INTEGRATED MANAGEMENT IN TISSUE CULTURED *Musa* spp. AGAINST FUSARIUM WILT DISEASE TROPICAL RACE 4 (VCG 01213)

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by

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LIST OF ABBREVIATIONS

A	Adenine
AAS	Atomic Absorption Spectroscopy
AFLP	Amplified Fragment Length Polymorphism
AM	Arbuscular Mycorrhizal
ANOVA	Analysis of Variance
AOS	Active Oxygen Species
ATP	Adenosine Triphosphate
A_{260}/A_{290}	Absorbance at 260/290 wavelengths
B	B genome (*Balbisiana*)
BAP	Benzylamino-6-purine
BBTV	Banana Bunchy Top Virus
B.C	Before Christ
BCA	Biocontrol Agents
BLS	Black Leaf Streak
B.P	Before Present (Present is A.D 1950 – anno Domini)
bp	Base pair
BW	Bunch Weight
C	Cytosine
Co.	Company
CARBAP	Centre Africain de Recherche sur Bananiers et Plantains
CLA	Carnation Leaf Agar
Cm	Centimetre

C: N	Carbon Nitrogen Ratio
ºC	Degree Celsius
C_3	Three Carbon Compound
DAMD	Direct Amplification of Minisatellite-region DNA
DaRT	Diversity Arrays Technology Markers
DNA	Deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
DOA	Department of Agriculture
E	East
EAHB	East African Highland Banana
E.C	Enzyme Commission
EDTA	Ethylenediaminetetraacetic acid
EMBRAPA	Empresa Basileira de Pesquisa Agropecuária
et al.	*et alia* (and others)
EtBr	Ethidium Bromide
F	Number of Fingers
FAA	Formaldehyde-Acetic acid - Alcohol
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	Food and Agriculture Organization Statistics
FHIA	Fundación Hondurena de Investigación Agricola
FLH	Functional Leaves at Harvesting
Foc	*Fusarium oxysporum cubense*
f. sp.	*formae specialis*
g	Gram
G	Guanine

GC-MS	Gas Chromatography – Mass Spectrometry
GML	Ground Magnesium Lime
H	Hydrogen
HCl	Hydrochloric Acid
HSD	Honest Significant Difference
HYT	High Yielding Technology ®
H_2SO_4	Sulphuric Acid
IAEA	International Atomic Energy Agency
IBM	International Business Machines
IITA	International Institute of Tropical Agriculture
INIBAP	International Network for the Improvement of Bananas and Plantains
ISR	Induced Systemic Resistance
ISSR	Inter Simple Sequence Repeats
K	Potassium
K_2O	Potassium Oxide
L	Laevorotation
LF	Length of Fingers
LS	Linsmaier and Skoog
M	Molarity
$MgCl_2$	Magnesium Chloride
M.F	Moisture Factor
Mg	Magnesium
MS	Murashige and Skoog
MSAP	Methylation-Sensitive Amplification Polymorphism

N	Nitrogen
NAOH	Sodium Hydroxide
NH_4	Ammonium
NH_4F	Ammonium Fluoride
NRCB	National Research Centre for Banana
ºN	Degree North
O	Oxygen
P	Phosphorus
PC	Petiole Collapse
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
PGPR	Plant Growth Promoting Rhizobacteria
pH	*pondus hydrogenii* (quantity of hydrogen)
PHH	Pseudostem Height at Harvesting
PGH	Pseudostem Girth at Harvesting
ppm	Parts Per Million
P_2O_5	Phosphorus pentaoxide
QTL	Quantitative Trait Loci
RAPD	Randomly Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
ROS	Reactive Oxygen Species
Rpm	Revolutions per Minute
S	Sulphur
SAR	Systemic Acquired Resistance

SCAR	Sequence Characterized Amplified Regions
Sdn. Bhd.	‘Sendirian Berhad’ (Private Limited)
SEM	Scanning Electron Microscope
SH	Sulfhydryl
Si	Silicon
SI	Similarity Index
SNP	Single Nucleotide Polymorphisms
SPAR	Single Primer Amplification Reaction
SP	Splitting of Pseudostem base
SPSS	Statistical Package for the Social Sciences
SR4	Sub-Tropical Race 4
SSR	Simple Sequence Repeats
ºS	degree South
T	Thymine
Taq	*Thermus aquaticus*
TBA	Tertiary-Butyl Alcohol
TBE	Tris-Borate-EDTA
TCW	Third Comb Weight
TE	Trace Elements
TLD	Thermoluminescent Dosimeter
T_M	Melting Temperature
TR4	Tropical Race 4
UP Bhd	United Plantations Berhad
UPRD	United Plantations Research Department
USA	United States of America

USD	United States Dollars
UV	Ultra Violet
var.	*varietas* (variety)
VCG	Vegetative Compatibility Grouping
Vic	Vegetative incompatibility
VNTR	Variable Number of Tandem Repeats
WF	Width of Fingers
WP	Wettable Powders
YF	Yellowing of Lower Foliage

LIST OF SYMBOLS

°	Degree
%	Percentage
µL	Micro litre
µM	Micro molar
µmol	Micro mol
cm	Centimetre
g	gram
ha	Hectare
kg	Kilogram
L	Litre
m	Metre
mL	Millilitre
mM	Millimolar
$m^{-2}s^{-1}$	Metre squared per second
n	Number of Chromosomes
N	Normality
V	Volt
w/v	Weight over Volume
W	Watt

PENGURUSAN BERSEPADU DALAM KULTUR TISU *Musa* spp. TERHADAP PENYAKIT LAYU FUSARIUM TROPIKA RAS 4 (VCG 01213)

ABSTRAK

Kepentingan ekonomi pisang dinyatakan daripada pendapatan luar negara yang berharga melalui eksport dan juga merangkumi ekonomi keluarga luar bandar di seluruh dunia. Fenomena 'bananageddon' merupakan istilah yang dicipta untuk menggambarkan kesan kemusnahan oleh kulat yang terkenal *Fusarium oxysporum f. sp. cubense* (Foc) Tropika Ras 4 (TR4) yang melanda industri pisang di seluruh dunia. Tujuan penyelidikan ini adalah untuk menentukan kawalan strategi yang boleh dilaksanakan melalui pengurusan bersepadu terhadap penyakit layu fusarium tropika ras 4 (VCG 01213). Bahan kultur tisu empat kultivar pisang, iaitu Berangan Intan (AAA), Grande Naine (AAA), Williams (AAA) dan Lemak Manis (AA) dipindahkan ke 'kawasan khas' Foc untuk menilai variasi somaklonal melalui tekanan pemilihan yang berulang sehingga tanaman layu dan daun terkulai atau sehingga tanaman dituai. 'HYT Chitinase' digunakan semasa penanaman dan juga semasa selang penananam di sepanjang pertumbuhan tanaman pisang. Penanaman selingan kultur tisu pisang dengan *Allium* spp. diuji di 'kawasan khas' Foc dan juga ditanam bersama di dalam polibeg sebelum ditanam di 'kawasan khas' Foc untuk memberikan toleransi penyakit. 'HYT Chitinase' tidak berjaya memberikan toleransi terhadap empat kultivar pisang yang dinilai di 'kawasan khas' Foc. Penanaman *Allium tuberosum* di dalam polibeg bersama kultur tisu pisang Berangan Intan telah memberikan toleransi terhadap Foc. Namun, *Allium* spp. perlu ditanam sekurang-kurangnya sebanayak 3 generasi di 'kawasan khas' Foc untuk mendapatkan tahap memadai insiden layu Fusarium. Kultivar Lemak Manis yang sebelum ini dilaporkan sebagai kultivar toleran 20 tahun yang lalu melalui pemilihan rintangan yang berulang adalah disahkan rintang terhadap penyakit layu Fusarium. Pusingan kedua pemilihan rintangan menghasilkan 8% Berangan Intan yang rintang. Sulur pisang Berangan ini diuji menggunakan teknologi kultur tisu dan pemilihan rintangan yang ketiga mengesahkan varian somaklonal toleran Intan berjaya diperoleh apabila varian ini bermandiri pada 78%. Penanda molekul mempamerkan polimorfisma sebanyak 20% - 36.4% di antara Berangan Intan dan varian toleran Intan. Kajian mikroskopi menunjukkan salur xilem yang lebih besar pada varian somaklonal toleran Intan manakala SEM mempamerkan penghasilan tilosa pada kedua-dua kultivar Lemak Manis dan varian toleran Intan mengesahkan mekanisme pertahanan yang serupa terhadap layu Fusarium pada tanaman pisang.

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ABSTRACT

The economic importance of bananas is expressed in valuable foreign revenue through export and also constitutes the economic livelihood of rural households worldwide. The ‘bananageddon’ phenomenon is a term coined to describe the decimation effect of an infamous fungus *Fusarium oxysporum f. sp. cubense* (Foc) Tropical Race 4 (TR4) that plagues the banana industry globally. The aim of this research was to determine a feasible control strategy in battling Fusarium wilt disease Tropical Race 4 (VCG 01213) through integrated management. Tissue culture materials from four cultivars, namely Berangan Intan (AAA), Grande Naine (AAA), Williams (AAA) and Lemak Manis (AA) were transferred to a Foc hotspot field to determine somaclonal variation through recurrent resistance selection until the plants withered and collapsed or until harvest. HYT Chitinase was applied during planting and also during intervals throughout the growth of the banana plants. Intercropping of banana tissue culture materials with *Allium* spp. was field tested as well as planted inside potted banana materials prior to field planting to confer disease tolerance. HYT Chitinase was not able to neither induce resistance nor confer tolerance for the four banana cultivars evaluated at the Foc hot spot area. The planting of *Allium tuberosum* inside the potted banana cultivars prior to field planting did to a certain extent confer tolerance to Berangan Intan cultivar. But, *Allium* spp. needs to be established for at least 3 generations in a Foc hot spot area in order to obtain negligible levels of Fusarium wilt incidences. Lemak Manis cultivar which was previously reported as a tolerant cultivar 20 years ago through recurrent resistance selection is resistant to Fusarium wilt disease. Second round of recurrent resistance selection produced 8% of tolerant Berangan Intan. The suckers were subjected to tissue culture technology and a third round of recurrent resistance selection confirmed the somaclonal variant tolerant Intan was successfully obtained when this variant survived at 78%. Molecular markers exhibited 20% - 36.4% polymorphism between the susceptible Berangan Intan and variant tolerant Intan. Ultrastructural studies revealed larger xylem vessels in somaclonal variant tolerant Intan whereas SEM exhibited tylose production in both the resistant Lemak Manis and variant tolerant Intan affirming its similar defence mechanism against Fusarium wilt of banana.

CHAPTER 1

INTRODUCTION

Fusarium wilt disease of banana (*Musa* spp.) is one of the most devastating diseases in agricultural history (Molina et al., 2016a). This disease was first reported in Panama, Central America in 1890, causing serious damage in its export plantation and since then has been interchangeably known as Panama disease (Ploetz, 2015a; Wang et al., 2017). *Fusarium oxysporum* Schlecht *f. sp. cubense* (E.F.Smith) Snyder & H. N. Hansen (Foc) is the asexual, root-invading soil borne causal agent (Czislowski et al., 2016a; Mostert et al., 2017). This pathogenic fungus invades through the radical and damaged rhizomes, migrating through the vascular bundles to the banana plant pseudostem and leaves, eventually causing the plant to collapse to its death, typically right after flowering (Czislowski et al., 2016b; Wang et al., 2017).

Since mid 19th century to the first half of the 20th century, this disease practically destroyed the agricultural, social, economy and political landscape in Central America where ‘Gros Michel’ cultivar (AAA genome) was cultivated primarily for export (Molina et al., 2016a; Mostert et al., 2017). The export trade heavily relied on the highly productive ‘Gros Michel’ cultivar up until 1960 but it was susceptible to race 1 of Foc (Ploetz, 2015b). Various Foc pathotypes are classified into 3 agronomically relevant races based on host cultivar affected – Race 1, Race 2 and Race 4 (Czislowski et al., 2016a, 2016b; Aguayo et al., 2017). Race 1 is the cause of the ‘Gros Michel’ epidemic (Ploetz, 2015b). When the costly shift to Foc race 1 resistant Cavendish cultivar (AAA genome) was adopted, Fusarium wilt vanished as a problem in export production and consequently saved the commercial banana industry from collapsing (Ploetz, 2015a; Molina et al., 2016b; Mostert et al., 2017). Not long after, a new threat emerged as Cavendish bananas were susceptible to Foc race 4, first in the subtropics and then in the tropics (Mostert et al., 2017). *Fusarium oxysporum f. sp. cubense* Race 4 is subdivided into 2 groups, Foc Subtropical Race 4 (SR4) and Foc Tropical Race 4 (TR4) where pathogenicity varies under different environmental conditions although being phylogenetically distantly related (Aguayo et al., 2017). Foc Tropical Race 4 severely affected Cavendish plantations in Taiwan, Indonesia and Malaysia in the 1990's. It had raised major concerns then and was intensified when the disease caused epidemics in Cavendish plantations in China and the Philippines come 21st century and more recently reporting incidences in the Middle East and Africa (Mostert et al., 2017). Foc tropical race 4 is a serious threat to global banana production (Molina et al., 2016a).

Historically, banana was mentioned in Ramayana (2020 B.C) and has always been an antiquity in India, the largest banana producing country in the world (Maharana et al., 2017). The generic name *Musa* is derived from the Arabic word *mouz* and appears in the Quran as the 'plant of paradise' (Robinson & Galán Saúco, 2010).

Banana the giant perennial herb is considered as the 'poor man's apple' in the tropical and sub tropical regions (Parkhe et al., 2018). It is ranked the fourth most widely consumed food crop throughout the world after rice (*Oryza sativa*), wheat (*Triticum spp.*) and maize (*Zea mays*), ranks among the top ten food commodities for Southeast Asia, Africa and Latin America and is the most popular fruit in the world. Around 85% of the global production is destined for local consumption whereas the balance 15% is for the international trade (Akila et al., 2011; Ordonez et al., 2015). The Musaceae is a family of monocotyledons which, through natural events and human selection and domestication over time immemorial, have come to hold a strong cultural significance in many countries where the diverse *Musa L*. species are grown (Moore et al., 2001).

1.1 Bananas in Malaysia

In Malaysia, banana production is an important economic revenue to the country and was ranked as the fifth major fruit export after melon, papaya, durian and star fruit (Zaffari et al., 2001). Banana plantations cover an estimated 26, 000 hectares of land area, which constitutes over 11% of the total fruit growing areas in the country (Suhaimi et al., 2017). Banana is recognised as one of the fruit types of special attention under the Economic Transfer Programme by the Malaysian government (Zulperi et al., 2014).

United Plantations Berhad (UP Bhd) is a Malaysian based oil palm plantation company principally in the business of oil palm, coconut and banana cultivation in a sustainable manner. The Company possesses considerable expertise and skills in plant breeding, agronomy and micro-propagation through its Research and Development facilities for the development of new and improved planting materials (United Plantations Berhad, 2018).

The Research Department (UPRD) was established in 1962 and banana micro-propagation started in 1988 at the Tissue Culture Laboratory, with Pisang Mas (AA) and Pisang Berangan (AAA). The first batch of ramets was field planted in 1987. Initially, the ramets were field planted to observe the agronomic traits of the planting materials produced from the tissue culture laboratory and selection of suckers for commercial production of banana planting materials. However, by 1989, Fusarium wilt became prevalent to the extent it destroyed the trial block. A concerted effort was pursued to combat the disease. In 1990, United Plantations Berhad embarked on a collaborative work with the International Atomic Energy Agency (IAEA), Austria and thereafter with Nuclear Technology Unit in Malaysia to explore the use of gamma irradiation to generate variability through mutation techniques for disease resistance. However, all the plants in this trial succumbed to Fusarium wilt disease by 1992. Since then, United Plantations Berhad has done several field evaluations on the same plot of land now referred to as ‘Foc hot spot area' through selection pressure. The surviving banana suckers which were more Fusarium tolerant was then brought in for tissue culture and besides commercialization; are replanted in the Foc hot spot area for further improved selection and the cycle is repeated. Following evaluations over the years, the following tolerant varieties has been identified and commercialized; Grande Naine (AAA) under the trade name ‘Novaria’, UP-Rastali (AAB) under the trade name ‘Mutiara’ and a diploid local dessert variety ‘Lemak Manis’ (AA) (Ho et al., 2001).

Pisang Berangan cultivar was patented and trade-named Intan in 2002. Hence, thereon it has been known as UP Bhd’s ‘Berangan Intan’ cultivar. This cultivar generally still succumbs to Fusarium wilt disease. Nonetheless, due to its popularity within the country, 90% of saleable ramets are from this cultivar. They are sold in the form of bare-root to nursery operators as well as acclimatized banana ramets in perforated polybags, ready for field planting are sold throughout the nation. The banana suckers of this banana cultivar which is planted within the Company, is selected, cloned, re-cloned and replanted as an intercrop planting with oil palm (Plate

1.1) and coconuts for several generations to improve its agronomic characteristics such as improved growth vigour, early flowering, uniform growth and bunch production, high bunch weight and fruits with clean peel (Singh & Ho, 2000).

Plate 1.1: **Berangan Intan intercrop planting with oil palm in United Plantations Berhad.**

1.2 Rationale of Study

Numerous researches had been carried out in battling the Fusarium wilt disease of banana over the last three to four decades. Various control strategies amongst them soil fumigation, fungicides, crop rotation, flood fallowing, heat sterilization, ground baring, N fertilization and organic amendments had been evolved and attempted (Ploetz, 2015b). None of the control strategies were effective. Hence, control against this virulent pathogen was confined to resistant or tolerant cultivars (Javed et al., 2004). However, these resistant or tolerant cultivars do not possess the marketability as commercial clones.

It is therefore hypothesized that a holistic approach in battling the disease through resistance selection for somaclonal variant towards Foc TR4 in popular local cultivars using tissue culture banana planting materials, in addition to intercropping systems and use of microbial based enhancement products may be effective control strategies.

1.2.1 Research objectives

The current study was conducted with the following objectives:

i. To assess the abiotic and biotic stresses at the Fusarium hot spot area,
ii. To investigate the effect of High Yield Technology (HYT Chitinase) efficacy on the suppression of Fusarium wilt disease,
iii. To study the effect of *Allium* spp. to control Fusarium wilt disease when intercropped with banana in the field and in nursery,
iv. To determine somaclonal variant towards Fusarium wilt disease tolerance of four different *in vitro Musa* spp. through resistance selection of Berangan Intan (AAA), Cavendish subgroup medium-dwarf Grande Naine (AAA), Cavendish subgroup tall Williams (AAA) and Lemak Manis (AA),
v. To detect polymorphism between somaclonal variant tolerant Intan and susceptible Berangan Intan using DAMD-DNA and ISSR markers,
vi. To analyse the ultrastructural characteristics between the resistant, susceptible, tolerant and somaclonal variant banana cultivar using histology and scanning electron microscopy (SEM).

CHAPTER 2

REVIEW OF LITERATURE

2.1 Botany of *Musa* spp.

The banana plant is a large, monocotyledonous, perennial herbaceous plant consisting of a branched, underground rhizome with roots and vegetative buds, and an erect *pseudostem* composed of tightly clasping leaf bases (Plate 2.1) (Stover & Simmonds, 1987). The leaves are very large and slightly waxy. The plant can spread via rhizomes, which is important in the propagation of these plants (Smith, 2015). The banana plant differs considerably from the majority of horticultural plants. It is herbaceous because the aerial parts die down to the ground after the fruit is harvested and there are no woody components. It is perennial because new suckers grow out of the base of the mother plant to replace the aerial parts which have died (Robinson & Galán Saúco, 2010).

The main function of the apical meristem is to build the inflorescence. The leaves are differentiated in regular succession during the vegetative phase. The leaf initials take the form of fitting cones which ultimately become the sheaths (Stover & Simmonds, 1987). At the transition from the vegetative to the floral stage, the meristem area becomes convex and rises above the surrounding leaf base. Then, flower bracts, appear in place of leaves several at a time. Once flowering has completed, the pseudostem dies, and new plants develop from suckers that arise freely from the underground rhizome (Stover & Simmonds, 1987). Generally, the plant crop begins to flower 180 - 210 days after planting. Shoot to harvest time is strongly influenced by temperature. Average flowering to harvest time of 70 - 125 days covers most tropical areas below 500 m altitude, and 110 - 250 days includes most subtropical areas (Stover & Simmonds, 1987).

Root systems are confined mostly to the upper 40 cm of the soil. There was a good correlation between bunch weight and quantity of roots produced. In general, alluvial soils had the best root growth. The laterally spreading roots can extend to 5.2 m from the plant descending commonly to a depth of 75 cm (Stover & Simmonds, 1987).

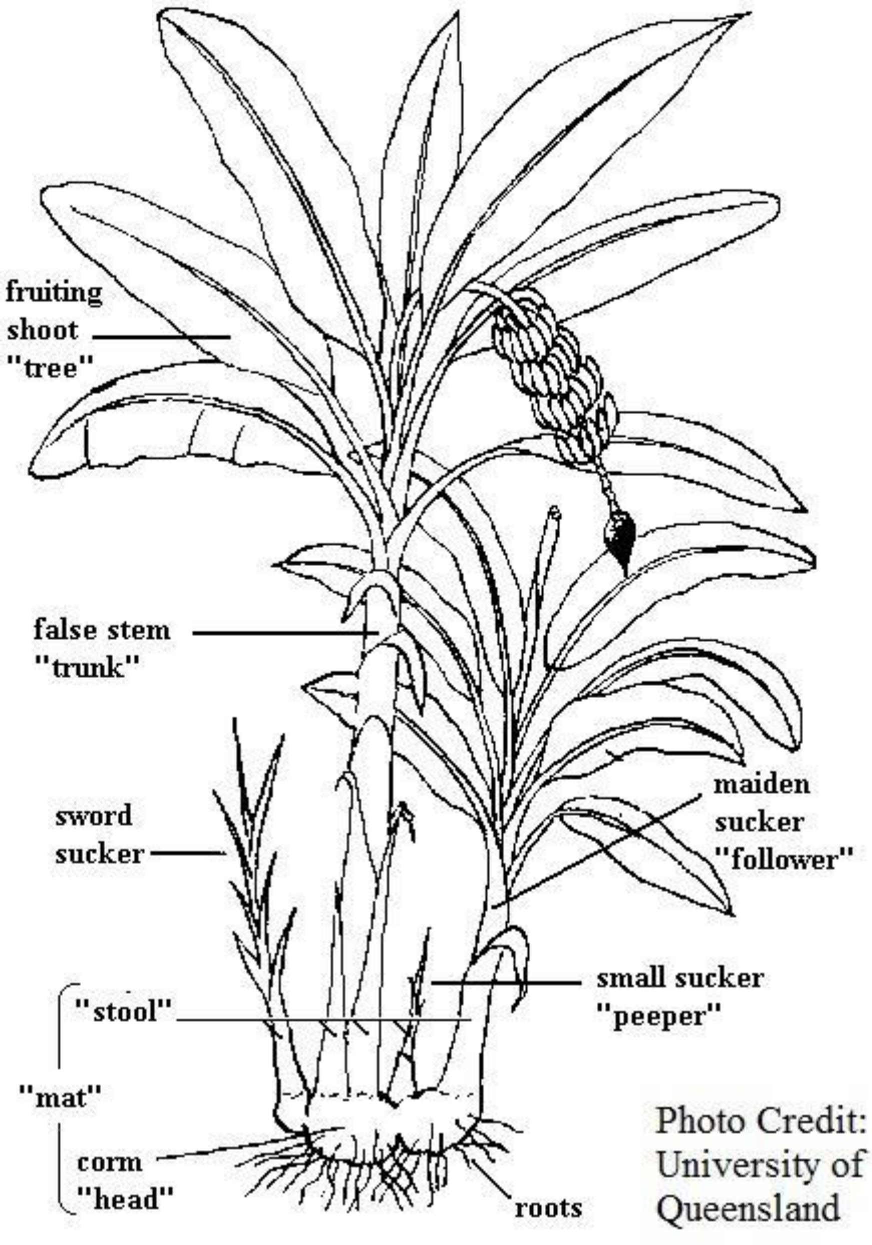


Plate 2.1: Drawing of a banana plant (University of Queensland, 2018).

2.1.1 Taxonomic classification

Bananas belong to the genus *Musa*, and form part of the Musaceae family in the order of *Zingiberales* (Simmonds, 1962). The genus *Musa* is composed of four taxonomic sections, namely *Australimusa, Callimusa, Musa* (formerly known as *Eumusa*) and *Rhodochlam* according to the number of chromosomes (Vilela et al., 2014). Thus, the genome with eleven chromosomes (2n = 22) is characteristics of *Musa* and *Rhodochlamys*, while ten chromosomes (2n = 20) is found in *Callimusa* and *Australimusa*. *Musa* is the largest and the most geographically widespread taxonomic section (Pereira & Maraschin, 2015).

Cultivated bananas are triploids cultivars originating from intra- and inter-specific hybridization between two diploid wild species of the section *Musa*: *Musa acuminata* (A genome) and *Musa balbisiana* (B genome) (Vilela et al., 2014; Ploetz, 2015a). Only two of the recognized 50 sub groups of banana produce most of these fruit, which is the Cavendish subgroup (over 40% of the global total), and the Plantain subgroup, which is next in importance, is responsible for an additional 21% (Ploetz et al., 2015).

2.1.2 Nomenclature

There are numerous names given to banana cultivars governed by morphological diversity and socio-linguistic diversity of the human population worldwide (Crichton et al., 2016). There are many synonyms (different names for the same cultivar) and homonyms (same name for different cultivars). The commercial cultivar ‘Poovan’ (AAB genome, Mysore subgroup) is synonym to ‘Rastali’ in India, whereas ‘Lady’s Finger’ is used as a homonym for Pome (AAB genome) cultivar in India and ‘Sucrier’ (AA genome) in Hawaii (Crichton et al., 2016).

2.2 Importance of banana plant

2.2.1 Banana as a food source

Banana is vital for food security in many tropical and subtropical countries and is the most popular fruit in industrialized countries (D’Hont et al., 2012). Dessert bananas form 43% of the world’s production of bananas and are eaten raw when ripe. The pulp is firm when unripe and upon maturation, the pulp becomes soft (Pereira & Maraschin, 2015). The ripe banana is utilized in a multitude of ways in the human diet. The fruit can be sliced and served in fruit cups and salads, sandwiches, custards and gelatins, banana boat ice cream, and being mashed and incorporated into bread, muffin and cakes (Abiodun-Solanke & Falade, 2011).

Bananas are a rich source of vitamins and minerals. It is also rich in fibre and carbohydrates, and low in fats with a caloric value of 67 calories / 100 g of pulp. It is also noted for its high nutritional value especially in potassium and folic acid which is needed during pregnancy. Bananas contain minerals such as calcium, phosphorus, nitrogen, magnesium and vitamins such as vitamin A, vitamin C, and vitamin B complexes which help to repair and regenerate body tissues (UNCTAD, 2016; Moradi et al., 2017; Sunarta & Trigunasih, 2017). The bananas contribute to the proper functioning of the energy metabolism and the nervous system and to maintain good digestive tract (UNCTAD, 2016).

2.2.2 Other uses

The banana fruit or pulp is used to make other industrial products such as ethyl alcohol and dye (DOA Philippines, 2011). There are many other uses that can be derived from other parts such as the leaves, pseudostem and peels. The most prevalent uses irrespective of plant parts has been for mulch and compost, feed for livestock, construction materials, ropes for tethering small ruminants and play items (Ngomuo et al., 2014). In a 2011 report by DOA Philippines, it was mentioned that the peel is used to make floor wax, paste and cork board. Banana pseudostem fibre has also been used for arts and crafts. The juice extracted from the stem is used in making paper bond and tissue paper (Ngomuo et al., 2014; Parida et al., 2017).

Besides that, banana waste has been utilized for the production of biogas, starch, biomass and lactic acid and lignolytic enzymes to generate energy (Gabhane et al., 2014). Lastly, banana plants may also be used as an ornamental plant (UNCTAD, 2016; Parida et al., 2017).

In some civilisations, banana is culturally interwoven in its heritage and all parts of this plant are auspicious in festive occasions and its social function or worship of gods (Ngomuo et al., 2014; Moradi et al., 2017).

2.3 Banana production worldwide

Banana is a staple food for nearly 400 million people across the globe and comprises about 10% of more than 80 million tonnes of food produced annually. It is a commodity that contributes to the food security of millions of people in the developing world, and when traded in local markets provide a means of income and employments in rural populations (Hussein, 2012; Moradi et al., 2017).

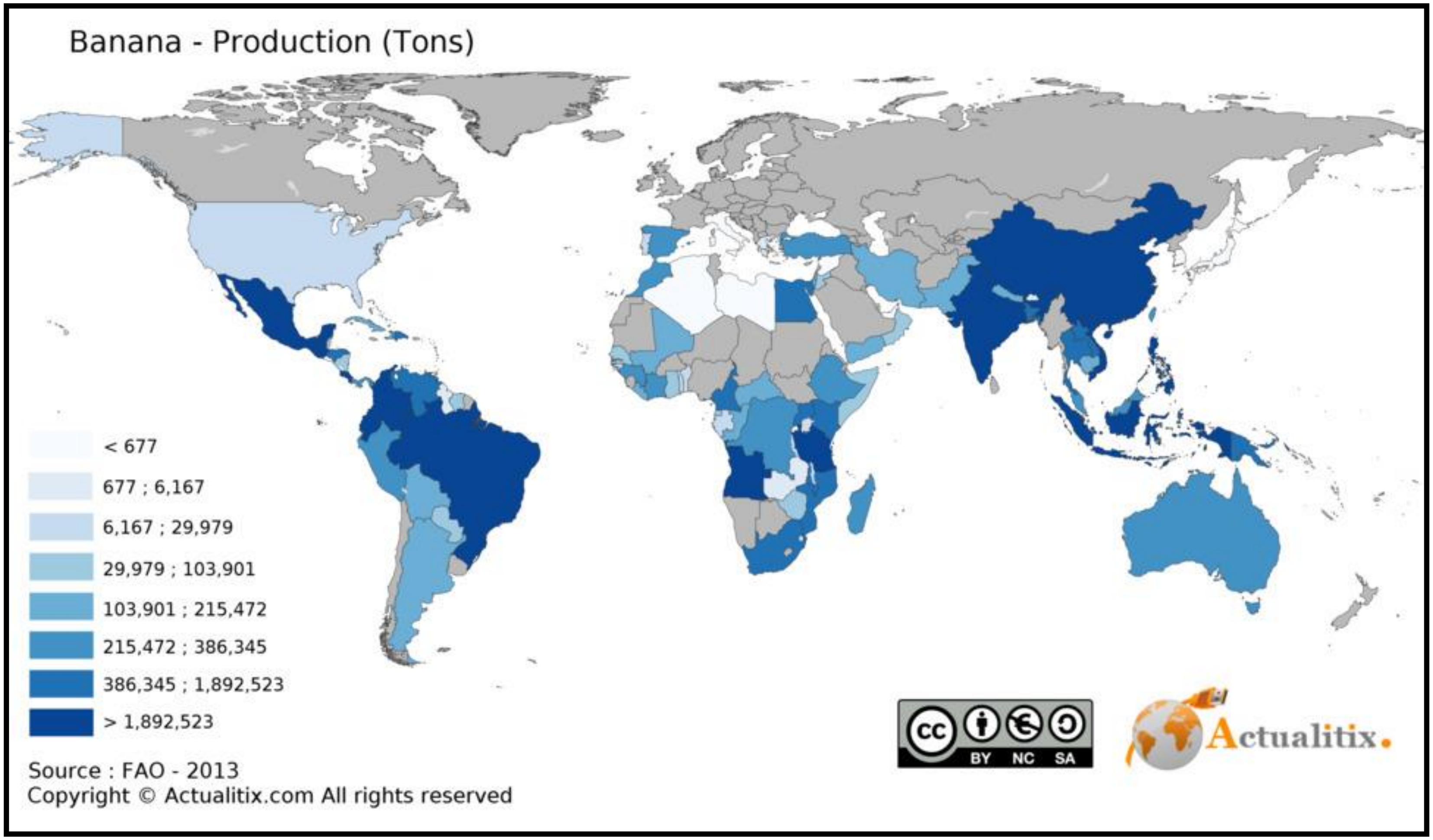
Banana and plantains are cultivated in more than 130 countries throughout the tropical and subtropical regions, over a harvested area of approximately 10 million hectares (Vilela et al., 2014). The annual world production is about 145 million tonnes (106 million tonnes for banana and 39 million tonnes for plantain) (FAO, 2017). Asian continent has the world's highest banana production and consumption (FAO, 2017). More than 150 banana cultivars are grown in Asia for domestic consumption and export, making up to 45% of all bananas grown globally (FAO, 2017). India is the largest producer at 29.7 million tonnes, with Maharastra and Gujarat together contributing 27% (Ghag & Ganapathi, 2017).

China is the world's largest producer of Cavendish bananas, with an annual production of approximately 11 million tonnes. Other major banana producers in Asia include the Philippines (8.9 million tonnes) and Indonesia (5.4 million tonnes). In 2011, 107 million metric tons of bananas were produced on 0.1 per cent of the world's agricultural area, with a total trade value of US$9 billion and a retail value of approximately US$25 billion (FAO, 2017).

Table 2.1 ranks the countries according to hectarage dedicated to banana cultivation worldwide. None of the fruit from these countries reach global markets with the exception of the Philippines and Ecuador (Ploetz et al., 2015a). Plate 2.2 illustrates the worldwide distribution of the banana belt cultivation area.

Table 2.1: Top ten banana producing countries according to hectarage dedicated to banana cultivation worldwide (FAO, 2013).

No.	Country	Banana Production (tonnes)
1	India	27,575,000
2	China	12,370,238
3	Philippines	8,645,749
4	Brazil	6,892,622
5	Ecuador	5,995,527
6	Indonesia	5,359,115
7	Guatemala	3,188,050
8	Angola	3,095,013
9	Tanzania	2,678,680
10	Burundi	2,235,697



Source : FAO - 2013

Plate 2.2: Map of banana producing countries in the world (FAO, 2013).

2.4 Banana import and export

The banana fruit is dubbed the world's most invaluable primary agricultural commodity (Ploetz, 2015b). The banana plant is considered as a lucrative cash crop yielding satisfactory returns (Ghag & Ganapathi, 2017). Bananas have a high rate of domestic consumption, with only about 17% of bananas exported to foreign markets annually. About two-thirds of bananas are exported from Latin America, with about the same amount destined for Europe and the United States (FAO, 2013). Cavendish bananas constitutes most of the total world export trade of 15 million tonnes amounting to about US$4.8 billion for exporting countries (Ghag & Ganapathi, 2017).

While most of Asia's bananas are consumed locally, the region also produces bananas for export. The Philippines is the world's second largest exporter, responsible for approximately 18% of all export fruit produced globally (FAO, 2012). Cultivars grown in Asia differ greatly from one country to another, depending on adaptability and market preferences. India, China and the Philippines have extensive Cavendish industries, whereas countries such as Thailand, Malaysia and Indonesia grow many local varieties (FAO, 2012).

Global banana exports were on an uphill trend from 2010 hitting almost 20 million tonnes until 2015. The decline in 2015 was largely due to the effects of *El Nino* as well as the spread of the systemic Fusarium wilt disease especially in the Philippines (FAO, 2017). Asian exports declined by 46% in 2015 due to the production drop experienced in the Philippines, the largest exporter in the region, which accounts for some 90% of the total export volume from Asia (FAO, 2017).

2.5 Production constraints

Since the 1990s, sustainable banana production has been promoted in order to be economically feasible, environmentally safe and socially acceptable (UNCTAD, 2016). However, there are five primary factors encompassing diverse biotic and abiotic factors that affect yields in export and smallholder productions, namely soils and plant nutrition, climatic conditions, drainage and irrigation, pest and disease as well as type of cultivar (Ghag & Ganapathi, 2017; Ploetz et al., 2015a). Soil nutrient losses due to increased banana commercialization acutely decline good quality bananas (Kahangi, 2010). Nonetheless, the main constraint to low banana productivity and high yield losses are pests and diseases, irrespective of region and production systems (Blomme et al., 2017).

It is imperative to tackle production constraints and ensure food security and sustainability of bananas although they are one of the least genetically improved crops in the world. Hence, biotechnological strategy for pests and disease tolerant, abiotic stress tolerant plants and increase productivity on available arable land is exploited (Ghag & Ganapathi, 2017).

2.5.1 Abiotic stress

Optimally, banana plants grow in crumbly and thick fertile soils with lots of humus, good aeration and drainage as well as sufficient water (Sunarta & Trigunasih, 2017). Abiotic stresses encroach where these conditions are compromised. The stresses include soil fertility, high temperature rates, cold weather, drought and salinity (Atkinson & Urwin, 2012).

2.5.1(a) Climatic requirements and problems

In tropical regions, climate change is a challenge for optimum crop productivity (Ghag & Ganapathi, 2017). Climatic events refer to droughts, heavy rainfall, and periods of low sunshine, cold fronts and winds. Climatic disruptions are generally caused by two worldwide phenomena, the *El Nino* and *La Nina* phenomenon. *El Nino* manifested every 2 to 7 years is characterised by increased temperatures in the south-east Pacific Ocean. These increased temperatures intensify droughts, tornadoes and cyclones. The reverse is characterised by the cooling of the Pacific waters (UNCTAD, 2016).

The major banana growing areas of the world are geographically situated between the Equator and latitudes 20°N and 20°S. Climatic conditions in these areas are mainly humid tropics with uniform warm climate (Robinson et al., 1993). Increased temperatures due to climate change disrupts water demand in this water loving evergreen, shallow rooted crop, incidentally affecting yield and productivity (Ghag & Ganapathi, 2017).

The banana plant requires temperatures between 25°C to 40°C with high levels of sunshine and approximately 180 mm of precipitation per month (UNCTAD, 2016). Temperatures below 10°C and above 38 °C give rise to growth cessation and physiological problems (Robinson et al., 1993). Banana plants also prefer a more sheltered environment to protect against high winds and prevent tattered leaves (Smith, 2015).

2.5.1(b) Site selection and soil requirements

Banana plants can grow in many places, from low grounds until highlands in various types of soil (Sunarta & Trigunasih, 2017). In the tropical countries, site selection is largely based on soil classification. ‘Banana soils’ are best grown in deep, well drained loams with high water holding capacity, high fertility and organic matter with less or without acidity and salinity. Whereas in the subtropics and the Mediterranean areas, site selection is largely based on long-term temperature data (Stover & Simmonds, 1987; Robinson et al., 1993). The most important key factor is soil pH as it plays a major role in nutrient efficiency which is often related to banana production (Segura et al., 2018).

2.5.1(c) Nutritional requirements

Banana requires large quantities of nutrients for its growth, development and yield. These requirements are mostly supplied by inorganic fertilizers which have adverse effects on soil, water and environmental conditions. Organic fertilizers are more consistent with slow release of nutrients, maintains ideal C: N ratio, improves water holding capacity and microbial biomass of soil profile. Every year, 50 tonnes of banana fruit in one hectare approximately removes 320 kg N, 32 kg P_2O_5 and 925 kg K_2O. Although inorganic fertilizer application is able to increase yield substantially, it is not able to sustain soil fertility status leaving adverse residual effects that eventually declines productivity in the long run. Regular, excessive and unbalanced usage of chemical fertilizers may cause health and ecological hazards, depletion of physico-chemical properties of soil and ultimately low yields (Hussain et al., 2017).

2.5.1(d) Water requirement and irrigation

Apart from temperature, rainfall is a pre-determinant where bananas and plantains may be grown. The crop has a high water demand, and 25mm / week is regarded as the minimum for satisfactory growth (Robinson & Galán Saúco, 2010). The humid tropics may receive a well distributed rainfall throughout the year of between 2500 mm to 4500 mm (Robinson et al., 1993).

Although the banana plant is a water-efficient C_3 crop, it still requires large amounts of water (Kissel et al., 2016). Tile drains, mechanically dug trenches or ridges and furrow planting systems are constructed in waterlogging areas. Conversely, lack of water causes salinity issues and periodic over irrigation may be done to leach salts out of the root zone (Robinson et al., 1993).

2.6 Biotic constraints

In general, plants are infected by insects, nematodes, viruses, bacteria, fungi and oomycetes and are mostly saturated at the aerial plant parts that include leaves, stems, flowers and fruits. Besides these areas, organisms also evolve to colonize below ground organs such as roots and tubers (Yadeta & Thomma, 2013). Due to monoculture cultivation, these diseases spread rapidly causing epidemics and are capable of causing famine in major banana producing countries (Ghag & Ganapathi, 2017).

Main biotic stresses to the banana plants include Fusarium wilt, Black Sigatoka, Yellow Sigatoka, Bunchy top virus, burrowing nematode and weevil borer (Atkinson & Urwin, 2012). Amongst these, Black Sigatoka and Fusarium wilt of banana are major global constraints on production worldwide (Dita et al., 2010).

2.7 Fusarium wilt of banana

The Fusarium wilt disease is a serious threat to a sustainable global banana industry as it occurs worldwide in all major banana producing countries (Wang et al., 2017).

The first description of Fusarium wilt of banana was published in 1876 by Joseph Bancroft who described diseased plants of the 'Sugar' (Silk / Pisang Rastali AAB) variety observed in Brisbane, Australia. The disease was fatal to 'Silk' and could not be effectively controlled and went on to destroy plantations of the susceptible Lady Finger cultivar (AAB) in Southern Queensland (Smith et al., 2008). It was Erwin Smith, who in 1910, recovered a fungus from diseased banana tissue from Cuba and named it *Fusarium cubense* E.F. Smith (Moore et al., 1995).

2.7.1 The pathogen

The word Fusarium is derived from the Latin word *fusus*, meaning 'spindle', i.e., a large genus of filamentous fungi found in soil and in association with plants (Dwivedi & Dwivedi, 2016). The life cycle of *Fusarium oxysporum* strains includes a saprophytic and a parasitic phase (Pan et al., 1997).

Fusarium oxysporum f. sp. cubense (Foc) is a member of the *F. oxysporum* species complex. The *Fusarium oxysporum* species is composed of a suite of asexual, morphologically similar, pathogenic and non-pathogenic, plant and animal pathogens. Plant pathogens often exhibit host specificity (Ordonez et al., 2015; Ploetz et al., 2015b).

This character is represented by variation of race and strain and then level of virulence (Riska & Hermanto, 2012; Ordonez et al., 2015). Further classifications of Foc populations have been analysed through vegetative compatibility group (Neill et al., 2011). The pathogen co-evolved with *Musa* spp. at the centre of its origin in Asia, although its notoriety was gained from across the other side of the globe (Ploetz, 2015a; Ordonez et al., 2015).

2.7.2 Taxonomic classification

The morphological taxonomy of species in the genus *Fusarium* is based primarily on the structure and abundance of asexual reproductive structures (chlamydospores, phialides, microconidia and macroconidia) and on cultural characteristics (colony texture, colour and cultural aroma) (Fourie et al., 2011; Azam et al., 2017).

Fusarium oxysporum is characterized mainly by non-septate microconidia formed in false heads on short monophiliades, 3-septate macroconidia formed from monophiliades on branched conidiophores in sporodochia, and chlamydospores with a smooth or rough wall appearance formed singly or in pairs (Fourie et al., 2011).

Once it invades its hosts, *Fusarium oxysporum* can persist in affected fields for an extended period of time on plant surfaces as macroconidia, or even survive on