Nutritional Value Assessment and Studies on the Genetic Diversity of Some Selected Wild *Musa* Species of Nagaland

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY in BOTANY

Submitted by
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नागालैंड विश्वविद्यालय

Nagaland University

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

Lumami 798 627, Nagaland, India

Department of Botany

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October 9th , 2025

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

(Prof. Chitta Ranjan Deb)
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The saying goes, “If you want to go fast, go alone. If you want to go far, go together.” The author intended to set out on this journey with the goal of going at it fast and alone, only to realise that a journey in search of knowledge was best travelled together, and indeed it has been a very long journey at that. Six years of that endeavour has led to this moment. A moment filtered through the lenses of the experiences endured, lessons learnt and bonds cherished. The author takes this moment to bring to mind everything and everyone instrumental in reaching this milestone with a sense of profound gratitude, without which this journey would not have seen its end.

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(Thejavitsu Noah Vupru)

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ABBREVIATIONS

Abbreviation	Full Form
%	Percentage
A0	Absorbance of the sample
A10	Absorbance of the sample at 10 min
Abs	Absorbance of sample
AFLP	Amplified Fragment Length Polymorphism
$AlCl_3.6H_2O$	Aluminum Chloride
AMOVA	Analysis of Molecular Variance
AT	Adenosine Tyrosine
Avg	Average
CRISPR	Clustered regularly interspaced short palindromic repeats
CTAB	Cetyltrimethylammonium bromide
DAMD	Directed Amplification of Minisatellite DNA
DAPC	Discriminant Analysis of Principal Components
DF	Dilution factor
DNA	Deoxyribonucleic Acid
DNS	3,5-Dinitrosalicylic acid
dNTPs	Deoxynucleotide Triphosphates
DPPH	2,2-diphenyl-1-picrylhydrazyl
DW	Dry Weight
g	Gram
GA	Gallic acid

GC	Guanine Cytosine
GPS	Global Positioning System
h	Hour
H	mean gene diversity
H2SO4	Sulphuric acid
HCL	Hydrochloric acid
IC50	Half maximal inhibitory concentration
INIBAP	International Network for the Improvement of Banana and Plantain
ISSR	Inter-simple sequence repeats
IUCN	International Union for Conservation of Nature
ITS	Internal Transcribed Spacer
Kcal	Kilocalorie
m	Metre
M15	*Musa itinerans* Cheesman
MA1	*Musa aurantiaca* Baker
MAS	Marker-assisted Selection
matK	Maturase K
Mb	Megabyte
MB2	*Musa balbisiana* Colla
MC3	*Musa cheesmanii* N.W.Simmonds
MEGA	Molecular Evolutionary Genetics Analysis
MF4	*Musa flaviflora* N.W.Simmonds
mg	Milli gram

mg/L	Milli gram per Litre
$MgCl_2$	Magnesium Chloride
MGIS	*Musa* Germplasm Information System
mL	Milli Litre
ML	Maximum Likelihood
mM	Milli molar
MM6	*Musa markkuana* (M.Sabu, A.Joe & Sreej.) Hareesh, A.Joe & M.Sabu
MN7	*Musa nagalandiana* S. Dey & Gogoi
MN8	*Musa nagensium* Prain
MUSCLE	Multiple Sequence Alignment
N	Normal
Na	Number of allele
Na_2CO_3	Sodium carbonate
$NaNO_2$	Sodium nitrate
NaOH	Sodium hydroxide
NCBI	National Center for Biotechnology Information
nm	Nanometre
NPB	Number of polymorphic band
NRCB	National Research Centre for Banana
ºC	Degree Celsius
PCR	Polymerase chain reaction
PIC	Polymorphic information content
PPB	Percentage of polymorphic band

PVA	Provitamin A
RAPD	Random amplified polymorphic DNA
rbcL	ribulose-1,5-bisphosphate carboxylase/oxygenase
RE	Retinol Equivalent
SD	Standard deviation
SNP	Single Nucleotide Polymorphism
Sp.	Species
SRAP	Sequence Related Amplified Polymorphism
SSR	Simple Sequence Repeat
TE	Total Ethanolic Extract
TEAC	Trolox Equivalent Antioxidant Capacity
TF	Total flavonoid
Tm	Melting temperature
TNB	Total number of bands
TP	Total phenol
UBC	University of British Columbia
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
UV-Vis	Ultraviolet-Visible Spectroscopy
V	Total extract volume
v/v	Volume/Volume
viz	Which
W_1	Pre-weighted dish
W_2	Pre-weighted dish with sample
W_3	Pre-weighted dish with samples after ignition

WGS	Whole-Genome Sequencing
WSC	Water-soluble carbohydrates
Wt	Sample weight
Y	Dry matter content of the sample
µg	Micro gram
µL	Micro litre

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Chapter - 1

Introduction

Banana, in the botanical sense, is the name given to a taxon that includes dessert bananas, cooking bananas, plantains and their wild relatives. The etymology of the term 'banana' is most probably derived from the West African term 'banaana'. It is not only representative of the sweet dessert fruits but is also a staple food of 400 million people in the underdeveloped and developing economies. Available data indicate that between 2000 and 2017, global production of bananas grew at a compound annual rate of 3.2 percent, reaching a record of 114 million tonnes in 2017, up from around 67 million tonnes in 2000, and the projection proceeds to 136 million tonnes in 2025. The biggest producers are India, which produced 30.5 million tonnes per year on average, and China at 12 million tonnes. Approximately 5.94 million hectares of land are dedicated to banana production globally (FAOSTAT). The rapid expansion of the banana industry is evident in the evolution of the harvested area over time, which amounted to 3.6 million hectares in 1993 and 4.6 million hectares in 2000 (FAOSTAT). The international trade, which involves just a few varieties of fruit, accounts for 15% of the total production. In addition to the edible species, *Musa textilis* (abacá, Manila hemp) is important for fibre production, and there are several ornamental species (Häkkinen, 2007).

India is a treasure chest of biodiversity that hosts a large variety of plants and animals. It has been one of the major centres of origin and distribution for both wild and cultivated bananas (*Musa* spp.), especially for *balbisiana*-derived hybrids. Due to the antiquity of bananas in India, their great diversity and long history of domestication, bananas are interwoven with national heritage and culture and have great socio-economic significance. The rich genetic diversity is an irreplaceable resource, providing materials for introduction, domestication and improvement

programmes as well as opportunities for the search and selection of *Musa* genotypes resistant to pests and diseases. In this regard, the northeastern region of India is the centre of origin of many a wild species of the genus *Musa* and therefore stands a good case for exploring the genetic diversity as well as the screening of possible value nutrition from various species within the genus. There is also the impetus that information on the physicochemical, nutritional properties and genetic diversity of the wild *Musa* spp. in the area under study is largely underexplored.

The presence of a good number of wild *Musa* species belonging to the sections *Rhodoclamys* and *Eumusa* (although members of the sections *Australimusa, Callimusa* and *Ingentimusa* have not been reported) has been indicated in Nagaland by various studies. These species exhibit a wide altitudinal habitat range from low-lying areas to high altitudes beyond 2000 AMSL, and their populations intermix freely. Variations between these species, as well as members of the same species occurring at different altitudinal ranges and niches, exhibit forms of phenotypic plasticity. Mixed populations of these species further decrease the distinction between these species because of possible crossing among the members of the population. The ambiguity among the species, therefore, necessitates assessment of its genetic diversity at a molecular level.

The broad genetic pool maintained by farmers can also be used for future banana crop improvement, as banana is essentially a clonally propagated crop with many sterile species, which makes progress through conventional breeding slow and difficult. Due to the limited number of landraces and commercial varieties available and their asexual reproduction, bananas have a narrow genetic pool that makes them vulnerable to pests and diseases. As a result, screening new cultivars and wild species with the potential to improve the existing varieties and new breeding methods and tools, including biotechnology and mutation breeding, will be helpful to develop resistant bananas for cultivation without the threat of genetic drift.

Taxonomy and Description

Bananas and plantains are large herbaceous plants that can confer the aspect of a tree. One wild species (*Musa ingens*) can reach 16 m tall, although most commercial types grow to between 2 m and 5 m. The main trunk is a pseudostem formed by the

concentric assembly of the leaf sheaths (modified petioles), which is crowned by a rosette of very large oblong to elliptic leaves. The leaf blade (up to 2 m) normally is transversely split (by wind) between parallel veins, which assists cooling and photosynthesis (Karamura and Karamura, 1995). Leaves are produced successively until the single inflorescence is cast (Allen *et al*., 1988) and are present in variable numbers (10 to 20 under healthy conditions) depending on the variety, the climate and cultivation practices. Each leaf takes 7-14 days to emerge (Turner *et al.,* 2007). The true stem is a subterranean organ (corm) which extends upwards at the core of the pseudostem until culminating in the inflorescence which emerges from the top. The meristem of the true stem produces all other parts of the plant (Swennen and Rosales, 1994). The many main roots emerging from the rootstock are rather straight, adventitious cords (2-10 mm in diameter) that extend up to 2-3 (-5) m outwards, from which (with a density of 8 to 10 per cm of cord root) branch lateral roots (0.3-4 mm in diameter) that extend up to 1 m and from which tertiary rootlets extend for several centimetres (Blomme et *al.,* 2000; Belalcázar *et al.,* 2005; Draye *et al.,* 2005; Turner *et al.,* 2007). There are two conspicuous main phases in the development of the banana plant: a vegetative phase characterised by the appearance of leaves and a reproductive phase during which the inflorescence emerges (Stover and Simmonds, 1987; Swennen and Rosales, 1994; Karamura and Karamura, 1995; Turner *et al.,* 2007). This is a simplification of the actual developmental process, as the apical meristem has already differentiated from its vegetative phase into a reproductive meristem before the appearance of the last new leaves. At the transition from the vegetative to the floral stage, the plant's growing point shifts mitotic activity, with the whole dome contributing to the aerial stem and inflorescence development. The inflorescence tip first appears at the top of a pseudostem, and the inflorescence continues to elongate and mature, in crop plants typically turning downward. Floral clusters appear, enlarge, and differentiate into flowers. This elongating aerial stem is totally dependent for its mechanical support upon the surrounding mass of leaf sheaths (the pseudostem). The large inflorescence is composed of flowering clusters which occur helicoidally along its axis (reproducing the phyllotaxy pattern of the leaf system) and are each covered by a large bract typically ranging from yellow over reddish to purplish. Each nodal cluster has 12-20 flowers, which are aligned in two tightly adjacent rows. The flowers

in a cluster are usually either functionally female or male but sometimes are hermaphroditic or neuter (Kwa and Tomekpé, 2000). The first (i.e., basal) 3-9 (to 18) clusters, or hands, are female and give rise to the edible fruits. Although the principal plant dies after producing fruit, the whole plant is perennial inasmuch as suckers successively replace the senescent aerial part without the need for replanting. Several suckers emerge consecutively from buds located at leaf axils on the rhizomatous rootstock (Kurien *et al.,* 2002).

Bananas and plantains are in the genus *Musa* L. of the small family Musaceae (which also includes the genera *Ensete* and *Musella*), in the monocot order Zingiberales (formerly Scitamineae) (Kress *et al.,* 2001; Kress and Specht, 2005, 2006). *Musa* has over 60 known wild species with known diploid (2n) chromosome numbers of 22, 20, or rarely 18 or 14 (Simmonds, 1962; Shepherd, 1999). The diploid chromosome number together with phenotypic characteristics of the inflorescence and seeds have been the basis for conventional division of the genus into four, or sometimes five, sections: section *Musa* (nomenclaturally correct but often traditionally still called *Eumusa*), *Rhodochlamys*, *Callimusa* and *Australimusa*, and sometimes also *Ingentimusa* (Simmonds, 1962; Stover and Simmonds, 1987; Simmonds and Weatherup, 1990; Häkkinen, 2004; De Langhe, 2009). However, researchers are finding some sections ill-defined, and that fewer sections may be more accurate (Jarret and Gawel, 1995; Shepherd, 1999; De Langhe, 2000; Wong *et al.,* 2002, 2003; Nwakanma *et al.,* 2003; Bartoš *et al.,* 2005; cf. Häkkinen *et al.,* 2007c; De Langhe *et al.,* 2009). The *Musa* section*,* as commonly circumscribed, includes over a dozen wild species. Among these are *Musa acuminata* Colla and *Musa balbisiana* Colla, which have provided the great majority of edible bananas and plantains through selection and crossing both intraspecifically (within and between *M. acuminata* subspecies and cultigens) and interspecifically (between these two species). Cheesman (1947, 1948a, 1948b) pioneered the modern scientific classification of *Musa* (Simmonds, 1966/1973). Most edible bananas (in the broad sense) derive either from one diploid species or from its diploid or triploid (or rarely tetraploid) hybrids with one other diploid species. The *Musa acuminata* haploid genome is designated with the letter A, and the *M. balbisiana* genome with B. The dessert or sweet bananas are often (but not always) triploids and diploids of *M. acuminata*, and the plantains and other starchy

cooking bananas are generally (but not always) triploids derived from hybridisations between *M. acuminata* and *M. balbisiana.*

The genus *Musa*, which is over 50 million years old (Kress and Specht, 2006), has diversified throughout much of the greater Southeast Asian region (*cf.* WWF and IUCN, 1995). *Musa acuminata* and *Musa balbisiana* diverged about 4.6 million years ago (Lescot *et al*., 2008). However, the taxonomy of *Musa* is far from settled at the sectional, species, and intraspecific levels, although it has been receiving considerable attention in recent years. The research and explorations are resulting in the discovery and description of many distinctive new species as well as many botanical varieties and in reclassifications of taxa. However, some taxa remain little known. The natural geographical range of some taxa is obscure because of limited exploration, and in some cases, the difficulty in distinguishing between natural populations and naturalised or adventive (i.e., free-living but non-native) plants (De Langhe, 2009). The nomenclature of some taxa is also unsettled (Häkkinen and Väre, 2008; Väre and Häkkinen, 2009a, 2009b). There have been many efforts to invent and classify the wealth of cultigens and cultivars of bananas and plantains in different *Musa* germplasm collections. Evaluation based on morphology had been the standard (Simmonds and Shepherd, 1955; Simmonds and Weatherup, 1990a; IPGRI-INIBAP and CIRAD, 1996; Ortiz, 1997a). Modern techniques to detect differences have employed isozyme polymorphism (Bhat *et al*., 1992a, 1992b) and various DNA molecular markers, such as RAPD (Howell *et al*., 1994; Bhat and Jarret, 1995; Bhat et *al.,* 1995; Damasco *et al.,* 1996); RFLP (Gawel *et al*., 1992; Jarret *et al.,* 1992; Carreel *et al*., 1993; Fauré *et al.,* 1993; Bhat *et al*., 1994); AFLP (Ude *et al*., 2002a, 2002b; Noyer *et al.,* 2005); and microsatellites (SSRs) (Lagoda *et al.,* 1996; Dussart, 2001; Creste *et al.,* 2004; Buhariwalla *et al.,* 2005). Chloroplast DNAs of A and B cytoplasms have been differentiated (Umali and Nakamura, 2003; Boonruangrod *et al.,* 2008), as well as their mitochondrial DNAs (Boonruangrod *et al.,* 2008), and the genomic distribution of nuclear ribosomal DNA has been studied in various species (Bartoš *et al.,* 2005; Boonruangrod *et al.,* 2009).

Musa taxonomy is still very obscure today, just as it has been throughout its history, despite several attempts at clarification (Baker 1893; Cheesman 1931, 1948a, b, 1949a, b, 1950a, b; Simmonds 1957, 1960; Champion 1967; Nasution 1991). Some

earlier typifications seem to have failed. Consequently, the usage of *Musa* names is very confusing. With the advances in the various fields stated above, distinctions at the sectional, interspecific, specific, intraspecific and varietal levels are currently being established, which will help clearly define the taxonomy of these otherwise obscure genera.

Genetic diversity studies and DNA sequence-based markers

Morphological data has suggested that *Musa* is diverse with well-defined characters (Pollefeys *et al.,* 2004), giving accurate indicators of genome constitution and ploidy. However, phenotyping for many physiological characters, including biotic and abiotic stress tolerance, particularly under controlled, contained, reproducible conditions, is difficult because of the size of the plants and long life cycle. The genome size of *Musa* was determined by Dolezel *et al.* (1994): the unreplicated haploid genome size is 550 Mbp in *M. balbisiana* and 600 Mbp in *M. acuminata* (Lysak *et al*., 1999), extended to a range of species in all sections of *Musa* and in *Ensete* by Bartos *et al.* (2005). In contrast to the genetic mapping of *Musa*, the study of genomics advanced rapidly in the first years of the 21st century. Several BAC clone libraries were developed from both A and B genome diploid *Musa* species (Vilarinhos et al., 2003; Safa´r *et al.,* 2004; Ortiz-Vazquez *et al*., 2005; Musagenomics, 2007) with a total genomic coverage of more than 30 times. Like all plant genomes, the *Musa* genome consists of repetitive DNA and single-copy sequences, and understanding the composition and organisation of the genome at the large-scale level is beneficial to allow gene isolation and to understand long-term and short-term evolutionary processes (Kubis *et al.,* 1998; Schmidt and Heslop-Harrison, 1998). Baurens *et al.* (1996) used a competitive PCR to show that some repetitive DNA sequence families represented more than 1% of the *Musa* genomes. Using labelled total genomic DNA from A and B genome diploid species to hybridise to chromosomes from hybrid cultivars (Osuji *et al.* 1997) showed that the repetitive DNA components of the two genomes were substantially different since the two or three chromosome sets in the hybrids were labelled differentially. Researchers widely use molecular marker methods that employ polymorphic anonymous markers (which are not known to have any functional importance to the plant) for germplasm characterisation. In the 1980s, isozyme and anthocyanin analysis

confirmed that *Musa* germplasm was indeed genetically diverse (Jarret and Litz, 1986; Horry and Jay, 1988). Soon after, DNA markers became available. Although little attention has since been paid to non-DNA-based biochemical markers, including antibodies, it is worth noting that they can be very high throughput, fast and accurate, with little requirement for skilled labour or advanced laboratory equipment compared to DNA markers. They may prove to be valuable in some plant diversity, selection and breeding contexts, allowing larger samples and complementing the use of DNA markers. Projects aiming to characterise and sample the diversity of *Musa germplasm,* including wild species and cultivars with worldwide (Tenkouano *et al.,* 1999) and regional (Pillay *et al.,* 2001) focus, have used most of the different classes of DNA markers, including RFLP, AFLP (Ude *et al.,* 2002a, b), PCR–RFLP (Nwakanma *et al.,* 2003a, b), microsatellites and retroelements (Teo *et al.,* 2005) markers, as well as the unreliable (Jones *et al.,* 1997) RAPD method. The data have been used to infer relationships between *Musa* accessions, including the investigation of the representation of wild species' diversity in cultivars, to build phylogenetic trees suggesting relationships and groupings of diverse germplasm. Methods using PCR-based techniques are particularly appropriate because of the high levels of polymorphism that can be detected efficiently. Primers flanking polymorphic simple sequence repeats (SSRs) have been used in several studies in *Musa,* where they provide co-dominant, chromosome-specific markers; SSRs have been successfully used for germplasm analysis and estimation of genetic relationships between accessions. As pointed out by Buhariwalla *et al.* (2005), SSR markers are routinely used in numerous studies in *Musa* (Crouch *et al*., 1999, 2000; Creste *et al.,* 2004), and a large number of markers have been published from both the A and B genomes; many of the markers amplify sequences from both genomes. An alternative marker system to SSRs exploits the abundance and polymorphic nature of retrotransposon insertions into the nuclear genome. By using PCR primers facing outwards from conserved regions of retroelements, DNA fragments can be amplified and inter-retroelement amplified polymorphisms (IRAPs) detected (Waugh *et al.,* 1997; Kalendar *et al.,* 1999); as with SSR markers, polymorphisms can be used to infer relationships and phylogenies, as the retroelements are a relatively rapidly evolving genomic component. IRAP methods can reveal high levels of polymorphism and have the advantage that no DNA digestion,

ligations or probe hybridisation are needed to generate the marker data (Nair *et al.,* 2005; Teo *et al.,* 2005). Phylogenetic analyses of the banana family (Musaceae) based on nuclear ribosomal (ITS) and chloroplast (*trnL*-F) evidence have also been carried out (Liu *et al*. 2010). Evidence from the molecular phylogenetic investigations highlights the evolutionary diversification and biogeographic context of this plant group and suggests additional taxonomic investigations of both *Musa* and *Ensete* are in order.

Since 2020, diversity studies on wild *Musa* species have gained considerable momentum, largely driven by the availability of high-quality genome assemblies. Notably, Martin *et al.* (2025) produced chromosome-scale assemblies across multiple wild genetic groups, revealing previously unknown ancestral contributors to cultivated bananas and demonstrating the significant role of structural variation in speciation events. Similarly, Liu *et al.* (2023) provided a haplotype-resolved, telomere-to-telomere assembly of *M. acuminata*, establishing a valuable reference for comparative genomic studies. Building on these efforts, Wang *et al.* (2023) generated a genome assembly of *M. beccarii,* uncovering extensive chromosomal fusions and fissions that have shaped genome evolution. Additional genomic resources have been expanded through assemblies of Australimusa lineages (Zhou *et al.,* 2024) and haplotype-resolved hybrids (Xie *et al.,* 2024), enriching the genetic landscape of wild *Musa.* Complementing these genomic advances, resequencing studies by Li *et al.* (2022–2024) revealed strong regional structuring within M. acuminata subspecies alongside recurrent introgression between wild and cultivated lineages, while Akech *et al.* (2024) highlighted the notably lower genetic diversity retained in breeding populations compared to their wild counterparts. Regional floristic research, such as the work by Vu *et al.* (2023) in northern Vietnam, has further contributed by mapping distribution patterns and identifying conservation priorities. Together, these studies illustrate that cultivated bananas are genetic mosaics derived from multiple wild lineages, emphasising the indispensable role of wild germplasm in broadening the genetic base of modern cultivars.

Alongside progress in diversity research, molecular barcoding approaches have been increasingly utilised to address taxonomic ambiguities within wild *Musa*. Dhivya *et al.* (2020) compared various barcode markers, including *rbcL, matK, ITS*, and *ITS2*, finding that *ITS2* alone offered the highest species resolution, although a multi-locus strategy was recommended for distinguishing closely related taxa. Regional investigations have also tested the applicability of plastid and nuclear markers, with Ardiyani (2023) effectively using *rbcL* sequences to differentiate wild and cultivated bananas in East Java, and Fajri *et al.* (2024) employing *matK*-based phylogenetic analysis to clarify species relationships among wild bananas in West Kalimantan. These studies collectively suggest that while plastid markers such as *rbcL* and *matK* are useful for broad taxonomic resolution, their discriminatory power is considerably enhanced when combined with nuclear ITS or ITS2 sequences. Despite persistent challenges, including the limited availability of comprehensive barcoding libraries featuring vouchered specimens, complete metadata, and global taxonomic coverage, barcoding remains a cost-effective and valuable method for species identification, germplasm management, and resolving wild-cultivar relationships, effectively complementing high-throughput sequencing approaches.

Nutritional value

Bananas and plantains are the world's 4th most prominent agricultural crop after rice, wheat, and maize (Ganapathi *et al.,* 1999) and are considered to be one of the most important sources of energy and starchy staple food for the people of tropical humid regions (Onwuka and Onwuka, 2005). Furthermore, bananas and plantains are rich in nutrients, starch, sugar and vitamins A and C, potassium, calcium, sodium and magnesium (Doymaz, 2010). Plantains are nutritionally low-protein food materials but relatively high in carbohydrates, vitamins, and minerals (Offem and Njoku, 1993).

Moreover, studies on bananas grown in Africa and South America have shown a relationship between yellow-to-orange flesh colouration and higher carotenoid content (Amorim et al., 2009; Newilah et al., 2008). Several studies (Englberger et al., 2003, 2006, 2010; Fungo et al., 2007, 2010; Fungo and Pillay, 2011) have identified carotenoid-rich banana cultivars. However, there is little published information on the variability in bananas' and plantains' micronutrient concentrations (Wall, 2006; Fungo

et al., 2007). This is important to initiate activities to identify the banana and plantain cultivars with a high level of provitamin A and micronutrients, which will encourage the growth and consumption of such acceptable cultivars in the region of vitamin A and micronutrient deficiency. Based on the above-mentioned comments, it is not surprising that the nutritional benefits of the *Musa* species have been attracting considerable interest. Banana, in general, and its popularity aside, offers excellent nutritional value **(Table 1.1).**

Table 1.1: Comparable composition of fresh banana and plantain

Constituents	**Unit**	**Banana *Musa* species**	**Plantain**
Energy	Kcal	89	122
Water	g	74	65
Protein	g	1.1	1.3
Total lipid	g	0.3	0.4
Carbohydrate	g	21.8	32
Fibre	g	2	2.0-3.4
Sodium	mg	1	4
Potassium	mg	385	500
Calcium	mg	8	3
Magnesium	mg	30	35
Iron	mg	0.4	0.6
Phosphorus	mg	22	30
Vitamin C (Ascorbic acid)	mg	11.7	20
Equivalent β. carotene	Mg	68	390-1035
Thiamin (Vitamin B1)	Mg	40	80
Riboflavin (Vitamin B2)	Mg	70	40
Niacin	Mg	610	600

Pantothenic acid (Vitamin B5)	Mg	280	na
Pyridoxine (Vitamin B6)	Mg	470	na
Folic acid	Mg	23	na

***Source*:** Modified table adopted from Aurore *et al*., (2009); na, data not available

Vitamin A deficiency remains one of the world's major public health problems despite food fortification and supplement strategies. Biofortification of staple crops with enhanced levels of provitamin A (PVA) offers a sustainable alternative strategy to both food fortification and supplementation. As a proof of concept, PVA-biofortified transgenic Cavendish bananas were generated, and a field trial was undertaken in Australia with the aim of achieving a target level of 20 µg/g of dry weight (DW) β-carotene equivalent (b-CE) in the fruit. Expression of a Fe'i banana-derived phytoene synthase 2a (MtPsy2a) gene resulted in the generation of lines with PVA levels exceeding the target level, with one line reaching 55 µg/g DW b-CE. Expression of the maize phytoene synthase 1 (ZmPsy1) gene, used to develop 'Golden Rice 2', also resulted in increased fruit PVA levels, although many lines displayed undesirable phenotypes. Constitutive expression of either transgene with the maize polyubiquitin promoter increased PVA accumulation from the earliest stage of fruit development. In contrast, PVA accumulation was restricted to the late stages of fruit development when either the banana 1-aminocyclopropane-1-carboxylate oxidase or the expansin 1 promoters were used to drive the same transgenes. Wild-type plants with the longest fruit development time also had the highest fruit PVA concentrations. The results from this study suggest that early activation of the rate-limiting enzyme in the carotenoid biosynthetic pathway and extended fruit maturation time are essential factors to achieve optimal PVA concentrations in banana fruit (Paul *et al.,* 2017).

The water-soluble carbohydrates (WSC) glucose, fructose and sucrose are well-known to the general public, but fructans represent another type of WSC that deserves more attention, given their prebiotic and immunomodulatory properties in the food context. Although the occurrence of inulin-type fructo-oligosaccharides (FOS) was proposed in the fruit of some banana accessions, little or no information is

available on either the exact identity of the fructan species or the fructan content in different parts of banana plants and among a broader array of banana cultivars.

High-performance anion exchange chromatography with integrated pulsed amperometric detection (HPAEC-IPAD) showed the presence of 1-kestotriose [GF2], inulobiose [F2], inulotriose [F3], 6-kestotriose and 6G-kestotriose (neokestose) fructan species in the pulp of mature fruits of different accessions, but the absence of 1,1-nystose and 1,1,1-kestopentaose and higher degree of polymerisation (DP) inulin-type fructans. This fructan fingerprint points to the presence of one or more invertases that are able to use fructose and sucrose as alternative acceptor substrates. Quantification of glucose, fructose, sucrose and 1-kestotriose and principal component analysis (PCA) identified related banana groups based on their specific WSC profiles. These data provide new insights into the biochemical diversity of wild and cultivated bananas and shed light on potential roles that fructans may fulfil across species during plant development and adaptation to changing environments. Furthermore, the promiscuous behaviour of banana fruit invertases (sucrose and fructose as acceptor substrates besides water) provides a new avenue to boost future work on structure-function relationships of these enzymes, potentially leading to the development of genuine banana fructosyltransferases that are able to increase fructan content in banana fruits (Cruz-Cardenas *et al.,* 2015).

The nutritional evaluation of wild *Musa* species has garnered increasing interest, driven by the growing focus on functional foods and dietary diversification. Meghwal *et al.* (2024) conducted a nutritional profile of *M. balbisiana*, highlighting its high content of dietary fibre and essential minerals such as calcium, magnesium, iron, and zinc, along with notable antioxidant properties. In a similar vein, Basumatary and Daniel (2024) developed flour from *M. balbisiana,* which demonstrated a rich composition of carbohydrates and micronutrients, indicating its potential application in functional food products. Reviews by Zou *et al.* (2022) and Maseko *et al.* (2024) further underscore the value of wild bananas and their by-products, including pseudostems and peels, as valuable sources of resistant starch, phenolic compounds, and other bioactive substances with promising health benefits. Collectively, these studies suggest that wild *Musa* species often exhibit higher nutritional density compared to cultivated bananas. Nevertheless, research in this area remains limited in

taxonomic scope, predominantly focusing on *M. balbisiana*, and is characterised by a variety of methodologies that complicate direct comparisons across studies.

Problem Statement

Bananas (*Musa* spp.) are an important staple crop for global food security. They are susceptible to many pests and diseases (e.g., Panama disease/*Fusarium* wilt), which greatly limit global production. Currently, over 90% of commercial export dessert bananas are produced from a single cultivar, namely 'Cavendish' (AAA). Unsurprisingly, this dependency on a single cultivar and the consequent lack of genetic variation in production systems have resulted in a crop that is potentially highly susceptible to disease pandemics. Pandemics are also not without precedent in bananas, and in the 1950s, 'Panama Disease' ('*Fusarium* wilt'), caused by the soil-borne fungus *Fusarium oxysporum,* was responsible for entirely wiping out commercial banana production, which at that time was dependent on the cultivar 'Gros Michel' (AAA). By necessity, 'Gros Michel' was replaced by the Fusarium-resistant cultivar 'Cavendish', but unfortunately, in the intervening years, new strains of *F. oxysporum* have evolved (*F. oxysporum* f. sp. Cubense race 4, 'Tropical race 4') that have overcome this natural Cavendish resistance. Currently, the other major disease threat is that posed by *Mycosphaerella fijiensis* ('Black Sigatoka'). Chemical control of *Fusarium* wilt is ineffective, and even though 'Black Sigatoka' can be controlled by frequent (up to 50) fungicide applications per year, these are socio-economically and environmentally inappropriate and require integrated strategies to avoid the development of fungicide resistance in the pathogen. Consequently, there is currently much interest in developing sustainable solutions to these disease threats through the introgression of novel resistance loci and the development of new, disease-resistant varieties. However, the largely sterile nature of most commercial varieties means that banana breeding is both lengthy and time-consuming.

Therefore, the collection, conservation, characterisation and breeding of cultivated *Musa* spp. and their wild relatives contribute to the preservation of their genetic diversity and are essential components in ensuring food security. That said, the above steps of collection, conservation, characterisation and breeding can only be achieved through various approaches (the molecular approach being one of them),

which presents with itself its various unique challenges. Next generation sequencing (NGS), for example, has the potential to change the way scientists deal with genetic resources by unlocking genetic diversity stored in gene banks. This technology also allows gene bank users to select germplasm material based not only on passport and phenotypic data but also on genomic information. However, NGS raises several unique challenges, including managing the volume and diversity of generated sequences, providing appropriate storage facilities and developing analytical and graphical visualisation tools.

Although commercial banana production is dominated by only a few genotypes, around the world collections of *Musa* germplasm contain >15000 accessions (i.e., unique samples in a germplasm collection) distributed among 56 collections. These collections represent a wide range of phenotypic variation and genome constitutions. Unravelling and exploiting genetic diversity in banana germplasm collections is critically important, as long-term sustainable crop cultivation depends on this diversity to adapt to the ever-changing agro environment.

There is also the fact that there is quite limited information about most wild *Musa* species' ranges and habitats (De Langhe, 2000b). Wild populations apparently tend to be isolated, maintaining significant genetic diversity among the dispersed populations rather than within each population (Asif Javed *et al*., 2002; Ge *et al*., 2005; Wang *et al*., 2007). Information on the physicochemical and nutritional properties and genetic diversity of the wild *Musa spp.* is also largely underexplored.

Developments

The world's largest banana germplasm collection is maintained at the Biodiversity International Transit Centre (ITC) in Belgium and is continuously expanded by new accessions of edible cultivars and wild species. It is an in vitro gene bank established in 1985 and contains 1566 accessions as of 8 April 2019, of which 937 are available for distribution. In a recent study by Lamare & Rao (2015), three single primer-based DNA marker techniques, viz., random amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR) and directed amplification of minisatellite DNA (DAMD), were used for diversity diagnostics among 25 genotypes of wild *M. acuminata* collected from Meghalaya province of northeast India. A total

of 58 primers (26-RAPD, 21-ISSR, and 11-DAMD) yielded 451 DNA fragments, of which 395 (87.58%) were found to be polymorphic in nature. The polymorphic information content (PIC) values were almost identical for each marker system. The resolving power of the marker system was found to be highest in RAPD (3.96), whereas ISSR resolved the highest marker index (16.39) in the study. Selected amplicon data obtained through single primer amplification reactions were utilised for the determination of diversity within and among the populations of *M. acuminata*. Nei's genetic differentiation (Gst) value (0.451) indicated a higher proportion of the genetic variation within the populations, which is supported by the AMOVA analysis (88%). The study provides insight into the efficacy of RAPD, ISSR and DAMD to analyse the genetic variation existing in the wild *Musa* germplasm, which can further be exploited for quality trait improvement and domestication of such important horticultural crops. The genetic diversity-based population structure may shed light on the genetic basis of speciation and evolution of various species within the genus *Musa*.

Knowledge about the genome of the plant in focus is essential for any molecular study, and progress has been made in this field as well. The genome of *M. acuminata* was the first genome to be sequenced in the genus *Musa.* The genome of the *M. acuminata* (subspecies *malaccensis,* DH Pahang) was sequenced in 2012 (D'hont *et al.,* 2012), followed by the sequencing of a draft sequence of *M. balbisiana* (PKW) in 2013 (Davey *et al.,* 2013). A recent genome sequencing of a wild *Musa* species was completed. One individual plant of *M. itinerans* collected from Baoting County on Hainan Island and transplanted in the South China Botanical Garden was sequenced by the whole-genome shotgun strategy on the Illumina Hiseq2000 platform. The assembled genome size was 462.1 Mb, covering 75.2% of the genome (615.2 Mb) and containing 32,456 predicted protein-coding genes. Since the approximate divergence around 5.8 million years ago, the genomes of *Musa itinerans* and *Musa acuminata* have shown conserved collinearity. Gene family expansions and contractions enrichment analysis revealed that some pathways were associated with phenotypic or physiological innovations. These include a transition from wood to herbaceous in the ancestral Musaceae, intensification of cold and drought tolerances, and reduced disease resistance genes for subtropical marginally distributed *Musa* species. The qualified assembly of the *M. itinerans* genome provides deep insights into

the lineage-specific diversification and also valuable resources for future banana breeding (Wu *et al.,* 2016).

Studies on the genetic diversity, taxonomy and phylogeny of *Musa* species based on molecular techniques are catching waves in recent years with the goal of understanding the wild species of the genus. One such study states, "*M. itinerans* species complex harbours high cryptic species diversity. We recommend that *M. itinerans* var. *Itinerans* and *M. itinerans var. Lechangensis* be elevated to subspecies status, and the extremely rare latter subspecies be given priority for conservation. We also recommend that the very recently diverged *M. itinerans var. Chinensis* and *M. itinerans var. Guangdongensis* should be merged under the subspecies *M. itinerans var. Chinensis*. Finally, we speculate that species delimitation of recently diverged lineages may be more effective using genome-wide bi-allelic SNP markers with BFD* than by using unlinked loci and BP&P."

With the advent of advanced molecular techniques, the need for storage, organisation and assessment of information generated as such is also required, for which software and databases are being developed. The Banana Genome Hub is a database based on Tripal—a construction toolkit for online genome databases—to facilitate the integration between various systems that are developed for plant genome analysis, which includes several major banana data sets. Several use cases illustrate how the Banana Genome Hub can be used to study gene families. Overall, with this collaborative effort, the importance of interoperability toward data integration between existing information systems is discussed (Droc *et al.,* 2013).

Another recent development is the creation of the MGIS by Ruas *et al.* (2017). The *Musa* Germplasm Information System (MGIS) is the largest database for banana genetic resources and includes passport and characterisation data, voucher images and genomic-based marker information generated for many accessions. Data associated with accessions in MGIS will help identify important agricultural traits of bananas and thus support targeted analyses and distribution of diverse germplasm to researchers, breeders and farmers.

Crop improvement of existing *Musa* cultivars is essential. Genetic solutions to postharvest crop loss can reduce cost and energy inputs while increasing food security, especially for banana (*Musa acuminata*), which is a significant component of

worldwide food commerce. A recent study functionally characterised two banana E-class (SEPALLATA3 [SEP3]) MADS box genes, MaMADS1 and MaMADS2, homologous to the tomato (*Solanum lycopersicum*) RIN-MADS ripening gene. Transgenic banana plants repressing both genes (via antisense or RNA interference [RNAi]) were created and exhibited specific ripening delay and extended shelf-life phenotypes, including delayed colour development and softening (Friedman *et al.,* 2016).

The use of bananas not only as a source of nutrition but also as an alternative bio-compound source is also gaining traction. New nanocomposites were prepared from a mixed suspension of banana starch and cellulose nanofibers using the casting method, and the effect of the addition of these nanofibers on the properties of the resulting nanocomposites was investigated. The cellulose nanofibers homogeneously dispersed in the starch matrix increased the glass transition temperature due to the strong intermolecular interactions occurring between the starch and cellulose. The nanocomposites exhibited significantly increased tensile strength, Young's modulus, water resistance, opacity, and crystallinity as the number of passages through the homogeniser augmented. In general, the cellulose nanofibers improved the features of the starch-based material and are potentially applicable as reinforcing elements in a variety of polymer composites (Pelissari *et al.,* 2017).

All aspects of the study on bananas are primarily focused on their commercial varieties and not so much on their wild relatives. The disproportionate focus, although justifiable, between the commercial and wild varieties creates a lacuna in our better understanding of the subject at hand, thereby presenting the need and the opportunity to explore all that is to be understood, as the collection, conservation, characterisation and breeding of the cultivated *Musa* and, more so, their wild relatives contribute to the preservation of biological diversity and are essential components in ensuring food security.

There are well over a thousand domesticated *Musa* cultivars, and their genetic diversity is high, indicating multiple origins from different wild hybrids between two principal ancestral species. The genetic diversity in the wild species and varieties is also still largely undetermined. However, the difficulty of genetics and sterility of the crop has meant that the development of new varieties through hybridisation, mutation

or transformation was not very successful in the 20th century. Knowledge of structural and functional genomics and genes, reproductive physiology, cytogenetics, and comparative genomics with other model species has increased our understanding of *Musa* and its diversity enormously. This advancement in the tools and approaches also presents a varied scope of study for the plant of interest.

Bananas are the fourth most important food crop in the world. As with all other crop species, banana production faces major challenges from biotic and abiotic stresses. As well as the critical need for better genetic disease resistance, there is the need for bananas to contribute to better food security through greater yields and more efficient production, greater yield stability, better use of water, decreasing toxic chemical input, and higher quality. About 85% of banana production is eaten as a staple food in the country of production, but this market is changing rapidly with urbanisation of the population and a smaller proportion of the crop being produced for homestead use.

In Southeast Asia, where wild bananas occur naturally, they are often in open disturbed areas such as on steep slopes, and they are often among the first colonisers when natural regeneration follows forest clearance (Tang *et al.,* 2003) — thus showing pioneer characteristics. Semi-wild or free-living populations occur in human-made habitats such as the edges of cleared land and on roadsides. *Musa* genetic resources are now found in an array of habitats such as forest edges and openings, fallow land, and home gardens and farms. The human communities living around such habitats maintain variability through cultivation of traditional cultigens and landraces and by conservation of natural areas and ecosystems (Sharrock and Engels, 1997; Uma and Buddenhagen, 2006). Both wild diploid progenitor species are still valuable genetic resources for banana improvement (De Langhe, 2000b). Their populations contain important traits such as resistance to various diseases and the ability to thrive in relatively dry and cool environments (Ude *et al.,* 2002b; Häkkinen and Wang, 2007; Wang *et al.,* 2007). Knowledge of *Musa* has been limited due to the complex and varied genomes between sections, but with modern molecular methods, great progress is being made.

The taxonomy of approximately 60 species within the genus *Musa* remains poorly resolved, not least because of the widespread vegetative reproduction and

natural occurrence of many hybrids. Most frequently, the genus is divided into four (sometimes five) sections: *Eumusa* and *Rhodochlamys* with a basic chromosome number of x ¼ 11, *Australimusa* (x ¼ 10), and *Callimusa* (x ¼ 10 or x ¼ 9) (Cheesman, 1947; Simmonds and Weatherup, 1990; Dolezel and Bartos, 2005). Various minor and major regroupings have been suggested (Wong *et al.,* 2002). At the species level, the number of species and the status of subspecies have been debated (Taxonomic Advisory Group for *Musa,* 2007). However, from the point of view of the taxa related to crops, the morphological features are well defined (*Musa* Germplasm Information System, MGIS; IPGRI–INIBAP/CIRAD, 1996). In the last decade, in conjunction with molecular studies of *Musa* accessions at the DNA level (Bartos *et al.,* 2005), aspects of the taxonomy have been clarified, but careful treatment of the complementary and contrasting data, along with judicious filling of gaps in the data, is required to resolve the relationships and phylogeny in the genus. Genomic approaches are now rapidly advancing in *Musa*. The Global *Musa* Genomics Consortium was established in 2001 to assure the sustainability of bananas as a staple food crop by developing an integrated genetic and genomic understanding, allowing targeted breeding, transformation and more efficient use of *Musa* biodiversity. The gene pool within wild *Musa* species and represented in the cultivars has the diversity to meet the challenges faced by banana breeders, and its characterisation is a key to making better use of the biodiversity. Effective plant breeding requires knowledge of *Musa* genome evolution and structure (Dolezel and Bartos, 2005), complemented with functional genomics, allowing bioinformatics and gene discovery. Understanding the evolution of the banana genome through the DNA changes, hybridisation and polyploidy in the past will allow prediction of how genomes will change in the future, whether under natural evolutionary processes or the accelerated processes of plant breeding. New data allows us to exploit comparative genomics to gain an understanding of *Musa* genetics, and the complete DNA sequence of a Musa species would be valuable for both fundamental research and for application in future improvement of the crop, enabling access to all the genes and their control sequences. The genetic understanding and development of tools means there are prospects for ‘new generations’ of super-domestication through breeding technology involving sexual hybridisation, mutation breeding and targeted transformation approaches.

There are also major challenges to banana production from virulent diseases, abiotic stresses and new demands for sustainability, quality, transport and yield. Within the gene pool of cultivars and wild species, there is genetic resistance to many stresses. Genomic approaches are now rapidly advancing in *Musa* and have the prospect of helping enable bananas to maintain and increase their importance as a staple food and cash crop through integration of genetic, evolutionary and structural data, allowing targeted breeding, transformation and efficient use of *Musa* biodiversity in the future. There is also the prospect of the use of bananas not just as a source of food security but also as a source of biocompounds in the drug industry and raw material in the textile industry, as well as their aesthetics as ornamental plants. Regarding bananas, the disappearance of wild bananas and how human presence and expansion affect their biology, especially in Southeast Asia, is concerning. This calls for greater use of genetic diversity for strengthening breeding programmes in developing countries and for promoting awareness of the inevitable consequences of a narrow genetic base in crops and the need for a broader genetic base, especially in the case of commercial bananas and the generation of new and improved commercial varieties from their wild relatives.

About Nagaland and *Musa* diversity

The area under study falling under this distribution region consists of a varied array of species belonging mostly to the section *Musa* being found as well as a single member of the genus *Ensete* (Deb *et al*., 2023). Members of this genus in the Indian sub-continent are mainly distributed in the Northeastern States and are rich in their diversity, with 19 endemic species out of about 30 taxa reported, representing about 81% of the total wild Musaceae diversity in India. The region is a microcentre for the evolution of the *Musa* species (Dey *et al.,* 2014; Lamare *et al.,* 2016). Worldwide, about 68 *Musa* species are reported (Lamare *et al.,* 2016). The earliest records of documentation of *Musa* in Nagaland can be traced back to 1904, from whence the type locality of *Musa nagensium* Prain has been obtained and its etymology thus derived (Prain, 1904). *Musa nagalandiana* (Dey *et al*., 2014), another species first reported from Nagaland, also derives its name thus.

A survey of all the agro-climatic regions of Nagaland revealed the presence of 12 wild *Musa* species. These species were identified based on their morpho-taxonomic characteristics. The 12 identified species of *Musa* L. include M. *aurantiaca, M. balbisiana, M. cheesmanii, M. flaviflora, M. itinerans, M. mannii, M. markkuana, M. markuii,* and *M. sikkimensis*. These additions to the Checklist of Flora of Nagaland (Mao *et al.,* 2017) expand the list of Musaceae species from the original three: *M. nagalandiana, M. velutina, and M. nagensium*. Joe and Sabu (2019) also listed 10 species of *Musa* L., including *Ensete glaucum, M. mannii,* and *M. sikkimensis*, from Nagaland.

The various species documented have been found in single populations or in association with other species. Their distribution depends on the varying altitudinal profile and the agro-climatic and topographical conditions of the environment. The wild *Musa* distribution is diverse, with the highest diversity found in the northern part of the state. Here, tropical and subtropical forests gradually decrease in the southern part of the state, where subtropical and temperate forests with higher altitudes are found. *Musa* is limited to the tropical lowlands, while *M. sikkimensis* inhabits areas above 1500 AMSL (Deb *et al*., 2023).

Objectives of the Study

Musa, as a taxon in Nagaland, represents an important yet underexplored resource, ethnobotanically as well as genetically, owing to its rather extensive use locally in households, not only as food but also for other domestic purposes, not forgetting its cultural significance. This use not only pertains to its cultivated varieties but also extends to its wild relatives as well, of which most of its plant parts can find some use by the otherwise resourceful local. Although much is known about the nutritional profile of the cultivated kind, not much has been done to study the nutritional profile of the wild *Musa* species.

There is also the fact that the area under study is sandwiched between two biodiversity hotspots: the Himalayas to its east and the Indo-Burma to its west. This region is considered the centre of origin of the taxon under study. This provides an impetus to explore the reservoir of genetic diversity present in this region. Following

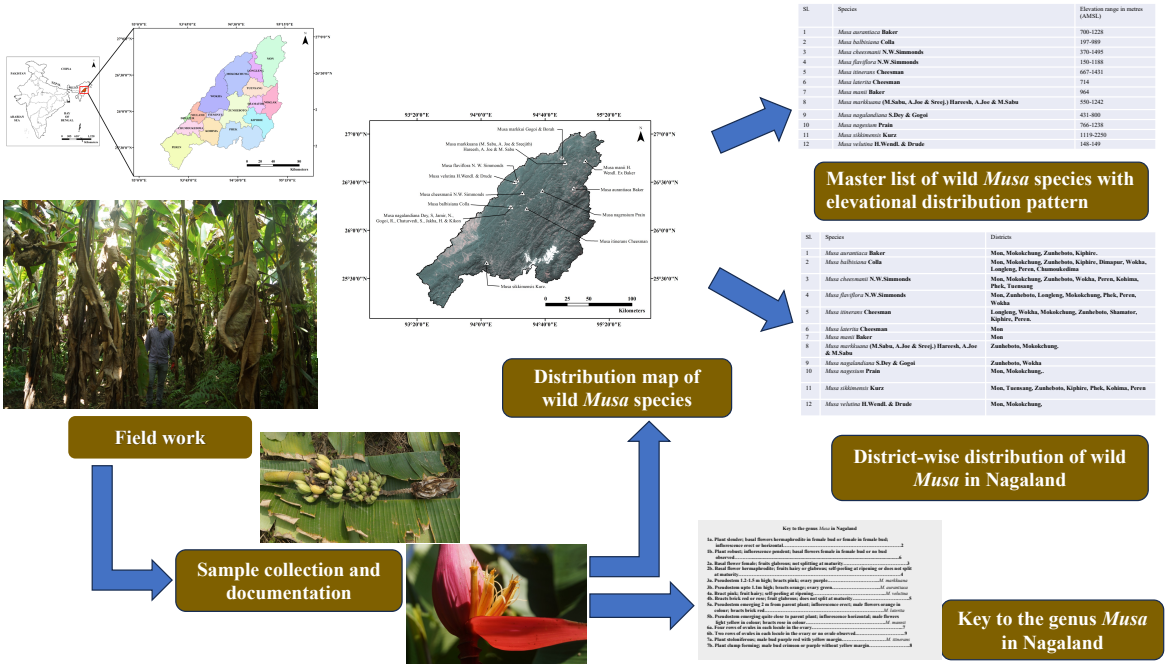
this rationale, this study commenced in November 2018 for doctoral research with the following objectives:

1. Field exploration and documentation of existing wild *Musa* species in Nagaland.
2. Nutritional assessment of selected wild *Musa* species (fruits and other edible parts).
3. Molecular characterisation of wild *Musa* species.
4. Genetic diversity study of selected species growing in different areas of Nagaland.

Chapter - 2

Morpho-taxonomic Diversity

Graphical Summary



Introduction

The genus *Musa* comprises a group of large herbaceous plants, which consists of bananas and plantains belonging to the genus *Musa* L. of the small family Musaceae, which also includes the genera *Ensete* and *Musella*, in the monocot order Zingiberales (Kress *et al*., 2001). The earliest classification of this genus, *Musa*, was given by Sagot (1887) and further carried on by Baker (1893). Chessman (1947) carried out a drastic shift in the classification of this genus by the addition of two sections. Another section was added to this genus by Argent (1976), resulting in five sections within this genus, namely *Australimusa*, *Callimusa, Ingentimusa, Musa,* and *Rhodochlamys*. Hakkinen (2013) reduced these five sections to two, namely *Musa* and *Callimusa* (Hakkinen, 2013).

This genus is mainly distributed in Africa, South and Southeast Asia and northern Australia. The area under study falling under this distribution region consists of a varied array of species belonging mostly to the section *Musa* being found. Members of this genus in the Indian subcontinent are mainly distributed in the Northeastern States and are rich in their diversity, with 19 endemic species out of about 30 taxa reported, representing about 81% of the total wild Musaceae diversity in India. The region is a microcentre of evolution of *Musa* species (Dey *et al.,* 2014; Lamare *et al.,* 2016). Worldwide, about 68 *Musa* species are reported (Lamare *et al.,* 2016). The earliest records of documentation of *Musa* in Nagaland can be traced back to 1904, from whence the type locality of *Musa nagensium* Prain has been obtained and its etymology thus derived (Prain, 1904). The genus Musa exhibits significant polymorphism; hence, the precise number of species remains undetermined. Häkkinen and Väre (2008) classified 92 species, 5 subspecies, 35 variations, 5 forms, and 1 hybrid from *Musa* records in the International Plant Name Index. Häkkinen (2013) identified 70 species in his revised taxonomy of *Musa*. Lamare *et al.* (2016) reported the existence of about 68 *Musa* species worldwide. In India, Joe and Sabu (2019) reported 34 taxa of Musaceae, of which 30 taxa occurred in the Northeastern states of India, out of which about two-thirds are endemic to the region (Tiatemsu *et al*., 2023).

Study Area: Nagaland

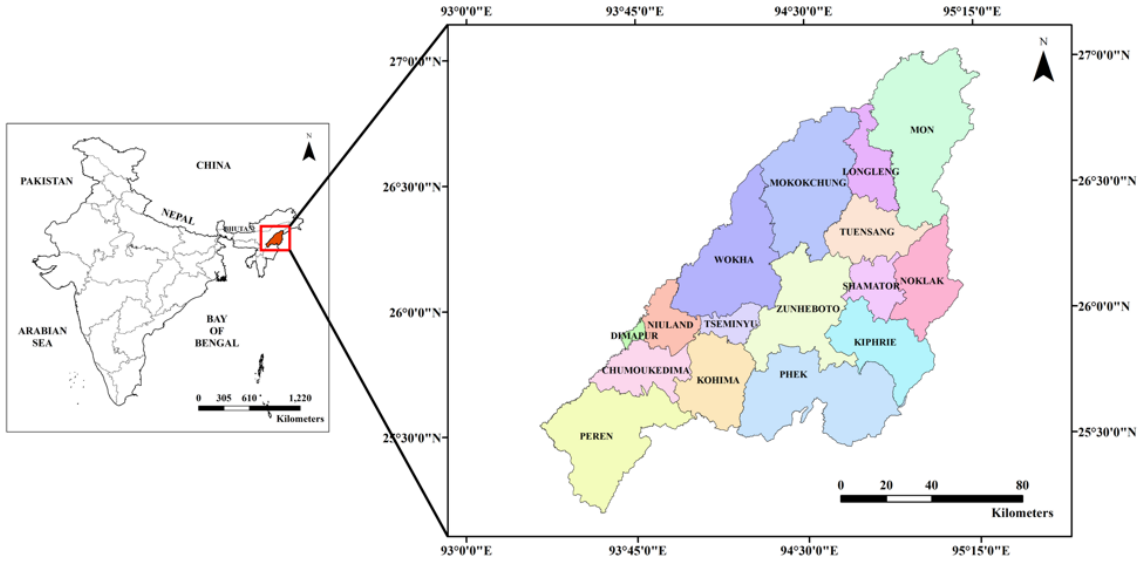


Figure 2.1: Map of the study area

Nagaland's longitude and latitude are 25°06'27°04' N and 93°20'95°15' E, and its area is 16,579 square kilometres (Figure 2.1). The state is bounded by Arunachal Pradesh to the north, Manipur to the south, Assam to the west, and Myanmar's international border to the east. Nagaland's geography is mostly mountainous with deep valleys and occasional plains. Elevations range from ~100 m in Dimapur lowlands to over 3,840 m at Mount Saramati in Kiphire District.

Depending on altitude, the state has a subtropical to temperate climate, with annual rainfall ranging from 1,800 to 2,500 mm, with the majority falling between May and October. Temperatures range from 21°C to 40°C in the lower regions and 4°C to 25°C in the higher altitudes. The varied topography, height, and climatic circumstances have resulted in a diverse range of flora types, from tropical evergreen and semi-evergreen forests in the foothills to subtropical pine and temperate broad-leaved forests at higher elevations. (Nagaland Statistical Handbook 2022)

Materials and methods

The classification of *Musa* is carried out using morphological descriptors. Morphological descriptions for *Musa* L. species are achieved using the Descriptors of International Network for the Improvement of Banana and Plantain (INIBAP), which provide useful information regarding the classification and characterisation of this genus. Extensive fieldwork involving the collection and documentation of members of *Musa* L. was conducted, covering different agro-climatic zones of Nagaland, India. Sampling was conducted along road transects, forest paths, jhum fallow regions, damp ravines, valleys, and specifically the riparian zone, to encompass the greatest morphological and biological diversity.

Morphometric analysis was done in the field, and the suckers were transferred to Nagaland University, Lumami, for maintenance and conservation of the germplasm. For the present study, the entire state was covered for exploration. For this purpose, explorations were made in different seasons and multiple visits to the same site based on the morpho-phenological cycles of the wild banana species during 2018-2022. The location coordinates were recorded using the Garmin Oregon 750t, while photographs from the field were taken with a Canon EOS 600D and Nikon DSLR 3200 camera. After collection, the GPS collection data were compiled and mapped using ArcGIS 10.8.

The species' authenticities were confirmed with various voucher specimens deposited in various herbaria, different floras, Monographs, and published papers (Gogoi and Borah, 2013; Joe & Sabu, 2019).

Field Logistics and Constraints

Field operations were executed with logistical assistance from the forestry department and village councils. Accessibility differed by terrain; the western and central districts, including Dimapur, Kohima, Mokokchung, and Wokha, were comparatively accessible via motorable roads, whereas the northern and eastern regions, such as Mon and Kiphire, necessitated extensive foot surveys through wooded inclines and unpaved mountain paths. Seasonal problems encompassed significant monsoon precipitation, resulting in road

obstructions, landslides, and leech infestations. Numerous trips throughout various seasons guaranteed thorough coverage of phenological stages (flowering and fruiting) for precise identification and documentation.

Results

During the extensive field explorations during the study period, a total of 14 *Musa* species were collected. The mature seeds from the berry as well as suckers from the parent plants, were collected **(Figures 2.2a-2.2c).** The collected germplasm is being maintained in the *Musa* Garden of the Department of Botany, Nagaland University, India, for reference and conservation purposes. Of the 14 samples collected from different parts of Nagaland, 12 samples were identified up to the species level, but the floral parts of two samples are yet to be collected. In the present study, we present these 12 identified species with their altitudinal distribution range **(Table 2.1)** and district-wise distribution in the state **(Table 2.2).**

Figure 2.2a : Suckers from wild parent plants for *ex-situ* germplasm conservation; Figure 2.2b : Ripe berries with matured seeds; Figure 2.2c : Matured seeds for preservation as seed germplasm;

Table 2.1: Distribution and occurrence of wild *Musa* species at variational altitude (in feet above mean sea level)

Sl. No.	*Musa* species	Elevation range (AMSL)
1	*Musa aurantiaca* Baker	700-1228
2	*Musa balbisiana* Colla	197-989
3	*Musa cheesmanii* N.W.Simmonds	370-1495
4	*Musa flaviflora* N.W.Simmonds	150-1188
5	*Musa itinerans* Cheesman	667-1431
6	*Musa laterita* Cheesman	714
7	*Musa manii* Baker	964
8	*Musa markkuana* (M.Sabu, A.Joe & Sreej.) Hareesh, A.Joe & M.Sabu *stat. nov*	550-1242
9	*Musa nagalandiana* S. Dey & Gogoi	431-800
10	*Musa nagesium* Prain	766-1238
11	*Musa sikkimensis* Kurz	1119-2250
12	*Musa velutina* H.Wendl. & Drude	148-149

Table 2.2: District wise distribution of Wild *Musa*

Sl. No.	Species name	Districts
1	*Musa aurantiaca* Baker	Mon, Mokokchung, Zunheboto, Kiphire
2	*Musa balbisiana* Colla	Mon, Mokokchung, Zunheboto, Kiphire, Dimapur, Wokha, Longleng, Peren, Chumoukedima
3	*Musa cheesmanii* N.W.Simmonds	Mon, Mokokchung, Zunheboto, Wokha, Peren, Kohima, Phek, Tuensang

4	*Musa flaviflora* N.W.Simmonds	Mon, Zunheboto, Longleng, Mokokchung, Phek, Peren, Wokha
5	*Musa itinerans* Cheesman	Longleng, Wokha, Mokokchung, Zunheboto, Shamator, Kiphire, Peren
6	*Musa laterita* Cheesman	Mon
7	*Musa manii* Baker	Mon
8	*Musa markkuana* (M.Sabu, A.Joe & Sreej.) Hareesh, A.Joe & M.Sabu	Zunheboto, Mokokchung.
9	*Musa nagalandiana* S. Dey & Gogoi	Zunheboto, Wokha
10	*Musa nagensium* Prain	Mon, Mokokchung,.
11	*Musa sikkimensis* Kurz	Mon, Tuensang, Zunheboto, Kiphire, Phek, Kohima, Peren
12	*Musa velutina* H.Wendl. & Drude	Mon, Mokokchung

The distinctive morphological variation between species is observed by the pseudostem colour, plant size and leaf habit **(Figures 2.3a-2.14a),** the variation in the fruiting body and the inflorescence **(Figures 2.3b-2.14b)** and the fruit shape and size. Brief descriptions of each of the species are also provided for easy reference.

Description of *Musa* species in Nagaland

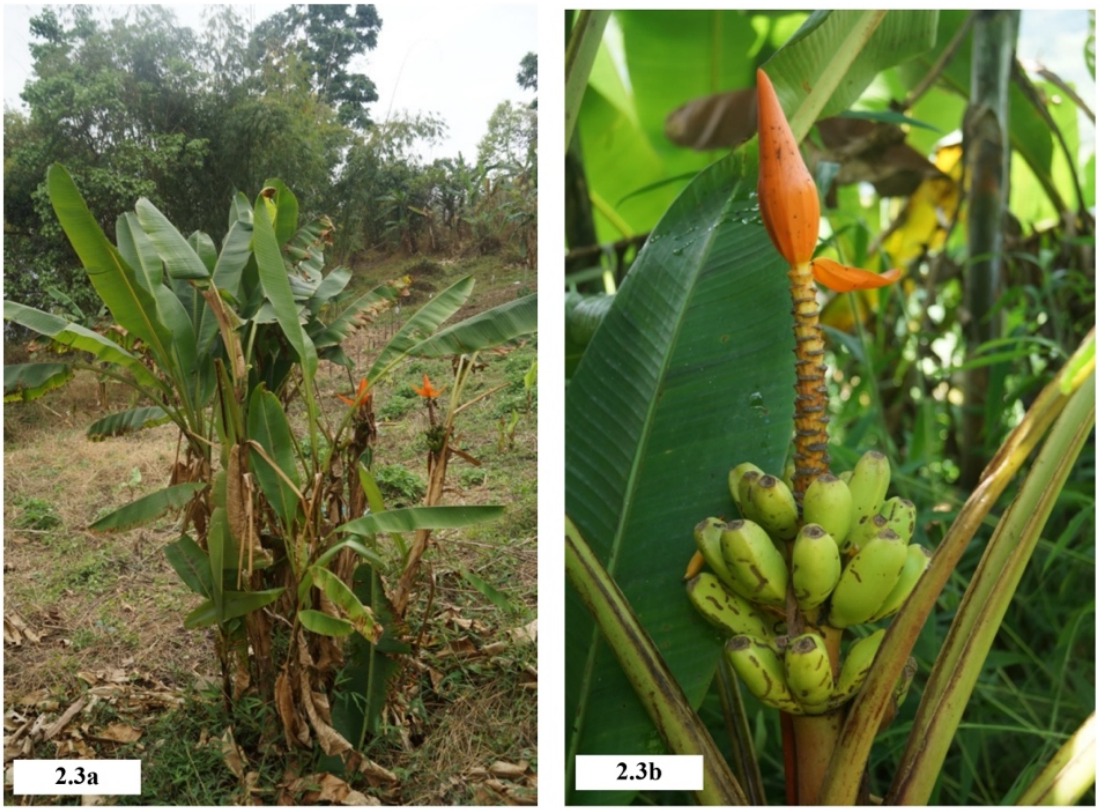
1. *Musa aurantiaca* Baker

Description

Plant thin to medium, with up to 10 suckers. The mature pseudo-stem reaches a height of 270 cm and a base diameter of 10.5 cm, infrequently adorned with dry sheaths; the underlying hue is yellowish-green with reddish blotches, exhibiting a glossy appearance; the sap is milky. Petiole measures 60–77 cm in length and 4.5 cm in diameter; canal borders are inwards rolled; base is green, non-waxy, and tightly clasping. Leaves upright; lamina elliptical, measuring up to 150 cm in length and 60 cm in width, with an

asymmetric base rounded on both sides; apex truncated, adaxially deep green, abaxially pale green; midrib yellowish green abaxially, occasionally featuring brown speckles. Inflorescence erect; peduncle up to 30 cm in length, exhibiting N-shaped bending for larger clusters, green, glabrous or slightly pilose, measuring up to 4 cm in diameter, non-waxy; one leafy bract; one sterile bract, persistent at the emergence of the initial female flowers. Female bud lanceolate, measuring up to 35 cm in length and 8 cm in diameter, with orange bracts on both sides that are convolute and acute, raising a few bracts at a time without revoluting before abscission; basal flowers are female, arranged in a single row of 4 to 6, reaching up to 11 cm in size. Ovary measuring 6.5 cm in length and 1.7 cm in diameter, yellowish-green, distinctly 3-ridged; ovules arranged in two rows per locule; style creamy, up to 3.6 cm long; stigma creamy or light orange, up to 4 mm in diameter; compound tepal approximately 4.2 cm long and 2.3 cm wide at the base, featuring two prominent keels, orange in colour; free tepal approximately 4.5 cm long, oblong, devoid of a keel, translucent orange with an acute apex; staminodes five in number, inconspicuous, creamy, approximately 1.5 cm long, lacking pollen. Male buds are lanceolate, measuring up to 23 cm in length and 6 cm in diameter; bracts are orange on both surfaces, convolute, and acute at the apex, elevating 1 or 2 bracts simultaneously, without revoluting before to abscission; male flowers typically number 4 to 6 per bract in a single row, detaching along with the bracts. Compound tepal measuring 5 cm in length, featuring two thickened keels, orange in colour; free tepal also 5 cm long, translucent white, with an acute apex; anthers numbering five, reaching 5.5 cm in length, dirty brown; filament extending to 3 cm; anther lobes measuring up to 2.3 cm; stamen prominently exerted; stigma exhibiting a dirty brown hue; ovary arched, white, devoid of additional pigmentation. Fruit clusters are upright to slightly horizontal, dense, comprising up to 10 hands with 4 to 6 fruits per hand arranged in a single row; fingers are upright; individual fruits measure up to 12 cm (including pedicel) in length and up to 3 cm in diameter, exhibiting a straight, ridged form; pedicel measures up to 2 cm and is glabrous; fruit apex is obtuse; immature peel is green and non-waxy; pulp is white; seeds are angular,

approximately 5 mm in diameter, with an average of 130 seeds per fruit **(Figures 2.3a and 2.3b).**



Figures 2.3a & 2.3b : Habit and Inflorescence of *Musa aurantiaca*

2. *Musa balbisiana* Colla

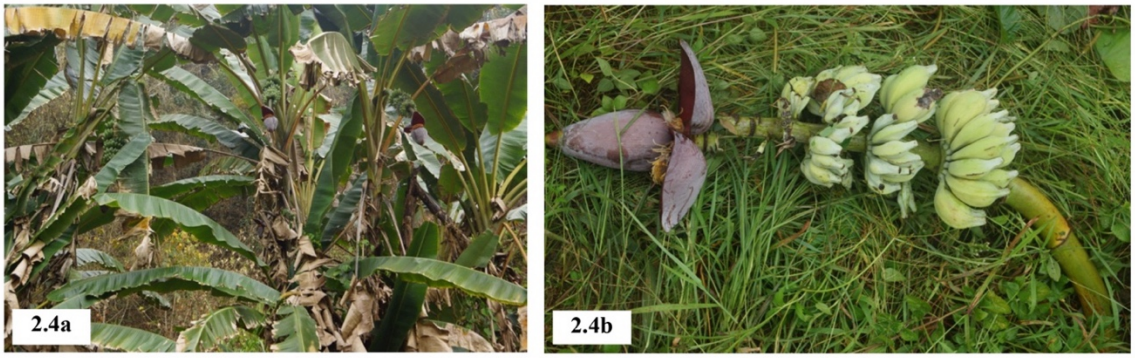
Description

Plants exhibit free stooling; pseudostems are sturdy, exceeding 6 meters in height, typically measuring at least 30 cm in diameter at the base, predominantly green or yellowish-green. The upper sections of the leaf sheaths frequently display black markings, while the lower portions may turn light reddish-brown with age. Leaf sheaths and petioles are generally glaucous or pruinose, occasionally to a significant degree. Leaf blades are oblong, exceeding 3 meters in length and 60 centimetres in width, truncate at the apex, and rounded or slightly cordate at the base. The upper surface is green, while the underside

is paler and somewhat glaucous, with midribs that are green or yellowish-green. Petioles measure 60 centimetres or more in length, exhibiting a pronounced concavity above, with edges nearly converging over the adaxial channel. The margins are inconspicuous above but more pronounced below, where the Inflorescence is pendulous, with a glabrous peduncle and rachis; basal flowers are female, with the number of female hands changing up to around 10 (10 - 15 according to Backer); higher hands are male. Male bud in advanced bloom, broadly ovoid to ellipsoidal, with bracts imbricate at the blunt apex. Bracts exhibit various shades of purple, broadly ovate, rounded at the apex, often greenish or yellow at the extreme tip; the outer surface is glaucous, typically longitudinally ribbed, while the inner surface ranges from dark crimson to the base, transversely corrugated between the ribs; multiple bracts are elevated concurrently, revealing several clusters of male flowers simultaneously; bracts are generally deciduous shortly after flowering but may occasionally persist in a withered state, particularly during the later phases of blooming. Male flowers consist of approximately 20 per bract, arranged in two rows; the compound tepal measures 4 to 5 cm in length and about 1.2 cm in width, exhibiting a whitish base colour but typically displaying a purplish hue internally, with pigmentation ranging from a pale blotch at the tepal's base to a vivid reddish-purple along most of its length, which is visible externally as a pink tint. The upper portion of the tepal, including the teeth, is yellow, varying from pale yellow to dark orange, with the teeth measuring about 5 mm in length, the two outer teeth possessing a dorsal filiform appendage of 1 to 2 mm. The free tepal is approximately half the length of the compound tepal, translucent white or pinkish, and generally boat-shaped, with an obtuse, truncate, or emarginate apex featuring a short mucronate apicula; the stamens initially match the perianth in length but later become exserted.

The fruit bunch is pendant and highly compact, with the densely packed fruits having limited space (save in the basal hand) to reflex geotropically, resulting in them predominantly protruding from the rachis. Individual fruits measure approximately 10 cm in length (ranging from 7 to 15 cm), with a diameter of 4 cm, exhibiting distinct angularity upon maturity. The base narrows abruptly into a pedicel measuring 1 to 2 cm, while the

apex tapers more gradually into a short, broad acumen. The pericarp is approximately 3 mm thick, pale yellow at full ripeness, and quickly darkens to black. The pulp is whitish in colour. Seeds are black, irregularly globose, somewhat flattened, and minutely warty, measuring 5 to 6 mm in diameter and 4 to 5 mm in height **(Figures 2.4a and 2.4b).**



Figures 2.4a & 2.4b : Habit and Inflorescence of *Musa balbisiana*

3. *Musa cheesmanii* N.W.Simmonds

Description

Plants are vigorous, producing suckers freely near the parent plant, typically 7–25 cm away, with 2–3 suckers arranged vertically; the mature pseudostem reaches a height of 3.3–6.5 m and has a circumference of 30–51 cm at the base, exhibiting a dark reddish-brown or reddish-black hue, while the upper portion is green and glaucous. The pseudostem is enveloped in old brown leaf sheaths, with an underlying colour that is greenish or reddish, shiny, and highly glaucous towards the apex, more pronounced in younger specimens. The sap is watery, and the pseudostem is notably hard, with colour variations observed in both young and older clumps, predominantly concentrated in the older ones. Leaf morphology is intermediate, with lamina dimensions of 75–185 × 50–61 cm, exhibiting an oblong-lanceolate shape, obliquely truncated at the apex. The adaxial surface is green and dull, while the abaxial surface appears light green or possesses a greyish hue due to a pronounced glaucous quality. The leaves are highly glaucous, with asymmetric bases; one side is rounded and the other auriculate. The midrib is yellow-green on the dorsal side and light yellow with purple pigmentation or lines on the ventral

side. Petiole measures 45–55 cm in length, exhibiting a greenish-yellow to purple hue, with edges that are closed and overlapping, adorned with reddish-black blotches at the base. The bases of the petiole are winged and embrace the pseudostem. Inflorescence is pendulous, with a peduncle of 45–80 cm in length and 3–5 cm in diameter, exhibiting a dull green hue, either glabrous or possessing a powdery look. Two sterile bracts, deciduous, measuring 48.5–63 × 4.5–11.5 cm, adaxially olive green in the centre, yellow-green towards the apex, with pink-purple at the base and margins; occasionally with or without an appendage at the apex, slightly glaucous, abaxially at the base and margins. Crimson-violet, verdant at the apex delineated by lines, lustrous. Feminine bud lanceolate. Bracts lanceolate, measuring 35–43 × 12.2–12.5 cm, smooth, adaxially dark brown.-purple transitioning to pink-purple, yellow at the apex, slightly glaucous, abaxially red-purple with a yellow apex, glossy, bract apex acuminate, bracts elevating individually, barely opening prior to abscission. Fundamental Female plants measure 3–14 hands in height, featuring 6–16 blooms per bract arranged in two rows, with a length of 11.5–13.6 cm and pedicellate structures. Compound tepal measuring 3.1–4 × 1.1–1.6 cm, exhibiting a cream to creamy yellow colouration, ribbed at the dorsal angles, with five lobes that are yellow and 0.6–0.8 cm in length; the two outer marginal lobes are bigger and possess horn-like appendages. Tepal dimensions are 2.4–3.1 × 1.7–2.3 cm, exhibiting a boat shape, translucent white or cream colouration with a yellow base, slightly corrugated at the apex, and featuring a little acumen. Staminodes five, measuring 1.1 to 1.7 cm in length, cream-coloured with a yellow base and a brown apex. Ovary measuring 7.7–9.7 cm in length, linear, pale green to yellow-green, containing 2 ovules per locule; style is linear, 3.2–3.7 cm long, cream-coloured; stigma is globose, exserted, and sticky, exhibiting grey to brown hues. Male buds are lanceolate and imbricate at the apex, with male mud continuing to form post-fruit maturity. The rachis descends vertically, remains green, and elongates to 1.3–2 m following fruit maturation. Bract scars are conspicuous and spatially organised, with occasional persistence of dry bracts. Bracts measuring 18–29.2 × 10.8–11.5 cm, lanceolate in shape, somewhat grooved, with an adaxial surface that is dark brown-purple and features a yellow apex, slightly glaucous; the abaxial surface is red-

purple with a yellow tip, having an acute apex, and lifts 1–2 bracts simultaneously, either just open or fully open and reflexed prior to abscission. Male flowers typically number 18–20 per bract, arranged in two rows, measuring 6.5–7.2 cm in length, and detach along with the bract. Compound tepal measuring 4.1–4.6 × 0.8–1.1 cm, cream-coloured, ribbed at dorsal angles, with five lobes that are creamy yellow, measuring 0.3–0.4 × 0.4–0.5 cm; the two outer marginal lobes are bigger and possess horn-like appendages. Free tepal measuring 2–2.4 × 1.5–1.7 cm, boat-shaped, bilobed at the apex, featuring a short acumen, and translucent white in colour. Five stamens, measuring 2.6–4.8 cm in length, with an exserted apex; filaments are cream-coloured and range from 1.8 to 2.3 cm long, while the creamy yellow anthers are 2.1–2.8 cm long, occasionally accompanied by a little extra stamen. Ovary either straight or curved, rudimentary, measuring 1.8–2.4 cm in length, cream in colour; style cream, inserted, measuring 4–4.6 cm in length; stigma brown and globose. Fruit clusters are loose, comprising 3 to 14 in an inflorescence. Fruits numbering between 6 and 12 per hand, exhibiting a curved, elongated, and angled morphology, with a backward curve and a pointed orientation towards the peduncle, arranged in two rows. They measure 9.8 to 14 cm in length and have a circumference of 8.7 to 11.4 cm. The apex is slightly pointed and devoid of floral remnants, measuring 0.5 to 1 cm in length. The pedicel ranges from 3 to 5 cm in length and is glabrous. The pericarp is dull green upon maturity, while the pulp is white. Seeds comparable in size to *Musa nagensium* and the genus *Ensete*, although exhibiting a warty surface akin to *Musa balbisiana*, subglobose in shape, measuring 0.6–1 × 0.5–0.7 cm, dull black, and brown when desiccated, with a quantity of 80–170 per fruit **(Figures 2.5a and 2.5b).**

Figures 2.5a & 2.5b : Habit and Inflorescence of *Musa cheesmanii*

4. *Musa flaviflora* N. W. Simmonds

Description

Plants are thin, with 3–4 suckers positioned vertically, approximately 10–15 cm from the main shoot. Mature pseudostems are 1.8–2.1 m in height and 20–30 cm in circumference at the base, exhibiting a green hue with occasional black spots. They are encased in old brown leaf sheaths, excluding the apex region, which is waxy, and the sap is watery. Leaf morphology is intermediate, with laminae measuring 150–155 × 36–51 cm, oblong-lanceolate in shape, and an apex that is oblique and truncate. The adaxial surface is dark green with black blotches or spots and has a dull texture, while the abaxial surface is medium green and lustrous in appearance. The leaf bases are symmetric and rounded, with the midrib appearing light green on the adaxial side and yellow-green on the abaxial side. Petioles about 43–50 cm in length, glaucous in appearance, with broad

erect margins featuring blackish-brown scarious edges and dark brown blotches at the base; the base of the petiole is winged and embraces the pseudostem. Inflorescences initially erect during the emergence stage, subsequently becoming horizontal to sub-horizontal; peduncle measures 13–15 cm in length, green, and glabrous. Sterile bracts 1–2, deciduous, approximately 30 × 10 cm, red on the adaxial surface and creamy pink on the abaxial surface, with a leafy appendage at the apex. Female buds are lanceolate, convolute, and have a somewhat imbricate tip. Female bracts are 15–23.3 × 8–12.4 cm, exhibit moderate grooves, are adaxially red with a yellow tip, slightly glaucous, and abaxially cream with a pink tint, possessing a shiny surface. The apex is acute, with bracts lifting individually, reflexing and revoluting prior to abscission. The cincinnus contains 12–20 flowers. Fundamental 5 to 8 hands, female. Female flowers, about 6.5–8.5 cm in length, are arranged in two rows, with 12–20 flowers per bract. Composite tepal Dimensions of 3.5–4 × 1.1–1.3 cm, yellow in colour, exhibiting ribbing at the dorsal angles, with five lobes of 0.4–0.6 × 0.2–0.3 cm, dark yellow, with the outer two lobes possessing tiny horns. Free tepal measuring 2.5–2.7 × 1.4–2 cm, boat-shaped, translucent cream with a yellow hue, apex corrugated, featuring a short acumen. Staminodes five, measuring 1.3 to 2.1 cm in length, cream-coloured with a brown tip. Ovary measuring 3.8–4.1 cm in length, linear, light green to yellow-green, containing ovules arranged in two rows per locule; style is straight, exserted, measuring 2.5–3.4 cm, cream-coloured with red speckles towards the apex, surface adorned with rigid trichomes; stigma is globular, creamy-grey, and adhesive. Male bud intermediate, apex imbricate, top-shaped, and convolute at full bloom, with a curved rachis. Male bracts measure 10–17 × 7–10.5 cm, exhibit moderate grooves, are red with a yellow tip on the adaxial surface, fairly glaucous, creamy pink and lustrous on the abaxial surface, with an acute apex. They lift one bract at a time, are reflexed and revolute prior to abscission, and the entire bud degenerates before the fruits reach maturity. Male flowers typically range from 14 to 32 per bract, arranged in two rows, measuring 5.8 to 6.2 cm in length, and detach along with the bract, leaving visible bract scars. Compound tepal measuring 3.3–4.5 × 1.2–1.6 cm, yellow in colour, ribbed along the dorsal angles, featuring 5-toothed dark yellow lobes,

each 0.3–0.4 cm in length. Free tepal measuring 1.9–2.6 × 1–1.6 cm, translucent cream with yellow hues, much darker at the apex and base, boat-shaped, corrugated at the apex, and featuring a little acumen at the tip. Five stamens, measuring 3.3–4.7 cm in length, are exserted; filaments are cream-coloured and range from 2 to 2.4 cm long, while the anthers are cream with a pink hue, measuring 1.1–2.3 cm long. Ovary linear, rudimentary, 0.9–1.5 cm in length, creamy white; style somewhat curled at the base, 2.9–3.5 cm long; stigma globose, yellow. Fruit clusters are loose, with 5–8 hands with 12–20 fruits per hand grouped in two rows, fingers orientated perpendicular to the rachis. The fruits are glabrous, straight, slightly ridged, with a pointed apex and devoid of floral remnants. Immature fruits have a dull yellow hue with black spots, and the pulp is cream-coloured **(Figures 2.6a and 2.6b).**

Figures 2.6a & 2.6b : Habit and Inflorescence of *Musa flaviflora*

5. *Musa itinerans* Cheesman

Description

The plant exhibits unrestricted stooling and produces elongated rhizomes that extend 2 meters or more from the parent stem; pseudostems reach heights of 4 meters or greater, with a base diameter of 20-25 cm, displaying a green colouration that varies in reddish-brown or blackish pigmentation based on age and exposure; leaf sheaths and petioles are free of wax. Leaf blades measuring up to 3 meters in length and 60 cm in width, truncate at the apex and rounded at the base; petioles ranging from 30 to 50 cm in length, with short, membranous margins that are upright above, closely embracing the pseudostem at the base and rapidly becoming scarious. Inflorescence semi-pendulous; peduncle velvety with thick minute pubescence; basal 2-4 (-6) "hands" female, higher hands male. The bract beneath the female flowers is ovate-lanceolate, measuring approximately 30 cm in length and 6 cm in width at the base, expanding to 13 cm at the centre. The apex of the lower bracts is obtuse and somewhat foliaceous; it has a pale yellow and lustrous inside, a dark purple exterior at the base, and green streaked with purple towards the apex. Tepals measure 4-5 cm in length, pale yellow, with appendages of 2-3 mm; the free tepal is translucent, boat-shaped, rounded at the back, and smooth. The five longer structures exceed the free tepal in length, are flattened, linear, white, fleshy, and acuminate. The style is slightly shorter, white, and 2-3 mm thick. The stigma is capitate, broad and deep, dorsiventrally compressed, with 3-5 angles, measuring 7-8 cm in length, including a whitish pedicel of approximately 2 cm, divided into three sections, with about 160 ovules per loculus arranged in scattered rows. The male bud is in an advanced stage of blooming, exhibiting a narrowly ellipsoidal shape with a convolute apex. Bracts exhibit a rich reddish-purple exterior, typically including a yellow edge and frequently displaying variegation with longitudinal stripes, while the inside is yellow, fading to nearly white at the base. Male flowers consist of 12-16 per bract arranged in two rows, about 6-8 cm in length. The compound tepal is whitish with lobes around 5 mm long; the free tepal is half the length of the compound tepal, boat-shaped, featuring minute stamens that are as long as the compound tepal and are eventually somewhat exserted. The fruit cluster is

somewhat loose, with the "fingers" extending and without pronounced geotropic curvature. Individual fruits measure approximately 10.3 cm in diameter, exhibiting an oblong-turbinate or obovoid shape, characterised by a broad apex that gradually tapers into a lengthy (5 cm) pedicel. The distinction is not sharply defined, presenting an obscure five-angled form at maturity, and culminates in a narrow apex with a short (5 mm) truncate acumen. Immature fruit exhibits a faint whitish-green hue, transitioning to yellow upon full ripeness, with a thick consistency; the pulp is a pale cream colour. Seeds are tuberculate, irregularly angulate-depressed, measuring 5-7 mm in length and 3 mm in height **(Figures 2.7a and 2.7b).**

Figures 2.7a & 2.7b : Habit and Inflorescence of *Musa itinerans*

6. Musa laterita Cheesman

Description

Perennial stoloniferous rhizomatous herbs that propagate through suckering rather than clumping, dispersing via running rhizomes, with suckers located 3–5 meters from the parent plant, orientated vertically. Pseudostems are cylindrical, either not or indistinctly swollen at the base, reaching a height of 30–220 cm and a circumference of 15–20 cm at the base. They are slender, light green with black blotches or dark reddish-brown patches, with an underlying colour of light green that may exhibit pigmentation or dark reddish-brown patches. The surface is shiny, and the sap is watery. The leaf habit is erect, with leaves forming a terminal tuft at the apex. The lamina measures 90–108 × 25–37 cm, is

oblanceolate, adaxially dark green with a brown scarious margin, and dull, while abaxially it is medium green and glabrous. The leaf bases are symmetric, tapering on both sides towards the base and extending to the petiole as a petiole margin, which narrows towards the apex, which is either acute or obtuse. At the young stage, there is a tendril-like appendage present. The midrib is light green to yellow-green on both adaxial and abaxial surfaces. Petiole measures 22–40 cm in length, glabrous, with open edges that spread and lack blotches at the base. The bases of the petiole are winged, featuring blackish-brown scarious margins, smooth and encircling the pseudostem. Inflorescence upright, peduncle 15–30 cm in length, green, densely covered with short white hairs, grooves not pronounced. Sterile bracts 1–2, persistent; the first is leaf-like with a widened petiole, exhibiting a brick red hue and leafy tip, while the subsequent bract resembles a real bract, being linear-lanceolate, brick red with a cream base, measuring 15–19 × 4.5–5.5 cm. Female bud lanceolate, imbricate at the apex. Female bracts lanceolate, measuring 12–17 × 6–8 cm, adaxially red to brick red with a yellow apex, oblique base, moderately grooved, slightly glaucous, abaxially somewhat paler, heavily grooved with transverse lines, shining, sharp apex, raising 1–2 bracts simultaneously, reflexed and revolute prior to abscission. Basal 4–6 hands, female. Bracts and flowers are individually attached to the axis. Female flowers, about 6.7–8 cm in length, are arranged in two rows, with 4–8 flowers per bract. Compound tepal measuring 2.8–3.3 × 1.8–2.1 cm, orange-yellow in colour, ribbed at the dorsal angles, split solely at the apex, with five lobes of dark orange-yellow hue. The free tepal is approximately half the size of the compound tepal, measuring 1.6–1.9 × 1.2–1.6 cm. It is boat-shaped, fused, and exhibits a cream hue tinged with yellow, becoming yellow towards the base and apex. The apex is slightly corrugated and features a short acumen, which is yellow and measures 0.2–0.4 cm in length. Staminodes five, measuring 1.2 to 1.6 cm in length, yellow with a cream-coloured tip. Ovary measuring 3.8–4.3 cm in length, straight, green, containing ovules arranged in two rows per locule; style straight, 3–3.8 cm long, cream with a yellow hue at the base and apex, inserted; stigma globose, exserted, cream-grey, sticky, around 4 mm in diameter. Male bud lanceolate, top-shaped in advanced bloom, imbricate at the tip, with an erect rachis. Male

bracts measure 12–15 × 6.5 cm, exhibiting pronounced grooves, adaxially red to brick-red with a yellow apex, and slightly glaucous. Abaxially, they are significantly darker and shiny, featuring prominent ridges with transverse lines. The apex is obtuse or rounded, lifting 1–2 bracts simultaneously, and may be either deciduous or persistent, reflexing prior to abscission, without being revolute. The entire bud deteriorates before the maturation of the fruits. Bracts and flowers are inserted individually along the axis. Male flowers, measuring 3–4.8 cm in length, are arranged in two rows of 6–8 per bract, detaching with the bracts or occasionally remaining attached, with a conspicuous bract scar. Compound tepal measuring 2.5–4 × 1.9–2.5 cm, orange-yellow in colour, split solely at the apex, ribbed at the dorsal angles, and featuring five-toothed orange lobes. Free tepal measuring 1.3–1.6 × 1–1.1 cm, translucent creamy orange, boat-shaped, fused, and corrugated at the apex with a short acumen. Five stamens, measuring 3–3.5 cm in length, are exserted; the filament is 1.7–1.9 cm long, exhibiting a greenish-white hue with an orange tint at the anther; the anther measures 1.3–1.9 cm in length and is creamy yellow in colour. Ovary linear, rudimentary, 0.7–1.2 cm in length, cream with yellow-green hues; style linear, 2.3–3.5 cm in length, cream, orange near the stigma, inserted; stigma globular, orange, adhesive, approximately 1 mm in diameter. The fruit cluster is compact, with 4–6 hands with 4–8 fruits per hand, arranged in two rows. The central hand is significantly developed, with fingers orientated upwards, exhibiting negative geotropism, and closely appressed to the rachis, with individual fruits present. Fruits measuring 3.5–7 cm in length, borne on a very short pedicel of 0.2–0.3 cm, are glabrous, straight, and distinctly ridged, with a slightly pointed apex measuring 0.3–0.6 cm. They lack floral remnants, exhibit a green peel when immature, transitioning to yellow upon maturity. The immature pulp is white, becoming soft and white at maturity, and the fruits do not self-peel upon ripening. Each fruit contains 50–60 seeds, measuring 0.6–0.9 cm by 0.3–0.4 cm, globular in shape, with a smooth surface, and exhibiting a colouration ranging from brown to black, except for the circum-hilar region **(Figures 2.8a and 2.8b).**

Figures 2.8a & 2.8b : Habit and Inflorescence of *Musa laterita*

7. *Musa mannii* H. Wendl. ex Baker

Description

Plants are slender and produce suckers freely near the parent plant, measuring 7–15 cm, with three or four suckers grouped vertically. The mature pseudostem measures 85–110 cm in height and has a circumference of 14–16 cm at the base. It exhibits a creamy green hue with reddish-brown patches, and its underlying colour is also creamy green with reddish-brown patches. The surface is shiny, and the sap is watery. Leaf morphology exhibits a drooping habit, with lamina dimensions of 98–100 × 42–44 cm, characterised as oblong-lanceolate and truncate at the apex. The adaxial surface is green with a yellow tinge, exhibiting a dull texture with transverse lines, while the abaxial surface is light green and shiny in appearance. The leaf bases are asymmetric and rounded on both sides. The midrib presents a yellow-green hue on the adaxial side and displays a purple colouration

when young, transitioning to a greenish tone with pink-purple accents on the abaxial side, also purple in its juvenile state. Petiole measures 43–44 cm in length, exhibiting a light green hue with pink pigmentation. The margins are erect, displaying black to reddish-brown blotches at the base. The bases of the petiole are winged, featuring a broad, wrinkled scarious margin in black to reddish-brown, which does not clasp the pseudostem. Inflorescence initially erect, subsequently inclined to one side; peduncle is 8–10 cm in length, crimson, and densely pubescent with short white hairs (velvety). Deciduous sterile bract, measuring 28–30 × 4–5 cm, lanceolate in shape, scarlet red, with an apex including a leaf appendage. Female bud is lanceolate and convolute. Female bracts are 14.5–20 × 4.5–5.2 cm, are lanceolate in shape, adaxially crimson red with minor hairiness, and abaxially smooth and crimson red. The apex is somewhat obtuse, with one to three bracts lifting simultaneously, reflexing before detachment and contacting the adaxial surface of the peduncle. Basal three or four-handed hermaphrodite, with flowers arranged in a single row, numbering two to six per bract, measuring 6.7–7 cm in length. Compound tepal measuring 3.8–4 × 1.4–1.6 cm, cream orange in colour, significantly darker at the base and apex, ribbed along the dorsal angles, featuring five lobes that are orange and 0.2–0.3 cm in length, with the outer two being bigger and possessing horn-like appendages. Free tepal measuring 3.2–3.5 × 1.5–1.7 cm, oval, translucent white, corrugated at the apex, with a truncate apex. Five stamens, measuring 2.6–3.2 cm in length, with white filaments of 1.1–1.2 cm, and cream-coloured anthers measuring 2–2.2 cm long. Ovary measuring 2.9–3.5 cm in length, linear, light green in colour, containing two ovules per locule; style is straight, with a rough surface, measuring 3.1–3.2 cm in length, cream-coloured; stigma is globose, exserted, sticky, and dull yellow. Male bud oblong, convolute, with a rachis inclined to one side, red in colour, and exhibiting pronounced bract scars. Male bracts are 13–14.8 × 4.3–4.8 cm, are lanceolate, smooth, and exhibit a crimson red colouration on both adaxial and abaxial surfaces. The apex is pointy, and they raise one to three bracts simultaneously, reflexing before detachment and contacting the rachis on the adaxial side. Male flowers typically number five per bract in a single row, measuring 5.1–5.4 cm in length, and detach together with the bract. Compound tepal measuring 4.2–4.5 × 1.3–1.4

cm, cream orange in colour, ribbed at the dorsal angles, with five orange lobes. Free tepal measuring 3.7–4 cm in length and 1.4–1.5 cm in width, translucent white with a golden apex, which is obtuse and oval in shape. Five stamens, measuring 4.2–5.3 cm in length, with an exserted apex; filaments are white and 2–2.4 cm long, while the cream-coloured anthers are 2.2–2.4 cm in length. Ovary linear, rudimentary, 0.8–1 cm in length, cream-coloured; style cream, inserted, 3.4–3.7 cm in length; stigma greenish-yellow, exserted. Fruit cluster loose, including three or four hands within an inflorescence. Fruits numbering two to six on the mid-hand, arranged in a single row, measuring 4.4–4.7 cm in length, straight, slightly ridged, glabrous, with a slightly pointed apex, devoid of any floral remnants. pedicel Measuring 0.5–0.6 cm in length, the glabrous, white immature fruit exhibits a light green hue, transitioning to light green or yellow-green and thin upon full ripeness. The pulp is white and delicious, with the fruits being deciduous and not self-peeling at maturity. Seeds are verrucose, numbering 12–17 per fruit, irregularly angular, spherical, dorsiventrally flattened, measuring 0.2–0.3 cm in diameter and height, dull black, and brown when desiccated **(Figures 2.9a and 2.9b).**

Figures 2.9a & 2.9b : Habit and Inflorescence of *Musa manii*

8. *Musa markkuana* M.Sabu, A.Joe & Sreej.

Description

Plants are slender, producing suckers abundantly but in proximity to the main branch, measuring 7–12 cm, with 2–3 vertically placed suckers. Globose rhizomes, measuring 9–11 cm in length and 8–11 cm in diameter, adorned with brown scale bracts. Developed pseudostems Height ranging from 1.1 to 1.6 meters, base diameter between 6 to 10 cm, exhibiting a light green hue, enveloped in aged brown waxy leaf sheaths, with an underlying light green colouration accented by pink-purple pigmentation, possessing a glossy appearance and watery sap. Leaf morphology is intermediate, with laminae measuring 107–129×45–47 cm, oblong-lanceolate in shape, and truncate at the apex. The upper surface is dark green and dull, while the lower surface is medium green and shiny. The leaf bases are asymmetric, with both sides rounded. The midrib is light green dorsally and red-purple ventrally, and young leaves exhibit a light green colouration with purple patches. Petioles measuring 55–66 cm in length, exhibiting a waxy texture, with inwardly curved margins adorned with purple-black scarious edges and little brown-black blotches at the base; the bases of the petioles are winged and embrace the pseudostem. Inflorescences are erect, with peduncles measuring 12–14 cm in length and 2 cm in diameter, exhibiting a maroon hue, slightly puberulent with short hairs, and featuring noticeable grooves. Deciduous sterile bract of 25–27 cm in length, with a pink colouration on both abaxial and adaxial surfaces. Female buds are lanceolate and convolute. Bracts measuring 16–18×7–8 cm, somewhat grooved, pink on both abaxial and adaxial surfaces, slightly puberulent externally, glossy inside, with a pointed apex, raising two bracts simultaneously, and revolute prior to abscission. Fundamental Four to five female hands beside one bisexual hand. Flowers arranged in a single row, 4 to 6 per bract, measuring 7.3 to 7.5 cm in length. Compound tepal measuring 4.3–4.5 cm by 1.7–1.9 cm, yellow in colour, ribbed at dorsal angles with a pink flush, with 5 lobes, orange in hue. Free tepal measuring 3.3–3.7 cm in length and 1.6–1.9 cm in width, oval-shaped, yellow-tinged, corrugated, with an obtuse tip. Staminodes five, measuring 1.4 to 1.7 centimetres in length, cream-coloured. Ovary measuring 3–4 cm in length, linear, initially creamy white,

subsequently transitioning to maroon, containing ovules arranged in two rows per locule; style is straight, inserted, 2–3 cm long, cream in colour; stigma is globular, creamy yellow, approximately 4 mm in diameter. Fifth-generation hermaphrodite. Male bud lanceolate, with erect rachises and convolute structure. Bracts measuring 12–15×6.5 cm, somewhat grooved, pink on the abaxial side and significantly darker on the adaxial side, with minimal wax present, pointed apex, raising two bracts simultaneously, revolute before to abscission, with the entire bud deteriorating before the maturation of the fruits. Male flowers typically number seven per bract in a single row, measuring 6 to 6.5 cm in length, and detach along with the bract, leaving visible bract scars. Compound tepal measuring 5.2–5.4 cm in length and 1.1–1.4 cm in width, yellow in colour, ribbed at the dorsal angles with a pink flush, and featuring five-toothed orange lobes. Free tepal measuring 4.3–4.5×1.2–1.6 cm, translucent cream with a golden hue, oval-shaped, corrugated, with an obtuse apex. Five stamens, measuring 4.6–4.8 cm in length, are exserted; the filaments are white and range from 2.2 to 2.4 cm, while the cream-coloured anthers measure 2.3–2.5 cm. Ovary linear, 1.1–1.2 cm in length, initially creamy white, subsequently transitions to maroon; style linear, inserted, 4.6–4.8 cm in length; stigma cream, approximately 1 mm in diameter. The fruit cluster is compact, consisting of 5 hands with 4 to 6 fruits per hand, arranged in a single row, with the fingers orientated towards the male bud, featuring distinct fruits. Pedicel measuring 6–10 cm in length. 1 cm in length, glabrous, fruits linear, distinctly ridged, apex blunt, devoid of floral remnants, immature fruit peel maroon, transitioning to light maroon or pink without black blotches. 3–4 mm thick, the juvenile fruit pulp is white, transitioning to white and soft at maturity; the fruits are deciduous and do not self-peel when mature. Seeds are verrucose, irregularly angular, measuring 4–6 mm in diameter and 2–3 mm in height, brown-black except for the circum-hilar region, with 70–80 seeds per fruit **(Figures 2.10a and 2.10b).**

Figures 2.10a & 2.10b : Habit and Inflorescence of *Musa markkuana*

9. *Musa nagalandiana* S. Dey &Gogoi

Description

Plant vigorous, producing up to 6 vertically placed suckers; mature pseudo-stem reaches 10 m in height and 30 cm in basal diameter, devoid of dead sheaths, exhibiting a yellowish-green hue with brown or black mottling, glossy; sap is aqueous. Leaves are intermediate; petiole measures up to 70 cm in length and approximately 2 cm in diameter, with canal margins curving inward and overlapping upon maturation. The bases are yellowish-green, mildly waxy, and tightly embrace the pseudo-stem. The lamina is either elliptic or oblong, reaching lengths of up to 140 cm and widths of 62 cm, exhibiting a deep green hue adaxially and a pale green hue abaxially. The base is asymmetric, with both sides rounded and auriculated. The midrib appears yellowish-green externally, devoid of pigmentation. Pendent inflorescence. The female buds are imperceptible. Male buds are

ovate, measuring up to 20 cm in length and 16 cm in diameter; bracts are ovate, acute at the apex, reaching up to 16 cm in length and 11 cm in width, exhibiting a yellowish-orange hue on both surfaces, non-waxy, imbricate, and lifting multiple bracts simultaneously, not revolute prior to abscission; male flowers average 20 per bract, approximately 5 cm in length, arranged in 2 rows, detaching with the bracts; the compound tepal is about 2.5 cm long, oblong, with a 5-partite apex and 2 thickened keels, creamy in colour; the free tepal is around 1 cm long, translucent white, oblong-ovate, with an acuminate apex; there are 5 anthers containing pollen, each 2.8 cm long, creamy, with filaments measuring 6 mm; anther lobes are 2.5 cm long; stamens are exerted; stigma is dirty white; the ovary is not arched, creamy, 1.5 cm long, devoid of additional pigmentation. Fruit cluster pendant, compact; peduncle measures up to 45 cm in length and 3.5 cm in diameter; comprises 13 hands, each with up to 17 fruits arranged in 2 rows; fruits are curved, reaching up to 10.5 cm in length and 3.5 cm in diameter, glabrous with a pointy apex; juvenile fruit skin is pale green and non-waxy; pulp is white. Each fruit contains up to 338 seeds, which are globular, smooth, and measure 4 mm in diameter **(Figures 2.11a and 2.11b).**

Figures 2.11a & 2.11b : Habit and Inflorescence of *Musa nagalandiana*

10. *Musa nagensium* Prain

Description

Plants are slender, somewhat suckering, in proximity to the parent plant, measuring 20–25 cm, with 3–4 vertically orientated suckers. Mature pseudostems measure 3.5–7 m in height and 50–60 cm in basal diameter, exhibiting a greenish-yellow or olive-green hue in their juvenile phase, transitioning to reddish-brown or nearly black upon maturity. They possess a glaucous appearance, becoming increasingly waxy towards the apex, with the most pronounced waxiness observed in young suckers. The underlying colouration is light green, featuring reddish-brown pigmentation or large reddish-brown patches, and exhibits a shiny finish. Leaf morphology exhibits a drooping habit, with increased spacing between individual leaves. The lamina measures 130–210 × 40–46 cm, characterised as oblong-lanceolate with a truncate apex. The upper surface is dull green with a reddish-brown margin, while the lower surface is minimally waxy, presenting a greyish hue. The leaf bases are asymmetric, featuring one rounded side and the other auriculated. The midrib displays a yellow-green colouration on both dorsal and ventral surfaces. Petioles are slender, measuring 45–55 cm in length, yellow-green, waxy, devoid of blotches at the base, with wings bases featuring crimson borders that clasp the pseudostem. Inflorescence semi-pendulous or initially horizontal then pendulous, peduncle measuring 20–45 cm in length and 3–5 cm in diameter, dark green with some purple pigmentation, glaucous, and devoid of grooves. Sterile bracts measuring 1–5, either deciduous or persistent, range from 20 to 30 cm in length, exhibiting a colour spectrum from green to yellow-orange, with a glaucous appearance on the adaxial surface. Female bud almost cylindrical or lanceolate, with imbrications either extensive or restricted to the apex. Bracts measuring 13–23 × 5–10 cm, oblong-lanceolate, smooth, adaxially yellow-orange interspersed with brick red or variations of red, yellow, and orange, with red, waxy borders, abaxially orange and lustrous, with an obtuse apex, raising one bract at a time without revoluting prior to abscission. Basal 4–8 female hands. Flowers ranging from 4 to 12 per bract, arranged in two rows. Male bud oblong to cylindrical, exhibiting great imbrication or imbrications restricted to the apex, with the rachis descending vertically, reaching a height of around 1

metre. The bracts, positioned far on the short rachis, confer a very imbricate quality to the bud. 9.5–19 × 5–8.5 cm, smooth, lanceolate, adaxially glaucous, interspersed with orange-yellow and brick red or yellow, margins red, apex green or orange, obtuse, adaxially creamy orange to orange, lustrous, elevating one bract at a time, partially open, deciduous or persistent, with the bud developing at the maturity of the fruits. Male flowers typically number 8–16 per bract, arranged in two rows, measuring 4.8–6.4 cm in length, and detach along with the bract, leaving visible bract scars. Compound tepal measuring 3.9–5 cm in length and 0.9–1.4 cm in width, exhibiting a creamy orange hue, ribbed at the dorsal angles, and featuring five-toothed orange lobes, with the outer two lobes significantly bigger, measuring 0.6–0.7 cm by 0.5 cm. Free tepal measuring 2.8–3.7 × 0.8–1.9 cm, translucent cream in colour, boat-shaped, corrugated at the apex, either incised along the margins or not, with an acuminate apex, possessing or lacking an acumen. Five stamens, measuring 3.5–4.5 cm in length, are exserted. The filaments are cream to white, ranging from 1.9 to 2.2 cm long, while the anthers are cream to golden yellow, measuring 1.4–2.7 cm long, with the apex curving backwards. Linear ovary, rudimentary. Measuring 1.5–1.7 cm in length, creamy white with a yellow hue, the style is straight and inserted, ranging from 3–4.1 cm long, with a globose stigma that is cream to creamy yellow in colour. The fruit cluster is loose, including 4–8 hands with 4–12 fruits per hand, arranged in two rows. The fruits are clavate-oblong, suspended from a slender rachis and orientated towards the male bud. They are straight, distinctively angular, and range in colour from green to dark green, often exhibiting a glaucous hue and occasionally featuring black dots. Each fruit is long-stalked and individual. Pedicel measuring 12–15 cm in length measuring 1.8–2 cm in length, glabrous, with a slightly pointed apex and devoid of floral remnants, the juvenile fruit pulp is white, transitioning to a soft white upon maturity; the fruits do not self-peel when mature. Seeds are large, irregularly angular, somewhat warty, measuring 0.9–1.4 mm in diameter and 0.5–0.6 mm in height, tapering towards the circum-hilar region. They

are brown-black, except for the white circum-hilar area, with 25–40 seeds per fruit **(Figures 2.12a and 2.12b).**

Figures 2.12a & 2.12b : Habit and Inflorescence of *Musa nagensium*

11. *Musa sikkimensis* Kurz

Description

Pseudostem reaches 4 meters in height, sturdy, with a diameter of 35 to 40 centimetres at the base, exhibiting a reddish hue. Leaf sheaths exhibit a blackish-brown colouration and are waxy only in their juvenile stage. Petiole around 65 cm, channelled, with upright borders that are narrowly blackish-scarious, creating a black line in the bottom section adjacent to the pseudostem. Leaves expansive, blades oblong-lanceolate, base rounded or slightly cordate, measuring 1.8 - 2.1 x 0.6 m., yellowish-green, glossy on both surfaces, typically purplish when juvenile, occasionally beneath when mature, midrib red or purplish on the underside. Bracts wide oval, obtuse, non-reflexed, deep purple to crimson, glaucous externally, concolorous inside, with 1 to 2 male bracts opening simultaneously. Male bud, approximately 12 x 8 cm, turbinate in shape. Male flowers approximately 14 per bract; compound tepal approximately 3.5 cm, creamy-orange; free tepal approximately 1.6 cm, transparent; stamens equal in length to compound tepal. Fruit clusters oblique, approximately 4 hands each containing 7 to 9 fruits arranged in two rows; fruits somewhat

loose, originating from huge, brown callosities on the axis, measuring 11 to 15 x 4 cm, angled at maturity, abruptly tapering into a substantial pedicel (approximately 2 x 1 to 2 cm), apex bluntly rounded and apiculate; pericarp green transitioning to brown. Numerous seeds, sharply angled, smooth, measuring 6 - 10.5 x 5 - 6 mm, black in colour, with sparse pulp that is dirty white to pale brownish-pink **(Figures 2.13a and 2.13b).**

Figures 2.13a & 2.13b : Habit and Inflorescence of *Musa sikkimensis*

12. *Musa velutina* H. Wendl & Drude

Description

The plant exhibits free stooling; pseudostems reach heights of up to 1.5 meters and have a base diameter of 7 cm, though they are often smaller. The colouration is yellowish-green and lacks wax. Leaf blades can extend up to 1 m in length and 35 cm in width, featuring a truncate apex and an asymmetrical base, with one side rounded and the other broadly wedge-shaped. The lamina is a lustrous dark green on the upper surface and a paler shade beneath, with minimal glaucousness. The inflorescence is notably erect, with a red peduncle measuring 2 cm in thickness, densely covered in white pubescence. The terminal leaf features a widened petiole that exhibits a red hue, succeeded by a persistent sterile bract measuring 15-20 cm in length. The bottom flowers are hermaphroditic, with

2-4 fertile "hands," while the higher flowers are male. Hermaphroditic flowers occur in clusters of 3-5 per bract, arranged in a single row; the ovary measures 3-3.5 cm in length and 1-1.5 cm in width, exhibiting a distinctly acute trigonous or quadrilateral shape depending on its orientation, and is densely pubescent. The compound tepal is 3 cm long, vibrant orange with a pink hue on the reverse side, featuring lateral lobes that are 4 mm long with a filiform dorsal appendage measuring 3 mm (or occasionally up to 5 mm), while the central lobe is broader, shorter, and lacks an appendage. The free tepal is nearly equivalent in length to the compound tepal, white, oval, and emarginate at the apex. Typically, there are fewer than five fertile stamens, with one or more being vestigial, and the fully developed stamens are as long as the style. The male bud is predominantly turbinate, either acute or acuminate, aborting after generating around twelve "hands" of convolute bracts at the apex, pale pink externally, distinctly sulcate, and darker internally; one is elevated at a time, significantly fading, and is early deciduous. Male flowers typically consist of approximately five per bract, usually arranged in a single row, but when more abundant, they may form two rows; they are compound, elongated, orange-yellow with a pink flush on the reverse side, and possess rudimentary pink lobes of tepal measuring nearly 2 mm in length, along with minute lateral dorsal appendages; the free tepal measures 3 to 5 cm in length and 1 cm in width, occasionally exhibiting jagged teeth towards the apex, with a short stamen that is ultimately exserted, the filaments of which are longer than the anthers. The fruit is brilliant pink, approximately 7 cm in length and 3-4 cm in diameter, resembling the peduncle, with a widely truncate apex and a rounded base. The pericarp is 3-4 mm thick, separating at maturity into uneven strips from apex to base, revealing the inner pulp and seeds. Seeds are black, tuberculate, irregularly angulate-depressed, measuring 2-3 mm broad (**Figures 2.14a and 2.14b).**

Figures 2.14a & 2.14b : Habit and Inflorescence of *Musa velutina*

A taxonomic key to the genus *Musa* in Nagaland is provided below to ensure ease of identification:

Table 2.3: Key to the genus *Musa* in Nagaland

1a	Plant slender; basal flowers hermaphrodite in female bud or female in female bud; inflorescence erect or horizontal	2
1b	Plant robust; inflorescence pendent; basal flowers female in female bud or no bud observed	6
2a	Basal flower female; fruits glabrous; not splitting at maturity	3
2b	Basal flower hermaphrodite; fruits hairy or glabrous; self-peeling at ripening or does not split at maturity	4
3a	Pseudostem 1.2-1.5 m high; bracts pink; ovary purple	*M. markkuana*
3b	Pseudostem upto 1.1m high; bracts orange; ovary green	*M. aurantiaca*
4a	Bract pink; fruit hairy; self-peeling at ripening	*M. velutina*

4b	Bracts brick red or rose; fruit glabrous; does not split at maturity	5
5a	Pseudostem emerging 2 m from parent plant; inflorescence erect; male flowers orange in colour; bracts brick red	*M. laterita*
5b	Pseudostem emerging quite close to parent plant; inflorescence horizontal; male flowers light yellow in colour; bracts rose in colour	*M. mannii*
6a	Four rows of ovules in each locule in the ovary	7
6b	Two rows of ovules in each locule in the ovary or no ovule observed	9
7a	Plant stoloniferous; male bud purple red with yellow margin	*M. itinerans*
7b	Plant clump forming; male bud crimson or purple without yellow margin	8
8a	Pseudostem densely covered with dead sheaths; male bud deep purple; fruit bunch oblique; peduncle densely brown-hairy; fruiting pedicel hair	*M. sikkimensis*
8b	Pseudostem not covered with dead sheaths; male bud crimson; fruit bunch straight; peduncle glabrous; fruiting pedicel glabrous	*M. balbisiana*
9a	Plant less than 6 m tall; pseudostem base to 20 cm in diameter; its underlying colour green with black or dark brown blotches; male bud pink reddish; fruit peel deep green	*M. flaviflora*
9b	Plant more than 7 m tall; pseudostem base to 35 cm in diameter; its underlying colour black or dark brown, reddish or yellowish green; male bud purple or scarlet or orange; fruit peel pale green or whitish	10
10a	Pseudostem dark brown, reddish or blackish; female bract greenish with purplish margin; male bud purplish; fruit recurved and tip pointing against gravity, fruit peel green	*M. cheesmanii*

10b	Pseudostem yellowish green; female bract flame scarlet or orange; male bud flame scarlet or orange; fruit drooping and tip always pointing towards gravity	11
10c	Pseudostem yellowish green; female bud unobserved; male bud orange or yellowish orange; fruit bunchy not densely compact; fruit tip pointing against gravity; fruit peel pale green	*M. nagalandiana*
11	Male bud flame scarlet; fruit apex less pointed; mature seeds black and smooth; fruit peel green to dark green in color; intense wax on the lower surface of the leaf	*M. nagensium*

Ethnobotanical documentation

A single member of the genus *Ensete*, namely *Ensete glaucum,* belonging to the same family, Musaceae, as the genus *Musa,* has been observed during the study as well **(Figures 2.15a and 2.15b).** It has not been enumerated in this study owing to its taxonomic dissociation from the taxon under study. This single member from the genus *Ensete* was observed in Wokha, Zunheboto, Kohima and Chumoukedima districts of Nagaland. It can be distinguished by its pale to almost pastel green coloured, snowy appearance. The pseudostem is distinguished by its bulbous base and its oxidised reddish sap; the bracts by their large, persistent, overlapping tile-like appearance.

Figures 2.15a and 2.15b : Habit and inflorescence of *Ensete glaucum*

Discussion

The research presents an overview of the diversity of wild *Musa* species in Nagaland. The study concluded with the identification of 12 wild *Musa* species in Nagaland, which is significant, and the discovery of other wild *Musa* species inside the state would enhance the existing richness of this genus. The recorded species reside in solitary populations or alongside other species, with their distribution contingent upon the altitudinal profile and the agro-climatic and topographical characteristics of their surroundings. The research area's topography features parallel hill ranges oriented southwest to northeast, interspersed with short valleys and multiple river systems, including the Dhansiri, Doyang, Tizu, and Dikhu. The altitudinal gradient considerably contributes to habitat heterogeneity, affecting the geographic distribution and natural variation of wild *Musa* species.

The majority of taxonomic members, with the exception of *Musa sikkimensis* Kurz, exhibit an altitudinal limit of 2000 m AMSL, above which they are not found. The predominant species identified is *Musa balbisiana* Colla, which flourishes at elevations below 1000 m AMSL and coexists with *Musa cheesmanii* N.W.Simmonds, *Musa flaviflora* N.W.Simmonds, *Musa itinerans* Cheesman, and *Musa velutina* H.Wendl. & Drude. The research noted that these wild *Musa* species flourished not just in undisturbed regions but also in disturbed environments, including roadsides, jhum fields, and fallow lands. The species distribution across the state is heterogeneous, with the highest concentration recorded in the Northern and Western regions, specifically in the Mon, Longleng, and Mokokchung districts. *Musa mannii* exhibits a limited range within the Sub-tropical forests of Mon, but *Musa nagensium* and *Musa markkuana* have a distribution that extends southward to the Zunheboto district and portions of the Tuensang region. The state's terrain is characterised by hills and rivers that function as obstacles to the dispersal of *Musa*. This is evidenced by the species diversity observed on the northern banks of the Doyang and Dikhu rivers, where up to ten species were documented in specific districts such as Mon, Tuensang, and Mokokchung. In contrast, the southern region, including Kiphire and Phek, with its elevated mountains, is primarily populated

by *Musa sikkimensis.* The species was documented at elevations of 2250 m in Chendang Saddle, Tuensang district; however, no other Mus species were detected at these sites. The most well-distributed species in the study area is *Musa balbisiana,* with dense populations occurring in altitudinal habitats at 700-800 m. It was found growing in large expanses along riparian zones, valleys and even in open dry fallow lands throughout the study area, which demonstrates drought-resistant features that were not detected in the other *Musa* species whose habitats are restricted to wet, damp and shaded areas. *Musa balbisiana* grows sympatrically with *Musa flaviflora* in the state's lower altitude zones. *Musa cheesmanii* and *Musa balbisiana* are also seen growing sympatrically at elevations ranging from 500 to 700 meters in various parts of the state. These are places of convergence, with *Musa balbisiana* growing at lower elevations and *Musa cheesmanii* at higher altitudes till around 1400 m.. *Musa itinerans* adapted to altitudes ranging from 400 to 1400 m in the majority of Nagaland's northern and western areas. It is found in huge clumps with dispersed individual plants owing to the stooling behaviour of the plant. It was discovered to be mostly growing in the shady wet western slopes.. It is seen to occur sympatrically with *Musa balbisiana* and *Musa flaviflora*, albeit the population forms a cluster within the larger population owing to its stooling habit. *Musa aurantiaca* seems to be the most broadly adapted species within the former Cheesman's section Rhodochlamys, as it was observed in clusters distributed across extensive areas at elevations of 800 to 1300 meters throughout the state, excluding the southern regions, which encompass the Dimapur, Kohima, Kiphire, and Phek districts. *Musa aurantiaca* was also found to grow sympatrically with *Musa nagensium* in Mokokchung district as well as with *Musa markkuana* in Mon district. *Musa nagalandiana* was found in a few clumps among the *Musa balbisiana* clumps along the Doyang riverbanks in Wokha district. The altitudinal profile of that particular population saw *Musa nagalandiana* sandwiched between *Musa balbisiana* and *Ensete glaucum*, both of which belong to different genera of the same family. *Musa balbisiana* occurring along the lower range and moving up till the *Musa nagalandiana* population, which then proceeded higher up to a cluster of *Ensete glaucum*. An observation noted in the field regarding this endemic and

rare species, i.e., *Musa nagalandiana*, was that despite the plant bearing a good quantity of fruits, none of the fruit produced viable seeds, which can only lead to the assumption that members of this species are naturally sterile and only reproduce vegetatively. This can, in a way, explain their limited distribution and rarity.

This member of the genus *Ensete*, although not a banana in the taxonomic sense, is a banana in the ethnobotanical sense, owing to the fact that the local populace in the state considers it and uses it as a wild banana variety. The nomenclature of this particular species in the local Tenyidie dialect is 'Thesükhwe', categorising it as a banana, with the suffix 'khwe' being ascribed to different types of bananas. It also has cultural significances and folklore associated with it, making it intrinsically banana in the ethnic culture.

The exploration revealed the ethnobotanical applications of wild species, including the use of *Musa balbisiana* leaves for packaging and roofing temporary shelters, dried glaucous leaves of *Musa nagensium* for waxing handloom tools, and the domestication of *Musa flaviflora* for its inflorescence and inner pseudostems as vegetables. Likewise, the inflorescences of *Musa sikkimensis* and *Musa aurantiaca* were consumed as vegetables. The inflorescence and pseudostem of *Musa itinerans*, in particular, is widely consumed in the districts of Mokokchung and the northern regions of Zunheboto, where it is the dominant species in the genus. The pseudostem of these wild species, depending on the region and availability, is also widely used as animal feed. *Musa velutina* was discovered in a few spots along riverbanks in low-lying areas bordering Assam in Mokokchung district. One of these spots was in Tuli under Mokokchung district, where *Musa velutina* occurring in the wild near riverbanks were brought home by locals to cultivate them as ornamental plants. **(Figures 2.16a and 2.16b)**

Figures 2.16a and 2.16b : Domestication of *Musa velutina* for horticultural applications

Numerous areas in Northeast India remain inadequately studied, resulting in the ongoing discovery of new species. Recent scientific investigations have resulted in the identification of new species: *Musa markkui (*Gogoi and Borah, 2013), *Musa puspanjaliae* (Gogoi and Häkkinen, 2013a), and *Musa kamengensis* (Gogoi and Häkkinen, 2013b) from Arunachal Pradesh, as well as *Musa nagalandiana* (Dey *et al.,* 2014) from Nagaland. The natural geographical distribution of certain taxa is unclear due to insufficient exploration, and certain taxa are poorly understood because of unresolved nomenclature (Häkkinen, 2013).

The unequivocal identification and classification of samples are crucial for the effective collection and preservation of banana diversity (Čížková *et al.,* 2015). The primary focus is the systematic discovery and establishment of robust ex situ collections of *Musa* genetic resources, with their conservation.

Summary and Conclusion

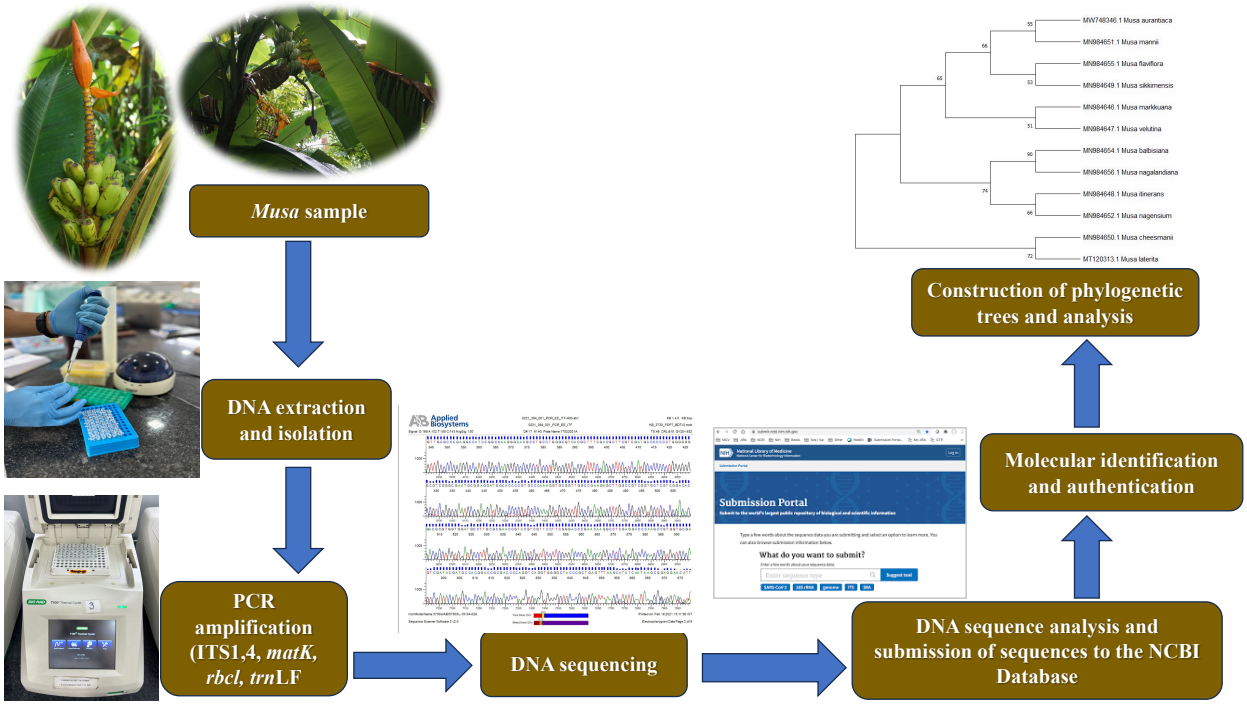
This study examined all agro-climatic zones of Nagaland, leading to the identification and collection of 12 wild *Musa* species based on morpho-taxonomic traits.

Digital field notes were documented, images were captured, and specimens were gathered in situ to furnish accurate descriptions of the plants in their native environment. Viable seeds of these wild *Musa* species are preserved at the Seed Bank of the Department of Botany. The germplasm is conserved in the *Musa* Garden of the Department of Botany at Nagaland University. A taxonomy key for the identification of *Musa* species in Nagaland was constructed for convenience in identification. The range of wild *Musa* exhibits significant diversity, with the northern region of the state exhibiting the highest diversity and is characterised by tropical and subtropical forests. This diversity progressively diminishes in the southern region, which features subtropical and temperate woods at elevated altitudes. *Musa* was restricted to the tropical plains and lowlands, but *Musa sikkimensis* thrived at elevations beyond 1500 m. The restricted population of *Musa mannii*, classified as critically endangered under criteria Blab(iii)+2ab(iii) by the IUCN Red List of Threatened Species and formerly deemed extinct in the wild, necessitates intervention through the implementation of conservation efforts to prevent its extinction in natural habitats. The many species present opportunities for developing cold resistance traits, as well as the potential for breeding smaller species for horticultural applications. The degradation of habitats caused by diverse natural and anthropogenic activities like jhum cultivation and the decimation of these wild *Musa* populations beside roads to make way for development, coupled with the removal of wild *Musa* species for land clearing and the harvesting of their inflorescence and stems for consumption as a local vegetable, were identified as principal factors contributing to population decline. These concerns require proper oversight and mitigation actions for the preservation of this taxon in the wild. A point of note, however, is the resilience of these plants in the observation that despite all the above-mentioned factors, these populations continue to survive owing to their rapid growth and their ability to propagate both sexually and asexually, and the hope is that they continue to do so until appropriate measures can be put in place or that they continue to do so despite of it all, which would be in itself an onus to their ability as pioneer species.

Chapter - 3

Molecular Characterisation of Wild *Musa* Species of Nagaland

Graphical Summary



Introduction

One of the most significant fruit crops in the world, bananas (Genus *Musa*, Family Musaceae) are prized for their cultural, nutritional, and economic worth. Originating in Southeast Asia and Oceania, bananas *(Musa* spp*.)* are currently grown extensively in tropical and subtropical climates worldwide. Due to hybridisation between two main wild species, *Musa acuminata* and *Musa balbisiana* Colla, most banana types currently cultivated are triploids (Cheesman, 1948; Deb *et al.,* 2023; Tiatemsu *et al.,* 2023; Jenny *et al.,* 2024). Wild species offer vital genetic resources for tackling issues like disease resistance and climate resilience, whereas cultivated bananas, created by hybridisation of wild species like *Musa acuminata* and *Musa balbisiana* Colla *,* are a staple sustenance for millions of people worldwide. Native to places like Southeast Asia and the Indian subcontinent, wild bananas are especially important because they are stores of genetic traits that can help with problems in banana farming, such as reduced productivity and disease susceptibility (Cížková *et al.,* 2015).

Although bananas affect people all around the world, areas like India, especially Nagaland, offer remarkable biodiversity that is essential to comprehending and protecting wild *Musa* species. For *Musa* spp., especially the wild species *Musa balbisiana* Colla *,* India is acknowledged as a significant centre of origin and diversity (Singh *et al.,* 2021). A wide variety of *Musa* germplasm, influenced by various agroclimatic conditions, can be found throughout the nation, ranging from wild kinds to cultivated variants. India has had a major role in the domestication of bananas throughout history, helping to create interspecific hybrids such as the widely grown AAB and ABB chromosomal groupings (Li *et al.,* 2013). However, issues including habitat loss, changed farming methods, and genetic erosion threaten India's *Musa* biodiversity. Protecting *Musa* biodiversity requires conservation measures that either keep species in their natural habitats (*in situ*) or in controlled settings, like as gene banks (*ex situ*). To preserve this priceless genetic resource, systematic research and better preservation techniques are still required, notwithstanding recent efforts. This diversity highlights India's crucial role in banana breeding, research, and conservation, making the nation a major contributor to world banana agriculture and biodiversity (Kallow *et al.,* 2022).

Wild *Musa* variety is especially abundant in the Andaman and Nicobar Islands, the Western and Eastern Ghats, and Northeast India (Subbaraya *et al.,* 2006). With over 30 taxa in Northeast India alone, two-thirds of which are endemic to the region, many species are endemic to these regions (Sabu *et al.,* 2014).

With species like *Musa nagalandiana* S. Dey & Gogoi and *Musa aurantiaca* Baker that flourish in the state's varied agro-climatic zones, which range from subtropical lowlands to mountain forests with elevations of up to 3,840 meters, Nagaland stands out among India's regions as a micro-centre of *Musa* evolution (Deb *et al.,* 2023). These bananas support a variety of plant and animal species in various habitats, which include woods, riverbanks, and shifting agriculture regions. This suggests that they play a part in maintaining ecological equilibrium.

Wild bananas are extremely important both ecologically and economically because their genetic diversity contributes traits like disease resistance, drought tolerance, and adaptability that are necessary to improve cultivated bananas (Fu *et al.,* 2022). In terms of economics, wild bananas can be used in traditional medicine, local food security, and as genetic resources for banana breeding programs. For example, *Musa balbisiana* Colla provides resistance to biotic and abiotic stressors such as Sigatoka leaf spot, Fusarium wilt, and drought conditions (Thingnam *et al.,* 2023; Mohite *et al.,* 2024) and is frequently used in breeding programs to increase resilience in cultivated varieties. Similarly, *Musa acuminata* subsp. *burmannica*, which is native to the Western Ghats, is another significant genetic source that confers disease resistance and adaptability (Padmesh *et al.,* 2012).

Understanding the evolutionary links among the *Musa* genus, which includes both wild and farmed bananas, depends heavily on phylogenetic research. These studies provide a clearer understanding of banana taxonomy and evolutionary history by tracking the origins, diversity, and adaptive features of the fruit through the analysis of genetic data. It has been established that the ancient wild progenitors of modern banana cultivars are *Musa acuminata* and *Musa balbisiana* Colla . Because of this, it is an essential tool for meeting the agricultural and ecological needs of this significant crop on a global scale.

The taxonomic classification of bananas remains complex, with ongoing debates regarding nomenclature and phylogenetic relationships among species (Noor and

Sholichah, 2019). Genetic lineages have been identified with the help of molecular markers such as ribosomal DNA, nuclear DNA, and chloroplast genomes. For example, studies have demonstrated the evolutionary relationships between farmed bananas (genomic groups AAA, AAB, and ABB) and their wild counterparts, *Musa acuminata* and *Musa balbisiana* Colla , emphasising instances of natural hybridisation and domestication (Christelova *et al.,* 2011; Dhivya *et al.,* 2020). The banana genetic reservoir has benefited greatly from the contributions of four wild species: *M. acuminata* (as a donor of the A genome), *M. balbisiana* (as a donor of the B genome), *M. schizocarpa* (as a donor of the S genome), and *M. textilis* (as a donor of the T genome) (Dhivya *et al.,* 2020). These species are widely dispersed across Asia's tropical and subtropical zones. Banksii, Burmannica, Burmannicoides, Errans, Malaccensis, Microcarpa, Siamea, Truncata, and Zebrina are the nine subspecies of *M. acuminata* that share morphological traits and are hybrids of *M. acuminata* and *M. balbisiana* (Simmonds and Weatherup, 1990).

Chloroplast genome comparisons have improved classifications and confirmed connections, such as the sister grouping of sections *Callimusa* and *Musa*, by shedding more light on conserved and variable areas among *Musa* species. In order to comprehend species adaptability and evolution, these analyses have also shown hotspots of genetic diversity and patterns of codon usage (Dhivya *et al.,* 2020; Song *et al.,* 2022).

Genetic and phylogenetic studies of wild *Musa* species in India, especially in areas with significant biodiversity like Nagaland, shed light on the region's importance as a secondary site for banana genesis as well as the species' unique adaptations. However, challenges including the lack of molecular data on endemic species and the inaccessibility of natural habitats impede research un these locations (Singh *et al.,* 2023). Although worldwide phylogenetic techniques such as Bayesian inference and maximum likelihood (ML) have advanced tremendously, their application to *Musa* species in India is still severely limited. Furthermore, high-resolution DNA barcoding methods and comprehensive analyses of chloroplast genomes, both crucial for species identification and conservation efforts, are rarely used (Dhivya *et al.,* 2020).

These findings have important applicability in real life. Nagaland's wild bananas are extremely important both ecologically and economically, and their genetic

characteristics can help solve the world's agricultural problems. Phylogenetics directs breeding programs and the preservation of genetic resources, for example, in wild bananas like *M. balbisiana*, which have genetic features like drought tolerance and disease resistance. Developing resilient cultivated variants and reducing threats to banana biodiversity require this information (Panwar *et al.,* 2022). In general, the sustainable management of banana resources, conservation initiatives, breeding advancements, and the resolution of intricate taxonomic problems are all signified by phylogenetic study.

Native to Northeast India, *Musa nagensium* Prain was completely overlooked by the botanical community for more than a century, and no samples from its natural habitat were recorded during this time (Joe *et al.,* 2014). Additionally, *M. cheesmanii* N.W. Simmonds was incorrectly identified as *M. nagensium* and misrepresented in photographic records. Both taxa were meticulously described in detail after the species was later rediscovered (Gogoi, 2013). Systematic investigation and documentation of *Musa* species are hindered by the inaccessibility and remoteness of several regions in Nagaland (Deb *et al.,* 2023).

The combination of molecular methods and conventional taxonomic techniques is especially important in areas with high biodiversity, like Nagaland, where *Musa* species show adaptations to various agro-ecological settings. Molecular techniques support these identifications and help to ensure precision, whereas classical methodologies lay the groundwork for field identification. According to Dhivya *et al.* (2020), molecular markers including ITS, *trnL, matK,* and *rb*cL are particularly effective at distinguishing between closely related *Musa* species and can help resolve ambiguities that might result from morphological traits alone. To help with taxonomy and conservation, phylogenetic methods like Bayesian Inference and molecular markers (such ITS and *matK)* offer crucial insights on the evolutionary relationships of *Musa* species (Probojati *et al.,* 2021). The combined sequences of the DNA sections ITS and *trnL* frequently exhibit excellent congruence with evolutionary assumptions, and they have been demonstrated to be helpful for reconstructing phylogenetic connections at the generic, sectional, and species levels. Phylogenetic analyses of this genus have also made extensive use of DNA sequencing methods. These methods

include chloroplast DNA fragments such *trnL, atpB-rbcL*, and *rps16,* as well as nuclear ribosomal internal transcribed spacers (Li *et al.,* 2013).

Traditional numerical and morphological methods of taxonomy identification are difficult, time-consuming, and frequently prone to conflicts due to the wide diversity of banana cultivars. Deforestation, agricultural growth, and Jhum (Slash and Burn) farming methods have caused substantial genetic erosion, all of which impede conservation (Deb *et al.,* 2023). Furthermore, problems like viral infections are common in India's field gene banks, highlighting the need for improved conservation techniques (Song *et al.,* 2022).

It is clearly necessary to combine classical taxonomy with modern molecular techniques in order to address these issues. In addition to improving species data, this integration is crucial for the preservation and sustainable use of wild *Musa* species. To preserve *Musa'*s genetic diversity and tackle global agricultural issues, cooperation between local people, academics, and politicians is crucial. Conservation efforts are further complicated by taxonomic issues. Because *Musa* species are polymorphic, traditional categorisation schemes that rely on morphological characteristics are frequently inadequate (Opara *et al*., 2010). To properly differentiate between species and hybrids, modern molecular tools are needed, however, they are not widely used in this area. The absence of *ex situ* and *in situ* methods specifically designed for wild bananas further impedes conservation efforts. The precise explanations of species relationships that are often masked by morphological variability, environmental factors, and hybridisation within traditional classification frameworks can be provided by molecular techniques like DNA barcoding and phylogenetic analysis (Ao *et al.,* 2020; Deb and Kamba, 2022; Longchar and Deb, 2022; Letsiou *et al.,* 2024; Sharma and Deb, 2024). In order to bridge the gap between these two approaches, interdisciplinary cooperation and molecular research investment are required. This will ultimately strengthen efforts to protect the genetic diversity of complex plant groups, including *Musa* species (Christelova *et al.,* 2011; Dhivya *et al.,* 2020). In order to properly authenticate species and manage *Musa* biodiversity sustainably, this study sought to investigate the phylogenetic relationships and molecular characterisation of wild *Musa* species from Nagaland using DNA sequences.

Materials and methods

The current study is an extension of Deb *et al*. (2023) which was the first investigation into the distribution and variety of wild *Musa* species in Nagaland, India. Establishing their molecular barcodes based on nuclear and chloroplast barcode markers, as well as researching the evolutionary relationships of these species, were the objectives of the study's second phase.

Study area and sample collection

Situated between 25°06′–27°04′N and 93°20′–95°15′E, the research region encompassed the state of Nagaland, with elevations varying from around 100 m to 3,840 m. Sample collections were conducted from all over the state of Nagaland between 2018 and 2022. *Musa* species from different agroclimatic zones with a range of morphologies were included in these collections. A Canon DS600 and Nikon DSLR 3200 were used for capturing pictures, and a Garmin GPS 72H was used to record the GPS coordinates of the collection sites. The voucher specimens were placed in the Herbarium of the Department of Botany at Nagaland University in Lumami.

Morphological documentation

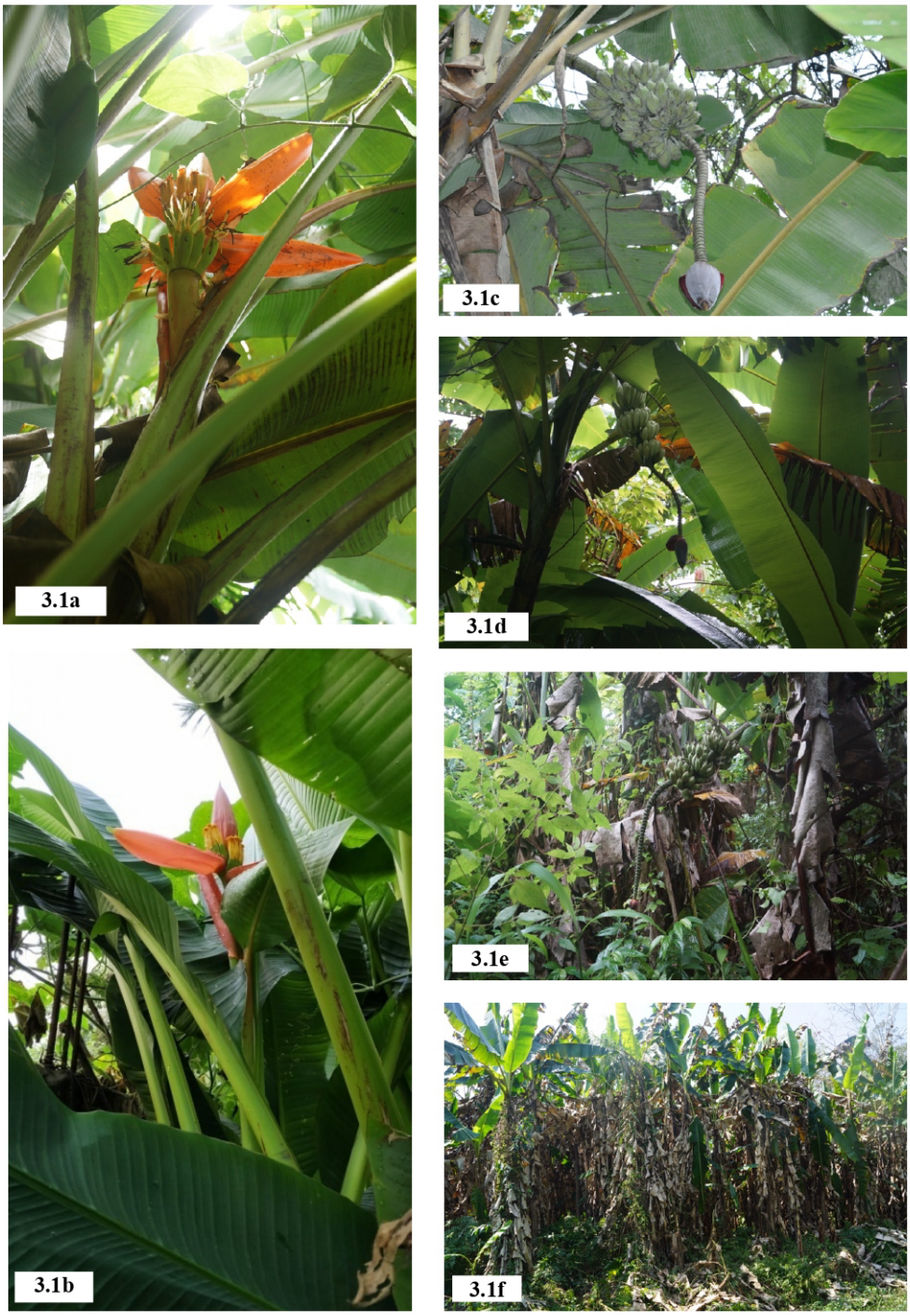
The International Network for the Improvement of Banana and Plantain (INIBAP, 1996) descriptors were used to document morphological data. Detailed measurements of plant parts, including pseudostems, leaves, flowers, and fruits, were among them. For appropriate representation, at least five individuals of each species were evaluated. For additional research, the suckers gathered for the first study were kept in Nagaland University's germplasm conservation garden.

DNA extraction, amplification and sequencing

Plant material

Fresh, tender leaves of the wild banana species in Nagaland were gathered in order to obtain DNA. Using the modified CTAB DNA isolation approach, the genomic DNA was extracted (Kamba and Deb, 2018; Sharma and Deb, 2024). This study examined the following wild banana species: *M. aurantiaca* Baker*, M. balbisiana* Colla*, M. cheesmanii* N.W.Simmonds*, M. flaviflora* N.W.Simmonds, *M. itinerans*

Cheesman, *M. laterita* Cheesman, *M. manii* Baker, *M. markkuana* (M.Sabu, A.Joe & Sreej.) Hareesh, A.Joe & M.Sabu, *M. nagalandiana* S. Dey & Gogoi, *M. nagensium* Prain, *M. sikkimensis* Kurz and *M. velutina* H.Wendl. & Drude. Spectrophotometric analysis and agarose gel electrophoresis were utilised for checking the quality and concentration of the DNA.



Figures 3.1a-3.1f : *M. aurantiaca* Baker, *M. laterita* Cheesman, *M. balbisiana* Colla, *M. cheesmanii* N.W.Simmonds, *M. flaviflora* N. W. Simmonds, *M. itinerans* Cheesman.

Figures 3.1g-3.1l : *M. manii* H. Wendl. ex Baker, *M. nagalandiana* S. Dey &Gogoi, *M. markkuana* M.Sabu, A.Joe & Sreej., *M. nagensium* Prain, *M. sikkimensis* Kurz, *M. velutina* H. Wendl & Drude.

PCR amplification

The barcode genes in the plastid and nuclear regions, specifically, *atpB-rbcL*, *rps16, trnL-F,* and ITS, were amplified using the isolated genomic DNA (Table 3.1). The PCR reaction mixture included 10X PCR buffer, 25mM $MgCl_2$, 2mM dNTPs, 10 µM of each primer, 20–30 ng of template DNA, and 2 units of DNA polymerase. The final reaction volume was 20 µL, and the water was nuclease-free.

Table 3.1: Primer sequences used in the study

Sl. No.	Barcode region	Primer	Sequence direction	No. of bases
1	ITS1-ITS4	ITS1	5′-TCCGTAGGTGAACCTGCGG-3′	19
		ITS4	5′-TCCTCCGCTTATTGATATGC-3′	20
2	*atpb-rbcL*	*atpb*	5′-ACATCKARTACKGGACCAATAA-3′	22
		rbcL	5′-AACACCAGCTTTRAATCCAA-3′	20
3	*rps16*	*rps16F*	5′-GTGGTAGAAAGCAACGTGCGACTT-3′	24
		rps16R	5′-TCGGGATCGAACATCAATTGAAAC-3′	24
4	*trnf-trnL*	*trnf*	5′-ATTTGAACTGGTGACACGAG-3′	20
		trnL	5′-GGTTCAAGTCCCTCTATCCC-3′	20

Thermal cycling conditions for the chloroplast genome markers (*atpB-rbcL, rps16,* and *trnL-F*) included a 5-minute initial denaturation at 94°C, 35 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 45 sec, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. An initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 45 sec, annealing at 52°C for 45 sec, extension at 72°C for 1 min, and a final extension at 72°C for 10 min comprised the thermal cycling conditions for the nuclear marker (ITS). A Chemi-Doc gel documentation system (Bio-Rad) was used to visualise the PCR products after they were separated using 1.2% TAE agarose gels stained with ethidium bromide (0.5

μg/mL). The amplified products were sequenced at Chromous Biotech Pvt. Ltd. in Bangalore, India, and 1st Base Laboratories in Singapore. Version 7.0.9.0 of the BioEdit Sequence Alignment Editor Software was then used to assess the quality of the generated sequence.

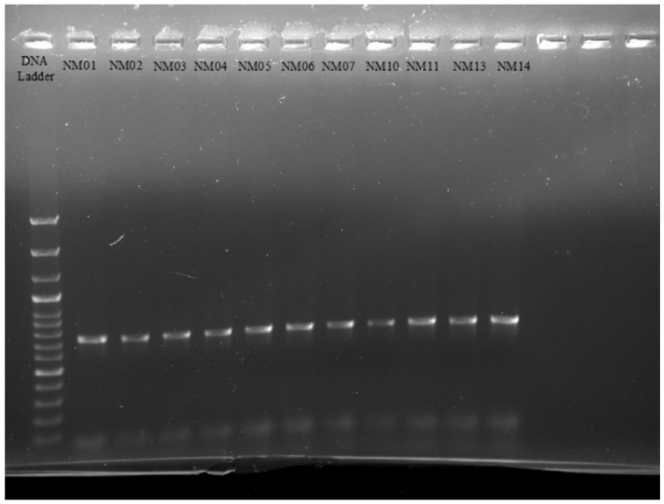


Figure 3.2a: Gel image showing PCR amplicons of primer pair ITS1 – ITS4

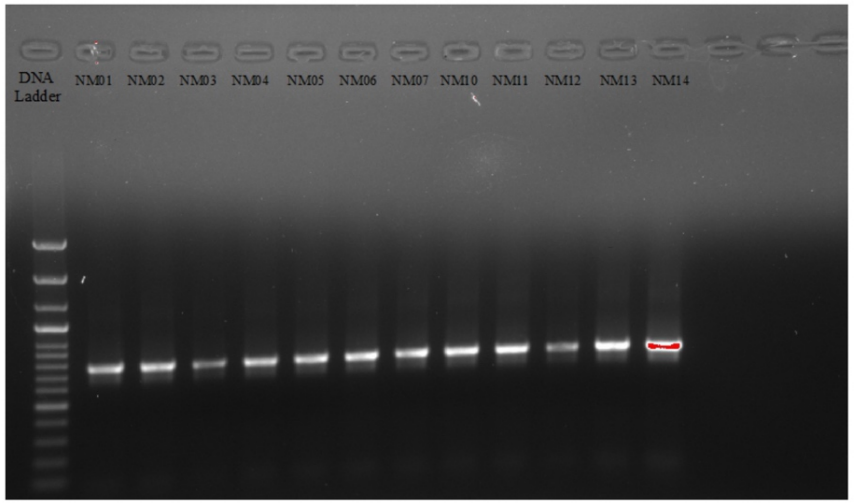


Figure 3.2b: Gel image showing PCR amplicons of primer pair *atpb-rbcL*

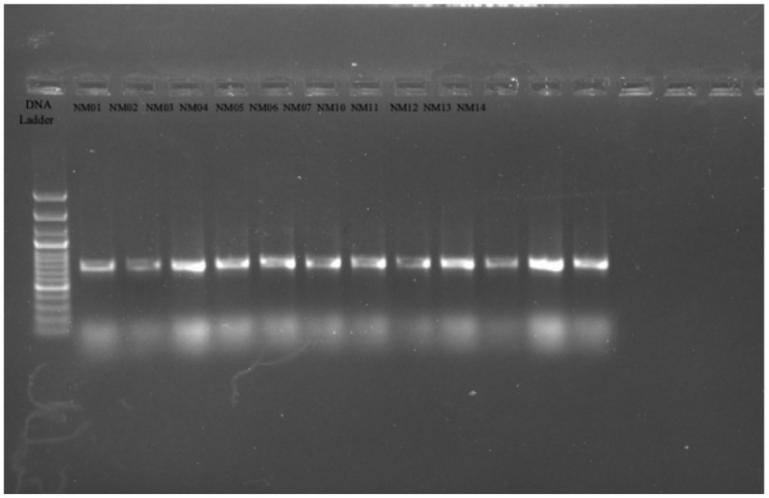


Figure 3.2c: Gel image showing PCR amplicons of primer pair *rps16*

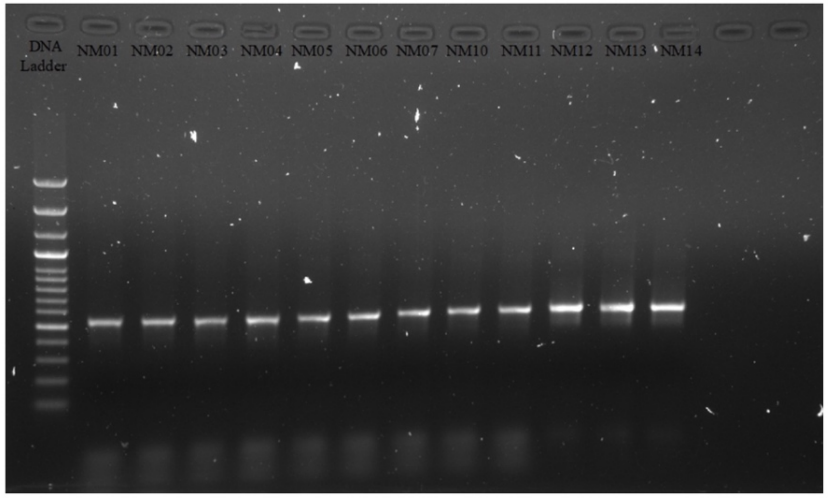


Figure 3.2d: Gel image showing PCR amplicons of primer pair *trnf-trnL*

Sequencing and data analysis

The nucleotide sequences of the *Musa* species for the primers *rps16, trnL-F*, *atpB-rbcL*, and ITS were submitted to the National Centre for Biotechnology Information's (NCBI) GenBank. Employing the Basic Local Alignment Search Tool (BLAST), these sequences were compared with nucleotide sequences previously recorded in GenBank. The MUSCLE function in MEGA-11 software was employed to align multiple sequences. Accession numbers were acquired by submitting the *atpB-rbcL, rps16, trnL-F,* and ITS sequences of the identified candidate species to GenBank.

Phylogenetic trees were constructed in the MEGA-11 software (Tamura *et al.,* 2021) utilising the Tamura-Nei model, a statistical approach commonly employed in Maximum Likelihood (ML) analysis to deduce evolutionary relationships from DNA sequence data. The Kimura 2-parameter approach in MEGA-11 was employed to calculate interspecific distances to evaluate interspecific variance. Consensus trees depicting the evolutionary history of the analysed taxa were generated for each phylogenetic study using 1000 bootstrap repeats.

Results

Genetic Data and Barcode Analysis

Twelve wild *Musa* species obtained from Nagaland were analysed utilising four DNA barcode regions: *atpB-rbcL, rps16, trnL-F*, and ITS. The nucleotide sequences of these markers were amplified, sequenced, and submitted to GenBank, receiving accession numbers for each marker (Table 3.2). The lengths of base pairs for the markers differed among species, indicating the genetic diversity present in these wild bananas.

The chloroplast marker *atpB-rbcL* had sequence lengths varying from 856 bp in *Musa balbisiana* Colla to 894 bp in *Musa laterita* Cheesman. The nuclear marker ITS exhibited considerable diversity, with sequence lengths varying from 616 bp in *Musa nagensium* Prain to 753 bp in *Musa laterita* Cheesman. The disparity in sequence lengths among the markers underscores the genetic diversity within the examined species. The amplified sequences were submitted to the National Centre for Biotechnology Information (NCBI) GenBank, guaranteeing their accessibility for subsequent research. The *rps16* region of *Musa flaviflora* N.W. Simmonds is

designated with the GenBank accession number MT044458 and has a length of 865 base pairs. These publicly accessible sequences offer a significant resource for comparative analyses and additional phylogenetic investigations of *Musa* species.

Each DNA barcode region contributed to elucidating the evolutionary relationships among the examined species. Chloroplast markers (*atpB-rbcL, rps16, trnL-F*) were successful in differentiating closely related species, whereas the nuclear marker ITS exhibited greater resolution for interspecific connections. These markers enabled a thorough investigation of genetic diversity and evolutionary links among wild *Musa* species. The results demonstrated significant interspecific differences in marker sequences. *Musa markkuana* (M.Sabu, A.Joe & Sreej.) Hareesh, A.Joe & M.Sabu, *Musa flaviflora* N.W.Simmonds, and *Musa laterita* Cheesman demonstrated substantial genetic variation across all four barcode markers. The nuclear marker ITS exhibited significant diversity, with sequence lengths varying from 616 bp in *Musa nagensium* Prain to 753 bp in *Musa laterita* Cheesman. These variances indicate the genetic uniqueness of the examined species.

Table 3.2 : List detailing GenBank accession numbers of marker sequences for *Musa* species of Nagaland

Sl. No.	Species	Voucher No.	Barcode markers	GenBank Acc. No.	Base pair length
1	*Musa markkuana* M. Sabu, A. Joe & Sreej.	NM01	*atpB-rbcL*	MN984646	889
			rps16	MT044449	818
			trnL-F	MT120317	428
			ITS	MN954312	630
2	*Musa aurantiaca* Baker	NM02	*atpB-rbcL*	MW748346	892
			rps16	MT120315	869
			trnL-F	MW264809	417
			ITS	MW255325	671
3	*Musa itinerans* Cheesman	NM03	*atpB-rbcL*	MN984648	856
			rps16	MT044450	847
			trnL-F	MT120318	389

			ITS	MN954314	623
4	*Musa sikkimensis* Kurz	NM04	*atpB-rbcL*	MN984649	868
			rps16	MT044451	861
			trnL-F	MT120319	408
			ITS	MN954315	680
5	*Musa cheesmanii i* N. W. Simmonds	NM05	*atpB-rbcL*	MN984650	856
			rps16	MT044452	861
			trnL-F	MT120320	388
			ITS	MN954316	664
6	*Musa manii* H. Wendl. ex. Baker	NM06	*atpB-rbcL*	MN984651	868
			rps16	MT044453	862
			trnL-F	MT120321	408
			ITS	MN954317	677
7	*Musa nagensium* Prain	NM07	*atpB-rbcL*	MN984652	874
			rps16	MT044454	850
			trnL-F	MT120322	388
			ITS	MN954318	616
8	*Musa balbisiana* Colla	NM10	*atpB-rbcL*	MN984654	856
			rps16	MT044457	843
			trnL-F	MT120325	387
			ITS	MW255326	629
9	*Musa flaviflora* N. W. Simmonds	NM11	*atpB-rbcL*	MN984655	865
			rps16	MT044458	865
			trnL-F	MT120326	408
			ITS	MW255327	680
10	*Musa nagalandiana* S. Dey & Gogoi	NM12	*atpB-rbcL*	MN984656	856
			rps16	MT044459	851
			trnL-F	MT120327	387
11		NM13	*atpB-rbcL*	MN984647	889
			rps16	MT120316	847

	Musa velutina H. Wendl. & Drude		*trnL-F*	MT120328	428
			ITS	MN954313	658
12	*Musa laterita* Cheesman	NM14	*atpB-rbcL*	MT120313	894
			rps16	MT044460	853
			trnL-F	MT120329	388
			ITS	MW737615	753

Phylogenetic tree analysis

The sequence data obtained from the barcode markers were utilised to create phylogenetic trees using Maximum Likelihood (ML) analysis based on the Tamura-Nei statistical model. The findings elucidated distinct evolutionary links among the examined species. Phylogenetic trees were constructed using 1000 bootstrap replicates to ensure robustness. The consensus trees effectively represented the evolutionary history of the examined taxa, with elevated bootstrap values affirming the clade reliability.

Phylogenetic Tree of *atpB-rbcL* Marker

The phylogenetic tree constructed from the *atpB-rbcL* marker **(Figure 3.3a)** examined the evolutionary relationships among 12 wild banana species from Nagaland. The analysis encompassed 953 nucleotide locations, accounting for all gaps and missing data. The cumulative branch length was determined to be 0.040, offering a comprehensive assessment of evolutionary divergence within the sample. The constructed phylogenetic tree categorised the examined taxa into separate groupings. Bootstrap values varied from 51% to 90%, reflecting differing degrees of support for particular branches. *Musa balbisiana* Colla and *Musa nagalandiana* S. Dey & Gogoi exhibited a strong evolutionary relationship, as evidenced by their clustering with substantial bootstrap support (90%). *Musa aurantiaca* Baker and *Musa manii* Baker constituted a cluster with a bootstrap value of 55%. Additional significant groupings comprised *Musa flaviflora* N.W.Simmonds and *Musa sikkimensis* Kurz (66%),

alongside *Musa markkuana* (M.Sabu, A.Joe & Sreej.) Hareesh, A.Joe & M.Sabu clustering with *Musa velutina* H.Wendl. & Drude *(*51%).

The Tamura-Nei model was found to be the most appropriate statistical framework for this study. The transitions-to-transversions ratio (Ts/Tv) was found to be 1.272, with substitution rates varying between nucleotide pairs. The transition rate from adenine (A) to guanine (G) was 0.112, whereas the rate of conversion from cytosine (C) to guanine (G) was 0.030. These rates reflect the genetic variation and evolutionary limits seen in the *atpB-rbcL* region of the investigated species. The found clustering patterns are consistent with the taxonomic classifications given for the tested species. Bootstrap values are high, especially within clades like *Musa balbisiana* Colla and *Musa nagalandiana* S. Dey & Gogoi, indicating evolutionary proximity and genetic cohesiveness among specific taxa. In contrast, lower bootstrap values (e.g., 51% for *Musa markkuana* (M.Sabu, A.Joe & Sreej.) Hareesh, A.Joe & M.Sabu and *Musa velutina* H.Wendl. & Drude) suggest potential divergence or insufficient lineage sorting within these species. The phylogenetic analysis emphasises the effectiveness of the *atpB-rbcL* marker in elucidating interspecific linkages, as its rather steady evolutionary rate provides a reliable measure of genetic divergence.

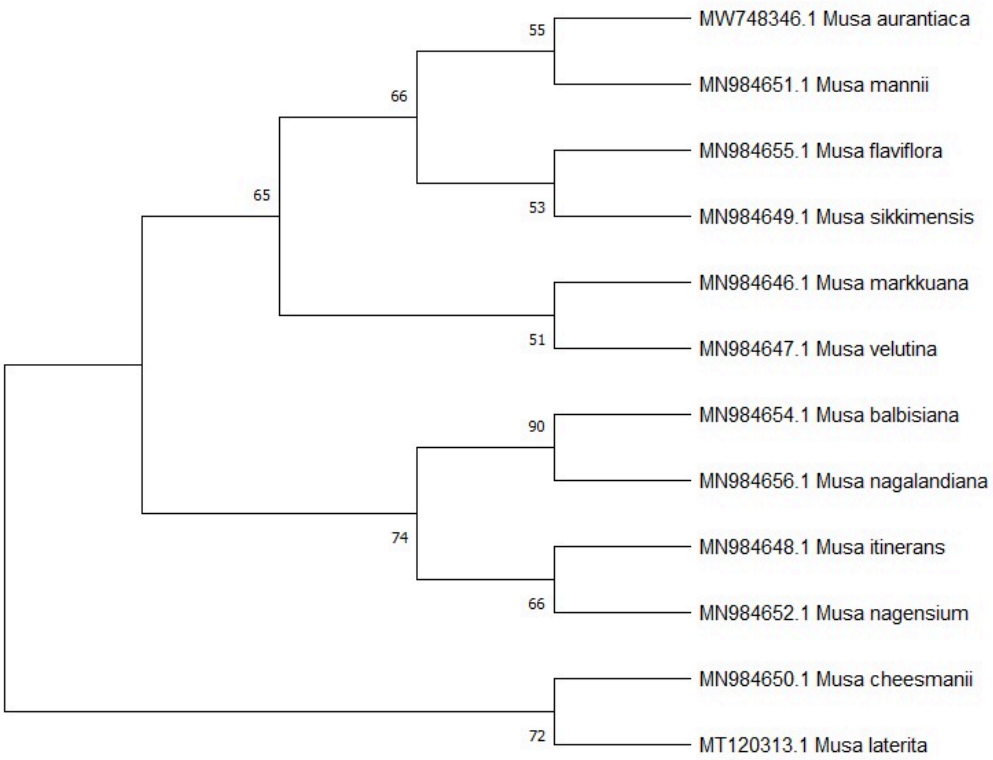


Figure 3.3a : Phylogenetic tree of *atpB-rbcL* marker

Phylogenetic Tree of *rps16* Marker

The generated phylogenetic tree (**Figure 3.3b)** displays two primary clades with bootstrap support values ranging from 37% to 89%, indicating varying levels of confidence in the branching patterns. The figure demonstrates that the primary significant clade encompasses species such as *Musa aurantiaca* Baker and *Musa velutina* H.Wendl. & Drude , which have strong genetic relationships supported by bootstrap values of 73% and 68%, respectively. *Musa markkuana* (M.Sabu, A.Joe & Sreej.) Hareesh, A.Joe & M.Sabu and *Musa manii* Baker are similarly classified within this clade, underscoring their evolutionary proximity. The second branch includes a diverse collection of species, such as *Musa laterita* Cheesman*, Musa sikkimensis* Kurz, and *Musa flaviflora* N.W.Simmonds. *Musa sikkimensis* Kurz demonstrates a robust bootstrap value of 89% with *Musa flaviflora* N.W.Simmonds*,* signifying a notable evolutionary relationship. The species *Musa nagensium* Prain and *Musa itinerans* Cheesman form an additional sub-cluster within this lineage.

Musa balbisiana Colla, a widely spread species, is closely linked to *Musa nagalandiana* S. Dey & Gogoi, evidenced by a bootstrap value of 87%, underscoring the potential genetic and ecological importance of *Musa nagalandiana* S. Dey & Gogoi in the area. The total branch length of the tree was measured at 0.043, with an estimated Ts/Tv ratio of 0.949, indicating balanced substitution rates. Evolutionary factors suggest that the *Musa* species in Nagaland have undergone distinct diversification events while maintaining close genetic relationships within certain groups. The analysis highlights the genetic distinctiveness of *Musa nagalandiana* S. Dey & Gogoi, a potentially novel or regionally adapted species.

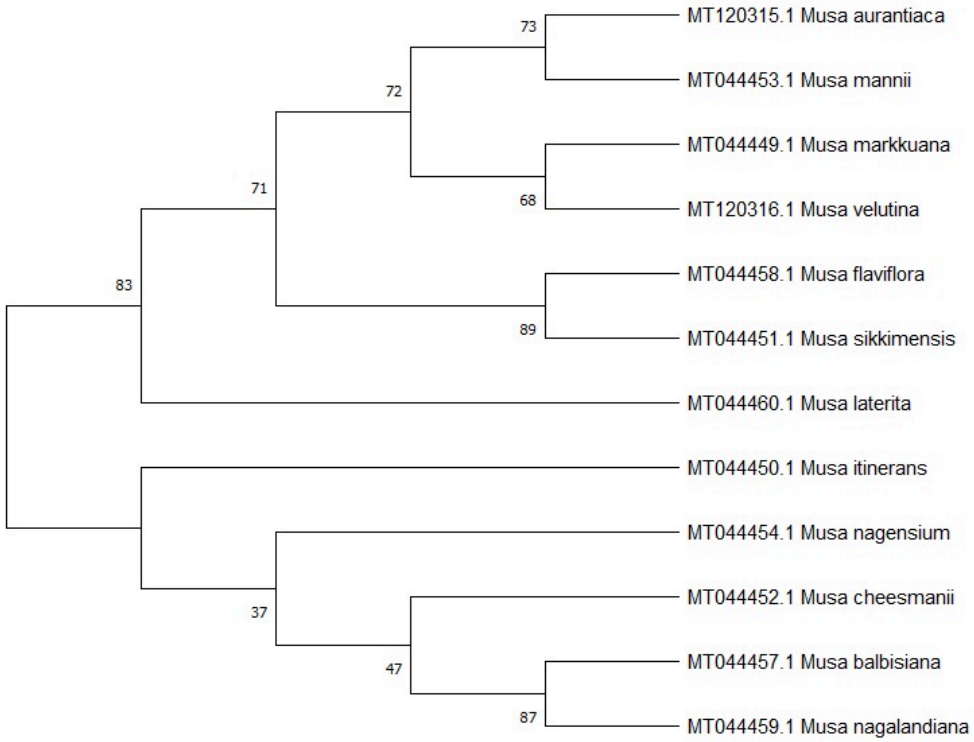


Figure 3.3b : Phylogenetic tree of *rps16* marker

Phylogenetic Tree of *trnL-F* Marker

The phylogenetic tree **(Figure 3.3c)** exhibited robust clades, including a cluster of *Musa itinerans* Cheesman and *Musa nagensium* Prain with a bootstrap value of 20%. A notable clade, corroborated by a bootstrap value of 42%, encompassed *Musa balbisiana* Colla and *Musa markuana* (M.Sabu, A.Joe & Sreej.) Hareesh, A.Joe &

M.Sabu. *Musa nagalandiana* S. Dey & Gogoi exhibited a close evolutionary relationship with *Musa cheesmanii* N.W.Simmonds, indicating their genetic affinity. *Musa flaviflora* N.W.Simmonds established a distinct lineage, underscoring its unique evolutionary path. The assemblage of *Musa aurantiaca* Baker, *Musa manii* Baker, and *Musa sikkimensis* Kurz was corroborated by moderate bootstrap values between 33% and 35%.

Short branch lengths in the phylogenetic tree signify little genetic difference among the species, illustrating their proximate evolutionary affiliations. The transition/transversion ratio (Ts/Tv) was determined to be 2.289, signifying a greater frequency of transition mutations. Substitution rates exhibited notable nucleotide alterations, with the transition rate between cytosine (C) and thymine (T) at 0.2098, and the transition rate between guanine (G) and adenine (A) at 0.2585, both of which were very noteworthy.

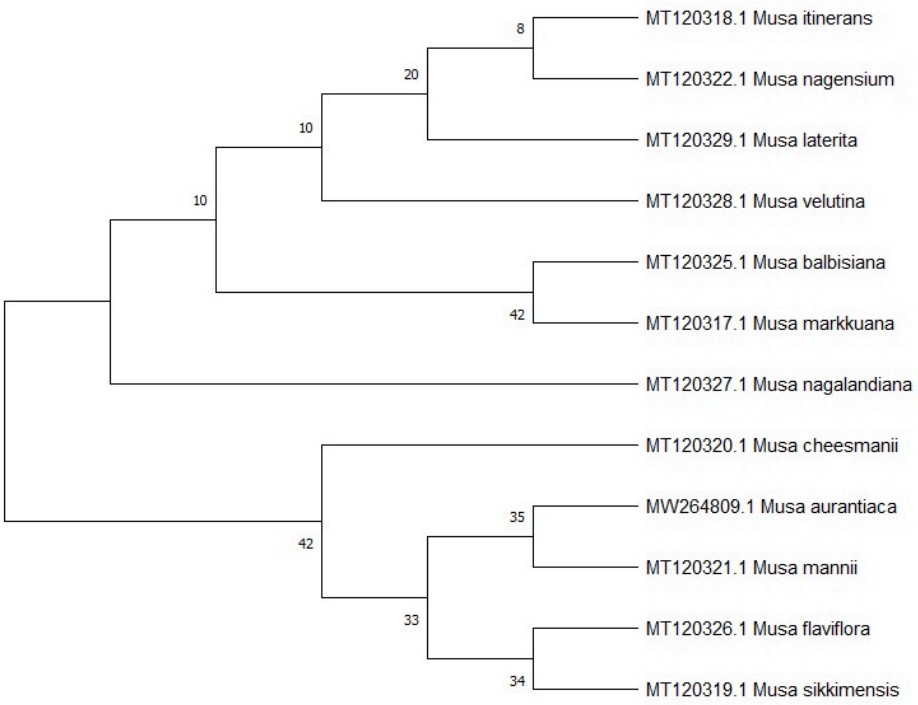


Figure 3.3c : Phylogenetic tree of *trnL-F* marker

Phylogenetic Tree of ITS Marker

The evolutionary relationships among 11 wild banana species from Nagaland were examined utilising the nuclear ribosomal DNA marker ITS **(Figure 3.3d).** A total of 770 nucleotide sites were aligned, and a Maximum Likelihood phylogenetic tree was generated utilising the Tamura-Nei substitution model in MEGA 11.

The phylogenetic tree demonstrated robust clades, with bootstrap values underscoring the dependability of the clusters. A separate clade of *Musa balbisiana* Colla and *Musa cheesmanii* N.W.Simmonds was corroborated by a robust bootstrap value of 92%, signifying strong genetic affinity. A notable clade, exhibiting a bootstrap value of 67%, encompassed *Musa itinerans* Cheesman and *Musa nagensium* Prain. *Musa flaviflora* N.W.Simmonds and *Musa laterita* Cheesman exhibited clustering, indicating evolutionary closeness. Moderate support values ranging from 33% to 49% were noted among the grouping of *Musa manii* Baker, *Musa markkuana* (M.Sabu, A.Joe & Sreej.) Hareesh, A.Joe & M.Sabu, and *Musa sikkimensis* Kurz, highlighting their close yet different evolutionary connections.

The short branch lengths noted across multiple clades indicate restricted genetic divergence among these wild banana species. The transition/transversion ratio (Ts/Tv) was determined to be 2.418, signifying a prevalence of transition mutations. Substitution rates revealed substantial nucleotide dynamics, with a transition rate of 0.2966 between thymine (T) and cytosine (C), and a transition rate of 0.1802 between cytosine (C) and thymine (T), indicating a notable frequency.

Each phylogenetic tree exhibited unique grouping patterns, affirming that the wild banana species possess significant genetic diversity. All markers indicated that taxa such as *Musa balbisiana* Colla , *Musa cheesmanii* N.W.Simmonds, and *Musa nagalandiana* S. Dey & Gogoi regularly grouped with high bootstrap values, indicating strong evolutionary ties. These findings demonstrate the genetic distinctiveness of Nagaland's bananas and underscore their potential as significant additions to *Musa* genetic resources. There is significant alignment among the trees produced using various markers, with species such as *Musa balbisiana* Colla and *Musa cheesmanii* N.W.Simmonds exhibiting tight relationships across all datasets. Nonetheless, little discrepancies in the placement of species such as *Musa manii* Baker and *Musa flaviflora* N.W.Simmonds illustrates the subtleties of marker-specific

evolutionary indicators. The ITS and *trnL-F* markers demonstrated superior resolution in differentiating closely related taxa, whereas the *rps16* and *atpB-rbcL* markers facilitated broader grouping.

The incorporation of *Musa nagalandiana* S. Dey & Gogoi, a species presumably native to the Nagaland region, underscores its persistent correlation with *Musa balbisiana* Colla across many markers. This highlights its likely evolutionary origin from major gene pools in the region. *Musa aurantiaca* Baker, exhibiting variety in clustering patterns, may signify a lineage shaped by environmental or adaptive factors in this region. Robust bootstrap support for principal clades across markers signifies robust evolutionary signals, suggesting ancient divergences within the *Musa* genus. The persistent clustering of species such as *Musa sikkimensis* Kurz, *Musa velutina* H.Wendl. & Drude , and *Musa flaviflora* N.W.Simmonds indicates a common lineage, perhaps shaped by ecological and geographical influences in the Northeastern region of India.

This analysis underscores the imperative necessity for the conservation of wild *Musa* species in Nagaland. Their genetic variety and distinct evolutionary paths offer potential for breeding initiatives, especially in creating disease-resistant and climate-adaptive banana varieties. The amalgamation of data from all four molecular markers enhances the comprehension of the evolutionary relationships among the wild bananas of Nagaland. The findings not only reinforce the region's significance as a biodiversity hotspot for the *Musa* genus but also underscore the imperative to conserve these genetic resources for future agricultural and ecological advantages.

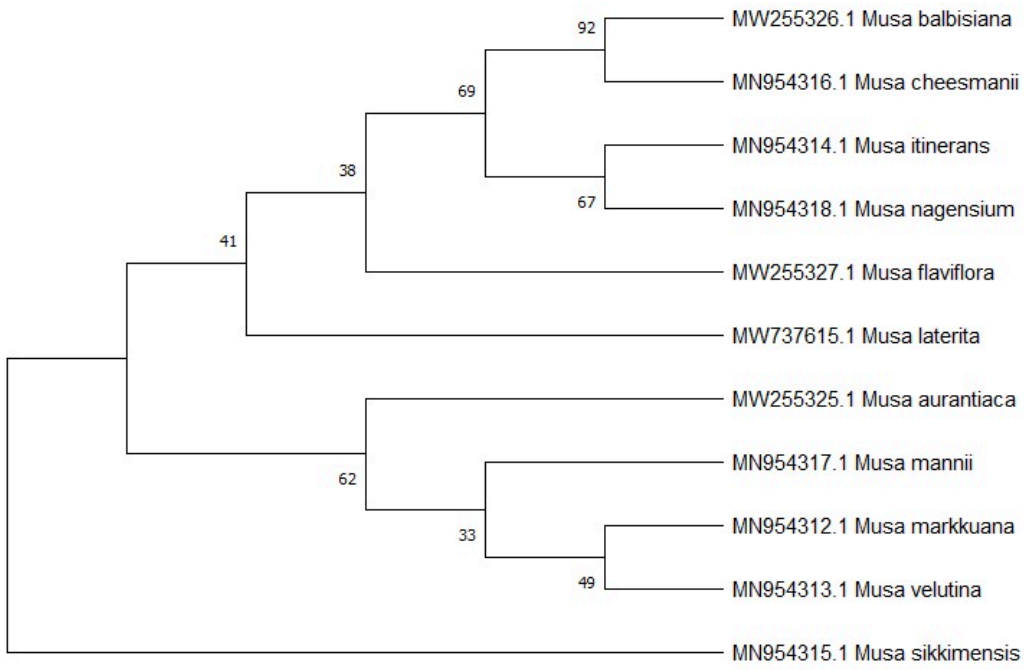


Figure 3.3d : Phylogenetic tree of ITS marker

Genetic distance

***atpB-rbcL* pairwise distance**

Musa aurantiaca Baker and *Musa flaviflora* N.W.Simmonds has the shortest genetic distance (0.003174), indicating a tight evolutionary relationship **(Table 3.3).** This points to either a recent divergence or shared evolutionary ancestry. Similarly, *Musa nagalandiana* S. Dey & Gogoi and *Musa balbisiana* Colla have a low genetic distance (0.003835), indicating phylogenetic proximity and probable ecological or geographic linkage. *Musa manii* Baker and *Musa markkuana* (M.Sabu, A.Joe & Sreej.) Hareesh, A.Joe & M.Sabu have a genetic distance of 0.005971, indicating moderate divergence between these species. *Musa sikkimensis* Kurz and *Musa velutina* H.Wendl. & Drude have a distance of 0.005219, which is consistent with their clumping as different but reasonably near species in the evolutionary tree.

Significant divergence is seen between *Musa itinerans* Cheesman and *Musa cheesmanii* N.W.Simmonds (0.007376), indicating that they are more distantly connected in evolutionary terms. This significant genetic divergence corresponds to their placement in distinct clades in the phylogenetic study. Another example of great divergence is *Musa nagensium* Prain against *Musa flaviflora* N.W.Simmonds (0.007089), indicating separate evolutionary routes.

These genetic distance patterns support the phylogenetic clustering shown in the tree built for the *atpB-rbcL* marker. *Musa aurantiaca* Baker and *Musa flaviflora* N.W.Simmonds are closely related species that belong to the same clade, as evidenced by minimal pairwise genetic distances. In contrast, species with larger genetic gaps, such as *Musa itinerans* Cheesman and *Musa cheesmanii* N.W.Simmonds, form different branches, showing significant evolutionary divergence.

Table 3.3: *atpB-rbcL* pairwise distance

	Musa aurantiaca	***Musa balbisiana***	***Musa cheesmanii***	***Musa flaviflora***	***Musa itinerans***	***Musa laterita***	***Musa mannii***	***Musa markkuana***	***Musa nagensium***	***Musa sikkimensis***	***Musa velutina***	***Musa nagalandiana***
Musa aurantiaca												
Musa balbisiana	0.0048500435											
Musa cheesmanii	0.0073185541	0.0073755580										
Musa flaviflora	0.0031744909	0.0081351707	0.0073535297									
Musa itinerans	0.0048557834	0.0024156398	0.0082303752	0.0081351707								
Musa laterita	0.0063944067	0.0064815222	0.0056524369	0.0096522499	0.0056707549							
Musa mannii	0.0007889899	0.0056607070	0.0081350547	0.0039697320	0.0056674090	0.0071966109						
Musa markkuana	0.0031699043	0.0064662005	0.0073162628	0.0047753307	0.0048372164	0.0063777048	0.0039639893					
Musa nagensium	0.0064700294	0.0048343482	0.0106736518	0.0097555146	0.0024064187	0.0080820509	0.0072817545	0.0064453300				
Musa sikkimensis	0.0007889899	0.0056607070	0.0081350547	0.0023798955	0.0056674090	0.0071966109	0.0015786245	0.0039639893	0.0072817545			
Musa velutina	0.0039674179	0.0056573508	0.0089757563	0.0055783166	0.0048429261	0.0080016751	0.0047628304	0.0030902045	0.0080868328	0.0047628304		
Musa nagalandiana	0.0048500435	0.0000000000	0.0073755580	0.0081351707	0.0024156398	0.0064815222	0.0056607070	0.0064662005	0.0048343482	0.0056607070	0.0056573508	

***rps 16* pairwise distance**

The pairwise genetic distance study using the *rps16* molecular marker revealed a wide range of divergence values among the *Musa* species studied, indicating various degrees of genetic relatedness **(Table 3.4).** The genetic distances varied from 0.0046, the lowest divergence between *Musa aurantiaca* Baker and *Musa flaviflora* N.W.Simmonds, to 0.0189, the greatest difference between *Musa aurantiaca* Baker and *Musa cheesmanii* N.W.Simmonds. This large range shows the evolutionary variety of Nagaland's wild banana species. *Musa nagalandiana* S. Dey & Gogoi showed significant divergence values with other species, including 0.0093 with *Musa balbisiana* Colla, 0.0111 with *Musa sikkimensis* Kurz, and 0.0134 with *Musa aurantiaca* Baker. These intermediate divergence values establish *Musa nagalandiana* S. Dey & Gogoi as a genetically separate taxon while retaining a moderate evolutionary affinity for these species. This contributes to its classification as a distinct wild *Musa* species peculiar to Nagaland.

Other tight genetic links were discovered, such as *Musa flaviflora* N.W.Simmonds and *Musa velutina* H.Wendl. & Drude, with a distance of 0.0074, indicating recent divergence. Similarly, *Musa sikkimensis* Kurz had lower divergence values with *Musa flaviflora* N.W.Simmonds (0.0081) and *Musa balbisiana* Colla (0.0076), indicating that these species had similar evolutionary histories. In contrast, species with larger divergence values, such as *Musa cheesmanii* N.W.Simmonds, followed divergent evolutionary paths. *Musa cheesmanii* N.W.Simmonds, for example, diverged significantly from *Musa aurantiaca* Baker (0.0189) and *Musa velutina* H.Wendl. & Drude (0.0147), highlighting its genetic uniqueness within the examined group.

Overall, the findings reveal a complicated pattern of evolutionary relationships among *Musa* species, with *Musa nagalandiana* S. Dey & Gogoi developing as an intermediate taxon and taxa such as *Musa aurantiaca* Baker, *Musa flaviflora* N.W.Simmonds, and *Musa cheesmanii* N.W.Simmonds exhibits different genetic characteristics. These results are compatible with the evolutionary tree topologies.

Table 3.4: *rps 16* pairwise distance

	Musa aurantiaca	***Musa balbisiana***	***Musa cheesmanii***	***Musa flaviflora***	***Musa itinerans***	***Musa laterita***	***Musa mannii***	***Musa markkuana***	***Musa nagalandiana***	***Musa nagensium***	***Musa sikkimensis***	***Musa velutina***
Musa aurantiaca												
Musa balbisiana	0.01212											
Musa cheesmanii	0.01894	0.00969										
Musa flaviflora	0.00465	0.00965	0.01769									
Musa itinerans	0.01073	0.00602	0.01079	0.00831								
Musa laterita	0.01193	0.01322	0.01684	0.01190	0.01311							
Musa mannii	0.00116	0.01335	0.02016	0.00585	0.01200	0.01200						
Musa markkuana	0.00246	0.01237	0.01237	0.00492	0.00992	0.00988	0.00246					
Musa nagalandiana	0.01213	0.00119	0.00973	0.00965	0.00602	0.01323	0.01219	0.01236				
Musa nagensium	0.01429	0.00724	0.01201	0.01192	0.00593	0.01675	0.01558	0.01494	0.00725			
Musa sikkimensis	0.00350	0.00849	0.01654	0.00117	0.00718	0.01081	0.00467	0.00370	0.00852	0.01076		
Musa velutina	0.00357	0.01337	0.01443	0.00598	0.01204	0.01321	0.00477	0.00123	0.01343	0.01564	0.00476	

trnL-F pairwise distance

The pairwise genetic distance study utilising the *trnL-F* marker demonstrated genetic distances ranging from 0.000 (showing no divergence) to 0.011, with a mean distance of 0.005 and a standard deviation of 0.003, indicative of low-to-moderate genetic diversity **(Table 3.5).**

A minimal genetic distance of 0.000 was recorded between the species *Musa velutina* H.Wendl. & Drude *and Musa laterita* Cheesman, signifying a high degree of genetic similarity. This indicates recent divergence or a common evolutionary ancestry. The highest genetic distance, 0.011, was observed between *Musa balbisiana* Colla and *Musa nagalandiana* S. Dey & Gogoi, indicating substantial genetic divergence and probable ancient evolutionary separations. Most species pairs displayed moderate genetic distances, with values centering around 0.005, signifying balanced divergence within the collection. The genetic distance between *Musa aurantiaca* Baker and *Musa flaviflora* N.W.Simmonds was 0.002, indicating a close kinship. The distance between *Musa cheesmanii* N.W.Simmonds and *Musa laterita* Cheesman was 0.008, signifying intermediate divergence. Species like *Musa aurantiaca* Baker and *Musa flaviflora* N.W.Simmonds constituted a closely related cluster, corroborated by consistently minimal genetic distances. *Musa nagalandiana* S. Dey & Gogoi demonstrated increased genetic distances from other species, highlighting its unique phylogenetic status.

The genetic distances indicated by the *trnL-F* marker underscore the genetic diversity and evolutionary links among the wild bananas of Nagaland. The minimal gaps among specific species indicate a common heritage, whilst greater distances in others highlight considerable diversity.

Table 3.5: *trnL-F* pairwise distance

	Musa aurantiaca	*Musa balbisiana*	*Musa cheesmanii*	*Musa flaviflora*	*Musa itinerans*	*Musa laterita*	*Musa mannii*	*Musa markkuana*	*Musa nagalandiana*	*Musa nagensium*	*Musa sikkimensis*	*Musa velutina*
Musa aurantiaca												
Musa balbisiana	0.0104237744											
Musa cheesmanii	0.0051790153	0.0053831148										
Musa flaviflora	0.0024578039	0.0077955371	0.0025821765									
Musa itinerans	0.0077987909	0.0077955371	0.0080845574	0.0051844039								
Musa laterita	0.0051844039	0.0051822568	0.0053738158	0.0025848539	0.0025848539							
Musa mannii	0.0024578039	0.0104031900	0.0051688648	0.0024532342	0.0077834369	0.0051742322						
Musa markkuana	0.0073995121	0.0025888608	0.0077753346	0.0049196982	0.0051742322	0.0025797998	0.0073856900					
Musa nagalandiana	0.0078109389	0.0025864718	0.0026891089	0.0051924601	0.0051924601	0.0025888608	0.0077955371	0.0051822568				
Musa nagensium	0.0077906565	0.0103923273	0.0107775702	0.0077753346	0.0077753346	0.0051688648	0.0051790153	0.0077600729	0.0077874096			
Musa sikkimensis	0.0024578039	0.0077955371	0.0025821765	0.0	0.0051844039	0.0025848539	0.0024532342	0.0049196982	0.0051924601	0.0077753346		
Musa velutina	0.0049288924	0.0051822568	0.0051790153	0.0024578039	0.0025848539	0.0	0.0049196982	0.0023385065	0.0025888608	0.0051688648	0.0024578039	

ITS pairwise distance

The pairwise genetic distance study utilising the ITS marker yielded extensive insights into the genetic relationships among the *Musa* species **(Table 3.6).** The genetic distances varied from 0.009 to 0.103, with a mean pairwise genetic distance of 0.049 and a standard deviation of 0.022, signifying significant genetic diversity across the examined species.

A genetic distance of 0.009 was recorded between *Musa velutina* H.Wendl. & Drude and *Musa sikkimensis* Kurz, indicating a significant level of genetic similarity. Furthermore, several species pairs, including *Musa markkuana* (M.Sabu, A.Joe & Sreej.) Hareesh, A.Joe & M.Sabu and *Musa flaviflora* N.W.Simmonds had minimal genetic distances of 0.014, thereby reinforcing their tight evolutionary affiliations. The highest genetic distance, 0.103, was observed between *Musa balbisiana* Colla and *Musa markkuana* (M.Sabu, A.Joe & Sreej.) Hareesh, A.Joe & M.Sabu, indicating significant genetic divergence. Other couples, like *Musa balbisiana* Colla and *Musa aurantiaca* Baker, had significant genetic distances of 0.097, underscoring their different evolutionary placement. Most species pairs displayed genetic distances between 0.03 and 0.07, signifying considerable divergence. The genetic distance between *Musa nagalandiana* S. Dey & Gogoi and *Musa itinerans* Cheesman was 0.053, and the gap between *Musa sikkimensis* Kurz and *Musa flaviflora* N.W.Simmonds was 0.043, indicating balanced genetic divergence.

According to the pairwise genetic distances, species such as *Musa velutina* H.Wendl. & Drude , *Musa sikkimensis* Kurz, and *Musa flaviflora* N.W.Simmonds exhibited closely related clusters, marked by little genetic divergence (ranging from 0.009 to 0.043). *Musa balbisiana* Colla exhibited consistently larger divergence from other species, with distances often surpassing 0.08, indicating its status as an outlier within the sample. These findings, in conjunction with phylogenetic tree analyses, underscore the ITS marker's efficacy in defining genetic links among wild Musa species. The identified genetic variation underscores the evolutionary intricacy of bananas in Nagaland, India, featuring a coexistence of closely related species and divergent lineages. This investigation is essential for comprehending the evolutionary processes and conservation methods pertaining to the genetic resources of wild bananas in this biodiversity hotspot.

Table 3.6: ITS pairwise distance

	Musa aurantiaca	*Musa balbisiana*	*Musa cheesmanii*	*Musa flaviflora*	*Musa itinerans*	*Musa markkuana*	*Musa laterita*	*Musa mannii*	*Musa nagensium*	*Musa sikkimensis*	*Musa velutina*
Musa aurantiaca											
Musa balbisiana	0.03995										
Musa cheesmanii	0.04940	0.03276									
Musa flaviflora	0.03394	0.04331	0.06373								
Musa itinerans	0.04529	0.05332	0.06021	0.05751							
Musa markkuana	0.01616	0.04704	0.05035	0.04136	0.03893						
Musa laterita	0.01986	0.03975	0.05040	0.03190	0.03682	0.02115					
Musa mannii	0.01362	0.04168	0.04573	0.03517	0.03995	0.01452	0.01814				
Musa nagensium	0.04389	0.04927	0.04733	0.05448	0.04619	0.05094	0.04196	0.04039			
Musa sikkimensis	0.07305	0.08799	0.09495	0.09046	0.10332	0.07072	0.07237	0.07226	0.08914		
Musa velutina	0.01694	0.04518	0.04447	0.03292	0.03899	0.01456	0.01699	0.00918	0.04732	0.06557	

Discussion

This research offers a thorough phylogenetic evaluation of wild *Musa* species from Nagaland, India, utilising four DNA barcode markers: *atpB-rbcL, rps16, trnL-F,* and ITS. The results indicate considerable genetic diversity, evolutionary connections, and conservation ramifications for these species, underscoring Nagaland's status as a biodiversity hotspot for *Musa* species. The findings correspond with numerous previous studies, underscoring the significance of molecular technologies in enhancing our comprehension of banana biodiversity and their utility in breeding and conservation efforts.

Chloroplast markers (*atpB-rbcL, rps16, trnL-F)* proficiently elucidated larger interspecific relationships, but the nuclear marker ITS offered enhanced resolution for differentiating closely related species. This methodology aligns with the results of Liu *et al*. (2010), who illustrated the effectiveness of ITS and *trnL-F* markers for taxonomic clarification within Musaceae. Workneh *et al.* (2022) underscore the significance of molecular markers in evaluating genetic diversity in bananas, reporting 187 highly polymorphic alleles among banana genotypes. This corroborates the genetic variability identified in this study, especially in species such as *Musa nagalandiana* S. Dey & Gogoi and *Musa balbisiana* Colla, which consistently grouped together.

Likewise, Ardiyani *et al.* (2023) employed three barcoding markers (*rbcL, matK*, and *trnL-F*) to reconstruct phylogenetic trees and evaluate genetic relationships among banana accessions, a method comparable to the four-marker approach utilised in this investigation. Both findings emphasise the varied resolution capabilities of various markers. Ardiyani *et al.* (2023) observed that *rbcL* is significantly conserved and efficient at the generic level, whereas *matK* and *trnL-F* provide superior resolution at the species level. This conclusion corresponds with our findings, indicating that chloroplast markers (*atpB-rbcL, rps16, trnL-F*) were proficient in elucidating broader interspecific relationships, whereas ITS was superior in differentiating closely related species. Furthermore, Ardiyani *et al.* (2023) discovered that phylogenetic trees derived from DNA barcodes exhibited two distinct clades, bolstered by high bootstrap values, paralleling the evident clustering identified in this study, wherein species such as *Musa*

laterita Cheesman and *Musa flaviflora* N.W.Simmonds constituted separate clusters, indicative of their evolutionary divergence.

The clustering patterns observed indicate the complexities of banana phylogeny, as noted by Workneh *et al.* (2022), who discovered that genetic relationships among banana genotypes did not entirely align with breeding history or genome composition, highlighting the necessity of considering evolutionary subtleties. The clumping of species such as *Musa aurantiaca* Baker and *Musa manii* Baker, indicated by modest bootstrap values, implies recent divergence or incomplete lineage sorting, a phenomenon previously seen by Wu *et al.* (2021), who recognised analogous difficulties in *Musa* phylogeny using genome sequencing. This underscores the significance of multi-marker methodologies in elucidating the genetic intricacies of *Musa.*

El-Khishin *et al.* (2009) addressed problems in banana genetics, including inter-hybridisation, heterozygosity, and polyploidy, which may result in cultivar ambiguity. This study highlights that molecular markers were crucial in elucidating the evolutionary relationships among wild *Musa* species from Nagaland. Similar to the approach taken by El-Khishin *et al.* (2009) in utilising AFLP markers to discriminate banana cultivars in Egypt, this study utilized DNA barcoding markers (*atpB-rbcL, rps16, trnL-F,* and ITS) to distinguish species and reconstruct phylogenetic trees. The capacity of molecular methods to reveal specific markers for cultivars, as illustrated by El-Khishin *et al.* (2009), corresponds with the study's identification of various clades, including the close grouping of *Musa balbisiana* Coll*a* and *Musa nagalandiana* S. Dey & Gogoi. Both results enhance the significance of molecular data in revising conventional morphological classifications.

Anu *et al.* (2019) emphasised the association between morphological characteristics and molecular markers, which can be beneficial in validating genetic distinctions among the wild banana species in this study and elucidating their evolutionary relationships. Kundu *et al.* (2018) showed that molecular characterisation facilitates the precise identification of banana clones, resolving challenges such as duplication in cultivation and research commonly faced with conventional morphological techniques. Their research highlighted the significance of RAPD markers in differentiating various cultivars, especially in polyploid *Musa,* which is

essential for enhancing banana breeding initiatives and ensuring genetic stability. Baysal and Ercişli (2022) investigated the application of morphological and molecular markers to evaluate genetic relationships among banana clones. Consistent with our research, they utilised RAPD and SRAP markers to elucidate genetic variation and develop a dendrogram for genetic classification. This underscores the importance of combining morphological data with molecular approaches to deepen the comprehension of genetic diversity and linkages in bananas, which is crucial for identifying closely related genotypes and enhancing breeding strategies.

Šimoníková *et al.* (2022) examined the molecular cytogenetics of bananas, emphasising chromosomal alterations and genome size variation within the Musaceae family. Their research corresponds with our investigation into the genetic variety of bananas, highlighting the need to comprehend chromosome architecture and genomic evolution to elucidate the genesis and diversification of banana species.

Our research underscores the evolutionary importance of *Musa nagalandiana* S. Dey & Gogoi, which seems to be genetically unique, highlighting its potential as a significant genetic resource for breeding initiatives. Ferreira *et al.* (2004) underscored the significance of genetic variety in banana breeding, especially for the creation of disease-resistant cultivars. Through the examination of genetic variety among 20 diploid banana accessions exhibiting differing resistance to Sigatoka illnesses, they illustrated the applicability of genetically diverse accessions for effective hybridisation. our finding corresponds with the results of our study, wherein species such as *Musa nagalandiana* S. Dey & Gogoi and *Musa balbisiana* Colla exhibit significant genetic variety and may be utilised as parental materials in breeding programs aimed at enhancing tolerance to biotic stressors. Álvarez-López *et al.* (2022) identified resistance gene candidates (RGCs) for Fusarium wilt in wild bananas, reinforcing the notion that wild *Musa* species, such as those from Nagaland, may possess beneficial features for enhancing banana cultivars.

SSR markers in genetic research, as illustrated by Čížková *et al.* (2015), offer significant insights into phylogenetic relationships and genetic variation within the species. Furthermore, genomic investigations of wild bananas, encompassing the examination of rDNA loci and ITS sequences, have elucidated the intricate evolutionary relationships among several *Musa* species, indicating possible

interspecific hybridisation occurrences (Čížková *et al.,* 2015). These findings highlight the significance of incorporating wild banana varieties in breeding strategies to tackle issues like disease resistance and environmental stress tolerance.

The research conducted by Wu *et al.* (2021) offers significant insights into banana phylogeny and organelle inheritance, directly correlating with the aims of our work. Through high-throughput sequencing of plastomic, rDNA, and mitochondrial data, Wu *et al.* (2021) discerned two principal clades within *Musa,* corroborating the evolutionary relationships elucidated in our study. Their research on increased inverted repeats (IR) in plastomes and the suggested paternal inheritance mechanism of mitochondrial genes provides significant insights into banana organelle heredity. This methodology may be used to examine the organelle inheritance patterns of the indigenous *Musa* species in Nagaland, thereby enhancing our comprehension of hybridisation and evolutionary mechanisms.

This discovery is particularly useful for the application of genome skimming to track genealogies and detect hybrid parentage, as established by Wu *et al.* (2021). This offers a promising approach for examining the lineage of wild *Musa* species, particularly those displaying distinctive characteristics, such as *Musa nagalandiana* S. Dey & Gogoi. The incorporation of plastomic and mitochondrial data into forthcoming phylogenetic analyses may significantly improve the clarity of genetic links, hence guiding conservation efforts for wild banana species.

This work underscores the significance of preserving wild *Musa* species, especially those with unique genetic characteristics such as *Musa nagalandiana* S. Dey & Gogoi. The strong association with *Musa balbisiana* Colla underscores the importance of conserving these species to sustain local genetic diversity. The results of Wu *et al.* (2021) and Álvarez-López *et al.* (2022), which highlight the conservation of species exhibiting considerable genetic difference, correspond with this study's suggestions to prioritise the safeguarding of distinct taxa. *Musa* species such as *Musa aurantiaca* Baker and *Musa flaviflora* N.W.Simmonds demonstrates particular adaptations to the region's ecological conditions, presenting potential for future breeding initiatives focused on enhancing banana resilience to climate change and diseases.

The research conducted by Saranya *et al.* (2021), utilising ISSR markers to evaluate 191 banana accessions, underscores the dependability of molecular markers in determining genetic diversity, as they remain impervious to environmental influences. Their findings, which categorised accessions into three primary groups based on genetic similarity, correspond with the grouping identified in our study, especially the elevated bootstrap values for *Musa balbisiana* Colla and *Musa nagalandiana* S. Dey & Gogoi. Saranya *et al.* (2021) underscored the significance of recognising genetically unique species for breeding initiatives, as evidenced by this study's conclusions about the conservation value of species such as *Musa nagalandiana* S. Dey & Gogoi. These species may function as genetic resources for the development of disease-resistant or climate-adaptive banana cultivars.

The persistent clustering of *Musa balbisiana* Colla and *Musa nagalandiana* S. Dey & Gogoi with substantial bootstrap support in our study underscores their strong evolutionary relationship, perhaps attributable to common ancestry or ecological interconnection in the region. Feng *et al*. (2022) underscored the value of chloroplast and mitochondrial DNA sequences in clarifying maternal and paternal lineages in *Musa* species, stressing the necessity for multi-marker strategies to encompass genetic complexity. Moreover, research by Christelová *et al.* (2017), which offered molecular and cytological characterisation of worldwide *Musa* germplasm, underscores the necessity for a thoroughly defined germplasm collection to facilitate breeding initiatives. This corresponds with the present study's results, which emphasise the genetic variety of Nagaland's wild bananas and the imperative to include molecular data in germplasm conservation. Kulkarni *et al.* (2012) investigated genetic variability in Musaceae with RAPD markers, uncovering significant diversity among 19 genotypes, comprising both wild and farmed bananas. Their research illustrated the efficacy of molecular markers in elucidating taxonomic connections, consistent with their study's application of DNA barcode markers (*atpB-rbcL, rps16, trnL-F*, and ITS) to evaluate genetic diversity and phylogenetic relationships among wild *Musa* species from Nagaland.

The clustering patterns in both research are congruent, with Kulkarni *et al.* (2012) categorising genotypes according to ploidy, taxonomic categorisation, and evolutionary divergence, akin to the clades reported in this work, including *Musa*

laterita Cheesman and *Musa flaviflora* N.W.Simmonds. Their distinctive grouping of Ensete species further substantiates the taxonomic categorisation of the Musaceae family into several genera.

The research conducted by Davey *et al.* (2013) on the *Musa balbisiana* Colla B-genome sequence offers significant insights into the genetic mechanisms governing the B-genome of contemporary *Musa* cultivars. The findings of this study regarding the B-genome's elevated heterozygosity and new miRNAs are pertinent, as they reflect the significant interspecific and intraspecific divergence noted in our research, especially in species such as *Musa laterita* Cheesman and *Musa flaviflora* N.W.Simmonds. Davey *et al.* (2013) investigated interspecific recombination between the A- and B-genomes, underscoring the intricate evolutionary processes within *Musa,* hence corroborating this study's findings on recent divergence events and the possibility of interspecific hybridisation among the wild *Musa* species in Nagaland.

Incorporating genomic resources such as the draft B-genome of *Musa balbisiana* Colla into forthcoming research will be essential for elucidating the genetic pathways that govern stress tolerance, adaptability, and variety in both wild and cultivated *Musa* species. These insights will facilitate the production of enhanced banana cultivars and establish a basis for expanding breeding strategies to tackle global agricultural concerns.

Dhivya *et al*. (2020) employed the ITS2 region as a DNA barcode to classify banana varieties and investigate their genetic affiliations. This methodology corresponds with the study's emphasis on the significance of molecular techniques in differentiating various banana cultivars and elucidating their phylogenetic connections. The research conducted by Anuradha *et al.* (2024) illustrates the application of molecular markers to guarantee genetic stability in Red bananas and their somaclonal variants, especially during tissue culture procedures. Their research underscores the significance of molecular methodologies for validating genetic homogeneity and differentiating among various banana cultivars or variations. The findings of this work on discovering particular markers associated with anthocyanin for distinguishing Red bananas from their green versions can be utilised to improve the role of molecular markers in banana classification and breeding. This is particularly

significant for the identification and conservation of banana germplasm, essential for sustaining genetic variety in breeding initiatives.

Fu *et al*. (2022) indicated that broadening geographic and taxonomic sampling, along with the inclusion of supplementary molecular markers such as plastome-wide data, could enhance the precision of phylogenetic relationships. Integrating molecular analysis with ecological and phenotypic investigations might augment our comprehension of the adaptive characteristics and ecological functions of wild *Musa* species. This methodology is crucial for precisely evaluating the genetic linkages within *Musa* and ensuring that conservation and breeding initiatives consider the complete range of genetic diversity and adaptability in these species. This study underscores the significance of molecular markers in elucidating the phylogeny and genetic diversity of wild *Musa* species from Nagaland, accentuating the necessity of conservation initiatives for species such as *Musa nagalandiana* S. Dey & Gogoi as genetic resources for future breeding endeavours.

Similarly, Ardiyani *et al.* (2023) used three barcoding markers (*rbcL, matK*, and *trnL-F*) to reconstruct phylogenetic trees and assess genetic relationships among banana accessions, a strategy akin to the four-marker approach in this study. Both studies highlight the varying resolution capabilities of different markers. Ardiyani *et al.* (2023) noted that *rbcL* is highly conserved and effective at the generic level, while *matK* and *trnL-F* offer better resolution at the species level. This observation aligns with our results, where chloroplast markers (*atpB-rbcL, rps16, trnL-F*) were effective for resolving broader interspecific relationships, and ITS excelled in distinguishing closely related species. Moreover, Ardiyani *et al.* (2023) found that phylogenetic trees constructed with DNA barcodes revealed two distinct clades supported by high bootstrap values, which mirrors the clear clustering observed in this study, where species like *Musa laterita* Cheesman and *Musa flaviflora* N.W.Simmonds formed separate clusters, reflecting their evolutionary divergence. The clustering patterns observed here reflect the complexities of banana phylogeny, as noted by Workneh *et al.* (2022), who found that genetic relationships in banana genotypes did not fully correlate with breeding history or genome composition, showing the importance of considering evolutionary nuances. Similarly, the clustering of species like *Musa aurantiaca* Baker and *Musa manii* Baker with moderate bootstrap values suggests

recent divergence or incomplete lineage sorting, a phenomenon also noted by Wu *et al.* (2021), who identified similar complexities in *Musa* phylogeny through genomic sequencing. This highlights the value of multi-marker approaches in capturing the genetic complexity within *Musa*.

El-Khishin *et al.* (2009) discussed challenges in banana genetics, such as inter-hybridisation, heterozygosity, and polyploidy, which can lead to cultivar confusion. These issues are echoed in this study, where molecular markers were essential in resolving the phylogenetic relationships among wild *Musa* species from Nagaland. Like El-Khishin *et al.* (2009) used AFLP markers to distinguish banana cultivars in Egypt, this study employed DNA barcoding markers (*atpB-rbcL, rps16, trnL-F*, and ITS) to differentiate species and reconstruct phylogenetic trees. The ability of molecular tools to uncover unique markers for cultivars, as demonstrated by El-Khishin *et al*. (2009), aligns with the study's identification of distinct clades, such as the close clustering of *Musa balbisiana* Colla and *Musa nagalandiana* S. Dey & Gogoi. Both studies deepen the role of molecular data in refining traditional morphological classifications.

Anu *et al.* (2019) highlighted the correlation between morphological traits and molecular markers, which can be valuable for confirming genetic differences in the wild banana species in this study and understanding their phylogenetic relationships. Similarly, Kundu *et al.* (2018) demonstrated that molecular characterisation enables the accurate identification of banana clones, addressing issues like duplication in cultivation and research, often encountered with traditional morphological methods. Their study emphasised the importance of RAPD markers in distinguishing between different cultivars, particularly in polyploid *Musa*, which is crucial for improving banana breeding programs and genetic stability. Baysal and Ercişli (2022) also explored the use of both morphological and molecular markers to assess genetic relationships among banana clones. Similar to our study, they employed RAPD and SRAP markers to reveal genetic variation and construct a dendrogram for genetic classification. This signifies the value of integrating morphological data with molecular techniques to enhance the understanding of genetic diversity and relationships in bananas, which is essential for identifying closely related genotypes and improving breeding programs.

Finally, Šimoníková *et al.* (2022) discussed the molecular cytogenetics of bananas, focusing on chromosomal changes and genome size variation across the Musaceae family. Their work aligns with our study on the genetic diversity of bananas, emphasising the importance of understanding chromosome structure and genome evolution to explain the origin and diversification of banana species.

Our study highlights the evolutionary significance of *Musa nagalandiana* S. Dey & Gogoi, which appears to be genetically distinct, emphasising its potential as a valuable genetic resource for breeding programs. Ferreira *et al*. (2004) emphasised the importance of genetic diversity in banana breeding, particularly for developing disease-resistant cultivars. By exploring the genetic variability of 20 diploid banana accessions with varying resistance to Sigatoka diseases, they demonstrated the utility of genetically distinct accessions for efficient hybridisation. This finding aligns with the results observed in this study, where species like *Musa nagalandiana* S. Dey & Gogoi and *Musa balbisiana* Colla show substantial genetic diversity and could serve as parental materials in breeding programs targeting resistance to biotic stresses. The identification of *Fusarium* wilt resistance gene candidates (RGCs) in wild bananas by Álvarez-López *et al*. (2022) further supports the idea that wild *Musa* species, like those from Nagaland, may harbour valuable traits that can be leveraged to improve banana cultivars.

The use of SSR markers in genetic analysis, as demonstrated by Čížková *et al.* (2015), provides valuable insights into the phylogenetic relationships and genetic variation within the species. Additionally, genomic studies of wild bananas, including the analysis of rDNA loci and ITS sequences, have revealed the complex evolutionary relationships between different *Musa* species, suggesting potential interspecific hybridisation events (Čížková *et al.,* 2015). These findings underscore the importance of utilising wild banana diversity in breeding programs to address challenges such as disease resistance and environmental stress tolerance.

The study by Wu *et al*. (2021) provides valuable insights into banana phylogeny and organelle inheritance, which directly aligns with the objectives of our study. Using high-throughput sequencing of plastomic, *rDNA*, and mitochondrial data, Wu *et al.* (2021) identified two major clades within *Musa*, supporting the evolutionary relationships revealed in this study. Their work on expanded inverted repeats (IR) in

plastomes and the proposed paternal inheritance mode of mitochondrial genes offers important perspectives on banana organelle inheritance. This approach could be adapted to investigate the organelle inheritance patterns of the wild *Musa* species in Nagaland, further refining our understanding of hybridisation and evolutionary processes.

The use of genome skimming to trace genealogies and identify hybrid parentage, as demonstrated by Wu *et al.* (2021), is particularly relevant for this study. It provides a promising method for investigating the parentage of wild *Musa* species, especially those exhibiting unique traits, such as *Musa nagalandiana* S. Dey & Gogoi. The integration of plastomic and mitochondrial data into future phylogenetic studies could further enhance the resolution of genetic relationships, informing conservation strategies for wild banana species.

This study emphasises the importance of conserving wild *Musa* species, particularly those with distinct genetic signatures like *Musa nagalandiana* S. Dey & Gogoi. Its close relationship with *Musa balbisiana* Colla highlights the significance of preserving these species to maintain local genetic diversity. Similarly, the findings of Wu *et al.* (2021) and Álvarez-López *et al.* (2022), which emphasise the conservation of species with significant genetic divergence, align with this study's recommendations to prioritise the protection of unique taxa. As *Musa* species like *Musa aurantiaca* Baker and *Musa flaviflora* N.W.Simmonds exhibits specific adaptations to the region's ecological conditions, which hold promise for future breeding programs aimed at improving bananas' resilience to climate change and diseases.

Additionally, the study by Saranya *et al*. (2021), which used ISSR markers to screen 191 banana accessions, highlights the reliability of molecular markers in assessing genetic diversity, as they are unaffected by environmental factors. Their findings, where accessions were grouped into three major clusters based on genetic similarity, align with the clustering observed in this study, particularly the high bootstrap values for *Musa balbisiana* Colla and *Musa nagalandiana* S. Dey & Gogoi. Saranya *et al.* (2021) also emphasised the importance of identifying genetically distinct species for breeding programs, which is reflected in this study's findings on the conservation importance of species like *Musa nagalandiana* S. Dey & Gogoi. These

species could serve as genetic resources for breeding disease-resistant or climate-adaptive banana cultivars.

The consistent clustering of *Musa balbisiana Colla* and *Musa nagalandiana* S. Dey & Gogoi with high bootstrap support in our study further reflects their close evolutionary relationship, potentially due to shared ancestry or ecological connectivity in the region. Similarly, Feng *et al.* (2022) highlighted the utility of chloroplast and mitochondrial DNA sequences in elucidating maternal and paternal lineages in *Musa* species, emphasising the need for multi-marker approaches to capture genetic complexity. Furthermore, studies such as Christelová *et al.* (2017), which provided molecular and cytological characterisation of global *Musa* germplasm, emphasise the need for a well-characterised germplasm collection to support breeding efforts. This aligns with the current study's findings, which highlight the genetic diversity of Nagaland's wild bananas and the critical need for integrating molecular data into germplasm conservation. Kulkarni *et al.* (2012) explored genetic variability in *Musaceae* using RAPD markers, revealing high polymorphism among 19 genotypes, including both wild and cultivated bananas. Their study demonstrated the effectiveness of molecular markers in resolving taxonomic relationships, aligning with this study's use of DNA barcode markers (*atpB-rbcL*, *rps16*, *trnL-F*, and ITS) to assess genetic diversity and phylogenetic relationships among wild *Musa* species from Nagaland.

The clustering patterns in both studies are consistent, with Kulkarni *et al.* (2012) grouping genotypes based on ploidy, taxonomic classification, and evolutionary divergence, similar to the clades identified in this study, such as *Musa laterita* Cheesman and *Musa flaviflora* N.W.Simmonds. Their unique clustering of *Ensete* species also supports the taxonomic classification of the *Musaceae* family into distinct genera.

The study by Davey *et al.* (2013) on the *Musa balbisiana* Colla B-genome sequence provides valuable insights into the genetic mechanisms underlying the B-genome of modern *Musa* cultivars. This study's findings on the B-genome's high heterozygosity and novel miRNAs are relevant, as they mirror the substantial interspecific and intraspecific divergence observed in our study, particularly in species like *Musa laterita* Cheesman and *Musa flaviflora* N.W.Simmonds. Davey *et al.* (2013)

researched interspecific recombination between the A- and B-genomes highlighting the complex evolutionary dynamics within *Musa*, supporting this study's findings on recent divergence events and the potential for interspecific hybridisation among the wild *Musa* species in Nagaland.

Integrating genomic resources like the draft B-genome of *Musa balbisiana* Colla into future studies will be critical for understanding the genetic mechanisms underlying stress tolerance, adaptation, and diversity in wild and cultivated *Musa*. These insights will aid in the development of improved banana cultivars and provide a foundation for advancing breeding strategies aimed at addressing global agricultural challenges.

The study by Dhivya *et al.* (2020) utilised the ITS2 region as a DNA barcode to identify banana cultivars and explore their genetic relationships. This approach aligns with this study's focus on the value of molecular techniques in distinguishing different banana varieties and understanding their phylogenetic relationships. The study by Anuradha *et al.* (2024) demonstrates the use of molecular markers to ensure genetic stability in Red bananas and their somaclonal variants, particularly during tissue culture. Their work emphasises the importance of molecular techniques for confirming genetic uniformity and distinguishing between different banana cultivars or variants. This study's findings on identifying specific markers linked to anthocyanin for differentiating Red bananas from their virescent variants can be referenced to enhance how molecular markers aid in banana classification and breeding. This is especially valuable for the identification and preservation of banana germplasm, which is critical for maintaining genetic diversity in breeding programs.

As Fu *et al.* (2022) pointed out, expanding the geographic and taxonomic sampling and incorporating additional molecular markers, such as plastome-wide data, could refine phylogenetic relationships further. Combining molecular analyses with ecological and phenotypic studies would enhance our understanding of the adaptive traits and ecological roles of wild *Musa* species. This approach is essential for accurately assessing the genetic relationships within *Musa* and ensuring that conservation and breeding efforts take into account the full spectrum of genetic diversity and adaptability in these species. This study emphasises the role of molecular markers in understanding the phylogeny and genetic diversity of wild *Musa* species

from Nagaland, highlighting the importance of conservation efforts for species like *Musa nagalandiana* S. Dey & Gogoi as genetic resources for future breeding.

Summary and Conclusion

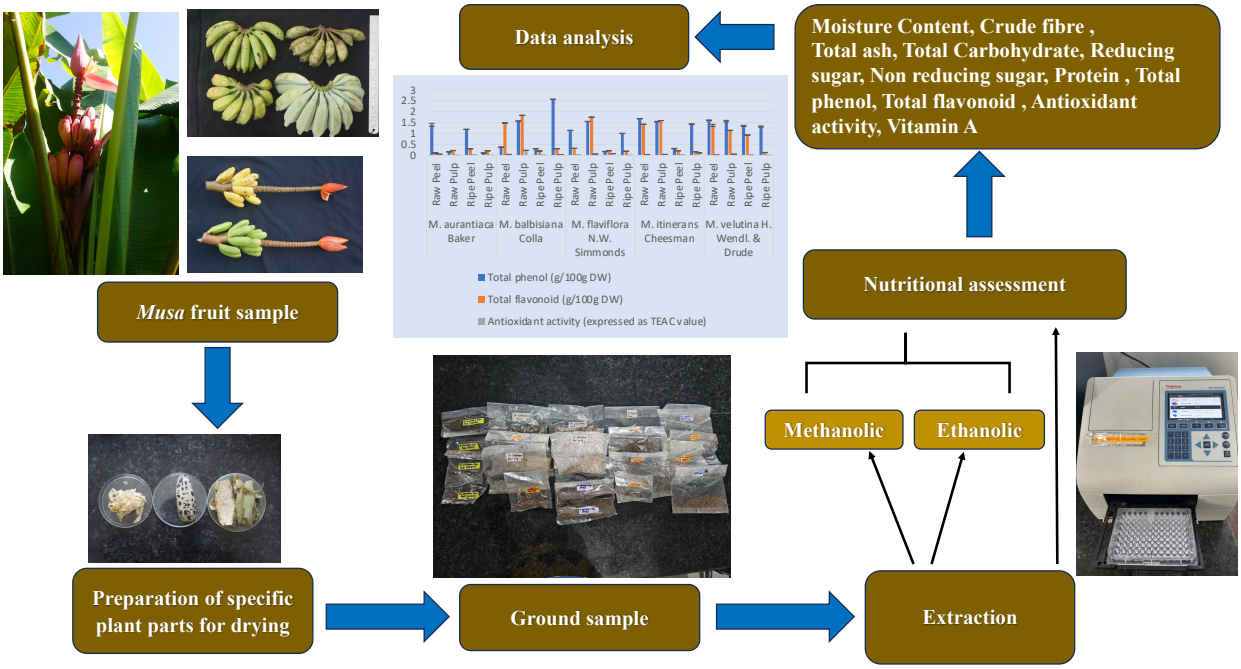
This study establishes a comprehensive phylogenetic framework for the wild *Musa* species native to Nagaland, highlighting their significant genetic diversity and evolutionary relationships. The genetic proximity of *Musa nagalandiana* S. Dey & Gogoi to *Musa balbisiana* Colla highlights its potential as a breeding resource for improving disease resistance and adaptability in farmed banana types. Phylogenetic clustering patterns reveal unique evolutionary trajectories influenced by ecological and geographical factors, with molecular markers like ITS and *trnL-F* providing improved resolution for distinguishing closely related taxa. The genetic distinctiveness of *M. nagalandiana* S. Dey & Gogoi as a separate taxon is established thereby contributing to its classification as a separate species. The detected genetic variations amongst the species highlight the evolutionary complexity of bananas in Nagaland, India, characterised by the coexistence of closely related species and divergent lineages.

The preservation of these taxa is crucial, especially considering the challenges posed by habitat loss and genetic erosion. The Musa species, such as *Musa flaviflora* N.W. Simmonds, *Musa laterita* Cheesman, and *Musa nagalandiana* S. Dey & Gogoi, are particularly significant due to their unique genetic traits and adaptive importance. This research promotes the integration of traditional taxonomic methods with DNA approaches to refine species identification and strengthen conservation efforts. This study's findings contribute to the preservation of genetic diversity in Nagaland's wild bananas, aiding in the alleviation of global agricultural issues and fostering sustainable management methods.

Chapter - 4

Nutritional Assessment on the Different Fruit Parts of Wild *Musa* Species

Graphical Summary



Introduction

In botanical terms, the term "banana" refers to a taxon encompassing dessert bananas, cooking bananas, plantains, and their wild relatives. The term 'banana' originates from the West African Woolof word ‘bananaa’. It serves as a basic diet for 400 million individuals in impoverished and developing economies. Bananas and plantains rank as the 4th most significant agricultural crop globally, following rice, wheat, and maize (Ganapathi *et al.,* 1999; Deb *et al.,* 2023), and are regarded as vital sources of energy and starchy staple food for populations in tropical humid regions (Onwuka and Onwuka, 2005).

India is a part of the centre of origin and spread for both wild and farmed bananas (*Musa* spp.). The Northeastern region of India, and Nagaland in particular, is situated within the Indo-Burma Biodiversity hotspot which forms the northwestern extreme of the centre of origin of *Musa* from where numerous wild species of the genus *Musa* has been reported making it a prime candidate for exploration and evaluation of potential nutritional value from various wild species within this genus (Janssens *et al.,* 2015; Chauhan and Jethva, 2016; Benjamin and Krishnan, 2020).

The northeastern states of India, adjacent to China and Myanmar, have major concentrations of wild *Musa* species (Ranibala *et al.,* 2018; Borborah *et al.,* 2020; Singh *et al.,* 2023). The genus *Musa* is divided into five sections, with the section *Eumusa* including 11 species, including the majority of edible bananas. India possesses around 8 of the 11 recognised species within the section *Eumusa*. *Musa balbisiana, Musa itinerans,* and *Musa flaviflora* are included among these eight species. *M. balbisiana* is a wild banana species that serves as the progenitor of numerous edible bananas by hybridisation with *Musa acuminata. Musa itinerans* is a wild banana species distinguished by its elongated rhizome; it was initially described by Ernest E. Cheesman. This species is extensively distributed in continental Southeast Asia, spanning from Northeast India to Vietnam and neighbouring islands. *Musa flaviflora* proliferates naturally in the Northeast region of India and is restricted to select areas within this region. A specialist in flowering plant taxonomy, Rajib Gogoi of the Botanical Survey of India in Itanagar, Arunachal Pradesh, described various species of *Musa* found in Northeast India (Ranibala *et al.,* 2018). *Musa nagalandiana* was named

after the state of Nagaland, its discovery location, and is exceedingly rare. *Musa markkui, Musa kamengensis, Musa argentii, Musa puspanjaliae, Musa aurantiaca,* and *Musa mannii* are additional *Musa* species identified in Northeast India (Taid *et al*., 2024; Dey *et al.,* 2014; Singh *et al.,* 2023).

Bananas are a major source of dietary calories and provide substantial nutritional value, including minerals, vitamins, proteins, and other essential elements (Ashokkumar *et al.,* 2018). They possess a high concentration of fibre, carbohydrates, potassium, phosphorus, magnesium, and an array of vitamins. Bananas are a substantial source of nutrients, and their biochemical makeup fluctuates with growth stage and maturity (Pieltain *et al.,* 1998; Pereira and Maraschin, 2015). The nutritional profile of *Musa* is characterised by elevated levels of sugar, fibre, vitamins, and minerals, alongside a minimal fat content (Ranjha *et al.,* 2020; Kumari *et al.,* 2023). Bananas are traditionally consumed for their rich nutritional content and exceptional taste, often enjoyed in desserts, puddings, and ice creams. They serve as flavouring additives in beverages, confections, and breakfast cereals. They are, moreover, utilised to prepare fritters, chips, and baked goods. In addition to the fruit, the pseudostem is exploited for fibre processing, which is applied in thatching, textiles, paper manufacturing, natural absorbents, and cordage (Kumari *et al.,* 2022). Nevertheless, the inedible banana peel, rich in health-promoting phytochemicals, is often discarded due to limited scientific knowledge. Traditional medicine asserts that banana peel can treat diabetes, anaemia, inflammation, snakebites, cough, and other conditions, or function as a health supplement (Vu *et al*., 2018). Upon thorough analysis, the peel component may function as a feasible and promising material for the development of nutraceutical products. Unripe bananas include higher concentrations of phytochemicals and lower sugar levels than ripe bananas, in addition to polyphenols, antioxidants, and resistant starch, all of which beneficially impact intestinal health and overall human well-being (Borges *et al*., 2020). Therefore, alternative techniques for maintaining the nutritional advantages of green bananas may include employing the unripe pulp as a vegetable and as flour for producing pancakes, bread, and analogous items. Moreover, both unripe and ripe bananas (encompassing pulp and peel) can be utilized by the food industry to produce products such as pickles, health-oriented fat-

free chips, banana cakes, cookies, nutraceuticals, and in the cosmetic industry for health-related items like supplements and medications, as well as creams, soaps, shampoos, and beauty powders, thereby converting waste into valuable commodities (Ngullie and Deb, 2025).

Wild edible plants garner significant study attention worldwide. Numerous investigations indicate that these unconventional plant diets may provide nutritional content comparable to or exceeding that of farmed crops. Nevertheless, many lesser-known wild foods are frequently disregarded, despite their significance for the food security of tribal and rural communities. In light of this context, the current investigation has been initiated (Dan and Thaha, 2014).

This research presents a comprehensive analysis of the composition and nutritional value of the pulp and peels of selected wild banana species in Nagaland. The assessment of total flavonoid and phenolic contents was also performed to examine their correlation with antioxidant activity.

Materials and methods

Sample collection

Five wild banana species, namely *Musa aurantiaca* Baker, *Musa balbisiana* Colla, *Musa flaviflora* N. W. Simmonds, *Musa itinerans* Cheesman, and *Musa velutina* H. Wendl. & Drude, were collected from all over the specified jurisdiction of the study area, packed in polythene bags, labelled, and transported to the laboratory. The fruits were collected as and when available in the wild to ensure fidelity to their natural state. The taxonomic identification of the collected banana specimen has been documented in a prior study (Deb *et al.,* 2023).

Preparation of the sample

The fruits were washed clean and left to dry completely at room temperature. The peel was separated from the pulp and the pulp from the seeds; the samples were then weighed, cut into small pieces, and dried in an oven for 30 hours at a temperature of 60°C **(Figure 4.6a)**. After drying, the materials were weighed and pulverised into a powdery consistency **(Figure 4.6b)**. The dried samples were extracted using 80% ethanol (v/v) (1 g of dry material in 10 ml of solvent) to yield the total ethanol extract

(TE). A subsequent fraction (1 g) of the dried sample was extracted successively with 80% methanol (10 ml) to obtain the whole methanol extract. All resultant extracts were preserved at −20°C until use.

The nutritional evaluation of the gathered samples encompasses the fruit pulp and peel of both the unripe and ripe fruit. The present analysis investigated numerous parameters, including moisture content, crude fibre, total ash content, total carbohydrate content, reducing sugars, non-reducing sugars, protein content, total phenol and flavonoid content, antioxidant capacity, and vitamin A.

Figures 4.1a & 4.1b: Raw and ripe fruits of *Musa balbisiana*; **Figures 4.2a & 4.2b**: Raw and ripe fruits of *Musa flaviflora*; **Figures 4.3a & 4.3b:** Raw and ripe fruits of *Musa itinerans.*

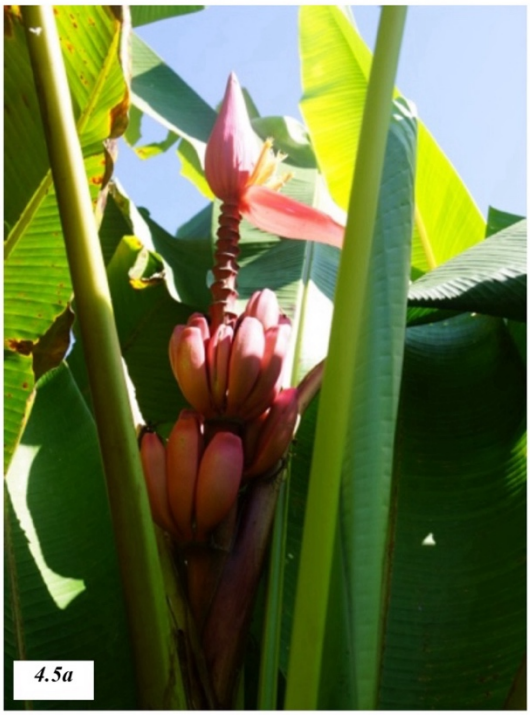


Figure 4.4a: Raw and ripe fruits of *Musa aurantiaca;*

Figures 4.5a & 4.5b: Raw and ripe fruits of *Musa velutina.*

Figure 4.6a: Wild *Musa* sample preparation for peel and pulp analysis

Figure 4.6b: Dried and ground wild *Musa* samples

Proximal analysis

The proximate components (moisture, ash) of banana samples were analysed according to standard methods established by the Association of Official Analytical Chemists (AOAC, 2019). The sample was dried in an oven at 105ºC until it reached a stable weight, and the moisture content was assessed using the gravimetric method. To ascertain ash content, the silica crucible was initially heated in a furnace for 1 hour, and its original weight was recorded after cooling in a desiccator until a stable weight was achieved. 2g dry sample was placed in a crucible of known weight and subjected to heating at 550ºC for 6 hours in a muffle furnace. The sample was cooled in the desiccator, and its final weight was recorded. The ash content was determined according to the formula provided below.

Ash (%) = [Weight of ash / Weight of sample] × 100.

Crude Fibre

For crude fibre analysis, the AOAC procedure (2019) was used. Samples were extracted (2g) with petroleum ether to eliminate fat (Initial boiling temperature 35-38°C). After extraction with petroleum ether, the resultant substrate was heated with 200 mL of H2SO4 for 30 minutes with bumping chips. It was then filtered and rinsed with hot water to eliminate the acid. The filtrate was then again heated with 200 mL of sodium hydroxide for 30 minutes. The following step involves filtration and washing with 25mL of boiling 1.25% H2SO4, three 50mL volumes of water and 25mL of alcohol. The residue was then transferred to an ashing dish (pre-weighted dish W1) and cooked for 2 hours at 130 ± 2°C. The dish was then chilled in a desiccator and weighed (W2). The dish was burned for 30 minutes at 600 ± 15°C, then cooled in a desiccator and reweighed (W3). The crude fibre was determined using the formula given below.

$$\% \text{ Crude fibre in ground sample} = \frac{\text{Loss in weight on ignition } (W_2 - W_1) - (W_3 - W_1)}{\text{Weight of the sample}} \times 100$$

Total Protein

The protein concentration was determined using the Bradford assay (Bradford, 1976). 0.0, 0.2, 0.4, 0.6, 0.8, and 1 ml of the working standard were pipetted out into a series of marked test tubes. Additionally, 1 ml of the provided sample was transferred into a separate test tube. The volume was then adjusted to 1 ml in each test tube using PBS. A tube containing 1 ml of water was taken to function as the blank. Then 5 ml of diluted dye solution was added to each test tube, including those designated as 'blank' and 'unknown'. Vortex or shake the tubes to mix their contents and permit the colour to develop for 20 minutes. The red dye transitions to blue when binding to the protein. Subsequently, the absorbance was measured at 595 nm relative to the blank. Then, the standard curve was plotted by plotting protein concentration on the X-axis and absorbance at 595 nm on the Y-axis. The protein concentration in the unknown sample was then determined using this standard curve.

Total Carbohydrate

The total carbohydrate was quantified using slight modifications of the Anthrone method (Hodge and Hofreiter, 1962). 100 mg of the sample was measured and placed into a boiling tube. The sample was hydrolysed by maintaining it in a boiling water bath for 3 hours with 5 mL of 2.5 N HCl, then allowed to cool to room temperature. The sample was then neutralised with solid sodium carbonate till the effervescence terminated. The volume was adjusted to 100 mL and centrifuged. The supernatant was then filtered and stored for estimation. Standards of varying concentrations were prepared by measuring 0, 0.2, 0.4, 0.6, 0.8, and 1 mL of the working standard. ‘0’ functioned as a blank. The volume was adjusted to 1 mL in all the tubes by adding deionised water, including the sample tubes, where 0.5 mL to 2 mL of the sample extract was introduced. Then 4 mL of anthrone reagent was added to each tube, and the tubes were heated for 8 minutes in a boiling water bath. The tubes were then cooled rapidly under running water, and the green to dark green solutions were measured at 630 nm. A standard graph was constructed by plotting the concentration of the standard on the X-axis against absorbance on the Y-axis. The quantity of carbohydrates in the sample tube was estimated based on the standard graph.

Reducing Sugar

The quantification of reducing sugar was carried out via modified Dinitrosalicylic acid (DNS) method (Miller, 1972). Sample extracts of volume 0.5 to 2 mL were added into test tubes, and the volume was adjusted to 3 mL with deionised water in each tube. 3 mL of DNS reagent was added to each tube. The tubes were subjected to a boiling water bath for 5 minutes, and then taken off the heat. Then, 1 mL of 40% Rochelle salt solution was added to the tubes while their contents were still hot. Absorbance readings of the solutions were taken at 510 nm. A series of standards utilising glucose (0-500µg) as a standard was constructed, and a graph was generated. The reducing sugar content of the samples was then estimated by plotting it against the standard graph.

Non-reducing Sugar

The quantity of reducing sugar was determined by subtracting the amount of reducing sugar from the total carbohydrate content.

DPPH Radical Scavenging Activity

The 2, 2-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity was assessed according to Brand-Williams *et al.* (1995) with slight modifications. The reaction was started when 5mL of diluted supernatant was mixed with 0.5 mL of 1 mM DPPH in methanol. The absorbance at 517 nm was promptly measured at 0 minutes, after which the mixture was maintained in darkness for 5-min. The ability to scavenge the DPPH free radical was determined using the equation.

$$\textbf{DPPH free radical scavenging activity (\%)} = [(A0 - A10) / A0] \times 100$$

Where A0 is the absorbance of the sample at 0 min and A10 is the absorbance of the sample at 10 min.

Total Phenolic Content

The same supernatant from the total antioxidant assay was utilised to quantify the total phenols (TP) in the banana. The total phenolic content was assessed using the methodology outlined by Slinkard and Singleton (1977). The reaction was performed

with the addition of 0.5 mL of the supernatant to 0.5 mL of water, followed by the incorporation of 1 mL of 50% (v/v) Folin-Ciocalteu reagent solution and 2 mL of saturated Na_2CO_3 solution. The solution was incubated at room temperature for 30 minutes before measuring the absorbance at 750 nm. The findings were presented as grams of gallic acid per 100 grams of dry weight (g GA/100g DW) by plotting a gallic acid standard graph.

Total Flavonoid Content

The same supernatant from the total antioxidant assay was utilised to ascertain the total flavonoids (TF) level in a banana. The TF content was evaluated using the assay by Zhishen *et al*. (1999). The reaction started with the addition of 0.25 mL of the supernatant to a mixture comprising 1.25 mL of water and 75 µL of 0.5% $NaNO_2$, followed by a 6-minute incubation at room temperature. 150 µL of 10% AlCl3·6H2O was incorporated into the mixture and allowed to stand for 5 minutes. A 0.5 mL aliquot of 1 M NaOH was subsequently added, and the absorbance at 510 nm was measured. The data were presented as grams of quercetin per 100 grams of dry weight (g quercetin/100g DW) by plotting a quercetin standard graph.

Vitamin A

The methodology of Aremu and Nweze (2017) was employed to ascertain ß-Carotenoid content. 10 mL of methanol was introduced to 1 g of the sample, which was thereafter placed in a shaker in the dark overnight. The β-carotene layer was isolated using a separating funnel and 10 ml of hexane, with moisture removed with the use of sodium sulfonate. An absorbance test was conducted at 436 nm using hexane as a blank in a UV-Vis spectrophotometer. The following formula was used to determine the ß-carotenoid content:

$$\textit{ß}\text{− carotenoid (µg/100g)} = \{(\text{Abs x V x DF})/\text{Wt}\} \text{ x Y}$$

Where,

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

A conversion factor of 0.167 µg RE [1 µg (-carotene 0.167 µg Retinol Equivalent)] was used to estimate the amount of vitamin A.

Statistical Analysis

All data is expressed as mean ± standard deviation (n=3). The figures were generated utilising Microsoft Excel 2019. Substantial differences were assessed via One-Way ANOVA in MS Excel 2019.

Results

The proximate content representing the nutritive components of the ripened and raw banana pulp and peel extracts is depicted in Table 4.1.

Table 4.1 : Proximate nutritive values of ripened and raw banana pulp and peel extracts												
***Musa* species**	**Plant part**	**Moisture content (%)**	**Crude fibre (% in dry wt.)**	**Total ash (% in dry wt.)**	**Total carbohydrate (g/100g DW)**	**Reducing sugar (g/100 g DW)**	**Non-reducing sugar (g/100g DW)**	**Protein (g/100 g DW)**	**Total phenol (g/100g DW)**	**Total flavonoid (g/100g DW)**	**Antioxidant activity (expressed as TEAC value)**	**Vitamin A(mg of RE/100 g DW)**
***M. aurantiaca* Baker**	**Raw Peel**	89.61±0.026	2.125±0.041	15.65±0.092	10.87±0.172	2.47±0.023	8.4±0.03	5.548±0.043	1.402±0.067	0.11±0.009	0.0544±0.0001	2.69±0.009
	Raw Pulp	86.32±0.094	0.34±0.007	4.35±0.059	36.67±0.08	2.39±0.056	34.28±0.09	3.782±0.021	0.164±0.002	0.225±0.006	0.0022±0.0002	1.444±0.014
	Ripe Peel	86.73±0.057	1.965±0.035	15.8±0.039	5.94±0.026	2.45±0.042	3.49±0.051	4.143±0.031	1.198±0.005	0.303±0.007	0.0076±0.0006	2.208±0.017
	Ripe Pulp	87.29±0.032	0.455±0.011	5.8±0.047	40.94±0.018	16.18±0.026	24.76±0.071	8.826±0.026	0.108±0.008	0.21±0.003	0.0013±0.0005	2.9±0.008
***M. balbisiana* Colla**	**Raw Peel**	87.79±0.009	18.63±0.059	12±0.019	11.98±0.03	4.21±0.016	7.77±0.023	3.53±0.051	0.38±0.001	1.486±0.008	0.0457±0.0002	1.632±0.011
	Raw Pulp	70.73±0.038	0.45±0.009	4.65±0.08	21.18±0.07	4.24±0.052	16.94±0.08	2.522±0.018	1.573±0.009	1.839±0.002	0.2319±0.0007	2.64±0.015
	Ripe Peel	81.17±0.008	4.73±0.019	12.05±0.021	11.03±0.074	5.77±0.062	5.26±0.027	3.531±0.038	0.306±0.005	0.201±0.006	0.0032±0.0001	2.062±0.013
	Ripe Pulp	74.31±0.005	0.5±0.007	11.7±0.011	43.17±0.083	26.79±0.073	16.38±0.061	2.269±0.018	2.575±0.012	0.307±0.004	0.0245±0.0005	2.078±0.018

M. flaviflora **N.W. Simmonds**	**Raw Peel**	89.68±0.036	6.805±0.028	5.3±0.016	10.51±0.019	2.47±0.09	8.04±0.015	4.791±0.136	1.146±0.008	0.326±0.003	0.0071±0.0003	1.012±0.008
	Raw Pulp	70.19±0.029	0.32±0.08	6.7±0.08	14.01±0.063	3.03±0.07	10.98±0.055	4.035±0.129	1.557±0.006	1.755±0.013	0.0601±0.0004	2.048±0.027
	Ripe Peel	72.62±0.071	2.105±0.049	18.3±0.041	11.12±0.095	4.49±0.08	6.63±0.22	3.026±0.182	0.178±0.009	0.215±0.006	0.0828±0.0005	0.347±0.007
	Ripe Pulp	73.68±0.007	0.1±0.005	25.7±0.018	36.03±0.137	22.65±0.019	13.38±0.065	3.53±0.153	1.01±0.004	0.193±0.008	0.0019±0.0008	1.627±0.009
M. itinerans **Cheesman**	**Raw Peel**	91.45±0.016	4.24±0.08	30.1±0.09	10.14±0.09	3.09±0.04	7.05±0.024	8.322±0.09	1.681±0.004	1.429±0.003	0.0294±0.0005	1.616±0.017
	Raw Pulp	83.49±0.041	0.425±0.04	10.65±0.021	28.89±0.032	2.86±0.07	26.03±0.077	5.548±0.07	1.548±0.011	1.596±0.004	0.0475±0.0004	2.569±0.014
	Ripe Peel	88.77±0.031	2.6±0.03	16.2±0.052	5.62±0.08	2.47±0.04	3.15±0.042	12.861±0.016	0.316±0.008	0.214±0.007	0.0075±0.0003	0.92±0.009
	Ripe Pulp	83.22±0.017	0.79±0.02	9.4±0.026	32.31±0.06	18.76±0.013	13.55±0.033	9.023±0.014	1.435±0.013	0.167±0.005	0.1173±0.0009	3.229±0.011
M. velutina **H. Wendl. & Drude**	**Raw Peel**	90.91±0.046	1.98±0.013	19.2±0.049	5.75±0.09	3.07±0.06	2.68±0.018	1.513±0.08	1.608±0.020	1.377±0.05	0.0147±0.0006	1.071±0.015
	Raw Pulp	80.46±0.021	0.28±0.005	8.2±0.08	28.12±0.062	7.57±0.018	20.55±0.035	6.052±0.021	1.571±0.035	1.148±0.009	0.0553±0.0008	3.016±0.028
	Ripe Peel	90.68±0.049	1.81±0.017	16.6±0.018	12.06±0.031	2.95±0.011	9.11±0.027	2.451±0.011	1.354±0.025	0.932±0.004	0.0081±0.0007	1.2±0.011
	Ripe Pulp	86.68±0.028	0.56±0.003	5.33±0.015	35.63±0.029	27.06±0.024	8.57±0.062	5.859±0.054	1.323±0.027	0.133±0.007	0.0024±0.0007	0.965±0.006

Moisture content

The moisture content of all 20 *Musa* samples varies from 70.19% to 91.45%, with an overall mean of 83.289%, signifying exceptionally high moisture levels. The raw peel of *Musa itinerans* Cheesman contains the maximum moisture content, measuring 91.45%. The raw pulp of *Musa flaviflora* N. W. Simmonds has the lowest moisture content at 70.19% as depicted in **Figure 4.7.** The moisture content in raw peel averages 89.888%, the highest among plant parts, while raw pulp exhibits the lowest at 78.238%. The mean moisture content of the ripe pulp was 81.036%, whereas that of the ripe peel was 83.994%.

Crude Fibre Content

The raw peel of *Musa balbisiana* Colla exhibited the maximum fibre concentration at 18.63%, while the ripe pulp of *Musa flaviflora* N.W. Simmonds displayed the lowest fibre level at 0.10%. The raw peels exhibit a 40% greater fibre density in comparison to the ripe peel, while the pulp samples demonstrate exceedingly low fibre density as depicted in **Figure 4.7.**

Total Ash Content

The raw peel of *Musa itinerans* Cheesman exhibits the highest total ash concentration at 30.1%, signifying a substantial mineral content; conversely, the raw pulp of *Musa aurantiaca* Baker shows a mere 4.35% as depicted in **Figure 4.7**. Peels regularly contain a higher concentration of ash than pulps. The sequence is as follows: raw peel > ripe peel > ripe pulp > raw pulp. The average percentage of raw peel is 16.45%, raw pulp is 6.91%, ripe peel is 15.79%, and ripe pulp is 11.59%. These values demonstrate significant variation among species and plant components.

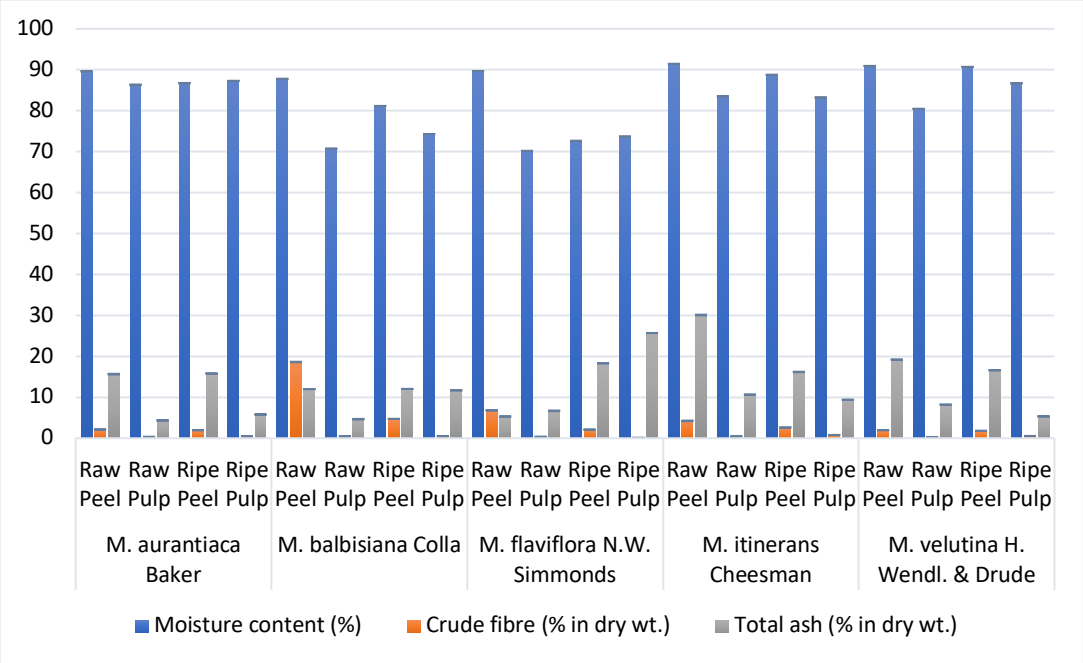


Figure 4.7: Bar graph depicting moisture, crude fibre and total ash content in the selected wild *Musa* species.

Total Carbohydrate Content

The pulps of all species contain a higher carbohydrate content than the peels at different stages of ripening. The maximum carbohydrate content was observed in the ripe pulp of *Musa balbisiana* Colla at 43.17 g/100 g DW, while the minimum was recorded in the ripe peel of *Musa itinerans* Cheesman at 5.62 g/100 g DW, as seen in **Figure 4.8**. The mean carbohydrate content, categorised by plant part, is 9.85 g/100g DW for raw peel, 25.77 g/100g DW for raw pulp, 9.15 g/100g DW for ripe peel, and 37.62 g/100g DW for ripe pulp.

Reducing and non-reducing sugar content

The concentration of non-reducing sugars was highest in the raw pulp of *Musa. aurantiaca* Baker at 34.28 g/100 g DW, while the minimal measurement was 2.68 g/100 g DW from the raw peel of *Musa velutina* H. Wendl. & Drude. The highest reducing sugar content is found in the ripe pulp of *Musa balbisiana* Colla, a progenitor species, at 26.79 g/100 g DW, while the lowest is in the ripe peel of *Musa aurantiaca*

Baker at 2.39 g/100 g DW, as depicted in **Figure 4.8**. In the 20 *Musa* samples, sugars are concentrated in the pulp, with ripening significantly increasing reducing sugars in the pulp while somewhat decreasing non-reducing sugars; sugars in the peel remain relatively unchanged.

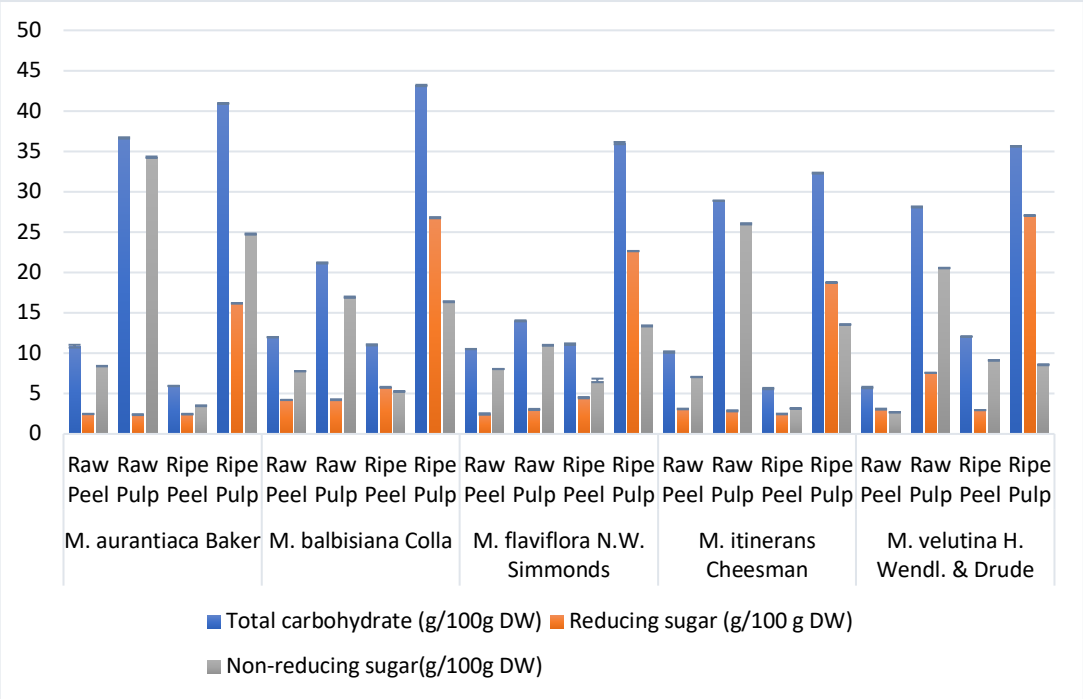


Figure 4.8: Bar graph depicting total carbohydrate, reducing and non-reducing sugar content in the selected wild *Musa* species

Total Protein Content

The protein concentration in the ripe peel of *Musa itinerans* Cheesman was notably high at 12.861 g/100 g DW, whereas the raw peel of *Musa velutina* H. Wendl. & Drude exhibited the lowest value at 1.513 g/100 g DW. The observed trend indicates that mature components yield, on average, greater than raw components, with raw peel averaging 4.74 g/100g DW, raw pulp 4.39 g/100g DW, ripe peel 5.20 g/100g DW, and ripe pulp 5.90 g/100g DW. The protein concentration in ripe peel across the five species demonstrates significant variety, ranging from 2.45 g/100g DW in the ripe peel of *Musa velutina* H. Wendl. & Drude to 12.86 g/100g DW in the ripe peel of *Musa itinerans* Cheesman as seen in **Figure 4.9**.

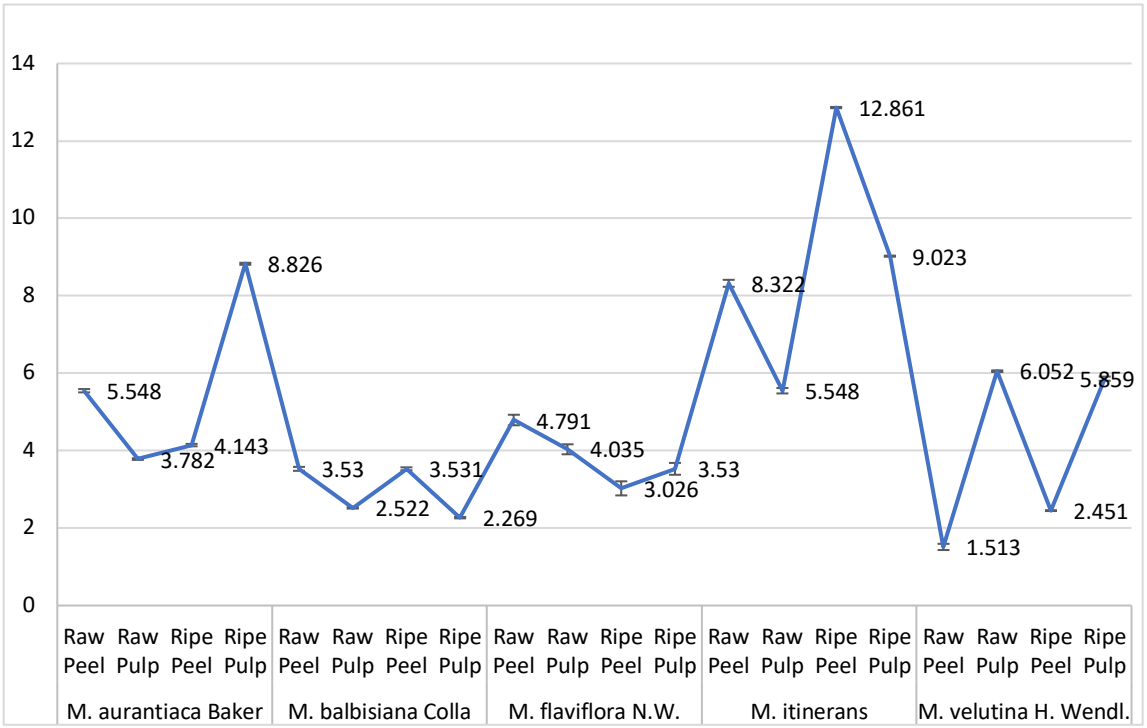


Figure 4.9: Line graph depicting protein content in the selected wild *Musa* species.

Total Phenol and Flavonoid Content

The total phenol concentration and total flavonoid content are highest in the ripe pulp and raw pulp of *Musa balbisiana* Colla, measuring 2.575 and 1.839 g/100 g DW, respectively. The total phenol concentration is lowest in the ripe pulp of *Musa aurantiaca* Baker at 0.108 g/100 g DW, whereas the total flavonoid content is lowest in the raw peel of *Musa aurantiaca* Baker at 0.11 g/100 g DW, as depicted in **Figure 4.10**. Phenol content is highest in ripe pulp, averaging 1.29 g/100g DW, while flavonoid content is highest in raw pulp, averaging 1.313 g/100g DW. The aforementioned values for total phenol and total flavonoid are species-specific, with *Musa balbisiana* Colla exhibiting the highest values in both parameters, while *Musa aurantiaca* Baker displays the lowest levels in both.

Antioxidant Activity

The antioxidant activity (expressed as TEAC value) is highest in the raw pulp of *Musa balbisiana* Colla at 0.2319 and lowest in the raw pulp of *Musa aurantiaca* Baker at 0.0002, as seen in **Figure 4.10.** Among plant parts, raw pulp exhibits the highest TEAC value, averaging 0.0794, followed by raw peel at 0.0303, ripe pulp at 0.0295, and ripe peel, which has the lowest TEAC value at 0.0218. This verifies that raw pulp possesses the highest antioxidant density among plant components.

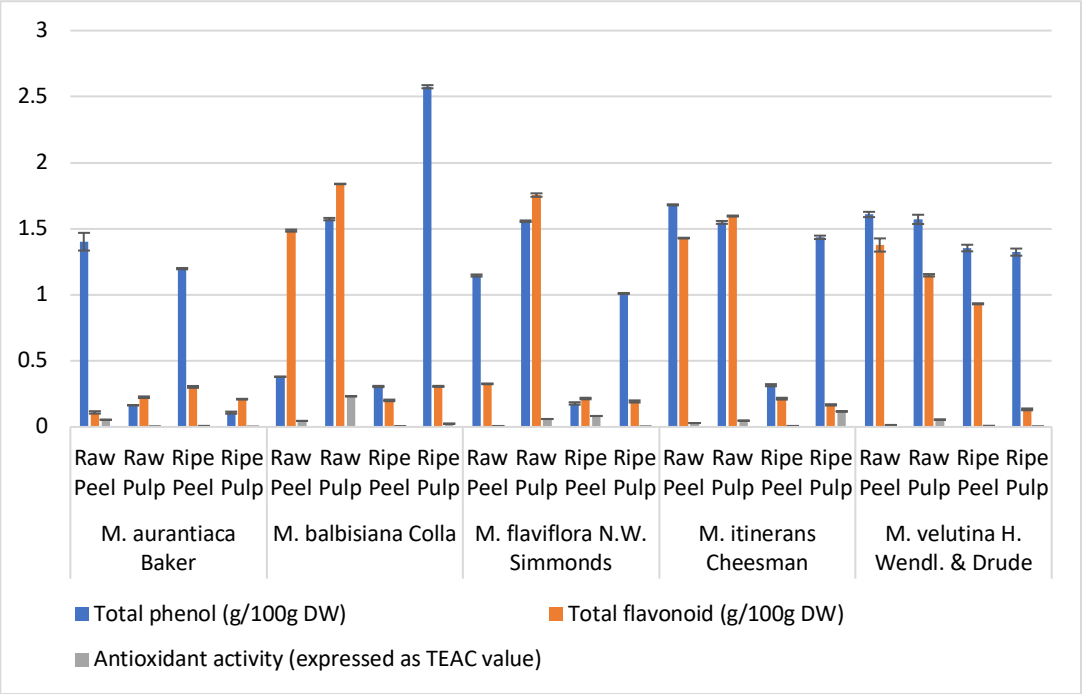


Figure 4.10 : Bar graph depicting total phenol, total flavonoid, and antioxidant activity in the selected wild *Musa* species.

Vitamin A Content

Vitamin A content, expressed in mg of RE per 100 g of dry weight, was highest in the ripe pulp of *Musa itinerans* Cheesman at 3.229 g RE/100g DW and lowest in the ripe peel of *Musa flaviflora* N.W. Simmonds at 0.347 g RE/100g DW as depicted in **Figure 4.11**. Raw pulp contains the greatest average vitamin A at 2.343 mg of RE/100g DW, followed by ripe pulp at 2.160 mg of RE/100g DW, although raw and

ripe peel had lower concentrations at 1.604 mg of RE/100g DW and 1.347 mg of RE/100g DW, respectively.

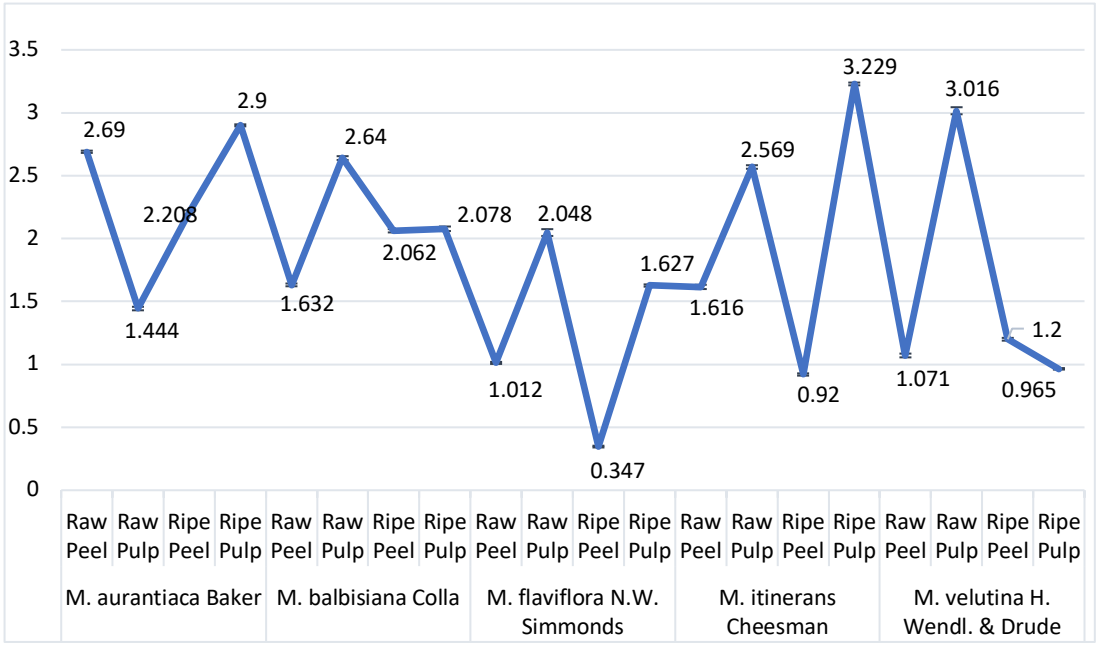


Figure 4.11 : Line graph depicting vitamin A content in the selected wild *Musa* species.

Discussion

This study delineates the nutritional value approximations of several fruit components of selected wild *Musa* species and shows significant disparities in nutritional profiles among different *Musa* species and between their raw and ripe stages. The following are several key observations from this study. The moisture content substantially affects the calorific value and determines the shelf life. Water is a vital element in numerous food items (Oyeyinka and Afolayan, 2019). The findings demonstrate that *Musa* possesses a significant moisture content, as evidenced by Ranjha *et al.* (2020). Samples with elevated moisture levels, particularly the raw peels, necessitated more intensive drying for effective dehydration and preservation of shelf stability.

Bananas are rich in fibre, which facilitates a healthy digestive system and encourages regular bowel motions. An increased fibre intake also expedites digestion (Anderson *et al*., 2009). The raw peel of *Musa balbisiana* Colla contains a significantly higher fibre content of 18.63±0.059% in dry weight, surpassing that of any other plant part or species mentioned, indicating its potential as a substantial source of dietary fibre. Raw peels exhibit a 40% greater fibre density than ripe peels or pulp, suggesting enhanced glycemic management potential. This corresponds with research indicating that green banana products enhance glucose regulation and elevate GLP-1 levels relative to mature variants (Wang *et al.,* 2022; Wani *et al.,* 2025).

Ash content serves as a quality indication. Elevated ash level may signify an abundance of minerals or impurities, whereas diminished ash content could imply a fruit with reduced mineral density (Gupta and Solanki, 2015). Ash serves as a proxy for total mineral residue; the persistently elevated levels in peels relative to pulp signify greater mineral density, but ripening reduces this peel-pulp gap. The ash concentration in the raw peel of *Musa itinerans* Cheesman, for one, is of significance.

Carbohydrates function as a principal energy source in the human body and are crucial for the brain, which predominantly depends on glucose for optimal operation (Nadeeshani *et al.,* 2021). Diabetic patients are frequently counselled to refrain from consuming ripe bananas due to their elevated carbohydrate and sugar content (Hermansen *et al.,* 1992). A consistent trend is noted in the pulp, where carbohydrate and reducing sugar levels significantly rise from the raw to the ripe stages, attributable to ripening-induced sugar transformation, leading to increased sugar escalation ratios across all five species and plant parts, underscoring a pronounced part and ripeness effect (Cordenunsi-Lysenko *et al.,* 2019). Nonetheless, the values in sugar levels exhibits considerable variability among samples and species (6.5% to 75.9%), signifying substantial genetic and developmental impacts on glycemic potential. This variation facilitates the precise selection of low-glycemic species for the production of diabetes-friendly dietary products and metabolic enhancement.

Dietary proteins are essential components in anabolic processes and tissue regeneration (Ranjha *et al*., 2020). Bananas are not regarded as a substantial source of protein in comparison to other foods. A medium-sized banana (about 118 grammes) contains around 1.3 grammes of protein (Peumans *et al.,* 2002). All species have

varying protein concentrations in various parts, lacking a consistent pattern. Among all combinations, peels exhibit the greatest protein variety, spanning from 2.45 g/100 g DW to 12.86 g/100 g DW across different species. This significant variance indicates distinct protein accumulation techniques during peel maturation, with *Musa itinerans* Chessman attaining remarkable protein concentration.

Saura-Calixto and Goni (2006) indicated that fruits encompass plant secondary metabolites, including carotenoids, phenolic acids, flavonoids, and vitamins C and E, which enhance antioxidant activity and imply significant therapeutic potential. Bravo (1998) emphasises that the polyphenol concentration in fruits and vegetables is significantly affected by genetic variables, environmental conditions, and their maturity stage. Various natural antioxidants have a wide range of biological activities, including antibacterial, antiviral, anti-inflammatory, antiallergenic, antithrombotic, and vasodilatory capabilities. Banana pulp is rich in several antioxidants, such as vitamins, carotenoids, and phenolic compounds (Reihani *et al.,* 2012). The ripe pulp of *Musa balbisiana* Colla exhibits the highest total phenol content, while the raw pulp exhibits the highest total flavonoid content and antioxidant activity values, indicating a correlation between elevated phenol and flavonoid levels and enhanced antioxidant activity, thereby establishing it as a strong source of antioxidants (Alothman *et al.,* 2009). Utilising a ripeness-part metric to assess its impact on antioxidant activity, raw pulp exhibits the highest mean TEAC value, greatly surpassing other combinations. This trend corresponds with research suggesting that the early ripening stage maintains elevated amounts of phenolic compounds, especially in pulp tissues, where enzymatic breakdown is reduced (Kritsi *et al.,* 2023). This ripeness-part metric exhibits no consistent universal pattern across the five species, indicating the potential influence of additional factors, including species-specific genetic traits and cultivation conditions, which may lead to the presence and varying concentrations of diverse bio-compounds, such as ascorbic acid, alpha and beta-carotenes, and various xanthophylls identified in bananas, potentially enhancing antioxidant activity (Subagio *et al*., 1996; Kondo *et al.,* 2005), thereby superseding general ripeness-part trends.

Animals are incapable of synthesising retinoids and must acquire vitamin A through their diet, which is available as retinyl esters from animal sources or provitamin A carotenoids from plant sources (Zhan *et al.,* 2010). The samples

exhibiting the greatest vitamin A concentrations are predominantly pulp: *Musa itinerans* Cheesman ripe pulp at 3.229 mg of RE/100 g DW, *Musa velutina* H. Wendl. & Drude raw pulp at 3.016 mg of RE/100 g DW, and *Musa aurantiaca* Baker ripe pulp at 2.900 mg of RE/100 g DW. This trend suggests that pulp, especially when raw or ripe, depending on the species, generally contains a higher concentration of vitamin A than the peel.

Wild bananas have traditionally been utilised in medicine to address numerous ailments. These plants possess a plethora of bioactive chemicals that are integral to their medicinal efficacy. Their significant antioxidant and anti-inflammatory characteristics underscore their potential in the formulation of herbal therapies (Pereira and Maraschin, 2015; Mathew and Negi, 2017; Mathew and Negi, 2021; Sethiya *et al.,* 2019). Researchers are investigating the utilisation of wild banana plant components in the creation of biodegradable packaging and natural antibacterial agents for food preservation, hence advocating for environmentally friendly alternatives to traditional chemical preservatives (Mostafa, 2021). Wild banana species are the subject of current conservation and domestication initiatives aiming at enhancing food security and safeguarding their future agricultural utilisation (Naikawadi *et al.,* 2022).

Summary and Conclusions

The nutritional evaluation of the specified *Musa* species offers a comprehensive understanding of their nutritional worth, facilitating the exploration of potential edible wild *Musa* species that may be integrated into current cultivars to improve nutritional content. The examination of the nutritional values of five wild *Musa* species from Nagaland were carried out, comparing the fruit's nutritional content encompassing both the peel and pulp of their raw and ripe forms for the following parameters: moisture, ash content, protein, crude fibre, reducing sugar, total carbohydrate and vitamin A, total phenolic, flavonoid content and DPPH radical scavenging activity. The study found that wild bananas' pulp and peel are good sources of antioxidants and provitamin A for phytomedicine. The results obtained from the baseline data indicate and point to several other possible avenues of exploration based on the performance of selected species on specific parameters. The peels of these fruits, especially the peel of *Musa balbisiana* Colla, show promise for their ideal sugar-

to-fibre ratio. *Musa itinerans* Cheesman is noteworthy due to its remarkable all-round comprehensive nutritional profile, enhancing its potential as a high-value functional food.

The consumable portion of the wild *Musa* significantly contributes to food security and is a fundamental aspect of the culinary heritage in Nagaland. Traditional medicine has utilised all components of the wild *Musa* plant, including the flower, pseudo-stem, corm, leaves, sap, and roots, to address numerous ailments. This creates an opportunity to investigate their nutritional and nutraceutical advantages. Bananas can serve as a source of food security and as a bio-compound resource for the pharmaceutical industry.

Chapter - 5

Nutritional Assessment of Inflorescence and Pseudostem of Few Wild *Musa* Species

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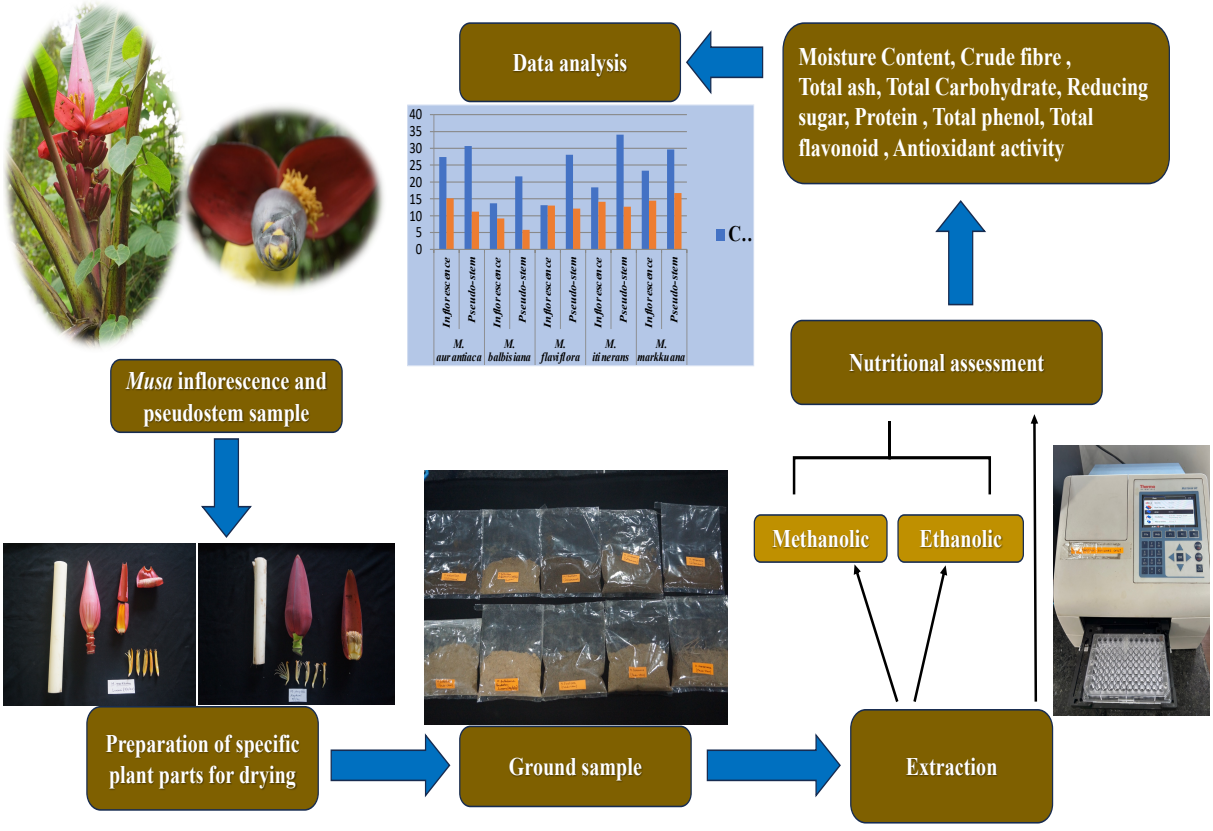
Graphical Summary



Introduction

The genus *Musa* (family Musaceae) encompasses both wild and cultivated bananas that play critical ecological, cultural, and nutritional roles in tropical regions. India, recognised as one of the centres of origin for *Musa*, harbours exceptional genetic diversity, with the Northeastern Hill region, including Nagaland, functioning as a micro-centre of evolution and diversification (Simmonds, 1962; Deb *et al*., 2023). In Nagaland, wild bananas not only constitute genetic resources but are incorporated into indigenous diets, ethnomedicinal practices, and agrarian livelihoods. Nevertheless, investigations into their nutritional attributes have predominantly concentrated on the cultivated fruits, whereas other consumable components, including the inflorescence (banana flower) and pseudostem, especially of their wild kin, have been insufficiently examined despite their extensive utilisation in traditional culinary practices and folk medicine.

Many Asian civilisations have historically eaten the pseudostem and flower of bananas. Pseudostem fibres are added to soups or utilised as functional components during times of food scarcity, while tribal groups in portions of Northeast India prepare banana blooms in stews or curries. However, there is a dearth of thorough nutritional analysis of these tissues, particularly in wild *Musa* species, in contrast to the majority of fruit-focused research. This discrepancy is important for two reasons. Firstly, because of variations in genetic makeup and environmental adaptation, wild species may have different nutrient and phytochemical compositions from cultivated bananas (Pillai, 2024); and secondly, because the valorisation of non-fruit tissues may help achieve the objectives of circular bioeconomy and nutrition security by lowering biomass waste and enhancing local livelihoods. The contrast in the domestic widespread utilisation of these globally underutilised banana parts and the attempts at the global level to bring into vogue the sustainable utilisation of these, so called, biowaste products can be partly seen in the local marketplaces of Nagaland wherein, these plants parts, i.e., pseudostem and inflorescence of wild *Musa* species are readily and widely available for consumption as vegetables. These edible parts collected from the wild can belong to different *Musa* species, as there are at least four species whose pseudostem and inflorescence are consumed. These vegetables are not only a source

_of nutrition but also a source of income for the vendors and thereby an integral part of the local economy **(Figure 5.0).**

Figure 5.0: Photo plate depicting edible pseudostem and inflorescence of different wild *Musa* species being sold in the local market

The nutritional and functional potential of banana inflorescences has garnered significant attention in the last ten years. Banana blossoms are consistently found to contain a variety of minerals, particularly potassium, magnesium, calcium, and iron, as well as moderate protein and significant dietary fibre (Lau, 2020; Senevirathna, 2024).

Sustainable extraction and processing techniques have also received a lot of attention lately. According to Senevirathna (2024), ethanol and aqueous extracts of the

inflorescence have potent radical scavenging activity, indicating their potential for use in nutraceutical compositions. Comparative studies between cultivars and wild Asian species, meanwhile, show notable compositional heterogeneity, highlighting the impact of processing, environment, and genotype on nutritional profiles (Kumari *et al.,* 2023). The need for region-specific nutritional studies is strengthened by this variability, especially in understudied yet biodiversity-rich environments like Nagaland.

Banana pseudostem, which is frequently wasted after harvest, has received notice for its nutritional and industrial potential in parallel with inflorescence studies. Although water (85–90%) and fibre make up the majority of pseudostem tissue, it also contains significant amounts of proteins, minerals, and carbohydrates (Pillai, 2024). According to recent studies, pseudostem flour can be used in baked goods as a fibre-rich additive, and extracts have been found to have bioactive components with antimicrobial activity (Zou *et al.,* 2022).

Pseudostem juice contains antioxidants, including gentisic acid, catechin, ferulic acid, and protocatechuic acid. Consequently, it possesses the capability to address gallbladder stone formation and urinary system disorders. It facilitates the dissolution of calcium oxalate, which contributes to the formation of kidney stones (Sharma *et al.,* 2017). Pseudostem juice from both naturally and greenhouse-cultivated sources can block α-glucosidase and postpone carbohydrate digestion. This reduces the likelihood of hyperglycemia (Nguyen *et al.,* 2017). The ability of extracts from different parts of the banana plant to inhibit enzymes such as glucosidase and amylase, responsible for carbohydrate digestion, has garnered interest regarding their effects on type I and type II diabetes mellitus in the past decade (Silvestre *et al*., 2016). The phytochemicals found in the banana pseudostem and the silver nanoparticles derived from them, particularly phenols, flavonoids, and tannins, have been demonstrated to possess anticancer properties and exhibit antimicrobial activity (Kumari *et al*., 2021).

In the past decade, research has progressed beyond proximal analyses to valorisation processes. Pseudostem sap has been investigated as a natural preservative owing to its phenolic constituents (Anju and Geetha, 2019), and dried pseudostem powder has been integrated into nutritional supplements. Furthermore, advancements in fibre extraction and nanocellulose manufacturing render the pseudostem a biomass

of nutritional and industrial significance. This dual-purpose potential matches with sustainability objectives by reducing waste and fostering new revenue sources for agricultural communities.

Notwithstanding global advancements, Northeast India, specifically Nagaland, continues to be inadequately represented in nutritional research concerning banana inflorescence and pseudostem. Ethnobotanical literature indicates that Naga tribes incorporate banana flowers into their traditional diets and utilise pseudostem preparations in folk medicine for gastrointestinal relief and detoxification. However, no published research has rigorously examined the proximate composition, mineral profiles, or bioactive components of various tissues in indigenous wild *Musa* populations. The absence of empirical evidence is problematic for three reasons. Initially, the absence of compositional baselines renders the nutritional importance of wild *Musa* parts questionable, hence constraining their advocacy in dietary diversification initiatives. Secondly, Nagaland's distinctive agro-ecology, characterised by high precipitation, acidic soils, and forest-edge farming, may affect the accumulation of micronutrients and the content of secondary metabolites, which cannot be generalised from studies conducted in other regions. Third, unacknowledged nutritional value may result in the underutilization or erosion of indigenous knowledge of these food resources, hence compromising conservation and sustainable development initiatives.

Furthermore, with ongoing dietary transitions in Nagaland, where traditional food systems are being replaced by processed foods, there is an urgent need to document and valorise indigenous, nutrient-rich plants. Wild *Musa* inflorescences and pseudostems represent underexploited resources that could support both nutrition-sensitive agriculture and community-based enterprises, such as flour production, functional beverages, and herbal nutraceuticals.

Gaps in existing research

Despite extensive research over the past decade on the nutritional and functional attributes of banana inflorescence and pseudostems globally (Lau, 2020; Zou *et al*., 2022; Pillai, 2024; Senevirathna, 2024), three significant gaps remain:

Species and regional specificity — The majority of research focuses on commercial cultivars, including Cavendish (AAA) and cooking bananas (ABB). The nutritional and phytochemical characterization of wild species is still inadequate.

Methodological comparability – Variations in drying techniques, extraction solvents, and reporting metrics (fresh versus dry weight) complicate cross-study comparisons. Integration of indigenous expertise: Ethnobotanical practices are hardly correlated with laboratory data, undermining the application of findings into culturally relevant interventions. Addressing these deficiencies is especially crucial for Nagaland, where wild *Musa* species constitute a distinctive reservoir of genetic and nutritional diversity, as well as a very real source of nutrition and food security.

Materials and methods

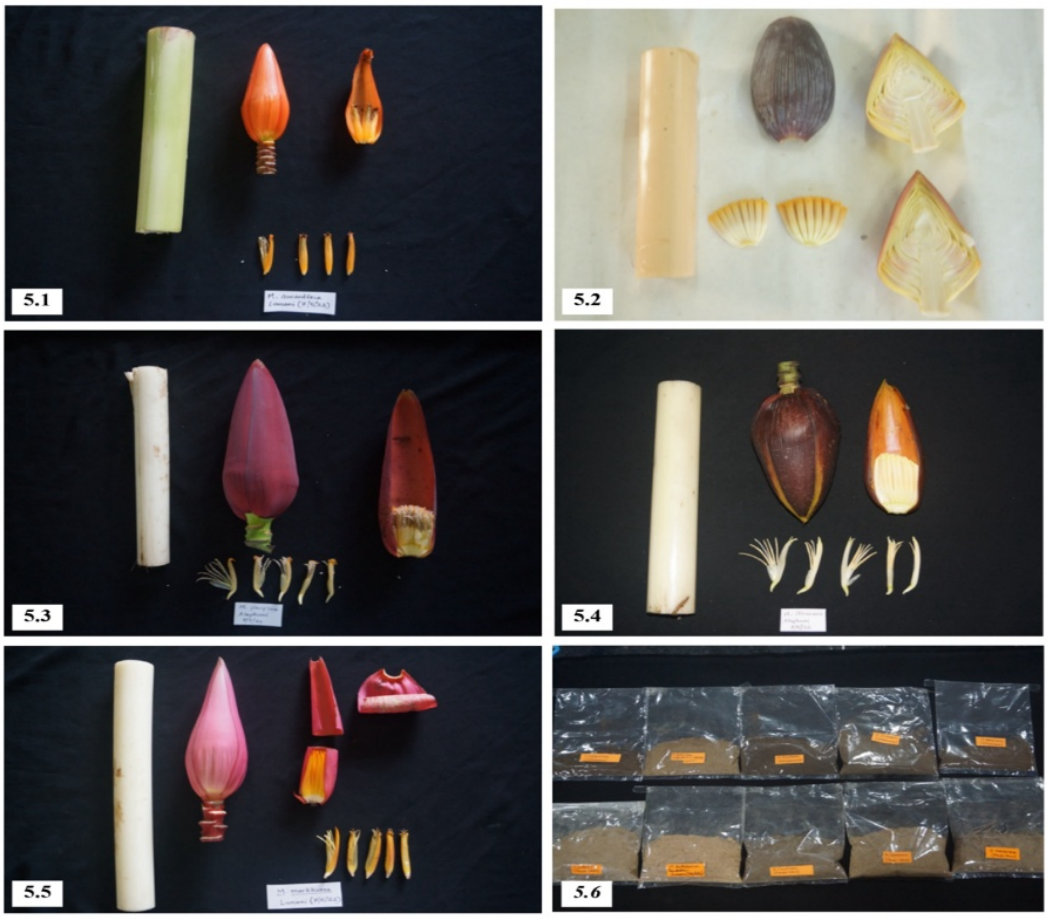
Sample collection

Five wild banana species, *Musa aurantiaca* Baker, *Musa balbisiana* Colla, *Musa flaviflora* N. W. Simmonds, *Musa itinerans* Cheesman, and *Musa markkuana*, were gathered from throughout the designated study area, placed in polythene bags, labelled, and brought to the lab. A previous study (Deb *et al.,* 2023) documented the taxonomic identification of the collected banana specimen.

Preparation of the sample

The samples were weighed, cut into small fragments, and dried in an oven for 30 hours at a temperature of 70°C. Following drying, the materials were weighed and ground into a powdery texture **(Figure 5.6).** The dried materials were extracted using 80% ethanol (v/v) (1 g of dry material in 10 ml of solvent) to produce the total ethanol extract (TE). A subsequent portion (1 g) of the dried material was repeatedly extracted with 80% methanol (10 ml) to yield the whole methanol extract. All resulting extracts were stored at −20°C until used.

The nutritional assessment of the collected samples includes the inflorescence and pseudostems of all five wild *Musa* samples. This analysis examined various parameters, including moisture content, crude fibre, total ash content, total carbohydrate content, reducing sugars, non-reducing sugars, protein content, total phenol and flavonoid content, antioxidant capacity, and vitamin A.



Figures 5.1–5.6: Inflorescence and pseudostem of *Musa aurantiaca* Baker, *Musa balbisiana* Colla, *Musa flaviflora* N. W. Simmonds, *Musa itinerans* Cheesman and *Musa markkuana* (M.Sabu, A.Joe & Sreej.) Hareesh, A.Joe & M.Sabu stat. nov; Figure 5.6 : Dried and ground samples

Proximal Analysis

Moisture content

The proximate content of moisture content in the samples was estimated following the standard methods established by the Association of Official Analytical Chemists (AOAC, 2019). The sample was dehydrated in an oven at 105°C until a stable weight was achieved, and the moisture content was assessed using the gravimetric method.

Total ash

The AOAC Method (2019) was implemented to ascertain the ash content. The silica crucible was initially heated in a furnace for one hour, and its initial weight was recorded after cooling in a desiccator until a consistent weight was achieved. The dry sample was subsequently heated in a muffle furnace at 550ºC for 6 h, with 2 g being deposited in a crucible. The desiccator was used to cool the sample, and its ultimate weight was recorded. The formula provided below was employed to determine the ash content.

Ash (%) = 100 x [Weight of ash / Weight of sample].

Crude fibre

Crude fibre analysis was conducted using the AOAC technique (2019). Petroleum ether was employed to extract the fat from the samples (2 g) at an initial vaporisation point of 35-38°C. After defatting, 2 g of the material was heated with 200 mL of H_2SO_4 for 30 min using bumping chips. The acid was subsequently removed by filtering and treating it with boiling water. The filtrate was subjected to a 30 minute boil with 200 mL of sodium hydroxide. Filtration and cleansing with 25 mL of boiling 1.25 M H_2SO_4, three 50 mL aliquots of water, and 25 mL of alcohol are carried out in the subsequent stage. The residue was heated at 130±2°C for 2 h in a pre-weighed ashing dish (W1). The dish was subjected to cooling in a desiccator, and measurements were taken (W_2). After cooling in a desiccator, the dish was reweighed (W3) and incinerated for an additional 30 minutes at 600±15°C. The formula presented below was used to determine the crude fibre.

$$\textbf{\% crude fibre in ground sample} = \frac{\textbf{Loss in weight on ignition } (W_2 - W_1) - (W_3 - W_1)}{\textbf{Weight of the sample}} \textbf{x100}$$

Total carbohydrate

The total carbohydrate was quantified using the Anthrone method (Hodge and Hofreiter, 1962). Measure 100 mg of the sample into a boiling tube. Hydrolyse by maintaining it in a boiling water bath for 3 hours with 5 mL of 2.5 N HCl, then allow

it to cool to room temperature. Neutralise it with solid sodium carbonate till the effervescence terminates. Adjust the volume to 100 mL and centrifuge. Obtain the supernatant and prepare 0.5 mL and 1 mL aliquots. Prepare the standards by measuring 0, 0.2, 0.4, 0.6, 0.8, and 1 mL of the working standard. '0' functions as a blank. Adjust the volume to 1 mL in all tubes, including the sample tubes, by adding distilled water. Add 4 mL of anthrone reagent. Heat for 8 min in a boiling water bath. Cool rapidly and measure the green to dark green at 630nm. Construct a standard graph by plotting the concentration of the standard on the X-axis against absorbance on the Y-axis. Determine the quantity of carbohydrates in the sample tube by plotting it against the standard.

Reducing sugar

The quantification of reducing sugar was conducted using the Dinitrosalicylic acid (DNS) reagent (Miller, 1972). Dispense 0.5 to 2 mL of the extract into test tubes and dilute each tube to a total volume of 3 mL with distilled water. Add 3 mL of DNS reagent. Immerse the materials in boiling water for 5 minutes. Introduce 1 mL of 40% Rochelle salt solution into the tubes while preserving their heated condition. Measure the intensity of the dark red colour at 510 nm. Conduct a series of standards utilising glucose (0-500µg) and generate a graph. Estimate the reducing sugar content in the samples by plotting the sample against the standard graph.

Non-reducing Sugar

The quantity of reducing sugar was determined by subtracting the amount of reducing sugar from the total carbohydrate.

DPPH radical scavenging activity

The scavenging activity of 2,2-diphenyl-2-picrylhydrazyl (DPPH) was evaluated following the methodology of Brand-Williams *et al*. (1995) with modifications. The reaction commenced with the incorporation of 5 mL of diluted supernatant and 0.5 mL of 1 mM DPPH in methanol. The absorbance at 517 nm was promptly recorded at 0 minutes, followed by a 10 minute period in darkness for the

combination. The ability to neutralise the DPPH free radical was evaluated using the equation.

DPPH free radical scavenging activity (%) = [(A0 – A10) / A0] × 100

Where A0 is the absorbance of the sample at 0 min and A10 is the absorbance of the sample at 10 min.

Total phenolic content

The same extract from the total antioxidant assay was utilised to quantify the total phenols (TP) in the sample. The total phenolic content was assessed using the method developed by Slinkard and Singleton (1977). The procedure entails combining 0.5 mL of the supernatant with 0.5 mL of water, followed by the addition of 1 mL of 50% (v/v) Folin-Ciocalteu reagent solution and 2 mL of saturated Na_2CO_3 solution. The solution was permitted to incubate at ambient temperature for 30 minutes prior to measuring the absorbance at 750 nm. The results were presented as grams of gallic acid per 100 grams of dry weight (g GA/100g DW).

Total flavonoid content

The same extract from the total antioxidant assay was employed to determine the total flavonoids (TF) concentration in the sample as well. The TF content was evaluated using the assay formulated by Zhishen *et al.* (1999). The reaction commenced with the introduction of 0.25 mL of the supernatant into a mixture of 1.25 mL of water and 75 µL of 0.5% $NaNO_3$, followed by a 6-minute incubation at ambient temperature. 150 µL of 10% $AlCl_3$, $6H_2O$ was incorporated into the mixture and permitted to rest for 5 min. A 0.5 mL aliquot of 1 M NaOH was subsequently added, and the absorbance at 510 nm was measured. The results were presented as grams of quercetin per 100 g of dry weight (g quercetin/100 g DW).

Statistical analysis

All data are presented as mean ± standard deviation (n=3). All data derived from the nutritional and phytochemical examinations of the pseudostem and inflorescence of wild *Musa* species underwent thorough statistical and graphical assessment to guarantee accurate interpretation of compositional variations. The

experimental results were gathered into raw data, and bar graphs were generated using Microsoft Excel 2019. The aggregated data was subsequently imported into Python. Descriptive statistics, encompassing means, standard deviations, and coefficients of variation, were computed to encapsulate the central tendency and dispersion of the nutritional parameters in both plant parts. A correlation matrix was created with Pearson's correlation coefficients and illustrated via a heatmap produced with Seaborn's heatmap function.

Results

This section delineates parameter-specific results from the nutritional analyses performed on wild *Musa* species gathered in Nagaland. The dataset included ten samples: five inflorescence samples and five pseudostem samples from five species: *Musa aurantiaca* Baker, *Musa balbisiana* Colla, *Musa flaviflora* N. W. Simmonds, *Musa itinerans* Cheesman, and *Musa markkuana* (M. Sabu, A. Joe & Sreej.) Hareesh, A. Joe & M. Sabu stat. nov. Descriptive statistics for each parameter, along with the species and plant component corresponding to the observed lowest and maximum values, as well as the outcomes of between-group comparisons (inflorescence vs. pseudostem), are presented. The proximate content representing the nutritive components of the inflorescence and pseudostem extracts is depicted in **Table 5.1**.

Table 5.1: Proximate nutritive values of Wild banana inflorescence and pseudostem

Musa species	Plant part	Moisture content (%)	Crude fibre (% in dry wt.)	Total ash (% in dry wt.)	Total carbohydrate (g/100g DW)	Reducing sugar (g/100g DW)	Non-Reducing sugar	Total phenol (g/100g)	Total flavonoid (g/100g)	DPPH Scavenging activity (%)
M. aurantiaca	Inflorescence	92.22 ±0.12	27.45 ±0.11	15.2 ±0.18	10.87 ±0.33	2.47 ±0.29	8.4 ±0.25	1.402 ±0.034	0.110 ±0.032	39.99 ±0.25
	Pseudo-stem	92.20 ±0.41	30.70 ±0.32	11.2 ±0.29	36.67 ±0.21	2.39 ±0.35	34.28 ±0.31	0.164 ±0.045	0.225 ±0.013	6.34 ±0.32
M. balbisiana	Inflorescence	86.89 ±0.21	13.65 ±0.21	9.15 ±0.26	11.68 ±0.34	4.21 ±0.40	7.47 ±0.38	0.380 ±0.032	1.486 ±0.02	8.83 ±0.34
	Pseudo-stem	92.72 ±0.24	21.70±0.24	5.85±0.30	20.08 ±0.27	4.24 ±0.34	15.84 ±0.25	1.573 ±0.032	1.839 ±0.047	40.33 ±2.5
M. flaviflora	Inflorescence	90.18 ±0.25	13.10 ±0.25	13.05 ±0.2	10.23 ±0.28	2.47 ±0.22	7.76 ±0.34	1.146 ±0.023	0.326 ±0.038	13.45 ±0.34
	Pseudo-stem	93.44 ±0.22	28.05 ±0.35	12.15 ±0.38	13.39 ±0.12	3.03 ±0.19	10.36 ±0.18	1.557 ±0.013	1.755 ±0.043	45.42 ±1.45
M. itinerans	Inflorescence	89.91 ±0.35	18.45 ±0.35	14.15 ±0.40	9.90 ±0.43	3.09 ±0.44	6.81 ±0.34	1.681 ±0.043	1.429 ±0.028	7.35 ±0.34
	Pseudo-stem	94.44±0.32	34.05 ±0.41	12.7 ±0.48	29.82 ±0.39	2.86. ±034	26.96 ±0.43	1.548 ±0.036	1.596 ±0.027	46.08 ±0.32
M. markkuana	Inflorescence	90.79 ±0.27	23.40 ±0.31	14.5 ±0.4	5.80 ±0.25	3.07 ±0.33	2.73 ±0.32	1.608 ±0.042	1.377 ±0.041	3.15 ±0.18
	Pseudo-stem	94.20 ±0.38	29.65 ±0.38	16.7 ±0.35	27.62 ±0.36	7.57 ±0.29	20.05 ±0.27	1.571 ±0.036	1.148 ±0.043	25.4 ±0.5

Moisture content

The data indicate that *Musa* possesses a significant moisture content. The average moisture content (%) for all samples was 91.699 (SD = 2.3052), with a range from 86.89 (lowest) to 94.44 (highest). Pseudostem samples had a markedly greater moisture content compared to inflorescence samples, with a mean moisture content of 93.400% for pseudostems and 89.998% for inflorescences. The minimum moisture content (%) recorded was 86.89, noted in the inflorescence of *Musa balbisiana* Colla, while the pseudostem of *Musa itinerans* Cheesman possesses the highest moisture content at 94.44%, rendering this specimen the highest overall **(Figure 5.7).**

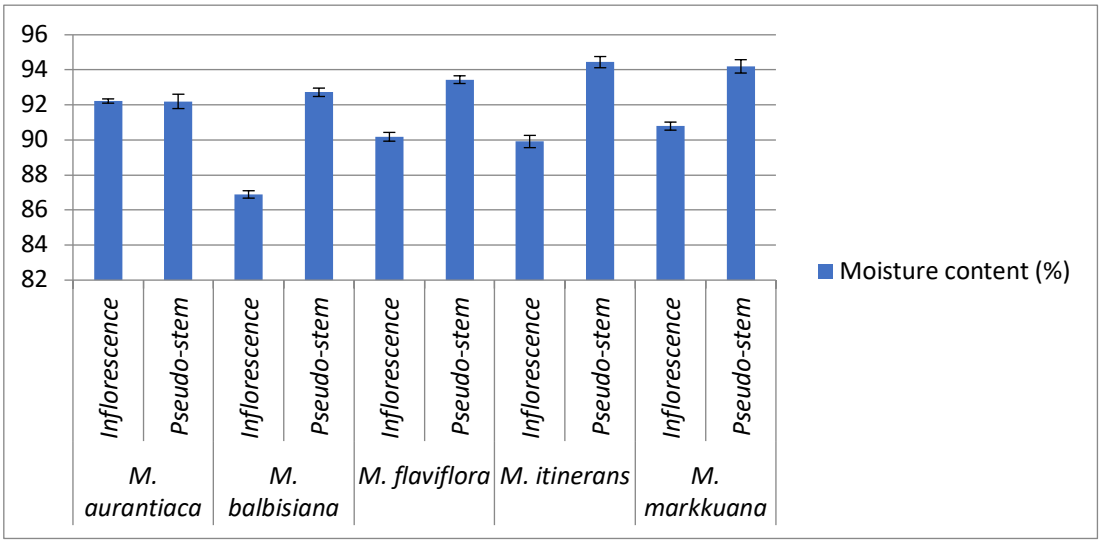


Figure 5.7: Bar graph depicting moisture content in the selected wild *Musa* species.

Crude fibre content

The crude fibre content in pseudostem samples was much greater than that in inflorescence, with mean values of approximately 28.830% for pseudostem and 19.210% for inflorescence. The specimen with the highest fibre content is the *Musa itinerans* Cheesman pseudostem with 34.05%, and the lowest fibre content is *Musa flaviflora* N. W. Simmonds inflorescence with 13.10% of fibre in it as seen in **Figure 5.8.**

Total ash content

The edible inflorescences of *Musa aurantiaca* Baker, *Musa balbisiana* Colla, *Musa flaviflora* N. W. Simmonds and *Musa itinerans* Cheesman exhibit a higher ash content than their pseudostems, indicating a greater mineral presence, with their mean value standing at 12.47%. Contrary to this trend, *Musa markkuana* (M.Sabu, A.Joe & Sreej.) Hareesh, A.Joe & M.Sabu stat. nov. pseudostem contains a higher ash percentage than its inflorescence, suggesting that the pseudostem possesses more minerals. The pseudostem of *Musa markkuana* (M.Sabu, A.Joe & Sreej.) Hareesh, A.Joe & M.Sabu stat. nov. contains the highest ash content of 16.7%, whereas the pseudostem of *Musa balbisiana* Colla has the lowest ash content of 5.85% as depicted in **Figure 5.8.**

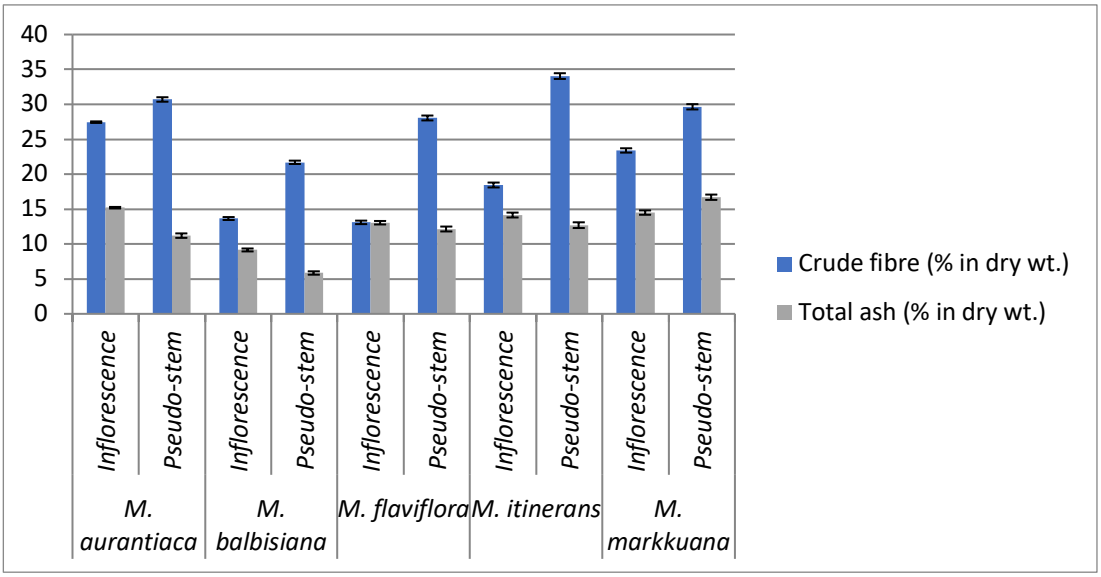


Figure 5.8: Bar graph depicting crude fibre and total ash content in the selected wild *Musa* species.

Total carbohydrate content

The edible part of the pseudostem of all the species has more carbohydrate than the edible part of the inflorescence: *Musa aurantiaca* Baker pseudostem with 36.67 g/100 g DW and inflorescence with 10.87 g/100 g DW, *Musa balbisiana* Colla pseudostem with 20.08 g/100 g DW and inflorescence with 11.68 g/100 g DW, *Musa flaviflora* N. W. Simmonds pseudostem with 13.39 g/100 g DW and inflorescence with 10.23 g/100 g DW, *Musa itinerans* Cheesman pseudostem with 29.82 g/100 g DW and inflorescence with 9.90 g/100 g DW, *Musa markkuana* (M.Sabu, A.Joe & Sreej.) Hareesh, A.Joe & M.Sabu stat. nov. with 27.62 g/100 g DW and inflorescence with 5.80 g/100 g DW. The pseudostem of *Musa aurantiaca* Baker has the highest carbohydrate content with 36.67 g/100 g DW. The total carbohydrate content in pseudostem samples was substantially higher (mean = 25.516 g/100 g DW) compared to inflorescences (mean ≈ 9.696 g/100 g DW) **(Figure 5.9)**.

Reducing and non-reducing sugar content

All the *Musa* species have less reducing sugar, with *Musa aurantiaca* Baker pseudo-stem exhibiting the lowest value at 2.39 g/100g DW and *Musa markkuana* (M.Sabu, A.Joe & Sreej.) Hareesh, A.Joe & M.Sabu stat. nov. pseudo-stem exhibiting the highest value at 7.57 g/100g DW. Overall, the mean reducing sugar (g/100g DW) across all samples was 3.54 (SD = 1.5613); the observed range was 2.39 (minimum) to 7.57 (maximum) with no significant part-wise or species-specific distribution. The part-specific means are as follows: Inflorescence = 2.574 g/100 g DW; Pseudostem = 4.507 g/100 g DW.

The pseudostem contains a higher amount of non-reducing sugars than the inflorescence. The *Musa aurantiaca* Baker pseudostem has the highest non-reducing sugar value at 34.28 g/100g DW and the lowest was observed in *Musa markkuana* (M.Sabu, A.Joe & Sreej.) Hareesh, A.Joe & M.Sabu stat. nov. inflorescence at 2.73 g/100g DW. The average Non-Reducing sugar across all samples was 14.066 g/100g DW (SD = 10.1329), with a range from 2.73 g/100g DW (minimum) to 34.28 g/100g DW (highest). Part-specific means are as follows: Inflorescence = 7.121 g/100 g DW**;** pseudostem = 21.009 g/100 g DW **(Figure 5.9)**.

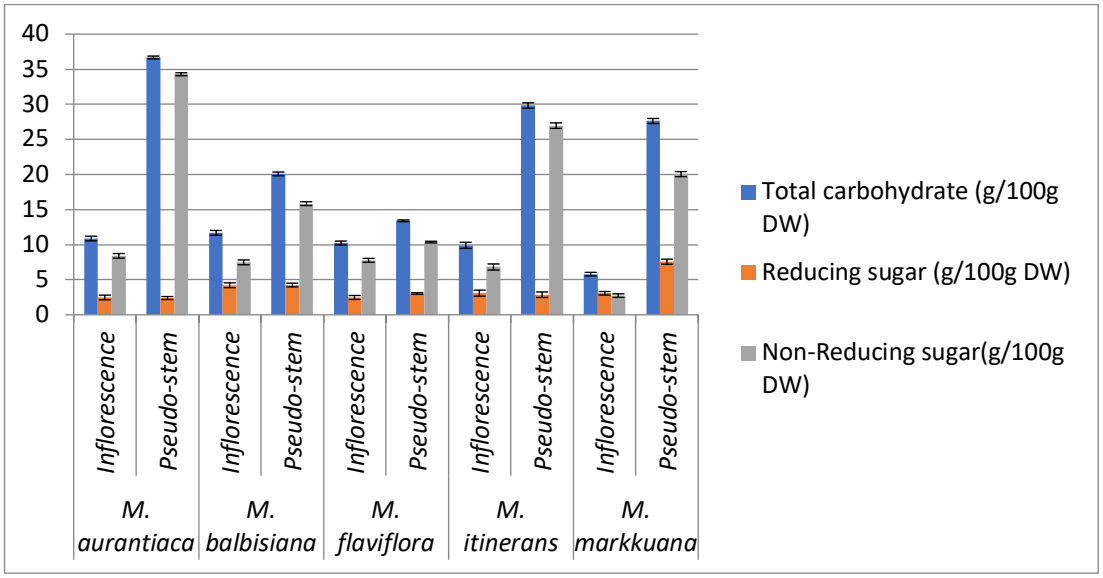


Figure 5.9: Bar graph depicting total carbohydrate, reducing and non-reducing sugar content in the selected wild *Musa* species

Total phenol content

The average total phenol (g/100g) across all samples was 1.263 g/100g DW (SD = 0.545), with a range from 0.164 g/100g DW (lowest) to 1.681 (highest). The lowest value for total phenol (g/100g DW) was 0.164, recorded in the pseudostem of *Musa aurantiaca* Baker. The highest concentration of total phenol (g/100g DW) was 1.681, recorded in the inflorescence of *Musa itinerans* Cheesman. The part-specific means are as follows: Inflorescence = 1.243 g/100 g DW; Pseudostem = 1.283 g/100 g DW **(Figure 5.10)**.

Total flavonoid content

The mean overall total flavonoid content across all samples was 1.1291 (SD = 0.6578) with an observed range of minimum 0.11 g/100 g DW and maximum 1.839 g/100 g DW. The total flavonoid content was highest in the pseudostem of *Musa balbisiana* Colla at 1.839 g/100 g DW and lowest in the inflorescence of *Musa aurantiaca* Baker at 0.11 g/100 g DW. The flavonoid content in the pseudostem across all five species scored a higher mean value of 1.313 g/100 g DW, and the inflorescence scored 0.946 g/100 g DW **(Figure 5.10)**.

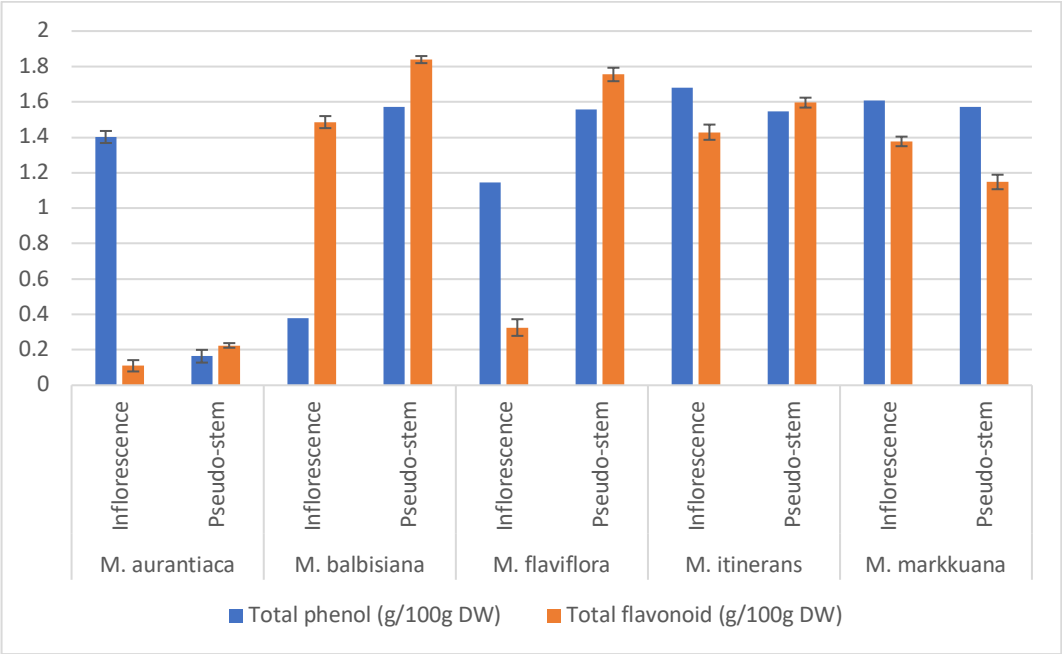


Figure 5.10: Bar graph depicting total phenol and total flavonoid in the selected wild *Musa* species.

DPPH Scavenging Activity

Scavenging activity among the samples showed a similar trend apart from *Musa aurantiaca* Baker where scavenging activity was higher in the inflorescence (39.99%) than the pseudostem (6.34%**),** unlike all the other species. The mean values expressed in percentage showed pseudostems with a value of 32.714% compared to the inflorescences at 14.554%. The minimum DPPH scavenging activity (%) recorded was 3.15%, reported in the inflorescence of *Musa markkuana* (M. Sabu, A. Joe & Sreej.) Hareesh, A. Joe & M. Sabu stat. nov. The peak DPPH scavenging activity (%)recorded was 46.08%, reported in the pseudostem of *Musa itinerans* Cheesman **(Figure 5.11)**.

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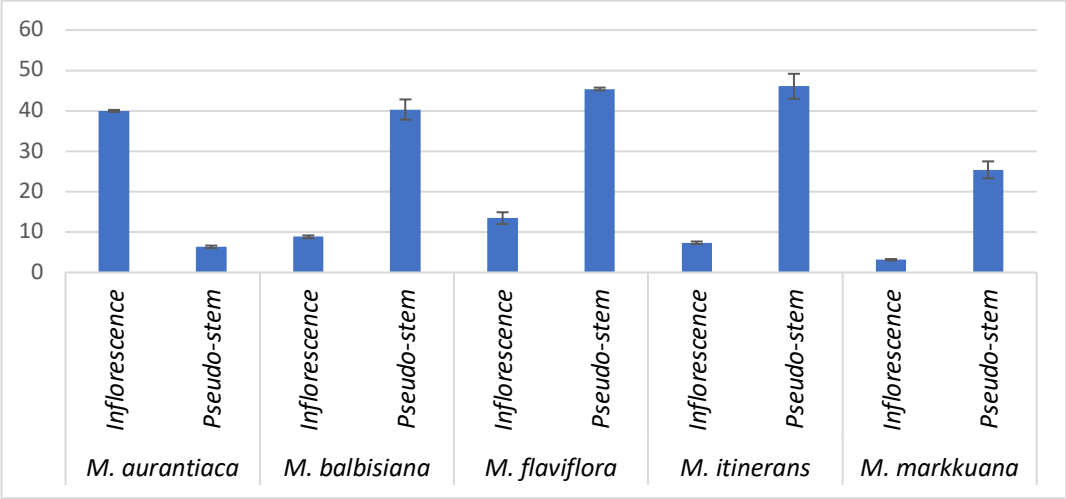

Figure 5.11: Bar graph depicting DPPH scavenging activity (%) in the selected wild *Musa* species.

Correlation studies

Correlation studies across different parameters show various parameters with strong positive links as well as negative relations:

- In inflorescence, crude fibre correlated positively with antioxidant activity (r = 0.56) and with total ash (r = 0.75) and showed a moderate positive correlation with total phenol (r = 0.60)
- Several parameters also show a strong positive correlation with antioxidant activity. Moisture content showed the highest correlation to antioxidant activity (r = 0.693), followed by crude fibre (r = 0.514). Total phenol and flavonoid content is also seen to positively correlate with antioxidant activity at r values of 0.462 and 0.266 (moderate), respectively.
- Moisture is strongly associated with fibre (r = 0.857) and moderately with carbohydrate (r = 0.575) and non-reducing sugar (r = 0.559).
- Carbohydrate is tightly coupled with non-reducing sugar with r = 0.989.
- Total ash shows a weak and slightly negative correlation with antioxidant activity, with r value at -0.127.

Given the small sample size, this model is regarded as exploratory and hypothesis generating rather than confirmatory.

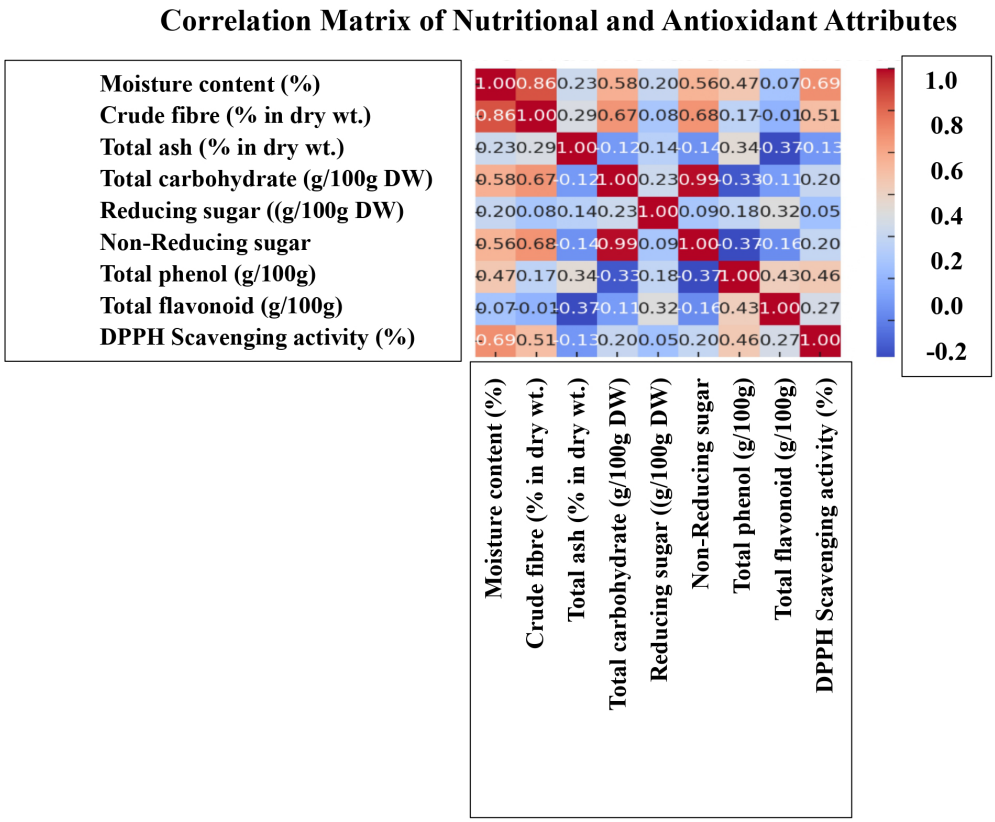

Figure 5.12: Correlation matrix heatmap representing parameter correlations

Discussion

The five *Musa* species examined were selected due to their accessibility and palatability. *Musa balbisiana* Colla, while edible, is often less preferred among edible *Musa* species compared to *Musa flaviflora* N. W. Simmonds and *Musa itinerans* Cheesman, whose pseudostems and inflorescences are the most popularly consumed species. *Musa aurantiaca* Baker, while edible and extensively spread, is not as widely consumed. *Musa markkkuana* (M. Sabu, A. Joe & Sreej.) Hareesh, A. Joe & M. Sabu stat. nov, conversely, is not utilised due to its overall rarity in availability and distribution in the wild.

Moisture content

The pseudostem tissue in the examined wild *Musa* specimens consistently exhibited a higher water content than the inflorescence. The observed difference is biologically justifiable due to the structural function of the pseudostem as a water-laden, turgid organ, and it holds analytical significance as moisture affects extraction yields of hydrophilic phytochemicals and may distort comparisons based on fresh weight (Zou *et al.,* 2022). The robust association between moisture and antioxidants seen subsequently (r = 0.693) implies that moisture-related extraction dynamics may partially elucidate the antioxidant effects obtained from fresh or wet extracts.

Total fibre content

Pseudostem samples exhibit a significantly higher fibre content than inflorescences, with a mean difference of around 9.62 percentage points, aligning with the established composition of banana pseudostem as a cellulosic and ligno-cellulosic tissue (Pillai, 2024; Zou *et al*., 2022). The elevated fibre content in the pseudostem enhances its viability as a raw material for fibre-enriched food products or for fibre extraction in industrial applications.

Total ash content

Ash content, reflecting the total mineral load, exhibited variability among samples but did not demonstrate a distinct influence based on plant parts in this dataset. The small to medium effect size indicates a slight inclination for the pseudostem to possess elevated mineral residue, corroborating certain studies that suggest the pseudostem can concentrate specific minerals (Kumari *et al*., 2023). However, comprehensive sampling and element-specific analyses are necessary for conclusive results. Another indication, on point, is the species-specific concentration of minerals on different plant parts.

Total carbohydrate content

Pseudostem samples exhibited much higher carbohydrate content on a dry-weight basis compared to inflorescences. The substantial and statistically significant disparity indicates that pseudostem is a superior candidate where carbohydrate content

is a sought-after functional characteristic (e.g., bulking agents, flours). The pseudostem's markedly elevated carbohydrate and fibre levels correspond with the findings of Zou *et al*. (2022) and Pillai (2024), who identified banana pseudostem as a potential fibre source for nutraceutical and feed applications. Kumari *et al.* (2023) have documented similar fibre levels for Northeast Indian *Musa* cultivars. This pattern also indicates carbohydrate allocation to the pseudostem and endorses its application for flour production and fermentative valorisation (Zou *et al*., 2022).

Reducing and non-reducing sugar content

No statistically significant difference was detected in the reducing sugar concentration among the various plant parts. The limited sample number and diversity necessitate cautious interpretation; however, the pseudostem exhibited a larger mean reducing sugar concentration, potentially indicating greater overall carbohydrate reserves. Non-reducing sugars were markedly elevated in the pseudostem, aligning with the trend noted for total carbohydrate content. This substantiates the assertion that pseudostem tissues possess a greater quantity of storage carbohydrates compared to inflorescences, indicating a distinct carbohydrate partitioning between organs.

Total phenolic content

The phenolic content was statistically comparable across the various plant parts in this dataset. This finding reveals that both the inflorescence and pseudostem of the examined wild *Musa* have similar concentrations of total phenolics. Literature presents inconsistent findings: certain research indicates elevated phenolic content in flowers (Lau, 2020), while others reveal significant phenolics in pseudostem extracts contingent upon the extraction method employed (Senevirathna, 2024; Zou *et al.,* 2022). The observed similarity indicates that, for these wild taxa from Nagaland, phenolic synthesis may be allocated among several organs or that the extraction methods effectively collected both free and bound phenolics comparably. The total phenol values of 1.681 g/100 g DW align with the literature ranges for banana inflorescences and pseudostem derivatives. Schmidt *et al.* (2015) observed that Cavendish inflorescences contain approximately 1690 mg GAE/100 g DW (oven-dried, 50% ethanol extraction), while recent studies on fresh inflorescences indicated

a range of 295-1239 mg GAE/100 g DW, contingent upon cultivar and extraction method. The independent ranges corroborate the reported scale and indicate that the extraction solvent, drying process, and cultivar substantially affect absolute levels. Schmidt *et al.* (2015)

Total flavonoid content

Despite the pseudostem exhibiting a greater mean flavonoid concentration, the difference lacked statistical significance. The moderate effect size indicates a potential biological difference that might be validated with a bigger sample size. Flavonoids enhance antioxidant activity and can exist in both soluble and wall-bound forms; the selection of procedure (hydrolysis, solvent polarity) significantly affects the observed values (Lau, 2020). Concurrently, the comparable quantities of phenolics and flavonoids indicate that the inflorescence possesses antioxidant potential, aligning with the findings of Senevirathna (2024) and Lau (2020), who emphasised that floral extracts are abundant in phenolics.

DPPH antioxidant activity

Although the pseudostem demonstrated a DPPH activity exceeding that of the inflorescence by more than twofold and exhibited a considerable effect size, the difference did not attain statistical significance, potentially due to a restricted sample size and intra-group variability. DPPH had a positive correlation with multiple variables: most significantly with moisture ($r = 0.6931$), followed by crude fibre ($r = 0.5144$) and total phenol ($r = 0.4617$). The favourable correlation between moisture and DPPH may indicate extraction circumstances (hydrophilic antioxidants are more readily extractable from fresher, moister material), co-variation between moisture and extractable phenolics in pseudostem, or the existence of water-soluble antioxidant molecules. The moderate connection between phenol and DPPH corroborates the anticipated relationship between phenolic content and radical scavenging capacity, while also suggesting that additional constituents (flavonoids, non-phenolic antioxidants, or matrix effects) influence the observed activity (Lau, 2020; Zou *et al.,* 2022).

Correlation studies

The moisture–DPPH correlation (r = 0.69) may indicate extraction effects in fresh-weight experiments (Zou *et al*., 2022). Increased moisture can improve the recovery of hydrophilic antioxidants, elucidating the pseudostem's elevated mean DPPH despite comparable phenolic contents. The limited sample size (n = 5/group) constrains statistical power; yet, the effect sizes indicate biologically significant differences. Pseudostem samples exhibited superior nutritional and antioxidant profiles compared to inflorescences, however, not all differences reached statistical significance. The superior antioxidant activity in pseudostems compared to inflorescences in most species corresponds with findings that structural tissues with elevated fibre content and bound phenolics exhibit enhanced radical-scavenging capacity following appropriate extraction. Our stratified correlations substantiate this mechanism: in inflorescences, DPPH exhibited a moderate positive correlation with crude fibre (r = 0.56) and total phenols (r = 0.60), aligning with the notion that fibre-bound or co-occurring phenolic matrices enhance the antioxidant signal.

The pseudostems of *Musa itinerans* Cheesman and *Musa flaviflora* N. W. Simmonds emerge as promising antioxidant sources for ingredient development, particularly when combined with extraction methods that focus on both bound and free phenolics. Inflorescences retain their value but exhibit greater variability, where cultivar selection and process optimisation are likely critical for achieving maximum yields.

Summary and Conclusions

The nutritional evaluation of the *Musa* species provides an in-depth analysis of their nutritional worth, enabling an assessment of the dietary intake of the local population and expanding the potential for the consumption of additional wild *Musa* species. The five selected species were assessed across nine different nutritional parameters. Assessments were carried out for moisture content, crude fibre content, ash content, total carbohydrate content, reducing and non-reducing sugar content, phenol and flavonoid content, and finally DPPH antioxidant activity of the edible parts

of the pseudostem and inflorescence. This study forms a baseline study into the prospect of these selected species. The samples of wild *Musa* demonstrate distinct differences among plant parts: the pseudostem has significantly higher levels of moisture, crude fibre, and carbohydrates compared to the inflorescence, and generally exhibits stronger mean antioxidant activity. Correlation analysis identifies moisture and flavonoids as predictors of DPPH activity in these samples. The findings support global evaluations of pseudostem as a fibre-rich, valuable by-product, while emphasising the necessity for extensive, methodologically standardised investigations to thoroughly characterise the nutritional value of wild *Musa* in Nagaland. The pseudostem of wild *Musa* in Nagaland demonstrates significant promise as a fibre and carbohydrate-rich resource with promising antioxidant properties, positioning it as a viable candidate for value addition in the form of flour, fibre concentrates, or functional food ingredients. The inflorescence maintains significant phenolic content and may be investigated for nutraceutical extracts that require flower-specific components.

Chapter - 6

Genetic Diversity Studies of Some Selected Wild *Musa* Species of Nagaland

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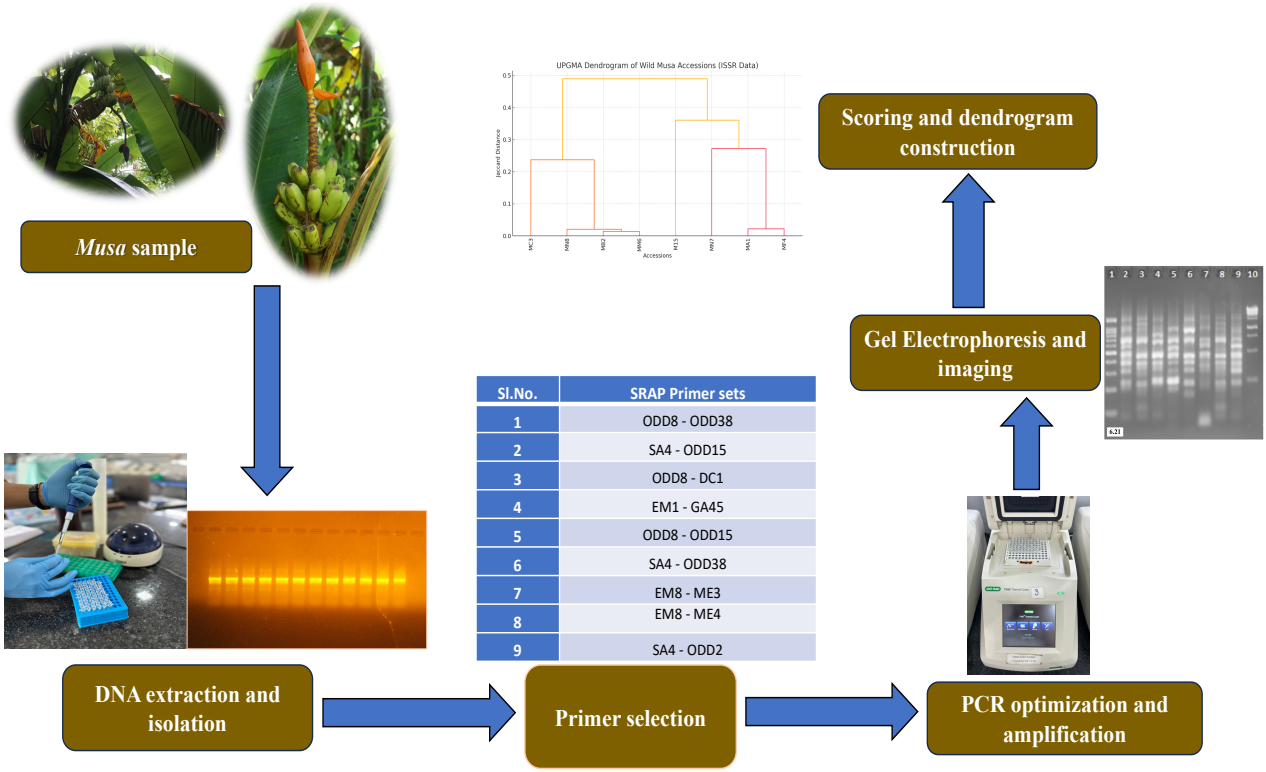
Graphical Summary



Introduction

Bananas and plantains (*Musa* spp.) are among the main fruit crops globally, providing sustenance and revenue for hundreds of millions in the humid tropics. The global banana business relies on a severely limited genetic foundation mostly drawn from two wild progenitors, *Musa acuminata* (A genome) and *Musa balbisiana* (B genome), whose ancient hybridisations, followed by polyploidization and clonal propagation, produced the current triploid cultivars. Centuries of vegetative propagation have resulted in agronomic uniformity while limiting genetic recombination, rendering cultivated bananas very vulnerable to diseases and climate stress (Drenth and Kema, 2021). The catastrophic proliferation of Fusarium wilt Tropical Race 4 and Black Sigatoka shows how restricted allelic variation compromises resilience. Wild *Musa* species, meanwhile, maintain unexploited stores of genetic diversity that include resistance genes, abiotic stress tolerance, and developmental characteristics vital for breeding and long-term sustainability.

Comprehending and protecting this diversity necessitates molecular methodologies that may reveal concealed variations beyond morphology, which is notoriously variable in *Musa*. Genetic diversity, defined as the totality of variations in DNA sequences within and between populations, arises from mutation, recombination, and gene flow, while its preservation is influenced by selection, genetic drift, and demographic history (Frankham *et al.,* 2010). Diversity patterns in clonally propagated crops illustrate sporadic sexual reproduction overlaying widespread clonal dissemination; allelic innovations produced during a few meiotic occurrences may endure for millennia if integrated into viable lineages. Thus, evaluating variance in wild relatives provides insights into evolutionary potential and guides conservation and enhancement initiatives.

Molecular markers have transformed diversity analysis in the last thirty years, facilitating genome-wide sampling independent of prior phenotypic knowledge. Inter-simple sequence repeats (ISSR) and sequence-related amplified polymorphisms (SRAP) are particularly effective multilocus systems in non-model taxa with limited genetic resources. ISSR markers amplify interspersed areas between microsatellite repeats utilising anchored primers, yielding consistent multilocus fingerprints that

reflect genome-wide heterogeneity (Zietkiewicz *et al.,* 1994; Reddy *et al*., 2002). In contrast, SRAP markers utilise primer pairs specifically crafted to preferentially amplify open reading frames, hence enriching genic areas where polymorphisms may contribute to adaptive features (Li and Quiros, 2001). When utilised together, ISSR and SRAP offer complementary insights into neutral and functional variation, respectively.

The Indo-Burma region, acknowledged as a significant biodiversity hotspot, possesses a remarkable *Musa* variety. Nagaland, located centrally and intersecting the Eastern Himalayan corridor, displays strong biological gradients from tropical valleys to temperate mountains, supporting various habitats for wild bananas. Floristic surveys document species including *Musa balbisiana, Musa sikkimensis, Musa nagensium, Musa laterita, Musa mannii,* and the newly identified *Musa nagalandiana* (Dey and Gogoi, 2014). These taxa exhibit significant variation in pseudostem pigmentation, bract colouration, fruit indumentum, and orientation, characteristics traditionally employed in classification but frequently complicated by hybridisation and environmental plasticity. Molecular characterisation is essential for defining species, assessing genomic organisation, and finding evolutionarily relevant units in this inadequately studied area.

ISSR technology is based on the prevalence and variability of microsatellites—short tandem repeats susceptible to replication slippage. Anchored primers (e.g., $(GA)_8YC$, $(AG)_8YT$) hybridise to complementary sequences; amplification transpires when two repeats are at a suitable distance, producing different bands. Each band signifies a locus evaluated for presence (1) or absence (0), producing multilocus genotypes appropriate for diversity metrics including percentage polymorphic loci (PPL), Nei's gene diversity (H), and Shannon's information index (I). ISSR markers are esteemed for their superior reproducibility compared to RAPD, minimal DNA needs, and ability to identify significant variability in both coding and non-coding regions. In *Musa*, researchers have clarified genome-group linkages, confirmed cultivar identities, evaluated somaclonal variation, and disclosed regional structure in wild accessions (Pachuau *et al*., 2014; Atom *et al.,* 2017).

The SRAP markers were developed to connect anonymous fingerprinting with functional genomics. GC-rich forward primers (e.g., Me1–Me10) preferentially anneal

to exonic regions, while AT-rich reverse primers (Em1–Em10) target promoters or introns, resulting in fragments primarily derived from open reading frames (Li and Quiros, 2001). Due to the influence of selection in these locations, SRAP polymorphisms may be associated with adaptive divergence. The system's simple primer design, elevated multiplex ratio, and codominant-like characteristics in some settings augment its attractiveness. In *Musa*, SRAP analyses have more distinctly discriminated closely related cultivars and clarified genome-group boundaries more precisely than ISSR alone (Robarts and Wolfe, 2014). Crucially, SRAP data can be amalgamated with functional annotations upon the availability of reference genomes, thereby associating fragment polymorphism with candidate genes.

Soraisham *et al.* (2025) conducted a crucial regional study utilising ISSR and SRAP to examine twenty-eight *Musa* accessions from Northeast India, encompassing the AAA, AAB, ABB, AB, and BB genomic groups. Polymorphism rates of 90% were recorded for both techniques, with SRAP providing superior cluster resolution. Bayesian structural analysis identified five primary groups aligned with cytological classifications, confirming the synergistic efficacy of ISSR (neutral, genome-wide) and SRAP (gene-associated) data. These findings highlight the effectiveness of dual-marker frameworks in elucidating intricate evolutionary links within *Musa*.

Worldwide, ISSR research has revealed diversity patterns in both wild and farmed bananas. Reddy *et al.* (2002) exhibited significant inter-group polymorphism among South Indian cultivars; Pachuau *et al*. (2014) utilised ISSR and IRAP to categorise Northeast Indian accessions, attaining consistency with morphological genome classifications; Atom *et al.* (2017) assessed differentiation among A-, B-, and AB-genome groups, presenting Gst values that reflect considerable inter-group structure. In other regions, ISSR analysis of Southeast Asian *Musa acuminata* subspecies identified domesticated lineages and gene flow pathways (Li *et al.,* 2013). These experiments collectively demonstrate ISSR's efficacy in distinguishing both interspecific and intraspecific variation in *Musa*.

The concurrent advancement of SRAP has substantiated these conclusions. Robarts and Wolfe (2014) emphasised the extensive applicability of SRAP across several plant taxa, whereas in *Musa*, its gene-targeted approach has enabled the identification of polymorphisms associated with agronomic features. Comparative

analyses indicate that SRAP frequently exceeds RAPD and ISSR in resolving power among closely related genotypes, due to its emphasis on coding areas where selection preserves relevant diversity. The amalgamation of SRAP with expression analysis additionally ensures the functional evaluation of diversity signals.

The environmental diversity of Nagaland serves as a natural laboratory for investigating the effects of geography and climate on genetic structure. Significant altitudinal gradients (200–3000 m asl) provide distinct heat and moisture variations, influencing species distributions. Populations situated along riparian corridors may undergo uninterrupted gene flow, while those isolated on alpine slopes may diverge due to genetic drift or local adaptation. Human activities, especially shifting farming (*Jhum*), deforestation, and infrastructure development, fragment habitats, thus diminishing effective population levels and hastening genetic degradation. Evaluating diversity under these circumstances will elucidate whether the observed divergence is attributable to historical biogeography or current human-induced stresses.

Population-genetic theory posits that outcrossing, animal-dispersed species such as wild *Musa* should maintain substantial within-population variety and moderate among-population divergence, contingent upon sustained connection. In contrast, fragmentation and restricted dispersal increase FST or ΦST values due to the predominance of genetic drift. Through the partitioning of variance via AMOVA and the assessment of isolation-by-distance utilising Mantel tests, ISSR-SRAP datasets may effectively quantify these dynamics. Complementary multivariate studies (PCoA, DAPC) illustrate clustering tendencies, whereas Bayesian assignment identifies admixture indicative of hybrid zones, prevalent in areas where the ranges of *Musa balbisiana* and *Musa acuminata* intersect.

From a conservation genetics perspective, it is essential to identify populations abundant in private alleles or situated at basal phylogenetic locations. Such units embody distinct evolutionary histories and merit prioritising for in situ conservation. In contrast, genetically depleted or recently bottlenecked populations may gain from regulated gene flow to enhance diversity. Integrating molecular findings with ecological data, habitat type, disturbance severity, elevation—facilitates refined management recommendations that balance genetic integrity and adaptability. In addition to conservation, clarifying wild *Musa* diversity directly affects breeding

efforts. Resistance to Fusarium wilt is linked to *Musa balbisiana*, whereas *Musa sikkimensis* provides cold tolerance, and *Musa laterita* imparts ornamental characteristics. Understanding genetic links informs pre-breeding by finding suitable donors and reducing linkage drag. ISSR and SRAP fingerprints aid in parental selection, hybridity verification, and the preservation of variability in germplasm banks.

Despite whole-genome sequencing already serving as the foundation for comparative genomics in *Musa* (D'Hont *et al*., 2012; Busche *et al.,* 2019), high-resolution population studies are still limited due to financial and technical challenges. Dominant multilocus markers remain an effective initial estimate of genetic organisation, particularly in biodiverse, data-scarce areas. Their cost-effectiveness, minimal DNA requirements, and simple methods facilitate implementation in regional laboratories, promoting capacity building and local stewardship of genetic resource management.

Collectively, these factors warrant a thorough evaluation of wild *Musa* diversity in Nagaland utilising ISSR and SRAP markers. The research seeks to measure intra- and inter-population variation, deduce genetic structure in relation to geography and ecology, and recognise unique lineages that serve as reservoirs of adaptive potential. The project will integrate classical taxonomy with molecular genetics to create a foundation for future genomic studies and evidence-based conservation strategies. Documenting the genetic structure of Nagaland's wild bananas surpasses mere academic curiosity. It tackles a strategic necessity: obtaining the evolutionary resources essential for global banana enhancement. As climatic instability and novel diseases jeopardise monocultural plantations, resilience will depend on alleles conserved in forest remnants and shifting-cultivation mosaics within biodiversity frontiers such as Nagaland. This chapter aims to reveal the concealed heritage through the integrative perspective of ISSR and SRAP markers and convert it into knowledge for sustainable utilisation.

Materials and methods

This study expands upon Deb *et al.*'s (2023) foundational research regarding the distribution and diversity of wild *Musa* species in Nagaland, India. This phase of the study aimed to investigate the genetic diversity among eight wild *Musa* species.

Study area and sample collection

The research area, located between 25°06′–27°04′N and 93°20′–95°15′E, covered the state of Nagaland, featuring elevations ranging from around 100 m to 3,840 m. Sample collecting was conducted throughout the state of Nagaland from 2018 to 2022. Collections included *Musa* species from various agroclimatic zones exhibiting diverse morphologies. A Canon DS600 and Nikon DSLR 3200 were employed for photography, while a Garmin Oregon GPS was used to document the GPS coordinates of the collection sites. Sample collection included cigar leaf rolls of young suckers or plantlets attached to flowering parents.

Morphological documentation

The descriptors from the International Network for the Improvement of Banana and Plantain (INIBAP, 1996) were utilised to record morphological data. Comprehensive measurements of plant components, such as pseudostems, leaves, flowers, and fruits, were included. To ensure adequate representation, a minimum of five individuals from each species were assessed. The specimens collected for the initial study were maintained in the germplasm conservation garden of Nagaland University for further research.

DNA extraction, amplification and sequencing

Plant material

Fresh, tender leaves of the wild *Musa* species in Nagaland were gathered in order to obtain DNA. The genomic DNA was extracted with the modified CTAB DNA isolation method (Kamba and Deb, 2018; Sharma and Deb, 2024). This study investigated the following wild banana species: *Musa aurantiaca* Baker (MA1)*, Musa balbisiana* Colla (MB2)*, Musa cheesmanii* N.W.Simmonds (MC3)*, Musa flaviflora* N.W.Simmonds (MF4), *Musa itinerans* Cheesman (M15)*, Musa markkuana* (M.Sabu, A.Joe & Sreej.) Hareesh, A.Joe & M.Sabu (MM6)*, Musa nagalandiana* S. Dey & Gogoi (MN7) and *Musa nagensium* Prain (MN8). Spectrophotometric analysis and

agarose gel electrophoresis were employed to assess the quality and concentration of the DNA.

PCR amplification

21 UBC-ISSR primers were originally assessed for PCR amplification of the genomic DNA from eight wild *Musa* accessions, of which ten primers demonstrating reproducibility and significant polymorphism were selected for the study (Lamare *et al.*, 2015) (Table 6.1). The PCR amplification was conducted using 10X PCR buffer, 25 mM MgCl2, 2 mM dNTPs, 10 µM of each primer, 20–30 ng of template DNA, and 2 units of DNA polymerase. The final reaction volume was 20 µL, and the water used was devoid of nucleases. The PCR protocol was set for 35 cycles, each comprising a denaturation phase at 94 ◦C for 5 min, followed by annealing at 35–55 ◦C for 35 sec, an extension phase at 72 ◦C for 1 min, and a final extension of 10 min at 72 ◦C. The amplified product was subsequently separated on a 1.5% TBE agarose gel.

Nine SRAP primer combinations were selected for the SRAP studies based on Valdez-Ojeda et. al. (2014) (Table 6.2 & 6.3) following preliminary screening for their reproducibility. The preliminary evaluation involved 15 SRAP primer sets, from which 9 were chosen for the PCR amplification of the extracted genomic DNA from 8 wild *Musa* accessions.

PCR amplification was performed using a BioRad Thermalcycler. The PCR reaction mixture comprised 10X PCR buffer, 25mM $MgCl_2$, 2mM dNTPs, 10 µM of each primer, 20–30 ng of template DNA, and 2 units of DNA polymerase. The final reaction volume was 20 µL, and the water used was free of nucleases.

The PCR was programmed with the Initial activation at 94 °C for 5 min; followed by 5 cycles of denaturation at 94 °C for 1 min, annealing at 35–55 °C for 45 sec, and extension at 72 °C for 2 min; subsequently, 31 cycles of denaturation at 94 °C for 1 min, annealing at 40–50 °C for 40 sec, and extension at 72 °C for 2 min; concluding with a final extension at 72 °C for 10 min. The amplified products were resolved on a 1.5% TBE agarose gel, with 100 bp and 1 kb DNA ladders as standards, stained with ethidium bromide, then visualised and photographed with the BioRad ChemiDoc MP Imaging System (XRS Plus).

Table 6.1: ISSR Primer sequences used in the study

Sl.No	Primer Name	Sequence direction	No. of base pairs
1	UBC810	5'-GAGAGAGAGAGAGAGAT-3'	17
2	UBC818	5'-CACACACACACACACAG-3'	17
3	UBC826	5'-ACACACACACACACACC-3'	17
4	UBC834	5'-AGAGAGAGAGAGAGAGYT-3'	18
5	UBC835	5'-AGAGAGAGAGAGAGAGYC-3'	18
6	UBC843	5'-CTCTCTCTCTCTCTCTRA-3'	18
7	UBC848	5'-CACACACACACACACARG-3'	18
8	UBC854	5'-TCTCTCTCTCTCTCTCRG-3'	18
9	UBC855	5'-ACACACACACACACACYT-3'	18
10	UBC873	5'-GACAGACAGACAGACA-3'	16

Table 6.2: SRAP Primer sequences used in the study

Sl.No	Primer Name	Sequence direction	No. of base pairs
1	ODD2	5'-TTAGTCTTCACTTCCAAACC-3'	20
2	ODD8	5'-CACAAGTCGCTGAGAAGG-3'	18
3	ODD38	5'-TACAGAAACTTAGAACCGAAA-3'	21
4	ODD15	5'-GCGAGGATGCTACTGGTT-3'	18
5	SA4	5'-TTCTTCTTCCTGGACACAAA-3'	20
6	DC1	5'-TAAACAATGGCTACTCAAG-3'	19
7	EM1	5'-GACTGCGTACGAATTCAAT-3'	19
8	EM8	5'-GACTCGATAGCAATTCAC-3'	18
9	GA45	5'-AGTGGTATTTTTGCAGTTCTA-3'	21
10	ME3	5'-TGAGTCCAAACCGGAAT-3'	17
11	ME4	5'-TGAGTCCAAACCGGACC-3'	17

Table 6.3: SRAP Primer sets

Sl.No.	SRAP Primer sets
1	ODD8 - ODD38
2	SA4 - ODD15
3	ODD8 - DC1
4	EM1 - GA45
5	ODD8 - ODD15
6	SA4 - ODD38
7	EM8 - ME3
8	EM8 - ME4
9	SA4 - ODD2

Data analysis

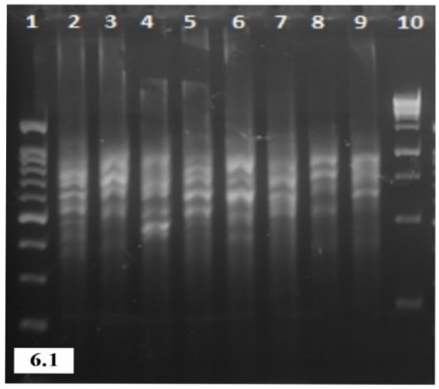
The resulting PCR products generated from the different banana accessions using the SRAP and ISSR markers were manually scored as 1 (band present) and 0 (band absent) as a binary data matrix. To assess the efficacy of the primers used, total number of bands (TNB), number of polymorphic band (NPB) and percentage of polymorphic band (PPB) were calculated based on the presence of clear and stable bands. Polymorphic information content (PIC), number of allele and gene diversity were calculated using PowerMarker program (version 3.51). Genetic diversity and genetic differentiation of the accessions were studied based on both the ISSR and SRAP data using the software POPGENE ver. 1.32 to generate percentage of polymorphism bands (PPB) and mean gene diversity (H). For generating the UPGMA cluster diagram with a display of >60 bootstrap values of the accessions studied, a pairwise genetic dissimilarity matrix was calculated based on the Jaccard's dissimilarity coefficient with 1000 iterations and principal coordinate analysis (PCoA) was carried out using DARwin software (version 6.0.021).

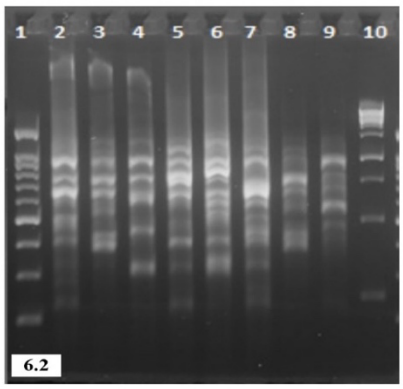
Results

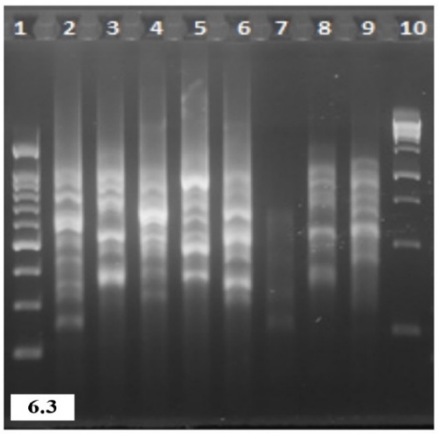
Inter-Simple Sequence Repeat (ISSR) Studies

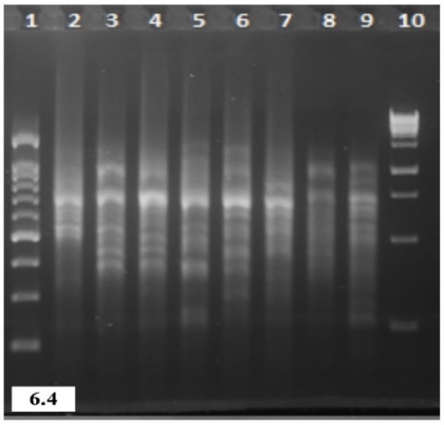
Banding Pattern and Marker Informativeness

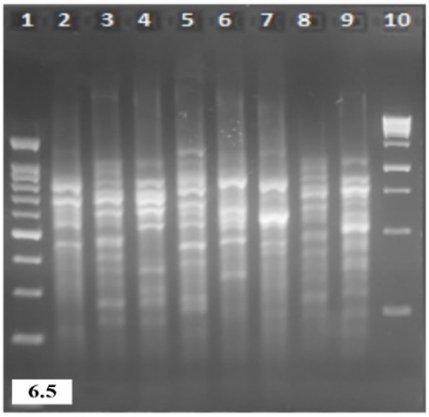
A total of ten ISSR primers were used, all of which generated clear and reproducible amplification profiles across the eight wild *Musa* accessions that were studied (MA1, MB2, MC3, MF4, M15, MM6, MN7, MN8). Band scoring was carried out manually, and distinct bands were assigned a score of 1 (present) or 0 (absent), resulting in a binary data matrix for subsequent analysis (Figures 6.1 - 6.10).

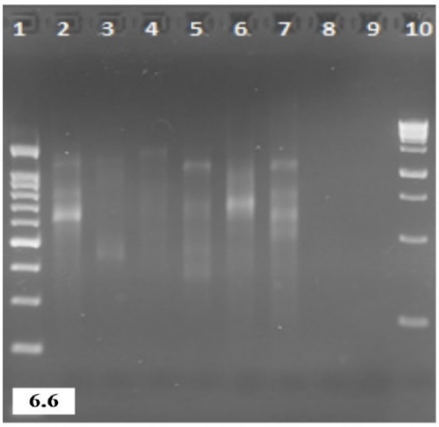


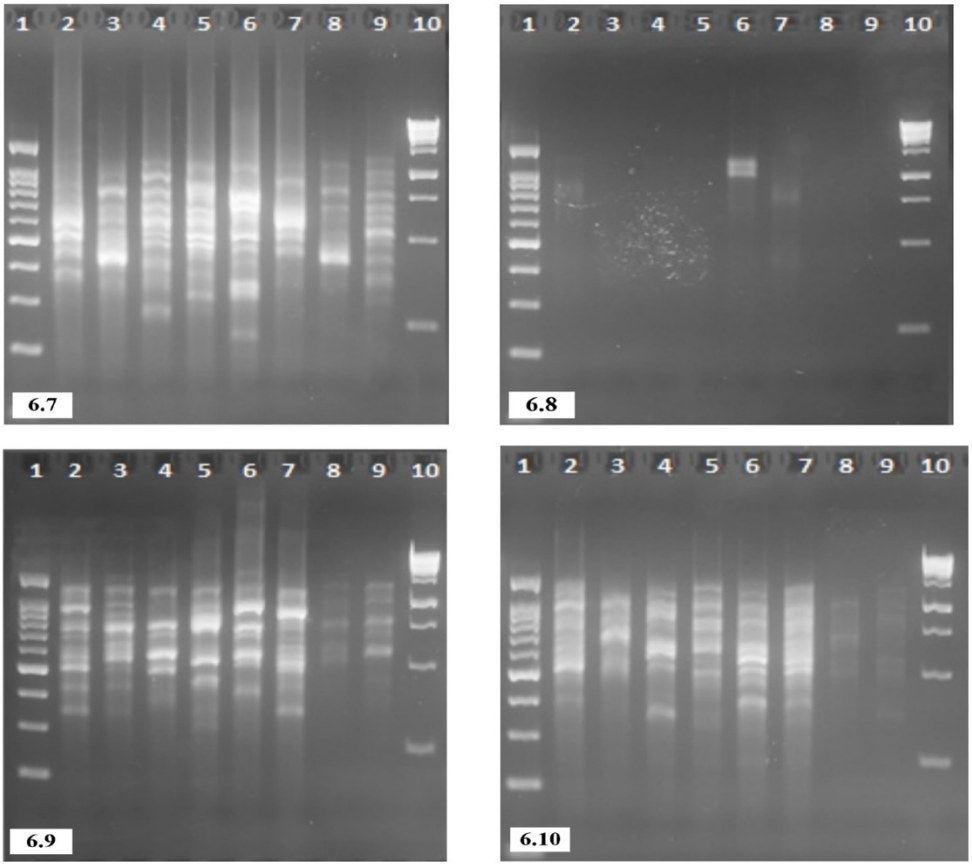












Figures 6.1 – 6.10: Gel image showing PCR amplicons of ISSR primers

6.1 : UBC810; 6.2 - UBC818; 6.3 - UBC826; 6.4 - UBC834; 6.5 - UBC835; 6.6 - UBC843; 6.7 - UBC848; 6.8 - UBC854; 6.9 - UBC855; 6.10 - UBC873
Lane identity : 1 – 100 bp ladder; 2 – MA1 ; 3 – MB2; 4 – MC3; 5 – MF4; 6 – M15; 7 – MM6; 8 – MN7; 9 – MN8; 10 – 1kb ladder

The number of bands per primer (TNB) had values ranging from 4 (UBC854) to 8 (UBC818, UBC826, UBC834, UBC835, UBC848, UBC855, UBC873), with an average of 7.1 bands per primer. The number of polymorphic bands (NPB) varied with values from 3 (UBC854) to 7 (UBC826, UBC834, UBC835, UBC848, UBC855, and UBC873), with an average of 6, and the percentage of polymorphic bands (PPB) varied from 71.43% (UBC810) to 87.50% (UBC826, UBC834, UBC835, UBC848, UBC855, and UBC873). This demonstrates the high polymorphic potential of the ISSR markers to detect genetic variations between accessions.

All of the primers showed mean PIC values between 0.32 (UBC854) and 0.41 (UBC855), with a mean value of 0.379. This suggests that the primers were moderately to highly informative. Mean gene diversity (H) values exhibited a comparable trend (0.32–0.41). Gene diversity exhibited a parallel trend to PIC, hence validating marker informativeness. The average number of alleles (Na) per locus was 1.829, reflecting the dominant and biallelic nature of the ISSR loci. Primers UBC826, UBC834, UBC835, UBC848, UBC855, and UBC873 exhibited the highest informativeness (PPB ≥ 87.5%), indicating their superior capacity to identify polymorphism within wild *Musa* populations. On the other hand, UBC854 had the lowest PPB (75.0%), but nevertheless contributed useful variability (Table 6.4).

Table 6.4: Summary of polymorphism and marker informativeness statistics for ISSR primers.

Primer	TNB	NPB	PPB (%)	Mean PIC	Mean Gene Diversity	Mean Na
UBC810	7	5	71.43	0.36	0.36	1.71
UBC818	8	6	75.00	0.38	0.38	1.75
UBC826	8	7	87.50	0.39	0.39	1.88
UBC834	8	7	87.50	0.38	0.38	1.88
UBC835	8	7	87.50	0.40	0.40	1.88
UBC843	5	4	80.00	0.35	0.35	1.80
UBC848	8	7	87.50	0.40	0.40	1.88
UBC854	4	3	75.00	0.32	0.32	1.75
UBC855	8	7	87.50	0.41	0.41	1.88
UBC873	8	7	87.50	0.40	0.40	1.88

Overall mean: TNB = 7.1, NPB = 6, PPB = 82.9%, PIC = 0.379, H = 0.38, Na = 1.829.

Genetic Similarity and Cluster Analysis

The Jaccard similarity coefficient among the eight accessions ranged from 0.54 to 0.88, with a mean of 0.71, reflecting moderate genetic similarity within the

population. The UPGMA dendrogram based on Jaccard's dissimilarity matrix grouped the accessions into two major clusters: Cluster I (MB2, MC3, MM6, MN8) and Cluster II (MA1, MF4, M15, MN7), suggesting a geographic or ecological basis for divergence (Figure 6.11).

Principal Coordinate Analysis (PCoA)

The PCoA demonstrated a comparable clustering trend. The initial two principal coordinates accounted for 89.67% of the overall genetic diversity (PCo1 = 77.95%, PCo2 = 11.72%). Accessions MA1, MB2, MC3, and MF4 clustered closely along positive PCo1 values, but M15, MM6, MN7, and MN8 were noticeably separated along the negative axis, signifying their genetic distinctiveness (Figure 6.12).

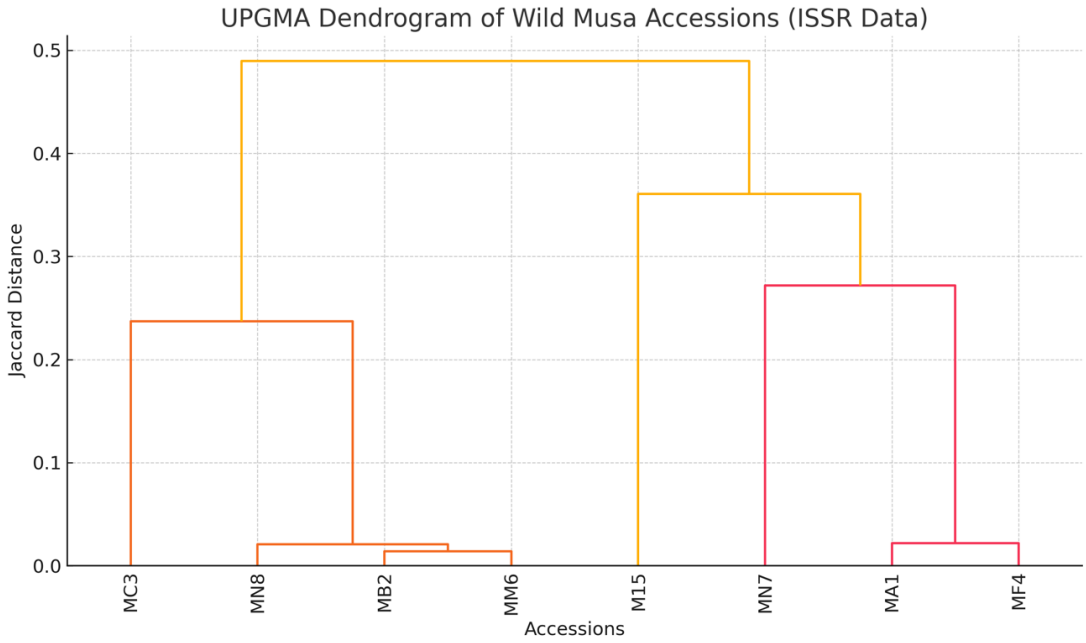


Figure 6.11 : UPGMA dendrogram showing genetic relationships among eight wild *Musa* accessions based on ISSR data.

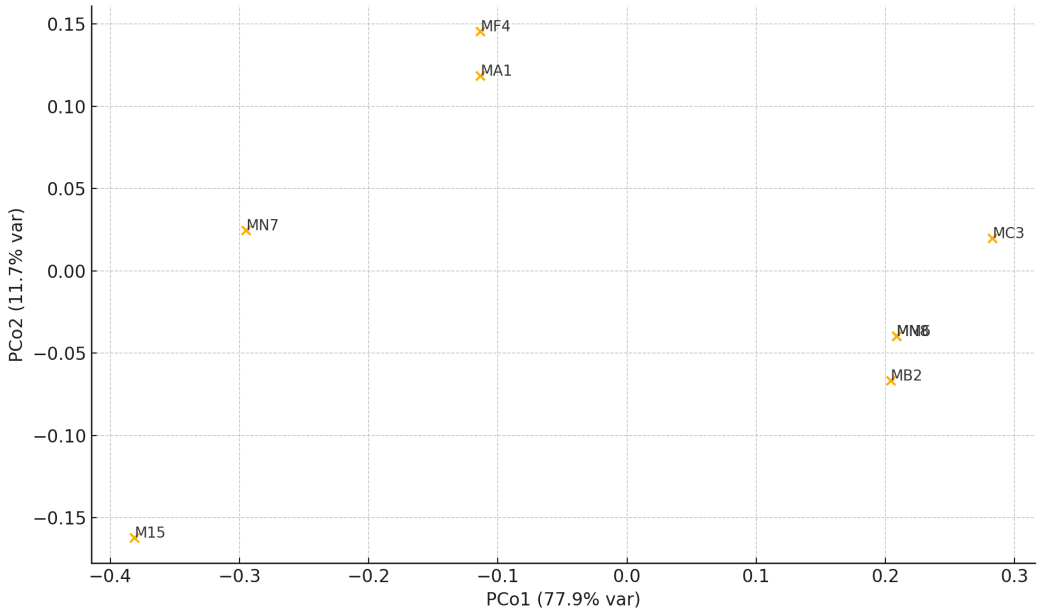
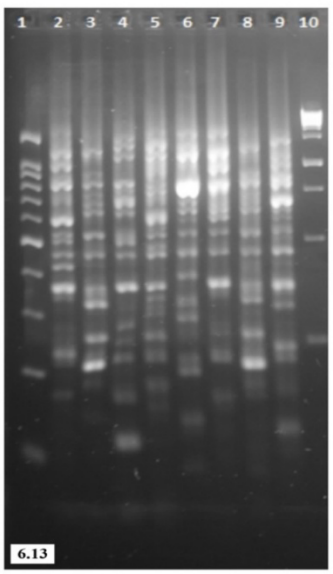


Figure 6.12: PCoA figure illustrating the distribution of wild *Musa* accessions according to Jaccard distances.

Sequence-Related Amplified Polymorphism Studies

Banding Pattern and Marker Informativeness

A total of nine SRAP primers were used, across the eight wild *Musa* accessions that were studied (MA1, MB2, MC3, MF4, M15, MM6, MN7, MN8). Band scoring was carried out manually, and distinct bands were assigned a score of 1 (present) or 0 (absent), resulting in a binary data matrix for subsequent analysis (Figures 6.13 - 6.21).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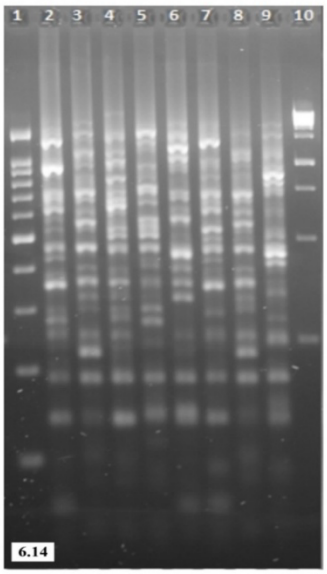
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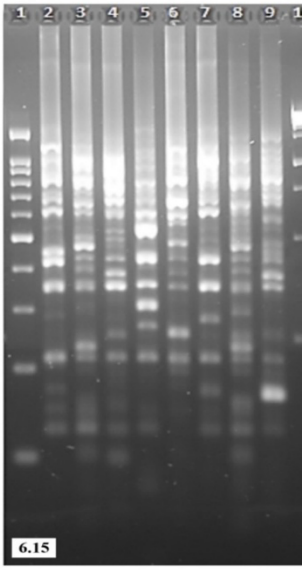
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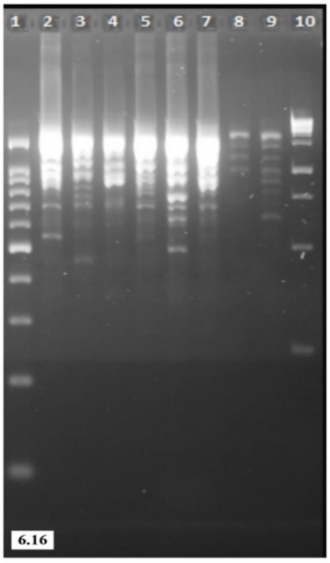
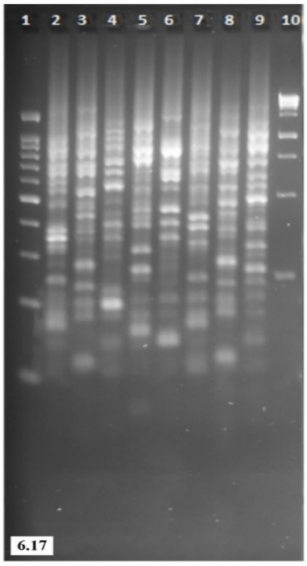

1 2 3 4 5 6 7 8 9 10
6.16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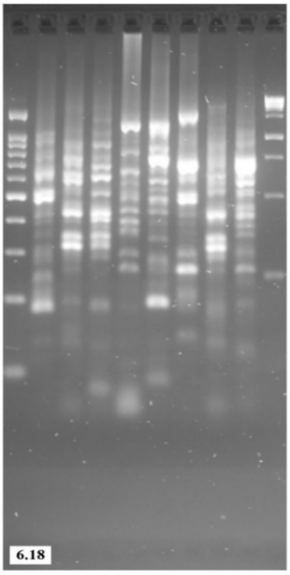
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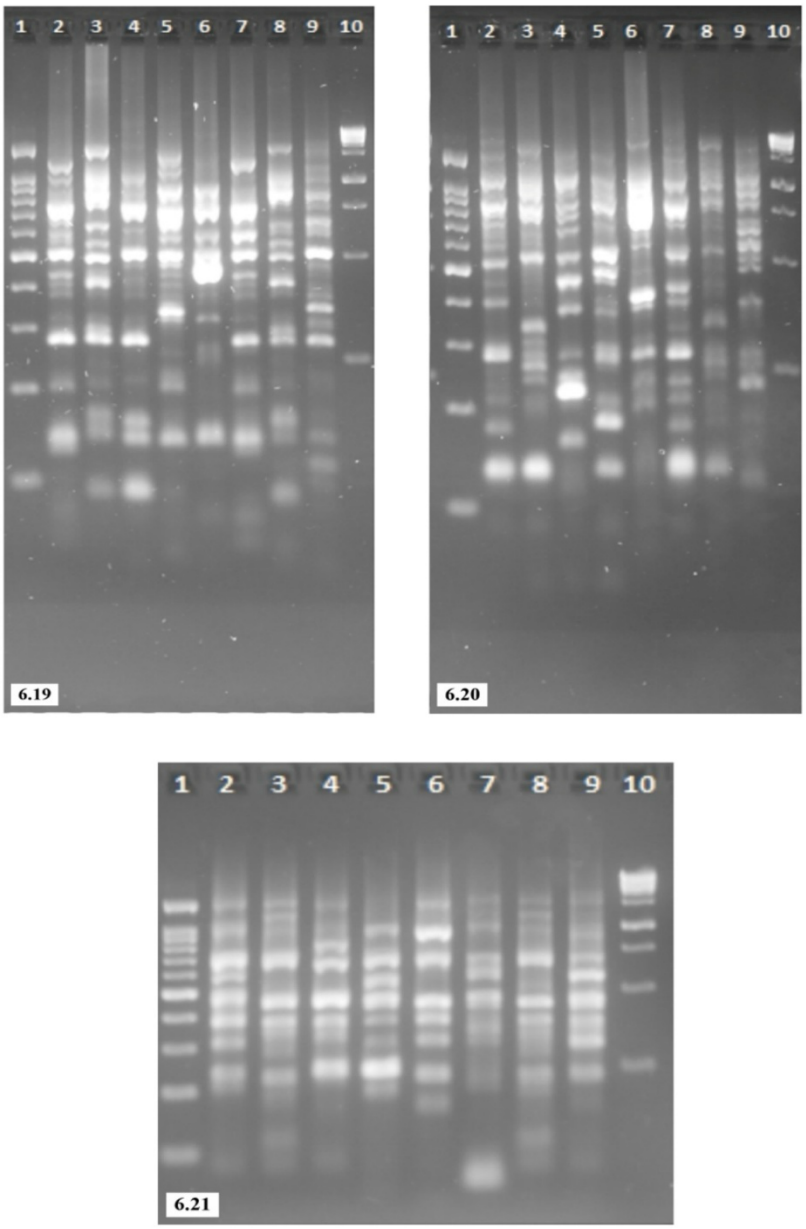

Figures 6.13 – 6.21: Gel image showing PCR amplicons of SRAP primers

6.13 : ODD8 - ODD38; 6.14 - SA4 - ODD15; 6.15 - ODD8 - DC1;
6.16 - EM1 - GA45; 6.17 - ODD8 - ODD15; 6.18 - SA4 - ODD38;
6.19 - EM8 - ME3; 6.20 - EM8 - ME4; 6.21 - SA4 - ODD2;
Lane identity : 1 – 100 bp ladder; 2 – MA1 ; 3 – MB2; 4 – MC3; 5 – MF4; 6 – M15; 7 – MM6; 8 – MN7; 9 – MN8; 10 – 1kb ladder

A total of 93 SRAP bands were scored across the 9 primer sets that were employed.

The percentage of polymorphic bands (PPB) across all loci averaged 72.04%. Mean PIC and gene diversity across primers were 0.28 and 0.28, respectively, with an average observed number of alleles (Na) of 1.72. Per-primer statistics (TNB, NPB, PPB%, mean PIC, mean gene diversity, mean Na) are tabulated in Table 6.5.

Table 6.5: Summary of polymorphism and marker informativeness statistics for SRAP primers.

Primer	TNB	NPB	PPB (%)	Mean PIC	Mean Gene Diversity	Mean Na
EM1-GA45	8	6	75.0	0.29	0.29	1.75
EM8-ME3	11	8	72.73	0.28	0.28	1.73
EM8-ME4	11	8	72.73	0.28	0.28	1.73
ODD8-DC1	11	8	72.73	0.28	0.28	1.73
ODD8-ODD15	11	8	72.73	0.28	0.28	1.73
ODD8-ODD38	10	7	70.0	0.28	0.28	1.7
SA4-ODD15	11	8	72.73	0.28	0.28	1.73
SA4-ODD2	10	7	70.0	0.27	0.27	1.7
SA4-ODD38	10	7	70.0	0.27	0.27	1.7

Genetic Similarity and Cluster Analysis

Pairwise Jaccard similarity coefficients among accessions were calculated from the SRAP dataset and converted to dissimilarity for grouping purposes. The UPGMA dendrogram (Figure 6.22) delineated two primary groups that align with the associations identified in the ISSR analysis. The resultant dendrogram categorised the accessions into discrete clusters, indicating their genetic relationships. Samples MB2, MM6, and MN8 had similiar SRAP banding patterns and were closely grouped. Additional accessions, such as MA1, MC3, MF4, M15, and MN7, established distinct branches, signifying differing levels of genetic divergence among the wild populations.

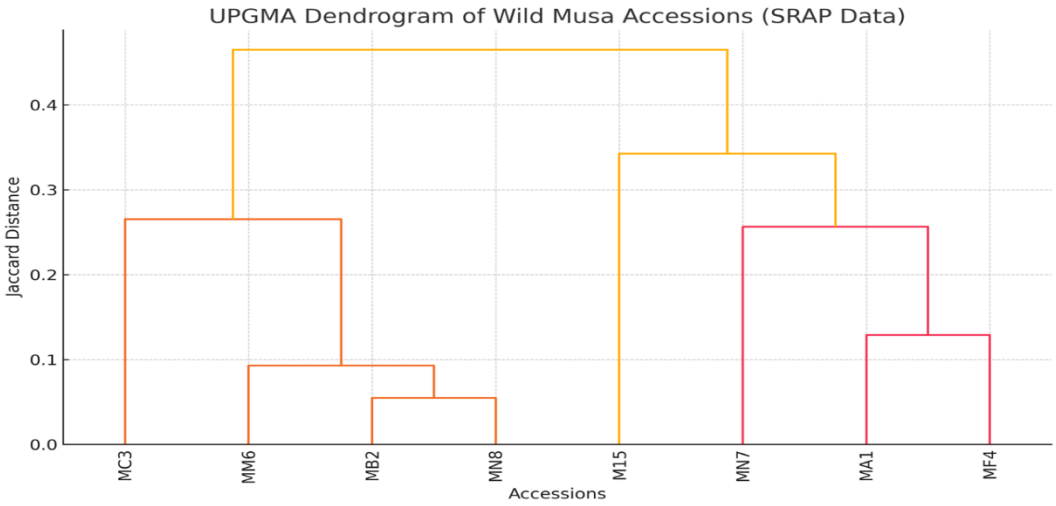

Figure 6.22: UPGMA dendrogram based on Jaccard distances from SRAP data.

Principal Coordinate Analysis (PCoA)

The PCoA of the Jaccard distance matrix elucidated the primary structure among accessions. The initial two axes accounted for 70.29% and 17.00% of the variance, respectively. The ordination confirmed the clustering trend, distinguishing closely related accessions along PCo1 (Figure 6.23).

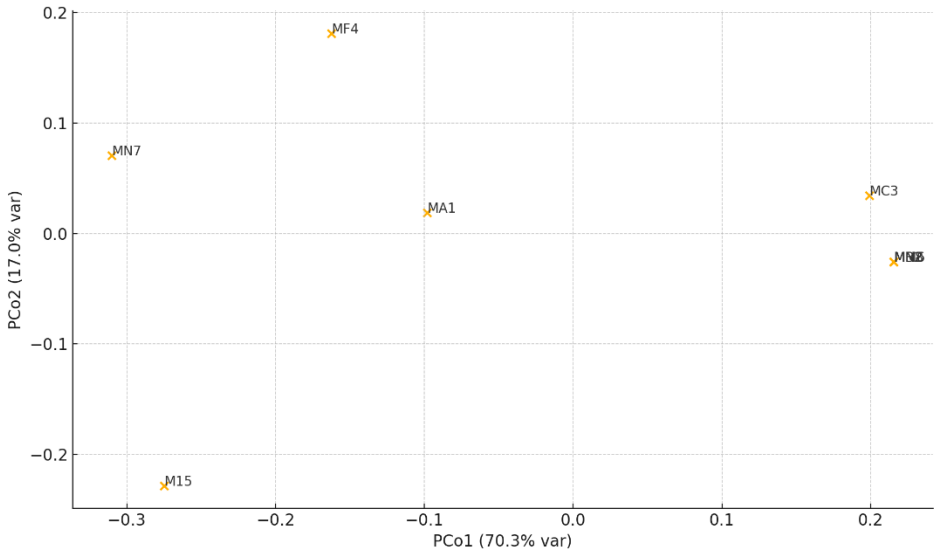

Figure 6.23: PCoA plot of eight wild *Musa* accessions based on SRAP markers.

Discussion

When it comes to determining intraspecific diversity among wild *Musa* species in Nagaland, the ISSR markers that were utilised in this study demonstrated a highly effective level of effectiveness. The overall PPB, which is 82.9%, indicates a significant degree of genetic diversity. This is in line with the heterogeneity that is expected to be present in wild *Musa* populations as a consequence of open pollination, vegetative propagation, and geographic isolation. The mean PIC (0.38) aligns with earlier studies on *Musa* using ISSR markers (Singh *et al.,* 2013; Kumar *et al.*, 2015; Nguyen *et al.,* 2017), where PIC values between 0.30 and 0.45 were considered moderately informative. High polymorphism suggests that ISSR markers, particularly UBC826, UBC834, UBC835, UBC848, UBC855 and UBC873, are suitable for genetic diversity assessment in wild *Musa* germplasm.

The moderate Jaccard similarity (0.71) implies that there is significant allelic variation, which may be the product of both clonal diversification and local adaptation. Cluster I and Cluster II may represent distinct genetic lineages that have adapted to different microclimatic or altitudinal environments. This genetic structure has been observed in the past in accessions of *Musa balbisiana* and *Musa acuminata* that were found in the Northeastern region of India (Kumar *et al.,* 2016; Panda *et al.,* 2021). The enormous diversity that was observed underlines the importance of protecting wild *Musa* populations in the environments in which they were originally found. Genotypes that have distinct ISSR profiles, such as *Musa nagalandiana* and *Musa nagensium*, should be given priority in ex situ germplasm collections and employed in breeding programmes in order to increase the genetic variety in cultivated bananas.

The genetic variation among wild *Musa* accessions from Nagaland was successfully identified using Sequence-Related Amplified Polymorphism (SRAP) markers. All primers produced 93 scoreable bands, 67 of which were polymorphic, indicating a 72.04% polymorphism rate. This considerable polymorphism highlights the usefulness of SRAP markers for analysing diversity in vegetatively propagated crops like bananas. Eight to eleven distinct bands were formed by each SRAP primer combination, with an average of eight polymorphism loci per primer. The mean gene diversity value of 0.28 and the average polymorphism information content (PIC) value

of 0.28 suggest that the SRAP primers were reasonably informative, successfully differentiating most accessions while preserving repeatability. Primers with slightly higher PIC and polymorphism values, such as EM1–GA45, demonstrated greater effectiveness in identifying chromosomal alterations. The presence of both monomorphic and polymorphic loci implies a balanced degree of genetic differentiation within the population, as indicated by the mean number of alleles per locus (Na = 1.72). The moderate PIC and gene diversity values are consistent with earlier studies on *Musa* and other clonally propagated crops, indicating that SRAP markers typically exhibit intermediate degrees of polymorphism compared to more variable systems like ISSR or SSR. While clonal reproduction and low levels of sexual recombination often limit genetic variability in *Musa*, somatic mutations, spontaneous hybridisation, and environmental selection forces enhance the diversity observed. These new discoveries highlight the ability of wild *Musa* populations to adapt to Nagaland's diverse ecological settings, ranging from mid-elevation forests to lowland lowlands. The UPGMA dendrogram's clustering pattern supports these ideas, grouping genetically related accessions and suggesting a common ancestor or adaptation to similar environments. The SRAP data show moderate to high genetic diversity in wild *Musa* accessions of Nagaland, crucial for their long-term conservation and banana improvement projects. SRAP markers target coding regions, complementing ISSR and SSR by revealing functional diversity. These molecular discoveries provide a comprehensive genetic landscape of wild *Musa* in this area when combined with plant appearance and ecology information. These new discoveries highlight the ability of wild *Musa* populations to adapt to Nagaland's diverse ecological settings, ranging from mid-elevation forests to lowlands. The UPGMA dendrogram's clustering pattern supports these ideas, grouping genetically related accessions and suggesting a common ancestor or adaptation to similar environments. The SRAP data shows moderate to high genetic diversity in wild *Musa* accessions of Nagaland, crucial for their long-term conservation and banana improvement projects. SRAP markers target coding regions, complementing ISSR and SSR by revealing functional diversity. These molecular discoveries provide a comprehensive genetic landscape of wild *Musa* in this area when combined with plant appearance and ecology information.

Summary and conclusion

Cultivated bananas are known for their low genetic diversity owing to their parentage via clonal propagation. Their wild relatives, however, are natural by-products of natural selection and hence are more diverse in their genetic composition. The following study was undertaken to ascertain and study the genetic diversity of 8 selected wild *Musa* species of Nagaland. Two dominant PCR-based DNA marker methods were employed to this end, i.e., ISSR and SRAP. Plant samples of all the selected species were collected from the wild and their DNA extracted. PCR amplifications were carried out for both ISSR and SRAP primers and the PCR amplicons were resolved onto 1.5% TBE gels. Gel images were obtained, from which scoring of DNA bands was carried out. The resultant binary matrix was used to calculate TNB, NPB, PPB%, mean PIC, mean gene diversity and mean Na. A UPGMA dendrogram as well as a PCoA illustration were constructed.

Significant genetic variety was observed among the wild *Musa* accessions in Nagaland, as shown by ISSR markers. The informative primers (UBC835, UBC848, and UBC855), along with the distinct clustering pattern, highlight the presence of divergent gene pools that are valuable for biodiversity conservation and crop improvement.

SRAP markers demonstrated efficacy in elucidating variation across wild *Musa* accessions, augmenting the ISSR findings. Moderate-to-high PPB% and PIC values signify that the selected SRAP primer combinations are effective for evaluating diversity. The alignment between SRAP-based clustering and ISSR results reinforces the conclusion of two primary genetic groupings or clusters within the panel, indicating potential ecological or geographic heterogeneity. These findings endorse the efficacy of SRAP for germplasm characterisation and for informing sampling and conservation initiatives.

Chapter - 7

Summary and Conclusion

Musa, as a taxon in Nagaland, is an important but not well-studied resource, both ethnobotanically and genetically. This is because it is widely used in homes, not just for food, but also for other household tasks, and it holds cultural significance. This use applies not only to its cultivated varieties but also to its wild relatives, from which most of its plant parts can be utilised by the resourceful local population. We know a lot about the cultivated *Musa* species, but not much has been done to study the wild *Musa* species.

The area being studied is also located between two biodiversity hotspots: the Himalayas to the east and the Indo-Burma to the west. This area is thought to be where the taxon being studied came from. This motivates the investigation of the genetic diversity reservoir in this region. Based on this reasoning, the study began in November 2018, and the following goals were set:

Field Exploration and Documentation of Existing Wild *Musa* Species in Nagaland

Conduct extensive field surveys throughout Nagaland to identify, collect, and document the diversity of wild *Musa* species in the area. This goal includes making maps of where species are found, noting the characteristics of their habitats, making

detailed morphological descriptions, giving each species a key, and taking pictures of each one. The goal is to create a current list of wild *Musa* species in Nagaland that can be used as a starting point for conservation and more research.

Nutritional Evaluation of Specific Wild *Musa* Species (Fruits and Other Consumable Components)

Examine the nutritional profile of specific wild *Musa* species, emphasising fruits and other consumable components, including pseudostems and inflorescence. This evaluation will encompass the quantification of macronutrients, dietary fibre, antioxidant properties, and bioactive compounds. The goal is to find species that are very nutritious and could be used to make functional foods and encourage people to eat a wider variety of foods.

Molecular Characterisation of Wild *Musa* Species

Use molecular methods like DNA barcoding and marker-based genetic profiling to describe the wild *Musa* species that were collected from Nagaland. This goal is to clear up any taxonomic confusion, make sure that species are correctly identified, and look at the genetic links between the species. Molecular data will augment morphological observations and aid in the establishment of a genetic reference database for the region's *Musa* germplasm.

Study of Genetic Diversity in Certain Species Cultivated in Various Regions of Nagaland

Examine the genetic diversity of specific wild *Musa* species across various geographic regions in Nagaland. This will necessitate the sampling of various species from distinct populations and the utilisation of molecular markers to evaluate inter-species genetic variation. The goal is to learn how much genetic diversity there is, find unique or isolated populations, and learn more about how these wild *Musa* species evolved and what conservation priorities should be.

This study provides a comprehensive analysis of the diversity, genetic relationships, and nutritional value of wild *Musa* species found across the diverse agro-climatic regions of Nagaland. The integration of morpho-taxonomic, molecular, and nutritional methodologies enhances our comprehension of the ecological distribution, evolutionary linkages, and nutritional significance of these wild banana species. This information is beneficial for their preservation and potential application.

Field observations throughout Nagaland led to the identification and collection of twelve wild *Musa* species, characterised by detailed morpho-taxonomic traits. Digital field notes, pictures, and herbarium specimens were employed to document each species for precise description in its natural habitat. The study revealed that the diversity of wild *Musa* species is profoundly influenced by ecological gradients, with the northern tropical and subtropical regions exhibiting the highest species richness, which gradually declines towards the southern, cooler temperate areas. *Musa sikkimensis* thrives at elevations beyond 1500 m, indicating its adaptability to colder environments. *Musa mannii* is classified as severely endangered by the IUCN Red List (criteria Blab(iii)+2ab(iii)), with its populations being exceedingly limited, necessitating urgent conservation measures. The primary factors contributing to the population decline were habitat destruction due to jhum farming, road construction, and the collection of flowers and pseudostems for local utilisation. Wild *Musa* populations persist despite these challenges due to their rapid growth and ability to reproduce both sexually and asexually. This demonstrates their resilience and pioneering spirit throughout their environments. The Department of Botany's Seed Bank maintains viable seeds of collected species, while the *Musa* Garden at Nagaland University preserves living germplasm for long-term conservation and future research. A taxonomy key was developed to assist individuals in the state in identifying these species.

Molecular investigations utilising ITS and *trnL-F* markers established a phylogenetic framework for the wild *Musa* species of Nagaland, confirming their genetic variety and evolutionary complexity. The research revealed several genetic lineages corresponding to geographical and ecological differences, highlighting both closely related and divergent individuals within the genus. The genetic affinity between *Musa nagalandiana* S. Dey & Gogoi and *Musa balbisiana* Colla suggests its potential as a genetic resource for the improvement of cultivated bananas, particularly for enhanced disease resistance and adaptability to diverse environmental circumstances. Molecular data also confirmed the genetic distinctiveness of *M. nagalandiana* as a valid, separate species, thereby improving the taxonomic understanding of *Musa* in the area. The molecular findings complement the morpho-taxonomic data and underscore the significance of integrating traditional taxonomy

with DNA-based methodologies for accurate classification and enhanced conservation management. The unique genetic signatures identified in species such as *M. flaviflora, M. laterita*, and *M. nagalandiana* affirm the evolutionary uniqueness of Nagaland's wild bananas and underscore their significance as reservoirs of untapped genetic resources vital for global banana breeding and improvement efforts.

The nutritional examination of five selected wild *Musa* species provided substantial baseline data on the nutritional attributes of their fruits, including both pulp and peel in raw and mature stages. We examined parameters such as moisture, ash, protein, crude fibre, reducing sugar, total carbohydrates, vitamin A, total phenolics, flavonoids, and DPPH radical scavenging activity. The results indicated that both the pulp and peel are rich in antioxidants and provitamin A, suggesting their potential use in nutritional improvement and phytotherapy. *Musa itinerans* Cheesman exhibited the most favourable nutritional profile among the species examined, indicating its potential as a high-value functional food. The peel of M. balbisiana demonstrated a beneficial sugar-to-fibre ratio, suggesting its potential for the creation of dietary fibre-based products or nutraceuticals. These findings demonstrate the nutritional and medicinal value of wild *Musa* species. They play a significant role in local cuisines and traditional medicine, with various plant parts, including flowers, pseudostems, corms, and roots, utilised to address numerous health issues. These species are nutritionally abundant, indicating their potential to enhance food and nutritional security and serve as ingredients in functional and therapeutic meals.

A comprehensive analysis of the pseudostem and inflorescence of the same species uncovered notable differences in their nutritional composition. The pseudostem included elevated quantities of moisture, crude fibre, and carbohydrates in contrast to the inflorescence, which displayed considerable phenolic content. The pseudostem had increased antioxidant activity. The correlation analysis demonstrated that moisture and flavonoid concentration significantly impacted DPPH radical scavenging activity, confirming their status as critical determinants of antioxidant capacity. The pseudostem of wild *Musa* represents a prospective source of fibre and antioxidants for enhanced products, including flour, fibre concentrates, and functional food components. The inflorescence, abundant in phenolic and flavonoid compounds, can be employed to generate nutraceutical extracts highlighting flower-derived

bioactive agents. The findings demonstrate that the underutilised parts of wild *Musa* plants, typically considered agricultural waste, can be converted into valuable bioresources. This fosters sustainability and the cyclical use of botanical resources.

The findings of this study indicate that the wild *Musa* species in Nagaland exhibit significant diversity, substantial genetic variation, and considerable nutritional potential. The integration of taxonomic, molecular, and nutritional research provides a comprehensive understanding of these species, highlighting their significance to the environment, evolution, and economy. The documenting of diversity establishes a foundation for further bioprospecting and conservation initiatives, while the genomic data clarify species relationships and their evolutionary processes. The nutritional studies provide a substantial scientific basis for exploring wild bananas as sources of nutrient-rich and health-promoting foods.

Utilising both in situ and ex situ conservation approaches is crucial due to challenges posed by habitat degradation, land-use alteration, and overexploitation. To ensure the long-term survival of these species, it is imperative to implement community-based conservation initiatives, awareness campaigns, and sustainable utilisation methods. Wild *Musa* species have benefits beyond local livelihoods due to their resilience, adaptability, and nutritional density. They may also prove beneficial for prospective agricultural and biotechnological applications.

This study concludes that the wild *Musa* species of Nagaland are essential to the region's biological variety, providing substantial prospects for genetic betterment, nutritional advancement, and sustainable resource development. The integration of morpho-taxonomic, molecular, and nutritional data presented herein establishes a robust foundation for forthcoming study and conservation planning. Protecting these species is crucial for maintaining ecosystem balance and genetic diversity, as well as addressing larger issues related to food security, health, and sustainable agriculture. The wild bananas of Nagaland exemplify the equilibrium between biodiversity conservation, human welfare, and sustainable development via continuous study, conservation efforts, and judicious utilisation.

Future Aspects of the Research

This research's results suggest several promising paths for future study, including conservation biology, genetic enhancement, nutraceutical development, and sustainable resource use. The thorough understanding acquired through morpho-taxonomic, molecular, and nutritional analyses establishes a robust foundation for subsequent research aimed at enhancing the scientific, ecological, and economic prospects of wild *Musa* species in Nagaland and beyond.

This study highlights the necessity for sustained structured conservation initiatives for wild *Musa* species in the region. The identification of uncommon and endangered taxa such as *Musa mannii,* which confronts severe threats in its native habitat, highlights the necessity for the establishment of specialised in situ conservation sites and community-managed gene sanctuaries in areas with substantial *Musa* variety. Furthermore, ex situ conservation strategies, such as the preservation of living germplasm in botanical gardens, seed banks, and tissue culture repositories, should be enhanced and disseminated more broadly. The *Musa* Garden at Nagaland University contains material that might serve as a foundation for future genetic and breeding research. Employing biotechnological methods such as cryopreservation and micropropagation would facilitate the long-term preservation of elite and endangered genotypes, thereby mitigating the risk of genetic loss.

An essential focus for the future is the molecular characterisation and genomic mapping of these wild *Musa* species. The present study utilised ITS and trnL-F markers for phylogenetic analysis; however, high-throughput sequencing technologies, such as whole-genome sequencing (WGS), transcriptomics, and chloroplast genome analysis, provide substantially enhanced understanding of evolutionary dynamics and adaptive mechanisms. Advanced genomic investigations can identify genes that regulate critical features including as cold tolerance, drought resistance, pest and disease resilience, and enhanced nutritional content. Subsequently, these genes can be utilised to develop superior banana varieties by marker-assisted selection (MAS) or contemporary gene-editing technologies like as CRISPR-Cas systems. Due to the escalating threat of climate change, the genetic diversity of Nagaland's wild *Musa* germplasm possesses significant promise for producing resilient variants capable of withstanding biotic and abiotic challenges.

This research's nutritional aspect offers substantial prospects for the progression of nutraceuticals and functional foods. The high concentrations of antioxidants, phenolics, and flavonoids present in both the edible and non-edible parts of wild *Musa* species suggest their potential use in creating dietary supplements, health foods, and natural antioxidant extracts. Future study may focus on the separation, purification, and structural characterisation of certain bioactive chemicals, including phenolics, carotenoids, and flavonoids, and evaluate their pharmacological properties using in vitro and in vivo models. The peel, pseudostem, and flower have significant nutritional and antioxidant properties. They may be utilised to produce value-added goods, such as baking flour, fibre-enriched dietary formulations, or phenolic-rich nutraceutical powders. Such applications will not only facilitate garbage utilisation but also enable individuals in rural regions to generate income by establishing small-scale processing enterprises.

A primary emphasis for future efforts involves the amalgamation of ancient knowledge with modern scientific validation. Given the significance of wild *Musa* species to the ethnobotanical and culinary practices of the Naga populations, the integration of ethnonutritional and ethnopharmacological documentation with biochemical and pharmacological research can substantiate traditional applications scientifically. Collaborative research with local communities can enhance participatory conservation and sustainable harvesting practices, thereby protecting cultural heritage alongside biodiversity.

Future study should emphasise ecological and population-level studies of wild *Musa* species across various altitudinal gradients and ecological niches. Such investigations can elucidate population structure, reproductive mechanisms, gene flow, and the impact of environmental stressors on genetic diversity. Employing molecular ecology methodologies like as population genomics and landscape genetics can enhance our comprehension of adaptation and speciation processes within the species. These findings are essential for formulating region-specific management strategies, particularly in places undergoing rapid habitat transformation due to developmental activity.

There are opportunities to explore the industrial and agricultural applications of wild *Musa* biomass. The fibres in the pseudostem can be converted into

biodegradable materials, paper pulp, or textile fibres. This is a more ecologically sustainable alternative to manufactured items. The sap and flower extracts may be examined for antibacterial and biofertilizer applications, while the carbohydrate-rich residues can be evaluated for bioethanol or biogas production. Such integrated approaches could facilitate the establishment of circular bioeconomy models in rural Nagaland, aligning biodiversity utilisation with sustainable economic development.

This thesis demonstrates the necessity for a comprehensive regional database of wild *Musa* species encompassing their taxonomy, genetics, and nutritional information. This database will serve as a valuable resource for scientists, policymakers, and environmentalists in the future. Additionally, the formation of collaborative networks with national and international banana research institutions, such as the National Research Centre for Banana (NRCB) in Trichy and Bioversity International, may augment advanced molecular research, enable germplasm exchange, and advance global conservation efforts.

The interdisciplinary nature of this work indicates that future research should adopt a multidimensional framework encompassing taxonomy, genomics, metabolomics, and emerging biotechnologies. By merging these techniques, researchers might uncover new insights into the evolutionary adaptations of wild *Musa s*pecies while exploring their untapped potential for food security, health, and sustainable resource management.

The future ramifications of this thesis extend beyond academic interest, with substantial consequences for conservation, agriculture, industry, and public health. The indigenous *Musa* species of Nagaland constitutes a significant component of the region's biodiversity and serves as a rich natural resource that can address global challenges such as nutrition, sustainability, and climate resistance. Continued study, guided by integrative and collaborative frameworks, will ensure the preservation, understanding, and appropriate use of this unique genetic and nutritional heritage for the benefit of present and future generations.

The present investigation represents one of the first comprehensive and integrative studies on wild Musa species of Nagaland, combining morpho-taxonomic documentation, molecular characterization, genetic diversity analysis, and detailed nutritional and nutraceutical profiling. Unlike earlier studies that primarily focused on

cultivated bananas or isolated aspects of wild species, this research systematically evaluates both genetic and functional attributes of multiple wild Musa taxa.

Key original contributions include: (i) extensive field-based documentation and mapping of wild Musa species across diverse agro-climatic zones of Nagaland; (ii) molecular authentication and phylogenetic clarification of wild species using DNA barcoding and marker-based approaches; (iii) assessment of genetic diversity and population structure using complementary multilocus marker systems; and (iv) comprehensive nutritional and nutraceutical evaluation of underutilized plant parts, including fruit pulp, peel, inflorescence, and pseudostem.

By integrating molecular diversity with nutritional functionality, this study establishes wild Musa species of Nagaland as valuable genetic and nutritional resources with implications for conservation, crop improvement, functional food development, and sustainable utilization.

Miscellaneous photo plate

Figure 7.1: The author standing in a cluster of stoloniferous *Musa itinerans* Cheesman

Figure 7.2: A population of *Musa balbisiana* Colla nestled in a valley just beyond Tamlu town, Longleng district.

Figure 7.3: A sympatric population of *Musa itinerans* Cheesman and *Musa markkuana* (M.Sabu, A.Joe & Sreej.) Hareesh, A.Joe & M.Sabu *stat. nov* in a damp ravine in Lumami, Zunheboto district.

Figure 7.4: A cluster of *Musa itinerans* Cheesman thriving in the riparian zone of Tishi river, Zaphumi, Zunheboto district.

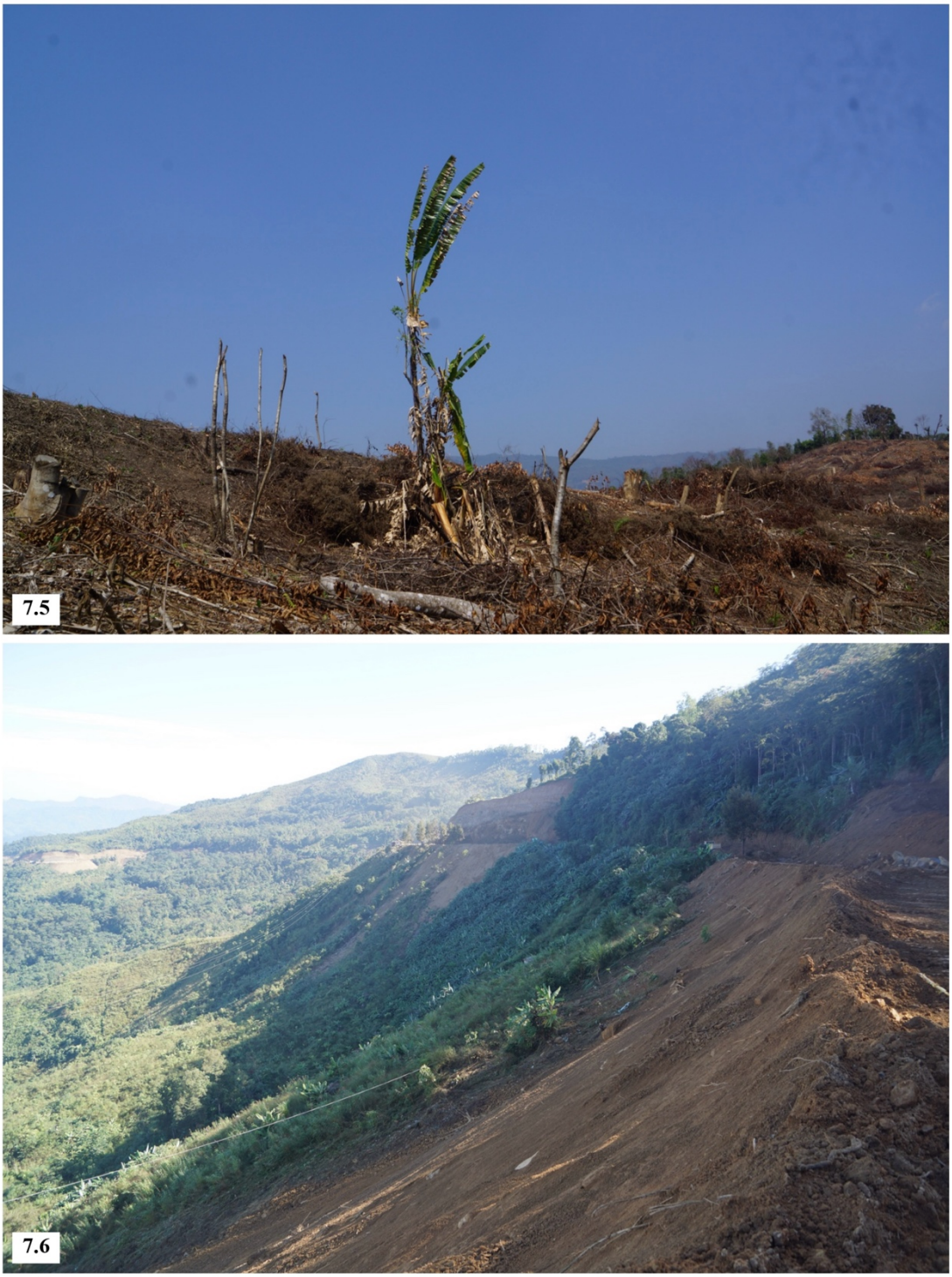

Figure 7.5 and 7.6: A contrast between a conscious practice and mindless development. The picture on top is a lone plant from a cluster of *Musa balbisiana* Colla, left by farmers after clearing of land for Jhum cultivation. The picture below is a sympatric population of *Musa aurantiaca* Baker and *Musa nagensium* Prain near Yisemyong, Mokokchung (which was most probably the biggest population of the rare *Musa nagensium* Prain observed during the study period) almost wiped out to make way for road extension project.

7.7 to 7.12: Photo plate depicting rachis orientation diversity in wild *Musa* of Nagaland.

7.7: Erect rachis of *Musa markkuana* (M.Sabu, A.Joe & Sreej.) Hareesh, A.Joe & M.Sabu *stat. nov*

7.8: Pendulous rachis characteristic of *Musa balbisiana* Colla.

7.9: A curious case of probable floral meristem duplication in *Musa aurantiaca* Baker leading to a rare and abnormal double inflorescence with an erect main rachis and a secondary branching rachis at its base.

7.10: Horizontal inclined rachis in *Musa flaviflora* N. W. Simmonds.

7.11: Diversity in rachis colour.

7.12: *Musa balbisiana* Colla exhibiting a serpentine rachis.

Figure 7.13: Inflorescence emerging from within the pseudostem during its early stages of development in *Musa manii* Baker.

Figure 7.14: Emerging inflorescence of *Musa flaviflora* N. W. Simmonds with barely developed bract colour.

Figure 7.15: Peeled ripe fruit of *Musa flaviflora* N. W. Simmonds full of seeds with barely any pulp, representative of all wild *Musa* species found in Nagaland.

Figure 7.16: L.S. of the fruit of *Musa nagalandiana* S. Dey & Gogoi showing fruit with no viable seeds, an exception to the rule.

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LIST OF PUBLICATIONS

RESEARCH PAPERS PUBLISHED / COMMUNICATED

1. 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, 315-337. https://doi.org/10.4236/ojf.2023.133019.

2. Vupru, T. N., Jamir, B., Paul, A. Sharma, T. I., Ngullie, T. & Deb, C. R. Phylogenetic relationships of wild *Musa* species in Nagaland, India, using multiple DNA barcoding markers (*atpB−rbcl, rps16, trnL−F*, and ITS). Gene (Communicated). (Manuscript ID: GENEJOURNAL-S-25-05998).

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Diversity and Distribution of Wild *Musa* in Nagaland, India

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Abstract

The state of Nagaland is located in a region known to be a micro-centre of *Musa* evolution. The prevailing agro-climatic conditions are known to support various members of the genus. This study documents 12 species, which is an ideal representation of the rich diversity of this genus in an area of 16,579 sq. km. The various species were classified using morphological descriptors based on INIBAP, 1996 following the subsequent collection of the live specimen. The various species documented have been found to exist in single populations or in association with other species and their distribution depends on the varying altitudinal profile as well as the agro-climatic and topographical condition of the environment.

Keywords

Descriptors, Wild *Musa* Diversity, *Musa* Distribution, Nagaland

1. Introduction

The genus *Musa* comprises of a group of large herbaceous plants which includes

LIST OF SEMINARS, CONFERENCES AND PAPERS PRESENTED

- Hands-on Training Workshop on "Gene Cloning, Protein Biochemistry, Structure Biology and Bioinformatics" held at Advanced Centre for treatment, Research an Education in Cancer (ACTREC), Kharghar, Navi Mumbai, India from 4th to 15th February 2019.
- National Conference of "Stakeholders on Conservation, Cultivation, Resource Development and Sustainable Utilisation of Medicinal Plants of North-Eastern India" held at Nagaland University, Lumami, Nagaland from 6th to 7th March 2019.
- Hands-on Training on "Molecular Taxonomy of Microbes and Higher Plants" organised by Advance Level Institutional Biotech Hub, Nagaland University, Lumami from 17th to 23rd July 2019.
- Workshop on "Research Ethics, Paper Writing & IPR" organised by Department of Botany and Advance Level Institutional Biotech Hub, Nagaland University, Lumami from 14th to 15th November 2019.
- International Conference on "Bioresources & Bioeconomy" *(ICBB-2022)* organised by Department of Botany, Nagaland University, in collaboration with Nagaland Forest Management Project, Department of Environment, Forest and Climate Change, Government of Nagaland, held at Nagaland University, Lumami from 19th to 21st September 2022.
- National Seminar on "Recent Advances in Herbal Drug Research in Reference to Ayurveda and Pharmacovigilance of ASU&H Drugs" organised by

Regional Ayurveda Research Centre (RARC), CCRAS, Ministry of AYUSH, Government of India, held at Dimapur, Nagaland on 4th March 2023.

- National e-Conference on "Bioresources and Sustainable Livelihood of Rural India" organised by the Department of Botany, Nagaland University, Lumami, Nagaland, held from 28th to 29th September, 2020. Presented a paper entitled *"Nutritional Assessment of Some Wild Musa of Nagaland, India"* (Abstract No. 35) in the Technical Session.
- National Workshop on "Techniques in Applied Plant Science Research" organised by the Advanced Level Institutional Biotech Hub, Department of Botany, Nagaland University, Lumami, Nagaland, held from 17th to 22nd March, 2025. Contributed as a member of the organising team.
- Research Development Programme for Young Researchers organised by the Internal Quality Assurance Cell (IQAC), Nagaland University, Lumami, Nagaland, held from 24th to 28th March, 2025.
- ACS Webinar on "ACS PhD Hour: Thinking Beyond Academia" organised by American Chemical Society on 20th December, 2022.
- One Day Workshop on "Importance of IPR in Academic Institutions" organised by the IPR Cell, Nagaland University, held on 29th May, 2019.
- Hands-on Training on "Functional Genomics" organised by Department of Biotechnology, Government of India sponsored Institutional Biotech Hub, Nagaland University, Lumami from 14th – 21st November. 2017.

International Conference on 'Bioresources & Bioeconomy' (ICBB-2022)

Organized By

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

In Collaboration With

Nagaland Forest Management Project, Department of Environment, Forest and Climate Change, Govt. of Nagaland, India.

September 19-21, 2022

CERTIFICATE OF PRESENTATION

This is to certify that

Mr./Miss/Dr. Thejavitsü Noah Vüprü of Nagaland University, Lumami, India

has presented a paper (Oral/~~poster~~) titled

'Nutritive Value Proximation of Pseudostem and Inflorescence of Some Wild Musa Species of Nagaland, India.'

in the ICBB-2022 organized by the Department of Botany, Nagaland University, Lumami 798627, Nagaland, India, held on September 19-21, 2022.

(Prof. M. S. Rawat)
Dean, School of Sciences

(Prof. Chitta Ranjan Deb)
Convener cum Organizing Secretary
ICBB-22

Ministry of Ayush
Government of India

आज़ादी का अमृत महोत्सव

REGIONAL AYURVEDA RESEARCH CENTRE

DIMAPUR, NAGALAND

(Under CCRAS, Ministry of AYUSH, Government of India)

Certificate of Merit

This is to certify that

THEJAVITSU NOAH VUPRU

has been awarded the 1st **Best Poster Award** for the poster entitled

presented in the National Seminar on **"Recent Advances in Herbal Drug Research in Reference to Ayurveda and Pharmacovigilance of ASU&H Drugs"** organized by RARC, Dimapur, Nagaland on 04th March 2023

Dr. Ravindra Singh
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Dr. Vilas Gangurde
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RARC Dimapur, Nagaland

PARTICULARS OF THE CANDIDATE

NAME OF CANDIDATE : **Thejavitsu Noah Vupru**

DEPARTMENT : **Department of Botany**

TITLE OF THESIS : **Nutritional Value Assessment and Studies on the Genetic Diversity of Some Selected Wild *Musa* Species of Nagaland**

DATE OF ADMISSION : **August 17, 2019**

APPROVAL OF RESEARCH PROPOSAL:

1. **DRC: 07. 09. 2000**
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