IN VITRO MULTIPLICATION AND PLANT REGENERATION OF RED BANANA (*Musa acuminata*) USING LIGHT-EMITTING DIODES (LEDS) SYSTEM

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by

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LIST OF ABBREVIATIONS

A	Absorbance
AdS	adenine sulphate
APX	Ascorbate peroxidase
BAP	6-Benzylaminopurine
CAT	Catalase
CBDP	CAAT-box derived polymorphism
cm	Centimetre
cv	Cultivar
DAMD	Direct Amplification of Minisatellite DNA
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
g/L	Gram per litre
H_2O_2	Hydrogen peroxide
HMDS	Hexamethyldisilazane
IBA	Indole-3-butyric acid
IRAPs	Inter-Retrotransposon Amplified Polymorphism
ISSR	Inter-Simple Sequence Repeat
LEDs	Light-emitting diodes
MDA	Malondialdehyde
mg/g	Milligram per gram
mM	Millimolar
MS	Murashige and Skoog
mT	meta-topolin
NAA	Naphthaleneacetic acid
nm	Nanometre

PCR	Polymerase chain reaction
PGRs	Plant growth regulators
PPF	photosynthetic photon flux
RAPDs	Random Amplified Polymorphic DNA
SCoT	Start Codon Targeted
SEM	Scanning electron microscopy
TDZ	Thidiazuron
U/g	Unit per gram
μg/g	Microgram per gram
μL	Microlitre
μm	Micrometre
μM	Micromolar
μmol	Mikromol

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PENGGANDAAN *IN VITRO* DAN REGENERASI POKOK PISANG MERAH (*Musa acuminata*) MENGGUNAKAN SISTEM DIOD PEMANCAR CAHAYA (DPC)

ABSTRAK

Pisang merah ialah tumbuhan serbaguna dengan pelbagai manfaat nutrisi, potensi pertanian, dan aplikasi dalam sains dan teknologi makanan. Mempunyai ciri unik yang menjadikannya sebagai topik kajian berterusan yang bertujuan untuk memaksimumkan kegunaan dan kemampanannya dalam pelbagai industri. Dalam kajian semasa, kesan pelbagai spektrum cahaya dan pengawalatur pertumbuhan tumbuhan (PPT) diselidik terhadap penanaman *in vitro,* ciri fisiologi, kestabilan genetik, dan aklimitasi *Musa acuminata* cv. Pisang Merah. Tujuan kajian ini adalah untuk menambahbaik regenerasi tunas, penunjuk pertumbuhan, pembangunan akar, dan kejayaan pemindahan anak pokok ke persekitaran ladang. Pada fasa pertama percubaan, thidiazuron (TDZ) dan 6-benzylaminopurine (BAP) didapati sebagai PPT paling berkesan untuk meningkatkan pembentukan tunas, dengan rawatan TDZ menghasilkan kadar regenerasi tunas tertinggi. TDZ meningkatkan penggandaan tunas, tetapi menghasilkan tunas yang lebih pendek berbanding dengan PPT yang lain. Asid indole-3-butyrik (AIB) didapati paling berkesan dalam pembentukan akar, dengan 1 µM meningkatkan pemanjangan akar dengan signifikasi. Asid naphthaleneacetic (ANA) pada 3 µM menghasilkan bilangan purata akar yang tertinggi. Fasa aklimitasi menunjukkan kejayaan yang tinggi, dengan kadar kemandirian hidup 100% dicapai melalui prosedur pemindahan yang teliti, menunjukkan keperluan rawatan PGR tertentu untuk pertumbuhan dan adaptasi. Kajian ini juga menyelidik kesan pelbagai spektrum cahaya diod pemancar cahaya

(DPC) terhadap penggandaan tunas, tindak balas fisiologi, dan kestabilan genetik. Cahaya biru+merah paling berjaya dalam mempromosikan penggandaan tunas dan pembentukan daun, manakala cahaya merah jauh menghasilkan tunas terpanjang tetapi kadar penggandaan terendah. Dari segi fisiologi, rawatan cahaya putih pudina dan biru+merah menghasilkan tahap klorofil dan karotenoid tertinggi, dengan cahaya biru+merah juga menunjukkan aktiviti antioksidan tertinggi, menunjukkan pengurangan tekanan oksidatif. Kajian anatomi mendapati bahawa cahaya biru dan biru+merah menghasilkan perkembangan tisu daun baik, tetapi cahaya merah jauh dan putih pudina menghasilkan sel mesofil yang rendah. Kestabilan genetik dan polimorfisme dinilai dengan menggunakan kajian penanda start codon targeted (SCoT), inter-simple sequence repeat (ISSR), dan direct amplification of minisatellite DNA (DAMD). Keputusan ini menunjukkan tahap kestabilan genetik yang baik, dengan lebih daripada 96% kesamaan dalam semua rawatan cahaya. Walau bagaimanapun, nisbah polimorfik diperolehi di bawah rawatan DPC tertentu, terutamanya pada anak benih yang disinari dengan LED merah dan biru menghasilkan lebih atau kurang jalur berbanding dengan kawalan. Kajian ISSR menunjukkan kestabilan genetik yang juga mendedahkan polimorfisme sederhana di bawah rawatan DPC biru dan merah. Kajian ini menunjukkan interaksi antara spektrum cahaya, PPT, dan kestabilan genetik dalam mengoptimumkan perkembangan *in vitro* anak benih pisang merah dan memberikan kerangka kerja yang kukuh untuk pertumbuhan anak pokok pisang merah yang baik dan berjaya dengan dengan penyesuaian di ladang. Penemuan ini memberikan pandangan berharga dalam mengoptimumkan penanaman *in vitro* dan keserupaan genetik anak pokok pisang merah, sekali gus membuka jalan ke arah peningkatan produktiviti pertanian dan penyesuaian ladang yang mampan merentasi pelbagai industri.

***IN VITRO* MULTIPLICATION AND PLANT REGENERATION OF RED BANANA (*Musa acuminata*) USING LIGHT-EMITTING DIODES (LEDS) SYSTEM**

ABSTRACT

The red banana is a versatile plant with numerous nutritional benefits, agricultural potential and applications in food science and technology. Its special characteristics make it the subject of ongoing studies aimed at maximising its benefits and sustainability in a variety of industries. In the present study, the effects of different light spectra and plant growth regulators (PGRs) on the *in vitro* cultivation, physiological properties, genetic similarity and acclimatisation of *Musa acuminata* cv. red banana were investigated. The aim of the study was to improve shoot regeneration, growth indicators, root development and the successful transfer of plantlets to the field. In the first phase of the trial, thidiazuron (TDZ) and 6-benzylaminopurine (BAP) proved to be the most effective PGRs for promoting shoot formation, with TDZ treatments resulting in the highest shoot regeneration rates. TDZ improved shoot multiplication but resulted in shorter shoot lengths than other PGRs. Indole-3-butyric acid (IBA) was found to be most effective in root formation, with 1 µM significantly increasing root elongation. Naphthaleneacetic acid (NAA) at 3 µM produced the largest average number of roots. The acclimatisation phase proved to be highly successful, with 100% survival achieved through rigorous transplanting procedures, demonstrating the need for specific PGR treatment for optimal growth and adaptation. The study also investigated the effects of different lighting-emitting diodes (LEDs) spectra on shoot multiplication, physiological responses and genetic similarity. Blue+red light was most successful in promoting shoot multiplication and leaf formation, while far-red light produced the longest shoots but the slowest rate of multiplication. Physiologically, the mint-white and blue+red light treatments produced the most chlorophyll and carotenoid levels, with blue+red light also having the strongest antioxidant activity, indicating lower oxidative stress. Anatomical studies revealed that blue and blue+red light produced well-differentiated leaf tissue, while far-red and mint-white light resulted in poorly defined mesophyll cells. Genetic similarity and polymorphism were assessed using start codon targeted (SCoT), inter-simple sequence repeat (ISSR) and direct amplification of minisatellite DNA (DAMD) marker studies. The results showed a remarkable degree of genetic similarity, with more than 96% similarity across all light treatments. However, polymorphic ratios were observed in certain LED treatments, particularly in plantlets irradiated with red and blue LEDs, which had more or fewer bands than controls. The ISSR study showed genetic similarity while revealing modest polymorphisms under blue and red LED treatments. These findings offer valuable insights into optimizing the *in vitro* cultivation and genetic similarity of red banana plantlets, paving the way for enhanced agricultural productivity and sustainable field adaptation across various industries.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

The term “banana” is derived from the Arabic word “banan”, which means “finger”. The banana, scientifically known as *Musa* spp. and belongs to the Musaceae family. It is a highly nutritious and easily digestible fruit that originated in South East Asia. Today, it is cultivated by millions of people in 150 countries for cultivation, processing and marketing, with an annual global production of about 153 million tonnes (Kagy et al., 2016; Abou Elyazid et al., 2021; Hanafi et al., 2022; Talla et al., 2022). As the world’s oldest staple food, bananas are widely exported to different parts of the world, making them the fourth most important global food crop after rice, wheat and maize (Ortiz & Swennen, 2014; Justine et al., 2022; Talla et al., 2022). The banana (*Musa* spp.) evolved from interspecific and intraspecific hybridisation between two wild diploid species of the genus *Musa acuminata* (AA) and *Musa balbisiana* (BB).

The red banana, scientifically known as *Musa acuminata* ("Red Dacca"), is characterised by its reddish-purple peel and luscious, creamy flesh. Apart from its remarkable appearance, this fruit is nutritionally superior to its yellow cousins as it has a higher content of beta-carotene, antioxidants and vitamin B6 (Sawardekar et al., 2024). The red banana, which is mainly grown in the tropics, is has been reported for its health benefits, including possible anti-ageing properties due to its high concentration of phenolic compounds and anthocyanins (Thiruppathi et al., 2023). These components not only give the fruit its bright colour, but also contribute to its antioxidant capabilities (Damián et al., 2022). The red banana plant is particularly tall, which makes it susceptible to lodging, where the plant collapses due to wind or rain

(Sawardekar et al., 2024). This susceptibility has led researchers to develop dwarf varieties that retain the attractive characteristics of red bananas while improving resilience (Sawardekar et al., 2024).

Furthermore, clonal propagation of the plant poses a problem for traditional breeding, emphasising the need for new genetic research to improve this triploid species (Sawardekar et al., 2024). In addition to their nutritional benefits, red banana peels are known for their high anthocyanin content, which is much higher than that of yellow banana peels (Rosalina et al., 2022). This characteristic not only enhances the appearance of the fruit, but also opens up opportunities for natural colouring applications and the development of smart packaging (Rosalina et al., 2022). The potential for bioactive substances in red banana peels emphasises the value of this variety for culinary and industrial applications (Damián et al., 2022).

Bananas are remarkable in that they reproduce vegetatively, resulting in a wide range of fruit characteristics such as size, shape and colour (Li et al., 2013). Cultivated bananas, especially Cavendish varieties such as Grande Naine and Williams, have become the main source of global banana exports (Were et al., 2023).

Bananas are not only important because of their fruit. Their cultivation produces important by-products such as pseudostems and leaves, which can be used for a variety of purposes, including the production of bioethanol. In addition, banana stem juice has been investigated as a feedstock for bioethanol production, demonstrating the adaptability of this fruit crop (Gupta et al., 2019). In addition, banana fibres produced from pseudostems have been investigated for textile applications, illustrating the possibility of sustainable use of banana plant components (Balakrishnan et al., 2019). There are a large number of cultivars with different characteristics, as shown by studies on the diversity of banana varieties in different locations based on morphological parameters (Lumowa et al., 2022). Understanding the genetic links between cultivated and wild bananas is crucial for conservation measures and breeding initiatives to improve banana varieties (Sunaryo et al., 2020). In addition, developments in somatic embryogenesis techniques have contributed to the propagation and regeneration of banana plants, creating new opportunities for banana cultivation and genetic improvement (Adero et al., 2023).

Photosynthesis and photomorphogenesis in plants are influenced by the quantity, quality and duration of light exposure. Fluorescent lamps, or FLs, have long been the most widely used artificial light source in plant tissue culture, despite the fact that their distinct emission spectra sometimes do not match the sensitivity range of plant photoreceptors (Gupta et al., 2013). Thanks to the uniform light intensity they provide, white light (in cool, daylight, neutral, or warm variants) or Grolux fluorescent lamps have been widely used to illuminate *in vitro* growth chambers until recently. Nowadays, these fluorescent lamps have given way to LED lamps, which, among many other options, allow us to select the exact wavelengths we want to deliver to the plants, for example, depending on the species or process of interest (Barceló-Muñoz et al., 2021; Nacheva et al., 2021).

The enormous number of articles published in recent years in which LED technology is used in plant tissue culture shows the growing interest in this type of light source. This is because the use of LEDs has opened up a wide range of possibilities, transforming light from a factor that determines photomorphogenesis, but can only be manipulated, into a component that can be configured very precisely, allowing the control and manipulation of plant growth and morphogenesis *in vitro* (Batista et al., 2018). However, the description of the experiments carried out shows a clear heterogeneity of configurations and approaches in relation to the lighting systems, even if the evaluation parameters of the results of the studies and investigations analysed are generally very similar or equivalent. This is in line with the information provided by Batista et al. (2018), who stated that the environmental elements, including light (in terms of quantity and, above all, quality), are the main reasons for the lack of repeatability of the laboratories.

1.2 Problem statement

Traditionally, banana plantations are established using suckers, but this method carries the risk of transmission of plant-parasitic nematodes and other diseases. Breeding new banana varieties that have characteristics such as resistance to pests and diseases, a short growing season, low height, high fruit yield, the ability to bear fruit without fertilisation (parthenocarpy) and a preferred quality by combining characteristics of different varieties remains a difficult undertaking.

Although the Cavendish variety Grand Naine (AAA) dominates the global banana trade, several indigenous varieties such as the red banana (AAA), which have different sensory and nutritional properties, are still largely unexplored. In contrast to the widely cultivated banana, the red banana is only grown in limited geographical regions worldwide.

1.3 Rationale of study

In an effort to overcome these obstacles, advanced technologies have been used for the large-scale propagation of other banana varieties. In particular, *in vitro* culture of shoot tips has gained importance as it produces high-yielding banana plants that outperform conventional scions and are characterised by vigorous growth, early ripening and higher yields. Nevertheless, the pursuit of cost-effective approaches remains a priority for commercial laboratories. Tissue culture has proven to be the linchpin for improving the quality of banana planting material and increasing overall productivity. Tissue cultures of banana plants that are free from pests are often used in commercial banana plantations. One of the most important elements for the growth and development of plants is light. Numerous studies have investigated how light conditions influence the development of secondary metabolites in plants. This research therefore aims to explore the influence of LED irradiation on red bananas.

1.4 Novelty

Various elicitation techniques have been employed to enhance banana micropropagation, particularly by optimizing protocols with cytokinins such as BAP, TDZ, BA, Kinetin, mT, and 2iP, which have proven highly effective. However, targeting specific light spectra to activate specialized plant photoreceptors, such as cryptochromes and phytochromes—responsive to blue and red light, respectively—presents a novel and innovative approach. This research is driven by the distinct abilities of these photoreceptors to respond to different light spectra, potentially influencing metabolic pathways and enhancing the growth and development of red banana, as well as the synthesis of bioactive compounds. To date, the effects of photoperiods and LEDs on *in vitro* cultures of red banana have not been explored. This study aims to fill this gap in knowledge and offer valuable insights into optimizing bioactive compound production in this medicinal plant through the use of photoperiods and LED lighting.

1.5 Objectives of the research

The objectives of the present study are:

i) To promote the development of multiple shoots and roots in the shoot tips of red bananas various cytokinins (6-benzylaminopurine, thidiazuron, meta-topolin and adenine sulphate) and auxins (IBA and NAA),

ii) To evaluate the effects of different LED light spectra on the morphogenetic and physiological properties of the red banana plants,

iii) To analyse the micromorphology and anatomy of *in vitro* cultivated red banana plants using histological and scanning electron microscopy (SEM) techniques,

iv) To carry out biochemical analyses, including the measurement of chlorophyll, carotenoids, and porphyrins from *in vitro* red banana leaves,

v) To determine genetic similarity of the *in vitro* red banana plants using SCoT, ISSR, and DAMD DNA markers.

CHAPTER 2

LITERATURE REVIEW

2.1 Botanical description and morphology of banana

Bananas are herbaceous plants that are sometimes also referred to as trees. The tree consists of a pseudostem that develops through an arrangement of leaf sheaths crowned by a rosette of large rectangular leaves. The leaves are produced sequentially until the inflorescence is cast (Beaton et al., 2023). It is a monocotyledonous plant that belongs to the Musaceae family and is mainly cultivated in the tropical and subtropical regions (Zhou et al., 2024).

Among the edible cultivated species of Musa spp, bananas and plantains belong to the genus *Eumusa* of *Musa*, family Musaceae and order Zingiberales (Safhi et al., 2023). In 2011, bananas and plantains were grown on 10.6 million hectares with an average yield of 13.6 tonnes ha^{-1} and the trend is increasing (Ortiz & Swennen, 2014). According to recent reports, bananas are grown on about one hundred to two million hectares in the humid tropics and subtropics of Africa, Asia, the Americas and Europe, with the area under cultivation extending to Australia and Europe. Several countries in Africa, Asia, Latin America and the Pacific Islands are highly dependent on banana production (Safhi et al., 2023; Subrahmanyeswari et al., 2023).

There are about 1000 banana varieties, including dessert, cooking and beer cultivars, which are the result of intra- or interspecific hybridisation of the wild diploid ($2n = 2x = 22$ chromosomes) ancestors *Musa acuminata* (A genome) and *Musa balbisiana* (B genome). The cultivated varieties (mostly triploid or $2n = 3x = 33$) show fruit parthenocarpy and often sterility, which makes hybridisation of this cultivated plant quite difficult (Ortiz & Swennen, 2014; Auliya et al., 2019).

The banana is a tasty and nutritious fruit and is one of the most produced, consumed and traded fruits in the world. A total of 88 million tonnes of bananas are selected for human consumption every year (Stragliotto et al., 2022). In many tropical and subtropical countries, different banana varieties are grown for consumption or for medicinal purposes (Justine et al., 2022; Al-Dairi et al., 2023). The "Cavendish" (*Musa acuminata*, AAA group, Cavendish subgroup) is the most popular and most commercialised variety and accounts for 40-50% of global banana production. Because of this, Cavendish is the most exported and abundant type of bananas (Al-Dairi et al., 2023).

The genus *Musa acuminata* was divided into different sections based on the number of chromosomes and morphological characteristics (Natarajan et al., 2023). The subgenus *Eumusa* includes edible bananas that are hybrids of *Musa acuminata* (genome A) and *Musa balbisiana* (genome B) (Pinheiro et al., 2019). Most cultivated banana varieties are triploid, although some are diploid and have either an *M. acuminata* genome or hybrids of *M. acuminata* and *M. balbisiana* genomes (Biswas et al., 2020). The taxonomy of triploid cultivars and genetic groupings were initially studied based on morphological traits that distinguish *M. acuminata* from *M. balbisiana* (Lanaud et al., 1992).

The genome of *M. acuminata* has been extensively studied and provides insights into genetic diversity, population structure and gene function. Complete sequencing of the banana genome has enabled the identification of candidate genes and molecules for genetic transformation and gene editing approaches (Soares et al., 2021). Studies have identified specific genes related to fruit ripening, stress response, lignin biosynthesis, flavonoid biosynthesis and *bZIP* transcription factors in *M. acuminata* (Alvarez et al., 2013; Hu et al., 2016; Pucker et al., 2020; Wang et al., 2023). In addition, the development of genetic microsatellite markers has facilitated the analysis of genetic diversity and population structure in banana (Biswas et al., 2020).

The red banana is of interest due to its unique properties and potential health benefits. Red bananas are rich in phenolic compounds, tannins, anthocyanins, minerals and other natural antioxidants that contribute to their distinctive colour, pleasant fragrance and potentially health-promoting properties (Thiruppathi et al., 2023), and are cultivated in South America and East Africa (Ghodake et al., 2023). In the Middle East, it is popular for its flavour as it is sweeter than bananas and has a raspberry taste (Kadam et al., 2023). These compounds are essential for the antioxidant effect of red bananas, which has been shown to be due to their phenolic and vitamin C composition (Proteggente et al., 2002). Studies have shown that red bananas have strong anti-ageing properties and could be considered a valuable dietary ingredient (Thiruppathi et al., 2023).

In terms of classification and distribution, red bananas belong to the broader category of bananas and plantains (*Musa* spp.) (Nzawele et al., 2013). The classification of bananas is based on naturally occurring relationships, with red bananas categorised together with other banana varieties such as Cavendish, Gros Michel, Lakatan and others (Hongyun et al., 2024). Red bananas are distinct from other banana varieties such as Cavendish, Ambon, Stone and others, with each variety having unique characteristics that can be categorised using various methods such as neural networks and support vector machines (Jinan et al., 2023).

2.2 The journey so far in red banana distribution

The red banana has a fascinating history that began in Southeast Asia. *Musa acuminata* and *Musa balbisiana*, the wild banana species from which the red banana is said to originate, are native to the tropical regions of Southeast Asia. Over time, the red banana has spread throughout the world, including Africa, South America and Central America. Red bananas are grown and eaten in these areas for their distinctive taste and colourful appearance (De Langhe et al., 2015; Mathew & Negi, 2017).

The red banana has become a popular fruit in many regions of the world, where it is used in a variety of culinary specialties, including smoothies, baked goods, savoury dishes and sweets. The red banana has a long history and is believed to have evolved from the wild banana species *Musa acuminata* and *Musa balbisiana* in Southeast Asia (Mathew & Negi, 2017). It has spread and been cultivated in various places, including Africa and South America (Subrahmanyeswari et al., 2023). The history of the red banana is extensive and complex and still not fully understood. However, the red banana has become popular due to its distinct flavour and colourful appearance.

2.3 Uses of red banana

Plantains and bananas are examples of rhizomatous herbs, with the terminal bud forming the flower. The cycles are perennial because they are repeated over a period of one to fifty generations or more (Tixier et al., 2004). According to Jayasinghe et al. (2022) the banana plant goes through four primary developmental stages: shoot formation, growth of foliage, blooming, and fruiting. The mature fruit of the banana plant is its most valuable component economically. Nonetheless, in many nations, unripe fruits, inflorescences, leaves, stems, and rhizome portions are utilised as cooked vegetables and animal feed. In addition, the banana can also be called an herbal plant as it is enriched with medicinal benefits. All parts of the banana have nutritional and therapeutic value (Kumar et al., 2012), and previous studies have shown that the banana is rich in bioactive compounds (carotenoids, flavonoids, phenols, amines, phytosterols, vitamins) that have antioxidant properties and can be used for pharmaceutical and health purposes (Pereira & Maraschin, 2015; Singh et al., 2016; Jayasinghe et al., 2022).

Red bananas, known for their distinctive flavour profile and nutritional benefits, have recently gained much attention in studies exploring various facets of this variety. Research has demonstrated the potential of red bananas as a rich source of anthocyanins, with a particular focus on the extraction of these beneficial compounds from the peel and flowers of red bananas (Rosalina et al., 2022). In addition, research has looked at the use of red banana fibres in lightweight composites, highlighting their exceptional tensile strength compared to other commonly used fibres (Yu & Xu, 2014). In addition, red bananas have been identified as one of the different banana varieties characterised by unique morphological properties that distinguish them from other banana species (Lumowa et al., 2022).

In food science, the incorporation of red banana flour into products such as biscuits has been studied in detail. Remarkable changes have been observed in various properties, including functional, thermal and physicochemical characteristics (Mabogo et al., 2021). In addition, the quality characteristics of banana milk smoothies enriched with red plum were investigated. This revealed significant differences in physicochemical properties and sensory profiles compared to conventional smoothies (Kumar et al., 2021). Similarly, researchers have investigated the effects of ingredient composition on artificial rice made from banana flour and found that the properties of the final product change depending on the recipe used (Kamsiati et al., 2022).

In addition, discussions on the potential of red bananas for biofortification have emphasised the variability of carotenoids in different banana varieties and the impact on increasing their nutrient content (Amah et al., 2019). In addition, studies have investigated the use of red bananas in indicator films developed to detect the degree of ripeness, showing innovative applications of this variety beyond traditional consumption (Santoso et al., 2023). Sensory evaluations of red bananas in various foods have also been conducted, showing their potential as a topping and ingredient in different culinary creations (Yunita et al., 2024).

2.4 Banana tissue culture

Tissue culture of bananas is a key technique in modern agriculture that offers a number of advantages, such as the rapid production of disease-free planting material with the desired genotypes. The challenges associated with banana tissue culture are being addressed through innovative methods. For example, Ngomuo et al. (2014) investigate the use of shoot tip cultures for banana propagation *in vitro* and show the potential of this method to overcome problems encountered with traditional propagation techniques (Ngomuo et al., 2014). In addition, Xiang et al. (2023) emphasise the routine use of tissue culture methods in banana propagation and point out the need to produce planting material that is free from pathogenic microorganisms (Xiang et al., 2023).

The development of efficient protocols for tissue culture of banana is of paramount importance to ensure the production of high-quality planting material while minimising production costs. Research by Priyanka (2020) highlights the influence of growth regulators on the *in vitro* growth of bananas and emphasises the need to optimise culture conditions for successful tissue culture (Mulugo et al., 2020). The adoption of micropropagation technology for banana production is influenced by various factors, including socio-economic considerations. Murongo et al. (2018) and Mulugo et al. (2020) examine the socio-economic factors that affect smallholder farmers' adoption of tissue culture banana technology, they reported that the uptake of tissue culture banana technology has been shown to be very low. These factors play a crucial role in farmers' decisions to integrate this technology.

In addition, the susceptibility of tissue-cultivated banana plants to infections such as banana bunchy top virus (BBTV) is a cause for concern. Jebakumar et al. (2018) emphasise the susceptibility of tissue-cultured banana plants to BBTV infection compared to plants grown from cuttings, highlighting the need for robust disease control strategies in tissue culture.

2.4.1 Explant type

The type of explant used in banana tissue culture is a crucial factor that significantly influences the success and efficiency of the propagation process. Various studies have shown the importance of selecting a suitable explant to optimise banana tissue culture techniques. For example, Ngomuo et al. (2014) highlighted the effectiveness of using shoot tip cultures in combination with bud division techniques to increase the number of explants during tissue culture. In this approach, younger plantlets are divided longitudinally to produce more viable explants and thus increase the overall success of propagation.

In addition, Mekonen et al. (2021) found that banana shoot tip meristem explants cultured on specific media showed the best response in shoot formation. This finding emphasises the importance of not only selecting the right type of explant, but also using the appropriate culture media to achieve optimal growth and development. The study shows that precise conditions tailored to the specific needs of the explant type can lead to significantly better results in tissue culture. In addition, the choice of explant can have a direct influence on the regenerative capacity of banana plants. Jekayinoluwa et al. (2020) reported the successful use of different explant types, such as embryogenic cell suspensions and meristematic tissue, in *Agrobacterium*-mediated transformation systems for banana. This shows that selecting the right explant type can enable successful genetic transformation processes, opening up opportunities for genetic improvement and expansion in banana cultivation.

The success of tissue culture in banana is also influenced by factors such as explant surface sterilisation protocols. Wong (1986) pointed out the importance of using specific explant types, such as longitudinally sliced tissue, to induce maximum numbers of primordia and shoots during tissue culture. The study emphasised that careful preparation and handling of the explants are crucial for high regeneration rates and successful tissue culture results. Furthermore, El-Mahrouk et al. (2019) emphasised the importance of developing efficient protocols for banana tissue culture to produce high-quality and pathogen-free planting material. Research has shown that the type of explant used can influence not only the efficiency of the propagation process but also the quality of the resulting seedlings. Efficient tissue culture protocols that incorporate the correct explant types are essential for the production of robust, disease-free banana plants suitable for large-scale cultivation.

2.4.2 Explant size

The size of the explants is a critical factor in banana tissue culture that influences the success of micropropagation and growth outcomes. Research has shown that explant size has a direct impact on the regeneration potential and overall development of banana cultures. For example, Ngomuo et al. (2014) have discussed the use of bud division techniques to increase the number of explants in tissue culture and emphasised the importance of selecting banana plantlets at a younger physiological age for optimal results. This technique involves dividing the buds of younger plantlets longitudinally to maximise the number of viable explants.

Kiruwa et al. (2024) observed that smaller explants, such as meristem domes, have slower regrowth and higher mortality compared to larger explants. This emphasises the importance of selecting explants of an appropriate size to ensure better survival and regeneration in culture. Smaller explants tend to have more problems in the early stages of growth, which can lead to increased failure rates in tissue culture processes.

In addition, Kethiri et al. (2017) have shown that the small size of the explant significantly affects the average expansion area, with larger explants having greater growth potential. Their study suggests that maintaining certain size thresholds can lead to more robust growth and development, improving the overall success of banana tissue culture. The results suggest that meeting certain size criteria is essential for achieving optimal expansion and regeneration results. Mekonen et al. (2021) substantiated these conclusions by showing that optimal shoot formation is achieved with explants of specific sizes grown under defined conditions. Their research found that when cultivating explants with precise size specifications, shoot formation rates and overall growth were significantly improved, resulting in more successful propagation.

2.5 Plant growth regulators

Plant growth regulators, especially phytohormones, play a crucial role in the growth and development of plants and their response to environmental stresses. Phytohormones such as auxins, cytokinins, gibberellins, abscisic acid and ethylene act as chemical messengers that regulate various physiological processes (Su et al., 2011; Bielach et al., 2017; ; Peres et al., 2019; Li et al., 2021). These hormones interact in complex signalling networks to coordinate processes such as seed dormancy, meristem development and root growth (Liu et al., 2013; Liu et al., 2017; Zhou et al., 2022).

The application of plant growth regulators such as paclobutrazol has been shown to increase crop yields, increase stress tolerance and improve the physiological characteristics of plants (Desta & Amare, 2021). In addition, bacterial volatiles and zinc oxide nanoparticles can influence the synthesis and metabolism of phytohormones and thus promote plant growth (Tahir et al., 201; Ahmed et al., 2023). Understanding the biosynthesis and signalling pathways of phytohormones is important to elucidate their role in plant development and stress responses (Zhao, 2010; Nascimento et al., 2019; Emamverdian et al., 2020).

Phytohormones are not only crucial for the regulation of plant growth, but also for the adaptation of plants to environmental changes and stress conditions (Sytar et al., 2019; Li et al., 2021; Orozco-Mosqueda et al., 2023). They modulate physiological processes, gene expression and metabolic pathways to promote plant growth and improve stress tolerance (Emamverdian et al., 2020). In addition, phytohormones are involved in the regulation of plant responses to heavy metal stress, further emphasising their importance in plant physiology (Sytar et al., 2019).

Research has investigated the effects of various cytokinins, such as metatopolin, meta-topolin riboside, metamethoxy-topolin and meta-methoxy-topolin riboside, on the micropropagation of banana varieties such as 'Williams' and 'Grand Naine' (Bairu et al., 2008). High levels of cytokinins during micropropagation have been associated with clonal variability in major crops such as banana (Venkatachalam et al., 2007). Moreover, the combination of different plant growth regulators in the media has been reported to influence *in vitro* propagation of different banana cultivars (Gübbük & Pekmezci, 2004; Rajoriya et al., 2018; Bhaya et al., 2019; Singh et al., 2024).

Maintaining genetic fidelity in micropropagation is of crucial importance. Techniques such as meristem culture at the shoot tip have been shown to be effective in maintaining genetic uniformity in micropropagated banana plants (Ray et al., 2006). In addition, studies on metabolite profiles during the different stages of banana micropropagation emphasise the importance of understanding the physiological changes throughout the process (Carrera et al., 2021). The use of light-emitting diodes (LEDs) has been found to increase stomata formation and chlorophyll content in banana *in vitro* plants, improving growth characteristics (Vieira et al., 2015).

2.6 Oxidative stress

Plants are exposed to oxidative stress, which has a significant impact on their growth and development. Several research studies have focused on the effects of oxidative stress on plants and the mechanisms they use to resist it. Savchenko & Tikhonov (2021) describe the changes in central metabolism that occur in plants during oxidative stress situations. They focus on the changes in metabolites and biochemistry caused by oxidative stress and provide insight into how plants respond to such conditions. Qamer et al. (2021) investigate the response of *Gossypium hirsutum* L. to harsh abiotic conditions and emphasise the increase in reactive oxygen species (ROS) during stress events and the resulting biochemical and morphophysiological changes in the plant. This work sheds light on how plants respond to abiotically induced oxidative stress.

In addition, Zulfiqar and Ashraf (2021) investigate the interactions of auxins and ROS in plants under oxidative stress conditions. They emphasise the complex link between these two components and their involvement in building tolerance to oxidative stress. This review helps to understand the different mechanisms underlying plant responses to oxidative stress. Yonny et al. (2023) investigate the use of Ilex paraguariensis extract in foliar treatments to reduce oxidative stress in horticultural crops. The work shows how abiotic stress causes the development of reactive oxygen species (ROS) in plant cells and emphasises the need to control oxidative stress in plants.

Oxidative stress and light exposure are inextricably linked in plants, with both high and low light intensities playing an important role in triggering oxidative stress responses. High light intensity has been shown to cause oxidative stress in plants, leading to increased production of reactive oxygen species (ROS) (Aarti et al., 2006). This increased ROS production under high light conditions can cause oxidative damage to plant cells and impair activities such as chlorophyll biosynthesis (Aarti et al., 2006). In addition, excessive light stress can exacerbate ROS formation and lead to light stress conditions that contribute to oxidative stress in plants (Chen & Gallie, 2005).

Exposure to low light, together with additional stress factors such as cold temperatures, can cause oxidative stress in plants, leading to photo inhibitory stress and oxidative damage (Szalai et al., 2018). This demonstrates the dual nature of light exposure in plants, with both high and low light conditions influencing the extent of oxidative stress and plant responses.

2.7 The role of LED lights in plant tissue culture

LED lighting systems have become an indispensable tool in plant tissue culture and offer numerous advantages over conventional light sources. Numerous studies have shown that LED lighting can significantly increase the production of important secondary metabolites in *in vitro* propagated plants (Hashim et al., 2021). The preference for LED lights in plant culture systems is based on their lower energy consumption, longer lifetime, higher efficiency and ability to deliver a broader spectrum of wavelengths than conventional light sources (Jung et al., 2021). These characteristics make LED lights more suitable for indoor plant growth and *in vitro* cultures.

The interaction of LED light with plants is significantly influenced by various photoreceptors that perceive light signals and mediate subsequent physiological responses. These photoreceptors, including phytochromes, cryptochromes, and phototropins, play crucial roles in regulating plant growth and development in response to different light qualities, particularly red and blue wavelengths emitted by LEDs. Phytochromes are red and far-red light receptors that exist in two interconvertible forms: Pr (inactive) and Pfr (active). The activation of phytochromes by red light leads to a cascade of signaling events that regulate numerous developmental processes, including seed germination, flowering, and stress responses

(Fichman et al., 2022) Qiu et al., 2023). For instance, phytochrome B (phyB) has been shown to regulate reactive oxygen species (ROS) signaling, which is essential for plant adaptation to both abiotic and biotic stresses (Fichman et al., 2022). Furthermore, phytochromes interact with various transcription factors, such as the basic helix-loop-helix (bHLH) proteins, to modulate gene expression in response to light (Zhong et al., 2012). Cryptochromes are blue light receptors that mediate several light-dependent processes, including phototropism and stomatal opening. They are crucial for the regulation of circadian rhythms and the modulation of growth in response to blue light (Gangappa & Botto, 2016; Yadav et al., 2020). Cryptochromes interact with the COP1 protein, a central repressor of light signaling, to promote the degradation of negative regulators and enhance the expression of positive regulators like HY5, which is essential for light-induced gene expression (Gangappa & Botto, 2016; Nawkar et al., 2017). This interaction highlights the intricate network of signaling pathways that integrate light perception and hormonal responses to optimize plant growth. Phototropins, another class of blue light receptors, are primarily involved in phototropism and chloroplast movement. They facilitate the bending of plants towards light sources, enhancing light capture for photosynthesis (Yadav et al., 2020; Тараканов et al., 2022). The activation of phototropins leads to the phosphorylation of downstream targets that regulate growth and development, demonstrating the importance of blue light in shaping plant morphology (Yadav et al., 2020). The interplay between these photoreceptors and various signaling pathways is critical for understanding how plants adapt to their light environment. For instance, the interaction between phytochromes and the PIF (phytochrome-interacting factor) family of transcription factors is essential for integrating light signals with other environmental cues, such as temperature and hormonal signals (Sun et al., 2018; Wang et al., 2015).

This integration allows plants to optimize their growth strategies under varying light conditions.

LED lights are remarkably versatile in horticulture. Their applications range from research under controlled conditions to the illumination of tissue culture and supplemental or photoperiodic lighting in greenhouses (Morrow, 2008). The advent of LED-based artificial lighting systems in plant tissue culture has significantly enhanced morphogenesis in micropropagated plants, leading to improved callus induction, shoot and root organogenesis and acclimatisation of *in vitro* propagated plants (Fan et al., 2022). Consequently, LED lights have proven to be a viable alternative to conventional lighting systems in plant tissue culture and have positively influenced plant growth and development (Mahdi & Kadhim, 2023).

Research has shown that LED lights with specific spectral characteristics can induce morphogenetic improvements in *in vitro* plants, emphasising their importance for tissue culture studies (Han, et al., 2021; Parab, et al., 2021). Standardisation of illumination conditions based on LED technology is now a common practise in plant tissue culture, indicating the widespread use of LED illumination in this field (Barceló-Muñoz et al., 2021). In addition, LED lighting has been integrated into the production processes for high-value plants *in vitro* to replace traditional light sources and achieve better results under tissue culture conditions (Gupta & Sood, 2023).

Numerous studies have elucidated the effects of LED light on various aspects of plant physiology, including the production of phenolic compounds, Amaryllidaceae alkaloids and antioxidant enzyme activities in plant cultures (Morańska et al., 2023). Phenolic compounds, which are crucial for plant defence and human health, have been shown to increase under certain LED light treatments. The increased activities of antioxidant enzymes indicate improved stress tolerance and overall plant health, further demonstrating the multiple benefits of LED light in tissue culture environments (Samuoliene et al., 2017; Rahman et al., 2021). In addition, the use of LED light has been shown to improve growth, phytochemical content and antioxidant enzyme activity in plants cultured *in vitro*, emphasising the positive effects of LED light on plant development (Manivannan et al., 2015). These improvements are attributed to the ability of LED lights to precisely control light quality, intensity and duration, which are critical factors in regulating plant metabolic pathways and developmental processes.

LED lights promote plant growth by emitting certain qualities of light, such as enhanced red and blue wavelengths, which contribute to increased leaf area and root length in plant tissue culture (Nguyen et al., 2020). Red light is known to promote photosynthesis and stem elongation, while blue light is important for chlorophyll production and overall plant morphology. This targeted light spectrum enables optimised growing conditions, resulting in more robust and healthier plants in tissue culture. LED luminaires are able to maintain uniform illumination in plant tissue culture rooms by balancing the fluctuations of natural light, ensuring stable light conditions essential for plant growth (VU et al., 2020). This reliability of light supply is crucial for experimental reproducibility and the standardisation of culture conditions, which are fundamental in research. In addition, the ability to fine-tune the light spectrum and intensity allows researchers to study the effects of specific wavelengths on plant growth and development, enabling a deeper understanding of plant physiology and the optimisation of tissue culture protocols (Breen et al., 2023; Stamford et al., 2023).

In addition, LED lights are particularly well suited for use in strictly controlled environments, making them the preferred choice for plant cultivation in research facilities (Astolfi et al., 2012). Their compact size, low heat output and installation flexibility, they can be integrated into various growth chamber designs and configurations, improving the efficiency and effectiveness of tissue culture laboratories. The low heat output of LED lights also reduce the risk of overheating, which can be harmful to sensitive plant tissues and crops (Vargas, 2014; Singh et al., 2015). The benefits of LED lighting go beyond growth and development. They also play a crucial role in the sustainability and profitability of plant tissue culture (Morrow, 2008). The energy efficiency of LEDs results in lower operating costs, making them a cost-effective choice for large tissue culture facilities (Manuel & George, 2018). The long lifespan of LED lights reduces the need for frequent replacement, further contributing to cost savings and reducing the environmental impact associated with the disposal of traditional lighting systems (Ganandran et al., 2014).

The integration of LED lights in plant tissue culture not only improves the physiological and morphological properties of plants, but also supports the sustainable and economical production of *in vitro* cultures. The positive effects on secondary metabolite production, stress tolerance and overall plant health emphasise the transformative potential of LED lighting in plant biotechnology and horticulture (Yavari et al., 2021). As research continues to develop, LED technology is expected to further revolutionise plant tissue culture practises and promote innovation and efficiency in the field.

2.7.1 Effects of LED lights on photosynthesis

The effects of LED lighting on photosynthesis in various plant species have been extensively researched. It has been found that the spectral composition of LED light has a significant influence on photosynthetic processes. LED lamps emitting red and blue wavelengths are often selected to increase the efficiency of photosynthesis in plants (Evlakov et al., 2021). Studies suggest that matching the spectral power distribution of LED lamps to the spectrum of photosynthetic efficiency is crucial for optimising photosynthetic performance (Oh et al., 2015). Furthermore, the use of a combination of red and blue LED light is beneficial for supporting photosynthesis in plant factories (Setiawati et al., 2023).

The effects of LED light on photosynthesis go beyond immediate reactions and also influence long-term processes. Research has investigated both the short-term and long-term effects of different LED spectra on the photosynthetic rates of pepper plants, emphasising the importance of considering both the immediate and long-term effects on photosynthetic activity (Murakami et al., 2013). In addition, studies emphasise that plants need to acclimatise to narrow-band LED lighting in order to use this light effectively for photosynthesis (Zakurin et al., 2020; Paradiso & Proietti, 2022).

In practical applications, optimising the light spectrum of LED lamps can significantly improve the photosynthetic performance of various plant species. Studies on different seedlings, for example, have shown that different light spectra (white, red, yellow, green and blue) of LED lamps have different effects on photosynthesis (Park & Runkle, 2018). These results emphasise the importance of selecting LED types that not only work efficiently but also promote effective photosynthesis in crops.