **RESULTS AND DISCUSSION**

### ***Effect of Temperature on Flower Initiation under Continuous Light***

The plants were grown at different temperatures under continuous light illumination to see if the growth temperature would affect flower initiation. As shown in Figure 1, the days required for detecting the first flower bud after sowing the seeds were dependent on the growth temperature being kept between 15°C and 35°C; the higher the temperature was, the faster the flower bud formation was. In the temperature range examined, the first flower bud could be detected less than nine days after sowing the seeds.

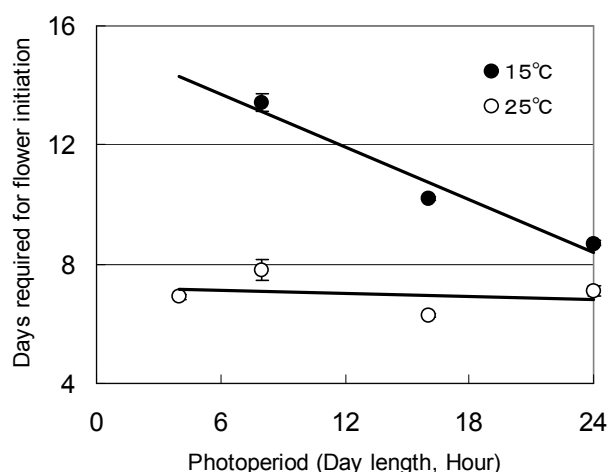


**Figure 1: The effect of temperature on flower initiation in the standard type of WFPs**

The vertical axis indicates the number of days required for detecting the first flower bud after placing the seeds under continuous light illumination (n = 50, four replications).

### Flower Initiation under Different Photoperiods

According to King and Kondra (1986), *B. campestris* (= *B. rapa*), as well as *B. napus*, shows photoperiodic responses. However, Weis (2015) reported that the standard purple-stemmed line of WFPs was insensitive to day length. As he did not mention the temperature examined, we examined the effects of day length on flower initiation at different temperatures of 15°C, 20°C and 25°C. As shown in Figure 2, at 15°C, the number of days required for detecting the first flower bud after sowing the seeds was dependent on the day length. Under continuous light at 15°C, the first flower bud was detected nine days after sowing the seeds (Fig. 1). However, under a short-day condition (8-hour light and 16-hour dark; L:D = 8:16), it took nearly two weeks to detect the first flower bud. On the other hand, at 20°C (data not shown) and 25°C, the number of days required for detecting the first flower bud after sowing the seeds was constant (about one week) (Fig. 2). From the results, the standard type of WFPs is considered to be a tempera-



**Figure 2: The effect of photoperiod (day length) on flower initiation in the standard type of WFPs at 15°C and 25°C**

The vertical axis indicates the number of days required for detecting the first flower bud after sowing the seeds ( $n = 50$ , four replications).

ture-dependent “quantitative long-day plant (see details in Appendices)” for which flowering is accelerated or delayed by the length of the photoperiod at lower temperatures. The critical temperature for its photoperiodic response is considered to be between 15°C and 20°C. Therefore, it is recommended that the temperature must be kept below 15°C for this experiment.

### Flower Initiation under Different Light Intensities

To see if the light intensity would affect flower initiation, plants were grown at 15°C under different light intensities in a long-day condition (L:D = 16:8). The light intensity which plants received was changed by placing each planter a different distance from the light source. Under light intensity ranging from 13  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (ca 1,500 lux) to 103  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (ca 13,000 lux), there was no significant difference in the length of the period (ca 10 days) until the first flower bud was detected. From the results, the experiment possibly can be carried out without considering the strength of the light. As the lowest light intensity examined in the present study is easily obtainable by using an ordinary fluorescent desktop light stand, no special light source is required for conducting the experiments.

### Effect of Gibberellic Acid on Flower Initiation

Whittwer and Bukovac (1957) firstly reported that a group of plant growth regulators, gibberellins, affected the temperature and photoperiodic requirements for flowering in some plants including *Brassica*. Thereafter, gibberellins have been shown to promote flower initiation in many plants (Lang, 1957; Boss *et al.*, 2004). Therefore, we examined whether GA<sub>3</sub> stimulates flower initiation in the standard type of WFPs. The flower bud formation in the plants treated with 0.01  $\mu\text{g}\cdot\text{mL}^{-1}$ , 1  $\mu\text{g}\cdot\text{mL}^{-1}$  GA<sub>3</sub> (Table 1) or

higher concentrations of GA<sub>3</sub> (data not shown) was faster than in the GA<sub>3</sub>-untreated plants. The application of 100 µg·mL<sup>-1</sup> GA<sub>3</sub> induced deformity of apical leaves, and so, such a high concentration of GA<sub>3</sub> may cause some negative effects on plant development. As treatment with a GA<sub>3</sub> concentration higher than 0.01 µg·mL<sup>-1</sup> resulted in the same stimulation effect on flower initiation, further examinations of GA<sub>3</sub> concentrations lower than 0.01 µg·mL<sup>-1</sup> are required.

#### ***Advantages of WFPs as an Experimental Material for Flower Initiation***

As well as *A. thaliana*, the WFPs are small in plant size and, therefore, many plants can be cultivated in a narrow space. The seeds of WFPs are non-dormant, so the seeds collected from mother plants can be used immediately. Flower initiation in WFPs does not require any vernalization treatment (pre-chilled treatment). Flower initiation starts just after the complete expansion of the cotyledons; the color of flower bud is pale yellow so that one can identify it with the naked eye more easily than in *A. thaliana*, whose flower bud is white. Flower initiation can be detected 1-2 weeks after sowing the seeds, as shown above, and the flower opening occurs 2-3 weeks after the seeds have been sown (See Appendices).

So far, plant materials for teaching flower initiation have mainly been short-day plants, such as the morning glory (*Ipomoea nil*) and *Xanthium occidentale*. By using WFPs, teachers can show flower initiation in a long-day plant to their students easily. In addition, the temperature dependence of photoperiodic responses and the involvement of plant growth regulators in photoperiodic responses can be taught (Our results may be helpful for the teachers). Compared with the other plants commonly used as experimental materials for flower initiation, the flower bud forma-

**Table 1: The effect of gibberellic acid (GA<sub>3</sub>) on flower initiation in the standard type of WFPs**

GA <sub>3</sub> concentration (µg·mL <sup>-1</sup> )	Days* (± SD)
0	11.8 ± 0.05
0.01	8.5 ± 0.09
1	8.5 ± 0.19

15°C, 13000 lux (103µmol·m<sup>-2</sup>·s<sup>-1</sup>), L:D = 8:16

\* Days after sowing the seeds when the first flower bud was detected (n = 20, four replications).

tion in WFPs occurs faster. Thus, inquisitive students can carry out experiments on flower initiation repeatedly.

In addition, both *Ipomoea* and *Xanthium* are “strict” short-day plants, which have an apparent critical night (dark) length for flower initiation. On the other hand, WFPs do not seem to have such a critical night length for flowering. The combination of these plants in teaching flower initiation will lead students to more effective learning of its mechanisms and its relationship to environmental factors.

#### **FURTHER EXPERIMENTS RECOMMENDED**

Further research should be carried out to examine the experimental conditions in preparation for more student laboratory experiments to provide students with stimulating activities. These experiments are as follows:

- (1) On the effect of light-break or dark-break on flower initiation;
- (2) On the determination as to whether the WFPs have the critical night length;
- (3) On the effects of light quality (blue, red or far-red light) on flower initiation (Friend 1968b);
- (4) On the determination of photosensitive plant parts by the excision of leaves, grafting, etc.;
- (5) On the effects of chemical substances, such as

sugars (Friend 1984) and plant growth regulators other than gibberellins, on flower initiation.

#### ACKNOWLEDGEMENTS

This work was supported in part by a Grant-in-Aid for Science Research (No. 12680174) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

#### REFERENCES

- Boss, P. K., Bastow, R. M., Mylne, J. S. and Dean, C. (2004) Multiple pathways in the decision to flower: enabling, promoting, and resetting. *Plant Cell* **16** (Suppl.), S18-31.
- Fujioka, S. (1986) Flower initiation in duckweeds. *Iden (The Heredity)* **40**(5): 45-51. (in Japanese)
- Friend, D. J. C. (1968a) Photoperiodic responses of *Brassica campestris* cv. Ceres. *Physiologia Plantarum* **21**: 990 -1002.
- Friend, D. J. C. (1968b) Spectral requirements for flower initiation in two long-day plants, rape (*Brassica campestris* cv. Ceres), and spring wheat (*Triticum X aestivum*). *Physiologia Plantarum* **21**: 1185-1195.
- Friend, D. J. C. (1984) Promotion of flowering in *Brassica campestris* L. cv Ceres by sucrose. *Plant Physiology* **75**: 1085 -1089.
- Friend, D. J. C. and Helson, V. A. (1966) *Brassica campestris* L.: floral induction by one long day. *Science* **153**: 1115–1116.
- Gotoh, S. (1998) Flowering in a test tube – The observation of life cycle using *Arasbidopsis* -. *Iden (The Heredity)* Suppl. **10**: 122-126. (in Japanese)
- Hafner, R. (1990) Fast plants – Rapid-cycling Brassicas. *The American Biology Teacher* **52**: 40-46.
- Harper, F. R. and Berkenkamp, B. (1975) Revised growth-stage key for *Brassica campestris* and *B. napus*. *Canadian Journal of Plant Science* **55**: 657-658.
- Hayama, R. and Coupland, G. (2003) Shedding light on the circadian clock and the photoperiodic control of flowering. *Current Opinion in Plant Biology* **6**:13-19.
- Hibbs, T. E. & Yokum, N. G. (1976) Bryophyllum: A versatile plant for the laboratory. *The American Biology Teacher* **38**: 281-283.
- Himmelblau, E., Lauffer, D., Teutonico, R., Pires, J. C. and Osborn, T. C. (2004) I-2 Rapid-cycling Brassica in research and education. In: Pua, E.-C. and Douglas, C. J. (eds.) *Brassica. Biotechnology in Agriculture and Forestry, Vol. 54*, pp. 13-28. Springer Verlag, Berlin, Heidelberg.
- Hinata, T. (1988) Flower-bud formation in *Bryophyllum daigremontianum* (*Kalanchoe daigremontiana*): Laboratory experiments with gibberellin. *Japanese Journal of Biological Education* **28**: 121-128. (in Japanese)
- Hinata, T. (1989) Laboratory experiments of flower-bud formation with gibberellin in *Bryophyllum pinnatum*. *Japanese Journal of Biological Education* **29**: 106-112. (in Japanese)
- Hollis, C. A. and Miller, H. A. (1968) A laboratory exercise demonstrating morphogenesis. *The American Biology Teacher* **30**: 393-396.
- Imamura, S. (1967) (ed.) *Physiology of Flowering in Pharbitis nil*. University of Tokyo Press, Tokyo.
- King, R. W. and Kondra, Z. P. (1986) Photoperiod response of spring oilseed rape *Brassica napus* L. and *B. campestris* L. *Field Crops Research* **13**: 367-373.
- Lang, A. (1957) The effect of gibberellin upon flower formation. *Proceedings of the National Academy of Science, USA* **43**:709-717.

- Lockard, D. J. and Shortess, D. K. (1960) Photoperiodism: Bringing classroom biology up-to-date. *The American Biology Teacher* **22**: 156-159.
- Madrazo, G. M. Jr., and Hounshell, P. B. (1978) Photoperiodic Treatments in Morning Glory: A Laboratory Investigation. *The American Biology Teacher* **40**: 480-497.
- Musgrave, M. E. (2000) Realizing the potential of rapid-cycling *Brassica* as a model system for use in plant biological research. *Journal of Plant Growth Regulation* **19**:314-325.
- Orr, A. R. (1978) Inflorescence development in *Brassica campestris* L. *American Journal of Botany* **65**: 466-470.
- Orr, A. R. (1981) A quantitative study of cellular events in the shoot apical meristem of *Brassica campestris* (Cruciferae) during transition from vegetative to reproductive condition. *American Journal of Botany* **68**: 17-23.
- Price, R. (1991) Perfect plants for projects. *Biological Sciences Review* **4**(1): 32-36.
- Price, R. and Harding, S. (1993) Genetics in the classroom: inheritance patterns of two mutant phenotypes in rapid-cycling *Brassica rapa* (syn. *campestris*). *Journal of Biological Education* **27**: 161-164.
- Rhodes, L. W. (1968) The Duckweeds: Their use in the high school laboratory. *The American Biology Teacher* **30**: 548-551.
- Rood, S. B., Pearce, D., Williams, P. H. and Pharis, R. P. (1989) A gibberellin-deficient *Brassica* mutant-rosette. *Plant Physiology* **89**: 482-487.
- Takimoto, A. and Kaihara, S. (1984) Photocontrol of flower-opening in *Pharbitis nil*. In: Vince-Prue, D., Thomas, B. and Cockshull, K. E. (eds.) *Light and the Flowering Process*, pp. 241-253. Academic Press, Orland, Florida.
- Tomkins, S. P. and Williams, P. H. (1990) Fast plants for finer science – an introduction to the biology of rapid-cycling *Brassica campestris* (*rapa*) L. *Journal of Biological Education* **24**: 239-250. <See also Website list>
- Weis, A. E. (2015) Inheritance of rapid cycling in *Brassica rapa* fast plants: dominance that increases with photoperiod. *International Journal of Plant Sciences* **176**: 859-868.
- Whittwer, S. H. and Bukovac, M. J. (1957) Gibberellin effects on temperature and photoperiodic requirements for flowering of some plants. *Science* **126**: 30-31.
- Williams, P. H. (1985) *CrGC Resource Book*. Department of Plant Pathology, University of Wisconsin - Madison, Madison WI 53706, USA.
- Williams, P. H. and Hill, C. B. (1986) Rapid-cycling populations of *Brassica*. *Science* **232**: 1385-1389.

## WEBSITES

- Wisconsin Fast Plants (WFPs): [http://www.fastplants.org/home\\_flash.html](http://www.fastplants.org/home_flash.html) <accessed March 27, 2018>
- Science & Plants for Schools (SAPS): <http://www.saps.org.uk/secondary/teaching-resources>  
<http://www.saps.org.uk/secondary/teaching-resources/282-fast-plants-for-finer-science-an-article-from-the-journal-of-biological-education> <accessed March 27, 2018>

## APPENDICES

### *What are WFPs?*

WFPs are also known as “Rapid-cycling brassicas (Hafner, 1990)” because of their short life cycle.

They are new varieties of rape (*Brassica rapa*, syn. *campestris*) developed by Professor Williams of the University of Wisconsin, Madison (Williams, 1985; Williams and Hill, 1986). These varieties were at first developed as experimental materials for studying genetics. So far, many strains, such as the dwarf type and the high-anthocyanins-producing type, have been obtained (Rood *et al.*, 1989).

WFPs have been used widely for plant biological research as one of the ideal model organisms (Musgrave, 2000). Furthermore, WFPs have been used as experimental materials from the primary level to the tertiary level in teaching not only genetics, but also morphology, physiology and reproduction (Tomkins and Williams, 1990; Price, 1991; Price and Harding, 1993; Himelblau *et al.*, 2004; WFPs' website; SAPS's website). However, there has been no report on the use of WFPs in students' laboratories on photoperiodism.

As well as *A. thaliana*, *B. rapa* is well known as a long-day plant (Friend and Helson, 1966; Friend, 1968a, 1968b, 1984; King and Kondra, 1986). Its floral development is induced by one long-day treatment (Friend and Helson, 1966). Its growth stages were defined well by Harper and Berkenkamp (1975), and its floral development process was studied well by Orr (1978, 1981). Therefore, we have enough information about the flower initiation of *B. rapa*, and thus, WFPs might be an ideal long-day plant material to study flower initiation. The following characteristics (Williams and Hill, 1986) make them a suitable experimental material for studying flower initiation: (1) Under optimal conditions, their life cycle (from seed germination to the seed maturation of the next generation) completes itself within about five weeks; (2) flower-opening is observed 13 days after sowing the seeds when the plants are grown under continuous illumination of a sufficient light intensity at a temperature of 22°C; (3) Even in a mature plant, its height is shorter than 20 cm, so one can cultivate many plants in a small space or in a growth cabinet.

### ***Quantitative long-day plants and qualitative long-day plants***

Long-day plants are those whose flowering is promoted by a long-day condition. Among them, the plants which can flower even under inappropriate photoperiods, though their flowering is delayed, are called “quantitative long-day plants” or “facultative long-day plants.” The plants whose flowering is absolutely dependent on a long-day condition are called “qualitative long-day plants” or “obligate long-day plants.” See details in the following websites:

Bareja, B. G. (2011) What is photoperiodism, crop types and significance

<https://www.cropsreview.com/photoperiodism.html> <accessed March 27, 2018>

Cox, D. (2009) Photoperiod and bedding plants

<https://ag.umass.edu/greenhouse-floriculture/fact-sheets/photoperiod-bedding-plants> <accessed March 27, 2018>

Wada, K. (2003) Physiology of flowering in *Pharbitis nil*

<https://www.sc.niigata-u.ac.jp/biologyindex/wada/english/index2.html> <accessed March 27, 2018>

At present, in biology textbooks for Japanese senior high school students, only qualitative long-day plants as well as qualitative short-day plants are described, and there is no reference to quantitative long-day plants and quantitative short-day plants. We consider that the difference between “qualitative” and “quantitative” is not all that essential for secondary students to understand photoperiodism.

***Light intensity conversion***

Few secondary schools have light meters (quantum-meters) which can measure photon flux density (PFD) because the equipment is expensive. On the other hand, schools can have illuminometers which are generally cheaper than any quantum-meter and can be purchased easily anywhere, *e.g.*, at camera shops or local science equipment suppliers. Therefore, in the present paper, we show light intensities in both PFD and illuminance for the convenience of teachers to convert the illuminance to PFD. However, the factor for conversion is not constant; it is dependent on the light source. For example, in the case of the fluorescent lamp we used in the present study, 1 klux equals  $9 \mu\text{mol m}^{-2} \text{s}^{-1}$ , while with light from a projector lamp (Phillips FP-10S, 100V, 300W), 1 klux equals  $18.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

---

**Biological Resource**

---

**Inquiry into the Onion****Teiko Nakamichi\****Tokyo Institute of Biology Education, Japan*

(Received: 29 October 2018; Accepted for publication: 29 October 2019)

In Japan, inquiry activities have been introduced into science subjects for upper secondary schools since 1994. Through these inquiry activities students are expected to address issues actively, think deeply by themselves, and feel pleasure in solving problems. However, so far, these activities have not been implemented widely. In the present paper, an example of inquiry activities which relates to the morphology and growth of the onion bulb is proposed. The process of the activity is as follows: First, the teacher gives the students a question, “Which part of the onion do you eat: root, stem, leaf, flower or fruit?” Most of the students may not give the correct answer. Teachers can use the gap between the students’ answers and the correct answer to foster students’ curiosity. This part is easy and inexpensive, and it can be carried out within one school hour. The next part is also inexpensive, but more challenging. The teacher gives the students another question, “How does the onion bulb get bigger?” To figure out their answers and verify them, students are asked to carry out a series of group activities: making a hypothesis which is an answer to the question, designing an experiment, carrying out the experiment and collecting data, analyzing and discussing the results, and finally deciding whether the hypothesis is acceptable. Then, each student is asked to write a report or give a presentation. Through these activities, students’ abilities of logical thinking, decision-making, and expression can be cultivated.

*Keywords: active learning, inquiry activity, morphology, onion bulb, plant growth, secondary school biology,*

\* **Mrs. Teiko Nakamichi**, Email: teikonakamichi@hotmail.com

**INTRODUCTION**

In Japan, inquiry activities have been introduced into science subjects for upper secondary schools since 1994 when the official school curriculum, the Course of Study (CS), revised by the Ministry of Education, Japan (MOE, presently Ministry of Education, Culture, Sports, Science and Technology, Japan: MEXT) was enforced. Since then, examples of inquiry activities, which are the result of the efforts of highly motivated biology teachers and researchers, have been provided in biology textbooks. In the textbooks that

followed each CS which was revised in and after 1989 (MOE, 1989, 1999; MEXT, 2009), each chapter includes at least one inquiry activity. However, there are quite a few teachers who feel that most of these inquiry activities are time-consuming and/or high cost. Thus, these inquiry activities have not been implemented widely.

In the newly revised CS, which was announced in 2018 and will be enforced from the 2022 school year, active learning, *i.e.*, student-centered instruction, is emphasized (Nakamichi and Katayama 2018). Under the revised

CS, science teachers are asked to allow students to learn through inquiry activities much more than at the present time. Students are asked to study through a process of inquiry activities, *i.e.*, to have a question, to decide the key issue, to make a hypothesis, to design a research plan, to carry out experiments, to analyze and discuss the experimental results, to make a decision whether the hypothesis is acceptable, and to write a report to be submitted to the teacher or to be presented in the class.

Here, the author would like to provide an example of inquiry activities which is easily carried out in any upper secondary school.

## INQUIRY INTO THE ONION

### 1. Which part of the onion do you eat?

The first part of this inquiry activity is composed of the teacher’s question and an observation activity by students. The time required for this part is one school hour. At first, the teacher gives the students a question, “Which part of the onion do you eat: root, stem, leaf, flower or fruit?” Students are requested to give their answers with reasons.

The author gave this question to the seventh grade (lower secondary school) and the tenth grade (upper secondary school) students and university students. As shown in Figure 1, most of the students thought they eat the stem or root of the onion.

In Table 1 the major reasons they gave are listed. Some students who answered “root” might have been misled by the Japanese term “kyukon” for bulb (“kyu” means round-shaped and “kon” means root). After examining these reasons together, students observed the morphology of the onion bulb to confirm which answer is correct. The gap between student answers and the correct answer may foster student interests.

How can the teacher lead the students to the correct answer? One possible way to proceed is

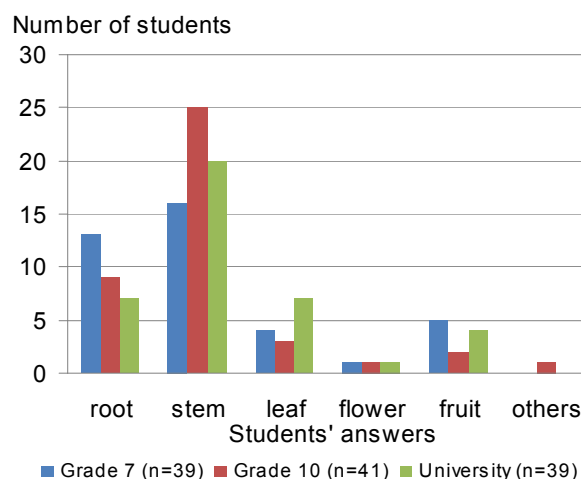
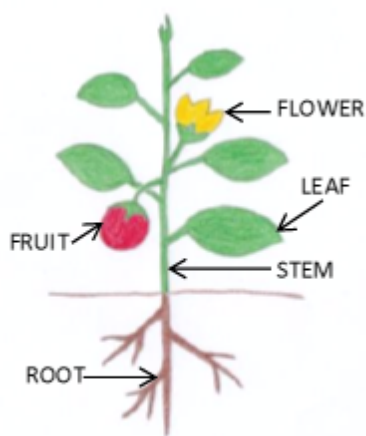


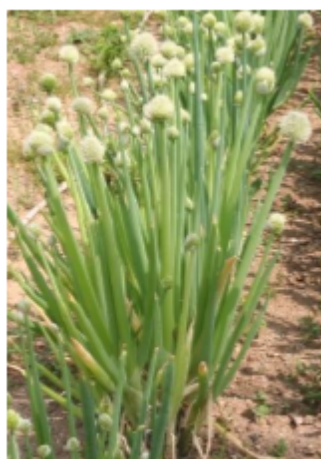
Figure 1: Results of student answers

Table 1: Major reasons of student judgements

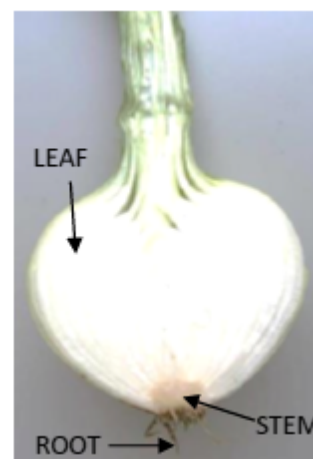
Answer	Reasons
Root	<ul style="list-style-type: none"> <li>➢ I have an image that onion bulb is under the ground and the plant part under the ground is root.</li> <li>➢ There are green leaves and stems above the bulb.</li> <li>➢ Onion bulbs resemble tulip bulbs and the Japanese term for bulb is “kyukon.”</li> </ul>
Stem	<ul style="list-style-type: none"> <li>➢ The onion bulb is between the roots and leaves.</li> <li>➢ It is above the root.</li> <li>➢ There are leaves above it and roots below it.</li> <li>➢ I learnt it when I was an elementary school child.</li> </ul>
Leaf	<ul style="list-style-type: none"> <li>➢ When we peel the onion bulb, the peeled part looks like a leaf.</li> <li>➢ It seems to have leaves overlapping.</li> <li>➢ It has the stem and the root at the bottom.</li> <li>➢ I heard the onion bulb was composed of many leaves.</li> </ul>
Flower	<ul style="list-style-type: none"> <li>➢ The onion bulb has roots under it, so I think it will be a flower.</li> <li>➢ It looks like a flower.</li> <li>➢ Because it is multi-layered.</li> </ul>
Fruit	<ul style="list-style-type: none"> <li>➢ I guess the answer from its round shape.</li> <li>➢ It has stems and leaves above, roots below, and no flower. Maybe, we eat the fruit.</li> <li>➢ Because we eat it after peeling.</li> <li>➢ We eat fruits.</li> <li>➢ It is the result of elimination.</li> </ul>



**Figure 2: Basic morphology of a plant**



**Figure 3: Growing onions with onion heads**



**Figure 4: Longitudinal section of an onion bulb**

as follows:

At first, let students recall the basic morphology of the plant, *i.e.*, roots, leaves and flowers (fruits) attach to the stem (Figure 2). Then, show a photograph of growing onions in a field (Figure 3), because most students have no experience of seeing onions grow. The teacher can point out that the onion head is a cluster of onion flowers. Figure 4 shows a longitudinal section of the onion bulb. Students can easily identify the roots in the figure. By careful observation, they can recognize that there is the stem above the roots. Since the edible part of the onion is the part attached to the stem, it can be understood that they are scale leaves. The morphology of the onion bulb can also be compared to cabbage or lettuce. The similarity of the morphology between the onion bulb and cabbage can be confirmed by cutting them in half longitudinally.

**2. How does the onion bulb get bigger?**

The second part of this inquiry activity is more challenging and needs two or three school hours. The teacher asks the students “How does the onion bulb get bigger?”

At first, students are asked to confirm the changes in the diameters of different parts (upper, middle and lower) of the onion bulb during its

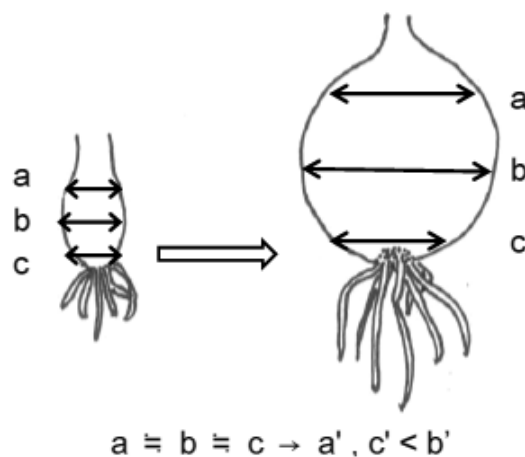
growth by using such pictures as shown in Figure 5.

Then, students need to continue the process of inquiry, which is composed of several sub-processes: making a hypothesis, designing an experiment or an observation activity, carrying out the experiment or making observations and collecting data, analyzing the results, and having a discussion on whether the hypothesis is acceptable. Students need logical thinking to analyze the data. It also requires the ability to make a decision on whether the hypothesis is supported by the results.

The inquiry activity may be organized in the following manner:

**(1) Making a hypothesis**

Students are asked to make a hypothesis ex-



**Figure 5: Growth pattern of the onion bulb**

plaining the difference in the growth of different parts of the onion bulb. They may make various hypotheses; for example, focus on the size of epidermal cells, or on the number of scale leaves, or on the hypertrophic growth of cells, and so on. I would like to show two simple examples of hypotheses which are related to the cells of scale leaves.

Hypothesis (I): The size of cells changes.

Hypothesis (II): The number of cells changes.

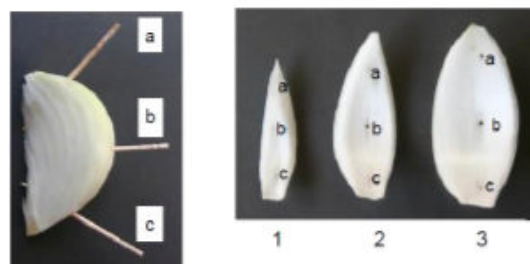
**(2) Designing an observation activity**

Students are asked to design an observation activity to verify their answers. One approach to prove the hypotheses is to observe cells in different parts of an onion bulb and measure the size of cells in each part.

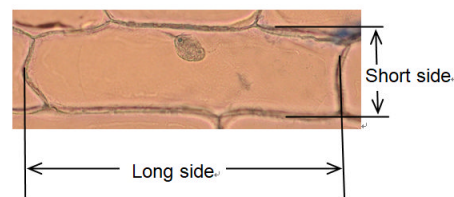
**(3) Making observations and collecting data**

- (i) After removing the dry brown skin, cut the onion bulb into six or eight wedges.
- (ii) Insert three toothpicks deeply into the upper (a), middle (b), and lower (c) areas of a wedge (Figure 6). Then remove the toothpicks. The remaining holes will mark the upper, middle, and lower areas.
- (iii) Separate the wedges into three pieces: inner, intermediate, and outer. (Figure 7)
- (iv) Take a small epidermal segment of inner (the abaxial) side from the three locations (upper, middle, lower) of each piece.
- (v) Put the epidermal segment on a glass slide, add a drop of methylene blue solution, and seal it with a cover slip. Soak up excess solution.
- (vi) Observe each specimen by microscope, take photographs, and measure the cell sizes. To find cell size, use a micrometer to measure the length of the long side and the short side of cells (Figure 8).

If a micrometer and a camera for the microscope are not available, students need to design another method of measurement. For example, the number of cells in a standardized area, such as the field of vision of the microscope, can be com-



**Figure 6 (right): Onion bulb wedge**  
**Figure 7 (left): Scale leaves of onion bulb**  
 1: inner leaf, 2: intermediate leaf, 3: outer leaf.



**Figure 8: Measurement of the long side and the short side of a cell**

pared, and the camera function of a smartphone can be substituted for an ordinary camera.

**(4) Analyzing the results**

After collecting the data, students need to compile and tabulate the results of the observations in order to analyze the data. An example of the results that was collected from my own research is shown in Figure 9 and Table 2. In Table 2, each figure in the column of long side and short side is the average length for 10 cells. Cell size could be shown simply as the area of the cross section of each cell which is a product of the length of long side by the length of short side. If possible, it is better to analyze the results statistically. By calculating the standard deviation for each value, it becomes possible for students to consider variations in cell size at each location. Then, they can verify whether the difference of the values is significant.

From the data, one can understand the following:

- \* The cell size increases at every location from inner to outer.
- \* Within the same piece, the cell size at the middle

(location b) is the largest.

\* At the lower area (location c), cell sizes show the least difference, and the length of the short side is the smallest and almost the same in all pieces.

(5) Having a discussion on whether the hypothesis is acceptable

By the microscopic observation, most students can notice the differences in cell size of different parts of the onion bulb. Then they have a discussion on the hypothesis they choose prior to beginning the observation activity.

From the results, students can come to see that hypothesis (I) is acceptable and hypothesis (II) is not acceptable. This well coincides with the findings of Aoba (1954) who reported that the enlargement of an onion bulb is caused by the increase in both the number of scale leaves and the thickness of each scale leaf. He mentioned that (i) the number of scale leaves increases during the bulb growing period, (ii) the number of scale leaf cells scarcely increases after the scale length comes up about 1 cm, and (iii) the thickness of

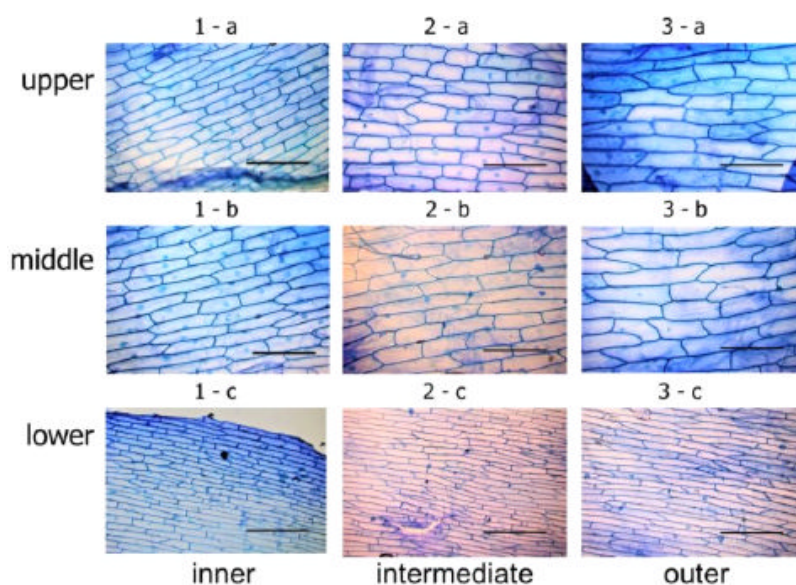


Figure 9: Epidermal tissue of onion scale leaf (Bars = 300 μm)

Table 2: Average of cell sizes at each area

Area of the wedge	Location*	Long side		Short side		Cell size (μm <sup>2</sup> /100)
		Length (μm)	SD**	Length (μm)	SD**	
1 Inner	a	336	32.3	61	4.3	206
	b	377	94.8	78	6.7	294
	c	256	49.1	36	6.0	92
2 Intermediate	a	280	29.5	86	6.8	242
	b	382	95.1	93	6.4	354
	c	280	50.9	37	3.4	103
3 Outer	a	421	117.8	96	4.5	406
	b	437	109.9	110	6.6	481
	c	369	50.0	40	2.0	147

\* a: upper, b: middle, c: lower

\*\* SD: standard deviation

bulbs is caused solely by the enlargement of cells in scale leaves.

#### (6) Writing a report

At the end of the activity, each student is asked to write a report or give a report by oral or poster presentation. Students can use ICT to prepare the report so that this process may nurture the ability of expression in the students.

#### **CONCLUDING REMARKS**

The latest revision of the CS by the MEXT was carried out with the aim of preparing students for society in the 2030s when they will be adults. In the new era, people will live in an information-based society because of the development of techniques of AI (artificial intelligence), IoT (the internet of things), and so on. They will need to respond to different environments with intellectual flexibility. The teacher-centered teaching style cannot cultivate such abilities. MEXT advocates the importance of proactive, interactive and deep learning for living in the forthcoming society.

The inquiry activities described in this article will be helpful for nurturing the following abilities specified by MEXT.

- \* Through careful observations of natural phenomena, students will have lots of questions. By having a question which is the first step of scientific inquiry, students can cultivate a proactive attitude.
- \* Through experiments, students can foster the ability to understand things deeply.
- \* Through discussion, students can deepen their understanding and develop dialogue skills.

Inquiry activities would be valued more than

ever, as these activities can help students develop the skills they must acquire for their future life. Teachers would be expected to introduce many more inquiry activities. I hope that the contents introduced in this article will encourage teachers to incorporate these activities into their lessons.

#### **ACKNOWLEDGEMENTS**

I would like to express my gratitude to Dr. Nobuyasu Katayama, the Director of Tokyo Institute of Biology Education, for his suggestions and comments.

#### **REFERENCES**

- Aoba, T. (1954) On bulb formation and dormancy in onions. II. On process of bulb formation and development of scales. *Journal of the Japanese Society for Horticultural Science* **23**:249-258. (in Japanese with English summary)
- Ministry of Education, Japan (1989) *The Course of Study for Upper Secondary Schools*. Printing Division, Ministry of Finance. (In Japanese)
- Ministry of Education, Japan (1999) *The Course of Study for Upper Secondary Schools*. Printing Division, Ministry of Finance. (In Japanese)
- Ministry of Education, Culture, Sports, Science and Technology, Japan (2009) *The Guidelines on Science Subjects of the Course of Study for Upper Secondary Schools*. Jikkyo Shuppan Co., Ltd. (in Japanese)
- Nakamichi, T. and Katayama, N. (2018) Biology Education in Upper Secondary Schools at Present in Japan. *Asian Journal of Biology Education* **10**: 7-16

---

**Biological Resource**

---

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

**Chansean Mam<sup>1)\*</sup>, Youhei Noda<sup>2)</sup>, Hiroyoshi Funai<sup>2)</sup>,  
Tsutomu Iwayama<sup>3)</sup>, Juntaro Kato<sup>3)</sup>**

<sup>1)</sup> Graduate School of Education, Aichi University of Education & Shizuoka University, Japan

<sup>2)</sup> Affiliated Senior High School, Aichi University of Education, Japan

<sup>3)</sup> Department of Science Education, Aichi University of Education, Japan

(Received: 31 October 2019; Accepted for publication: 05 August 2020)

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*

\***Author for correspondence:** E-mail: mamchansean@gmail.com

### **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 prac-

ticed 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  $\Omega$  and 51  $\Omega$  (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 K $\Omega$  (“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.

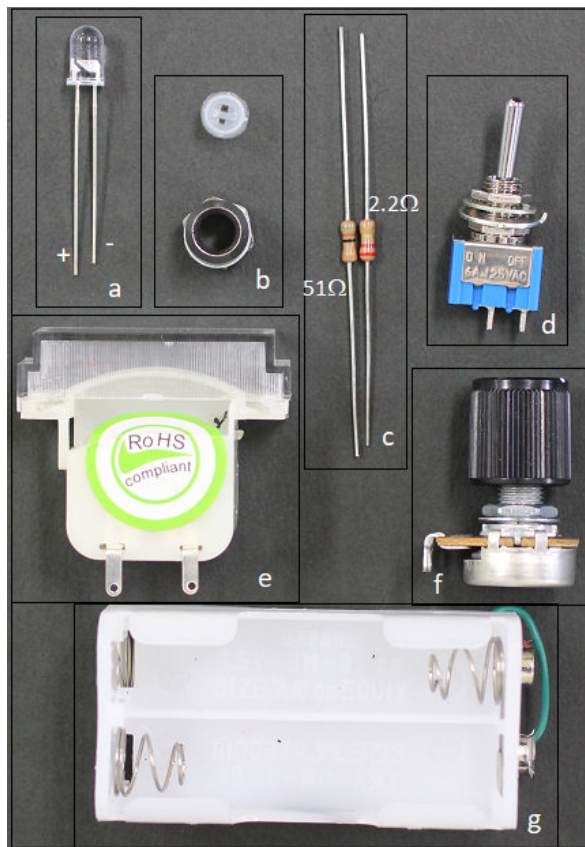


Figure 2: Essential parts for setting up an LED-attached box

**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).

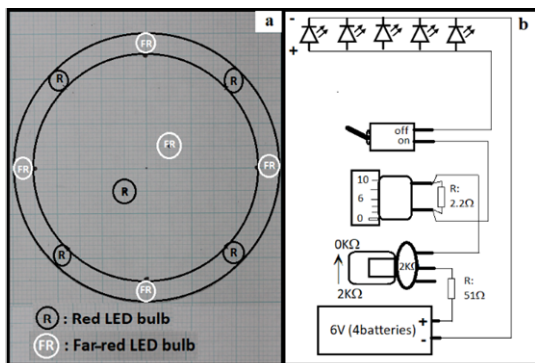


Figure 3: a: Distribution of LED bulbs on the box cover, b: The diagram of each LED circuit.

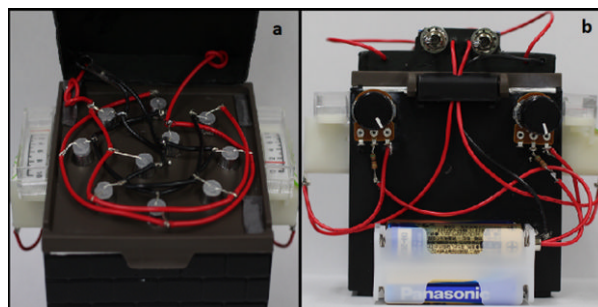


Figure 4: a: Top view of an LED-attached box, b: Side view of an LED-attached box.

**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) ( $\mu\text{mol}/\text{m}^2/\text{s}$ )	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) ( $\mu\text{mol}/\text{m}^2/\text{s}$ )	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) ( $\mu\text{mol}/\text{m}^2/\text{s}$ )	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) ( $\mu\text{mol}/\text{m}^2/\text{s}$ )	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-mediated 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 generate 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, “Fururu (frill)” lettuce (Sakata Seed Co. Ltd., Japan), out of 25 cultivars commercialized in Japan.

Gibberellic acid ( $\text{GA}_3$ ) and abscisic acid (Sigma-Aldrich Co. Ltd., USA) were used for  $\text{GA}$  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**

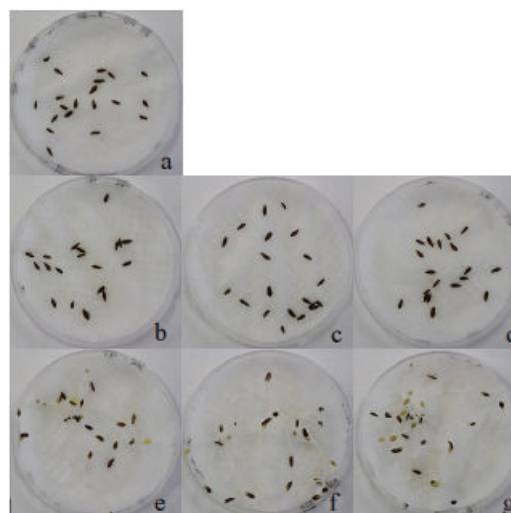
A total of 20 Fururu lettuce seeds were put on each prepared Petri dish, then the preparation was placed into the LED-attached box. Two milliliters of tap water were added to the Petri dish in the box while the acclimation light, R or FR, was irradiating, and the box was closed immediately. The acclimation 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 accli-

mation 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.

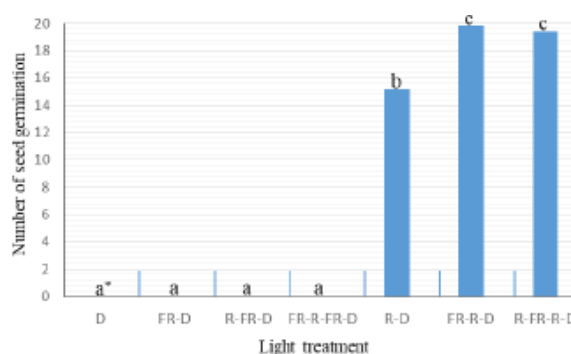
Experiments which observe the effects of plant hormones on lettuce seed germination were also conducted for showing the results to students in the classroom. Instead of tap water, 2 ml of 10 ppm ABA or GA was added to each Petri dish containing 20 Furiru lettuce seeds, and the seeds were treated with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



**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

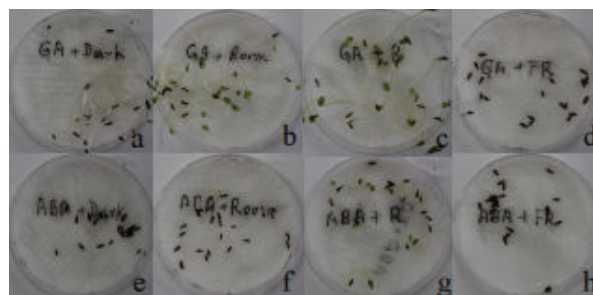
\*Different letters on the bars indicate significant differences among treatments by Real Statistics Using Excel (Charles Zaiontz) one factor Anova follow-up Turkey HSD,  $p$ -value <0.05.

sponse 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-

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 responds 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 Fururu 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 Fururu 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 Fururu 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. Fururu 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  $\mu\text{mol}/\text{m}^2/\text{s}$ ) and to 6 (PFD-FR = 15.30  $\mu\text{mol}/\text{m}^2/\text{s}$ ), 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 germina-

tion 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  $2.25 \pm 0.60$  (pre-lesson) to  $2.74 \pm 0.61$  (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  $3.08 \pm 0.76$  and post-lesson average score was  $3.43 \pm 0.58$  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?	$2.25 \pm 0.60$	$2.74 \pm 0.61$
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)?	$3.08 \pm 0.76$	$3.43 \pm 0.58$
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?	$2.71 \pm 0.79$	$3.04 \pm 0.62$
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?	$2.42 \pm 0.86$	$2.74 \pm 0.85$
Q-5: Totally, to what extent did you understand the contents of this class?		$2.96 \pm 0.62$
Q-6: Was this class interesting for you?		$2.78 \pm 0.72$
Q-11: How useful is this experimental apparatus for you to understand the phytochrome response?		$3.24 \pm 0.53$

score was  $2.71 \pm 0.79$  and post-lesson average score was  $3.04 \pm 0.62$  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  $2.42 \pm 0.86$  (pre-lesson) to  $2.74 \pm 0.85$  (post-lesson). In general, students expressed that they could understand the contents of the lesson (the average score for Question 5 was  $2.96 \pm 0.62$ ). 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  $2.78 \pm 0.72$ ). 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  $3.24 \pm 0.53$ ).

**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  $2.96\pm 0.46$ ), and this experimental class was remarkably interesting for them (the average score was  $3.61\pm 0.49$ ). The trainees expressed that this LED-attached box is useful for biology education in Cambodia (the average score for Question 4 was  $2.91\pm 0.50$ ), but they were not sure whether they

can set up the apparatus by themselves (the average score for Question 5 was  $2.26\pm 0.44$ ). The view that “This equipment is not dangerous for students” was shared by all the trainees (the score  $4.00\pm 0.00$  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?	$2.96\pm 0.46$
Q-2: Is this science class interesting for you?	$3.61\pm 0.49$
Q-3: Did you get new knowledge or new ideas from this lecture?	$2.83\pm 0.38$
Q-4: Do you think that this LED-attached box is useful for biology education?	$2.91\pm 0.50$
Q-5: Do you think that you can set up this apparatus by yourself if there are enough materials available?	$2.29\pm 0.44$
Q-6: Do you think that this apparatus is dangerous for students?	$4.00\pm 0.00$

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) Dai-ichi 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) Jikyo-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
- Question 3: 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)

## Publications

*Biology Education for Social and Sustainable Development* (ISBN: 978-94-6091-925-1) was published in 2012 by Sense Publishers, Rotterdam, Netherlands (<http://www.sensepublishers.com/>). Some papers presented at **the 23rd Biennial Conference of the AABE** which was held in Singapore in October 2010 were compiled in this book by Dr. Mijung Kim and Dr. C. H. Diong. You can refer to the abstracts of these papers in **the sixth volume of the *Asian Journal of Biology Education*** (2012).

*Biology Education and Research in a Changing Planet (2015)* (ISBN 978-981-287-523-5) was published by Springer (<http://www.springer.com/in/book/9789812875235>). Some papers presented at **the 25th Biennial Conference of the AABE** which was held in Malaysia in October 2014 were compiled in this book by Dr. Esther Gnanamalar Sarojini A Daniel. The abstracts of these papers were included in **the eighth volume of the *Asian Journal of Biology Education*** (2015).

### From the Editor-in-Chief

At first, we planned to publish this issue, the twelfth volume of the *Asian Journal of Biology Education* (AJBE), at the end of this year, 2020, because the 28th Biennial Conference of the AABE (AABE28) was planned to be held in Tianjin, China, in October of this year and the conference report of AABE28 would be included in this issue. However, because of the COVID19 pandemic, the conference had to be postponed, possibly until October 2021. Therefore, this volume is published a few months ahead of schedule without including the AABE28 conference report, though there are only three reports on biological resources.

During last two years, the manuscripts contributed to AJBE have been reviewed by the following persons as well as the Editorial Board members: Dr. Hideo Kitano (Tokyo Gakugei University, Japan), Professor Kim Kyounggho (Gongju National University of Education, Korea), Dr. Fumi Nakanishi (Tokyo Gakugei University, Japan), Dr. Danny Ng (The Chinese University of Hong Kong, China), Dr. Jason Orozco (Philippine Normal University, Philippines), Dr. Takayuki Sato (Hirosaki University, Japan), and Dr. Shigeyoshi Watanabe (Kumamoto University, Japan). I am very thankful to them for their efforts to review the manuscripts.

Everyone can contribute their research paper, practical report, or a report on biological resources to AJBE. So, I would like to ask the readers to prepare their manuscripts referring to the “Instructions to Contributors” and send them to me.

**Dr. Nobuyasu Katayama** ([katayama@u-gakugei.ac.jp](mailto:katayama@u-gakugei.ac.jp))