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Full Paper

FLUORESCENT LIGHT (FL), RED LED AND BLUE LED SPECTRUMS EFFECTS ON IN VITRO SHOOTS MULTIPLICATION

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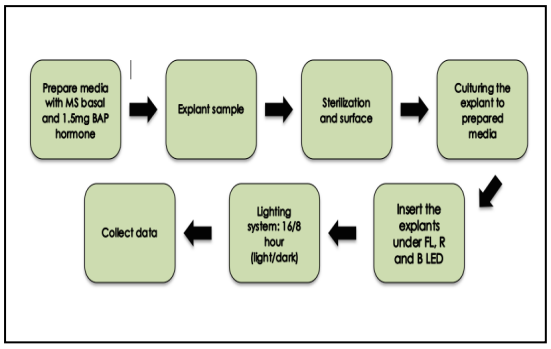

Abstract

Tissue culture in ornamental plants is one of the relevant factors that beat production of vegetables and fruit production worldwide. It has been recognized as an effective tool to enhance large scale of plant multiplication. However, the conventional lighting system may contain unnecessary wavelength that are low quality to promote growth. In this study, experiment was conducted by using Light Emitting Diodes (LED) as an alternative source of lighting. Red and blue LEDs along with fluorescent light (FL) were applied to determine the best source of light in multiplication of rose. Under the same media regimes which are MS media basal and BAP shoot hormone, blue LED had shown more shoots and leaves.

Keywords: Tissue culture, rose, LED, fluorescent light, red and blue LED

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1.0 INTRODUCTION

Ornamental plants like roses are usually used as a cut flower, flowering potted plants, garden and landscape. Roses are originated in Iran, Iraq and China [1]. Roses can be processed to produce aromatic oil and scents [2]. To further expand its potential, a model system for growth development can be further applied for *in vitro* breeding [3]. Tissue culture is a method by transferring an explant to high in nutrition agar medium in environmental control chamber. Parts of explants include leaf tissue, peduncles, tuber segment and floral parts [4]. Conventionally, rose plants were bred with vegetative method like cutting, layering, grafting and budding of plants. But, this method does not promised disease free plants [5]. Furthermore, the problem in rose plants is also linked with inter-specific breeding, which includes low percent of seed set and seed germination [6].

Lights are energy source of most plants during photosynthesis by using their photosensitive mechanism [7, 8]. Normally, fluorescent light is used for *in vitro* culture, but it contains low quality and unnecessary wavelength. However, plant does not have to absorb this full mixture of light wavelength [9-10]. Unlike LED, it has been utilized globally in agriculture and attracts lot of interest because of its wavelength specificity, small mass and volume, long life and minimum heating [9-11]. As reported in [12], light spectrum within the range 400-700nm does provide energy for plant photosynthesis. Importantly, spectrum in the said range was known as photosynthetically active radiation or PAR that parallels to nearly the visible spectrum of the human eye [13] these spectrums range are actually at blue to red color spectrums. Red spectrum LED acts by

hoarding starch through photosynthesis and blue spectrum LED is in chloroplast development, chlorophyll formation and stomata opening [14]. The objective of this study is to analyze the effects of fluorescent light (FL), monochromic blue LED (B) and monochromic red LED (R), on the growth and morphogenesis of rose plantlets *in vitro* and to select the best light source for this cultivation system.

2.0 METHODOLOGY

2.1 Culture Condition

Media formula were based on 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, 0.1g/L myo-inositol and 0.1g/L of Ferrum. The pH was adjusted to 5.8 prior to autoclaving at 121 °C at 103 kPa for 15 minutes. The apparatus for culturing such as forceps, scalpels, and culture bottle were sterilized by autoclaving for 20 minutes at the same temperature and pressure.

2.2 Shoot Multiplication

The aseptic shoots of rose explants were trimmed until 2cm from the base and sub cultured into the multiplication MS medium supplemented with 1.5mg?l concentration of BAP. The explants were incubated at 26±1°C with 16/8-hour photoperiod (light/dark) (60 μmol m^{-2} s^{-1}) under fluorescent light (FL), monochromic red LED (R) and monochromic blue LED (B) separately for 30 days.

2.3 Experimental Design and Data Analysis

The culture growth was examined periodically and the morphological changes were recorded. The growth was observed and determined according to the following parameters; 1) mean of shoots number per explant, 2) mean of leaf number per explant 3) mean plant height per explant. The statistical significance was determined at $p<0.05$. Figure 1 shows the block diagram of the processes of the system

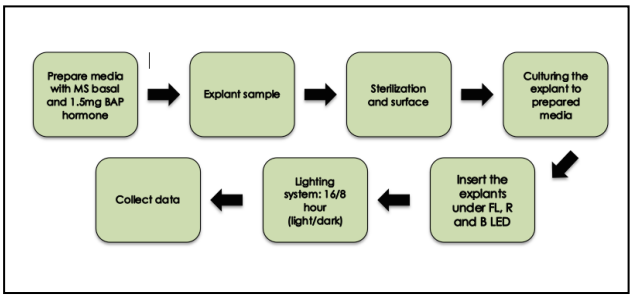

Figure 1 Block diagram of the processes of the system

3.0 RESULTS AND DISCUSSION

3.1 Shoots and Physical Measurement

Light is one of the important environmental conditions that have an impact on growth and development of a plant [15], [16]. Light certainly has an effect on stem elongation, leaf expansion, pigmentation and photosynthetic activity [17]. Red and blue LEDs have influence on plant growth as they are the major energy sources for photosynthesis process [18] particularly red wavelength has influence in accumulating starch for photosynthesis and blue wavelength in chloroplast development [19]. In contrast, ordinary FL which had various wavelengths and heat factor will bring different responses to the plant as plant shows different reaction to different wavelength [20][21].

Table 1 Effects of light treatment under FL, R and B on physical parameters.

Week	Treatment	Physical parameter		
		Plant height	No of shoots	No of leaves
2	FL	0.88 ± 0.87^{a}	1.56 ± 0.11^{abc}	8.22 ± 1.82^{a}
	R	1.13 ± 0.13^{b}	3.22 ± 0.11^{abc}	10.33 ± 1.02^{b}
	B	1.07 ± 0.15^{c}	7.22 ± 0.22^{abc}	11.22 ± 0.78^{c}
4	FL	2.24 ± 0.23^{a}	12.00 ± 0.192^{ac}	28.89 ± 2.92^{ac}
	R	2.08 ± 0.31^{b}	16.44 ± 0.97^{b}	25.33 ± 3.60^{bc}
	B	2.12 ± 0.13^{c}	19.00 ± 2.41^{ac}	44.11 ± 3.11^{ab}

*Results were expressed as mean ± standard error (SE)
*Mean results for each parameters followed by the different letters are significantly different ($p< 0.05$)
*FL: Fluorescent light
*R: Red LED

According to Figure 2, shoot multiplication was best grown under blue LED with mean of shoots 19.00±2.41. From previous study by (22), marigold stem were 3 times higher under blue LED than FL treatment which in agreement with this study. Based from the Table 1, the explants shown greater stem elongation under blue LED light until week 2, but slightly shorter than FL in week 4. However, there is no significant difference on plant height after 4 weeks. On Figure 4, during week 2, shoots were well respond towards both red and blue LEDs as compared to FL. As experiment continued, red LED produced more shoots but less leaves. The experiments showed good results up until week 4 in which blue LED influenced shoot growth impressively by producing more leaves and numbers of shoots.

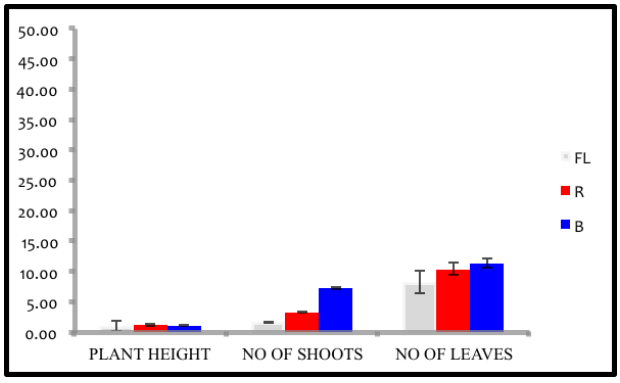

Figure 2 Effects of light treatment under FL, R and B on physical parameters

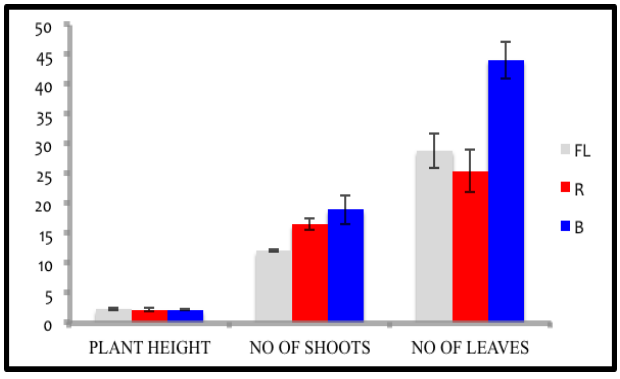

Figure 3 Multiple shoots and leaves start to grow after 2 weeks (upper picture) and growth progress after 4 weeks (below picture)

As in Figure 3, plant height of the explants was almost at the same height for all three treatments during the four weeks of experiment. The results are in disagreement with [23], where it reported that red light promoted plant elongation in lettuce. However, the study is agreement with [22] in which it reported that marigold elongation was inhibited under monochromic red but was the highest elongated in monochromic blue LED. This is due to the fact that it was possibly related to the radiation time, growing energy in blue and red spectrums in the spectral distribution and also plant species [22].

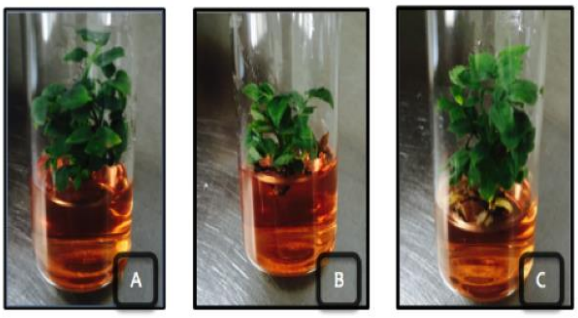

Figure 4 Explant under Fluorescent light (A); Red LED (B) and Blue LED (C).

Moreover, previous research on potato and radish [24] showed the need of blue spectrum to obtain high biomass and leaf expansion which was in agreement with this report, which displayed in Figure 3, blue LED treatment showed bigger leaves compared to the other 2 light treatments. Furthermore, in agreement with this study, [25] stated that certain plant species and light intensity response in blue light other than others light. *In vitro* study in potato by [26], enlightened that plantlets growth under FL and red LED had no significance differences in leaf area was in agreement with this investigation. Many findings [27] on Antihirnum, [28] on chrysanthemum, [29] on baby leaf lettuce, [30] on Doritaenopsis and [31] on upland cotton, and [32] on *Alternanthera brasiliana Kuntze* reported that blue light had decreased the number, length and area of leaves, which totally disagreed with this report. It is proved that the function of light is species dependent [33], [34].

Moving forward, report by [35], was in agreement with this study by stated that blue LED shown the highest influence on shoot production of Dendrobium. Plus, [36] stated that number of shoots in *Anthurium* was increased under higher percentage of blue LED. Higher differentiation was recorded on *Oncidium* by [37] under blue LED. [21], affirmed that photoreceptor of plant gets light to regulate their diversity and growth of plants. Other than that, several reports claimed that LED light shown higher nutritional value such as vitamin C [38] and carotenoid [39]. Based on Figure 3, explant under monochromic red LED in this study was not as compact as compared [40] on 'Green Oak Leaf' lettuce.

As reported [41], light quality indeed effects on morphological characteristics such as stem elongation, leaf size and plant anatomy. [10], testified that FL has always been selected as a light source for tissue culture, but this study showed that blue LED light is the alternative source for tissue culture. This was strongly agreed by [31] stated that FL was less suitable for the growth of upland cotton.

4.0 CONCLUSION

This study was able to narrowing the objective which is analyze the effects of fluorescent light (FL), monochromic blue LED (B) and monochromic red LED (R), on the growth and morphogenesis of rose plantlets *in vitro* by successfully increased the multiplication rate by using blue LED. This study shown that, FL can be replaced by blue LED with the right plant species and suitable cultivation environment; it is possible that it will increase the maximum yield to provide good economical production.

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