

Methods for Teaching Light Wavelength Dependencies on Seed Germination and Seedling Elongation Applicable for High School Experimental Class in Developing Countries

Chansean Mam¹, Youhei Noda², Hiroyoshi Funai², Tsutomu Iwayama³,
and Juntaro Kato³

¹Cooperative Doctoral Course in Subject Development in the Graduate School of Education,
Aichi University of Education & Shizuoka University

²Affiliated Senior High School, Aichi University of Education

³Department of Science Education, Aichi University of Education

ABSTRACT

Simple teaching methods to teach science experiments were considered in order to raise the quality of science education in developing countries. Students tend to prefer experimental classes than textbook-based classes. However, developing countries do not have enough budget to purchase experimental equipment for high school, so science experimental classes seem not to become practice in those countries. In this study, we introduced a teaching method of light wavelength dependencies on seed germination and seedling elongation by using simple LED experiment boxes that teachers can produce by themselves. The appropriate experimental methods and crop cultivars were selected for high school experiments. The concept of seed germination regulated by phytochrome under the effect of red and far-red wavelength, and the suppression of seedling elongation by blue wavelength were described in the biology textbooks in Japan. However, developing countries such as Cambodia did not integrate these concepts in school curriculum yet. School curriculum reform in developing countries is generally done by referring to the curriculum of developed country. Therefore, the concept of light dependencies on seed germination and seedling elongation might be integrated in biology curriculum of developing country in the future. Through the result of pilot experimental classes by using the simple LED-attached experimental boxes, students could understand the effect of light wavelength on seed germination and seedling elongation which were described in Japanese textbooks and other articles.

Keywords

LED experimental box, seed germination, seedling elongation, experimental class, high school education

1. INTRODUCTION

Science education at general education, especially at high school level, is essential to prepare students for their further study and work. Science experiment is a method of teaching students to understand the science concepts clearly and to help them to confirm the finding of researchers. Laboratory experiences have the potential to help students attain some important learning goals such as mastery of science subject matter, increasing interest in science, and development of scientific reasoning skills (Singer *et al.*, 2005). However, in developing countries such as Cambodia, laboratories have not existed in all high schools yet. In Cambodia, science experiments were introduced to high school teachers by Japanese

International Cooperation Agency (JICA) under the project called Secondary School Teacher Training Project in Science and Mathematics (STEPSAM), collaborated with the Ministry of Education Youth and Sports (MoEYS) of Cambodia from 2000 – 2004 (Seng *et al.*, 2006). In that project, science experimental teaching methods were introduced in a classroom without using a laboratory. Simple teaching materials produced by the project and other materials those are easy to get in the local areas or markets were used for science experimental teaching in classrooms in all senior high schools in Cambodia during the project period, 2000 - 2004.

The content of plant growth and development is very important to be understood survival strategy of

plants in ecology and physiological phenomena to apply for agriculture by students. Especially, the cause-effect relation between light and plant growth had researched and necessity of light for plant growth is well known not only by farmer but also general people. Moreover, an integration of Physics content in Biological study were introduced partially in developed countries such as Japan integrated the effects of red and far-red light on phytochrome response to seed germination in biological textbook of senior high school (Akasaka *et al.*, 2014, Agata *et al.*, 2015, Baba *et al.*, 2015 & Asashima *et al.*, 2018), but developing countries such as Cambodia has not integrated this concept yet. However, school curriculum reforms in developing countries are generally used school curriculum of developed countries as references. In this study, we aim to introduce the integration of physics content “light wavelength” in biological study “seed germination and seedling elongation”, and methods of producing simple experimental apparatus to teach science which are applicable for high school in developing countries.

Different light wavelengths have different effects on plant growth and development. Blue (B) light is important not only in chloroplast development, but also in regulating plant growth by the functions of Cryptochrome and Phototropin, B light receptors (Lin *et al.*, 1998 and Chen *et al.*, 2014). B light inhibited elongation of cucumber hypocotyls (Spalding & Cosgrove, 1988), growth of Arabidopsis seedlings (Folta, 2004). There were no reports described the effect of orange (O) light on seed germination and seedling elongation.

Photoblastic-lettuce seed germination was promoted by red (R) wavelength and inhibited by far-red (FR) wavelength, and there were reversible effects of R and FR on the germination (Borthwick *et al.*, 1952). Phytochrome, a receptor for R and FR light, regulated germination of lettuce seeds and these receptors function acted alterations in the levels of gibberellin (GA) which induces seed germination, and abscisic acid (ABA) which inhibits germination (Seo *et al.*, 2006 & Sawada *et al.*, 2008). Some researchers have researched on phytochrome reaction on seed germination, but almost all researches found irregular reaction of phytochrome in seed germination at dark or FR (Borthwick *et al.*, 1952 & Jomori *et al.*, 2010). Since Choi and Takahashi (1979) had researched

photosensitivity using both photoblastic cultivars and non-photoblastic cultivar, the presence of non-photoblastic cultivars might be known among lettuce cultivars. Selecting lettuce cultivars with complete inhibition by phytochrome reaction are very important for class experiment of phytochrome reaction in order to avoid student confusing. However, there are no researches to find complete photoblastic cultivar or these inhibiting condition until now.

Seeds used in agriculture is convenience for using experimental class because biological teacher can get stable at garden shop. Some home pages of agriculture, Ex. Sakata seeds co Ltd., were written that carrot is photosensitive species. Although there are some researches on the effect of temperature on carrot seed germination (Pereira *et al.*, 2008, Nascimento *et al.*, 2008, and Dias *et al.*, 2015), the reports showed the effect of light wavelength on carrot seed germination and seedling elongation could not be found. We preliminary researched on many species using for agriculture but the most of all species had not shown clear photoblastic reaction. In this study, we produced LED irradiating handmade equipment to search how to use this equipment. After set up, we got data in experimental class of photosensitive reaction followed by description of text book in high school in Japan.

2. MATERIALS AND METHODS

2.1. LED-box Development and Wavelength

Analysis

Six different Light Emitting Diode (LED) bulbs of diameter 5 mm including W, B, G, O, R, and FR were used in this study. LED-attached experimental boxes were produced manually with designing simple LED circuit based on the size of the boxes by using electrical wire to connect from one LED to another LED as series and then connect to the electrical supplier. Only one resistor of 54Ω was used in this LED circuit (Figure 1a). Black box with width of 190mm, length of 260mm and the height of 115mm with low price of 108-yen each were used as experimental containers. Box covers were holed for 11-LED holders and LED bulbs were arranged in 3 rows with the distance from one LED bulb to another approximately 6cm (Figure 1b). The decided number of LED bulbs and their arrangement is to make the light irradiating in the whole boxes. Paper board was folded to make the outer cover of LED circuit on the

experimental box. Aluminum foil was stuck inside the box in order to make light reflexing and to prevent outside light into the box. Figure 1c is a complete

LED-attached experimental box ready to be used. Six LED boxes were connected to one adaptor of DC 6V, 2A from AC100-240V.



Figure 1: LED experimental box development, **a.** LED circuit in the experimental box composed of 11 LEDs with connecting to 54Ω resistor and the electrical wire for connecting to electrical supplier, **b.** the arrangement of LED bulbs on the cover of the experimental box, and **c.** outside view of experimental box

Table 1: Light features in each experimental box (analyzed by LA-105)

Light features	White LED	Blue LED	Green LED	Orange LED	Red LED	Far Red LED
LUX (lx)	1338	768	5710	251	2662	10.9
Lambda D (nm)	485	477	527	607	618	690
Lambda P (nm)	454	474	520	612	626	732
PFD-FR (700-780nm)	0.4252	0.0322	0.2038	0.024	0.4319	15.9
PFD-R (600-700nm)	4.02	0.0265	0.3736	3.22	56.8	0.607
PFD-G (500-600nm)	9.03	2.10	47.9	0.6226	2.06	0.0535
PFD-B (400-500nm)	6.23	27.9	3.37	0.0176	0.2643	0.0535
PFD-UV (380-400nm)	0.0195	0.0285	0.0431	0.0051	0.0707	0.0139

Light analyzer LA-105 (NK-system, JAPAN) was used to measure the different LED light colors in the experimental boxes. In the measurement, the light illuminance (LUX) is high in W LED, more than 120 times compared with FR LED, and also much higher than other LED-light colors (Table 1). The information of each LED wavelength was not attached in each package so the information of each wavelength such as dominant wavelength (Lambda D) and peak wavelength (Lambda P) were measured by the LA-105. LED wavelengths increase orderly from W, B, G, O, R, and FR-LED lights. All LED wavelengths were confirmed by spectrum analysis by LA-105 and W LED had not only B, G and R spectrum, but also other spectrum with a dominant of blue spectrum (Table 1). Photon Flux Density (PFD) were divided among FR, R, G, B, and ultra violet (UV) and consisted in each LED light sources (Table 1).

2.2 Experimental Methods

Ten-carrot cultivars and ten-lettuce cultivars were used in these experiments (Table 2a and Table 2b). On

the packages of all cultivars indicated germination rate from 55% to over 85%. These seeds were purchased from garden shops in Japan. Petri-dishes of 5.5 cm diameter were used as culture containers and were layered the bottom with six layers of paper towel containing 50% fibers which is widely used in Japan. This paper towel is easy to absorb water. Ten seeds of each cultivar were placed on the dry paper towel in each petri-dish. Ten petri-dishes with 10-different cultivars were set in each LED experimental box. Fill approximately 5 ml of tap water in each petri-dish and then the imbibed seeds were irradiated by each color LEDs continuously until the date of result checking. In our preliminary study, there was no difference of germination rate between supplying water in completely dark condition by putting a tube of 2ml water on petri-dish and then shake the box to release water on the seed in the petri dish, and the supply of water to the seeds on petri-dish with opening the experiment box and then close the box cover immediately. So, water supply method were selected the latter one. The experimental boxes was kept in

room temperature around 24°C (measured by electrical thermometer placed outside the experimental boxes) during the day time and the temperature in the room is expected not to change a lot at night time because it was controlled by air conditioner for plant growth. Seed germination was checked in 5 days and 3 days after the irradiation started for carrot and lettuce respectively. The germination check was done under room light condition by opening one experimental box for a time, and the number of seed germination in each petri-dish was counted immediately. The petri-dishes with seedlings were placed back to the same LED-box after counting seed germination in all petri-dishes in one box, and the seedlings were irradiated with the same light wavelength continuously until the seedling elongation check in 10 days from seed sowing for both carrot and lettuce cultivars. The seedling-stem segment between roots and leaves were measured with a ruler. Since the scope of this study is focused on seedlings elongation, not seedling growth so the size of leaves and the length of roots were not measured. The experiments on phytochrome control on seed germination of photoblastic-lettuce seed germination were done by irradiating a period of R or FR, and then kept the treated seeds in dark condition. However, we do not focus only photoblastic seed germination, we want to confirm the strong effect of many light wavelengths on many lettuce and carrot cultivars so we decided to irradiate light continuously. The experiments were confirmed for 3 times for each cultivar. Therefore, 30 seeds of each cultivar were used in this experiment. Statistical significances were analyzed by free software Real Statistics Using Excel (Charles Zaionts) or free software R Console and one factor ANOVA followed up option Turkey HSD with $p\text{-value} < 0.05$.

2.3 Performance Practice Check

LED-attached boxes and experimental methods about the effect of different light wavelengths on seed germination were piloted with senior high school students in Japan and senior high school teacher trainees in Cambodia. The detailed processes will be explained in the following part of the application to classroom.

3. RESULT AND DISCUSSION

3.1. Seed Germination Experiment

Seeds of carrot and lettuce cultivars did not need light to induce germination because seeds of all cultivars germinated in dark condition except Fururu lettuce as 0% (Table 2a and Table 2b). Fururu lettuce is a photoblastic-lettuce cultivar in this experiment. Referring to photoblastic germination phenomenon, our experimental apparatus and methods could inhibit seed germination in dark condition completely (Table 2b), but seed germination rate in dark condition was still high in previous researches, 8.5% (Borthwick *et al.*, 1952) and 29% (Jomori *et al.*, 2010). All wavelengths had similar effect on carrot and lettuce seed germination, except the lettuce cultivars in FR light (Table 2a and Table 2b). Carrot seed germination rate was slightly inhibited by B, especially on 'Aikou Tokinashi 5 sun' (Table 2a). However, the germination rate of lettuce cultivars in B irradiation was not suppressed in comparing to other wavelengths, except for Fururu lettuce (Table 2b). Seed germination of Fururu lettuce was strongly suppressed by the B light irradiation, only 37% germinated comparing to other treatments with germination rate from 73 to 100% (Table 2b). Since each one cultivar of carrot and lettuce cultivars were suppressed to germinate by the B light irradiation, the B light might have effects to suppress seed germination of specific genotype in some of crops. Wareing and Black (1958) also indicated that B inhibited light-sensitive lettuce seed germination.

It was known that seeds of lettuce, carrot and so on favored light irradiation for germination (Sadhu, 1989). The R light irradiation promoted seed germination comparing to FR light for both carrot and lettuce seed germination (Table 2a and 2b). It was reported R light promotes seed germination and FR inhibits seed germination of lettuce mediated by Phytochrome (Kendrick & Russell, 1975). The irradiation of FR continuously could inhibit lettuce-seed germination, but could not inhibit carrot-seed germination (Table 2a and 2b).

Different light wavelengths have different effect on lettuce-seed germination. It is clear that FR inhibited lettuce seed germination, and one lettuce cultivar "Fururu lettuce" did not germinate in dark condition. Jomori *et al.* (2008 and 2010) performed

Table 2a: Average seed-germination rate of carrot cultivars under continuously irradiation of different light wavelengths (5 days after seed sowing)

Cultivar Name ¹	Germination rate (%) in different light wavelength ²						
	D	W	B	G	O	R	FR
Kokubu senkouonaga (Takii)	83	77	80	83	73	77	60
Grand prix (Takii)	80	67	73	87	83	83	73
Yellow Harmony (Marutane) ²	33	63	57	63	73	77	63
AikouToki nashi 5sun (Aisan)	83	77	37	73	90	73	70
Kuroda 5sun (Fukukaen)	73	77	73	87	80	80	77
Chihama 5sun (Asahi)	77	77	73	73	77	77	70
Yō mei 5 sun (Takii)	70	73	63	87	83	80	80
Kō shoku 5sun 2gou (Asahi)	57	43	70	63	70	70	53
Kō shoku 5sun 2gou (coated) (Asahi)	73	57	47	80	63	73	60
Kōyō 2 gou (coated) (Asahi)	70	73	63	87	83	80	80
Average germination rate of all cultivars	70	68	64	78	78	77	69

¹Cultivar name in Japanese and the name in bracket next to cultivar name is the name of seed-production company

²Light treatment: D (dark), W (white), B (blue), G (green), O (orange), R (red), FR (far-red)

lettuce seed germination tests among 12 cultivars under several LED irradiating conditions and reported that only two cultivars, “Fururu lettuce” and “Green oak” kept nature of light sensitivity and B wavelength tended to inhibit lettuce seed germination. Between results of Jomori *et al.* (2008 and 2010) and our results, different germination rate of “Fururu lettuce” were found, and both germination rate in R and inhibition rate in FR or dark of our experiments were better than their results. To perform experimental classes, clear reaction is very important to avoid misunderstanding of students. Since not only Jomori’s results (2008 and 2010) but also Borthwick’s results (1958) were detected germinate-able seeds in both dark and FR irradiated condition, it was considered that the phytochrome reaction was difficult to introduce for experimental class of high school. However, since our equipment was achieved almost 0% germination rate in dark and FR condition, the phytochrome experiment should be considered to introduce to high school experimental class by using our handmade equipment.

Table 2b: Average seed-germination rate of lettuce cultivars under continuously irradiation of different light wavelengths (3 days after seed sowing)

Cultivar Name ¹	Germination rate (%) in different light wavelength ²						
	D	W	B	G	O	R	FR
Fururu lettuce (Sakata)	0	93	37	97	100	97	0
Green leaf lettuce (Sakata)	77	97	90	97	93	93	3
Income lettuce (Mikado)	73	100	100	97	93	90	0
Sunny lettuce (Sakata)	97	100	93	97	93	97	0
Vitamin- lettuce (Aisan Syubyo)	100	97	97	97	97	100	0
Miniko lettuce (Yamato Farm)	70	100	100	100	97	97	0
Otegaru lettuce (Sakata)	100	93	100	93	90	100	0
Romain lettuce (Sakata)	93	97	97	90	97	90	0
Red-sun-star lettuce (Asahi Farm)	80	90	97	90	97	80	0
Red fire lettuce (Takii)	77	93	93	97	93	93	0
Average germination rate of all cultivars	77	96	90	95	95	94	0

3.2. Seedling Elongation Experiment

Light is an important factor for plant growth. In this experiment, different wavelengths affected on seedling elongation of both carrot and lettuce cultivars. Seedlings of both species in dark condition elongated the longest, and then following by in O, R, G and G, O, FR, R for carrot and lettuce cultivars respectively. Seedlings elongation of both carrots and lettuces were inhibited the most by B irradiation, and then the seedlings elongation increased orderly in FR, W and W, R for carrots and lettuces respectively (Figure 2 & Figure 3 and Table 3 & Table 4). The seedlings did not elongate longer in W wavelength might be caused by PFD-B dominant in W light (Table 1) and W light with the supplemented of B increases net photosynthetic rate in plants (Chen *et al.*, 2017). B light wavelength had strong inhibition on Fururu lettuce seedling elongation (Figure 3 & Figure 4 and Table 4) which are the same as the effect of B on seedling elongation of other cultivars disseminated by previous researchers (Spalding & Cosgrove, 1988, Folta, 2004, and Hui *et al.*, 2017). G light did not inhibit elongation of carrot and lettuce seedlings (Table 3 & Table 4) and this result confirmed the previous researches that G light at 531nm did not inhibit the lettuce hypocotyl and stem elongation (Steinitz *et al.*, 1985, and Park & Runkle, 2018).

Table 3: Average length of carrot seedlings under continuously irradiation of different light wavelengths as centimeter (cm) at 10 days after seed sowing

Cultivar Name ¹	Average seedling length (cm) in different light wavelength						
	Dark	White	Blue	Green	Orange	Red	Far-red
Kokubu senkouoonaga (Takii)	6.1±2.0 a ²	3.8±1.2 bc	3.7±1.1 b	4.7±1.3 bce	5.0±1.4 acd	5.1±1.5 ae	3.9±1.0 bd
Grand prix (Takii)	6.8±1.9 a	3.7±1.4 be	3.2±0.8 b	4.8±1.3 de	5.1±1.3 cd	5.3±1.3 cd	3.9±1.3 be
Yellow Harmony (Marutane)	2.9±1.0 abc	3.0±0.7 abc	2.6±0.8 abc	3.2±1.0 b	3.2±1.3 b	2.9±1.4 ab	2.0±1.0 ac
Aikou Toki nashi 5sun (Aisan)	6.0±1.9 a	3.9±0.8 bd	2.6±0.9 b	4.7±1.9 cde	5.5±1.2 ae	4.2±1.9 cd	4.0±1.5 bcd
Kuroda 5sun (Fukukaen)	4.4±1.6 a	2.8±1.3 bc	2.1±0.9 b	4.5±1.3 a	4.2±1.7 a	4.0±1.3 a	3.3±1.4 ac
Chihama 5sun (Asahi)	4.7±1.4 ad	4.0±1.1 bc	3.2±0.9 ab	4.9±1.3 cd	4.9±1.3 cd	4.9±1.7 cd	3.3±1.1 b
Yō mei 5 sun (Takii)	6.4±1.7 ad	4.3±1.5 bc	3.4±1.2 b	5.3±1.3 ce	5.8±1.4 de	5.8±0.9 de	4.0±1.1 b
Kō shoku 5sun 2gou (Asahi)	5.2±1.7 acd	3.9±1.3 a	3.8±1.0 ac	4.5±2.2 acd	5.3±2.1 acd	5.7±1.4 bd	4.2±1.5 acd
Kō shoku 5sun 2gou (coated) (Asahi)	4.8±2.3 a	3.8±1.2 ac	3.3±1.0 bc	4.8±1.3 ac	4.6±1.8	5.1±1.8 a	3.7±0.8 ac
Kōyō 2 gou (coated) (Asahi)	6.3±2.0 a	4.8±1.1 b	4.3±0.9 b	6.3±1.2 a	6.6±1.6 a	6.5±1.4 a	4.3±1.4 cb
Average seedling length of all cultivars	5.4±2.1 a	3.8±1.3 b	3.2±1.4 ce	4.8±1.6 d	5.1±1.7 d	5.0±1.8 d	3.7±1.4 be

¹Cultivar name in Japanese and the name in bracket next to cultivar name is the name of seed-production company

²It was shown Mean ±STD, different letters within the row indicate the significant differences of all cultivars by Real Statistics Using Excel (Charles Zaiontz) One Factor Anova follow-up option Turkey HSD p-value < 0.05

Table 4: Average length of lettuce seedlings under continuously irradiation of different light wavelengths as centimeter (cm) at 10 days after seed sowing

Cultivar Name ¹	Average seedling length (cm) in different light wavelength						
	Dark	White	Blue	Green	Orange	Red	Far-red
Fururu lettuce (Sakata) ²	3.2±0.3 ae ³	2.2±0.6 b	0.8±0.5 c	3.4±0.8 ae	3.6±0.4 a	3.0±0.6 de	3.5±0.3 a
Green leaf lettuce (Sakata)	3.6±0.8 ae	2.5±0.8 bd	1.6±0.5 c	3.4±0.8 ae	3.4±0.6 ae	2.9±0.6 ad	3.3±0.7 e
Income lettuce (Mikado)	4.4±1.2 a	2.4±0.7 b	1.9±0.5 b	4.1±0.8 ad	3.7±0.5 cd	3.2±0.8 c	3.7±0.9 cd
Sunny lettuce (Sakata)	4.9±0.8 a	2.6±0.6 b	1.7±0.7 c	4.5±0.7 ae	3.7±0.9 d	3.3±0.4 d	3.8±0.4 de
Vitamin lettuce (Aisan Syubyo)	5.0±1.2 a	2.5±0.5 b	2.3±0.5 b	4.2±1.0 c	3.8±0.6 ce	3.3±0.6 de	3.7±0.7 ce
Miniko lettuce (Yamato Farm)	4.8±0.8 a	2.2±0.6 b	1.7±0.5 b	3.7±0.7 c	3.3±0.6 ce	3.1±0.4 de	3.1±0.6 d
Otegaru lettuce (Sakata)	5.4±1.3 a	2.5±0.7 b	1.8±0.6 c	4.5±0.8 dg	4.1±1.2 eg	3.9±0.5 fg	3.5±0.9 ef
Romain lettuce (Sakata)	4.9±0.8 a	2.4±0.6 b	2.0±0.6 b	3.9±0.9 c	3.8±0.5 c	3.4±0.6 c	3.8±0.8 c
Red-sun-star lettuce (Asahi Farm)	4.4±1.3 a	2.9±0.7 b	1.9±1.0 c	4.3±0.9 a	3.8±0.6 ad	3.2±1.0 bd	3.4±1.1 bd
Red fire lettuce (Takii)	4.9±1.0 a	2.4±0.9 b	1.9±0.6 b	4.4±0.7 ad	3.8±0.6 cd	3.3±0.3 c	3.5±1.0 c
Average seedling length of all cultivars	4.5±1.2 a	2.4±0.7 b	1.8±0.7 c	4.0±0.9 d	3.7±0.7 eh	3.2±0.7 f	3.5±0.8 gh

¹Cultivar name in Japanese and the name in bracket next to cultivar name is the name of seed-production company

²Seed germinated under room-light condition and kept for 3 days from sowing (average seedlings about 1cm) were kept in dark box for a week

³It was shown Mean ±STD, different letters within the row indicate the significant differences of all cultivars by Real Statistics Using Excel (Charles Zaiontz) One Factor Anova follow-up option Turkey HSD p-value < 0.05

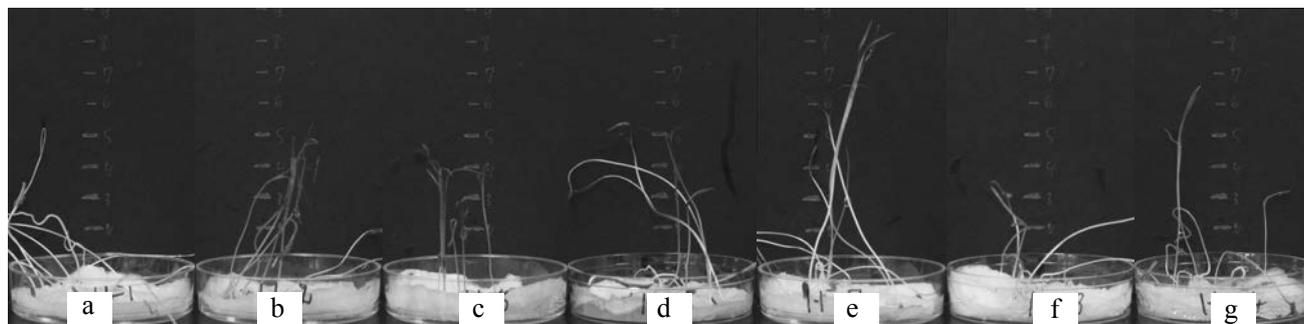


Figure 2: Carrot seedlings, Aikou Toki nashi 5 sun, at 10 days after seed sowing in different light condition: a. dark, b. white, c. blue, d. green, e. orange, f. red, and g. far-red LED light.

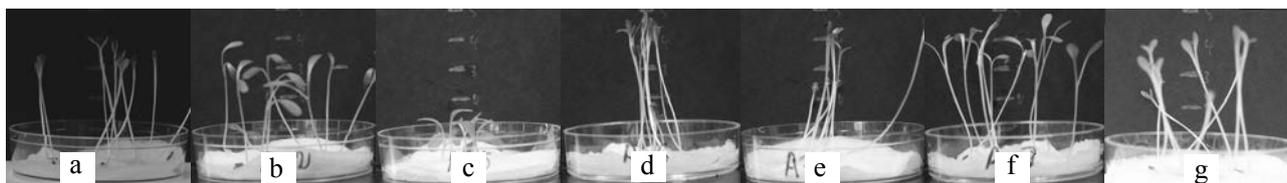


Figure 3: Fururu lettuce seedlings in 10 days after seed sowing in different light condition: a. dark, b. white, c. blue, d. green, e. orange, f. red, and g. far-red LED light

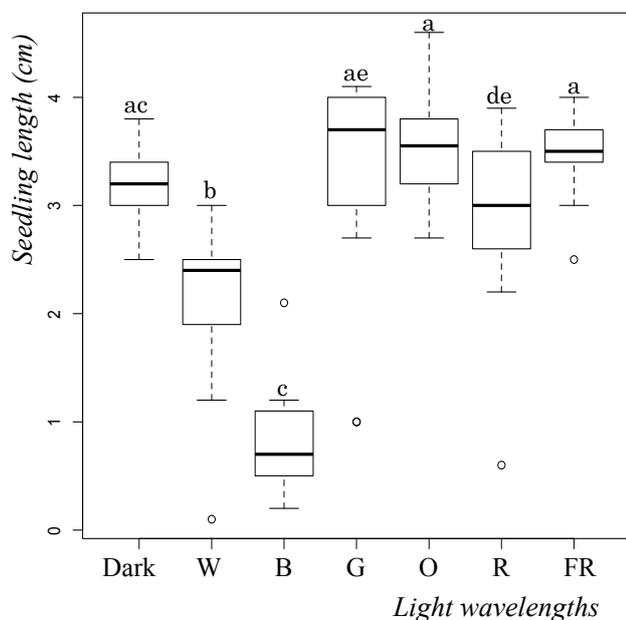


Figure 4: Average seedling length of Fururu lettuce for 3-time experiments analyzed by R Console. The different letters on bars indicate significant differences with p -value < 0.05

For statically analysis, there are significant differences of seedling elongation under the irradiation of different light wavelengths. The result of our experiments on the effect of different wavelengths on seedling elongation could confirm the finding by previous researchers and the result also showed the effects of some other wavelengths on seedling elongation which were not described by previous researchers.

4. APPLICATION TO CLASSROOM

We used different teaching methods for piloting in Japan and Cambodia. Students and teacher trainees were asked to complete questionnaires at the end of the lesson. Questionnaires composted of two kinds of questions, multiple-choice questions to evaluate their understanding or impression, and questions required for them to describe or explain. Multiple-choice questions were scored from 1 (the lowest) to 4 (the highest).

In Japan

Smaller type of LED-attached box made of kitchen canister of 430ml (8cm³), and 5 R-LEDs and 5 FR-LEDs in each box was used to conduct experiment with 2^{yr} as 11th grade students at affiliated senior high school to Aichi University of Education in 2019. The purpose of the experiment was to make students understanding of survival strategy under trees condition described in the text book. We used 10 kinds of seed cultivars, 3 lettuces, 4 radishes, 1 celery, 1 edible chrysanthemum, and 1 mizuna. Carrot cultivar was not included because its germination length longer than 3 days. Two experimental class periods of 50 min were used in the trial experimental lesson. The first period, students conducted experiment by using 20 seeds of each cultivar to place on paper towel prepared in a petri-dish, put the prepared petri-dish in the LED-attached box, put a tube containing of 2 ml of tap water in the petri-dish, closed the box cover completely, shook the box to pour the water from the tube, irradiated the first light, FR or R, continuously for 10 min, and then changed to second light, R or FR, for 10min respectively before keeping the treated seeds in the dark condition in the box. The experimental settings were kept in room temperature around 25⁰C in July for 3 days until the result checking in second period of the experimental class. The experimental result showed that seeds of 9 cultivars of crops germinated in R light-end or FR light-end, except one lettuce cultivar “Fururu lettuce” did not germinate in FR light-end. Students were asked to fill in pre-lesson questionnaires at the beginning of the first experimental period and post-lesson questionnaires at the end of the second experimental period.

At the end of the experimental classes, students have changed their understating related to following: (1) wavelength concept, the light color of rainbow are composted of different light wavelengths, average score 2/4 for pre-lesson and average score 3/4 for post-

lesson questionnaire, (2) necessary elements (water, temperature, oxygen) required for seeds to germinate, pre-lesson 2/4 and post-lesson 3/4, and (3) the effect of light on seed germination, pre-lesson:1/4 and post-lesson:3/4. The most of 3 knowledge that students received from the lesson are related to (1) seed germination is controlled by different kinds of light, (2) R wavelength promotes seed germination, and (3) FR wavelength suppresses seed germination. These are the main knowledge students were expected to receive through this experimental class. They understood throughout the lesson, and the lesson was interesting for them (3/4). However, students have raised up some points that they did not understand in lesson such as germination phenomenon, the advantage of seeds dormancy when they are under the tree, the reason of FR has longer wavelength not promote seed germination. Even though some points were not well understood, but the students showed their good impression to the experimental classes. Students said that they learned many things from this experiment class, even though it was difficult to interpret the experiment result. They could understand the relation between light and seed germination through this small experiment. In general, students said that this kind of experimental class is fun and very interesting. One student said that “I am glade that the experiment was easier to understand and more enjoyable than reading the textbook”. Last but not least, students evaluated that the experimental boxes they used can helps them to understand the relation between light and seed germination (3/4) and one student said that he/she wanted to make this experimental box.

In Cambodia

We also introduced the methods of teaching experiment on the effect of light on seed germination and seedling growth to teacher trainees at National Institute of Education in Cambodia in January 2018. The purpose of this pilot class was to introduce new biological content “regulation of phytochrome on lettuce seed germination” and the LED-attached box for conducting this experiment. Two periods of class lecturing were used in this pilot teaching. The first period, trainees were asked to discuss about light source for conducting experiment and the methods to produce experimental boxes. In this pilot teaching, we

used the same experimental boxes as those used in affiliated senior high school in Japan. But at this time, 7 experimental boxes were used including dark box, W, B, G, O, R, and FR LED-attached boxes. In these experiments, we used only one kind of lettuce cultivar exported from Japan “Fururu lettuce”. Twenty lettuce seeds were placed on dry paper and then fill approximately 5 ml of water in each box. The imbibed seeds were irradiated continuously with different light wavelength for one week until the date of checking the experimental results. The results of seed germination were the same as those in Table 2b that Fururu lettuce did not germinate in dark and FR irradiating condition.

Through questionnaires, the trainees said that they could understand this science lesson well (3/4) and it was very interesting for them (4/4). They could get new knowledge and the ways of thinking from this science lesson well (3/4) and they thought that these LED-attached experimental boxes are useful for biology experimental classes in Cambodia (3/4) from junior high school to university level in the chapter of plant growth and response, and photosynthesis. Teacher trainees did not think to use this experimental box for seed germination or phytochrome control on seed germination because this concept is not yet integrated in biology curriculum in Cambodia. Trainees evaluated that they are not sure to be able to produce these experimental boxes by themselves (2/4).

5. CONCLUSION

Through the result of our experiment, we could evaluate that our experimental apparatus and methods could be used to confirm the research result of previous researchers and they are simple to be applied to school experimental class. Therefore, the use of simple LED-attached experimental boxes to teach wavelength dependencies on seed germination and seedling elongation is one of the methods of teaching science experiments at senior high school in developing countries when this concept is integrated in biology curriculum or it is useful to teach students as extra curriculum activities. Even though, industrial plant growth chambers or experimental apparatus attached with LED bulbs are available, but they are too expensive to equip to senior high schools in developing countries. Teachers are able to produce these experimental boxes by themselves with different sizes and purposes of using by following the LED

diagram as in Figure 1a. The LED-attached boxes used for piloting in both Japan and Cambodia are smaller than the experimental box used for conducting experiment in this article. However, the result of the experiment is not based on the size of the experimental box, it depends on the light wavelength irradiating in the boxes. On the other hand, the selection of plant materials is another essential factor to achieve lesson objective. Lettuce seeds, especially Fururu lettuce seeds, are good plant material for this study. Teachers in developing countries should search for good lettuce cultivars in their areas for their experimental class. However, it might be difficult to select photoblastic seeds from commercial crops because high germination rate seeds are good for commerce.

In this study, two phenomena depended on different wavelengths, seed germination and seedling elongation, were targeted. Since Fururu lettuce seed, which is complete photoblastic, did not germinate in dark or FR irradiation condition in our handmade equipment, we performed trial of seed germination in high school experimental class. Although student could observe a cultivar, 'Fururu lettuce' did not germinate in dark or in last irradiation of FR, these observations could not be connected to the presence and characters of phytochrome in student mind directly. Seedling elongation results also could not be connected the presence of receptors in student mind. Further researches are needed in order to teach the presence of these receptors in experimental class.

References

- Chen X., Guo W., Xue X., Wang L., and Qiao X., Growth and quality responses of Green Oak Leaf lettuce as affected by monochromic or mixed radiation provided by fluorescent lamp (FL) and light-emitting diode (LED), *Scientia Horticulture* Vol. 172, 2014, pp. 168-175
- Chen X., Yang Q., Song W., Wang L., and Guo W., Growth and nutrient properties of lettuce affected by different alternating intervals of red and blue LED irradiation, *Scientia Horticulture* Vol. 223, 2017, pp. 44-52
- Choi K., and Takahashi, N., Studies on the lettuce seed germination with special reference to light response (English Summary). *Japan J. Breed.* Vol. 29, 1979, pp.197-204
- Dias M. A., Lopes J. C., Guin G. A. M., Neto J. D. S., and Bernardes C. O., Carrot seed germination in different conditions of salinity and temperature, *IDESIA (Chile)* Vol. 33, 2015, pp. 41-46
- Folta K. M., Green light stimulates early stem elongation, antagonizing light-mediated growth inhibition, *Plant physiology* Vol. 135, 2004, pp. 1407-1416
- Hui X., Yan-nan F., Tian-lai L., and Rui W., Effects of different LED light wavelengths on the resistance of tomato against *Botrytis cinerea* and the corresponding physiological mechanisms, *Journal of integrative agriculture* Vol. 16 (1), 2017, pp. 106-114
- Jomori H., Kanazawa T., Yui S., Takamura S. and Takamuta Y., Effect of LED photosensitivity on lettuce seed germination. *Hort. Res.*, 7 suppl. 1, 2008, pp 182 (in Japanese)
- Jomori, H., Kanazawa, T., Akutsu, K., Kikuchi, S. and Fujita, T., Effect of LED light irradiation on lettuce seed germination. *Hort. Res.*, 9 suppl. 1, 2010, pp 178 (in Japanese)
- Kendrick R. E., and Russell J. H., Photomanipulation of phytochrome in lettuce seeds, *Plant physiology* Vol. 56, 1975, pp. 332 - 334
- Lin C., Yang H., Guo H., Mockler T., and Chen J., Enhancement of blue-light sensitivity of *Arabidopsis* seedlings by blue light receptor cryptochrome 2, *Proc. Natl. Acad. Sci. USA*, Vol. 95, 1998, pp. 2686-2690
- Nascimento W. M., Viera J. V., Silva G. O., Carrot seed germination at high temperature: Effect of genotype and association with ethylene production, *Hort. Science* Vol. 43, pp. 1538-1543
- Park Y., and Runkle E. S., Spectral effects of light-emitting diodes on plant growth, visual color quality, and photosynthetic photon efficacy: White versus blue plus red radiation, *PLOS ONE* <https://doi.org/10.1371/journal.pone.0202236>, 2018, pp.1-14
- Pereira R. S., Nascimento W. M., and Vieira J. V., Carrot seed germination and vigor in response to temperature and umbel orders, *Sci. Agric. (Piracicaba, Braz.)*, Vol. 65, 2008, pp. 145-150
- Sadhu M. K., Light in plant propagation, New age international publisher, New Delhi, India, 1989, pp. 65-66
- Sawada Y., Aoki M., Nakaminami K., Mitsunashi W., Tatematsu K., Koshiro T., Koshib T., Kamiya Y., Inoue Y., Nambara E., and Toyomasu T.,

- Phytochrome- and gibberellin-mediated regulation of abscisic acid metabolism during germination of photoblastic lettuce seeds, *Plant physiology* Vol. 146, 2008, pp. 1386 – 1396
- Seo M., Hanada A., Kuwahara A., Endo A., Okamoto M., Yamauchi Y., North H., Marion-Poll A., Sun T. P., Koshiha T., Kamiya Y., Yamaguchi S., and Nambara E., Regulation of hormone metabolism in *Arabidopsis* seeds: phytochrome regulation of abscisic acid metabolism and abscisic acid regulation of gibberellin metabolism, *The plant journal* Vol. 48, 2006, pp. 354 – 366
- Seng S., Miyoshi M., and Kita M., Development of teaching materials for upper secondary school: study lesson on detergent concept in Cambodia, *Bulletin of the center for educational practice, Okayama University* Vol. 6, 2006. pp. 11-20
- Singer, S. R., Hilton, M. L., Schweingruber, and Heidi A., Investigations in high school science, *America's lab report, National academic of science*, 2005, pp. 254
- Spalding E. P., and Cosgrove D. J., Large plasma-membrane depolarization precedes rapid blue-light-induced growth inhibition in cucumber, *Planta* Vol. 178, 1988, pp. 407-410
- Steinitz B., Ren Z., and Poff K., Blue and green light-induced phototropism in *Arabidopsis thaliana* and *Lactuca sativa* L. seedlings, *Plant physiology* Vol. 77, 1985, pp. 248-251
- Wareing P.F. and Black M., Similar effects of blue and infra-red radiation on light-sensitive seeds, *Nature Publishing Group* No.4620, 1958, pp.1420-1421
- 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) Shinkoshuppansha 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) Jikkyo-Shuppan Co. Ltd, pp. 240-241

【論文】

発展途上国の高等学校の実験授業に適応可能な種子発芽と苗の伸長に対する光の波長依存性を教える方法

チャンセン マム¹ ・ 野田 陽平² ・ 船井 裕由² ・ 岩山 勉³ ・ 加藤 淳太郎³¹愛知教育大学大学院・静岡大学大学院教育学研究科共同教科開発学専攻²愛知教育大学附属高等学校³愛知教育大学理科教育講座

要約

発展途上国の理科教育の質を高めるために、実験の授業における簡便な教育方法について検討した。生徒は、教科書を中心とした授業よりも実験を実施する授業を好む傾向が見られるが、カンボジアを含む発展途上国では、高等学校において予算的な制約から十分な実験装置を購入できず、理科の教育方法として実験を行うことが極めて困難である。本研究では、教師が自分自身で作製可能な簡易な LED 付き実験ボックスを用いる種子発芽と苗の伸長に対する光の波長依存性に関する教育方法を提案する。本研究では、高等学校で実験を行うために適した実験条件および品種の選択を行った。赤色および遠赤色波長の影響下でフィトクロムによる種子発芽の調節、および青色波長による苗の伸長抑制は、日本の高等学校の生物学の教科書には記載されているが、カンボジアなどの発展途上国の教科書にはこれらの内容の記載は含まれていない。発展途上国の高等学校のカリキュラム改革は、一般的に先進国のカリキュラムを参照することにより行われる。したがって、種子の発芽と苗の伸長に対する光の波長依存性の内容は、将来的に発展途上国の生物学カリキュラムに含まれてくる可能性が高い。簡易な LED 付き実験ボックスを使用した授業実践の結果により、生徒が日本の教科書などに記載されている種子の発芽と苗の伸長に対する光の波長依存性を理解するために、このボックスは有効であることが明らかになった。

キーワード

LED 実験ボックス、種子発芽、苗の伸長、実験授業、高等学校教育