In vitro flowering of tobacco induced by light emitting diode

Tilak Raj Maity, Aveek Samanta, Debanjan Jana, Babita Saha and Siraj Datta*
Department of Biotechnology, Haldia Institute of Technology, Haldia 721 657, India

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The influence of light quality on the in vitro flowering of tobacco (Nicotiana tabacum L.) var. Jayasri was investigated. A number of different light conditions, namely, fluorescent, red light-emitting diodes (LEDs), blue LEDs, and red and blue (1:1) LEDs, were used in in vitro cultures. It was found that in vitro flowering percentage increased and days to flowering were reduced significantly in the presence of red and blue (1:1) LEDs compared to fluorescent light. Growth parameters, like fresh and dry wt, stem length, leaf area, chlorophyll and carotenoid content were the highest when plants were grown under red and blue (1:1) LEDs. In contrast, soluble sugar content was lower in plantlets under red and blue (1:1) LEDs. These finding signifies that red and blue (1:1) LEDs may be triggering the metabolic activity of tobacco. The present study thus suggests that red and blue (1:1) LEDs significantly promote the in vitro flowering of tobacco, representing a potentially new in vitro experimental platform to study the molecular mechanism of flowering.

Keywords: Blue LEDs, in vitro flowering, Nicotiana tabacum L., red LEDs

Over the past several decades, tobacco plant has become a model system to provide insight on the different physical and chemical factors that regulate the growth and differentiation in in vitro culture of plants. In vitro flowering provides the fundamental knowledge for flower initiation and development that can be further applied for in vitro breeding. The ability of explants to initiate in vitro flowering depends on numerous external, internal, physical and chemical factors, which interact with each other in various complex ways.

Light (spectral quality, photon flux & photoperiod) is an important factor to control morphogenesis, growth and differentiation as well as in vitro flowering of plants in in vitro culture system. Light emitting diode (LED) is a solid-state light source with various advantages like ability to control spectral composition and specificity in wavelength with cool emitting surface. The wave length emitted by LEDs can be matched with plant photoreceptors and thus might modulate plant morphogenesis as well as its metabolic activity when it is used as an alternative light source for in vitro culture. Thus, in vitro system may be used as a controlled system to study the effect of light spectrum necessary for flower induction by using the LEDs illuminated light system. In the present study, an attempt has been made to determine the effect of fluorescent, monochromatic blue, monochromatic red, mixture of monochromatic blue and red LEDs lights on in vitro flowering of tobacco plants.

Tobacco (Nicotiana tabacum L.) var. Jayasri seeds were surface sterilized and transferred to jars containing 50 mL MS solid medium for production of aseptic seedlings. The shoots were cut from 15-d-old seedlings and cultured in MS medium containing 0.5 mg L⁻¹ indole-3 acetic acid (IAA) and treated with one of the four light sources for 35 d. Cultures were maintained at 25-27°C, under 12 h photoperiod.

The light emitting sets were created by parallel connection of LED lights. The LED lights were aligned in such a way that 528 LEDs (48×11) formed a rectangular light emitting set (60×40 cm) for 11 tissue culture jars. Experimental plants were randomly assigned to each light treatment. The treatments of LEDs were arranged as: Control = fluorescent lamp; Red LEDs = 100% red LED lights (660 nm); Blue LEDs = 100% blue LED lights (460 nm); Red and blue (1:1) LEDs =50% blue LED + 50% red LED lights.

After 35 d of growth, the fresh wt (FW) of plantlets was measured, and dry weight (DW) was determined by drying the plantlets at 85°C until a constant mass was reached. Flowering time was recorded by observing the first flower opening date of a plantlet. Plant height was measured from base to top of the plantlet by a ruler, and leaf area (cm²) was measured using mm graph paper. Leaf chlorophyll (Chl) and carotenoids were extracted using 80% acetone and estimated by Arnon’s methods. Total soluble protein was extracted by 10 mL extraction buffer (0.1 M phosphate buffer, pH 7.6 containing 0.5 mM EDTA). The protein concentration of the supernatant was estimated using the method of Bradford and the
results were expressed as mg protein g⁻¹ FW. Extraction of total soluble sugar was carried out by the method of Martin et al.¹⁰ Soluble sugar content was determined by the sulphuric acid anthrone method¹¹ and measured at 630 nm. Stomata were observed by taking the peeled lower epidermises of fully expanded leaves.

Different light sources showed variable effects on growth, morphogenesis and metabolic activity of tobacco plants under in vitro condition (Fig. 1). The combination of red and blue (1:1) LEDs had a significant influence on in vitro flowering (days to flowering, 14±1 d; as well as flowering percentage, 87.3%) (Fig. 2A). The percentage of in vitro flowering was least in blue LED (1.2%) and a similar trend was followed by fluorescent light (2.8%) and red LEDs (16.1%). After 5 wk of culture, total soluble sugar content was observed lowest in plantlets under red and blue (1:1) LEDs (19.76 mg g⁻¹ FW) and the highest in fluorescent light (29.33 76 mg g⁻¹ FW). But the content of total soluble protein was higher in red and blue (1:1) LEDs (8.63 mg g⁻¹ FW), followed by blue LEDs, red LEDs and fluorescent light treatment. The FW, DW, stem length, leaf area, total Chl, Chl a, Chl b and carotenoids were the highest in plantlets under the red and blue (1:1) LED lights (Table 1). Growth was severely inhibited in plantlets under red LEDs, with lowest FW, DW, stem length, leaf area, Chl and carotenoids. However, the Chl a/b ratio did not vary significantly under all light treatments. Different light sources affected the frequency of stomata as well as the size of stomata (Figs 2B-E). The frequency of stomata was the highest in plantlets under red LEDs (167.39±3.23 mm⁻²), followed by fluorescent (153.74 ± 3.85mm²) and red and blue (1:1) LED (127.65 ± 4.37 mm²), while it was the lowest in plantlets under blue LEDs (123.73 ± 2.92 mm²). The size of the stomata was bigger in blue LEDs treatment, intermediate in red and blue (1:1) and larger when the plantlets were under fluorescent and red LEDs light treatments. Various academic quarters suggest that LEDs may be an attractive alternative light source in near future for in vitro propagation of plants.⁵

In the present study, we have tried to explore the significance of LEDs in in vitro plant tissue culture for stimulating in vitro flowering. Red light is responsible for inter conversion of phytochrome (Pr to Pfr). Pfr may alter gene expression and different physiological responses, such as, flowering, seed germination, leaf expansion, chlorophyll development

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Fig. 1—Effect of red, blue, red and blue (1:1) LEDs and fluorescent light on days to flowering, flowering percentage, total soluble sugar and soluble protein content in in vitro culture of tobacco.[Bars mean±SE]

Fig. 2—(A-E) The LED light setup for the experiments and the effect of different lights sources on leaf stomata of tobacco plantlets cultured in vitro: A. In vitro flowering in red and blue (1:1) LED setup; Inset, Picture of an in vitro tobacco flower; B. Stomata of in vitro cultured tobacco leaves lighted by fluorescent lamp; C. Stomata of red LED lighted leaf; D. Stomata of blue LED lighted leaf; & E. Stomata of red and blue (1:1) LED lighted leaf. [Stomatal areas were the maximum in plantlets cultured under blue LED (D) and the minimum in red LED (C). Ep: Epidermis, Sto: stomata, bar represent 25 µm.]
and stem elongation\textsuperscript{12}. It was also reported that combination of red and blue (1:1) LEDs was also effective for increasing the FW and DW of plants, Chl and carotenoid synthesis\textsuperscript{5} and induction of flowering in \textit{in vitro} plants. These results showed that a mixture of blue and red light source might combine the advantages of monochromatic blue and red light. The quality of light seemed to play an important role in photosynthesis as well as morphogenesis of plants\textsuperscript{13,14}. Fluorescent lighted plantlets showed the presence of higher amount of total carbohydrate after 35 d of culture, while least amount was observed in the plants lighted by red and blue (1:1) LEDs. Thus, it shows that LEDs treated plants consume more of the produced carbohydrates compared to plants exposed to the fluorescent light treatment. During shifting from vegetative to reproductive state, metabolic activity of the plants become much higher than the normal, for up regulation of several genes and their morphogenic conversion\textsuperscript{15}. It was also reflected in total soluble protein content of the red and blue (1:1) LEDs lighted plants, as it had higher soluble protein in comparison to other light source treated plantlets. The presence of blue LEDs as well as red and blue (1:1) LEDs activated the signalling cascade of phototropism and thus resulted into faster opening of stomata\textsuperscript{16} with faster transpiration rate. It might have direct correlation with increased rate of photosynthesis and morphogenic conversion.

Tobacco is used as a model plant to understand the basic developmental physiology of initiation of flowering. LED light initiated \textit{in vitro} flowering and can further pave the way for an alternative platform for detection of the common master-control gene(s) associated with the initiation of floral morphogenesis in a wide range of angiosperms.

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\begin{table}[h]
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\begin{tabular}{|c|c|c|c|c|c|c|c|}
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Light treatments & Chlorophyll (mg g\textsuperscript{-1} FW) & Chlorophyll a/b ratio & Carotenoids (mg g\textsuperscript{-1} FW) & Fresh wt (mg) & Dry wt (mg) & Stem length (cm) & Leaf area (cm\textsuperscript{2}) \\
\hline
Fluorescent & 1.52\textsuperscript{b} & 0.61\textsuperscript{b} & 2.13\textsuperscript{b} & 2.49\textsuperscript{a} & 0.22\textsuperscript{b} & 358.4\textsuperscript{a} & 32.55\textsuperscript{b} & 8.57\textsuperscript{b} & 1.67\textsuperscript{c} \\
Red LED & 1.37\textsuperscript{a} & 0.50\textsuperscript{a} & 1.87\textsuperscript{a} & 2.74\textsuperscript{a} & 0.2\textsuperscript{b} & 363.1\textsuperscript{a} & 32.89\textsuperscript{b} & 9.12\textsuperscript{b} & 1.86\textsuperscript{b} \\
Blue LED & 1.62\textsuperscript{b} & 0.67\textsuperscript{b} & 2.29\textsuperscript{b} & 2.42\textsuperscript{b} & 0.23\textsuperscript{b} & 401.6\textsuperscript{b} & 33.27\textsuperscript{b} & 11.07\textsuperscript{b} & 2.13\textsuperscript{ab} \\
Red+Blue LED & 2.21\textsuperscript{a} & 0.82\textsuperscript{a} & 3.03\textsuperscript{a} & 2.69\textsuperscript{a} & 0.32\textsuperscript{a} & 496.9\textsuperscript{a} & 35.45\textsuperscript{a} & 11.53\textsuperscript{a} & 2.41\textsuperscript{a} \\
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\end{tabular}
\caption{Effect of red, blue, red and blue (1:1) LEDs and fluorescent lights on chlorophyll and carotenoid contents, fresh and dry wt, stem length and leaf area of tobacco plantlets (after 35 d \textit{in vitro} culture)}
\end{table}

Different letters within a column indicate significant differences at p=0.05 by Duncan’s multiple range test

\begin{thebibliography}{99}
\item Atron D L, Copper enzyme in isolated chloroplast polyphenol oxidase in \textit{Beta vulgaris}, \textit{Plant Physiol}, 24 (1949) 1-15.
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