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Effects of Red and Blue (RB) LED on the *in vitro* Growth of *Rosa Kordesii* in Multiplication Phase

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Abstract. Light is an important need in plant growth. In in-vitro culture, generally fluorescent light is used as the main source for plant to undergo photosynthesis. The effect of Red (R) and Blue (B) LED light on the in vitro of *Rosa sp.* using BAP hormone were used in this study and responses were compared with explant grown under 16/8h (light/dark) fluorescent light. Nodes from matured plants were sterilized and cultured for 30 days under two light treatments; fluorescent light (FL), as T1 and combination of 16 R and 4 B LED light, as T2. The explant were cultured in MS (Murashige and Skoog, 1962) medium supplemented with 3% (w/v) of sucrose, 0.25% Gelrite^{tm} (Duchefa), 1.5 mg/L of BAP hormone for growth with 0.1 mg/L of Indole-3-butyric acid (IBA) for induce rooting, 100mg/L myo-inositol and 100mg/L of Ferrum. Stem elongation under both treatment towards the end of the experiment shows parallel result compared to the early week of cultured, however, plantlets under RB LED (16:4) show where the number of shoots and number of leaves of RB LED (16:4) shows greatest growth.

Keywords: Rose, LED, Multiplication rate

1. Introduction

Roses are one of the most important ornamentals and are most often used for ornamental, medicinal, and aromatic purposes [1]. The trend is expected to continue in the future with growing affluence of the local population and that of the developed countries as well as improved market opportunities [2].

In-vitro culture method has been practiced as a technique to various types of ornamental plant especially for flower colour, plant morphology and some physiological characters [3]. Primary phases for in vitro involve sterilization, multiplication and hardening [4] [5]. Moreover, it is important to produce disease-free explant with rapid multiplication rate to ensure successful cultivation of particular crop [6]. According to Preil *et al.*, [7], in vitro culture probably shorten breeding cycles and decrease the development cost. Light source such as fluorescent lamps, metal halide lamps and high-pressure sodium lamps are generally used for in vitro cultures. However, these lights contains unnecessary wavelength that are low in quality for stimulating growth [8] [9]. Furthermore, the use of these lights consumed a lot of electricity and it was reported that tissue cultured lab consumed 65% of total electricity [10]. Light Emitting Diode also known as LED light has been utilized globally in agriculture as an alternative light source for plant growth and photosynthesis. LEDs have attracted considerable interest because of their wavelength specificity and narrow bandwidth, small mass and volume, long-life and minimum heating [11][12].

Morphology and physiology of in vitro grown plants are regulated by various micro-environmental factors such as light, temperature, humidity and carbon dioxide [12][13]. Inadequate or insufficient light may cause harmful for plant growth or led to excessive growth respectively [14]. Light quality has effect on morphological characteristics such as stem elongation, leaf size, plant anatomy that shows the important of light as a promising source [15].

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The previous research had successfully showed the significance of red (R) and blue (B) LED lights on plant photosynthesis. R and B color of LED has been widely used to enhance plant growth. However, there were few studies involved in vitro plant and rose as a subject. Past studies had done in vitro technique under RB ratio 1:1. Xue Fan et al, [16] reported to apply RB with ratio 1:1 for leaf development of young tomato and Li et al., [9] applied RB with ratio 1:1 with wavelength 660nm and 460nm respectively on rapeseed. Different spectrum of light truly gave an impact in term of anatomy, physiology and morphology of leaves [16]-[18]. Nevertheless, the spectrums emitted from both colours vary too much with the sunlight. Within the range 400-700nm, light spectrum is able to provide the most important energy for plant photosynthesis among the solar energy. Studies by Matsuda et al., [19] rice plants that grown under combination of blue (470nm) and red (660nm) shows greater leaf photosynthetic rates compared to single colour LED.

A study by Harun et al., [20] reported that, treatment under 16:4 RB ratio, it is more effective to promote higher photosynthesis activity which lead to increase number of shoots and leaf. High photon level of blue and red LED and their wavelength have certainly given an advantage [15]. Therefore, the objective of this study is to examine the effect of red blue LED with ratio 16:4 in multiplication of *Rosa kordesii* in tissue culture as suggested by Tanaka, et al., [21] that LED has improved the quality of explant to increase shoot multiplication.

2. Materials and Method

2.1. Plant material

Explants are from *Rosa sp.* with the scientific name of Rosa *Kordesii*. Stem with nodes from matured plant were excised from mother plant approximately about 15- 20 cm with 10- 20 nodes, washed in 1L of sterilized distilled water for few minutes. Then, a beaker with a few drops of Dettol® in 1L of sterilized distilled water was prepared and stirred for 30 minutes. 1 ml of Bavestin in 1L of sterilized distilled water was prepared inside a beaker and then clean stem was placed inside the solution and stirred for about 45 minutes.

Then, the stem was washed thoroughly with sterilized distilled water and placed in a beaker covered with aluminum foil before entering the laminar flow. The stem was brought to laminar air flow cabinet and sterilized with 60% ethanol thoroughly. The stem was then dipped into 95% ethanol and immersed in 30% Clorox® for about 15 minutes and added with two drops of Tween 20 (Merck Schuchardt, Hobenburn, Germany) followed by five times rinsed with sterilized distilled water. The stem was trimmed to 2 cm in length for every nodes. Finally, the nodes were dried on sterilized paper and placed vertically on culture media [22].

The explant was then being incubated at 26 ± 1^{o}C with 16/8-hour photoperiod (light/dark) 70 µmol $m^{-2}s^{-1}$ provided by i) combinations of red and blue LED light and ii) white fluorescent tubes as a control. Cultures were maintained in the same media for about 30 days.

2.2. Culture conditions and lighting system

The culture media of shoot induction contained of MS [22] medium supplemented with 3% (w/v) of sucrose, 0.25% Gelrite™ (Duchefa), 1.5 mg/L of BAP hormone for growth with 0.1 mg/L of Indole-3-butyric acid (IBA) for induce rooting, 100mg/L myo-inositol and 100mg/L of Ferrum. The pH was adjusted to 5.7 to 5.8. Cultures will be maintained in the same media for about 30 days. Light treatment consisted of combinations of red and blue LED (T2) with ratio 16 R: 4 B and white fluorescent (T1) as a control.

2.3. Data collection and analysis of results

Each treatment consisted of four replications with five samples each. The plants were measured at the end of second and fourth week in each treatment for destructive measurements. The physical measurement involves plant height, number of shoots and number of leaf. Statistical analyses were conducted with statistical product and service solutions for Windows, version 16.0 (SPSS). All measurements were evaluated for significance by an analysis of T-test with significance different test at $p < 0.05$.

3. Result and Discussion

3.1. Physical Plant growth

All data were recorded on physical plant growth, which are plant height, number of shoots and number of leaf. This study is focusing on multiplication phase where it is an early development of in vitro plantlet (Fig. 1). Plants structure is controlled by light signals from the surroundings [16]. The function red LED is to induce chlorophyll so plant can make food from photosynthesis, as for blue LED is for plant morphology [23]. Ratio of red LED is 4 times more than blue is perhaps to encourage plant to make food and induce more shoots to multiply in laboratory. During first week of culture, all sample started to develop shoots. Data was recorded at second week and end of week 4. Number of plant height in, shows not significance, but for number of shoots and leaf shows significance with the study in week 2 (Table 1).

In week 4, number of shoots and leaf in T2 increase which agree with previous study reported by Kim et al, [8] stated that proper LED system will promoted or inhibited of shoot and root growth, formation and growth of bulbs and control of flowering. Number of leaf under T2 was greater in number for both weeks than T1 and significantly difference. According to Xiao *et al*., [24], red and blue light spectrum strongly shaped plant morphology and red light increases leaves. However, plantlet height was insignificant from week 2 to week 4 possibly cause by plant growth hormone (PGR) that was used in this study is Benzylaminopurine (BAP) which act as growth hormone and multiplication [25]. This agree with the purpose of this study to induce more shoots for multiplication.

The use of red LED light is to enhance the photosynthesis process has been accepted extensively. According to Massa *et al*.,[26], red wavelength between 600 to 700nm is competent to be absorbed by plant pigments. Moreover, optimum absorption peak of chlorophyll were red. As for blue LED, the light has significant roles in plants including water relations, gas exchange, stem elongation and stomatal control [12][27].

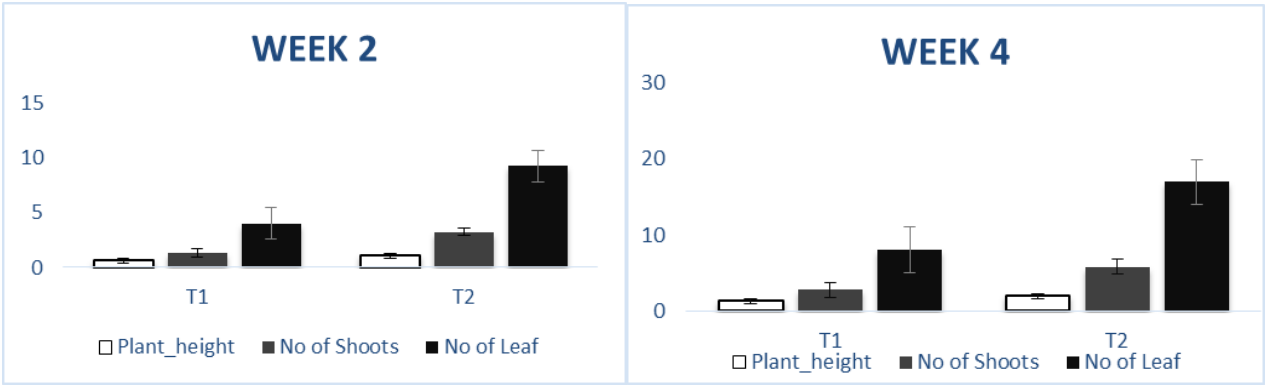

Fig. 1: Comparison of Plant height, no of shoots and no of leaf between treatment for week 2 and week 4 (70 µmol $m^{-2}s^{-1}$).

Table 1: Different lettershows within columns indicate significant different $p<0.05$ according T-test.

Week	Treatment	Physical Parameter		
		Plant height	No of shoots	No of Leaf
2	1	0.70±0.13a	1.31±0.14a	4.00±1.00a
	2	1.31±0.14a	3.25±0.34b	9.25±1.03b
4	1	1.32±0.22a	2.83±0.35a	8.08±1.77a
	2	1.97±0.26a	5.83±0.91b	16.92±2.38b

4. Conclusion

This result clearly demonstrates combination two colors of LED light has greater influence on rose in vitro plant growth. Based on this study, rose that grow under the combination of LED promote more shoots compared to conventional way in vitro. The mixed light colour of LED (Red and blue) were beneficial for cultivations of ornamental plant as general and roses specifically. As in this study the purpose to increase multiplication rate is succeed by using LED, more analysis should be done such as dry and fresh weight as well as chlorophyll content to in order to get more accurate result.

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