Conference Paper

The Effects of Light Color on Seed Germination of *Markhamia stipulata* (Wall.) Seem

Sri Ulie Rakhmawati and Arief Noor Rahmadiyanto

Center for Plant Conservation Botanic Garden, Indonesian Institute of Sciences, Jl, Ir. H. Juanda No.13, Bogor 16003, Indonesia

Abstract

Markhamia stipulata (Wall.) Seem. (Bignoniaceae) is one of collections in Bogor Botanical Garden that potential as a medicine. Information about germination of these species were not known yet. This research was aimed to examine the effect of light color on germination process of *Markhamia stipulata* (Wall.) Seem. The research method used a Randomized Complete Block Design (RCBD) that consist of three treatments (red light, far red light, dark) and one control (open air) with four replications for each group. Each group consist of 10 seeds. Precentage of seed germination; seedling height; cotyledon width; length, width and number of leaves were measured. The result showed that seed germination start at third day after sowing. Germination rate and germination rate coefficcient showed there is no significant difference between all treatments even though far red light showed highest number (100%; 0.755). However, light color influence on seedling growth. Far red light showed affect on internodes distance. Dark light showed affect on leaves and cotyledon size. Red light and control showed similarity.

Keywords: dark; far red light, *Markhamia stipulata* (Wall.) Seem; red light; seed germination.

Corresponding Author:
Sri Ulie Rakhmawati
ulierakhmawati@yahoo.com

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1. Introduction

Markhamia stipulata (Wall.) Seem (Bignoniaceae) is one of collection in Bogor Botanic Garden. It is a medium size tree, approximately 15 m to 30 m tall, with a trunk diameter up to 50 cm. The bark is grey with many thin inner layers that are yellow. It has low separate branches that contain bifoliate pinnate leaves, opposite and 40 cm to 45 cm long. *M. stipulata* flower is bisexual, brown-yellow in color, bilabiate and stamens have two calycine-stamens in a compound ovarium [1]. As like other Bignoniaceae, *M. stipulata* also has wafer-thin, winged seeds. This winged seeds enable traveled for hundreds of meters in favourable condition [2].

This species can be found in sparse forests and humid places at elevations from 300 m to 1 700 m, growing in evergreen forest on limestone mountains in southern China [3]. Based on observation in Herbarium Bogoriense showed that *M.stipulata* also found in Laos and Vietnam. We need to preserve *M. stipulata* because this species has many potential. Tradionally, a lot of genus Markhamia used as medicines. Ali et al.

showed that *M. stipulata* leaves and bark contain of phenylpropanoids that describes as markhamiosides (A-E). Phenylpropanoids are large group of secondary plant metabolites, mainly produce in response to wound, infection, UV irradiation or any other stressfull condition attribute to their free radical scavenging capability [4]. *M. stipulata* leaves also harvested as a vegetable in Laos and the tree used as timber [1].

However, less of study of *M. stipulata*, especially for seed germination. Seed germination is light sensitive. The germination process involves some kinds of light-controlled behaviour that help the young shoot out of the ground [5]. Information about effect of light on *M. stipula* seed germination is lack of data. So, this study aims are (i) to find out the fruit and seed characteristic of *Markhamia stipulata*; (ii) to observe effect of color on *M. stipulata* seed germination.

2. Materials and Methods

2.1. All collecting materials and methods

Fruits and seeds were collected from Bogor Botanical Garden on Mei 8, 2015. After that, the fruits and seeds were characterized on size, weight and color. Color characterization was using RHS color chart. Fruits and seeds examination were conducted in Treub Laboratory and Seed Bank Unit of Bogor Botanical Garden while observation of seed germination conducted in Seed Bank Unit of Bogor Botanical Garden from Mei to June, 2015. Three fruits of *M. stipulata* were used as fruits examination, and 30 seeds used as seeds examination, that were choosen randomly.

Randomized Complete Block Design (RCBD) with total three treatments of light color (red light, far red light and dark) and one control was used to seed germination test. Each treatment had four replications and each replication contained 10 seeds. All the seeds were immersed in the water for 24 h to encourage imbibition before sowing. Seeds was immersed for control, red light and far red light treatments using a jar and put it in open air room, but for dark treatment was put it in storage room without any of light. Sowing of the seeds conducted in polybag with bamboo as media and put it on each treatment boxes (red light, far red light and dark), and control were put in open air. After 16 d of germination, the amount of seedling; seedling height, cotyledon width and length, width and number of leaves was measured.

2.2. Data analysis

For analysis, we measure water moisture content (%) (i), seed germination rate (ii), germination rate coefficient (iii) and coefficient of simultaniety grow (4). The formulation as bellow [6]:

$$\text{Water moisture content (\%)} = \frac{\text{dried weight}}{\text{moist weight}} \times 100\% \quad (1)$$

$$\text{Seed germination rate} = \frac{\text{amount of seeds that germinate}}{\text{amount of sowing seeds}} \times 100\% \quad (2)$$

$$\text{Germination seed coefficient} = \frac{\sum n}{n \times t} \quad (3)$$

$$\text{Coefficient of uniformity of germination} = \frac{\sum n}{\sum\{(T-t)^2 \times n\}} \quad (4)$$

$$T = \frac{\sum(t \times n)}{\sum n} \quad (5)$$

Description: t: germination day; n: amount of seeds that germinate.

Data will be checked on normality then followed by F test on 0.05 level using Minitab 16.0 software. If data not normal, we have to normalized first. After that, data will be test with Duncan Multiple Range Test (DMRT) 0.05 if there is significant treatments.

3. Result and Discussion

3.1. Fruit and seeds description

Based on the examination, the fruit of *Markhamia stipulata* (Wall.) Seem (see Fig.1) was long, oblongate and flat as like as sword [7]. Color of the fruit is greyed brown group (199 C-D) based on RHS color chart. It contain hundreds of winged seed for each fruit (between 150 to 400 seeds). When it is matured, the fruits dried and dehisce on the line of dehiscence into two carpels, then the winged seeds will release. The fruit has smooth hairy like fur covered all the mesocarp. The average size of fruits are 64 cm long, 28 mm width and 77 g of weight.

The seed of *M. stipulata* (see Figure 2) catagorized as a winged seed with two opposite wings in both sides. Its color is white group (155 B-D) based on RHS color chart. The average size of seed are 46 mm long, 10 mm width and 0.05 g of weight. The embrio was in midle front of the seed. The seed is ortodox type, because it is tolerant with desication [8].

3.2. Imbibition rate

Germination starts with imbibition, a process of water uptake of seed, caused by different water concentration between seed and the environment. It changes the metabolic process of the seed and induce cell diferrentiation of the embryo. It is because water uptake can activate the enzymatic activity. More water uptake encourage colloidal material such as protein inflated and causing the split of seed husk [9].

Before imbibition, the weight was 0.89 g (for 10 seeds) with water moisture content 6.01%. After 24 h imbibition showed that a lot of water uptake increased the weight of seeds. Figure 3 showed that after imbibition, the weight was increasing until 2.74 g

Figure 1: Fruits of *Markhamia stipulata* (Wall.) Seem.

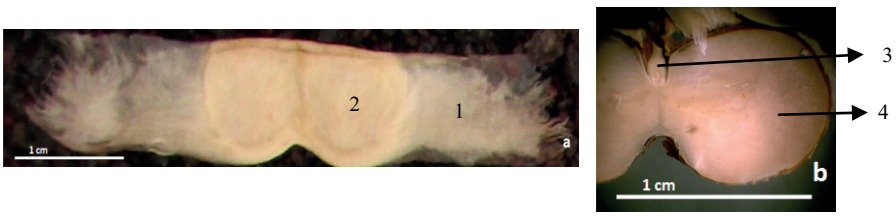

Figure 2: Seed of *Markhamia stipulata* whole body (a). consist of two opposite wings (1) and body (2). cross section of seed (b) showed embryo in midle front of the seed (3) and endosperm (4).

or about 300% from earlier (for 10 seeds). At this point, we assumed that *M.stipulata* would be germinating fast.

Germination type of *M. stipulata* (Wall.) Seem is epigeal. The first growth are radicules (primary roots) appearence and followed by lengthwise of hypocotyl that bring cotyledons and plumulae (foliage leaves) to upground [5]. This process happend on 3 d. On 5 d, cotyledon already realease from the husk. The 6 d cotyledon open perfectly and shows plumulae. After 7 d, appear first leaves (two leaves every appearence). The second leaves (two more leaves) appear on 9 d. Figure 4 shows all process of seed germination on *M. stipulata*.

Seed germination induce by many factors, one of them are light colors. Germination is regulated by a pigment, phytochrome, which is sensitive to different wavelenghts of light. When far red light is receive the phytochrome equilibrium changes towards the

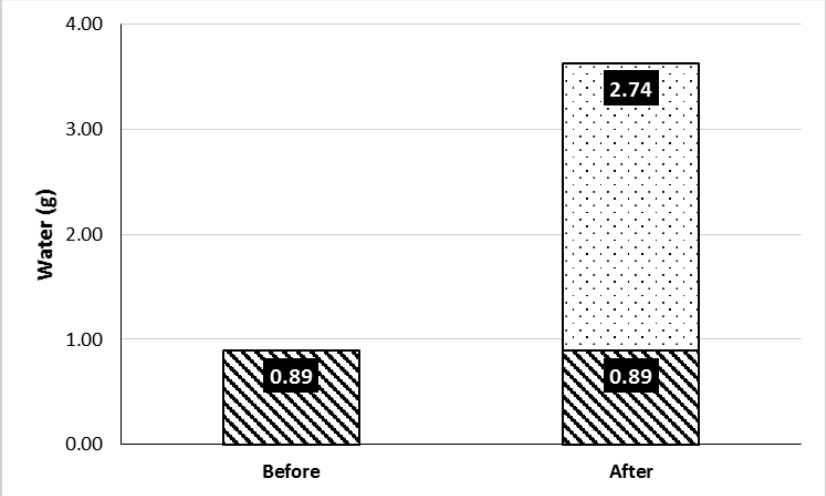

Figure 3: Imbibition rate after 24 h Seed germination.

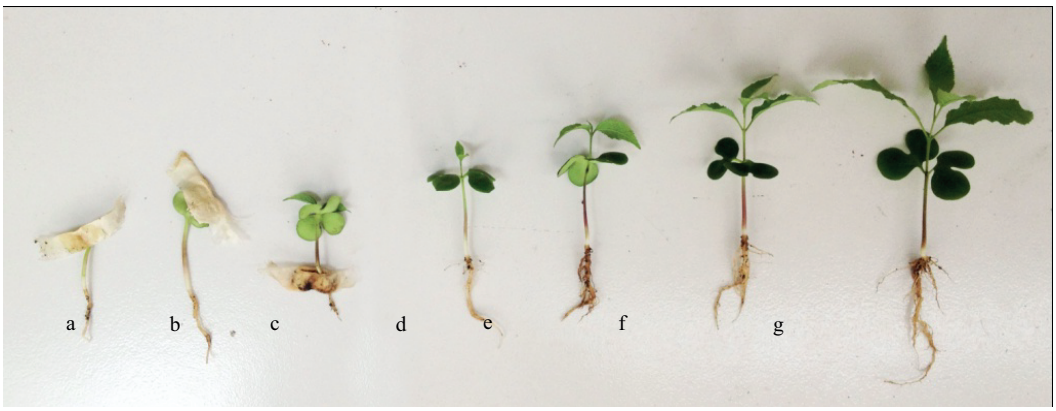

Figure 4: Seed germination process on *Markhamia stipulata* since 3 d until 9 d. (a) 3 d; (b) 4 d; (c) 5 d; (d) 6 d; (e) 7 d; (f) 8 d; (g) 9 d.

red light absorbing form. The reverse occurs when red light is receive. Phytochrome has function in stimulating or inhibiting of germination seeds [9].

In this study, we used red light (about 650 mμ), far red light (about 730 mμ) and dark. Based on observation, color of light doesn't affect the germination rate (Table 1). Seeds germinate after 3 d, due to radicules appearence, and by 12 d all seeds are germinated. The highest germination rate is far red light (100%) then followed by dark light (97.5%). Control and red light show the same germination rate (95%). Germination rate coefficcient also showed no significant difference between all treatments even though far red light showed highest number (0.755). At this point, we assume that far red light absorbing form works as trigger for sprouting, while the red light absorbing form of the pigment prevents germination.

Red light was indicate a gap canopy. So, when red light has the highest coefficient of uniformity of germination (0.998), it indicates that seeds can be germinating well on gap canopy. In forest vegetations this only takes place when the canopy is opened and the light is enough to germinate [10]. Opposites of that, far red light has the lowest

Treatments	Germination rate	Germination Rate Coefficient	Coefficient of uniformity of germination
Control	95.0	0.657	0.122
Dark light	97.5	0.652	0.115
Far red light	100.0	0.755	0.095
Red light	95.0	0.736	0.998
Description: Number followed by the same letter showed no significant difference at 0.05 DMRT test.			

Table 1: Seed viability of Markhamia stipulata (Wall.) Seem.

	Height (cm)	Hipocotyl (cm)	Epicotyl (mm)	Leaves length (mm)	Leaves width (mm)	Cotyledon length (cm)	Cotyledon width (mm)
Control	16.99	14.35	2.23	35.18	15.83	27.93	14.83
Dark light	16.84	14.00	2.67	11.32	2.99	22.93	13.00
Far red light	19.38	13.64	5.06	12.94	5.23	24.20	14.48
Red light	15.99	12.50	3.22	16.74	6.00	23.60	13.17

Table 2: Seedling growth of Markhamia stipulata (Wall.) Seem.

coefficient of uniformity of germination (0.095). This condition showed that seeds on far red light needs more time to germinate all.

3.3. Seedling growth

Most seed which germinate posses a more or less large amount of food, which stored in the cotyledons, endosperm, or more rarely in the hypocotyl. The food source is rather long persistent on the seedlings, and support the development of strong root system and a stem with two or more developed leaves. When two opposite leaves are produced, the cotyledons are soon dropped after the former have attained full size. After that, the plant is dependent on the assimilates produced by these leaves. This growth is also influenced by light.

Based on observation, seedling in the dark treatment (see Figure 5a) are pale; leaves do not expand but remain small, rudimenter and yellowish; cotyledon small and yellowish. Seedling in far red light treatment are longer than others, leaves are small and yellowish-green; cotyledon are green. Far red light treatment (see Figure 5b) has internodes elongate many times more than normal and cause the plant becomes very tall and splindly (Table 2). This condition because of far red light activated auxin hormone more than others [11].

Seedling on red light (see Figure 5c) and control (see Figure 5d) showed similarity, except internodes on red light is more longer than control. Leaves and cotyledon are green, full size and grow normally, even though leaves on red light are smaller than control. Red light can inhibitate auxin, so other cells can be growth, not only roots and axillary buds [12]. This condition indicate that both red and far red light are needed on seed germination to germinate normally.

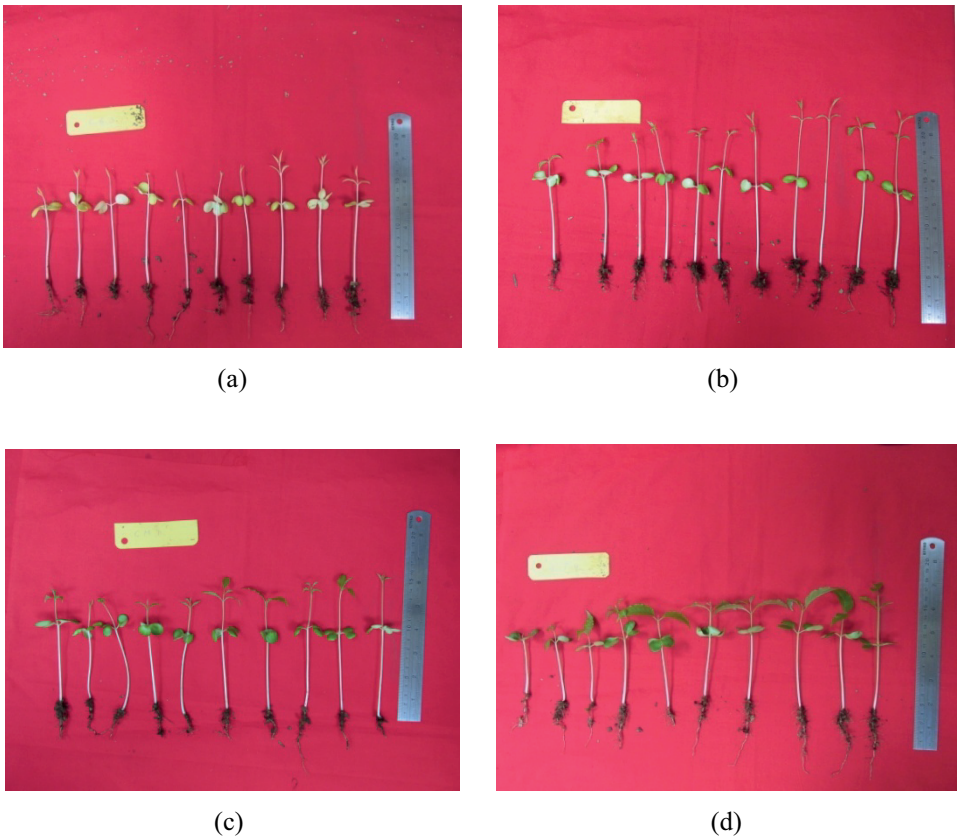

Figure 5: Seedling growth of *Markhamia stipulata* (Wall.) Seem in different light color. (a) seedling of dark treatment; (b) seedling of far red light treatment; (c) seedling of red light treatment; (d) seedling of control (open air).

4. Conclusions

Fruit of *Markhamia stipulata* (Wall.) Seem was long, oblongate, flat as like as sword and greyed-brown. The seed was catagorized as winged seed with two opposite wings on both sides. Light color doesn't influence on seed germination of *M. stipulata*. Germination rate and germination rate coefficient showed there is no significant difference between all treatments even though far red light showed highest number (100%; 0.755). However, light color influence on seedling growth. Far red light affect internodes elongate many times more than normal and cause the plant becomes very tall and splindly. Dark light affect leaves and cotyledon small, rudiment and yellowish. Red light and control showed similarity, except internodes on red light is longer than control.

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