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Effects of Different Light Treatments on the Germination of *Nepenthes mirabilis*

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ABSTRACT

Seeds of *Nepenthes mirabilis* were germinated *in vitro* on composted medium. All seeds were germinated under white (fluorescent), red, green, blue and yellow light. Over a period of 27 days, some Nepenthes seeds under white and red light germinated first, and those under green light were the last ones to germinate. The highest average speed of emergence was recorded for seedlings under red light. All healthy and complete seedlings were counted after 60 days. Seedlings under yellow light were the most vigorous with the highest germination index and average height of 0.79 cm. Seedlings under yellow light and white light exhibited the highest average number of roots and light green leaves as well as greatest root length, but seedlings under green light had few roots and pale green leaves. There were more young leaves on seedlings under yellow light (average 5.2) and red light (average 5.0) compared with those under green light and blue light.

1 Introduction

Nepenthes has a common name, Tropical Pitcher Plant. It is found in diverse habitats throughout Southeast Asia : Thailand, Malaysia, Indonesia. There are about 200 species in the tropical area. Nepenthes can be propagated by growing seeds, stem cutting, and tissue

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culturing. It can be grown as a commercial hanging plant. Nepenthes can grow in different environments(Chawan et al.,1971).

Nepenthes mirabilis be found in all parts of Thailand (Catalano (2010),Chawan et al. (1971) and Cheek and Jebb (2009)) . It is a climbing plant that grows to about 10 meters in height with single lanceolate spiral leaf arrangement, parallel vein, and long green pitcher. Some pitchers have red vein at the pitcher peristome flowering from November to December. It has long seeds, average about 1 cm. Nepenthes is a dioecious plant. Rischer (2000) reported that Nepenthes seeds were sown on media, sprayed with pure water, and the pots were placed in plastic bags in warm temperature and 100 % RH. It takes one month for seeds to germinate.

Nepenthes is one of the plant species protected by CITES (1992). Its survival in the natural environment is increasingly endangered by the growing demand for land for farming and housing. Another threat to its survival is the sale of the plant at Sunday markets. Many varieties of Nepenthes will be lost forever unless attempts are made to protect and conserve the species.

Growing Nepenthes from seed is one method for increasing the number of plants. Germination can take from 4 weeks to almost a year. Little is known about the factors that promote seed germination. The study was designed to discover how different light treatments can facilitate seed germination.

2 Materials and Methods

The study was conducted in the laboratory of Plant Biotechnology, Faculty of Science and Technology, Thammasat University, Rangsit Campus, Thailand, from November 2009 to August 2010. Seeds of *Nepenthes mirabilis* were used as experimental materials. The experiment was designed to assess the effect of different types of light: (white (fluorescent), red light with peak emission of 660 nm, green light with peak emission of 550 nm , blue light with peak emission of 490 nm ,and yellow light with peak emission of 600 nm light) (all of electric light were from Philips company in Thailand) on germination of *Nepenthes mirabilis*.

The seeds were sprinkled onto the surface of the sterile composted medium, then sprayed

with sterile water, and placed the pots in plastic bags. These pots were placed under different lights with 25 ± 2º C. There were 5 treatments in this experiment including the control (white light). The experiment was undertaken in completely randomized design with six replications, and 100 seeds were tested for each replication. Data on different germination parameters were recorded after germination at 5-day intervals until no further germination occurred. The seedlings were evaluated as described in seedling Evaluation Handbook (AOSA,1983).

The germination Index (GI) was calculated as described in the Association of Official Seed Analyst (AOSA,1983) by following formula:

$$\text{Germination Index (GI)} \quad GI = \Sigma \left(\frac{GT}{Tt} \right)$$

$$\text{Or} \quad \left[\frac{\text{No.of germinated seed}}{\text{Days of final or last count}}\right] + \ldots\ldots + \left[\frac{\text{No.of germinated seed}}{\text{Days of final or last count}}\right]$$

The vigor index was calculated according to ISTA, [12] following formula :

$$\text{Seedling Vigor Index (SVI)} = \left[\frac{\text{seedling length (cm)} \times \text{Germination percentage}}{100}\right]$$

The speed of emergence was calculated according to following formula

$$\text{Speed of emergence} = \left[\frac{\text{No.of germinated seed 30 day after sowing}}{\text{No.of seeding emergence 50 day after sowing}}\right] \times 100$$

The number of days taken for first germination was counted from the data of treatment. The number of days from first to last germination for each trial was observed. Moreover, measurements were made of the percentage of germinated seeds. Final germination percentage (%), seedling length, was recorded after 30 days of planting. For statistical analysis, experimental data were analyzed by a statistical package SAS,(2000). Treatments means were compared using Duncan's multiple Range Test at 5 % level of probability (Steel and Torrie, 2010).

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Table 1: Effect of different light (blue, green, red, yellow, and white light) treatments on germination parameters of *Nepenthes mirabilis*.

Light	NDFG (Days)	GP (%) *	Color of cotyledon and seedling leaves
White	28	57±7.71bc	Light green
Red	28.67	60.75±8.518b	Green
Blue	37	31.63±11.066d	Green
Green	30	36.4±2.93cd	green
Yellow	30	83.69±3.372a	Light green

NDFG - number of days to first germination

GP - germination percentage

a b c - Figures not sharing the same letters in the same column differ significantly at $p < 0.05$

3 Result and discussion

It was revealed from this study that different light treatments can have various effects on different seed germination parameter of *Nepenthes mirabilis* (Table 1).It has been reported earlier that light is an important factor affecting germination and seedling growth. *Nepenthes mirabilis* seeds which were exposed to red and white light started to germinate within 28 days after sowing. Research works from Ellis and Robert (1981), Hangarter (1997), Wapeha and Kaufman (1989) and Winslow (1999) reported that many plants species responded to the environment with optimal growth and development according to the light they received and Colbach (2002) reported that some seeds germinated under different lights. In this experiment, the earliest germination was observed in white and red light treatments. Germination of seeds of *Ruellia tuberosa* (Borthwick,1957) , *Asteracantha longifolia* (David and Chawan ,1970) and *Cucumis callosus* (Bansal and David,1978) was promoted when irradiated with red light. These reports supported the findings of David and Chawan (1970) , and Shyam and David (1975) that the red region of spectrum (590 and 680µm) was most effective for the germination of light requiring seeds. *Nepenthes mirabilis* seeds germination started 28 days after sowing and *Nepenthes mirabilis* seeds under blue light stared to germinate within 37 days. David and Chawan (1970), and Shyam and David (1975) also

reported that the seedling growth of some *Merremia* species was the least in blue light. This was similar to the findings by Wareing and Black (1958) and Gwynn and Scheibe (1972) with regard to lettuce seeds. *Nepenthes mirabilis* seeds under white and red light showed the fastest germination. Shyam and David (1975), and Steel and Torrie (2010) reported that the highest percentage of some *Merremia* sp. was found in red light. After 80 days, all seedlings were counted for germination percentage. Seedlings under yellow light gave the highest percentage (83.69 %). Their leaves were light green like leaves in white light. Leaves under red, green, and blue lights were green.

After 45 days, germination index, speed of emergence, seedling vigor index and number of seedlings that survived are shown in Table 2. There was significant difference ($p<0.05$) among their parameters. Under yellow light, average number of seedlings was the highest (83.69%); their germination index was 2.384 and seedling was vigor (0.661) also. Seedlings under red light and white light were 60.75% and 57.0 % respectively. Saebo et al. (1995) reported that red light was important for the development of the photosynthetic apparatus of plant and blue light was important in the formation of chlorophyll. Speed of emergence from seeds of Nepenthes was the highest under red and white light (49.38 and 49.122% respectively).

Table 2: Average percentage of germination and characteristic of *Nepenthes mirabilis* seedling after growing 80 days.

Light	SVI *	GI *	SE (%) *
White	0.399 bc	1.65 bc	49.122 a
Red	0.419 b	1.823 b	49.38 a
Blue	0.227 d	0.733 d	33.003 c
Green	0.251 c	0.949 cd	32.96 cd
Yellow	0.661 a	2.384 a	39.825 b

SVI - Seedling Vigor Index

GI - Germination Index

SE - Speed of Emergence

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a,b,c - Figures not sharing the same letters in the same column differ significantly at $p < 0.05$

All seedlings were transferred and cultured under room temperature with sun shade for 30 days. Maximum number of root and their length of *Nepenthes mirabilis* in different lights were also recorded. Nonetheless, for Nepenthes under white light the longest root and their length (fig. 1) were obtained from seedlings after culturing 30 days (Table 3). Significant ($p<0.05$) effect of seed priming was observed on the final germination percentage in different lights. Maximum height of seedling was seen in yellow light followed by seedling under the other light (blue, red, green and white lights). The different lights had effects on the number of roots and root length. Seedling from yellow and red lights gave the highest average number of leaves and their leaves were green and light green respectively. Winslow and Eva (1999) reported that blue light was important in the formation of chlorophyll and Warpepha and Kaufman(1989) reported that blue light was effect to epicotyl elongation of *Pisum sativum*; Saebo et al.(1995) reported that red light was important for the development of the photosynthetic apparatus of plant.

Table 3: Average number of roots, root length, seedling height, and number of leaves per seedling after culturing 30 days.

light	No. of roots (root)*	Root length (mm.) *	Seedling height (cm.) *	No. of leaves per seedling (leaves)*
White	3.4a	3.42a	0.7b	4.2 b
Red	2.6c	2.36b	0.69b	5.2 a
Blue	1.1d	1.24c	0.72b	3.2 c
Green	1.1d	1.20c	0.69b	2.8 cd
yellow	3.1ab	2.79b	0.79a	5 ab

a,b,c - Figures not sharing the same letters in the same column differ significantly at $p < 0.05$

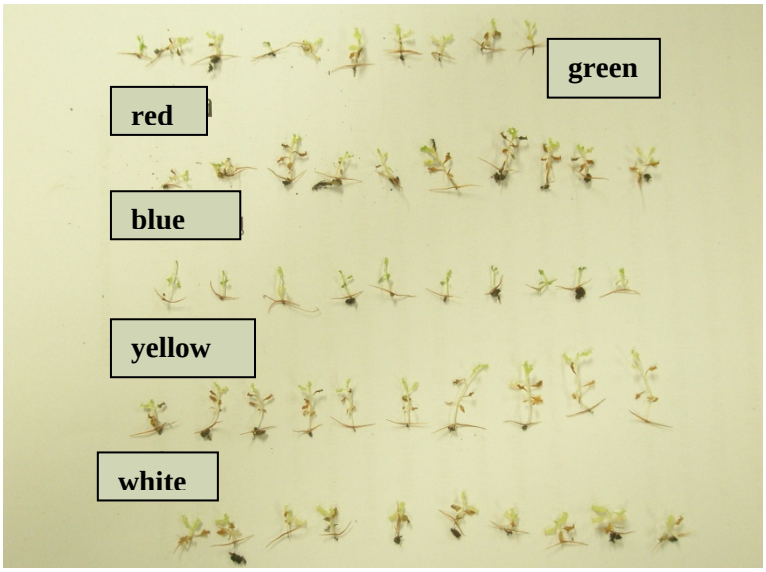

Figure 1: Seedling of Nepenthes mirabilis in different light after culturing 30 days.

4 Conclusion

Light is also important for seed germination. *Nepenthes mirabilis* seeds responded to the different lights (white (fluorescent), red, green, blue, and yellow) with optimal growth and development according to the light they received. Seedlings under white and red lights experienced the fastest in germination, but under the blue light germination was slowest. Seedlings under yellow light gave the highest average germination percentage, while seedlings under white and red lights were the second fastest and their leaves were green and light green respectively. Seedling vigor index and germination index were highest under yellow light, followed by red light. The highest average speed of emergence was achieved under white and red lights. The lowest was under green light. After all seedlings had been transferred to room temperature with sun shade for 30 days, the maximum number of roots was observed on seedlings under white and yellow lights. The greatest root length was observed on seedlings under yellow light. The average maximum number of leaves was found on the seedlings which had been under red and yellow lights.

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