

Influence of Light Emitting Diode on *in vitro* rooting of *Moringa oleifera* Lam. shoots

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Abstract

The present study aimed to evaluate the influence of light emitting diode (LED) on *in vitro* rooting of *Moringa oleifera* Lam shoots. To achieve this, the vitroplants were grown in MS mod 3B basal medium, supplemented with 20 g/l sucrose and without phytohormones, and subjected to white and red LED light for 4 weeks. At the end of the experiment, it was observed that there were significant differences between the LED lights used in terms of the number of roots and the weight of the callus obtained. The white LED gave vitroplants with many roots and reduced callus. Thus, the basic medium MS mod 3B, with 20 g.L⁻¹ sucrose and without phytohormones, and the white LED could be used for a good rooting of *M. oleifera in vitro* plants.

Keywords: Micropropagation, callus, LED, *Moringa oleifera*, vitroplants and wavelength.

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1. INTRODUCTION

The species *M. oleifera* of the family Moringaceae is considered as a tree of life or miracle tree by virtue of its multiple properties both nutritional and medicinal [1-4]. However, until now, its micropropagation presents serious problems of hyperhydricity, early senescence, excessive callus production, thus making it difficult to root *ex vitro*. Although micropropagation techniques preserve the genotypes and phenotypes of elite *M. oleifera* plants [5-7], the optimal propagation conditions for this species are not yet well defined. For [8], *in vitro* growth plants allow the production of pathogen-free plant material and rapid propagation, and this technique is widely used to obtain secondary metabolites.

Several studies conducted have shown the influence of light on plant growth and development in *in vitro* culture [9-15]. According to [16], light is a key environmental factor that affects almost all aspects of plant life. However, light quality refers to the spectral quality, quantity (photon flux) and photoperiod, which

significantly influence the morphogenesis and growth of plant cell, tissue and organ cultures [17, 18]. For [19], plant species respond differently to light quality.

Currently, one way to improve the quality of light for plant tissue culture is the use of light emitting diode (LED) lamps as light sources. They are considered the most economical and powerful available light sources that accelerate the growth and physiological development of seedlings [12, 20]. Budiarto states that LED can be used to enhance shoot and root induction during *in vitro* culture [21].

Apart from the study of [8] who determined the influence of light quality on growth and accumulation of phenolic compounds in *M. oleifera* grown *in vitro* while using MS basal medium which is less preferred in micropropagation, there is no information regarding the influence of the use of LED light on multiplication, growth and rooting of seedlings in *in vitro* culture.

In the context of optimizing the conditions for micropropagation of *M. oleifera*, the present study seeks to determine the influence of LED light on its rooting in order to evaluate its effectiveness.

2. MATERIALS AND METHODS

2.1. Material

The plant material consisted of *M. oleifera* vitroplants obtained after shoot multiplication in *in vitro* culture from MS mod 3B medium supplemented with mTR and MemTR topolines at 2 μ M without auxin.

2.2 Growing media and conditions

For the present experiment, the vitroplants were subjected to the basic medium MS mod. 3B, with 20 g.L^{-1} sucrose added and without phytohormones. It should be noted that the MS mod. 3B medium obtained was solidified with agar (7 g.L^{-1}), its pH was adjusted to 5.8 before autoclaving for 15 min at 121°C. Thus, the cultures were placed in a culture chamber at $25^\circ\text{C} \pm 1^\circ\text{C}$ under the different LED wavelengths (white and red) under a 16h/8h photoperiod. After 4 weeks of *in vitro* culture, data on shoot length, number and length of roots and callus weight were collected.

2.3. Data analysis

The data obtained were processed using Graphpad Prism software (version 5.03). After checking the homogeneity of the data variances by Fischer's test, Student's parametric test was applied to compare the influence of different LED wavelengths on the number and length of roots; on the other hand, Mann Whitney's non-parametric test was used to compare the influence of different LED wavelengths on the length of shoots and callus weight. The differences are considered significant at the 5% level.

3. RESULTS

At the end of this study, the results obtained showed the influence of different LED wavelengths (white and red) on the measured *in vitro* rooting parameters of *M. oleifera*. The vitroplants submitted under the red LED presented slightly higher size compared to those submitted under the white LED (Figure 1). On the other hand, the vitroplants submitted to the white LED presented slightly longer roots than those submitted to the red LED (figure 4). However, no significant difference was observed in terms of shoot and root length of the vitroplants using the white and red LEDs ($U=265$ and $t=0.46$; $p\text{-value} > 0.05$). In addition, white LED was effective in root number and callus weight compared to red LED (Figures 2 and 3). Student's and Mann Whitney statistical tests showed significant and highly significant differences between vitroplants subjected to white LED and those subjected to red LED ($t=2.093$ and $U=186.5$; $p\text{-value} < 0.05$).

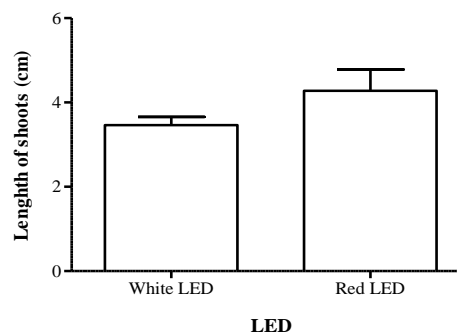


Figure 1: Influence of different LED wavelengths on shoot length of *M. oleifera*

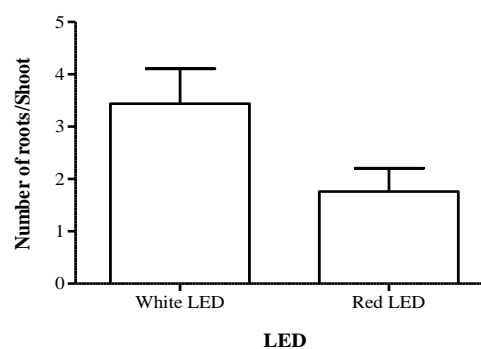


Figure 2: Effect of different LED wavelengths on the number of roots per shoot of *M. oleifera*

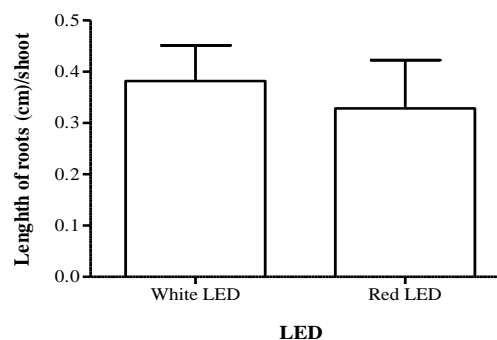


Figure 3: Influence of different LED wavelengths on root length per shoot of *M. oleifera*

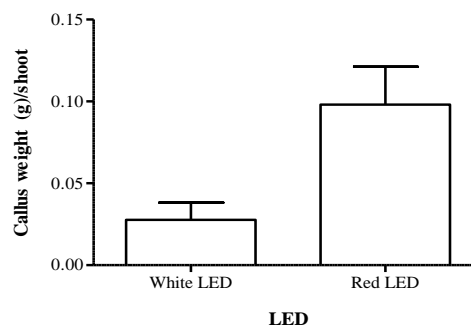


Figure 4: Effect of different LED wavelengths on callus weight of *M. oleifera*

In order to solve the difficulty related to the excessive production of callus which significantly inhibits the process of root development of *M. oleifera* vitroplants, a study was carried out to evaluate the influence of LED light on their rooting. At the end of this study, the results obtained showed the influence of LED light on the measured parameters. According to the literature, light is one of the conditions that influence the growth, development and morphogenesis of different plants in *in vitro* culture [9, 15, 22-24]. As a result, the quality of light has a significant effect on the micropropagation of several plant species [25, 26]. By eliminating light wavelengths that are inactive for photosynthesis, LED light has been shown to be the most effective quality for micropropagation of most plant species [8, 9, 12, 15, 18, 27]. For [9], LED light is valued over fluorescent light due to its specificity of their wavelengths, low degradation, low amount of thermal emissions, and long lifetime. Note that white light (wavelength=420 nm), red light (wavelength=660 nm), blue light (wavelength=460 nm), and a combination of blue and red remain the most commonly used LED lights in *in vitro* culture. These lights influence plant morphology, physiology and morphogenesis in a species-specific manner [9].

Although no significant difference was observed between the vitroplants subjected to white and red LEDs in terms of shoot and root lengths, those subjected under the red LED were slightly long with slightly short roots compared to those subjected under the white LED. The results obtained on shoot length are in agreement with those obtained by [12, 28, 29] who found the sensitive elongation of shoots in *in vitro* culture using the red LED or the combination of blue and red LED.

However, it was observed an influence of the used LED wavelengths on the number of roots and callus weight of *M. oleifera* shoots. The white LED improved the quality of the vitroplants of this species by reducing the callus weight with the production of more roots. Evaluating the effects of different LEDs on the growth, development and physiology of *Vanilla planifolia in vitro* seedlings, [12] obtained significant differences in the growth, development and physiology of seedlings grown under LED and fluorescent lights. Blue and red LEDs promoted seedling elongation and photosynthesis in the multiplication phase which corroborates to our results on shoot length. On the other hand, the blue LED stimulated shoot elongation, the number of roots formed and the number of leaves per shoot in the rooting phase.

According to the literature, red light positively influences the development of seedlings in *in vitro* culture in some species, yellow light promotes shoot elongation and leaf formation, blue light reduces the growth of seedlings *in vitro* [27].

4. CONCLUSION

Finally, there is no reliable standardized protocol in place for the optimal production of healthy *Moringa oleifera* seedlings in *in vitro* culture. There are still serious unresolved problems regarding its best micropropagation. In order to find the best conditions for *in vitro* culture of *M. oleifera*, a study was carried out on the influence of light emitting diodes (LED) on the *in vitro* rooting of shoots of his species. The plant material consisted of *M. oleifera* vitroplants obtained after shoot multiplication in *in vitro* culture. To evaluate the influence of LED light on rooting, the vitroplants were grown in MS mod 3B basal medium, supplemented with 20 g/l sucrose and without phytohormone, and subjected to white and red LED light. At the end of this study, the results showed the significant influence of LED light wavelengths on the number of roots and callus weight obtained (p-value<0.05). However, no significant difference was recorded between white and red LED light in terms of shoot and root length (p-value>0.05).

In short, the use of white LED light and MS mod 3B basal medium with 20 g/l sucrose and without phytohormones could be interesting to reduce the excessive callus production that significantly inhibits the root development process of *M. oleifera* vitroplants.

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