Aichi University of Education & Shizuoka University (Japan)

Doctoral Thesis

カンボジアの生物教育における物理的アプローチによる教材開発
Development of Teaching Materials through Integration of Physical Approach for Biology Education in Cambodia

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ABSTRACT

This doctoral dissertation aims (1) to produce biological experimental apparatus with the integration of physical approaches and other teaching materials related to biological contents, and (2) to use experiments as a method to teach biology in high school level.

This dissertation consisted of 9 chapters which Chapter 1 described the situation of biological education in Cambodia, the research purposes, the research methodology, and so on. Chapter 2 described the school quality assurance framework and the main problem of science education in Cambodia; and the following chapters address the problem described in chapter 1 and chapter 2.

Chapter 3 described the methods to select plant seeds for biological experiments. The seeds of 25 lettuce cultivars, 18 carrot cultivars, 5 radish cultivars, and 11 other vegetative cultivars were searched for their germination phenomenon in the dark and far-red wavelength irradiation. Carrot and lettuce cultivars were selected for the experiments, and especially "Furiru lettuce" is only photoblastic seed germination cultivar.

Chapter 4 titled "Methods for teaching light wavelength dependencies on seed germination and seedling elongation applicable for high school experimental class in developing countries" aims to introduce the methods to produce light emitting diode (LED) attached-experimental apparatus, and the experimental methods using this apparatus. An experimental box (190mm(W) x 260mm(L) x 115mm(H)) attached with 11 LED bulbs of the same wavelength was introduced. Each box was attached with white LED (major peak: 485nm), blue LED (477nm), green LED (527nm), orange LED (607nm), red LED (618nm), and far-red LED (690nm). Carrot and lettuce seeds of 10 cultivars each were used as plant materials. The result showed that the seeds of all carrot and lettuce cultivars germinated in dark conditions, except "Furiru lettuce". Continuously irradiation of far-red wavelength suppressed lettuce seed germination, but it did not suppress seed germination of all carrot cultivars. Seedlings of both carrot and lettuce cultivars elongated the longest in dark condition, but the seedling elongations were inhibited the most by blue light irradiation.

Chapter 5 titled "Development of an LED-attached box for phytochrome response experiments on lettuce seed germination in senior high school biology" aimed to introduce the methods to develop an apparatus specified for phytochrome response experiments and the experiments using this experimental apparatus. A smaller LED-attached box with the combination of red (624nm) and far-red (690nm) was developed, and Furiru lettuce seeds were used. The result showed that Furiru lettuce seeds did not germinate in dark conditions or the last irradiation of far-red light, and the high germination rates were observed in the last irradiation of red light. Gibberellic acid (GA) and Abscisic acid (ABA) solutions were treated on lettuce seed to explain to students about seed germination phenomena.

Chapter 6 titled "The use of dwarf tomato cultivar for genetic and physiology study applicable for school education" aimed to introduce the methods to conduct biological experiments in high school. GA does not affect only seed germination but also on plant physical growth. GA_3 of 1mg/L, 10mg/L and 100mg/L were applied exogenously to dwarf and normal stem tomatoes. Moreover, the dwarf type tomato was crossed with the normal type tomato. The results of the experiments showed that 100mg/L GA_3 enhanced stem elongation of both dwarf and normal type tomatoes. The result of the cross followed Mendel's 1^{st} and 2^{nd} laws of inheritance. Normal stem was the dominant trait.

Chapter 7 titled "Genetics materials for experimental class of Mendel's 3^{rd} law using dihybrid crosses of lettuce cultivars in high school" aimed to introduce experimental methods and teaching materials for Mendel's Law of Independent Assortment. In chapter 6, while dwarf tomato was used for the educational materials of Mendel's 1^{st} and 2^{nd} laws, but using tomatoes to conduct experiments to study Mendel's 3^{rd} law was difficult because a big space is needed to keep approximately 100 tomato plants. Lettuce can be cultivated in small spaces. Reciprocal crosses between 'Furiru lettuce'lettuce and 'Red sunstar', 'Sunny' or 'Vitamin' with red lobed leaf were performed. The segregation of F_2 populations was the same as the results of Mendel's dihybrid crosses with the ratio: 9: 3: 3: 1.

Chapter 8 titled "Genetics of photoblastic seed germination and seed color using lettuce cultivars for biological experiments in high school" aimed to introduce the experimental methods used to trace Mendel's law of inheritance. Furiru lettuce which is a photoblastic seed germination cultivar was crossed with 3 non-photoblastic seed germination cultivars. While the result indicated that violet flowers and black color seeds were dominant on yellow flowers and white seeds respectively, the genetics of photoblastic phenomena showed complicated inheritance.

Chapter 9 aimed to make a general discussion, conclusion and recommendation of the dissertation to contribute to development of biology education in Cambodia. The experimental apparatus developed in this study is useful for biological experiments in high schools. The experimental methods introduced in this study are suitable for the teachers and students. Teachers and relevant partners should consider developing this kind of experimental apparatus for their experimental classes in high schools.

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GENERAL INTRODUCTION

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GENERAL INTRODUCTION

1.1. BIOLOGY EDUCATION IN CAMBODIAN HIGH SCHOOL

1.1.1. Biology Textbooks and Curriculum Development

The biology curriculum as well as other subject curriculum were developed based on the special requirements of the Ministry of Education, Youth and Sports (MoEYS) and the support of international counter partners. There are no definite policies related to the time frame to reform curriculum and to renew textbooks for using in high school. The current biology textbooks used in high school were developed and authorized by MoEYS in 2007, 2008, and 2009 for 10^{th} grade, 11^{th} grade, and 12^{th} grade textbooks respectively (Yihoop *et al.*, 2007, 2008, and 2009). This biology curriculum reform was supported by the Japan International Cooperation Agency (JICA) project. The biology curriculum from 10^{th} grade to 12^{th} grade were developed with technical support of Japanese experts. The 10^{th} grade textbook writing was fully supported by Japanese experts, but 11^{th} grade and 12^{th} grade textbooks were responded by Cambodian partners without support from Japanese experts.

1.1.2. The Content in the Textbooks

Senior high school biology textbooks in Cambodia were organized for each grade (Table 1.1). The 10^{th} grade textbook included 5 chapters. Chapter 1, Diversity of Organisms, described about the classification of organisms, viruses, bacteria, fungus, plants, and animals. Chapter 2, Unity of Organisms, described about the kinds of cells and their structure. Chapter 3, Metabolism, described photosynthesis and cell respiration. Chapter 4, Human Biology, described about skeleton system and muscle

Table 1. 1: Contents in biology textbooks at senior high school in Cambodia

Grade level	Chapter number	Chapter titles
10th	1	Diversity of Organisms
	2	Unity of Organisms
	3	Metabolism
	4	Human Biology
	5	Biology for Agriculture
11th	1	The Cell
	2	Reproduction and Growth
	3	Reproduction
	4	Food and Food Digestion
	5	Gas Exchange and Excretion
	6	Interaction between Animals and Environment
	7	The Structure of Vascular Plants
	8	Biology and Human Health
12th	1	Gymnosperm and Angiosperm
	2	Plant Growth and Response
	3	Regulation of Organisms
	4	Function of Protein in Organisms
	5	Genetic Information and Gene Expression
	6	Evolution of Organisms
	7	Group and Community
	8	Ecology

system. And the last chapter, Biology for Agriculture, described about fertilizer utilization and plant breeding. (Yihoop *et al.*, 2007).

The 11th grade textbook included 8 chapters. Chapter 1, The Cell, described the chemical elements in cell, cell structure and function, and cell division. Chapter 2, Reproduction and Growth, described reproductive cell formation and embryonic development in human and animals. Chapter 3, Reproduction, described Mendel's Laws of Inheritance that applied for animals and plant reproduction, and chromosomes. Chapter 4, Food and Food Digestion, described the nutrients in food, food energy, and food digestion process in human. Chapter 5, Gas Exchange and Excretion, described respiration and the excretion in animals and humans. Chapter 6, Interaction between Animals and Environment, described about the blood circulation system and immunity, and the animal response to the environment. Chapter 7, The Structure of Vascular Plants, described about plant tissue, root, stem, and leaf. And the last chapter, Biology and Human Health, described the methods to prevent diseases and the food for health. (Yihoop *et al.*, 2008).

The 12th grade textbook included 8 chapters. Chapter 1, Gymnosperm and Angiosperm, described the characteristics of gymnosperm and angiosperm plants including their structure and life cycle. Chapter 2, Plant Growth and Response, described the processes of plant growth and development and the responses of the plants to plant growth hormones and light. Chapter 3, Regulation of Organisms, described the nerve regulation and nerve system of animals and human, human sense organs, human endocrine system. Chapter 4, Function of Protein in Organisms, described the different kinds of amino acids, protein synthesis, and enzymes. Chapter 5, Genetic Information and Gene Expression, described about DNA structure, DNA replication, gene expression to synthesize protein, and biotechnology in plants and animals crossing. Chapter 6, Evolution of Organisms, described about Darwin theory of evolution, evidence of evolution, and fossil record. Chapter 7, Group and Community, described about the development of animals in community, interaction of organisms. The last chapter, Ecology, described the energy transformation in the food chain, and the chemical element cycles. (Yihoop *et al.*, 2009).

1.1.3. Biology Teaching

MoEYS has encouraged teachers to teach biology with experimental practice in order to make students understand the biology concepts well. The use of experiment as a teaching methodology is a part of Inquiry-Based Lesson (IBL) which is the teaching method that helps students to think a lot in order to discover the lesson content by themselves. However, teachers in Cambodia might teach based on the textbooks only. They might not think to produce new experiments and experimental apparatus by themselves in accordance to the curriculum or contents in the textbooks. Therefore, there are not many experimental practices or research activities at their schools.

1.2. RATIONAL

The current biology curriculum and textbooks have been used for more than 10 years. The trends of education in the world are approaching Science Technology Engineering and Math (STEM) education. Therefore, the biology curriculum in Cambodia needs to be reformed to update and to follow the world trend of education. The teaching apparatus to serve for STEM education might be expensive to equip to schools in Cambodia. Experimental apparatus produced by teachers might be good materials to start STEM education gradually. The apparatus produced in this study can be used to conduct biology experiments which is one of the methods to teach biology by referring to STEM education. The simple experimental methods and teaching materials are also considered to raise the quality of biology education in Cambodia.

The experimental apparatus equipped with different light wavelengths were considered for biological experiments. This apparatus can be used to conduct the experiments related to chapter 2 of the 12th grade textbook. Some experiments such as the effects of light wavelengths on seed germination, seedling growth, as well as plant development can be prepared for the experimental classes. The chapter 2 of the 12^{th} grade textbook also described the response of plants to plant growth hormone so the experimental apparatus attached with different light wavelengths are used with the combination of plant hormone treatments to study seed germination. Plant growth hormones do not affect only on seed germination, they have a strong effect on plant growth, so the experiments on the effects of plant growth hormones on physiology plant growth are considered. This experimental apparatus is also used to study the inheritance of photoblastic seed germination phenomenon to trace Mendel's Law of Inheritance which included in chapter 3 of the 11^{th} grade textbook. Mendel's Laws of Inheritance are the main genetic concepts studied in high school level. Therefore, other experiments related to the study of Mendel's Laws of Inheritance are also considered. Mendel studied the seven characteristics in garden peas including seeds, pods, flowers, and stem characters (Weaver and Hedrick, 1992). Lettuce and tomato cultivars are suitable plant materials to study the characteristics of seed, flower, fruit, leaf, and stem characters to explain Mendel's Laws of Inheritance.

Therefore, the development of experimental apparatus and the creation of experimental methods were decided in my study in order to contribute to improve biology education in Cambodia to attract students learning biology and to follow the trends of science education in the world.

1.3. RESEARCH PURPOSE AND QUESTIONS

The main purposes of this study are to produce handmade experimental apparatus with the integration of light wavelength, to produce teaching materials for biology lesson, and to use experiments as a method to teach biology in high school level. In order to achieve the purpose, 3 research questions are set:

1. What kinds of experimental apparatus can be produced with the integration of physical approaches?
2. How to use these experimental apparatus to conduct biology experiments suitable for high school education?
3. What are biological experiments and experimental methods suitable for biology education in high school?

1.4. RESEARCH METHODOLOGY

The research methods were generally composed of 4 steps:

Step 1: Experimental topic determination

Both Japanese and Cambodian biology textbooks for high school levels were read in order to determine the appropriate experimental topics to teach students related to the content written in the textbooks.

Step 2: Experimental apparatus development

The experimental apparatus appropriated for the experimental topics selected in step 1 were developed through using low-cost materials. The experimental apparatus were used to conduct trial experiments several times in order to check the stable result and usability.

Step 3: Experimental method creation

The experimental methods suitable for high school experimental classes were considered. The experimental methods with the use of the apparatus developed in step 2 were considered first and then the experimental methods related to the experiments used the apparatus were also considered in order to improve biology teaching at the high school level.

Step 4: Application to school

The experimental apparatus and methods were applied in the classroom with students in order to get their perception about the experimental apparatus and the experimental methods. Students were asked to complete pre- and post-questionnaires. For the experimental topics those are not able to conduct experiments in class, the alternative application methods are considered such as to be used for extra activities outside class and so on.

1.5. LIMITATIONS

The selection of experimental topics in this doctoral dissertation focused on the biology content related to light wavelength and some others extended to genetics and physiology study. Even though this research focuses on the development of biological education in Cambodia, some experimental topics are not related to the contents in biology textbooks in Cambodia yet. I hope that these contents will be integrated in Cambodian biology textbooks in the future.

Some experimental topics were able to be piloted in classroom teaching, but some others were not able to be piloted in classroom because the experiment process required a long time to get the results.

1.6. OUTLINE OF THIS DISSERTATION

This dissertation is composed of 9 chapters structuring as following:

Chapter 1 focused on the general information about biology curriculum and textbook development as well as biology teaching in Cambodia. This introduction chapter also described the overview and the outline of the dissertation.

Chapter 2 described the overview of school quality assurance through school inspection in Cambodia. Systemic school inspection has been implemented in Cambodia since 2015. This chapter indicated the current school performance from primary to high schools. First, the finding in this research tried to explain the general school performance including: leadership and management, teaching and learning, students' results and achievements, and school self-assessment. These are the 4 components defined by MoEYS in order to ensure quality of education at school. Then, the specific school performance on science subjects were indicated. This part shows inspectors' evaluation on science teaching and students' result on science subject improvement from a few years ago. The reasons that make students not interested in science lessons and some interventions to solve the problem were also indicated in this chapter.

Chapter 3 focused on the selection of plant materials usable for experimental class at school. The length of seed germination, the germination rate in dark or far-red light conditions of crops and ornamental plants commercialized in Japan were researched. The plants which were good for genetic study are also selected.

Chapter 4 focused on the research to find the methods for teaching light wavelength dependencies on seed germination and seedling elongation applicable for high school experimental class in developing countries where the experimental apparatus are not enough. The methods to produce LED-attached experimental boxes and the methods to teach experiments in high school were introduced in this chapter. Seeds of 10 carrot cultivars and 10 lettuce cultivars were used to check their germination and seedlings elongation under dark, white, blue, green, orange, red, or far-red wavelengths. The students' perception on the experimental apparatus and the teaching methods were also shown. This chapter and the following chapters are the key factors to solve the problem found in chapter 1 and chapter 2.

Chapter 5 described the methods to develop an LED-attached box for conducting the experiment on phytochrome response to lettuce seed germination. The confirmation to phytochrome response on lettuce seed germination to red and far-red light written in Japanese biology textbook was experimented by using the LED-attached box. Gibberellic acid (GA) and Abscisic acid (ABA) solutions were treated on lettuce seed to explain to students about the seed germination phenomenon. The method to select appropriate lettuce seeds for the success of this experiment was also described. The students' perception on the experimental boxes and teaching methods, and the possibility to apply these experimental boxes for biology education in Cambodia were also shown.

Chapter 6 was the extended study from chapter 5. GA and ABA do not affect only on seed germination, but also on plant physiological growth. Different concentrations of GA were applied exogenously to dwarf and normal stem tomatoes. The effect of GA on tomato stem elongation was designed to confirm a theory written in biology textbooks of Cambodia and Japan. These tomato cultivars have different stem length "dwarf and normal" that are good for the genetic inheritance study. Therefore, the experiments to trace Mendel's Law of Dominant and Law of Segregation was also conducted by crossing between dwarf and normal tomatoes. This chapter aimed to introduce the experimental methods which are suitable for biology experimental class in high school.

Chapter 7 focused on the use of lettuce cultivars to conduct experiments to study Mendel's Law of Independent Assortment which was included in the 12^{th} grade biology textbook in Cambodia. Tomato cultivars were used in chapter 6, but it is difficult to keep many tomato plants to study the Law of Independent Assortment. Lettuce plants need small space to grow so they are usable in this study. The experimental box attached with red LED bulbs was used to enhance seed germination. The possibility to implement this experiment in biology education in high school was also proposed.

Chapter 8 focused on the inheritance of the lettuce reproductive organs including flowers and seeds. This chapter completed to study in chapter 6 and chapter 7 about Mendelian genetics. While the characteristics of flower color and seed color were studied to trace Mendel's Laws of Inheritance, seed germination phenomena showed complicated inheritance. Photoblastic lettuce was crossed between non-photoblastic lettuce cultivars. The experimental apparatus were used to conduct experiments about seed germination response in dark or light conditions in order to study the heredity of photoblastic germination seeds.

Chapter 9 was the general discussion, conclusion and recommendation chapter of this dissertation. The discussion and conclusion focused on experimental apparatus development, the use of the experimental apparatus, the use of teaching materials which were the results of the experiments in this study, and the use of experiments as the teaching methods to teach biology in Cambodia. The recommendation suggested to teachers and other relevant partners to improve biology education in Cambodia.

Chapter II

SCHOOL PERFORMANCE EVALUATION THROUGH SYSTEMIC SCHOOL INSPECTION IN CAMBODIA

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2.1. INTRODUCTION

2.1.1. Inspection History and Reform in Cambodia

School inspection is today a common and widely used method to check the quality of formal education. In Cambodia school inspection has been used since the French protectorate in the 1930's. Some primary schools were inspected annually since 1935 by one French educational officer who played a role as an inspector, and some pagoda schools were inspected by a delegated person of the King. In 1947, the school inspections were conducted by two teams, one team inspected pagoda schools and another team inspected French-Khmer educational schools. There were two ranking-category inspectors: (1) primary school inspectors who provided advice on teaching of French language, pedagogy, autonomy and punctuality, and (2) secondary inspectors who were specialized subject inspectors and provided advice on for example teaching and learning Khmer literature. This kind of school inspection was used until 1970 (MoEYS, 2015a). From 1970, the inspection work as well as education decreased gradually because of the civil war. During the regime of Pol Pot, 1975-1979, Cambodia had no definite education system.

The formal education system was re-established in 1979, after the country was rescued from the Pol Pot regime. Since then, the system has been reformed several times in order to enhance the quality of schools and develop the capacity to meet the needs of education in Cambodia. In 1994, the Ministry of Education Youth and Sport (MoEYS) established an inspection framework to monitor school performance and recruited primary education inspectors and secondary education inspectors for this purpose (MoEYS, 2015a). However, in order to catch up with the global world education reform, the MoEYS decided to change the inspection perspective from subject inspection conducted by secondary education inspectors, and primary school inspection conducted by primary education inspectors to a “Systemic School Inspection”. This was done through a Memorandum of Understanding (MoU) between the MoEYS and the Swedish School Inspectorate in 2012 (MoEYS, 2015a). Through the MoU, Swedish inspectors and experts had supported the training of Cambodian inspector trainers and the development of inspection tools. The inspector trainers organized training for existing inspectors (primary education and secondary education inspectors) and newly recruited inspectors. The inspectors in the systemic school inspection have mandates to inspect public and private schools, from pre-schools to secondary schools. The systemic school inspection has been widely implemented in Cambodia from 2015, after the existing inspectors finished their training on the new inspection system.

2.1.2. Systemic School Inspection Definition and Methods

Systemic school inspection is different from subject-based inspection which inspects only specific subjects, it evaluates school performance on the whole from management to teaching and learning. In the systemic school inspection, the inspection can be performed in two ways, internal inspection and external inspection. The internal inspection conducted at school-level through school self-evaluation, and conducted by District Training and Monitoring Team (DTMT) in line with Child-Friendly Schools (CFS). In Cambodia, CFS has been supported by United Nations Children’s Fund

(UNICEF) including 6 dimension: (1) All children have access to schooling, (2) Effective teaching and learning, (3) Health, safety and protection of children, (4) Gender responsiveness, (5) The participation of children, families and communities in the running of their local school, and (6) The National Education System supports and encourages schools to become more child-friendly (Shaeffer and Heng, 2016). The external inspection has two forms, thematic inspection and regular inspection. Thematic inspection is conducted by inspectors of the Educational Quality Assurance Department (EQAD) of MoEYS while the regular inspection is conducted by inspectors of the Provincial Office of Education (POE). The cycle of regular inspection is three to five years for a school while the thematic inspection is conducted when schools are at-risk in a certain area, for example chemistry teaching and learning at 12^{th} grade, or the priority topic defined by MoEYS. A regular inspection of one school takes for 1-2 days with two inspectors. The Swedish School Inspectorate and the MoEYS have defined the following, (MoEYS, 2015a).

Regular inspection means a systematic examination and assessment of all schools, to ensure that they comply with the laws and regulations applicable to its business. The assessment is based on an interpretation of national regulations. The audit results in a breakdown of what works well and what must be developed.

Thematic inspection means a systematic examination and assessment of the quality of a business within a defined area. The assessment is based on an interpretation of national goals and guidelines, supported by research findings and best practices. The audit results in a breakdown of what works well and what needs to be developed.

In this research, I focused on the external school inspections. Inspection tools such as questionnaires and assessment areas and indicators, have been developed by EQAD with the support from other relevant institutions of MoEYS and the experts from the Swedish School Inspectorate. The regular inspection is conducted focusing on four areas: leadership and management, teaching and learning, students' result and achievements, and school self-assessment. The developed questionnaires and assessment tools are used by inspectors in all POEs. The questionnaires and assessment tools for the thematic inspections have been developed based on the inspection topics. In a regular inspection, inspectors are required to write a school inspection report for each school and send it to the District Office of Education (DOE) within two weeks after the inspection date. They are also required to write a provincial inspection report which is a summarized report of the quality of all the inspected schools in the province, to EQAD. (MoEYS, 2015b).

2.1.3. Research Objective and Questions

The systemic school inspection has just been implemented in Cambodia. The inspection cycle is at least 3 to 5 years why there is still no result when it comes to improvement due to systemic inspection of schools. The study, therefore, will explore the possibility of the new inspection system to contribute to the development of school performance in Cambodia. In order to achieve the objective, I raised the following research questions: (1) what are the current school performances found by systemic school inspection?, and (2) what are necessary actions to improve school performance in Cambodia?

2.2. LITERATURE REVIEW

Evaluation and accountability are perceived as a key to ensure educational quality, and in most of Europe, school inspection is an important instrument of educational evaluation and accountability (Gustafsson *et al.,* 2015). Harris (2009) has mentioned that school inspections are used as the means of steering quality improvement, they often form the basis for targets agreed between education authorities and individual schools, and the sanctions will be applied if schools fail to meet the target (Gaertner and Pant, 2011). Gustafsson *et al*. (2015) indicated that some studies have suggested that sanctions and rewards have a positive effect on educational quality in schools, performance criteria and feedback alone may not be sufficient to motivate schools to perform high standard. However, inspection only will not improve school quality, good interaction characteristics of inspection and school lead to school improvement (Klerks, 2012). Educational efficiency and productivity can be increased by inspection system and the purpose of inspection is to identify the defects with the aim of correcting and preventing them from happening again, but should not focus on only the faults, defects, and errors of employees (Aysun and Baris, 2009). Koklu had underlined the purpose of inspection is to develop the teaching and learning process, and Bradly and Kottler had emphasized the objective of inspection on ensuring personal and professional development as well as competencies of those who inspected (Aysun and Baris, 2009). Jassens said that supervision has played an important role in the practice of inspection since 2002 in the Netherland and the poorly performing schools or at risked schools were prioritized to be inspected (Janssens and Gonnie, 2008). In Europe, external evaluations by inspectorates provide information to policy makers and the public about the state of education system, compliance with regulations, and quality differences among schools. Inspection reports provide feedback to schools about their strengths and weaknesses, and indicate the way to the internal evaluation (Janssens and Gonnie, 2008). Janssens and Gonnie (2008) also indicated some researchers have recognized that inspectorates that carry out independent evaluations make contribution to improve the education sector. The ideas that the education system can be developed through inspection is the concept in Swedish education policy and has been conducted in various organizational forms since the late 1800s (Lindström and Perdahl, 2014).The regular school inspection in Sweden focuses on four main areas such as attainment of objectives and results, pedagogy leaderships and development, learning environment, and individual pupils' right (Segerholm and Hult, 2018). Inspection processes, including school visits with inspectors observing lessons, interviewing with relevant people such as school leaders, teachers, parents, students; and questionnaires completed by those people; and the study of school documents are necessary to evaluate one school (Gaertner and Pant, 2011 and Segerholm and Hult, 2018). External and internal evaluation can be regarded as two interrelated areas for school reform and school-self-evaluation (SSE), inspectors use the result of SSE in some ways to improve the quality of schools (Janssens and Gonnie, 2008). Head teachers considered school inspection reports to be positive support for change and an opportunity to push through modifications and served as an eye-opener concerning student performance and the school actors were very positive with the inspection in Sweden (Segerholm and Hult, 2018).

In Cambodia, POE leaders and high school directors expressed that Systemic School Inspection should be implemented in order to ensure educational quality because the educational problems at school level is often due to a number of interrelated problems. Inspectors using Systemic School Inspection can point out strengths and weaknesses in several relevant areas that together make up a good quality of education. One high school director said that "*systemic school inspection should be implemented because it can help a lot of teachers, which is different from subject inspection that one inspector can observe only his or her specialized subject, not others*". The educational officers also confirmed that the systemic school inspection can ensure high education quality because it investigates in several important educational areas related to educational quality. Moreover, all of them desire to implement Systemic School Inspection in order to strengthen the quality of education. (Mam *et al.,* 2017).

2.3. METHODOLOGY

The participants in this research were 87 inspectors who received training on systemic school inspection from the National Institute of Education (NIE) of the Ministry of Education Youth and Sport (MoEYS) of Cambodia. Most of them (71%) were new inspectors who just finished their pre-service training from NIE in 2016 and 2017, and the others were existing inspectors who received in-service training on systemic school inspection from the NIE in 2015. The inspectors in 18 out of 25 provinces and EQAD inspectors completed the questionnaires. It should be noted that five provinces did not have inspectors yet during my research (Source: EQAD, May 2018).

Two kinds of questionnaires were used in the research, one for regular inspectors and another for thematic inspectors. A total of 78 out of total 104 POE inspectors and 9 out of a total of 15 EQAD inspectors were involved in the research. The indicators officially used in regular inspection were used in the questionnaire for the inspectors working at inspection offices of POEs while the questionnaire for EQAD inspectors were designed in relevant to the regular inspection indicators. The questionnaires were piloted before distributing to the participants through their Facebook messengers or telegrams. The questionnaires were required to be printed out for completion. The completed questionnaires were sent individually or through a group's representative to me. The questionnaires were sent and collected in April and May 2018.

The collected data and information was analyzed statistically for the quantitative data, and using Grounded Theory Method, the generation of theories from data, for the qualitative data. However, some qualitative information was grouped together and calculated as quantitative data.

2.4. FINDING AND ANALYSIS

The result of this research was divided into four parts, *the 1st part* was about school performance based on regular inspection, *the 2nd part* was about school performance based on thematic inspection, *the 3rd part* was about specific school performance on science subjects, and the *4th part* was about the systemic school inspection implementation and implication.

2.4.1. School Performance Found by Regular Inspection

Regular Inspection on schools is the duty of inspectors working in the inspection offices of the POEs. New inspectors with their experience from 1 to 2 years had inspected 10 primary schools, 4 lower secondary schools and 2 high schools as the average for one inspector. The inspectors who have 3 years-or-more experience of school inspection had inspected 29 primary schools, 8 lower secondary schools, and 4 high schools as the average for one inspector. Based on the experience of inspection, they evaluated school performance in a majority of inspected schools as in table 2.1.

As can be seen in the table 2.1, school directors in Cambodia were not considering much on equipment, books and other relevant materials for efficient teaching and learning, only 14% of the inspectors indicated that the majority of schools they inspected have the teaching materials they need (Table 2.1). The directors seemed to focus on their administrative works rather than the quality of teaching and learning. They have considered a lot on the preparing of the organizational structure with defining the responsible of vice directors and some other staff (84%), followed by the focusing more on school environment which is friendly for students (78%) and the effectively and efficiently organize the work in schools (67%) respectively (Table 2.1). The results indicated that there was not enough focus on technical support for teaching and learning. Only 14 percent of inspectors evaluated that the inspected schools had enough equipment, books and other relevant material for teaching and learning. And about half of inspectors evaluated the inspected schools needed to improve their pedagogical methods (47 %) and improve their teaching methods (50 %). From this result the MoEYS and other relevant partners should consider how to improve the school performance in Cambodia.

In the teaching and learning category, the table 2.1 showed that nearly half of the inspectors evaluated the inspected schools needed to improve their pedagogical methods (47%), and they also evaluated that 50 % of schools needed to improve their teaching methods to adapt to students' needs. Teachers might not be offering variation in teaching and learning methods in relation to different lessons or different student

Table 2. 1: School performance evaluated by inspectors working at POEs (% inspectors)

School performance evaluation indicators	Positive evaluation (%)
1. Leadership and management	
1.1 There is a clear organizational structure with defined procedures and responsible persons	84
1.2 The school director effectively and efficiently organizes the work in the school	67
1.3 The school has equipment, books and other relevant material for efficient teaching and learning	14
1.4 The school has a safe and favorable environment for learning and development	78
2. Teaching and learning	
2.1 Teaching and learning is according to the curriculum and other steering documents	88
2.2 Adequate pedagogical methods are used in relation to the aim of the class/ lesson	47
2.3 Teaching is adapted to students' needs	50
2.4 The teachers create a stimulating learning environment in class	86
2.5 The teachers continuously work to improve their teaching	73
3. Students' results and achievements	
3.1 Students' results and achievements improve over time	78
3.2 The school has a functioning program to assure students' attendance	84
3.3 Assessment and grading is equitable and fair	95
4. School self-assessment	
4.1 The school has functioning system for monitoring and evaluation the work in the school	43
4.2 There is a school development plan which is based on the reports of self-assessment results and external inspection results	53
4.3 There is good cooperation and communication with the community and other stakeholders	92

backgrounds. Teachers might use the same teaching methods for nearly all the lessons. That is “students read the textbooks and teachers asked questions for students to pick up the answer from the textbook”. About ninety percent (90%) of the inspectors evaluated that teachers teach according to the curriculum and other steering documents provided by the MoEYS and/or POE. These indicated that the teachers follow the law and other regulations of MoEYS or the government. Teachers created a good learning environment to students (86%). The good learning environment refers mainly to teachers encouraging students to think and create a welcoming atmosphere and, not so much on teaching methods and the use of teaching materials in class. Teachers always asked for help from their colleagues about the lessons which they did not understand, and some of teachers tried to pursue their education in higher degree. These means that they continuously work to improve their teaching.

Inspectors evaluated high on school performance in the students' results and achievements category, from 78 to 95% (Table 2.1). This was a good indicator to show that education quality was improving gradually. The students' results have improved gradually in comparison to previous years. Many schools had a functioning program to assure students' attendance and identify the reasons for students' absence and why some students drop out of school. The assessment and grading section indicated that assessment and grading was equitable and fair for all students with providing the reasons for their certain grade points. This will encourage students to learn because their result can be reflected to their knowledge and their learning efforts.

In the school self-assessment category, the results showed that the inspected schools had good cooperation and communication with the community and other stakeholders (95%) (Table 2.1). This indicated that schools had good cooperation with community people, local authority, students' parents, and other relevant stakeholder which was a good chance to develop the education in their schools. However, schools were not yet good in the areas of effectively management to improve work in school (43%) and the development plan which was based on the report of self-assessment results and external inspection results (54%) (Table 2.1). This means that the management teams of the school did not yet use the inspection result, especially school-self assessment, as a means to develop their schools.

School performances found by the regular inspection indicated several areas that MoEYS and other relevant partners need to consider in order to raise school performance in Cambodia. They could take the right intervention to improve the school performance in certain areas based on the priority and risk indicated by regular inspection reports.

2.4.2. School Performance Found by Thematic Inspection

Thematic inspection was conducted by EQAD inspectors in the whole country while regular inspection was conducted by the inspectors working at POEs in their province. According to EQAD inspectors, 7 topics were chosen for thematic inspection in the whole country from 2016 to 2018: (1) 12^{th} grade teaching and learning, (2) 12^{th} grade physics teaching and learning, (3) 9^{th} grade teaching and learning, (4) 6^{th} grade writing, (5) 6^{th} grade completion rate, (6) 4^{th} grade English teaching and learning, and (7) pre-school teaching and learning.

Based on these thematic inspections, inspectors evaluated school performance related to the majority findings as follows. Only 11% of the inspectors have agreed that the students' result on the inspected topics have improved over time and that

Table 2. 2: School performance evaluated by inspectors working at EQAD (% inspectors)

School performance evaluation indicators	Positive evaluation (%)
1. Students' results on the inspected topics improved over time (from 80%)	11
2. Schools have functioning program to encourage students to study on the topics inspected	33
3. Schools have effective apparatus and teaching materials to teach the topics inspected	11
4. Teachers have adequate pedagogy methods to teach the topics inspected	44
5. Teachers created a good environment to encourage students to study the topics inspected	89
6. Schools have functioning system to monitor and evaluate the educational quality on the topics inspected	22
7. Schools have development plan to improve educational quality on the topics inspected	33

schools have an effective apparatus and teaching materials to teach the topics inspected (Table 2.2). This indicated that the quality of education in the inspected topics were not strong enough, and that schools did not yet have enough teaching materials to assist students in understanding the lesson content related to the topics inspected. This result confirmed the results from regular inspection-that there was a lack of relevant teaching materials in order to support positive student achievement. Table 2.2 also indicated that only 33% of the inspectors have agreed that schools had a functioning program to encourage students to study and had a development plan to raise the quality of education in their schools. Only 44% of inspectors evaluated that teachers have adequate teaching methods to teach their students. These results also confirmed to the findings found by regular inspection. Teachers created a good and stimulated environment for students to study were found by both regular and thematic inspection

(86%) & (89%) respectively (Table 2.1 and Table 2.2). Thematic and regular inspections can thereby be seen as complementary to evaluate school performances.

Through the thematic inspection, inspectors can diagnose the specific problem related to the topic selected. For example, in the inspection on 12^{th} grade physics teaching and learning, the inspectors could see the critical and specific problem related to the teaching and learning in relation to students' result in this subject. This result can be sent to schools and to other relevant partners in order to take relevant action to develop that specific area as part of raising the school performance for improving the quality of education.

2.4.3. Specific School Performance on Science Subjects Found by Systemic School Inspection

Science subjects are common and essential for general education worldwide. I, therefore, conducted further investigation on school performance in science subjects in addition to general school performance found by the systemic school inspection. Table 2.3 indicated that the result in science subjects did not improve much yearly, only 47% of POE inspectors and 56% of EQAD inspectors had agreed that the students' result on science subjects have improved over time. It seemed that science lessons were not interesting to students or they were difficult for students to study. Most inspectors, 88% of POE inspectors and 100% of EQAD inspectors, evaluated that one of the reasons could be that schools did not teach experiments to their students which they found out as a part of their inspection at schools (Table 2.3).

Table 2. 3: School performance on science experiment and materials (% inspectors)

Questions (and answers)	**Positive evaluation** by POE inspectors (%)	**Positive evaluation** by EQAD inspectors (%)
1. Did students' result on science subject improve over time on the schools you inspected?	47	56
2. Did you see any teacher teach experimental science to students during your inspection period?	12	0
3. Why did not teachers teach experiments to their students? *Reasons (provided by inspectors and categorized by authors)*:		
1. The school doesn't have experimental materials	65	44
2. The teachers do not have enough knowledge to teach experiments	32	11
3. The school doesn't have a laboratory	31	22
4. The teachers do not have enough time to teach experiments	5	11
4. What kind of teaching materials are necessary for teaching science subjects in Cambodia at present time? *Reasons (provided by inspectors and categorized by authors)*:		
1. Teaching materials related to lesson theories, school curriculum, and/or textbooks	45	67
2. The simple, easy to be used, and/or low cost teaching materials	42	44
3. High quality and standard teaching materials	30	44
4. Chemicals	3	11

The inspectors provided different reasons, based on their ideas, that teachers did not use experiment as a method to teach their students. However, the most common reasons indicated by inspectors are as following. Most of the inspectors (62%) said that teachers did not use experiment to teach their students because the schools did not have experimental apparatuses and materials, and followed by 30% of them gave the reasons that teachers did not have enough knowledge to use experiments as a teaching method or schools did not have a laboratory while only 6% mentioned that teachers did not have enough time to prepare experiments. Teaching materials and teachers' competent knowledge on experiments are essential for teaching experiments at schools. Even though, science laboratory is required to conduct most of science experiments but some science experiments in general education can be conducted in a classroom by using simple experimental materials. All inspectors thought that science experimental apparatus is essential for developing science education in Cambodia. Nearly half of inspectors (47%) mentioned about the teaching materials related to curriculum and textbooks at schools and 41% of the inspectors mentioned about the simple, easy to be used, and/or low-cost materials are required to teach science experiments in general education at schools in Cambodia in the present time. And 31% of those inspectors said that high-quality-standard teaching materials are required to provide to school at the present time.

This investigation revealed that teaching materials and experimental apparatus are essential for teachers to use experiment as a method to teach science. Experimental apparatuses produced by industrial company are needed, but since most of the science teachers in Cambodia are not yet familiar to use experiment in science teaching, simple and low-cost experimental apparatuses should be considered in order to encourage teachers to use experiment as a means to teach science.

2.4.4. Systemic School Inspection Implementation and Implication

Systemic school inspection is expected to be implemented well to strengthen and improve the quality of education in Cambodia because it is the main focus of educational reform. It was also confirmed in this research that educational leaders at all levels are supporting the inspection work. Through the questionnaire, 78% of the POE inspectors indicated that POE directors of their province had strong support to implement the inspection in their province through allocation inspection budget, follow up inspection plan, nomination to conduct inspection while 100% of EQAD inspectors said that they received strong support from the MoEYS in order to conduct thematic inspections in the whole country. Moreover, both regular and thematic inspections were well recognized by relevant partners including school directors, teachers, and students' parents. Through the questionnaire, 95% of POE inspectors and 89% of EQAD inspectors said that the relevant partners have cooperated with them well during their inspection at schools. However, inspectors in Cambodia had no power or authority to put sanctions or rewards to schools. This was the limitation of inspection to improve school performance because the findings through inspection were not considered or taken action on by the schools and inspectors cannot put sanctions to those schools. One inspector suggested to the MoEYS to provide authority to inspectors to punish teachers or school directors who did not commit their task well, and to provide rewards or praising certificate to good performance teachers or directors. Some inspectors have suggested that the problems found through inspection should be considered and taken actions by the schools and/or relevant partners in order to provide the value to inspection work and also to make schools trust the inspection. The inspectors will be appreciated by school director and teachers if the problem found and reported by the inspectors were solved by POE, MoEYS and/or relevant development partners. If the inspection is reliable and valuable, it will serve as a key to ensure quality of education. The inspection reports are important for policy makers and the public in considering the improvement of educational quality.

Inspectors had some comments and suggestions in order to make the systemic school inspection become functioning and valuable. Some inspectors mean they need to be independent from the POE in order to report the real problems occurring in schools are under the administration of the POE to the MoEYS or the schools. Some inspectors suggested more training on systemic school inspection is needed for school directors, DTMTs, and the inspectors themselves. Other suggestions were related to the provision of budget, transportation means, and other materials that serve the inspection work were also requested.

2.5. DISCUSSION AND CONCLUSION

Systemic school inspection, recognized as the new inspection system in Cambodia, is a good means to measure school quality, the same as school inspection in other countries which has been concluded in earlier research (Janssens and Gonnie, 2008; Aysun and Baris, 2009; Gaertner and Pant, 2011; Gustafsson *et al.*, 2015). Both regular and thematic school inspections indicated the strong and weak points of school performances and they were complementary. Regular school inspection indicated the overall school performance while specific problems can be targeted through thematic school inspections. The inspections point out the main weaknesses of the schools which this article on systemic inspection in Cambodia shows was material, teacher competence and leading pedagogical development. The main problem shown by the inspections was that many schools lack enough competence as well as relevant material and resources to give high quality education. To improve the science education for example, the regular and thematic inspections showed that there was a need for experimental equipment in schools as well as teachers competence development when it comes to using experiments as a teaching method in teaching science. In order to raise school quality and student performance there is a need for political decision in order to give better conditions for school directors and teachers to improve the quality of the schools. According to Klerks (2012), inspection only will not improve school quality, good interaction characteristics of inspection and school lead to school improvement. This article showed that systemic school inspection in Cambodia was a good approach to find out the problems – but additional measures need to be taken in order to solve the problems. Therefore, based on the research findings presented in this article MoEYS as well as development partners should consider how to raise the knowledge of teaching as well as how to supply schools with relevant learning and teaching materials and equipment as a basic priority in order to enhance the quality of education in the Cambodian schools. MoEYS and other technical departments should focus on helping schools to produce teaching materials related to the curriculum and easy to be used for present situation in Cambodia and then gradually use the high quality teaching materials produced by industrial company.

There are also place for improvements when it comes to implementing systemic school inspection in Cambodia. Today there is a lack of inspectors, inspectors did not have authority on schools, and school inspection was not yet highly appreciated by teachers and schools. Some researches revealed that inspection alone did not make change at schools, good interaction between inspectors and schools, and sanction on schools was required in order to improve quality of education at schools (Klerks, 2012; Gustafsson *et al.*, 2015). POEs, MoEYS and schools should take actions to improve the poor performance in schools indicated in the inspection reports in order to make sure the inspection work has a function to contribute to raising school performance. In Sweden, inspection was highly appreciated by school actors and inspection report was considered as positive support for changes at schools (Segerholm and Hult, 2018). On the other hand, inspectors should strengthen the capacity of the inspections in order to be appreciated by schools and other relevant partners, and they also need to develop an inspection plan in order to receive budget from the government.

Even though, the Swedish Schools Inspectorate had terminated the project to support inspection in Cambodia, NIE and EQAD should work closely with UNICEF to build the capacity in inspection. UNICEF in Phnom Penh might be able to contribute in both budget and technical support in inspection work. Cambodia has done a lot and succeeded in raising the quality of the schools but there are still many challenges. And the solutions to these challenges must be met by political decisions and in collaboration with national and international help of different kind.

Chapter III

SELECTION OF PLANT MATERIALS APPLICABLE FOR EXPERIMENTAL CLASSES IN HIGH SCHOOL

Chapter III

SELECTION OF PLANT MATERIALS APPLICABLE FOR EXPERIMENTAL CLASSES IN HIGH SCHOOL

3.1. INTRODUCTION

Selection of plant materials is essential for the success of biological experimental classes in school. In biological textbooks, physiological parts of a plant such as leaves are used for the experiments to check the product of photosynthesis, and roots are used to check the number of chromosomes and so on and so forth. Seeds are origins for growing new plants and there are many experiments done using seeds. The period needed for each seed cultivar to germinate should be known in order to apply with school curriculum so the seeds germinated in 2 or 3 days after sowing might be easy to be adapted to school curriculum. This study focused partially on the effects of different light wavelengths on seed germination and seedling growth, so the selection of seeds to achieve my experimental purpose was needed. For a specific experiment on phytochrome responses on seed germination, the selection of photoblastic seeds is needed in order to prepare for biological experimental class at high school. This chapter aimed to find out the period of seed germination of some plant cultivars and to also select photoblastic seeds to be used for phytochrome responses experiments.

3.2. MATERIALS AND METHODS

Materials

Commercial crop seeds sold in Japanese garden shops were used in the research. Seeds of 25 lettuce cultivars, 18 carrot cultivars, 5 cultivars of *Brassica* and *Raphanus*, and 11 vegetative plant cultivars were checked for the period of seed germination and the germination phenomenon in the dark or far-red light wavelength irradiation. In Carrot and lettuce, cultivars with artificial coated seeds (same as pellet seeds; that is coated with an inert material to prevent seed germination or to keep seeds for a long time with agricultural materials) were also used.

A Petri dish of 5.5 cm in diameter was used with four layers of kitchen paper towel at the bottom. Black plastic box with a width of 190 mm, length of 260 mm and the height of 115 mm with cover was used as the dark box or it attached with 11 far-red (FR) LED bulbs was used for checking seed germination under far-red light irradiation.

Experimental Methods

The experiments were divided into 2 stages:

Stage 1: The experiment to check the length of seeds germination: In this stage, 20 seeds of each plant cultivars described above were placed on the kitchen paper towel in a Petri dish, and 2 ml of tap water was poured on the seeds in the Petri dish. The Petri dish was covered with cover and then it was sealed with a laboratory PARAFILM tape. The experiment was kept under room light condition with control temperature around 24^0C to check germination period. The germination of each seed cultivar was checked every day. The experiments were not repeated.

Stage 2: The experiment to check seed germination phenomenon in dark or FR light wavelength irradiation: This experiment was done after the period of seed germination of all seed cultivars were known. In this stage, the seeds of each cultivar were sown in two Petri dishes. Twenty seeds of each cultivar were placed on a paper towel in a Petri dish, and 2 ml of tap water was poured on the seeds. One Petri dish with the seeds was placed in a dark box and another was placed in the box with continuous irradiation of FR light. The experiments were kept in a room with the control temperature around 24°C. The experimental results were checked based on the length of seed germination of each cultivar and plus one more day in order to observe clear germination phenomenon. The experiments were not repeated.

3.3. RESULTS

3.3.1. Screening of Photoblastic Cultivars in Lettuces

The seeds of all lettuce cultivars including coated seeds used in this experiment germinated in 2 days after seed sowing. The seeds of all lettuce cultivars germinated in dark conditions, except a lettuce cultivar 'Furiru lettuce'. Continuously irradiation of FR light inhibited seed germination in uncoated lettuce seeds, but that could not inhibit seed germination in coated lettuce seeds. Among 20 uncoated seed lettuce cultivars, while germination of 14 cultivars was completely inhibited by continuous irradiation of FR, 6 cultivars could germinate with germination rate from 5 to 30% in continuous irradiation of FR light. However, the coated seed cultivars showed high germination rate from 65 to 100% in the condition of continuous irradiation of FR light (Table 3.1).

Table 3. 1: Germination rate of lettuce cultivars in dark or FR light condition, and the period needed for seed germination

No.	Cultivar Names in Japanese (Company name)	Seed germination period(days)	Germination rate in dark condition (%)	Germination rate FR light (%)
1	Bachus 8 (Nantes)[1]	2	85	15
2	Berkeley (Takii)	2	75	0
3	Furiru (Sakata)	2	0	0
4	Horoniga (Sakata)	2	50	0
5	Income (Mikado Kyowa)	2	95	0
6	Minikko (Yamamoto Noen)	2	100	0
7	Noble SP (Tohoku)	2	75	5
8	Olympia (Mikado Kyowa)	2	100	0
9	Otegaru (Sakata)	2	85	30
10	Polar (Asahi)	2	45	10
11	Red-fire (Takii)	2	95	0
12	Red sunstar (Asahi)	2	90	0
13	Green leaf (Sakata)	2	85	5
14	Red leaf (Sakata)	2	95	0
15	Romaine (Sakata)	2	100	0
16	Sunny (Sakata)	2	100	0
17	Shakishaki (Sakata)	2	95	0
18	Sisco (Takii)	2	100	0
19	Smile (Asahi)	2	100	0
20	Vitamin (Aisan Syubyo)	2	100	5
21	Dancing (Takii) (Coated)	2	80	70
22	Furifurika (Sakata) (Coated)	2	100	65
23	Green jacket (Takii) (Coated)	2	100	100
24	Red falter (Takii) (Coated)	2	100	65
25	Sunny (Sakata) (Coated)	2	100	95

[1]Name in bracket next to cultivar name is the name seed production company

3.3.2. Screening of Photoblastic Cultivars in Carrots

The carrot seeds used in this experiment germinated in 5 to 6 days after seed sowing. The germination rate of carrot seeds was not very different between dark condition and continuous irradiation of FR. In generally, the germination rate in dark was a little bit higher than those in FR condition (Table 3.2).

Table 3. 2: Germination rate of carrot cultivars in dark or FR light condition, and the period needed for seed germination

No.	Cultivar Names in Japanese (Company name)	Seed germination period(days)	Germination rate in dark condition (%)	Germination rate FR light (%)
1	Aikoutoki Nashi 5-sun (Aisan)[1]	5	80	40
2	Chihama 5-sun (Asahi)	5	50	40
3	Cream Harmony (Marutane)	6	80	70
4	Dark Purple (Asahi)	6	90	90
5	Fresh Scale (Yamauchi)	5	90	90
6	Kinko 4-sun(Sakata)	5	80	80
7	Kokubu Senkou Oonaga (Takii)	5	90	50
8	Koshoku 5-sun 2-gou (Asahi)	5	40	30
9	Kuroda 5-sun (Fukuhanen)	5	70	60
10	One dish (Marutane)	5	50	50
11	Piccolo (Takii)	6	60	90
12	Shimakogane (Uchina)	6	50	50
13	Toginashi 5-sun (Fukuhanaen)	6	70	60
14	Yellow Harmony (Marutane)	5	30	70
15	Grand Prix (Takii) (coated)	5	60	40
16	Koshoku 5-sun 2-gou (Asahi) (coated)	5	50	30
17	Koyo 2-gou (Takii) (coated)	5	50	60
18	Yomei 5-sun (Takii) (coated)	5	80	100

[1]Name in bracket next to cultivar name is the name seed production company

3.3.3. Screening of Photoblastic Cultivars in *Brassica* and *Raphanus*

Cultivars of *Brassica* and *Raphanus* being used in this experiment germinated in 2 days after seed sowing. *Brassica* and *Raphanus* seeds did not need light to germinate. Nearly all seeds germinated in dark conditions. The continuous irradiation of FR light did not suppress *Brassica* and *Raphanus* seed germination. The germination rate in dark and FR condition were mostly the same (Table 3.3).

Table 3. 3: Germination rate of cultivars of *Brassica* and *Raphanus* in dark or FR light condition, and the period needed for seed germination

No.	Cultivar Names in Japanese (Genus; Company name)	Seed germination period(days)	Germination rate in dark condition (%)	Germination rate FR light (%)
1	Aka-chan (*Brassica*; Asahi)[1]	2	100	100
2	Aska-Akane Kabu (*Brassica*; Nanto)	2	100	100
3	Fuku-komachi (*Brassica*; Takii)	2	100	90
4	Hinona Kabu (*Brassica*; Takii)	2	100	100
5	Shima Daikon (*Raphanus*; Futaba)	2	90	90

[1]Name in bracket next to cultivar name are the names of genus and company seed Production Company

3.3.4. Screening of Photoblastic Cultivars in other Vegetative Plants

The seeds of 11 cultivars (in Table 3.4) germinated from 2, 3, 5, 6, or 7 days after seeds sowing. The seeds of these vegetative plant cultivars germinated in both dark and FR irradiation condition (Table 3.4).

Table 3. 4: Germination rate of some vegetative plant cultivars in dark or FR light condition, and the period needed for seed germination

No.	Cultivar Names in Japanese (Genus; Company name)	Seed germination period(days)	Germination rate in dark condition (%)	Germination rate FR light (%)
1	Horenso by Takii cross (*Spinacia*; Takii)[1]	2	90	85
2	Wase-salada akari (*Spinacia*; Takii)	2	65	70
3	Sensuji-kyo-mizuna (*Brassica*; Tohoku)	2	90	90
4	Kyo-kanade-mizuna (*Brassica*; Takii)	2	100	100
5	Kanamachi-kokabu (*Brassica*; Takii)	5	70	60
6	Pasrlei paramount (*Petroselinum*; Takii)	3	60	60
7	Topseller celeri (*Apium*; Takii)	8	50	60
8	Mini-white celeri (*Apium*; Takii)	6	50	40
9	Soup celeri (*Apium*; Sakata)	6	80	75
10	Shirokuki-Mitsuba (*Cryptotaenia*; Takii)	8	50	60
11	Kiku-no-suke (*Glebionis*; Takii)	2	80	90

[1]Names in bracket next to cultivar name are the names of genus and company seed Production Company

3.4. DISCUSSION

Lettuce cultivar 'Furiru lettuce' only showed a complete photoblastic reaction. So, this cultivar is a good plant material for the experiment class of the light wavelength dependencies on seed germination including response of phytochrome reaction. However, the other cultivars which could germinate in 2 to 3 days after seed sowing are also appropriate for the experiments on the effect of different light wavelengths on seed germination and seedling growth.

In this chapter, I found only a lettuce cultivar 'Furiru lettuce', which showed strong photoblastic phenomena and most of all cultivars did not show photoblastic reaction. These results were considered that photoblastic gene resources might be eliminated in agricultural cultivars because commercial seeds cultivar required high germination rate. I also used coated seeds in this study and all coated seeds can germinate in dark condition. I considered that to germinate coated seeds which was covered by agricultural chemicals which interfered the light to pass through, photoblastic reaction might be eliminated from agricultural genetic resources.

Chapter IV

METHODS FOR TEACHING LIGHT WAVELENGTH DEPENDENCIES ON SEED GERMINATION AND SEEDLING ELONGATION APPLICABLE FOR HIGH SCHOOL EXPERIMENTAL CLASS IN DEVELOPING COUNTRIES

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4.1. 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 that 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 understand 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 has been researched and the necessity of light for plant growth is well known not only by farmers 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 Cambodia has not integrated this concept yet. However, school curriculum reforms in developing countries generally used school curriculums of developed countries as references. In this study, I aimed 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; Chen *et al.,* 2014). B light inhibited elongation of cucumber hypocotyls (Spalding and Cosgrove, 1988), growth of *Arabidopsis thaliana* seedlings (Folta, 2004).

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 cultivars, 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 confusion. However, there are no researches to find the condition being shown complete photoblastic reaction by now.

Seeds of crops are convenient for using in experimental class because biological teachers can always purchase at garden shops. However, some home pages of agriculture, Ex. Sakata seeds co Ltd., written that carrots are photosensitive species. Although there are some researches on the effect of temperature on carrot seed germination (Pereira *et al.*, 2008; Nascimento *et al.*, 2008; Dias *et al.*, 2015), the reports showed the effect of light wavelength on carrot seed germination and seedling elongation could not be found. In chapter 3, I preliminary researched on many species used for agriculture but the most of all cultivars in each species, except 'Furiru lettuce', had not shown strong photoblastic reaction. In this study, I produced LED irradiating handmade equipment to search how to use this equipment. After setting up, I got data in an experimental class of photosensitive reactions followed by descriptions of textbooks in high school in Japan.

4.2. MATERIALS AND METHODS

4.2.1. LED-box Development and Wavelength Analysis

In chapter 3, I had produced FR LED (Light Emitting Diode) attached prototype model for photoblastic cultivar screening. In this chapter, I extended to use other experimental boxes with different light wavelengths. Six different LED light wavelength bulbs of 5 mm diameter including W, B, G, O, R, and FR were used. LED-attached experimental boxes were produced manually with designing a 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

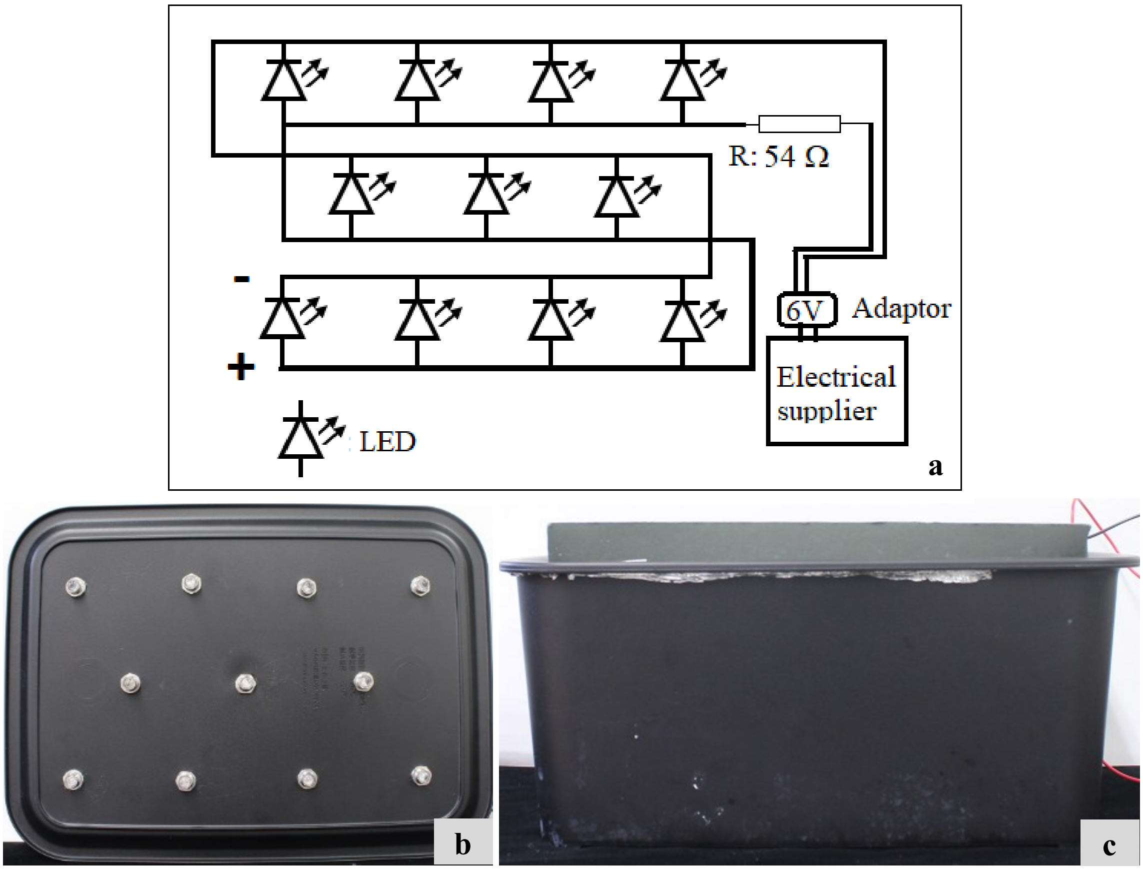


Figure 4. 1: LED experimental box development **a**. LED circuit in the experimental box composed of 11 LEDs with connecting to 54Ω resistor and the electrical wire for connecting to electrical supplier, **b**. the arrangement of LED bulbs on the cover of the experimental box, and **c**. outside view of experimental box

of 54Ω was used in this LED circuit (Figure 4.1a). Black boxes with width of 190 mm, length of 260 mm and the height of 115 mm 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 6 cm (Figure 4.1b). The decided number of LED bulbs and their arrangement is to make the light irradiating in the whole box. Paper board was folded to make the outer cover of the LED circuit on the experimental box. Aluminum foil was pasted inside the box in order to make light reflex and to prevent outside light into the box. Figure 4.1c 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.

Light analyzer LA-105 (NK-system, JAPAN) was used to measure the LED light feature 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 4.1). 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 B, G, O, R, and FR-LED lights with mono peak and LED wave length of W showed wide wavelength range with 1strong peaks of B and 1 wide range peak between B and R. 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 blue spectrum (Table 4.1). Photon Flux Density (PFD) was divided among FR, R, G, B, and ultra violet (UV) and consisted in each LED light source (Table 4.1).

Table 4. 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	11
Lambda D (nm)	485	477	527	607	618	690
Lambda P (nm)	454	474	520	612	626	732
PFD-FR (700-780nm) (μmol/m^2/s)	0.43	0.03	0.20	0.02	0.43	15.90
PFD-R (600-700nm) (μmol/m^2/s)	4.02	0.03	0.37	3.22	56.80	0.61
PFD-G (500-600nm) (μmol/m^2/s)	9.03	2.10	47.90	0.62	2.06	0.05
PFD-B (400-500nm) (μmol/m^2/s)	6.23	27.90	3.37	0.08	0.26	0.05
PFD-UV (380-400nm) (μmol/m^2/s)	0.02	0.03	0.04	0.01	0.07	0.01

4.2.2 Experimental Methods

Each 10 cultivars of carrot and lettuce were used in these experiments (Table 4.2 and Table 4.3). On the commercial 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 my preliminary study, there was no difference of germination rate between supplying water in completely dark condition by putting a tube of 2 ml 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, the water supply method was selected the latter one. The experimental boxes were kept at room temperature around 24^{0}C (measured by an 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 an 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, I do not focus only on photoblastic seed germination, I want to confirm the strong effect of many light wavelengths on many lettuce and carrot cultivars so I decided to irradiate light continuously. The experiments were repeated for 3 times for each cultivar. Therefore, total 30 seeds of each cultivar were used in this experiment. Statistical significance in seedling elongation was 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-value < 0.05.

4.2.3 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 the classroom.

4.3. RESULTS AND DISCUSSION

4.3.1. Seed Germination Experiment

To use LED apparatus producing in this chapter, seeds of all carrot and lettuce cultivars, except 'Furiru lettuce', were also reconfirmed not to require light for inducing germination because seeds of all cultivars germinated in dark conditions (Table 4.2 and Table 4.3). 'Furiru lettuce' was screened as only cultivar with photoblastic cultivars and other cultivar did not have photoblastic in chapter 3 and photoblastic feature of 'Furiru lettuce' and other cultivars were confirmed by three times repeat in this chapter. Referring to photoblastic germination phenomenon, while seed germination rate in dark condition was still high in previous researches, 8.5% (Borthwick *et al.*, 1952) and 29% (Jomori *et al.*, 2010), my experimental apparatus and methods could inhibit seed germination of 'Furiru lettuce' in dark condition completely (Table 4.3). All wavelengths had similar effect on carrot and lettuce seed germination, except the lettuce cultivars in FR light (Table 4.2 and Table 4.3). Carrot seed germination rate was slightly inhibited by B, especially on 'Aikou Tokinashi 5 sun' (Table 4.2). However, the germination rate of lettuce cultivars in B irradiation was not suppressed in comparing to other wavelengths, except for 'Furiru lettuce' (Table 4.3). Seed germination of 'Furiru lettuce' was strongly suppressed by the B light irradiation, only 37% germinated comparing to other treatments with germination rate from 73 to 100% (Table 4.3). 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 a specific genotype in some of crops. Wareing and Black (1958) also indicated that B light inhibited light-sensitive lettuce seed germination.

Table 4. 2: 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 senkouoonaga (Takii)	83	77	80	83	73	77	60
Grand prix (Takii)	80	67	73	87	83	83	73
Yellow Harmony (Marutane)	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

[1]*Cultivar name in Japanese and the name in bracket next to cultivar name is the name of seed-production Company*
[2]*Light treatment: D (dark), W (white), B (blue), G (green), O (orange), R (red), FR (far-red)*

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 compared to FR light for both carrot and lettuce seed germination (Table 4.2 and 4.3). It was reported that R light promotes seed germination and FR inhibits seed germination of lettuce mediated by phytochrome (Kendrick and Russell, 1975). The irradiation of FR continuously could inhibit lettuce seed germination, but could not inhibit carrot seed germination (Table 4.2 and 4.3).

Table 4. 3: 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
Furiru 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 sunstar 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

[1]*Cultivar name in Japanese and the name in bracket next to cultivar name is the name of seed-production Company*

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

Different light wavelengths have different effects on lettuce seed germination. It was clear that FR inhibited lettuce seed germination, and one lettuce cultivar "Furiru lettuce" did not germinate in dark condition. Jomori *et al.* (2008 and 2010) performed lettuce seed germination tests among 12 cultivars under several LED irradiating conditions and reported that only two cultivars, "Furiru lettuce" and "Green oak" kept nature of light sensitivity and B wavelength tended to inhibit lettuce seed germination.

Between the results of Jomori *et al.* (2008 and 2010) and my results, different germination rates of “Furiru lettuce” were found, and both germination rate in R and inhibition rate in FR or dark of my experiments were better than their results. To perform experimental classes, clear reaction is very important to avoid misunderstanding of students. Since not only Jomori *et al.*’s results (2008 and 2010) but also Borthwick *et al.*’s results (1952) were detected germinate-able seeds in both dark and FR irradiated condition, it was considered that the phytochrome reaction had been difficult to introduce for the experimental class in high school. However, since my equipment achieved almost 0% germination rate in dark and FR condition, the phytochrome experiment should be considered to be introduced to high school experimental class by using my handmade equipment.

4.3.2. 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 followed 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 4.2 & Figure 4.3 and Table 4.4 & Table 4.5). The seedlings did not elongate longer in W wavelength might be caused by PFD-B dominant in W light (Table 4.1) 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 ‘Furiru lettuce’ seedling elongation (Figure 4.3, Figure 4.4 and Table

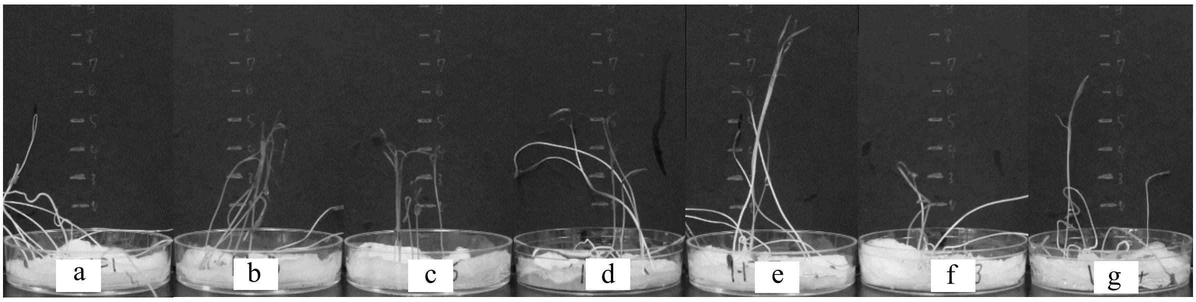


Figure 4. 2: Carrot seedlings, Aikou Toki nashi 5 sun, at 10 days after seed sowing in different light condition: **a**. dark, **b**. white, **c**. blue, **d**. green, **e**. orange, **f**. red, and **g**. far-red LED light.

Table 4. 4: 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
AikouToki 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

[1]*Cultivar name in Japanese and the name in bracket next to cultivar name is the name of seed-production Company*

[2]*It 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*

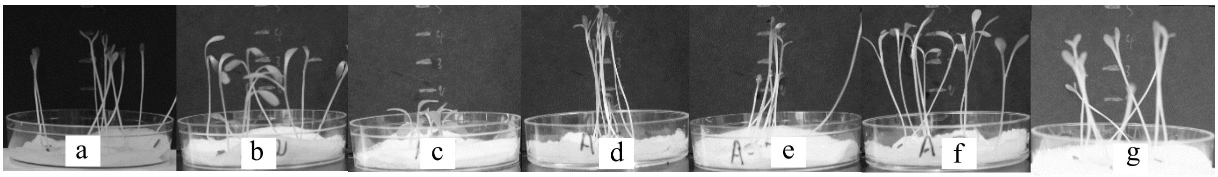


Figure 4. 3: Furiru lettuce seedlings in 10 days after seed sowing in different light condition: **a**. dark, **b**. white, **c**. blue, **d**. green, **e**. orange, **f**. red, and **g**. far-red LED light

Table 4. 5: 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
Furiru 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±08 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 sunstar 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

[1]*Cultivar name in Japanese and the name in bracket next to cultivar name is the name of seed-production Company*

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

[3] *It 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*

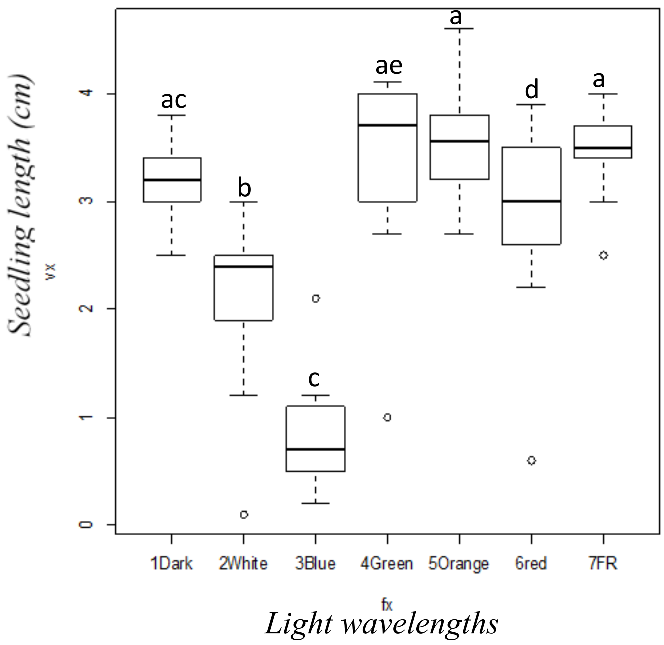


Figure 4. 4: Average seedling length of Furiru lettuce for 3-time experiments analyzed by R Console. The different letters on bars indicate significant differences with p-value < 0.05

4.5) which were the same as the effect of B on seedling elongation of other cultivars disseminated by previous researchers (Spalding and Cosgrove, 1988; Folta, 2004; Hui *et al.*, 2017). G light did not inhibit elongation of carrot and lettuce seedlings (Table 4.4 and Table 4.5) and this results were confirmed the previous researches that G light at 531nm did not inhibit the lettuce hypocotyl and stem elongation (Steinitz *et al.*, 1985; Park and Runkle, 2018).

For statistical analysis, there are significant differences of seedling elongation under the irradiation of different light wavelengths. The result of my 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.

4.4. APPLICATION TO CLASSROOM

I 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 composed 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).

4.4.1. In Japan

Smaller type of LED-attached box made of kitchen canister of 430ml (8cm^3), 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 understand the survival strategy under trees condition described in the textbook. I 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 period was 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 a 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 at room temperature around 25^0C in July for 3 days until the result checking in the 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 "Furiru 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 composed 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 some points that they did not understand in the lesson such as germination phenomenon, the advantage of seeds dormancy when they are under the tree, the reason for FR has longer wavelength not promoting seed germination. Even though some points were not well understood, 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 glad 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 help 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.

4.4.2. In Cambodia

I 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 light sources for conducting experiments and the methods to produce experimental boxes. In this pilot teaching, I used the same experimental boxes as those used in the 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, I used only one kind of lettuce cultivar exported from Japan "Furiru lettuce". Twenty lettuce seeds were placed on dry paper and then filled approximately 5 ml of water in each box. The imbibed seeds were irradiated continuously with different light wavelengths for one week until the date of checking the experimental results. The results of seed germination were the same as those in Table 4.3 that 'Furiru lettuce' seeds 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 had 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).

4.5. CONCLUSION

Through the result of my experiment, I could evaluate that my 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 in senior high school in developing countries when this concept is integrated in biology curriculum or it is useful to teach students as extracurricular activities. Even though industrial plant growth chambers or experimental apparatus attached with LED bulbs are available, 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 4.1a. The LED-attached boxes used for piloting in both Japan and Cambodia are smaller than the experimental box used for conducting experiments 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 the lesson objective. Lettuce seeds, especially Furiru 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 Furiru lettuce seed, which is complete photoblastic, did not germinate in dark or FR irradiation condition in my handmade equipment, I performed trial of seed germination in high school experimental class. Although students could observe a cultivar, 'Furilu 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 the 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.

Chapter V

DEVELOPMENT OF AN LED-ATTACHED BOX FOR PHYTOCHROME RESPONSE EXPERIMENTS ON LETTUCE SEED GERMINATION IN SENIOR HIGH SCHOOL BIOLOGY

Chapter V

DEVELOPMENT OF AN LED-ATTACHED BOX FOR PHYTOCHROME RESPONSE EXPERIMENTS ON LETTUCE SEED GERMINATION IN SENIOR HIGH SCHOOL BIOLOGY

5.1. 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 5.1. This content is included in 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 germination (Piskurewicz *et al.*, 2009). The treatment of R light on a photoblastic lettuce seed causes the conversion of Pr to Pfr, 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).

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 5. 1: Germination responses of photoblastic lettuce seed to light treatment with red light (R) and far-red light (FR)

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. The light sources for the experiment were usually contrived from incandescent or fluorescent lamps together with cellophane or gelatin color 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 experimental apparatuses of this kind might be too expensive for high schools, especially in developing countries such as Cambodia, phytochrome experiments have not yet been practiced extensively in biology education at schools. Jomori (2010) used commercial panels with many LEDs for phytochrome experiments and he got results mostly similar to those of phytochrome experiments reported by Borthwick *et al.* (1952). Nowadays, R- and FR-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 3) and seedling growth (Website 4). 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 to set up the apparatus and reports its usefulness for the experiments.

5.2. DEVELOPMENT OF AN LED-ATTACHED BOX AND ITS APPLICATION TO PHYTOCHROME RESPONSE EXPERIMENTS

5.2.1. Development of an 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 5.2a), five sets of LED bulb holders (Figure 5.2b), resistors of 2.2 Ω and 51 Ω (Figure 5.2c), an on-off switch (Figure 5.2d), an electric current meter (Figure 5.2e), a volume dial with a variable resistor from zero to 2 KΩ (Figure 5.2f) are needed to set up one LED circuit. A 4-battery case (Figure 5.2g) 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 a100-yen shop was used as the container box.

Figure 5. 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 completely block the light from outside. Holes were drilled on the cover of the plastic box for inserting LED bulb holders. Five R LEDs and the other five FR LEDs were attached on the cover of the box as indicated in Figure 5.3a. The wiring diagram of one LED circuit, which includes five bulbs of R LED or FR LED is shown in Figure 5.3b. Using electrical wire, a handmade LED-circuit to connect one LED to another

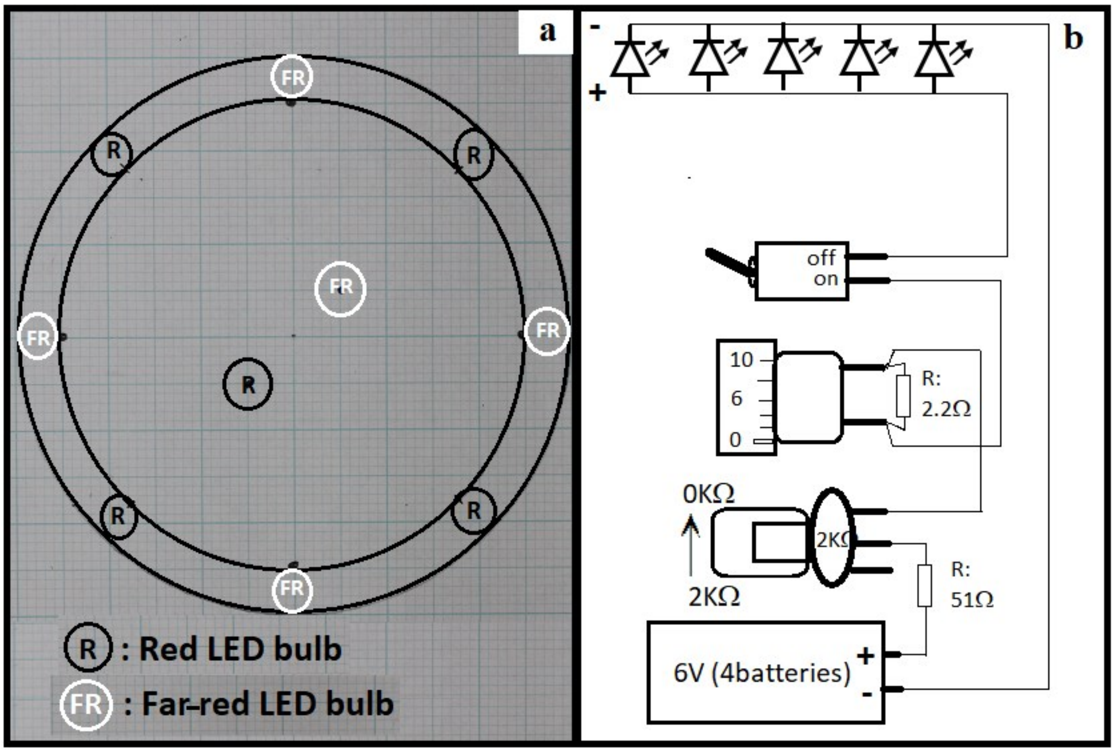


Figure 5. 3: Diagram of LED, **a.** Distribution of LED bulbs on the box cover, **b.** The diagram of each LED circuit.

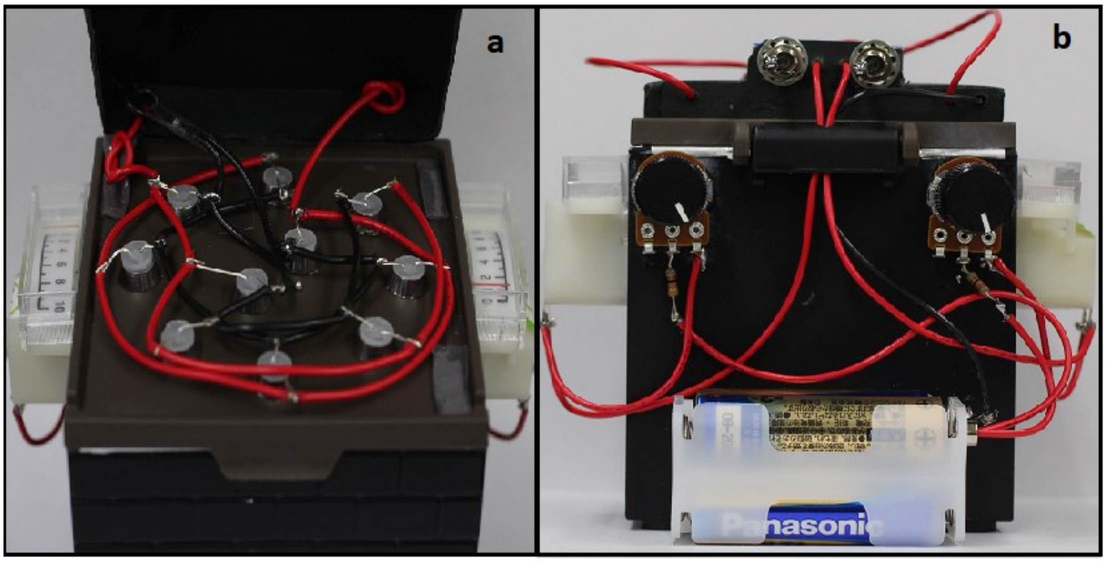


Figure 5. 4: The view of LED-attached box , **a.** Top view of an LED-attached box, **b.** Back view of LED-attached box

LED following the wiring diagram in Figure 5.3b was shown in Figure 5.4a. Black paper board was folded to make the outer cover of the LED circuit. A completed LED-attached box is shown in Figure 5.4b.

Analysis of Light Spectrum

To ensure the correct light spectrum emitted from the light apparatus, 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 revealed by the analyzer include illuminance (LUX), dominant wavelength (Lambda D), and photon flux density (PFD). In the box, the PFD-R was higher than PFD-FR in R-LED light and the PFD-FR was higher than the PFD-R in FR-LED light (Table 5.1 and Table 5.2). Lambda D of the light from the red LED bulbs was 623 or 624nm and that from the far-red LED bulbs was 690nm.

Table 5. 1: Features of light from red LED bulbs in the LED-attached box at each scale of intensity of electric current pointed in an electric current meter measured by LA-105

Light features	Intensity of electric current pointed in an electric current meter									
	1	2	3	4	5	6	7	8	9	10
LUX (lx)	35.90	95.40	143.00	190.00	259.00	306.00	376.00	476.00	585.00	694.00
Lambda D (nm)	624	623	623	623	623	623	623	623	623	623
PFD-FR (700-780nm) (µmol/m^2/s)	0.06	0.08	0.10	0.11	0.14	0.16	0.18	0.22	0.26	0.30
PFD-R (600-700nm) (µmol/m^2/s)	**0.80**	**2.29**	**3.48**	**4.69**	**6.41**	**7.63**	**9.46**	**12.10**	**14.90**	**17.80**

Table 5. 2: Features of light from far-red LED bulbs in the LED-attached box at each scale of intensity of electric current pointed in an electric current meter measured by LA-105

Light features	Intensity of electric current pointed in an electric current meter									
	1	2	3	4	5	6	7	8	9	10
LUX (lx)	7.27	10.00	12.00	15.20	17.40	21.20	26.50	30.30	35.40	41.20
Lambda D (nm)	690	690	690	690	690	690	690	690	690	690
PFD-FR (700-780nm) (µmol/m^2/s)	**1.06**	**4.11**	**5.98**	**8.95**	**11.30**	**15.30**	**19.70**	**24.20**	**29.00**	**35.00**
PFD-R (600-700nm) (µmol/m^2/s)	0.10	0.21	0.29	0.40	0.49	0.64	0.81	0.97	1.14	1.35

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 button-switches that can allow users to switch on one type or both types of light at one time. The combination of the variable resistor and the electric current meter allows users to determine the intensity of LED light as shown in Table 5.1 and Table 5.2. Turning the variable resistor switch (knob) from low to high values results in reducing the resistance which would then generate a higher intensity of electricity current. However, if the batteries were low, the indicator of the electric current meter could not reach the maximum reading point 10 even though the knob (switch) was turned to maximum.

5.2.2. 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 my preliminary experiments, I had selected only one lettuce cultivar out of the 25 cultivars commercialized in Japan. The seed of "Furiru lettuce (Sakata Seed Co. Ltd., Japan)" demonstrates photoblastic phenomena. Gibberellic acid (GA_3) and abscisic acid (Sigma-Aldrich Co. Ltd, USA) were used for GA and ABA treatment respectively.

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

Methods

A total of 20 'Furiru lettuce' seeds were put on each prepared Petri dish, then the preparation was placed into the LED-attached box. Two milliliter of tap water was added to the Petri dish in the box while the acclimation light, R or FR, was irradiating; and then the box was closed immediately. The acclimation light continuously irradiated for 10 minutes. In the case that only one kind of light was used, the acclimation light was switched off to keep the seeds in the dark in the box. In the case of alternative light treatment, the treatment light, R or FR, was irradiated immediately after the acclimation 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. There were 7 different light treatments for this 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 5.1). 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 compare the effects of plant hormones on lettuce seed germination were also conducted for showing the results to students during the laboratory class. 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.

5.3. 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 when they received R or the last irradiation of R before being kept in the dark (Figure 5.5a-g and Figure 5.6). In 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 3) also indicated that lettuce seed germination was inhibited in their dark box. However, their box was not developed for phytochrome response experiments because it was equipped with neither R-LED nor FR-LED. Although it is not possible simply to compare my results with those of the previous studies because the lettuce cultivars they used and their experimental conditions are different from my 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 germination phenomenon which was 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, I used simple materials and methods, such as a small dark box and the seeds were sown on wet paper, which are more suitable to apply to student laboratories.

The mode of action of phytochrome, which responses to R irradiation to produce GA and, to FR irradiation to produce ABA, can be explained by the results of the experiment using the respective plant hormones. The lettuce seeds treated with GA could germinate in dark conditions as well as they were irradiated with R before being

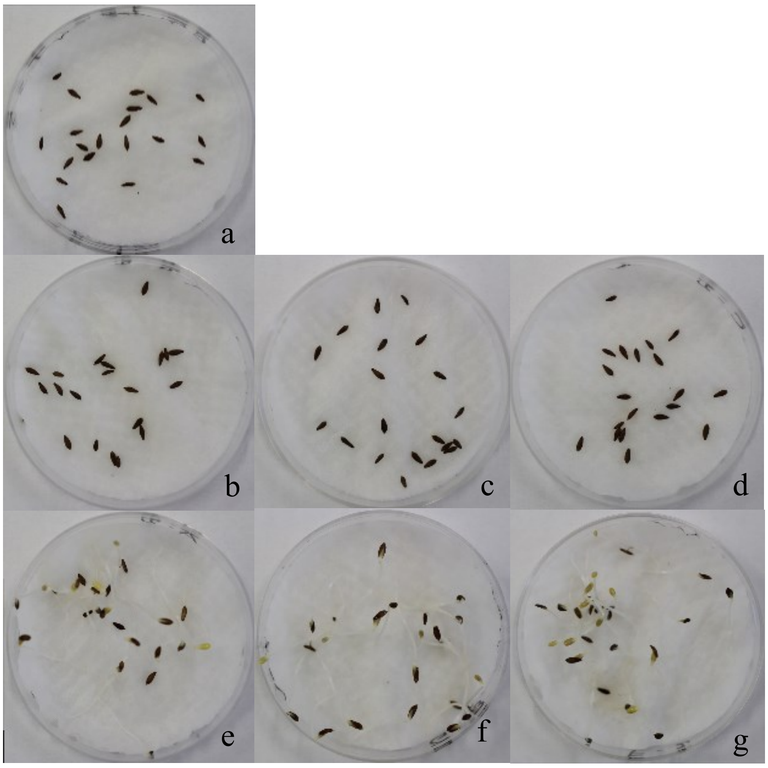


Figure 5. 5: Germination responses of Furiru lettuce seeds to different light treatments 3 days after experiment started, **a.** dark (D), **b.** FR-D, **c.** R-FR-D, **d.** FR-R-FR-D, **e.** R-D, **f.** FR-R-D, **g.** R-FR-R-D.

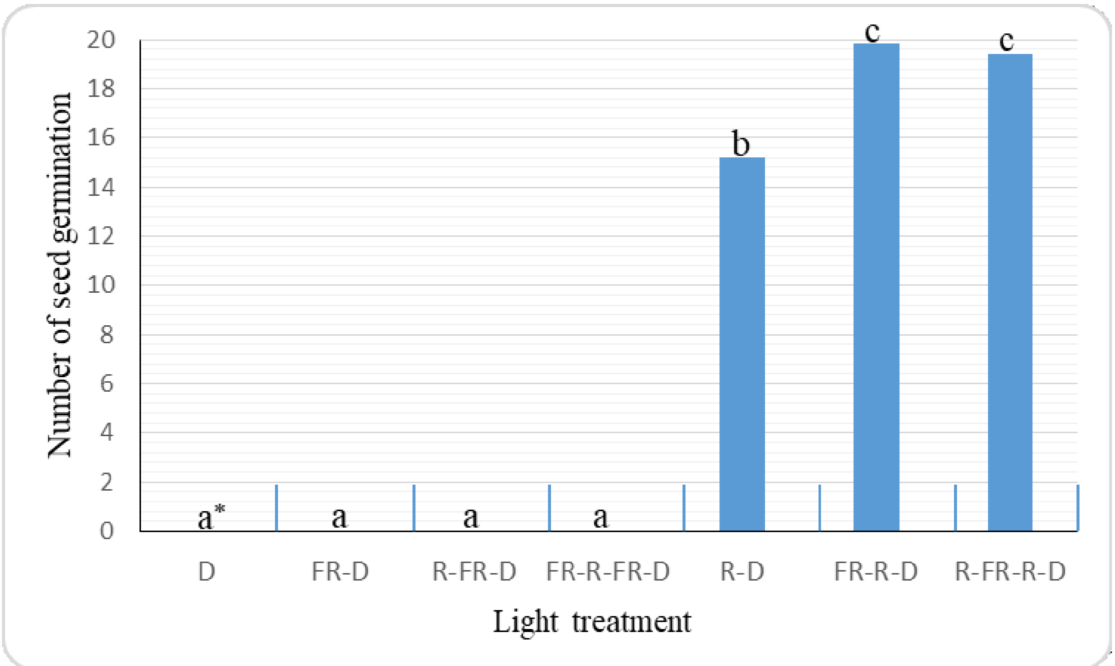


Figure 5. 6: Germination of 'Furiru lettuce' seeds on the 3rd day after different light treatments, D: dark, FR: far-red, R: red.

*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.

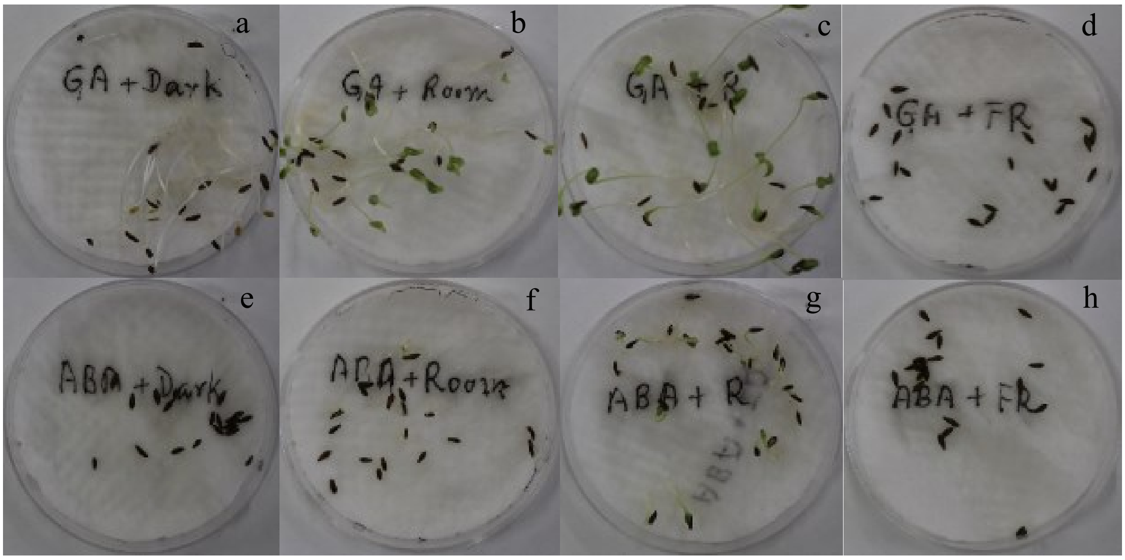


Figure 5. 7: Germination of Furiru lettuce seeds on the 4th day after different light treatments with GA or ABA, a to d: 10ppm of GA was added to seeds, and kept in the dark (a), room light (b), R light (c), and FR light (d), e to h: 10ppm of ABA was added to seeds, and kept in the dark (e), room light (f), R light (G), and FR light (h).

kept in the dark. The seeds, however, did not germinate when they were treated with ABA in the dark or in room conditions as well as they were irradiated with FR before being kept in the dark (Figure 5.5, Figure 5.6 and Figure 5.7).

At around 24°C, the 'Furiru lettuce' seed germination generally started within 2 days after being imbibed. However, the results, whether the seeds have germinated, can be checked one week after the experiment was started. Therefore, the 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, further examinations of the effects of temperature on the phytochrome-mediating germination of ‘Furiru lettuce’ seeds should be needed.

5.4. APPLICATION TO CLASSROOM

The LED-attached box developed was piloted with senior high school students in biology laboratory classes at the Senior High School Affiliated to Aichi University of Education in Japan and with teacher trainees in a pre-service teacher training course at the National Institute of Education in Cambodia. ‘Furiru lettuce’ seeds were used for examining the seed germination. In this experiment, the intensity of electric current for LED-R was adjusted to reading 8 (PFD-R= 12.10 µmol/m2/s) and that for LED-FR was adjusted to reading 6 (PFD-FR=15.30 µmol/m2/s) in order to make the PFD values of R and FR almost similar.

5.4.1. Situation in Japan

The first trial was carried out in a biology laboratory class having 24 students of 2^{nd} -year senior high school (11^{th} grade) in 2018. An experiment was designed to confirm the theory about the phytochrome-mediated lettuce seed germination which was explained in the biology textbook that students used. In the first lesson on this topic, students carried out the experiment. Seeds were irradiated with R or FR for 5 minutes immediately after being soaked, and then they were irradiated with FR or R, respectively, for 5 minutes before being kept in the dark. The results were checked 3 days later in the second lesson. The results obtained were consistent with the theory written in the textbooks as well as the results of previous studies shown in Figure 5.1, 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 changed to treatment light, R or FR, 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). Students were asked to fill in pre-lesson and post-lesson questionnaires (Appendixes 5.2 and 5.3).

In comparing the results of a pre-lesson questionnaire with that of a post-lesson questionnaire, students' comprehension did not change considerably after carrying out the experiment (Table 5.3). The high average scores of pre-lesson indicate that students still remembered the phenomena that R promotes and FR suppresses seed germination which they had learnt in the biology laboratory class in the previous year (2018). However, as the average score for Question 1 rose from 2.25±0.60 (pre-lesson) to 2.74±0.61 (post-lesson), students' understanding of the relation between wavelength and light was improved by the lesson. Although students understood well about the effects of R and FR on seed germination (pre-lesson average score was 3.08±0.76 and

Table 5. 3: The results of multiple-choice questions given to the students (n=24)

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

post-lesson average score was 3.43±0.58 for Question 2), their understanding of the mode of action of light, that causes the change in phytochrome structure which results in the promotion or suppression of seed germination, seemed to be insufficient even after carrying out the experiment (pre-lesson average score was 2.71±0.79 and post-lesson average score was 3.04±0.62 for Question 3). The lesson also improved students' understanding that plant hormones promote or suppress seed germination since the average score for Question 4 rose from 2.42±0.86 (pre-lesson) to 2.74±0.85 (post-lesson). These evaluated results indicated that the experimental apparatus developed can help students understand the wavelength concepts, the effects of R to promote and FR to suppress seed germination because teachers used the light feature data in table 5.1 and 5.2 to explain students and the experimental boxes were used for all experiments including the experiment about the effects of plant hormones on seed germination. In general, students expressed that they could understand the contents of the lesson (the average score for Question 5 was 2.96 ± 0.62), and they also replied that the experiment was interesting for them (the average score for Question 6 was 2.78±0.72). This indicated that experimental apparatus, experimental methods, and the way of teaching were interesting to them.

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 5.4). Further activities which students wanted to do after taking this class were an experiment using shorter period of light irradiation, an experiment with increasing light intensity, and so on (Table 5.5). Only a few students could not understand some aspects of this class: one student could not understand why the experiment similar to that in the last year was conducted, one

Table 5. 4: Pieces of knowledge which students obtained from the class (n=24)

Piece of Knowledge	*Number of Students*
Red light promotes seed germination, and far-red light suppresses seed germination	16
Number of seed germination affected by the duration of red and far-red light irradiation	13
The change of phytochrome structure by red and far-red light irradiation	8
The relation between light and wavelength	4

Table 5. 5: Further activities which students wanted to do (n=24)

Further Activities	*Number of Students*
To conduct experiments on shorter periods of light irradiation (in seconds)	15
To conduct experiments by increasing light intensity	3
To conduct experiments using seeds of other cultivars	1
To conduct the same experiment using plant hormones	1
To grow lettuce	1
No ideas	3

student could not understand about phytochrome, and another student could not understand why the reading point in the electric current meter was adjusted to 8 for R and to 5 for FR. Only three students were not interested in this class: one student said that it was difficult, one student said the experiment was similar to that in the previous year, and another student said that the experimental result was not the same as his/her predicted result. The pieces of knowledge that students obtained from this experimental class were mostly related to experimental results obtained from the use of the experimental box and some further activities they wanted to do were also related to the use of the experimental box to conduct an experiment.

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 result of the experiment was different their expected one, and three students could not understand clearly the mode of action of phytochrome. Students evaluated that the LED-attached box was helpful for them to understand the phytochrome response phenomenon (the average score for Question 11 was 0.24±0.53).

5.4.2. Situation in Cambodia

The LED-attached box was applied to a biology teacher training course in a laboratory class with 23 trainees at the National Institute of Education. The teacher trainees will be high school teachers after they finish their study at this teacher training institution. The concept of the phytochrome-mediated 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 another 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 5.4. They could understand the contents of the lesson well (the average score of Question 1 was 2.96±0.46) and this experimental class was very interesting for them (the average score for Question

Table 5. 6: The results of multiple-choice questions given to the teacher trainees (n=23)

Question	*Average Score*
Q-1. Can you understand this science lesson?	2.96±0.46
Q-2. Is this science class interesting for you?	3.61±0.49
Q-3. Did you get new knowledge or new ideas from this lecture?	2.83±0.38
Q-4. Do you think that this LED-attached box is useful for biology education?	2.91±0.50
Q-5. Do you think that you can set up these apparatus by yourself if there are enough materials available?	2.26±0.44
Q-6. Do you think that this apparatus is dangerous for students?	4.00±0.00

2 was 3.61±0.49) (Table 5.6). The trainees expressed that this LED-attached box is useful for biology education in Cambodia (the average score for Question 4 was 2.91±0.50), but they were not sure whether they can set up the apparatus by themselves (the average score for Question 5 was 2.26±0.44). The view that "This equipment is not dangerous for students" was shared by all the trainees (4.00±00). The trainees thought that this experimental box is appropriate for biology education in university (3 trainees provided the answer), 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 available materials for the apparatus set up (6 trainees), the explanation of the effect 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).

5.5. CONCLUSION

The LED-attached box, which has been developed in this study, is suitable to be introduced to biological education at the high school level. By means of this apparatus, students can study the effects of R or FR on phytochrome-mediated 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. Small batteries used can provide a stable electric current and they are appropriate for any school setting including those without 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.

Chapter VI

THE USE OF DWARF TOMATO CULTIVAR FOR GENETIC AND PHYSIOLOGY STUDY APPLICABLE FOR SCHOOL EDUCATION

Chapter VI

THE USE OF DWARF TOMATO CULTIVAR FOR GENETIC AND PHYSIOLOGY STUDY APPLICABLE FOR SCHOOL EDUCATION

6.1. INTRODUCTION

Plant physiology and genetic heredity were integrated in biology study in general education in many countries. Cambodian integrated the effect of plant hormones on plant growth and development in the 12th grade biology textbook, and Mendel's law of heritance in the 11th grade biology textbook (Yihoop *et al.*, 2008 and Yihoop *et al.*, 2009), while Japanese integrated plant hormone in senior high school level (Akasaka *et al.*, 2014 and Agata *et al.*, 2015) and Mendel's law of heritance in junior high school level (Arima *et al.*, 2016 and Tsukada *et al.*, 2016). Gibberilic Acid (GA) is one of the major plant hormone groups studied in general education in Cambodia and Japan (Akasaka *et al.*, 2014; Agata *et al.*, 2015; Yihoop *et al.*, 2009). GA was discovered by Japanese scientists (Kurosawa, 1926), and crystalized by Yabuta and Sumiki (1938). Currently GA is commercially used to enhance growth and productivity of many crops and vegetables. GA_3 has been used to conduct experiments by various ways of application. Different concentrations of GA_3 were applied on plants through different methods such as Miceli *et al.* (2019) applied in hydroponic solution for lettuce growth, Khan *et al.* (2006) sprayed on tomatoes, and Pal *et al.* (2016) sprayed on cucumber experiment. All parts of plants including height, branches, leaves per plant, leaf size, fresh weight, dry weight as well as flower and fruit, were measured in the study of the effect of GA_3 on plant growth and development (Khan *et al.*, 2006; Pal *et al.*, 2016; Miceli *et al.*, 2019). Khan *et al.* (2006) and Pal *et al.* (2016) reported that plants received GA_3 elongated longer than those in control. GA controls plant development by regulating plant physiological mechanism (Hooley, 1994). Lower internal GA level was well known for inducing some kinds of dwarf phenotype. Semi-dwarf mutant of rice, IR-8, was known as green revolution rice and this dwarf phenotype was responded sensitively to exogenous GA. Ashikari *et al.* (2002) revealed that this dwarf phenotype was caused by mutation of GA biosynthesis enzyme, GA20-ox2. However, dwarf phenotype was also introduced by mutation associated Brassinosteroid (BR). The dwarf gene of Rice d61 (Yamamuro *et al.*, 2000) and Barley Uzu (Chono *et al.*, 2003) were revealed as receptor mutation of BR. However, this mutant phenotype was known as pleiotropic effect expressed dwarf and wide leaf.

Mendel was a famous genetic scientist through his law of heritance, First law: Law of Dominance; Second law: Law of Segregation; and Third law: Law of Independent Assortment (Yihoop *et al.*, 2008; Gautam, 2018). There were many researchers conducting experiments to trace Mendel's law such as Smykal *et al.* (2016) cited that in the early 1900s de Vries, Correns, and Tschermak had published each research paper to confirm Mendel's second law (3:1). Even though there have been many papers published so far, there are not any papers that have yet been rejected Mendel's law of heredity.

The effect of GA on plant growth and Mendel's law of heredity were integrated in biology textbooks in many countries, but students might learn from the theories written in the textbooks without experimental practices. In my study, I introduced the experiments which are applicable for school education. Tomato is a good plant material for pupils to grow easily in kindergarten and to do simple research in primary schools in Japan. It is an important crop worldwide, and a model system for genetic studies in plants (Barone *et al.*, 2007). Yui *et al.* (2011) suggested that tomato is a good material to study genetics because many characters followed Mendel's law can be observed. In my preliminary research, I purchased 25 cultivars of tomato seedlings sold in Japanese garden shops and then all cultivars were transplanted to the field for physiological growth examination. As a result, I found that one cultivar "Regina" was a dwarf tomato and other tomatoes were normal stem tomato plants. Among the normal type tomato plants, "Momo" showed the different stem height and fruit color. Therefore, I selected Regina and Momo for my study materials.

6.2. MATERIALS AND METHODS

6.2.1. Plant Materials

In this research, self-fertilized plants of cultivar 'Regina' (dwarf type) and cultivar 'Momo' (normal type) were used as plant materials.

6.2.2. Physiology Study

Gibberellic acid (GA_3: Sigma-Aldrich) was dissolved in distilled water to make three different concentrations, 1mg/L, 10mg/L and 100mg/L. Seedlings with 3 true leaves of normal and dwarf types were used for exogenous GA_3 treatment in this study. Four seedlings were used in each concentration GA_3 treatment including water treatment as control. Each seedling was sprayed on the whole plant with 1.5 mL of distilled water, 1mg/L, 10mg/L, or 100mg/L GA_3 solution at 2 weeks after transplanting and the second treatment was done 2 weeks after the first treatment. The length of stem and internode were measured every week from the starting of GA_3 treatment by a ruler and caliper respectively.

6.2.3. Genetic Study

The dwarf type plant was crossed with the pollen of the normal type plant. The crossing techniques were done as follows. From female plant (dwarf type): (1) selected young flower with green anthers at not full blooming stage (Figure 6.1a); (2) emasculation was done to pick up the anthers carefully by forceps without damaging of stigma (Figure 6.1b), and remained all parts of flower after removing anthers (Figure 6.1c). From the male plant (normal type): selected a full-bloom flower and used another forceps without pollen to break down the anthers in order to make pollen grain attached to the forceps (Figure 6.1d). The forceps with the pollen grain was tapped on the stigma of the female plant (Figure 6.1e). The flower pollinated were labelled (Figure 6.1f).

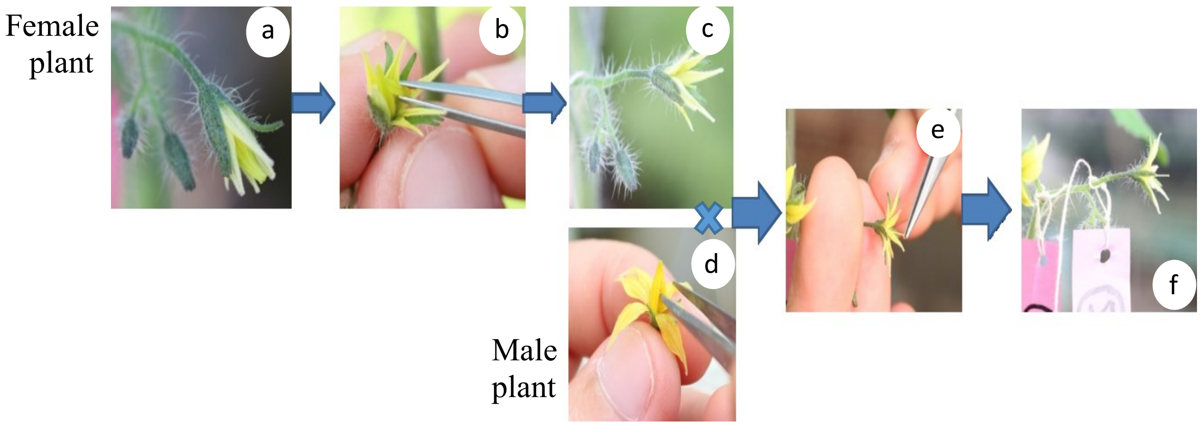


Figure 6. 1: The method to cross tomato, **a**. young flower; **b**. emasculation, **c**. flower after removing anthers, **d**. breaking down the anthers, **e**. tapping pollen on stigma, and **f**. flower after crossing.

The success of crossing could be confirmed by the youngest fruit observed in one week, and the mature fruits were obtained in 5 to 6 weeks from the crossing. The matured fruit was cut into small pieces, placed the pieces on a net, poured tap water to wash the pieces to remain the F_1 seeds on the net, and then the seeds were placed on

Kimwipes paper for 3 days to dry. F_1 seeds were sown to check their phenotypes and some of F_1 plants were transplanted to the field and kept for self-fertilization in order to produce F_2 seeds. F_2 seeds were sown in order to check their seedling phenotypes. F_2 populations were counted by pulling them out from the soil. The experiments were conducted for 2 times repeatedly by counting the seedlings within different stages.

6.2.4. Statistical Analysis

Statistical significances of GA treatment were analyzed by free software Real Statistics Using Excel (Charles Zaionts) with one factor ANOVA followed up option Turkey HSD with p-value < 0.05.

The statistical test of F_2 segregations, probabilities of Chi-square was calculated by free online software for Calculation for the Chi-square test by Kristoper J. Preacher ©2010-2020.

6.3. RESULTS AND DISCUSSION

6.3.1. Physiological Study

Tomato plants responded to GA_3 treatment generally enhanced stem elongation compared to the control treatment in my experiments. Both dwarf type and normal type responded to 100mg/L GA_3 elongated the longest among other treatments, except normal type 2 weeks after treatment (week-2: Figure 6.2). This elongating result indicated the same effect of GA on plant growth written in biology textbooks and previous researchers (Hooley, 1994; Khan *et al.*, 2006; Yihoop *et al.*, 2009; Akasaka *et al.*, 2014; Agata *et al.*, 2015 and Pal *et al.*, 2016). The dwarf type tomato could elongate twice longer than the control treatment (Figure 6.2). This result suggested

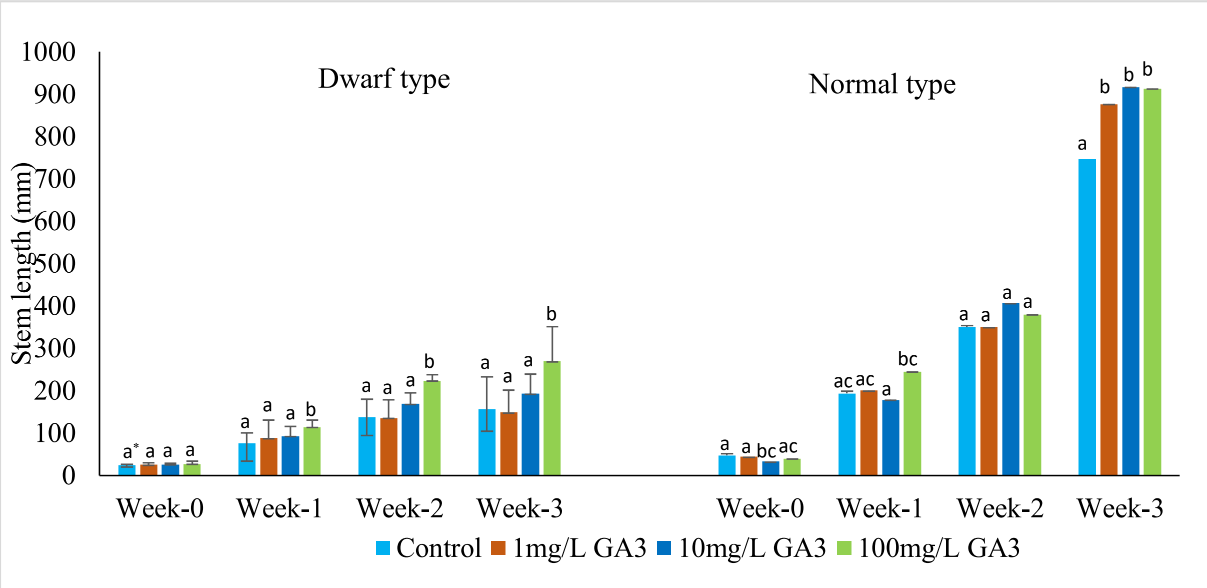


Figure 6.2: Average length of tomato plants 'Regina and Momo' under treatment of different GA_3 concentration. Week-0 is the started point of GA_3 treatment.

*Different letters on the bars show significant differences between treatments at a certain week by Real Statistics Using Excel (Charles Zaiontz) One Factor Anova follow-up option Turkey HSD p-value < 0.05

that GA_3 has a strong effect on dwarf plant recovery. In my research, I also conducted the experiment on the effect of Brassinosteroid on the dwarf type tomato by applying the same method as those of applying GA_3, but Brassinosteroid did not have any effect on stem elongation of this dwarf type. However, Brassinosteroid mutants with dwarf phenotype often expressed pleiotropic effect as dwarf and short length leaf. Because the leaf of dwarf type used in this study showed normal character, this dwarf character did not look like as a Brassinosteroid mutant. Recently, genes of Brassinosteroid dwarf of rice and barley were isolated and this dwarf was regulated by the gene of Brassinosteroid receptor (Yamamuro *et al.*, 2000; Chono *et al.*, 2003). In tomato, cultivar 'Micro-tom' expressed a Brassinsteroidic dwarf phenotype. The gene of this dwarf revealed that this mutation phenotype was caused by mutation of brassinosteroid biosynthesis (Marti *et al.*, 2006). Because both leaf mutant and receptor mutant expressed short length leaf in the brassinosteroid, I thought that dwarf type ‘Regina’ was caused by mutation of GA biosynthesis.

The effects of GA_3 on internode elongation were paralleled to those effects on stem growth. GA_3 had strong effects on internode elongation during the period of internode growing stage. The period of internode with potential growth was within one week after its induction. This growth was gradually decreased in the following weeks ‘week-2’ and some internodes were shortened in week-3 such as internode number 2 of dwarf type responded to 1.5mL of distilled water, 1mg/L GA_3, and 10mg/L GA_3 increased a lot at week-1, but this growth increased a little at week-2, and then they

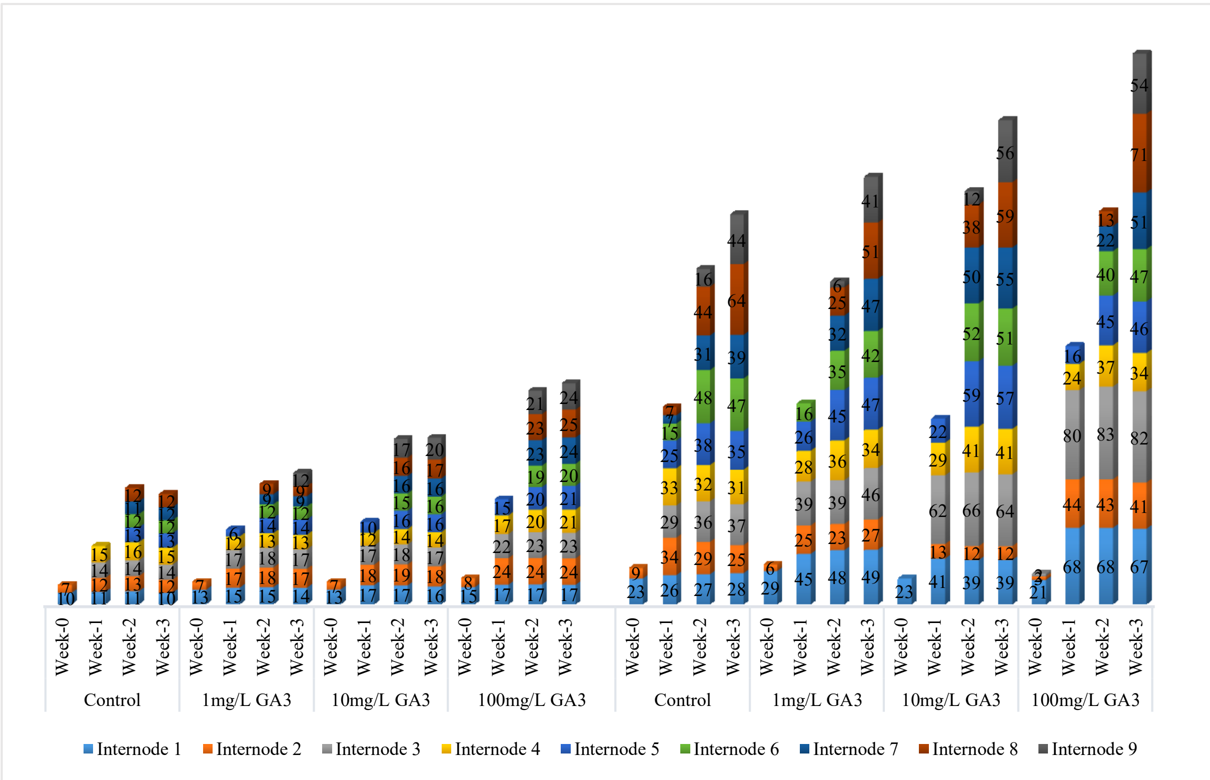


Figure 6.3: Average length of internode of tomato plants “dwarf and normal types” under treatment of different GA_3 concentration or distilled water from start point to 3 weeks later. The figure value in each internode image showed the average length (mm).

decreased a little at week-3 (Figure 6.3). The internode number 2 of normal tomatoes responded to 1.5 mL of distilled water or 100mg/L GA_3 were also a little shorten in week-3 (Figure 6.3). The internode number 2 of normal tomato responded to 1.5 mL of distilled water or 100mg/L GA_3 were also a little shortened in week-3 (Figure 7.3). The shortening of internode indicates that stem or internode grows when it is young that cells have possibility to divide and the node is also enlarged during the time of internode elongation stop. GA_3 affected young internode elongation, but this effect could not be observed in older internodes such as internode number 1 (Figure 6.3). The experiment on the effect of GA_3 on stem elongation did not only explain the effect of GA_3 on stem elongation, but also could explain students about the growing stage of the plant. Plant height increases depending on the growth of meristem at the top of the plant, not depending on the older parts of the plants.

6.3.2. Genetic Study

F_1 plants resulting from the cross between dwarf type and normal type were all expressed as the phenotype of normal type character in stem height. This result can confirm to Mendel's 1st Law of Dominance "the characters appear in F_1 generation are called dominant characters and the characters remain hidden are called recessive characters", "if the parents are homozygotes, all F_1 hybrids have the same phenotypes" and "tall is dominant on short" (Yihoop *et al.*, 2008; Gautam, 2018).

Figure 6.4: F_2 population resulted from the cross between Regina (short) and Momo (height), the photo was taken 3 weeks from seed sowing.

There were segregations among F_2 population obtained from the cross between dwarf type and normal type, and normal-stem types were shown larger numbers than dwarf ones at seedling stage (Figure 6.4). The F_2 segregation can be used to confirm Mendel's law of segregation, 3 dominant phenotypes and 1 recessive phenotype (3:1). F_2 population obtained from four F_1 plants in table 7.1 and six F_1 plants in table 7.2 showed a high tendency to reach the ratio 3:1. Too young plants were more difficult to identify than the plants at 3rd leaf stage or over. The F_2 populations obtained from six F_1 plants in table 7.1 mostly had only 1st true leaf that caused more difficult to identify between the normal stem and dwarf stem. The F_2 segregation of tomato plants having 3rd true leaves were easier to identify between normal stem and dwarf stem, and had high probabilities to reach 3:1 ratio (Table 6.2). In table 6.2, even though F_2 segregation received from other four F_1 plants had low Chi-square p-value, 0.102 to 0.290, but this value also did not have statistic differences ($p>0.05$) to explain

Table 6. 1: F_2 segregation of normal stem and dwarf stem tomato at 1st true-leaf stage in F_2 population obtained from the cross between dwarf tomato 'Regina' and normal tomato 'Momo'

Parents of F_2 (F_1 Plant)	Number of F_2 Plants	F_2 Phenotypes (Number of plants)				Probabilities of Chi-square (p-value)
		Normal stems		Dwarf stems		
		Observed	Expected	Observed	Expected	
1	55	42	41.25	13	13.75	0.815
2	44	32	33.00	12	11.00	0.734
3	44	32	33.00	12	11.00	0.734
4	46	32	34.50	14	11.50	0.395
5	58	39	43.50	19	14.50	0.172
6	66	43	49.50	23	16.50	0.064
7	52	33	39.00	19	13.00	0.054
8	68	41	51.00	27	17.00	0.005
9	60	35	45.00	25	15.00	0.003
10	57	31	42.75	26	14.25	0.0003

Table 6. 2: F_2 segregation of normal stem and dwarf stem tomato at 3rd true-leaf stage in F_2 population obtained from the cross between dwarf tomato 'Regina' and normal tomato 'Momo'

Parents of F_2 (F_1 Plant)	Number of F_2 Plants	F_2 Phenotypes (Number of plants)				Probabilities of Chi-square (p-value)
		Normal stems		Dwarf stems		
		Observed	Expected	Observed	Expected	
1	50	37	37.50	13	12.50	0.870
2	63	48	47.25	15	15.75	0.827
3	72	52	54.00	20	18.00	0.586
4	65	46	48.75	19	16.25	0.431
5	69	55	51.75	14	17.25	0.366
6	74	59	55.50	15	18.50	0.347
7	67	54	50.25	13	16.75	0.290
8	66	54	49.50	12	16.50	0.201
9	73	60	54.75	13	18.25	0.156
10	72	60	54.00	12	18.00	0.102

Table 6. 3: F_2 segregation of Mendel's experiments on garden peas

Characteristics	F_2 Phenotypes				Probabilities of Chi-square (p-value)
	Observed	Expected	Observed	Expected	
Stem height	Tall stems		Short stems		0.436
	787	798	277	266	
Flower color	Purple flowers		White flowers		0.544
	705	697	224	232	
Seed color	Yellow seeds		Green seeds		0.897
	6022	6017	2001	2006	
Seed character	Round seeds		Wrinkled seeds		0.608
	5475	5494	1850	1831	
Pod color	Green pods		Yellow pods		0.502
	428	435	152	145	

Source of data: Cambodian biology textbook (Yihoop et al., 2009) and Genetics Second Edition (Weaver R.F. and Hedrick P.W., 1992)

the F_2 segregation to follow Mendel' s law. Moreover, Mendel's experiment Chi-square is also low in term of stem height, p-value: 0.436 (Table 6.3). In my experiment, F_2 segregation obtained from some F_1 plants were higher Chi-square value than those of Mendel's experiment with the highest p-value: 0.870 (Table 6.1, Table 6.2, and Table 6.3).

6.4. POSSIBILITY SCHOOL EDUCATION APPLICATION METHODS

The experiments on the effects of GA_3 on tomato physical growth and the use of tomatoes to trace Mendel's law needs a few months to finish so these experiments should be applied as extra-research activities for students at school. These experiments are applicable for school education because students are familiar with tomato, GA_3 source could be purchased at a chemical store or online shop, and the crossing methods are also easy for students to handle. The implication of the application is that students need a long time to take care of their tomato plants. The method of spraying GA_3 on stem is a good technique to apply GA_3 because the students in developing countries including Cambodia are not familiar with hydroponic growing. Even though experiment practice is preferable, the use of these experimental results in classroom teaching is another method to apply the use of tomatoes to teach physical growth and genetic study. The data in these experiments are evidence to help students understand the effect of GA_3 on plant growth and to confirm Mendel's law of heredity. While Chi-square test is too difficult for high school students, teachers can know that students counted the data match the expected value. When p-value is small but bigger than 0.05, students may misunderstand that their observed data does not follow Mendel's Law. In that case, I propose teachers to ask their students to compare between observed value and expected value by themselves. They will understand that the observed value is not so different from expected value.

6.5. CONCLUSION

Dwarf tomato induced by mutation of GA biosynthesis is a good plant material for physiology and genetic study which are applicable for school education. Normal stem tomatoes are observed in many farms, but dwarf tomatoes could not be observed a lot at a farm or daily life. Therefore, the selection of tomato plants including dwarf and normal plants are essential for the study. The theories describing the effect of GA on plant growth and Mendel's law of dominance and segregation could be confirmed by the experiments using tomato plants and they are applicable for school education because the experiments can be conducted by using simple equipment that students can find in their daily-life equipment.

Chapter VII

GENETICS MATERIALS FOR EXPERIMENTAL CLASS OF MENDEL'S 3RD LAW USING DIHYBRID CROSSES OF LETTUCE CULTIVARS IN HIGH SCHOOL

Chapter VII

GENETICS MATERIALS FOR EXPERIMENTAL CLASS OF MENDEL'S 3RD LAW USING DIHYBRID CROSSES OF LETTUCE CULTIVARS IN HIGH SCHOOL

7.1. INTRODUCTION

Lettuces were classified in the family Asteraceae, genus *Lactuca* L. Lettuces are leaf vegetables and famous plant materials for genetic study such as Chin *et al.* (2001) used lettuce to study recombination and spontaneous resistance genes, Jeuken *et al.* (2002) investigated the genetics and specificity of *Bremia* resistance in *L. saligna*, Christopoulou *et al.* (2015) dissected two complex clusters of resistance genes in *L. sativa*, and other articles reported their study on disease resistance genes in lettuce (Landry *et al.*, 1987; Kuang *et al.*, 2006). Lettuces have many traits derived from natural and induced mutations that are useful in genetic studies (Mou, 2011).

The use of lettuce to trace Mendel's law of inheritance was also performed by some researchers. Lindqvist (1960) crossed lettuce to trace Mendel's law of segregation (2nd law). Truco *et al.* (2007) reported the result of intra-specific crosses of lettuce species to study on integrated genetic linkage map that the majority of markers segregated match to Mendelian expectations. Even though Mendel used garden peas to conduct experiments that led to the definition of the law of inheritance, genetics experiments for junior high school or senior high school in the world should use usable plant materials. Recently, I introduced the use of tomato cultivars to trace Mendel's 1st and 2nd laws (Mam *et al.* 2020b). This study suggested that the dwarf tomato plant was a good plant material to be used by students to conduct research to study genetic inheritance following Mendel's 1st and 2nd laws because the dwarf trait was expressed at an early stage of tomato growth and it was easy to be recognized by students. Mendel's laws of inheritance including the 3rd law, "Law of Independent Assortment", are being studied in high school in Japan and Cambodia (Yihoop *et al.*, 2008; Arima *et al.*, 2016; Tsukada *et al.*, 2016). However, only Mendel's experiment results are being studied in these biology textbooks, there were no other experiments used to explain Mendel's Law of Independent Assortment in those biology textbooks, and there was no experimental class introduced in the textbooks. The phenotypic ratio of Mendel's 3rd law is 9:3:3:1 in the combinations of alleles of two different genes. The lowest rate is 1/16 as double recessive type in F_2 segregation. Theoretically, when teachers want students to observe 5 double recessive plants, teachers have to keep more than 80 plants until plants expressed the target characters. In tomato plants, while several fruit character differences were introduced for genetic study (Yui *et al.*, 2011), they are very difficult to keep more than 80 tomato plants until getting fruits in the schools.

In this study, I performed dihybrid crosses using lettuce cultivars to produce study materials for the experimental class of Mendel's Law of Independent Assortment. Lettuces were used in this study because lettuce leaf characters could be identified easily, their growth cycles were short and also many seeds can be obtained in a lettuce plant.

7.2. MATERIALS AND METHODS

7.2.1. Plant Materials

Lettuce cultivars (cv.) purchased in Japan were used in this experiment. ‘Furiru lettuce’ with green oak leaf and other 3 cultivars, ‘Red sunstar’, ‘Sunny’, and ‘Vitamin’, with red lobed leaf were used in this dihybrid cross experiment.

7.2.2. Crossing Techniques

Reciprocal crosses between ‘Furiru lettuce’ and ‘Red sunstar’, ‘Sunny’ or ‘Vitamin’ were performed. The crossing method followed the YouTube Video (Work with Nature, 2014). From female plant, the flower bud was selected in the early morning at around 6:00 AM (Figure 7.1a), the yellow part of the flower was cut (Figure 7.1b), the flower cut was kept for about 3 hours to make style elongate (Figure

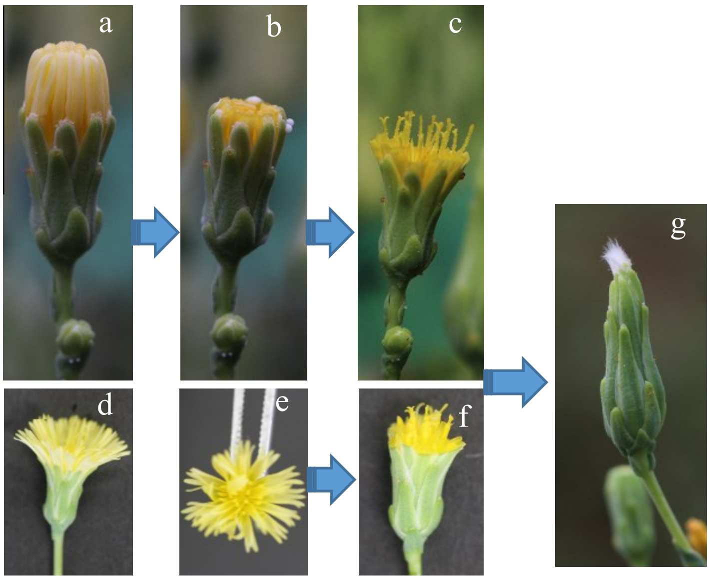


Figure 7. 1: Crossing technique : **a.** flower bud, **b.** cut flower bud, **c.** pistil elongated from flower bud, **d.** full bloomed flower, **e.** top view of bloomed flower showing pistil with pollen, **f.** bloomed flower after cutting petal, **g.** lettuce fruit.

7.1c) and then the flower was sprayed or soaked in water to remove their pollens. From male plant, at the crossing time, a full-bloomed flower was selected (Figure 7.1d and Figure 7.1e), the petals of the flower were cut to make naked style with pollen (Figure 7.1f), and then this flower was tapped on the female flower (Figure 7.1c) to pollinate. The fertilized fruit could be observed a few days after crossing (Figure 7.1g).

7.2.3. F_1 and F_2 Plant Cultivation

F_1 seedlings obtained from the cross combinations between 'Furiru lettuce' and the 3 lettuce cultivars were transplanted to the 20cm diameter pots with soil. Totally, twenty five F_1 seedlings obtained from 'Furiru lettuce' used as the male parent and 25 F_1 seedlings obtained from the 'Furiru lettuce' used as female parent in each cross combination of each cultivar were transplanted and the F_1 plants were kept to produce F_2 seeds by self-pollination.

Approximately 300 F_2 seeds of an F_1 plant were sown on kitchen paper towels in 10 Petri dishes and then these seeds were irradiated with red light in order to maximize the seed germination. The seedlings germinated under red light irradiation in a room with a controlled temperature around 24°C, 3 days after seed sowing, were adapted to sunlight and outside temperature for one day before transplanting to seedling trays. Around 230 to 250 seedlings were transplanted to seedling trays for one week before transplanting to the land in the total area of about 3 m^2 in a field.

7.2.4. Statistical analysis

The statistical test, probabilities of Chi-square was calculated by free online software for Calculation for the Chi-square test by Kristopher J. Preacher ©2010-2020.

7.3. RESULTS AND DISCUSSION

Lettuce leaf traits, leaf color and leaf form, could be identified from 3 weeks after transplanting to the field when the growth of lettuce had 4 to 5 true leaves. The leaf size was also different between parents but I did not adopt leaf size for genetic study because the leaf size is generally affected by the environment and the plant growth itself. The F_1 lettuce plants were transplanted in a plastic greenhouse in early November and the leaf characters were checked 6 months after transplanting. Leaf of 'Furiru lettuce' expressed a green oak leaf with the deeply curled margins (Figure 7.2, left) and the leaves of 'Red sunstar' (Figure 7.2a, right), 'Sunny' (Figure 7.2b, right) and 'Vitamin' (Figure 7.2c, right) expressed red lobed leaves. All F_1 plants survived, around 25 F_1 plants of each cross combination, expressed traits of red oak leaves with the deeply curled margins (Figure 7.2a, Figure 7.2b, and Figure 7.2c, middle). These results indicated that red leaf color might be the dominant trait inherited from 'Red sunstar', 'Sunny' and 'Vitamin', while the oak leaf might be the dominant trait inherited from 'Furiru lettuce'. The leaf form trait inheritance in this study was the same as those reported by Lindqvist (1958), *i.e.* the oak leaf trait was dominant on the lobed leaf character. The F_1 leaf color character in this study was the same as those reported by Thompson (1938). Both leaf traits inherited in this experiment followed Mendel's Law of Dominance that all F_1 offspring inherited one character from a female

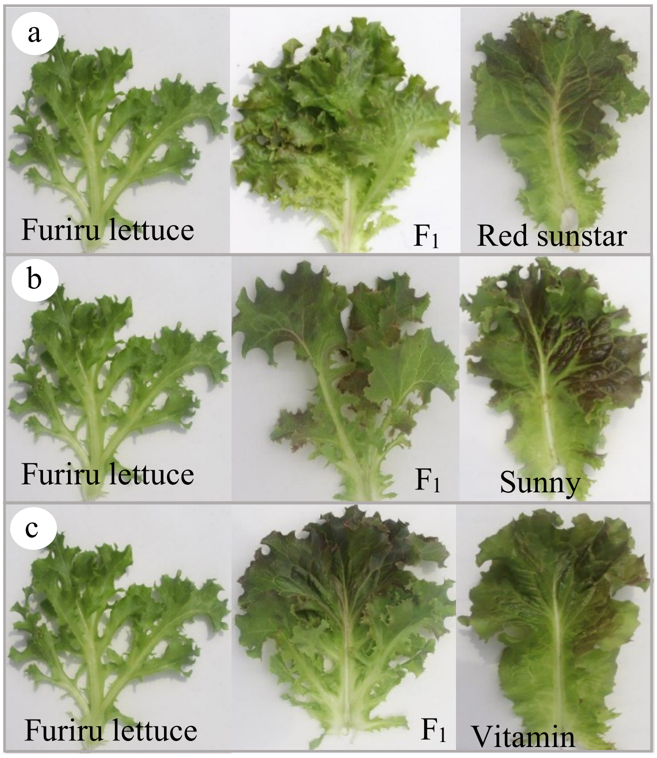


Figure 7. 2: Leaf traits of F_1 plants and their parents at 6 months after transplanting to pots. A reciprocal F_1 lettuce leaf (middle) and the parents' leaf (left and right).

or male plant. The red and oak leaf characters that appeared in F_1 offspring are called dominant characteristics and the green and lobed characters remain hidden are called recessive characters.

In the F_2 populations, four different phenotypes: red oak leaf (RO), red lobed leaf (RL), green oak leaf (GO) and green lobed leaf (GL) appeared from each F_1 derived from each reciprocal cross combination (Figure 7.3). F_2 segregation ratios of all cross combinations matched as RO: GO: RL: GL= 9: 3: 3:1 (Table 7.1) which are the same as the results of Mendel's dihybrid crosses and statistical analysis of all combinations between 'Furiru lettuce' and 3 cultivars were confirmed without deviation from 9:3:3:1 by Chi square test (Table 7.1). Mendel crossed between round yellow seed and wrinkled green seed peas. He obtained only round yellow seed plants in the F_1

generation; and round yellow, wrinkled yellow, round green and wrinkled green seed plants in the phenotypic ratio 9: 3: 3: 1 in F_2 generation (Figure 7.4).

The dihybrid cross between the green oak leaf lettuce and red lobed lettuces obtained only red oak leaf plants in F_1 generation so the parents using this cross were homozygotes in leaf color alleles and leaf form alleles. In this lettuce leaf color, because Red leaf color expressed dominant and Green leaf color expressed recessive, the former allele was represented as "*R*" and the latter allele was represented as "*r*", tentatively. In the lettuce leaf form, because Oak leaf expressed dominant and Lobed leaf expressed recessive, the former allele was represented as "*O*" and the latter allele was represented as "*o*", tentatively. Therefore, the genotype of 'Furiru lettuce' with the green oak leaf becomes "*rrOO*" and the genotype of the three cultivars with the red lobed leaf lettuces becomes "*RRoo*". The reciprocal F_1 offspring which had the red oak leaf had the genotype "*RrOo*". According to the Law of Independent Assortment, combinations of alleles of two different genes gave phenotype ratio of 9: 3: 3: 1. Because my lettuce leaves in F_2 segregations were also approximately same as 9: 3: 3: 1 ratio, statistically, inheritance of either leaf color gene or leaf form gene was independent. The segregation of "*R*" and "*r*" is independent of the segregation of "*O*" and "*o*" in gametes formation. Therefore the F_1 hybrid formed 4 different genotypes of gametes, *RO*, *Ro*, *rO*, and *ro*. In the fertilization time, these gametes paired up to form the progenies of 9 different genotypes in F_2 generation and 4 different phenotypes including red oak leaf, green oak leaf, red lobed leaf, and green lobed leaf plants in the ratio 9: 3: 3: 1 (Figure 7.4). The results of lettuce dihybrid crosses in this experiment were the same as those of Mendel's dihybrid cross of pea plants (Figure 7.4).

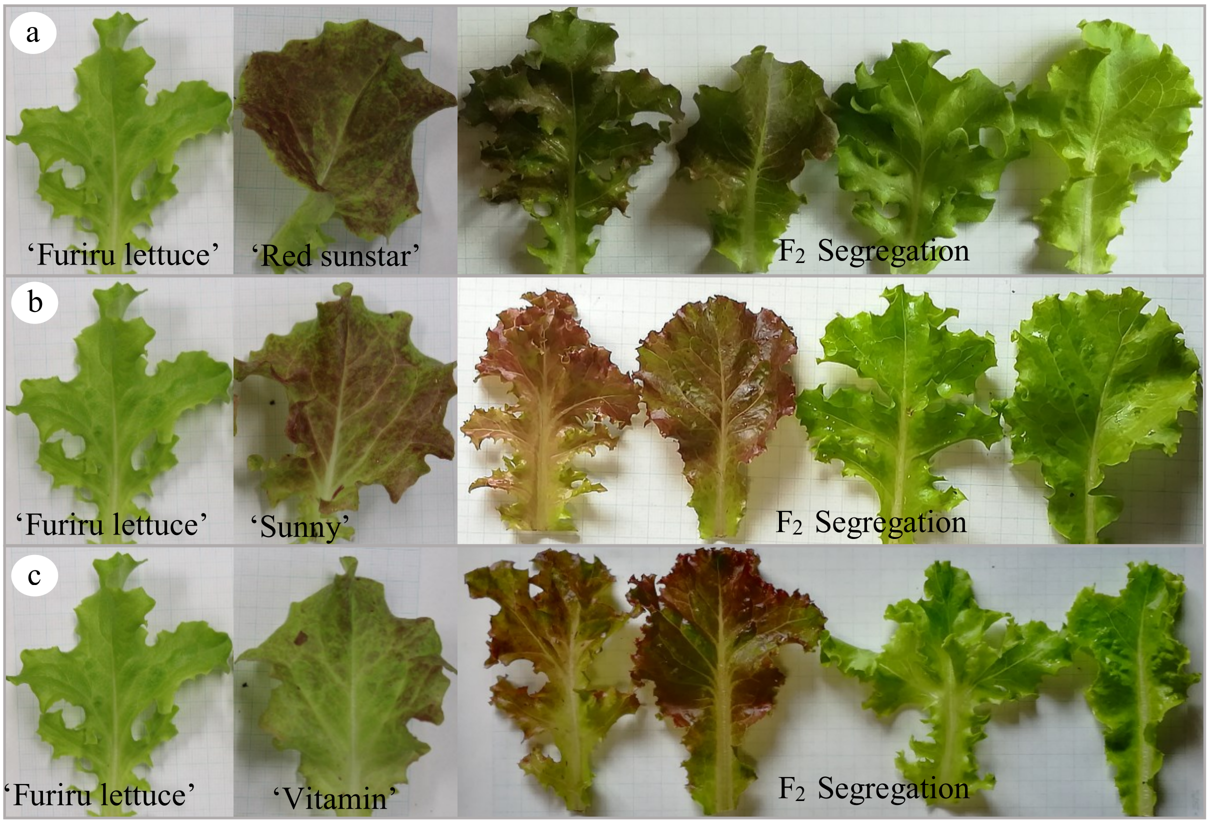


Figure 7. 3: Segregation of F_2 plants' leaf traits comparing to their parents' leaf traits at 3 weeks after transplanting to pots for parents' plant (in winter), and to field for F_2 plants (in summer). Two leaves at the right side of each figure are the parent plants' leaves. Four leaves at the left side are F_2 plants' segregated leaves.

Table 7. 1: The segregation of F_2 lettuce plants (Dihybrid characters), the ratio 9: 3: 3: 1

Reciprocal F_1 hybrid with 'Furiru lettuce'	Number of F_2 Plants	Segregation of F_2 plants								Chi-square test		
		Red oak leaf		Red lobed leaf		Green oak leaf		Green lobed leaf		Chi-square	Degree of freedom	p-value
		Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected			
Red sunstar	245	140	137.8	35	45.9	54	45.9	16	15.3	4.085	3	0.252
Sunny	103	52	57.9	21	19.3	22	19.3	8	6.4	1.529	3	0.676
Vitamin	172	85	96.8	37	32.3	37	32.3	13	10.8	3.254	3	0.354

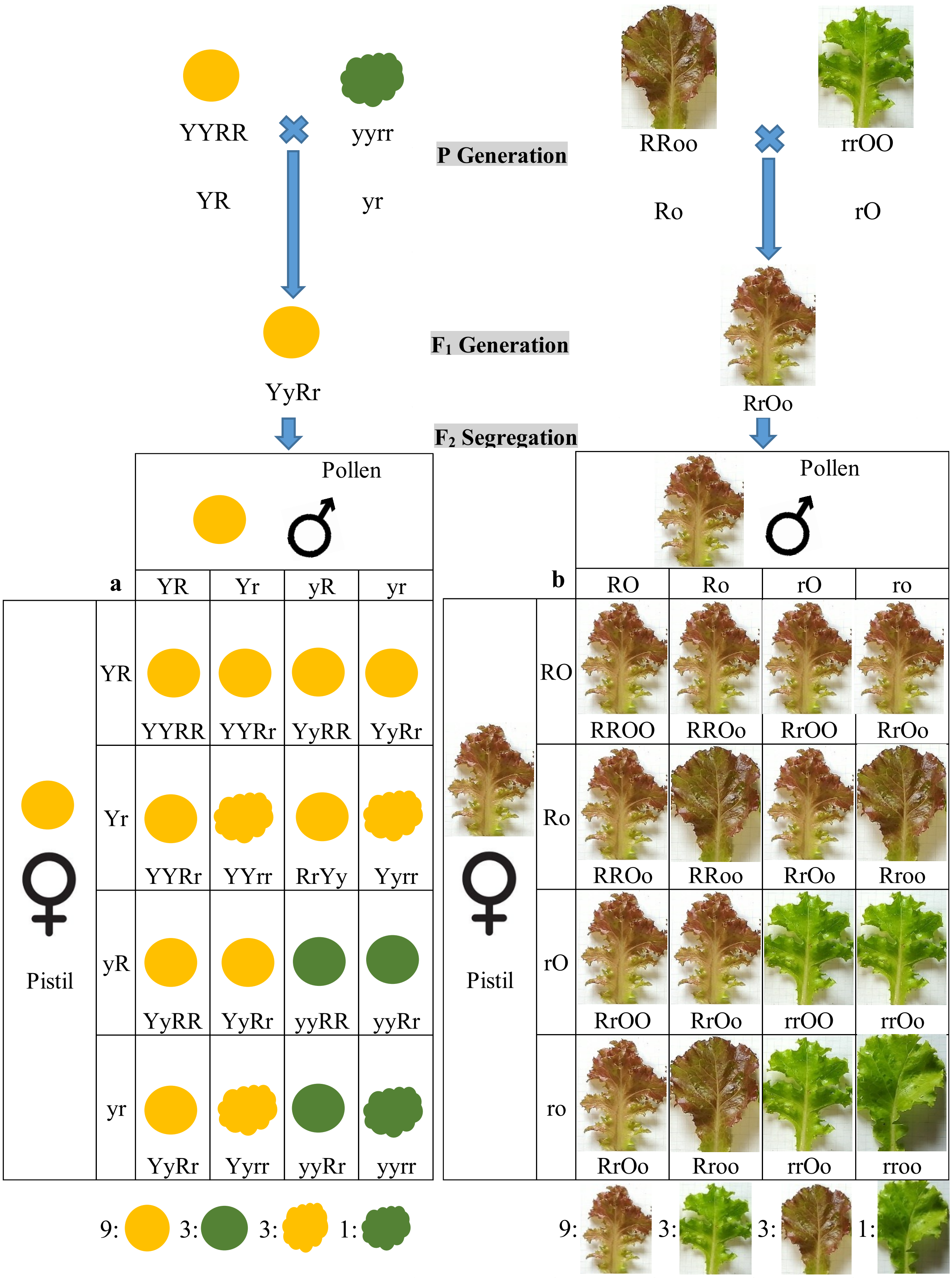


Figure 7. 4: Dihybrid crosses and their F_2 segregation, **a.** Mendel's cross between yellow round seed pea and green wrinkled seed pea (Data source from Yihoop *et al.*, 2009 and Gautum, 2018), **b.** A cross between the red lobed leaf lettuce and green oak leaf lettuce.

Mendel crossed between pure line pea plants of dominant traits in a plant “yellow round, *YYRR*” and recessive traits in another plant “green wrinkled, *yyrr*”. But in my study, I crossed lettuce plants of both dominant and recessive traits in a plant, “red lobed leaf, *RRoo*” and “green oak leaf, *rrOO*” (Figure 7.4). The dihybrid crosses in this study and Mendel’s dihybrid cross could obtain the same results of F_2. These results can confirm Mendel’s Law of Independent Assortment that during a dihybrid cross, an assortment of each pair of traits is independent of the other. The gametes of lettuce plant having both dominant and recessive alleles “*Ro* and *rO*” while the gametes of pea plants having the dominant or recessive alleles “*YR* and *yr*”, the F_1 of these crosses were heterozygous for both traits, *i.e. RrOo* and *YyRr* for a red oak leaf lettuce and a yellow round pea respectively (Figure 7.4).

7.4. POSSIBILITY SCHOOL EDUCATION APPLICATION METHODS

The use of experiments as a teaching methodology in science lessons is preferable, but not all experiments are suitable to be applied to the classroom. The experiments introduced in this study are applicable to biology education as a research activity in fieldwork, and the experimental data and figures are the biological source for studying Mendel’s Law of Independent Assortment.

7.4.1. Application to field-work

The use of lettuce to trace Mendel’s law of inheritance is not difficult for students and teachers, but they should pay attention to 4 factors leading to the success of their experiments including lettuce cultivar selection, temperature, light, and number of F_2 plants.

1. The selection of lettuce cultivars is an essential factor for the success of the experiment and to achieve the objective. For example, if teachers want students to study the dihybrid traits, parent plants that have two different leaf traits such as leaf color and leaf form are required. In this study, I selected 'Furiru lettuce' with green oak leaves and other 3 cultivars with red lobed leaves to conduct experiments. Lettuce cultivars with homozygous alleles for target traits might not be difficult to select at the market because most of commercial cultivars might be produced by self-fertilization in order to keep the same traits.

2. The appropriate seasons are required to start lettuce growing. In my study lettuce grew well, vegetative growth, in a plastic greenhouse in winter which has cool temperature and short day length in Japan. This result was the same as Lee *et al.* (2015), and the optimum temperature for lettuce growing is 18.5^{0}C (Wallace *et al.*, 2012). In my study of lettuce inheritance, the vegetative growth is not a preference factor. I need lettuce plants to induce flowers as soon as possible. Lettuce plants elongated stems and induced flowers within 3 months in summer and it did not induce flowers in the winter. Wallace *et al.* (2012) also reported that lettuce induced flowers in the temperature between 21 to 27^{0}C, and Waycott (1995) reported that lettuce elongated stem and initiated flowers in the long day length and high temperature. Therefore, lettuce plants cultivation should be started at the end of winter because the plants will grow well at that time and they will induce stem and followers in the following 2 or 3 months and the cultivation of F_1 and F_2 plants will be continued consequently in order to finish the field activities within one year. In a tropical country such as Cambodia, the temperature and day length might not be the problem because the temperature did not change a lot within the year varying from 22 to 35^{0}C and the

day length varied from 11.30 to 13.00 hours. Therefore, students and teachers might be able to start their lettuce cultivation any time in the year.

3. Light affects on red leaf color. In this study the red leaf color was difficult to identify when the plants were grown in a plastic greenhouse, but the red leaf color could be observed clearly when the plants were moved to the outside. Ohashi-Kaneko *et al.* (2007) reported that the pigment content in lettuce leaves was affected by different kinds of light. Therefore, lettuce plants should be grown in the field that plants can be exposed to sunlight.

4. The larger number of F_2 plants are better for this experiment because the result should meet the 9:3:3:1 ratio. The number of F_2 plants should remain at least around 100 at the time of leaf identification. Therefore, about 120 to 150 F_2 plants should be transplanted to the field. Lettuce plants can grow in a small space. I can grow up to 250 lettuce plants in the land area about 3 m^2 in a field in this study (Figure 7.5). Because lettuce can be grown indoors with LED (Light Emitting Diode) illumination in Japan, it is a good plant to be able to cultivate in a small space.

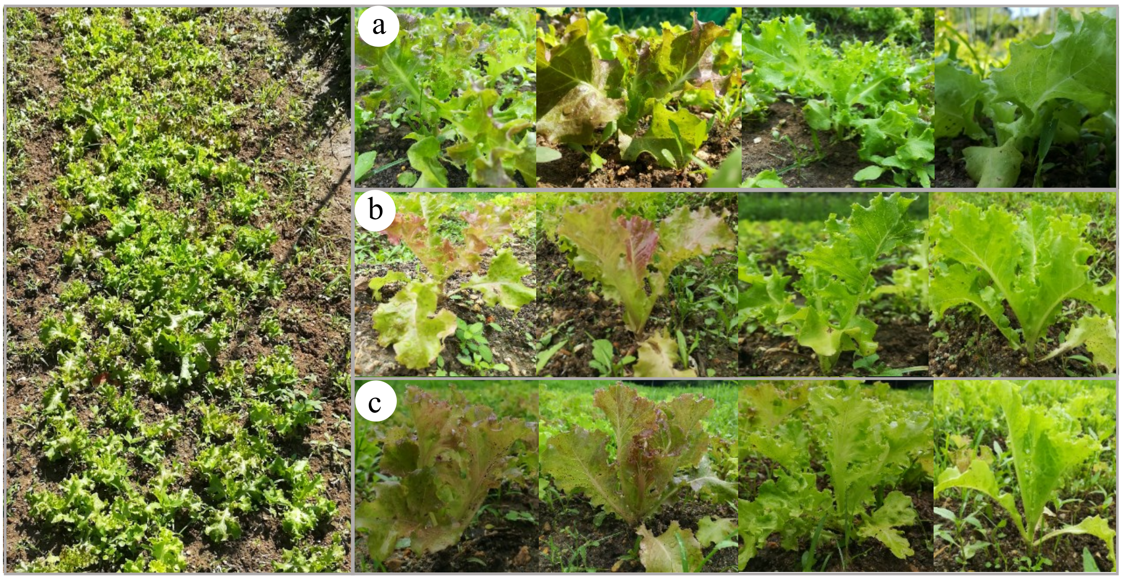


Figure 7. 5: F_2 plants cultivation at the 3 weeks after transplanting, **a.** Red sunstar segregation, **b.** Sunny segregation, and **c.** Vitamin segregation

7.4.2. Application to classroom

The data and figures in this study can be used as a reference for Mendel's law lesson in addition to the content in the biology textbooks. The results of this experiment are additional information to students understanding Mendel's Law of Independent Assortment. A teacher should ask students to compare Mendel's dihybrid cross and lettuce dihybrid cross in Figure 7.4. The teacher should propose two questions for students to make an inquiry: "What are the different genotypes at parent generation of the lettuce cross in this experiment and Mendel's pea cross?", and "Why the F_2 result of the lettuce cross and Mendel's cross have the same phenotypic ratio?"

7.5. CONCLUSION

The experimental methods and the crossing technique used in this study are suitable to be introduced to biological experiments in high school levels. Lettuce is an easy plant to grow and they are also easy to fertilize. The results of these dihybrid crosses are good evidence to explain Mendel's Law of Independent Assortment.

Chapter VIII

GENETICS OF FLOWER COLOR, SEED COLOR, AND PHOTOBLASTIC SEED GERMINATION USING LETTUCE CULTIVARS FOR BIOLOGICAL EXPERIMENTS IN HIGH SCHOOL

Chapter VIII

GENETICS OF FLOWER COLOR, SEED COLOR, AND PHOTOBLASTIC SEED GERMINATION USING LETTUCE CULTIVARS FOR BIOLOGICAL EXPERIMENTS IN HIGH SCHOOL

8.1. INTRODUCTION

Mendel studied about 7 characteristics, the stem length, flower position and color, fruit shape and color, seed shape and color, and fruit characteristics in garden peas (Weaver and Hedrick, 1992). In chapter 6 and 7, tomatoes were used to study stem length and lettuce were used to study leaf traits. To study phytochrome response on seed germination, to use photoblastic seed is essential and lettuce cultivar with photoblastic characteristics is a good plant material for this study. Photoblastic seed germination phenomenon under phytochrome control; red light promotes and far-red light inhibits seed germination, and the reversible effect of red and far-red light on seed germination was reported in their researches (Borthwick *et al.*, 1952; Sawada *et al.*, 2008). Mam *et al.* (2020a) introduced the methods for teaching light wavelength dependencies on seed germination and seedling elongation which is applicable for experimental class in developing countries by using photoblastic lettuce cultivar and other non-photoblastic lettuce cultivars. The use of photoblastic lettuce as genetics materials has yet not been reported. In this study, the photoblastic lettuce could be used to cross with non-photoblastic lettuce cultivars in order to study genetics of the photoblastic seed germination phenomenon, flower color, and seed colors. The application of this experiment to biology education at the high school level is also concerned.

8.2. MATERIALS AND METHODS

8.2.1. Plant Materials

Lettuce cultivars purchased in Japan were used in this experiment. One photoblastic and 3 non-photoblastic cultivars were used. In chapter 3, I could select only one lettuce cultivar 'Furiru lettuce' as a photoblastic lettuce from 25 commercial lettuce cultivars sold in Japan. 'Furiru lettuce' also has different flowers color and seed color from other lettuce cultivars used in this study. 'Furiru lettuce' has yellow petals (yellow flower) and black color seeds. 'Red sunstar', 'Sunny', and 'Vitamin' have violet petals (violet flower) and white color seeds. The crossing techniques and transplanting methods were explained in chapter 7.

8.2.2. Seed Germination Experiments

Seed germination experiments were done by using dark box and white light LED-attached box. Each box can store up to 11 Petri dishes with 5.5 cm in diameter each. F_1 and F_2 seeds were used to conduct experiments as follows.

F_1 seed germination experiment

F_1 seeds resulting from the cross between 'Furiru lettuce' and non-photoblastic lettuce cultivars, and self-fertilization seeds of non-photoblastic cultivars were checked for stability of their photoblastic germination phenomenon under white light or dark condition. Ten seeds of each cultivar or an F_1 plant were sown on a wet kitchen paper towel in a Petri dish. Two Petri dishes were prepared for each cultivar or F_1 seed germination experiment. The seeds in one Petri dish were placed in a dark box and the seeds in another Petri dish were placed in the box with white light LED attachment.

After all Petri dishes were placed in the box, about 2 ml of tap water was poured in each Petri dish. The box cover was closed immediately after pouring water to all Petri dishes for the dark box, and the white LED light was irradiated immediately after pouring water in each Petri dish. The experiment setting was kept in the room with a control temperature of 24°C.The results were checked 3 days after the experiments started. The same experiments were conducted 4 times.

F_2 seed germination experiment

F_2 seeds were experimented to check their photoblastic germination phenomenon. Totally 200 seeds from each F_1 plant were sown on wet kitchen paper towels in 10 Petri dishes in a dark box, one Petri dish 20 seeds. Twenty seeds of the same F_1 plant were sown on a Petri dish as those of 200 seeds but this Petri dish was kept under white light condition and it is used as the control treatment. The experiment setting was kept in a room with control temperature around 24°C. The result was checked 3 days later. The F_2 seeds of 10 reciprocal F_1 plants of each cross combination were used to check their photoblastic germination.

8.2.3. Statistical Analysis

Statistical significance was analyzed by free software Real Statistics Using Excel (Charles Zaionts) with one factor ANOVA followed up option Turkey HSD with p-value < 0.05).

The statistical test, probabilities of Chi-square was calculated by free online software for Calculation for the Chi-square test by Kristopher J. Preacher ©2010-2020.

8.3. RESULTS AND DISCUSSION

8.3.1. Flower Color Traits

F_1 flower color

The F_1 plants around 25 of the reciprocal crosses between 'Furiru lettuce' and 'Red sunstar', 'Sunny', or 'Vitamin' induced violet flowers (Figure 8.1). Mendel crossed the violet flower pea plant with the white flower pea plants, he received all F_1 plants with violet flowers (Weaver and Hedrick, 1992). Therefore, violet flower trait is the dominant trait for both pea and lettuce plants. The results of this lettuce crosses can be used to explain Mendel's Law of Dominant that the violet flower appeared in F_1 is called the dominant trait and the yellow flower did not appear in F_1 is called recessive trait.

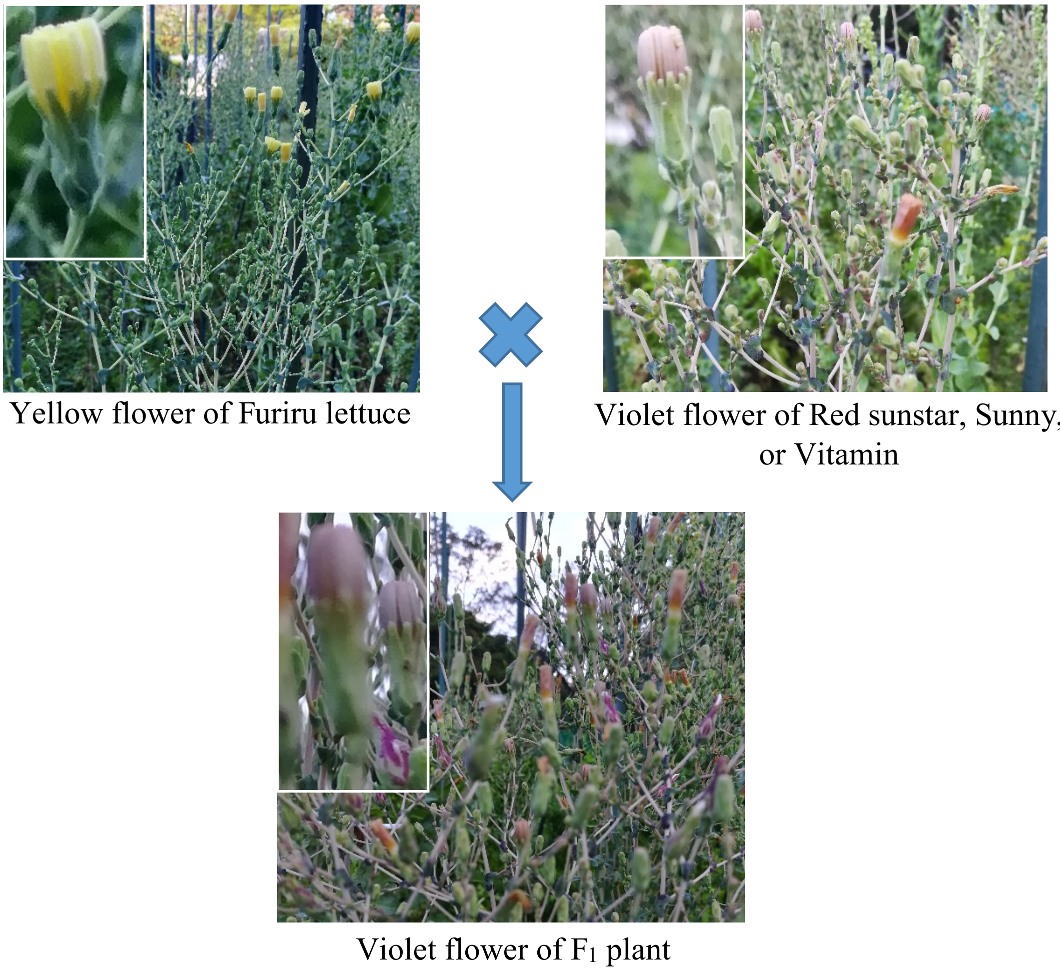


Figure 8. 1: F_1 lettuce plants' flower color and their parent plants' flower color

F_2 flower color

The segregation of F_2 plants' flower characteristics meet the principle of Mendel's segregation 3:1 of violet flower: yellow flower ratio. The chi-square values in table 8.1, 0.012 for 'Red sunstar', 0.02 for 'Sunny', and 0.013 for 'Vitamin' are much less than the critical value needed for statistical significance defined in probabilities of different theoretical chi-square value for one degree of freedom at 3.84 (Weaver and Hedrick, 1992). Therefore, the observed number of F_2 lettuce plants were consistent with the expected number considering the flower color under the principle of segregation. The chi-square value in this lettuce crosses was less than Mendel's chi-square (0.10) in the cross between a dominant violet flower pea and a recessive white flower pea plant (Weaver and Hedrick, 1992). This experimental result is good data to use as reference to teach students about Mendel's law of segregation.

Table 8. 1: Segregation of F_2 plants with flower color traits

Reciprocal F_1 hybrid with Furiru lettuce	Number of F_2 Plants	Segregation of F_2 plants				Chi-square test		
		Violet flower plants		Yellow flower plants		Chi-square	Degree of freedom	p-value
		Observed	Expected	Observed	Expected			
Red sunstar	110	82	82.5	28	27.5	0.012	1	0.912
Sunny	66	50	49.5	16	16.5	0.02	1	0.887
Vitamin	102	77	76.5	25	25.5	0.013	1	0.909

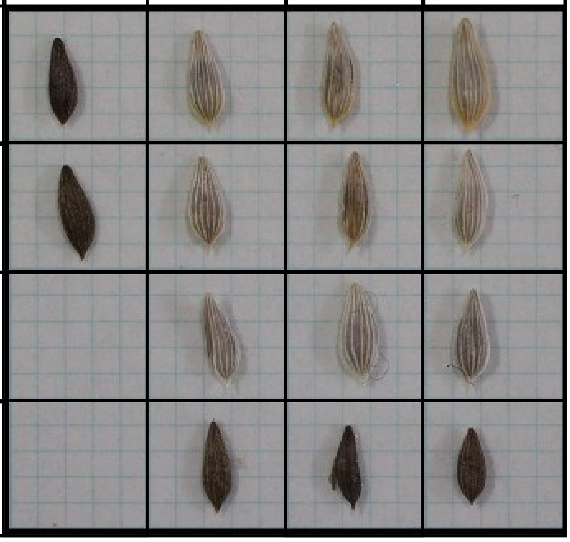
8.3.2. Seed Color Traits

F_1 seed color

The seed color of parents using in this genetics study showed black type and white type, and 'Furiru lettuce' was the only black seed color cultivar. The results of the experiments showed that the color of F_1 seed was the same as the seed color of the female parents. When F_1 seeds obtained from the crosses of 'Furiru lettuce' used as female parent showed black seed color; when F_1 seed obtained from the crosses of

‘Furiru lettuce’ used as male parent showed white seed color (Table 8.2). These results suggested that F_1 seeds color were depended on maternal parents’ seed color. The pigments that determine the seed color seem to be localized in pericarp tissues which develop from the ovary wall around the seed after fertilization (Thompson, 1948).

Table 8. 2: F_1 seed color comparing to commercial and self-fertilized seeds

Seed sources	Furiru	Red sunstar	Sunny	Vitamin
Commerial seeds				
Self-fertilized seeds				
F_1 seeds from Furiru pollen				
F_1 seeds from Furiru ovule				

F_2 seed color

The reciprocal F_1 hybrids obtained from the cross between ‘Furiru lettuce’ and the 3 lettuce cultivars were grown to produce F_2 seeds. The color of seeds produced from all reciprocal F_1 lettuce plants were shown black (Table 8.3). These results indicated that the black seed color inherited from ‘Furiru lettuce’ is dominant over white seed color of other cultivars. Because seed color of F_2 did not segregate, seed color might be depended on the hetero genotype of F_1 plants. These results were the same as the results of the old lettuce genetics experiments reported by Durst (1930) and Thompson (1938) that black color seed characteristic is dominant on white color characteristic.

Table 8. 3: F_2 seed color produced by reciprocal F_1 hybrid obtained from the cross of Furiru lettuce

Lettuce cultivars and seed color	Number of reciprocal F_1 plants	F_2 seed color
Red sunstar (white seed)	22	All black
Sunny (white seed)	23	All black
Vitamin (white seed)	25	All black

Note: The color of Furiru lettuce cultivar is black.

F_3 seed color

Although each F_2 plants produced seeds with uni-color, the F_2 plants were segregated of black color seed plants and white color seed plants in the ratio 3:1. The chi-square values of the crosses between 'Furiru lettuce' and the 3 cultivars were less than 3.84 so these results indicated that the observed numbers are consistent with the numbers expected under the principle of segregation. These results can be used for additional reference to teach students about Mendel's law of segregation.

Table 8. 4: F_3 seed color produced by reciprocal F_2 plants obtained from the cross of Furiru lettuce

Reciprocal F_1 hybrid with Furiru lettuce	Number of F_2 Plants	Segregation of F_2 plants				Chi-square test		
		Black seed plants		White seed plants		Chi-square	Degree of freedom	p-value
		Observed	Expected	Observed	Expected			
Red sunstar	82	65	61.5	17	20.5	0.797	1	0.372
Sunny	66	52	49.5	14	16.5	0.505	1	0.477
Vitamin	102	72	76.5	30	25.5	1.059	1	0.303

8.3.3. Photoblastic Seed Germination Phenomenon

F_1 seed germination

'Furiru lettuce'-self-fertilized seeds kept nature as photoblastic seeds. These seeds did not germinate completely in dark conditions. However, most of F_1 seeds obtained from reciprocal crosses between 'Furiru lettuce' and other 3 cultivars germinated in dark conditions, even though germination rates were similarly lower than those of under the white light condition (Table 8.5). Self-fertilized seeds of non-photoblastic cultivars had high germination rate in both light and dark conditions and higher than F_1 seeds of its cultivar. Even though F_1 germination rate of all cultivars in dark condition was lower than those of light condition, this germination rate was higher than the germination rate of 'Furiru lettuce' seeds in the dark condition. The average seed germination of F_1 seeds of each cultivar crossing with 'Furiru lettuce' using as both pollen and ovule parent showed higher seed germination rate than commercial seeds of 'Furiru lettuce' in dark condition. And there was a significant differences by statistical analysis between seed germination of self-fertilized 'Furiru lettuce' and F_1 seed germination (Figure 8.2). The seed germination phenomenon of the F_1 seeds looked like the dominance or the incomplete dominance of non-photoblastic phenomenon over photoblastic seed germination phenomenon characters (Table 8.5).

Table 8. 5: Average germination rate of F_1 seeds comparing to commercial and self-fertilized seeds (n=40)

Lettuce plants	F_1 seeds obained from the crosses				Self-fertilized seeds		Commercial seeds	
	Furiru Ovule[1]		Furiru Pollen[2]					
	Dark[3]	Light	Dark	Light	Dark	Light	Dark	Light
Furiru lettuce	-	-	-	-	0.00	92.50	0.00	95.00
Red sunstar	80.00	100.00	75.00	90.00	100.00	95.00	-	-
Sunny	65.00	90.00	80.00	77.50	80.00	95.00	-	-
Vitamin	72.50	85.00	40.00	77.50	92.50	95.00	-	-

[1]Seeds from the cross using pollen of the cultivar in the same column with Furiru lettuce ovule
[2]Seed from the cross using Furiru lettuce pollen with the ovule of the cultivar in the same column
[3]Light treatment on seed germination

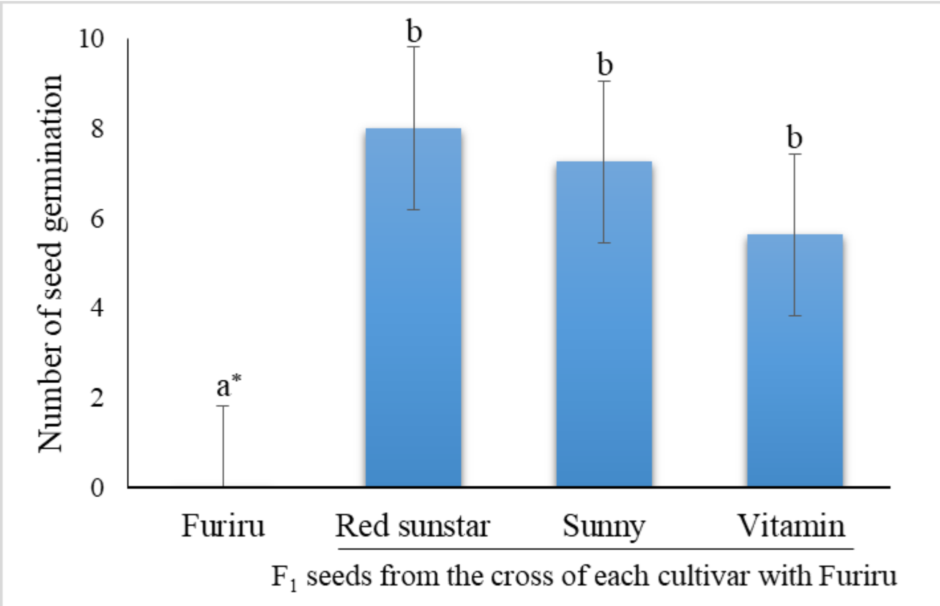


Figure 8. 2: Average seed germination of F_1 seeds resulting from crossing between Furiru lettuce and 3 lettuce cultivars in the dark.

*Different letters on the bars show significant differences between seed germination of cv. Furiru and their F_1 seeds by Real Statistics Using Excel (Charles Zaiontz) One Factor Anova follow-up option Turkey HSD p-value < 0.05.

F_2 seed germination

While the germination rate of F_2 seeds of all cross combinations was high under white light irradiation, F_2 seeds germinated very few in the dark (Figure 8.3 and Figure

8.4). These results indicated that F_2 seeds obtained from self-fertilization of F_1 plants with heterozygote genotype between photoblastic gene and non-photoblastic gene showed the dominant inheritance of F_1 seed germination characteristic as photoblastic. However, it is different of the F_1 seed characteristic like as dominance on non-photoblastic seed germination plants. The stronger photoblastic germination phenomenon was observed in the reciprocal F_1 plants obtained from the cross of 'Red sunstar' and the reciprocal F_1 plants obtained from the cross of 'Vitamin', only 0.3% and 0.8% germinated in the dark respectively. The germination rate was a little bit

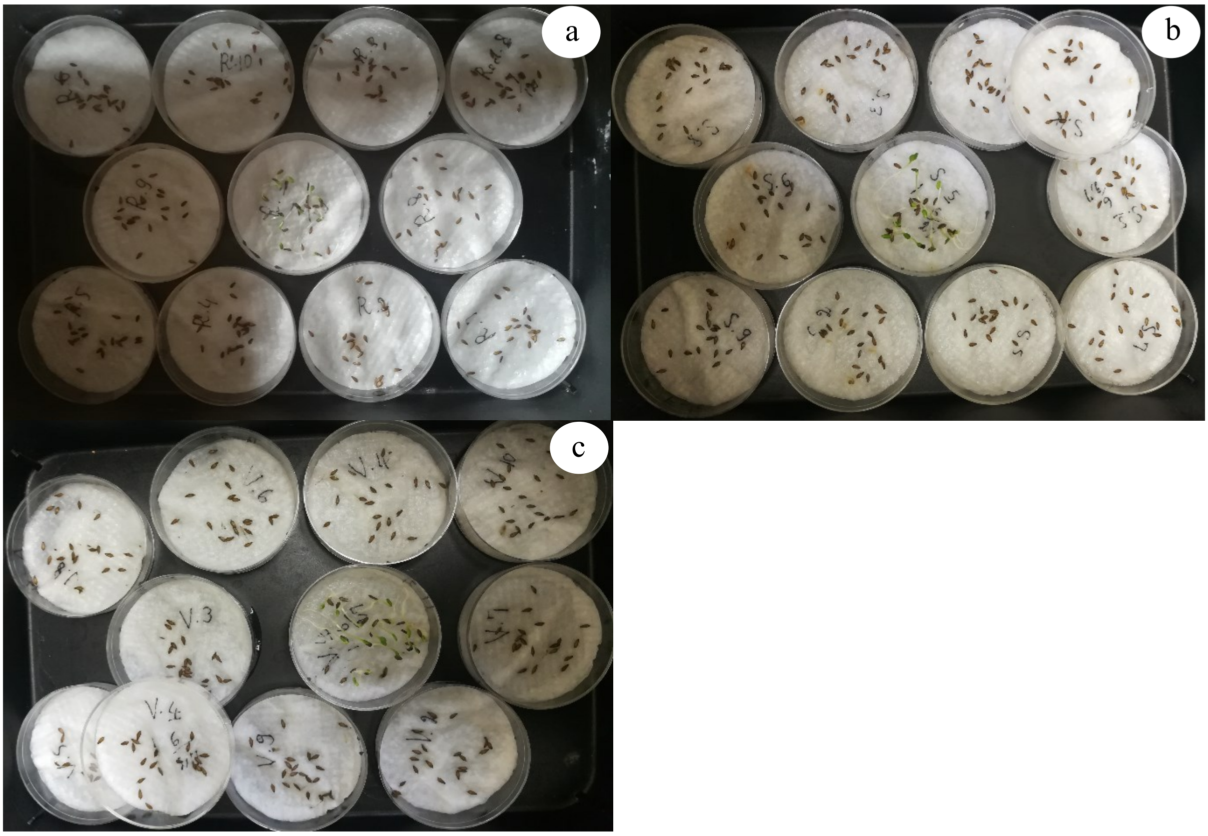


Figure 8. 3: F_2 seed germination in the dark and white light condition. The germinated seeds in the Petri dish in the middle of each figure were kept in the white light condition in the experimental box and the seeds in the other 10 Petri dishes in each figure were kept in the box in the dark condition after imbibing tap water. The result was checked 3 days after the experiment started. The seeds in each figure were produced by one reciprocal F_1 plant of the Furiru lettuce with **a:** 'Red sunstar', **b:** 'Sunny', and **c:** 'Vitamin'.

higher in the reciprocal F_1 of 'Sunny', 2.10% (Figure 8.4). The germination rate of F_2 seeds of all cross combinations were much higher compared to those in the dark. These results indicated that the photoblastic germination phenomenon was dominant on non-photoblastic phenomena in seeds produced by F_1 plants.

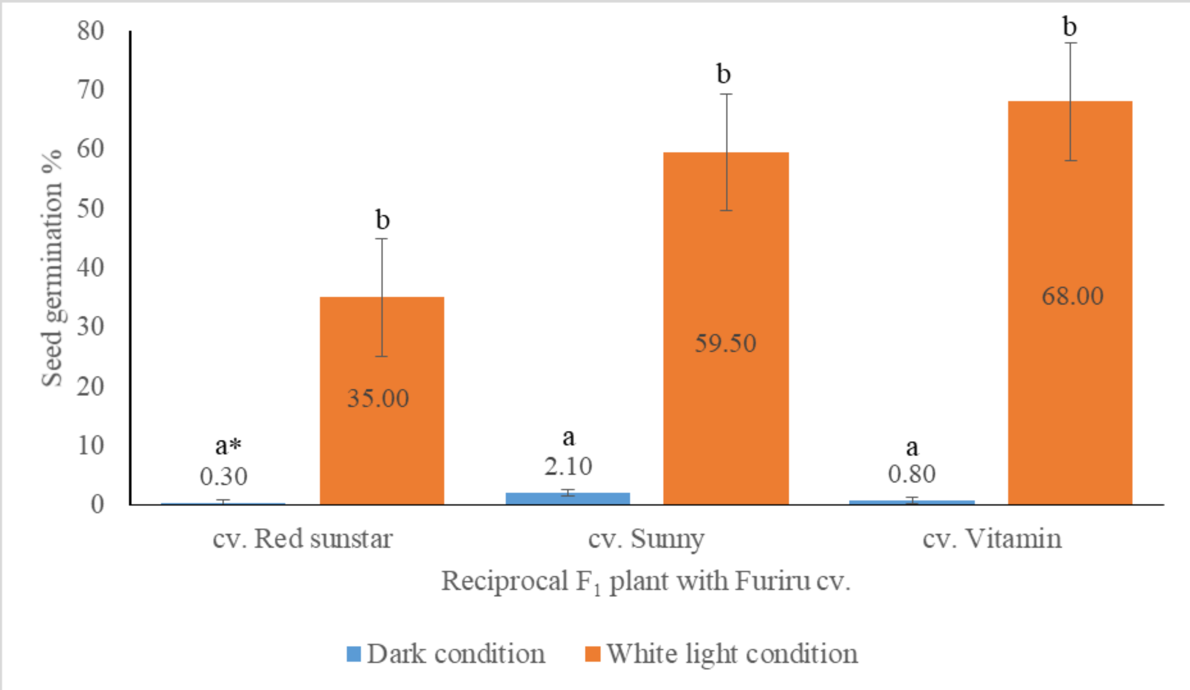


Figure 8. 4: Percentage of F_2 seed germination in the white and dark condition. There are 10 reciprocal F_1 hybrids between cv. Furiru and each cultivar. The number of F_2 seeds in each cross combination were 200 seeds in the white light condition and 2 000 seeds in the dark condition.
*Different letters on the bars show significant differences of seed germination in the dark and white light condition of each F_2 cultivar by Real Statistics Using Excel (Charles Zaiontz) One Factor Anova follow-up option Turkey HSD p-value < 0.05.

Because these results were difficult from the explanation of inheritance way of photoblastic, I am trying to checked F_3 seed photoblastic character. If the photoblastic inheritance is dominant and controlled by one gene, F_2 plants should be segregated between 3/4 photoblastic seed germination plants and 1/4 non photoblastic seed plants. The experiments are in progress.

8.3.4. Linkage Gene

Table 8.6 indicated that there was a complete linkage between leaf color and flower color, but there was independent between leaf color and seed color, statistically. The lettuce plants with red leaves always had violet flowers while the plant with green leaves had yellow colors. In this experiment, there were no recombinant between flower color gene and leaf color gene. The red leaf was a dominant trait on green leaf (chapter 7) and the violet flower was also a dominant trait on yellow flower (Figure 8.1, Table 8.1). Red leaf trait and green leaf trait are co-segregated violet flower and yellow flower, respectively. The allele for red leaf represented as "*R*", the allele for green leaf represented as "*r*", the violet flower (dominant trait) represented as "*V*" and the allele for yellow flower (recessive) represented as "*v*". While the cross between green leaf-yellow flower plant (*rrvv*) and the red leaf-violet flower plant (*RRVV*) was expected F_2 population with the phenotypic ratio: 9: 3: 3: 1of red leaf-violet flower*(R_V_)*: red leaf-yellow flower(*R_vv*): green leaf- violet flower(*rrV_*): green leaf-yellow flower (*rrvv*) if this result followed Mendel's law of segregation, my results showed red leaf-violet flower*(R_V_)*: green leaf-yellow flower (*rrvv*) =3:1. The results of F_2 plants did not segregate independent, these two genes were the same gene or closely linked.

The cross between the green leaf-yellow flower lettuce (*rrvv*) and the red leaf-violet flower lettuce (*RRVV*) indicated a linkage genes explained by William Bateson and R.C. Punnett (Weaver and Hedrick, 1992). They conducted the cross between purple flower-long pollen shape (*PPLL*) and red flower-round pollen shape (*ppll*), they obtained F_2 population with the majority as the parent plants and less number of segregated plants. From their result Bateson and Punnett suggested that there might be a physical connection between the parental alleles- the dominant alleles from one parent and the recessive allele from the other parent. This physical connection was also explained by Thomas Hunt Morgan through his *Drosophila* cross experiments. Based on his observation result, Morgan suggested that the genes having the alleles for the connection traits are on the same chromosome (Weaver and Hedrick, 1992). Based on Morgan's suggestion, the alleles determine the leaf color and flower color in this experiment might be located in the same chromosome. The results of this experiment can be used to teach students at 11th grade in Cambodia in chapter 3 about reproduction related to linkage genes.

Table 8. 6: Linkage between leaf color, flower color, and seed color of F_2 plants obtained from the cross between 'Furiru lettuce' and the 3 cultivars

F_2 plants obtained from the cross between Furiru lettuce	Leaf color plants	Flower color plants		Seed color plants	
		Violet	Yellow	Black	White
Red sunstar (n=82)	Red leaf, n=62	62	0	48	14
	Green leaf, n=20	0	20	17	3
Sunny (n=66)	Red leaf, n=50	50	0	41	9
	Green leaf, n=16	0	16	11	5
Vitamin (n=102)	Red leaf, n=77	77	0	55	22
	Green leaf, n=25	0	25	17	8

8.4. POSSIBILITY SCHOOL EDUCATION APPLICATION METHODS

This study included field work activity and the experiments. The field work activity took a long time in cultivation from parent plants to F_2 plants. The experiments took only 3 days to keep the lettuce seeds germinate in dark or white light. These experiments can be done with students in the biology classes. The figures and tables in this study can be used to teach students about Mendel's Law of Dominance, Mendel's Law of Segregation and the theory of linkage gene.

8.5. CONCLUSION

The results of this study are good additional data and materials for students and teachers to study Mendel's Laws of Heredity and the linkage gene impact in high school biology in Cambodia. The flower and seed color was studied by Mendel, but the inheritance of photoblastic seed germination phenomenon has yet not been reported in any article. The dominant characteristic of the photoblastic seed germination phenomenon in this experiment might be new biology concept of high school and it is good for students to inquire more. The experiments in this study can be organized by students and teachers because daily life use materials are needed. The experimental boxes produced in chapter 4 are needed to conduct the experiments on photoblastic seed germination.

Chapter IX

GENERAL DISCUSSION, CONCLUSION, AND RECOMMENDATION

Chapter IX
GENERAL DISCUSSION, CONCLUSION, AND RECOMMENDATION

This chapter attempts to provide the general discussion, conclusion, and conclusion related to the overall dissertation in this study. The discussion and conclusion will be provided related to the experimental apparatus development, experimental methods, experimental result, and the contribution of this study to improve biology education in Cambodia. The recommendation will be provided for teachers and relevant partners to consider to start any intervention to improve biology education at the high school level.

9.1. GENERAL DISCUSSION

9.1.1. Development of Experimental Apparatus

Science experiments are considered as good teaching methods to make students understand the science concepts well and the experiments make students interested in science subjects (Singer *et al.*, 2005). Production of the experimental apparatus by not only teachers but also students themselves might attract students to do this experiment because they can generally use easily. Even though Japan is a developed country that has enough ability to buy expensive industrial products for conducting the experiment, students still developed their experimental apparatus for their experiment (Website 3 and Website 4). Moreover, when students produce the experimental apparatus by themselves, it will increase their inquiry in science and technology, and it will also encourage them to become an engineer in the future. Simple experimental apparatus produced by teachers were recommended for science experimental class at high school level in Cambodia (Seng *et al.*, 2006). Cambodian education inspectors indicated that simple experimental apparatus should be introduced for science experimental classes in Cambodia (Mam *et al.*, 2019).

The apparatus developed in this study was limited to the use of physical approach for biology experiments. The use of physics concepts for biology experiments will attract the students to study both biology and physics because the students good at biology are not generally good at physics and the students good at physics are not generally good at biology. Light wavelengths are the physics concept, the experimental box attached with different light wavelengths of LED bulbs were produced. To produce this experimental apparatus, the general knowledge related to light circuits is needed. This general knowledge was also included in physics subject. Moreover, producers should pay high attention when producing this apparatus because the electricity is used and it can cause the electric shock to death if the alternative current is used. To practice with a light circuit, the power source from batteries should be used because it will not cause the danger even if the wrong circuit is made.

The materials needed to produce this apparatus including LED bulbs, sets of LED bulb holders, resistors and so on. The resistors of different resistances are used in order to determine appropriate electrical intensity to supply to LED bulbs and the resistors vary in according to the number of LED bulbs used. The resistance capacity of the resistor is according to the number of LED bulbs used. The appropriate resistance capacity of the resistor used is that it provides high light intensity and it is not hot when electricity passes through it.

9.1.2. Experimental Methods for Cambodia High School

The experimental methods which are good to be applied to biology experimental classes in high school should be simple, but can ensure the accurate result following scientific methods. The biological experiments introduced in this study were (1) the effect of different light wavelengths on seed germination and seedlings growth by using carrot and lettuce seeds, (2) phytochrome response on photoblastic lettuce seed germination, (3) the effects of plant growth hormones, GA and ABA, on lettuce seed germination, (4) the effects of plant growth hormones on plant growth by exogenous application of GA_3 on dwarf and normal stem tomatoes, and (5) the study of Mendel's Laws of Inheritance by using lettuce and tomato cultivars. The experiments were conducted by using the LED-attached box and other materials such as Petri dishes, kitchen paper towel, and the chemical products for GA_3 which can be purchased at markets as an agricultural chemical. Daily life equipment such as spray bottle, ruler, and caliper were used in the experiments on the effects of GA_3 on tomato plant growth. Simple crossing techniques and experimental procedures were introduced in the experiments on the study of Mendel's Laws of Inheritance.

The experimental procedures used to conduct the above experiments were operated manually with simple materials use. The experiments were conducted at the field or in a room without using a science laboratory.

9.2. GENERAL CONCLUSION

This study can help teachers in high school to use experiments as a teaching method to teach biology in order to attract students to be interested in studying biology as well as other science subjects. The experimental apparatus development, the experimental methods, and the result presentation are suitable for students and teachers in high school level to follow.

9.2.1. Development of Experimental Apparatus

The experimental apparatus developed in this study was simple, inexpensive, applicable by students, and provided stable results. Therefore they are good for biology experiments in developing country such as Cambodia where teacher's capacity and school budget for laboratory equipment are limited. Moreover, the experimental apparatus developed in this study can help biology teacher to implement STEM education which is advised by the Ministry of Education Youth and Sport for education reform in Cambodia. The process of producing the experimental box in this study can encourage students to like science subjects and even encourage them to become an engineer in the future. LED bulbs and other electrical equipment are required to produce the experimental box. LED bulbs of 5 mm diameter of different wavelengths are good light sources for conducting experiments on the effects of different light wavelengths on seed germination and seedling growth as well as the experiments on phytochrome response on photoblastic lettuce seed germination. Plastic boxes of both kinds used in this study are good experimental containers to provide dark conditions. The handmade LED circuit introduced in this study is a good method to determine the position of LED on the cover of the plastic box in order to make light distribute in the whole box and allow producers to determine the number of LED bulbs according to the size of the box. The LED-attached box equipped with electric meters and switches with variety resistances is a good apparatus for conducting experiments about different light intensity on seed germination and seedling elongation. The use of batteries as an electricity source provides stable current and there is no danger to students.

The process of setting up LED-attached boxes described in this study can help teachers to set up an LED-attached apparatus for laboratory class in their schools. Two kinds of boxes produced in this study are appropriate for students and teachers to conduct experiments on the effects of light wavelength on seed germination and seedling growth with their experiment setting.

9.2.2. Experimental Methods for Cambodia High School

The experimental methods used in this study are suitable to be introduced to biology laboratory class in high school level in Cambodia because (1) the experimental topics were determined referring to biology curriculum in high school level, (2) the experimental procedures were not difficult to be handled by high school students, and the materials used in the experiments are available in Cambodia with low price. The experimental methods on seed germination and seedling elongation can be done by using the materials found in daily life-used tools. Kitchen paper towel is a good material for seed sowing because it can absorb water well, and it can allow seed to germinate and seedling to grow well. Students can do these experiments by themselves after an explanation from their teachers or reading the articles in this study.

The experiment about the effect of plant hormone “GA” on plant growth can be done in high school level because the source of GA can be purchased at garden shops. The exogenous treatment on plants is an easy method for students to do. In this experiment, students will improve their measuring skill.

The crossing techniques, both carrot and lettuce, introduced in this study are suitable to be applied in school to conduct monohybrid and dihybrid crosses. Lettuce crossing methods are easy for students to do themselves. However, tomato crossing is a little bit difficult for students to handle since the emasculation stage should be taken care of a lot.

9.2.3. Experimental Results for Cambodia High School

The experimental results are the additional data and information used to explain the content written in biology textbooks. The data was presented in tables and figures which are easy to use as teaching materials. The ways of result presentation in this study are suitable for high school students and teachers to understand. The results of experiments in chapter 4 and chapter 5 can be used to confirm the contents written in biology textbooks as well as descriptions reported by previous researchers. These results indicate that the experimental methods and apparatuses used in my study are good and applicable to biology education at high school because the handmade apparatus and simple experimental methods were used. The results of experiments in chapter 6, 7 and 8 are good for students to learn about the effects of plant hormone "GA" on plant growth and more over the results of crossing experiments are the good information for students to learn 1st, 2nd, and 3rd Mendel's Laws of Inheritance, which included in both biology textbooks in Cambodia and Japan.

9.3. RECOMMENDATION

In order to make the result of this study applicable to biology education in high school level, the authors provided some recommendations as follows.

9.3.1. Development of Experimental Apparatus

School principals and teachers should consider producing the LED-attached box for biology laboratory class in their school. The use of batteries as an electric power supply is recommended for teachers to produce LED-attached boxes. The use of batteries is safe for both students and teachers. The size of the box is dependent on the experiments designed for the laboratory class. The complete dark box with pasting an aluminum foil along the wall inside the box is an important factor for the success in the experiment of the response of phytochrome on photoblastic lettuce seed germination. The LED-attached box used in chapter 5 is expensive and complicated. Teachers can make a simpler LED-attached box without using two electric current meters and two volume switches with variable resistor dial from zero to 2KΩ. Teachers can produce this kind of LED-attached box with one button-switch for alternative irradiation of R and FR light and a 100Ω resistor to each LED-circuit.

9.3.2. Plant Materials Selection

Seed selection is an essential factor in the experiment of the effect of different light wavelengths on seed germination and seedling growth. Seeds of plant cultivars need a short period of time to germinate are good materials for this experiment. Seeds of lettuce cultivars are good because the seeds germinate in two days after seed sowing. The selection of photoblastic lettuce seed is the most essential factor for conducting the experiment on the effect of R and FR on phytochrome response on seed germination. Therefore, teachers should conduct experiments to search for photoblastic lettuce seeds in their areas. It might be difficult for teachers to find photoblastic lettuce seeds because I could select only one photoblastic lettuce seed plant among 25 lettuce cultivars commercialized in Japan.

In the study of Mendel's genetics of inheritance, the tomato and lettuce plants with different traits should be selected for the experiment. The lettuce plants with different leaf color and leaf form should be selected because the students can study both monohybrid and dihybrid crosses at the same time.

9.3.3. Curriculum Development in Cambodia Biology

Curriculum developers should consider to conclude the concept of light wavelength on seed germination and phytochrome response on photoblastic seed germination in biology education in Cambodia. A developed country such as Japan concluded these concepts in biology textbooks in senior high school.

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WEBSITES

1. Work With Nature, plant breeding/how to cross new lettuce varieties
https://www.youtube.com/watch?v=ATMYpmmy7c&fbclid=IwAR1nqEpN_sAEZf3okccAzw1-6-FoGz7OQNNKVT6XX9tvmFyKEj0Aq0G6e7c

2. Kristopher J. Preacher ©2010-2020, Calculation for the Chi-square test
http://www.quantpsy.org/chisq/chisq.htm

3. Experiments on the effect of light on seed germination
https://www.nagano-c.ed.jp/omc-shin/gakka/risuuka/2015/se-2.pdf

4. Experiments on the effects of light on seedling growth
https://school.gifu-net.ed.jp/ena-hs/ssh/H24ssh/sc3/31216.pdf

APPENDIXES

Appendix 2. 1: Questionnaire for inspectors in Provincial Office of Education

Note: A Khmer version was prepared for the participants.

Research topic:
SCHOOL PERFORMANCE EVALUATION THROUGH SYSTEMIC SCHOOL INSPECTION IN CAMBODIA

Questionnaire for Inspectors of Provincial Office of Education (POE)

I am Mam Chansean, deputy head of the Planning and Management Department of the National Institute of Education (NIE) of the Ministry of Education Youth and Sport of Cambodia. I am now a doctoral student of Cooperative Doctoral Course in Aichi University of Education & Shizuoka University in Japan. I am conducting research on "School Performance Evaluation Through Systemic School Inspection in Cambodia". The systemic inspection has been implemented since 2015 after the inspectors and school principals received training organized by NIE. This research is a part of my doctoral thesis. I would like to ask your kindness to fill in the questionnaire as follows. Your information is confidential and is used for the purpose of research only. Thank you very much in advance for sharing your valuable time to complete this questionnaire.

Note: Please print out this questionnaire. Fill in the questions and then please send to me through: *e-mail:* mamchansean@gmail.com , telegram:+855-12670294

Facebook messenger: https://www.facebook.com/chansean.mam

I. Personal Information

1. Working place (province/city) :……………………………………………………
2. Current position :……………………………………………………
3. Experience working as an inspector:……………………………………………………

II. General information about inspection implementation

1. How many schools have you inspected since 2014?
   A. Primary schools:…………….. Total primary schools in the province:…………
   B. Lower secondary schools:…. Total lower secondary schools in the provinces:…
   C. High Schools:………………… Total high schools in the province:……………

Note: If you are a chief of inspection office or the representative in each province, please continue to question 2. If Not, please continue to question 3.

2. How many schools (in total) were inspected in your province since 2014?
   A. Primary schools:…………………
   B. Lower secondary schools:…………
   C. High schools: …………….

3. Did you get strong support from your Provincial Office of Education management team in inspection?

☐ Yes ☐ No

Reason to your answer:…………………………………………………………………..

…………………………………………………………………………………………

…………………………………………………………………………………………

4. Did the relevant target people such as school principals, community representative, local authority cooperate with you well during inspection?

☐ Yes ☐ No

III. Impact of Systemic Inspection on the School Performance

1. How many schools have you inspected 2 times or more since 2014?
   A. Primary schools:……………………
   B. Lower secondary schools:…………
   C. High schools:…………..
2. In case you have inspected one school from two times or more please continue to <u>question number 3</u>. If Not, please skip question 3 and continue at <u>part IV</u>.
3. Please tick in the box under "Yes" or "No" based on the majority of the schools you inspected.

No.	Indicators to be observed for the evaluation of school performance	1st time inspection		2nd time inspection		3rd time inspection	
		Yes	No	Yes	No	Yes	No
1	Students' results and achievements improve over time.						
2	The school has a functioning program to assure students' attendance.						
3	Assessment and grading is equitable and fair.						
4	There is a clear organizational structure with defined procedures and responsible persons.						
5	The school director effectively and efficiently organizes the work in the school.						
6	The school has equipment, books and other relevant material for efficient teaching and learning.						
7	The school has a safe and favorable environment for learning and development.						

8	Teaching and learning is according to the curriculum and other steering documents (i.e. Education law, Teacher policy).						
9	Adequate pedagogical methods are used in relation to the aim of the class/ lesson.						
10	Teaching is adapted to students' needs.						
11	The teachers create a stimulating learning environment in class.						
12	The teachers continuously work to improve their teaching.						
13	The school director effectively manages improvement work in the school.						
14	There is a school development plan which is based on the reports of self-assessment results and external inspection results.						
15	There is good cooperation and communication with the community and other stakeholders.						

4. Did you observe science teachers teaching experiment during inspection?

   A. Yes B. No

   If you chose answer "A", how many percentage? ……………………………………..

   Why did not teachers teach experiment to students? …………………………………..

   ……………………………………………………………………………………..

   ……………………………………………………………………………………..

5. Did you think that experimental apparatus needed for science subject?

   A. Yes B. No

   If you chose answer "A", please go to question 6.

6. What kinds of experimental apparatus for teachers to teach science subject in Cambodia?

………………………………………………………………………………………

………………………………………………………………………………………

………………………………………………………………………………………

IV. Comment or Suggestion

Please give comment or suggestion about Systemic Inspection

………………………………………………………………………………………

Appendix 2. 2: Questionnaire for inspectors in Educational Quality Assurance Department

Note: A Khmer version was prepared for the participants.

Research topic:
SCHOOL PERFORMANCE EVALUATION THROUGH SYSTEMIC SCHOOL INSPECTION IN CAMBODIA

Questionnaire for Inspectors of Educational Quality Assurance Department (EQAD)

I am Mam Chansean, deputy head of the Planning and Management Department of the National Institute of Education (NIE) of the Ministry of Education Youth and Sport of Cambodia. I am now a doctoral student of Cooperative Doctoral Course in Aichi University of Education & Shizuoka University in Japan. I am conducting research on "School Performance Evaluation Through Systemic School Inspection in Cambodia". The systemic inspection has been implemented since 2015 after the inspectors and school principals received training organized by NIE. This research is a part of my doctoral thesis. I would like to ask your kindness to fill in the questionnaire as follows. Your information is confidential and is used for the purpose of research only. Thank you very much in advance for sharing your valuable time to complete this questionnaire.

Note: Please print out this questionnaire. Fill in the questions and then please send to me through: *e-mail:* mamchansean@gmail.com , telegram:+855-12670294

Facebook messenger: https://www.facebook.com/chansean.mam

I. Personal Information

1. Current position:……………………………………………………………………..
2. Experience working as an inspector:………………………………………………………

II. General information about inspection implementation

1. How many schools have you inspected (thematic or topic inspection) since 2015?
   A. Primary schools:…………
   B. Lower secondary schools……………
   C. High Schools:…………………

Note: If you are a chief of inspection office or the representative in the office, please continue to question 2. If Not, please continue to question 3.

2. How many schools (in total) were inspected (thematic or topic inspection) since 2015?
   A. Primary schools:…………
   B. Lower secondary schools……………
   C. High Schools:…………………

3. Please write down your inspection topic and school level
   A. Topic:……………………………………   School level:……………………
   B. Topic:……………………………………   School level:……………………

C. Topic:…………………………………… School level:……………………
D. Topic:…………………………………… School level:……………………
E. Topic:…………………………………… School level:……………………

4. Did you get strong support from the Ministry of Education Youth and Sport for the thematic inspection?

 Yes No

5. Did the relevant target people such as school principals, community representative, local authority cooperative with you well during inspection?

 Yes No

III. Impact of Systemic Inspection on the School Performance

1. How many schools have you inspected 2 times or more since the systemic inspection be implemented?

A. Primary schools:……………………
B. Lower secondary schools:………….
C. High schools:…………..

2. Please tick in the box under “Yes” or “No” based on the majority of the schools you inspected.

No.	Indicators to observe for the evaluation of school performance	2nd time inspection		3rd time inspection		Not sure
		Yes	No	Yes	No	
1	Students’ results on the inspected topics improved over time (from 80%).					
2	Schools have functioning program to encourage students to study on the topic inspected.					
3	Schools have effective apparatus and teaching materials to teach the topics inspected.					
4	Teachers have adequate pedagogy methods to teach the topics inspected.					
5	Teachers created a good environment to encourage students to study the topics inspected.					
6	Schools have functioning system to monitor and evaluate the educational quality on the topics inspected.					
7	Schools had development plant to improve educational quality on the topics inspected.					

Note: If you had inspected on science topic, please continue to question 3. If Not, please continue at question 5.

3. Did the students' achievements on science subject improve continuously (in most schools you have inspected)?

   A. Improved B. Not improved C. Not sure

4. Did you observe science teachers teaching experiment during inspection?

   B. Yes B. No

   If you chose answer "A", how many percentage? ……………………………………..

   Why did not teachers teach experiment to students? …………………………………..

   ……………………………………………………………………………………………..

   ……………………………………………………………………………………………..

5. Did you think that experimental apparatus needed for science subject?

   B. Yes B. No

   If you chose answer "A", please go to question 6.

6. What kinds of experimental apparatus for teachers to teach science subject in Cambodia?

………………………………………………………………………………………………

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IV. Comment or Suggestion

Please give comment or suggestion about Systemic Inspection

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………………………………………………………………………………………………

………………………………………………………………………………………………

Appendix 4. 1: Light spectrum test report in each LED-attached box

SPECTRUM TEST REPORT

White LED

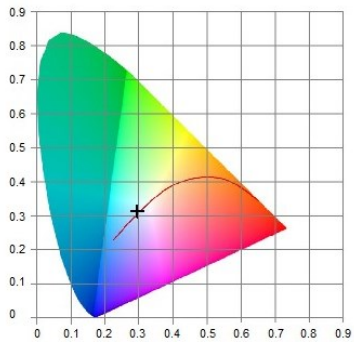
■ Information

User : Chansean	Measure Time : 2019/06/05 14:07:08
Model NO. : LA-105	Light Source : White LED box
Memo :	

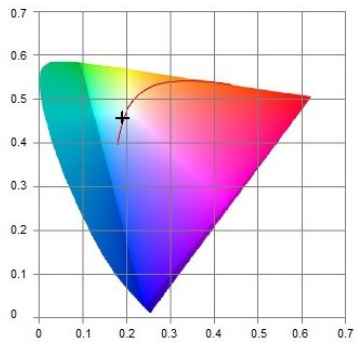
■ BASIC

CCT (K)	: 7807
x	: 0.2949
y	: 0.3140
LambdaD (nm)	: 485

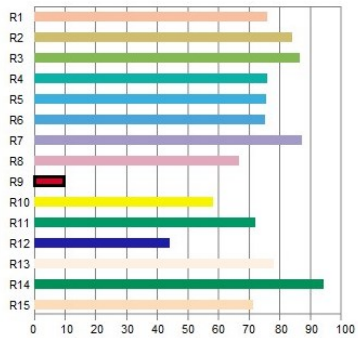
■ CIE1931



■ CIE1976



■ CRI



■ Spectrum

■ Features

CCT (K) : 7807	x : 0.2949	deltau : -0.0043	PFD-FR (700~780nm) : 0.4252	PFD (380~780nm) : 19.6	R5 : 75.5	R11 : 72.0
LUX (lx) : 1338	y : 0.3140	deltav : 0.0038	PFD-UV (380~400nm) : 0.0195	IRR (Wm-2) : 4.39	R6 : 75.0	R12 : 44.0
ETime (ms) : 173	u' : 0.1909	LambdaD (nm) : 485	PFD-R (600~700nm) : 4.02	R1 : 75.8	R7 : 87.0	R13 : 77.9
Purity (%) : 14.5	v' : 0.4574	LambdaP (nm) : 454	PFD-G (500~600nm) : 9.03	R2 : 83.7	R8 : 66.5	R14 : 94.0
fc (lmft-2) : 124	deltax : -0.0022	LambdaPV (mWm-2nm-1) : 52.1	PFD-B (400~500nm) : 6.23	R3 : 86.2	R9 : -9.85	R15 : 71.1
Duv : 0.0050	deltay : 0.0071	CRI (Ra) : 78.2	PPFD (400~700nm) : 19.1	R4 : 75.8	R10 : 58.2	

NKsystem

SPECTRUM TEST REPORT

Blue LED

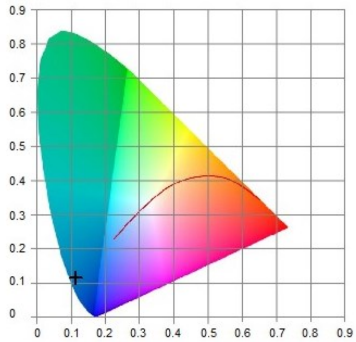
Information

User : Chansean	Measure Time : 2019/06/05 14:02:58
Model NO. : LA-105	Light Source : Blue LED box
Memo :	

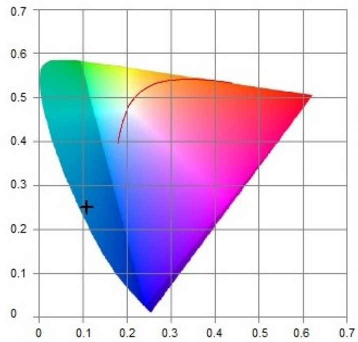
BASIC

CCT (K)	: 0.0000
x	: 0.1132
y	: 0.1159
LambdaD (nm)	: 477

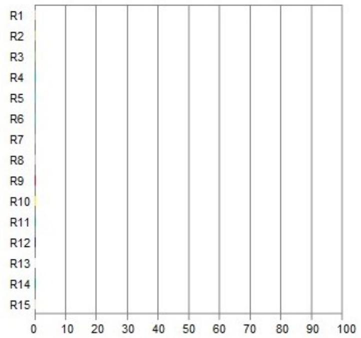
CIE1931



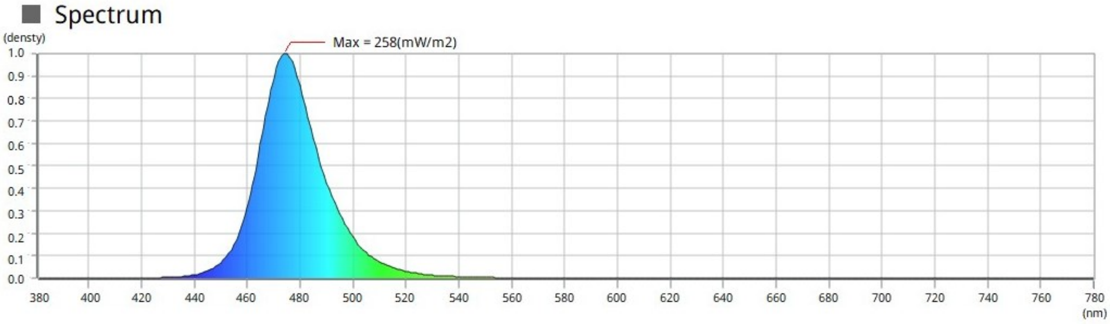
CIE1976



CRI



Spectrum



Features

CCT (K)	: 0.0000	x	: 0.1132	deltau	: 0.1087	PFD-FR (700~780nm)	: 0.0322	PFD (380~780nm)	: 29.9	R5	: 0.0000	R11	: 0.0000
LUX (lx)	: 768	y	: 0.1159	deltav	: 0.2505	PFD-UV (380~400nm)	: 0.0285	IRR (Wm-2)	: 7.50	R6	: 0.0000	R12	: 0.0000
ETime (ms)	: 32.0	u'	: 0.1087	LambdaD (nm)	: 477	PFD-R (600~700nm)	: 0.0265	R1	: 0.0000	R7	: 0.0000	R13	: 0.0000
Purity (%)	: 94.9	v'	: 0.2505	LambdaP (nm)	: 474	PFD-G (500~600nm)	: 2.10	R2	: 0.0000	R8	: 0.0000	R14	: 0.0000
fc (lmft-2)	: 71.4	deltax	: 0.1132	LambdaPV (mWm-2nm-1)	: 258	PFD-B (400~500nm)	: 27.9	R3	: 0.0000	R9	: 0.0000	R15	: 0.0000
Duv	: 0.1993	deltay	: 0.1159	CRI (Ra)	: 0.0000	PPFD (400~700nm)	: 29.9	R4	: 0.0000	R10	: 0.0000		

NKsystem

SPECTRUM TEST REPORT

Green LED

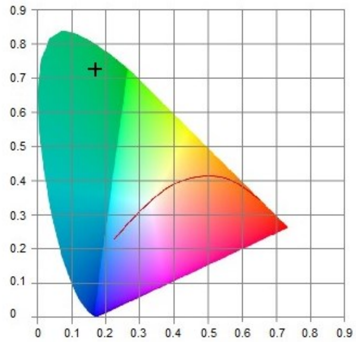
Information

User : Chansean	Measure Time : 2019/06/05 13:59:18
Model NO. : LA-105	Light Source : Green LED box
Memo :	

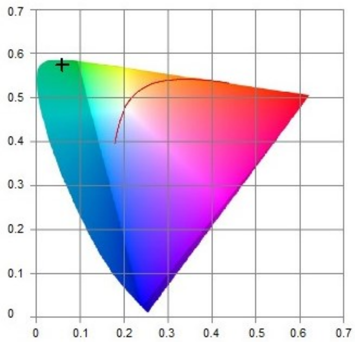
BASIC

CCT (K)	: 7798
x	: 0.1708
y	: 0.7266
LambdaD (nm)	: 527

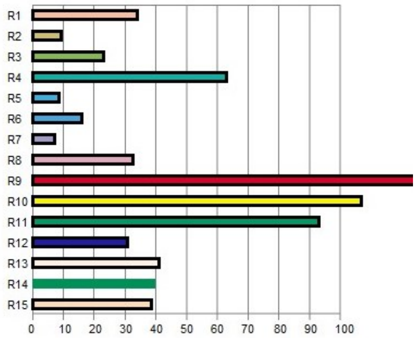
CIE1931



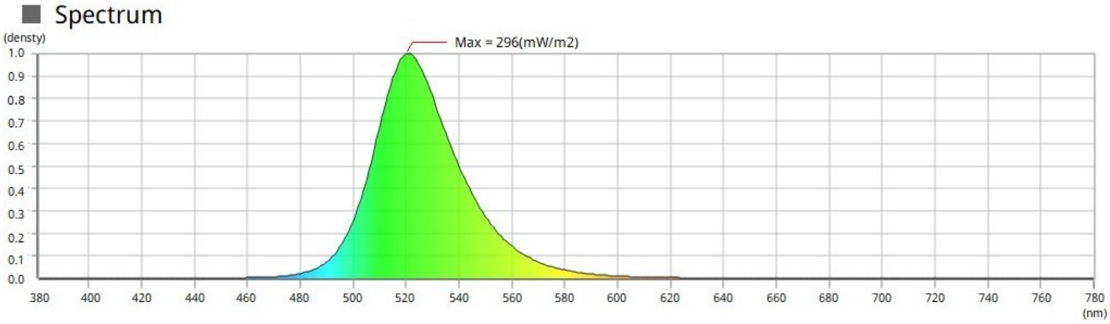
CIE1976



CRI



Spectrum



Features

CCT (K)	: 7798	x	: 0.1708	deltau	: -0.1352	PFD-FR (700~780nm)	: 0.2038	PFD (380~780nm)	: 51.4	R5	: -8.56	R11	: -92.9
LUX (lx)	: 5710	y	: 0.7266	deltav	: 0.1211	PFD-UV (380~400nm)	: 0.0431	IRR (Wm-2)	: 11.7	R6	: -16.1	R12	: -30.8
ETime (ms)	: 24.0	u'	: 0.0600	LambdaD (nm)	: 527	PFD-R (600~700nm)	: 0.3736	R1	: -34.1	R7	: -7.38	R13	: -41.1
Purity (%)	: 80.9	v'	: 0.5748	LambdaP (nm)	: 520	PFD-G (500~600nm)	: 47.9	R2	: -9.31	R8	: -32.5	R14	: 39.8
fc (lmft-2)	: 531	deltax	: -0.1264	LambdaPV (mWm-2nm-1)	: 296	PFD-B (400~500nm)	: 3.37	R3	: -23.2	R9	: -360	R15	: -38.6
Duv	: 0.1574	deltay	: 0.4196	CRI (Ra)	: 0.0000	PPFD (400~700nm)	: 51.2	R4	: -62.9	R10	: -107		

NKsystem

SPECTRUM TEST REPORT

Orange LED

Information

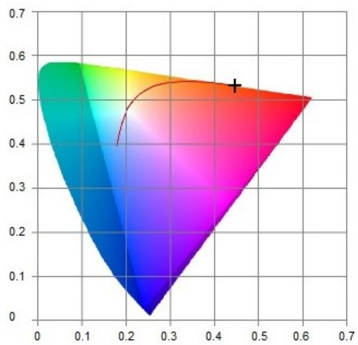
User : Chansean	Measure Time : 2019/06/05 14:01:05
Model NO. : LA-105	Light Source : Orange LED box
Memo :	

BASIC

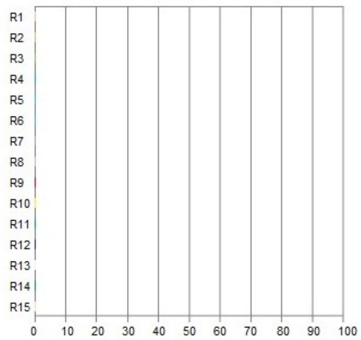
CCT (K)	: 0.0000
x	: 0.6511
y	: 0.3444
LambdaD (nm)	: 607

CIE1931

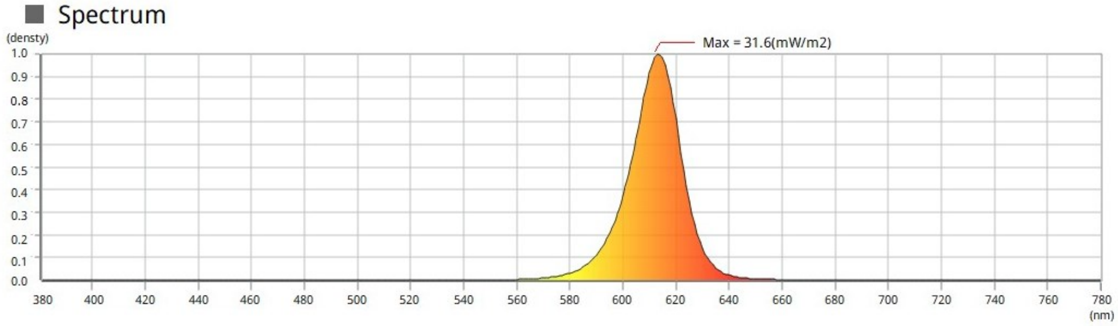
CIE1976



CRI



Spectrum



Features

CCT (K)	: 0.0000	x	: 0.6511	deltau	: 0.4467	PFD-FR (700~780nm)	: 0.0240	PFD (380~780nm)	: 3.81	R5	: 0.0000	R11	: 0.0000
LUX (lx)	: 251	y	: 0.3444	deltav	: 0.5316	PFD-UV (380~400nm)	: 0.0051	IRR (Wm-2)	: 0.7479	R6	: 0.0000	R12	: 0.0000
ETime (ms)	: 266	u'	: 0.4467	LambdaD (nm)	: 607	PFD-R (600~700nm)	: 3.22	R1	: 0.0000	R7	: 0.0000	R13	: 0.0000
Purity (%)	: 98.6	v'	: 0.5316	LambdaP (nm)	: 612	PFD-G (500~600nm)	: 0.6226	R2	: 0.0000	R8	: 0.0000	R14	: 0.0000
fc (lmft-2)	: 23.4	deltax	: 0.6511	LambdaPV (mWm-2nm-1)	: 31.6	PFD-B (400~500nm)	: 0.0176	R3	: 0.0000	R9	: 0.0000	R15	: 0.0000
Duv	: 0.5702	deltay	: 0.3444	CRI (Ra)	: 0.0000	PPFD (400~700nm)	: 3.78	R4	: 0.0000	R10	: 0.0000		

NKsystem

SPECTRUM TEST REPORT

Red LED

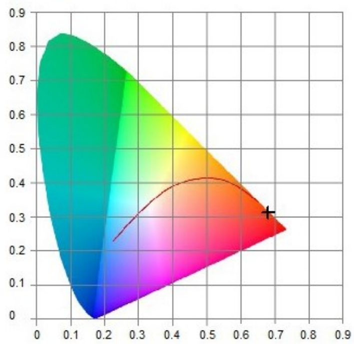
Information

User : Chansean	Measure Time : 2019/06/05 14:13:48
Model NO. : LA-105	Light Source : Red LED box
Memo :	

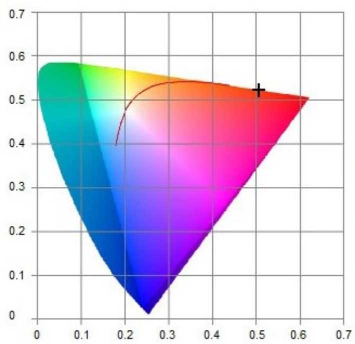
BASIC

CCT (K)	: 0.0000
x	: 0.6817
y	: 0.3126
LambdaD (nm)	: 618

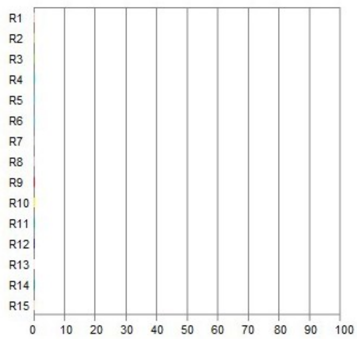
CIE1931



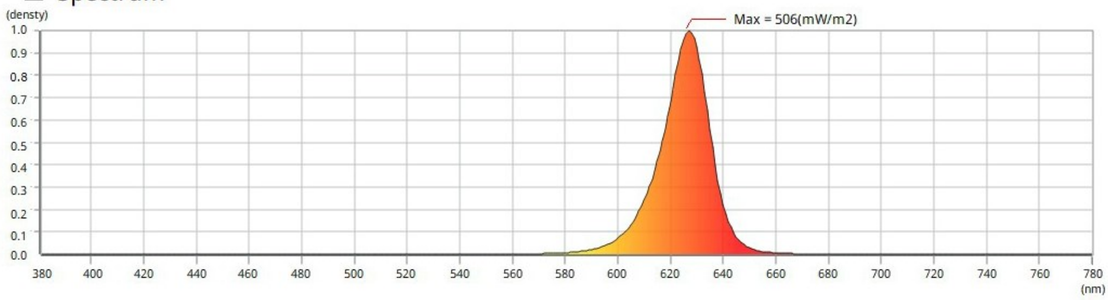
CIE1976



CRI



Spectrum



Features

CCT (K)	: 0.0000	x	: 0.6817	deltau	: 0.5061	PFD-FR (700~780nm)	: 0.4319	PFD (380~780nm)	: 59.4	R5	: 0.0000	R11	: 0.0000
LUX (lx)	: 2662	y	: 0.3126	deltav	: 0.5222	PFD-UV (380~400nm)	: 0.0707	IRR (Wm-2)	: 11.4	R6	: 0.0000	R12	: 0.0000
ETime (ms)	: 16.0	u'	: 0.5061	LambdaD (nm)	: 618	PFD-R (600~700nm)	: 56.8	R1	: 0.0000	R7	: 0.0000	R13	: 0.0000
Purity (%)	: 98.4	v'	: 0.5222	LambdaP (nm)	: 626	PFD-G (500~600nm)	: 2.06	R2	: 0.0000	R8	: 0.0000	R14	: 0.0000
fc (lmft-2)	: 247	deltax	: 0.6817	LambdaPV (mWm-2nm-1)	: 506	PFD-B (400~500nm)	: 0.2643	R3	: 0.0000	R9	: 0.0000	R15	: 0.0000
Duv	: 0.6143	deltay	: 0.3126	CRI (Ra)	: 0.0000	PPFD (400~700nm)	: 58.9	R4	: 0.0000	R10	: 0.0000		

NKsystem

SPECTRUM TEST REPORT

Fare-red LED

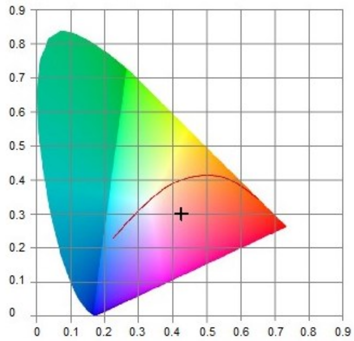
Information

User : Chansean	Measure Time : 2019/06/05 14:04:56
Model NO. : LA-105	Light Source : Far Red LED box
Memo :	

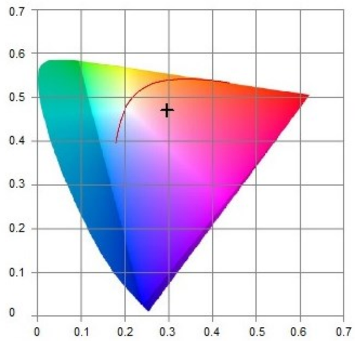
BASIC

CCT (K)	: 2200
x	: 0.4270
y	: 0.3016
LambdaD (nm)	: 690

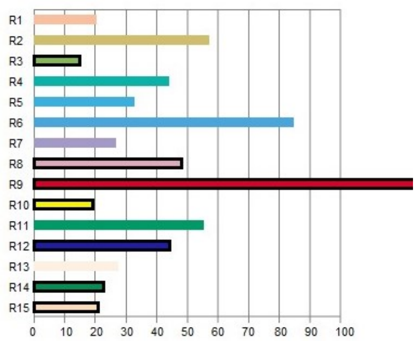
CIE1931



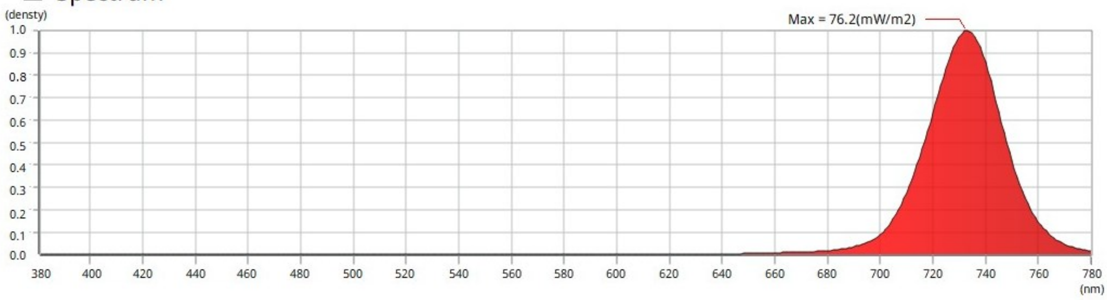
CIE1976



CRI



Spectrum



Features

CCT (K)	: 2200	x	: 0.4270	deltau	: 0.0062	PFD-FR (700~780nm)	: 15.9	PFD (380~780nm)	: 16.6	R5	: 32.6	R11	: 55.2
LUX (lx)	: 10.9	y	: 0.3016	deltav	: -0.0652	PFD-UV (380~400nm)	: 0.0139	IRR (Wm-2)	: 2.72	R6	: 84.4	R12	: -44.3
ETime (ms)	: 137	u'	: 0.2963	LambdaD (nm)	: 690	PFD-R (600~700nm)	: 0.6070	R1	: 20.4	R7	: 26.7	R13	: 27.3
Purity (%)	: 24.3	v'	: 0.4708	LambdaP (nm)	: 732	PFD-G (500~600nm)	: 0.0535	R2	: 56.9	R8	: -48.1	R14	: -22.6
fc (lmft-2)	: 1.01	deltax	: -0.0786	LambdaPV (mWm-2nm-1)	: 76.2	PFD-B (400~500nm)	: 0.0535	R3	: -14.9	R9	: -191	R15	: -20.9
Duv	: -0.0439	deltay	: -0.1137	CRI (Ra)	: 25.2	PPFD (400~700nm)	: 0.7130	R4	: 43.8	R10	: -19.3		

Appendix 4. 2: Worksheet for piloting with 2nd year students in affiliated high school to AUE

Note: A Japanese version was prepared for the students.

Topic: The Relation between Light and Seed Germination

Question

What is the effect of red and far-red light on seed germination?

Hypothesis

Do the seeds germinate or not in following condition?

N.	Light treatment	Result
1	Red → Far-red → Dark	
2	Far-red → Red → Dark	

Experiment

Seeds of cultivars: 3 lettuce cultivars, 4 radish cultivars, 1 celery cultivar, 1 edible chrysanthemum cultivar, and 1 mizuna cultivar.

Experimental procedure:

1. Place 4 layers of kitchen paper towel at the bottom of 5.5cm Petri dish
2. Place 20 seeds of each cultivar on the prepared Petri dish
3. Put each prepared Petri dish in each LED-attached box with red and far-red LEDs
4. Put a tube containing of 2 ml of tap water in the petri-dish
5. Close the box cover completely and shook the box to pour the water from the tube
6. Irradiate the first light, FR or R, continuously for 10 min, and then changed to second light, R or FR, for 10min respectively
7. Turn off the last light to keep the treated seeds in the dark condition in the box.

Experimental Result

Record your result in the table below and calculate the percentage of seed germination.

	Red → Far-red → Dark		Far-red → Red → Dark	
	Number of seed germination	Seed germination (%)	Number of seed germination	Seed germination (%)
Otegaru lettuce				
Sunny lettuce				
Furiru lettuce				
Asuka- Akena radish				
Shima radish				
Fukukomachi radish				
Hinonakabu radish				
Celery				
Chrysanthemum				
Mizuna				

Discussion

1. What did you learn about the relation between light and seed germination from this experiment?

………………………………………………………………………………………………
………………………………………………………………………………………………
………………………………………………………………………………………………
………………………………………………………………………………………………

2. What did you understand the effect of light on seed germination of different types of plant seeds?

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………………………………………………………………………………………………

Appendix 4. 3: Worksheet for piloting with teacher trainees at NIE in Cambodia

Note: A Khmer version was prepared for the trainees.

Topic: The effects of different light wavelengths on seed germination and early stage of seedling growth

There are many factors effecting seed germination and plant growth. Plants need water, mineral, temperature, humidity and light in the appropriate amount to grow and reproduce. Sunlight is the main light source for plants. Sunlight is composed of many light colors that make us observe as white light. Blue, green, and red light are the main light in sunlight.

Key Question

What are the effects of light wavelength on seed germination and early stage of seedling growth?

Hypothesis

Light condition	Seed germination		Seedling growth	
	Germinate	Not germinate	Grow well	Not grow well
Dark				
White light				
Red light				
Far-red light				
Green light				
Blue light				
Orange light				

Experiment

Experimental materials: lettuce seed ‘Furiru’, paper towel, water, LED-attached boxes of 6 different colors and dark box.

Experimental procedure:

1. Clean inside box and check LED light box whether the light bright well
2. Prepare 4 layers of paper towel and cut to fit the bottom of the box
3. Place 20 seeds of lettuce on the paper in the box
4. Light on the experimental box
5. Use plastic pipette or other tubes to absorb water and then pour on the paper in the box to make it wet
6. Close the box with irradiating the light continuously and keep until the day of result check with at least 3 days (without opening the box cover).

Experimental Result:

Fill the result in the table after seed sowing (at least 3 days):

Light condition	Number of Seeds		Number and length of seedlings	
	Germinate	Not germinate	Number	Average length (cm)
Dark				
White light				
Red light				
Far-red light				
Green light				
Blue light				
Orange light				

Analysis and Conclusion

Please analyze and make conclusion about lettuce seed germination and seedling growth. And then make temporary conclusion about other plants.

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………………………………………………………………………………………………

………………………………………………………………………………………………

Appendix 4. 4: Pre-lesson questionnaire for 2nd year students in affiliated high school to AUE

Note: A Japanese version was prepared for the students.

Answer the following questions by ticking.

Question 1: I understand that the seven colors of the rainbow are the light of different wavelengths.

Understand well	Understand	Do not understand well	Do not know
4	3	2	1

Question 2: The three elements of seed germination are well understood (water, temperature, oxygen).

Understand well	Understand	Do not understand well	Do not know
4	3	2	1

Question 3: I know that seeds have a phenomenon of "dormancy", and even when all three elements of germination are present, seed germination may not occur even if seeds that have just produced are sprinkled immediately.

Know well	Know	Do not know well	Do not know
4	3	2	1

Question 4: I understand that there is relation between "dormancy" and light wavelengths.

Understand well	Understand	Do not understand well	Do not know
4	3	2	1

Appendix 4. 5: Post-lesson questionnaire for 2nd year students in affiliated high school to AUE

Note: A Japanese version was prepared for the students.

Answer the following questions by ticking.

Question 1: I understood that the seven colors of the rainbow are the light of different wavelengths.

Understood well	Understood	Did not understand well	Do not know
4	3	2	1

Question 2: The three elements of seed germination were well understood (water, temperature, oxygen).

Understood well	Understood	Did not understand well	Do not know
4	3	2	1

Question 3: I knew that seeds have a phenomenon of "dormancy", and even when all three elements of germination are present, seed germination may not occur even if seeds that have just produced are sprinkled immediately.

Understood well	Understood	Did not understand well	Do not know
4	3	2	1

Question 4: I understood that there is relation between "dormancy" and light wavelengths.

Understood well	Understood	Did not understand well	Do not know
4	3	2	1

Question 5: How well did you understand this science lesson?

Understood well	Understood	Did not understand well	Do not know
4	3	2	1

Question 6: Was this class interesting for you?

Very interesting	Interesting	Not interesting	Boring
4	3	2	1

Question 7: Write three things you have learnt in this lesson. It doesn't matter you confirm it.

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Question 8: Please write the activities you want to do or to know after you took class.

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…………………………………………………………………………………………
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Question 9: Please write some things that you do not understand in this lesson.

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…………………………………………………………………………………………
…………………………………………………………………………………………
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Question 10: If you answered in question 6 that the lesson in this science class was not interesting or boring, please write the reason.

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Question 11: Do you think that the experimental apparatus you used help you understand the relation between light and seed germination?

Very interesting	Interesting	Not interesting	Boring
4	3	2	1

Question 12: Write down comment or suggestion

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Appendix 4. 6: Questionnaire for teacher trainees at NIE in Cambodia

Note: An English version was prepared for the trainees.

Please tick and answer the following questions

Question 1: Can you understand this science lesson?

☐ Very well ☐ Well ☐ Some ☐ Not at all

Question 2: Is this science class interesting for you?

☐ Very interesting ☐ Interesting ☐ A little interesting ☐ Not interesting

Question3: Did you get new knowledge or new thinking from this lecture?

☐ Very well ☐ Well ☐ A little ☐ Nothing

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

☐ Very useful ☐ Useful ☐ Not useful ☐ Bad

Question 5: Do you think that you can produce these apparatuses by yourself if there is enough materials available?

☐ Very sure ☐ Sure ☐ Not sure ☐ Not at all

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

☐ Not at all ☐ Dangerous ☐ Very dangerous ☐ Cannot use at school

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

☐ University level Chapter:..

☐ Senior high school level Chapter:..

☐ Junior high school level Chapter:..

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: Comment and suggestion

Appendix 5. 1: Light spectrum test report of red or far-red in the LED attached box

SPECTRUM TEST REPORT

Red LED

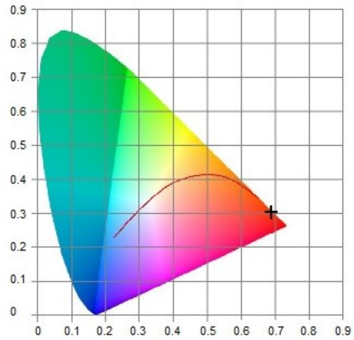
■ Information

User : Chansean	Measure Time : 2018/12/04 15:28:48
Model NO. : LA-105	Light Source : Red 100% volume
Memo :	

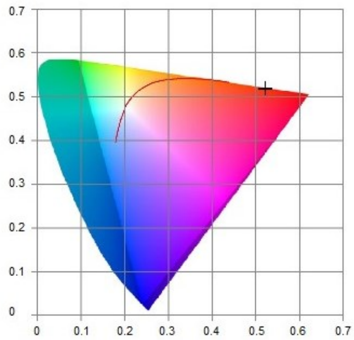
■ BASIC

CCT (K)	: 0.0000
x	: 0.6884
y	: 0.3041
LambdaD (nm)	: 623

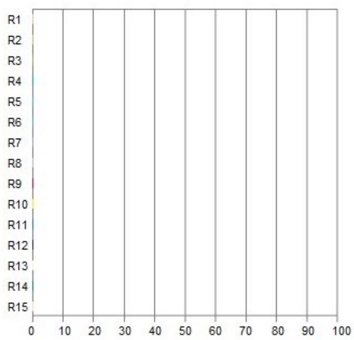
■ CIE1931



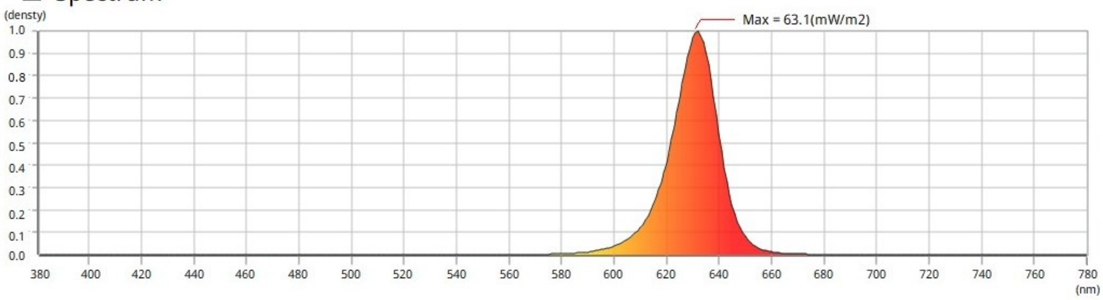
■ CIE1976



■ CRI



■ Spectrum



■ Features

CCT (K)	: 0.0000	x	: 0.6884	deltau	: 0.5222	PFD-FR (700~780nm)	: 0.0605	PFD (380~780nm)	: 7.46	R5	: 0.0000	R11	: 0.0000
LUX (lx)	: 281	y	: 0.3041	deltav	: 0.5191	PFD-UV (380~400nm)	: 0.0105	IRR (Wm-2)	: 1.42	R6	: 0.0000	R12	: 0.0000
ETime (ms)	: 138	u'	: 0.5222	LambdaD (nm)	: 623	PFD-R (600~700nm)	: 7.21	R1	: 0.0000	R7	: 0.0000	R13	: 0.0000
Purity (%)	: 97.6	v'	: 0.5191	LambdaP (nm)	: 631	PFD-G (500~600nm)	: 0.1627	R2	: 0.0000	R8	: 0.0000	R14	: 0.0000
fc (lmft-2)	: 26.1	deltax	: 0.6884	LambdaPV (mWm-2nm-1)	: 63.1	PFD-B (400~500nm)	: 0.0364	R3	: 0.0000	R9	: 0.0000	R15	: 0.0000
Duv	: 0.6265	deltay	: 0.3041	CRI (Ra)	: 0.0000	PPFD (400~700nm)	: 7.39	R4	: 0.0000	R10	: 0.0000		

NK system

SPECTRUM TEST REPORT

Fare-red LED

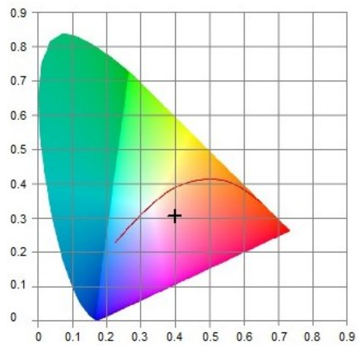
Information

User : Chansean	Measure Time : 2018/12/04 15:49:18
Model NO. : LA-105	Light Source : Far Red 100% Volume
Memo :	

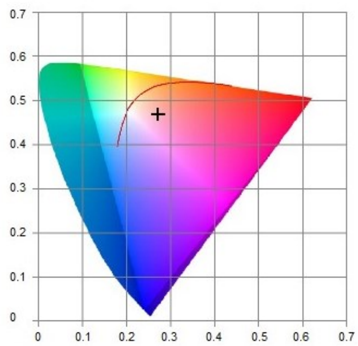
BASIC

CCT (K)	: 2746
x	: 0.3994
y	: 0.3056
LambdaD (nm)	: 690

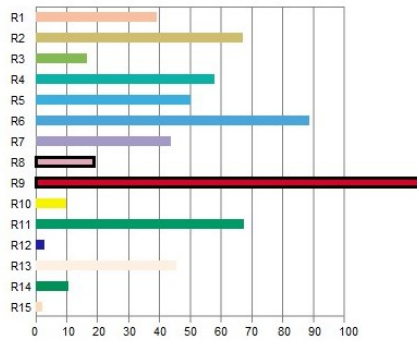
CIE1931



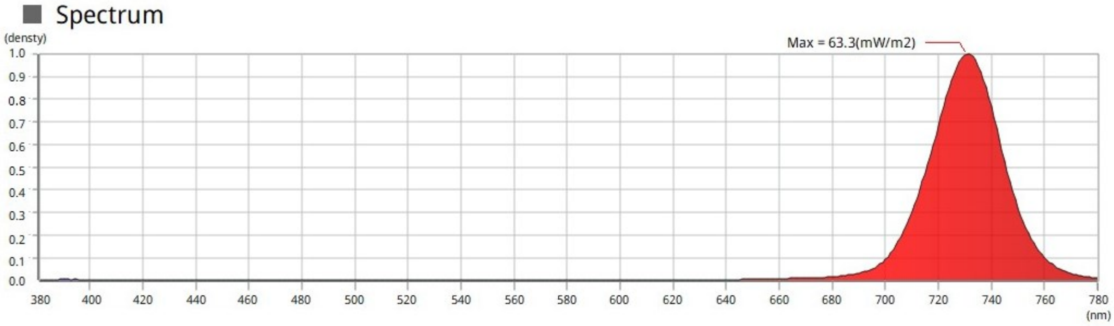
CIE1976



CRI



Spectrum



Features

CCT (K) : 2746	x : 0.3994	deltau : 0.0117	PFD-FR (700~780nm) : 12.6	PFD (380~780nm) : 13.2	R5 : 49.8	R11 : 67.3
LUX (lx) : 13.6	y : 0.3056	deltav : -0.0578	PFD-UV (380~400nm) : 0.0181	IRR (Wm-2) : 2.19	R6 : 88.4	R12 : 2.55
ETime (ms) : 166	u' : 0.2722	LambdaD (nm) : 690	PFD-R (600~700nm) : 0.5234	R1 : 39.2	R7 : 43.8	R13 : 45.2
Purity (%) : 17.6	v' : 0.4687	LambdaP (nm) : 730	PFD-G (500~600nm) : 0.0727	R2 : 66.9	R8 : -18.9	R14 : 10.5
fc (lmft-2) : 1.27	deltax : -0.0568	LambdaPV (mWm-2nm-1) : 63.3	PFD-B (400~500nm) : 0.0702	R3 : 16.4	R9 : -150	R15 : 2.05
Duv : -0.0403	deltay : -0.1041	CRI (Ra) : 42.9	PPFD (400~700nm) : 0.6648	R4 : 57.8	R10 : 9.58	

NKsystem

Appendix 5. 2: Worksheet for piloting with 3rd year student at affiliated senior high school to AUE

Note: A Japanese version was prepared for the students.

Topic: The Relation between Light and Seed Germination

Review

Seed germination control

Plant hormone: (1) ⇒ suppress seed germination (dormancy)

Plant hormone: (2) ⇒ promote seed germination

Photoblastic seed germination

Photoblastic seed cultivar: (3) in Arabidopsis

Do seeds germinate under following conditions?

N.	Light treatment	Result
1	Red → Dark	
2	Far-red → Dark	
3	Red → Far-red → Dark	
4	Far-red → Red → Dark	

Experiment

Seeds: Furiru lettuce seeds Experimental apparatus: R-FR LED attached boxes
Experimental procedure as in the table:

Procedure	Red → **Far-red** → Dark	Far-red → **Red** → Dark
1	Set red light to 8 and far-red to 5 to make the light intensity similar.	
2	Put water on the seeds and then place in the LED-attached box.	
3	Irradiate red light for 10 minutes	Irradiate far-red light for 10 minutes
4	Turn off the red light, and then irradiate far-red light with different period of time as follows respectively. 1min, 2min, 3min, 4min, 5min 6min, 7min, 8min, 9min, 10min	Turn off the far-red light, and then irradiate red light with different period of time as follows respectively. 1min, 2min, 3min, 4min, 5min 6min, 7min, 8min, 9min, 10min

5	Keep the treated seeds in the dark condition

Experimental result prediction

Set the germination rate (%) on the vertical axis and the period of time (minutes) on the horizontal axis to plot the expected result.

Graph 1: Red → **Far-red** → Dark

Graph 2: Red → **Far-red** → Dark

Experimental result

Fill the result in the table bellows:

Table 1: Red → **Far-red** → Dark

FR irradiation (min)	1	2	3	4	5	6	7	8	9	10
N. of seed germination										
Seed germination (%)										

Table 2: Far-red → **Red** → Dark

R irradiation (min)	1	2	3	4	5	6	7	8	9	10
N. of seed germination										
Seed germination (%)										

Plot the result in Graph 1 and Graph 2 above.

Appendix 5. 3: Worksheet for piloting with teacher trainees at NIE in Cambodia

Note: A Khmer version was prepared for the trainees.

Topic: The Effects of Red and Far-red Light on Seed Germination

Sunlight is the main light source for plants. Sunlight is composed of many light colors that make us observe as white light. Blue, green, and red light are the main light in sunlight. In the experiment, different light source can be irradiated on seed such as red or far-red light wavelength.

Key Question

What is the effect of red or far-red light on seed germination?

Hypothesis

Light condition	Seed germination	
	Germinate	Not germinate
Red light 30min → Far-red light 30min →Dark		
Far-red light 30min → Red light 30min →Dark		

Experiment

Experimental materials: lettuce seed 'Furiru', paper towel, water, LED-attached boxes of red and far-red

Experimental procedure:

1. Clean inside box and check LED light box whether the light bright well
2. Prepare 4 layers of kitchen paper towel and cut to fit the bottom of the box
3. Place 20 seeds of lettuce on the paper in the box

4. Turn on the first light (red or far-red)

5. Use plastic pipette or other tubes to absorb water and then pour on the paper in the box to make it wet

6. Close the box and continue to irradiate the first light for 30min and then change to second light (Red or Far-red) for 30min

7. Close the second light and keep until the day of result check with at least 3 days (without opening the box cover).

Experimental Result

Fill the result in the table after seed sowing (at least 3 days):

Light condition	Number of seeds	
	Germinated	Did not germinate
Red light 30min → Far-red light 30min →Dark		
Far-red light 30min → Red light 30min →Dark		

Analysis and Conclusion

Please analyze and make conclusion about the effect of Red or Far-red light on lettuce seed germination.

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Appendix 5. 4: Pre-lesson questionnaire for 3rd year student in affiliated high school to AUE

Note: A Japanese version was prepared for students.

Answer the following questions by ticking the number of the applicable item.

Question 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?

Understand well	Understand	Do not understand so well	Do not understand at all
4	3	2	1

Question 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)?

Understand well	Understand	Do not understand so well	Do not understand at all
4	3	2	1

Question 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?

Understand well	Understand	Do not understand so well	Do not understand at all
4	3	2	1

Question 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?

Understand well	Understand	Do not understand so well	Do not understand at all
4	3	2	1

Appendix 5. 5: Post-lesson questionnaire for 3rd year students in affiliated high school to AUE

Note: A Japanese version was prepared for students.

Please 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?

Understood well	Understood	Did not understand so well	Did not understand at all
4	3	2	1

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

Understood well	Understood	Did not understand so well	Did not understand at all
4	3	2	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?

Understood well	Understood	Did not understand so well	Did not understand at all
4	3	2	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?

Understood well	Understood	Did not understand so well	Did not understand at all
4	3	2	1

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

Understood well	Understood	Did not understand so well	Did not understand at all
4	3	2	1

Question 6: Was this class interesting for you?

Very interesting	Interesting	Not interesting	Boring
4	3	2	1

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

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

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

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………………………………………………………………………………………………
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Question 10: Please write reason if you answered not interesting or boring for this science class on question 6.

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………………………………………………………………………………………………
………………………………………………………………………………………………

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

Very useful	Useful	Not very useful	Textbooks enough
4	3	2	1

Question 12: Comments and impressions (if any)

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………………………………………………………………………………………………
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Appendix 5. 6: Questionnaire for teacher trainees at NIE in Cambodia

Note: An English version was prepared for the trainees.

Please tick and answer the following questions

Question 1: Can you understand this science lesson?

☐ Very well ☐ Well ☐ Some extent ☐ Not at all

Question 2: Is this science class interesting for you?

☐ Very interesting ☐ Interesting ☐ A little interesting ☐ Not interesting

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

☐ A lot ☐ Some ☐ A little or a few ☐ Not at all

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

☐ Very useful ☐ Useful ☐ Not useful ☐ Not at all

Question 5: Do you think that you can produce these apparatuses by yourself if there is enough materials available?

☐ Very sure ☐ Sure ☐ Not sure ☐ Not at all

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

☐ Not at all ☐ Some attention should be required ☐ Dangerous ☐ Very dangerous

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?

☐ University level Chapter:..

☐ Senior high school level Chapter:..

☐ Junior high school level Chapter:..

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?

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Question 10: Comments and Suggestions (if any)

………………………………………………………………………………………………………