



Impact of photoperiodism on *in vitro* propagation of Indigenous *Musa*

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ABSTRACT

This research aimed at *in vitro* propagations of bananas originating from India. Banana (*Musa spp.*) is a nutritious fruit but shows susceptibility to specific diseases. A traditional method of its propagation is through the separation of suckers, although it may culminate in the transmission of nematodes, parasitic organisms, and viral diseases. In the past two decades, plant tissue culture techniques have facilitated the production of disease-free plantlets. Tissue culturing bananas can involve different explants, including shoot tips, suckers, leaves, and flower buds. Each responds differently to the presence and absence of light. In the current research, explant cultures were placed in light or dark incubation for identical durations, i.e., one to six months, to monitor their growth and development. The color intensity of the explants changed in response to different photoperiods. Young flower buds, mature flower buds, and suckers developed a higher color intensity when placed in light than in dark conditions. However, the opposite occurred in leaf explants that grew optimally in the dark. The results indicated that banana growth occurred productively from sucker explants in different light conditions and variable durations. The results were optimal when employing sucker explants, which exhibited the fastest growth.

Introduction

Plant growth can commonly change by several factors, such as light, temperature, water availability, heat, soil nutrition, and plant hormones. It is essential to perceive how these factors impact plant growth and development. With a fundamental knowledge of these components, plant growth may become more controllable and directed towards specific objectives, e.g., leaf expansion, flower development, or secondary metabolite biosynthesis (Seif et al., 2021).

Plants have a combination of developmental and physiological responses to the intensity and duration of exposure to light. In phototropism, a plant curves or grows directionally towards light. Fundamentally, shoots move towards light, whereas roots move away from it. Exposure to

different photoperiods significantly controls plant blossoming and other developmental procedures (Moosavi-Nezhad et al., 2022). Plant reactions to light depend on the plant-driven ability to detect light intensity and duration. The mechanism of this detection involves the participation of molecules called photoreceptors, comprised of proteins connected to chromophores, i.e., light-engrossing pigments (Javadi Asayesh et al., 2021). When chromophores intercept light, they cause protein-based adjustments, thereby changing protein function through biosynthetic pathways (Hosseini et al., 2019). These pathways initiate biochemical reactions to light, which may adjust growth, development, and hormone biosynthesis. More importantly, photosynthesis sustains plant survival by generating carbohydrates for

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respiration and cellulose for structural composition (Solouki et al., 2023).

Plant development receives stimuli from three primary light-driven variables, i.e., intensity, quality, and photoperiod. Light intensity varies in different seasons, with the strongest available in summer and the weakest in winter. The more sunlight a plant receives, the greater its ability to sustain photosynthesis. Low light intensity may occur when plants are under shade, either naturally or as a treatment (Esmaeili et al., 2022). Light quality insinuates the concealing or recurrence of light. Sunlight contains a full spectrum of frequencies. Blue and red light are necessary for plants to attain optimal growth. Blue light is essential for vegetative growth. With blue light, red light promotes flower formation and blossoming. Deciding which light source to use is crucial in controlling plant growth and development. For example, fluorescent light, known as cool white, is a blue spectrum. It promotes vegetative growth and triggers seedling emergence. Fluorescent light creates a reflective mixture of red and blue frequencies, but they may incur a high cost of operation.

The temporal exposure of plants to light, or photoperiod, is the amount of time a plant receives light. Photoperiod is responsible for the occurrence of blooming in various plants. Fundamental research has indicated that photoperiod controls blooming and triggers several other plant responses. Along these lines, they determine whether plants are short-day or long-day, depending on what conditions they blossom under. The length of the light photoperiod is not a criterion, but that of darkness is fundamental to the plant response (Ashroostaghi et al., 2022). Plants appear in three categories, i.e., short-day (long-night), long-day (short-night), and day-neutral plants, depending on their response to light and darkness. Short-day plants bloom when the day length does not exceed 12 hours. Many spring- and fall-blooming plants, for instance, chrysanthemum, poinsettia, and Christmas thorny plant, are in this category. Strangely, long-day plants bloom when the day length exceeds 12 hours. The timeframe of light and its effect on plants may vary considerably (Fig. 1). Most summer flowering plants, e.g., Rudbeckia, California poppy, and aster, are similar to vegetables such as beet, radish, lettuce, spinach, and potato in this class. Day-neutral plants bloom irrespective of day length. Several examples are tomato, corn, cucumber, and some strawberry cultivars. There are exceptions among plants that do not fit into any arrangement, and yet, they may respond to a mixture of day lengths. Petunias, for example, bloom without receiving

stimuli from day length but blossom earlier and more profusely when exposed to long days (Ghorbanzadeh et al., 2021). Of course, the photoperiod can change in favor of blooming. Chrysanthemums consistently bloom from spring to fall, but their vegetative growth can increase in midsummer by providing shade-covering to keep out the sunlight for 12 hours.

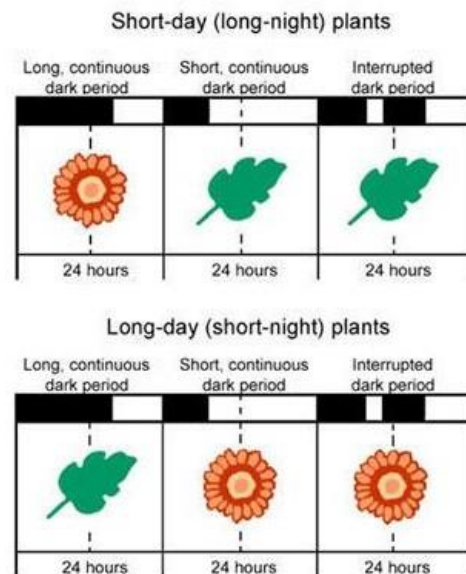


Fig. 1. Photoperiod requirement for plants. Two different categories of plants are classified based on short-day and long-day classification.

Bananas (*Musa spp.*) are a member of the Musaceae family and are one of the most significant crops because of their nutritious significance. In 2013, the world produced nearly 10704 million tons of bananas and 39 million tons of plantain across 135 nations (FAO, 2016). Ordinarily, bananas propagate by suckers, but this technique might cause the transmission of nematodes, parasitic infestations, and viral diseases. In the past two decades, plant tissue culture methods have enabled the production of disease-free plantlets. These methods have benefited banana propagation likewise, with advantages such as the rapidity of propagation, ease of transport after propagation, and the acquisition of high yields at harvest (Bairwa et al., 2015; Shukla et al., 2016). Banana production is a profitable business that currently benefits more than 132 countries. India is the most productive in this regard, with an annual production of 26.2 million tons in 2008 (Naeem et al., 2018; Raman et al., 2018) and a yield/area ratio of 3,698 kg ha⁻¹ (INIBAP, 2000; FAOSTAT, 2008). *In vitro*, micro-

propagation is a widely accepted practice in the clonal propagation of bananas. It allows large quantities of homogenous plantains and bananas to grow in a short timeframe (Pierik, 1987; Rowe and Rosales, 1996; Vuylsteke et al., 1997; Kalimuthu et al., 2007; Al-Amin et al., 2009). The method is increasingly becoming prevalent in countries where banana cultivation has prospects (Rowe and Rosales, 1996; Vuylsteke et al., 1997). Suitable explants reportedly assisted in the micropropagation of bananas and plantain. Shoot tip culture is usually regarded as the most suitable explant for *in vitro* propagation of profitable cultivars (Kulkarni et al., 2004a, 2006b).

The pervasiveness of pathogens and the requirement for creating perfect planting stock in enormous amounts have improved prospects for banana clonal micropropagation in aseptic miniaturized scales. Some of the tissue-cultured plants appear in Fig. 2. Tissue-cultured plants have the advantage of being homogenous and uniform in blossoming, shortened harvest length, and enhanced levels of profitability (Taramla et al., 2019).



Fig. 2. An example of banana micropropagation via explants.

The nature of light and optical standards can significantly contribute to shading, vision, and the extent of shading. It indicates how shading affects synthetic and organic levels of components while demonstrating the natural significance of

phytochromes. It comprises the various effects of shading on leaves, stems, flowers, and organic products. Explants behave differently in the presence and absence of light. Some examples of banana explants are shoot tips, suckers, leaves, and flower buds.

Male inflorescences can serve as explants for the rapid micropropagation of *Musa spp.* Researchers placed the explants on the Murashige and Skoog (MS) medium (Murashige and Skoog, 1962), enhancing it with 1 mg L⁻¹ thidiazuron (TDZ), 6-benzyl amino purine (BAP), kinetin (Kin), zeatin (Zea) and 2-isopentenyl adenine (2-ip), 30 g L⁻¹ sucrose and 3 g L⁻¹ gelrite. The pH changed to 5.7 before autoclaving at 121 °C for 17 min. Zea and 2-ip were added to the MS media while autoclaving by filtration. The samples remained at 25±2 °C, with a photoperiod of 16 h day⁻¹ (Mahdavi Darvari et al., 2010).

In using the 'Udhayam' banana variety for micropropagation, researchers enriched the MS media with different growth regulators. Following the inoculation procedure, the culture tubes remained in a culture room at 25±2 °C for growth. The explants grew for shoot proliferation under white light fluorescence tubes, 25±2 °C, photoperiod of 16 hours of light (2000-3000 lux), and 8 hours in the dark in a culture room (Shukla et al., 2018).

Material and Methods

Plant materials, media, and laboratory conditions

We selected banana plants from a local plant population in the north and northeast of India. The plants included young flower buds, mature flower buds, leaves, and suckers. The plant materials were sterilized and inoculated on MS media containing various growth regulators. The experiments were carried out in the Applied Plant Tissue Culture Facility at Amity University, Noida, India. The inoculated cultures were maintained in sterile conditions at 25 °C under 16 h of light (60 μmols m⁻² s⁻¹) and 8 h of darkness per day. Conditions for incubation were provided on a 24-hour basis. The cultures were placed in light and dark incubation at the same time and in the same timeframe, i.e., one month, during which their growth and development were monitored (Table 1 and Fig. 3, 4, 5, and 6). The effect of light was observed, and the explants were monitored for further growth to produce plantlets.

Table 1. Media composition of different indigenous banana (*Musa* spp.) varieties for shoot proliferation.

Local Name	Genome	Scientific name	Place	Media
Basrai	AAA	<i>M. acuminata</i>		MS + 6 mg L ⁻¹ BAP (Muhammad et al., 2007)
Gopi	AAA	<i>M. sapientum</i>	Tripura, India	MS + 8 mg L ⁻¹ BAP (Kumar Sinha et al., 2018)
Grand Naine	AAA	<i>M. sapientum</i>		MS + 4 mg L ⁻¹ BAP + 2 mg L ⁻¹ IAA (Ahmed et al., 2014)
Giant-Cavendish			Ethiopia	MS + 3 mg L ⁻¹ BAP + 0.4 mg L ⁻¹ IAA (Dagneu et al., 2012)
Dwarf-Cavendish			Ethiopia	MS + 3 mg L ⁻¹ BAP (Dagneu et al., 2012)
Poyo			Ethiopia	MS + 2 mg L ⁻¹ BAP + 0.2 mg L ⁻¹ IAA (Dagneu et al., 2012)
Kluai Sa	AA	<i>M. acuminata</i>	Thailand	MS + 3 mg L ⁻¹ BAP (Srngsam and Kanchanapoom, 2007)
Kluai Leb Mue Nang	AA	<i>M. acuminata</i>	Thailand	MS + 3 mg L ⁻¹ BAP (Srngsam and Kanchanapoom, 2007)
Agnishwar	AAA	<i>M. sapientum</i>	Bangladesh	MS + 4 mg L ⁻¹ BAP (Rahman et al., 2013)
Amritasagar	AAA	<i>M. sapientum</i>	Bangladesh	MS + 5 mg L ⁻¹ BAP (Ferdous et al., 2015; Hoque et al., 2018)
Sabri	AAA	<i>M. sapientum</i>	Bangladesh	MS + 5 mg L ⁻¹ BAP (Ferdous et al., 2015)
Basrai		<i>M. sapientum</i>	Pakistan	MS + 5 mg L ⁻¹ BAP (Muhammad et al., 2004)
Meitei Hei			Manipal, India	MS + 1 mg L ⁻¹ NAA + 0.2 mg L ⁻¹ BAP (Lahrinsanga et al., 2013)
Rasthali		<i>M. sapientum</i>	Tamil Nadu, India	MS + 2 mg L ⁻¹ BAP (Govindaraju et al., 2012)

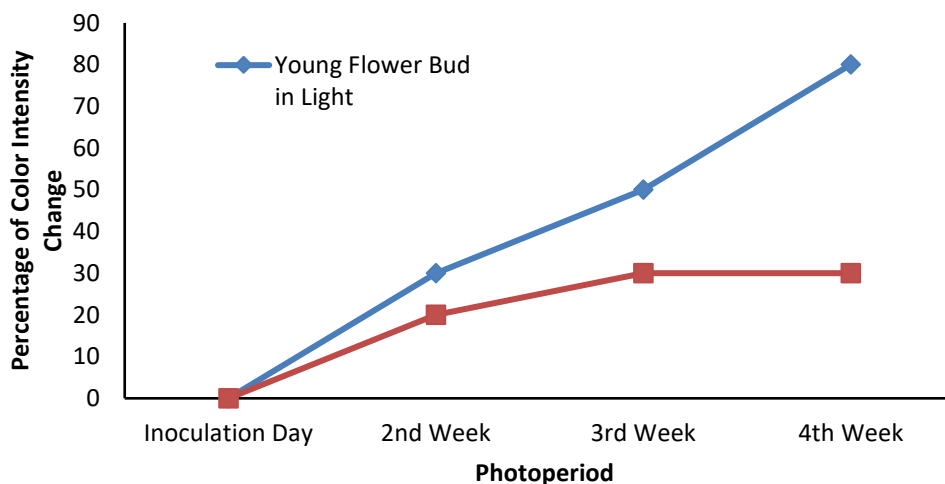


Fig. 3. Changes in color intensity (%) with photoperiod in young flower buds in the dark and light conditions.

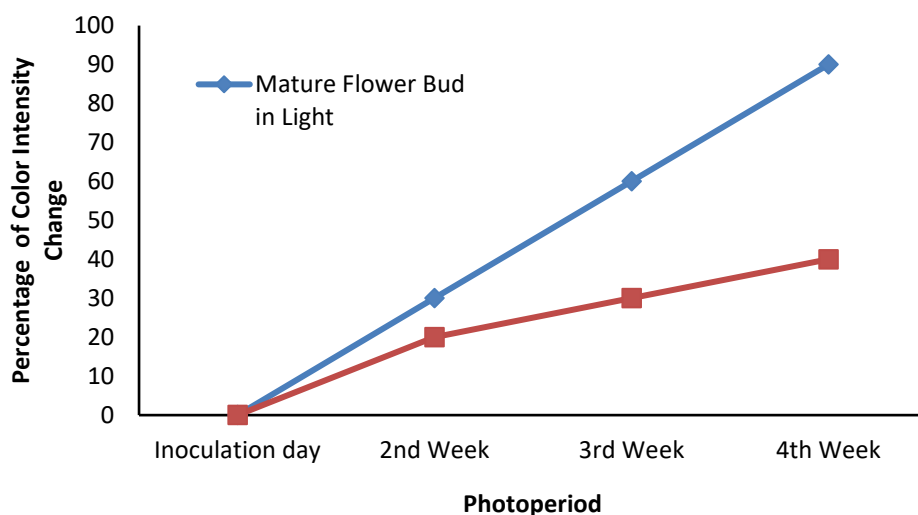


Fig. 4. Changes in color intensity (%) with photoperiod in mature flower buds in the dark and light conditions.

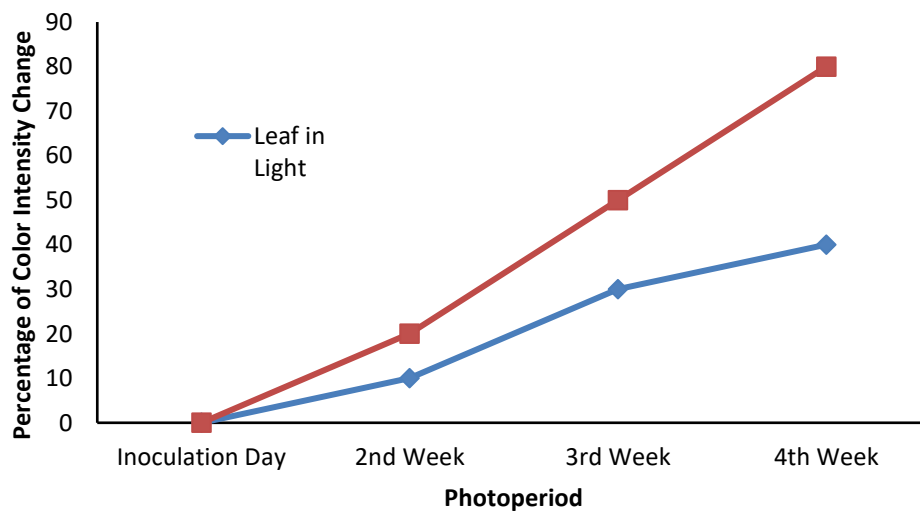


Fig. 5. Changes in color intensity (%) with photoperiod in leaf explants in the dark and light conditions.

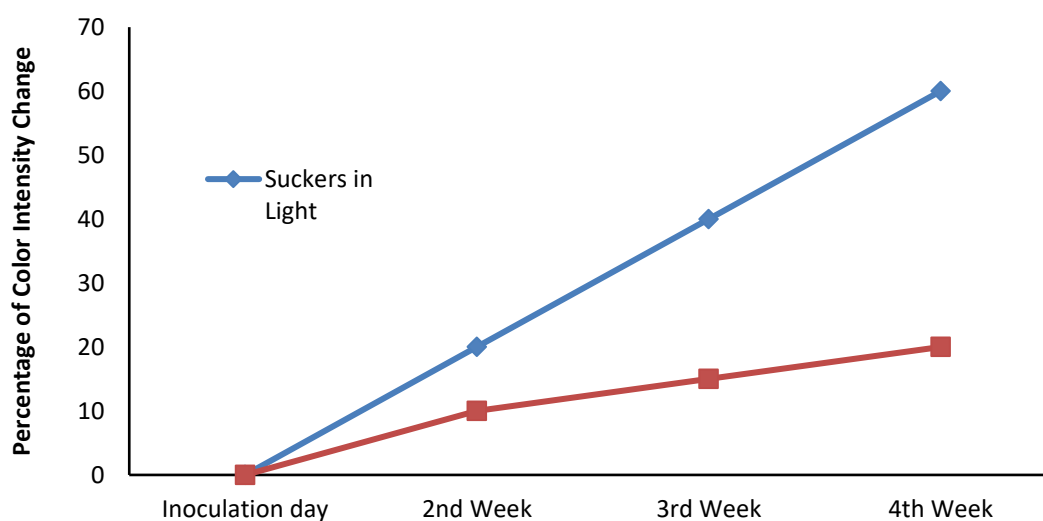


Fig. 6. Changes in color intensity (%) with photoperiod in sucker explants in the dark and light conditions.

In a photoperiodic plant tissue culture experiment, measurable parameters can conclude the effects of light on plant growth and development, including growth rate, morphology, and color intensity. Growth rate is the amount of plant growth over a specific time. It is compared among plants to determine the difference in photoperiods and measure the influence of light on plant growth. Morphology is the shape and structure of plants, examinable to describe the effects of photoperiod on morphological parameters. Color intensity is recognized visually and varies among plants, depending on the photoperiod (Table 2). By measuring these parameters and analyzing the results, it is possible to specify how light intensity and photoperiod affect plant growth and development in plant tissue culture.

Statistical analysis

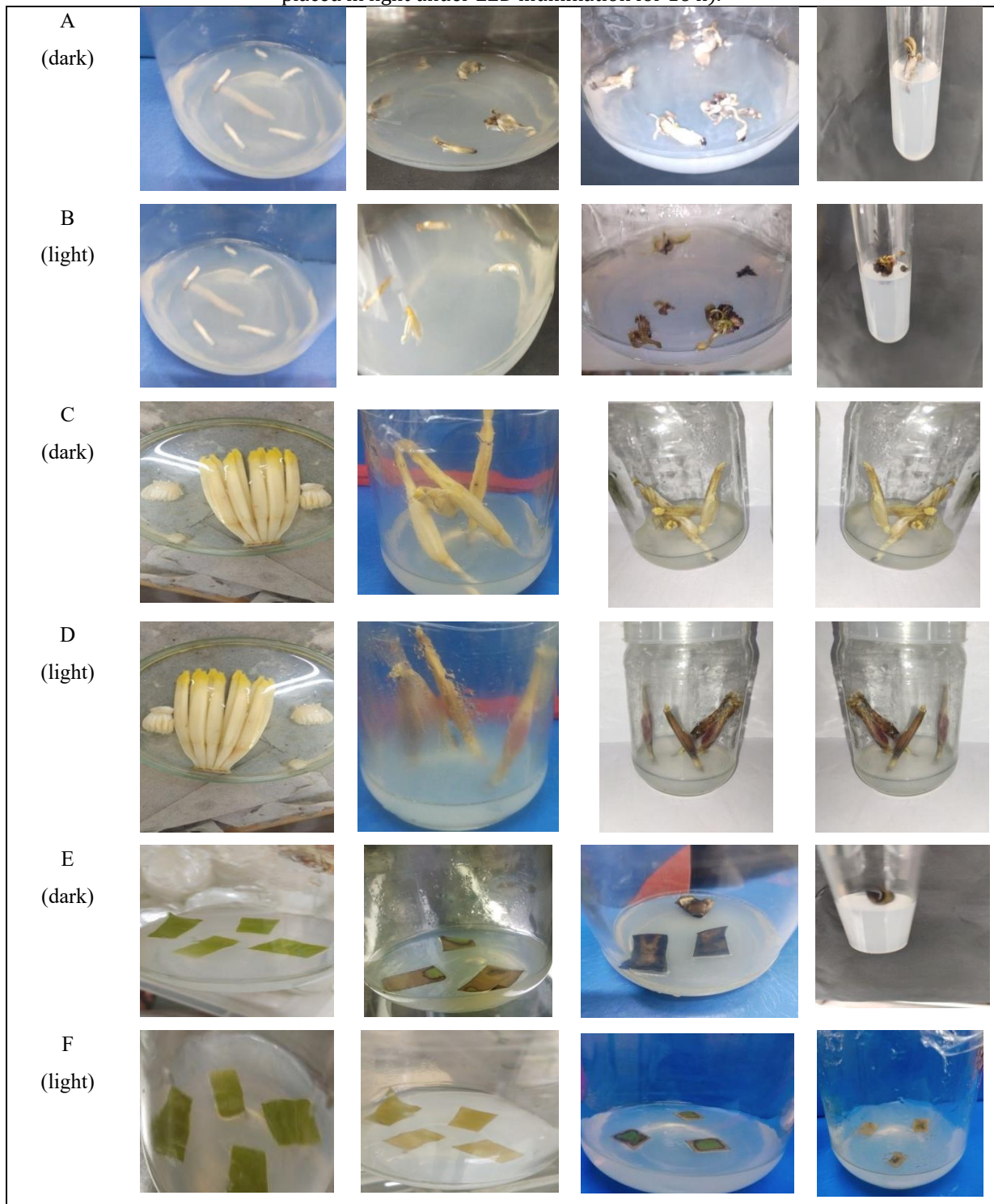
Experiments were conducted using a completely randomized design with five treatments and three replications per treatment. Data were collected from each explant during its growth and development. To determine the effect of different photoperiods on plant growth and development, a one-way analysis of variance (ANOVA) was performed for each parameter measured. The results were analyzed for statistical significance ($p < 0.05$).

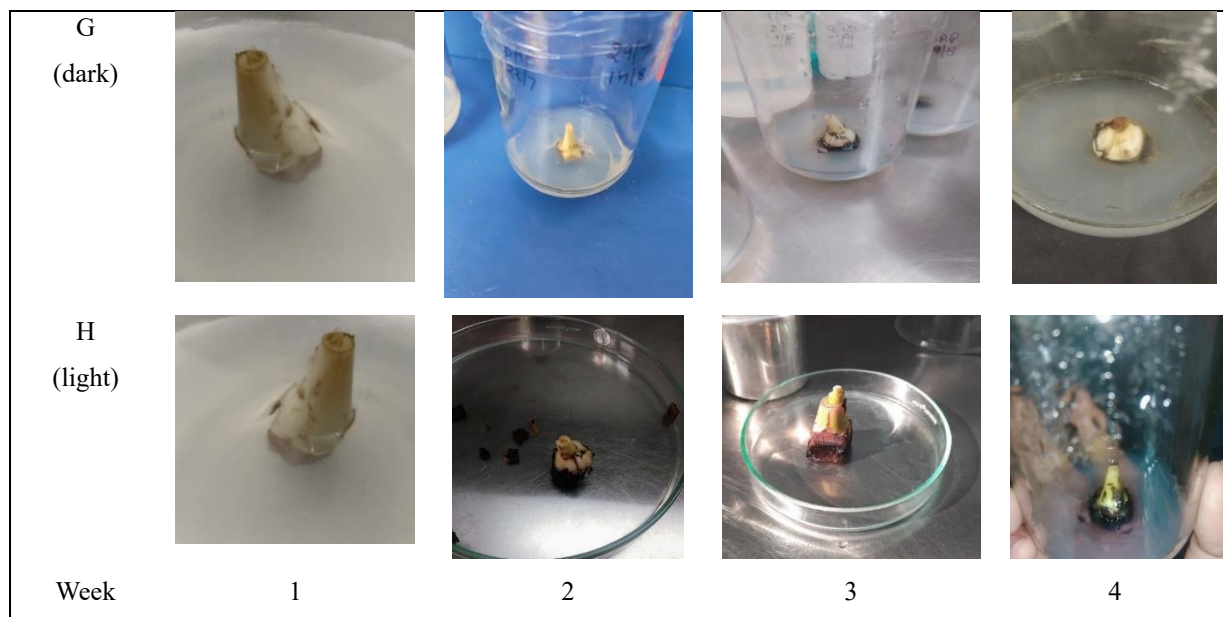
Results

In this study, observations aimed at understanding the effect of photoperiod and light

source. These variables were significantly affected while determining plant growth and development in plant tissue culture. Different explants responded differently, changed color over time, and showed different growth rates. This experiment also assists in the standardization of the perfect environment for banana micro-propagation. The suckers were used as explants and inoculated directly in light. Their growth rate was lower than explants cultured in the dark and light (both for a month) simultaneously. The color chart of sucker growth and the changes in their outer layer hues appear in Table 3. The photoperiod required for plants shows different categories of plants based on short-day and long-day classification (Fig. 2). An example of banana micro-propagation can be observed from the images of plants in the plant tissue culture laboratory (Fig. 3-6). Changes in color intensity (%) in response to photoperiod occurred in young flower buds. Exposure to light caused a higher color intensity in the flower buds than in dark conditions. The growth of various explants, i.e., young flower buds, mature flower buds, leaf, and suckers, was studied over six months. The most optimal outcome resulted from suckers because they showed a fast growth rate and were more suitable for banana micro-propagation than the other explants.

Table 2. Banana explant growth and development through time (A, C, E, G are placed in dark- B, D, F, H are placed in light under LED illumination for 16 h).





(A1, B1) Freshly young flower bud inoculated on media in the first day, (A2, B2) young bud after 2 weeks, (A3, B3) young bud after 3 weeks, (A4, B4) young bud after 4 weeks, (C1, D1) freshly mature bud, (C2, D2) mature bud after 2 weeks, (C4, D4) mature bud after 4 weeks, (E1,F1) fresh banana leaf placed on media, (E2, F2) leaf after 2 weeks, (E3, F3) leaf after 3 weeks, (E4, F4) leaf after 4 weeks, (G1, H1) young sucker day of inoculation in media, (G2, H2) sucker after 2 weeks, (G3, H3) sucker after 3 weeks, (G4, H4) sucker after 4 weeks.

Table 3. Color chart of sucker germination in the dark and light.

Color in dark							
Color in light							
Color in dark and light (1 month each)							
Photoperiod	1 st day	7 th day	14 th day	21 st day	28 th day	35 th day	42 nd day

The study focused on shoot proliferation, color intensity changes, and photoperiod effects. Thus, a summary of the results and findings is presented.

Media composition and growth of different indigenous Musa varieties

Media compositions for shoot proliferation in different *Musa* varieties were determined. Various factors, such as the concentration of BAP (1-5 mg L⁻¹) and IAA (0.2-2 mg L⁻¹), were used to stimulate shoot growth in the respective varieties.

Ex-plant Growth and Development

The growth and development of *Musa* ex-plants (flower buds, mature buds, leaves, and suckers)

were observed over four weeks under different light conditions (dark and light). The results were recorded over several weeks. Fresh young flower buds inoculated on media showed growth progress over the weeks, as indicated by images A2, A3, and A4. Similar observations were made for mature buds (C2, C4), banana leaves (E2, E3, E4), and suckers (G2, G3, G4) under varying light conditions.

Color Intensity Changes and Germination

We used a color chart to document the color changes in the germination process of suckers under different light conditions. The color intensities were recorded as percentages (Table 4).

Table 4. Changes in color intensity (%) during different time periods.

EXPLANT	DARK/LIGHT	PERCENTAGE COLOUR INTENSITY (%)	TIME PERIOD (weeks)
Young Bud	Dark	20-30	2-4
Young Bud	Light	30-80	2-4
Mature Flower Bud	Dark	20-40	2-4
Mature Flower Bud	Light	30-90	2-4
Leaf	Dark	20-80	2-4
Leaf	Light	10-40	2-4
Sucker	Dark	10-20	2-4
Sucker	Light	20-60	2-4

Percentage color intensity change

Percentages of changes in color intensity were measured over 2-4 weeks for different explants under dark and light conditions. Young buds, mature flower buds, leaves, and suckers exhibited varying degrees of color intensity change, with light generally resulting in higher percentages.

Photoperiod effects

Photoperiod effects on plants were discussed, with the classification of short-day plants and long-day plants based on their photoperiod requirements.

Micro-propagation example

An example of micro-propagation of *Musa* was shown through images depicting controlled plant tissue-culture laboratory settings.

The study demonstrated that light conditions significantly influenced the growth and color intensity of *Musa* explants. In general, plants in

light exhibited higher color intensity and better growth than those in dark conditions. This study also highlighted the importance of specific media compositions for promoting shoot proliferation in different *Musa* varieties.

Discussion

Micropropagation has vitally assisted banana and plantain production worldwide (Reddy et al., 2014). Researchers have examined diverse explants for banana and plantain propagation, with shoot tips being the most well-known for *in vitro* propagation of profitable cultivars. Fast-growing meristems in shoot tips of the inflorescence apices could grow sufficiently for micropropagation (Hasnain et al., 2022). Assorted explants may act in unexpected ways and change color intensity through time. This analysis additionally aids the normalization of an ideal condition for banana proliferation in small-scale applications through sucker explants

(Lanoue et al., 2022). They receive conditions for growth appropriately in the light where plant growth and development are not as vigorous as suckers in either the dark or light for several months. Despite progress in using different explants like flower buds, leaves, and suckers, the most optimal results occurred in sucker explants. These explants had a fast growth rate and were suitable for developing explants in the small-scale proliferation of bananas (Chandel et al., 2023). The impact of photoperiod on the *in vitro* propagation of bananas has received extensive research. Photoperiodic conditions reportedly affected the growth and development of banana plants *in vitro*. The photoperiodic conditions can affect various physiological and morphological characteristics, such as shoot and root growth, leaf area, chlorophyll content, and the accumulation of phytohormones (Altaf et al., 2022). Previous studies have shown that greater photoperiods with higher light intensity promote faster and more vigorous growth in banana plantlets. However, shorter photoperiods with lower light intensity can lead to slower growth and development. The type of light, such as blue or red, can also affect the growth of banana plantlets *in vitro* (Jung et al., 2021). The impact of different photoperiods on the *in vitro* propagation of bananas can vary depending on the variety and genotype of the banana. Therefore, it is necessary to consider the specific requirements of each banana variety in propagation when designing protocols for *in vitro* proliferation (Mekonen et al., 2021). In conclusion, different photoperiods affected the *in vitro* propagation of bananas in various ways. It is necessary to carefully consider the photoperiodic conditions when designing protocols for the *in vitro* propagation of banana plants.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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