Development of Protocols for Production of Clonal Planting Materials of Two Commercially Viable *Musa* Cultivars, Nutritional Assessment and Genetic Fidelity Assessment of Regenerates

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Submitted By

Thejano Ngullie

Ph. D. REGN NO.: Ph. D/BOT/00155 dated August 18, 2018

Department of Botany

School of Sciences, Nagaland University

Lumami 798627, Nagaland

Nagaland, India

December, 2024

Development of Protocols for Production of Clonal Planting Materials of Two Commercially Viable *Musa* Cultivars, Nutritional Assessment and Genetic Fidelity Assessment of Regenerates

Submitted By

Thejano Ngullie
Department of Botany

Name of the Supervisor
Prof. Chitta Ranjan Deb

Submitted

In Partial Fulfillment of the Requirement of the Degree of Doctor of Philosophy in Botany of Nagaland University

CONTENTS

Chapter	Title	Page No.
	Certificate and Declaration	i-ii
	Acknowledgment	iii
	Plagiarism Self Declaration Certificate and report	iv-v
	Abbreviations	vi-vii
	List of Tables	viii
	List of Figures	ix - x
1	Introduction	1-24
2	Nutritional Assessment of Different Parts of Raw and Ripe Fruits of Two Commercially Viable Banana Cultivars 'Grand Nain' and 'Sabri'	25
	Graphical Summary	25
	Introduction	26-27
	Material and Methods	27-31
	Results	31-38
	Discussion	39-43
	Conclusions and Summary	44
3	Macropropagation in Banana Cultivars Grand Nain and Sabri	45
	Graphical Summary	45
	Introduction	46-47
	Material and Methods	48-54
	Results	55- 74
	Discussion	74-82
	Conclusions and Summary	82-83
4	*In Vitro* Micropropagation in Banana Cultivars Grand Nain and Sabri	84
	Graphical Summary	84
	Introduction	85-86
	Material and Methods	86-89
	Results	89-113
	Discussion	114-121
	Conclusions and Summary	122-123
5	Summary and Conclusions	124-128

	References	**129-152**
	List of Publications	**153**
	Screen shots of Published Papers	**154-156**
	List of Seminar, Conferences Attended and Presented Papers	**157-158**
	Particulars of the Candidate	**159**

नागालैण्ड विश्वविद्यालय

Nagaland University

(A Central University Estd. By the Act of Parliament No. 35 of 1989)

Lumami 798 627, Nagaland, India

Department of Botany

Certificate

December 12, 2024

I, Prof. Chitta Ranjan Deb, hereby certify that the subject matter of this Thesis is the record of work done by the Scholar and the contents of this Thesis did not form the basis for the award of any research degree to the best of my knowledge. Further, this is to certify that the thesis has not been submitted by her for any research degree in any other University/Institute.

This is Ph. D. thesis being submitted to Nagaland University for the Degree of Doctor of Philosophy in the Department of Botany.

(Prof. Chitta Ranjan Deb)
Supervisor
Prof. Chitta Ranjan Deb
Department of Botany
Nagaland University
Lumami-798627. Nagaland, India

नागालैण्ड विश्वविद्यालय

Nagaland University

(A Central University Estd. By the Act of Parliament No. 35 of 1989)

Lumami 798 627, Nagaland, India

Department of Botany

DECLARATION

December 12, 2024

I, Ms. Thejano Ngullie, hereby declare that the subject matter of this Thesis is the record of work done by me, that the contents of this Thesis did not form basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/Institute.

(Thejano Ngullie)

Scholar

(Prof. Chitta Ranjan Deb)

Supervisor

Prof. Chitta Ranjan Deb
Department of Botany
Nagaland University
Lumami-798627. Nagaland, India

(Prof. Talijungla)

Head, Dept. of Botany

Head
Department of Botany
Nagaland University
Lumami- 798627

Acknowledgement

I would like to honor my Almighty God for His unwavering love and guidance, which have sustained me throughout this journey.

I am deeply grateful to my supervisor, Prof. C.R. Deb, for his constant support, invaluable guidance, patience, and financial assistance throughout this research. His mentorship has been instrumental in shaping the direction of this work, and I am truly thankful for the opportunity to work under his expertise.

I extend my sincere thanks to the Head of the Department of Botany and the entire faculty, both teaching and non-teaching staff, for their continuous support and encouragement during my research.

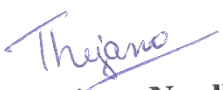
This research would not have been possible without the generous financial and institutional support provided by the UGC NON-NET, GBPNIHESD Sponsored NMHS R&D Project, DBT Sponsored Advance Level Institutional Biotech Hub, UGC-SAP (DRS-III), DST FIST (Level 1), and the Botany Department, Nagaland University. I am truly appreciative of the resources and facilities they provided, which were crucial for the success of this research.

I would also like to express my heartfelt gratitude to my seniors, Dr. Bendangnaro Jamir, Dr. Temjenokcha B. Longchar, Dr. Aolemla Pongener, and my Biotech lab colleagues Dr. Maman Megu, Ms. Bendangsenla Pongener, Dr. Mum Tatung, Ms. Ibeeka Sharma, Mr. Thejavitsii Noah Vuprii, Ms. Rokono Nagi, Ms. Narola Jamir, Ms. Debashree, Mr. Kaushik, Mr. Mayang, and Mr. Puro for their guidance, support, and friendship throughout my research journey.

My sincere thanks also goes to Amu Hekato and Amu Shiwoto for their constant help and support.

I am profoundly grateful to my family, friends, Exodus Home, Releiki hostellers, and the NUCF family for their unwavering moral support, constant prayers, and for always believing in me. Their encouragement, love, and faith in my abilities have been a constant source of strength throughout this journey.

Finally, I would like to express my deepest gratitude to everyone who has contributed to the success of this work. This research would not have been completed without your unwavering support, and for that, I am truly thankful.



(Thejano Ngullie)

NAGALAND UNIVERSITY

Plagiarism Self Declaration Certificate

Name of Scholar	Thejano Ngullie	
Registration Number	Ph. D/BOT/00155	
Title of PhD Thesis	Development of Protocols for Production of Clonal Planting Materials of Two Commercially Viable *Musa* Cultivars, Nutritional Assessment and Genetic Fidelity Assessment of Regenerates	
Name &Institutional Address of the Supervisor/Co-Supervisor	Prof. Chitta Ranjan Deb, Nagaland University, Lumami-79862, Nagaland, India	
Name of the Department and School	Department of Botany, School of Sciences	
Date of submission	2024-12-12	
Date of plagiarism check	2024-12-04	
Name of the software used	DrillBit Plagiarism Detection Software	
Percentage of similarity detected by the software	**Core Areas**	4.73%
	Non-Core areas	3.63%

I hereby declare/certify that the Ph.D. Thesis submitted by me is complete in all respect, as per the guidelines of the UGC/NU for this purpose. I also certify that the Thesis (soft copy and print version) has been checked for plagiarism using DrillBit similarity check software. Copy of the Report generated by the software is also enclosed.

Thejano

(Thejano Ngullie)

Ph. D. Scholar

Place: Lumami

Date: 12/12/24

(Prof. Chitta Ranjan Deb)

Supervisor

Prof. Chitta Ranjan Deb
Department of Botany
Nagaland University
Lumami-798627. Nagaland, India

(Prof. Talijungla)

Head, Department of Botany

Head
Department of Botany
Nagaland University
Lumami- 798627

DrillBit

DrillBit Similarity Report

8	131	A
SIMILARITY %	MATCHED SOURCES	GRADE

A-Satisfactory (0-10%)
B-Upgrade (11-40%)
C-Poor (41-60%)
D-Unacceptable (61-100%)

LOCATION	MATCHED DOMAIN	%	SOURCE TYPE
1	spandidos-publications.com	<1	Internet Data
2	www.dx.doi.org	<1	Publication
3	moam.info	<1	Internet Data
4	Thesis Submitted to Shodhganga Repository	<1	Publication
5	Thesis Submitted to Shodhganga Repository	<1	Publication
6	www.dx.doi.org	<1	Publication
7	pdfcookie.com	<1	Internet Data
8	recentscientific.com	<1	Publication
9	kubanni.abu.edu.ng	<1	Publication
10	Thesis submitted to shodhganga - shodhganga.inflibnet.ac.in	<1	Publication
11	bioresources.cnr.ncsu.edu	<1	Internet Data
12	Thesis Submitted to Shodhganga Repository	<1	Publication
13	academicjournals.org	<1	Publication
14	alldokument.com	<1	Internet Data

Abbreviations

Abbreviation	Full Form
µM	Micro molar
µg	Micro gram
µl	Micro litre
abs	Absorbance
$AlCl_{3,}$	Aluminum Chloride
Avg	Average
BAP	Benzyl amino purine
CRD	Completely randomized design
CTAB	Cetyltrimethylammonium bromide
Cv	Cultivar
DF	Dilution factor
DNA	Deoxyribonucleic Acid
DNS	3,5-Dinitrosalicylic acid
dNTPs	Deoxynucleotide Triphosphates
DPPH	2,2-diphenyl-1-picrylhydrazyl
DW	Dry Weight
G9	Grand Nain
GAE)	Gallic acid equivalent
H_2SO_4	Sulphuric acid
HCL	Hydrochloric acid
IAA	Indole acetic acid
IC_{50}	Half maximal inhibitory concentration
ISSR	Inter simple sequence repeat
LAF	Laminar air flow
mg	Milli gram
mg/L	Milli gram per Litre
$MgCl_2$	Magnesium chloride
mM	Milli molar
mm	Milli metre
Mp	Monomorphic bands
NAA	Naphthalene acetic acid
NaOH	Sodium hydroxide
P_1	Garden soil + Straw + Dried cow dung manure
P_2	Saw dust
P_3	Forest soil manure
P_4	Sand
P_5	Saw dust + Sand
P_6	Sand+ Tea manure
P_7	Sand + Forest soil manure
pb	Poly morphic bands
PCR	Polymerase chain reaction
PGPRs	Plant growth promoting rhizobacteria
PIF	Plants Issus des Fragments de Tige

RAPD	Random amplified polymorphic DNA
RE	Retinol Equivalent
RGP	Ripe Grain Nain pulp
RGPL	Ripe Grain Nain peel
ROS	Reactive oxygen species
Rpm	Revolutions Per Minute
RS	Reducing Sugar
RSP	Ripe Sabri pulp
RSPL	Ripe Sabi
SCoT	Start Codon Targeted
SD	Standard deviation
T_0	Incomplete decapitation
T_1	Complete decapitation/ decortications
T_2	Split corm method
T_3	Plants Issus des Fragments de Tige
T_4	Excised bud method
T_5	Modified combination of split corm + PIF method
T_C	Conventional method (Control)
TDZ	Thidiazuron
TFC	Total flavonoid content
Tm	Melting temperature
TPC	Total phenol content
UGP	Unripe Grand Nain Pulp
UGPL	Unripe Grand Nain Peel
USP	Unripe Sabri pulp
USPL	Unripe Sabri peel
UV-Vis	Ultraviolet-Visible Spectroscopy
viz	Which is

Lists of Tables

Table No.	Table Legend	Page No.
2.1	Comparative proximate compositions of unripe and ripe pulps and peels of banana cultivars Grand Nain and Sabri	33
2.2	Comparative values of antioxidant activity, β-carotenoid and Vitamin A contents in unripe and ripe banana pulps and peels of banana cultivars Grand Nain and Sabri	37
3.1	Effect of different corm fragmentation techniques on morphogenic response in Cv. G9	56
3.2	Effect of different corm fragmentation techniques on morphogenic response in Cv. Sabri	59
3.3	Effect of different potting mix and corm fragmentation on morphogenic response Cv. G9	62
3.4	Effect of different potting media on morphogenic response in Cv. Sabri	65
3.5	RAPD and SCoT primers data analysis for evaluation of genetic stability of regenerated plants in Cv. Grand Nain	69
3.6	RAPD and SCoT primers data analysis for evaluation of genetic stability of regenerated plants in Cv. Sabri.	71
3.7	Comparative and combined presentation RAPD and SCoT primers results in Cv. Grand Nain	72
3.8	Comparative and combined presentation RAPD and SCoT primers results in Cv. Sabri	72
4.1	Effect BAP, IAA and NAA on morphogenic response of rhizome segments of banana Cv. Grand Nain	92-93
4.2	Effect KN, IAA and NAA on morphogenic response of rhizome segments of banana Cv. Grand Nain	94-95
4.3	Effect TDZ and IAA on morphogenic response of rhizome segments of banana Cv. Grand Nain	95
4.4	Effect BAP, IAA and NAA on shoot proliferation in banana Cv. Sabri	99
4.5	Effect KN, IAA and NAA on shoot proliferation in banana Cv. Sabri	100
4.6	RAPD and ISSR primers data analysis for evaluation of genetic stability of regenerated plants in Cv. Grand Nain	107
4.7	Comparative and combined presentation RAPD and ISSR primers results in Cv. Grand Nain	108
4.8	RAPD and ISSR primers data analysis for evaluation of genetic stability of regenerated plants in Cv. Sabri	109
4.9	Comparative and combined presentation RAPD and ISSR primers results in Cv. Sabri	110

List of Figures

Figure No.	Figure Legend	Page No.
1.1	Pictures of banana fruits in *Musa* cultivars. a) Grand Nain, b) Sabri	24
2.1	Showing the DPPH scavenging activity and IC_{50} value in different parts and stages of fruits; a. Cv. Grand Nain or G9, b. Cv. Sabri.	35
2.2	Total phenolic and flavonoid contents in different parts and stages of fruits; a. Cv. Grand Nain or G9, b. Cv. Sabri.	36
2.3	Correlation between total phenol and flavonoid contents. Correlation coefficient, r = 0.7738, 95% confidence interval = 0.1518 to 0.9568, Coefficient of determination, r^2 =0.5988.	43
3.1	Pictures of potting media and their mixtures, along with recycled containers used in the experiment. P_1: Garden Soil+ Straw + Dried cow dung manure, P_2: Saw dust, P_3: Forest soil manure, P_4: Sand, P_5: Sand + Saw dust, P_6: Sand+ Tea manure, P_7: Sand + Forest soil manure, B: Polyethene covering the pot for retaining humidity.	50
3.2	Different macropropagation techniques used for planting of rhizome parts. a. Incomplete decapitation (T_0); b. Conventional method (T_c); c. Complete decapitation (T_1); d. Split corm method (T_2); e. PIF method (Plants Issus des Fragments de Tige) (T_3); f. Excised bud method (T_4); g. Split corm and PIF method (T_5).	51
3.3	Schematic presentation of experimental steps	53
3.4	Morphogenic responses in different corm splitting techniques. a. Conventional method (T_C); b. Incomplete decapitation (T_0); c. Complete decapitation (T_1); d. Split corm method (T_2); e. PIF method (Plants derived from stem fragments) (T_3); f. Excised bud method (T_4); g. Split corm+PIF method (T_5); h. Plantlets separated for hardening; i. Plantlets kept in green house for acclimatization.	57
3.5	Morphogenic responses in different corm splitting techniques of Cv. Sabri. a. Conventional method (Control, T_c); b. Incomplete decapitation (T_0); c. Split corm method (T_2); d. Plants Issus des Fragments de Tige (T_5); e. Excised bud method (T_4); f. Split corm and PIF method (T_5); g. Regenerated plantlets ready for field establishment.	60
3.6	Graphical representation of the time taken in days for emergence of shoot buds in different potting media.	63
3.7	Reponses obtained from different potting mixtures in Split +PIF method (T_5) in Grand Nain. a. Split+PIF method (T_5); b. Garden soil + Straw + Dried cow dung manure (P_1); c. Saw dust (P_2); d. Forest soil manure (P_3); e. Sand (P_4); f. Sand + Saw dust (P_5); g. Sand+ Tea manure (P_6); h. Sand + Forest soil manure (P_7).	64
3.8	Reponses obtained from different potting mixtures in PIF method (T_3) in Grand Nain. a) PIF method (T_3); b) Garden soil + Straw + Dried cow dung manure (P_1); c) Saw dust (P_2); d) Forest soil manure (P_3); e) Sand (P_4); f) Sand + Saw dust (P_5); g) Sand+ Tea manure (P_6); h) Sand + Forest soil	65

	manure (P_7).	
3.9	Pictures of the responses in the three potting media in Cv. Sabri. a. Garden soil + Straw + Dried cow dung manure (P_1); b. Saw dust (P_2); c. Forest soil manure (P_3).	68
3.10	Field established regenerates. a. Cv. Grand Nain, b. Cv. Sabri.	69
3.11	RAPD and ScoT markers profiles of mother plant (M), macropropagated plantlets (R_1 to R_7 lanes), showing in primers, in Cv. Grand Nain (a-d) and Sabri (e-h) along with DNA ladder (100bp). a) OPA- 13, b) OPA-12, c) S10, d) S11 in cv. Grand Nain; e) OPA-11, f) OPD-20, g) S6, h) S5 in cv. Sabri.	70
4.1	Graphical representation of the effect of BAP and KN in Cv. Grand Nain and Sabri on plant regeneration. a. Effect of BAP on shoot regeneration of G9; b. Effect of BAP on shoot regeneration of G9; c. Effect of KN on shoot regeneration of G9; d. Effect of KN on shoot regeneration of Sabri.	90
4.2	Effect of different stages of *in vitro* morphogenic response of G9 on MS medium supplemented with different adjuncts. a. Explants cultured on MS medium; b. Initial morphogenic response marked with swelling followed by invoking loci and green colored initial response; c-d. Shoot buds formation; e-f. Development of globular structures; g. Development of shoot buds from the base of the cultured; h-i. Multiple shoots formation.	91
4.3	Different stages of *in vitro* propagation of banana Cv. Sabri. A. Initial regeneration response from the cultured explants; b. Initiation of shoot proliferation; c-e. Multiple shoots formation; f. A regenerated shoot with well developed leaves showing ignition of root formation; g-h. Rooted plants under primary hardening.	92
4.4	Represents different stages of plant regeneration and culture proliferation of Cv. Grand Nain. a. Regeneration plantlets with well expanded leaves; b-d. Multiple shoot development; e. Rooted regenerates; f. Regenerates under primary hardening condition.	98
4.5	a-b. Cultures with abnormal curling of leaves at higher concentrations of TDZ in Cv. Grand Nain.	100
4.6	Primary hardened regenerates ready for transplantation. a. Grand Nain; b. Sabri; c. G9 regenerates transplanted in cocopeat under secondary acclimatization; d. Sabri regenerates transplanted in cocopeat under secondary acclimatization.	106
4.7	Acclimatization and filed established regenerates of G9 and Sabri. a. Acclimatized regenerates of Cv. Grand Nain maintained in polyhouse; b. Acclimatized regenerates of Cv. Sabri maintained in polyhouse; c. Field established transplant of Cv. Grand Nain; d. Field established transplant of Cv. Sabri.	107
4.8	Survival performance of regenerates in different stages transplantation	108
4.9	RAPD and ISSR markers agarose profiles of mother plant (M) and tissue culture raised plantlets (R_1 to R_9 lanes) of Cv. G9 and Cv. Sabri. a-d. Cv. Grand Nain, e-h. Cv. Sabri. a. RAPD primer OPA- 12; b. RAPD primer OPA-15; c) ISSR 18 primer; d. ISSR 816 primers; e. RAPD primer OPA-11; f. RAPD primer OPA-04; g. ISSR 841 primer; h. ISSR 816 primer.	113

Chapter – 1

Introduction

Bananas are very popular, widely distributed, economically viable fruit crop with the highest consumption reported worldwide (Pereira and Maraschin, 2015; Singh et al., 2016). They have been referred to as "Common Man's Fruit" due to their extensive availability and affordability and, therefore an economical dietary option for low-income households (Parmar and Vinod, 2018). Bananas rank as the fourth most important food commodity in the world, exceeded only by rice, wheat, and milk/milk products (Chada and Sahijram, 2000; Sahijram et al., 2003; Alemu, 2017). In global fruit production, they rank third, surpassing citrus fruits and even apples, making them one of the most produced fruits (Hailu et al., 2013; Dotto et al., 2019).

Bananas were among the first crops to be domesticated and are now widely distributed throughout the subtropics, where they constitute a major staple food for millions of people and serve as a balanced diet (Davey et al., 2013). They are especially important in Africa, particularly in Ghana and Uganda, and also play a significant role in the diets of Caribbean countries (Haiti and the Dominican Republic) and Latin American countries (Singh et al., 2011; Pereira and Maraschin, 2015).

In addition to their role as a staple food, bananas are becoming increasingly significant as cash crops, serving as a major source of income for rural communities. They thrive in diverse habitats and yield fruit throughout the year, offering security during famine periods amid harvest. Their biomass also provides nutrients to other crops in intercropping farming practices (Hailu et al., 2013).

In India, banana rank as second after mango in the list of most important fruit crop with 37 percent production of the total fruit production (Kookal and Thimmaiah, 2018). With about 13 percent of the agricultural area is dominated by banana farming and about 600 varieties of bananas are known in India. Documentation by Tamil Nadu Agricultural University also recorded more than 100 varieties (Das, 2010; Kumari et al., 2022). The Northeastern states of India have the highest documented Musaceae diversity, comprising of 81% of Indias total diversity, and are home to 30 Musaceae taxa, with 20 endemic to the region (Sabu et al., 2016; Deb et al., 2023). Banana is also the one of the most important fruit plants of Manipur, Assam which is related with the culture and are used for different purposes (Singh et al., 2014; Borborah, 2016).

Global Production of Banana and Export Trends

Bananas are cultivated in approximately 130 countries globally, with 99% of production originating from developing nations. The primary banana-producing continents are Asia (56%), Africa (15%), and Latin America (26%), covering an area of 4.84 million hectares and producing 95.6 million tonnes. Although no definitive statistics are available, nearly 60% of the contribution comes from small farms and home gardens (Singh et al., 2011; Evans et al., 2020).

Bananas have emerged as one of the most valued commercially important agricultural commodities, ranking fourth and fifth in fruits and food crops globally, with an annual production of more than 72.5 million metric tonnes worldwide (Singh et al., 2016; Subagyo and Chafidz, 2018). Additionally, besides banana fruit, the banana textiles that utilize banana pseudostems are expected to be the next emerging economy, with their annual production escalating to 100 thousand tonnes in 2010 **(**Subagyo and Chafidz, 2018).

As per the most recent FAO estimates from 2019, global banana production increased from 67 million tonnes in 2000 to 114 million tonnes in 2017. From 2000 to 2017, India accounted for over 25% of global banana production annually, establishing itself as the largest producer, followed by China, the Philippines, Ecuador, and Brazil (FAO, 2019). Globally, banana production surpassed 116 million metric tonnes in 2019, cultivated over an area of 5.15 million hectares. FAO (2023) estimates that banana production will reach 140 million tonnes throughout the following decade (MoFPI, 2021; Maseko et al., 2024). The production of bananas in India is projected to remain

stable and continue to rise, driven by robust local demand from a high population, with an anticipated output of 35 million tonnes by 2032 (Maseko et al., 2024).

The total global banana export is estimated to reach 19.2 million tonnes in 2023 (FAO, 2023, and India's annual production is said to reach 34.5 million metric tonnes (Worldstats, 2024) showing promising export prospects with a 38% growth in exports from 2022 to 2023, reaching 500,000 metric tonnes (FAO, 2024). Fresh Fruit Portal (2015) reports stated that the United Arab Emirates (UAE) and Saudi Arabian markets imported approximately 63,000 metric tonnes of Grand Nain from India in 2014. However, banana production and export reportedly decreased by 0.9 million tonnes, from 20.5 million tonnes in 2021 to 2022 (Maseko et al., 2024).

According to the 2024 reports by World Stats, Andhra Pradesh is the major producer of bananas, followed by Maharashtra, Karnataka, Tamil Nadu, and Uttar Pradesh, which collectively account for approximately 67% of India's banana production tones (Worldstats, 2024). India grows bananas on 878 thousand hectares of cultivated land, but despite its reputation as the largest producer, it is still behind other major producers like China, the Philippines, etc. in productivity, ranking twenty-third at 35.17 MT per hectare (MoFPI, 2021).

Bananas are commercially farmed in tropical and subtropical environments across all Indian states, excluding those with severe winter climates, such as Himachal Pradesh and Jammu & Kashmir. Northeast India, comprising eight states, contributes significantly to the nation's production, with Assam leading among them, producing 913.27 thousand MT in 2017-2018, placing 11th nationally, according to NHB (2018-2019). Nagaland ranks third in the northeast and fifteenth nationally in banana production, yielding 94.781 thousand tonnes in 2024 (NHB, 2018-2019). The peak banana production in the state occurred in 2012, totalling 166.430 thousand tonnes, while the lowest production was recorded in 2013 at 80 thousand tonnes (Department of Agriculture & Farmers Welfare 2012 to 2024, Production: Horticulture Crops: Fruits: Banana: Nagaland | Economic Indicators CEIC).

Nagaland has an ideal climate for the cultivation of several tropical and subtropical fruits, with a total land area of 1,657,587 hectares (Survey on Horticulture Fruit Crops in Nagaland-Directorate of Economics and Statistics, Nagaland 2015). The state's economy largely depends on agriculture, with horticulture fruit crops playing a

vital role, yielding approximately 35.658 thousand metric tonnes from a cultivated area of 34,861.5 hectares, as reported for the years 2020-2021 (Kumar and Konyak, 2023).

There are around 22 horticultural fruits produced in the state, including pineapple, litchi, orange, banana, papaya, jackfruit, plum, passion fruit, guava, kiwi, and more, of which banana ranks fifth in terms of output (Survey on Horticulture Fruit Crops in Nagaland-Directorate of Economics and Statistics, Nagaland, 2015), with 53,900 MT produced annually on approximately 6,690 hectares cultivated land (Murry and Das, 2019). The 2015 Survey on Horticulture Fruit Crops in Nagaland indicated that Peren district achieved the highest banana production, followed by Dimapur and then Wokha district (Directorate of Economics and Statistics, Nagaland 2015). In the period spanning 2012 to 2013, Kohima district exhibited the most extensive area dedicated to banana cultivation within the state, followed closely by Mokokchung and subsequently Wokha (Murry and Das, 2019).

Taxonomy and Diversity of Banana Species

Bananas are classified within the genus *Musa*, constituting the largest monocotyledonous herbaceous plants, with several wild species attaining heights of 15 meters (Teisson and Côte, 1997). They belong to the Musaceae family, which is the most economically significant family within the Zingiberales order, located in the commelinids clade of monocots, classified under the Zingiberidae subclass, in the Liliopsida class, and Magnoliophyta division. The Musaceae family includes three genera: *Musa, Ensete*, and *Musella* (De Langhe et al., 2009). The *Ensete* genus, first recognized by Cheesman (1948), comprises 25 species that produce inedible fruits; however, recent research has shown only 8 species are currently recognized in the genus.

The genus *Musa* contains 50 species, including all edible banana varieties. The family comprises large, rhizomatous perennial or monocarpic herbs with advanced aerial shoots, with the exception of the species in *Ensete* (Pillay and Tripathi, 2007; De Langhe et al., 2009; Joe and Sabu, 2016; Sabu and Sreejith, 2016). Taxonomists have divided the genus *Musa* into several subgenera (sections), including *Physocaulis, Eumusa*, and *Rhodoclamys*. However, the most widely accepted classification comprises five sections: *Australimusa* (2n=20), *Callimusa* (2n=20), *Musa* (*Eumusa*) (2n=22), *Rhodoclamys* (2n=22), and *Ingentimusa* (2n=14), based on chromosome

number and morphological traits (Joe and Sabu, 2016). The *Ingentimusa* genus was subsequently incorporated as a fifth section by Argent (1976), containing only one species (Christelová et al., 2011).

The sections *Callimusa* and *Rhodochlamys* contain species with non-edible fruits, typically used as ornamental plants. The *Australimusa* is characterized by its erect edible fruit bunches and red-coloured sap, setting it apart from other banana varieties (Pillay and Tripathi, 2007). *Eumusa* is the largest and most significant section, with the highest diversity of edible bananas, primarily originating from two wild species: *Musa acuminata* (genome A) and *Musa balbisiana* (genome B) (Arvanitoyannis and Mavromatis, 2008).

These two wild species of *Musa* produce seeded fruits with minimal starch and negligible edible pulp, making them unsuitable as crops (Osuji et al., 2006). However, edible cultivars typically arise from inter- and intraspecific hybridizations between the diploid species *M. acuminata* (AA) and *M. balbisiana* (BB), leading to triploid cultivars (AAA, AAB, ABB), which are commonly known as commercial cultivars, along with a few diploid landraces (AA and AB genome types) or tetraploid (AAAA, AAAB) cultivars. Through chromosome restitution and hybridization, the process has given rise to: (a) autoploids and homogenomic hybrids, such as (AAA) dessert and beer bananas, and (b) alloploids and heterogenomic hybrids, comprising plantains (AAB) and cooking bananas (ABB) (Arvanitoyannis, 2008).

Bananas are categorized into dessert bananas (sweet, seedless), which are the common edible bananas from *Musa acuminata* (AAA), and plantains or cooking bananas, which are hybrids like AAB and ABB (starchier, requiring cooking). *Musa acuminata* grows in clumps, while *M. balbisiana* has a massive trunk and is important in breeding for disease resistance (Anyasi et al., 2013; Hailu et al., 2013; Mohamad Said et al., 2016). The term "plantain" refers specifically to a category of cooking bananas, while sweeter versions are commonly referred to as dessert bananas. However, this distinction does not include all cooking bananas, as not every cooking banana qualifies as a plantain. The notion that "plantains" refer to cooking bananas and "bananas" denote dessert types is an oversimplification, as other varieties of *M. balbisiana* are also used in cooking but are not classified as plantains (Cheesman, 1948). While plantains and dessert bananas look alike in their unripe stage, the primary distinction lies in their pulp or flesh. Plantains have larger bananas with starchier, less

sweet pulp that require cooking prior to consumption (Mohamad Said et al., 2016). Plantains are consumed in both ripe and unripe stages, often cooked, and serve as a staple food in many African cuisines (Anyasi et al., 2013). These genotypes are virtually or completely seed-sterile (Marroquin et al., 1993; Simmonds and Shepherd, 1955; Matsumoto, 2003).

Musa acuminata is the most abundant species of the *Eumusa* genus. Structural alterations in chromosomes, whether arising spontaneously or as a consequence of recombination events, led to the establishment of natural reproductive barriers within the species, hence facilitating subspecies separation and enhancing genetic diversity within the species overall (Arvanitoyannis et al., 2008).

Many experts have debated the distinction between plantains and bananas, and the most common classification is to use *M. paradisiaca* for plantains and *M. sapientum* for bananas (Cheesman, 1948). *Musa paradisiaca* and *Musa balbisiana* are distinguished by their genotype and morphology. *Musa paradisiaca* has both diploid and triploid clones, with diploids generally exhibiting a slenderer form, akin to wild *M. acuminata*. The leaves exhibit chocolate-brown spots, even on young suckers that are exclusively found in *M. paradisiaca*. The fruits are elongated and slender, typically exhibiting curvature towards maturation. While, *M. balbisiana* diploids are more robust, with larger fruits in relation to their length. Only the pedicel exhibits geotropic responsiveness, leading to straight fruit formation **(**Hailu et al., 2013)**.** Culinary banana (*Musa* ABB) which is known as *kachkal* in local language of Assam is the only cooking banana found in entire North Eastern region and Assam (Khawas et al., 2014).

In India, 34 taxa of the family Musaceae have been recorded, and the specific number of species in the genus *Musa* is unclear due to its considerable variability (Deb et al., 2023). Northeast India, consisting of eight states, is regarded as the most abundant source of natural banana diversity in the nation. This region hosts a multitude of indigenous *Musa* cultivars and wild or semi-wild species, flourishing in diverse agro-climatic conditions, with 30 taxa documented, of which 20 are endemic (Mathukmi et al., 2022; Deb et al., 2023). Prominent species including *Musa nagalandiana, M. balbisiana, M. cheesmani, M. flaviflora, M. itinerans, M. nagensium, M. puspanjaliae,* and ornamental species from the Rhodochlamys section have been documented in the states of Nagaland, Arunachal Pradesh, and Tripura, emphasizing the region's significance as a biodiversity hotspot for bananas (Mathukmi et al., 2022). *Musa*

nagalandiana was initially spotted in Nagaland, thriving on the mountainous slopes of tropical semi-evergreen forests adjacent to the Doyang River in the district of Zunheboto (Dey et al., 2014). Additionally, the state also reported two critically endangered wild banana species namely, *Musa manii* and *Musa markkui* (Tiatemsu et al., 2023).

History and Origin of Banana Cultivation

They are known to be one of the initial food crops to be domesticated and are predominantly grown efficiently and extensively scattered in humid tropical and subtropical regions serving as a primary staple meal for millions (Davey et al., 2013). Bananas are said to have originated from the dense forests of Southeast Asia, particularly Malaysia, Indonesia, and the Philippines. The earliest recorded mentions of bananas date back to 500 to 600 BC in texts written by ancient Greeks, Romans, Chinese, and Hindus, leading some horticulturists to assume that bananas were the first fruits on Earth (Pillay and Tripathi, 2007; Arvanitoyannis and Mavromatis, 2008). They were among the initial crops to be domesticated and are now extensively scattered in the subtropics, serving as a primary staple meal for millions (Davey et al., 2013).

Bananas were first cultivated around 8000 B.C. in the Kuk Valley of New Guinea, following their domestication approximately 10,000 years ago. Throughout the subsequent two millennia, merchants and voyagers played a vital role in spreading bananas to regions such as Australia, Indonesia, India, and Malaysia. By 600 B.C., bananas were being cultivated in India, with references appearing in Mediterranean texts around 300 B.C. Eventually, bananas spread throughout Southeast Asia and the South Pacific, reaching a variety of tropical regions worldwide (Singh et al., 2016). The genus name *Musa* is associated with Antonius *Musa*, a Roman physician, and is believed to originate from the Greek deity Muses, while the term "banana" originally referred to the peel of the fruit (Arvanitoyannis and Mavromatis, 2008; Singh et al., 2016).

Nutritional Value and Medicinal Properties of Bananas

Bananas are highly nutritious fruits valued for their significant contributions to both human diets and pharmaceutical applications. A medium-sized banana is reported to have an average of approximately 105 calories (Kothawade, 2019). The fruit contains

high carbohydrates (22.2%), fiber (0.84%) and protein (1.1%) with less fat (0.2%) and water (75.7%) (Ali et al., 2011). They are prized for being a rich source of many essential nutrients which are health beneficial, such as fibre, starch, vitamins like A, B, C and E, as well as minerals such as calcium, magnesium, and iron, and various antioxidants and phytonutrients (Kothawade, 2019). Due to its role as an energy-rich, starchy staple food, it is considered to be one of the most important sources of energy and starchy staple food for the people of tropical humid regions (Doymaz, 2010; Khawas et al., 2014). Multiple investigations on banana pulp and peel have recorded the presence of a significant amount of high-value nutritional components. These includes vitamins A, B, C, and E, carotene, and phenolic chemicals containing gallocatechin, dopamine, lignin, and tannins, along with phytosterols and triterpenes such as cycloeucalenol and cycloartenol (Mohamad Said et al., 2016). These bioactive chemicals possess significant potential for utilization in the pharmaceutical sector. Additionally, bananas are traditionally used for both their culinary and medicinal properties (Kumari et al., 2022).

Ripe bananas are an excellent addition to a balanced diet, offering a nutrient-dense composition that includes natural sugars (Sucrose, Fructose, glucose and maltose), low fat, protein, potassium, and vital vitamins. These components contribute significantly to their numerous health benefits (Adubofuor et al., 2016). The ripened bananas are noted for their high carbohydrate content (approximately 20 g per 100 g), fibre (2 g), and potassium (4.10–5.55 mg), in addition to significant minerals such as phosphorus, magnesium, calcium, iron, sodium, and manganese, as well as essential vitamins, including A, C, and B-complex (thiamine, riboflavin, niacin, and B6), rendering them a nutrient-dense fruit (Anyasi et al., 2013).

The unripe banana (green stage) of banana is considered to be a functional food with prebiotic properties owing to its higher resistant starch content. This resistant starch comprises granules or their degradation products that evade digestion and absorption in the small intestine, yet are fermented in the large intestine. As a result, green bananas are esteemed for their health advantages, especially in enhancing intestinal health. Their use is advised for the management of several disorders, such as colitis, ulcerative colitis, gastric ulcers, uremia, nephritis, gout, cardiovascular diseases, and celiac disease (Bezzera et al., 2013; Khoza et al., 2021).

Although banana peels are a major by-product during processing, numerous studies confirmed the high amounts of bioactive compounds like polyphenols, carotenoids etc., which have beneficial effects on human health (Arvanitoyannis and Mavromatis, 2008; Khawas and Deka, 2016). Banana peels also have higher fibre quality compared to other sources, characterised by high levels of total and soluble fibre, exceptional water and oil retention capabilities, and lesser caloric and phytic acid content (Budhalakoti, 2019). The raw pulp and peels of bananas are particularly rich in flavonoids such as epicatechin, prodelphinidin dimer, gallic acid, and epigallocatechin (found in the pulp), while the peels contain high levels of dopamine and catecholamines (Anyasi et al., 2013).

The banana flower (inflorescence) possesses significant nutritional content and is utilized as a culinary ingredient in various Asian nations, including Sri Lanka, Indonesia, and Thailand (Sheng et al., 2010). Banana pulp is rich in bioactive components, which include phenolic acids and flavonoids, which offer strong antioxidant properties (Borges et al., 2014). Banana intake offers substantial quantities of potassium and iron, advantageous for muscular health and patients who have diverse ailments. Additionally, bananas have been demonstrated to aid in blood pressure regulation and are particularly advantageous for persons managing diabetes (Singh et al., 2016).

Phytochemicals are biologically active naturally occurring compounds located at surfaces of the plant tissues, which act as a natural defense for plants protecting them from environmental stress, and attack from microbes and insects. They are termed secondary metabolites having therapeutic value, especially in antioxidant activity (Obiageli et al., 2016). Polyphenols have been explored for their notable antioxidant properties and epidemiological studies indicate that diets rich in flavonoids can lower the risk of diabetes, prevents cardiovascular disease, cancer, type 2 diabetes and neurodegenerative diseases (Anyasi et al., 2013).

Resistant starch (RS) lowers blood glucose levels owing to its restricted digestion. Microbial fermentation in the stomach generates short-chain fatty acids (SCFAs), such as butyrate, propionate, and acetate, from resistant starch (RS). These SCFAs are recognized for their anti-inflammatory characteristics, enhancement of gut barrier functions, and reduction of colon cancer risk. (Anyasi et al., 2013; Khoza et al., 2021).

Dietary fibre is an essential component of the human diet and its consumption reduces or helps prevent many diseases such as cardiovascular diseases, colon cancer, diverticulosis, diabetes, constipation, and irritable colon (Budhalakoti, 2019). Potassium plays a crucial role in regulating blood pressure and supporting proper kidney function, and studies have identified bananas as a significant dietary source of this essential mineral (Kothawade, 2019). Also, high amounts of potassium and iron, are beneficial for the muscle function, in patients and proven to help regulate blood pressure and are particularly beneficial for individuals with diabetes (Singh et al., 2016).

Bananas can be consumed in generous amounts to meet daily vitamin A and C requirements, while also helping to prevent chronic conditions like cardiovascular issues, age-related muscle degeneration, and muscle cramps in athletes, as well as ideal for those sensitive to salt due to low sodium and fat (Mohapatra et al., 2010).

Bananas also help combat intestinal problems such as ulcers by neutralizing gastric acid and coating the stomach lining to minimize inflammation. They promote healing and relieve ulcer pain, as well as treat burns and wounds when applied as a paste or using banana leaves as a cool compress. Bananas also aid in relieving constipation, diarrhea, and arthritis, and can assist in treating anaemia (Kumar et al., 2012). The fruit has antimicrobial and therapeutic properties, rich in ascorbic acid, β-carotene, citric acid and malic acid (Mohapatra et al., 2010).

Bananas are commonly recommended in daily diets for their health-boosting and medicinal benefits (Kumari et al., 2022). Bananas are thought to aid in the prevention of depression, kidney cancer, diabetes and anaemia (Mohamad Said et al., 2016; Alemu, 2017). Bananas are traditionally used for both their culinary and medicinal properties. Additionally, every part of the banana plant offers medicinal benefits, Imam and Akter (2011) reported the traditional use of bananas for treating various conditions. Both unripe and ripe pulp are consumed for dysentery, intestinal lesions in ulcerative colitis, diabetes, uremia, nephritis, gout, hypertension, cardiac disease, and excessive menstruation. Banana leaves (ashes) are applied to eczema, blisters, and burns, while flowers are used for dysentery and menorrhagia. The stem juice treats diarrhea, dysentery, diabetes, and menorrhagia. The root is used as an anthelmintic and to treat blood disorders, venereal diseases, inflammation, pain, and snakebites. In addition, Subagyo and Chafidz (2018) also reported that the banana flower is usually cooked and consumed by individuals with diabetes, bronchitis, dysentery, and ulcers the sap from

the pseudo-stem can be ingested or applied externally to treat stings and bites; young leaves are used as a poultice for skin irritations; and in some countries, the roots, leaf ashes, peels, and seeds are also utilized for various medicinal purposes.

Imam and Akter (2011) reported several successful pharmacological activities of bananas, including antidiarrheal, antiulcer, antimicrobial, hypoglycemic, hypocholesterolemic, antihypertensive, diuretic, wound healing, and antimalarial effects.

Banana Processing and its diverse Commercial Applications

Bananas are multipurpose fruits that can be consumed raw, cooked, or transformed into many food items. Post-harvest processing is essential in bananas to reduce losses due to inappropriate handling, insufficient storage, and limited shelf life. This method enhances the utility of excess produce while simultaneously increasing the value of the fruit. The banana food processing industry plays an important role in converting the surplus produce as well as the entire parts of bananas into nutritious and healthy delicacies and other value-added products, thereby increasing the economy of the country (Chukwu et al., 2011).

Bananas are extensively consumed every day for their health benefits, both in their raw form and in combination with other food products such as cereals, ice cream, and desserts (Kumari et al., 2022). The fruit of bananas are among the most esteemed fruits globally and are regarded as a significant commodity and are consumed in unripe as well as ripe stages. They are enjoyed fresh, cooked or processed into various products such as dried fruit, smoothies, flour, bread, and ice cream (Subagyo and Chafidz, 2018).

The ripe bananas are processed into several products, in fresh, as a puree for use in a variety of products including ice cream, yoghurt, cake, bread, nectar and baby food, and used in bakery products, fruit salads, and toppings, and are also preserved as dried fruit, packed as canned fruit with syrup, dehydrated ripe banana jam, jelly, fruit bar (Chukwu et al., 2011); and beverages products like shakes, juice, beer, alcohol, wine, vinegar etc. (Kookal and Thimmiah, 2018; Kumari et al., 2022). The unripe banana pulp is also processed in many products such as banana chips, flour, banana starch powder, pickles, etc. (Chukwu et al., 2011; Kookal and Thimmiah, 2018). The whole banana fruit is also processed as banana flour and other fermented beverages and snacks

(Chukwu et al., 2011). In addition to the edible part of banana fruit, there's a major opportunity for food processing in bananas with the utilization of the entire banana plant, benefiting both national and global economies (Kookal and Thimmiah, 2018). Bananas have been categorized as one of the most multipurpose plants globally, as practically all parts of the plant can be utilized, including the fruit, peel, pseudostem, leaves, rhizome, and inflorescence (Subagyo and Chafidz, 2018).

Banana leaves are often traditionally used as plates and food wrappings in rural regions (Mohamad Said et al., 2016), and are also processed for food packaging and aesthetics (Subagyo and Chafidz, 2018). The inflorescence (flower) of bananas is consumed as a food (dish) in numerous rural households, particularly as flower thokku, a South Indian seasoning, which is commonly paired with idly, dosa, chapatti, and rice dishes (Subagyo and Chafidz, 2018; Kothawade, 2019).

The banana pseudostem is valued for its fibre, which serves as a raw material for the textile industry. The processed fibre is transformed into various products, including diapers, sanitary pads, napkins, high-quality marine ropes, paper, tea bags, filter cloths, reinforcement fibres for plaster, disposable fabrics, and lightweight woven fabrics etc., (Subagyo and Chafidz, 2018). In addition to its success in textiles, the core of the pseudostem is also enjoyed as a delicacy in many regions, both as a prepared dish and in the form of packaged pickles (Kothawade, 2019).

Bananas are widely utilized across various industries, including food, nutraceutical, pharmaceutical, and beauty. In the food industry, they are used as natural colourants, flavouring agents, and thickeners, while in the nutraceutical and pharmaceutical sectors, they are incorporated into dietary supplements and other health products such as prebiotics, probiotics etc. (Mohapatra et al., 2010). The beauty industry also leverages bananas in cosmetic formulations such as creams, shampoos, face masks, powders, and fragrances (Mohamad Said et al., 2016; Subagyo and Chafidz, 2018; Kothawade, 2019). Additionally, they serve as valuable sources of macro- and micro-nutrients, livestock feed, fibres, bioactive compounds, and organic fertilizers (Chukwu et al., 2011; Subagyo and Chafidz, 2018).

Cost-Effective Macropropagation Techniques for Banana Seedling Production

Macro-propagation has been suggested as an effective and cost-effective alternative for generating significant numbers of high-quality banana seedlings, requiring reduced costs and skills (Ntamwira et al., 2017). This simple technique can significantly enhance banana production and is highly suitable for farmers, as it demands minimal skill and expertise, with macropropagated plantlets performing equally well as tissue-cultured plants (Manju and Pushpalta, 2020). The process involves the repression of the apical meristem, which stimulates/activates the regeneration of underdeveloped suckers/lateral meristems (Sajith et al., 2014; Bhende and Kurein, 2015).

Macropropagation techniques typically involve manipulating corms to alter the apical growing points, suppressing apical dominance and stimulating the lateral meristems for regeneration. This process enhances the corm's ability to produce a large number of healthy plantlets from secondary buds in a short period (Kindimba and Msogoya, 2014). This technique offers a simple and cost-effective approach, yielding many clonal plantlets with an estimated production of 40 to 60 shoots within a few months (Thiemele et al., 2015).

Common macropropagation techniques include complete decapitation and partial decapitation (also referred to as false decapitation). Complete decapitation involves the removal of the pseudostem to eliminate the central growing point through direct cutting, while partial decapitation achieves the same goal by drilling a hole into the pseudostem (Bhende and Kurien, 2015). Another widely used method is the PIF (Plant Issus de Fragments de Tige) approach, which entails creating a crisscross incision in the apical region to stimulate growth (Sajith et al., 2014; Opata et al., 2020). This alternative method necessitates cost-effective strategies utilizing simple methodologies, affordable equipment, and a minimal skilled labour force to generate healthy planting materials. This propagation technique can be performed either on-field or off-field, requiring the acceleration of the lateral meristem to stimulate the formation of lateral buds and shoots. As a result, a single corm can produce 10 to 30 plantlets in four months under controlled temperature and humidity conditions (Ntamwira et al., 2017).

Besides corm manipulation, other factors also influence plantlet production greatly. Temperature and relative humidity significantly influence key plant growth

processes, including respiration, transpiration, photosynthesis, protein synthesis, and translocation, while also breaking seed dormancy, inducing germination, and accelerating plant maturation to achieve optimal multiplication rates (Kwa, 2003; Ntamwira et al., 2017).

Additionally, Kwa (2003) emphasized the significance of using humidity chambers in macropropagation techniques to maintain elevated temperatures and relative humidity, ensuring optimal results are achieved. The substrate used in macropropagation techniques influenced the production greatly. Many investigations have been performed using various substrates, including garden soil, rice hulls, sand, and others, with sawdust being identified as one of the most effective substrates for promoting plantlet growth (Sajith et al., 2014). Ngullie and Deb (2024) also demonstrated the significance of substrates, with forest manure soil and sawdust being best for plantlet generation in banana Cv. G9. The corm of the banana functions as a nutrient reservoir, providing a continual supply of nutrients for plantlet regeneration (Ntamwira et al., 2017).

The age of suckers is crucial, and typically two types are used: sword suckers (characterized by a well-developed base, pointed tip, conical shape, and narrow, sword-like leaves in the early stages) and water suckers (featuring a small, undersized pseudostem, slender structure, and small leaves) (Bhende and Kurien, 2020).

Ahmad et al. (2016) demonstrated the application of organic and inorganic fertilizers like NPK, farm manure etc., for the propagation of plantlets. Additionally, numerous investigations, coupled with corm modifications, utilized plant growth regulators such as 6-benzylaminopurine (BAP), naphthalene acetic acid (NAA), and indole-3-acetic acid (IAA), yielding favourable outcomes (Nardi et al., 2000; Sajith et al., 2014; Ramirez, 2020).

Several researchers have successfully incorporated microorganisms such as *Trichoderma viride*, *Azospirillum*, *Bacillus subtilis*, Vesicular Arbuscular Mycorrhiza, and coconut water, acting as biofertilizers, plant growth-promoting rhizobacteria (PGPR), and antifungal agents for disease control, along with manipulated corms in various ways to enhance production (Baruah et al., 2017; Langford et al., 2017; Opata et al., 2020; Mintah and Arhin, 2020). Viswanath et al. (2021) reported variability in plantlet regeneration among cultivars, with the B genome showing the lowest multiplication rate, while the AAA group exhibited the highest.

Several studies on rhizome/corm manipulation techniques, including complete decapitation, decortication, false decapitation, and the PIF stem fragment method, have shown that macro propagation is a simple and efficient method. It is particularly advantageous for smallholder farmers and rural regions, where the adoption of modern technologies may be hindered by a lack of skills and financial resources (Njukwe et al., 2013; Sajith et al., 2014; Thiemele et al., 2015; Thungon et al., 2017; Langford et al., 2017; Suryanarayana et al., 2018; Opata et al., 2020; Izaile et al., 2021). Macropropagated plantlets demonstrate reduced post-establishment mortality owing to their superior adaptability to field conditions, as they are regenerated under active photosynthetic in vivo conditions, whereas micropropagated plantlets are more fragile and encounter difficulties in successful establishment in the field (Sajith et al., 2014). The macropropagation approach presents a viable strategy to address the gap between the demand for and the supply of affordable, high-quality banana seedlings (Ntamwira et al., 2017). Some studies reported the total expenditures for constructing growth chambers for the propagation of plantlets which was about USD 462.5 per unit and an overall set up of USD 2,301 (Njukwe et al., 2007; Ntamwira et al., 2017; Adjei et al., 2021), which is multiple times lower than the micropropagation techniques, aiding its farmer-friendly nature.

Mass Production of Banana Planting Propagules through Tissue Culture

Conventional banana propagation is highly limited, making *in vitro* micropropagation the preferred method due to its rapid multiplication, consistency, and production of disease-free plantlets (Lalrinsanga et al., 2013). *In vitro* propagation is a technique that involves the cultivation of plant cells, tissues, or totipotent cells or tissues on synthetic nutritional media in sterile conditions. This approach facilitates the rapid proliferation of plants while preserving genetic consistency, providing a more effective alternative to seed-based and traditional clonal propagation methods, particularly for species that exhibit limited responsiveness to conventional approaches (Thrope, 2007; Subrahmanyeswari and Gantait, 2022). *In vitro* culture is a biotechnological technique that utilizes plant cell totipotency to produce large numbers of uniform, disease-free clones, conserve genetic diversity, and is employed to facilitate shoot regeneration in bananas through organogenesis, somatic embryogenesis, and protoplast culture (Khalid and Tan, 2016).

The development of tissue culture technology has been the foundation of high-quality, disease-free planting material production at a mass scale, particularly in vegetatively propagated crops (Singh et al., 2011). *In vitro* micropropagation is an advanced technique designed to overcome challenges in producing clean and healthy multiple plantlets, as highlighted by Ferdous et al. (2015). This technology facilitates the rapid mass propagation of banana plantlets, offering significant advantages over conventional sucker propagation, including enhanced yields, plant uniformity, disease-free planting material, and true-to-type specimens. Furthermore, it is more economically viable for transportation compared to traditional suckers (Nguomuo et al., 2014). With a proven success rate and yield improvements of up to 39% over traditional methods, *in vitro* micropropagation has established itself as a promising solution for banana production (Ferdous et al., 2015).

Establishing *in vitro* cultures in bananas requires careful selection of explants (suckers), effective sterilization, appropriate media composition, optimal culture conditions (temperature, light and relative humidity) plant growth regulators (PGRs), and additives to support the initiation, shoot proliferation, and rooting (Subrahmanyeswari and Gantait, 2022). Maintaining aseptic conditions is crucial, as the initiation phase is highly susceptible to contamination and mortality, necessitating strict sterile procedures and equipment (Goswami and Handique, 2013).

A major issue in the *in vitro* culture of banana explants is the uncontrolled darkening of tissues caused by the exudation of polyphenolic substances (secondary metabolites) and contamination, which is influenced by various factors, including plant species, age, explant source, and environmental conditions (Goswami and Handique, 2013; Safwat et al., 2015).

The polyphenol exudates create a barrier between the exposed and damaged tissues, leading to oxidation, which impedes nutrient absorption and inhibits the onset of growth and development, ultimately resulting in explant mortality (Ngomuo et al., 2014; Kishor et al., 2017). Numerous studies have concentrated on sterilization and the management of lethal browning. Explants obtained from field-cultivated plants exhibit significant vulnerability to contamination; hence, widely utilized sterilants such as sodium hypochlorite and low doses of mercuric chloride have been identified as effective solutions (Goswami and Handique, 2013; Subrahmanyeswari et al., 2022).

Furthermore, research has indicated that the use of antioxidants is advantageous in reducing tissue browning in cultures (Kishor et al., 2017).

Micropropagation of banana has also been achieved using Shoot tips meristem (little suckers, parental pseudo stem and peepers), Lateral buds, male floral apices or inflorescence (anthers) and Seeds (Rahman et al., 2002, Mahadev et al., 2011; Avvaru 2016; Ngomuo et al., 2014; Kumar et al., 2017). There are also reports of somatic embryogenesis and regeneration in liquid medium, (Muhammad et al., 2004). The earliest application of micropropagation for *Musa* was Cox et al. (1960) who successfully cultured zygotic embryos (Altman and Loberant, 1997). While the *in vitro* banana propagation was first successfully established and reported in the 1970s in Taiwan, where shoot tip meristematic tissue with a few leaf primordia was identified as a suitable and easy method for propagating many banana genotypes. Since then, numerous protocols have been established for the propagation of *Musa* cultivars (Kishor et al., 2017).

Regeneration of banana plantlets through meristem culture offers a unique scope of developing disease-free planting materials against the bunchy top, cucumber mosaic virus and Panama wilt (Al-amin et al., 2009). Several *in vitro* plant propagation methods, including axillary and adventitious shoot induction, meristem culture, and regeneration via organogenesis or somatic embryogenesis, have been developed (Iliev et al., 2010). Rehana et al. (2009) also reported successful multiple plantlets grown from shoot tip meristem culture (Apical meristem culture) which is a well-established method for producing disease-free, clonal multiple planting. Jatoi et al. (2001) successfully demonstrated callus induction in shoot apex, nodal segments, and root segments, highlighting their effectiveness in plant regeneration. Many investigations confirmed direct shoot regeneration (without callus formation) at varied doses and combinations of growth regulators (Paulos et al., 2013). The micropropagation technique also addresses the challenges of low and inconsistent germination rates in banana seeds. Israeli et al. (1995) generated several clones from parthenocarpic green fruit pulp via *in vitro* cultures in their study. Banana plants are currently commercially cultivated via micropropagation, utilising tissue culture techniques and several biotechnological methods to generate disease-free specimens (Wiyono and Widono, 2013).

Plant growth regulators (PGRs) are critical media components in determining the developmental pathway of the plant cells (Khatun et al., 2017). However, the requirement of cytokinin and auxins depends on the variety of bananas and culture conditions (Cronauer and Krikorian, 1984; Al-Amin et al., 2009). The plant growth regulators in different concentrations and combinations, play an important role in the *in vitro* propagation of plants. Most common PGRs, such as Cytokinin (6-Benzylaminopurine, Kinetin) are associated with suppressing apical dominance while stimulating shoot proliferation from the lateral and axillary meristems, and Auxins (Indole-3-Acetic Acid, Indole-3-Butyric Acid, Naphthalene acetic acid) enhance root formation in the regenerated shoots (Al-Amin et al., 2009). Benzylaminopurine (BAP), a cytokinin, is known to reduce apical meristem dominance and promote the formation of both axillary and adventitious shoots from meristematic explants in bananas (Madhulatha et al., 2004). Consequently, the propagation of bananas depends on varying concentrations and combinations of plant growth regulators as well as banana variety (Ferdous et al., 2015). Several studies reported the successful propagation of bananas, using different PGRs and their varying concentrations. Khatun et al. (2017) carried out a study to check the effect of Benzyl aminopurine (BAP) and Indole-3-butyric acid (IBA), which obtained optimum response with MS medium supplemented with 5mg BAP and 2.5mg IBA. Another study by Rahman et al. (2002) in MS medium supplemented with 5mg of BAP along with NAA produced the highest number of shoots and roots. Sahoo et al. (2015) determined that the most effective combination for *Musa sp.* cv. Grand Nain involved supplementation of MS medium with 5 mg of BAP and 1 mg of IAA, while a study by Huq et al. (2012) on *Musa sp.* cv. Sabri found optimal banana propagation results utilizing MS medium supplemented with 4 mg of BAP and 2 mg of IAA, along with 13% (v/v) coconut water. A study by Gübbük and Pekmezc (2004) in three selected superior bananas resulted in the best combinations with 2 µM TDZ, and 1 µM IAA or 20 µM BAP and 1 µM IAA on MS medium, followed 5 g l-1 charcoal at the rooting stage for the *in vitro* micropropagation. BAP has been shown to outperform other cytokinins in promoting shoot tip multiplication in banana cultivars, as reported by Ali et al. (2011).

Micropropagation technique provides distinct and significant benefits compared to traditional approaches. It functions independently from seasonal variations incontrolled conditions, necessitates less plant tissue as the explant source, generates

uniform clones, and achieves superior productivity and yield relative to alternative approaches (Lalrinsanga et al., 2013).

Challenges and Problems in Banana Production and Utilization

Bananas are an essential crop worldwide, valued for their nutrition, economic significance, and versatility. Studies show that bananas are a nutritious food that promotes good health, earning the nickname "humble food" or "poor man's food" due to their affordability and availability (Englberger et al., 2006). The fruit banana contains beneficial nutrients that are required by the human body for daily bodily functions, such as carbohydrates, reducing sugars, proteins, fibre, and health supplements such as minerals and vitamins. In addition to that, it also contains bioactive compounds like carotenoids, polyphenols, and antioxidants, which have numerous positive health effects on human health, such as diabetes and anti-cancer activities (Someya et al., 2002; Arora et al., 2008; Deshmukh et al., 2009; Borges et al., 2020; Tan et al., 2024). The nutritious fruit is also known for its palatable, flavourful taste and is enjoyed as fresh fruit desserts or processed as yoghurts, ice creams, pudding, dried chips, wine/beer, juice, banana grits, and figs, pancakes, noodles, etc.

The remaining parts of the banana plant after harvest, i.e., the pseudostem of the banana, are processed, and the fibre is used for thatching, fabric production, paper, natural absorbent, cordage, etc., (Deshmukh et al., 2009; Pragati et al., 2014; Anyasi et al., 2015; Kumari et al., 2022; Kumar et al., 2023). However, despite high banana production in the country, there is growing concern about organic waste, with approximately 30% of bananas lost as by-products, primarily due to mishandling and poor post-harvest practices. The European Union's 1999 council directives called for reducing organic waste in landfills due to its negative impact on environmental quality, the spread of diseases that pose health risks to humans and animals, nutrient loss, and economic losses (Sardá et al., 2016; Sharma et al., 2019; Popoola, 2022). One significant by-product of bananas is the inedible peel, often discarded after consumption, yet it is rich in phytochemicals, dietary fibre, and antioxidants, offering essential nutrients that can enhance human health, leading to the development of innovative methods to utilize this waste product as a food ingredient and add value to it (Arora et al., 2008; González-Montelongo et al., 2010; Bhaskar et al., 2012; Bezerra et al., 2013; Joshi and Varma, 2015; Budhalakoti, 2019).

Another factor contributing to the production of waste is the short shelf life of bananas, which limits their full potential. Therefore, to intensify the maximum utility of the produce and enhance economic status, different innovative approaches have been integrated with the use of unripe banana pulp as raw material for the enrichment of various value-added products, such as the production of banana flour, banana cookies, bread, pickles, healthy fat-free chips, etc. (Muzanila and Mwakiposa, 2003; Joshi and Varma, 2015). Unripe green bananas have distinct physicochemical properties compared to ripe fruit, with lower sugar content, higher polyphenols, moderate antioxidants, and a significant amount of resistant starch, all of which benefit human health, particularly digestion and gut health, making them ideal for value-added products (Aurore et al., 2009; Menezes et al., 2011; Anyasi et al., 2015; Hoffmann Sardá et al., 2016; Borges et al., 2020).

Although about 95% of bananas are propagated through natural regeneration, this process cannot meet the growing demands of farmers for sufficient healthy planting materials due to numerous biotic and abiotic factors, such as viruses, which adversely affect banana yield and production. As a result, the traditional clonal propagation method is unable to satisfy the increasing need for disease-free and healthy planting materials (Al-Amin et al., 2009; Manju and Pushpalatha, 2022). Conventional methods of banana propagation through suckers have many limitations, such as low multiplication rate, clonal degradation, and the perils of spreading disastrous diseases (Ngamou et al., 2014; Uzaribara et al., 2015; Safarpour et al., 2017). The vegetative or natural regeneration of cultivated bananas through suckers is very slow due to the hormone-mediated apical dominance of the mother plant, with each plant producing only 5–20 suckers over its 12–14-month lifespan (Singh et al., 2011; Sajith et al., 2014). The propagation of various *Musa* cultivars (both banana and plantain) by conventional methods has been described by several authors over the past decades, but these techniques are laborious and time-consuming, particularly when it comes to producing a large number of homogeneous plants (Banerjee and Langhe, 1985).

Banana propagation is hindered by various microbial attacks, including common diseases such as BBTV (Banana bunchy top virus), CMV (Cucumber mosaic virus), BSV (Banana streak virus), and BBMV (Banana bract mosaic virus), bacterial Moko disease (*Pseudomonas solanacearum*), Panama disease Race 4 (*Fusarium oxysporum* f. sp. *cubense*), and Sigatoka leaf spot (*Mycosphaerella fijiensis*) (Singh et al., 2011;

Tripathi et al., 2016; Alemu, 2017; Thiagarajan et al., 2024). Additionally, nematodes such as *Pratylenchus coffeae, Meloidogyne incognita, Helicotylenchus multicinctus,* and *Radopholus similis*, as well as pests like banana weevils (*Odoiporus longicollis* and *Cosmopolites sordidus*), make bananas highly vulnerable and reduce their population (Singh et al., 2011). Several investigations report that between 20%−40% of the worldwide banana harvest is wasted annually, significantly reducing the global banana supply chain (Ismaila et al., 2023).

Scope of the Study

In vitro techniques address the limitations of conventional banana propagation, which is slow, reduces production, and contributes to disease spread, causing significant losses. Tissue culture provides a solution by enabling rapid production of homogeneous, disease-free plantlets in controlled environments, preventing contamination by viruses, bacteria, and fungi. Additionally, biotechnology facilitates the introduction of desirable traits in banana crops, overcoming challenges in conventional breeding, where few diploid clones produce viable pollen and commercial clones are sterile (Ganapathi et al*.,* 2001). The selection of nutrient-rich, commercially viable banana species and the development of efficient *in vitro* methods for high production will benefit farmers and producers, contributing to the economic development of regions like Nagaland, India, where banana cultivation holds considerable potential.

There has been limited research in Nagaland on banana *in vitro* techniques, which presents an opportunity to develop large-scale, commercially viable species. Some successful studies, such as the *in vitro* regeneration of banana cv. Grand Nain (Jamir and Maiti, 2014), demonstrate the potential for large-scale production. To meet the growing demand for disease-free plantlets, proper *in vitro* techniques and standardized protocols need to be developed, along with cost-effective methods for tissue culture.

Micronutrient deficiencies are a significant global issue, particularly in emerging and underdeveloped countries, with approximately 2 billion people affected. In India, about 190.7 million people suffer from nutritional deficiencies due to poor diets and poverty, which restrict access to essential nutrients and contribute to stunted growth and illness, especially among children (Gonmei and Toteja, 2018). A comprehensive understanding of the nutritional differences between ripe and unripe bananas, as well as

the edible pulp and inedible peel, is crucial for developing value-added products. This research could greatly enhance the economic value of bananas while addressing micronutrient deficiencies.

Bananas are highly nutritious and hold substantial market demand, so more emphasis should be placed on increasing their mass production. By focusing on biotechnology and *in vitro techniques*, it will be possible to meet nutritional needs, reduce waste, and contribute to economic growth.

Of the various commercially viable banana cultivars, two important cultivars are Grand Nain and Sabri. A brief about both the cultivars are given below:

Musa acuminata (AAA) Cv. Grand Nain

The cultivar 'Grand Nain' (G9) also spelled 'Grande Naine' is a popular Cavendish banana, originated from *Musa acuminata* and is a triploid variant of *M. acuminata* (AAA). It is known as Large Dwarf (translated from French) since its height measures 6-8 ft. i.e., comparatively taller than the Dwarf Cavendish and shorter than the Giant Cavendish and popularly also known as G9 (Manokari et al., 2022). The genome 'AAA' is known to have 33 chromosomes, and produces sterile, seedless fruits through parthenocarpy (Ploetz, 2007). The G9 cultivar is widely grown and highly popular across Central America, Africa, India, and Southeast Asia. In India, it holds significant commercial and economic importance and is extensively cultivated. Fresh Fruit Portal (2015) reports stated that United Arab Emirates (UAE) and Saudi Arabian markets imported approximately 63,000 metric tons of G9 from India in 2014.

The delicious fruit of the G9 measures up to 7-10 inches (**Figure 1.1a)**, and a fruit bunch typically contains about 10 to 12 hands and turns into an appetizing rich yellow when ripened (Shankar et al., 2014). It is packed with nutrition, with a high content of carbohydrates, fibre, vitamins, potassium, calcium, sodium, and magnesium (Doymaz, 2010; Ali et al., 2011; Khawas et al., 2014). Due to its flavor, the fruit is used as a delicacy on many occasions as desserts, fruit dressings, smoothies, juices, etc, and in addition, even the raw pulp is consumed and used in many aspects, like banana flour, banana chips, candy, pickles, etc. Besides its delicacies, the entire banana plant aids in value addition, in terms of textiles industries, banana fibres, fabric, etc.

With over 1000 types of cultivars consumed and grown, the Cavendish group dominates the market, with cultivar G9 dominating 47 % of the global market production (FAO, 2023). Among the 20 cultivars in *Musa acuminata* Cv. G9 is favoured for its robust growth, resilience to biotic stressors, and high-quality yield, characterized by an extended shelf life and superior quality relative to other cultivars (Safarpous et al., 2017).

***Musa paradisiaca* (AAB) Cv. Sabri**

The banana cultivar Sabri belongs to *Musa paradisiaca* to the silk subgroup with genome AAB. The Sabri banana cultivar is a regional banana variety from Tripura in India and Bangladesh, known for its special flavour and high nutritional value. The fruits of Sabri are sweet, and flavourful, medium-sized with an ivory-yellow colour, firm texture, and thin peels (Huq et al., 2012) (**Figure 1.1b**). It is a commercial cultivar, acknowledged for providing livelihood and nutritional security in the region, with approximately 70 per cent of the land under cultivation for Sabri bananas, making it the most extensively cultivated in the region (Mitra et al., 2022; Uma et al., 2023). The ripe fruit of the Sabri banana contains a good source of carbohydrates, vitamin C (ascorbic acid), vitamin B6, potassium and iron, and is recognized to be devoid of compounds that produce tri-uric acid, rendering it beneficial for individuals with gout or arthritis (Huq et al., 2012). Numerous banana cultivars are cultivated, but among them, *Musa paradisiaca* cv. 'Sabri' is regarded as one of the most popular and superior varieties in Bangladesh, distinguished as a prominent and exceptional cultivar. It is highly valued in both Bangladesh and Tripura, with a production value of 102,000.00 TK per hectare in Bangladesh (Tabassum et al., 2018; Mohiuddin et al., 2020). However, the cultivar has reportedly declined in recent years due to the spread of numerous diseases, including Fusarium wilt and Sigatoka leaf spot, among others (Mitra et al., 2022; Uma et al., 2023).

Figure 1.1: Pictures of banana fruits in *Musa* cultivars. a) Grand Nain, b) Sabri.

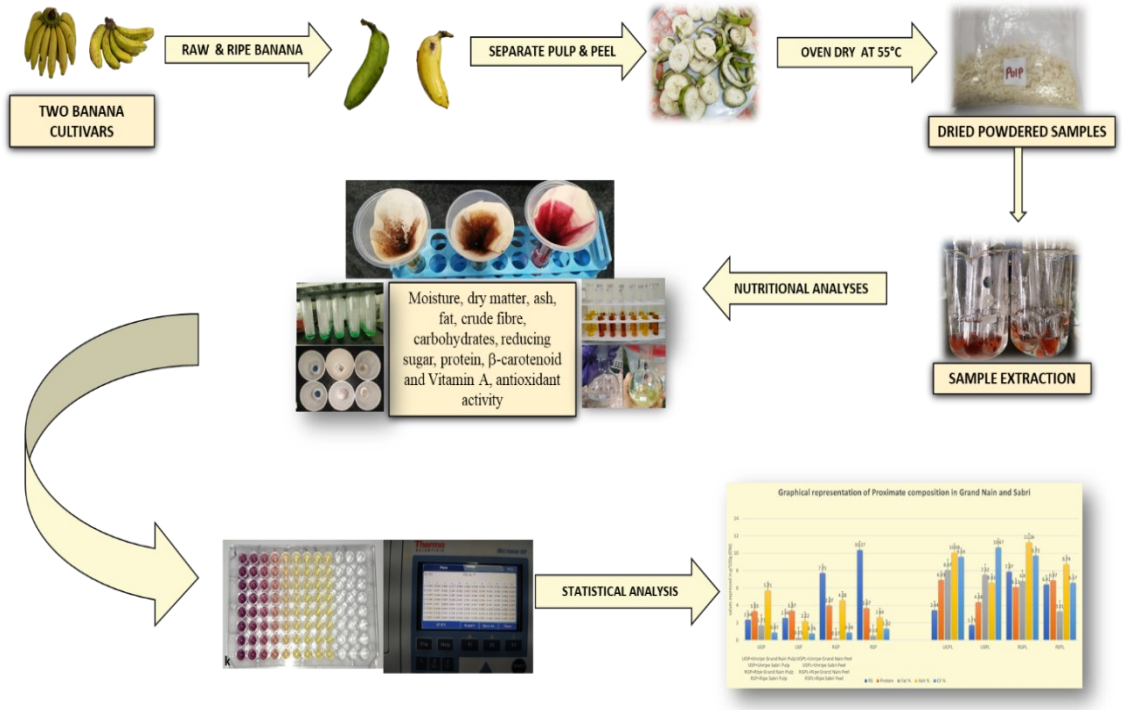
Based on the review of the challenges, opportunities, and potential applications in banana propagation and utilization I have worked for my Doctoral research on the above two cultivars with the following objectives:

- Comparative nutritional assessment of the selected *Musa* cultivars.
- Development of low-cost efficient macropropagation of *Musa* cultivars.
- *In vitro* micropropagation of the *Musa* cultivars
- Genetic fidelity assessment of the regenerated plantlets and field establishment.

Chapter – 2

Nutritional Assessment of Different Parts of Raw and Ripe Fruits of Two Commercially Viable Banana Cultivars 'Grand Nain' and 'Sabri'

Graphical Summary



Introduction

Banana is herbaceous perennial plant produces delicious and nutrient-packed fruits making it one of the most produced fruits worldwide and ranked fourth most valuable agricultural commodity after rice, wheat, and maize, with a yearly estimated annual volume of 125 million tons and 13.6 billion USD in trade value (Kumari et al., 2022). According to FAO (2012) statistical data, India is one of the leading banana producers, producing 139 million metric tons annually, contributing ~18% of the world production. Banana holds great significance in India, as it serves as a major fruit crops providing livelihood stability for thousands of people and also contributes ~37% of the nation's total fruit production (Kookal and Thimmaiah, 2018).

In India, ~190.7 million people suffer from nutritional deficiency as reported by FAO 2014 to 2016 (Gonmei and Toteja, 2018), due to a lack of balanced meals and poor choice of food (Nutritional Problems in India: Types & Symptoms of Nutritional Deficiencies, acko.com). Banana is known as “poor man’s food” due to their widespread availability and affordably. Due to their high nutritive values and potential to treat celiac disease, also known as ‘Super Food’ validated by the American Medical Association and recommended as a health food for children (Gonmei and Toteja, 2018). Reports suggest that bananas to be a nutritious choice due to their abundant essential nutrients, such as carbohydrates, proteins, fibre, minerals, vitamins, and bioactive compounds (carotenoids, polyphenols, and antioxidants etc.) that have the potential to prevent diseases like diabetes and cancer (Borges et al., 2020).

Though, banana is among the largest produced crops, ~30% of bananas are lost as major waste/by-products, especially due to the shorter shelf life, mishandling, and non-ideal practices in the post-harvest process. The major by-products like over-ripe bananas, non-edible parts like peels and pseudostem contributes to the increased organic wastes (Vu et al., 2018) which warrants the necessity to develop/incorporate novel approaches for management of these wastes and converting into value-added products.

Traditionally, banana is consumed due to its nutritious aspects and flavour and enjoyed in fresh fruit desserts, yoghurts, ice creams, pudding, dried chips, etc. In addition, to fruit peel, pseudostem is processed for fibre used for thatching, fabric, paper, natural absorbent, cordage, etc. (Kumari et al., 2022). However, the inedible banana peel, rich in health-boosting phytochemicals, is often discarded due to a lack of

scientific knowledge. According to traditional medicine, banana peel can treat diabetes, anaemia, inflammation, snakebite, cough, and other diseases or be used as a health supplement (Vu et al., 2018). Through proper analysis, the peel component could be a suitable and potential material for development of nutraceutical products.

Unripe bananas are known to contain higher phytochemicals and lesser sugar than ripe fruits, and additionally contain polyphenols, antioxidants, and resistant starch, which are all beneficial for healthy gut and overall human health (Borges et al., 2020). Therefore, alternative measures for trapping the nutritional values of green bananas could be using the unripe pulp as vegetables and as flour used in baking pancakes, bread, etc. In addition, the whole banana in raw and ripe stages (pulp and peel) can be utilized by food industries for the production of products such as pickles, healthy fat-free chips, banana cakes, cookies, nutraceuticals and cosmetic industries, health products such as supplements, medicines etc., and as creams, soaps, shampoo, beauty powder etc., thereby converting the waste into suitable usable products.

Grand Nain (G9) is a Cavendish group banana cultivar popular globally because of its excellent nutritional value, productivity, and desirable fruit quality (Manokari et al., 2022); while, Sabri, a silky subgroup banana cultivar very popular in Bangladesh and parts of North East India. It is widely farmed in Tripura as a key source of revenue and security of nutrition because of its favoured taste and dietary values (Mitra et al., 2022).

Though, the two well-established commercial cultivars (G9 and Sabri) are very popular, mostly the pulps of ripe fruits are being consumed. There are no data available on comparative nutritional values of edible and non-edible parts i.e., peel and pulp of both green and ripe fruits. The present study was undertaken to understand the food and nutraceutical values of different parts (peel and pulp) of green and ripe bananas of these two *Musa* cultivars i.e., Grand Nain and Sabri.

Materials and Methods

Sample collection and preparation

The green/vegetable stage and ripe banana of selected *Musa* cultivars Grand Nain and Sabri were collected from Nagaland University campus, Nagaland, India and Agartala, Tripura, India respectively. The samples were washed properly in pure water and allowed to air dry. The peel and pulp were separated, and thinly sliced (3-5mm) using a sterile knife and oven dried at 55°C for 12h. The dried samples were then

grounded using an electric grinder, and sieved to obtain fine particles. The samples were then labelled into two categories; a) *Musa* Cv. Grand Nain – unripe pulp (UGP), unripe peel (UGPL), ripe pulp (RGP), and unripe peel (RGPL), b) *Musa* Cv. Sabri - unripe pulp (USP), unripe peel (USPL), ripe pulp (RSP), and unripe peel (RSPL), and stored in airtight sealed plastic bags at room temperature (25±3°C) for further analysis.

Proximate analyses of the samples

Moisture Content: Ten g of freshly sliced samples were taken as initial weight and kept at 60°C for 10-12 h until consistent weight was achieved, and final weight was measured. Moisture content was determined by AOAC (1990) and was calculated by using the formula:

$$\boldsymbol{MC\%} = \left\{\frac{\boldsymbol{initial\ wt - final\ wt}}{\boldsymbol{initial\ wt}}\right\} * \mathbf{100}$$

Dry matter content: Dry matter content was determined by weighing the final weight of the sample that has been oven-dried, at 60±2°C for 10-12 h and calculated using the formula:

$$\boldsymbol{DMC}\ (\%) = \left\{\frac{\boldsymbol{Final\ Weight}}{\boldsymbol{Initial\ weight}}\right\} * \mathbf{100}$$

Total carbohydrate: Carbohydrate was estimated following the Anthrone method (Sadasivam, 1996) using glucose as the standard. 100 mg of powdered sample from each cultivar with 5 ml of 2.5 N HCl were hydrolyzed in a boiling water bath for 3 h, cooled to room temperature and neutralized with solid sodium carbonate until bubbling stopped. After neutralization, 100 ml of pure H_2O was added and centrifuged for 10 min at 10000 rpm. After taking 100 µl of the sample extract (supernatant), adjust the volume to 1ml with ultra-pure water, add 4 ml of Anthrone reagent, and incubate for 8 min in boiling water. After boiling, absorbance was measured at 630 nm and concentration was calculated from the standard graph in g/100 g dry weight equivalent to glucose.

Reducing sugar: Reducing sugar was estimated using the Dinitrosalicylic Acid method (Sadasivam, 1996), using glucose as the standard. Using preheated 80% ethanolic solvent, one gram of powdered sample was centrifuged at 10000 rpm for 10 min. Mixed 200 µl sample extract with 2ml ultra-pure water, 4ml DNS reagent, and incubated for 5 min in boiling water. Post boiling 500 µl of 40% Rochelle salt solution was added to the mixture, mixed well, and absorbance was read at 510 nm using a UV-Vis

spectrophotometer. Concentration was g/100 g dry weight equivalent to glucose standard.

Protein content: Nitrogen content was estimated using micro Kjeldahl method. Three g of dried sample was digested in acid, distilled, and titrated for colour change (green to pink). The amount of nitrogen was calculated using the formula:

$$\boldsymbol{Nitrogen\ estimation}\ \% = \mathbf{1.4}\boldsymbol{V} * \boldsymbol{NW}$$

Where, V= volume of acid used in titration (ml), N= Normality of the standard acid, and W= weight of the sample (g). Then, Protein content was calculated by multiplying the nitrogen content by 6.25 (Sadasivam, 1996).

Crude fat: Crude fat was determined using the Soxhlet apparatus following the AOAC method (1990) using petroleum ether as the solvent. Pre-oven dried and cooled 5 g of sample was put in the cellulose extraction thimble, covering it with cotton, then place it inside the thimble and put it back in the Soxhlet extractor. Petroleum ether was added in the flask and extract for 6 h at 60-80°C. After extraction, the flask with its contents was oven-dried and cooled in a desiccator. The crude fat content was calculated as the disparity between the extraction flask's initial and final weights. The formula used for calculating crude fat:

$$\boldsymbol{Crude\ fat}\ (\%) = \left\{\frac{\boldsymbol{Final\ weight\ of\ flask - initial\ weight\ of\ flask}}{\boldsymbol{weight\ of\ sample}}\right\} * \mathbf{100}$$

Ash content: The ash content was measured by incineration of pre-weighed grounded dried samples in muffle furnace at 550°C for 5h, until white or greyish ash was obtained (AOAC, 2000). And ash content was calculated by using formula:

$$\boldsymbol{AC}\% = \left\{\frac{\boldsymbol{Initial\ wt - Final\ wt}}{\boldsymbol{sample\ wt}}\right\} * \mathbf{100}$$

Crude fibre: Crude fibre content was determined following Madhu et al. (2017). Defatted 3 g sample was boiled in 200 ml H_2SO_4 (1.25%) for 30 min, filtered with muslin cloth and washed off acid remnants with boiling water, then boiled with 200 ml sodium hydroxide (1.25%) for 30 min, filtered and wash off with boiling water and alcohol. Transfer residue to a pre-weighed crucible (W1) and dry at 100±3°C for 4 h, cool in desiccator and weigh (W2). Ignite dried sample at 600°C for 1 h, cool in desiccator and weighed (W3).

$$\%\ \boldsymbol{Crude\ fibre} = \frac{\boldsymbol{(W2 - W1) - W3 - W1}}{\boldsymbol{Weight\ of\ sample}} * \mathbf{100}$$

Antioxidant activity by DPPH (2, 2- diphenyl-1-picrylhydrazyl free radical Antioxidant activity) assay: The antioxidant activity was determined using DPPH free radical scavenging assay (Baliyan et al., 2022), where Trolox was used as standard. The samples were extracted overnight in cool, dark 80% methanolic solvent. Centrifuging at 10000 rpm for 15 min yielded sample extracts. Ten concentrations of sample extracts from 0.5 mg/ml to 5 mg/ml were prepared by diluting it with 80% methanol to 1 ml and adding 3 ml of 0.1 mM DPPH solution. The mixtures were properly mixed and incubated for 30 min in dark room temperature and measured in a UV-Vis spectrophotometer at 517 nm. IC_{50} values were calculated using the formula:

$$\textbf{\% DPPH radical scavenging} = \frac{\textbf{(abs of control-abs of sample)}}{\textbf{abs of control"}} * \mathbf{100}$$

IC_{50} value is the concentration of sample required to scavenge 50% of the DPPH free radical and is calculated by plotting % DPPH activity against sample concentration.

Total phenol content: Total phenol content was estimated by Folin-Ciocalteau Reagent colorimetric method (Fu et al., 2011), using gallic acid as standard. 10 g of powdered samples were incubated overnight using 80% methanolic solvent kept in a shaker and dark place. The supernatant was collected after centrifuging for 15 min at 10000 rpm and filtration. 20 µl sample extract was mixed in pure H_2O, making up to 3 ml; then vigorously mixed. Add 500 µl Folin-Ciocalteu phenol reagent, then 2 ml of 7% sodium carbonate and incubated for 90 min in the dark. The contents were measured in UV-Vis spectrophotometer at 765 nm and the TPC values were calculated using formula:

$$\mathbf{TPC} = \frac{\mathbf{C} * \mathbf{V} * \mathbf{DF}}{\mathbf{Wt}} * \mathbf{100}$$

TPC values were expressed as gallic acid equivalent (GAE) gram per 100 gram dry weight, where C= Concentration of the sample derived from the standard graph, V=Volume of the extract, DF= Dilution Factor, Wt = Weight of the dried Sample.

Total flavonoid content: Total flavonoid content was estimated using the aluminium chloride colorimetric method (Sahreen et al., 2010), with quercetin as the standard. One gram of powdered sample was incubated overnight with 80% methanol in a shaker, then centrifuged at 10000 rpm to extract sample. 10 µl of the sample extract was mixed in 30% methanol making up to 4 ml volume, and addition

of 150 µl each of $AlCl_3$, $6H_2O$ (0.3 M) and sodium nitrite (0.5 M), then mixed vigorously and incubated for 5 min, then 1 ml of 1M NaOH was added. The reaction mixture was measured at an absorbance of 510 nm, and values expressed as Quercetin equivalent gram/100 g dry weight.

ß-Carotenoid content*:* ß-carotenoid was determined by using the method described by Aremu and Nweze (2017). One g of the sample was incubated in 10 ml of methanol overnight in a shaker in the dark. To separate the β-carotene layer, 10 ml of hexane was used with a separator funnel, and sodium sulphonate was added to remove moisture. Absorbance was taken at 436 nm in UV-Vis spectrophotometer using hexane as a blank. ß-carotenoids were calculated using the formula:

$$\boldsymbol{\beta - Carotenoid}\left(\frac{\boldsymbol{\mu g}}{\boldsymbol{100g}}\right) = \frac{\boldsymbol{Abs * V * DF * 100 * 100}}{\boldsymbol{Wt}} * \boldsymbol{Y}$$

Abs = Absorbance of sample, V = Total extract volume, DF = Dilution factor; Wt = Sample weight; Y = Dry matter content of the sample.

Vitamin A content was estimated by multiplying it with the conversion factor 0.167 µg RE (1 µg β-carotene= 0.167 µg Retinol Equivalent).

Statistical Analysis

All data results were expressed as Mean±standard deviation (n=3). Results expressed in the figures were made using Origin software 2021. Significant differences were analysed using One Way ANOVA (MS Excel 2019) and, Pearson's correlation test for determination of correlation using Graph Pad Prism 10.

Results

Proximate composition

The nutritional compositions of both pulp and peel of raw and ripe fruits of both the cultivars, i.e., G9 and Sabri, are presented in **Table 2.1**. The significant differences (p> 0.05) within the cultivars and between the cultivars (intra and inter-cultivars) were found to be non-significant in the parameters *viz., m*oisture content, dry matter, total carbohydrate, protein, ash, and crude fibre. Whereas, significant differences were found in reducing sugar (intra and inter-cultivars) and fat content within the cultivars (intra-cultivars); however, were found non-significant between the cultivars (inter-specific).

Moisture content

The moisture content in the cultivars, as shown in **Table 2.1**, the moisture percentage ranged from 70.2-87.85% in G9 and 69.67-85.58% in Sabri. The highest moisture content was observed in the ripe peel of G9 (87.85%) and Sabri (85.58%) and lowest in the unripe pulp of G9 (70.21%) and Sabri (69.67%). The peels were found to have higher moisture than the pulp in both cultivars. There is also a notable increase in the moisture content as the fruit ripens observed in both cultivars.

Dry matter content

The dry matter content in the banana samples ranged from 12.16-29.79% in G9 and 16.56 and 14.64 to 30.33% in Sabri. The highest dry matter was observed from the raw pulp of both the cultivars (29.79% in G9 and 30.33% in Sabri) and lowest in the ripe peel of G9 (16.56%) and raw peel of Sabri (14.64%) (**Table 2.1**). A gradual decline in dry matter content in peel and pulp in G9 was observed as the fruits ripened. The dry matter reduced from 29.79 to 24.67% in pulp and 16.56 to 12.16% in peel. Similarly, in Sabri, dry matter in pulp reduced from 30.33 (unripe) to 27.76% (ripe), but there was a slight increase in dry matter in the peel (14.64%, unripe to 16.42%, ripe).

Carbohydrate content

The Carbohydrate content (**Table 2.1**) ranged from 12.29 to 80.40 g/100 g in G9 and 20.46 to 79.65 g/100 g in Sabri. The highest carbohydrate content was observed in the unripe pulp of Grand Nain (80.40 g) and Sabri (79.65 g) and lowest in ripe peel G9 (12.29 g). The carbohydrate content in the pulp of both the cultivars was found to be similar but, in the peels, the cultivar Sabri had higher carbohydrate content than the cultivar G9. It was also observed that carbohydrate content decreased gradually as it ripened in both the pulp and peel.

Table 2.1: Comparative proximate compositions of unripe and ripe pulps and peels of banana cultivars Grand Nain and Sabri

Parameters	Cv. Grand Nain					Cv. Sabri				
	Unripe Pulp	Unripe Peel	Ripe Pulp	Ripe Peel	P value	Unripe Pulp	Unripe Peel	Ripe Pulp	Ripe Peel	P value
Moisture Content (%)	70.21 ±2.23	83.44 ±2.16	75.33 ±0.85	87.85 ±3.21	2.6E-07	69.67 ±1.12	85.36 ±1.46	72.23 ±2.32	85.58 ±2.42	3.21E-06
Dry Matter Content% (DW)	29.79 ±2.23	16.56 ±2.16	24.67 ±0.85	12.16 ±3.21	2.6E-07	30.33 ±1.21	14.64 ±1.46	27.76 ±2.32	16.42 ±2.42	3.52E-06
Total Carbohydrate (g/100g in DW)	80.40 ±6.26	33.55 ±4.21	39.58 ±2.79	12.29 ±1.64	3.91E-06	79.65 ±4.36	53.28 ±8.28	42.16 ±6.12	20.46 ±1.68	1.3E-05
Reducing Sugars (g/100g in DW)	2.34 ±0.79	3.44 ±1.11	7.71 ±1.67	7.87 ±1.25	0.010115	2.56 ±1.19	1.73 ±1.17	10.37 ±1.45	6.41 ±0.34	0.014034
Protein (g/100g in DW)	3.33 ±0.30	6.93 ±0.23	3.97 ±0.33	6.11 ±0.08	3E-07	3.37 ±0.25	4.38 ±0.15	3.67 ±0.19	6.87 ±0.53	1.77E-06
Fat content% (DW)	1.71 ±0.14	8.07 ±1.32	0.17 ±0.11	6.80 ±0.35	0.000824	0.25 ±0.02	7.52 ±3.44	0.53 ±0.25	3.31 ±0.72	0.000216
Ash Content (%) (DW)	5.71 ±0.19	10.08 ±0.44	4.58 ±0.21	11.26 ±0.36	1.43E-06	2.22 ±0.91	6.61 ±0.82	2.64 ±0.11	8.74 ±0.15	3.08E-06
Crude Fibre (%) (DW)	0.87 ±0.13	9.59 ±0.64	0.88 ±0.09	9.72 ±0.43	2.23E-09	0.76 ±0.07	10.67 ±2.40	1.32 ±0.15	6.57 ±1.12	1.81E-05

Note: * : Data represents mean of three replicates ± standard deviation

Reducing sugars content

The Reducing sugar content ranged from 2.34 to 7.87 g/100 g in G9 and 1.73 to 10.37 g/100 g in Sabri. The RS content was found highest in ripe pulp Sabri (10.37 g) and lowest in raw peel Sabri (1.73 g) (**Table 2.1**). The RS content in both the cultivars was observed to have significantly increased as the bananas ripened. The values of RS in the ripe pulp and peel of G9 were found to have almost similar values.

Protein content

Though not very high, both the cultivars were found to contain good protein (3-7%). As shown in (**Table 2.1**), protein content in G9 ranged from 3.33 to 6.93 g/100 g and 3.37 to 6.87 g/100 g in Sabri. The protein values ranged similarly in both the cultivars and the highest protein content was found in peels of unripe G9 peel (6.93 g) and ripe Sabri peel (6.11 g) and the least was found in the unripe pulp of Sabri (3.38 g) and G9 (3.33 g).

In both the cultivars, there was a slight increase in protein content in ripe pulp compared to the raw stage. Though, in G9 the protein content declined in the ripe peel against its unripe counterpart, but there was a significant increase in protein content in the ripe peel of Cv. Sabri (6.87% against 4.38% in unripe peel).

Fat content

The Fat content as shown in **Table 2.1**, ranged from 0.17-8.07 % in G9 and 0.25-7.52 % in Sabri. The peels of both the cultivars were found to be rich in fat content both in green as well as in ripe stage with G9, 8.07 and 6.8 % in green and ripe stage respectively; while, in Cv. Sabri, it was 7.52 and 3.31 %.

Ash content

As in **Table 2.1**, the ash content ranged from 4.58-11.26 % in G9 and 2.22-8.74 % in Sabri. The present study revealed that G9 peel contains a high ash content (10-11 %) against 6.6-8.74 % in Sabri. The peels of both cultivars had higher ash content than the pulp.

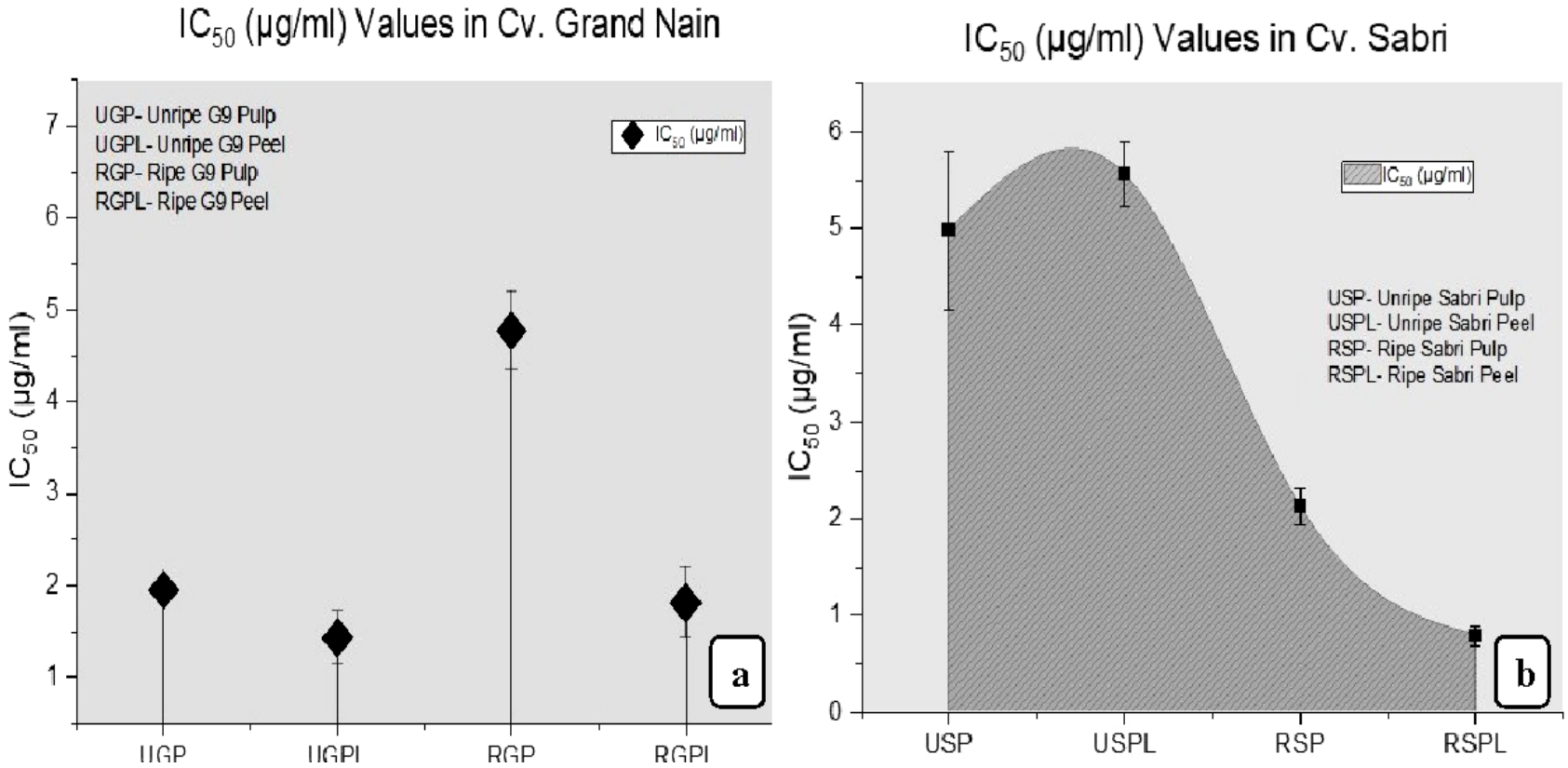


Figure 2.1: Showing the DPPH scavenging activity and IC_{50} value in different parts and stages of fruits; a. Cv. Grand Nain or G9, b. Cv. Sabri.

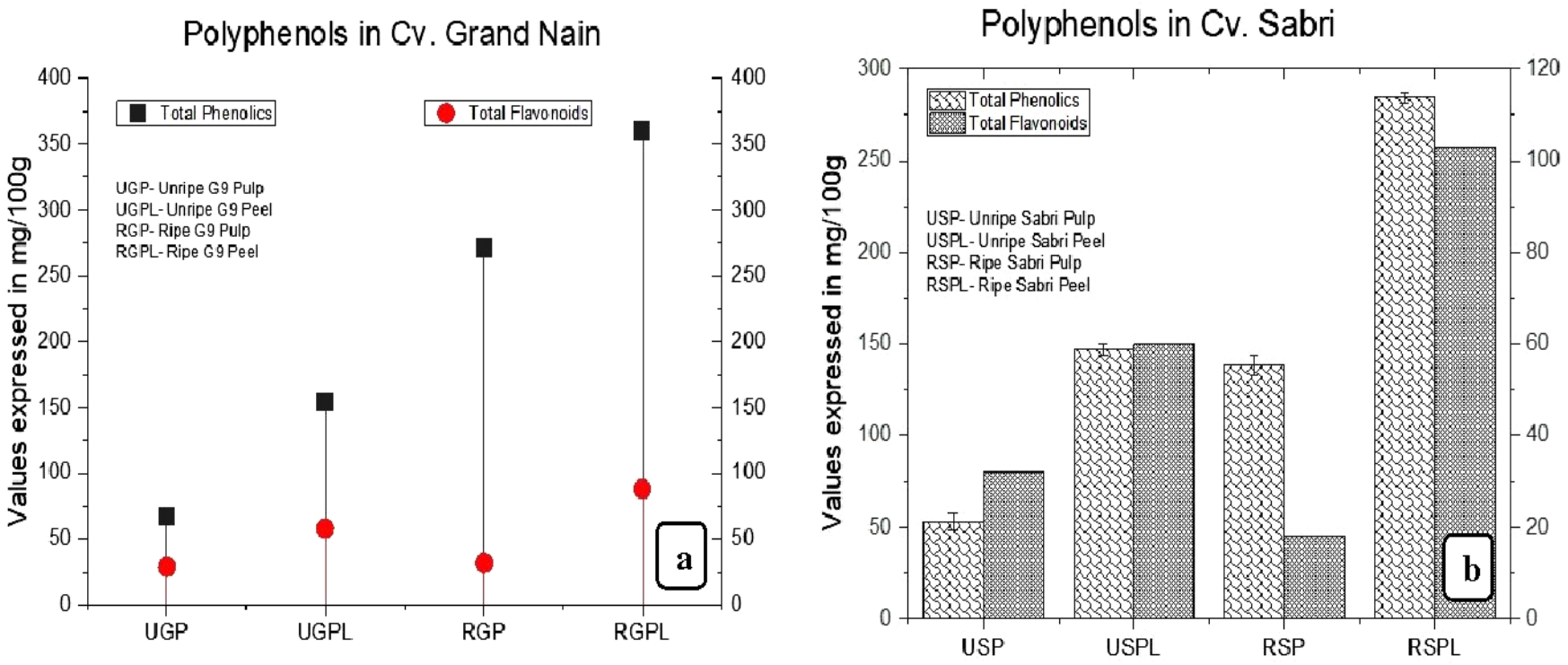


Figure 2.2: Total phenolic and flavonoid contents in different parts and stages of fruits; a. Cv. Grand Nain or G9, b. Cv. Sabri.

Table 2.2: Comparative values of antioxidant activity, β-carotenoid and Vitamin A contents in unripe and ripe banana pulps and peels of banana cultivars Grand Nain and Sabri

Parameters	Cv. Grand Nain					Cv. Sabri				
	Unripe G9 Pulp*	Unripe G9 Peel*	Ripe G9 Pulp*	Ripe G9 Peel*	P-Value	Unripe Sabri Pulp*	Unripe Sabri Peel*	Ripe Sabri Pulp*	Ripe Sabri Peel*	P-Value
Antioxidant activity (IC_{50} µg/ml)	1.95 ±0.12	1.43 ±0.29	4.77 ±1.82	1.81 ±0.39	0.0730	4.98 ±3.82	5.56 ±0.33	2.13 ±0.19	0.79 ±0.1	0.15723
β-Carotenoid (µg/100g)	53.7 ±5.03	465.0 ±18.61	60.29 ±3.04	275.4 ±29.6	1.11E-08	68.41 ±0.09	550.7 ±35.1	65.29 ±3.07	255.2 ±5.2	1.1E-11
Vitamin A (µg/100g)	8.62 ±0.98	84.81 ±3.11	9.82 ±0.51	48.95 ±7.1	1.16E-08	10.90 ±1.66	91.97 ±8.35	10.88 ±0.51	42.46 ±0.87	1.16-11

Note: * : Data represents mean of three replicates ± standard deviation.

Antioxidant activity and nutraceutical compounds

The results of the bioactive compounds of G9 and Sabri are shown in **Table 2.2**, and **Figure 2.1**. The difference in the values (p>0.05) were observed from bioactive compounds such as the total phenol, total flavonoid, and antioxidant activity. In contrast, in Vitamin A no significant difference (p<0.05) was obtained from the values of both intra and inter-cultivars.

Antioxidant activity

The IC_{50} value or the radical scavenging antioxidant activity was found to be between 1.43-4.77 µg/ml in G9 (**Figure 2.1a**) and 0.79-5.56 µg/ml in Sabri **(Figure 2.1b**). The highest scavenging activity was obtained from peels of both cultivars, G9 unripe peel (1.43 µg/ml) and Sabri ripe peel (0.79 µg/ml), which surpassed the amount of antioxidants present in the edible part of the banana.

Total phenolic content

Total phenolics ranged from 67.28-359 mg/100 g in G9 (**Figure 2.2a**), and 52.86-284.4 mg/100 g in Sabri (**Figure 2.2b**), and the highest were obtained in the ripe peel of G9 (359.8 mg) and Sabri (284.4 mg). The total phenolic content in peels was significantly higher than in the pulp. Further, a gradual increase in TPC values was observed in the ripened fruits of both cultivars.

Total flavonoid content

The total flavonoid content **(Figure 2.2**) in the present study ranged between 29-88 mg/100 g in G9 and 18-103 mg/100 g in Sabri, and the highest TFC were found in the ripe peel of G9, (88 mg/100 g) and Sabri (103 mg/g). The pulp and peels had significant differences in their values, with peels containing higher flavonoid content. The flavonoid content was also observed to increase gradually as the fruits ripened.

ß-carotenoid and vitamin A

Beta carotenoid (**Table 2.2**) ranged from 53.7-465 µg/100 g (Vitamin A-8.62-84.81 µg/100 g) in G9 and 65.29-550.7 µg/100 g (Vitamin A-10.88-91.97 µg/100 g) in Sabri. The highest content of Vit A was found in the unripe peel of G9 (84.81µg/100g) and Sabri (91.97 µg/100 g). The peels of both cultivars exhibited higher values than the pulp, and a slightly notable decrease in values was observed in the ripened stage.

Discussion

Proximate composition

Moisture content

A notable increase in the moisture content in the ripe fruits was observed in both cultivars as the moisture level in banana elevates as they ripen, primarily due to breakdown of carbohydrates. The peels of banana contain higher moisture content than in pulp, due to an osmotic transfer of water from the peel to the pulp, all resulting in elevated moisture and sugar levels. Increase in sugar content in ripe banana has a great affinity for water, making them hydrophilic (Kookal and Thimmaiah, 2018). The findings of the present study in both the cultivars are in consonant with the report of Hapsari and Lestari (2016) in ripe dessert (AAA) and cooking bananas (ABB) and Dotto et al. (2019) in unripe cooking banana. While, Khawas and Deka (2016) reported ~57-65 moisture content in culinary banana (ABB) which is lower than G9 and Sabri cultivars.

Dry matter content

The present study observed a gradual decline in the dry matter content of the fruits as they ripened. Chauhan and Jethva (2016) also reported similar findings, suggesting that the reduction in dry matter during ripening may be attributed to the transfer and conversion of moisture and starch.

Carbohydrate content

Different forms of carbohydrates are essential components of a healthy diet as they provide the body with energy. Upon digestion, the complex carbohydrates convert to glucose, a primary fuel for the brain, with an optimal daily intake of 200-300 g/day. The value obtained in the pulp of G9 in the present study is in line with the findings of Khoza et al. (2021). Further, a study conducted on Cavendish and other bananas by Sarda et al. (2016), reported that Sao Paulo had similar values (76.24 g and 81.43 g) comparable to the finding of the present study. There was a sharp decline in the total carbohydrate content of peel and pulp of raw and ripe bananas of both the cultivars in the present study, but there was an increase in the reducing sugar content.

Reducing sugars content

In both the cultivars in the present study, there was a gradual increase in total sugar content in ripened fruits and this was also observed by Kookal and Thimmaiah (2018), in their study. An increase in reducing sugar during ripening was primarily due

to degradation or hydrolysis of starch into soluble sugars by several enzymes leading to transforming the starch reserves into soluble sugars and making the pulp of the ripe banana sweet to taste and easily digestible (Khawas and Deka, 2016; Kookal and Thimmaiah, 2018).

Protein content

Protein is crucial for overall health, immunity, maintains pH balance, regulate fluid balance, tissue repair, and cell regeneration and promoting body growth and structure. The present study has similar results with Kumar et al. (2019) and Khoza et al. (2021) in raw G9 pulp. Similar results were also obtained to support the present study (Baiyeri et al., 2011; Bezerra et al., 2013; Sardá et al., 2016). However, in another study in G9 by Thatayaone et al. (2022), protein content was lower in comparison with the present study. The finding of the present study with relation to increase in the protein content as it ripened was also supported by past studies (Akaninwor and Sodje, 2005; Adegunwa et al., 2017; Kookal and Thimmaiah, 2018). Protein synthesis is crucial for the ripening of fruits, due to which protein content increases due to an increase in conversion of enzymes and synthesis of proteins (Onwuka and Onwuka, 2005).

Fat content

In general, banana is not good source of fat. Fats are the largest class of lipids known as triglycerides and are in small portion in our diet to support various bodily functions, such as, aiding in vitamin absorption, producing hormones like estrogens, testosterone, and cortisol, storing energy in the form of fat etc. The fat content in the edible part of bananas is significantly low and sometimes negligible. The peels had rich fat content which can be used to extract fats for commercial purpose. In the past, Sardá et al. (2016) and Thatayaone et al. (2022) reported similar results with pulp of Cavendish banana and G9 and the findings of the present study with Sabri is in agreement of the report. The study in green flour of G9 by Khoza et al. (2021) reported a comparatively lower fat content.

Ash content

The ash is an inorganic residue that remains after the samples have been exposed to high ignition, removing organic matter. The ash residue denotes the composition of numerous minerals, mainly potassium, sodium, calcium, magnesium, copper, manganese, zinc, etc. The peels in the present study had higher ash content,

indicating their potential to be used as a food supplement (**Table 2.1**). The findings of the present study are similar to some reports of the pulp of G9 (Khoza et al., 2021; Thatayaone et al., 2022). Studies conducted on Cavendish banana by Sardá et al. (2016), showed similar results with Cv. Sabri, but it was very low in comparison to G9. The study by Kookal and Thimmaiah (2018) and Dotto et al. (2019) in dessert as well as in cooking bananas showed comparatively low ash contents in contrast to the present study.

Antioxidant activity and nutraceutical compounds

Antioxidant activity

Antioxidants are compounds that neutralize and reduce or prevent oxidative damage to target molecules, usually known as Free radicals or Reactive oxygen species (ROS). The free radicals or ROS are unpaired electrons that are very reactive and can instantly attack the cells, DNA, molecules etc. and can cause harmful effects and damage to the human body causing many chronic illnesses (Ao and Deb, 2019; Baliyan et al., 2022).

Studies in Malaysian cultivars conducted by Sulaiman et al. (2011) obtained values similar to those of the present study. On the contrary, studies also reported lower values in antioxidants in comparison to the present study (Khawas and Deka, 2016; Siji and Nandini, 2017). In the present study, the peels had a higher number of antioxidants than the pulp, due to which it can be used as a source of antioxidants and can be extracted and processed into health-beneficial products, the findings are in line with the findings of Someya et al. (2002).

Total phenolic content

Phenolic compounds are diverse bioactive secondary metabolites with multiple biological activities. They have anti-inflammatory, antibacterial, and antioxidant properties linked to several health benefits and are scientifically proven to prevent various diseases like diabetes and degenerative disorders like cancer, cardiovascular disease, Alzheimer's and Parkinson's diseases, etc. (Sulaiman et al., 2011; Singh et al., 2016; Elbagoury et al., 2022).

The total phenolic content in peels was significantly higher than the pulp and is supported by the studies conducted by (Someya et al., 2002; Sulaiman et al., 2011). Study conducted by Elbagoury et al. (2022) on G9 reported similar results as the present study. Similar to the finding of the present study, Bashmil et al. (2021) in their

study with six banana cultivars reported that the phenol content ranged from 54-132 mg/100 g in peels and 38-128 mg/100 g in pulp. Further, in the present study, it was found that there was a gradual increase in polyphenol content in the ripened fruits of both cultivars.

A study conducted by Khoza et al. (2021) on G9 green flour reported higher TPC (524.87 mg/100g) content; while, in contrast, Bashmil et al. (2021) reported slightly low amounts of TPC in their study on Australian-grown bananas. The variations in the TPC values may be due to limitations in the handling and maintenance during postharvest, prolonged storage, oxidative reactions during storage, temperature, or selection and drying methods of samples (Kookal and Thimmaiah, 2018; Elbagoury et al., 2022).

Total flavonoid content

Flavonoids are the most prominent class of phenolic compounds found in almost all plants and are usually also known for the occurrence of colours in fruits and vegetables. They are scavengers that protect against reactive oxygen species (ROS) and free radicals, which causes many illnesses and aging (Singh et al. 2016; Bashmil et al. 2021).

The flavonoid content was observed to increase gradually as it ripened. Ali et al. (2019), in their study on G9, the ripe pulp reported values that are similar to the ripe pulp of Sabri and a reasonable difference in the ripe G9 pulp of the present study; however, significantly lower peel values in comparison to the present study. The peels of both cultivars have significantly higher amounts of flavonoids than the pulp, correlating with the TPC (**Figure 2.3**) and antioxidant value (Someya et al., 2002; Sulaiman et al., 2011).

The TFC in some of the studies reported significantly higher values (Khoza et al., 2021) (407.08 mg/100 g) in raw G9 flour and (110 to 131 mg/100 g) in 3 cultivars of ripe bananas (Kookal and Thimmaiah, 2018). The differences in the TFC from study to study can be associated with samples, extraction, solvent, time, temperature, etc. (Bashmil et al., 2021).

ß-carotenoid and vitamin A

Carotenoids are precursors of vitamin A found naturally and abundantly in natural foods and are called pro-vitamin A. They are fat-soluble pigments imparting the characteristic yellow, orange, or red colours that enhance flavour and aroma and also

attract pollinators. They are renowned for possessing anti-ageing properties and numerous health benefits, including the prevention of leukemia, retinitis pigmentosa, lung and breast cancer, and skin conditions etc.

The studies from different workers reported beta carotenoid content on Cavendish and some selected banana cultivars, which ranged from 5 to 117.2 µg/100 g in the pulp and 49 to 241 µg/100 g in peels, which aligned with the present study (Arora et al., 2008). In a study by Siji and Nandini (2017), beta carotenoid was reported to be significantly low (2.19 µg/100 g and 21.19 µg/100 g). The notable differences in the carotenoid content are due to the destruction of carotenoids during storage and also repeated thawing and transferring of samples from one place to another, carotenoids can be destroyed during post-harvest activities.

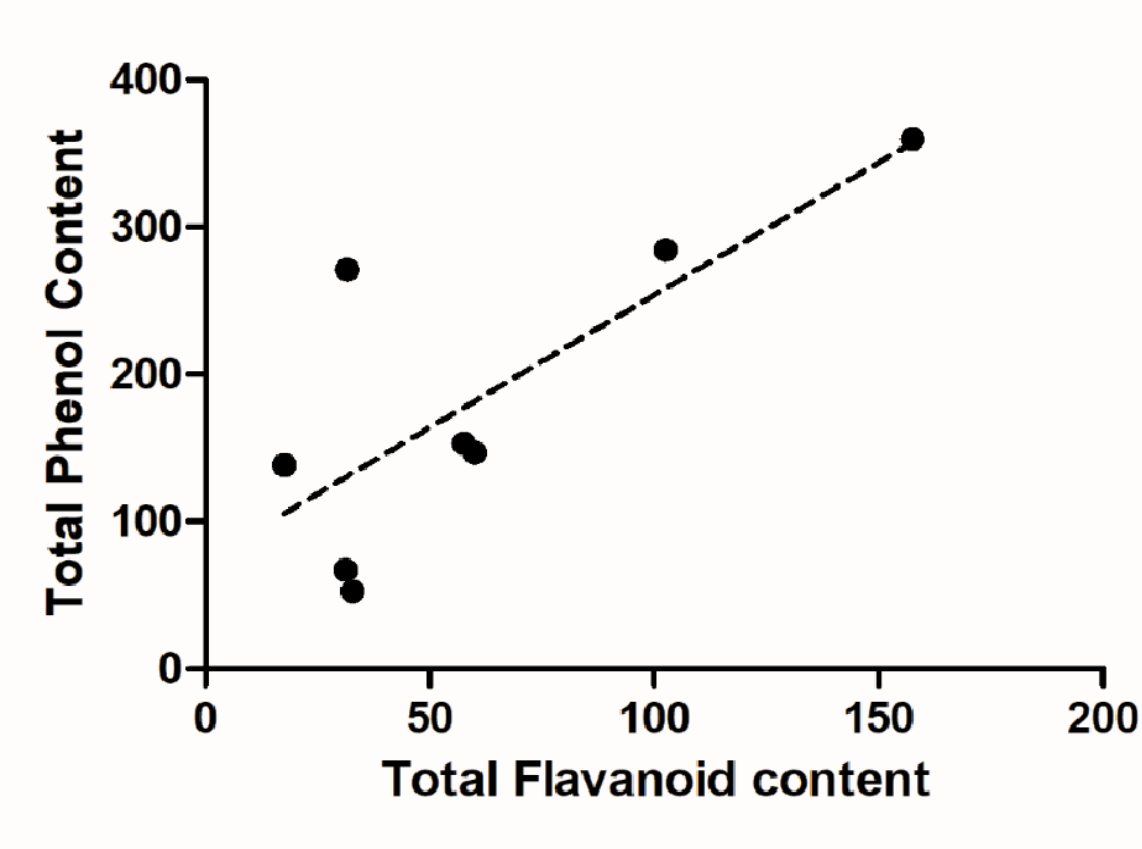


Figure 2.3: Correlation between total phenol and flavonoid contents. Correlation coefficient, r = 0.7738, 95% confidence interval = 0.1518 to 0.9568, Coefficient of determination, r^2 =0.5988.

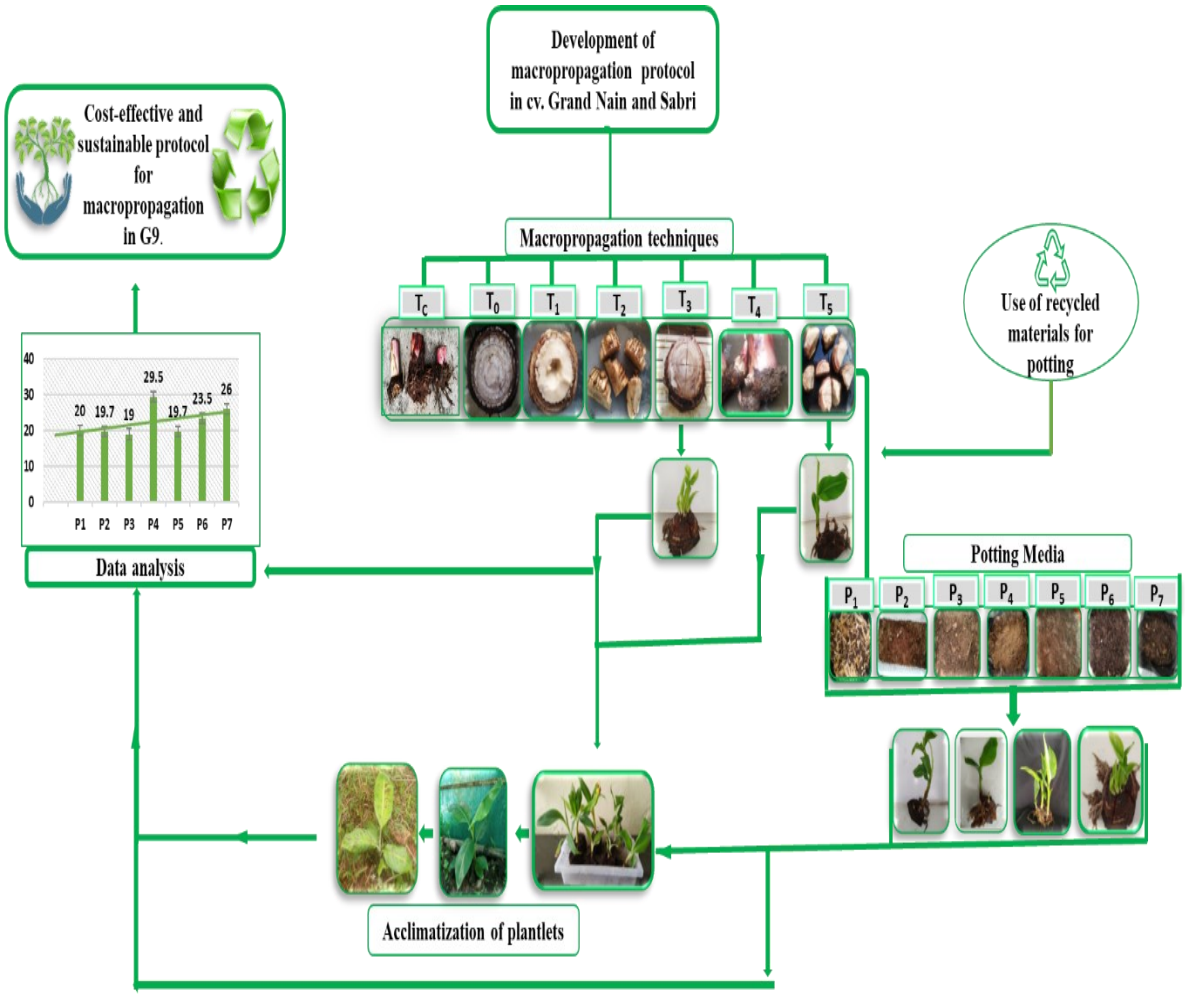
Summary and Conclusions

The present study carried out on two *Musa* cultivars 'Grand Nain' and 'Sabri' examined the unripe and ripe stages of both the pulp and peel. The study concludes that the comparative analysis highlights the importance of nutrient distribution. It also provides a comprehensive dataset of the cultivars in two stages of banana (ripe and unripe) as well as in pulp and peel, which will further facilitate research and enhance our understanding of how to utilize the entire banana and also determining the best utilization stage for specific purposes. Both cultivars in the study exhibited the presence of nutrients that can mitigate nutrient deficiency problems encountered by many countries and regions. The cultivars also demonstrate similar nutrient composition; however, Cv. Sabri had comparatively higher carbohydrate and reducing sugar content; this disparity explains the contrasting texture and sweetness between the two cultivars. Further, the study demonstrated the significance of unripe bananas and their inedible peel, maximizing banana use. Unripe pulp had high carbohydrate content, indicating the presence of resistant starch, which is renowned for its advantageous effects on the digestive system. The peels are also nutrient-rich with high fibre, protein, minerals, antioxidants, and bioactive compounds essential to the body. Therefore, pharmaceutical companies can use them to extract and formulate health supplements, drugs, food additives, etc. The unripe pulp and peels of bananas can be used to advance value-added products and a complementary component to the existing products. The unripe pulp can also serve as a vegetable, and both the unripe pulp and peels can enhance the current products with superior quality, nutrient-rich, gluten-free, fibre-rich products. Thus, by utilizing the whole banana fruit, we can create more valuable products and contribute to a healthier environment.

Chapter – 3

Macropropagation in Banana Cultivars Grand Nain and Sabri

Graphical Summary



Introduction

Bananas rank as the fourth most essential food crop globally, following rice, wheat, and maize, owing to their year-round availability, affordability, wide range of varieties, and significant nutritional and medicinal value (Kumari et al., 2022). They are an excellent source of essential nutrients, including carbohydrates, vitamins, and minerals, and are also rich in bioactive compounds such as carotenoids, flavonoids, and amine, which are known to provide various health benefits, including antioxidant and anticancer properties (Tan et al., 2024). In addition to being consumed as fresh fruit, bananas have diverse uses as processed products, such as dried chips, wine, beer, flour, and juice, as well as applications in thatching, textiles, cordage, and mulch (Deshmukh et al., 2009).

However, the increasing commercial demand for bananas has led to a rising need for clonal planting propagules. Traditionally, bananas are propagated by vegetative means, such as rhizome splitting, a slow process that produces only 5-20 suckers in its lifetime. Furthermore, cultivated banana cultivars are highly susceptible to soil-borne pathogens and other diseases, which can be spread through contaminated suckers during transplanting from infected mother plants or areas (Singh et al., 2011; Sajith et al., 2014; Manju and Pushpalatha, 2022; Ngullie and Deb, 2024). Due to these the natural regeneration process of cultivated banana cannot meet the growing demands of clonal planting materials. *In vitro* micropropagation technology is a viable solution to meet the demands of large-scale planting materials; however, the technology is beyond the limits of marginal unskilled farmers living in extremely rural areas deprived of sophisticated facilities, and even lack of required financial support. Due to the higher cost of quality planting materials, poor and marginal farmers are often unable to afford the costly planting materials, which are 4-8 times costlier than the vegetative propagated suckers (Suryanarayana et al., 2018), hence are not an option for the framers. Therefore, it is crucial to develop simple, effective and sustainable methods of propagation banana enabling the unskilled remote farmers to produce their own planting materials with ease for small to medium scale cultivation.

A viable alternative approach to *in vitro* propagation is macropropagation techniques, requiring simple methods, inexpensive tools, and a less skilled workforce

can produce planting materials they need for their small cultivation plots. This propagation technique is either done on-field or off-field, where it necessitates the acceleration of the lateral meristems, resulting in the formation of lateral buds and shoots and thereby can producing 10-30 plantlets by a single corm in a short period of time under semi-controlled conditions (Kasyoka et al., 2010; Ngullie and Deb, 2024). In the past many studies were undertaken for macropropagation of banana using different rhizome/corm manipulation techniques like complete decapitation / decortications, false decapitation, PIF stem fragmentation method etc. and demonstrated that these macropropagation techniques are simple and quite effective, especially for small and rural stakeholders (Njukwe et al., 2013; Sajith et al., 2014; Thiemele et al., 2015; Langford et al., 2017; Thungon et al., 2017; Suryanarayana et al., 2018; Opata et al., 2020; Izaile et al., 2021). Some studies reported the total expenditures for constructing growth chambers for the propagation of plantlets which was about USD 462.5 per unit and an overall set up of USD 2,300 (Njukwe et al., 2007; Ntamwira et al., 2017; Adjei et al., 2021), which is multiple times lower than the micropropagation techniques, aiding its farmer-friendly nature.

Although, several reports are available on macropropagation of banana, but there are challenges in the practicality of the experiments (Langford et al., 2017) along with lack of awareness amongst the remote and poor farmers. With this background, the present study was undertaken to develop a simple and effective sustainable macropropagation technique for two commercially established cultivars Grand Nain (G9) and Sabri following different technique of sucker fragmentation along with formulation of effective potting mixture using locally available substrata to facilitate the poor sub-urban farmers and small stakeholders. The experiments carried out had two main aspects: 1. Using different corm splitting methods of macropropagation; 2. Using different potting mix or substrata. The study was conducted in the eco-friendly and budgetary way by using only available items like reusable containers, clay pots, biofertilizers, and naturally obtained potting substrates found readily in the University campus.

Materials and Methods

Collection and preparation of the planting materials

The study was conducted during 2020 to 2024. The healthy banana sword suckers of G9 and Sabri cultivars were collected from Nagaland University, Lumami campus during pre-monsoon season (March-April) every year. The collected suckers were washed and cleaned thoroughly with running tap water to eliminate adherent dirt particles followed by sun-dried for 1-2 h. The suckers pruned carefully to remove the roots and outer leaf foliage. The primarily processed corms were subjected to different corm manipulation, according to the said techniques described below and then treated with 0.1% Bavistin (w/v) (BASF India Ltd., India) for 30 min, then potted in different potting mixtures, maintained in the poly-house Ca. 50% shade where humidity was maintained using concealed perforated transparent polythene bags, and at ~25-30°C temperature.

The present investigation comprises macropropagation techniques and use of different potting media. The suckers, after undergoing subsequent cleaning in the running water, are sundried for few hours. Then the following corm manipulations were employed followed by planting in pot containing different potting mixture/media (**Figure 3.1)**. The pots were kept in polyhouse with ca.50% shade, and each pot was sealed with thin plastic sheets to conserve moisture and promote sprouting. Watering and monitoring of the pots were conducted at regular intervals every third day, accompanied by the documentation of observations for two generations during a period of three months.

Macropropagation Techniques

Six different corm manipulation methods were employed in the macropropagation techniques with traditional method as control (T_C).

1. Conventional method (Control) (Tc) (**Figure 3.2 a, Tc**): The conventional method is a traditional method of banana propagation by sucker propagation. In this technique, 2-4 months old young sucker is detached from the mother plant and the upper leaf sheaths are chopped off for easy transfer of suckers.

2. Incomplete decapitation (T_0) **(Figure 3.2b, T_0)**:

The roots of the cleaned suckers were pruned minimally, the lower portion of the rhizome was cut, and the overlapping leaf sheaths were removed, exposing the apical region of the sucker.

3. Complete decapitation/ decortications (T_1) **(Figure 3.2c, T_1)**: Like the previous method (T_0), the lower portion and the overlapping leaf sheaths were removed. Further, the exposed apical region was removed up to 4-5 cm depth destroying the apical meristem/ shoot apex.

4. Split corm method (T_2) **(Figure 3.2d, T_2)**: In this method, the initial process of removal of the basal portion and leaf sheaths was done similarly to the previous methods. Subsequently the corm was split longitudinally into 4 equal fragments, where the apical meristem is evenly distributed in all the fragments.

5. Plants Issus des Fragments de Tige (PIF) (Plants resulting from stem fragments) (T_3) **(Figure 3.2e, T_3)**: This method is also partly similar to the split corm method (T_2) since it partially divides the apical meristem portion into four parts. In this method, a crosswise partial incision was made in the apical portion of the corm up to a depth of 3-6 cm, not fully dividing the corm.

6. Excised bud method (T_4) **(Figure 3.2f, T_4)**: In this method, young suckers was not used, instead, the lateral buds sprouted from the sides of the mother plant rhizomes were used. The lateral buds were unnaturally detached from the mother plant with a sterile knife and undergo cleaning and Bavistin treatment. The lateral buds later grow into a shoot.

7. Modified combination of split corm + PIF method (T_5) **(Figure 3.2g, T_5)**: This method is a modified combination of both the split corm (T_2) and the PIF method (T_3), where a corm was split into two equal halves and a straight incision is made in the middle of the apical portion at a depth of 3-6 cm in both the halves, dividing the apical portions equally.

Figure 3.1: Pictures of potting media and their mixtures, along with recycled containers used in the experiment. P_1: Garden Soil+ Straw + Dried cow dung manure, P_2: Saw dust, P_3: Forest soil manure, P_4: Sand, P_5: Sand + Saw dust, P_6: Sand+ Tea manure, P_7: Sand + Forest soil manure, B: Polyethene covering the pot for retaining humidity.

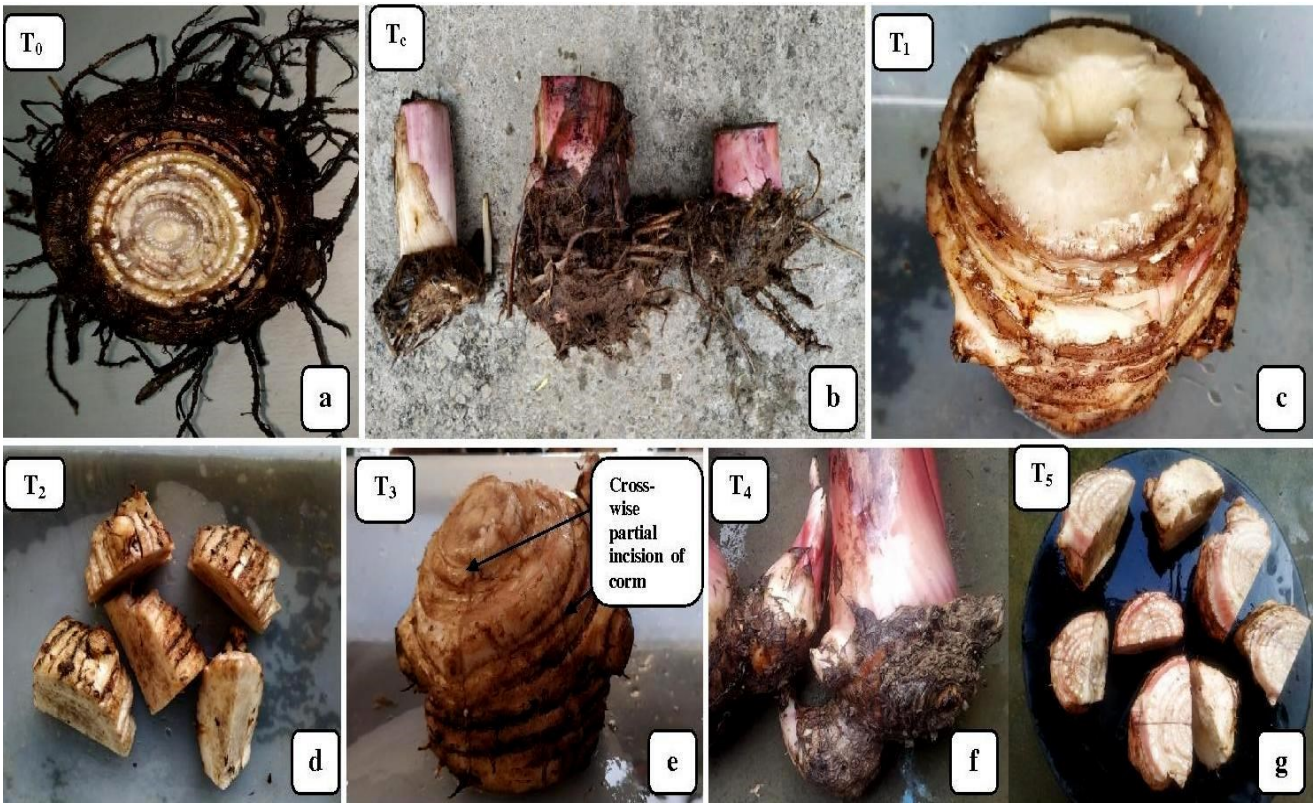


Figure 3.2: Different macropropagation techniques used for planting of rhizome parts. a. Incomplete decapitation (T_0); b. Conventional method (T_c); c. Complete decapitation (T_1); d. Split corm method (T_2); e. PIF method (Plants Issus des Fragments de Tige) (T_3); f. Excised bud method (T_4); g. Split corm and PIF method (T_5).

Potting mixtures / media

The substrata for potting mixtures were collected from the University campus and are commonly found in any place at either free of cost or at a very low cost. The substrata were garden topsoil, forest soil, sand, sawdust, straw, dried cow dung manure, and tea manure. Collected substrata were sundried properly for 2-5 days. From the preliminary initial study, it was found that of the five corm splitting techniques, PIF and split corm methods were better choices, hence used for the optimization of the potting mixture combinations. The cultivar Sabri due to its insufficient sample was used in three potting mixtures garden soil (P_1) saw dust (P_2) and forest soil (P_3) in the PIF method. To make different potting mixtures, the substrata were mixed in different combinations and ratios. Seven types of potting mixtures/combinations were used in this study (**Figure 3.1, P_1** to **P_7**):

***1. Garden soil + Straw + Dried cow dung manure (P_1)*:** The P_1 potting mixtures consisted of garden top soil, paddy straw and dried cow dung at 1:1:1 ratio. The top most soil was collected from the experimental garden of the Department of Botany. Straw was chopped into smaller pieces and the clumped cow dung was granulated properly. The substrates were then mixed properly in the same ratio and used as a potting medium.

***2. Saw dust (P_2)*:** In P_2 saw dust was singly used as the potting medium. To prepare the sawdust for potting, the collected sawdust was soaked in boiling water for an hour followed by rinsed several times with tap water, drained off the excess water, sundried until dry, and distributed in the pots for the experiment.

***3. Forest soil manure (P_3)*:** In P_3, the top forest soil consisting of mulch and soil was collected from the surrounding forests and sundried for a day and used solely as a potting medium.

***4. Sand (P_4)*:** In P_4, the unwanted derbies were removed from the collected sand and sundried before using as a potting medium.

***5. Saw dust + Sand (P_5)*:** The P_5 potting medium was prepared by mixing sawdust and sand at a 1:1 ratio. The substratum was prepared using the same preparation method as P_2 and P_4.

***6. Sand+ Tea manure (P_6)*:** In P_6, the substrata tea manure and sand were used, where the tea manure was collected from the used disposed of tea leaves and composted for 2-

3 months. To form a potting medium, the composted tea manure was sun-dried and mixed with sand at 1:1 ratio and used as a potting medium.

7. Sand + Forest soil manure (P_7): In P_7, sand and forest soil were used as the potting medium. Both the substrate preparation methods were same as stated in P_3 and P_4. The substrata were mixed in a ratio of 1:1 and used as potting medium.

Experimental steps

The collected healthy suckers were carefully treated and prepared according to the various techniques described above. The modified corm/sucker were planted in different potting mixtures and maintained in the poly-house under ca.50% shade. The pots were covered with holed transparent polythene for invoking shoot meristems. The pots were watered twice weekly and progression was recorded at weekly interval for 3 months. Once the plantlets developed, attained a certain height and developed leaves and roots, the young plantlets were then transferred to another pot with the same potting mixture and maintained in the poly-house for another month under diffused sunlight. Following this, the young plantlets were maintained in the nursery bed in the poly-house for two months before transferring to the field. The flow chat of the experiment is given in **Figure. 3.3.**

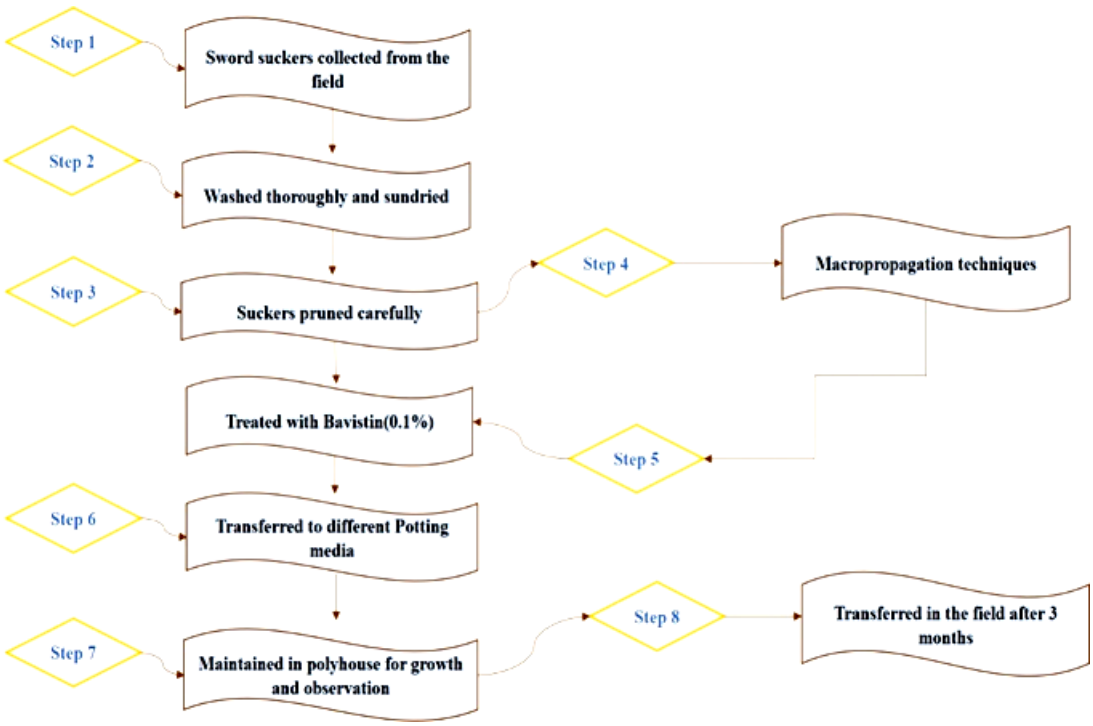


Figure 3.3: Schematic presentation of experimental steps

Genetic variability assessment

To evaluate the genetic stability of the regenerated plantlets, two markers i.e., RAPD and SCOT makers were used. For the purpose, donor plant (M) and seven regenerated plants (R_1-R_7) was randomly selected from the two cultivars, Grand Nain and Sabri and genomic DNA was extracted from young and fresh leaf tissues following modified CTAB protocol by Shankar et al. (2011). The extracted DNA templates were amplified using RAPD and SCoT makers. Initially a total of 25 primers from RAPD and SCoT markers were screened and 7 best performing primers for each marker were used for the study. The PCR mixture contained 5X PCR buffer, 2mM $MgCl_2$, 0.5mM dNTPs, 1µM primer, 1unit of *Taq* DNA polymerase. The PCR reaction was performed in a Thermal Cycler (BioRad) with the initial step of denaturation at 95ºC for 5 min, followed by 30 cycles of denaturation at 94ºC for 1 min, annealing Tm at 32-36ºC for 30 sec for RAPD and for SCoT analysis, initial denaturation at 94ºC for 4 min, with 30 cycles of the subsequent steps: 94ºC for 1 min, annealing Tm at 52ºC for 1 min, extension at 72ºC 2 min, final extension for 8 min. The amplified products were separated using 1.5% agarose gel and observed in a gel image system. The amplification products were scored between samples and recorded in binary layout in the presence and absence of bands in the gel.

Experimental design and statistical analysis

The study consisted of two sets of experiments, each having its response, impact, and observations. Studies on macropropagation techniques and potting mixtures were conducted in three replicates in a completely randomized experimental design. For evaluation of experimental outcome, data collection was done weekly for three months for each set on primary regeneration only. The data collection consisted of 14 independent variables and 5 dependent variables, *viz.,* a) time taken for the first emergence of bud sprouts (in days), b) number of shoots harvested per corm, c) shoot height (in cm), d) number of leaves per shoot and e) number of roots per shoot was considered. The data analysis was done using One Way ANOVA (MS Excel 2019), Graph Pad Prism 10.2.0 and Origin 6.4.

Results

Effect of different rhizome fragmentation on morphogenic response in Cv. Grand Nain

The different techniques of corm splitting exhibited significantly different morphogenic responses in cv. Grand Nain and it was noted that many of the dependent factors had both positive and negative responses (**Figure 3.4)**. All the corm splitting techniques exhibited positive responses in the sprouting of shoot buds within 3-4 weeks of potting (**Table 3.1, Figure 3.4 b-g**). In the control treatment (Tc) with intact shoot apex started sprouting within one day of treatment. Besides control, the initial sprouting of shoot bud(s) was first recorded in the PIF method (T_3) after 12.3 days of potting, followed by Split + PIF method (T_5) (17.7 days), Excised bud (T_4) (19.7 days), and Split corm (T_2) (19.7 days) where similar results were recorded; while the Incomplete (T_o) (23.2 days) and complete decapitation (T_1) (24.3 days) treatments were the slowest in morphogenic response (**Table 3.1**).

Length of the emerged shoots was recorded and found that there was a significant difference in shoot length in different corms splitting techniques. Higher shoot length, number of leaves, number of roots was observed in the Tc (40.1 cm, 5.0 and 32.7 respectively) compared to other techniques. Among the different corm splitting techniques, higher shoot length was recorded from Split + PIF method (T_5) (27.1 cm) and PIF method (T_3) (25.7 cm) and the lowest from Split corm (T_2) (8.0 cm) (Table 1). The number of leaves was also recorded a maximum number of leaves in Control (T_C) (5.0) and Split + PIF method (T_5) 5 leaves, although no significant differences were observed between all the techniques and it had an average of 3-4 leaves. The number of roots had significant differences in values between the techniques, and the highest number of roots was recorded in Split + PIF method (T_5) (39.0) (**Figure 3.4 g**) followed by Control (T_C) (32.7) (**Figure 3.4a),** PIF (T_3) (29.7) and least was recorded in Excised bud (T_4) (12.3). Though higher number of roots was recorded in T_5 technique, in control treatment the roots were thicker and healthier.

Significant differences in the $p<.05$ values in the factors between the techniques were obtained in the four techniques except in the number of leaves. Pearson's correlation factor also yielded a negative correlation between the parameters, bud emergence time and shoot height, and positive correlation between parameters bud

emergence and shoot bud number, shoot height and roots, and, a number of leaves and shoot height.

Table 3.1: Effect of different corm fragmentation technique on morphogenic response in Cv. G9

Corm splitting techniques*	Time for emergence of bud sprouts (in days) (±SD)**	No of shoot buds formed/ segment (±SD)**	Location of initial meristematic loci sprouting	Average shoot height (in cm) (±SD)**	No of leaves per shoot (±SD)**	No of roots per shoot (±SD)**
T_C	1.0 ±0.0^{e}	1.0 ±0.0^{a}	Apical meristem	40.1 ±1.2^{e}	5.0 ±1.6^{cd}	32.7±4.1^{cd}
T_0	23.2 ±1.6^{d}	1.0 ±0.0^{a}	Apical meristem	12.7 ±2.51^{bc}	3.3 ±0.5^{ab}	22.2 ±3.2^{bc}
T_1	24.3 ±4.1^{d}	1.3 ±0.6^{ab}	Lateral meristem	11.1 ±2.61^{b}	2.5 ±0.7^{a}	20.0 ±1.5^{b}
T_2	19.7 ±1.5^{c}	6.7 ±2.3^{d}	Apical and lateral meristem	8.0 ±2.17^{a}	3.6 ±2.6^{ab}	19.3 ±2.5^{b}
T_3	12.3 ±2.5^{a}	5.7 ±3.7^{cd}	Apical meristem	25.7 ±3.2^{d}	4.3 ±0.5^{bc}	29.7 ±4.3^{c}
T_4	19.7 ±1.5^{c}	1.3 ±0.3^{ab}	Apical meristem	10.2 ±1.2^{ab}	2.5 ±0.9^{a}	12.3 ±2.5^{a}
T_5	17.7 ±2.5^{bc}	4.0 ±2^{c}	Apical and lateral meristem	27.1 ±3.7^{d}	5.0 ±0.5^{cd}	39.0 ±4.9^{d}

* T_C: Conventional method; T_0: Incomplete decapitation; T_1: Complete decapitation; T_2: Split corm method; T_3: PIF method; T_4: Excised bud method; T_5: Split corm and PIF method; **±SD: Standard deviation (n=3); the values with different superscript letters in the same column are significantly different (p<0.05).

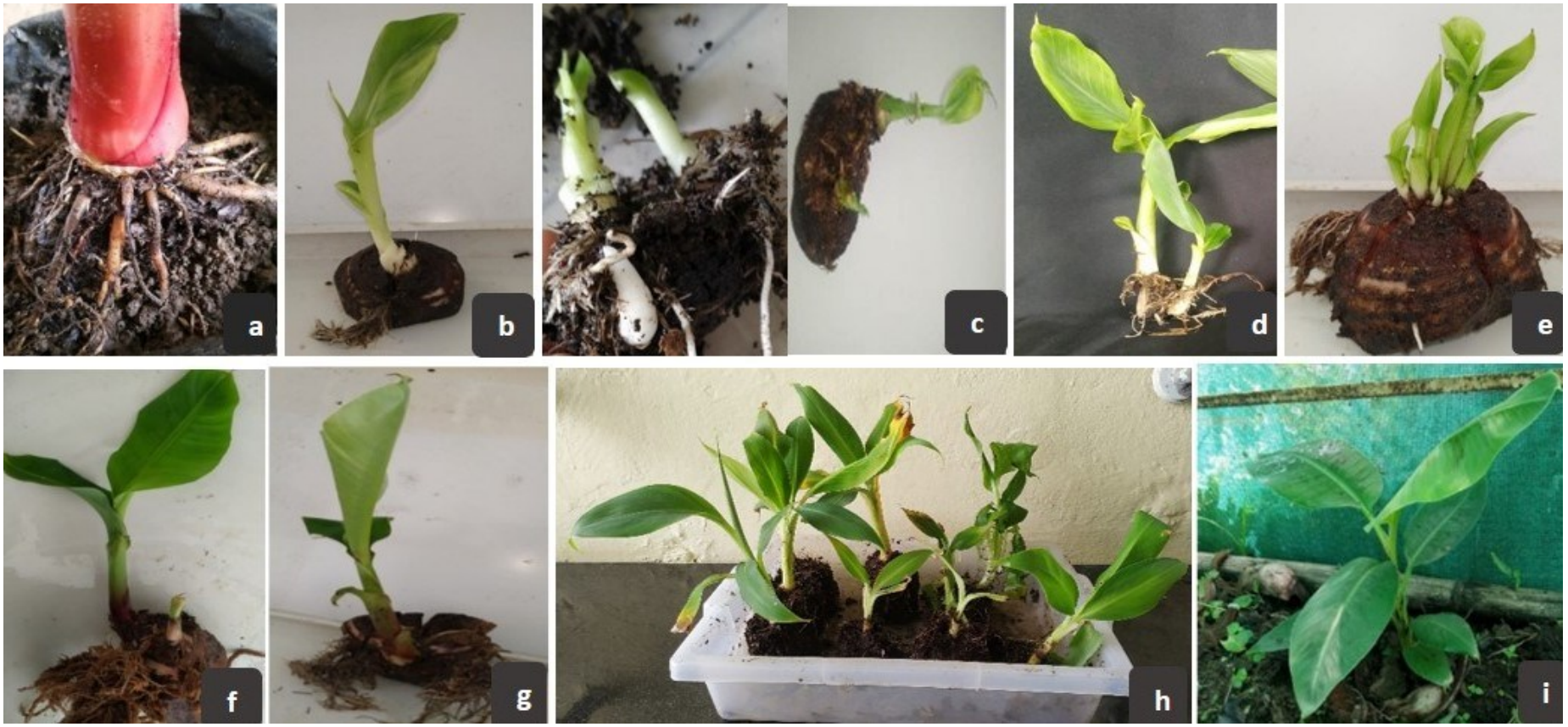


Figure 3.4: Morphogenic responses in different corm splitting techniques in Cv. Grand Nain. a. Conventional method (T_C); b. Incomplete decapitation (T_0); c. Complete decapitation (T_1); d. Split corm method (T_2); e. PIF method (Plants derived from stem fragments) (T_3); f. Excised bud method (T_4); g. Split corm+PIF method (T_5); h. Plantlets separated for hardening; i. Plantlets kept in green house for acclimatization.

It was observed that in the techniques where the apical dominance was either fully or partially manipulated, corms resulted more lateral buds and shoots compared with control and the incomplete decapitation (T_o) (**Table 3.1, Figure 3.4 a-g**). The split corm (T_2) and PIF (T_3) methods resulted the higher numbers of lateral shoots and followed by split + PIF method (T_5) and similar results were obtained in complete decapitation (T_1) and excised bud (T_4) **(Table 3.1, Figure 3.4 c-g).**

The location of the invocation of meristematic loci was also differed with the splitting techniques. The corm segments where the apical meristems were undisturbed (T_0, T_C and T_4), supported apical meristem growth and adversely affected the lateral shoot bud stimulation; while, the complete removal of the apical meristem (T_1) led to lateral shoots development. The partial inactivation of apical dominance resulted higher shoot bud formation as seen in the T_3 method, and complete division or splitting in T_2 and T_5 resulted both apical shooting and lateral shoots.

Effect of different rhizome fragmentation on morphogenic response of cv. Sabri

For Cv. Sabri, the experimental parameters/ treatments were selected from the leads obtained from the full scale experiments with Cv. Grand Nain. For Cv. Sabri, only the lead treatments both for corm splitting technique and potting media were used to verify the reproducibility of the technique optimized with Cv. G9. The growth response of cultivar Sabri to various corm manipulation techniques was assessed based on five parameters: emergence of bud sprouts (in days), number of shoots, shoot heights, leaf count, and roots. **Table 3.2** indicates that the traditional approach or control (T_C) attained the longest shoot height (65.32 cm), the highest the number of leaves (6.5), and a substantial root number (13.67); nevertheless, it was unable to produce multiple shoots **(Table 3.2, Figure 3.5).**

Table 3.2: Effect of different corm fragmentation technique on morphogenic response in Cv. Sabri

Corm splitting techniques*	Time for emergence of bud sprouts (in days) (±SD)**	No of shoot buds formed/ segment (±SD)**	Location of initial meristematic loci sprouting	Average shoot height (in cm) (±SD)**	No of leaves per shoot (±SD)**	No of roots per shoot (±SD)**
T_C	1.0±0.0^{d}	1.0 ±0.0^{d}	Apical meristem	65.32 ±0.25^{a}	6.5 ±0.5^{a}	13.67 ±1.52^{b}
T_0	19.5 ±0.81^{1}	2.6 ±0.62^{c}	Apical and lateral meristem	9.2 ±0.18^{e}	2.67 ±0.63^{d}	5.5 ±0.21^{d}
T_1	0	0	-	0	0	0
T_2	19.8 ±1.5^{a}	4.33 ±0.57^{b}	Apical and lateral meristem	8.77 ±1.36^{f}	3.33 ±0.57^{c}	5.1 ±0.64^{d}
T_3	11.8 ±0.71^{c}	5.2 ±0.7^{a}	Apical meristem	43.2 ±0.5^{b}	3.4 ±0.58^{c}	17.8 ±1.1^{a}
T_4	15.8 ±1.01^{b}	1.0 ±0^{d}	Apical meristem	17.83 ±0.11^{c}	4.1 ±0.31^{b}	5.5 ±0.5^{d}
T_5	20.67 ±0.58^{a}	4.1 ±0.06^{b}	Apical and lateral meristem	11.67 ±0.57^{d}	3.33 ±0.45^{c}	6.33 ±0.31^{c}

* T_C: Conventional method; T_0: Incomplete decapitation; T_1: Complete decapitation; T_2: Split corm method; T_3: PIF method; T_4: Excised bud method; T_5: Split corm and PIF method; **±SD: Standard deviation (n=3); the values with different superscript letters in the same column are significantly different (p<0.05).

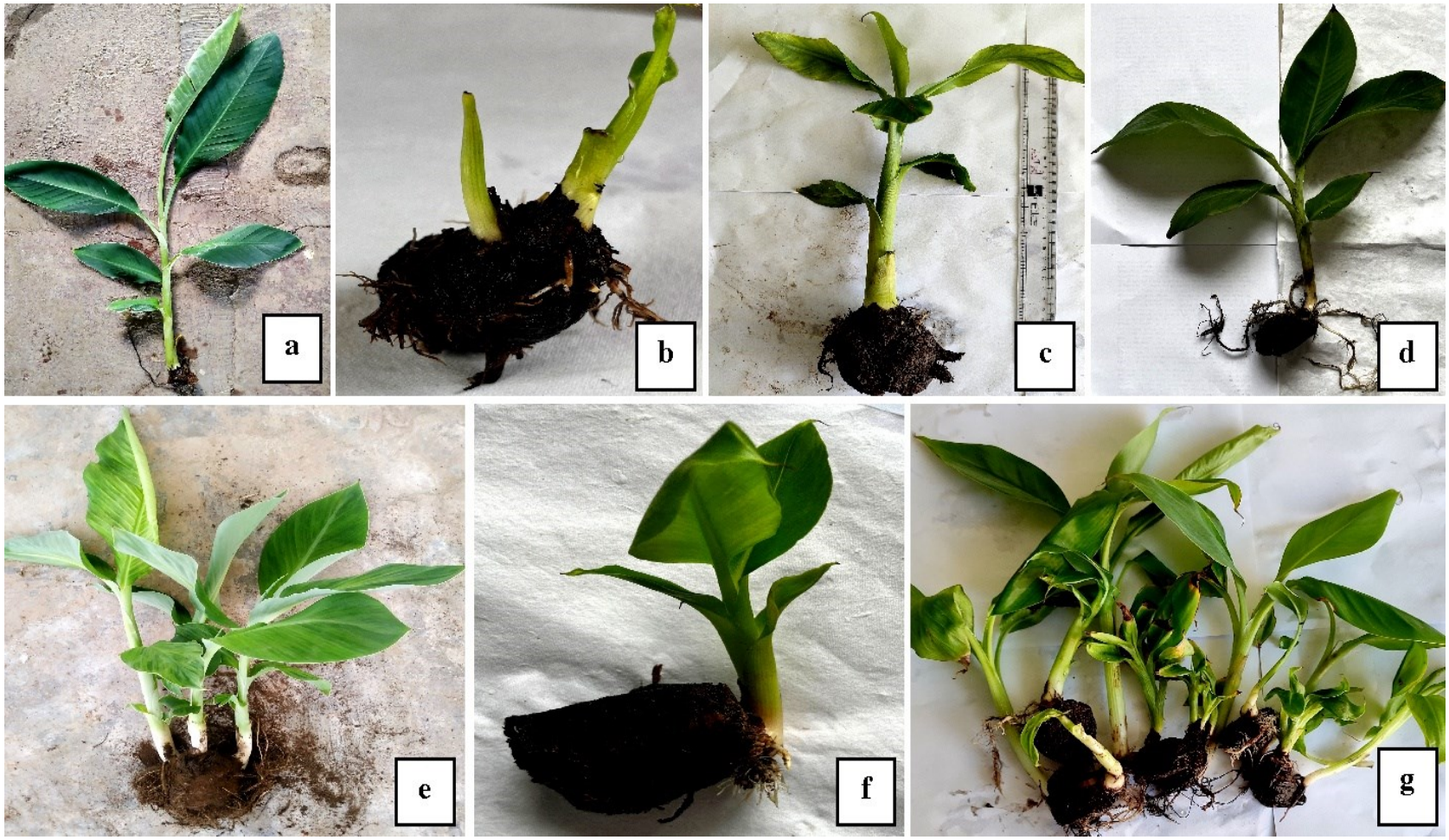


Figure 3.5: Morphogenic responses in different corm splitting techniques of Cv. Sabri. a. Conventional method (Control, T_c); b. Incomplete decapitation (T_0); c. Split corm method (T_2); d. Plants Issus des Fragments de Tige (T_3); e. Excised bud method (T_4); f. Split corm and PIF method (T_5); g. Regenerated plantlets ready for field establishment.

Although control (T_C) accomplished an optimal response for the majority parameters, corm approaches resulted in significantly more effective results. The quickest emergence of shoot buds occurred in 11.8 days using the PIF method (T_3), followed by the Excised bud method (T4) at 15.8 days, and the slowest in the split + PIF method (T_5) at 20.67 days. The corm technique, PIF, generated the highest outcomes in multiple shoots (5.2), shoot height (43.2 cm), and roots (17.8) (**Table 3.2, Figure 3.5),** while the excised bud (T_4) produced the most number of leaves (4) **(Table 3.2, Figure 3.5 b).** The techniques that gave the least responses were observed in split (T_2) and split + PIF method (T_5) in the emergence of shoots buds, multiple shoots in excised bud (T_4), shoot height and root number in split method (T_2), number of leaves in incomplete decapitation, while the complete decapitation method (T_1) gave no signs of growth or development and the planted corms was observed to decay further. There were no significant variations in the $p<.05$ values for all factors across the techniques. The Pearson's correlation factor revealed negative correlation between the parameters day of emergence and shoot height, while positive correlation was seen among the rest of the parameters.

Effect of different potting mixtures on morphogenic response in Cv. Grand Nain

The study also aimed to investigate the impact of 7 potting mixtures (P_1-P_7) on the morphogenic response and growth of regenerates in two corms splitting technique (T_3 and T_5) in Cv. Grand Nain, and for evaluation purpose, several metrics were considered *viz*., number of shoots, plantlet height, number of leaves developed, number of roots per corm (**Table 3.3, Figure 3.7 & 3.8**).

Table 3.3: Effect of different potting mix and corm fragmentation on morphogenic response Cv. G9

Potting mixture Code*	Avg. No. of shoots		Avg. shoot height (cm)		Agv. No. leaves per shoot		Avg. No. of roots per corm	
	PIF (T_3) ±SD#	Split+PIF (T_5) ±SD#	PIF (T_3) ±SD#	Split+PIF (T_5) ±SD#	PIF (T_3) ±SD#	Split+PIF(T_5) ±SD#	PIF (T_3) ±SD#	Split+PIF(T_5) ±SD#
P_1	3.1 ± 0.4^{ab}	4.0 ± 1.0^{b}	22.2 ± 2.08^{b}	20.2 ± 3.4^{c}	4.5 ± 1.8^{c}	6.0 ± 1.0^{d}	23.5 ± 2.6^{b}	30.1 ± 2.1^{c}
P_2	5.1 ± 0.3^{c}	4.0 ± 1.4^{b}	22.6 ± 4.8^{b}	18.2 ± 1.1^{b}	3.08 ± 0.16^{b}	3.3 ± 1.1^{b}	31.75 ± 2.87^{c}	31.2 ± 3.1^{c}
P_3	4.8 ± 1.1^{bc}	5.3 ± 1.5^{c}	18.29 ± 3.1^{a}	21.0 ± 1.1^{c}	4.3 ± 1.1^{c}	4.2 ± 0.5^{c}	30.5 ± 3.6^{c}	48.1 ± 3.4^{e}
P_4	1.33 ± 0.17^{a}	1.5 ± 0.7^{a}	30.88 ± 4.6^{d}	20.5 ± 2.4^{c}	2.75 ± 0.5^{a}	2.2 ± 0.3^{a}	34 ± 2.7^{d}	44.1 ± 3.41^{d}
P_5	8.67 ± 1.53^{d}	4.7 ± 0.3^{b}	31.0 ± 2.5^{d}	11.1 ± 1.7^{a}	2.1 ± 0.2^{a}	3.6 ± 0.1^{b}	30.3 ± 5.5^{c}	48.3 ± 3.3^{e}
P_6	2.33 ± 0.28^{b}	2.5 ± 0.4^{a}	18.17 ± 3.1^{a}	17.5 ± 1.7^{b}	2.3 ± 0.3^{a}	2.2 ± 0.4^{a}	14.7 ± 1.3^{a}	13.2 ± 2.8^{a}
P_7	6.33 ± 0.58^{c}	2.0 ± 0.4^{a}	25.63 ± 3.7^{c}	18.5 ± 1.7^{b}	2.5 ± 0.57^{a}	2.1 ± 1.9^{a}	22.63 ± 3.6^{b}	18.1 ± 2.1^{b}

* P_1: Garden soil + Straw + Dried cow dung manure; P_2: Saw dust; P_3: Forest soil manure; P_4: Sand; P_5: Sand + Saw dust; P_6: Sand + Tea manure; P_7: Sand + Forest soil manure; P_8: Garden soil + Straw + Dried cow dung manure + Saw dust + Forest soil manure + Sand + Tea manure. # ±SD: Standard deviation (n=3); the values with different superscript letters in the same column are significantly different (p<0.05). Note: Only selected corm fragments/fragmentation treatments are computed where responses were optimum under the given conditions.

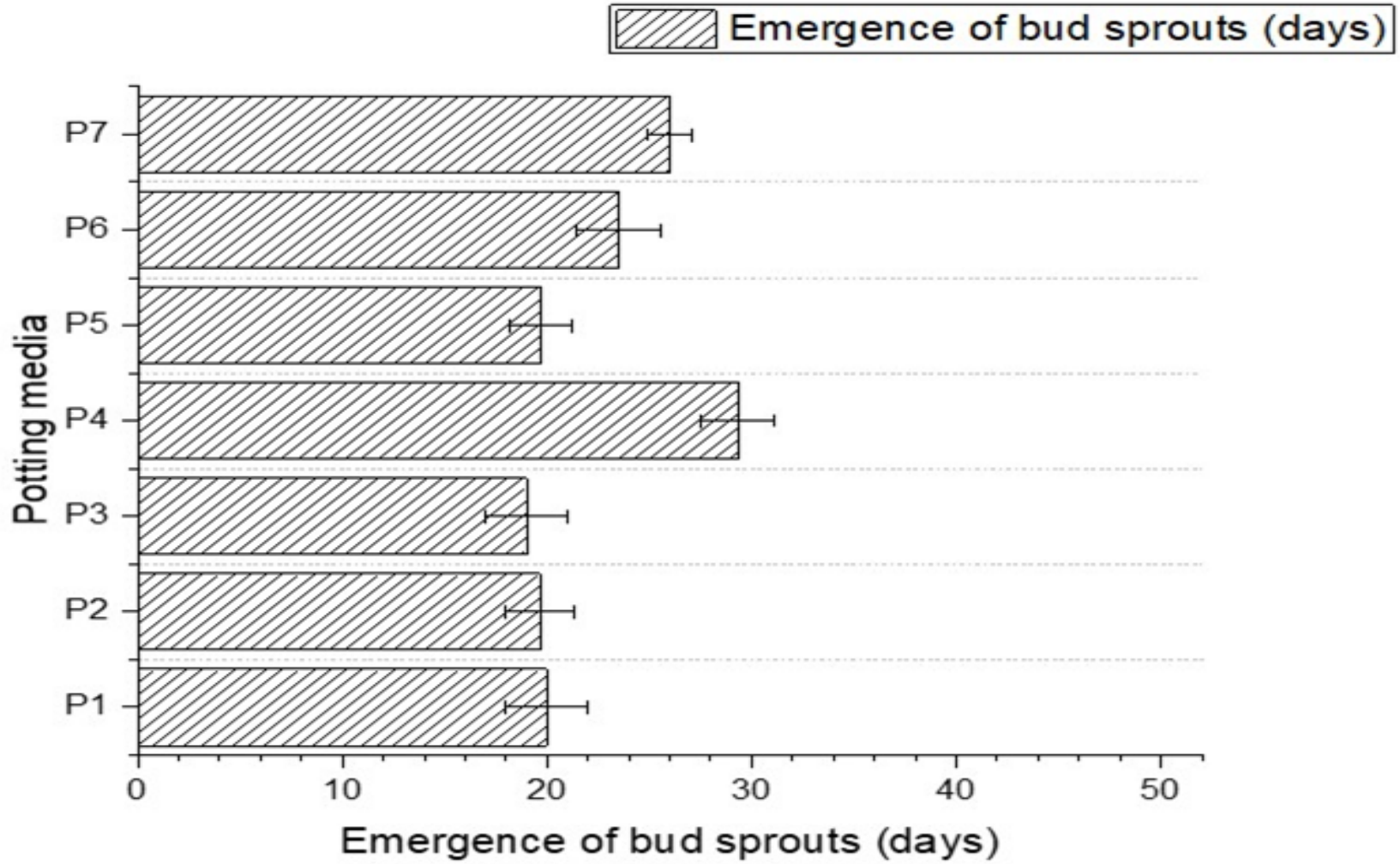


Figure 3.6: Graphical representation of the time taken in days for emergence of shoot buds in different potting media.

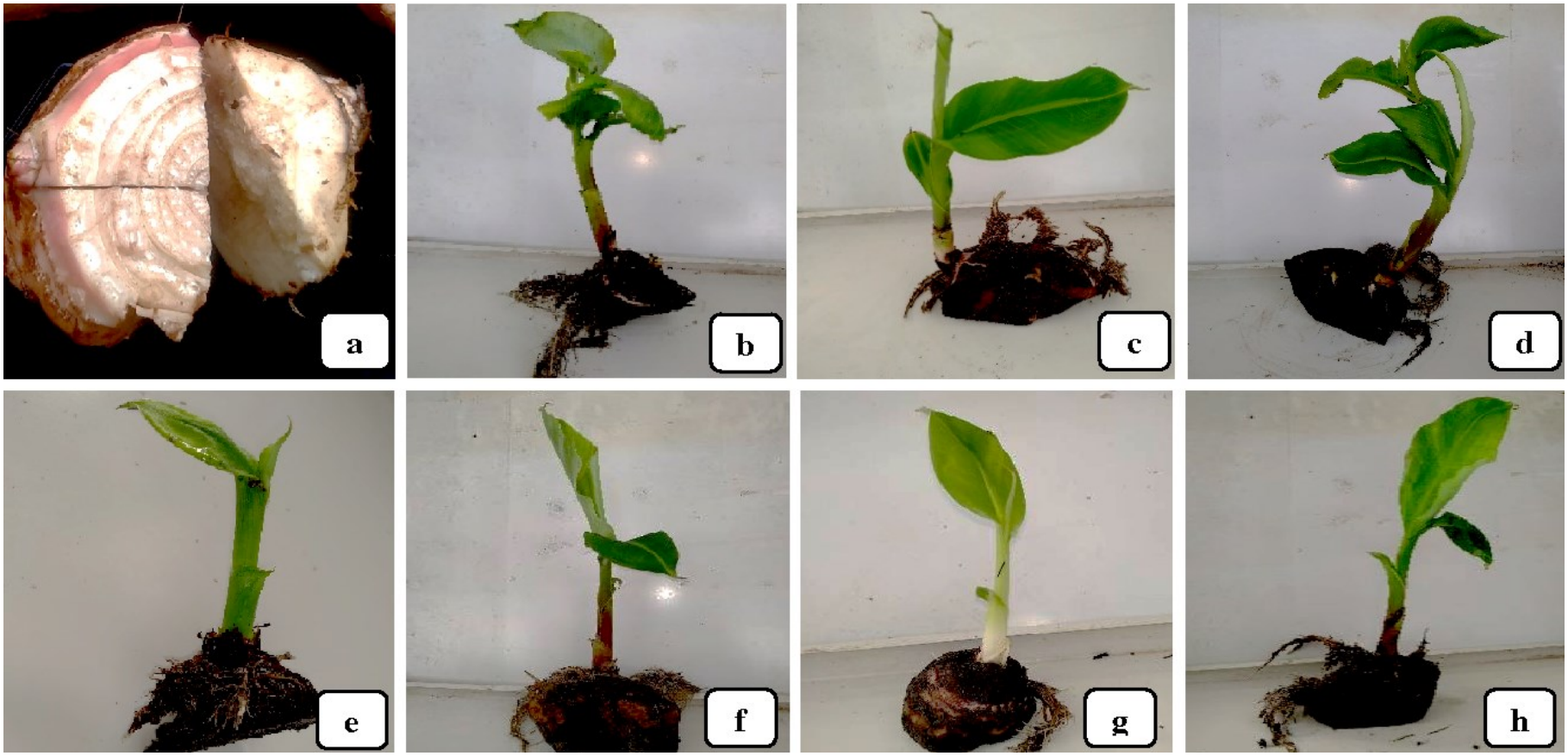


Figure 3.7: Reponses obtained from different potting mixtures in Split +PIF method (T_5) in Grand Nain. a. Split+PIF method (T_5); b. Garden soil + Straw + Dried cow dung manure (P_1); c. Saw dust (P_2); d. Forest soil manure (P_3); e. Sand (P_4); f. Sand + Saw dust (P_5); g. Sand+ Tea manure (P_6); h. Sand + Forest soil manure (P_7).

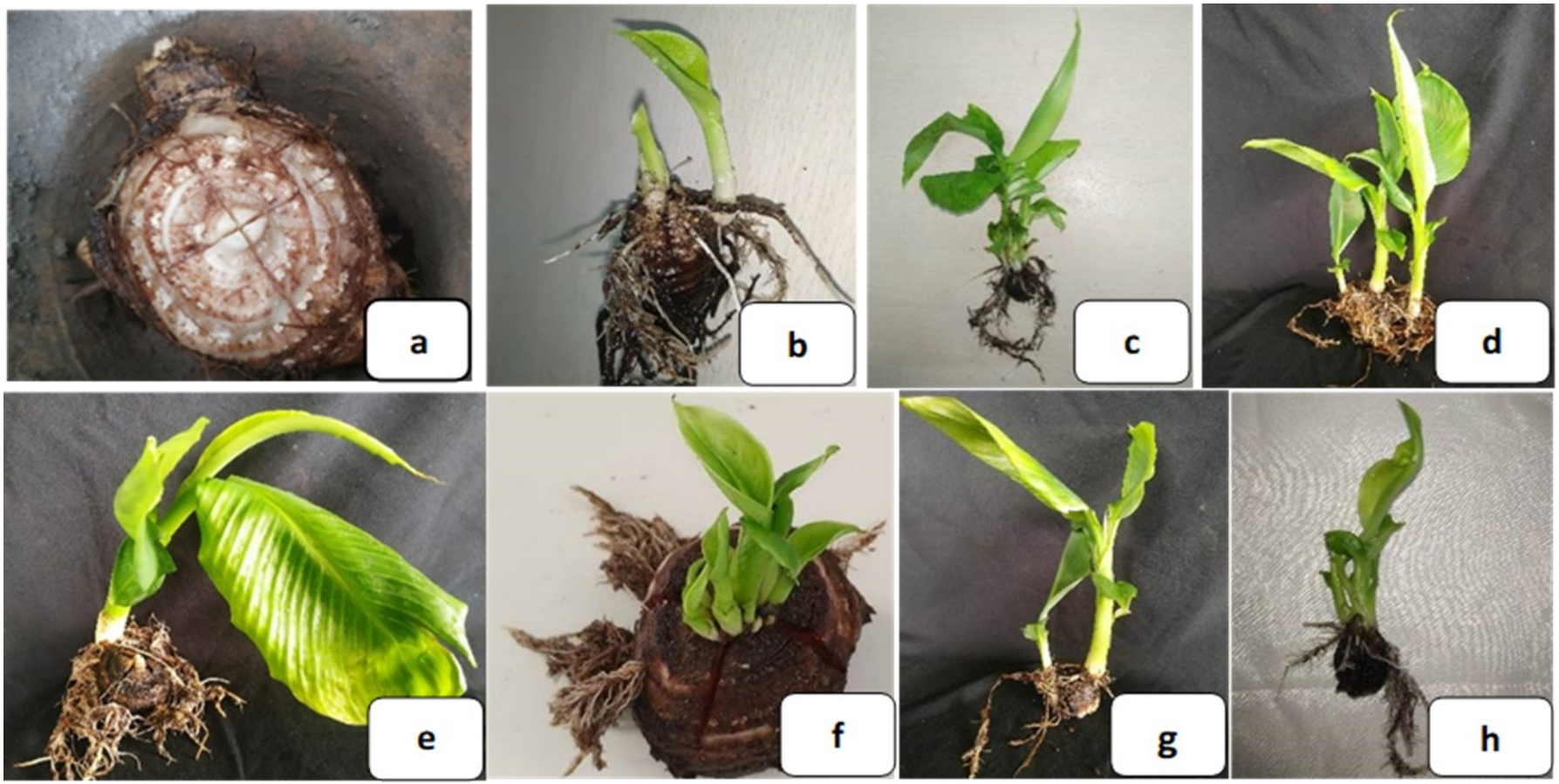


Figure 3.8: Reponses obtained from different potting mixtures in PIF method (T_3) in Grand Nain. a) PIF method (T_3); b) Garden soil + Straw + Dried cow dung manure (P_1); c) Saw dust (P_2); d) Forest soil manure (P_3); e) Sand (P_4); f) Sand + Saw dust (P_5); g) Sand+ Tea manure (P_6); h) Sand + Forest soil manure (P_7).

There were differential morphogenic responses on different potting media and corm fragmentation methods. Significant differences were recorded in the parameters especially in number of shoot buds formation and shoot heights. When considered the corm splitting methods, Split+PIF (T_5) method was found to support higher number of leaves (3.6) compared to rest. In other treatments, though there were differences in shoot buds formation, plantlet height, number of roots, the number of leaves was similar in all the treatments. Among the different potting mixtures tested, first emergence of shoot bud sprout was recorded in forest soil manure (P_3) followed by saw dust (P_2) and sand + saw dust (P_5) after ~19 days of potting and last bud emergence was observed in sand (P_4) after 29 days and the average emergence was after 22 days after potting **(Tabl3 3.3, Figure 3.6).**

Figure 3.7 and **3.8** present morphogenic response of different corm segments and potting mixtures combinations. Highest number of shoot formation was recorded in potting mix P_5 (8.67) and PIF (T_3) method followed by potting mix P_3 (5.3) and Split+PIF method (T_5) (**Figure 3.7, 3.8**). Similar to number of shoot buds, highest plantlet height was also recorded with potting mix P_5 (31 cm) and PIF (T_3) corm splitting followed by P_3 (21 cm) and T_5; however, higher number of roots formation was recorded with P_4 potting mix (34) and T_3 corm fragmentation. The Pearsons correlation factor indicated a negative correlation between shoot height and roots (weak) as well as leaves and shoot height, and a positive correlation with the remaining parameters.

Effect of different potting mixtures on morphogenic response in cv. Sabri

In this experiment, the cultivar Sabri was limited to three techniques due to insufficient availability of planting materials. The previously selected potting medias - Garden soil + Straw + Dried cow dung manure (P_1), Saw dust (P_2) and Forest soil manure (P_3), were utilized and observations were documented regarding shoot height, leaf, roots, shoots, and the emergence of shoot buds.

There was not a significant distinction in the emergence of bud sprouts between the three substrates, which was observed on the 11^{th} day after post-potting on the two substrates Forest soil manure (P_3) and Saw dust (P_2) (**Table 3.4, Figure 3.9**). The potting medium, forest soil manure (P_3) generated the highest quantity of multiple shoots (4.89) and roots (17); while, saw dust (P_2) resulted in the highest shoot height (43.65 cm) and leaves (4.2) (**Table 3.4**).

Table 3.4: Effect of different potting media on morphogenic response in Cv. Sabri

Potting mixture Code*	Time for emergence of bud sprouts (in days) (±SD)**	Avg. No. of shoots	Avg. shoot height (cm)	Avg. No. leaves per shoot	Avg. No. of roots per corm
P_1	11.0±0.81	1.7±0.18	22.75±0.45	3.0±1.53	10.1±1
P_2	11.33±0.47	4.7±0.08	43.65±0.23	4.2±0.57	13.7±0.76
P_3	14.0±0.77	4.89±0.15	43.1±0.6	3.85±0.57	17.1±1.0

* P_1: Garden soil + Straw + Dried cow dung manure; P_2: Saw dust; P_3: Forest soil manure. **±SD: Standard deviation (n=3).

The least response amongst the potting media was exhibited by GS (P_1). Significant differences were seen across the potting media in all four parameters ($P<0.05$) of number of shoots, shoot height, leaves number and roots and no significant results were obtained from the emergence of shoot buds ($P>0.05$). The Pearsons correlation test obtained positive correlations between all the parameters.

Once the plantlets developed, attained a certain height and developed leaves and roots, the young plantlets were then transferred to another pot with the same potting mixture and maintained in the poly-house for another month under diffused sunlight. Following this, the young plantlets were maintained in the nursery bed in the poly-house for two months before transferring to the field (**Figure 3.10 a, b)**. For both the cultivars, ~150 regenerates each were field established. After three months of field transfer, Cv. G9 transplants registered ~89% survival; while, transplants of Cv. Sabri registered ~85% survival.

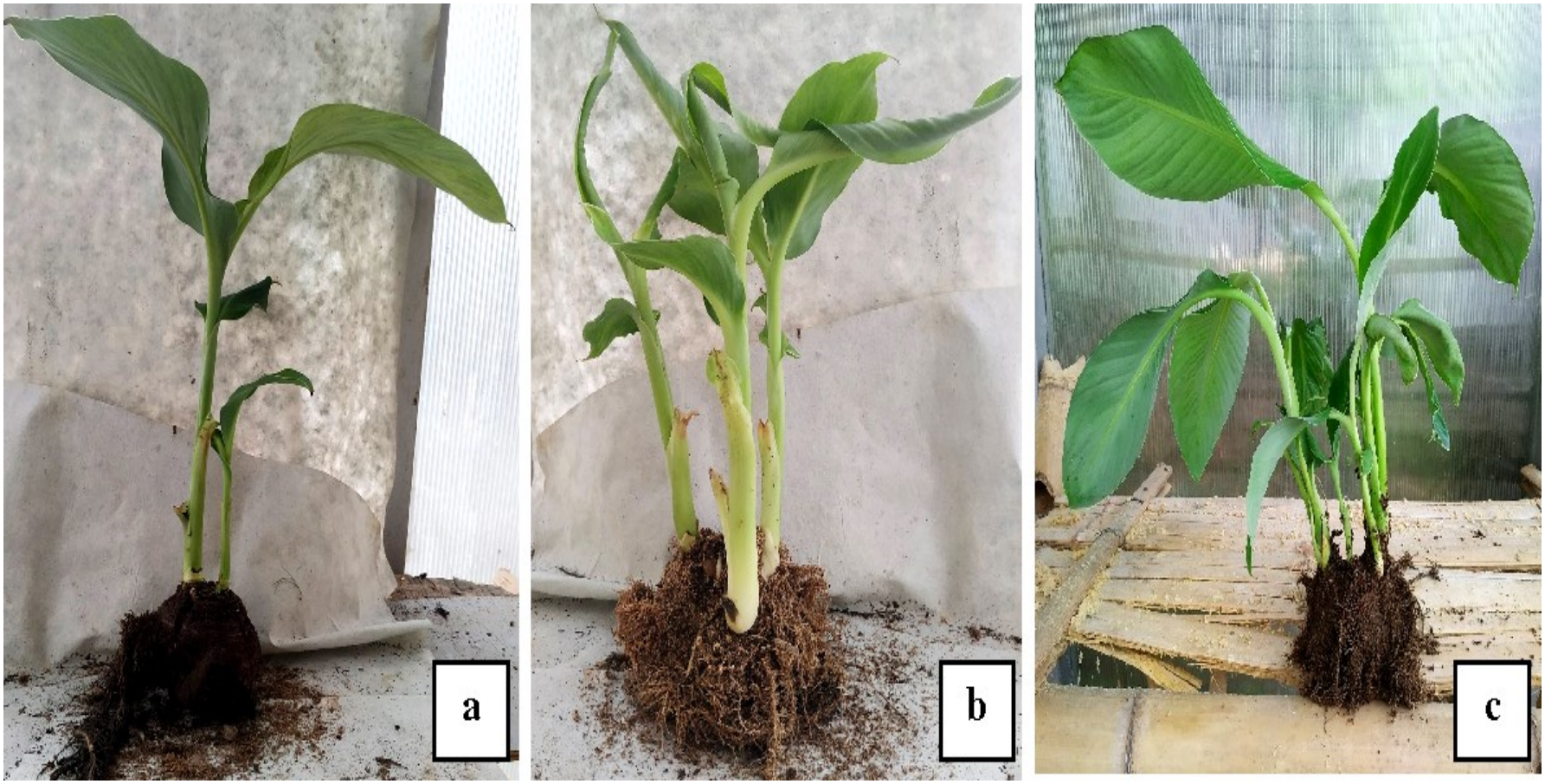


Figure 3.9: Pictures of the responses in the three potting media in Cv. Sabri. a. Garden soil + Straw + Dried cow dung manure (P_1); b. Saw dust (P_2); c. Forest soil manure (P_3).

Figure 3.10: Field established regenerates. a. Cv. Grand Nain, b. Cv. Sabri.

Genetic stability assessment

The evaluation of genetic fidelity was conducted on the macropropagated regenerated plantlets of cultivars Grand Nain and Sabri. The study employed two markers, RAPD and SCoT, with preliminary screening conducted on 25 primers, resulting in the selection of 14 primers, comprising 7 from each marker. Seven randomly selected macropropagated regenerated plantlets (R_1 to R_7) and one mother plant (M) were chosen for this study (**Table 3.5, 3.6, 3.7, 3.8, Figure 3.11**).

In the present study, the RAPD markers resulted 100% monomorphic bands across all seven primers for both cultivars, Grand Nain and Sabri. The maximum number of bands, six each, was recorded for OPA-13 and OPK-04, while the lowest, three bands, was noted for OPD-20 in Grand Nain (**Table 3.5**). In Sabri, OPA-11 yielded the highest count of eight bands, but OPD-20 and OPK-04 each produced the lowest count of three bands (**Table 3.7**). The SCoT markers in Grand Nain revealed 100% monomorphism across 6 primers and 90% monomorphism in 1 primer (S11), producing 1 polymorphic band. The maximum number of bands was recorded in S5, S6, and S10, each with 6 bands, while the remaining primers produced 5 bands each. The cultivar Sabri exhibited 100% polymorphism across 5 primers, yielding one polymorphic band each from S5 and S12. The most number of bands was recorded for primers S5 and S12, each producing 5 bands, while the lowest was observed in S6 and S9, each generating 3 bands.

The total number of bands generated in RAPD and SCoT were 34 and 33, respectively, and 38 and 28 bands in SCoT from G9 and Sabri, with an average of 4.7 to 4.8 bands per primer in RAPD and 4 to 5.8 bands in SCoT. The study yielded 72 bands in G9 and 61 in Sabri, with the most number of bands produced by RAPD at 67 and SCoT marker at 66, while the amplified bands ranged from 200 to 1500 base pairs. The average number of bands per primer was determined to be 5.14 in G9 and 4.36 in Sabri.

Table 3.5: RAPD and SCoT primers data analysis for evaluation of genetic stability of regenerated plants in Cv. Grand Nain

RAPD primer								
Sl. No.	**Primer code**	**Primer sequence (5′-3′)**	**Total bands**	**Monomorphic bands (mp)**	**% of mp**	**Polymorphic bands (pb)**	**% of pb**	**Range of amplicons (bp)**
1	OPA-05	AAAAGTCTTG	4	4	100	-	-	300-1000
2	OPA-11	CAATCGCCGT	5	5	100	-	-	400-1500
3	OPA-12	TCGGCGATAG	5	5	100	-	-	250-900
4	OPA-13	CAGCACCCAC	6	6	100	-	-	500-1500
5	OPAA-8	TCCGCAGTAG	5	5	100	-	-	250-1000
6	OPD-20	ACCCGGTCAC	3	3	100	-	-	700-1500
7	OPK-04	CCGCCCAAAC3	6	6	100	-	-	500-1400
		Total	**34**	**34**	**100**	-	-	
SCoT primer								
1	S5	CAACAATGGCTACCACGA	6	6	100	-		300-900
2	S6	CAACAATGGCTAGCACGA	6	6	100	-		300-1000
3	S9	CAACAATGGCTACCACGT	5	5	100	-		300-1000
4	S10	CAACAATGGCTACCAGCA	6	6	100	-		300-1000
5	S11	CAACAATGGCTACCACGCG	5	4	80	1	20	300-1000
6	S12	ACGACATGGCGACCAACG	5	5	100	-	-	200-900
7	S17	ACGATGGCTACCACCGAG	5	5	100	-	-	300-1000
		Total	**38**	**37**	**97.37**	**2.63**		

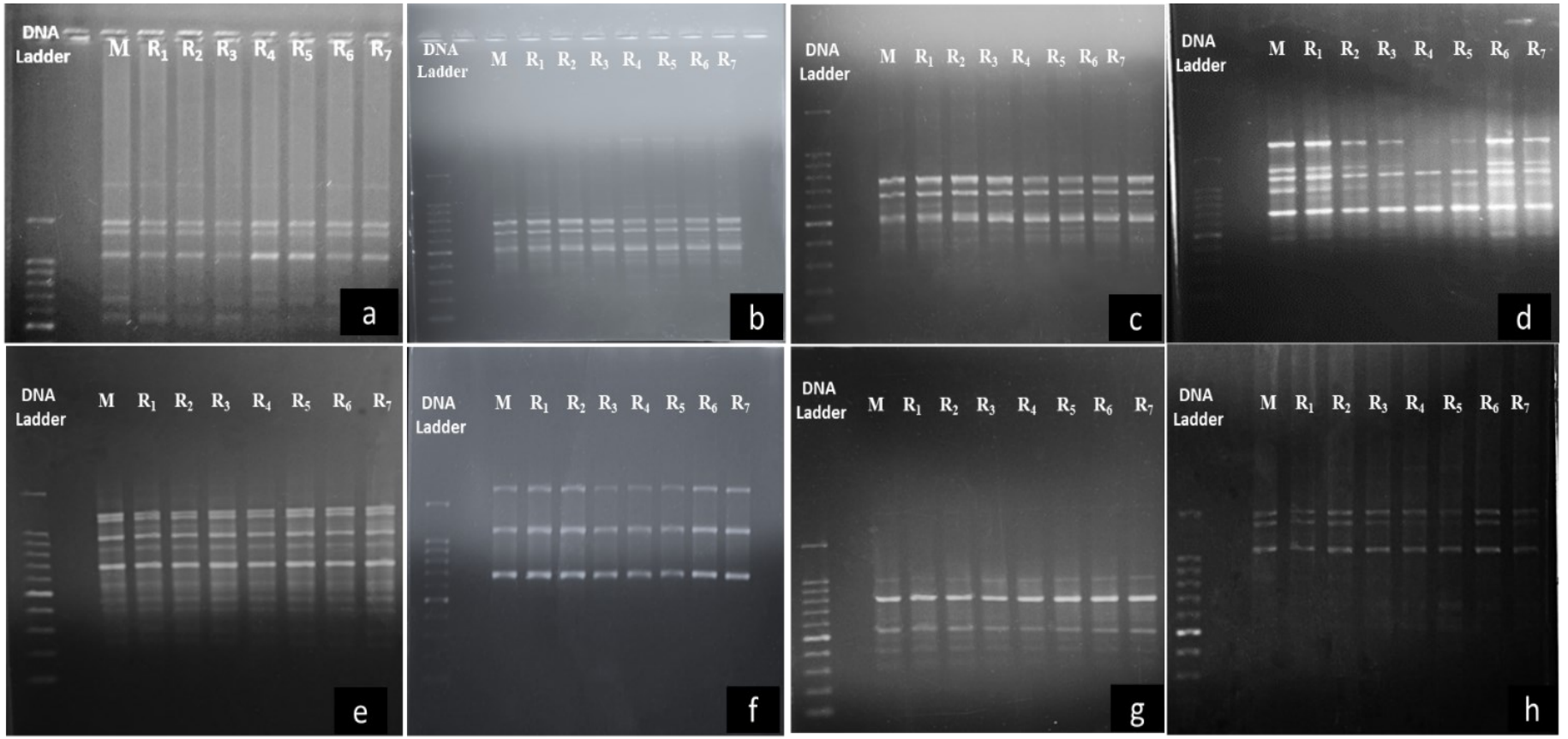


Figure 3.11: RAPD and ScoT markers profiles of mother plant (M), macropropagated plantlets (R_1 to R_7 lanes), showing in primers, in Cv. Grand Nain (a-d) and Sabri (e-h) along with DNA ladder (100bp). a) OPA- 13, b) OPA-12, c) S10 ,d) S11 in Cv. Grand Nain; e) OPA-11, f) OPD-20, g) S6, h) S5 in Cv. Sabri.

Table 3.6: RAPD and SCoT primers data analysis for evaluation of genetic stability of regenerated plants in Cv. Sabri.

RAPD primer								
Sl. No.	**Primer code**	**Primer sequence (5´-3´)**	**Total bands**	**Monomorphic bands (mp)**	**% of mp**	**Polymorphic bands (pb)**	**% of pb**	**Range of amplicons (bp)**
1	OPA-05	AATCGGGCTG	4	4	100	-	-	500-1500
2	OPA-11	CAATCGCCGT	8	8	100	-	-	300-1000
3	OPA-12	TCGGCGATAG	5	5	100	-	-	400-1000
4	OPA-13	CAGCACCCAC	4	4	100	-	-	400-1500
5	OPAA-8	TCCGCAGTAG	6	6	100	-	-	500-1500
6	OPD-20	ACCCGGTCAC	3	3	100	-	-	900-1200
7	OPK-04	CCGCCCAAAC3	3	3	100	-	-	500-1500
		Total	**33**	**33**	**100**	-	-	
SCoT primer								
1	S5	CAACAATGGCTACCACGA	5	4	80	1	20	800-1000
2	S6	CAACAATGGCTAGCACGA	3	3	100	-	-	500-1000
3	S9	CAACAATGGCTACCACGT	3	3	100	-	-	300-1000
4	S10	CAACAATGGCTACCAGCA	4	4	100	-	-	300-1000
5	S11	CAACAATGGCTACCACGCG	4	4	80	-		300-1000
6	S12	ACGACATGGCGACCAACG	5	4	80	1	20	200-900
7	S17	ACGATGGCTACCACCGAG	4	4	100	-	-	300-1000
		Total	**28**	**26**	**92.86**	**2**	**7.14**	

Table 3.7: Comparative and combined presentation RAPD and SCoT primers results in Cv. Grand Nain

Markers	No of primers	Total No. of bands	Average band per primer	Total (%)		Genetic stability (%)
				Monomorphism	Polymorphism	
RAPD	7	34	4.857	100	0	100
SCoT	7	38	5.428	97.37	2.63	97.37
RAPD + SCoT	14	72	5.142	98.685	1.315	98.685

Table 3.8: Comparative and combined presentation RAPD and SCoT primers results in Cv. Sabri

Markers	No of primers	Total No. of bands	Average band per primer	Total (%)		Genetic stability (%)
				Monomorphism	Polymorphism	
RAPD	7	33	4.71	100	0	100
SCoT	7	28	4	92.86	7.14	92.86
RAPD + SCoT	14	61	4.36	96.72	3.28	96.72

Discussion

Effect of different rhizome fragmentation on morphogenic response in Cv. Grand Nain

The rhizome/sucker of banana is a modified true stem, and serve as storage organ for reserve food and nutrients for growth and vegetative reproduction; it also functions as a root system supporting elevation and transport of nutrients to the rest of the plant (De Langue et al., 1983). The stem i.e., the rhizome of banana functions as a reproductive organ in reproducing new shoots laterally, although it is said to be slow in nature. The modification and manipulation of banana rhizomes in this study demonstrated the enhancement of growth and development of new shoots. The emergence of bud sprouts occurred on various days, indicating that it was only influenced by corm manipulation. In

Control (T_C), the lack of interference with the sucker did not hinder its development into a mature plant (Datta et al., 2020).

Though the apical meristem was tempered in the PIF (T_3) method, but the fastest emergence of shoot bud was recorded in this corm technique. The recorded response was probably due to disturbing the apical dominance, which allowed the invocation of lateral meristematic loci. Though the apical meristem was decapitated, the reserve nutrient pool was undisturbed in the corm which contributed in the initiation of shoot buds making it grow earlier than the other methods (De Langhe et al., 1983). Studies by Malemba et al. (2021) reported that there was delay in sprouting of shoot buds in G9 when the corms were fragmented into 2 and 4 halves which is in contrary with the findings of the present study; however, result obtained with incomplete decapitation of corm was in line with the findings of the present study.

Mathew et al. (2000) reported that tempering the banana corm adversely affect the regenerated shoot height and argued that the growth in the apical dominance is controlled by growth factors that are found in the rhizome, and therefore inhibits the growth of lateral buds thus leading to decreased in shoot height. In contrary, in the present study manipulated corms, the PIF (T_3) and Split+PIF (T_5) resulted better shoot buds formation and plantlets height even when the apical meristem was divided into halves. Malemba et al. (2021) reported that the whole corm and two halves split corm supported better plant height.

In the present study, it was observed that corm modifications hampered the leaf growth compared to control method (T_c) where broad and matured leaves were resulted. Suryanarayana et al. (2018) and Nayak et al. (2020) argued shoot buds developed from the whole corm results broader leaves due to non-alternation of corm apical meristem; but in the present study there was no significant change in the number of leaves in different corm splitting treatments in G9 after 3 months of growth.

The present study also found that the Split corm+PIF method (T_5) supported more roots formation than the control; however, root growth was healthier in the control treatment. Malemba et al. (2021) in their study also reported smaller and thinner roots in the plants obtained from the fragmented corms and higher numbers of roots formed from the ¼ split corms. Among the different fragmentations of corms tested in the present study, higher roots development was recorded with three techniques i.e., PIF (T_3), Split (T_2), and Split+PIF (T_5) compared to control.

Apical dominance plays an important role in inhibiting for sprouting the lateral buds. When analyzed the data on lateral shoot buds formation from the differentially fragmented corms, it was observed that in those corm fragmentations where the apical meristem was either partially or completely removed, resulted more numbers of lateral shoot buds. Though, the shoots developed from the apical meristem were taller and comparatively healthier, the shoots developed from the lateral buds were shorter, but quickly picked up the normal growth and attained normal height subsequently. Ntamwira et al. (2017) in their study suggested that removal of apical meristem completely results emergence to more shoots. In contrary, in the present study better morphogenic response was recorded from the partially tampered apical meristem without destroying the apical portion entirely (i.e., Split corm T_2, and PIF, T_3); but in treatments where the apical meristem was completely removed supported poor response. The location of the emergence of shoot buds differed with the techniques. The unrevoked or unhampered apical meristem (T_C, T_0 and T_4) led to apical growth from the corm and did not respond to lateral stimulation; whereas, the complete removal of the apical meristem (T_1) led to lateral shoot formation. The partial suppression of apical dominance resulted gave rise to numerous shootings in the T_3 method, and complete division or splitting in (T_2) and T_5 supported both apical and lateral shoot buds formation.

The findings also revealed significant differences in the four dependent factors, the emergence of buds, number of shoots, shoot height, and number of roots and while, there was no significance difference in number of leaves development. The corm modification techniques like PIF method (T_3) and Split+PIF method (T_5) supported optimum responses under the given conditions with reference to early emergence of shoot buds, number of shoots; shoot height, number of leaves and roots.

In the past several reports published reflecting that corm manipulations resulted better response compared to the conventional method in terms producing more planting materials, disease-free healthy plantlets, good yields etc. (Njau et al., 2011; Sajith et al., 2014; Manju and Pushpalatha, 2020).

Effect of different rhizome fragmentation on morphogenic response in Cv. Sabri

The subsequent methods were compared with the control, which demonstrated an optimal response in the overall plant growth. Due to the fact that the control was particularly intact and did not undergo any modifications, there were no restrictions associated with the nutrient supplement from the corm. The corm is well-known for its

nutrient reserves that are necessary for the growth and development of shoots (Baiyeri and Aba, 2005).

However, in contrast to the other methods, the control method did not produce plantlets within the specified time frame, despite the fact that it responded particularly well with good plant height. Elevated shoot growth due to apical dominance is often achieved due to the contribution of the auxin naturally found in the corms of bananas, due to which, it suppresses the development of new shoots Kindimba and Msogoya (2014). The study by Bhende and Kurien (2020), also showed good response in plant height, girth, leaf and leaf area obtained from full corm in comparison to the divided corms, aligning with the present observations.

Among the corm techniques PIF method gave the fastest shoot emergence although apical dominance was hindered which was similar to the findings of Opata et al. (2020). The findings of the present study in PIF with maximum shootings (5.2 shoots) were surpassed by the findings of the study carried out by Firew et al. (2024), which used the PIF method in conjunction with BAP and IBA. This may be owing to the fact that no PGRs were used in the present study with the corm manipulation techniques. In addition, Kindimba and Msogoya (2014) found that the addition of BAP and organic manure to the Cv. Itoke (AAB) plant resulted in a greater response in shoots (13 to 17.1 nodes) when compared to our study.

According to a study by Langford et al. (2017), the efficacy of the PIF method, despite damage to the apical meristem, can be attributed to the reduced auxin levels responsible for apical dominance, while not affecting cytokinin production, thereby facilitating the growth of axillary shoot buds. Contrary to our findings Opata et al. (2020) in their study in PIF technique did not produce good number of shoots. The study conducted by Dzomeku et al. (2014) obtained 65% of the overall shoot production, from apical meristem similar to the present study.

Additionally, the excised bud method demonstrated a better response to the split corm method in terms of height and shoot emergence. However, there was no response in multiple shooting, primarily due to the high apical dominance that resulted in low induction of lateral shoot buds (Kindimba and Msogoya, 2014; Langford et al., 2017). The split corm, however, produced a greater number of shoot buds compared to the control and excised bud, likely due to the suppression of apical dominance, which promotes lateral bud development (Patel and Rath, 2018).

The roots serve as an association between the plant and the soil and facilitates the absorption of water and nutrients, providing stability, and serving as the site for the synthesis and storage of specific phytohormones essential for plant development (Baiyeri and Aba, 2007; Bodjona et al., 2020). This elucidates the findings from the control and PIF methods, which yielded optimal root development and favourable shoot height.

Effect of different potting mixtures on morphogenic response in Cv. Grand Nain

Studies confirmed the presence of microorganisms and organic matter, and the usage of organic fertilizer, and composted bio-char in loamy, sandy, or degraded soil can alleviate and increase soil fertility; however, the usage of fertilizers needs to be optimized to reduce environmental damage and also to maintain sustainable soil fertility (Fischer and Glaser, 2012; Schulz et al., 2013; Schjoerring et al., 2019; Yadav et al., 2021). The different types of organic substrates potentially enhance plantlet growth, and organic matters play a key role in plantain maintenance.

In the present study, garden soil mixed with dried cow dung manure supported better response with more leaves, number of shoots, plant height and number of roots compared to other potting mix. Livestock manure is said to be a good source of organic fertilizer that contains essential micro-nutrients which are beneficial and needed for healthy plant growth (Ahmad et al., 2016; Malemba et al., 2021). Vyas et al. (2020) in their survey reported that the application of farm yard manure enhanced the growth rates and yield of bananas and were found to be very suitable for organic as well as economical production of bananas. It has been estimated that ~60% of the cultivated lands have deficiencies in nutrients leading to growth-limiting issues (Schjoerring et al., 2019). Therefore, the addition of animal manure and compost contributes richly in carbon sequestration and enriching the soil for cultivation (Schulz et al., 2013).

Sawdust was found to be a good substratum for potting mix which supported overall good response and initial shoot buds growth. The plant height obtained by Malemba et al. (2021) (23.3cm) and Manju and Pushpalatha (2022) (21.53cm) are similar with the findings of the present study. The results obtained by Sajith et al. (2014); Thungon et al. (2017) and Malemba et al. (2021) in Cv. Malbhog and G9 cultivars were poorer compared to the outcome of the present study. Sawdust being good biodegradable, high porosity, high water retention capacity, moderate water drainage, and bacterial tolerance has a favorable effect on plant growth (Maharani et al., 2010; Agboola et al., 2018).

Use of forest soil (P_3) supported the sprouting of higher numbers of shoot buds and healthy growth of regenerates indicating as a better choice as potting medium. A study by Patel et al. (2024) with G9 successfully used green manure to improve the banana yield. The Forest soil is known for its natural mineral deposits and rich humus content that affect plant growth by behaving as growth hormones (Vaughan et al., 1985; Muscolo et al., 1998; Nardi et al., 2000). The forest soil organic matter provides nutrients and moderate water retention capacity it suitable for the growth and development of plants (Jones and Jacobsen, 2005; White et al., 2013; Schjoerring et al., 2019).

The incorporation of sand in the potting medium gave a fair response in shoot height and roots. However, no nutrient composition was recorded in the sand, indicating the dependency of the endo-nutrients present in the corm reserves and the porosity of the sand particles leading to the growth of the plants and better rooting. The study conducted by Malemba et al. (2021) had higher shoots (3.16) than the present study, however the plant height (21.6 cm) and roots (9.4) was comparatively lower than the current study.

Banana plants are tolerant to altitude, drought, and adverse soil conditions, due to their supply of water and nutrients from the stem (Pusponegoro et al., 2018). However, in the case of leaves and shoot buds, poor response was obtained, indicating that these factors may be dependent on external nutrient supplements. The sand has very little ability to hold water and nutrients which are crucial components for plants, due to its large pore spaces between particles and also low surface area (Jones and Jacobsen, 2005). However, soil characteristics alone do not dictate the capacity to provide nutrients. Soils also need to sustain root growth so that the growing plants can capture a sufficient proportion of the available nutrients (White et al., 2013; Schjoerring et al., 2019).

In the present study, tea manure along with sand was found to be a poor choice as potting medium as the mixture did not support good morphogenesis and plant growth. While, Gomez-Brandon et al. (2015) reported the effectiveness of tea compost in suppressing diseases and also enhances plant growth. Though the nutrient composition of tea has balanced compositions of macro and micronutrients, antioxidants, trace metals etc., the presence of aluminum and fluoride retards the growth of plants (Lunkes and Hashizume, 2014). The pH of the potting mix also plays important role in either enhancement or discouragement of plant growth. The collective studies recorded that the pH of the tea is either 2.8 to 5.17 or 6.79 to 7.89 which are either too low or too high pH for the banana plant to grow (Lunkes and Hashizume, 2014; Allameh and Orsat, 2024;

Kumadoh et al., 2024). Further, slow process of the breakdown of the tea manure and also the presence of toxic substances hinder growth in bananas (White et al., 2013; Schjoerring et al., 2019).

Ramirez (2020) in his study on Saba banana reported that addition of BAP in the potting mix promote early sprouting of shoot buds (15.44 days) (corms when treated with plant hormone BAP produced bud sprouts after 15.44 days, which is closer with the finding of the present study with potting mix containing forest soil (P_3) without BAP. Sajith et al. (2014) in their study on the Malbhog banana reported a lower number of shoots formation in growth regulator free potting mix. In the past many reports indicated that incorporation of different plant hormones such as BAP, NAA, IAA and microorganisms like *Trichoderma viride*, *Azospirillum*, *Bacillus subtilis* and Vesicular Arbuscular Mycorrhiza, coconut water which act as biofertilizers, PGPR's, antifungal agents etc. necessary for better response from the corm fragments (Langford et al., 2017; Mintah and Arhin, 2020; Opata et al., 2020; Ramirez, 2020). However, in the present study no other additives besides the stated potting mixtures was added, but recorded better morphogenic response compared to past reports on locally available substrata. Ntamwira et al. (2017) also concluded that locally available materials are good alternatives for seedling production in banana for small-scale farmers.

Effect of different potting mixtures on morphogenic response in Cv. Sabri

The role of potting media in the growth and development of plantlets is attributed to their moisture retention and porosity, which facilitate the distribution of water and promote proper root growth (Baiyeri and Aba, 2005; Viswanath et al., 2021). The study by Firew et al. (2024) used saw dust and Farm yard manure, resulting in 7 plantlets, which is more than the findings of the current study values.

The shoot emergences were obtained first in Forest (P_3) and Saw Dust (P_2) media between three potting media and least in Garden soil (P_1), however no significant differences were obtained statistically. The present study's results in the emergence of shoots within two weeks are slightly comparable to those of Dzomeku et al. (2014), despite the fact that the present study obtained results three days prior to theirs.

The study by Sajith et al. (2014) reported (Malbhog banana, AAB) number of shoots ranging from 1.23 to 3.33 nos in Saw Dust lower than the present findings. Thungon et al. (2017) reported in their study on Malbhog banana that Saw Dust resulted in primary

shoots averaging 3.63 nos and secondary shoots 7.40 nos, which is comparable to the current study findings.

The Forest (P_3) and Sawdust media (P_2) were found to be competitive in all responses, yielded negligible differences between the two, resulting in both achieving optimal responses across all parameters. Significant differences in results were observed in the garden soil compared to the other two, rendering it the least effective of the three. According to Bhende and Kurien (2020), the application of organic fertilizers in banana cultivation is advantageous as it supplies essential nutrients to the parent plant, leading to enhanced growth and increased yield, as well as the production of suckers. The disintegration of sawdust is beneficial in enhancing plant growth, and well-composted sawdust is predominantly favoured in nurseries. Furthermore, the composted rich organic substrate stimulates lateral shoot bud development, leading to increased shoot production. It provides significant advantages over traditional topsoil by offering an improved rooting substrate relationship with sufficient moisture (Baiyeri and Aba, 2007).

The survivability rate of the post-transplantation of the plantlets ranged from 80 to 85% in the plants regenerated in different potting mixtures and corm splitting techniques in both cultivars. However, the regenerates from potting mixtures of forest soil, sawdust, and forest soil + sand outperformed overall and exhibited a marginal edge where 85-90% of transplants survived. The use of sawdust and forest soil in combination with sand; sand + forest soil, and sand + saw dust resulted better response and hence, can be recommended for adoption.

Cost effective analysis

The estimated cost of this study is 8.5 US$ for producing ~300 plantlets and estimated cost of per plantlet is 0.028 US$. In the study there was no separate production unit construction as presented in the past reports (Njukwe et al., 2007; Sajith et al., 2014; Ntamwira et al., 2017). As presented in the supplementary data 3, the average production 0.31-0.84 US$. Considering the sizes of that experimental setup and their cost, the current study presents a viable option for cost efficiency, sustainability and reproducibility. Though no separate equipped production unit was used in the present study, but could produce healthy plantlets and needed for poor, marginal and unskilled farmers who can endeavor to produce clonal planting materials for a small to medium scale cultivation plot without any additional infrastructure.

Genetic stability assessment

Several molecular markers are being used for testing the genetic stability of the regenerated plants especially when produced clones and also for genetic diversity study among species and populations (Khan et al., 2011). The RAPD assessment as used in the present study do not require prior knowledge of the organism's genome sequence, while, SCoT markers are gene-specific and bind specifically on the conserved regions, making them more reliable. Both RAPD and SCoT markers are being widely used in different species (Hromadová et al., 2023). In the present study, though the SCoT markers produced more scorable bands in Grand Nain, RAPD primers produced more monomorphic bands and the finding is in alignment with Gorji et al. (2011). In the present study the RAPD markers resulted 100 % monomorphic band against 97.37 % and 92.86 % with SCoT markers and overall analysis of genetic stability indicates that the regenerates were 98.685 % and 96.72 % comparable with the mother stock. The genetic variation of 1.315 to 3.28 % does not go unnoticed although very low, though it does not affect the main objective and this negligible variation may be attributed to different factors like stress driven by different corm manipulations techniques, available of adequate or inadequate nutrients, or extended experimental duration.

Summary and Conclusions

This study entailed with development of simple and sustainable macropropagation protocols for two well established commercial banana cultivars using locally available low-cost resources. The study can be easily followed by the semi-skilled and unskilled farmers in cost-effective manner who wish to cultivate banana in a small to medium scale. However, for larger-scale production, one must take into account the need for more space, additional resources, and increased manpower etc. The methods developed are reproducible and the outcomes will exhibit minor variations depending upon the cultivars and the chosen planting materials. The experiments yielded favourable results, with the macropropagation techniques that produced the highest number of plantlets being Split Corm (T_2), followed by PIF (T_3), and then Split Corm + PIF (T_5) in both the cultivars. Additionally, the healthiest shoots with the highest survival rates were observed in the PIF (T_3) treatment, followed by Split Corm + PIF (T_5), and then Split Corm (T_2).

To achieve sustainable reproduction and create robust and thriving plantlets, it is recommended to utilize the techniques PIF (T_3) and Split Corm + PIF (T_5) in the process

of plantlet reproduction. These approaches are straightforward and efficient for propagating bananas using basic materials, in contrast to *in vitro* propagation which necessitates costly equipment and significant expertise.

The investigations on potting substrates also determined that the incorporation of propagation techniques, careful selection of planting materials, timely delivery of nutrients, and the choice of substratum had a significant impact on the growth and reproduction of plantlets. The potting media, including garden soil mixed with straw and cow dung (P_1), sand (P_4), and sand mixed with sawdust (P_5), exhibited a consistent and average response overall in Cv. Grand Nain. Nevertheless, the most favourable outcomes from both cultivars were achieved using forest soil (P_3), sawdust (P_2) and a mixture of sawdust and sand (P_5), making them suitable for the production and growth of banana plantlets.

The studies were conducted in a cost-effective and sustainable manner to provide inexpensive planting materials. This was achieved by substituting artificial fertilizers with recycled materials, resulting in reduced expenses. The study also introduced new approaches to macropropagation by combining forest soil manure and adding organic manures to enhance growth and productivity. Furthermore, the combination of locally available materials with macropropagation techniques such as PIF and split corms gave good outcome like the standard macropropagation units.

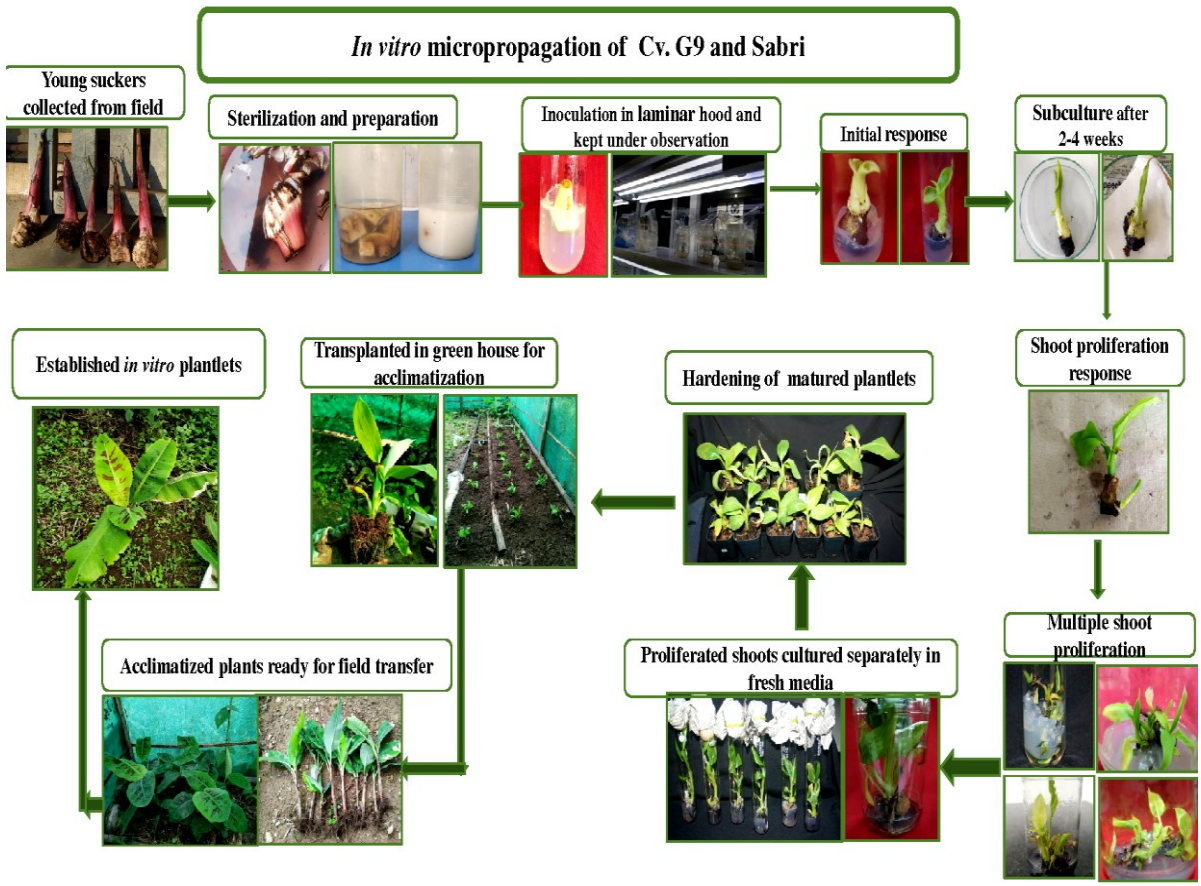
The genetic fidelity assessments of regenerates using 14 primers of RAPD and SCoT markers in both cultivars demonstrated that the regenerated plantlets exhibited genetic homogeneity rates of 96.72 % in G9 and 98.69 % in Sabri, respectively, thereby qualifying them as suitable clonal planting materials.

Chapter – 4

In Vitro Micropropagation in Banana Cultivars Grand Nain and Sabri

---

Graphical Summary



Introduction

Banana, a popular tropical fruit, ranks as the fourth most essential global food commodity after rice, wheat and milk (Karule et al., 2016; Ngullie and Deb, 2024). It is farmed in over 150 countries across 5.3 million hectares by farmers around the world (Uma et al., 2023). Banana belongs to the family Musaceae, and is an ancient food plant contributing to food security as well as serving as a staple food for millions in many countries Hossain et al. (2016). The world banana production rose from 67 million tonnes in 2000 to 114 million tonnes in 2017, of which India produced 29 million tonnes yearly from 2000 to 2017, according to FAO (2019). India leads in global banana production and contributed 34.5 million metric tonnes in production alone (Worldostats, 2024), and exhibited significant export potential, with a 38% increase in exports from 2022 to 2023, totalling 500 thousand tonnes (FAO, 2024).

Bananas are rich in nutrients containing high amounts of carbohydrates, and minerals such as calcium, phosphorus and potassium along with various vitamins (A, C etc.) rendering them the most valued fruit in the temperate regions even surpassing apples (Mohiuddin et al., 2020). The edible bananas known as cultivars are often sterile meaning they are parthenocarpic and are reproduced vegetatively. The natural propagation of banana plantlets is slow and produces a limited number of banana plants (Azad and Amin, 2001).

The natural or conventional propagation method that propagates from the underground rhizomes of the mother plants is often susceptible to many diseases for generations and adversely impacts yield, thereby affecting the economy with lowered harvest (Hossain et al., 2016). The growth and expansion of banana production in the country or region is constrained due to the lack of healthy planting materials for the farmers, therefore there is a dire need for the production of healthy plantlets to enhance yield and quality (Huq et al., 2012). *In vitro* micropropagation techniques are such an advanced technique which addresses the challenges associated with the production of clean and healthy multiple plantlets. The *in vitro,* micropropagation is an established technique promising success rates with 39 per cent more yield than the traditional methods (Ferdous et al., 2015). Multiple plantlets are grown from a small plant tissue within a year by using different culture techniques, and one such is shoot tip meristem culture (Apical meristem culture) which is a well-established method for producing disease-free, clonal multiple planting (Rehana et al., 2009).

The plant growth regulators in different concentrations and combinations, play an important role in the *in vitro* propagation of plants. Most common PGRs, such as Cytokinin (BAP, KN) are associated with suppressing apical dominance while stimulating shoot proliferation from the lateral and axillary meristems, and Auxins (IAA, IBA, NAA) enhance root formation in the regenerated shoots (Al-Amin et al., 2009) Subsequently, depending on the type of bananas different PGR concentrations and combinations are required for propagation (Ferdous et al., 2015).

Two prominent banana cultivars, Grand Nain and Sabri, are renowned for their economic significance. The G9 cultivar is highly popular, extensively produced, and cultivated globally, particularly in Central America, Africa, India, and Southeast Asia. It is commercially and economically significant and extensively cultivated in India.

The Sabri banana cultivar is a local variety from Tripura, India, and Bangladesh, distinguished by its unique flavour and high nutritional content. The cultivar is one of the most common in Tripura, classified under the silk subgroup with a genome of AAB.

However, it has been claimed that the production of both cultivars has decreased in recent years as a result of the spread of numerous diseases, such as Fusarium wilt and Sigatoka leaf spot, amongst other (Mitra et al., 2022; Uma et al., 2023).

Therefore, considering the limitations and the necessity to provide quality clonal planting material for restoring the cultivar population, this study aimed to assess the optimal protocol for the regeneration of plantlets and thereby provide a solution. With this back ground the present study aimed to develop *in vitro* propagation protocols and the optimization of factors influencing the production of clonal planting materials of these cultivars.

Materials and Methods

The present study on *in vitro* micropropagation of two *Musa*. Cv. Grand Nain (AAA) and Sabri (AAB) were carried out during 2018 to 2023. The healthy and diseased-free banana suckers aged 3-6 months were procured for the experiment. The suckers of cultivar G9 were collected from the experimental garden of Department of Botany; whereas, the cultivar Sabri was collected from a local garden in Agartala, Tripura.

Explants preparation and sterilization and nutrient media preparation

The healthy suckers were thoroughly washed under running tap water with Labolene to eliminate all extraneous dirt, and the external dead tissues and roots were meticulously excised without causing any injury; subsequently, the suckers were sun-

dried for one day to eradicate excess moisture and pathogens. The sundried suckers were subsequently treated with 0.2% Bavistin (fungicide) for an hour following the segmentation and dissection of the rhizome into blocks. The processed rhizome segments were sterilized under aseptic condition in the Laminar Air Flow (LAF) Chamber. The rhizome segments were treated with 0.1% mercuric chloride (v/v) for 2 min, rinsed off several times with autoclaved pure water. Before inoculation, the initially sterilized rhizome segments were re-sterilized with 0.1% $HgCl_2$ for 2 min in the LAF chamber for 30 sec and subsequently rinsed 5-5 times with autoclaved water, before being sliced to a final size of 1-2x2-3 cm size blocks and rinsed in 70% (v/v) alcohol for 20 sec. and washed twice with autoclaved pure water. The surplus moisture in the explants was removed with sterile absorbent paper and cultured on differentially fortified nutrient medium.

The Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) was augmented fortified with sucrose (3%) (w/v), PVP (0.1%, w/v) and agar (0.8%, w/v) and pH of the media were adjusted to 5.8 with 0.1N HCl and NaOH. The basal nutrient medium was fortified differentially with different plant growth regulators (PGPRs) as follows:

1. Benzyl amino purine (BAP) (1-6.5 mg/L) either singly or in combination with indole 3-acetic acid (IAA) (0.5-1.5 mg/L) or naphthalene acetic acid (NAA) (0.5-1.5 mg/L).
2. Kinetin (KN) (1-6.5 mg/L) either singly or in combination with IAA (0.5-1.5 mg/L) or NAA (0.5-1.5 mg/L).
3. Thidiazuron (TDZ) (0.5-1.5 mg/L) either singly or in combination with IAA (0.5-1.5 mg/L).

Tissue culture

The appropriately sterilized (apical shoot) explants were inoculated on fortified nutrient MS medium supplemented with different doses of BAP and kinetin concentrations to facilitate shoot bud initiation. The cultures are incubated in a sterile culture room at 25±2°C, and subjected to a photoperiod of 3000 Lux for 12/12 h (light/dark) photoperiod.

The cultures that exhibited growth responses were carefully sub-cultured at intervals of 4–5 weeks, depending on the specific requirements and developmental stages of the cultures. Additionally, the elongated apical shoot tips were trimmed carefully to

induce multiple shoot proliferation. Observations regarding the growth, development, and overall condition of the cultures were systematically recorded weekly.

After the first subculture, the initiated shoots (regenerated explants) that had begun multiple shoot proliferation were carefully separated from the parent explants under sterile conditions. These detached shoots were subsequently transferred to fresh nutrient media optimized for their further development and proliferation. To maintain continuous growth and avoid nutrient depletion, the cultures were periodically sub-cultured onto fresh media at shorter intervals of 3–4 weeks, facilitating vigour and health of the growing shoot along with sustained proliferation.

Following the generation of multiple shoots, the proliferated shoots were carefully separated without causing tissue injury and transferred individually into fresh growth media without trimming the apical shoot tip. This facilitated the development of leaves, roots, and shoot elongation, resulting in fully developed plantlets ready for hardening.

The matured plantlets, with well-developed leaves and roots, were transferred to a primary hardening medium composed of cocopeat. The transplanted plantlets are kept away from direct sunlight for 3 to 4 weeks, and covered with thin transparent polythene bags to minimize moisture loss. After primary hardening, the plantlets with well-hardened roots and leaves were transplanted into potting media consisting of soil. They were then placed under 50–70% shade in a greenhouse for approximately 3 to 4 weeks. The acclimatized plantlets after 3 to 4 weeks that are fully developed are then transferred to the field for establishment and further trials.

Genetic fidelity assessment

Genomic DNA was isolated from young fresh leaf tissues of *in vitro* regenerates of both the cultivars and donor plant (M) using a modified CTAB method as described by Shankar et al. (2011). For evaluation of genetic fidelity of the regenerates, two different markers (RAPD and ISSR) were used. For each marker, 7 primers were used. The PCR reaction was carried out using a total volume of 25µL, using 5X PCR buffer, 2mM $MgCl_2$, deionized water, 0.5mM dNTPs, 1µM primer, and 30ng genomic DNA template and a unit of *Taq* polymerase. The PCR reaction was conducted using a Thermal Cycler (BioRad), beginning with an initial denaturation step at 95ºC for 5 min. This was followed by 35 cycles comprising denaturation at 94ºC for 1 min, followed by annealing at 32-36ºC for 30 sec for RAPD analysis and in the case of ISSR, annealing at 45 to 60ºC for 30 sec and extension at 72ºC for 2 min and a final extension for 8 min.

The PCR products were separated on 1.5 % agarose gel at a constant voltage of 80 V for duration of 90 min and then visualized in a gel image documentation system. The gel images were evaluated, scored and documented in a binary format indicating the presence and absence of bands in the gel. Seven primers each from the two markers RAPD and ISSR were used for genetic fidelity assessment. Randomly selected 9 regenerated (R_1-R_9) plantlets and one mother plant (M) were used in this study.

Data collection and statistical analysis

In all experiments completely randomized experimental design (CRD) was followed with five replications. Observations were recorded on four parameters *viz.,* the number of shoots formed per explants, shoot height (cm), number of leaves per shoot and number of roots per shoot was considered. The collected data were analysed using One-way ANOVA (MS Excel 2019), Graph Pad Prism 10.2.0 and Origin 6.4.

Results

Initiation of cultures

The cultures were initiated following sterilization with Bavistin and mercuric chloride, which proved highly effective, resulting in 80-90% contamination free cultures. The initial cultures faced contamination and lethal browning causing necrosis and death of explants. For culture initiation from both the cultivars, MS nutrient medium was used supplemented with sucrose (3%) and fortified differently with different PGRs like BAP, KN, TDZ either singly or in combination with IAA and NAA. The cytokinins BAP and KN were adjunct with 1-6.5 mg/L. The most significant shoot initiation response was registered on BAP supplemented medium in G9, achieving an overall average of 71%, with peak responses at 2.5 and 4.5 mg/L, reaching 87% **(Figure 4.1a, c**). On the other hand, in Sabri, the initiation rate was superior with KN compared to BAP, averaging 43.8%, with the highest response observed at 2.5 and 5.5 mg/L in Kinetin (**Figure 4.1b, d**).

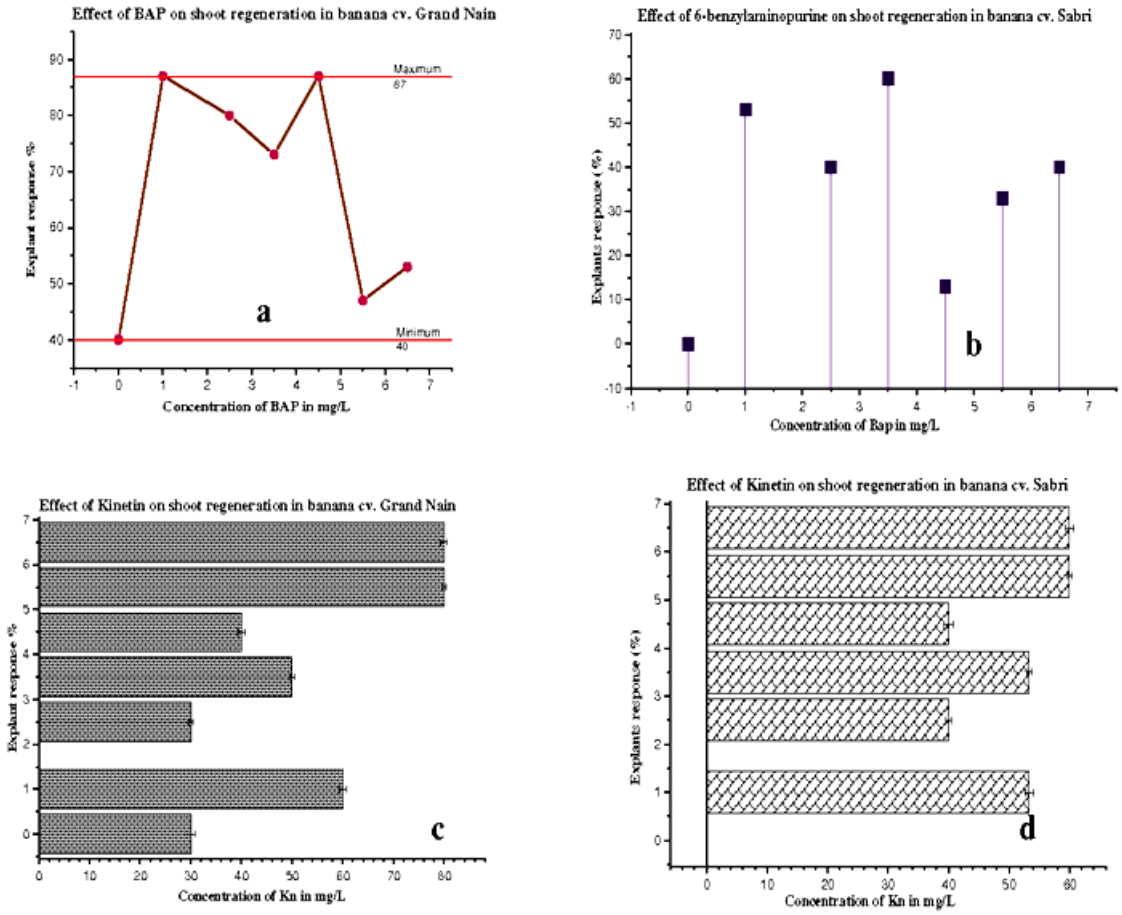


Figure 4.1: Graphical representation of the effect of BAP and KN in Cv. Grand Nain and Sabri on plant regeneration. a. Effect of BAP on shoot regeneration of G9 ; b. Effect of BAP on shoot regeneration of Sabri ; c. Effect of KN on shoot regeneration of G9; d. Effect of KN on shoot regeneration of Sabri.

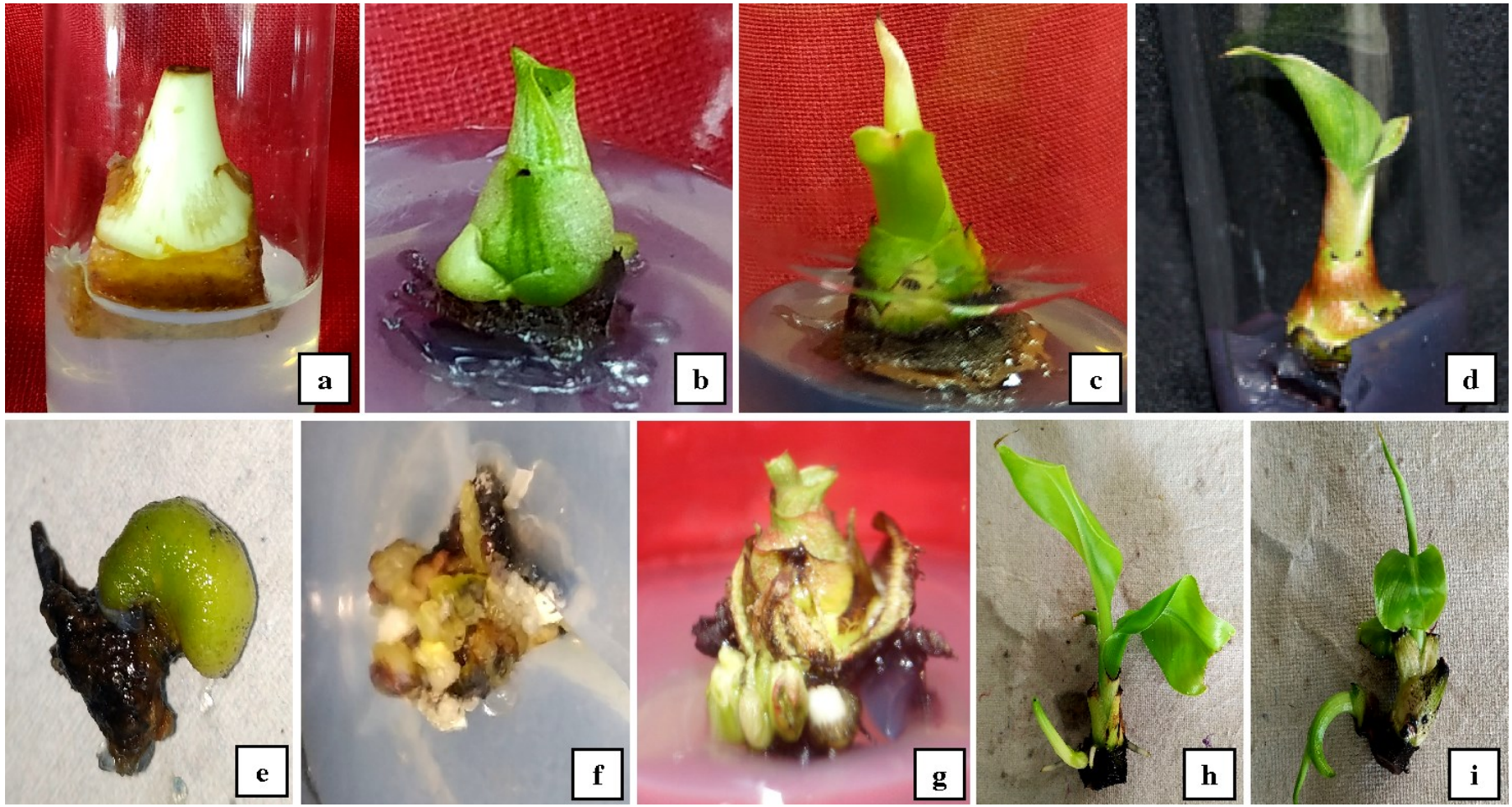


Figure 4.2: Effect of different stages of *in vitro* morphogenic response of G9 on MS medium supplemented with different adjuncts. a. Explants cultured on MS medium; b. Initial morphogenic response marked with swelling followed by invoking loci and green colored initial response; c-d. Shoot buds formation; e-f. Development of globular structures; g. Development of shoot buds from the base of the cultured; h-i. Multiple shoots formation.

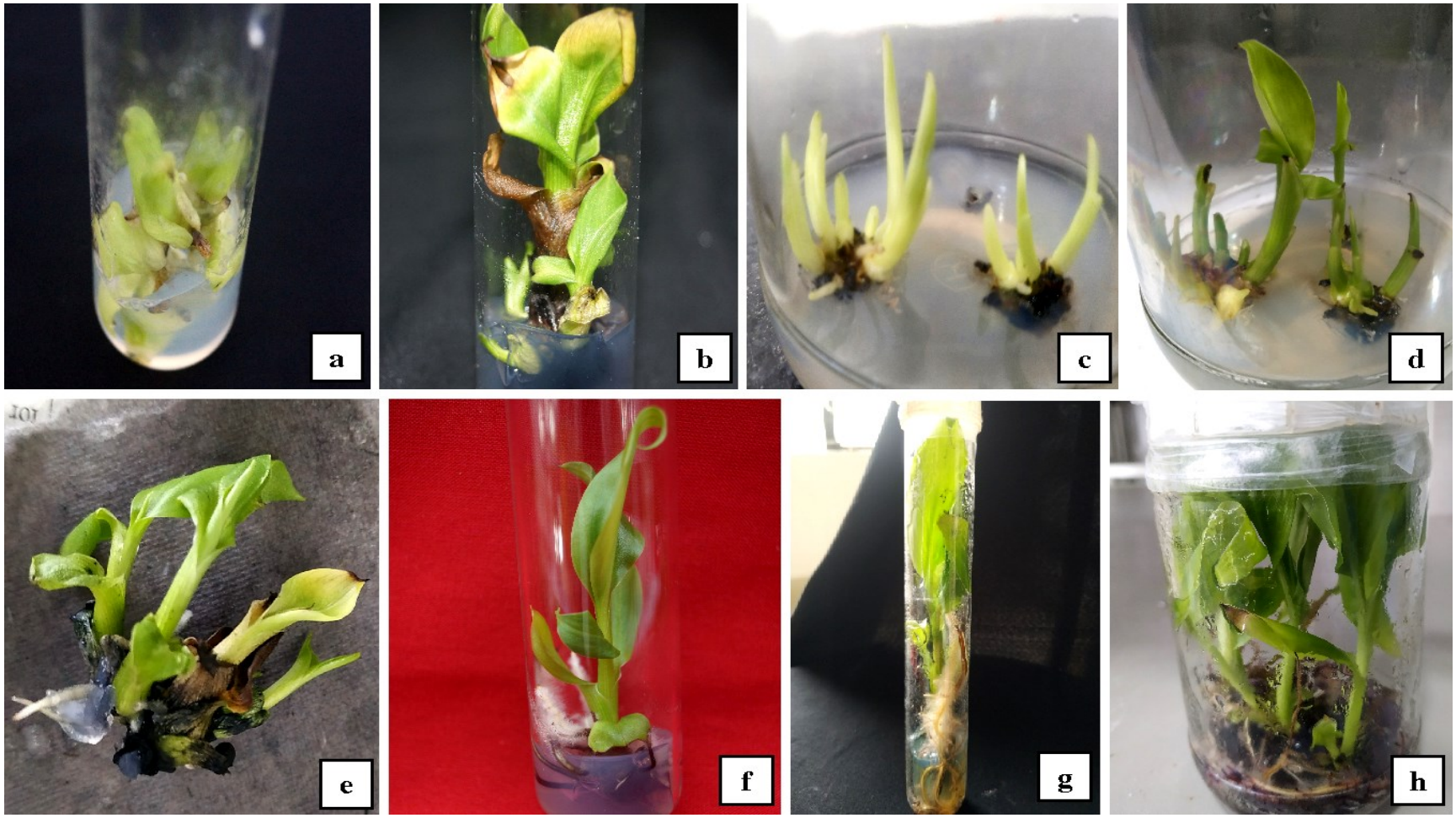


Figure 4.3: Different stages of *in vitro* propagation of banana Cv. Sabri. A. Initial regeneration response from the cultured explants; b. Initiation of shoot proliferation; c-e. Multiple shoots formation; f. A regenerated shoot with well developed leaves showing ignition of root formation; g-h. Rooted plants under primary hardening.

The *in vitro* morphogenic process and plantlets development processes of both the cultivars are presented in **Figure 4.2** and **4.3**. In both the cultivars, within 2-3 weeks of culture initiation the responsive explants exhibited of sign of morphogenic response through initial swelling of the explants at the basal parts followed by sprouting of meristematic loci and shoot buds. The resultant shoot buds were subsequently separated and cultured on the respective optimum initiation media for culture proliferation and differentiation. In the studied cultivars, explants cultured on PGR free medium failed to register any morphogenic response; while, the administered different PGRs either singly or in combination registered different responses.

Cytokinins like BAP and KN and the cytokinin-like plant hormone TDZ were tested at various concentrations ranging from 1 to 6.5 mg/L for BAP and Kinetin, and 0.5 to 1.5 mg/L for TDZ. The cultures registered responses within 2-3 weeks of culture and within 4-6 weeks distinct responses were recorded. Following the initial responses from the sucker segments, the resulting shoots were sub-cultured on MS medium supplemented with plant growth regulators for proliferation of multiple shoots.

Initiation of *in vitro* culture, plant regeneration and culture proliferation were greatly influenced by different factors in both the cultivars especially quality and quantity of PGR incorporated in the nutrient medium. Cultivar-wise role of factors are presented below:

Cultivar Grand Nain

Effect of PGRs on culture initiation and culture proliferation

In the present study different types of PGRs both auxins and cytokinins were used for culture initiation and culture proliferation from the cultured sucker segments. When BAP used singly at 1-6.5 mg/L resulted 100% of shoot induction, with the highest number of shoots (8.67) recorded on medium supplemented with 3.5 mg/L (**Table 4.1, Figure 4.4**). The multiplication rate escalated markedly in the mid-concentration range; while, the lower and higher concentrations exhibited comparatively poorer response and supported ~4 nos. of shoot buds (**Table 4.1**). The combination of the BAP with either IAA or NAA exhibited contrasting effects on shoot proliferation. The IAA adjunct nutrient medium supported better shoot induction and culture proliferation where an average of 7.5 shoots developed at 3.5 mg/L BAP + 1 mg/L IAA, and 6.5 shoots at 3.5 mg/L BAP + 1 mg/L IAA. However, in general NAA failed to induce shoot proliferation **(Table 4.1)**.

Table 4.1: Effect BAP, IAA and NAA on morphogenic response of rhizome segments of banana Cv. Grand Nain

PGR Conc. (mg/L)	Number of shoot buds invoked per segment	Length of shoots (cm)	Number of leaves per shoot	Number of roots per shoot
Control	0	0	0	0
BAP_1	4.33 ± 0.53^{d}	5.5 ± 0.71^{d}	2.67 ± 0.57^{g}	5.5 ± 0.71^{i}
$BAP_{2.5}$	1.5 ± 0.21^{g}	5.1 ± 0.79^{d}	8.33 ± 2.31^{a}	9.33 ± 1.52^{f}
$BAP_{3.5}$	8.67 ± 0.48^{a}	4.03 ± 0.47^{e}	7.0 ± 1.41^{b}	8.67 ± 0.82^{f}
$BAP_{4.5}$	2.33 ± 0.53^{f}	4.56 ± 0.21^{e}	7.67 ± 1.53^{b}	6.33 ± 0.57^{h}
$BAP_{5.5}$	3.1 ± 0.65^{e}	5.55 ± 0.35^{d}	6.0 ± 1.73^{c}	4.1 ± 0.32
$BAP_{6.5}$	4.0 ± 0.47^{d}	4.56 ± 0.41^{e}	4.33 ± 0.58^{e}	1.67 ± 0.57
$BAP_1 + IAA_{0.5}$	4.1 ± 0.41^{d}	3.5 ± 0.5^{f}	7.0 ± 1.05^{b}	4.0 ± 0.41
$BAP_1 + IAA_1$	2.67 ± 0.57^{f}	3.53 ± 0.35^{f}	5.33 ± 0.57^{d}	4.33 ± 0.57
$BAP_1 + IAA_{1.5}$	1.67 ± 0.28^{g}	3.3 ± 0.1^{f}	4.66 ± 0.52^{e}	5.0 ± 0.10
$BAP_{2.5} + IAA_{0.5}$	2.1 ± 0.29^{f}	2.55 ± 0.21^{g}	6.0 ± 0.34^{c}	2.5 ± 0.31
$BAP_{2.5} + IAA_1$	2.1 ± 0.70^{f}	2.4 ± 0.28^{g}	4.0 ± 0.41^{e}	1.67 ± 0.17^{m}
$BAP_{2.5} + IAA_{1.5}$	5.1 ± 0.9^{c}	2.45 ± 0.07^{g}	5.0 ± 0.41^{d}	2.5 ± 0.31^{l}
$BAP_{3.5} + IAA_{0.5}$	4.0 ± 09.73^{d}	5.33 ± 0.37^{d}	5.67 ± 0.53^{d}	2.33 ± 0.37^{l}
$BAP_{3.5} + IAA_1$	6.5 ± 1.09^{b}	4.63 ± 0.26^{e}	5.67 ± 0.57^{d}	3.33 ± 0.47^{k}
$BAP_{3.5} + IAA_{1.5}$	7.5 ± 1.12^{a}	4.86 ± 0.51^{e}	4.0 ± 0.41^{e}	1.67 ± 0.58^{m}
$BAP_{4.5} + IAA_{0.5}$	3.0 ± 0.5^{e}	2.4 ± 0.14^{g}	3.66 ± 0.57^{f}	2.67 ± 0.57^{l}
$BAP_{4.5} + IAA_1$	5.0 ± 0.5^{c}	3.9 ± 0.56^{f}	5.33 ± 0.52^{d}	3.33 ± 2.08^{k}
$BAP_{4.5} + IAA_{1.5}$	4.67 ± 0.57^{c}	2.7 ± 0.28^{g}	5.0 ± 1.41^{d}	1.67 ± 0.27^{m}
$BAP_{5.5} + IAA_{0.5}$	2.2 ± 0.41^{f}	3.8 ± 0.42^{f}	7.0 ± 0.66^{b}	2.5 ± 0.41^{l}
$BAP_{5.5} + IAA_1$	4.1 ± 0.73^{d}	3.85 ± 0.49^{f}	6.5 ± 0.71^{c}	3.0 ± 0.41^{k}
$BAP_{5.5} + IAA_{1.5}$	3.0 ± 0.3^{e}	2.65 ± 0.21^{g}	3.66 ± 0.57^{f}	-
$BAP_{6.5} + IAA_{0.5}$	3.1 ± 0.41^{e}	4.25 ± 0.35^{e}	5.33 ± 0.57^{d}	-
$BAP_{6.5} + IAA_1$	2.0 ± 0.26^{f}	1.6 ± 0.14^{h}	2.5 ± 0.31^{g}	-
$BAP_{6.5} + IAA_{1.5}$	3.0 ± 0.41^{e}	4.93 ± 0.51^{e}	6.1 ± 0.41^{c}	2.67 ± 0.57^{l}
$BAP_1 + NAA_{0.5}$	1.3 ± 0.02^{g}	3.9 ± 0.85^{g}	4.0 ± 0.76^{e}	4.5 ± 0.45^{j}
$BAP_1 + NAA_1$	1.0 ± 0.0^{g}	4.25 ± 0.36^{e}	5.5 ± 0.66^{d}	11.5 ± 1.41^{e}
$BAP_1 + NAA_{1.5}$	1.0 ± 0.0^{g}	7.75 ± 0.41^{b}	7.0 ± 0.31^{b}	11.0 ± 1.44^{e}
$BAP_{2.5} + NAA_{0.5}$	1.3 ± 0.02^{g}	5.75 ± 0.95^{d}	4.5 ± 1.06^{e}	7.5 ± 1.11^{g}
$BAP_{2.5} + NAA_1$	1.0 ± 0.0^{g}	3.05 ± 0.77^{f}	4.5 ± 0.71^{e}	3.5 ± 0.3^{k}
$BAP_{2.5} + NAA_{1.5}$	4.5 ± 0.58^{c}	5.67 ± 0.36^{d}	6.0 ± 1.33^{c}	16.5 ± 1.12^{c}
$BAP_{3.5} + NAA_{0.5}$	3.5 ± 0.67^{e}	6.5 ± 1.05^{c}	6.5 ± 0.70^{c}	18.5 ± 1.4^{a}
$BAP_{3.5} + NAA_1$	1.0 ± 0.0^{g}	7.6 ± 0.36^{b}	4.0 ± 1.03^{e}	12.0 ± 1.71^{e}
$BAP_{3.5} + NAA_{1.5}$	3.1 ± 0.27^{e}	9.0 ± 0.6^{a}	5.0 ± 1.56^{d}	9.5 ± 1.5^{f}

$BAP_{4.5} + NAA_{0.5}$	2.1 ± 0.12^{f}	5.63 ± 0.78^{d}	5.67 ± 0.55^{d}	14.0 ± 1.23^{d}
$BAP_{4.5} + NAA_{1}$	3.5 ± 0.57^{e}	5.3 ± 0.33^{d}	5.67 ± 1.08^{d}	17.5 ± 1.76^{b}
$BAP_{4.5} + NAA_{1.5}$	0.5 ± 0.07^{h}	4.25 ± 0.35^{e}	5.1 ± 0.89^{d}	7.0 ± 0.44^{g}
$BAP_{5.5} + NAA_{0.5}$	1.0 ± 0.0^{g}	6.53 ± 0.66^{c}	6.66 ± 0.34^{c}	5.5 ± 1.11^{i}
$BAP_{5.5} + NAA_{1}$	2.1 ± 0.31^{f}	7.23 ± 0.84^{b}	8.0 ± 1.09^{a}	12.5 ± 1.64^{e}
$BAP_{5.5} + NAA_{1.5}$	1.0 ± 0.0^{g}	6.95 ± 0.34^{b}	7.0 ± 1.02^{b}	6.3 ± 1.36^{h}
$BAP_{6.5} + NAA_{0.5}$	2.0 ± 0.11^{f}	2.95 ± 0.07^{f}	4.0 ± 0.61^{e}	4.0 ± 1.48^{j}
$BAP_{6.5} + NAA_{1}$	$1.3\pm.33^{g}$	5.57 ± 1.55^{d}	5.5 ± 1.33^{d}	9.0 ± 1.66^{f}
$BAP_{6.5} + NAA_{1.5}$	2.5 ± 0.31^{f}	6.25 ± 0.35^{c}	7.0 ± 1.15^{b}	7.0 ± 1.41^{g}

Note: Data represents the mean of three replicates ± Standard deviation from the mean. * *P* value has been calculated using one-way ANOVA and values with the same superscript letters in the column do not differ significantly ($P \leq 0.05$).

The singly treatment of KN was found to be inferior compared to BAP for morphogenic response (**Table 4.2**) where ~67% explants registered response with 2 shoot buds per explants at 5.5 mg/L concentration. However, in combination with IAA found to be promotive where as many as 4.5 shoot buds formed at 2.5 mg/L KN+1.5 mg/L IAA. In contrast, the combining effect of KN with NAA was ineffective in 94.5% of cultures responding solely at a concentration of 6.5 mg/L KN +1.5 mg/L NAA, resulting in just 1 shoot per explants.

In the present study TDZ was found to be not supportive for morphogenic response in the concentration ranges (0.5 to 1.5 mg/L); however, its combination with IAA resulted in 22% shoot induction with 1.67 shoots per explants (**Table 4.3**).

Table 4.2: Effect KN, IAA and NAA on morphogenic response of rhizome segments of banana Cv. Grand Nain

PGR Conc. (mg/L)	Number of shoot buds invoked per segment	Length of shoots (cm)	Number of leaves per shoot	Number of roots per shoot
Control	0	0	0	0
KN_1	1.0 ± 0.0^{e}	5.0 ± 0.05^{h}	8.0 ± 0.65^{c}	10.33 ± 1.63^{f}
$KN_{2.5}$	1.0 ± 0.0^{e}	4.35 ± 0.44^{h}	9.0 ± 1.56^{b}	11.0 ± 1.41^{e}
$KN_{3.5}$	1.0 ± 0.0^{e}	5.53 ± 0.69^{g}	9.0 ± 1.07^{b}	11.0 ± 1.49^{e}
$KN_{4.5}$	1.0 ± 0.0^{e}	2.75 ± 0.21^{i}	6.67 ± 0.58^{e}	8.0 ± 0.76^{h}
$KN_{5.5}$	2.0 ± 0.20^{cd}	7.75 ± 0.76^{e}	5.67 ± 0.58^{f}	11.5 ± 0.85^{e}
$KN_{6.5}$	1.0 ± 0.0^{e}	8.1 ± 1.68^{d}	6.5 ± 0.71^{e}	12.5 ± 1.35^{d}
$KN_1 + IAA_{0.5}$	0.5 ± 0.01^{f}	3.9 ± 0.85^{h}	4.1 ± 0.76^{g}	4.5 ± 0.45^{l}
$KN_1 + IAA_1$	1.0 ± 0.0^{e}	4.25 ± 0.86^{h}	5.5 ± 0.66^{f}	11.5 ± 0.41^{f}
$KN_1 + IAA_{1.5}$	1.0 ± 0.0^{e}	7.75 ± 0.41^{e}	7.0 ± 0.31^{d}	11.0 ± 0.44^{e}
$KN_{2.5} + IAA_{0.5}$	1.0 ± 0.0^{e}	5.75 ± 0.95^{g}	4.5 ± 1.06^{g}	7.5 ± 0.81^{h}
$KN_{2.5} + IAA_1$	1.0 ± 0.0^{e}	3.05 ± 0.77^{h}	4.5 ± 0.71^{g}	3.5 ± 0.3^{l}
$KN_{2.5} + IAA_{1.5}$	4.5 ± 0.18^{a}	5.67 ± 0.36^{f}	6.1 ± 0.33^{e}	16.5 ± 1.12^{b}
$KN_{3.5} + IAA_{0.5}$	3.5 ± 0.31^{b}	6.5 ± 1.05^{f}	6.5 ± 0.70^{e}	18.5 ± 1.4^{a}
$KN_{3.5} + IAA_1$	1.0 ± 0.0^{e}	7.6 ± 0.36^{e}	4.0 ± 0.23^{g}	12.0 ± 1.71^{d}
$KN_{3.5} + IAA_{1.5}$	3.0 ± 0.27^{b}	9.0 ± 0.6^{c}	5.0 ± 0.56^{f}	9.5 ± 0.5^{g}
$KN_{4.5} + IAA_{0.5}$	2.0 ± 0.12^{cd}	5.63 ± 0.78^{f}	5.67 ± 0.55^{f}	14.0 ± 1.23^{c}
$KN_{4.5} + IAA_1$	3.5 ± 0.57^{b}	5.3 ± 1.33^{f}	5.67 ± 1.08^{f}	17.5 ± 1.76^{b}
$KN_{4.5} + IAA_{1.5}$	0.5 ± 0.07^{f}	4.25 ± 0.35^{g}	5.0 ± 0.89^{f}	7.0 ± 0.44^{i}
$KN_{5.5} + IAA_{0.5}$	1.0 ± 0.0^{e}	6.53 ± 0.66^{e}	6.66 ± 1.34^{e}	5.5 ± 1.11^{k}
$KN_{5.5} + IAA_1$	2.0 ± 0.11^{cd}	7.23 ± 1.04^{e}	8.0 ± 1.09^{c}	12.5 ± 1.64^{d}
$KN_{5.5} + IAA_{1.5}$	1.0 ± 0.0^{e}	6.95 ± 1.34^{e}	7.0 ± 1.02^{d}	6.0 ± 1.36^{j}
$KN_{6.5} + IAA_{0.5}$	2.0 ± 0.21^{cd}	2.95 ± 0.07^{h}	4.0 ± 0.61^{g}	4.0 ± 1.48^{l}
$KN_{6.5} + IAA_1$	1.0 ± 0.0^{e}	5.57 ± 0.55^{f}	5.5 ± 1.33^{f}	9.0 ± 1.66^{g}
$KN_{6.5} + IAA_{1.5}$	2.5 ± 0.71^{c}	6.25 ± 0.35^{ef}	7.0 ± 1.15^{d}	7.0 ± 1.41^{i}
$KN_1 + NAA_{0.5}$	1.0 ± 0.0^{e}	9.93 ± 0.85^{c}	7.1 ± 0.65^{d}	19.0 ± 1.53^{a}
$KN_1 + NAA_1$	1.0 ± 0.0^{e}	7.6 ± 0.38^{d}	7.1 ± 0.66^{d}	15.0 ± 1.35^{c}
$KN_1 + NAA_{1.5}$	1.0 ± 0.0^{e}	8.5 ± 0.85^{c}	7.33 ± 1.15^{d}	17.67 ± 1.08^{b}
$KN_{2.5} + NAA_{0.5}$	1.0 ± 0.0^{e}	10.77 ± 1.62^{b}	7.67 ± 0.58^{d}	18.67 ± 1.02^{a}
$KN_{2.5} + NAA_1$	1.0 ± 0.0^{e}	6.2 ± 1.05^{e}	8.1 ± 0.77^{c}	12.5 ± 1.94^{e}
$KN_{2.5} + NAA_{1.5}$	1.0 ± 0.0^{e}	10.47 ± 1.34^{b}	8.1 ± 0.43^{c}	14.33 ± 1.35^{c}
$KN_{3.5} + NAA_{0.5}$	1.0 ± 0.0^{e}	7.7 ± 0.89^{d}	6.0 ± 0.34^{e}	10.67 ± 1.78^{f}
$KN_{3.5} + NAA_1$	1.0 ± 0.0^{e}	7.13 ± 0.40^{d}	9.7 ± 1.51^{b}	11.33 ± 1.53^{e}
$KN_{3.5} + NAA_{1.5}$	1.0 ± 0.0^{e}	5.8 ± 0.63^{f}	6.0 ± 1.15^{e}	8.0 ± 0.73^{g}

$KN_{4.5} + NAA_{0.5}$	1.0 ± 0.0^{e}	5.7 ± 0.42^{f}	8.0 ± 1.46^{c}	5.33 ± 0.58^{k}
$KN_{4.5} + NAA_{1}$	1.0 ± 0.0^{e}	7.45 ± 0.49^{d}	10.33 ± 0.58^{a}	10.67 ± 1.15^{e}
$KN_{4.5} + NAA_{1.5}$	1.0 ± 0.0^{e}	9.57 ± 1.60^{c}	7.67 ± 1.53^{d}	12.67 ± 0.62^{d}
$KN_{5.5} + NAA_{0.5}$	1.0 ± 0.0^{e}	6.9 ± 0.56^{e}	7.0 ± 1.82^{d}	9.0 ± 1.41^{f}
$KN_{5.5} + NAA_{1}$	1.0 ± 0.0^{e}	8.23 ± 1.19^{d}	7.67 ± 0.58^{d}	11.33 ± 0.57^{d}
$KN_{5.5} + NAA_{1.5}$	1.0 ± 0.0^{e}	4.57 ± 0.73^{g}	9.0 ± 1.73^{b}	5.0 ± 0.3^{k}
$KN_{6.5} + NAA_{0.5}$	1.0 ± 0.0^{e}	1.7 ± 0.07	0.67 ± 0.89^{i}	7.0 ± 1.73^{h}
$KN_{6.5} + NAA_{1}$	1.0 ± 0.0^{e}	5.6 ± 0.23^{f}	5.67 ± 0.15^{f}	-
$KN_{6.5} + NAA_{1.5}$	1.0 ± 0.0^{e}	12.3 ± 1.83^{a}	6.33 ± 0.15^{e}	17.67 ± 1.24^{b}
Note: Data represents the mean of three replicates ± Standard deviation from the mean. * *P* value has been calculated using one-way ANOVA and values with the same superscript letters in the column do not differ significantly ($P \leq 0.05$).				

Table 4.3: Effect TDZ and IAA on morphogenic response of rhizome segments of banana Cv. Grand Nain

PGR Conc. (mg/L)	Number of shoot buds invoked per segment	Length of shoots (cm)	Number of leaves per shoot	Number of roots per shoot
Control	-	-	-	-
$TDZ_{0.5}$	-	2.4 ± 0.14^{f}	3.5 ± 0.71^{f}	3.8 ± 0.41^{g}
TDZ_{1}	-	2.55 ± 0.64^{f}	3.5 ± 0.32^{f}	5.5 ± 0.32^{f}
$TDZ_{1.5}$	-	2.9 ± 0.14^{f}	1.67 ± 0.58	2.5 ± 0.33^{h}
$TDZ_{0.5} + IAA_{0.5}$	-	6.0 ± 0.67^{d}	7.5 ± 0.71^{b}	9.0 ± 0.68^{e}
$TDZ_{0.5} + IAA_{1}$	1.33±0.15a	6.85 ± 0.44^{d}	6.67 ± 0.66^{e}	12.5 ± 1.78^{c}
$TDZ_{0.5} + IAA_{1.5}$	1.67±0.28a	9.4 ± 0.23^{b}	6.33 ± 0.53^{e}	12.67 ± 1.01^{c}
$TDZ_{1} + IAA_{0.5}$	-	5.15 ± 0.74^{e}	5.5 ± 0.54^{e}	5.5 ± 0.36^{f}
$TDZ_{1} + IAA_{1}$	-	1.7 ± 0.28	1.33 ± 0.33^{g}	-
$TDZ_{1} + IAA_{1.5}$	-	8.17 ± 0.62^{c}	6.0 ± 0.65^{c}	10.0 ± 1.31^{d}
$TDZ_{1.5} + IAA_{0.5}$	-	11.25 ± 0.35^{a}	9.0 ± 0.41^{a}	17.5 ± 1.53^{a}
$TDZ_{1.5} + IAA_{1}$	-	6.0 ± 0.65^{d}	7.5 ± 0.53^{b}	11.5±1.02
$TDZ_{1.5} + IAA_{1.5}$	-	5.5 ± 0.32^{e}	6.0 ± 0.24^{c}	13.5 ± 1.02^{b}
Note: Data represents the mean of three replicates ± Standard deviation from the mean. * *P* value has been calculated using one-way ANOVA and values with the same superscript letters in the column do not differ significantly ($P \leq 0.05$).				

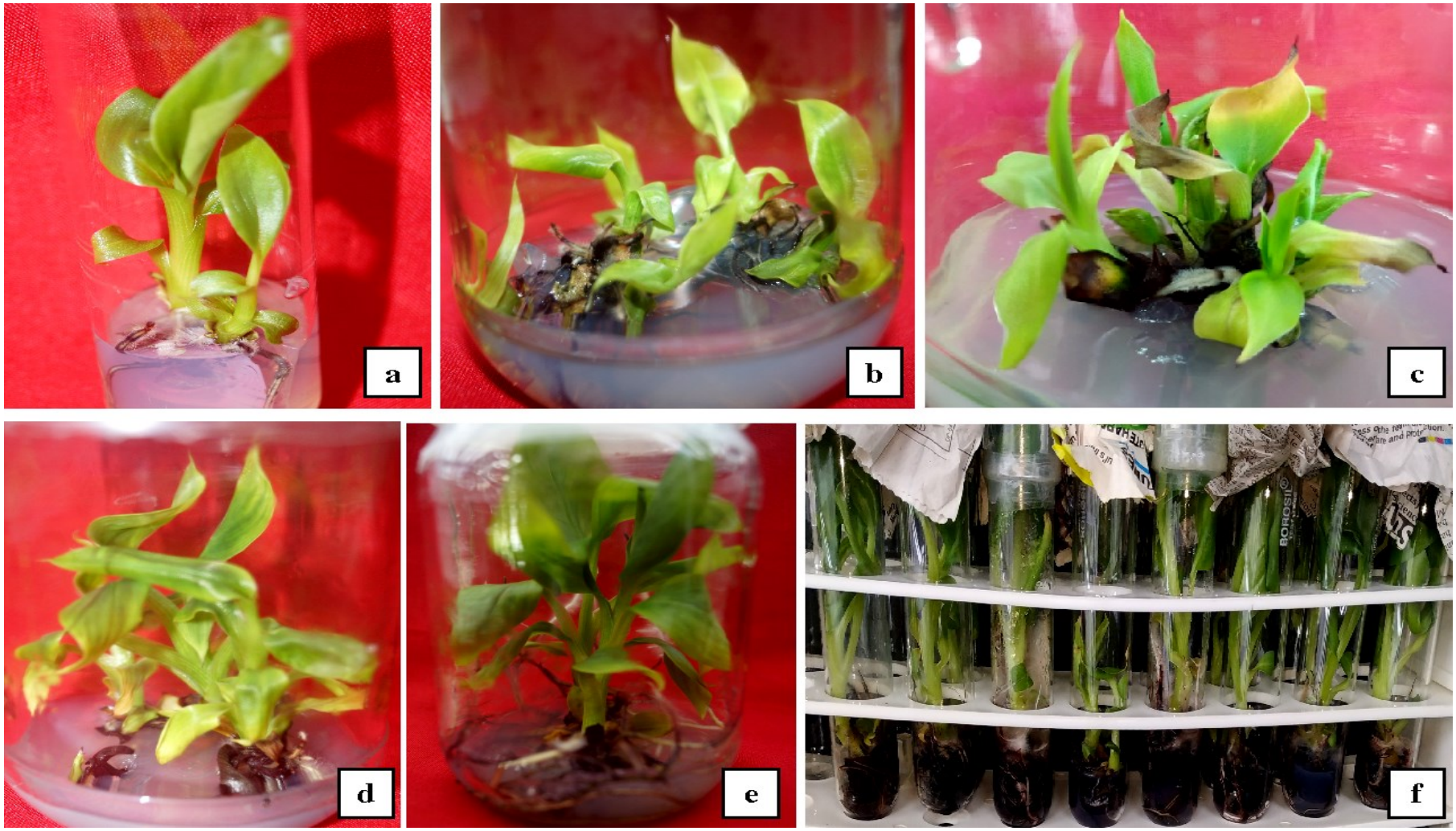


Figure 4.4: Represents different stages of plant regeneration and culture proliferation of Cv. Grand Nain. a. Regeneration plantlets with well expanded leaves; b-d. Multiple shoot development; e. Rooted regenerates; f. Regenerates under primary hardening condition.

Effect of PGRs on shoot length

Incorporating PGRs into the nutrient medium was obligatory. On PGR free medium there was no plant regeneration. When PGRs were used singly in the range 1-6.5 mg/L (BAP and KN) registered 100% morphogenic response. The KN fortified medium supported formation of taller shoots (5.58 cm) against 4.79 cm on BAP fortified medium (**Table 4.1, 4.2**); while, TDZ was not found to be promotive for multiple shoot formation as well as shoot length. On TDZ fortified medium optimum shoot length was recorded was 2.61cm (**Table 4.3**).

On BAP enriched medium, the tallest shoot length was 5.5 cm on medium contained 5.5 mg/L; on medium enriched with KN (6.5 mg/L), the average height of the shoots was 8.1 cm (**Table 4.1, 4.2**). Further, it was noted that with increase in KN concentrations, there was an increase in shoot length, but there was not much change on BAP enriched medium.

When the auxins were added in conjunction with KN and TDZ supported multiple shoot formation, however, there was no significant add on the values on BAP rich medium. The optimum response under the given conditions was registered on medium fortified with KN and NAA (6.5 mg/L KN +1.5 mg/L NAA) in combination where plant height was 12.3cm followed by 3.5 mg/L KN+1.5 g/L IAA with a shoot height of 9 cm (**Table 4.2**). The PGR TDZ exhibited a significant increase in shoot height when combined with IAA resulting shoot length of 11.25 cm at 1.5 mg/L TDZ and 1 mg/L IAA in contrast with the single treatment (**Table 4.3**). The application of TDZ at higher concentrations beyond 2mg/L registered abnormal shoot growth and shots curled downwards **(Figure 4.5**).

Effect of PGRs on the number of leaves per shoot

Quality and quantity of PGR influenced significantly the leaf development. Of the different treatments of BAP, KN and TDZ, higher number of leaved developed on medium fortified with KN (7.47 leaves) followed by BAP (6 leaves) and TDZ (2.8 leaves) (**Table 4.1, 4.2, 4.3**).

Effect of PGRs on number of roots

Number of roots per shoot varied significantly in different concentrations of BAP, KN and TDZ in the present study. The singly treatment with BAP, KN, and TDZ resulted in 100% root induction, with Kinetin supported height number of roots ranging from 8-12.5 roots per shoot followed by BAP (2.67-8.33 roots) and TDZ supported lease (1.67-3.5 roots) (**Table 4.1, 4.2, 4.3**). But, when the cytokinins were conjunct with IAA supported better root development. About 3.5-18.5 roots per shoot developed on medium conjunct with KN and IAA followed by TDZ+IAA ranging from 1 to 11 roots, and least in BAP+IAA ranging from 1.67 to 4.33 roots per culture.

Cultivar Sabri

Effect of PGRs on multiple shoots proliferation

As in the case of Cv. G9, in Cv. Sabri the morphogenic responses varied significantly along with quality and quantity of PGR investigated. From the various PGR treatments it was observed that singly BAP and KN incorporation in the range 1-6.5 mg/L supported invoking meristematic loci (**Table 4.4, 4.5**). On BAP fortified medium on an average of 6.3 shoots per explants developed. While, when BAP was conjunct with NAA resulted only 4.8 shoots. Further, KN in conjunct with IAA supported only 2.8 shoots development (**Table 4.5**).

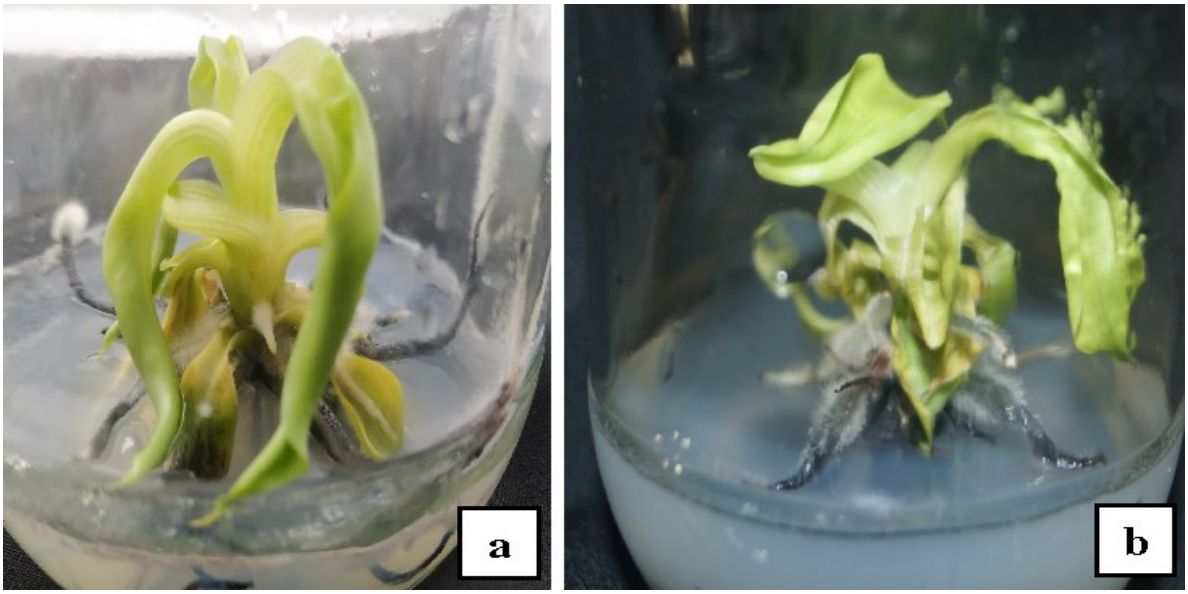


Figure 4.5: a-b. Cultures with abnormal curling of leaves at higher concentrations of TDZ in Cv. Grand Nain.

Table 4.4: Effect BAP, IAA and NAA on shoot proliferation in banana Cv. Sabri

PGR Conc. (mg/L)	No. of shoot buds invoked per segment	Length of shoots (cm)	Number of leaves per shoot	Number of roots per shoot
Control	-	-	-	-
BAP_1	7.67 ± 0.20^{c}	2.5 ± 0.17^{g}	4.0 ± 0.07^{e}	2.1 ± 0.06^{h}
$BAP_{2.5}$	5.67 ± 0.08^{g}	3.1 ± 0.19^{f}	5.5 ± 0.15^{c}	4.0 ± 0.05^{d}
$BAP_{3.5}$	8.66 ± 0.15^{a}	7.3 ± 0.25^{a}	5.0 ± 0.74^{d}	5.2 ± 0.07^{b}
$BAP_{4.5}$	6.33 ± 0.21^{f}	3.4 ± 0.16^{e}	6.1 ± 0.26^{b}	4.6 ± 0.19^{c}
$BAP_{5.5}$	6.3 ± 0.45^{f}	2.5 ± 0.05^{h}	4.1 ± 0.16^{e}	3.5 ± 0.25^{e}
$BAP_{6.5}$	3.33 ± 0.53^{j}	3.1 ± 0.07^{f}	5.1 ± 0.27^{d}	4.1 ± 0.17^{d}
BAP_1+ $IAA_{0.5}$	0.57 ± 0.01^{l}	4.1 ± 0.21^{cd}	$4.2\pm.06^{e}$	3.1 ± 0.18^{f}
$BAP_{2.5}$+ $IAA_{0.5}$	0.28 ± 0.06^{m}	2.2 ± 0.16^{h}	3.2 ± 0.26^{f}	4.6 ± 0.19^{c}
$BAP_{3.5}$+ $IAA_{0.5}$	8.1 ± 0.26^{b}	2.2 ± 0.11^{h}	3.2 ± 0.12^{f}	2.1 ± 0.16^{h}
$BAP_{4.5}$+ $IAA_{0.5}$	0.14 ± 0.02^{m}	3.7 ± 0.11^{e}	5.3 ± 0.21^{c}	4.1 ± 0.25^{d}
$BAP_{5.5}$+ $IAA_{0.5}$	7.1 ± 0.19^{d}	1.9 ± 0.13^{i}	3.2 ± 0.22^{f}	1.5 ± 0.06^{i}
$BAP_{6.5}$+ $IAA_{0.5}$	0.42 ± 0.03^{l}	2.7 ± 0.26^{g}	4.1 ± 0.13^{e}	4.1 ± 0.15^{d}
BAP_1+ $NAA_{0.5}$	4.2 ± 0.39^{i}	1.1 ± 0.08^{k}	2.0 ± 0.07^{h}	4.7 ± 0.18^{c}
$BAP_{2.5}$+ $NAA_{0.5}$	6.75 ± 0.77^{e}	1.73 ± 0.07^{i}	2.25 ± 0.07^{g}	3.1 ± 0.21^{f}
$BAP_{3.5}$+ $NAA_{0.5}$	5.38 ± 0.6^{g}	1.36 ± 0.05^{j}	2.13 ± 0.09^{g}	2.8 ± 0.09^{g}
$BAP_{4.5}$+ $NAA_{0.5}$	5.33 ± 0.63^{g}	4.5 ± 0.17^{c}	4.3 ± 0.15^{e}	2.1 ± 0.12^{h}
$BAP_{5.5}$+ $NAA_{0.5}$	4.67 ± 0.21^{h}	1.25 ± 0.16^{j}	2.1 ± 0.13^{g}	3.8 ± 0.09^{e}
$BAP_{6.5}$+ $NAA_{0.5}$	2.5 ± 0.07^{k}	1.3 ± 0.06^{j}	3.1 ± 0.18^{f}	2.55 ± 0.25^{g}

Note: Data represents the mean of three replicates ± Standard deviation from the mean. * *P* value has been calculated using one-way ANOVA and values with the same superscript letters in the column do not differ significantly ($p \leq 0.05$).

Table 4.5: Effect KN, IAA and NAA on shoot proliferation in banana Cv. Sabri

PGR Conc. (mg/L)	Number of shoot buds invoked per segment	Length of shoots (cm)	Number of leaves per shoot	Number of roots per shoot
Control	-	-	-	-
KN_1	1.33 ± 0.07^{f}	3.5 ± 0.33^{bc}	2.83 ± 0.76^{e}	3.1 ± 0.34^{d}
$KN_{2.5}$	1.1 ± 0.69^{f}	1.5 ± 0.26^{e}	1.6 ± 0.51^{f}	1.8 ± 0.23^{e}
$KN_{3.5}$	1.5 ± 0.53^{f}	4.13 ± 0.54^{b}	2.4 ± 0.22^{e}	3.66 ± 0.22^{d}
$KN_{4.5}$	4.2 ± 0.43^{c}	1.9 ± 0.61^{e}	1.8 ± 0.55^{f}	4.3 ± 0.62^{c}
$KN_{5.5}$	3.66 ± 0.09^{d}	2.33 ± 0.65^{d}	2.6 ± 0.47^{e}	4.2 ± 0.21^{c}
$KN_{6.5}$	1.2 ± 0.18^{f}	1.76 ± 0.44^{e}	1.83 ± 0.50^{f}	2.33 ± 0.28^{e}
$KN_1 + IAA_{0.5}$	0.42 ± 0.16^{g}	4.0 ± 0.8^{b}	4.0 ± 0.00^{c}	2.2 ± 0.21^{e}
$KN_{2.5} + IAA_{0.5}$	0.14 ± 0.04	2.1 ± 0.21^{d}	3.1 ± 0.26^{d}	0
$KN_{3.5} + IAA_{0.5}$	8.2 ± 0.42^{a}	2.2 ± 0.25^{d}	3.1 ± 0.26^{d}	4.2 ± 0.24^{c}
$KN_{4.5} + IAA_{0.5}$	0.44 ± 0.06^{g}	3.7 ± 0.11^{bc}	5.1 ± 0.37^{b}	3.2 ± 0.22^{d}
$KN_{5.5} + IAA_{0.5}$	7.1 ± 0.29^{b}	1.9±0.11e	3.1 ± 0.32^{d}	1.5 ± 0.16^{f}
$KN_{6.5} + IAA_{0.5}$	0.66 ± 0.03^{g}	2.7 ± 0.16^{d}	4.2 ± 0.23^{c}	4.1 ± 0.15^{c}
KN_1+ $NAA_{0.5}$	0.33 ± 0.03^{g}	4.2 ± 0.14^{b}	3.5 ± 0.45^{d}	3.4 ± 0.17^{d}
$KN_{2.5}$+ $NAA_{0.5}$	2.1 ± 0.04^{e}	3.4 ± 0.07^{bc}	4.2 ± 0.12^{c}	4.0 ± 0.05^{c}
$KN_{3.5}$+ $NAA_{0.5}$	0.5 ± 0.05^{g}	2.9 ± 0.26^{cd}	2.5 ± 0.12^{e}	5.1 ± 0.12^{b}
$KN_{4.5}$+ $NAA_{0.5}$	0.16 ± 0.02^{h}	3.1 ± 0.37^{bc}	3.2 ± 0.14^{d}	7.2 ± 0.36^{a}
$KN_{5.5}$+ $NAA_{0.5}$	0.17 ± 0.01^{h}	4.1 ± 0.38^{b}	3.7 ± 0.24^{d}	3.5 ± 0.16^{d}
$KN_{6.5}$+ $NAA_{0.5}$	2.16 ± 0.17^{e}	3.3 ± 0.37^{bc}	4.5 ± 0.32^{c}	5.2 ± 0.25^{b}

Note: Data represents the mean of three replicates ± Standard deviation from the mean. * *P* value has been calculated using one-way ANOVA and values with the same superscript letters in the column do not differ significantly ($p \leq 0.05$).

The influence of BAP and NAA on the explant during the initiation phase resulted in a globular-shaped explant, from which tiny globular shoots emerged **(Figure 4.4a**). Most shoots resulted on medium fortified with BAP (3.5 mg/L) where as many as 8.66 shoots developed. Further, it was recorded that with increase in concentration of BAP beyond 3.5 mg/L, there was a declining **(Table 4.4)**. The combination of BAP with IAA and NAA individually at concentrations of 3.5 mg/L BAP + 0.5 mg/L IAA and 2.5 mg/L BAP + 0.5 mg/L NAA produced 8.1 and 6.75 shoots, respectively, a noticeable decrease in a number of shoots was observed in the lowest and highest concentrations (**Table 4.4**).

When the data recorded from the KN treatments, it was observed that medium fortified with 4.5 mg/L resulted 4.2 shoots followed by 3.66 shoots on medium containing 3.5 mg/L. The resulted shoots were comparatively smaller in size. The combined treatments of KN with IAA and NAA separately exhibited better morphogenic response against singly treatment of KN. Under the given conditions, KN (3.5 mg/L) and IAA (0.5 mg/L) supported 8.2 shoots against 2.16 shoots on KN (6.5 mg/L) and NAA (0.5 mg/L) fortified medium (**Table 4.5, Figure 4.4**).

Effect of PGRs on shoot length

The single PGR application of BAP and KN in the concentration ranged from 1 to 6.5 mg/L supported 100% response in the subcultures. Among the two PGR tested, BAP supported optimum response under the given conditions where the shoot length was 7.3 cm against 2.16 cm in KN under otherwise identical conditions. Among the combination treatments, better plant height (4.1 cm) was registered on medium fortified with BAP and IAA (1mg/L and 0.5mg/L respectively).

Effect of PGRs on formation of leaves

For development of leaves BAP was found to be a better choice over KN. Medium with 4.5 mg/L BAP supported 6.1 leaves per regenerate. However, when BAP was used in combination with IAA or NAA found to be not supportive for lead development as number of leaves declined (**Table 4.4**).

Effect of PGRs on number of roots

Similar to plant height, BAP was also found to be better choice for root formation against KN. There were 5.2 roots per shoot on BAP (3.5 mg/L) treated culture against 3.23 roots on KN (4.5 mg/L) enriched medium (**Table 4.5**). Additionally, the combination of auxins (IAA and NAA) with BAP resulted 100% response; however, KN with auxins supported only 83.3% root induction. Highest response from BAP with IAA and NAA were obtained at concentrations 3.5 mg/L BAP +0.5 mg/L IAA resulting in 4.6 roots, while 4.5 mg/L BAP + 0.5 mg/L NAA yielded 4.7 roots (**Table 4.4**).

Hardening and field establishment

The proliferated shoots obtained from the cultures were transferred to the medium enriched with the nutrients as mentioned earlier, and the matured plantlets were obtained from the cultures and subsequently placed in cocopeat hardening media.

The PGRs that yielded the optimum results in the overall development of plantlets (shoot height, leaves and roots) are consequently utilized in the plantlet's comprehensive growth and maturation.

The PGRs (Plant Growth Regulators) and their concentrations that yielded the optimum response in G9 are as follows:

- BAP with NAA - 1 mg/L BAP + 1-1.5 mg/L NAA, 2.5 mg/L BAP + 1.5 mg/L NAA, 3.5 mg/L BAP+ 0.5-1.5 mg/L NAA, 4.5 mg/L BAP + 0.5-1.5 mg/L NAA, 5.5 mg/L BAP + 0.5-1.5 mg/L NAA and 6.5 mg/L BAP + 1-1.5 mg/L NAA.
- KN- Applied singly 1 to 6.5 mg/L.
- KN with IAA- 1 mg/L KN + 1-1.5mg/L IAA, 2.5 mg/L KN + 1.5 mg/L IAA, 3.5 mg/L KN+ 0.5-1.5 mg/L IAA, 4.5 mg/L KN+ 0.5-1 mg/L IAA, 5.5 mg/L KN+ 1-1.5 mg/L IAA, 6.5 mg/L KN+ 1-1.5 mg/L IAA.
- KN with NAA- 1 mg/L KN+ 0.5-1.5 mg/L NAA, 2.5 mg/L KN+ 0.5-1.5 mg/L NAA, 3.5 mg/L KN+ 0.5-1 mg/L NAA, 4.5 mg/L KN+ 1-1.5 mg/L NAA, 5.5 mg/L KN+ 0.5-1 mg/L NAA, 6.5 mg/L KN + 1.5 mg/L NAA.
- TDZ with IAA 0.5 mg/L TDZ + 0.5-1.5 mg/L IAA, 1 mg/L TDZ + 1.5 mg/L IAA and 1.5 mg/L TDZ + 0.5 mg/LIAA.

Whereas in Sabri,

- BAP single application - 2.5 to 6.5 mg/L
- BAP with IAA - 4.5 mg/L BAP + 0.5mg/L IAA
- KN single application - 3.5 mg/L
- KN with IAA - 4.5 mg/L KN + 0.5 mg/L IAA
- KN with NAA - 1mg/L KN +0.5 mg/L NAA, 2.5 mg/L KN + 0.5 mg/L NAA, 4.5 mg/L KN + 0.5 mg/L NAA, 5.5 mg/L KN + 0.6 mg/L NAA, 6.5 mg/L KN + 0.5 mg/L NAA.

After their growth and development, the mature plantlets were transferred to a primary hardening phase using cocopeat as the potting medium. The selected concentrations acclimatized successfully with cocopeat (without any external nutrients), showing excellent results and proving to be an effective substrate (**Figure 4.6 c, d**).

During primary hardening, a 100% survival rate was recorded across all PGR treatments (**Figure 4.6 a, d**). Cocopeat provided optimal conditions for growth, with shoot elongation averaging 9.75 cm per plantlet. The average number of leaves per plantlet was

6.05; while, rooting exhibited significant growth, with an average of 15.34 roots per plantlet. The roots grew profusely, indicating strong overall development.

To ensure proper acclimatization and maturity, the plantlets underwent secondary hardening in soil, preparing them for successful field establishment (**Fig. 4.7 a, b**). During this phase, the plantlets were transplanted into soil, achieving a survival rate of 96%. Following 3 to 4 weeks of acclimatization in the greenhouse, the hardened plantlets were transferred to the field, where they exhibited a survival rate of approximately 90% (**Fig. 4.7 c, d, Figure 4.8**).

Genetic fidelity assessment

The genetic fidelity assessment of regenerates of both the cultivars was evaluated by comparing the donor/mother plants (M) and *in vitro* micro propagated regenerated plantlets. The study used two markers, RAPD and ISSR, with a preliminary screening on 25 primers, resulting in the selection of 14 primers, seven from each marker. This study used nine randomly selected *in vitro* regenerated plantlets (R_1–R_9) alongside one mother plant (M) in both G9 and Sabri. The investigation revealed a significant degree of monomorphism, with G9 *in vitro* plantlets exhibiting monomorphism rates of 96.08% in RAPD and 96.55% in ISSR primers, yielding a total of 80 scorable bands and an average of 5.71 bands per primer (**Table 4.6, Table 4.7**). The highest number of bands in RAPD and ISSR was recorded by OPA-12 and ISSR-816, yielding 11 and 7 scorable bands, respectively. Polymorphism was seen in OPA-11 (1) and OPA-15 (1) for RAPD and in ISSR-816 (1) for ISSR (**Table 4.6, Table 4.7 and Figure 4.9 a-d**).

The genetic fidelity evaluation in Sabri revealed 100% monomorphism in ISSR and 97.92% in RAPD, with an average of 5.57 scorable bands and an overall total of 78 bands (**Table 4.8, Table 4.9**). The highest number of scorable bands was achieved in OPA-11 and ISSR-816, yielding 12 and 8 scorable bands respectively, while polymorphism was observed solely in RAPD for OPD-20 (1) (**Table 4.8, Table 4.9, Figure 4.9 e-h**). Among the two genetic markers, the RAPD markers yielded the highest scorable bands, with G9 producing 51 bands and Sabri 48 bands, indicating that G9 generated a greater number of bands than Sabri. Furthermore, the percentage of monomorphisms. The investigation produced a total of 158 bands, comprising 99 bands from RAPD and 59 bands from ISSR, with amplified bands ranging from 300 to 1500 base pairs.

Figure 4.6: Primary hardened regenerates ready for transplantation. a. Grand Nain; b. Sabri; c. G9 regenerates transplanted in cocopeat under secondary acclimatization; d. Sabri regenerates transplanted in cocopeat under secondary acclimatization.

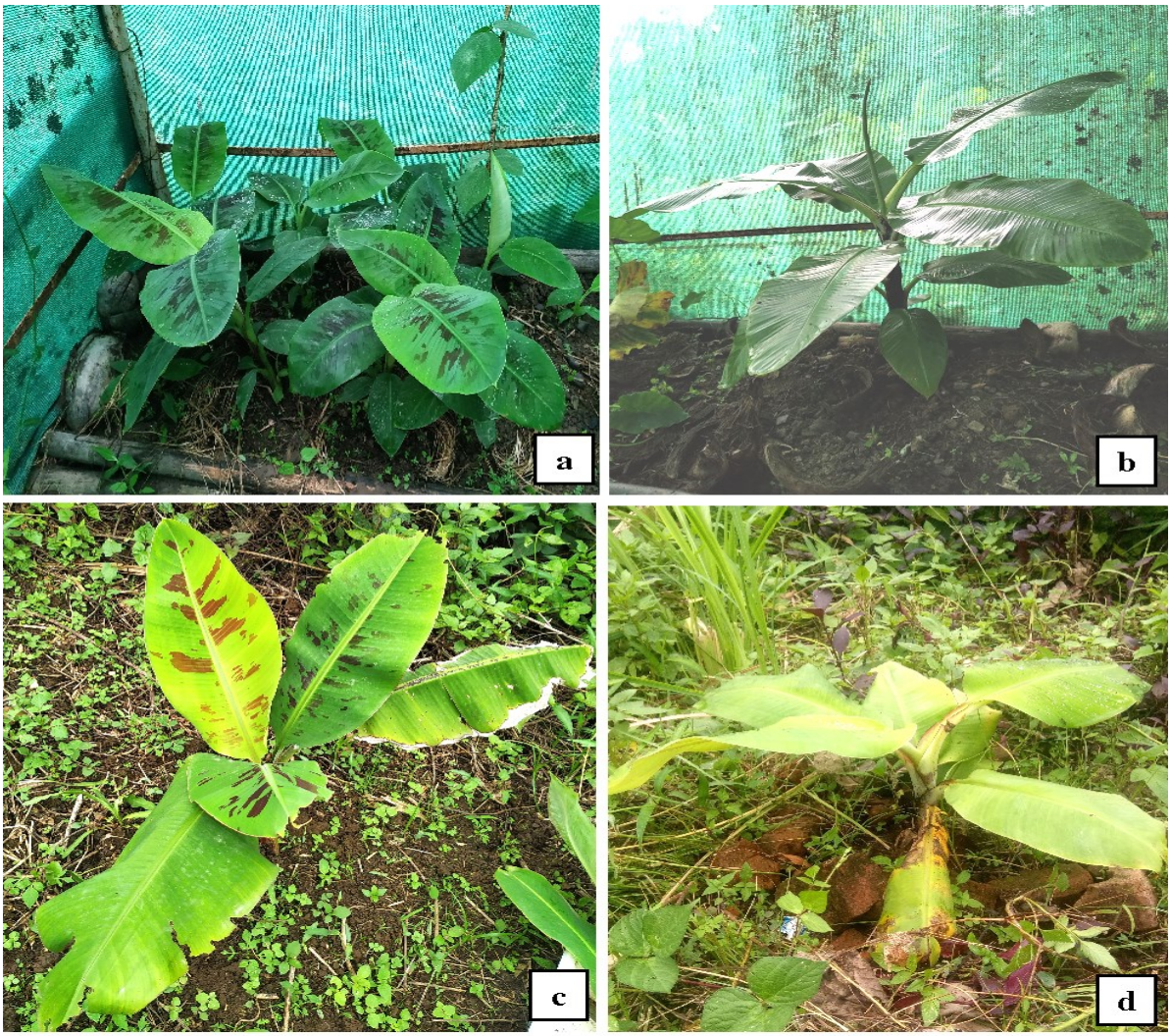


Figure 4.7: Acclimatization and filed established regenerates of G9 and Sabri. a. Acclimatized regenerates of Cv. Grand Nain maintained in polyhouse; b. Acclimatized regenerates of Cv. Sabri maintained in polyhouse; c. Field established transplant of Cv. Grand Nain; d. Field established transplant of Cv. Sabri.

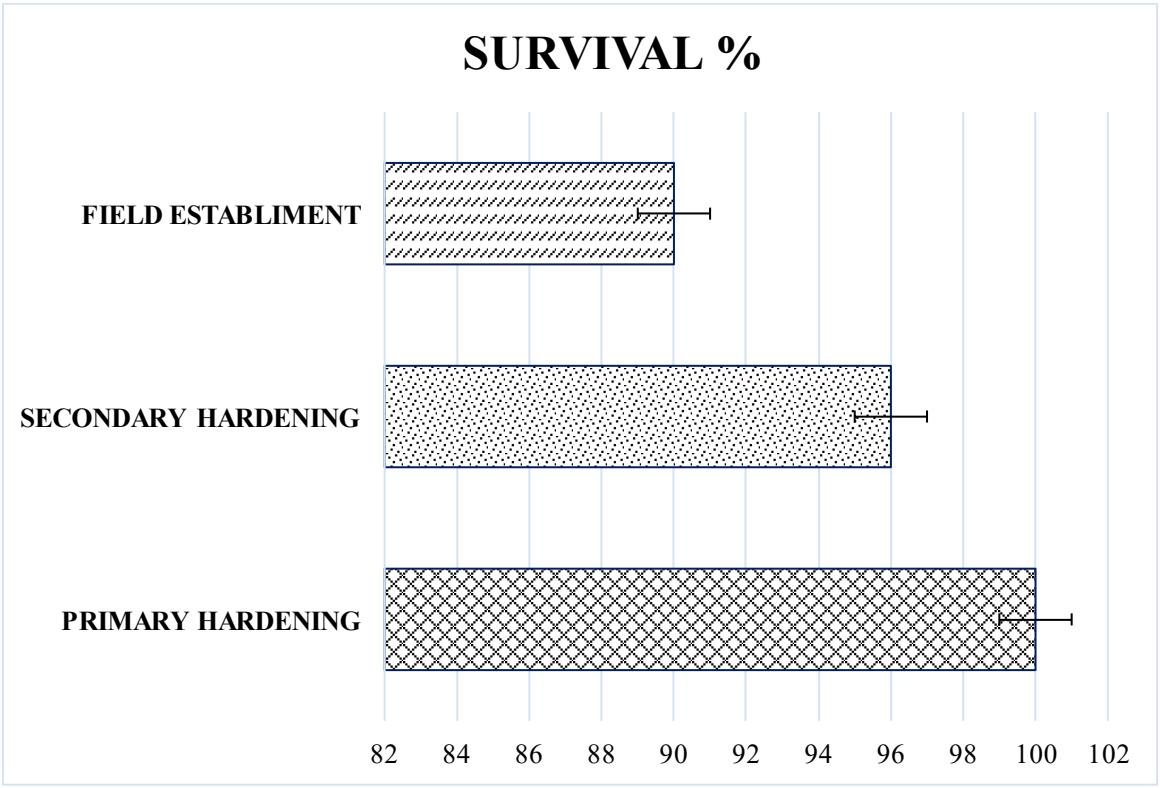


Figure 4.8: Survival performance of regenerates in different stages of transplantation

Table 4.6: RAPD and ISSR primers data analysis for evaluation of genetic stability of regenerated plants in Cv. Grand Nain

Sl. No.	Primer code	Primer sequence (5′-3′)	Total bands	Monomorphic bands (mp)	% of mp	Polymorphic bands (pb)	% of pb	Amplicon length (bp)
RAPD primers								
1	OPA-04	AATCGGGCTG	6	6	100	-	-	500-1500
2	OPA-11	CAATCGCCGT	10	9	90	1	10	400-900
3	OPA-12	TCGGCGATAG	11	11	100	-	-	300-1000
4	OPA-13	CAGCACCCAC	4	4	100	-	-	400-1500
5	OPA-15	TTCCGAACCC	8	8	100	1	12.5	500-1500
6	OPD-20	ACCCGGTCAC	8	7	87.5	-	-	500-1500
7	OPK-04	CCGCCCAAAC3	6	6	100	-	-	400-1500
		Total	**51**	**49**	**96.08**	**2**	**3.92**	-
ISSR primers								
1	ISSR-05	GGAGAGGAGAGGAGA	4	4	100	-	-	300-900
2	ISSR-08	CTCTCTCTCTCTCTCTG	3	3	100	-	-	300-1000
3	ISSR-12	AGAGAGAGAGAGAGAGY	3	3	100	-	-	400-900
4	ISSR-18	GATCATCATCATCATCATC	5	4	80	1	20	400-1000
5	ISSR-816	CACACACACACACACAT	7	7	100	-	-	500-1500
6	ISSR-841	GAGAGAGAGAGAGAGAYG	3	3	100	-	-	500-800
7	ISSR-857	ACACACACACACACACA	4	4	100	-	-	500-1500
		Total	**29**	**28**	**96.55**	**1**	**3.45**	-

Table 4.7: Comparative and combined presentation of RAPD and ISSR primers results in Cv. Grand Nain

Marker	No. of primers	No. of bands	Average band per primer	Percent (%)		Genetic stability (%)
				Monomorphism	Polymorphism	
RAPD	7	51	7.28	96.08	3.92	96.08
ISSR	7	29	4.14	96.55	3.45	96.55
RAPD + ISSR	14	80	5.71	96.25	3.75	96.25

Table 4.8: RAPD and ISSR primers data analysis for evaluation of genetic stability of regenerated plants in Cv. Sabri

RAPD primers								
Sl. No.	**Primer code**	**Primer sequence (5′-3′)**	**No. of bands**	**Monomorphic bands (mp)**	**% of mp**	**Poly morphic bands (pb)**	**% of pb**	**Amplicon length (bp)**
1	OPA-04	AATCGGGCTG	9	9	100	-	-	500-1500
2	OPA-11	CAATCGCCGT	12	12	100	-	-	400-1000
3	OPA-12	TCGGCGATAG	8	8	100	-	-	300-900
4	OPA-13	CAGCACCCAC	4	4	100	-	-	400-1500
5	OPA-15	TTCCGAACCC	4	4	100	-	-	500-1500
6	OPD-20	ACCCGGTCAC	6	5	83.33	1	16.66	500-1500
7	OPK-04	CCGCCCAAAC3	5	5	100	-	-	400-1500
		Total	48	47	97.92	1	2.08	-
ISSR primers								
1	ISSR-05	GGAGAGGAGAGGAGA	3	3	100	-	-	300-1000
2	ISSR-08	CTCTCTCTCTCTCTCTG	3	3	100	-	-	300-1000
3	ISSR-12	AGAGAGAGAGAGAGAGY	3	3	100	-	-	400-900
4	ISSR-18	GATCATCATCATCATCATC	5	5	100	-	-	400-1000
5	ISSR-816	CACACACACACACACAT	8	8	100	--	-	500-1500
6	ISSR-841	GAGAGAGAGAGAGAGAYG	4	4	100	--	-	400-900
7	ISSR-857	ACACACACACACACACA	4	4	100	-	-	500-1500
		Total	**30**	**30**	**100**	**-**	**-**	**-**

Table 4.9: Comparative and combined presentation of RAPD and ISSR primers results in Cv. Sabri

Markers	No of primers	No. of bands	Average band per primer	Percent (%)		Genetic stability (%)
				Monomorphism	Polymorphism	
RAPD	7	48	6.86	97.92	2.08	97.92
ISSR	7	30	4.29	100	-	100
RAPD + ISSR	14	78	5.57	98.72	1.28	98.72

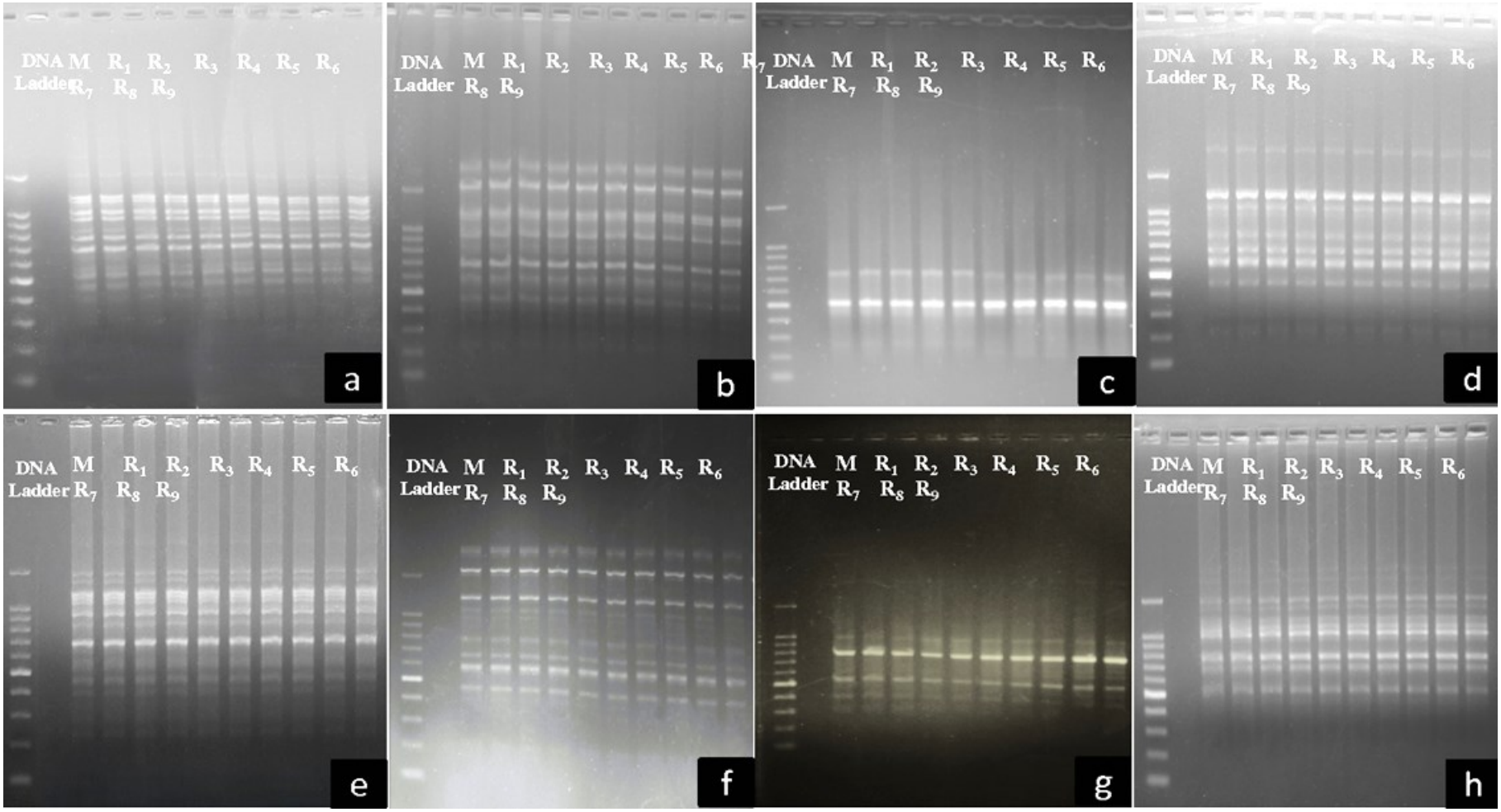


Figure 4.9: RAPD and ISSR markers agarose profiles of mother plant (M) and tissue culture raised plantlets (R_1 to R_9 lanes) of Cv. G9 and Cv. Sabri. a-d. Cv. Grand Nain, e-h. Cv. Sabri. a. RAPD primer OPA- 12; b. RAPD primer OPA-15; c) ISSR 18 primer; d. ISSR 816 primer; e. RAPD primer OPA-11; f. RAPD primer OPA-04; g. ISSR 841 primer; h. ISSR 816 primer.

Discussion

Cytokinin commonly diminishes the dominance of apical meristems and stimulates the development of axillary and adventitious shoots from meristematic explants. In addition to genetic influence, shoot proliferation and elongation were impacted by cytokinins and their concentrations (Smitha et al., 2014).

The prevalent issue in the *in vitro* culture of banana explants is the uncontrolled blackening of tissues caused by the exudation of polyphenolic compounds (secondary metabolites), which creates a barrier between exposed and damaged tissues. This barrier induces oxidation, impedes nutrient absorption, and obstructs the initiation of growth and development in explants, ultimately resulting in mortality (Ngomuo et al., 2014; Safwat et al., 2015; Kishor et al., 2017). The blackening of tissues is frequently mitigated by the constant subculturing of explants in fresh media during the initial 2 to 6 weeks; therefore, applying antioxidants is advantageous in reducing blackening tissues (Kishor et al., 2017).

Initiation response in shoot development in the two cultivars

Establishing aseptic cultures in bananas presents a significant challenge, as the explants from the soil carry a considerable contamination risk. Additionally, the elevated phenolic exudates released from the cut tissues of bananas contribute to this issue. Saha-Roy et al. (2010) reported contamination rates as high as 50% and as low as 20% during September to October months.

The efficacy of *in vitro* growth of plant material was greatly affected by the stage of development of the tissue or organ utilized as explants (Smitha et al., 2014). The response of explants in shooting is affected not only by the application of PGRs but also greatly by the initial size of the explant. Initial explants measuring 0.5 cm with 3 to 5 leaf primordia producing 2 to 3 times more shoots, as noted by Strosse et al. (2008), in comparison to 0.1 cm explants with a single leaf primordium.

The preliminary reaction of cultures was initially noted through the swelling and greening of the explants, which were deemed good responses, but explants that became brown or black or failed to swell did not indicate a positive response (Prabhuling and Sathyanarayana, 2017). All the positive cultures in the initiation media responded with single elongated shoots as their initial response (Strosse et al., 2008). Reddy et al. (2014) reported an outstanding explant response of 95% in a medium enriched with 2

mg/L BAP, closely resembling the findings of the current investigation. The explants exhibited a colour change from green to dark green in both G9 and Sabri, concluding that this variation was dependent on time, PGR content, and explant type (Hossain et al., 2016). Direct shoot regeneration was found in this study in response to several plant growth regulators (PGRs), their concentrations, and combinations, as validated by Paulos et al. (2013). The findings of the present investigation regarding optimal initiation response were further supported by Hossain et al. (2016) in both G9 and Sabri cultivars, where the highest shoot induction occurred at BAP 5 mg/L.

The study conducted by Dahari et al. (2017) indicated that decreased levels of BAP led to early shoot initiation, but higher concentrations caused delayed shoot emergence, aligning with the findings of the current study. The initiated cultures after subculturing obtained shoot growth responses, where the new shoots were derived from the base of the initial shoot, resulting in a multitude of shoots. Thereafter the multiple shoots are separated and subsequently transplanted into new media sustaining shoot multiplication throughout regular subculturing (Ali et al., 2011). The continuous application of the same concentration for two generations is employed for shoot multiplication. Strosse et al. (2008) reported lethal blackening associated with bigger explants sizes (shoot tips). The elevation of concentration promotes shoot growth and induces a mutagenic response in the cultures (Dahari et al., 2017).

The control treatment exhibited no response in shoot multiplication, responding only in terms of shoot height, potentially due to its endogenous plant hormones influencing this trait. Studies by Paulos et al. (2013), Uzaribara et al. (2015) and Sahoo et al. (2015) reported lowest values in the MS media without PGR (control) amongst the other culture media supplemented with PGRs. In the present study the response from PGR BAP achieved 100% shoot multiplication in comparison to PGR Kinetin when administered alone, was validated by study conducted by Ali et al. (2011).

The study by Paulos et al. (2013) and Hossain et al. (2016) reported the highest shoot proliferation response at 5 mg/L BAP in G9, yielding 2.6 and 3.5 shoots, respectively, which is comparable to the current study, obtaining similar concentration range of 4.5 to 5.5 mg/L, resulting in 2.33 to 3 shoots. However, the present study achieved a superior response, with 8.67 shoots at 3.5 mg/L BAP. Sazdur et al. (2013) reported a maximum response of 5.9 shoot buds in cv. Agnishwar at a concentration of 4 mg/L BAP, which supported the present study findings in optimum response in same

concentration; however, the present findings multiple shoots response achieved more shoots of 8.67 shoots in comparison. The study conducted by Uzaribara et al. (2015) indicated that the optimal concentration for shoot proliferation was 4 mg/L BAP combined with 1 mg/L IAA, which closely aligns with the present study's findings of 3.5 mg/L BAP and 1 to 1.5 mg/L IAA. However, their observed response of 8.8 shoots surpassed the current study's results of 6.5 to 7.5 shoots.

The rise of BAP concentration reduces shoot proliferation, and the combination of NAA with BAP did not further enhance shoot proliferation in the present study, is supported in the study conducted by Ali et al. (2011). The research conducted by Prabhuling and Sathyanarayana (2017), which utilized 2 mg/L BAP in agar medium, resulted in increased multiple shoot formation and reduced shoot height and leaf count compared to the current study. The minimum shoot number recorded was 1.2 shoots in 1 mg/L BAP according to Hossain et al. (2016); however, the current investigation observed the lowest at 2.5 mg/L BAP but with a similar number of shoots (1.5 shoots). Studies conducted by Paulos et al. (2013) and Dahari et al. (2017) reported a maximum response of 3 and 4.64 shoots at 5 mg/l BAP and 2 mg/L BAP, which contradicts our findings on optimum response. However, their highest values obtained were much lower than the current study's findings of 8.67 shoots at 3.5 mg/L BAP. Similarly, the findings of Sahoo et al. (2015) in the cultivar Grand Nain in MS medium supplemented with 5.0 mg/l BAP and 1.0 mg/l IAA greatly enhanced the number of numerous shoots per explant (4.47), contradicting the optimum response (3.5 mg/L BAP with 1.5mg/L IAA) in the present findings as well as obtaining significantly lower values than the present findings of 7.5 shoots per explants.

In the present study findings TDZ could not outperformed other cytokinin in respect to multiple shootings both in single application as well as in combination. However, studies by Strosse et al. (2008) and Smitha et al. (2014) indicated that TDZ was the most effective PGR to stimulate multiple shoot formation among other PGRs. Strosse et al. (2008), in their study demonstrated the strength and efficacy of TDZ at lower doses (10 μM), yielding a greater number of plantlets than BAP at higher concentrations while kinetin displayed the least efficacy amongst them. However, in the current study, BAP obtained the best response overall, and also kinetin generated better results in shoot multiplication compared to TDZ. This distinction may be attributed to the broader concentration range employed for kinetin (1 to 6.5 mg/L) versus the

restricted concentration range for TDZ (0.5 to 1.5 mg/L), which may not have reached the optimal response concentration. With the increase in the concentration of TDZ, the extent of abnormal shoot proliferation is detected; therefore, TDZ should be utilized at low doses due to its high cytokinin activity even at minimal concentrations (Shirani et al., 2009; Roy et al., 2010).

The incorporation of cytokinin in the nutrient medium significantly affected both the regeneration response as well as the means of regeneration greatly, emphasizing its critical role in the shoot proliferation of bananas, moreover, the use of BAP is considered the best for shoot proliferation (Ali et al., 2011; Smitha et al 2014; Uzaribara et al., 2015). The study conducted by Uzaribara et al., 2015, demonstrated the variations between the two cytokinin KN and BAP with BAP inducing double times more shoots than KN when used singly in the same concentration. Several workers observed multiple shoot development in medium enriched with lower amounts of BAP and IAA in diploid cultivars Suman et al. (2013). The PGR BAP exhibited superior performance in shoot multiplication relative to other cytokinin in this investigation, supported by findings from other researchers Ali et al. (2011).

The present investigation achieved the maximum shoot height of 5.5 cm in single application of BAP at 1 and 5.5 mg/L concentrations, was found to be comparable to the findings of Paulos et al. (2013). Additionally, the findings of Sahoo et al. (2015) with 5 mg/l BAP and 1 mg/l IAA yielded comparable response in plant height with the present study. Rahman et al. (2013) reported a maximum height of 4.9 cm at a dose of 5 mg/L, which was equivalent to the 4.56 cm recorded at the same concentration. However, contrasting the optimal maximum plant height in the present study was obtained in 2.5 and 6.5 mg/L BAP with 5.5cm.

The outcomes of BAP with NAA at 4.5 mg/L and 1 mg/L were comparable to those reported by Paulos et al. (2013) using 5 mg/L BAP and 2 mg/L NAA in terms of shoot length, while the concentrations varied slightly in the present study. The maximum recorded in our study is achieved with BAP+NAA (3.5 mg/L and NAA 1 mg/L), measuring 9 cm, comparable to 4.5 mg/L BAP, which ranges from 5.3 to 5.63 cm.

A study conducted by Uzaribara et al. (2015) reported the maximum shoot height of 4.36 cm at 4 mg/L BAP combined with 1 mg/L IAA, which is lesser to the shoot height of 5.33 cm observed in the present study using 3.5 mg/L BAP and 0.5

mg/L IAA. Additionally, Dahari et al. (2017) reported the highest shoot height of 5.99 cm at 2 mg/L BAP, surpassing the maximum height of 5.5 cm seen in the current study at 1 and 5.5 mg/L.

The present study findings showed increased shoot length in the PGR KN than in BAP and TDZ in G9, which was supported by the findings of Wong (1986). However, contrary to our findings Uzaribara et al., 2015, reported increased shoot length in BAP than in KN. Reddy et al. (2014), reported a response that differed from the current investigation, which showed a decrease in shoot lengths with an increase in the concentration of BAP. The combination of TDZ and IAA resulted in superior shoot elongation compared to TDZ alone, as evidenced by the research conducted by Gübbük and Pekmezc (2004).

The application of BAP at 3.5 mg/L yielded the highest response compared to other concentrations, as agreed by the findings of Dahari et al. (2017). Nevertheless, the current investigation revealed an increased number of leaves compared to their study, which reported 3.25 leaves. Similarly, the maximum of leaves was achieved with the combination of PGRs at 1.5 mg/L NAA and 1 mg/L BAP, as confirmed by Paulos et al. (2013). Meanwhile the number of leaves recorded in their study (3.4) was significantly lower than the current results (7 leaves).

Paulos et al. (2013) and Hossain et al. (2016) found that the highest number of leaves were obtained in a single application of BAP at 5.0 mg/L, with 4.4 and 5.5 leaves, respectively, which opposed the current findings' optimum response (3.5 mg/L). However, the current study's findings yielded a much higher number of leaves (8.33 leaves) than previous studies.

Hossain et al. (2016) in their study found that PGR NAA at 4mg/L produced the highest response (5.11 roots) in root formation, which was comparable to the values obtained (4.67 roots) from the current study's NAA values; however, in the current study, the concentration used was 1.5 mg/L along with various cytokinin concentrations. The investigation conducted by Dahari et al. (2017) reported a maximum of 2.48 roots at 1.5 mg/L IBA, which is considerably lower than the maximum number of roots achieved in the present investigation using 0.5 mg/L NAA combined with 3.5 mg/L BAP achieving 18.5 roots.

From the findings of the present investigation, it was noted that IAA performed more effectively than NAA in root development. The study conducted by Uzaribara et

al. (2015) stated that NAA (1 µM) was more effective than IAA, which contradicted the present study findings. Saha-Roy et al. (2010) noted, contrary to our findings, that variations in KN and NAA concentrations during rooting did not enhance root multiplication; however, the current study found that the application of NAA effectively induced root multiplication, with a lower dose proving more efficacious than a higher dose. Gübbük and Pekmezc (2004) and Prabhuling et al. (2017) determined that NAA was superior in root induction when compared to other auxins, aligning with the present investigation.

Cultivar Sabri

The cultured shoot tip response in supplied BAP concentration of BAP + NAA obtained a green-coloured swelled globular meristematic shoot with firm coating, which later produced lateral globular shoots, were also observed and reported by many studies (Al-Amin et al., 2009; Khatun et al., 2017).

The PGR BAP performs a critical role in bananas, helping to induce shoot proliferation in the diverse banana genotypes Khan et al. (2021). The findings of the current investigation demonstrated a progressive increase in value in shoot proliferation followed by a declining trend as the concentration increased, which was validated by the findings by Ferdous et al. (2015) in the single application of BAP (1 to 7 mg/L). In BAP, Ali et al. (2011) exhibited the highest response at 1.5mg/L in BAP with 5.8 shoots which is comparable to 2.5 mg/L in the current study. Habib et al. (2016) in their study, noted the maximum shoot responses at 3.0 mg/L BAP in liquid media, in line with the current investigation; however, the present study recorded a higher number of shoots, totaling 8.66, in contrast to 4 shoots seen previously. Additionally, the combination of 2 mg/L BAP with 0.25 mg/L NAA yielded the optimal response of 4.2 nos similar to the present study findings, which were obtained by combining of 1 mg/L BAP and 0.5 mg/L NAA. Rehana et al. (2009) also exhibited the highest shoot proliferation at 4mg/L in BAP among the concentrations (2 to 8 mg/L), with a decline noted at the highest concentration backing the current findings. In contrast, Ferdous et al. 2015 reported a significant gap in values (0 to 2 nos) in BAP 0 to 7mg/L compared to the present study (0 to 8.66 nos) at (0 to 6.5 mg/L BAP). The previous studies done by (Rehana et al., 2009; Hossain et al., 2016; Khatun et al., 2017) reported also less differences, ranging from 1 to 3 nos when compared to the current findings. Shalini (2019) observed a decline in the proliferation of shoots in the higher concentrations of

BAP and NAA, which is consistent with the observations of the present study. Kalimutha et al. (2007) observed the highest response at 3 mg/L BAP with 0.2 mg/L NAA, which is similar to the present study, which utilized concentrations ranging from 2.5 to 3.5 mg/L BAP with 0.5 mg/L NAA. However, the maximum number of shoots produced was 5 shoots per explant, which is comparable to the results obtained in the present study, which ranged from 5.38 to 6.75 shoots. The study by Al Amin et al. (2009) contradicts its findings with the current study about shoot proliferation at higher concentrations of BAP combined with NAA at 7.5 and 1.5 mg/L. Huq et al. (2012) achieved a higher count of shoots (10 nos) using a combination of 4 mg/L BAP, 2 mg/IAA and 13% coconut water, surpassing the results of the present findings that involve combination treatment (BAP and IAA). Additionally, in alignment with the present study, Khatun et al. (2017) reported maximum shoots from a single application of BAP. Moreover, the lowest and highest concentrations obtained less shoot height in BAP along with other combinations of IAA.

A study by Ferdous et al. (2015) reported findings resembling the present observations, indicating that the lowest and highest values exhibited reduced height relative to the intermediate concentration range, achieving a shoot height of 2.16 cm at 5 mg/L BAP, consistent with the present study's results. The investigations carried out by Azad and Amin (2001) and Hossain et al. (2016) obtained values of shoot height that were observed to be significantly lower in comparison to the findings of the present study. According to Ali et al. (2011) in their study, peak values of 3.4 cm were recorded at 0.5 and 1.5 mg/L BAP, while 3 cm was reported at 2 mg BAP with 0.25 mg NAA. These values contradict the present study findings and surpass the findings obtained.

Hossain et al. (2016) reported a similar result to the present study with 3.5 numbers of leaves in BAP 5mg/l which was similar to the present study findings. Furthermore, Ferdous et al. (2015) obtained a maximum number of leaves at 5mg/Lin BAP which aligns with the present findings in BAP concentration of 4.5 mg/L.

The research conducted by Anbazhagan et al. (2014) yielded optimal results with BAP at 3 mg/L and 0.5 mg/L IAA in the proliferation of roots validating the current study’s findings in BAP with IAA. However, Khan et al. (2021) found a maximum of 4.67 roots in BAP 5mg/L and IAA 0.5mg/L, which is lower than the findings of the present investigation, which contrast the findings of the present study.

Furthermore, contrary to the present study findings, a study conducted by Huq et al. (2012) reported that the single application of BAP at concentrations ranging from 1 to 5 mg/L failed to generate any type of response from the cultures.

The influence of plant growth regulators on the study significantly affected its growth and development in both positive and negative ways. Cytokinin is recognized for its ability to suppress apical dominance, whereas auxin plays a crucial role in inducing apical dominance and promoting lateral root growth. Nonetheless, the interaction between these two growth hormones has yielded both beneficial outcomes (such as multiple plantlets) and adverse effects (including growth retardation or a lack of multiple shoots), particularly regarding plant growth, as their functions often counteract one another Kindimba and Msogoya (2014).

The differences in the outcomes between the PGRs and their combinations can be attributed to differing translocation and uptake rates to meristematic regions, as well as metabolic processes that may cause disintegration or combination with sugars or amino acids, resulting in biologically inactive compounds (Uzaribara et al., 2015).

Genetic homogeneity of regenerated plantlets

Many studies were done on many markers out of which RAPD and ISSR have been said to offer advantages in terms of affordability, simple skills, and reliability. RAPD is said to amplify by binding randomly on any part of the DNA segment and ISSR amplifies by binding between simple sequences repeats. The use of these two markers enhances the amplification in many regions and allows better possibility in the observation of genetic mutations in the regenerates (Das et al., 2009). RAPD is said to be efficient for cultivar identification, due to its similar banding type for specific genotypes (Das et al., 2009). Genetic fidelity studies by Choudhary et al. 2015 in RAPD and ISSR obtained good results of 100% genetic homogeneity which differs from our study of about 1.28-3.75% but is negligible due to its lower variability. The present study showed 96.25 to 98.72 % genetic stability is supported by the study conducted by Borse et al. (2011). The study by Uma et al. (2023) also exhibited high genetic stability in their *in vitro* propagation protocol in bananas. The research findings have indicated that the propagation strategies are reliable and may be utilized for clonal production regeneration.

Summary and Conclusions

The present study conducted on two commercially significant banana cultivars, Grand Nain and Sabri, highlights the successful regeneration, shoot proliferation and the critical role of plant growth regulators (PGRs) in establishing and proliferating *in vitro* cultures. The cultures were initiated following surface sterilization using a standardized method involving 0.1% Bavistin and 0.1–0.2% mercuric chloride, resulting in 80–90% contamination-free cultures. In the shoot initiation and regeneration, the cytokinin BAP proved to be the most effective in G9, with an overall response rate of 71%, while, the Sabri cultivar exhibited a higher initiation rate with Kinetin, achieving an average response of 43.8%.

Multiple shoot proliferation in both cultivars achieved optimal response in BAP alone with 100% shoot induction. In G9, the best shoot multiplication was observed with BAP at concentrations of 1 mg/L, 3.5 mg/L, and 6.5 mg/L, as well as in combination treatments of BAP (3.5 mg/L) with IAA (1 mg/L) and KN (2.5 mg/L) with IAA (1.5 mg/L). While, Sabri cultivar exhibited optimal shoot proliferation with single BAP applications of 1 mg/L, 3.5 mg/L, and 4.5–5.5 mg/L, as well as combinations of BAP (3.5 mg/L and 5.5 mg/L) with IAA (0.5 mg/L) and BAP (2.5–4.5 mg/L) with NAA (0.5 mg/L).

For the successful establishment of plantlets into well-developed/ mature plantlets, the overall most effective results were obtained in the combinations of cytokinin (BAP, KN and TDZ) and Auxins (IAA and NAA). For G9, the optimum results included BAP (1-6.5 mg/L) with NAA (0.5-1.5 mg/L), single application of KN (1-6.5 mg/L), KN (1-6.5 mg/L) with IAA (0.5-1.5mg/L), and TDZ (0.5-1.5 mg/L) with IAA (0.5-1.5 mg/L). In Sabri, the optimum results include, single applications of BAP (2.5–6.5 mg/L) and KN (3.5 mg/L), along with combinations of BAP (4.5 mg/L) with IAA (0.5 mg/L), KN (4.5 mg/L) with IAA (0.5 mg/L) and KN (1-2.5 and 4.5-6.5 mg/L) with NAA (0.5 mg/L).

During primary hardening in cocopeat, all treatments achieved a 100% survival rate, with an average shoot elongation of 9.75 cm, 6.05 leaves, and 15.34 roots per plantlet, indicating healthy development of plantlets. Cocopeat proved an excellent substrate for the primary hardening phase, requiring no external nutrients. The secondary hardening phase achieved a successful survival rate of 96%, and the

acclimatized plantlets were subsequently transplanted to the field, and achieving a successful survival rate of 90%.

The assessment of genetic fidelity in regenerates, using 14 primers of RAPD and ISSR markers across both cultivars, indicated genetic homogeneity rates of 96.25% in G9 and 98.72% in Sabri. This confirms their suitability as clonal planting materials.

The findings of this study confirm that the optimized *in vitro* protocols (sterilization, initiation, multiplication and field establishment) are effective in promoting high shoot proliferation, strong survival rates, and successful establishment of banana cultivars Grand Nain (G9) and Sabri in field conditions. This highlights their potential as reliable methods for large-scale propagation, enabling the efficient clonal production and regeneration of healthy, robust plantlets suitable for commercial cultivation.

Chapter – 5

Summary and Conclusions

Bananas are popularly known for their nutritious and flavorful fruit, making them one of the most consumed fruits globally, and a popular dietary choice, as well as a highly economically viable crop. Due to its high economic value, the demand for planting propagules has increased. However, their availability remains limited due to the slow production rate in conventional propagation methods, coupled with the high susceptibility to biotic and abiotic factors. Additionally, the shelf life of bananas is short and their peels and pseudo stems often go to waste, contributing to approximately 30% of total wastes. To address these challenges, it is essential to implement various alternative approaches. Bananas are the country's leading crop in terms of global production being the lead in the global production in the country, hence this study focuses on two economically viable cultivars that have been carefully selected to investigate and document their potential. The cultivar G9 is a popular Cavendish, and cultivar Sabri a popular silk subgroup banana, both cultivars known for their sweet, appetizing and nutritious fruit.

This study focused on the nutritional aspects and propagation of two selected *Musa* cultivars. The first objective involves a detailed nutritional analysis of both the edible pulp and inedible peel at the ripe and unripe stages. The aim is to evaluate the food and nutraceutical value of different parts and stages of the banana fruit, thereby exploring its potential for value-added applications. The comparative analysis underscores the significance of nutrient distribution across the banana cultivars. While both cultivars exhibit similar nutrient profiles, Cv. Sabri stands out with higher carbohydrate and reducing sugar content, which accounts for the differing texture and

sweetness between the two. The study highlights nutritional values obtained in all parts of the fruit, particularly in terms of Vitamin A, antioxidants, and polyphenolic compounds such as phenols and flavonoids. Additionally, the study demonstrates the significance of unripe bananas and their inedible peel, maximizing the use of bananas. The unripe pulp is rich in carbohydrates, particularly resistant starch, which is known for its beneficial effects on the digestive system. The peels, also nutrient-dense, are packed with fibre, protein, minerals, antioxidants, and bioactive compounds, showcasing their value for potential applications in nutraceutical, pharmaceutical, and other industries. This study offers a detailed dataset of the two cultivars, in all parts of banana fruit, at two stages of banana (ripe and unripe) as well as in pulp and peel, which will further facilitate research and deepen our understanding of how to fully utilize the entire banana, while also assisting in the selection of the most suitable stage for utilization in specific applications.

To mitigate the rise in demands, two kinds of propagation methods were employed in this study, macropropagation and *in vitro* micropropagation techniques. Macropropagation technique is a viable alternative approach to expensive technology requiring basic materials and simple skills. The study used different corm manipulation techniques as well as potting substrates and materials which were easily available in the campus surroundings. The macropropagation study obtained optimal results, with the corm techniques that gave the highest number of plantlets being Split Corm (T_2), followed by PIF (T_3), and then Split Corm + PIF (T_5) in both the cultivars. Among the techniques, the highest survival percentage with healthy plantlets was obtained in the PIF (T_3) treatment, followed by Split Corm + PIF (T_5), and then Split Corm (T_2). The overall optimal method for sustainable, and thriving plantlets, was obtained in the techniques PIF (T_3) and Split Corm + PIF (T_5) considering their overall performances. Another aspect in this study is the experiments done on potting substrates which, also determined that the potting substrates along with the corm techniques, as well as selection of planting material, and routine supply of nutrients impacts greatly on proliferation and growth of plantlets. The optimal response from both the cultivars were obtained in potting media, forest soil (P_3), saw dust (P_2) and sawdust and sand (P_5), which yielded over all good response making them suitable for the production and growth of banana plantlets. The regenerated plantlets were further assessed for genetic

homogeneity, using RAPD and SCoT markers in 14 primers. The assessment resulted in genetic homogeneity percentage of 96.72% in G9 and 98.69% in Sabri, respectively, thus qualifying them as suitable clonal planting materials. This study was conducted in a sustainable and cost-effective way by substituting artificial fertilizers with recycled materials, resulting in reduced expenses. Additionally, it also produced new approaches and techniques which yielded better results than traditional methods, making it a viable option for simple propagation method especially for poor resources and small-scale farmers.

In vitro micropropagation is the tested technology used for addressing the natural propagation challenges. This method is efficient and facilitates rapid production of high quality and diseased free clonal planting materials. This study focused on successful regeneration, shoot proliferation and the critical role of plant growth regulators (PGRs) in establishing and proliferating *in vitro* cultures. Optimization of sterilization in the explants was the initial step, which involved 0.1% Bavistin and 0.1–0.2% mercuric chloride, resulting in 80–90% contamination-free cultures, standardizing the surface sterilization protocol. Following which, the Initiation of cultures for shoot regeneration and establishment was performed, where the cytokinin BAP proved to be the most effective in G9, with an overall response rate of 71%, while, the Sabri cultivar exhibited a higher initiation rate with Kinetin, achieving an average response of 43.8%.Both the cultivars achieved 100% shoot induction in multiple shoot proliferation, where the optimal response in G9, was obtained in BAP at concentrations of 1 mg/L, 3.5 mg/L, and 6.5 mg/L, as well as in combination treatments of BAP (3.5 mg/L) with IAA (1 mg/L) and KN (2.5 mg/L) with IAA (1.5 mg/L). While in Sabri, optimal shoot proliferation was achieved with single BAP applications of 1 mg/L, 3.5 mg/L, and 4.5–5.5 mg/L, as well as combinations of BAP (3.5 mg/L and 5.5 mg/L) with IAA (0.5 mg/L) and BAP (2.5–4.5 mg/L) with NAA (0.5 mg/L).The successful response for establishment of plantlets (well-developed/ mature plantlets), were obtained in the combinations of cytokinin (BAP, KN and TDZ) and Auxins (IAA and NAA). With cultivar G9 achieving the overall optimal response in BAP (1-6.5 mg/L) with NAA (0.5-1.5 mg/L), single application of KN (1-6.5 mg/L), KN (1-6.5 mg/L) with IAA (0.5-1.5mg/L), and TDZ (0.5-1.5 mg/L) with IAA (0.5-1.5 mg/L). While in Sabri, the optimum results include, single applications of BAP (2.5–6.5 mg/L) and KN (3.5

mg/L), along with combinations of BAP (4.5 mg/L) with IAA (0.5 mg/L), KN (4.5 mg/L) with IAA (0.5 mg/L) and KN (1-2.5 and 4.5-6.5 mg/L) with NAA (0.5 mg/L) for the establishment of plantlets into well-developed plantlets. After the success of the plantlets primary and secondary hardening is done to achieve mature and robust plantlets for the next step and final step that is the field establishment. Primary hardening was done in cocopeat, and all treatments achieved a 100% survival rate, with an average shoot elongation of 9.75 cm, 6.05 leaves, and 15.34 roots per plantlet, indicating healthy development of plantlets. The use of cocopeat with no external nutrients during the primary hardening phase demonstrated to be an excellent substrate for the initial hardening phase. Secondary hardening phase also achieved a good response of 96% successful survival rate. In the final step, the survived acclimatized plantlets were subsequently transplanted to the field for establishment, and the establishment of the plantlets responded successful with 90% survival rate. Genetic fidelity assessment was performed in the regenerates of both cultivars, by using 14 primers of RAPD and ISSR markers. The results indicated genetic homogeneity rates of 96.25% in G9 and 98.72% in Sabri, confirming the genetic stability; thereby the protocols are suitable for propagation of clonal planting materials.

The findings of the study shows that selected cultivars show much potential for further utilization, due to their high nutrient content, especially in the raw pulp and peels of bananas containing high nutrient content paving the way in food processing technology as well as pharmaceutical and other companies. Furthermore, the successfully optimization of propagation protocols underlined the potential of these protocols for large-scale banana propagation, reducing the dependency on traditional propagation methods. In addition, these protocols provide valuable insights into efficient banana propagation techniques, which are crucial for both commercial cultivation and sustainable agriculture.

Future Prospects

Present research outcomes warrants future research directing towards the exploration of sustainable use of unripe pulp and peels of banana. The study also offers a basis for development of new, gluten-free, fiber-rich, and nutrient-dense products that can cater to growing consumer demand for healthy food options. Furthermore, the pharmaceutical companies, cosmetic industries and biotechnology sectors can capitalize from these findings to develop several health beneficial supplements, therapeutic formulations, cosmetics extracted from the bioactive compounds present in both the pulp and peels, which may lead to new product lines focused on health, and overall wellness. Also, more research can be conducted on optimizing further propagation protocols of these cultivars in order to establish large scale and sustainable production of high-quality bananas. Furthermore, these findings will help in creating new income generating avenues for banana growers and the overall economy of the region where bananas are grown to a large extent, for instance Nagaland, India.

References

Adjei, A.E., Tetteh, E.N., Darkey, S., Dzomeku, B.M., Mintah, P., Anti, M. 2021. Extrapolating technology from research plots to commercial scale and challenges involved: case study of plantain macro-propagation technique. Journal of Agricultural Extension and Rural Development. 13(4), 225–231. https://doi.org/10.5897/jaerd2021.1258.

Adubofuor, J., Amoah, I., Batsa, V., Agyekum, B.P., Buah, A.J. 2016. Nutrient composition and sensory evaluation of ripe banana slices and bread prepared from ripe banana and wheat composite flours. American Journal of Food and Nutrition. 4, 103-111.

Agboola, O.O., Oseni, O.M., Adewale, O.M., Shonubi, O. 2018. Effect of the use of sawdust as a growth medium on the growth and yield of Tomato. Annals of West University of Timisoara, Series of Biology. 21(1), 67–74.

Ahmad, A.A., Radovich, T.J.K., Nguyen, H.V., Uyeda, J., Arakaki, A., Cadby, J., Paull, R., Sugano, J., Teves, G. 2016. Use of organic fertilizers to enhance soil fertility, plant growth, and yield in a tropical environment. In: Larramendy, M.L., Soloneski, S. (eds.). Organic Fertilizers. Basic Concepts to Applied Outcomes. InTech Publisher. 85-108. https://doi.org/10.5772/62529.

Al-Amin, M.D., Karim, M.R., Amin, M.R., Rahman, S., Mamun, A.N.M. 2009. *In vitro* micropropagation of banana. Bangladesh Journal of Agricultural Research. 34(4), 645-659. https://doi.org/10.3329/bjar.v34i4.5840

Alemu, M.M. 2017. Banana as a cash crop and its food security and socioeconomic contribution: the case of Southern Ethiopia, Arba Minch. Journal of Environmental Protection. 8(3), 319-329.

Ali, A., Sajid, A. , Naveed, H.N., Majid, A. , Saleem, A., Khan, A.U., Jafery, I.F., Naz S. 2011. Initiation, proliferation and development of micropropagation system for mass scale production of banana through meristem culture. African Journal of Biotechnology. 10, 15731-15738. https://doi.org/10.5897/AJB11.2079.

Ali, A., Sajid, A., Naveed, N.H., Majid, A., Saleem, A., Khan, U.A., Naz, S. 2011. Initiation, proliferation and development of micro-propagation system for mass scale production of banana through meristem culture. African Journal of Biotechnology. 10(70), 15731-15738. https://doi.org/10.5897/AJB11.2079.

Ali, M. A., Awad, M. A., Al-Qurashi, A.D., El-Shishtawy, R. M., Mohamed, S. A. 2019. Quality and Biochemical Changes of "Grand Nain" Bananas during Shelf Life. JKAU: Meteorology, Environment and Arid Land Agriculture Sciences. 28(1), 41–56. https://doi.org/10.4197/Met.

Ali, M. A., Awad, M. A., Al-Qurashi, A.D., El-Shishtawy, R. M., Mohamed, S. A. 2019. Quality and Biochemical Changes of "Grand Nain" Bananas during Shelf Life. JKAU: Meteorology, Environment and Arid Land Agriculture Sciences. 28(1), 41–56. https://doi.org/10.4197/Met.

Allameh, M., Orsat, V. 2024. Effects of time, ultrasonic treatment and *p*H during extraction on L-theanine and caffeine yields from white tea leaves. Future Foods. 9, 100304. https://doi.org/10.1016/j.fufo.2024.100304.

Altman, A., Loberant, B. 1997. Micropropagation: clonal plant propagation *In Vitro*. Agricultural Biotechnology. 19-22. https://doi.org/10.1201/9781420049275.

Amri, F.S., Al, Hossain, M.A. 2018. Comparison of total phenols, flavonoids and antioxidant potential of local and imported ripe bananas. Egyptian Journal of Basic and Applied Sciences. 5(4), 245–251. https://doi.org/10.1016/j.ejbas.2018.09.002.

Anbazhagan, M., Balachandran, B., Arumugam, K. 2014. *In vitro* propagation of *Musa* sp (banana). International Journal Current Microbiology and Applied Sciences. 3(7), 399-404.

Anyasi, T.A. Jideani, A.I.O., Mchau, G. A. 2015. Morphological, physicochemical, and antioxidant profile of non commercial banana cultivars. Food Science and Nutrition. 3(3), 221–232. https://doi.org/10.1002/fsn3.208.

Anyasi, T.A., Jideani, A.I., Mchau, G.R. 2013. Functional properties and postharvest utilization of commercial and non-commercial banana cultivars. Comprehensive Reviews in Food Science and Food Safety. 12(5), 509-522.

Anyasi, T.A., Jideani, A.I.O., Mchau, G.A. 2015. Morphological, physicochemical, and antioxidant profile of non-commercial banana cultivars. Food Science and Nutrition. 3(3), 221–232. https://doi.org/10.1002/fsn3.208.

AOAC. 1990. Official methods of analysis, 15th edition. Washington DC. Association of Official Analytical Chemists.

AOAC. 2000. Official methods of analysis, 17th edition. The Association of Official Analytical Chemists, Gaithersburg. Methods 925.10, 65.17, 974.24, 992.16

Ao, T., Deb, C. R. 2019. Nutritional and antioxidant potential of some wild edible mushrooms of Nagaland, India. Journal of Food Science and Technology. 56 (2), 1084-1089. https://doi.org/10.1007/s13197-018-03557-w.

Aremu, S.O., Nweze, C.C. 2017. Determination of vitamin A content from selected Nigerian fruits using spectrophotometric method. Bangladesh Journal of Scientific and Industrial Research. 52(2), 153-158.

Arora, A., Choudhary, D., Agarwal, G., Singh, V. P. 2008. Compositional variation in β-carotene content, carbohydrate and antioxidant enzymes in selected banana cultivars. International Journal of Food Science and Technology. 43(11), 1913–1921. https://doi.org/10.1111/j.1365-2621.2008.01743.x.

Arvanitoyannis, S.I., Mavromatis, G.A., Avgeli, G.G., Sakellariou. M. 2008. Banana: cultivars, biotechnological approaches and genetic transformation. International Journal of Food Science and Technology. 43, 1871–1879.

Aurore, G., Parfait, B., Fahrasmane, L. 2009. Bananas, raw materials for making processed food products. Trends in Food Science & Technology. 20(2), 78-91.

Avvaru, S. 2016. Plant tissue culture of banana in laboratory, research and reviews. Journal of Botanical Sciences. 5(3), 54-62.

Azad, M.A.K., Amin, M.N. 2001. Rapid clonal propagation of banana (*Musa* sp.) using *in vitro* culture of floral bud apex. Plant Tissue Culture. 11(1), 1-9.

Baiyeri, K.P., Aba, S.C. 2005. Response of *Musa* species to macro-propagation. I: Genetic and initiation media effects on number, quality and survival of plantlets at prenursery and early nursery stages. African Journal of Biotechnology. 4(2), 223-228.

Baiyeri, K.P., Aba, S.C. 2007. A review of protocols for macropropagation in *Musa* species. Fruit, Vegetable and Cereal Science and Biotechnology. 1(2), 110-115.

Baiyeri, K.P., Aba, S.C., Otitoju, G.T., Mbah, O.B., 2011. The effects of ripening and cooking method on mineral and proximate composition of plantain (*Musa* sp. AAB cv. 'Agbagba') fruit pulp. African Journal of Biotechnology. 10(36), 6979–6984. https://doi.org/10.5897/AJB11.607

Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R.P., Chang, C.M. 2022. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. Molecules. 27(4). https://doi.org/10.3390/molecules27041326

Banerjee, N., De Langhe, E. 1985. A tissue culture technique for rapid clonal propagation and storage under minimal growth conditions of *Musa* (banana and plantain). Plant Cell Reports. 4, 351-354.

Bashmil, Y.M., Ali, A., Bk, A. Dunshea, F.R., Suleria, H.A.R. 2021. Screening and characterization of phenolic compounds from australian grown bananas and their antioxidant capacity. Antioxidants. 10(10), 1521. https://doi.org/10.3390/antiox10101521.

Begashaw, T., Dagne, A., Yibeltal, D. 2023. Review on phytochemistry, medicinal properties, and toxicities of the genus *Musa*. Traditional Medicine. *4*(2), 1-24.

Bezerra, C.V., Rodrigues, A.M. da C., Amante, E.R., Silva, L.H.M. da. 2013. Nutritional potential of green banana flour obtained by drying in spouted bed. Revista Brasileira de Fruticultura. 35(4), 1140–1146. https://doi.org/10.1590/S0100-29452013000400025.

Bhaskar, J.J., Chilkunda, N.D., Salimath, P.V. 2012. Banana (*Musa* sp. var. elakki bale) flower and pseudostem: dietary fiber and associated antioxidant capacity. Journal of Agricultural and Food Chemistry. 60(1), 427-432.

Bhende, S.S., Kurien, S. 2016. Sucker production in banana. Journal of Tropical Agriculture. 53(2), 97-106.

Bhende, S.S., Kurien, S. 2020. Sucker invigoration in banana: techniques and strategies. Lap Lambart Academic Publishing (Member of Omni Scriptum Publishing Group) 17 Meldrum Street, Beau Bassin 71504, Mauritius. ISBN: ISBN: 978-620-0-480036-1.

Bodjona, B.P.I.T., Odah, K., Pitekelabou, R., Bokobana, A. 2020. Macro-propagation of Dessert Bananas (Dankodu and Tsikodu) and Plantain (Save) (*Musa*) spp. by PIF Technique in Togo, West Africa. Agricultural and Biological Sciences Journal. 6(4), 195-201.

Borborah, K., Borthakur, S.K. Tanti, B. 2016. *Musa balbisiana* Colla-taxonomy, traditional knowledge and economic potentialities of the plant in Assam, India. Indian Journal of Traditional Knowledge. 15, 116-120.

Borges, C.V., de Oliveira, A.M.V.B., Ramlov, F., da Silva, L.C.A., Donato, M., Maraschin, M., Amorim, E.P. 2014. Characterisation of metabolic profile of banana genotypes, aiming at biofortified *Musa sp*. cultivars. Food chemistry. 145, 496-504. https://doi.org/10.1016/J.FOODCHEM.2013.08.041

Borges, C.V., Maraschin, M., Coelho, D. S., Leonel, M., Gomez, H. A. G., Belin, M. A. F., Diamante, M. S., Amorim, E. P., Gianeti, T., Castro, G. R., Lima, G. P. P. 2020. Nutritional value and antioxidant compounds during the ripening and after domestic cooking of bananas and plantains. Food Research International. 132, 109061. https://doi.org/10.1016/j.foodres.2020.109061.

Borse, N., Chimote, V.P., Jadhav, A.S. 2011. Stability of micropropagated *Musa acuminata* cv. Grand Naine over clonal generations: A molecular assessment. Scientia Horticulturae. 129(3), 390-395. https://doi.org/10.1016/j.scienta.2011.04.001.

Budhalakoti, N. 2019. Formulation and standardisation of banana peel extracted insoluble dietary fibre based buns. Current Journal of Applied Science and Technology. 32(2), 1–9. https://doi.org/10.9734/cjast/2019/45832.

Chauhan, N., Jethva, K.R. 2016. Drying Characteristics of Banana Powder. Indian Journal of Science. 23(77), 75–88.

Cheesman, E.E. 1948. Classification of the bananas. Kew Bulletin. 3 (1), 17-28. https://doi.org/10.2307/4118909

Choudhary, D., Kajla, S., Poonia, A.K., Brar, B., Duhan, J.S. 2015. Molecular assessment of genetic stability using ISSR and RAPD markers in in vitro multiplied copies of commercial banana cv. *Robusta*. Indian Journal of Biotechnology. 4, 420–424.

Christelova, P., Valarik, M., Hribova, E., De Langhe, E., Dolezel J. 2011. A multi gene sequence-based phylogeny of the Musaceae (banana) family. BMC Evolutionary Biology. 11, 103.

Chukwu, O., Sunmonu, M.O., Egbujor, E.C. 2011. Effects of storage conditions on nutritional compositions of banana. QualityAssurance and Safety of Crops & Foods. 3(3), 135-139.

Cronauer, S.S., Krikorian, A. D. 1984. Multiplication of *Musa* from excised stem tips. Annals of Botany. 53, 321-328.

Dahari, P.H., Nizamani, G.S., Ali, M., Khatri, A., Nizamani, M.R., Yasmeen, S., Mari, S.N. 2017. Micropropagation of banana cultivar basrai under different concentrations of n 6-benzylaminopurin for shoot and indole-3-butyric acid for root induction. Pakistan Journal of Biotechnology. 14(4), p761.

Das, J.L. 2010. Medicinal and nutritional values of banana cv.NENDRA. The Asian Journal of Horticulture. 5, 11- 14.

Datta, H.S., Baruah, K., Bora, S.S., Borah, K. 2020. Effect of sucker weight on growth and yield of banana (*Musa* spp.) cv. Barjahaji (AAA). International Journal of Current Microbiology and Applied Sciences. 9(6), 456–465. https://doi.org/10.20546/ijcmas.2020.906.060.

Davey, W.M., Gudimella, R., Harikrishna, A.J., Sin,W.L., Khalid,N., Keulemans, J. 2013. A draft *Musa balbisiana* genome sequence for molecular genetics in polyploid, inter- and intra-specific *Musa* hybrids, Biological Modified Crops. 14, 683.

De Langhe, E., Swennen, R., Wilson, G. 1983. Hormonal aspects of plantain offspring. Fruit. 38(4), 318-325.

De Langhe, E., Vrydaghs, L., deMaret, P., Perrier, X., Denham, T. 2009. Why bananas matter: an introduction to the history of banana domestication. Ethnobotany Research & Applications. 7,165-177.

Deb, C.R., Tiatemsu, P., Vupru, T.N., Paul, A. 2023. Diversity and distribution of wild *Musa* in Nagaland, India. Open Journal of Forestry. 13(3), 315-337.

Department of Agriculture & Farmers Welfare 2012 to 2024, Production: Horticulture Crops: Fruits: Banana: Nagaland | Economic Indicators | CEIC.

Deshmukh, M.H., Pai, S.R., Nimbalkar, M.S., Patil, R.P. 2009. Biochemical characterization of banana cultivars from Southern India. International Journal of Fruit Science. 9(4), 305–322. https://doi.org/10.1080/15538360903241336.

Dey, S., Jamir, N.S., Gogoi, R., Chaturvedi, S.K., Jakha, H.Y., Kikon, Z.P. 2014. *Musa nagalandiana* sp. nov. (Musaceae) from Nagaland, Northeast India. Nordic Journal of Botany. *32*(5), 584-588.

Dotto, J., Matemu, A.O., Ndakidemi, P.A. 2019. Nutrient composition and selected physicochemical properties of fifteen Mchare cooking bananas: a study conducted in northern Tanzania. Scientific African. 6. https://doi.org/10.1016/j.sciaf.2019.e00150.

Doymaz, I. 2010. Evaluation of mathematical models for prediction of thin-layer drying of banana slices. International Journal of Food Properties. 13, 486–497. https://doi.org/10.1080/10942910802650424.

Dzomeku, B.M., Darkey, S.K., Wunsche, Jens-Norbert., Bam, R.K. 2014. Response of selected local plantain cultivars to PIBS (Plants Issus De Bourgeons Secondaires) Technique. Journal of Plant Development. 21(1), 117–123.

Elbagoury, M.M., Turoop, L., Runo, S., Sila, D.N., Madivoli, E.S. 2022. Postharvest treatments of banana (*Musa acuminata* cv. 'Grand Nain', AAA) during cold and ripening temperatures with chitosan and chitosan nanoparticles to alleviate chilling injury and maintain antioxidant activity. Horticulture Environment and Biotechnology. 63(5), 677–699. https://doi.org/10.1007/s13580-022-00436-4.

Englberger, L., Schierle, J., Aalbersberg, W., Hofmann, P., Humphries, J., Huang, A., Lorens, A., Levendusky, A., Daniells, J., Marks, G.C., Fitzgerald, M.H. 2006. Carotenoid and vitamin content of Karat and other Micronesian banana cultivars. International Journal of Food Sciences and Nutrition. 57(5–6), 399–418. https://doi.org/10.1080/09637480600872010.

Evans, E.A., Ballen, F.H., Siddiq, M. 2020. Banana production, global trade, consumption trends, postharvest handling, and processing. Handbook of Banana Production, Postharvest Science, Processing Technology, and Nutrition. 1-18.

Ferdous, M.H., Billah, A.M., Mehraj, H., Taufique, T., Uddin, A.F.M.J. 2015. BAP and IBA pulsing for *in vitro* multiplication of banana cultivars through shoot-tip culture. Journal of Bioscience and Agriculture Research. 3(2), 87-95.

Firew, T., Aragaw, D., Geto, G., Ayele, A., Girma, N. 2024. Study of Macropropagation Techniques and Economic Evaluation of Banana (*Musa* spp.) in Amhara, Ethiopia. Journal of Oasis Agriculture and Sustainable Development. 14-21. https://doi.org/10.56027/JOASD.172024.

Fischer, D., Glaser, B. 2012. Synergisms between compost and biochar for sustainable soil amelioration. In: Kumar, S., Bharti, A. (eds.), Management of Organic Waste. IntechOpen, Rijeka. 167-198. https://doi.org/10.5772/31200.

Fontes, S.M., Cavalcanti, M.T., Candeia, R. A., Almeida, E.L. 2017. Characterization and study of functional properties of banana starch green variety of Mysore (*Musa* AAB - Mysore). Food Science and Technology. 37(2), 224–231. https://doi.org/10.1590/1678-457x.18916.

Food and Agricultural Organizations (FAO). 2019. Banana facts and figures- Markets and Trade. Retrieved from http://www.fao.org/economic/est/est-commodities/oilcrops/bananas/bananafacts/en/.

Food and Agricultural Organizations (FAO). 2024. Banana Market Review 2023. Rome. Retrieved from https://www.fao.org/markets-and-trade/publications/detail/en/c/1709184.

Fresh Fruit Portal. 2015. India: Gulf markets go bananas for Grand Nain from Tamil Nadu. India: Gulf markets go bananas for Grand Nain from Tamil Nadu. FreshFruitPortal.com. Accessed on 09-09-24.

Fu, L., Xu, B.T., Xu, X.R., Gan, R.Y., Zhang, Y., Xia, E.Q., Li, H.B. 2011. Antioxidant capacities and total phenolic contents of 62 fruits. Food chemistry. 129(2), 345-350.

Ganapathi, T.R., Higgs, N.S., Balint-Kurti, P.J., Arntzen, C.J., May, G.D, Eck Van J.M. 2001. *Agrobacterium* -mediated transformation of embryogenic cell suspensions of the banana cultivar Rasthali (AAB). Plant Cell Reports. 20,157–162.

Gomez-Brandon, M., Vela, M. Martinez- Toledo, V.M., Insam, H., Dominguez, J. 2015. Effects of compost and vermicompost teas as organic fertilizers. Fertilizer Technology I: Synthesis. 300-318.

Gonmei, Z., Toteja, G.S. 2018. Micronutrient status of Indian population. Indian Journal of Medical Research. 148(5), 511-521. https://doi.org/10.4103/ijmr.IJMR_1768_18.

González-Montelongo, R., Gloria Lobo, M., Gonzalez, M. 2010. Antioxidant activity in banana peel extracts: Testing extraction conditions and related bioactive compounds. Food Chemistry. 119(3), 1030–1039. https://doi.org/10.1016/j.foodchem.2009.08.012.

Goswami, N.K., Handique, P.J. 2013. *In vitro* sterilization protocol for micropropagation of *Musa* (AAA group) 'Amritsagar' *Musa* (AAB group) ‘Malbhog’ and *Musa* (AAB group) 'Chenichampa' Banana. Indian Journal of Applied Research. *3*(6), 51-54.

Gübbük, H., Pekmezci, M. 2004. *In vitro* propagation of some new banana types (*Musa* spp.). Turkish Journal of Agriculture and Forestry. 28(5), 355-361.

Gulcin, I. 2020. Antioxidants and antioxidant methods: An updated overview. Archives of toxicology. 94(3), 651-715. https://doi.org/10.1007/s00204-020-02689-3.

Habib, S.E., Mohamed, S.M., Ali E.M., Allam, I. 2016. Effect of medium and cytokinin types on banana micropropagation during multiplication stage. Hortscience Journal of Suez Canal University. 5(1), 1-7.

Hailu, M., Workneh, T.S., Belew, D. 2013. Review on postharvest technology of banana fruit. African Journal of Biotechnology. 12(7).

Halliwell, B. 1996. Antioxidants: the basics-what they are and how to evaluate them. Advances in Pharmacology. 38, 3-20.

Hapsari, L., Lestari, D.A. 2016. Fruit characteristic and nutrient values of four Indonesian banana cultivars (*Musa* spp.) at different genomic groups. Agrivita. 38(3), 303–311. https://doi.org/10.17503/agrivita.v38i3.696.

Hasanah, R., Daningsih, E., Titin, T. 2017. The analysis of nutrient and fiber content of banana (*Musa paradisiaca*) sold in Pontianak, Indonesia. Biofarmasi Journal of Natural Product Biochemistry. 15(1), 21–25. https://doi.org/10.13057/biofar/f150104.

Hossain, M.A., Rubel, M.H., Nasiruddin, K.M., Evamoni, F.Z. 2016. Influence of BAP and NAA on *in vitro* plantlet regeneration of local and exotic banana cultivars. Journal of Bioscience and Agriculture Research. 6(2), 553-564.

Huq, A., Akter, S., Islam, S., Khan, S. 2012. *In vitro* plant regeneration in Banana (*Musa* sp.) cv. sabri. Bangladesh Journal of Scientific and Industrial Research. 47(2), 143-146.

Iliev, I., Gajdoˇsova, A., Libiaková, G., Jain, S.M. 2010. Plant micropropagation. Plant Cell Culture: Essential Methods. 1, 1-23.

Ismaila, A.A., Ahmad, K., Siddique, Y., Wahab, M.A.A., Kutawa, A.B., Abdullahi, A., Zobir, S.A.M., Abdu, A. Abdullah, S.N.A. 2023. Fusarium wilt of banana: current update and sustainable disease control using classical and essential oils approaches. Horticultural Plant Journal. 9(1), 1-28.

Israeli, Y., Lahav, E., Reuveni, O. 1995. *In vitro* culture of bananas. In Bananas and plantains. Dordrecht: Springer Netherlands. 147-178. https://doi.org/10.1007/978-94-011-0737-2_6.

Izaile, K., Ariina, M.S., Lohe, V., Pertin, M., Anna, Y. 2021. Advances in propagation techniques of banana. Just Agriculture. 2(2), 1-14.

Jamir, S., Maiti, S.C. 2014. Effect of various levels of cytokinin and auxin for *in vitro* regeneration of banana cultivars. International Journal of Agriculture Innovations and Research. 1160-1163.

Joe, A., Sabu, M. 2016. Wild ornamental bananas in India: an overview. South Indian Journal of Biological Sciences. 2, 213-22.

Jones, C., Jacobsen, J. 2005. Plant nutrition and soil fertility. Nutrient Management Module. 2(11), 1-11.

Joshi, P., Varma, K., 2015. Assessment of Nutritional and Physiochemical properties of Banana Flour. Research Journal of Family, Community and Consumer Sciences. 3, 3-4.

Kalimutha, K., Saravanakumar, M., Senthilkumar, R. 2007. *In vitro* micropropagation of *Musa sapientum* L. (Cavendish Dwarf). African Journal of Biotechnology. 6 (9), 1106-1109.

Karthikeyan, G., Vidya, A.K., Bhuvaneswari, A. 2019. A Study on proximate mineral composition of plantain (*Musa Paradisiaca*) Wastes; A Potential Nutritive Source in the formulation of animal feeds. International Journal of Development Research. 9(1), 25310-25314.

Karule, P., Dalvi, V., Kadu, A., Chaudhari, R., Subramaniam, V.R., Patil, A.B. 2016. A commercial micropropagation protocol for virupakshi (AAB) banana via apical meristem. African Journal of Biotechnology. 15(11), 401-407. https://doi.org/10.3329/bjsir.v47i2.11444.

Kasyoka, M.R., Mwangi, M., Kori, N., Gitonga, N., Muasya, R. 2010. Evaluating the macropropagation efficiency of banana varieties preferred by farmers in Eastern and Central Kenya. Research Application Summary. Second RUFORUM Biennial Meeting, 20-24th September, Entebbe, Uganda. 499-503.

Khalid, N., Tan, B.C. 2016. A to Z on banana micropropagation and field practices. Plant Tissue Culture: Propagation, Conservation and Crop Improvement. 101-118.

Khan, A., Bashir, A., Khatak, J.Z.K., Erum, S., Muhammad, A. 2021. Effects of 6-benzylaminopurine and indole-3-acetic acid on growth and root development of banana explants in micropropagation. Sarhad Journal of Agriculture. 37(1), 9-13. https://doi.org/10.17582/journal.sja/2021/37.1.9.13.

Khatun, F., Hoque, M., Huq, H., Adil, M., Ashraf-Uz-Zaman, K., Rabin, M. 2017. Effect of BAP and IBA on *in vitro* Regeneration of Local Banana Variety of Sabri. Biotechnology Journal International. 18(1), 1-10. https://doi.org/10.9734/BJI/2017/31592.

Khawas, P., Das, J.A., Sit, N., Badwaik, S.L., Deka, C.S. 2014. Nutritional composition of culinary *Musa* ABB at different stages of development. American Journal of Food Science and Technology. 2, 80-87. https://doi.org/10.12691/ajfst-2-3-1.

Khawas, P., Deka, S.C. 2016. Comparative nutritional, functional, morphological, and diffractogram study on culinary banana (*Musa* ABB) Peel at various stages of development. International Journal of Food Properties. 19(12), 2832–2853. https://doi.org/10.1080/10942912.2016.1141296.

Khoza, M., Kayitesi, E., Dlamini, B.C. 2021. Physicochemical characteristics, microstructure and health promoting properties of green banana flour. Foods. 10(12), 2894. https://doi.org/10.3390/foods10122894.

Kindimba, G.V., Msogoya, T.J., 2014. Effect of benzylaminopurine on *in vivo* multiplication of French plantain (*Musa* spp. AAB) cv.'Itoke sege'. Journal of Applied Biosciences. 74, 6086-6090. http://doi.org/10.4314/jab.v74i1.1.

Kishor, H., Abhijith, Y.C., Manjunatha, N. 2017. Micropropagation of native cultivars of Banana-A Critical Review. International Journal of Pure and Applied Bioscience. 5(5), 1559-1564.

Kookal, S.K., Thimmaiah, A. 2018. Nutritional composition of staple food bananas of three cultivars in India. American Journal of Plant Sciences. 9(12), 2480–2493. https://doi.org/10.4236/ajps.2018.912179.

Kothawade, C.V. 2019. Medicinal and nutritional importance of banana. Acta Scientific Agriculture. 3(5), 29-33.

Kowsalya, J., Rajkumar, M.,2019. Effect of Chemicals and Growth Regulators on Shelf Life and Quality of Banana Cv. Grand Naine. Plant Archives. 19(2), 832-834.

Krishnan, A., Sinija, V.R. 2016. Proximate composition and antioxidant activity of banana blossom of two cultivars in India. International Journal of Agriculture and Food Science Technology. 7(1), 13-22.

Kumadoh, D., Kyene, M.O., Archer, M.A., Yeboah, G.N., Adase, E., Sakyiamah, M.M., Oteng-Mintah, S., Adi-Dako, O., Osei-Asare, C., Oppong, E.E. 2024. Evaluation of a tea bag formulation of *Tapinanthus bangwensis* (Engl. and K. Krause) Danser

leaves, meant for the management of diabetes. Scientific African. 23. https://doi.org/10.1016/j.sciaf.2023.e02025.

Kumar, G.K., Krishna, V., Venkatesh, Shashikumar, V, Arunodaya, H.S. 2017. Production of haploids plants from anther culture of *Musa Paradisiaca cv.* 'Puttabale'. International Journal of Research and Scientific Innovation. 4, 3.

Kumar, K. S., Bhowmik, D., Duraivel, S., Umadevi, M. 2012. Traditional and medicinal uses of banana. Journal of pharmacognosy and phytochemistry. 1(3), 51-63.

Kumar, P Suresh, Keran, D Amelia, Shiva, K N, Sivananth, C, Kamaraju, K, Uma, S. 2023. Banana products for new business horizons. ICAR-National Research Centre for Banana. Thongamalai road, Tiruchirappalli-620 102, Tamil Nadu, India.

Kumar, P.S., Saravanan, A., Sheeba, N., Uma, S. 2019. Structural, functional characterization and physicochemical properties of green banana flour from dessert and plantain bananas (*Musa* spp.). LWT, 116, 108524. https://doi.org/10.1016/j.lwt.2019.108524

Kumari, S., Gogoi, S.S., Shamim, M.Z., Laskar, I., Mohanta, T.K., Penna, S., Mohanta, Y.K. 2022. Physicochemical characterization, antioxidant activity and total phenolic content of value-added products from indigenous banana varieties of Assam, India. Measurement: Food 7, 100040. https://doi.org/10.1016/j.meafoo.2022.100040

Kwa, M. 2003. Activation de bourgeons latents et utilisation de fragments de tige du bananier pour lapropagation en masse de plants en conditions horticoles *in vivo*. Fruits. 58, 315–328.

Lalrinsanga, R., Vanlaldiki, H., Meitei, W.I. 2013. *In vitro* shoot tip culture of banana cultivar Meitei Hei. The Bioscan, 8(3), 839-844.

Langford, E., Trail, P.J., Bicksler, A.J., Burnette, R. 2017. An evaluation of banana macropropagation techniques for producing Pig fodder in Northern Thailand. Sustainable Agriculture Research. 6(2), 48. https://doi.org/10.5539/sar.v6n2p48.

Lunkes, L.B.F., Hashizume, L.N. 2014. Evaluation of the pH and titratable acidity of teas commercially available in Brazilian market. RGO – Revista Gaucha de Odontologia. 62(1), 59–64.

Madhu, C., Krishna, K.M. Reddy, K.R. Lakshmi, P.J., Kelari, E., Kumar. 2017. Estimation of Crude Fibre Content from Natural Food Stuffs and its Laxative

Activity Induced in Rats. International Journal of Pharma Research and Health Sciences. 5(3), 1703–1706. https://doi.org/10.21276/ijprhs.2017.03.04.

Madhulatha, P., Anbalagan, M., Jayachandran, S., Sakthivel, N. 2004. Influence of liquid pulse treatment with growth regulators on in vitro propagation of banana (*Musa* spp. AAA). Plant Cell, Tissue and Organ Culture. 76, 189-192.

Maharani, R., Yutaka, T., Yajima, T., Minoru, T. 2010. Scrutiny on physical properties of sawdust from tropical commercial wood species: effects of different mills and sawdust's particle size. Indonesian Journal of Forestry Research. 7(1), 20-32.

Malemba, R.R., Ramjan, M., Der, M.L., Wangchu, L., Devachandra, N., Pattanaaik, S.K., Bhutia, N.D., Shantikumar, L. 2021. Effect of media and corm preparation methods on banana macro-propagation. The Pharma Innovation Journal. 10(10), 131-140.

Manju P.R., Pushpalatha P.B. 2020. Macropropagated plantlets in banana: performance evaluation with suckers and tissue culture plants in Grand Naine and Nendran. Journal of Tropical Agriculture and Food Science. 58(2), 263–268.

Manokari, M., Badhepuri, M.K., Cokulraj, M., Rajput, B.S., Dey, A., Faisal, M., Shekhawat, M.S. 2022. High-throughput in vitro propagation and evaluation of foliar micro-morpho-anatomical stability in *Musa acuminata* cv. 'Grand Nain' using 6-benzoyladenine (BOA) in the nutrient medium. Scientia Horticulturae. 304, 111334

Marroquin, C.G., Paduscheck, C., Escalant, J.V. 1993. Somatic embryogenesis and plant regeneration through cell suspensions in *Musa acuminata*. *In Vitro* Cellular and Development Biology. 29, 43-46.

Maseko, K.H., Regnier, T., Meiring, B., Wokadala, O.C., Anyasi, T.A. 2024. *Musa* species variation, production, and the application of its processed flour: a review. Scientia Horticulturae. 325, 112688.

Mathew, B., Hasan, M.A., Mazumder, D., Chattopadhyay, P.K. 2000. Performance of first ratoon crop in banana as influenced by parent pseudostem and sucker management. Indian Journal of Agricultural Sciences. 70(9), 558-588.

Mathukmi, K., Sarkar, A., Ariina, M. S., Pratap, A. 2022. Variability studies on banana (Musa spp.) genotypes of Nagaland. The Pharma Innovation Journal. 169-172.

Matsumoto, K., da Silva, N.S.P. 2003. Micropropagation of Bananas. In Micropropagation of Woody Trees and Fruits. Dordrecht: Springer Netherlands. 353-380.

Mbabazi, R., Harding, R., Khanna, H., Namanya, P., Arinaitwe, G., Tushemereirwe, W., Dale, J., Paul, J.Y. 2020. Pro-vitamin A carotenoids in East African highland banana and other *Musa* cultivars grown in Uganda. Food Science and Nutrition, 8(1), 311–321. https://doi.org/10.1002/fsn3.1308.

Mekaunint, T.F., Aragaw, D., Geto, G., Ayele, A., Girma, N. 2024. Evaluation of Macro Propagation Techniques for Multiplication of Banana (*Musa* spp.). Journal of Oasis Agriculture and Sustainable Development. 6(02), 14-21. https://doi.org/10.56027/JOASD.172024.

Menezes, E.W., Tadini, C.C., Tribess, T.B., Zuleta, A., Binaghi, J., Pak, N., Vera, G., Dan, M.C.T., Bertolini, A.C., Cordenunsi, B.R., Lajolo, F M. 2011. Chemical composition and nutritional value of unripe banana flour (*Musa acuminata,* var. Nanicão). Plant Foods for Human Nutrition. 66(3), 231–237. https://doi.org/10.1007/s11130-011-0238-0

Ministry of Food Processing Industries, Government India (2021). Diagnostic Report - Assessment Study for identifying gaps in infrastructure & processing facilities for development of banana value chain under Operation Greens Scheme. https://www.mofpi.gov.in/sites/default/files/study_on_infrastructure_gaps_-_banana.

Mintah, L.O., Arhin, L. 2020. Development of appropriate *in vivo* technique for rapid field multiplication of plantain (*Musa* AAB) using coconut (*Cocos nucifera* L.) water and indole-3-acetic acid. African Journal of Food, Agriculture, Nutrition and Development. 20(5), 16309-16324. https://doi.org/10.18697/AJFAND.93.18580.

Mitra, S., Debnath, P., Rai, R., Srivastava, N., Rao, G.P., Baranwal, V.K. 2022. Identification of 'Ca. *Phytoplasma asteris*', banana bunchy top virus and banana streak MY virus associated with Champa and Sabri banana cultivars in Tripura, a North Eastern state of India. European Journal of Plant Pathology. 163(4), 907–922. https://doi.org/10.1007/s10658-022-02528-4.

Mohapatra, D., Mishra, S., Sutar, N. 2010. Banana and its by-product utilisation: an overview. Journal of Scientific and Industrial Research. 69. 323-329.

Mohiuddin, M., Hasan, M.R., Miah, M.M. 2020. Profitability analysis of cultivation of banana cultivars sabri and sagar in some areas of Narsingdi District. Bangladesh Journal of Agricultural Research. 45(2), 175-183. https://doi.org/10.3329/bjar.v45i2.59865.

Muhammad, A., Hussain, I., Naqvi, S.M.S., Rashid, H. 2004. Banana plantlet production through tissue culture. Pakistan Journal of Botany. 36, 617-620.

Murry, N., Das, S. 2019. An economic analysis of banana cultivation in Wokha district of Nagaland, India. Indian Journal of Pure & Applied Biosciences.7(6), 140-145.

Muscolo, A., Cutrupi, S., Nardi, S., 1998. IAA detection in humic substances. Soil Biology and Biochemistry. 30(8), 1199-1202.

Muzanila, Y.C., Mwakiposa, V. 2003. Assessment of nutritional status of traditionally prepared banana flour "Khenyangwa." African Crop Science Conference Proceedings. 564–567.

Nadeeshani, H., Samarasinghe, G., Silva, R., Hunter, D., Madhujith, T. 2021. Proximate composition, fatty acid profile, vitamin and mineral content of selected banana varieties grown in Sri Lanka. Journal of Food Composition and Analysis, 100, 103887. https://doi.org/10.1016/j.jfca.2021.103887.

Nardi, S., Pizzeghello, D., Reniero, F., Rascio, N. 2000. Chemical and biochemical properties of humic substances isolated from forest soils and plant growth. Soil Science Society of America Journal. 64(2), 639-645. https://doi.org/10.2136/sssaj2000.642639x.

National Horticultural Board. 2018. Horticultural statistics at a glance. https://www.nhb.gov.in/statistics/Publication/Horticulture%20Statistics%20at%20a%20Glance-2018.

Nayak, P.K., Pavani, K., Vani, V.D., Panda, C.M. 2020. Influence of different propagation methods in vegetative characters of some commercial varieties of banana. International Journal of Current Microbiology and Applied Sciences. 9(11), 2904-2911. https://doi.org/10.20546/ijcmas.2020.911.352.

Ngomuo, M., Mneney, E. Ndakidemi, P. 2014. The *in vitro* Propagation Techniques for Producing Banana Using Shoot Tip Cultures. American Journal of Plant Sciences. 5, 1614-1622. https://doi.org/10.4236/ajps.2014.511175.

Ngullie, T., Deb, C. R. 2024. Development of a simple macropropagation technique of banana through corms splitting and formulation of effective potting media. South

African Journal of Botany, 174, 870-875. https://doi.org/10.1016/j.sajb.2024.10.002.

Njau, N., Mwangi, M., Kahuthia-Gathu, R., Muasya, R., Mbaka, J. 2011. Macropropagation technique for production of healthy banana seedlings. The African Crop Science Journal Conference Proceeding. 10, 469–472.

Njukwe, E., Ouma, E., Asten, P.V., Muchunguzi, P., Amah, D. 2013. Challenges and opportunities for macropropagation technology for *Musa* spp. among small holder farmers and small-and medium-scale enterprises. In: Blomme, G., Asten, P.V., Vanlauwe, B., (eds.) Banana Systems in the Humid Highlands of Sub-Saharan Africa: Enhancing Resilience and Productivity. CABI. 66-71. https://doi.org/10.1079/9781780642314.0066.

Njukwe, E., Tenkouano, A., Amah, D., Sadik, K., Perez, M., Nyine, M., Dubois, T. 2007. Macropropagation of Banana and Plantain: Training Manual. Entebbe: ASARECA. 1–23. https://hdl.handle.net/10568/91415.

Ntamwira, J., Sivirihauma, C., Ocimati, W., Bumba, M., Vutseme, L., Kamira, M., Blomme, G. 2017. Macropropagation of banana/plantain using selected local materials: a cost-effective way of mass propagation of planting materials for resource-poor households. European Journal of Horticultural Science. 82(1), 38–53. https://doi.org/10.17660/eJHS.2017/82.1.5.

Onwuka, G.I., Onwuka, N.D. 2005. The Effects of Ripening on the Functional Properties of Plantain and Plantain Based Cake. International Journal of Food Properties. 8(2), 347–353. https://doi.org/10.1081/JFP-200059489.

Opata, J., Skala, J., Hegele, M., Dzomeku, B.M., Wunsche, J.N. 2020. Macropropagation of banana (*Musa* AAA): responses to hormonal and mechanical corm manipulation. Fruits. 75(2), 78–83. https://doi.org/10.17660/TH2020/75.2.3.

Osuji, J O., Okoli, B.E., Edeoga, H.O. 2006. Karyotypes of the A and B genomes of *Musa* L. Cytologia. 71, 21–24.

Oyeyinka, B.O., Afolayan, A.J. 2020. Potentials of *Musa* species fruits against oxidative stress-induced and diet-linked chronic diseases: *In vitro* and *in vivo* implications of micronutritional factors and dietary secondary metabolite compounds. Molecules. 25(21), 5036. https://doi.org/10.3390/molecules25215036.

Parmar, H C. Vinod, B.M. 2018. Quality of banana influenced by different varieties and planting materials. International Journal of Current Microbiology and Applied Science. 7(6), 2227-2231. https://doi.org/10.20546/ijcmas.2018.706.264.

Patel, B., Ahlawat, T.R., Jadhav, S., Pandey, A.K. 2024. Effect of Green Manuring and Nutrient Management on Growth and Yield of Banana CV. Grand Nain. International Journal of Plant and Soil Science. 36(5), 893-902.

Patel, M.K., Rath, S.S. 2018. Standardization of Macro Propagation in Banana cultivars - A Review. International Journal of Current Microbiology and Applied Sciences. 7(9), 390-400. https://doi.org/10.20546/ijcmas.2018.709.048.

Paulos, M., Joshi, V.R., Pawar, S.V. 2013. Effect of BAP and NAA on *in vitro* shoot establishment and proliferation of banana (*Musa paradisiaca*) cv Grand naine. International Journal of Scientific Research. 2, 318-323.

Pereira, A., Maraschin, M. 2015. Banana (*Musa spp*) from peel to pulp: Ethno pharmacology, source of bioactive compounds and its relevance for human health. Journal of Ethno Pharmacology. 160, 149–163.

Pillay, M., Tripathi, L. 2007. Banana. In Fruits and Nuts. Berlin, Heidelberg: Springer Berlin Heidelberg. 4, 281-301. https://doi.org/10.1007/978-3-540-34533-6_15.

Ploetz, R.C., Kepler, A. K., Danniells, J., Nelson, S.C. 2007. Banana and plantain- an overview with banana on Pacific island cultivars (Musaceae) banana family. Species Profile for Pacific Island Forestry. 1, 21-32.

Pongprasert, N., Sekozawa, Y., Sugaya, S., Gemma, H. 2011. A novel postharvest UV-C treatment to reduce chilling injury (membrane damage, browning and chlorophyll degradation) in banana peel. Scientia Horticulturae. 130(1), 73–77. https://doi.org/10.1016/j.scienta.2011.06.006.

Popoola, B.M. 2022. Biodegradable waste. In Recycling Strategy and Challenges Associated with Waste Management Towards Sustaining the World. Intech Open. 1–10. https://doi.org/10.5772/intechopen.107910.

Prabhuling, G., Rashmi, H., Babu, A.G. 2017. Protocol for tissue culture propagation of banana cv. Rajapuri bale (AAB). International Journal of Science and Nature. 8 (4), 892-897.

Prabhuling, G., Sathyanarayana, B.N. 2017. Liquid medium culture method for rapid multiplication of banana (*Musa acuminata*) cv. 'Grand naine' through tissue

culture. International Journal of Plant Sciences. 12 (1), (85–89). https://doi.org/10.15740/HAS/IJPS/12.1/85-89

Pragati, S., Genitha, I., Ravish, K. 2014. Comparative study of ripe and unripe banana flour during storage. Journal of Food Processing & Technology. 5(11), 1-6.

Pusponegoro, I.H., Mujiyo, M., Suntoro, S., Herawati, A., Widijanto, H. 2018. Planning of banana plant development based on the land conservation aspect in Jenawi District. Journal of Degraded Mining Lands Management. 5(4), 1319-1326. https://doi.org/10.15243/jdmlm.2018.054.1319.

Rahman, M M., Rabbani, M.G., Rahman, M.A., Uddin, M.F. 2002. *In vitro* shoot multiplication and rooting of banana cv. Sabri. Pakistan Journal of Biological Sciences. *5*(2), 161-164.

Rahman, S., Biswas, N., Hassan, M. M., Ahmed, M.G., Mamun, A.N. K., Islam, M.R., Haque, M.E. 2013. Micro propagation of banana (*Musa* sp.) cv. Agnishwar by *in vitro* shoot tip culture. International Research Journal of Biotechnology. 4(4), 83-88.

Ramirez, J.S. 2020. Generation and proliferation rate assessment of saba banana (*Musa balbisiana*) as affected by irrigation levels and plant growth enhancers under glasshouse condition. Journal of Biodiversity and Environmental Sciences. 16(1), 37-41.

Reddy, D.R.D., Suvarna, D., Rao, D.M. 2014. Effects of 6-benzyl amino purine (6-BAP) on *in vitro* shoot multiplication of Grand Naine (*Musa* sp.). International Journal of Advanced Biotechnology and Research. 5, 36-42.

Rehana, S., Alam, M.S., Islam, K.S., Samad, M.A. 2009. Influence of growth regulators on shoot proliferation and plantlet production from shoot tips of banana. Progressive Agriculture. 20(1-2), 9-16. https://doi.org/10.3329/pa.v20i1-2.16840.

Rop, O., Sochor, J., Jurikova, T., Zitka, O., Skutkova, H., Mlcek, J., Salas, P., Krska, B., Babula, P., Adam, V., Kramarova, D., Beklova, M., Provaznik, I., Kizek, R. 2010. Effect of five different stages of ripening on chemical compounds in medlar (*Mespilus germanica* L.). Molecules. 16(1), 74–91. https://doi.org/10.3390/molecules16010074.

Roy, O.S., Bantawa, P., Ghosh, S.K., da Silva, J.A.T., Ghosh, P.D., Mondal, T.K. 2010. Micropropagation and field performance of 'Malbhog' (*Musa paradisiaca*, AAB

group): a popular banana cultivar with high keeping quality of North East India. Tree and Forestry Science and Biotechnology. 4(1), 52-58.

Sabu, M., Joe, A., Sreejith, P.E. 2014. Brief overview of diversity of wild Indian Musaceae. In *XXIX* International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): IX 1114 (pp. 75-80).

Sabu, M., Joe, A., Sreejith, P.E. 2016. Brief overview of diversity of wild Indian musaceae. Acta Horticulturae. 1114, 75-80. https://doi.org/10.17660/ActaHortic.2016.1114.10.

Sadasivam, S. 1996. Biochemical methods. New age international.

Safarpour, M., Sinniah, U.R., Subramanian, S., Swamy, M. K. 2017. A novel technique for *Musa acuminata* Colla 'Grand Naine' (AAA) micropropagation through transverse sectioning of the shoot-apex. *In-vitro* Cellular and Developmental Biology Plant. https://doi.org/10.1007/s11627-017-9809-6.

Safwat, G., Rahman, A.F., El Sharbasy, S. 2015. The effect of some antioxidants on blackening and growth of *in vitro* culture of banana (*Musa* spp. cv. Grand naine). Egyptian Journal of Genetics and Cytology. 44(1), 47-59.

Sahijram, L., Soneji, J.R., Bollamma, K.T. 2003. Analyzing somaclonal variation in micropropagated bananas (*Musa* spp.). *In Vitro* Cellular & Developmental Biology-Plant. 39, 551-556.

Sahoo, C., Beura, S., Rout, S., Beura, R. 2015. High frequency in vitro cloning of banana (*Musa acuminata*) cv. Grande Naine. International Journal of Agriculture, Environment and Biotechnology. 8(4), 943-950. https://doi.org/10.5958/2230-732X.2015.00107.2

Sahreen, S., Khan, M., Khan, RA. 2010. Evaluation of antioxidant activities of various solvent extracts of *Carisa apaca* fruits. Food Chem. 122, 1205–1211.

Said, K.A.M., Radzi, Z., Yakub, I., Amin, M.A.M. 2016. Extraction and quantitative determination of ascorbic acid from banana peel *Musa acuminata* Kepoka. IIUM Engineering Journal. 17(1), 103-114.

Sajith, K.P., Uma, S., Saraswathi, M.S., Backiyarani, S., Durai, P. 2014. Macropropagation of banana-effect of bio-fertilizers and plant hormones. Indian Journal of Horticulture. 71 (3), 299–305.

Sardá, F.A.H., de Lima, F.N., Lopes, N.T., Santos, A.D.O., Tobaruela, E.D.C., Kato, E.T., Menezes, E.W. 2016. Identification of carbohydrate parameters in

commercial unripe banana flour. Food Research International. 81, 203-209. https://doi.org/10.1016/j.foodres.2015.11.016.

Schjoerring, J.K., Cakmak, I., White, P.J. 2019. Plant nutrition and soil fertility: synergies for acquiring global green growth and sustainable development. Plant and Soil. 434, 1-6. https://doi.org/10.1007/s11104-018-03898-7.

Schulz, H., Dunst, G., Glaser, B. 2013. Positive effects of composted biochar on plant growth and soil fertility. Agronomy for Sustainable Development. 33, 817-827. https://doi.org/10.1007/s13593-013-0150-0.

Shahidi, F. 2000. Antioxidants in food and food antioxidants. Food Nahrung. 44(3), 158–163.

Shalini, S. 2019. Low cost media development for *in vitro* micropropagation of banana cultivars Karpuravalli (ABB) and Grand Naine (AAA). Department of Biotechnology, School of Bio Science and Technology, Vellore Institute of Technology, University. http://hdl.handle.net/10603/306921

Shankar, C.S., Balaji, P., Sekar, D.S. 2014. Mass propagation of banana (*Musa* spp) cv. Grand Naine through direct organogenesis by benzyl adenine purine and kinetin. Journal of Academia and Industrial Research.3, 92-97.

Shankar, K., Chavan, L., Shinde, S., Patil, B. 2011. An improved DNA extraction protocol from four *in vitro* banana cultivars. Asian Journal of Biotechnology. 84–90. doi:10.3923/ajbkr.2011.84.90

Sharma, B., Vaish, B., Monika, S., Singh, P., Singh, R.P. 2019. Recycling of Organic Wastes in Agriculture: An Environmental Perspective. International Journal of Environmental Research. 13(2), 409–429. https://doi.org/10.1007/s41742-019-00175-y.

Sheng, Z.W., Ma, W.H., Jin, Z.Q., Bi, Y., Sun, Z.G., Dou, H.T., Li, J.Y. and Han, L.N., 2010. Investigation of dietary fiber, protein, vitamin E and other nutritional compounds of banana flower of two cultivars grown in China. African Journal of Biotechnology. 9(25), 3888-3895.

Shirani, S., Mahdavi, F., Maziah, M. 2009. Morphological abnormality among regenerated shoots of banana and plantain (*Musa* spp.) after *in vitro* multiplication with TDZ and BAP from excised shoottips. African Journal of Biotechnology. 8(21), 5755-5761.

Siji, S., Nandini, P.V. 2017. Antioxidants and antioxidant activity common eight banana varieties in Kerala. International Journal of Advanced Engineering Research and Science. 4(7), 118–123. https://doi.org/10.22161/ijaers.4.7.18

Simmonds, N.W., Shepherd, K. 1955. The taxonomy and origins of the cultivated bananas. Botanical Journal of the Linnean Society. 55(359), 302-312.

Singh H.P., Uma S., Selvarajan R., Karihaloo J.L. 2011. Micropropagation for production of quality banana planting material in Asia pacific. Asia-Pacific Consortium on Agricultural Biotechnology. 1-81.

Singh, B., Singh, J.P., Kaur, A., Singh, N. 2016. Bioactive compounds in banana and their associated health benefits - a review. Food Chemistry. 206, 1–11. https://doi.org/10.1016/j.foodchem.2016.03.033.

Singh, R.W., Singh, S.S., Shrivastava, K. 2014. Analysis of banana genome groups of wild and cultivated cultivars of Manipur, India using sScore card method. South Indian Journal of Biological Sciences. 2, 213-221.

Smitha, P.D., Binoy, K.R., Nair, A.S. 2014. Effect of TDZ on direct shoot regeneration from whole male inflorescence of four diploid banana cultivars from South India. Plant Science International. 1(1), 24-32. https://doi.org/10.12735/psi.v1n1p24.

Someya, S., Yoshiki, Y., Okubo, K. 2002. Antioxidant compounds from bananas (*Musa* cavendish). Food Chemistry. 79, 351–354.

Strosse, H., Andre, E., Sági, L., Swennen, R., Panis, B. 2008. Adventitious shoot formation is not inherent to micropropagation of banana as it is in maize. Plant Cell, Tissue and Organ Culture. 95, 321-332. https://doi.org/10.1007/s11240-008-9446-1.

Subagyo, A., Chafidz, A. 2018. Banana pseudo-stem fiber: preparation, characteristics, and applications. Banana Nutrition-Function and Processing Kinetics, 20(4), 1-19.

Subrahmanyeswari, T., Gantait, S. 2022. Biotechnology of banana (*Musa* spp.): multi-dimensional progress and prospect of in vitro–mediated system. Applied Microbiology and Biotechnology, 106(11), 3923-3947

Subrahmanyeswari, T., Gantait, S., Sarkar, S., Bhattacharyya, S. 2022. Accelerated mono-phasic *in vitro* mass production of banana propagules and their morpho-cyto-genetic stability assessment. South African Journal of Botany. 146, 794-806.

Sulaiman, S.F., Yusoff, N.A.M., Eldeen, I.M., Seow, E.M., Sajak, A.A.B., Supriatno, Ooi, K.L. 2011. Correlation between total phenolic and mineral contents with antioxidant activity of eight Malaysian bananas (*Musa* sp.). Journal of Food Composition and Analysis. 24(1), 1–10. https://doi.org/10.1016/j.jfca.2010.04.005.

Suman, S., Rajak, K.K., Kumar, H. 2013. Micropropagation of banana cv. BB Battisa. Biochemical and Cellular Archieves. 13, 249-254.

Survey on Horticulture Fruit Crops in Nagaland. 2015. Directorate of Economics and Statistics, Nagaland. https://www.statistics.nagaland.gov.in/storage/statistical_data/2016/2131601161690.

Suryanarayana, P., Panda, C., Mishra, S. 2018. Morphological and yield attributing parameters of macro-propagated cultivars of banana (*Musa* spp L.). The Pharma Innovation Journal. 7(8), 240-245.

Tabassum, P., Khan, S.A.K.U., Siddiqua, M., Sultana, S., 2018. Effect of guava leaf and lemon extracts on postharvest quality and shelf life of banana cv. Sabri (*Musa sapientum* L.). Journal of the Bangladesh Agricultural University. 16(3), 337–342. https://doi.org/10.3329/jbau.v16i3.39489.

Tan, L., He, Y., Li, S., Deng, J., Avula, B., Zhang, J., Pugh, N. D., Solis-Sainz, J. C., Wang, M., Katragunta, K. 2024. Proximate composition and nutritional analysis of selected bananas cultivated in Hainan, China. Journal of Food Composition and Analysis. 125, 105798. https://doi.org/10.1016/j.jfca.2023.105798.

Teisson, C., Côte, F.X. 1997. Micropropagation of *Musa* species (bananas). In High-Tech and Micropropagation V. Biotechnology in Agriculture and Forestry; Springer, Berlin, Heidelberg. 39, 103-126. https://10.1007/978-3-662-07774-0_7.

Thatayaone, M., Saji, G., Meagle, J., Netravati, Bintu, K. 2022. Biochemical and nutritional characteristics of some commercial banana (*Musa* spp.) cultivars of Kerala. Plant Science Today. 9(3), 681–686. https://doi.org/10.14719/pst.1733.

Thiagarajan, J.D., Kulkarni, S.V., Jadhav, S.A., Waghe, A.A., Raja, S.P., Rajagopal, S., Subramaniam, S. 2024. Analysis of banana plant health using machine learning techniques. Scientific Reports. 14(1), 15041.

Thiemele, D.E.F., Issali, A.E., Traore, S., Kouassi, K.M., Aby, N., Gnonhouri, P.G., Kobenan, J.K., Yao, T.N., Adiko, A., Zakra, A.N. 2015. Macropropagation of plantain (*Musa* spp.) cultivars PITA 3, FHIA 21, ORISHELE and CORNE 1: effect of benzylaminopurine (BAP) concentration. Journal of Plant Development Sciences 22, 31–39.

Thorpe, T.A. 2007. History of plant tissue culture. Molecular Biotechnology. 37(2), 169–180. https://doi.org/10.1007/s12033-007-0031-3.

Thungon, S.C., Hazarika, D.N., Langthasa, S., Goswami, R.K., Kalita, M.K. 2017. Standardization of growing media for macropropagation of malbhog (AAB) banana. Journal of Food Composition and Analysis. 12 (6), 270-277.

Tiatemsu, P., Deb, C.R., Asossi, P. 2023. Extended distribution of two wild bananas for the flora of Nagaland. Plant Science Today. 10(3), 328-334. Tripathi, S., Patil, B.L., Verma, R. 2016. Viral diseases of banana and their management. Plant Viruses: Evolution and Management. 289-308.

Uma, S., Karthic, R., Kalpana, S., Backiyarani, S., Kumaravel, M., Saranya, S., Saraswathi, M.S., Durai, P. 2023. An efficient embryogenic cell suspension culture system through secondary somatic embryogenesis and regeneration of true-to-type plants in banana cv. Sabri (silk subgroup AAB). Plant Cell, Tissue and Organ Culture. 155(1), 313–322. https://doi.org/10.1007/s11240-023-02570-y.

United Fruit Company. 2018. Food Value of the Banana: Opinion of Leading Medical and Scientific Authorities. Create Space Independent Publishing Platform. https://books.google.co.in/books?id=ZqvmswEACAAJ.

Uzaribara, E., Nachegowda, V., Ansar, H., Sathyanarayana, B.N., Taj, M. 2015. *In vitro* propagation of red banana (*Musa acuminata*). The Bioscan. 10(1), 125-130.

Vaughan, D., Malcolm, R.E., Ord, B.G. 1985. Influence of humic substances on biochemical processes in plants. In: Vaughan, D., Malcolm, R.E., (eds) Soil Organic Matter and Biological Activity. Developments in Plant and Soil Sciences. Vol. 16. Springer, Dordrecht. https://doi.org/10.1007/978-94-009-5105-1_3.

Viswanath, M., Mangalore, C.N., Roy, A., Chatterjee, S., Nanda, S.P. 2021. Effect of different plant growth regulators on mass propagation of banana cv. Kovvur Bontha (ABB) through macropropagation. The Pharma Innovation Journal. 10(6), 713-716.

Vu, H.T., Scarlett, C.J., Vuong, Q.V. 2018. Phenolic compounds within banana peel and their potential uses: A review. Journal of Functional Foods. 40, 238–248. https://doi.org/10.1016/j.jff.2017.11.006.

Vyas, P.H., Kanzaria, D.R., Butani, A.M. 2020. Organic Farming In Banana And Guava. International Review of Business and Economics. 4(2), 118-123. https://doi.org/10.56902/IRBE.2020.4.2.26.

Wall, M.M. 2006. Ascorbic acid, vitamin A, and mineral composition of banana (*Musa* sp.) and papaya (*Carica papaya*) cultivars grown in Hawaii. Journal of Food Composition and Analysis. 19(5), 434–445. https://doi.org/10.1016/j.jfca.2006.01.002.

White, P.J., George, T.S., Dupuy, L.X., Karley, A.J., Valentine, T.A., Wiesel, L., Wishart, J. 2013. Root traits for infertile soils. Frontiers in Plant Science. 4, 193. https://doi.org/10.3389/fpls.2013.00193.

Wiyono, H., Widono, S. 2013. Vigor of plantlet from micro plantlet treated by filtrate and cell suspension of some isolates of bacillus and resistance to banana wilt pathogen after acclimatization. International Journal Plant Pathology. 2, 70-75.

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 Organ Culture. 6, 159-166.

World Data and Statistics. 2024. Banana Production by Country. Retrieved from https://worldostats.com/banana-production-by-country-2024/.

Yadav, A.N., Kour, D., Ahluwalia, A.S. 2021. Soil and phytomicrobiomes for plant growth and soil fertility. Plant Science Today 8 (sp1), 1-5. https://doi.org/10.14719/pst.1523.

List of Publications

Research Papers Published /Communicated

- Ngullie, T., Deb, C. R. 2024. Development of a simple macropropagation technique of banana through corms splitting and formulation of effective potting media. South African Journal of Botany, 174, 870-875. https://doi.org/10.1016/j.sajb.2024.10.002.
- Ngullie, T. and Deb, C. R. In-sight into the nutritional values of different parts of raw and ripe fruits of two commercially viable banana cultivars 'Grand Nain' and 'Sabri' exploring for value addition. Journal of Experimental Biology and Agricultural Sciences. (Under Review).
- Ngullie, T. and Deb, C. R. Development and Optimization of Macro and Micropropagation Protocols for *Musa* Cultivar 'Sabri' for Enhanced Commercial Production. Current Agriculture Research Journal. (Under Review).
- Ngullie, T. and Deb, C. R. *In vitro* Clonal Propagation and Evaluation of Clonal Plantlets and Genetic Variability Assessment in Banana cv. Grand Nain.
- American Journal of Plant Sciences. (Under Review).

Patent Filed/ Published

- Ngullie, T. and Deb, C. R. A Novel Biofertilizer for Pseudostem and Process There off. Indian Patent (Indian Patent Application No. 202431028203 A, dated 19/04/2024). (Patent Published).
- Ngullie, T. and Deb, C. R. A Novel Biofertilizer for Pseudostem and Process There off. (South African Patent Application No. 2024/09105, dated 28.11.2024). (Filed)

South African Journal of Botany 174 (2024) 870–875

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Development of a simple macropropagation technique of banana through corms splitting and formulation of effective potting media

Thejano Ngullie, Chitta Ranjan Deb*

Department of Botany, Nagaland University, Lumami 798627, Nagaland, India

ARTICLE INFO

Article History:
Received 7 April 2024
Revised 30 September 2024
Accepted 1 October 2024
Available online xxx

Edited by Dr P. Bhattacharyya

Keywords:
Banana
Potting mixture for macropropagation
Sustainable agriculture
Macropropagation
Potting media

ABSTRACT

The banana is very popular globally for its flavour, nutrition, and economic value. However, slow regeneration from suckers, and diseases, there is shortfall of clean planting materials. Though, the *in vitro* micropropagation is effective for rapid production of planting materials, but due to high cost, the small-scale poor farmers unable to afford. The macropropagation technique offers an alternative and cost effective way to produce planting materials. This study present a simple, efficient and cost efficient macropropagation protocol of banana for formulation of effective potting mix. The experimental design was based on different corm/pseudostem splitting technique and different combinations of potting mix substrata. The overall optimum response in macropropagation techniques was found in PIF (T_3) and Split corm + PIF method (T_5) and Forest soil (P_3) and Saw dust + Sand (P_5). For the study banana cultivar Grand Nain (G9) was taken as a model plant. The regenerated plantlets was assessed for genetic stability using two markers i.e., RAPD and SCoT and found that the regenerates were 97.37–100 % genetically stable. Protocol developed is simple, reproducible, cost effective and sustainable and can be followed by the unskilled farmers. Though, in this study the G9 cultivar was used as model, the technique can be effectively used for other banana cultivars.

1. Introduction

Though there is an ever increasing demand for clonal planting materials of cultivated banana, but natural regeneration through rhizome splitting of the mother plant is too slow and susceptible to different diseases and thus fails to meet the commercial demand. The viable alternative to the natural regeneration process is *in vitro* propagation technique to meet the demands of large-scale planting materials. But, the technology is beyond the limits of marginal unskilled poor farmers living in extreme rural areas deprived of sophisticated facilities. Due to the higher cost of quality planting materials, poor and marginal farmers are often unable to afford the costly planting materials, which are 4–8 times costlier than the vegetative propagated suckers (Suryanarayana et al., 2018), hence are not an option for the framers. Therefore, it is crucial to develop simple, effective and sustainable methods of propagation banana enabling the unskilled remote farmers to produce their own planting materials with ease for small to medium scale cultivation.

A viable alternative approach to *in vitro* propagation is macropropagation techniques, requiring simple methods, inexpensive tools, and a less skilled workforce and can be adopted by the marginal farmers for producing clonal planting materials by themselves for a small to medium scale cultivation plot. This propagation technique is either done on-field or off-field, where it necessitates the acceleration of the lateral meristem, resulting in the formation of 10–30 plantlets by a single corm in a short period of time (Kasyoka et al., 2010). In the past many studies were undertaken for macropropagation of banana using different rhizome/corm manipulation techniques and found effective especially for small and rural stakeholders (Izaile et al., 2021; Opata et al., 2020; Suryanarayana et al., 2018; Thungon et al., 2017). Although, several reports are available on macropropagation of banana, but there are challenges in the practicality of the experiments (Langford et al., 2017) along with lack of awareness amongst the remote and poor farmers. With this background, the present study was undertaken to develop a simple and effective sustainable macropropagation technique of banana following different sucker fragmentation technique and formulation of effective low-cost potting medium. For the study, a well-established commercial banana cultivar Grand Nain (G9) was considered as sample banana crop.

The experiments carried out had two main aspects: 1. Using different corm splitting methods of macropropagation; 2. using different potting mix or substrata. The study was conducted in the eco-friendly and budgetary way by using only available items like reusable containers, clay pots, biofertilizers, and naturally obtained potting substrates found readily in the University campus.

* Corresponding author.
E-mail addresses: debchitta@rediffmail.com, debchitta@gmail.com (C.R. Deb).

https://doi.org/10.1016/j.sajb.2024.10.002
0254-6299/

(12) PATENT APPLICATION PUBLICATION

(19) INDIA

(22) Date of filing of Application :05/04/2024

(21) Application No.202431028203 A

(43) Publication Date : 19/04/2024

(54) Title of the invention : A NOVEL BIOFERTILIZER FOR PSEUDOSTEM AND PROCESS THERE OFF

(51) International classification :A01H0004000000, G09B0019000000, A01K0001010000, A22C0025140000, C10L0005420000

(86) International Application No :NA
Filing Date :NA

(87) International Publication No : NA

(61) Patent of Addition to Application Number :NA
Filing Date :NA

(62) Divisional to Application Number :NA
Filing Date :NA

(71)**Name of Applicant :**
1)Nagaland University
Address of Applicant :Nagaland University, Lumami Headquarters, Zunheboto district, Nagaland, India Zunheboto ---------- ----------
Name of Applicant : NA
Address of Applicant : NA
(72)**Name of Inventor :**
1)Thejano Ngullie
Address of Applicant :Nagaland University, Lumami Headquarters, Zunheboto district, Nagaland India Zunheboto ---------- ----------
2)Chitta Ranjan Deb
Address of Applicant :Nagaland University, Lumami Headquarters, Zunheboto district, Nagaland India Zunheboto ---------- ----------

(57) Abstract :
Grand Nain is a well-liked Cavendish banana cultivar that is valued for its nutritional and commercial qualities. Nevertheless, there is a lack of clean planting materials because of diseases and steady natural regeneration. Micropropagation offers a solution, but it is inconvenient for small-scale, impoverished farmers who live in remote areas. As a result, macropropagation provides farmers with an alternate method of producing their own planting materials. Therefore, the goal of this study is to create a basic, low-cost macropropagation protocol that even inexperienced farmers can follow. The Conventional Method (TC), Incomplete Decapitation (T0), Complete Decapitation (T1), Split Corm Method (T2), PIF method (T3), Excised Bud (T4), and Split Corm + PIF Method (T5), as well as potting substrates like Garden Soil + Straw + Dried Cow Dung Manure, Sawdust (P2), and Forest Soil, were compared with each other.

No. of Pages : 20 No. of Claims : 4

FORM P1

REPUBLIC OF SOUTH AFRICA
PATENTS ACT, 1978

APPLICATION FOR A PATENT AND ACKNOWLEDGEMENT OF RECEIPT

[Section 30 (1)-Regulation 22]

The granting of a patent is hereby requested by the undermentioned applicant on the basis of the present application.

Official Application No.			Applicant's or Agent's Reference
21	01	**2024/09105**	PT_CP_ZA00011038

71	Full Name(s) of Applicant(s)
	Nagaland University Lumami Headquarters, Zunheboto district, Nagaland, Nagaland, 798627, India

54	Title of invention
	A NOVEL BIOFERTILIZER FOR PSEUDOSTEM AND PROCESS THERE OFF

The applicant claims priority as set out on the accompanying Form P.2. The earliest priority claimed is
COUNTRY: IN NUMBER: 202431028203 DATE: 2024/04/05

~~This application is for a patent of addition to patent application No.~~

21	01	

~~This application is a fresh application in terms of section 37 and based on Application No.~~

21	01	

This application is accompanied by:

X	1.	A single copy of a complete specification of 19 pages.
	2.	Drawings of _____ sheet(s).
X	3.	Publication particulars and abstract(Form P8)
	4.	A copy of a figure of the drawing (if any) for the abstract
	5.	Assignment of invention
X	6.	Certified priority document(s)
	7.	Translation(s) of the priority document(s)
	8.	Assignment of priority rights
	9.	A copy of the Form P.2 and the specification of S.A Patent Application (if applicable).
X	10.	A declaration and power of attorney on Form P3
X	11.	Statement on the use of indigenous Biological Resource, Genetic Resource, Traditional Knowledge or Use on Form P26
	12.	Translation(s) of the specifications document(s)
X	13.	Deed of Assignment

74	Address of Service:
	Sibanda and Zantwijk Oaktree Corner, 9 Kruger Street, Oaklands (PO Box 1615 Houghton 2041), Johannesburg, 2192 SOUTH AFRICA

Dated this 28th day of November 2024

Digitally signed by : Dirk Antonie Van Zantwijk

.....
Signature of Applicant(s)

This is returned to the applicant's
address for service as proof of lodging.

RECEIVED
Official Date Stamp
.....
Registrar of Patents

List of Seminar, Conference attended and Presented papers

- Hands on training on "genomics and gene expression analysis". Organised by Department of Biotechnology, Govt. Of India sponsored Advance Level Institutional Biotech Hub, Department of Botany, Nagaland University, Lumami 798627, Nagaland. July 18th -23rd, 2018.

- National conference of Stakeholders on 'Conservation, Cultivation, Resource Development and Sustainable Utilization of Medicinal Plants of North- Eastern India' jointly organized by Department of Botany (UGC- SAP DRS-III, FIST), Nagaland University Lumami and Society for Conservation and Resource Development of Medicinal plants (SMP) New Delhi at Nagaland University, Lumami, held on March 6-7, 2019.

- One-Day Workshop on 'Importance of IPR in Academics Institutions' organized by IPR Cell, Nagaland University, held on 29th May 2019.

- Hands on training on 'Molecular Taxonomy of Microbes and Higher Plants' Organized & Sponsored by Department of Biotechnology, Govt. of India sponsored by Advance Level Institutional Biotech Hub, Department of Botany, Nagaland University, Lumami 798627, Nagaland, July 17-23, 2019.

- Workshop on 'Research Ethics, paper writing & IPR' organized and sponsored by UGC-SAP(DRS-III), Department of Botany& Department of Biotechnology, Govt. of India sponsored Advance Level Institutional Biotech Hub, Nagaland University, Lumami, Nagaland, November 14-15, 2019.

- Presented paper on 'Macropropagation of *Musa acuminata* cv Grand Nain by Rhizome Splitting' in National e-conference on 'Bioresources and Sustainable Livelihood of Rural India' Department of Botany, Nagaland University, Lumami 798627, Nagaland, September 28-29, 2020.

- Presented paper on “Development of a Cost-Effective Technique for Production of Clonal Planting Materials of Grand Nain (G9) Cultivar of Banana” in an International Conference (Online) On “Novel Approaches In Life Sciences” Guru Nanak Khalsa College of Arts, Science and Commerce, Matunga, Mumbai dated 8th and 9th April 2022.

- Hands-on Training on ‘Techniques in Applied Biology’ [Under DST Sponsored ‘STUTI’] Organized by Advance Level Institutional Biotech Hub, April 21-27, 2022.

- Presented paper on “Analysis of Polyphenols, Anti-oxidant Activity and Anti-microbial Property of Pseudo-stem Sap of *Musa sp*. Cv. Grand Nain and Sabri” in an International Conference on ‘Bioresources & Bioeconomy’ (ICBB-2022) organized by Department of Botany, Nagaland university, dated: - September 19-21, 2022.

PARTICULARS OF THE CANDIDATE

NAME OF CANDIDATE: **Thejano Ngullie**

DEPARTMENT: **Department of Botany**

TITLE OF THESIS: "**Development of Protocols for Production of Clonal Planting Materials of Two Commercially Viable *Musa* Cultivars, Nutritional Assessment and Genetic Fidelity Assessment of Regenerates"**

DATE OFADMISSION: **August 18. 2018.**

APPROVAL OF RESEARCH PROPOSAL:

1. DRC : **10/05/2019**
2. BOS : **14/05/2019**
3. SCHOOL BOARD: **30/05/2019**

Ph.D. REGISTRATION NO. & DATE: **Ph. D/BOT/00155 dated August 18, 2018**

EXTENSION: **06 months w.e.f. August 18, 2024 to February 17, 2025**