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# Monochromic radiation through light-emitting diode (LED) positively augments *in vitro* shoot regeneration in Orchid (*Dendrobium sonia*)

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## Abstract

Monochromatic lights emitted by light-emitting diodes (LEDs) have generated great interest for efficient and controlled growth *in vitro*, especially of plants which are endangered or require specific intensity and wavelength of light. In the present study, we have evaluated the effect of monochromatic LEDs on *in vitro* morphogenesis: growth, proliferation of shoot cultures, and rooting of *Dendrobium sonia*. Different light sources viz. white LEDs (W), blue LEDs (B), yellow LEDs (Y) and red LEDs (R) were tested under photoperiod of 16 h of exposure and 8 h of dark. The frequency of morphogenesis depended on the wavelength of the applied monochromatic light. Higher wavelength monochromatic light (yellow light) was observed to induce higher shoot proliferation (98%), early PLB (protocorm-like bodies) formation, differentiation into green buds and shoot initiation as compared to red, blue and white light treatments. Yellow light also yielded higher number of shoots per explants (29 shoots/explant) than red, blue and white light treatments. The results suggest that the monochromatic light sources stimulate morphogenic effects on *in vitro* culture of *Dendrobium sonia*, and that yellow light treatment can be used to enhance the efficiency of micropropagation.

**Keywords**: *Dendrobium* sp., Plant regeneration, Protocorm-like bodies, *In vitro* culture, Light Emitting Diodes (LEDs)

**Abbreviations**: BAP - 6-Benzylaminopurine, IAA - Indole 3- Acetic Acid, MS - Murashige and Skoog medium, PLB - Protocorm-like bodies

## Introduction

Orchids are the monocotyledon ornamental potted plants belonging to the family Orchidaceae. They exhibit an incredible range of diversity in size, shape, and color of flowers and foliage. Orchids are categorized as threatened and endemic species of largest botanical family of higher plants by International Union for Conservation of Nature (IUCN) [1]. It is estimated that about 1,300 species (comprising 140 genera) of orchids can be found in India [2]. Orchids are well known for their economic importance and are widely cultivated for ornamental purposes and the production is now assumed at industrial level because of high commercial value [3]. Usually, orchids are germinated through seeds but only in the presence of appropriate host [4]. Plant tissue culture technique has become very useful for the propagation and production of orchid plants of high commercial value [5]. Out of these, there are about 300 species available in India.

The genus *Dendrobium* is the largest genus belonging to Orchidaceae, with most members being epiphytic. *Dendrobium* has approximately 1500 species and almost one fourth of them are used for high ornamental value [6]. *Dendrobium chrysanthum* Wall. ex Lindl. is one of the valuable ornamental orchids available in the Northeast India because of its herbal medicinal value [7]. Efficient protocols for *in vitro* plant regeneration have been developed for many

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species of Orchidaceae and effects of important factors affecting plant growth such as light wavelength (quality), light intensity (quantity) and duration of light have been reported [8]. However, only very few reports studied effect of light-emitting diodes on *Dendrobium* micropropagation [9]. Light quality and quantity initiate signaling cascade of specific photoreceptors, such as phyto-chromes and crypto-chromes which alter the expression of a large number of genes [10, 11]. White light is the major source for the plant growth but certain plants need its component light rays of variable wavelengths for their growth and development [12].

In the past decade, considerable progress has been made on the application of LED as a potential alternative light source for *in vitro* plant growth and morphogenesis [12]. However, despite the economic importance of *Dendrobium sonia* orchid, the effect of different LED wavelengths on the morphogenesis induction and growth of this species *in vitro* has not been evaluated. The objective of this research work is to study the effects of different light wavelengths on growth, propagation, multiplication and survival of *in vitro* regenerated shoot cultures of *Dendrobium sonia* orchid.

## Materials and Methods

*Ex vitro* grown *Dendrobium sonia* orchid plants were maintained at the green house (Maharaja Ranjit Singh College Of Professional Sciences, Dept. of Life Science, Indore, India) at a temperature of 22-25°C and 75-80% relative humidity. Rhizome buds from these *ex vitro* plants were collected and used as explants to initiate shoot cultures for the experiment. The explants were washed in running tap water for 10 min. The explants were taken into laminar air flow and treated with 0.1% mercuric chloride for 3 min. These were then washed with sterile distilled water and then rinsed with 70% (v/v) alcohol for 30 sec. This was followed by rinsing three times in sterile distilled water to remove the traces of chemicals. The surface sterilized explants were cultured in glass bottles containing MS basal medium [13] fortified with sucrose (3% w/v) and 6-Benzylaminopurine (BAP 11.1 µM), and gellified with 0.6% (w/v) agar-agar. The pH of the medium was maintained at 5.8. The medium was gelled with 0.6% agar (HiMedia) before autoclaving at a pressure of 15 psi and 121°C temperature for 20 min. The cultures were kept under 16 h light and 8 h dark photoperiod under white LED at 22±2°C.

### *In vitro* shoot initiation and multiplication

The surface sterilized rhizome buds were inoculated in MS medium fortified with 3% sucrose and 11.1 µM BAP for bud proliferation. Preliminary studies with a range of BAP concentrations ranging from 2.22 µM – 22.19 µM were tested for shoot induction (Data unpublished) out of which best response was observed in 11.1 µM BAP, and hence this concentration was selected for the study. After four weeks of inoculation, mature protocorm-like bodies were sub cultured in the same medium for further growth. The shoots initiated from PLBs were transferred on to multiplication medium fortified with 11.1 µM BAP and 11.42 µM indole-3-acetic acid (IAA) for shoot multiplication. These shoot cultures were further sub-cultured after every two weeks for bud initiation, faster proliferation and multiplication.

### Exposure of shoots to monochromatic light regime

The mature PLBs collected as explants from the stock cultures were maintained in white light and were incubated in four chambers illuminated with different monochromatic lights viz. blue (450 nm), yellow (590 nm), red (680 nm) and white light (400-700 nm) for the study. All these lights were provided with cool LED's with fixed intensities (Table 1).

**Table 1:** Experimental Setup

| Light | Intensity (µmol/$m^2$/s) |
|---|---|
| White | 17.7 |
| Blue | 22.5 |
| Yellow | 24.6 |
| Red | 15.6 |

The experiments were performed in a culture room with relative humidity of 80±5%, photoperiod of 16 h light and 8 h dark, and temperature of 22±2ºC. In all the stages of shoot induction, shoot proliferation, rooting and primary hardening, cultures were maintained under different LED light treatment conditions. The radiation intensity of artificial light was set to 15-25 µmol/$m^2$/s. Light sources used in this study were:

1. W: White LEDs (control): at intensity of 17.7 µmol/$m^2$/s (400-700 nm at broad wavelengths)
2. B: Blue LEDs: at intensity of 22.5 µmol/$m^2$/s (470 nm wavelength)
3. Y: Yellow LEDs: at intensity of 24.6 µmol/$m^2$/s (590 nm wavelength)
4. R: Red LEDs: at intensity of 15.6 µmol/$m^2$/s (660 nm wavelength)

After eight weeks of culture, data was recorded on growth parameters like survival rate (%), shoot proliferation %, fresh weight (gm), leaf area ($mm^2$), shoot length (cm) and root length (cm). All the experiments were performed with a minimum of 10 replicates per treatment.

### Root induction

Preliminary studies were performed with a range of concentrations of IAA starting from 11.42 µM to 28.55 µM

for root induction and root growth (data unpublished), out of which 28.55 µM IAA was found to be the best for rooting. The individual shoots measuring 1-2 cm long were transferred onto MS medium containing 3% sucrose fortified with 28.55 µM IAA for root induction.

**Hardening and acclimatization**

Plantlets were transferred to MS basal medium (without any plant growth regulators) for one week for primary hardening under the same light conditions. The plantlets were then removed from the culture bottles and were washed thoroughly with sterile double distilled water to remove any traces of the medium and then treated with 0.1% (w/v) bavistin (fungicide) and again washed with sterile distilled water. The rooted plantlets were transplanted in the small pots containing coco-peat covered with plastic bags for 15 days and maintained under 70-80% relative humidity at 24-28°C in controlled white light conditions. The plants were then transferred to large sized pots containing coco-peat under greenhouse conditions. The plants were sprayed with water twice a day (morning and evening). Data was recorded in percentage of survival of plantlets after 30 days of transfer to greenhouse.

**Data Analysis**

The experiments were done in completely randomized design (CRD), with three explants per ten replicates for each treatment. The results are expressed as a mean ± SE. All the data were subjected to one-way analysis of variance (ANOVA) and the significant differences between treatment means were assessed by Least Significant Difference (LSD) test at $p \leq 0.001$ using Statistics 7.1.

## Results and Discussion

**Induction of protocorm-like bodies and plant regeneration**

The explants viz. rhizome buds derived from the *ex vitro* plants were induced under normal white light LED illumination into shoot cultures through the formation of protocorm-like bodies (PLBs). The PLBs were observed in the form of globular shiny or oval shaped structures (Fig. 1A). It was observed that after 20 days of growth, large numbers of PLBs were induced which gave rise to a bunch of healthy shoots (Fig. 1A, B). Multiplication medium fortified with 11.1 µM BAP and 11.42 µM IAA proved to be the best medium combination for development of PLBs and shoots along with rooting (Fig. 1C, D). The early development of such morphogenic structures could be attributed to the presence of pre-existing meristematic tissue composed of densely blemished cells stimulated in the form of proliferating epidermal tissues which develop, divide and give rise to mass of meristematic region further developing into nodular mass of pro-embryo structures, as previously described [14].

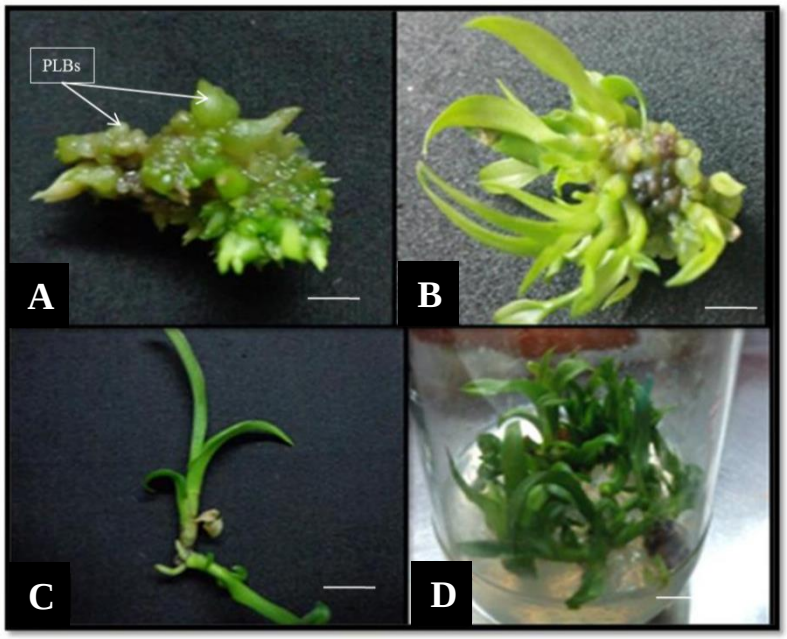


**Fig. 1:** Effect of white light treatment on *in vitro* propagation through PLBs in *Dendrobium sonia*. **(A)** Shoot initiation from PLBs, **(B)** shoot proliferation, **(C)** root induction and **(D)** multiple shoots. Bar = 10 mm.

**Influence of different monochromatic light spectra on shoot initiation and proliferation**

Subsequent to the establishment of *in vitro* cultures, the influence of monochromatic light regimes on shoot initiation and proliferation of PLBs was investigated. The different LED wavelengths induced a positive effect on the *in vitro* formation of shoots in *Dendrobium sonia*. After 4 weeks of shoot initiation, it was observed that the highest rate of shoot proliferation was induced under yellow light (98%) followed by red and blue light (Fig. 2). After three subcultures, shoot multiplication under yellow light treatment yielded 290 shoots (per 10 explants) followed by red, blue and white light treatments (266, 186 and 168 shoots per 10 explants, respectively). Leaf area was also higher (32.1±1.22) under yellow light as compared to other treatments and control (white light). Longer shoot length and root length were observed under blue and red light compared to other treatments (Table 2). The highest fresh weight of shoots was recorded in yellow light treatment (5.5 gm) followed by blue and red light treatment (5.2 and 5.1 gm, respectively) and control (Table 2). However, difference in fresh weight among monochromatic lights was not significant. The results suggested that different LED treatments produced a higher number of shoots per explants compared to white light suggesting a positive effect on *in vitro* morphogenesis in *Dendrobium sonia*.

In our study, Blue (470 nm) and Red light (660 nm) sources were found effective in increasing shoot length (Table 2). Blue light significantly increased the shoot length and root length, as reported previously in lettuce [15]. Shoot proliferation percentage was also less under blue light treatment where only 80% proliferation was observed (Fig. 2, 3B). 186 shoots were formed. PLB formation under blue light treated explants was compact in appearance, with dark greenish texture and more nodular compared to other spectral treatments (Fig. 3A), which may be due to high chlorophyll content, as reported by other researchers [9, 16-18]. In *Cattleya intermedia* × *C. aurantiaca*, callus morphology and number of shoots regenerated from PLBs were stimulated by blue light [19]. On the other hand in *Dendrobium kingianum*, average number of PLBs and chlorophyll content were highest under blue LEDs in contrast to the explants cultured under red LEDs where percentage of shoot formation and fresh weight were found to be maximum [20].

After four weeks of transfer to rooting medium, significant differences were observed in rooting responses under different LED wavelengths (Table 2). Blue and red light induced increased root length (Fig. 3C, 5C) followed by yellow light treatment (Fig. 4C). However, white light treatment induced shorter root length than all LED treatments (Fig. 1). This is suggestive that different light treatments

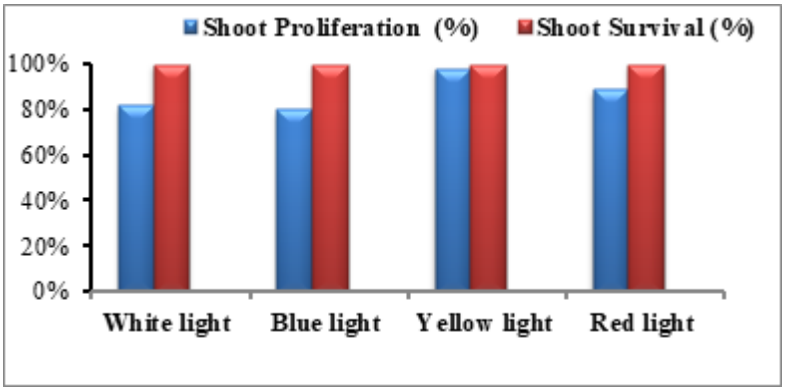


**Fig. 2:** Effect of different light treatments on % shoot survival and % shoot proliferation in *Dendrobium sonia*.

**Table 2:** Effect of different monochromatic light treatments on Shoot length, Root length, Leaf Area and Fresh weight in *Dendrobium sonia* (Values are the mean ± S.E. n=10). Different letters in each column indicate significant difference at $p \leq 0.01$.

| Monochromatic Lights | Shoot length (cm) | Root length (cm) | Leaf area ($mm^2$) | Fresh weight (gm) |
|---|---|---|---|---|
| **White light** | $4.7\pm0.461^{b}$ | $1.5\pm0.577^{b}$ | $19.7\pm0.986^{b}$ | $3.9\pm0.577^{b}$ |
| **Blue light** | $5.4\pm0.392^{a}$ | $2.1\pm0.333^{a}$ | $15.9\pm0.578^{c}$ | $5.2\pm0.230^{a}$ |
| **Yellow light** | $3.6\pm0.404^{d}$ | $1.7\pm0.145^{b}$ | $32.1\pm1.22^{a}$ | $5.5\pm0.404^{a}$ |
| **Red light** | $4\pm0.493^{c}$ | $2.0\pm0.033^{a}$ | $15.1\pm1.18^{c}$ | $5.1\pm0.233^{a}$ |

influence *in vitro* rhizogenesis in *Dendrobium sonia*.

In the present study, yellow light treatment was found to be the most significant for shoot regeneration in response of *Dendrobium* as PLBs formed under this treatment were found to be loose and friable in nature. Yellow light treated cultures showed early light green coloured PLB formation, differentiation into small shoot initiation (Fig. 4A) and proliferation (Fig. 4B). Similar result of yellow light elicited *in vitro* response of callus multiplication was also observed in *Vitis venifera* [21]. Comparatively not much research has been done on the effect of yellow light on *in vitro* regeneration; therefore, here we have reported the positive role of yellow light on plant regeneration in Orchids which could be of commercial use. There is also evidence that such attributes might be due to the presence of some photosensors and spectral overlaps that switch between green, orange and red spectral regions that can be selectively toggled to control plant growth, development, physiology and morphogenesis [22]. Green light effects have also been shown to share several attributes that typically oppose the action of red or blue light regulated plant processes [23]. The wavelengths ranging between 500-600 nm also represent the photosynthetically active radiation (PAR) [24] which could be useful to manipulate plant stature, color, nutrients and other attributes.

Red light significantly enhanced the adventitious bud formation and stem elongation in Petunia and Gerbera [25-28]. In our study, the red light was found to be effective for shoot proliferation (Fig. 5B) and shoot and root elongation (Fig. 5C) accompanied with gain in fresh weight (Table 2). Continuous red radiation significantly stimulated shoot elongation of sweet potato plantlets *in vitro* [29]. PLBs formed under red light were also loose and friable in nature (Fig. 5A). In *Cattleya intermedia*, the texture of PLBs formed under red light was also loose and friable in nature [19].

LEDs appear to be the best light source which can provide true flexibility of controlling spectral composition [30]. This attribute facilitates the use of LEDs as photosynthetic radiation sources with the possibility of selecting the peak wavelength emission that most closely matches the absorption peak of a selected photoreceptor. Using LED-based light sources, it is possible to regulate and control the physiological aspects of plant growth, such as photosynthesis and/or photomorphogenesis [31]. Light-emitting diodes are well suited for commercial plant production because of their

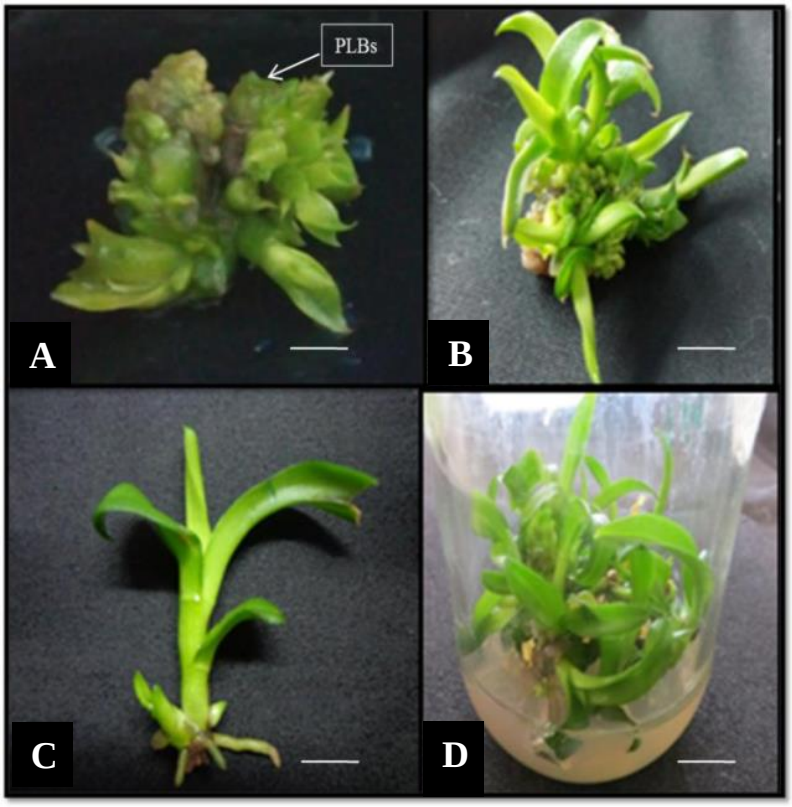


**Fig. 3:** Effect of blue light treatment on *in vitro* propagation through PLBs in *Dendrobium sonia*. **(A)** shoot initiation from PLBs, **(B)** shoot proliferation, **(C)** root induction and **(D)** multiple shoots. Bar= 10 mm.

high energy efficiency and spectral specificity [32, 33]. When operated at favorable temperatures, well-constructed LEDs have an operating lifetime of 50,000 h or more, which is at least two times longer than conventional high-pressure sodium or fluorescent lamps [30].

The LEDs have been shown to induce increase in length of roots per explant in different plant species grown *in vitro* [34]. In *Dendrobium sonia*, the different LED treatments, except blue light (Fig. 3D), positively influenced the *in vitro* shoot multiplication response (Fig. 4D, 5D). The PLBs of *Oncidium* and *Dendrobium officinale* cultured under red LED showed lower productivity rate, while the application of blue LED resulted in the enhanced productivity rate [9, 35]. Some reports indicate that blue LEDs stimulate *in vitro* formation and elongation of roots in various plant species [36] which is in conformity with our study.

**Plantlet regeneration and acclimatization**

Healthy plantlets with well-formed leaves and roots were transplanted into small pots containing coco peat (Fig. 6). All the plantlets regenerated through different treatments showed 100% survival rate (Fig. 2). After 2-4 weeks, the plants were transferred to larger pots for growth. In general, morphology of plantlets was quite heterogeneous, showing the variation in leaf morphology and growth between treatments. In the present study, yellow light treatment was the most suitable for overall growth of micro propagated plantlets which is in agreement with the results of Brazaitye *et al.* [37]. Such improved *in vitro* propagation system can be useful not only for large scale micropropagation of orchids but also for genetic manipulation studies [38].

## Conclusion

This study demonstrates that the monochromatic light source (spectral quality) can be an effective factor for rapid propagation, growth and survival of shoots of orchid plant species. Yellow light was observed to induce high frequency of shoot proliferation and higher number of shoots per explant. It is suggested that selective activation of specific light-sensing path could be used to develop efficient and novel protocols for mass production of economically important ornamental plants.

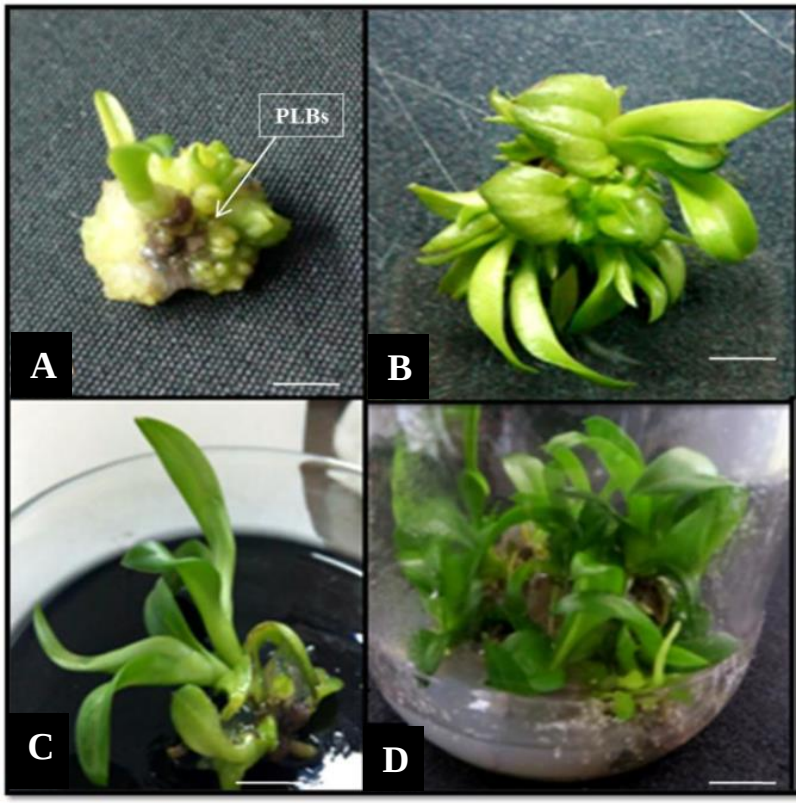


**Fig. 4:** Effect of yellow light treatment on *in vitro* propagation through PLBs in *Dendrobium sonia*. **(A)** Shoot initiation from PLBs, **(B)** Shoot proliferation, **(C)** Root induction and **(D)** Multiple shoots. Bar= 10 mm.

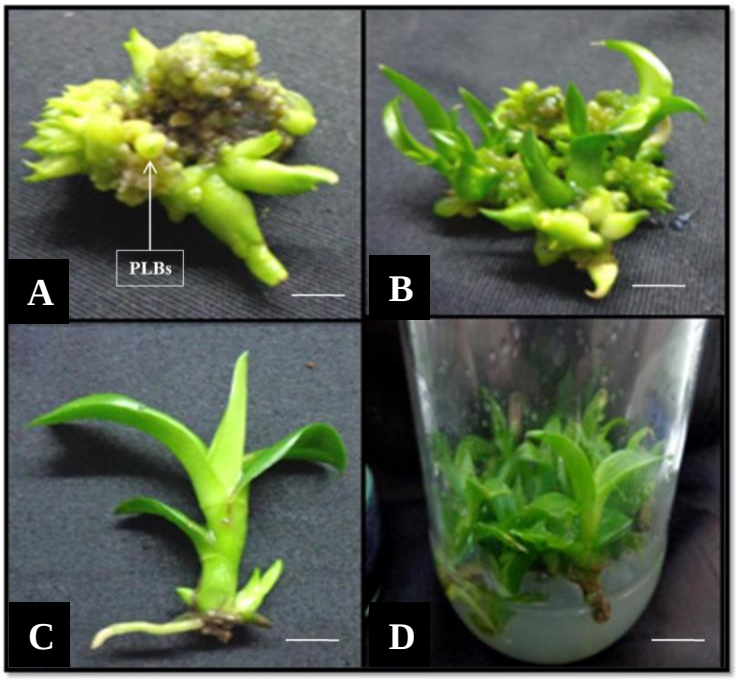


**Fig. 5:** Effect of red light treatment on *in vitro* propagation through PLBs in *Dendrobium sonia*. **(A)** Shoot initiation from PLBs, **(B)** Shoot proliferation, **(C)** Root induction and **(D)** Multiple shoots. Bar= 10 mm.

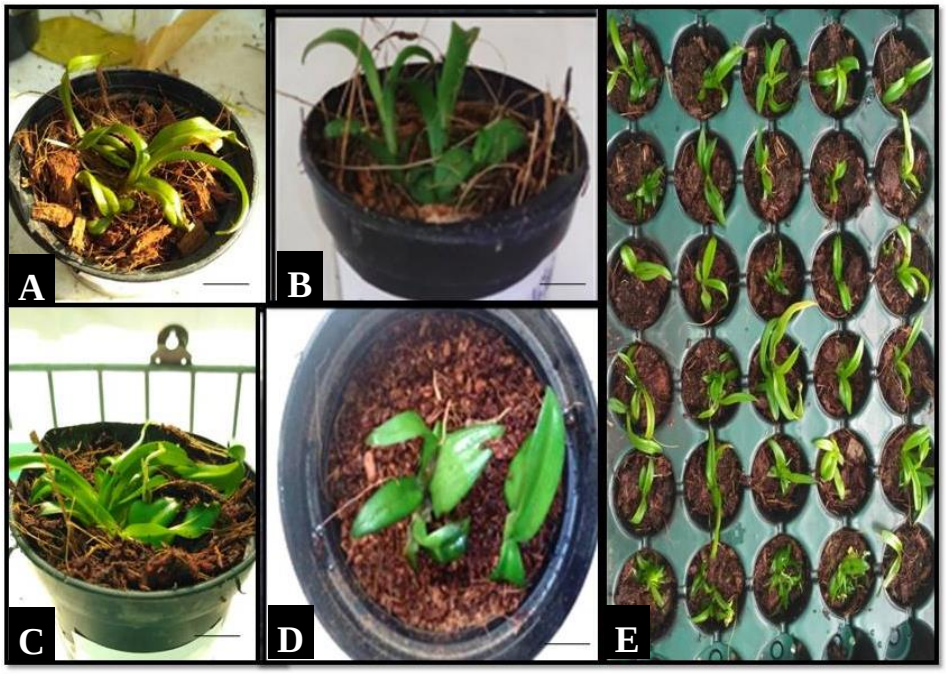


**Fig. 6:** Hardening and Acclimatization process of *Dendrobium sonia* plantlets derived from treatments with **(A)** White light, **(B)** Blue Light, **(C)** Yellow light and **(D)** Red light. **(E)** Plants derived from yellow light treatment undergoing hardening. Bar= 10 mm.

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## Conflict of Interest

There is no conflict of interest among the authors or institutions.

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