EVALUATION OF LIGHT QUALITY AND LIGHT LEVELS FOR IN VITRO PRODUCTION OF ORNAMENTAL BANANAS (MUSA SP.)

By

CASSANDRE FEUILLE

A THESIS PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2019

1
To my loving and wonderful parents Jean-Robert Feuillé and Vilaine Sinclaire whose endless love, proper education and support guided me throughout my life, and made me the person I am today. You are the best parents in the world and role models. I owe everything to you.
ACKNOWLEDGEMENTS

To the only and true God to guide me day after day in all my paths and give me health and the opportunity for all my undertakings to be a success.

To my advisor Dr. Wagner A. Vendrame and co-advisor Dr. Michael E. Kane for their guidance, time, patience, dedication, understanding, suggestions, generosity, contributions, for the knowledge and experiences transmitted in these years of study and above all for the friendship dedicated to my professional training.

Many thanks to Dr. Kimberly Moore for her professionalism, human quality, contributions and for her great willingness to review this thesis.

My sincere thanks go also to Dr. Wilson B. Sandra and Dr. Perez E. Hector for their expert, sincere and valuable guidance and encouragement extended to me.

Acknowledgement also goes to my lab-mates for their support and Mr. David Beleski for his assistance during my experiment at the laboratory.

Special thanks to Rocheteau Daréus, for being there for me since the beginning, for being so patient, for being always available when I need his help no matter when, for his advice, and for being an integrant part of my life.

A huge thanks to Dr. Van Santen and Mr. Riley for the data analysis and interpretation.

I thank my peers that helped me throughout this process, beginning with my fellow graduate students: Lynhe Demesyeux and Victoria O. Adelaye, who shared ideas, provided moral support, advice and comfort. You were an integral part of my graduate experience.

To my two precious sisters Daphkar and Djémie Feuillé for their love, their affection, their friendship, their support and for being so special to me. And also to Natacha and Judenscia.

Last but not least to USAID and the AREA project for their financial support.
# TABLE OF CONTENTS

ACKNOWLEDGEMENTS .............................................................................................................. 4

LIST OF TABLES .......................................................................................................................... 7

LIST OF FIGURES ......................................................................................................................... 8

LIST OF ABBREVIATIONS ............................................................................................................ 9

ABSTRACT .................................................................................................................................. 10

CHAPTER

1 INTRODUCTION .......................................................................................................................... 11

2 LITERATURE REVIEW ............................................................................................................ 14

   Botanical Classification ........................................................................................................... 14
   Origin and Distribution ............................................................................................................. 14
   Banana Production (Propagation) ............................................................................................ 14
   Limitation of Traditional Propagation of Banana ................................................................. 15
   Banana Economic and Social Importance ............................................................................. 16
   Ornamental Bananas ................................................................................................................ 16
   Banana Production in Haiti ...................................................................................................... 17
   Micropropagation ..................................................................................................................... 18
   Developmental Pathways of Micropropagation ..................................................................... 18
      Shoot Apical Meristems ........................................................................................................ 18
      Shoot and Node Culture ....................................................................................................... 18
      Shoot Organogenesis .......................................................................................................... 19
         Direct shoot organogenesis ............................................................................................. 20
         Indirect shoot organogenesis .......................................................................................... 20
      Nonzygotic Embryogenesis ................................................................................................. 21
         Direct nonzygotic embryogenesis .................................................................................... 21
         Indirect nonzygotic embryogenesis .................................................................................. 22
   Micropropagation Stages ......................................................................................................... 22
      Stage 0 ................................................................................................................................. 23
      Stage I: Establishment of Aseptic Cultures ....................................................................... 24
         Culture Media Components ............................................................................................... 26
         Lighting ............................................................................................................................. 36
         Stabilization ..................................................................................................................... 40
         Indexing ............................................................................................................................ 41
      Stage II: Multiplication of Propagules .............................................................................. 41
      Stage III: Rooting Stage ....................................................................................................... 44
      Stage IV: Acclimatization ..................................................................................................... 46
3 MATERIAL AND METHODS

Experiment Location .................................................................................................................. 50
Experimental Design and Statistical Analysis ............................................................................ 50
Plant Material and Culture Establishment .................................................................................. 50
In Vitro Treatments .................................................................................................................... 51
  Light Conditions ..................................................................................................................... 51
Growth and Biomass Parameter Analysis .................................................................................. 51
  Shoot Length .......................................................................................................................... 51
  Root Length and Number ........................................................................................................ 52
  Plantlet Fresh Weight .............................................................................................................. 52
  Shoot Fresh Weight ................................................................................................................ 53
  Root Fresh Weight .................................................................................................................. 53
  Shoot Dry Weight .................................................................................................................... 53
  Root Dry Weight ..................................................................................................................... 53
Chlorophyll Analysis .................................................................................................................. 54
Stomatal Count .......................................................................................................................... 54
Stomatal Measurements ............................................................................................................. 55
Anatomical and Histochemical Studies ...................................................................................... 55

4 RESULTS

Growth and Biomass Parameter Analysis .................................................................................. 56
  Effect of Light Quality and Quantity on Stem Diameter ......................................................... 56
  Effect of Light Quality and Quantity on Plantlet Fresh Weight ............................................. 56
  Effect of Light Quality and Quantity on Shoot Fresh Weight ................................................ 57
  Effect of Light Quality and Quantity on Root Fresh Weight ................................................ 57
  Effect of Light Quality and Quantity on Leaf Number .......................................................... 57
  Effect of Light Quality and Quantity on Shoot Length .......................................................... 57
  Effect of Light Quality and Quantity on Root Length ............................................................ 58
  Effect of Light Quality and Quantity on Root Number .......................................................... 58
  Effect of Light Quality and Quantity on Shoot Dry Weight ................................................... 58
  Effect of Light Quality and Quantity on Root Dry Weight .................................................... 59
Anatomical Studies ...................................................................................................................... 59
Stomatal Count .......................................................................................................................... 59
  Effect of Light Quality and Quantity on Stomata Size ........................................................... 60
  Effect of Light Quality and Quantity on Leaf Anatomy ......................................................... 60
Chlorophyll Content .................................................................................................................. 61

5 DISCUSSION ........................................................................................................................... 76

6 SUMMARY AND CONCLUSIONS ............................................................................................. 81

LIST OF REFERENCES ............................................................................................................... 82

BIOGRAPHICAL SKETCH ........................................................................................................... 94
## List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Evaluation of light quality and light levels on growth and development of in vitro production of two ornamental banana varieties.</th>
<th>page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-1</td>
<td>Evaluation of light quality and light levels on growth and development of in vitro production of two ornamental banana varieties.</td>
<td>63</td>
</tr>
<tr>
<td>4-2</td>
<td>Evaluation of light (quality and quantity) on stomata formation of in vitro production of two ornamental banana varieties.</td>
<td>64</td>
</tr>
<tr>
<td>4-3</td>
<td>Evaluation of light quality and quantity leaf anatomy of in vitro production of two ornamental banana varieties; Musa ‘Little Prince’ (LP) and ‘Truly Tiny’ (TT).</td>
<td>65</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
<td>------</td>
</tr>
<tr>
<td>4-1</td>
<td>Measurements of the spectral energy distribution of high LED (white LED lamps, top graph)</td>
<td>66</td>
</tr>
<tr>
<td>4-2</td>
<td>Stomatal impressions of abaxial and adaxial leaf surfaces of banana plantlets under fluorescent, high LEDs and low LEDs light sources. Bar 50 µmol</td>
<td>67</td>
</tr>
<tr>
<td>4-3</td>
<td>Microscopy of cross sections of banana leaves (Musa Little Prince and Truly Tiny) cultivated in vitro under different light sources (low and high LED)</td>
<td>68</td>
</tr>
<tr>
<td>4-4</td>
<td>Effect of interaction between light sources (fluorescent light, white and pink LED) and banana varieties (Musa Little Prince and Musa Truly Tiny)</td>
<td>69</td>
</tr>
<tr>
<td>4-5</td>
<td>Effect of interaction between light sources (fluorescent light, low and high LED) and banana varieties (Musa Little Prince and Musa Truly Tiny)</td>
<td>70</td>
</tr>
<tr>
<td>4-6</td>
<td>Effect of interaction between light sources (fluorescent light, low and high LED) and banana varieties (Musa Little Prince and Musa Truly Tiny)</td>
<td>71</td>
</tr>
<tr>
<td>4-7</td>
<td>Effect of interaction between light sources (fluorescent light, low and high LED) and banana varieties (Musa Little Prince and Musa Truly Tiny)</td>
<td>72</td>
</tr>
<tr>
<td>4-8</td>
<td>Effect of interaction between light sources (fluorescent light, low and high LED) and banana varieties (Musa Little Prince and Musa Truly Tiny)</td>
<td>73</td>
</tr>
<tr>
<td>4-9</td>
<td>Effect of interaction between light sources (fluorescent light, low and high LED) and banana varieties (Musa Little Prince and Musa Truly Tiny)</td>
<td>74</td>
</tr>
<tr>
<td>4-10</td>
<td>Effect of interaction between light sources (fluorescent light, high and low LED) on chlorophyll content of banana varieties.</td>
<td>75</td>
</tr>
</tbody>
</table>
LIST OF ABBREVIATIONS

2,4_D 2,4-dichlorophenoxyacetic acid
BAP  Benzylaminopurine
cm  Centimeters
FAO  Food and Agricultural Organization of the United Nations
g L$^{-1}$  Gram per litter
IAA  Indole-3-acetic acid
IBA  Indole-3-butyric acid
LED  Light Emitting Diodes
NAA  Naphthaleneacetic acid
nm  Nanometer
MAP  Mono-ammonium phosphate
mg L$^{-1}$  Milligram per litter
ml  Milliliters
MS  Murashige and Skoog (medium)
mT  $meta$ topolin
PPFD  Photosynthetic Photon Flux Density
PGF  Plant growth fluorescent
PSI  Pounds per Square Inch
TDZ  Thidiazuron
EVALUATION OF LIGHT QUALITY AND LIGHT LEVELS FOR IN VITRO PRODUCTION OF ORNAMENTAL BANANAS (MUSA SP.)

By

Cassandre Feuillé

August 2019

Chair: Wagner A. Vendrame
Major: Horticultural Sciences

Several reports claim that light emitting diodes (LEDs) can be an alternative lighting source to not only increase the quality of vitro plantlets but also to minimize the per plant production costs, which makes it suitable for commercial micropropagation. The main objective of this study was to develop a suitable protocol for the micropropagation of banana using traditional techniques that address low energy inputs.

Light quality and intensity of three different light sources (two LED lighting and one fluorescent), were evaluated to determine the lowest light conditions (intensity, quality and photoperiod) for proper banana in vitro growth and development. To examine the effect of the LEDs, low intensity LED (90 μmol m\(^{-2}\) s\(^{-1}\)) and high intensity LED (116 μmol m\(^{-2}\) s\(^{-1}\)) were compared with fluorescent lamps (100 μmol m\(^{-2}\) s\(^{-1}\)). Significant increases in length and biomass of plantlets were observed under the higher intensity LED (116 μmol m\(^{-2}\) s\(^{-1}\)) but were not significantly different from the lower level of LED. For the fresh and dry weight of shoots and roots, number of leaves, number and length of roots there were no significant difference between treatments. Chlorophyll content was greater under both LED lightings. Regarding leaf, stomata number and size were longer and wider on plant cultured under fluorescent lamps.
CHAPTER 1
INTRODUCTION

Bananas evolved in South-east Asian and Western Pacific regions many years ago (Ploetz and Pegg, 1997; Robinson and Sauco, 2010; Lui et al., 2013) between natural crosses among wild diploid subspecies of *Musa acuminate*, resulting in the production of numerous intraspecific hybrids (Robinson and Sauco 2010). Edible seedless bananas (parthenocarp), female sterile and triploid in genomic structure resulted from some of these hybrids (Robinson and Sauco 2010). Banana fruits, genus *Musa*, are named more than 100 different manners worldwide (Arvanitoyannis and Mavromatis, 2009). Banana (*Musa* spp AAA), are among the most valuable fruit crop of the Musaceae family (Kacar et al., 2010). Banana worldwide production ranked fourth, after rice, wheat and maize in terms of total incomes of production (Singh et al., 2011).

Presently production is spread across five continents (Bakry et al., 2009), and grown around 140 countries across the world (FAOSTAT 2017). Banana is widely grown in the tropics and sub-tropics in all types of agricultural system from small-scale, mixed, subsistence gardens to large commercial monocultures.

Banana is cultivated in an area of 5.6 million hectares ha and annual production of 2018 is estimated at 114 million tons (FAO 2019). Banana is a major staple food crop for millions of people as well as provides income through local and international trade. Worldwide trade of banana is mainly dominated by the Cavendish group (*Musa* AAA), (Robinson and Sauco 2010).

Banana production in Haiti has been recorded since 1961 (FAOSTAT 2017). Production has declined continuously in recent years (FAOSTAT 2017), as growers have experienced problems such as nematodes, viruses and fungal diseases. Some of these problems are associated with banana propagation techniques.
In Haiti, bananas and plantains are propagated by vegetative means only. Banana production in Haiti is negatively impacted by diseases (Blake Sigatoka, Panama Disease, Cigar end rot, Cordona Leaf Spot Disease, Bunchy Top) and nematodes which cause production losses estimated at 322,500 tons in 2000 to 264,528 tons in 2017 (FAOSTAT, 2017). These losses contribute to the high level of food insecurity in Haiti with a reported 40% undernourished households and 30% of Haitian children suffering from chronic malnutrition (USAID, 2017).

One way to reduce the spread of banana diseases and nematodes is to improve the quality of planting materials. For decades, micropropagation techniques have alleviated some of the challenges associated with vegetative propagation materials (Ahirwar et al., 2012; Yusnita et al., 2015). Compared to micropropagation methods, vegetative methods take longer periods of time, produced less plants and are prone to the spread of fungal, bacterial, viral and nematode diseases. Plants derived from micropropagation techniques have the potential to improve banana and plantain production as an alternative method in Haiti (Ahmed et al., 2014).

There are a number of challenges to develop functional micropropagation facilities in Haiti. These include lack of technical expertise in micropropagation techniques, supplies, equipment and the reliability of the electrical grid. Training laboratory technicians, purchasing supplies and equipment are challenges that are relatively easy to solve. However, the lack of a reliable energy source poses a greater challenge because the equipment and environmental conditions necessary to produce high quality plants need a consistent, continual supply of energy.

In 2012, approximately 38% of Haitian households had access to electricity (Gustafson, 2017). Of the total of electricity produced, 70% is consumed by the residential sector and only 15% is dedicated to the industrial production (Lucky et al., 2014). Electricity in Haiti is
generated from burning imported fossil fuels (85%) and hydraulically (15%) (Lucky et al., 2014). These two sources of energy are non-suitable and insufficient to supply the whole country. Even in Port-au-Prince, Haiti’s capital, electricity supplied by the local government is not reliable. For example, during the rainy season when hydroelectric power is more readily available, the public utility supplies approximately 12 hours of electricity per day. In contrast, during the dry season, supply is limited to approximately 7 hours per day. Outside the capital, the average hours of public electricity available is estimated to be between 5 to 9 hours per day. Therefore, finding ways to utilize low consumption energy equipment is necessary for the viable production of banana. Considering the unreliable power grid in Haiti and the need to produce clean banana material, the development on banana micropropagation protocols for production under alternative light sources might be one approach towards successful banana production in Haiti.

For that reason, the main objective of this study was to develop a suitable protocol for the micropropagation of banana using traditional techniques that forwarded low energy inputs. Because of Haiti’s unreliable energy grid and frequent electricity interruptions during the day, this study had as a specific objective the evaluation of light quality and intensity to determine the lowest light conditions (intensity, quality, and photoperiod) for proper banana in vitro growth and development. The effects of three light intensities were evaluated for the micropropagation of two ornamental banana varieties, Musa ‘Little Prince’ and Musa ‘Truly Tiny’. Light sources included two LED types and one fluorescent type. Growth and development, leaf anatomy, stomata formation (size and number) and chlorophyll content were evaluated.
CHAPTER 2
LITERATURE REVIEW

Botanical Classification

Bananas (*Musa* sp.), including plantains are monocotyledonous herbaceous plants belonging to the Musaceae sensu stricto (s.str.) family (Nakai, 1941; Hutchinson, 1967; Cronquist, 1981; Takhtajan, 1997). Revisions of the taxonomic relationships among this economically important crop based on molecular markers appear to be more reliable than earlier studies using morphological characteristics (mainly inflorescence orientation and chromosomal number). Recent phylogenetic analyses of the banana family using nuclear ribosomal (ITS) and chloroplast (*trnL-F*) sequences suggest that the family consists of three genera, *Musa* L., *Ensete* Horan. and *Musella* C.Y. Wu ex H.W. Li, with 41 currently accepted species (Wu and Kress, 2001; Lui et al., 2013).

Origin and Distribution

The edible bananas belong to the genus *Musa* which originated in tropical Asia with three major centers of diversity in 1) Assam, SW China, Myanmar and Thailand; 2) Borneo and Indonesia; and 3) New Guinea (Ploetz and Pegg, 1997; Robinson and Sauco, 2010; Lui et al., 2013). Edible bananas, originated from intra and interspecific hybridization between the wild species, of *Musa acuminata* Colla (A genome) and *Musa balbisiana* Colla (B genome) (Paull and Duarte, 2011), are cultivated in more than 130 countries around the world (De Langhe et al., 2009; Arvanitoyannis and Mavromatis, 2009; Li et al., 2010; FAOSTAT, 2017).

Banana Production (Propagation)

Bananas and plantains are large herbaceous plants that are grown in tropical and subtropical regions of the world (Harrison and Schwarzacher, 2007; Arvanitoyannis and Mavromatis, 2009). Their upright main stem, referred to as a pseudostem, produces a bunch of
fruits resulting from a single inflorescence made up of many individual flowers collectively called a branched spadix (http://agritech.tnau.ac.in/expert_system/banana/botany.html).

Individual fruits are formed in layers called “hands” on a single fruiting stalk. Depending on the cultivar, there may be 6 to 15 hands on a stalk with 10-20 bananas per hand (Arvanitoyannis and Mavromatis, 2009). Individual bananas and plantains called “fingers” range in size depending on the variety. Musa is a monocarpic perennial meaning that each pseudostem flowers, produces fruit and then dies. New suckers or daughter plants arise from the corm at the base of the plant (Arvanitoyannis and Mavromatis, 2009). In many countries including Haiti, these suckers serve as the primary propagule in which farmers establish new production fields of banana or plantains.

**Limitation of Traditional Propagation of Banana**

All cultivated bananas and plantains are triploid and their fruits develop by “vegetative parthenocarpy” which results in a seedless fruit surrounded by edible pulp (http://agritech.tnau.ac.in/expert_system/banana/botany.html). The most common vegetative materials used to establish new production fields are corms and suckers (Wong, 1986; Arvanitoyannis and Mavromatis, 2009). Using vegetative materials as the primary source for new crop establishment poses several production constraints. These include a lack of planting materials that are free of diseases, nematodes and pests (Wong, 1986; Makara et al., 2010). In addition, the traditional propagation ratio with suckers and corms is very slow, estimated at 5 to 10 suckers per year (Wong, 1986; Makara et al., 2010; Sadik et al., 2012). Vegetative planting materials lack physiological uniformity and may be difficult to transport (Wong, 1986; Yusnita et al., 2015) added to the fact of low conservation of genetic traits of the mother plants (Hussein, 2012) dedicated to large-scale propagation.
**Banana Economic and Social Importance**

Worldwide, bananas and plantains rank fourth in terms of economic value and they are a major food and export crop in numerous developing countries (Ploetz and Evans, 2015). According to the FAO (2019), banana worldwide production is 114 million tons produced on approximately 5.6 million hectares. Worldwide exports of banana and plantain are estimated at 19,205.8 thousand tons with Ecuador being the largest export country (6,646.2 million tonnes). The European Union is the largest importer of bananas and plantains (8,274.6 million tonnes) (FAO, 2019). Bananas and plantains are a source of carbohydrates, minerals and vitamins (Makara, 2010). Besides its edible value, banana is also cultivated for industrial production, including chips, flour, juice, wine extraction and as a starch source (Mohapatra et al., 2001; Waliszewski et al., 2003). In Uganda, some varieties of banana (Nsowe, Kabula, and Kayinja) are used exclusively to produce beer (Davies, 1993; Gold et al., 1999; Abera et al., 2000).

**Ornamental Bananas**

Ornamental bananas belong to the genus *Musa* particularly to the subgenera Rhodoclamys and the section Calimusa. These species normally have somewhat colored fruits and are valued (notable) for their highly colored (vivid, deep-colored) bracts, a characteristic that outstand them as ornamental plants (Häkkinen, 2007). Banana plants are globally perceived (globally known) as one of the most beautiful ornamentals (Carvalho et al., 2011). Various ornamental banana hybrids have been developed at Embrapa Cassava and Fruits, which already preserves a collection of more than 250 banana accessions in Cruz das Almas, in the northeastern part of Brazil (Promusa, 2011). Musa Little Prince, one of the varieties of banana used herein is an ornamental banana. Musa Little Prince is a genetically mutated dwarf sport banana obtained naturally of the supposed parent Musa acuminata ‘Dwarf Cavendish’. Musa ‘Little Prince’ was
found in the inventor's nursery in Raleigh, N.C. The stem height of banana Musa Little Prince ranges between 18 and 24 inches (1/2-21/2 feet).

Musa Truly Tiny is the other variety used herein. Musa ‘Truly Tiny’ is commonly used for both, edible and ornamental purposes. Musa 'Truly Tiny' is an extremely dwarf banana and is believed to be the tiniest edible fruit producing banana worldwide (Agristarts; https://www.agristarts.com). Musa ‘Truly Tiny’ grows 2 to 4 feet tall and have red variegated leaves, which make them desirable as ornamental plants (Agristarts; https://www.agristarts.com).

Banana Production in Haiti

According to FAOSTAT (2017), banana production and plantains ranked fourth in Haiti after sugarcane, mangoes, and sweet potatoes. A continuous increase in banana production was recorded from 1961 (110,000 tons) to 2000 (322,500 tons). Banana exportation in Haiti stopped in the late 1940s, after which time, the vast majority of the production is for local consumption (CFI, 2015). There has been limited interest and investment in the exportation of bananas and plantains. In a news article published in 2013, Dole Food Company reported that Haiti produced 290,000 tons of bananas and 200,000 tons of plantain. The company was exploring the potential of investing in banana plantations for export (Knowles, 2013). Haiti’s current president Jovenel Moïse, launched an organic banana production company (Agritrans) in 2014. The $27 million project focused on export production and involved approximately 3,000 farmers on over 1,000 hectares in northern Haiti (Saint-Pré, 2015). However, this project has become controversial. Peasants that owned farms which now are controlled by Agritrans claimed that their land was seized and alleged that it is senseless exporting banana to Germany while they have to buy the same product from the Dominican Republic (Steckley & Bell, 2016).
Micropropagation

Micropropagation is defined as the true-to-type propagation of cells, tissues or organs from selected genotypes aseptically on defined media contained in culture vessels maintained under controlled conditions of light and temperature using in vitro culture techniques (Debergh and Read, 1993; Evans et. al., 2003; Gaba, 2005; Kane, 2011).

Developmental Pathways of Micropropagation

There are three developmental pathways for plant regeneration through which micropropagation can be achieved. Depending on the species and cultural conditions, in vitro propagation can be achieved by the following: (1) Propagation from pre-existing meristems, such as shoot or node culture (Kane, 2011), (2) Shoot organogenesis, directly or indirectly via callus (Geneve, 2011), and (3) Non-zygotic embryogenesis, directly or indirectly on explants (Murashige, 1974; Kane, 2005; Gray, 2011; Kane, 2011; Thorpe, 2012).

Shoot Apical Meristems

Apical meristems are the growth tissues encountered at the tips and axillary buds of a plant (Kane, 2005). Shoot and node culture are the two techniques of shoot apical meristem used depending on the genotypes. Both methods depend on the stimulation (growth/sprout) of lateral shoot development from axillary buds after apical dominance is disrupted (Kane, 2011). Due to their highly organized structure, propagation from apical and axillary meristems tend to be genetically stable and are therefore a reliable technique for micropropagation of numerous species (Kurtz et. al., 1991; Kane, 2011).

Shoot and Node Culture

Shoot culture refers to the in vitro culture of lateral shoots from shoot apices or axillary buds on medium containing plant growth regulators, generally a cytokinin (George and Debergh, 2008). Lateral buds are either divided into shoots or nodal sections for subsequent shoot
multiplication or are rooted as microcuttings. Shoot culture in comparison to the other propagation techniques (1) produce reliable amount of bud proliferation following stabilization; (2) are less prone to genetic variation; and (3) may result in periclinal chimeras for clonal propagation (Kane, 2011). Shoot culture is the most effective and the most widely used method for micropropagation of banana (Safarpour et al., 2017; Waman and Bohra, 2019). Propagation of banana through in vitro techniques using shoot culture has been previously reported (Nandwani et al., 2000; Hussein, 2012; Karule et al., 2016; Prabhuling and Sathyanarayana, 2017; Rodrigues et al., 2017). Mendes et al. (1996), reported the efficacy of banana in vitro plantlet production using shoot tip culture. Long term genetic stability in micropropagated banana through shoot culture has been reported. Banana in vitro propagated via enhanced shoot culture over 10 years displayed high genetic stability (Lakshmanan et al., 2007).

Node culture, a simplified type of shoot culture, is another technique of micropropagation from preexisting meristems. While node culture is the simplest technique, it is less related to somaclonal variation (Kurtz et. al., 1991; Kane, 2011).

As mentioned previously, in addition to shoot culture, there are two other methods of micropropagation: shoot organogenesis and non-zygotic embryogenesis.

**Shoot Organogenesis**

Shoot organogenesis is one of the developmental pathways used to propagate plants in vitro (Zhang and Lemaux, 2005). Organogenesis is the de novo development of plant organs, typically shoots and/or roots, in vivo or in vitro, from cells and tissues that would not normally form them, using either meristematic or non-meristematic tissues (Geneve, 2001; Schwarz et. al., 2005) and can occur via two developmental sequences; indirect or direct (Hicks, 1980; Schwarz et. al, 2005). The aforementioned pathways differ in the existence or nonexistence of a callus stage in the organogenic sequence of events (Schwarz et. al., 2005).
Direct shoot organogenesis

Direct shoot organogenesis refers to propagation from explants without pre-existing meristems through production and subsequent rooting of adventitious shoots (Shen, 2007). Direct organogenesis can be achieved in in vitro regeneration of banana through shoot tip culture (Kishor et al., 2017). Micropropagation of banana through direct organogenesis have been successfully demonstrated (Venkatachalam et al., 2006; Huang et al., 2006). Nonetheless, somaclonal variation through direct shoot organogenesis have been reported (Giménez et al., 2001).

Although quantitative data are scarce it is often reported that plants generated from the adventitious meristems are genetically different from the mother plant (Hvoslef-Eide and Munster, 2007). The fact that genetic variation is more common in plants regenerated from adventitious shoots than from axillary shoot proliferation, variation in the regenerants is obviously a concern and needs to be continually monitored during commercial micropropagation in a clonal propagation system (Geneve, 2011).

Indirect shoot organogenesis

Indirect Shoot Organogenesis is the development of shoot meristems indirectly from an intermediary callus that first develops on the explant (Schwarz et al., 2005). De novo organ formation via indirect organogenesis carries a high risk of genetic variation in the chromosomal constitution of the plants produced (e.g., ploidy change), of the cells in the callus stage and, hence, the possibility for both physiological and morphogenic variation in the resulting organs (Schwarz et al., 2005). This type of variation has been termed somaclonal variation (Norton and Skirvin, 2011).

Since the introduction of the term somaclonal variation (Larkin and Scowcroft, 1981) to describe the variation observed among tissue culture propagated plants, the phenomenon has
been extensively reported for several crop species propagated through indirect organogenesis like *Vanilla planifolia* (Ramírez-Mosqueda and Iglesias-Andreu, 2015), rice (Kabir et al., 2008), Dieffenbachia (Shen, 2007), strawberry (Nehra et al., 1992), and banana (Cullis et al., 2007). While somaclonal variation may be a source of new desirable clones/variants with better agronomic traits (Bairu et al., 2011), it is unwanted for mass propagation (Hvoslef-Eide and Munster, 2007). Hence, entire elimination of propagation through unorganized growth (callus) has been suggested (Hicks, 1980).

**Nonzygotic Embryogenesis**

Nonzygotic embryogenesis, also termed somatic embryogenesis (Lydiane et al., 2013) is the formation of embryos from cells other than the zygote, but otherwise similar to their zygotic equivalent (Gray, 2005). Somatic embryogenesis can be either direct or indirect (Yang and Zhang, 2011). Two different methods of nonzygotic embryogenesis can be identified: direct and indirect nonzygotic embryogenesis (Pierik, 1987; Neumann et al., 2009). Direct or indirect nonzygotic embryos are differentiated from the explant with or without an intervening embryogenic callus phase (Yang and Zhang, 2011). After the possibility of pro-embryos development in carrot (*Daucus carota*) callus was proposed by Stewart et al. (1958), Reinert (1959) observed that plantlets in his carrot cultures were somatic embryos derived from single cells.

**Direct nonzygotic embryogenesis**

Direct somatic embryogenesis involves formation of a somatic embryo from a single cell or group of cells from an explant without an intervening callus stage. Epidermal cells that give rise to adventitious embryos directly are pre-embryonically determined cells (PEDCs) (Pierik, 1987). Since Stewart et al. (1958) first observed that nonzygotic embryos could be grown directly from somatic cells, repeated attempts have been made to regenerate plants via this
pathway (Reinert, 1959), and direct somatic embryogenesis has now been achieved in several plant species like *Phalaenopsis amabilis* (Chen and Chang, 2006), *Cymbidium* (Mahendran and Bai, 2012), *Curcuma longa* (Raju et al., 2015), *Zea mays* (Lowe, 2018), *Malaxis densiflora* (Mahendran and Bai, 2016), and banana (Uma et al., 2011). Although direct somatic embryogenesis is a more desirable approach to obtain regenerated plants, identical to the parent plants, as callus formation may result in somaclonal variation (Mizukami et al., 2008). The high risks of somaclonal variation associated with nonzygotic embryogenesis still represent a major concern (Standardi and Micheli, 2013).

**Indirect nonzygotic embryogenesis**

Indirect somatic embryogenesis involves the formation of somatic embryos from a single cell or group of cells from an intervening callus stage. Despite the potential abnormalities and the genetic instability of plants regenerated through nonzygotic embryogenesis (Vendrame, 1999), this technique has been used in many plant species (Homhuana et. al., 2008; Bairu et al., 2011; Linacero et al., 2011; Chin et. al., 2014). When plants were obtained, the recovery rate was very low or not reported, suggesting that a majority of nonzygotic embryos were too abnormal to germinate (Gray, 2011).

**Micropropagation Stages**

Many things have changed since three basic stages (I-III) were initially conceptualized by Murashige (1974) for successful micropropagation. Debergh and Maene (1981) concerned by the issue of microbial contamination frequently related to inoculation of primary explants, proposed a Stage 0. This stage outlined specific cultural practices to maintain the stock plants which decrease the contamination rate during explant establishment (Kane, 2011). A fourth stage (IV) is now considered as an integral part of the process and consists in the acclimatization of in vitro generated plantlets (Krikorian, 1995). Based on the increasing database in micropropagation, it is
now agreed that there are five stages (0–IV) critical to successful micropropagation (Debergh and Read, 1993; Kane 2011).

Stage 0

Stage 0 focuses on the selection of explants for micropropagation from donor plants. The objective of Stage 0 is to limit the contamination as much as possible prior to Stage I. Hence, the choice of donor tissues should be done carefully since adequate material is required as start material for micropropagation (Debergh and Read, 1993). The phytosanitary and physiological conditions of the stock plant affect considerably subsequent propagation efficiency of explants in vitro. (Debergh and Maene, 1981).

Generally, reduction of disease incidence and easier explant establishment is accomplished when the donor plants are maintained in a controlled environment. (Read, 1988; Kane, 2011). Reduction of contamination in candidate explants have been proven effective when specific pathogen-tested stock plants were treated with antibiotic sprays, drip irrigated, and maintained under conditions of relatively lower humidity (Kane, 2005). Consequently, larger and more responsive explants can be excised with low possibility of contamination (Kane, 2011).

Influence of the donor plant on in vitro culture has been demonstrated through multiple studies. Nutrition (Garton et al., 1983; Read, 1988; Haapala, 2004), light, temperature (Puddephat et al., 1997; Read, 1988), genotype (Youssef et al., 2010; Shelake et al., 2011), application of plant growth regulators (Read, 1988), physical treatments (Read and Yang, 1989; Barceló-Muñoz 1999) and season of the year (Thimmappaiah and Sadhana, 2002; Dangi et al., 2009; Waman et al., 2015) have all been reported to influence the success rate of micropropagation (Read and Preece, 2003; Kane 2011).

Modification of the physiological condition of the stock plant to improve explant responsiveness can be done through the following techniques: (1) pruning to stimulate axillary
shoot growth (Andreu and Marín, 2005), (2) pretreatment sprays containing cytokinin (Sanchez et al., 1997) or gibberellic acid (Anand, 1999), induction of bud break, usually during the dormant season, aided with forcing solutions containing 2% sucrose and 200 mg/L 8-hydroxyquinoline citrate (8-HQC), sucrose and sometimes PGRs (Read, 1988; Preece and Read, 2003).

The aforementioned parameters greatly influence the Stage 0 of banana micropropagation. For example, fungal and bacterial contaminations increased in banana micropropagation during rainy season and complete elimination of fungal contaminations and reduction of bacterial contaminations occurred when cultures were initiated during the dry period (Waman et al., 2015).

**Stage I: Establishment of Aseptic Cultures**

Induction and sterile establishment of pathogen-eradicated explants in vitro is the goal of Stage I (Murashige, 1974; Kane, 2011). Successful explant establishment results from the proper interaction among a variety of factors, which include the physiological and ontogenic age of the explant (Waithaka, 1992; Debergh and Read, 1993; Krishna et al., 2008; Youssef et al., 2010), explant source, type and size (Debergh and Read, 1993; Thimmappaiah et al., 2002, Krishna et al., 2008; Dangi et al., 2009; Waman et al., 2010,), explant position (Puddephat et al., 1997), type and concentration of PGRs (Dangi et al., 2009, Waman et al., 2010, 2011a), media components (Waman et al., 2011a; Bohra et al., 2014,), kind and concentration of carbon source (Silva, 2004; Roels et al., 2005) and culture environment (Vuylsteke, 1998; Waman et al., 2015).

Great variation exists among the published methodologies employed to obtain aseptic banana explants. Different explant types and sizes have been used depending on the objective of the studies. This often leads to different responses. The variation in explant response during the establishment stage depends on various factors. In addition to size and type of the explants, the
time of collection and the condition of media also affect in vitro response. The type of media used, and growth regulators added vary according to species and cultivars.

Surface sterilization of explants in general is essential for successful establishment of in vitro cultures, as it prevents culture contamination by bacteria, fungi or viruses. Consequently, survival and successful in vitro establishment of the explants can be adversely affected. Surface sterilization varies among published studies. Mante and Tepper (1983) trimmed the outer leaves of their banana explants and the soil was washed to clean their basal portion. After cleaning, the first 2 to 3 leaves which formed the pseudostems were removed. They were further placed in ascorbic/trihydrate citric acid solution (1.0/1.5% w/v). They were then sterilized in a solution of sodium hypochlorite (10 ml of 4-6% NaOCl in 100 ml distilled water). Two drops of 1% Triton x 100 were the added to the solution.

Hussein (2012) thoroughly washed banana buds for 5 minutes under running tap water. The explants were further placed in a solution of ascorbic acid (100 mg/L) for 1 hour. Subsequently, they were washed with sterile distilled water and surface sterilized with Clorox (20% v/v) with few drops of Tween-20. The explants were placed on a shaker for 15 min and eventually rinsed for an additional 15 min. Surface sterilization was performed in a laminar flow hood. Surface sterilization with ethanol for 30 s was mentioned only in a study conducted by Madhulatha et al. (2004). Subsequently, the explants were soaked for 20 min in a solution of 1.5% calcium hypochlorite and rinsed a few times with sterile water prior to in vitro inoculation. The number of times the explants were washed was not reported. Saraswathi et al. (2016) washed the explants with 200 µl/L Tween-20 (Hi-Media) followed by a 20-30 min rinse under running tap water. Subsequently, the explants were surface sterilized in 4.0% (v/v) sodium
hypochlorite followed by surface sterilization in 0.10% (w/v) mercuric chloride. Each treatment was followed by three rinses in distilled water.

Depending on the type of explants, and the objective of the study, the size of explant used varies as well. Explant lengths have been reported to vary from 2 mm to 1.5 cm long. Mante and Tepper (1983) used 2-4 mm explants, while Wong (1986) used explants that were 10 x 15 mm. In contrast, Bhagyalakshmi and Singh (1995) used larger explants, consisting of 1 cm shoots.

**Culture Media Components**

Murashige and Skoog (1962) is the most widely used medium for banana in vitro culture (Daud et al., 2011; Loyola-Vargas and Ochoa-Alejo, 2012). Nonetheless, the different supplements of the media influence greatly the success of in vitro regeneration (Hildebrandt et al. 1946). PGR’s, type and level of carbon sources, gelling agent, anti-phenolic agent (antioxidant), pH, and other compounds are among the main supplements (Waman and Bohra, 2019).

**Plant growth regulators.** Plant growth regulators (sometimes erroneously called hormones) are numerous essential chemical substances that are used in small concentrations added to the culture media to influence the growth and differentiation of explant (plant cells, tissues and organs) in vitro (Kyte, 1987; Vuylsteke, 1998). Their level and ratio in the medium frequently influence the pattern of development in culture and must be added selectively to the culture media. Plant growth regulators affect greatly the morphogenesis of developing tissues from the explants since they influence not only the growth of some cultured slow growing tissues, but also regulate the developing pathway of the plant cells (Vuylsteke, 1998). Hence, selection of nutrient medium, including chemical composition and physical form mostly determine successful in vitro micropropagation (Murashige, 1974).
There are currently five recognized groups of plant growth regulators: auxins, gibberellins, cytokinins, abscisic acid (ABA) and ethylene. Auxins and cytokinins, or their synthetic analogues, are the most commonly used plant hormones in media to regenerate plant tissue and organ cultures in vitro (Kyte, 1987; Beyl, 2011).

**Cytokinin.** Cytokinins are growth regulators that are key components in tissue culture media for cell division, shoot multiplication, and axillary bud proliferation (Kyte, 1987). Cytokinins are mainly categorized into two groups: naturally occurring and synthetic (Waman and Bohra, 2019). More commonly used cytokinins are: BA (6-benzyladenine; C₁₂H₁₀N₅O) or BAP (benzylamino purine), 2-ip (isopentenyl-adenine), kinetin (furfurylamino purine), TDZ (thidiazuron) and zeatin (Kyte, 1987; Bhojwani and Razdan, 1983).

Various types of cytokinins have been used for the in vitro propagation of banana varieties (Arinaitwe et al., 2000; Roels et al., 2005). Shoot multiplication rate is notably affected by the kind of cytokinin, their concentration and banana cultivars (Wong, 1986; Arinaitwe et al., 2000; Gubbuk and Pekmezci, 2004; Roels et al., 2005). BAP (Benzylaminopurine) or BA (6-benzyladenine) has been accepted to be by far the most common choice of cytokinin (Banerjee and De Langhe, 1985; Kyte, 1987; Vuylsteke, 1998; Gubbuk and Pekmezci, 2004). Wong (1986) reported a greater effect of BAP on 22 banana varieties as compared to kinetin. Waman et al. (2016) claimed that BAP is better than kinetin and zeatin for banana multiplication. Other cytokinin like kinetin (Arinaitwe et al., 2000; Muhammad et al., 2007) and zeatine (Vuylsteke and De Langhe, 1985) are rarely used for banana micropropagation. Enhancement of shoot multiplication in some bananas and plantains through incorporation of an auxin at lower level along with cytokinin have been reported (Resmi and Nair, 2007; Jafari et al., 2011; Ngomuo et al., 2013).
Lately, aromatic group of cytokinins designated as topolins are becoming popular amongst researchers. Reports have been made regarding meta topolin (mT), one of the most valuable members of the group, which shows promising results in tissue culture of several crops (Waman and Bohra, 2019). Favorable results include improved ex vitro survival (Valero-Aracama et al., 2010), enhanced histogenic stability and reverse effects of senescence (Bogaert et al., 2004), improved proliferation and decreased hyperhydricity (Bairu et al., 2007). Limited experiments have been conducted in banana with diversification in specific response. Use of mT improved multiplication rate in banana (Bairu et al., 2008, Waman et al., 2016) and plantain (Roels et al., 2005), whereas it did not promote proliferation in Ney Poovan (Musa AB) and Silk Banana var. Nanjanagud Rasabale, when isolated bud clumps were used (Bohra et al. 2016b; Waman et al. 2016a).

**Auxins.** Auxin are plant growth regulators that impact cell expansion, root formation, and adventitious bud development. Auxins are often used in tissue culture media, either combined with cytokinins during the multiplication stage (Stage II) or without cytokinins for the rooting stage (Stage III). Auxins are also utilized in somatic embryogenesis (Kyte, 1987). Auxins are extensively used in micropropagation and are usually essential component to the culture media. Different responses (calli formation, cell suspensions, organ formation and direction of morphogenesis) can be obtained depending on the auxin/cytokinin ratio. The most commonly used auxins in micropropagation are IAA (indole-3-acetic acid; C_{10}H_{9}NO_{2}, IBA (indole-3-butyric acid; C_{12}H_{13}NO_{2}), NAA (1-naphthaleneacetic acid; C_{12}H_{10}O_{2}) and 2,4-D (2,4-dichlorophenoxyacetic acid; C_{8}H_{6}C_{12}O_{3}) (Bhojwani and Razdan, 1983)

The state of the media (liquid vs. semi-solid) also affects explant responses in vitro (Bhagyalakshmi and Singh, 1995). Liquid culture media reduce the time of stabilization as
nutrient uptake generally increases in liquid media while the gelling agent in semi-solid media can block nutrient uptake. Nevertheless, explants established in liquid media are more prone to hyperhydricity than those established on solid media. The types and composition of Stage I media has varied among published studies. Mante and Tepper (1983) compared two types of media (MS medium and MS inorganic salts). Nonetheless, their second culture medium was subdivided into 5 additional types of media. However, the reported difference between the two types of media (MS medium and MS inorganic salts) is confusing. Different cytokinins types (BAP, kinetin and 2iP) and concentrations (0, 5, 10 and 20 mg/L) were evaluated. Some of the cytokinins were combined with edamin, auxin, and adenine sulphate. Bhagyalakshmi and Singh (1995) established in vitro banana explants in MS basal medium. The medium was supplemented with BA (4.4 – 22.2 μM) and IBA (0.98 μM) with (semi-solid) or without (liquid) agar. The liquid culture media used were static or agitated with a gyratory shaker (90 rpm). Maximum shoot proliferation and length were obtained at 13.3 μM BA while a decrease was registered at higher concentrations. It was concluded that explants cultured in liquid medium respond better than in semi-solid medium. However, semi-solid medium was best for multiplication of in vitro banana shoots as explants derived from liquid medium failed acclimatization.

Wong (1986) used a basal medium (BM) supplemented with PGRS to develop an initiation medium (IM). The BM consisted of inorganic MS salts supplemented with glycine (2 mg.l⁻¹), meso-inositol (100 mg.l⁻¹), thiamine HC1 (0.5 mg.l⁻¹), pyridoxin-HCl (0.5 mg.l⁻¹), nicotinic acid (5 mg.l⁻¹), sucrose (20 mg.l⁻¹), and bacto-agar (7 g/L). The IM consisted of BM supplemented with BA (5 mg.l⁻¹) and IBA (0.1 mg.l⁻¹). Similar to Mante and Tepper (1983), Wong (1986) concluded that BA is more effective than kinetin and that shoot proliferation decreased at higher cytokinin concentrations. Banerjee and Langhe (1985) evaluated a medium
containing macro and modified micronutrients (containing no CuSO₄·5H₂O and no ZnSO₄·4H₂O). The medium was then supplemented with different types and concentrations of vitamins (2.0 mg/l glycine, 0.1 mg/l thiamine HCL, 0.5 mg/l nicotinic acid and 0.5 mg/l pyrooxidine) and plant growth regulators (2.3 mg/L BA and 0.18 mg/L IAA). Hussein (2012) established explants in culture media containing MS basal salts, supplemented with sucrose (0.0, 15, 30 or 60 g/L), myo-inositol (0.0, 50, 100, 200, 400, or 1000 mg/L), agar (7g/L) and (0.0, x 0.25, x 0.5, x 1, and x 1.25) the normal concentration of white’s organics. Best results were observed in media supplemented with 30 g/L of sucrose. No effect of inositol and white’s organics was noted. Except that 1.25 x white’s organics had a positive effect on plantlet length. Madhulatha et al. (2004) tested liquid pulse treatment with a combination (1:1) of cytokinins (BA and kinetin) and auxins (NAA and IBA) on in vitro shoot proliferation. The basal media were supplemented with sucrose (30 g/L) and agar (8 g/L). The media were then supplemented with different concentrations (0, 50, 100 and 200 mg/L) of cytokinins and auxins diluted in 1N NAOH. The pH of the medium was adjusted to 5.5. The duration of liquid pulse treatments was 5, 15, 30 and 60 min. Subsequently, excess solution was removed from the explants by placing them on sterile filter paper before transferring them to MS basal medium solidified with agar (8 g/L) and sucrose (30 g/L) only. The control treatment consisted of explants that did not receive liquid pulse treatments. Cultures were maintained at 25 ± 2 °C with 60-65% relative humidity, under a 16-hour photoperiod supplied by constant cool-white fluorescent lights (50 μmol m⁻² s⁻¹).

**Carbon sources.** Low light intensity, limited gas exchange in the culture vessels and high relative humidity restrict photosynthetic responses (Kozai, 1991). Hence, energy is provided to the excised tissue to develop and proliferate via carbon sources (Buah et al., 2000). Moreover,
it has been demonstrated that photosynthetic ability (Langford and Wainwright, 1987), quantity and size of starch granule (Capellades et al., 1991; Conner et al., 1993) are greatly influence by carbon level. Kind and concentration of carbon sources affect root induction (Nhut et al., 2001), and ex vitro survival (Zimmerman, 1983; Jo et al., 2009). The common choices of carbon sources for in vitro propagation of banana include sucrose, glucose, fructose, lactose, maltose, mannitol, sorbitol, etc. with sucrose being the most frequently used (Buah et al. 2000b; Madhulatha et al. 2006; Jo et al., 2009; Vora and Jasrai, 2011; Hussein, 2012; Ahmed et al., 2014 Bohra et al., 2016). Type and concentration of carbohydrate sources depends mainly on the variety (Yaseen et al., 2013), culture stage (Gurel and Gulsen 1998), and the type of expected response.

**Gelling agent.** Different types of gelling agents are used for support in semi-solid media. The brand, purity and consistency of the gelling agents influence properties of the media, therefore can have different effects on growth response and tissue proliferation (Debergh, 1983). Agar is the most commonly used gelling agent in media for plant tissue culture (Jain and Babbar, 2002; Kacar et al., 2010). Among the characteristics that make agar a gelling agent of choice are its non-toxic nature, stability, high clarity and resistance to metabolism during culture (McLachlan, 1985; Jain and Babbar, 2002; Saraswathi et al., 2016). Nonetheless, is claimed to be the most expensive component of plant tissue culture media (Kacar et al., 2010). Due to the high price of tissue culture grade agar, attempts have been made to identify suitable alternatives (Jain and Babbar, 2002; Kacar et al., 2010). Different gelling substitutes, cassava starch (Kuria et al., 2008) corn starch (Vorpsi et. al, 2012), sago (Naik et al., 2001), guar gum, isubgol (Jain and Babbar, 2005), have been tried for different plant spices including banana (Kodym and Zapata-Arias, 2001, Saraswathi et al., 2016).
In a study conducted by Saraswathi et al. (2016), the effect of gelling agent, water and carbon sources on three commercial banana varieties were examined. All the studies reported here used agar, oxoid agar or bacto-agar. The concentration of gelling agents used in the studies assessed range from 0.45 to 0.8 %.

Saraswathi et al. (2016) established explants from 3 different varieties of banana (Ratshali, Grand Naine and Udhayam) on full strength MS media supplemented with BAP (3 mg/L) and IAA (1 mg/L). They evaluated three types of water (reverse osmosis water, bore-well water, or single-distilled water), which they compared with double distilled water. The water pH (5.20 to 6.52) was adjusted to the similar pH (5.8) as the medium using NaOH and/or HCl. Three concentrations; 3%, 4 %, 5% (w/v) of three different carbon sources (table sugar, rock candy and small candy) were also studied. In addition, three different gelling agents (sago, isabgol and corn flour) were evaluated in different combinations and were compared with conventional agar. No differences were observed between double distilled water and reverse osmosis water. Table sugar provided an equivalent response as sucrose and was the best between the 3 carbon sources tested. The control (agar) was the best gelling agent. Nonetheless, a combination of isagbol with sago responded similarly to the agar on two of the varieties (Ughayam and Ratshali) used.

Waman et al. (2014) cut clean banana suckers into 15 cm² shoot tips and washed them for 1 hour in a solution of 5% (w/v) Benfil. Subsequently, the explants were soaked for 12 hours in a solution containing 0.5% (w/v) Benfil and 500 mg/L Krosin AG. Thereafter, the explants were dipped for 1 hour in cetrimide solution (500 mg/L). A thin cell layer was removed from each treatment. The explants were then surface sterilized for 12 min with 5% (v/v) sodium hypochlorite solution followed by 12 min of washing in 0.1% (w/v) mercuric chloride. The
explants were rinsed 4 times with sterile distilled water between each treatment. After surface sterilization, the explants were cultured onto MS medium. The medium was supplemented with BAP (17.76 µM), IAA (5.71 µM), ascorbic acid (10 mg/L), sucrose, (3% w/v), and agar (0.6% w/v). The pH was adjusted to 5.8. The cultures were maintained for one week in darkness before transferred to a culture room. The temperature in the culture room was 28 ± 2 °C. The plants were inoculated under 14-h photoperiod provided by white fluorescent light (40 µmol m−2 s−1). The explants were stabilized for one month prior transfer to Stage II. The best response (20.3 shoots/explant) was obtained when 2% fructose was used. Manitol was the less effective carbon source at all concentrations.

The process of surface sterilization used by Waman et al. (2015) was the same employed earlier (Waman et al., 2014). Waman et al. (2014) evaluated the MS medium supplemented with BA (4 mg/L), IAA (1 mg/L), ascorbic acid (10 mg/L), agar (0.6 g/L) and sucrose (10, 20 and 30 g/L). The pH was adjusted to 5.8. Explants approximately 1.5 × 1.0 × 1.0 cm³ were established in the medium and incubated in dark for one week. After that time, they were cultured under 14-h photoperiod at 28 ± 2 °C. Light quality and intensity were not provided. The culture was stabilized for 4 weeks prior transfer to Stage II. The size and the developmental stage of explant affected the establishment in vitro. Less contamination (30%) was obtained when smaller explants were used. There was no fungal contamination on explants established during the dry season and bacterial contamination decreased to about 12.54%. Bacterial contamination was higher at higher sucrose levels. Bacterial contamination fluctuated from 15.39% (2% sucrose) to 22.22% (3% sucrose). Browning was observed and it was closely related with incubation time and stage of explant development.
Mbanaso et al. (2006) performed studies with 4-week in vitro-plantlets and 6 different genotypes; two plantain hybrids (TM3X15108-6 and TMPX548-9), Obino lewai (ABB), two cooking banana hybrids (TMBX 612-74 and TMBX 5295-1, ABB), and Cardaba (ABB). The shoot tips were removed and fragmented into 4 different positions. The first treatment consisted of shoots fragmented evenly and vertically in half. Shoot tips of the second treatment were cut evenly and vertically in 4 position. The third and fourth treatments consisted of shoot tips incised respectively at the apical and basal region of the shoot tips. The control consisted of whole shoots. Thereafter the whole or split shoots were cultured on MS medium solidified with gelrite (2 g/L). The use of plant growth regulators was not reported. The pH was adjusted to 5.8. Cultures were incubated at 27 ± 2°C under a 14-hour photoperiod supplied by constant cool-white fluorescent lights (30 to 40 μmol m⁻² s⁻¹). Cultures were stabilized for one month. Higher survival rate and number of shoots were obtained when incision was made at the base of the explants.

Ahirwar et al. (2012) performed studies with semi-solid MS medium supplemented with different concentrations (0, 2.5, 5.0, 7.5 and 10 mg/L) of cytokinins (BAP and Kinetin) alone or BAP in combination with NAA (0:0.3, 2.5:0.3, 5.0:0.3, 7.5:0.3, 10:0.3 and 0:0.5, 2.5:0.5, 5.0:0.5, 7.5:0.5, 10:0.5 mg/L). Agar at 8 g/L and sucrose at 30 g/L were added, before the pH was adjusted to 5.8. The explants were then inoculated and cultured at 25 ± 2 °C, under a 16-h photoperiod and cool fluorescent lights at an intensity of 4000 lux. Shoot explants were stabilized for 3 to 4 weeks while the inflorescences were stabilized for 21 days prior to subculturing. Shoot length and number was higher in medium supplemented with BAP.

Mondal et. al. (2012) cultured 5 x 8 mm shoot tip explants on semi-solid MS medium supplemented with BA (5 mg/L). Different levels of coconut water (0, 50, 100, 150 and 200
ml/L) and ascorbic acid (0, 25, 50, 75 and 100 mg/L) were added to the medium. The pH was adjusted to 5.8, after adding 8 g/L agar and 30 g/L sucrose. Explants were incubated under the same conditions as described by Ahirwar et. al. (2012). Mondal et al. (2012) reported that 50% of explants regenerated on medium without coconut water. Shoot proliferation, elongation, and multiplication were enhanced to 75% when 100 ml/L of coconut water was added. Further increase in coconut water level inhibits shoot regeneration. Shoot regeneration was induced in 48.50% from the explants in the control medium. Shoot proliferation also improved to 68.50% when ascorbic acid was present in the medium. However, additional increase in ascorbic acid concentration hampered shoot regeneration.

Gupta (1986) excised 6 to 8 mm diameter banana explants and surface sterilized in 10% Clorox. After 10 min, explants were rinsed (3-5 times) with distilled water containing ascorbic acid (50 mg/L). Meristematic domes containing 1 to 2 leaves primordia (1.5-2.0 mm long) were cut. The meristem tips were then cut longitudinally into 6 to 8 (1.5-2.0 mm) sections through the apex. Thereafter, the explants were inoculated in basal media with MS mineral salts and vitamins, supplemented with BAP and kinetin (1.0 and 5.0 mg/L) alone, BAP (3.5; 1.5; 2.5; 0.7 mg/L) combined with kinetin (1.5; 3.5; 2.5; 0.7 mg/L), BAP (2.5 mg/L) with NAA (0.5 mg/L) and BAP (0.5 mg/L) with NAA(0.5 mg/L) and 2,4-D (0.5 mg/L). The cultures were then incubated at 3 temperatures (22 ±1 °C, 26 ± 1 °C or 29 ± 1 °C). The best results were obtained when BA (5 mg/L) was used alone, nonetheless, percent of rooting and survival was higher when kinetin was used alone (1mg/L) or in combination with BA (0.7 mg/L BA and Kn).

**pH.** The pH at which the medium is adjusted is very important and may affect growth responses. The pH is species specific and sometimes is affected by the gelling agent used. The pH range generally goes from 4.5 to 6 with a mean of 5.7. The pH determines whether or not
salts will stay in solution. It influences the gelling efficiency and most importantly, it affects nutrient uptake.

The pH of the studies reviewed herein ranged from 5.5 to 5.8. Only Mante and Tepper (1983) mentioned the base (KOH) and acid (HCL) used to balance the pH of their media. All of the media was supplemented with 30g.l\(^-1\) of sucrose. Except Wong (1986) utilized 20 mg.l\(^-1\) sucrose. The temperature range at which the explants were cultured was between 25 ± 1°C to 32 ± 1°C. The photoperiod (16 hour) and the type of light used (cool-white fluorescent tubes) was the same in all of the studies assessed.

**Lighting**

Light is one of the primary factors needed for plant morphogenesis (Rajapakse and Shahak, 2008; Batista et al. 2018). Cool-white fluorescent lamps are mainly used as light source for plant tissue culture (Miyashita et al., 1995, Kodym and Zapata-Arias, 1998). Lighting of the shelves accounts for 65% of the total electricity cost (Standaert de Metsenaere, 1991) and is one of the highest non-labor costs in a tissue culture laboratory (Dooley, 1991).

Hence, studies have been conducted to reduce production costs by reducing the use of fluorescent light or eliminating completely the use of artificial light in culture rooms. Using sunlight in a greenhouse with shade or a sunlit room was one alternative that has been proposed (Kodym and Zapata-Arias, 1998). A study carried out by Kodym & Zapata Arias (2001), showed that sunlight instead of artificial lighting for banana propagation allowed savings on costs for electricity of US$3 m\(^2\) week\(^-1\) in Austria, comparing to a standard growth chamber (controlled light intensity and temperature regimes). Despite being economical, Kyte (1999) stated that the use of natural energy is not recommended because of its fluctuations and difficulties to manage.

Commercial application of micropropagation technology is restricted because of high production costs and consequently a novel approach is required to reduce production costs.
The utilization of light emitting diodes (LEDs) as lighting source for plants has received considerable attention recently because of their broad potential for commercial application and low energy consumption (Nhut et. al, 2003).

LED lighting is considered one of the cost-effective methods to reduce the cost of light, and consequently, the cost of the end product. It is believed that LED lighting consume 70% less electrical energy compared to fluorescent light (Royal Philips N.V, 2015). Since less heat is released when using LED lighting, the space between shelves can be reduced, increasing production up to 33%, such as in ferns (Royal Philips N.V, 2015). Reports with several plant species, such as sugarcane (Ferreira et, al.2017), vanilla (Bello-Bello et, al. 2016), and potato (Miyashita et al., 1995) indicate positive results when using LEDS for either shoot culture or embryogenesis. Nhut et al. (2003) studied different irradiation levels and ratios of blue and red to reduce the micropropagation cost of banana plantlets in vitro.

Tanaka et al. (1998) suggested to use LEDs for improving the quality of plant mother stock for shoot multiplication because of better photosynthetic ability of plantlets cultured under LEDs as compared to the ability under PGF (plant growth fluorescent) lamps. Light conditions influence plant growth responses in vitro. Hence, light intensity and light quality and photoperiod at which the in vitro plants are cultured are very important. Light condition is species specific.

**Light quantity (intensity/irradiance).** Light intensity, or photon flux density (µmol m\(^{-2}\) s\(^{-1}\)) available for in vitro plantlets, is controlled by light quality, the space between the cultures and the light source, the design and kind of material of the culture vessel (Fujiwara and Kozai, 1995). Photosynthetic responses of plants are primarily driven by wavelengths between 400 and 700 nm. Conversely, photomorphogenic response of plants is driven by light with wavelengths
close to ultraviolet (300-380 nm), blue (430-490 nm), red (640-700 nm) and far-red (700-760 nm) regions (Fujiwara and Kozai, 1995). Hence, light intensity is considered one of the main parameters of the environmental conditions, particularly when it comes to photosynthesis of cultures (Fujiwara and Kozai, 1995). Some researchers used light intensity ranging from 1000-5500 lux (Mante and Tepper, 1983; Banerjee and Langhe, 1985). In contrast, other researchers measured PPF (Photosynthetic Photon Flux) in µEm$^2$s$^{-1}$ ranging from 22 to 60 (Gupta, 1986; Bhagyalakshmi and Singh, 1995; Wong, 1986; Madhulatha et al. 2004; Resmi and Nair, 2007; Saraswathi et al., 2016). The light intensity required is also species specific and varies between the different micropropagation stages. Normally, higher light intensity is commonly used during Stage III to increase acclimatization survival. Two different type of light units (lux and µEm$^2$s$^{-1}$) were used to quantify light intensity in the studies evaluated. Those differences between units, therefore made it difficult to compare results of these studies with results of studies employing photon flux density.

There have been many studies on the effects of light flux density on the growth and development of cultures on proliferation an regeneration of orchids (Mengxi et al., 2011), growth and development of lettuce (Lin et al., 2013), on callus growth of olive (Lavee and Messer, 1969), on micropropagation of strawberry (Nhut et al., 2003), on growth of banana (Navarro et al. 1994). Navarro et al. (1994) cultured banana (Musa AAA cv. Petite Naine) plantlets in vitro under two different PPFD's (45µmol m$^{-2}$s$^{-1}$ and 380 µmol m$^{-2}$ S$^{-1}$). Better results were obtained under high light intensity. Significant differences in plantlets weight in the growth phase were higher comparing to low light intensity. In the same way, increase in chlorophyll content was more pronounced under high light intensity and the plantlets exposed to a high PPFD showed the best ex vitro development. Nhut et al. (2003) compared the same light intensity (45 µmol m$^{-2}$ s$^{-1}$)
for different light sources (fluorescent and LEDs) on banana growth in vitro. Despite the importance of light, some researchers simply state the environmental conditions but failed to describe the light condition during their study (Hussein, 2012).

**Light quality (light sources).** Light quality is also very important, and it can be affected by the light source used during micropropagation. Spectral quality of light is the relative intensity and quantity of the different wavelengths emitted by a light source and perceived by photoreceptors within the plant (Seabrook, 2005). Fluorescent lamps are commonly used for banana vitro culture (Vuylsteke, 1998). Navarro et al. (1994) used fluorescent lamps: TLD83, TL40W03 (PHILIPS, 40 W, white and blue cool light respectively) and GRO-LUX (SYLVANIA, 20 W) as light source for in vitro incubation of Banana (*Musa* AAA cv. Petite Naine). Nhut et al. (2003) examined the effect of light sources on the growth of banana plantlets under different LED ratios: (1) 100% red, (2) 90% red + 10% blue, (3) 80% red + 20% blue, (4) 70% red + 30% blue and (5) 100% blue, and PGF light was used as control. The highest total fresh weight of plantlets was obtained under 80% red + 20% blue LED and the value was equivalent to that under PGF. Studies have been performed aiming at reducing production costs by eliminating the use of light in the growth chambers. Using sunlight in a greenhouse with shade or a sunlit room has been suggested as an alternative (Kodym and Zapata-Arias, 1998).

**Photoperiod.** Overall, most plants need a photoperiod of 12 to 16 hours of light. Banana plantlet cultures are usually maintained under 16-h photoperiod (Madhulatha et al., 2004; 2006; Lakshmanan et al., 2007; Jain, 2008; Ahirwar et al., 2012). Notwithstanding, some researchers also incubated banana plantlets under an 12h/12h (day/night) (Navarro et al., 1994), 14 hours (Mbanaso et al., 2006; Waman et al., 2014) photoperiod.
Blackening of tissues is a frequent problem with in vitro culture of bananas. It is caused by oxidation of polyphenols contained in the tissues. Generally, oxidation is lethal to the explants. Those effects can be prevented by washing the explants with antioxidant and polyphenol absorbers (ascorbic acid, citric acid, or activated charcoal) or by adding them to the media. It is also reversible by frequent transfer of the explants to fresh media. Mante and Tepper (1983) placed their explants in ascorbic/trihydrate citric acid solution (1.0/1.5% w/v), but with no reference to duration of immersion in the solution. In contrast, Banerjee and Langhe (1985) added ascorbic acid (10 mg/L) in the media. A combination of ascorbic acid and periodic transfer (every two weeks) of the cultures to fresh media was proposed for a better response. Gupta (1986) added 25 mg/L ascorbic acid, filter sterilized to the media. Citric acid and activated charcoal were also added separately, but the amounts were not reported. The explants were also washed 3 to 5 times with distilled water containing 50 mg/L of ascorbic acid during surface sterilization.

**Stabilization**

After banana explants are established, they are transferred to Stage II media for shoot multiplication. Generally, the explants are transferred from media containing lower to higher cytokinin concentrations to disrupt apical dominance. However, culture stabilization should precede Stage II to enhance explant survival and decrease potential mutation rate. Omission of this step could adversely and irreversibly affect shoot survival, growth and multiplication rate.

Mante and Tepper (1983) allowed 7 to 28 days (They didn’t mention if there were differences or not) for cultures to stabilize. As mentioned previously, two different types of media were used for Stage I and II, respectively. There was no survival in one of their Stage II media. They proposed that it is due to the type of media used. Nonetheless, the time of stabilization was possibly the cause. Allowing only 7 days for 1-cm explants to stabilize is
obviously too short. Indexing was not mentioned in their paper. The time during which the explants were stabilized in a study conducted by Wong (1986) was not mentioned. Nevertheless, Wong cleansed the produced shoots by transferring them from the initiation medium to a basal medium for 14 days before Stage II. This amount of time should be sufficient for stabilization to occur. In contrast, Madhulatha et al., (2004) and Hussein (2012) stabilized cultured explants for 1 month prior to transferring them to Stage II.

**Indexing**

Multiplication rates can also be adversely affected by the presence of latent or non-culturable microbial contaminants that frequently remain inside the explants. Hence, it is crucial that explants be indexed prior to transfer onto Stage II multiplication. Indexing is an essential contaminant screening process used to detect possible existence of media microbial contaminants in cultures in cases where plants were well adapted in Stage I media. Nevertheless, this method is often neglected or determined using visual methods only. Among all the studies reviewed, only Gupta (1896) mentioned indexing via visual determination and bioassay. The bioassay consisted of crushing the banana leaves in 60mM sodium phosphate buffer (pH7.7). In addition to stabilization and indexing, culture cleansing is also a technique that could be used prior to Stage II. This technique consists of culturing pre-established culture on growth regulator-free medium. This procedure is commonly used to avoid carry over effect of any type of growth regulators that have been used during Stage I. Among all the papers reviewed, this technique was used in only one study. Wong (1986) transferred shoots proliferating from Stage I to the basal medium described above for 14 days prior to Stage II multiplication.

**Stage II: Multiplication of Propagules**

The goal of this Stage is quick proliferation of tissues, organs and other structures that can eventually induced high rate of new propagules (Murashige, 1974). The Stage II media used
by Mante and Tepper (1983) is the implementation of the media that worked better during Stage I. Two types of media (A and B) were evaluated. The first medium (A) consisted of MS medium supplemented with BAP (5mg/L), and sucrose (30g/L). However, the second medium (B) contained different concentrations of BAP (1-6 mg/L) supplemented with adenine sulphate (160 mg/L0 with 0.1 mg/L of IBA. This significant decrease in BAP concentration suggests that high BAP concentration is unnecessary. Explants failed to multiply on Stage A medium reason why the next subcultures were made using only Stage B media. The optimum concentrations of Stage B media were the ones supplemented with 3 to 5 mg/L and 160mg/L of adenine sulphate.

Wong (1986) evaluated the effects of 5 concentrations (0, 2.5, 5, 10, 15 mg l⁻¹) of two types of cytokinins (BAP and kinetin). Comparing growth regulators by mass unit is not a fair comparison. Banerjee and Langhe (1985) evaluated the same media for Stage II as Stage I over 7 consecutive subcultures. However, the time between each subculture was not provided. They reported that the low rate of shoot proliferation occurred at low BA levels with suppression of root elongation at high levels. They also noticed shoot proliferation between the 3rd and 6th subcultures. Madhulatha (2004) experimented with various combinations (0, 50, 100, and 200 mg/L) of kinetin and BA in liquid pulse treatment. The control did not receive liquid pulse treatments. The frequency of the liquid pulse treatment was 5, 15, 30, and 60 min. Thereafter, the explants were placed on the same media as Stage I for 30 days. They found best results with combination of BA and kinetin (1:1) at 50 mg/L.

Hussein (2012) screened different concentrations of BA (0, 1, 3, 5, 7, and 9 mg/L) to optimize a Stage II medium. The explants were split longitudinally in halves and inoculated in the fore-mentioned media. As other studies cited herein, Hussein observed higher shoot proliferation when BA concentration was enhanced. However, the best results were achieved at
7g/L for intact explants and at 3g/L of BA when buds were split in half. Those results suggested that the bud splitting technique could be an alternative to using higher BA concentrations. This means lower cost and higher multiplication rate. Although Bhagyalakshmi and Singh (1995) concluded that liquid culture works better for multiplication than solid medium, all Stage I established explants were transferred to semi-solid medium. This is due to low survival rate resulted in the plantlets from liquid medium during acclimatization. Three subcultures were examined during Stage II. Each subculture was of 4 weeks duration to eliminate carry-over effects of Stage I media prior to acclimatization.

Waman et al. (2014) cultured shoot tips on Stage II MS media containing 3 different concentrations (1, 2, and 3%) and 4 types (sucrose, glucose, fructose, and mannitol) of carbon sources. The basal medium was also supplemented with BAP (13.32 µM), kinetin (4.65 µM) and adenine sulfate (70 mg/L). Whether the cytokinins were used in combination or alone was not mentioned. The shoot tips were maintained under the same environment as Stage I and were subcultured after one month and a half. Waman et al. (2015) used the same method as Mbanaso et al. (2006) for Stage II. The preestablished shoot tips were trimmed and the basal shoots were either fragmented or incised. The control consisted of intact shoots. The medium used was not mentioned. The conditions at which the shoots were cultured were not provided. Waman et al. (2015) encountered the higher number of shoots from decapitated shoots divided into four, followed by shoots divided into halves, in contrast to Mbanaso et al. (2006). The lowest response was obtained in the control. Ahiwar et al. (2012) subcultured propagules from the shoot explants after 15 days and propagules from inflorescences explants after every 30 days. No additional information was provided about the Stage II. Mondal et. al. (2012) did not provide any information about Stage II.
Stage III: Rooting Stage

According to Murashige (1974), preparation of propagules for effective reestablishment in soil is the purpose of the Pretransplant Stage. During this Stage, individual propagules that have been previously propagated during Stage II are rooted. Root inducing substances are usually used during this Stage. Stage III, in some cases, helps the plants for a better adaptation ex vitro as they will be subjected to environmental changes. Typically, the plants are transferred from high humidity and low light to lower relative-humidity and higher light intensity, signaling a switch from the heterotrophic to the photoautotrophic phase. However, this stage is usually avoided for commercial production whenever possible as it adds around 35% to 75% to the final product cost.

The chosen auxins can affect positively or negatively the survival rates. Thus, in case that the Stage III cannot be bypassed for the cultivar used, the selection of auxins must be done carefully. Consequently, the type of auxin used can be dependent on various factors, including plant species/cultivars, type of shoot, and objective of the experiment, among many others. Long roots are undesirable as they are prone to damage during transplanting. Consequently, multiple and short roots are favored. The Stage III media used by Mante and Tepper (1983) was supplemented with sucrose (10-15g/L). The types of auxin used were either NAA (0.1 mg/L) or IBA (2-10 mg/L). They concluded that root formation and size were correlated with the plantlets age and the concentration and types of auxin used. However, the best auxin and the best concentration were not stated in the results.

As stated before, some plants can bypass rooting prior to Stage IV. Those plants are more suitable for commercial propagation. Of the studies examined, Stage III media was skipped in studies conducted by Wong (1986) and Singh (1995). Wong (1986) did not provide any information about their Stage III media. Based on their results, root growth was observed within
15 to 20 days in presence of cytokinins. Hence, they skipped Stage III media, and evaluated root produced during Stage II media. Thus, they concluded that auxin is not necessary for root induction. Similarly, Bhagyalakshmi and Singh (1995) stated that Stage III is optional for survival of banana ex vitro. Hence, treatments to which the shoots were subjected prior to acclimatization for root induction ex vitro were not mentioned.

Banerjee and Langhe (1985) reported rooting of shoots on half strength MS semisolid medium supplemented with 0.2 mg/L of IBA. Root induction occurred after 2 weeks culture. Madhulatha (2004) used different concentrations (0, 50, 100, 200 mg/L) of NAA and IBA combined during Stage III. They adjusted the pH at 5.5. The time of the liquid pulse treatment was similar as the one employed during Stage II. After liquid pulse treatment, the explants were also placed in the same culture as Stage I and II (MS basal medium supplemented with sucrose (30g/L) and agar (8g/L). Combination of NAA and IBA (1:1) for 60 min resulted in higher root induction. Nonetheless, the number of roots produced was insufficient to qualify the Stage III as root length also matter. Hussein (2012) induced root formation in media supplemented with NAA (0.0 to 1.2mg/L) with or without BA (0.2 and 0.4 mg/L), activated charcoal (0.0 to 2.0mg/L) and phosphate (0.0 to 255.0). Higher root lengths were obtained in media supplemented with 0.4 mg/L NAA while higher root numbers per microcuttings were achieved in media supplemented with 0.6 mg/L of NAA.

Waman et al., (2014) rooted 3cm long shoots on half strength MS medium containing NAA (2.69 µM), sucrose (2.5 %) and agar (0.6%). Subsequently, the plantlets were placed in the same conditions as Stage I and II. Root formation (100%) was obtained in shoots grown on medium containing sucrose. The poorest roots (80%) formation was observed on shoots originated from medium containing fructose. The rooting percentage was 100% independently of
the type and concentration of carbon source used, except for plantlets from fructose-containing (2%) treatment in which rooting formation was 80%. The thickest roots were obtained in media supplemented with fructose and sucrose (1%). Waman et al. (2015) did not experiment with Stage III. Ahiwar et al. (2012) transferred shoots to half strength MS medium supplemented with sucrose (15g), agar (8g) and different concentrations of NAA 0, 0.1, 0.2, 0.5, 1.0 and 1.5 mg/L). Mondal et. al. (2012) experimented with half strength MS medium without cytokinins for in vitro rooting. The medium was supplemented with 1mg/L of NAA, 15 g/L sucrose, and 8g/L agar.

**Stage IV: Acclimatization**

Stage IV is one of the most critical micropropagation stages. The success of in vitro micropropagation depends on the adaptability of the vitro plantlets ex-vitro. Thus, the condition at which the explants would be transferred is relevant. Commonly, the plantlets are rooted during Stage III to increase adaptation ex-vitro. However, due to cost, several studies have reported skipping the pretransplant Stage (Stage III). Hence, rooted microcuttings are transferred to Stage IV after Stage II or Stage III media. Usually, the acclimatization involves the opposite of the in vitro conditions. As mentioned previously, the plants are transferred from high humidity and low light to lower relative-humidity and higher light intensity. Unfortunately, several studies did not provide proper relevance to such stage, including Banerjee and Langhe (1985), Wong, (1986), Bhagyalakshmi and Singh (1995), and Hussein (2012).

Mante and Tepper (1983) transferred their rooted microcuttings to the greenhouse when the roots were over 15mm long. They transferred them into pots (10 x10cm) containing a substrate composed by top soil, peat moss, vermiculite (1: 1: 1). Medium was rinsed off the roots prior to potting. The pots were then wrapped with plastic bags and maintained in a shaded area for 7 days. After that, the plantlets where then introduced under direct sunlight. The plastic bags were removed after 14 days. Considering the high importance of this stage, limited information
has been provided about it. The conditions (temperature, humidity, light intensity, photoperiod) at which the plantlets were maintained in the greenhouse were not described. The watering frequency and the survival rate were not stated. Banana’s roots are very fragile. The longer the roots the more prone they are to damage. Considering those facts, prohibition of root elongation should be considered to prevent damage during transplanting (Kane, 2011).

Bhagyalakshmi and Singh (1995) stated that Stage III was not required for micropropagation of banana. They transferred shoots from Stage II media to Stage IV. Except the fact that the shoots were potted in soil for 4 weeks, Bhagyalakshmi and Singh (1995) failed to describe their Stage IV media. No information was provided about the acclimatization stage, such as the type of soil used, place, temperature, photoperiod, and humidity at which the plantlets were acclimatized. The survival percentage is not known either. Nonetheless, they concluded that the rate of survival was higher in shoots cultured on agar solidified medium as opposed to liquid media. As mentioned above, it is not worthy propagating plants in vitro if they could not be acclimatized. Wong, (1986) did not provide any information about their Stage IV procedures, except for the fact that the survival percentage was around 100%. Likewise, this was the situation in the studies conducted by Banerjee and Langhe (1985) and by Hussein (2012).

Waman et al., (2014) rinsed rooted plantlets in water to remove the adhering agar. After, the explants were first placed in a solution of Benfil (0.5%; w/v), followed by Krosin AG (500 mg/L). The plantlets were hardened off in polypropylene containers (150 ml). Sterilized coco peat was used as a substrate. Each pot was wrapped with a transparent polythene bag and transferred to a poly-tunnel to maintain high relative humidity. After 2 days of hardening off, the plantlets were sprayed with Mineral Salts solution (5 ml) to increase survival rate. After 1 month, plantlets were transferred to black polybags (15 x10cm) for the second phase of acclimatization.
The substrate used in this phase was a mixture of red earth: sand: coco peat (1:1:1; v/v/v). The plantlets were maintained in a shade house (50% shade) and moisturized when required.

Waman et al. (2015) experimented with two hardening stages. During the primary hardening, 5-cm rooted plantlets were acclimatized in 200 ml polypropylene pots. Different types of substrates were used during the first hardening i.e., coir pith, vermicompost, red earth + sand (1:1, v/v), red earth + perlite (1:1, v/v). The plantlets were then covered with PE plastic to retain high relative humidity. The containers were then placed in polytunnels for 4 weeks. The survival percentage was 100% in all of the media used. Nevertheless, the performance of plantlets cultured on lighter textured media was significantly higher than heavier textured media. Coir pith was described as the best media. During both, the primary and secondary hardening, the plantlets were fed either with 5 ml of MS or mono ammonium phosphate solution (0.2%). The application with both nutrient supplies (MS, MAP) was done either once biweekly (M\textsubscript{15}, P\textsubscript{15}) or once a month (M\textsubscript{30}, P\textsubscript{30}). The control treatment was watered with distilled water only. The watering schedule was not mentioned. Highest plant and the maximum number of leaves were obtained when MAP (P\textsubscript{30}) was applied once during acclimatization. The maximum number of roots were obtained in the control but did not differ statistically with the P\textsubscript{30} application. Thickest plants were found in the control.

Plantlets from the primary hardening were divided into two groups and used for the secondary hardening. One group were transferred to polybags containing same media as the primary hardening. The second group was transferred to the commercial mixture of red earth: sand: coir pith (1:1:1, v/v) for one month and a half. Plantlets from coir pith media gave better results in all of the media experimented during the secondary hardening. Hence, coir pith alone or the commercial mixture of red earth: sand: coir pith (1:1:1, v/v) were suggested for the
secondary hardening. Application of MAP once a month was also the best nutrient supply for secondary hardening (87.5%). To produce concurrent ex vitro rooting and hardening, bases of plantlets (ca 5cm) were immersed in IBA solution (250, 500, 750 and 1000 mg/L) for 30 s. 5ml of 0.2 % mono-ammonium phosphate (MAP) were applied as another treatment. The control was watered with distilled water only. From the control treatments, 80% of the plantlets rooted without any nutrient supply. Rooting percentage enhanced to 100% when MAP was applied once and increased from 70% to 100% when IBA concentration was increased from 250 ml to 750 ml.
CHAPTER 3
MATERIAL AND METHODS

Experiment Location

The study was performed at the Laboratory of Ornamental Horticulture and Biotechnology at the Tropical Research and Education Center (TREC) of the University of Florida (UF), in Homestead, Florida.

Experimental Design and Statistical Analysis

The experimental design was completely randomized and consisted of 6 treatments, consisting of 5 replications with each replication consisting of 3 baby food jars containing one in vitro plantlets, for a total of 90 experimental units. Data were collected and submitted to analysis of variance (ANOVA) using the R statistical analysis program, with means compared by LSD (Least Significant Difference) test at the 5% level of significance.

Plant Material and Culture Establishment

Two varieties of ornamental banana (Musa ‘Little Prince’ and Musa ‘Truly Tiny’) were used, obtained from AgriStarts, Inc. (Apopka, FL). The medium used was full strength MS basal medium (Murashige and Skoog, 1962) supplemented with BAP (8.8 µM), sucrose (30 g L⁻¹). The pH was adjusted to 5.8 with 1N NaOH and 1N HCl, solidified with agar (8g L⁻¹), and 50 ml of medium was dispensed in each baby food jars (15/treatments). The medium was autoclaved at 121 °C and 20 psi for 30 min.

Banana plant materials were obtained from AgriStarts and were not indexed. The plant materials were received in magenta vessels. The vessels were sprayed with alcohol 70% before placing inside a laminar floor hood, previously disinfected with hypochlorite and ethanol 700%. The banana plantlets were removed one by one and placed inside a sterile petri dish. The leaves and the roots of the banana plantlets were trimmed to 2 cm aided by forceps and scalpels.
(previously autoclaved and sterilize in a glass bead sterilizer after touching each plantlet).

Explants of banana shoot-tips (Musa ‘Little Prince’ and Musa ‘Truly Tiny’) 2 cm long were cut and inoculated into baby food jars. The vessels were then sealed with sealing film to overcome the problem of exogenous contamination, labeled with the date, banana variety, and repetition number and then placed in the culture room. Cultures were incubated in a controlled environmental growth chamber at $27 \pm 2 \, ^\circ\text{C}$ under 16h photoperiod. Regenerated shoots were propagated on the prementioned media at four-week intervals, then cut into 2 cm again and placed back in the culture room after repeating the aforementioned procedures.

**In Vitro Treatments**

**Light Conditions**

Three different light sources and intensities were evaluated; two provided by LED lighting (Philips™); low intensity LED (90 μmol m$^{-2}$ s$^{-1}$) and high intensity LED (116 μmol m$^{-2}$ s$^{-1}$), and the control provided by fluorescent (Philips™) lighting (100 μmol m$^{-2}$ s$^{-1}$). Photoperiod was 16/8 h (light/dark). The high LED was supplied by white LED lamps and the low LED by a combination of blue and red LED lamps. Fluorescent lamps served as the control. For the white LED lamps, the blue LED had a peak emission at 440 nm, red LED at 650 nm, and far-red LED at 720 nm. The spectral energy distribution of the LEDs is shown in figure 4-1. For the mix of blue LED lamps, the blue LED had a peak emission at 440 nm, red LED at 670 nm, and far-red LED at 720 nm. For the White LED lamps, the ratio of Blue to red was 20% to 100% while the ratio of blue to red in the mixture of blue and red light was 10% to 100% (Figure 4-1).

**Growth and Biomass Parameter Analysis**

**Shoot Length**

Shoot length was determined by placing a ruler under a petri dish inside the laminar flow hood. Each plantlet was then placed in a petri dish and measured. The shoot height was measured
from the main stem base to the tip of each plantlet. The height of each plantlets was recorded. Biweekly observations were recorded. Cultures were monitored closely for contamination. Cultures were allowed to proliferate for 4 weeks, during the time of this experiment. After each month, the number of shoots were counted. Growth characteristics, including plant size, shoot and root length and number, and fresh weight were evaluated. This experiment was repeated 4 times. Following each evaluation, shoots were then decapitated, cut back to 2cm, and transferred to fresh medium (same as the beginning of the experiment) and placed under the same aforementioned environmental (culture) conditions. The evaluations were the same after each culture (month). At the end of the experiment, shoot and root fresh and dry weight were also recorded.

**Root Length and Number**

Root number was determined by counting each root produced per plant. Root length was determined by cutting the longest root from each plantlet and placing them directly on the ruler. Root length was measured from the part cut to the main stem base to the top of the root tip.

**Plantlet Fresh Weight**

Plantlet fresh weight was determined by placing an autoclaved petri dish on a balance inside of the laminar flow hood. The balance was then tared. Each plantlet was placed inside the petri dish and weight measurements was taken. Each petri dish was discarded after being used. Five plantlets were randomly selected for biomass analysis within each treatment. The fresh and dry weight of the plant materials was recorded. The number of leaves and roots, plant height and root length, plantlets fresh weight, shoot and root fresh weight, shoot and root dry weight of the plantlets were recorded after the fourth subculture of one month each. Fresh weight of shoot and roots of the plantlets were determined separately. To determine their dry weight, the shoots and roots were dried at 77°C until a constant weight was reached.
Shoot Fresh Weight

Shoot fresh weight was determined by placing a petri dish on a scale. The scale was then tared. The basal part of each plantlet was washed in tap water to remove remaining medium adhering to the roots. Subsequently, the weight of each plantlet was taken. The roots were then removed. Each shoot was placed separately inside the petri dish and weight measurements was taken.

Root Fresh Weight

Root fresh weight was determined by placing a petri dish on a balance. The balance was then tared. After the remaining medium was washed out from the roots, the roots were removed from each plantlet. The roots from each plantlet were placed separately inside the petri dish and weight measurements was taken.

Shoot Dry Weight

The same five plantlets randomly selected within each treatment for biomass analysis of shoot fresh weight were used for shoot dry weight. To determine their dry weight, after shoot fresh weight was taken, the shoots were then placed in an envelope previously labeled with the variety, the repetition number of each plantlet and the date. The dry weight of the shoots was determined after oven drying at 77°C for 24 hours. After 24 hours, shoot dry weight was determined by placing a petri dish on a balance. The balance was then tared. Each shoot was placed inside the petri dish and weight measurements was recorded. Thereafter, the shoots were put back in the envelopes and placed back in the dryer.

Root Dry Weight

After root fresh weight was taken, the roots were then placed in the same envelope as the shoots that they were removed from. The dry weight of the roots was determined after oven drying at 77°C for 24 hours. After 24 hours, root dry weight was determined by placing a petri
dish on a balance. The balance was then tared. The roots from each were placed separately inside
the petri dish and weight measurements was recorded. Thereafter, the roots were put back in the
envelopes and placed back in the dryer. Data were recorded again after 2 more hours (26h total),
and weight was constant.

Additional parameters evaluated included chlorophyll content and leaf anatomy.

**Chlorophyll Analysis**

To evaluate this variable, completely expanded leaves were selected in 5 plantlets per
treatment. Chlorophyll content was evaluated as SPAD value by placing the third leaves, counted
from top downwards, of each plantlet in a portable SPAD-502 chlorophyll meter (SPAD-502,
Minolta Co., Ltd., Japan).

**Stomatal Count**

Stomata analysis was assessed on five randomly selected banana plantlets within each
treatment. The middle third of the third and fourth fully expanded leaves were cut. Fingerprints
of the leaves were prepared on glass slides. A thin layer of super glue was previously spread on
the microscope slides. Each slide was labeled with the banana variety, date, repetition number
and the adaxial and abaxial surface. After the glue was completely dry, the leave slices were
removed. The visible stomata were visualized in an optical Leica DMLB microscope (Leica
microsystems, Buffalo, NY, USA), at 200x magnification. Stomates number were counted on the
abaxial and adaxial surface epidermis in a diameter of 5 mm under the microscope from three
different field for each replicate in plantlets of each lighting treatments (Table 4-2) and a
morphological overview of these plantlets is shown in figure 4-2. Results are expressed as means
of counts per mm2.
Stomatal Measurements

Five randomly selected plantlets were chosen within each treatment for stomata analysis. The length and breadth of 3 randomly chosen stomata were measured on the adaxial and abaxial surface of each leaf in three different section of the leaf. The slides were examined and photographed under a light Leica DMLB microscope (Leica microsystems, Buffalo, NY, USA), coupled to a SPOT 4.7 idea digital camera and software. The images were taken and analyzed with the microscope imaging software SPOT basic.

Anatomical and Histochemical Studies

The anatomical studies were conducted using the middle third of the second completely developed leaf collected from five different plants per treatment, previously fixed in 70% FAA (formaldehyde - acetic acid - ethyl alcohol 70%) for 48 hours and then preserved in ethanol 70% (v v⁻¹). Leaf cross sections were obtained from free hand sectioning using a steel blade, using printing techniques. Following discoloration in sodium hypochlorite (1-1.25% active chlorine), triple-rinsing in distilled water, and staining in toluidine blue (0.05%, w/v), and subsequently fixed on semi-permanent slides with glycerinated water (Kraus and Arduin, 1997). The slides were examined and photographed under a light Leica DMLB microscope (Leica microsystems, Buffalo, NY, USA), coupled to a SPOT 4.7 idea digital camera and software. The images were evaluated using the basic image analysis software SPOT, with the assessment of five fields per repetition for each variable analyzed. The thickness of the abaxial surface epidermis (ASE), adaxial epidermis (ABE), abaxial hypodermis (AH), adaxial hypodermis (AbH), palisade parenchyma (PP), spongy parenchyma (SP) were determined (Figure 4-3).
CHAPTER 4
RESULTS

Growth and Biomass Parameter Analysis

Although light sources had no effects on some of the growth parameters and morphology of the banana varieties (Table 4-1) evaluated herein; plantlets produced under high and low LED intensities were apparently larger in size compared to the control. The different growth parameters evaluated (stem diameter, shoot and root length, shoot and root fresh weight, shoot and root dry weight, root number and leaf number) herein acted differently regardless of the light sources.

Effect of Light Quality and Quantity on Stem Diameter

The analysis of variance (Table 4-1) indicated that stem diameter showed no statistically significant differences between the light sources for the two banana varieties. Overall, the greatest means for stem diameter (Table 4-1) were obtained for banana variety Musa ‘Little Prince’ cultured under high (5.32 ± 0.50) and low LED (5.24 ± 0.44) light conditions.

Effect of Light Quality and Quantity on Plantlet Fresh Weight

The analysis of variance (Table 4-1) for plant fresh weight showed significant effect of the light sources on the two banana varieties. Overall, Musa Little Prince performed better than Musa Truly Tiny under the different light sources and intensities and the best light was high LED intensity (white LED) (Table 4-1). But within variety, when it comes to the variety Musa Little Prince, high LED intensity (white LED) was better but not significantly different from low LED intensity (mix of blue and red LED). When it comes to the variety Musa Truly Tiny, high LED intensity (white LED) had a significant effect on the mass of the plantlets but was not significantly different from the control (fluorescent light). In addition, there was interaction between light sources and banana varieties (Figure 4-4). The data (Table 4-1) suggest that there
is an interaction effect, which the significant p-value (0.0157) for the
light_sources*banana_variety term confirms. The effect of light quality and intensity depends on
the banana variety. Greatest plant fresh weight was observed when Musa Little Prince were
cultured under high LED intensity (2.33 ± 0.64).

**Effect of Light Quality and Quantity on Shoot Fresh Weight**

The banana varieties behaved differently under the different light sources. The analysis of
variance (Table 4-1) showed no significant effect of light (quality and quantity) on shoot fresh
weight. The lowest plant fresh weight (1.30 ± 0.26) was obtained when banana variety Musa
Truly Tiny was cultured under high LED intensity, while the greatest fresh weight (2.33 ± 0.64)
was obtained when Musa Little Prince was cultured under the same light.

**Effect of Light Quality and Quantity on Root Fresh Weight**

Results of ANOVA (Table 4-1) indicated that light had no significant effect on the root
fresh weight of both banana varieties studied herein. Even though there was no significant effect
between the varieties there was significant interactions between banana varieties and light
sources.

**Effect of Light Quality and Quantity on Leaf Number**

Results of ANOVA (Table 4-1) indicated that light (quality and quantity) had no
significant effect on leaf number regardless of banana varieties studied herein. Although the leaf
number was the same, leaf developed under the LED treatments were much larger and greener
than those developed under fluorescent light.

**Effect of Light Quality and Quantity on Shoot Length**

Results of ANOVA (Table 4-1) indicated that light quality and quantity had significant
effect on shoot length of banana (Figure 4-5). Overall, light quality and intensity affected the
variety of Musa Little Prince more than Musa Truly Tiny but low LED intensity (mix of red and
blue LED) significantly affected both banana varieties. Within varieties, regarding the variety Musa Truly Tiny, high LED intensity (white LED) had a positive effect on shoot length but was not significantly different from low intensity LED (mix of red and blue LED). However, when it comes to Musa Little Prince, both light intensities, high and low LED significantly affected shoot length comparing to the control (fluorescent light). Moreover, subsequent LSD mean separation analysis indicated the interaction between light sources and banana varieties had a significant effect on shoot length (Table 4-1, Figure 4-5).

**Effect of Light Quality and Quantity on Root Length**

Results of ANOVA (Table 4-1) indicated that light (quality and quantity) had no significant effect on the root length of plantlets. However, Musa Truly Tiny had the greatest mean (4.60 cm) when subjected to fluorescent light, while Musa Little Prince had the greatest root mean (3.70 cm) when cultured under low intensity LED (mix of red and blue LED).

**Effect of Light Quality and Quantity on Root Number**

The number of roots was not significantly different (Table 4-1). Nonetheless Musa Little Prince had the greatest mean (8.2) of root number when subjected to high LED intensity (white LED lamps), while Musa Truly Tiny had greatest number of roots (6.0) when cultured under low intensity LED (mix of red and blue LED).

**Effect of Light Quality and Quantity on Shoot Dry Weight**

Shoot dry weight were not significantly affected (Table 4-1) by light sources (fluorescent light, low and high LED). However, Musa Little Prince had the greatest mean (0.28 g) when subjected to high intensity LED (mix of red and blue LED) while Musa Truly Tiny had the greatest dry mass (0.21g) when cultured under the control (fluorescent light).
Effect of Light Quality and Quantity on Root Dry Weight

Results of ANOVA (Table 4-1) indicated that light had no significant effect on the root dry weight of the banana varieties studied herein. The greatest mean of dry mass (0.25 g) was obtained when Musa Little Prince was cultured under low intensity LED (mix of red and blue LED lamps), while the greatest mean of dry mass (0.24 g) for the variety Musa Truly Tiny was obtained when cultured under the control (fluorescent light).

Anatomical Studies

Although differences in growth and development of the plantlets were not affected by light sources (Table 4-1), leaf anatomy was greatly affected by light quantity and quality (Table 4-2).

Stomatal Count

The presence of stomata on both sides of the leaves, with a higher number of stomates on the abaxial than the adaxial leaf is called amphistomatic (Nunes et al., 2017). In this study, stomata were present in both adaxial and abaxial leaf surfaces (Figure 4-2), which characterized the banana plantlets as amphistomatic. The stomata were unevenly distributed over the leaf surface though sometimes they appeared arranged in lines (Table 4-2). Light quality and quantity influenced stomata number of both banana varieties. The stomates of the abaxial and adaxial leaf surfaces were numbered in plantlets of each lighting treatments (Table 4-2; Figure 4-2), and a morphological overview of these stomates is shown in figure 4-2. Significant differences were observed between lighting treatments, following the same pattern observed in chlorophyll contents.

Overall, there was no significant differences of light quality and intensity on both banana varieties when it comes to the adaxial number of stomata. However, both banana varieties performed better under white and pink LED (Figure 4-6). Within varieties, high LED intensity
(white LED lamps) was the best but not significantly different from low intensity LED (mix of red and blue LED lamps) for Musa Truly Tiny (Figure 4-6). But for Musa Little Prince, both high LED intensity (white LED lamps), and low intensity LED (mix of red and blue LED lamps) positively affected adaxial number of stomates (Figure 4-6). For the adaxial surface, the fluorescent lamps treatment resulted in a stomata average number per mm$^2$ of 30.0 and 32, while in high LED treatment it was counted 22.8 and 24.4 stomata and 28.4 and 25.8 for low LED (for Musa Little Prince and Musa Truly Tiny respectively). The same trend was observed on the abaxial leaf surface. The stomates number per mm$^2$ in high (75.4 and 92) and low LED (67.6 and 94.2) treatments were lower than observed in fluorescent lamps treatment (83.6 and 101.6). No significant differences, for both leaf surfaces (adaxial and abaxial), were recorded between LED lighting treatments (Table 4-2).

**Effect of Light Quality and Quantity on Stomata Size**

Results of ANOVA (Table 4-3) indicated that light had significant effect on the stomate size of both banana varieties (Figures 4-7 to 4-9). Except for the length and width of Musa Truly Tiny under low LEDs, stomates from fluorescent light treatments were much longer (Figures 4-7 and 4-8) and much wider (Figure 4-9) in both the abaxial and adaxial leaf surface regardless of the banana variety (Table 4-2). Most of the stomata from fluorescent light had the guard cells wide open (Figure 4-2) while the stomata from the other light treatments (low and high LED) remained closed in most of the leaf printing analyzed. This suggests that banana produced under fluorescent light had probably lost their ability to open and close the stomata, thus regulate transpiration.

**Effect of Light Quality and Quantity on Leaf Anatomy**

Cross-sections of the leaves were measured for adaxial and abaxial epidermis and hypodermis thickness. Thickness of palisade and spongy mesophyll were also measured. The
analysis of variance (Table 4-3) for leaf anatomy showed no significant effect of the light sources on leaf anatomy regardless of the banana variety. As for the organization of mesophyll, the banana is a species classified as dorsiventral or bifacial, with the palisade parenchyma oriented toward the adaxial epidermis and immediately below the adaxial hypodermis, and the spongy parenchyma facing the abaxial epidermis (Figure 4-3). The palisade parenchyma was made of closely packed cylindrical cells, exhibiting one to two layers of cells. On the other hand, in the spongy parenchyma, there were about two to three layers of spongy mesophyll cells, packed with inconspicuous air spaces. The cells were not well defined and had more or less a round shape (Figure 4-3).

The characteristics evaluated associated to tissue thickness indicated no significant effects for any of the parameters (Table 4-3). Regarding the epidermis, the abaxial surface was significantly thinner than the abaxial surface (Table 4-3). Similarly, the hypodermis of the abaxial surface was also significantly thinner than the adaxial hypodermis for both varieties. Although there were no statistical differences between the thickness of the different tissues measured, visually, the anatomy of the plants cultured under both LED lightings were more defined compared to fluorescent light (Figure 4-3). For instance, the cells of palisade parenchyma have an elliptical shape while the cells from the spongy parenchyma are not really define but are more or less roundish in shape. The prementioned features were well defined and could be observed in the leaf cross sections of plantlets cultured under LED lightings but not the cells of the palisade parenchyma were not as define in plantlets cultured under fluorescent light.

**Chlorophyll Content**

Significant differences were shown in chlorophyll contents among light levels (Figure 4-10). The range of total chlorophyll content of LED lighting treatments, both low and high LED showed greater SPAD values comparing to fluorescent lamps, resulting in SPAD values of
36.84 and 41.54 for high LED, 40.92 and 31.62 for low LED, and 24.12 and 24.56 for fluorescent lamps (for Musa Little Prince and Musa Truly Tiny respectively). Nonetheless, the amount of chlorophyll in LED lighting treatments were not significantly different. The leaves from the LED (low and high) treatments were a dark green color while the leaves under the fluorescent light had a yellowish-green color; explaining why the SPAD value of leaves under both LED intensities were greater than that of fluorescent light.
Table 4-1. Evaluation of light quality and light levels on growth and development of in vitro production of two ornamental banana varieties; Musa ‘Little Prince’ (LP) and ‘Truly Tiny’ (TT).

<table>
<thead>
<tr>
<th>BV</th>
<th>LS</th>
<th>SD</th>
<th>PFW</th>
<th>SFW</th>
<th>RFW</th>
<th>LN</th>
<th>SL</th>
<th>RL</th>
<th>NR</th>
<th>SDW</th>
<th>RDW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WL</td>
<td>5.32±0.50</td>
<td>2.84±0.14a</td>
<td>1.63±0.25</td>
<td>0.19±0.08</td>
<td>3.6±0.89</td>
<td>7.45±0.67a</td>
<td>3.02±0.30</td>
<td>6.4±2.07</td>
<td>0.24±0.03</td>
<td>0.01±0.005</td>
</tr>
<tr>
<td>LP</td>
<td>PL</td>
<td>5.24±0.44</td>
<td>2.62±0.42ab</td>
<td>1.94±0.54</td>
<td>0.34±0.09</td>
<td>4.0±1.00</td>
<td>7.33±0.76a</td>
<td>3.70±0.82</td>
<td>8.2±2.17</td>
<td>0.28±0.07</td>
<td>0.025±0.008</td>
</tr>
<tr>
<td></td>
<td>FL</td>
<td>5.08±0.71</td>
<td>2.21±0.39c</td>
<td>1.44±0.05</td>
<td>0.16±0.07</td>
<td>4.2±0.84</td>
<td>5.87±0.82b</td>
<td>3.21±0.25</td>
<td>7.8±3.19</td>
<td>0.24±0.02</td>
<td>0.016±0.007</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>4.28±0.55</td>
<td>2.31±0.16bc</td>
<td>1.27±0.19</td>
<td>0.25±0.10</td>
<td>3.6±0.89</td>
<td>6.67±0.30ab</td>
<td>4.21±0.34</td>
<td>5.6±1.52</td>
<td>0.18±0.03</td>
<td>0.015±0.007</td>
</tr>
<tr>
<td>TT</td>
<td>PL</td>
<td>3.94±0.37</td>
<td>1.79±0.21d</td>
<td>1.01±0.27</td>
<td>0.27±0.07</td>
<td>3.6±1.14</td>
<td>6.46±0.67b</td>
<td>4.38±0.92</td>
<td>5.2±1.79</td>
<td>0.12±0.03</td>
<td>0.018±0.008</td>
</tr>
<tr>
<td></td>
<td>FL</td>
<td>4.36±0.92</td>
<td>1.91±0.38ed</td>
<td>1.39±0.62</td>
<td>0.34±0.16</td>
<td>4.0±1.00</td>
<td>6.02±0.47b</td>
<td>4.60±0.36</td>
<td>6.0±2.12</td>
<td>0.21±0.10</td>
<td>0.024±0.012</td>
</tr>
</tbody>
</table>

Means within a column followed by the same letter for each treatment are not significantly different at P ≤ 0.05 according to the LSD (Least Significance Difference) test. The tests were conducted separately within each group (stem diameter, plant, shoot and root fresh weight, leaf number, shoot and root length, root number, shoot and root dry weight). WL = high LED; PL = low LED; FL = fluorescent light.

BV = banana variety; LS = light source; SD = stem diameter; PFW = Plant Fresh Weight; SFW = Shoot Fresh Weight; RFW = Root Fresh Weight; LN = Leaf Number; SL = Shoot Length; RL = Root Length; NR = Number of Roots; SDW = Shoot Dry Weight; RDW = Root Dry Weight.
Table 4-2. Evaluation of light (quality and quantity) on stomata formation of in vitro production of two ornamental banana varieties; Musa ‘Little Prince’ (LP) and ‘Truly Tiny’ (TT).

<table>
<thead>
<tr>
<th>BV</th>
<th>LS</th>
<th>AdSN</th>
<th>AbSN</th>
<th>AdSSL</th>
<th>AdSSW</th>
<th>AbSSL</th>
<th>AbSSW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP</td>
<td>WL</td>
<td>22.8±7.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75.4±14.08&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>25.18±2.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.13±0.94&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21.41±3.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.35±1.25&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>PL</td>
<td>28.4±7.67&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>67.6±12.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>25.63±1.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.13±0.91&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>27.08±2.47&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>11.35±1.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>FL</td>
<td>30.0±6.20&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>83.6±11.35&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>28.51±3.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.87±0.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.16±1.15&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>11.47±1.58&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TT</td>
<td>WL</td>
<td>24.4±2.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92.0±5.74&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>25.00±1.89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.16±0.61&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.81±1.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.11±1.72&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>PL</td>
<td>25.8±4.66&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>94.2±4.32&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>27.72±2.19&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>10.00±1.79&lt;sup&gt;c&lt;/sup&gt;</td>
<td>27.68±2.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.05±0.68&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>FL</td>
<td>32.0±3.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>101.6±21.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.45±0.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.02±1.20&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>25.64±1.44&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9.97±1.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means within a column followed by the same letter are not significantly different at P ≤ 0.05 according to the LSD (Least Significance Difference) test. The tests were carried out independently within each (adaxial and abaxial stomate number, adaxial and abaxial stomate size).

**BV** = banana variety; **LS** = light source; **AdSN** = Adaxial Stomate Number; **AbSN** = Abaxial Stomate Number; **AdSSL** = Adaxial Stomate Length; **AdSSW** = Adaxial Stomate Width; **AbSSL** = Abaxial Stomate Length; **AbSSW** = Abaxial Stomate Width.
Table 4-3. Evaluation of light quality and quantity leaf anatomy of in vitro production of two ornamental banana varieties; Musa ‘Little Prince’ (LP) and ‘Truly Tiny’ (TT).

<table>
<thead>
<tr>
<th>BV</th>
<th>LS</th>
<th>ASE</th>
<th>ABE</th>
<th>AdH</th>
<th>AbH</th>
<th>PP</th>
<th>SP</th>
</tr>
</thead>
<tbody>
<tr>
<td>LP</td>
<td>WL</td>
<td>15.53±4.89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.63±5.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.17±5.69&lt;sup&gt;c&lt;/sup&gt;</td>
<td>59.02±14.25&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>35.87±9.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.91±9.38&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>PL</td>
<td>17.70±2.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.40±5.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.31±13.52&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>52.38±39.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.63±10.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.12±10.29&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>FL</td>
<td>20.66±6.31&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>20.55±5.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.33±7.75&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>59.82±4.46&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>42.71±11.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.40±11.25&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>TT</td>
<td>WL</td>
<td>19.68±7.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>15.07±2.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.90±14.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.61±7.71&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>43.71±11.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.10±8.66&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>PL</td>
<td>17.73±5.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.08±4.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78.79±20.14&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>69.01±18.48&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>35.39±7.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.88±9.39&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>FL</td>
<td>25.10±5.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.15±3.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.96±15.37&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>62.76±14.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.95±13.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.33±17.81&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means within a column followed by the same letter are not significantly different at P ≤ 0.05 according to the LSD (Least Significance Difference) test. The tests were carried out independently within each group (adaxial surface epidermis, abaxial surface epidermis, adaxial hypodermis, abaxial hypodermis, palisade parenchyma, spongy parenchyma).

BV = banana variety; LS = light source; ASE = Abaxial Surface Epidermis; ABE = Adaxial Epidermis; AH = Abaxial Hypodermis, AbH = Adaxial Hypodermis; PP = Palisade Parenchyma; SP = Spongy Parenchyma.
Figure 4-1. Measurements of the spectral energy distribution of high LED (white LED lamps, top graph), Low LED (mix of red and blue LED lamps, middle graph) and fluorescent light (bottom graph). Photo courtesy of author.
Figure 4-2. Stomatal impressions of abaxial and adaxial leaf surfaces of banana plantlets under fluorescent, high LEDs and low LEDs light sources. Bar 50 µmol. Photo courtesy of author.
Figure 4-3. Microscopy of cross sections of banana leaves (Musa Little Prince and Truly Tiny) cultivated in vitro under different light sources (low and high LED, fluorescent light). Ad_E = adaxial epidermis; Ab_E = abaxial epidermis; Ad_H = adaxial hypodermis; Ab_H = abaxial hypodermis; PP = palisade parenchyma; SP = spongy parenchyma. Bars = 50 μm. Photo courtesy of author.
Figure 4-4. Effect of interaction between light sources (fluorescent light, white and pink LED) and banana varieties (Musa Little Prince and Musa Truly Tiny) on plantlets fresh weight after 4 weeks of culture. Colored horizontal bars represent interactions between light sources and banana varieties. Error bars indicate positive standard deviation of treatment means. Within each graph, treatments denoted with identical letter are not significantly different at \( \alpha = 0.05 \) level based on LSD mean separation. WL = high LED; PL = low LED; FL = fluorescent light; LP = Musa Little Prince; TT = Musa Truly Tiny.
Figure 4-5. Effect of interaction between light sources (fluorescent light, white and pink LED) and banana varieties (Musa Little Prince and Musa Truly Tiny) on shoot length after 4 weeks of culture. Colored horizontal bars represent interactions between light sources and banana varieties. Error bars indicate positive standard deviation of treatment means. Within each graph, treatments denoted with identical letter are not significantly different at $\alpha = 0.05$ level based on LSD mean separation. WL = high LED; PL = low LED; FL = fluorescent light; LP = Musa Little Prince; TT = Musa Truly Tiny.
Figure 4-6. Effect of interaction between light sources (fluorescent light, white and pink LED) and banana varieties (Musa Little Prince and Musa Truly Tiny) on adaxial stomates number after 4 weeks of culture. Colored horizontal bars represent interactions between light sources and banana varieties. Error bars indicate positive standard deviation of treatment means. Within each graph, treatments denoted with identical letter are not significantly different at $\alpha = 0.05$ level based on LSD mean separation. WL = high LED; PL = low LED; FL = fluorescent light; LP = Musa Little Prince; TT = Musa Truly Tiny.
Figure 4-7. Effect of interaction between light sources (fluorescent light, white and pink LED) and banana varieties (Musa Little Prince and Musa Truly Tiny) on abaxial length of stomates after 4 weeks of culture. Colored horizontal bars represent interactions between light sources and banana varieties. Error bars indicate positive standard deviation of treatment means. Within each graph, treatments denoted with identical letter are not significantly different at $\alpha = 0.05$ level based on LSD mean separation. WL = high LED; PL = low LED; FL = fluorescent light; LP = Musa Little Prince; TT = Musa Truly Tiny.
Figure 4-8. Effect of interaction between light sources (fluorescent light, white and pink LED) and banana varieties (Musa Little Prince and Musa Truly Tiny) on adaxial length of stomates after 4 weeks of culture. Colored horizontal bars represent interactions between light sources and banana varieties. Error bars indicate positive standard deviation of treatment means. Within each graph, treatments denoted with identical letter are not significantly different at $\alpha = 0.05$ level based on LSD mean separation. WL = high LED; PL = low LED; FL = fluorescent light; LP = Musa Little Prince; TT = Musa Truly Tiny.
Figure 4-9. Effect of interaction between light sources (fluorescent light, white and pink LED) and banana varieties (Musa Little Prince and Musa Truly Tiny) on abaxial width of stomates after 4 weeks of culture. Colored horizontal bars represent interactions between light sources and banana varieties. Error bars indicate positive standard deviation of treatment means. Within each graph, treatments denoted with identical letter are not significantly different at $\alpha = 0.05$ level based on LSD mean separation. WL = high LED; PL = low LED; FL = fluorescent light; LP = Musa Little Prince; TT = Musa Truly Tiny.
Effect of interaction between light sources (fluorescent light, white and pink LED) and banana varieties (Musa Little Prince and Musa Truly Tiny) on chlorophyll content after 4 weeks of culture. Colored horizontal bars represent interactions between light sources and banana varieties. Error bars indicate positive standard deviation of treatment means. Within each graph, treatments denoted with identical letter are not significantly different at $\alpha = 0.05$ level based on LSD mean separation. WL = high LED; PL = low LED; FL = fluorescent light; LP = Musa Little Prince; TT = Musa Truly Tiny.
CHAPTER 5
DISCUSSION

To guarantee the quality and quantity of plantlets, the growing environment of plantlets must be maintained at the optimum condition (Chen, 2005). The main factors influencing growth and development of in vitro plantlets include light (quality, intensity and photoperiod), temperature, relative humidity, and carbon dioxide (CO2) concentration (Aitken-Chrisie et al., 2013).

In this study, no significant effects of light were shown on stem diameter. Since the intensity of low LED was lower than the high LED, this implies that low LED intensity may be better to promote stem growth. However, the greatest stem diameter was obtained under fluorescent light for Musa ‘Truly Tiny’. In this study, the response of stem diameter to light seemed to be correlated with banana variety. Similarly, Nhut et al. (2003) reported that the stem diameter of banana plantlet was biggest under a mixture of red to blue LED ratio (60 μmol m-2s-1) as compared to that of the other light intensities (45, 75 μmol m-2s-1) and quality (fluorescent lamps). Tanaka et al. (1998) reported that stem diameter of Cymbidium plantlet was not affected by the different light sources used in their study. Results obtained in this study were inconsistent with the studies of Li et al. (2013), whereby stem diameter of rapeseed plantlets was greater in plantlets under B and B:R = 1:3 light than those under FL. Those differences had proven that the influences and mechanisms related to light quality, intensity and photoperiod in plants is rather specific to plant species or cultivars.

Plantlets fresh weight appeared to be greatest under high LED but the differences were not statistically significant from plantlets grown under low LED. Similar results were obtained when banana plantlets were cultured under a mix of red and blue LED (Nhut et al., 2003). Conversely, results reported by Li et al. (2013) indicated that there were no significant
differences among the fresh weight of plantlets under B: R = 3:1, B: R = 1:1, or B: R = 1:3 LED light, although fresh weight appeared numerically greater in plantlets grown under B: R = 3:1 LED light.

The shoot fresh and dry weight of banana plantlets were not significantly different. This is probably because data were collected after growth had probably plateaued. These results contrasted with resulted reported by Tanaka et al. (1998) who reported that shoot fresh and dry weight of plantlets were significantly higher under LEDs than under fluorescent light.

Different light sources (fluorescent light, high and low LED) had the same effects on shoot and root fresh and dry weight of both banana varieties evaluated in this study. The results showed no statistical differences among those variables. These results were consistent with the studies of Nhut et al. (2017) who obtained the highest shoot and root fresh weight of plantlets under a mix of red and blue LED (80% red + 20% blue LED). Similarly, Shin et al. (2008) reported that fresh weight/dry weight of roots was increased in plants grown under red and blue combined light.

Since the spectral energy distribution of red and blue lights consists with that of chlorophyll absorption, it is generally accepted that red and blue LED lighting ratio improves plant growth and development by enhancing net photosynthetic rate (Goins et al., 1997). Highest plantlet growth in our experiment also confirmed the effect of mixed red and blue LED lighting (referred herein as low LED). However, the effect of high LED on shoot length was not significantly different from low LED. Results of this study are consistent with the data reported for strawberry by Kim et al. (2004) who obtained highest stem length of chrysanthemum under mixed red and blue LED. These observations contrasted with results of Nguyen et al. (2001), who noted no differences in banana plantlets height.
There were no statistically significant differences among plantlet root length under the different light sources (fluorescent light, high and low LED) evaluated herein. However, the root length was longest in plantlets cultured under fluorescent light followed by low LED (mixed red and blue light). Similar results were obtained in root length of rapeseed in study conducted by Li et al. (2013), where root length was longest under a mix of blue and red light (B:R=3:1), although there were no significant differences between the different light sources. Results reported by Li et al. (2013) differed from those obtained in this study. Li et al. (2013) observed that there were no significant differences among plantlet stem lengths under any of the six light treatments (fluorescent lamps (FL), monochromic blue light-emitting diodes (LEDs) (B), monochromic red LED (R), and three mixtures of B plus R (3:1, 1:1, 1:3) LED) evaluated.

The general morphology of plantlets proved quite homogeneous in all lighting treatments evaluated, showing the number of leaves similar between treatments. This study is in agreement with results reported for banana by Vieira et al. (2015). In addition, these observations corroborate results of Tanaka et al. (1998), who noted no differences in leaf number of Cymbidium plantlets grown under the three different LED radiations (red, blue, red plus blue) and in the control fluorescent light source. Similarly, Nhut et al. (2003) reported that the number of leaves of banana plantlets cultured under 60 μmol m-2 s-1 and 80% red + 20% blue LED was higher than that under 45, 75 μmol m-2s-1 and PGF. Similarly, Nguyen et al. (2001) reported no significant differences in number of leaves of banana plantlets. In contrast, Nhut et al. (2007) reported higher number of leaves of banana plantlets under fluorescent lamps than under LED lighting.

Light quality and quantity are essential for photosynthesis, and influence light absorption by chlorophyll (Hoenecke et al., 1992; Tripathy and Brown, 1995). Results of this study showed
that the chlorophyll content of banana plantlets grown under high and low LED lighting treatments were higher as compared to fluorescent lamps. This study is in agreement with the data reported for banana plantlets by Vieira et al. (2015) who confirmed that higher chlorophyll values were obtained when banana plantlets were cultured under LED lights compared to fluorescent light. Kim et al. (2004) also found higher chlorophyll contents when chrysanthemum plantlets were grown under a mix of red and blue LED. Similarly, results reported by Li et al. (2013) confirmed that chlorophyll concentrations appeared greater in plantlets under blue and red (3:1) LED lighting than those under red LED light and fluorescent lamps. Similarly, the amount of total chlorophyll obtained was higher in in vitro cultured Doritaenopsis plants cultured under a mixture of red plus blue LED, followed respectively by blue LED and fluorescent light treatments (Shin et al., 2008). These results are in contradiction with those found by Tanaka et al. (1998) where highest SPAD value was obtained under fluorescent lamps in Cymbidium grown plantlets.

Light sources (quality and intensity) influenced the leaf stomata. Stomata of plantlets grown under the LED lightings were more or less ellipsoid and closed like those of the field-grown plants. Nonetheless, banana plantlets grown under fluorescent light presented open stomata and guard cells with roundish shape. Similar observations were made by Romano and Martins-Loução (2001) in leaves of cork oak. Banana plantlets grown under high and low LED had the smallest number and size of stomata whereas those under fluorescent light had the largest number and biggest stomata size. Comparable results were obtained by Kim et al. (2004) regarding stomata number. Nonetheless, Kim et al. (2004) reported biggest stomata size when using mix of red and blue LED. Results of this study contrasted with observations of Vieira et al. (2015) who reported increased formation of stomata were recorded on both leaf surfaces
(adaxial and abaxial) when banana plantlets were submitted to LED lighting (deep red/white and white LED) comparing to fluorescent light. In general, the presence of stomata was observed on both sides of the leaves, with more stomata on the abaxial than the adaxial leaf surface. These results corroborate with observations of Nunes et al. (2017) who observed greater presence of stomata on the lower epidermis. Amphistomatic leaves were also observed in E. heterophylla (Ferreira et al., 2003).

The results obtained in the present study showed that the epidermis of the adaxial face was always thicker, compared to the abaxial epidermis. These results corroborate with observations of Costa et al. (2009). Similarly, Ferreira et al. (2003) observed that the adaxial surface of the leaf cross sections was thicker than that of the abaxial surface in leaves of Amaranthus deflexus, Amaranthus spinosus, Alternanthera tenella and Euphorbia heterophylla. However, most of the leaf anatomical features measured in the current study were similar among the different light sources regardless the banana variety. These observations corroborate results of Schuerger et al. (1997), who noted a similar thickness in the anatomical features of pepper leaves, and of Li et al. (2013), who noted no statistically significant differences in the thickness of the palisade tissue between any plantlets cultured under six different light treatments. Nonetheless, results of this study regarding the similarity of thickness of the spongy mesophyll differs from observations made by Li et al. (2013), who noticed that the thickness of the spongy tissue was greater in plantlets under red and a mix of blue and red (B:R = 3:1) LED light than plantlets under fluorescent light.
CHAPTER 6
SUMMARY AND CONCLUSIONS

Our results revealed that shoot mass and length could be promoted by controlling light quality and intensity of different lights. However, effect of light quality and intensity related to plant growth and development (stem diameter, shoot and root fresh weight, shoot and root dry weight, root length, root number and leaf number) has not been fully clarified despite some claim of effects from other researchers’ experiments. Therefore, there still remains work to be done in this area. Although number shoot produced per plantlets were not taken into account during this study, some of the plantlets grown under the LED lightings produced multiple shoots while no shoots were formed in plants grown under the control (fluorescent light). That could be an interesting parameter to look at in subsequent studies.

Different interactions between the two banana varieties (Musa Little Prince and Musa Truly Tiny) indicated that effect of light quality and intensity is likely to differ according to plant species, and environmental conditions. Therefore, more studies are required regarding the correlations between light quality and growth.

The results of the present study indicate that the LED lighting affected the concentrations of chlorophyll as well as stomates sizes in banana in vitro plantlets. Higher chlorophyll contents were obtained when banana plantlets were grown under both intensities of LED lighting.

Based on the responses of both banana varieties to the different light intensity observed in this study, we can recommend the intensity LED (90 μmol m$^{-2}$ s$^{-1}$) used herein for micropropagation of banana.
LIST OF REFERENCES


[Agristarts Plant Laboratories](https://www.agristarts.com/index.cfm/fuseaction/plants.plantDetail/plant_ID/86/index.htm)


Li, L. F., Häkkinen, M., Yuan, Y. M., Hao, G., & Ge, X. J. (2010). Molecular phylogeny and systematics of the banana family (Musaceae) inferred from multiple nuclear and chloroplast DNA fragments, with a special reference to the genus Musa. Molecular Phylogenetics and Evolution, 57(1), 1-10.


Wong, W. C. (1986). In vitro propagation of banana (Musa spp.): initiation, proliferation and development of shoot-tip cultures on defined media. Plant Cell, Tissue and Organ Culture, 6(2), 159-166.


BIOGRAPHICAL SKETCH

Cassandre Feuillé was born in Port-Salut, Haïti, in 1992. She earned a bachelor’s degree in agronomy engineering in 2015, from Universidad ISA, in Santiago de los Caballeros, Dominican Republic. During her study in Dominican Republic, she discovered her passion for plant tissue culture which she thinks could be the answer to lower the level of food insecurity in Haiti. She decided to pursue a master’s degree in the aforementioned field. In 2017, Cassandre pursued a master’s degree in environmental horticulture at the University of Florida, under the supervision of Dr. Wagner A. Vendrame and Dr. Michael E. Kane. She successfully completed her Master of Science in horticultural sciences in the summer of 2019. She would like to build a tissue culture laboratory to collaborate with other peers and provide the opportunity to lower production costs for the farmers, as well as increase food production in Haiti. She is confident that the skills and experience acquired at the University of Florida will pave the way to sharpen her goal for her country.