DNA FINGERPRINTING AND NUTRITIONAL PROFILING OF DIFFERENT CULTIVARS OF BANANA (*Musa sp* L.)

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AUGUST, 2018

DNA FINGERPRINTING AND NUTRITIONAL PROFILING OF DIFFERENT CULTIVARS OF BANANA (*Musa sp* L.)

Thesis submitted to the
University of Horticultural Sciences, Bagalkot
in partial fulfillment of the requirements for the
Degree of

Master of Science (Horticulture)
In

BIOTECHNOLOGY AND CROP IMPROVEMENT

By

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CERTIFICATE

This is to certify that the thesis entitled **"DNA FINGERPRINTING AND NUTRITIONAL PROFILING OF DIFFERENT CULTIVARS OF BANANA (*Musa sp* L.)"** submitted by **Ms. B R CHAITRA, ID No. UHS16PGM736** in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE (HORTICULTURE)** in **BIOTECHNOLOGY AND CROP IMPROVEMENT** to the University of Horticultural Sciences, Bagalkot, is a record of bonafide research work carried out by her during the period of her study in this University under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associate-ship, fellowship or other similar titles.

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ACKNOWLEDGEMENT

The realization of this piece of work would not have been possible without God's grace and the contribution of many people who rendered kind help in many ways.

*It is a great privilege to express my heartfelt thanks and deep sense of regard to **Dr. Sayeed Wajeed R Mulla,** Chairman of my Advisory Committee for his inspiring guidance, unflinching support, close supervision and constant encouragement throughout the course of investigation as well as the preparation of this manuscript.*

*I express my gratitude to the members of the advisory committee **Dr. Raghavendra S.**, Assistant Professor, Department of Biotechnology and Crop Improvement**,** COH, Bagalkot., **Dr. Suma R.,** Assistant Professor, Department of Soil Science,COH Bagalkot, **Dr. Babu A. G.,** Assistant Professor, Plant Physiology, D R Office, UHS Bagalkot, **Dr.Raghavendra G..,** Assistant Professor, Department of Biotechnology and Crop Improvement**,**COH, Bagalkot.*

*I extend my sincere gratitude to the HOD **Dr. R.C. Jagadeesha**, Professor and Head, Department of Biotechnology and Crop Improvement, College of Horticulture, Bagalkot ,**Dr.Sarvamangala Chloin,** Assistant Professor, Department of Biotechnology and Crop Improvement, COH, Bagalkot, **Mrs. Rekha Chittapur,** Assistant Professor, Department of Biotechnology and Crop Improvement, COH, Bagalkot and **Dr. Bapurayagowda B. Patil,** Assistant Professor, Department of Biotechnology and Crop Improvement, COH, Bagalkot.*

*I wish to express my profound indebtedness and heartfelt thanks to **Karnataka Biotechnology and Information Technology Services (KBITS), Govt. of Karnataka,** for their support and help during my research work.*

*I wish to express with great pleasure my deep sense of gratitude and sincere thanks to **Dr. B.R. Gurumurthy,** Professor, Department of Crop Physiology, UAHS Shivamogga, for their support and help during my research work.*

*I avail myself of this opportunity to express my thanks to **Dr. H. B. Patil,** Dean, College of Horticulture, Bagalkot and **Dr. N. Basavaraj**, Dean, PGs, UHS, Bagalkot.*

I am thankful to Sarvajna library, COH, Bagalkot for providing me timely information regarding my studies.

These acknowledgments would not be complete without thanking my family for their constant support and care. Today I feel that my parent's hard work and dreams have been blossomed. I thank my father ***Sri. B. Rudrappa,*** *my mother* ***Smt. B. R. Lalitha,*** *my brothers* ***B.R. Pradeep*** *and* ***B.R. Sandeep,*** *and all relatives for their boundless love, unflagging interest and constant encouragement put confidence in me to reach this level. It's my pleasure to thank* ***Mrs. Drakshayani*** *my care taker, Banashankari girls hostel, COH, Bagalkot, for her constant help and support during my stay in hostel.*

Colourful blossoms would not have bloomed without the company of my friends Manasa C N., Hanumant M Hugar, Praveen Yadav, Gurumurthy, Akshay, my seniors Chaitra C K., Chaitra A.P., Mahesh D. and junior Divyashree N. for their moral support, guidance and help during my study.

I must thank all the scientists and staff members of the Division of Biotechnology and Crop Improvement for their co-operation in availing lab facilities during my research work.

The presentation that follows is the work assisted by many seen and unseen hands and minds, I am thankful to all of them. End is inevitable for any kind of work. Though acknowledging is an endless task, I end by saying infinite thanks to all those whom I am able to recall here and also to those whom I might have left unknowingly.

BAGALKOT
AUGUST, 2018 **(B. R. CHAITRA)**

Affectionately Dedicated

to

My Beloved Parents

Sri. B. Rudrappa, Smt. B. R. Lalitha

and brothers Pradeep and Sandeep

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1. INTRODUCTION

Banana (*Musa* sp.) is an edible fruit, botanically a berry, produced by large herbaceous flowering plants. Taxonomically banana belongs to the genus *Musa* of the family Zingiberales (Abdullah *et al.,* 2012). The genus *Musa*, which has about 40 species, is divided into five sections, Emusa, Rhodochlamys, Australimusa, Callimusa and Ingentimusa. Emusa and Rhodochlamys contains species which have 22 (n=11) chromosomes. Australimusa and Callimusa contains species which has 20 (n=10) chromosomes. Ingentimusa contains one species which has 14 (n=7) chromosomes. Emusa is the largest and most wide ranging section of genus and contains *Musa acuminate* and *Musa balbisiana*, which are principle progenitors of the most edible banana cultivars (Allen *et al.,* 1988).

India is one of the centres of origin of banana. There are four wild progenitors of domesticated banana, out of them three from section Emusa, *i.e*, *Musa acuminata* (A genome), *Musa balbisiana* (B genome) and to much lesser extent, *Musa schizocarpa* (S genome) and one from section Australimusa *i.e*, *Musa textilis* (T genome) (Bhat *et al.,* 1995). The scientific names of bananas are *Musa acuminata, Musa balbisiana* or natural hybrids between *M.acuminata*×*M.balbisiana*, depending on their genomic constitution (Purseglave *et al.,* 1972). Almost all modern edible partehnocarpic bananas comes from *M.acuminata* (AA) and *M.balbisiana* (BB). There exists a lot of diversity especially in 'B' rich genomes of north-eastern India. B genome being the source of resistance to different diseases, which is a potential candidate genome for developing resistance characters and can be used as a donor plant in banana breeding programmes (de Jesus *et al.,* 2009).

Banana ranks fourth most important food crop after rice, wheat and maize. India is the largest producer of banana in the world with 28 percent share of world's production. India has an area of 8,58,000 hectares for banana cultivation and produces around 2,97,79,000 MT of bananas every year with the average productivity of 34.7 tonnes per hectare (Crouch *et al.,* 2000). The top five banana producing countries are India, Brazil, Ecuador, China and Philippines. India is 20th biggest exporter of bananas globally due to the fact that the country also consumes bananas in great quantity (Cheesman, 1947). Tamil Nadu, Maharashtra, Gujarat, Andhra Pradesh and Karnataka

are biggest banana producing states of India, among which Maharashtra stands the largest exporting state with 4,55,730.34 MT of bananas worth 9,156.78 lakhs during 2016-17 (Brown *et al.,* 2009). Major destinations of Indian bananas are UAE, Saudi Arabia, Iran, Kuwait, Bahrain and many other middle-east countries. Major banana growing districts of Karnataka are Shimoga, Dakshin Kannada, Tumkur, Bangalore, Udupi, Uttara Kannada, Belgaum, Chikkamagalur, Hassan, Mysore and Mandya (Ashalatha *et al.,* 2005).

Banana is a fruit that is commonly available across the globe. Dessert bananas are used for making sweets like kheer, malpua, halwa, payasam, preparation of baby foods and consumed fresh. The cooking bananas are used in preparation of curries, chips and some traditional dishes (Arora, 1997). Even banana peels are used as cattle feed and also natural fertilizers. Banana plant stems are edible, healthy and rich in fibres. Stem fibres are natural craft materials or natural threads. Banana leaves can be used as natural leaf platters, long enough to serve a full meal with lots of dishes. Even banana flowers are edible that contains fibres. Banana suckers or rhizomes are mentioned in ancient Ayurveda for their medicinal properties and also they are the propagating materials (Chakravorty, 1951).

Bananas are the good source of total carbohydrates (22.85 g), calories (88.9), dietary fiber (5.9g), fat (2.75 g), proteins (3.65 g), vitamin A (144 IU), vitamin C (19.6 mg), vitamin E (0.2 mg), vitamin K (1.1 µg), B-complex vitamins, water (75 %) (Arnaud and Horry, 1997). Medicinal properties of bananas treats anemia, blood pressure, constipation, depression, heartburn, ulcers, relieves stress, manages body temperature, drives away from smoking addiction, etc. (Almajali *et al.,* 2012).

The banana production in India is hampered by various devastating biotic and abiotic stresses and also production has been seriously threatened by decreasing soil fertility and yield decline phenomena. The use of resistant cultivars is considered the most effective, economical and environmentally friendly approach to controlling diseases and pests (Ning *et al.,* 2007). Breeding programmes make use of knowledge from several scientific disciplines, including genetics, agronomy, horticulture, crop protection, biotechnology, molecular biology and many others that are an essential part of plant breeding (Nsabimana and Staden, 2005). The exploitation of plant genes and

genomes for plant breeding is now becoming better recognized. Although the genus *Musa* has benefited immensely from such studies, there is scope for greater in depth research into cytogenetic and molecular genetics of genus. The multipurpose use has attracted global attention which prompted us to conduct preliminary studies on genetic diversity in *Musa* sp. (Opara *et al.,* 2010).

Molecular markers are the important component of molecular biology which plays an important role in physical mapping, gene tagging, SNP mapping, identification of genome, allelic diversity, genetic diversity, evolutionary, taxonomical, phylogenic and genetic studies of plant sciences (Mohamad, 2006). DNA markers offer numerous advantages over conventional phenotype based markers as they are stable and detectable in all tissues regardless of growth, differentiation, development of the cell in a specific environment, pleiotropic and epistatic effects (Karamura, 1999). SSRs have proven useful for species identification, elucidation of genetic relationships of numerous plant species, and parentage testing (Nair *et al.,* 2005). SSR is a very fast way to obtain information about genetic variation and for discrimination of the genomic composition of *Musa* triploids beside their varietal identification (Nsabimana and Staden, 2006). SSR has also been used to study patterns of diversity amongst germplasm collections from different geographic regions.

Genetic variability among different varieties is the pre-requisite for evaluation of their performance in different agro climatic conditions and to assess their yield and quality attributes. Also this information on genetic architecture contributes in planning the breeding programmes for developing elite cultivars (Ortiz, 1997). Very few cultivars satisfy standards of fruit quality and clonal fidelity; thus, accurate verification of cultivar identity for checking propagation material and patent protection is important. Traditional methods for testing genetic variability in fruit crops are based on morphological or time consuming physiological assays. But the presently existing biochemical and molecular techniques are more advance to improve these fruit crops (Pillay *et al.,* 2004).

DNA fingerprinting can be used to detect parental genotypes with reduced number of progeny populations that need to be generated during hybridization. More

importantly, DNA fingerprinting techniques have been used for various applications, including but not limited to identification of species and cultivars, identification of duplication among accessions in the field and in tissue culture germplasm banks, monitoring of genetic stability in micropropagated material, selection of key markers suitable for breeding programmes, and to protect rights for newly bred cultivars (Padmesh *et al.,* 2012).

Nutritional profiling of elite cultivars was done to assess the nutritional differences between the genotypes. Nutritional profiling is the science of classifying the foods according to their nutritional composition to prevent diseases and to promote health (Argent, 1976).

Keeping these in view the present investigation was carried out entitled "DNA fingerprinting and nutritional profiling of different cultivars of banana *Musa* sp. L" with the following objectives:

1. Collection of local ecotypes of banana from different ecological/geographical regions in Karnataka.
2. Morphological and genetic diversity analysis using suitable molecular markers.
3. Nutritional Profiling of selected banana cultivars and their comparative nutritive value analysis in Rajapuri, Elakki bale, Nanjangud Rasabale and Red banana.

2. REVIEW OF LITERATURE

Musa is a genus of giant perennial herbs belonging to the Family Musaceae of the order Scitamineae. Majority of *Musa* is under the section *Emusa* which included of all edible cultivated bananas. Current breeding efforts for the improvement of bananas relay on introgression of useful genes from the wild and cultivated diploid progenitors, especially in the *B* genome, which are related to resistance to pests and diseases (Anon., 1995) and determination of the starch type, while the *A* genome is related to susceptibility to diseases (Bhat *et al.,* 1994). The genome composition of the banana showed some correlation to a grouping by use; so that bananas derived from the *M. balbisiana* genome were cooking bananas and bananas derived from the *M. acuminata* genome were dessert bananas (Creste *et al.,* 2003).

Polymorphisms of genomic DNA are very useful for tagging genetic traits and studying biological diversity among species, such as random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP). These methods have been used to investigate the genetic variability present in *Musa* germplasm (Crouch *et al.,* 1998). In our study we have used simple sequence repeats (SSR) markers, which are extensively employed in plant genetic studies using both low and high throughput genotyping approaches.

Mostly, the edible cultivated bananas and plantains are polyploidy from two species, which have been classified into 2 genome types, A-genome, a representative of *M. acuminata* and B-genome, a representative of *M. balbisiana* (Cruz *et al.,* 2007). At present, the classification of edible cultivated bananas and plantains is based on ploidy. Therefore, there is a need to study genetic diversity and relationships among the wild and cultivated diploid accessions and their natural polyploids. Molecular technology has been used to investigate the phenotype and genotype of gene expression, genetic variation, genetic diversity, genetic relationship and classification of species of flora and fauna because it provides more accurate results compared to other methods(Daniells *et al.,* 2001).

A sincere effort has been made to collect the available literature on the topic "DNA fingerprinting and nutritional profiling of different cultivars of banana *Musa sp* L." and literature on morphological diversity, molecular diversity analysis and nutritional composition analysis in banana is reviewed in this chapter and presented on the following aspects.

2.1. Morphological diversity in banana

2.2. DNA fingerprinting using different molecular markers

2.3. Nutritional profiling

2.1 Morphological diversity in banana

Robinson and Nel (1985) observed that the pseudostem height increased progressively for the cultivars William and Dwarf Cavendish with advancement of age.

William produced significantly larger leaves and leaf area index (36%) than dwarf Cavendish.

Biju and Kurien (1994) stated that the bunches were produced in Robusta , when plants produce 31 numbers of 9 functional leaves but in case of Red banana it was 36 leaves. The leaf area of third functional leaf was maximum in Robusta than in Red banana during bud differentiation.

Medhi (1994) studied the performance of 15 banana cultivars and reported that the growth parameters like pseudostem height, stem girth, shooting period was significantly more in banana cv. Athiakal and the number of leaves were the highest in Malbhog banana.

Madhusmita *et al*. (1998) found that in banana cultivar Basrai and Srimanti, the height of the plant was maximum in micro-propagated plants than the sucker propagated plant. The vegetative growth of the micro-propagated plant ceases after 300 days but it continue to 450 days in sucker propagated plants.

Ram Kumar (1999) stated that maximum pseudostem height and leaves were recorded in Athiakal while the stem girth was highest in Kathia. Baiyeri and Ortiz

(2000) reported variation in plant height, girth and sucker weight in 17 banana cultivars under Wigerian condition.

Hasan *et al*. (2002) found that banana variety like Krishna Kanthali, Red banana, Agniswara showed maximum plant vigour, plant height and girth. Largest leaves were produced in Red banana and Agniswara. He also reported that cooking Banana type-3 was short stature having large leaf area and produce 33 leaves at shooting stage.

de Jesus *et al.* (2009) studied characterization of recommended banana cultivars using morphological and molecular descriptors. The aim of the research was to characterize recommended banana varieties using qualitative morphological and molecular descriptors. Twelve genotypes were analyzed using 61 morphological descriptors where 17 were related to the plant, 24 to the bunch and 20 regarding the flower. Eighty-one molecular markers; 47 RAPD primers and 34 SSR primers were used. The morphological and molecular descriptors were efficient in the characterization and identification of specific characteristics for most of the varieties evaluated. Plant and inflorescence descriptors presented the greatest variability of characteristics that can facilitate its use for cultivar protection and registration.

Abdellatif *et al.* (2012) conducted experiment on morphological and molecular characterization of somaclonal variations in tissue culture derived plants. In this study, 40000 tissue culture-derived banana plants (vitro plants) at different growth stages, *i.e*. acclimatization, nursery and open field of banana (Musa spp.) cultivar ‘Grand Naine’ were screened for somaclonal variations using morphological investigations and molecular characterization. The total detected variants were grouped into 25 off-types (two of them died) in addition to the normal plant. Random Amplified Polymorphic DNA (RAPD) was carried out to study the differences among the normal cultivar ‘Grand Naine’ and its 23 variants using 17 arbitrary primers. Cluster analysis results revealed that ‘winged petiole’ and ‘deformed lamina’ were more related to the normal plant. However, ‘Giant plant’ and ‘weak plant’ related to each other and clustered with normal plant. According to principal coordinate analysis, most of the variants were aggregated nearly, whereas ‘variegated plant’ was separated apart from the other variants. This may reflect the genetic difference between ‘variegated plant’ and the other variants.

Aquino *et al.* (2017) studied physical, chemical and morphological characteristics of banana cultivars depending on maturation stages. The objective of this work was to morphologically characterize 15 banana cultivars and assess the physical and chemical characteristics of their fruits at two maturation stages, unripe (pre-climacteric) and ripening. The plants were evaluated regarding their pseudostem height and diameter, petiole length, leaf blade length, width and length-to-width ratio. The cultivar Ouro had fruits with lower diameter, total length, market weight and fresh weight at both stages and also firmer pulp when they were unripe. The cultivar Caru-Roxa had higher fresh fruit and pulp weights, and the cultivar Terrinha had the highest percentage of pulp dry weight percentage in unripe and ripe fruits. The cultivars Maca and Ouro had higher pulp-to-peel ratio in unripe fruits. The ripe peels had lower fresh weight and thickness and higher dry weight percentage compared to unripe peels. The fruit peel of the cultivar Marmelo had the highest fresh weight at both stages. The cultivars Marmelo and Maca had higher percentage of peel dry weight percentage at both stages. The unripe pulp had lower soluble solids. The titratable acidity in the pulp increased with ripening. The average plant height ranged from 2.25 to 6.15 m. The cultivars that had the largest pseudostem diameters had also the highest heights, except the Prata-Anã and Prata-Grauda. The cultivar and maturity stage influenced all the characteristics evaluated in fruits, except the total and market lengths, which did not vary with the ripening of fruits.

Adheka *et al.* (2018) studied the morphological diversity of plantains in the democratic republic of Congo. This work focused on the morphological characterization of plantain cultivars collected in the period 2005–2014 in 280 villages across 9 provinces of the Democratic Republic of Congo. Most of the collected cultivars were French plantains (64 out of 98), followed by False Horn (23) and Horn (10) plantains. The bunch type was the main striking difference which allows the quick separation of plantain cultivars into three main types. Other striking differences within plantain were the size of the pseudostem (giant, medium-sized and small-sized) and the bunch orientation (which was generally pendulous or subhorizontal, and rarely horizontal and erect). These three descriptors were considered as main descriptors. Other descriptors (pseudostem colour, immature fruit peel colour, fruit shape, fruit apex, fruit position, number of hands, fruit size, number of fingers per hand and flower relicts at the fruit

apex) allowed the differentiation of one cultivar from another within the same main group of bunch type, pseudostem size or bunch orientation. These descriptors are considered as secondary descriptors. Rare descriptors allowed to differentiate one cultivar from all the others in the subgroup. This approach makes the cultivar description logical and faster because it moves from general to particular characteristics, and it offers a platform for reflections on the Pan-African scale of plantain diversity.

2.2 DNA Fingerprinting

Pillay *et al.* (2000) conducted experiment on identification of RAPD markers linked to A and B genome sequences in *Musa* L. Eighty operon primers were used to amplify DNA from *M. acuminata* subsp. *burmannicoides* clone 'Calcutta 4' (AA genomes) and *M. balbisiana* clone 'Honduras'(BB genomes). Three primers (A17, A18 and D10) that produced unique genome specific fragments in the two species were identified. These primers were tested in a sample of 40 genotypes representing various genome combinations. The RAPD markers were able to elucidate the genome composition of all the genotypes.

Jordan *et al.* (2001) conducted experiment on aromatic profile of aqueous banana essence and banana fruit by gas chromatography-mass spectrometry (GC-MS) and gas chromatography-olfactometry (GC-O). Totals of 43 and 26 compounds were quantified in commercial banana essence and fresh banana fruit paste, respectively. Five new components in commercial banana essence were identified as methyl butyrate, 2, 3-butanediol diacetate, 2-hydroxy-3-methylethylbutyrate,1-methylbutyl isobutyrate, and ethyl 3-hydroxyhexanoate. A total of 42 components appear to contribute to the aromatic profile in banana. Isoamyl acetate, 2-pentanol acetate, 2-methyl-1-propanol, 3-methyl-1-butanol, 3-methylbutanal, acetal, isobutyl acetate, hexanal, ethyl butyrate,2-heptanol, and butyl butyrate had high concentrations and were most detected by GC-O panelists in the commercial banana essence. Volatile components found only in fresh banana fruit paste that were detected by aroma panelists include *E*-2-hexenal, limonene, and eugenol.

Wong *et al.* (2001) conducted experiment on genetic diversity of wild banana *Musa acuminate* Colla in Malaysia as evidenced by AFLP. AFLP was employed to distinguish among three subspecies of *Musa acuminata* (sub sp truncate and sub sp malaccensis from peninsular Malaysia and sub sp microcarpa from Borneo) and to examine whether sub sp truncata is a distinct taxon. Eight primer combinations revealed molecular markers specific for each of the three taxa. UPGMA cluster analysis showed the three taxa were distinct. Subspecies malaccensis which is endemic in peninsular Malaysia and subsp. Microcarpa which is endemic in Borneo were found to be similar to each other.

Noyer *et al.* (2005) conducted experiment on plantain diversity assessed by SSR, AFLP and MSAP (Methylation-sensitive Amplified Polymorphism) markers. Using both SSR and AFLP markers, the genetic diversity of 30 plantains constituting a representative sample of the phenotypic diversity was assessed. The results confirmed a very narrow genetic base of this cultivar group. SSR and AFLP data support the hypothesis that these cultivars may have arisen from vegetative multiplication of a single seed. MSAP were used to survey cytosine methylation status at CCGG sites in order to obtain an alternative source of diversity data. A higher degree of polymorphism was revealed allowing the classification of the samples into three clusters. No correlation was observed between the phenotypic classification and methylation diversity.

Oriero *et al.* (2006) conducted experiment on analysis of B genome derived SSR markers in *Musa spp.* This study was conducted to investigate the genetic variability between 40 *Musa* genotypes, maintained at Nusa germplasm collection of International Institute for Tropical Agriculture, Ibadan using 9 B genome derived SSR markers. The nine primers produced reproducible and discrete fragments and generated a total of 23 alleles with an average of 2.1. Cluster analysis showed clusters of diploid cultivars separate from triploid ones.

Ning *et al.* (2007) conducted experiment on genome composition and genetic diversity of *Musa* germplasm from China revealed by PCR-RFLP and SSR markers. In this study, 216 banana accessions, 184 from the National Banana Germplasm Collection of China (NBGCC) and 32 from the International Network for the Improvement of

Banana and Plantain (INIBAP), were used to determine the genome composition of banana plants in these collections and to estimate their genetic diversity. The genome composition was examined using PCR-RFLP markers. Microsatellite (SSR) markers were used to investigate the genetic variability and relationships among these banana accessions. Ten of the 47 primer pairs tested consistently produced reproducible and discrete fragments. We identified a total of 92 alleles, ranging from 5 to 15 per locus. The genetic similarity between the accessions ranged between 0.1 and 1, when estimated using Jaccard's coefficient. The UPGMA method based on genetic similarities, grouped the NBGCC accessions according to those containing the 'A' and 'B' genomes.

Mattos *et al.* (2010) conducted experiment on agronomical and molecular characterization of banana germplasm. Thirteen microsatellite primers revealed an average of 7.23 alleles, which showed high variability. A dendrogram was prepared using the Gower algorithm for the distance matrices obtained from the agronomical, physical and physicolchemical analysis of fruit and SSR markers. Adopting the average genetic divergence as the cut-off point, three clusters were found: G1, formed by the diploids 'Jaran', 028003-01 and M-48; G2, by the diploids 'Malbut' and 'Ido 110'; and G3, by 21 tri-and tetraploid accessions, including one diploid, 'Tuugia'. The triploids with the B genome 'Thap Maeo', 'Walha', 'Pacha Nadan' and 'Champa Madras' were grouped in G2. Results from this work can be used for breeding hybrids with good agronomical traits and fruit quality.

Opara *et al.* (2010) conducted experiment on analysis of genetic diversity in banana cultivars (*Musa* cvs.) from the south of Oman using AFLP markers and classification by phylogenetic, hierarchical clustering and principal component analyses. Using 12 primer combinations, a total of 1094 bands were scored, of which 1012 were polymorphic. Eighty-two unique markers were identified, which revealed the distinct separation of the seven cultivars. The results obtained show that AFLP can be used to differentiate the banana cultivars. Further classification by phylogenetic, hierarchical clustering and principal component analyses showed significant differences between the clusters found with molecular markers and those clusters created by previous studies

using morphological analysis. Based on the analytical results, a consensus dendrogram of the banana cultivars was constructed.

Lu *et al.* (2011) conducted experiment on molecular assessment of genetic identity and genetic stability in banana cultivars (*Musa* spp.) from China using ISSR markers. This study was carried out to identify of different cultivars and monitor somaclonal variations of banana during rapid mass micropropgation using Inter simple sequence repeats (ISSR) marker. DNA templates from 30 Banana cultivars were evaluated using 45 primers. Genetic diversity is analyzed by one statistical procedure: hierarchical classification. Total number of bands varied between the various cultivars from 5~9. The percentage of total polymorphism is about 85.1%. Four large group were obtained.

Abdullah *et al.* (2012) conducted experiment on genetic relationship among Musa genotypes revealed by microsatellite markers. 44 *Musa* genotypes were collected from various locations in Malaysia. The microsatellite profiles of 44 *Musa* genotypes of various origins were detected by 130 alleles at nine microsatellite loci. Genetic distances and relationships were determined with one locus designated as MaOCEN08 being most polymorphic. The highest percentage of similarity observed was 43%. This was between *Musa* sp. *cv.*Raja and *Musa sp.cv.* Mas and this indicated that these two genotypes are the closest relatives. The analysis also revealed that an unknown cultivar was 100% dissimilar from the rest of *Musa* genotypes. It was therefore concluded that all accessions in the germplasm collection are of different genotypes and none are duplicates. Seven clusters were established to group the genotype.

Changadeya *et al.* (2012) conducted experiment on molecular characterization of *Musa* L. cultivars cultivated in Malawi using microsatellite markers. Genetic diversity and relationships were assessed in 141 locally named banana cultivars growing in five different districts of Malawi at 12 microsatellite loci. High allelic diversity (174) attributable to high frequency of duplicated alleles was observed. Primers discriminating power was high with mean polymorphism information content (PIC) of 0.74. The low genetic diversity estimation could be due to loss of co-dominance by SSR loci in polyploids which leads to underestimation of allelic relationships in populations. The results also reveal that the genetic diversity of bananas from Chitina, Karonga,

Nkhata Bay, Thyolo, Mulanje, BRS local collection and BRS gene bank is not significantly different. Pooled northern region population (Chitina, Karonga and Nkhata Bay) is as genetically diverse as pooled southern region population (Thyolo, Mulanje, Local collection and Gene bank). Cluster analysis showed that most cultivars were dissimilar probably due to multiplicity of mutations generated by high rate of cultivar multiplication by farmers. However, genetic relationships among some cultivars showed some possible synonyms.

Das *et al.* (2016) conducted experiment on molecular diversity study on dessert banana genotypes (*Musa* spp.) from Odisha using ISSR markers. The present investigation was done to assess the molecular diversity present amongst the local dessert banana genotypes of Odisha along with some national released cultivars based on Inter Simple Sequence Repeats (ISSR) markers. For the present study ten ISSR primers were used to differentiate 22 banana genotypes. Total seventy six scorable fragments were obtained, out of which 36 (47.4%) were polymorphic and 39 (51.3%) were monomorphic.

Islam *et al.* (2016) conducted experiment on analysis of genetic variation between five banana fruit varieties by RAPD markers. DNA fingerprinting was conducted on five banana varieties using three RAPD primers (OPA-3, OPD-04 and OPE- 20). RAPD analysis revealed 49 scorable bands on amplification and their sizes ranged between 300bp to 1500bp. Among the 49 RAPD bands, 7 bands were monomorphic and 42 bands were polymorphic. Maximum polymorphism was found by primer OPE-20. Study revealed that all five varieties are closely related with each other in the samples studied and providing clear informations for any future genetic manipulation.

Ubi *et al.* (2016) conducted experiment on molecular and genetic diversity studies among elite cultivars of plantain (*Musa paradisiac* L.) in Southern Nigeria using RAPD and SSR fingerprints. Fourteen (14) elite plantain cultivars were characterized for molecular variability and genetic diversity using random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) markers. Out of the 21 RAPD and 15 SSR primers designed and tested, 5 RAPD and 6 SSR primers that showed distinct and fragments varying from 50 bp to 3.0kbp in size of polymorphic bands were selected and

used for molecular characterization and fingerprinting of the elite plantain cultivars. The total number of amplified bands (TNB), mean percentage polymorphism for primer (%P), mean polymorphic information content (PIC), average marker indices (MI) and mean gene diversity for RAPD primers were 47,67.14%, 0.51, 4.04 and 0.80, the respective values for the SSR assay were 59, 70.24%, 0.79, 3.74 and 0.832. The results for the combined analysis of RADP and SSR fingerprints showed 55 TNB, 65.47% polymorphism, 0.67 PIC, 3.54 MI and 0.832 gene diversity. The genetic similarity coefficient was calculated using the Jaccard coefficient. The unweighted pair group method with arithmetic averages (UPGMA)-based clustering pattern was done for the combined RAPD and SSR data. The range of genetic distance of the elite plantain cultivars was from 0.11 – 0.91. The genotypes Enugu black and Kenkwa showed lowest genetic distance (coefficient = 0.11) indicating high genetic similarity and close relationship while Bakpri and Ikpobatashowed highest genetic distance(coefficient = 0.91) indicating lowest genetic similarity and more distant relationship than other elite cultivars.

Das *et al.* (2018) studied evaluation of genetic diversity in some banana hybrids using ISSR markers. A total of 10 primers were evaluated for banana hybrids based on ISSR polymorphism. Diversity analysis carried out by using ISSR markers in some Banana hybrids revealed the confirmation of the hybridization along with the phylogenetic relationship of the hybrids with their parents. Out of the 10 primers studied, six primers exhibited scorable markers. A total of 54 markers were produced. The markers ranged from 5 to 8 in different primers. Of the 54 markers produced, 30 were polymorphic which account to 53.83 per cent polymorphism. Among the six primers studied ISSR 812 (UBC- 812) produced maximum number of polymorphic bands among the banana genotypes and are useful for genetic diversity or DNA finger printing studies.

Karunambika *et al.* (2018) conducted analysis of genetic variation in different banana varieties by RAPD. The current study was focused to study the DNA fingerprint and to assess the genetic diversity among the varieties of banana. A total of five banana cultivars were collected in and around Coimbatore. The DNA was isolated from samples and confirmed by agarose gel electrophoresis with respective markers. The dendrogram was constructed using ID advanced software D Gel DAS (Digital Gel

Documentation Analysis Software). The co-efficient ranged for Rasthali and Poovan is 0.749. Red banana and Nendran falls between 0.857. Highest co-efficient is shown by Grand Naine *i.e.*, 0.873.

2.3 Nutritional profiling

Jordan *et al.* (2001) conducted experiment on aromatic profile of aqueous banana essence and banana fruit by gas chromatography-mass spectrometry (GC-MS) and gas chromatography-olfactometry (GC-O). Totals of 43 and 26 compounds were quantified in commercial banana essence and fresh banana fruit paste, respectively. Five new components in commercial banana essence were identified as methyl butyrate, 2,3-butanediol diacetate, 2-hydroxy-3-methylethylbutyrate,1-methylbutyl isobutyrate, and ethyl 3-hydroxyhexanoate. A total of 42 components appear to contribute to the aromatic profile in banana. Isoamyl acetate, 2-pentanol acetate, 2-methyl-1-propanol, 3-methyl-1-butanol, 3-methylbutanal, acetal, isobutyl acetate, hexanal, ethyl butyrate,2-heptanol, and butyl butyrate had high concentrations and were most detected by GC-O panelists in the commercial banana essence. Volatile components found only in fresh banana fruit paste that were detected by aroma panelists include *E*-2-hexenal, limonene, and eugenol.

Arora *et al.* (2008) conducted experiment on compositional variation in β-carotene content, carbohydrate and antioxidant enzymes in selected banana cultivars. in the present study, proximate composition, carotenoids, beta-carotene and some antioxidative enzymes as well as carbohydrate content of selected Indian banana varieties were determined. Karpooravalli cultivar of banana showed the maximum accumulation of carotenoid content in the non-edible (68 μg^{-1} d.w.) portion of banana, while being the second highest in beta-carotene content (143.12 µg per 100 g). However, Red banana ranked highest in total carotenoid contents for pulp (4 $\mu g\ g^{-1}$ d.w.) and beta-carotene was estimated to be the highest in the case of peels (241.91 µg per 100 g) and in pulp (117.2 µg per 100 g). Karpooravalli cultivar of banana is also rich in carbohydrate content in terms of total starch (1786.0 $\mu g\ g^{-1}$ d.w. in peels and 544.85 $\mu g\ g^{-1}$ d.w. in pulp) and sugars (53.53 $\mu g\ g^{-1}$ d.w. in peels and 39.05 $\mu g\ g^{-1}$ d.w. in pulp). The catalase enzyme activity in these peels ranged from 5.66 to 35.57 nmol

min^{-1} mg^{-1} proteins and was found at a higher level in cultivar Poovan, while the ascorbate peroxidase showed the range of 2.25 to 6.22 μmol min^{-1} mg^{-1} proteins. The peels of cultivars Red banana and Karpooravalli are rich source of bioactive compounds, such as carotenoids (beta-carotene), antioxidative enzymes and carbohydrate contents.

Adeyemi and Oladiji (2009) conducted experiment on compositional changes in banana fruits during ripening. The mineral elements analyzed included magnesium (Mg), manganese (Mn), zinc (Zn) and cobalt (Co). Their composition was found to be 0.68, 0.66 and 0.60% in unripe, ripe and overripe banana fruits, respectively. The moisture content and ash values for the selected mineral elements were 73.47 and 0.68%; 77.19 and 0.80%; 79.22 and 0.78% in unripe, ripe and overripe banana fruits, respectively. The results showed that the nutritional composition of banana pulp was diversely affected by ripening. Changes in mineral composition varied and were not consistent with the stages of ripeness. Bananas are considered a good source of Mg in the diet, and the data obtained herein support these assertions.

Sheng *et al.* (2010) conducted experiment on investigation of dietary fibre, protein, vitamin E and other nutritional compounds of banana flower of two cultivars grown in China. Flower samples were collected and extracted according to methods of Association of Official Analytical Chemists (AOAC). Results showed that banana flowers contained abundant dietary fiber (4.96-5.74 g/100g) and proteins (1.62-2.07 g/100 g). The major amino acids are glycine, leucine, alanine, and aspartic acid. Lysine had a lowest chemical score of 64% among the essential amino acids. In both species, flowers contained a higher composition of unsaturated fatty acids (65-66%), mainly the linoleic acid, while saturated fatty acids (mainly palmitic acid) is low. The contents of vitamin E, total saponin and flavonoids were 0.87-1.07, 0.12 and 5.27–5.90 mg/100 g, respectively. This study provides a fundamental nutritional data of banana flowers which can be essential in food science.

Fungo and Pillay (2011) conducted experiment on estimation of beta carotene content of selected banana genotypes from Uganda. This study used high-performance liquid chromatography (HPLC) to determine the β-carotene content of 47 banana genotypes from the International Institute of Tropical Agriculture (IITA) germplam collection in Uganda and used a color meter to assess the correlation between pulp color intensity and β-carotene levels. There was wide variability in β-carotene levels within and among the different groups of banana studied. Banana genotypes from Papua New Guinea (PNG) had the highest levels of β-carotene with values as high as 2594.0 µg/100 g edible pulp. A positive correlation existed between pulp color intensity and β-carotene concentration. Accessions with relatively high levels of β-carotene, especially the PNG genotypes, could be deployed to regions with high vitamin A deficiency and/or be used as parents for development of vitamin dense varieties. The PNG genotypes could be useful in genetic studies related to vitamin A in banana.

Menezas *et al*. (2011) conducted experiment on chemical composition and nutritional value of unripe banana flour (*Musa acuminata*, var Nanicao). The unripe banana flour (UBF) presented a high amount of total dietary fiber (DF) (56.24 g/100 g), which consisted of resistant starch (RS) (48.99 g/100 g), fructans (0.05 g/100 g) and DF without RS or fructans (7.2 g/100 g).

The contents of available starch (AS) (27.78 g/100 g) and soluble sugars (1.81 g/100 g) were low. The main phytosterols found were campesterol (4.1 mg/100 g), stigmasterol (2.5 mg/100 g) and β-sitosterol (6.2 mg/100 g). The total polyphenol content was 50.65 mg GAE/100 g. Antioxidant activity, by the FRAP and ORAC methods, was moderated, being 358.67 and 261.00 µmol of Trolox equivalent/100 g, respectively. The content of Zn, Ca and Fe and mineral dialyzability were low. The procedure used to obtain UBF resulted in the recovery of undamaged starch granules and in a low-energy product (597 kJ/100 g).

Babu *et al.* (2012) conducted experiment on varietal impact on phytochemical contents and antioxidant properties of *Musa acuminata.* The differences in the

phytochemical composition and antioxidant properties among four different varieties of banana rasthali, karpooravalli, manjal vazhapazham (yellow) and pachai vazhapazham (green) were analysed. Taking into account the flavonoid content and, metal chelating activity the cultivar rasthali stood superior in comparison to other three varieties under investigation whereas green variety hold the highest concentration of total phenolics, free radical scavenging activity and reducing power activity. Thus the cultivars rasthali and green was considered to be more beneficial to health in terms of antioxidant potential in comparison to other two cultivars.

3. MATERIALS AND METHODS

The present investigation on "DNA fingerprinting and nutritional profiling of different cultivars of banana (*Musa sp* L.)" was carried out at College of Horticulture, Bagalkot, during 2017-18. The details of material used, methodologies adopted in this experiment are briefly explained in this chapter.

3.1 Materials

3.1.1 Location of the Experimental field

The field experiment was conducted at College of Horticulture, Bagalkot falls under agro-climatic zone-III (northern dry zone) of region-2 of Karnataka. It is situated at 16°10' Northern latitude, 75°42' East longitude and at an altitude of 542.00 meters above the mean sea level.

3.1.2 Weather and climate

Bagalkot is situated in Northern dry zone (zone-III) of Karnataka state at 16^0 10' North latitude 75^0 42' East longitudes and at an altitude of 542.00 meters above the mean sea level. Bagalkot which comes under zone-III of region-2 has benefit of both South-West and North-East monsoons. The average annual rainfall of South-West monsoons is about 360 mm, which is mostly received during June to September. The average rainfall of North-East monsoon is about 646.40 mm. The meteorological parameter during the crop season such as minimum and maximum temperature, rainfall, number of rainy days and relative humidity were recorded at meteorological observatory of the MHREC, Bagalkot and is presented in Appendix I.

3.2 Experimental details

The experiment was laid out in an randomized block design for morphological diversity with 4 treatments (Rajapuri, Elakki, Red banana and Rasabale) and 6 replications. These four treatments were also used for carrying out nutritional profiling. For molecular diversity analysis *i.e*, DNA fingerprinting, 40 local ecotypes of four genotypes (Rajapuri, Elakki, Red banana and Rasabale) were used.

3.2.1 Experimental materials

Total 40 local ecotypes of four genotypes were collected from different locations in Karnataka were included as experimental material for the molecular diversity analysis. The local ecotypes along with their data of collection are listed below in Table 1.

Table 1. List of banana local ecotypes used for the experiment

Sl.No	Ecotype	Farmer's Name	Address
1	ELK 1	Vishwanath Ningappa	Gokak, Belgaum
2	ELK 2	Aravind Hattipuradar	Savadatti, Belgaum
3	ELK 3	Suresh Kumar	Nanjangud, Mysore
4	ELK 4	Suma Bhat	K.R. Pete, Mandya
5	ELK 5	Rajanna Singayya	Nanjangud, Mysore
6	ELK 6	Shekharajappa	T.Narasipur, Mysore
7	ELK 7	Chandrashekhar Patil	Kalaghatagi, Dharwad
8	ELK 8	Abhijeet Panisai	Hospet, Bellary
9	ELK 9	Thotappa	Varuna, Mysore
10	ELK 10	B.M. Jagadeesh	K.R. Nagar, Mysore
11	RJP1	Ramesh Siddappa	Gokak, Belgaum
12	RJP2	Pradeep Pattar	Gokak, Belgaum
13	RJP3	Basappa Mallappa	Savadatti, Belgaum
14	RJP4	Ramappa Sathyappa	Gokak, Belgaum
15	RJP5	Vasanth Maruthi	Gokak, Belgaum
16	RJP6	Shreeshail Mahadev	Gokak, Belgaum
17	RJP7	Chandrashekhar Patil	Kalaghatagi, Dharwad
18	RJP8	Hanumanthappa Talwar	Savadatti, Belgaum
19	RJP9	Ramappa Adiveppa	Gokak, Belgaum
20	RJP10	Vishwanath Ningappa	Gokak, Belgaum
21	RED 1	Abhijeet Panisai	Hospet, Bellary
22	RED 2	Krishi Vigyan Kendra	Brahmavar, Uttarakannada

23	RED 3	Suraj Patil	Kamalapur, Gulbarga
24	RED 4	Sanjay Patil	Kamalapur, Gulbarga
25	RED 5	Mahesh Patil	Rajanal, Gulbarga
26	RED 6	Chandrakanth	Rajanal, Gulbarga
27	RED 7	Nagannavar Shivpujari	Rajanal, Gulbarga
28	RED 8	Basavaraj Patil	Rajanal, Gulbarga
29	RED 9	Hucchanna Biradar	Rajanal, Gulbarga
30	RED 10	Ramesh Patil	Rajanal, Gulbarga
31	RSB 1	Rajanna Singayya	Nanjangud, Mysore
32	RSB 2	Basavayya Swamy	Hospet, Bellary
33	RSB 3	Krishi Vigyan Kendra	Brahmavar, Uttarakannada
34	RSB 4	Krishnamurthy	Nanjangud, Mysore
35	RSB 5	Lakshmikanth	Nanjangud, Mysore
36	RSB 6	Thotappa	Varuna, Mysore
37	RSB 7	Venkatesh	Varuna, Mysore
38	RSB 8	Gavigowda	K.R. Nagar, Mysore
39	RSB 9	Gangadhar	Golur, Tumkur
40	RSB 10	Chandrappa	Kunigal, Tumkur

3.2.2 Preparation of the experimental plot

The land area selected for experimental field was ploughed to a fine tilth. Twenty five tonnes of farm yard manure (40 t/ha) was incorporated in the soil. The fertilizers were applied at the rate of 400:240:500 kg N:P:K/ha as per the package of practices, UHS, Bagalkot. Suckers were planted by maintaining spacing of 1.5m×1.5m. Entire field was irrigated immediately after planting.

3.3 Observations recorded

The observations were recorded from five randomly selected plants in each treatment for all characters. The mean of observations recorded on these selected five

Plate 1: General view of the experimental plot

plants was calculated and used for statistical analysis. The details of the observations recorded are given below.

3.3.1 Growth parameters

3.3.1.1. Pseudostem length (cm)

The pseudostem length was measured from ground level to the tip of fully opened leaves with the help of measuring scale and average was worked out. Height of the five randomly selected and tagged plants was measured at 30, 60, 90, 120,150,180, 210 and 240 days after planting.

3.3.1.2. Pseudostem girth (cm)

The pseudostem girth was measured above the base of the plant with the help of measuring scale and average was worked out. Girth of five randomly selected and tagged plants was measured at 30, 60, 90,120,150,180,210 and 240 days after planting.

3.3.1.3. Leaf length (cm)

Leaf length of the third leaf from the apex is taken using the measuring scale and average was worked out. Leaf length of five randomly selected and tagged plants was measured at 30, 60, 90,120,150,180,210 and 240 days after planting.

3.3.1.4. Leaf breadth (cm)

Leaf breadth of the third leaf from the apex is taken using the measuring scale and average was worked out. Leaf breadth of five randomly selected and tagged plants was measured at 30, 60, 90,120,150,180,210 and 240 days after planting.

3.3.1.5. Number of leaves per plant

The number of leaves of selected plants was counted and average was taken out at 30, 60, 90,120,150,180,210 and 240 days after planting.

3.3.2 Yield parameters

3.3.2.1. Fruit length (cm)

Length of the individual fruit was measured from the five randomly selected and tagged plants with the help of measuring scale and average was taken.

3.3.2.2. Fruit weight (g)

Individual fruit weight was measured from the five randomly selected and tagged plants with the help of weighing balance and the average was taken.

3.3.2.3. Number of fruits per hand

Total number of fruits in the individual hands were counted from the selected bunches of five randomly selected plants and the average was taken.

3.3.2.4. Number of hands per bunch

Total numbers of hands in the bunch from the selected plants were counted and the average was taken.

3.3.2.5. Weight of bunch (kg)

Total weight of the bunch from the selected plants was recorded and the average from five plants was taken.

3.3.3 Nutritional parameters

3.3.3.1. Total Carbohydrates

Estimation of carbohydrates by Phenol sulphuric acid method (de Souza *et al.,* 2011).

Materials

Phenol 5%: Redistilled (reagent grade) phenol (50g) dissolved in water and diluted to 1 lit. Sulphuric acid: 96% reagent grade. Standard Glucose: Stock – 100 mg in 100mL of water.

Working standard: 10mL of stock diluted to 100mL with distilled water.

Procedure

1. Pipette out 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard into a series of test tubes.
2. Pipette out 0.1 and 0.2 ml of the sample solution in two separate test tubes. Make up the volume in each tube to 1 ml with water.
3. Set a blank with 1 ml of water.
4. Add 1 ml of phenol solution to each tube.
5. Add 5 ml of 96% sulphuric acid to each tube and shake well.
6. After 10 min shake the content in the tubes and place in a water bath at 25-30°C for 20 min.
7. Read the color at 490 nm.
8. Calculate the amount of total carbohydrate present in the sample solution using the standard graph.

   Absorbance corresponds to 0.1 ml of the test = 'x' mg of glucose

   100 ml of the sample solution contains = $\frac{\text{'x'}}{0.1} \times 100$ mg of glucose

3.3.3.2. Reducing Sugars:

Estimation of reducing sugars by Di-nitro salicylic acid method: (De Langhe *et al.,* 2005).

Materials:

DNS reagent: Dissolve 1 g dinitrosalicylic acid, 200 mg crystalline phenol and 50 mg sodium sulphate in 100ml 1% NaOH.

40 % Rochelle salt (Potassium sodium tartarate)

Standard glucose: Dissolve 100 mg in 100 ml water.

Working standard: 10 ml stock diluted to 100 ml with distilled water.

Procedure:

1. Pipette out 0.4, 0.8, 1.2, 1.6 and 2ml of the working standard into a series of test tubes.
2. Pipette out 0.1ml and 0.2ml of the sample extract in two other test tubes (unknown).
3. Make up the volume to 2ml with water in all the test tubes. A tube with 2ml water serves as blank.
4. Pipette out 0.5 to 2ml of the extract in test tubes and equalize the volume to 2ml with water in all the tubes.
5. Add 2ml of DNS reagent.
6. Heat the contents in a boiling water bath for 5 minutes.
7. When the contents of the tubes are still warm, add 1ml of 40% rochelle salt solution.
8. Cool and read the intensity of dark red colour @ 510nm.
9. Draw a standard graph by plotting concentration of the standard on the x-axis versus absorbance on the y-axis.
10. From the graph calculate the amount of reducing sugar present in the sample tube.

3.3.3.3 Total soluble solids (TSS)

The total soluble solids were determined by using ERMA hand refractometer and expressed as ^{0}Brix.

3.3.3.4 Mineral nutrients estimation

Analysis of phosphorous, potassium, calcium, magnesium, sulphur, boron, copper, iron, manganese, zinc was carried out using Inductively Coupled Plasma Optical Emission Spectroscopy (ICPOES) at University of Agricultural and Horticultural Sciences, Shivamogga.

Procedure:

1. Sample preparation: 1g fruit sample is digested using di-acid mixture (HNO_3:$HClO_4$ =9:4) and diluted to 100ml using distilled water.
2. Standards preparation: Aliquots of ICP-OES multi-element stock standard solutions (Thermofisher) were used in preparation of calibration solutions. Working standard solutions were prepared by dilution of stock standard solutions to desired concentrations (0.5, 1, 2, 4, 8 and 10) in 10% HNO_3.
3. Feed the standards sequentially and then feed the samples using the probe.
4. Readings shown in the computer are noted (sample concentration).
5. Calculate using formula: $\frac{\text{(Sample conc. - Blank conc.)}}{\text{Sample weight}} \times \text{volume made}$

3.4 Statistical Analysis

The experimental data for various characters, recorded in the course of this investigation were subjected to statistical analysis using suitable technique for different characters. The observations recorded were subjected to statistical scrutiny. The results of the following parameters were analyzed.

3.4.1. Mean

It was calculated by using following formula: Mean= $\Sigma x/n$

Where, Σx = The sum of all the observations

n = Number of observations

3.4.2. Analysis of variance

The data based on the mean of individual plants selected for observation were statistically analyzed as described by (Dens *et al.*, 2002) to find out overall total variability present in the material under study for each character and for all the populations. The first and foremost step is to carry out analysis of variance to test the significance of differences among the populations. The skeleton of analysis of variance was used as follows:

Table 2. ANOVA for Randomized Block Design

Source of variation	D.F.	Sum of square	Mean sum of square	F value	Ft 5 % or 1 % table value
Replication	r-1	RSS	RMS	RMS/EMS	-
Genotypes	t-1	TrSS	TrMS	TrMS/EMS	-
Error	(r-1)(t-1)	ESS	EMS	-	-
Total	rt-1	TSS	-	-	-

Where, r = Number of replications

t = Number of treatments

D.F. = Degree of freedom

RSS = Replication sum of square

TrSS = Genotypes sum of square

ESS = Error sum of square

TSS =Total sum of square

RMS = Replication mean sum of square

TrMS = Genotypes mean sum of square

EMS = Error mean sum of square

A significant value of "F" test indicates that the test entries differ significantly among themselves, which requires computing.

3.5 DNA fingerprinting of banana ecotypes

3.5.1. DNA isolation from plant tissue (Doyle and Doyle, 1990)

For genomic DNA extraction, 100 mg leaf tissue was ground in morter and pestle with 500 µl of 2 per cent DNA extraction buffer [100 mMTris-HCI, 1.4 M NaCl, 30 mM EDTA, and 2 per cent (w/v) CTAB pH; 8.0]. A pinch of polyvinylpyrrolidone (PVP) and 10 µl of β-mercaptoethanol were added during grinding. Again 500 µl of 2 per cent DNA extraction buffer was added until sample was finely grinded. Then sample was taken in two micro-centrifuge tubes. Samples were incubated in water bath at 65^0C for 45 min with occasional shaking at 10 min interval. After incubation, 500 µl of chloroform: isoamyl alcohal (24:1, v/v) was added and mixed well for 10 min and centrifuged at 12,000 rpm for 10 min. Supernatant was carefully separated and transferred to fresh tube. This was subjected to again extraction in presence of chloroform: isoamylalcohal. Supernatant was collected and equal volume of isopropanol was added and kept overnight at -20^0C. Centifuge the mixture for 15 min at 12000 rpm. Supernatant was discarded and pellet was washed with 500 µl of 70 per cent of chilled ethanol. Discard the ethanol and sample was air dried and finally the pellet was dissolved in 50 µl of 10:1 TE (10 mM tris, 1 mM EDTA). Quality and quantity of DNA sample were examined by the Nano drop (Thermo Scientific). The diluted DNA samples were stored at -20^0C freezer.

3.5.2. Quantification of DNA

The DNA was quantified using Nano drop. Then the absorbance was observed at 260 nm and 280 nm. The DNA concentration was calculated using the formula,

$$\text{DNA (mg/ml)} = \frac{\text{A260} \times 50 \times \text{dilution factor}}{1000}$$

3.5.3. SSR -PCR amplification

Primers for SSR markers were collected from literature. 20 short listed primers used in this study are presented in table 3 with sequence of forward primer and reverse primer.

The following parameters were optimized for PCR analysis:

1. Template DNA concentration
2. Primer concentration
3. Primer annealing temperature

3.5.3.1 Polymerase Chain Reaction (PCR)

Diluted DNA samples were subjected to PCR amplification, using the selected SSR primers in automated thermo cycler (Eppendorf mastercycler model nexus). The total reaction volume was 10 µl containing Master mix (3.0 µl), Primer (1 µl), Template DNA (1.0µl) and double distilled water (5 µl). The tubes were firmly placed in the wells of the thermo cycler and the following temperature programme was set to perform the reaction. Reaction condition was programmed as initial hold at 94^0C for 3 min followed by 30 cycles of denaturation at 94 ^{0}C for 30s, annealing temperature of primer based on primer for 45s (Table 2.) and extension at 72 ^{0}C for 1 min, final elongation step are 72 ^{0}C for 10 min followed by holding the samples at 4 ^{0}C for 1 min. The amplification products were stored at -20^0C freezer till further analysis through agarose gel electrophoresis.

Contents	Concentrations of the contents	Volume in 10 µL reaction mixture
DNA Sample	50 ng/µl	1 µl
Master mix (dNTP's Buffer Taq)	1 mM	3 µl
	10X	
	3U/µl	
De-ionized nuclease free Water	-	5 µl
Primer	10 pM/µl	1 µl

Table 3. SSR markers used for DNA fingerprinting of 40 ecotypes of banana genotypes

Markers	Forward primers	Reverse primers	Annealing temperature (˚C)
CNPMF 21	TGAACTCTTGCTACCCCAGC	TTAGTGGCTTCTGTCCCAGG	55.5
CNPMF 26	TGGAGATGAAGAAGATCGCC	TCATCAAGTGCGTTGCATTC	55.5
CNPMF 31	AGCGGAAGAGGGTAGAGAGC	ATCTTCTGCTGGTTCATGGC	57.5
CNPMF 32	AGGCTTCGACCACAAACTCC	AGCGTTCTCGTTCCAATCAC	57.5
CNPMF 38	TCGCAAGAATCTCACCTTCC	TGGTCTTCAGGTTCCGTTTC	57.5
CNPMF 43	AAACCCTCCACCAACACCTC	GTTTGGTGCTCATTGCTGTG	55.5
CNPMF 53	GGAACACAAACACGATGCAG	TTTGCACTTTGTTCAGGCAG	57.5
MABN 01	CCACTGAAGCTGAAAGGAGG	GGATTGTAGGTGACGGGAGA	59
MABN 06	GCAACCATCAACCAAAAACC	TTTGCAAGAAAATCGTGCTG	58
MABN 08	TTACCGTAAACGGAGCCAAC	GAAATCGAGGAAAACCGACA	58
MABN 20	AAGAAGTGCAACAGATGGGC	GCCAAAGGAATCATGCTGTT	57.5
MABN 22	GTCGCAGAGATCAAGGAACC	GGACCTCCTATGTTTGCTGC	58
MABN 30	CAGCCGTTGATGTTCAAATG	CGTTACGGTGGATCGTCTTT	59
EVG 22	CCACCAAAGGGCTCCTCG	ACTCTTCTCTTCGCCTGTGG	50
MA 1/24	GAGCCCATTAAGCTGAACA	CCGACAGTCAACATACAATAC	55.5
MA 1/27	TGAATCCCAAGTTTGGTCAAG	CAAACACATGTCCCCATCTC	55.5
MaOCEN 03	GGAGGAAATGGAGGTCAACA	TTCGGGATAGGAGGAGGAG	55.5
Mb 1-100	TCGGCTGGCTAATAGAGGAA	TCTCGAGGGATGGTGAAAGA	55.5
STMS 1	TGAGGCGGGGAATCGGTA	GGCGGGAGACAGATGGAGTT	57
STMS 7	AAGAAGGCACGAGGGTAG	CGAACCAAGTGAAATAGCG	57

3.5.4. Gel electrophoresis

Amplification products were subjected to agarose gel electrophoresis in 3 per cent (W/V) agarose gel. For this 3 g of agarose was weighed and poured into a conical flask containing 100 ml of water and 1 ml of 10X TAE buffer (Tris base : Glacial acetic acid: EDTA). This was allowed to melt with frequent shaking, followed by cooling the gel to 65 ^{0}C, 6 µl of ethidium bromide (10 mg/ml) was added, mixed by gentle swirling and then poured into gel tray to solidify. Amplification products were loaded and run for 1.30 hr at fixed voltage (80 V). Banding pattern were visualized, photographed and scored on gel Doc. Along with samples 100 bp DNA ladder was loaded on each gel approximate allele size in each sample were scored in bp.

Genotype data was analyzed using Power Marker V.3.0 and phylogenetic tree was constructed using Darwin’s cluster software.

4. EXPERIMENTAL RESULTS

Genetic variability among different varieties is the pre-requisite for evaluation of their performance in different agro climatic conditions and to assess their yield and quality attributes. Also this information on genetic architecture contributes in planning the breeding programmes for developing elite cultivars. Very few cultivars satisfy standards of fruit quality and clonal fidelity; thus, accurate verification of cultivar identity for checking propagation material is important. The major concern of plant breeder is to improve the best available genotypes for various traits, which contribute to high economic yield.

The raw material on which plant breeding procedures depend is the genetic variability. If large variation is present, then there are better chances of developing improved genotypes through selection. Besides, other parameters like coefficient of variation, predicted genetic advance, heritability and correlation between various characters are considered helpful in deciding the breeding strategy. Also nutritional profiling of fruits classifies the genotypes based on their composition of nutrients and the elite cultivar among the genotypes can be identified. This becomes another effective tool for selection of cultivars. The results obtained from the present investigation "DNA fingerprinting and nutritional profiling of different cultivars of banana" are presented under the following heads.

4.1. Analysis of variance

4.2. DNA fingerprinting

4.3. Nutritional profiling

4.1 Analysis of variance

The analysis of variance was conducted to test the significance of differences among the genotypes studied. The mean sum of squares due to various sources for different characters is presented in table 3. and it revealed highly significant (P=0.01) difference among genotypes for pseudostem length at 120 and 210 DAP, pseudostem

Plate 2a. Different banana genotypes

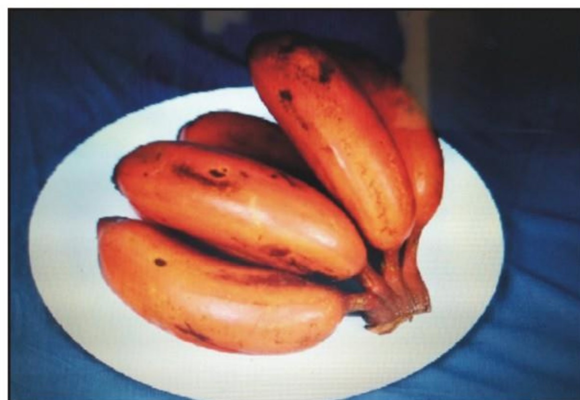


Plate 2b. Different banana genotypes

Table 4. Analysis of variance (mean sum of squares) for growth and yield parameters in banana genotypes

Source of Variance	D.F.	Pseudostem length		Pseudostem girth	
		120 DAP	210 DAP	120 DAP	210 DAP
Replications	5	2.356	4.658	0.234	0.900
Genotypes	3	120.255**	3371.588**	52.668**	72.689**
Error	15	1.347	4.95	0.319	0.629

Source of Variance	D.F.	Leaf length	Leaf breadth	No. of leaves	Fruit length
Replications	5	2.519*	0.003	0.038	0.071
Genotypes	3	113.259**	34.776**	10.362**	47.867**
Error	15	0.833	0.051	6.072	0.039

Source of Variance	D.F.	Fruit weight	No. of fruits per hand	No. of hands per bunch	Wt. of bunch
Replications	5	6.875	0.003	0.032	0.179
Genotypes	3	3162.155**	3.031**	39.473**	42.513**
Error	15	9.652	0.031	0.032	0.381

* And ** indicate significant at 5 and 1 per cent probability level, respectively

Table 5. Mean performance of banana genotypes for quantitative characters

Genotypes	Pseudostem length (cm)		Pseudostem girth (cm)		Leaf length (cm)	Leaf breadth (cm)	Number of leaves
	120 DAP	210 DAP	120 DAP	210 DAP			
Rajapuri	107.1667	195.5000	41.8000	57.7667	106.5333	43.2000	17.8000
Elakki	112.6333	233.6000	40.3333	53.8667	113.9667	47.5667	15.5000
Red banana	116.0333	239.4333	47.0000	62.1333	113.7000	48.5000	16.5000
Rasabale	106.7667	195.8333	41.5000	56.2333	106.1000	45.0667	18.4333
Mean	**110.650**	**216.091**	**42.658**	**57.500**	**110.075**	**46.083**	**17.058**
S. Em.±	**0.4737**	**0.9085**	**0.2306**	**0.3238**	**0.3726**	**0.0927**	**0.1098**
C.D.@5%	**1.4278**	**2.7384**	**0.6950**	**0.9759**	**1.1230**	**0.2794**	**0.3310**

Genotypes	Fruit length (cm)	Fruit weight (g)	Number of fruits per hand	Number of hands per bunch	Weight of bunch (kg)
Rajapuri	12.4667	81.5000	12.6667	7.5333	19.4667
Elakki	10.6000	65.3333	14.3333	12.1333	17.3333
Red banana	17.2000	118.5000	13.0667	7.9333	23.7333
Rasabale	12.4000	77.1667	13.4000	12.2000	20.3333
Mean	**13.166**	**85.625**	**13.367**	**9.950**	**20.217**
S. Em.±	**0.0803**	**1.2684**	**0.0720**	**0.0730**	**0.2521**
C.D.@5%	**0.2420**	**3.8233**	**0.2171**	**0.2201**	**0.7599**

girth at 120 and 210 DAP, leaf length, leaf breadth, number of leaves, fruit length, fruit weight, number of fruits per hand, number of hands per bunch and weight of bunch.

4.2 Range and mean performance

4.2.1. Pseudostem length (cm)

Pseudostem length showed large variation with respect to genotypes at each growth phase of 120 and 210 DAP and it is varied from 106.76 to 116.63 cm and 195.5 to 239.43 cm with overall mean performance of 110.650 and 216.091 cm, respectively. Genotype Red banana recorded highest pseudostem length 239.43 cm however, genotype Rajapuri recorded lowest pseudostem length 195.50 cm at 210 DAP.

4.2.2. Pseudostem girth (cm)

Pseudostem girth per plant ranged from 40.33 to 47 and 53.86 to 62.13 with grand mean performance of 42.65 and 57.5 cm at 120 and 210 DAP, respectively. Genotype Red banana recorded highest pseudostem girth 62.13 however, genotype Elakki recorded lowest pseudostem girth 53.86 at 210 DAP.

4.2.3. Leaf length (cm)

Highest leaf length was recorded in genotype Elakki (113.97) followed by Red banana (113.7). However lowest leaf length was observed in Rasabale (106.1). The average leaf length was 110.075 cm.

4.2.4. Leaf breadth (cm)

Highest leaf breadth was recorded in genotype Red banana (48.5) followed by Elakki (47.57). However lowest leaf breadth was observed in Rajapuri (43.2). The average leaf breadth was 46.083 cm.

4.2.5. Number of leaves

Number of leaves ranged between genotypes of Elakki recorded with the minimum 15.5 and Rasabale recorded with the maximum of 18.43, with an overall mean of 17.058 leaves.

4.2.6. Fruit length (cm)

Range of fruit length lied between the genotypes of Elakki and Red banana were recorded minimum (10.6 cm) and maximum (17.2 cm), respectively with an average mean of 13.16 cm.

4.2.7. Fruit weight (g)

Genotype Red banana (86.55 g) recorded maximum fruit weight followed by Rajapuri (81.5 g) and Rasabale (77.16 g) and it was recorded minimum in the genotype of Elakki (65.33 g). The average fruit weight was 85.62 g.

4.2.8. Number of fruits per hand

Number of fruits per hand varied from 14.33, 13.4 and 13.06 fruits was recorded in Elakki, Rasabale and Red banana respectively and lowest 12.66 fruits was recorded in Rajapuri with an overall mean performance of 13.37 fruits per hand.

4.2.9. Number of hands per bunch

Highest number of hands 12.2, 12.13 and 7.93 were exhibited in genotypes Rasabale, Elakki, Red banana receptively and lowest number of hands 7.53 was recorded in Rajapuri, with mean performance of 9.950 hands per bunch.

4.2.10. Weight of bunch (kg)

Red banana was recorded maximum bunch weight of 23.733 kg followed by Rasabale and Rajapuri *i.e.* 20.33 and 19.46 kg, respectively. Whereas, Elakki was recorded with minimum bunch weight *i.e.*17.33 kg, with an overall mean performance of 20.217 kg.

4.3 DNA fingerprinting

Assessment of genetic diversity is important for cultivar classification in plant breeding. The assessment of genetic diversity using molecular markers has been generally superior to morphological markers. The genetic diversity is commonly assessed by genetic distance with comparison of genetic similarity or dissimilarity. Genetic diversity in 40 ecotypes of four genotypes of banana was assessed using SSR

markers and a phylogenetic tree was constructed based on dissimilarity index (Creste *et al.,* 2004).

In this study banana ecotypes were analyzed using 20 SSR markers, out of 20 markers 14 markers are showed polymorphic between genotypes (Plate 3a, 3b, 3c, 3d, 3e, 3f and 3g.) and 6 primers were monomorphic hence were not considered for further analysis. 14 primers were used for genetic diversity analysis on the basis of scorable amplified bands.

The number of alleles per locus, genetic diversity and polymorphic information content (PIC) were determined by using Power marker V.3.0 and phylogenetic tree was constructed using Darwin's cluster software.

The number of alleles per locus ranged from 2 to 6 with an average of 2.35. The highest number of alleles (6) was detected in primers CNPMF 32 (Plate 2a). The polymorphic information content (PIC) value ranged from 0.19 to 0.79 with mean value of 0.31 (Table 6). The observed heterozygosity (H_0) based on polymorphic data is varied from 0.00 to 1.00 with a mean of 0.31. In order to see the inter-relationship among the banana genotypes used in this study, a phylogenetic tree was constructed from the pairwise distance matrices. Cluster analyses of 40 ecotypes fingerprinted with 20 SSR markers was done. Genetic diversity of banana genotypes were analyzed using Darwin's software with 10,000 boot straps (Fig. 2).

The dendrogram based on UPGMA cluster analysis separated the genotypes into four major clusters. The cluster I consisted of Elakki. Whereas, Rajapuri was located in cluster II, and Red banana located in cluster III. Cluster IV consisted of Rasabale.

4.4 Nutritional profiling of four cultivars (Elakki, Rajapuri, Red banana and Rasabale) of banana

Planning for nutritional profiling is helpful in evaluation, the genotype selection for future breeding programmes so as to develop nutritionally elite cultivar. Nutritional profiling is the science of classifying the foods according to their nutritional composition to prevent diseases and to promote health (Javed *et al.,* 2002). Nutritional profiling of these four elite cultivars (Rajapuri, Elakki, Red banana and Rasabale) was

Table 6. Molecular diversity of banana genotypes based on SSR markers

Marker	Major allele frequency	Number of alleles	Gene Diversity	Heterozygosity	PIC
CNPMF 32	0.25	6.00	0.81	1.00	0.79
CNPMF 38	0.38	4.00	0.72	1.00	0.67
CNPMF 43	0.50	3.00	0.59	0.25	0.51
CNPMF 53	0.75	2.00	0.38	0.00	0.30
MA1-24	0.75	3.00	0.41	0.50	0.37
MA1-27	0.50	2.00	0.50	0.50	0.38
MABN06	0.63	3.00	0.53	0.25	0.47
MABN 08	0.50	3.00	0.63	0.00	0.55
MABN 20	0.50	3.00	0.63	0.00	0.55
MABN 22	0.75	3.00	0.41	0.50	0.37
MAOCEN 03	0.50	2.00	0.50	1.00	0.38
Mb1-100	0.75	2.00	0.38	0.50	0.30
STMS 1	0.88	2.00	0.22	0.25	0.19
STMS 7	0.75	3.00	0.41	0.50	0.37
Mean	**0.72**	**2.35**	**0.35**	**0.31**	**0.31**

PIC= Polymorphism Information Content

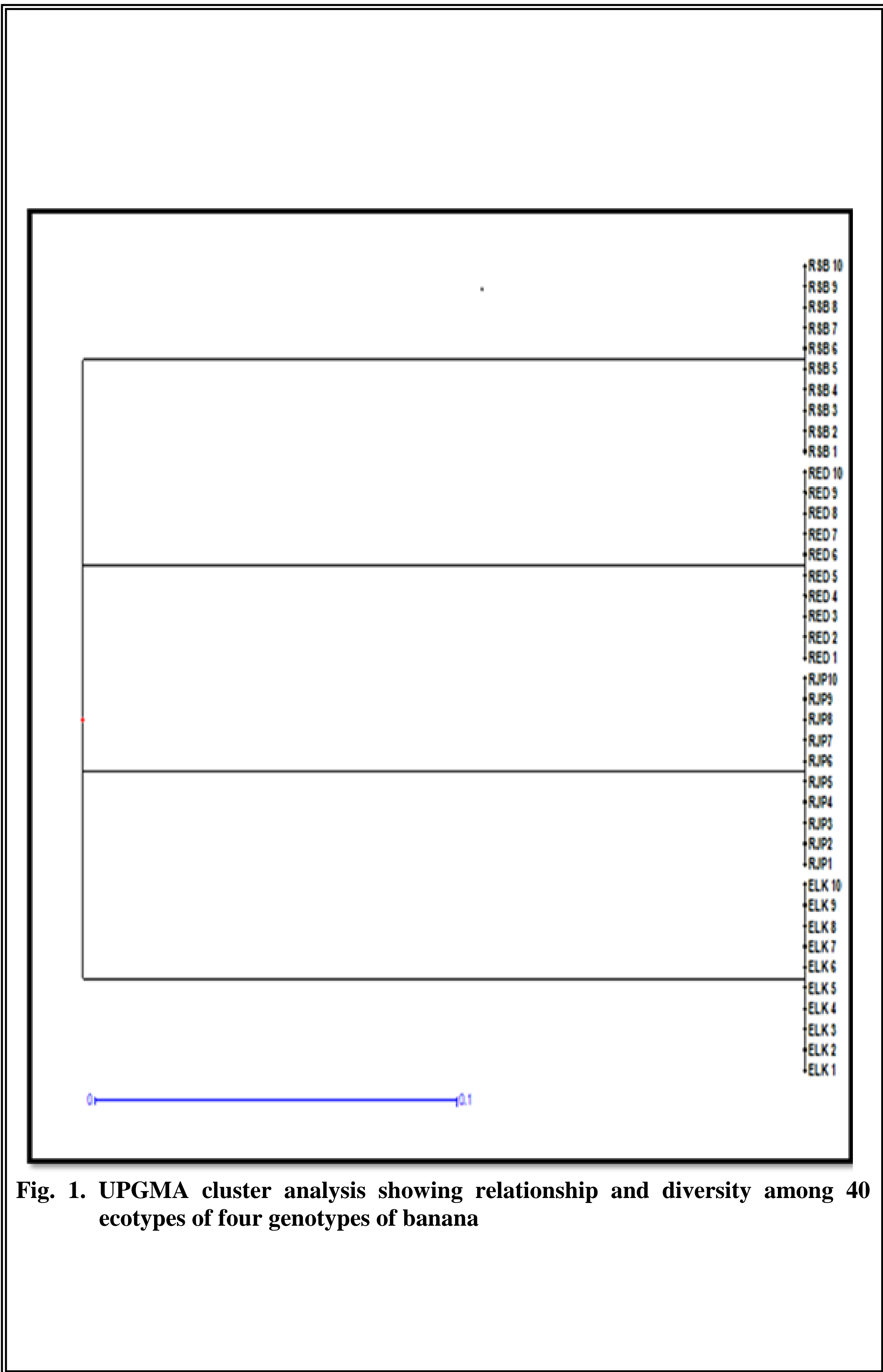


Fig. 1. UPGMA cluster analysis showing relationship and diversity among 40 ecotypes of four genotypes of banana

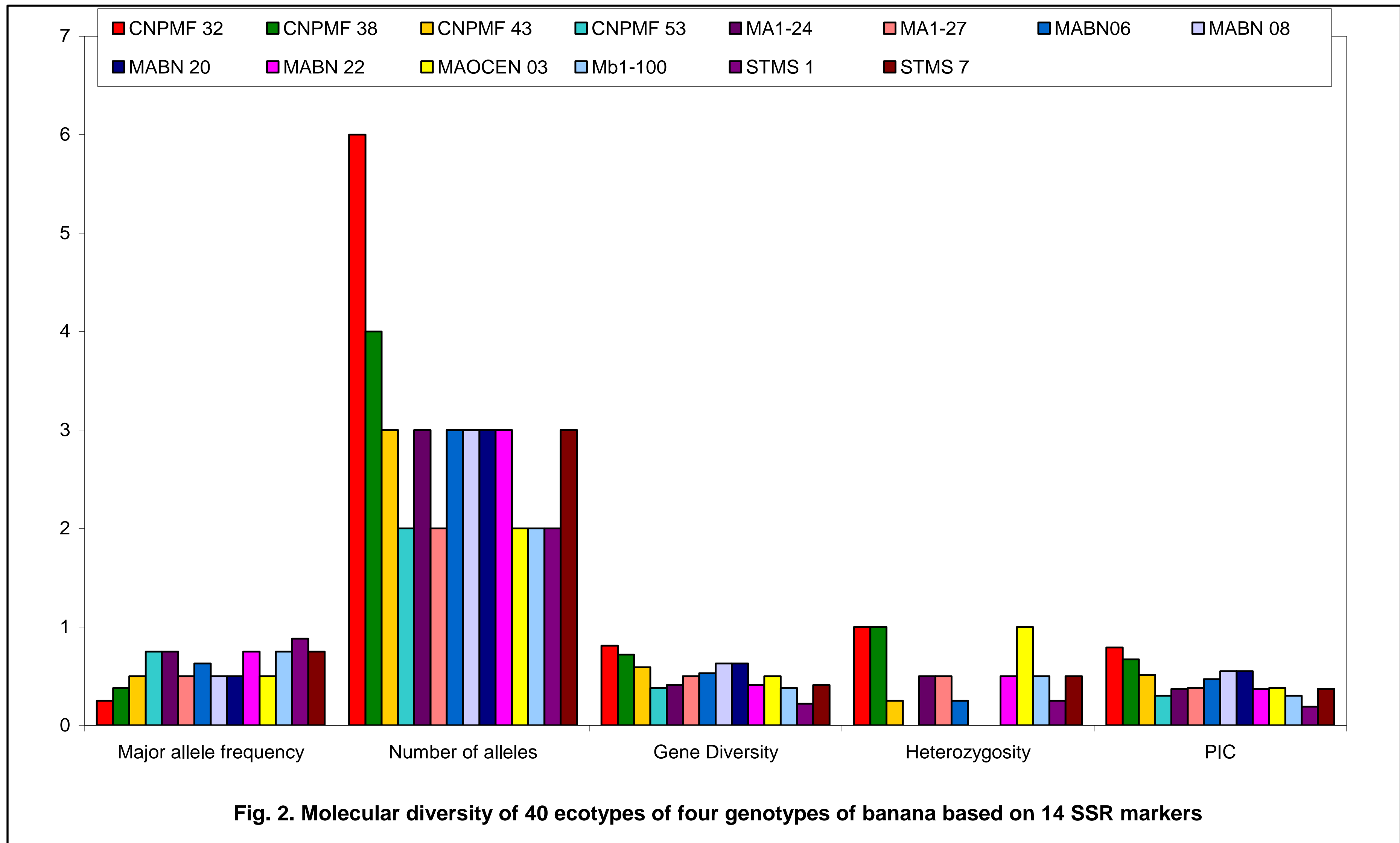


Fig. 2. Molecular diversity of 40 ecotypes of four genotypes of banana based on 14 SSR markers

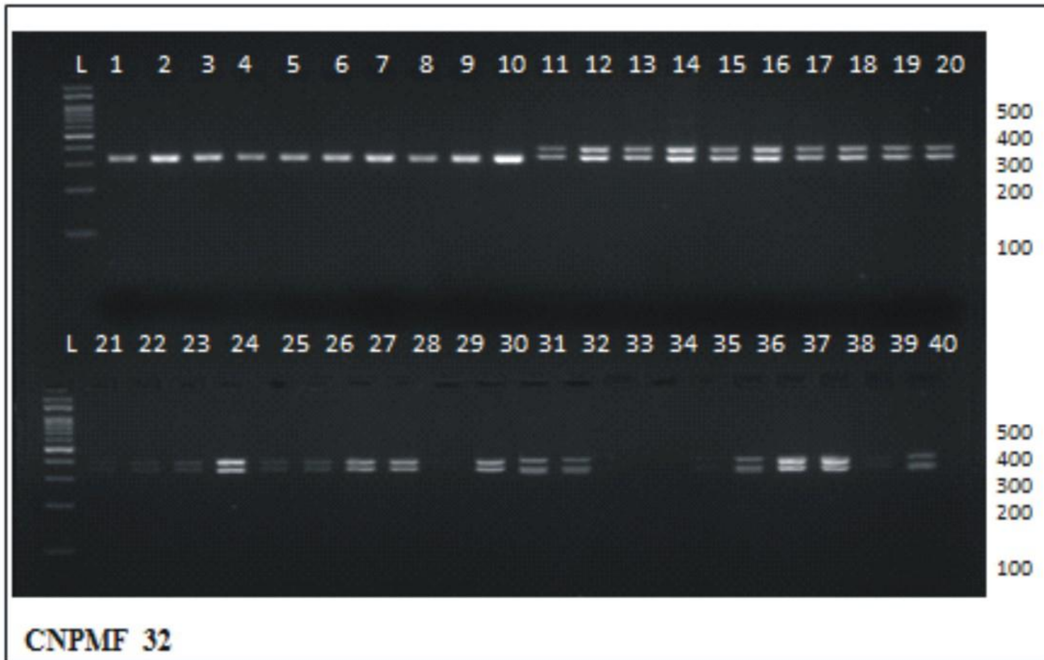


Plate 3a. Banding profile of CNPMF 32 obtained on agarose gel of banana genotypes

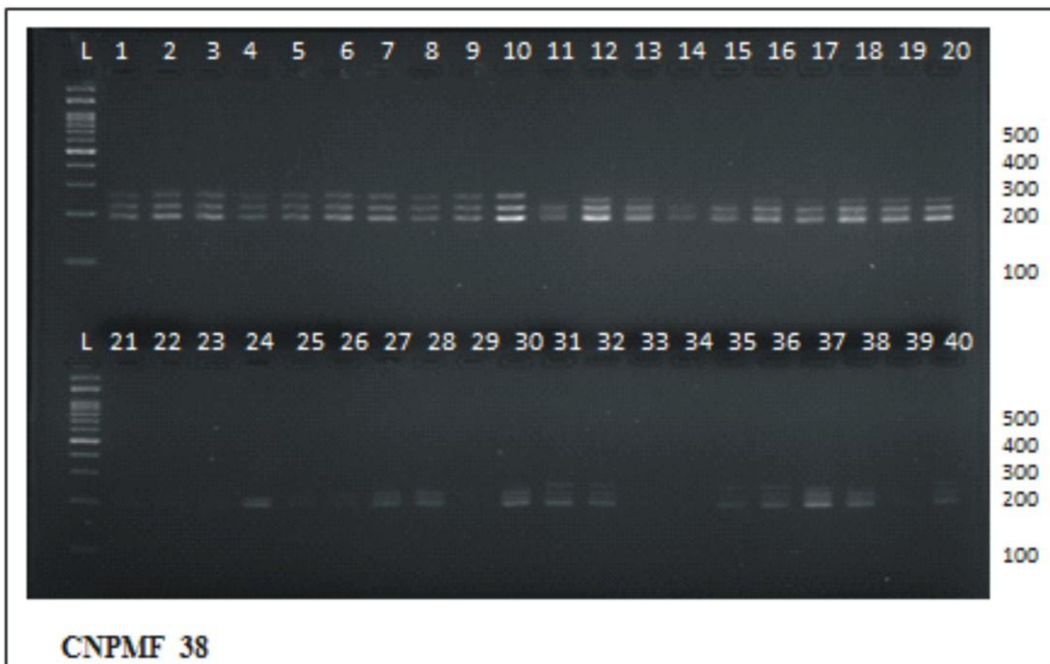


Plate 3b. Banding profile of CNPMF 38 obtained on agarose gel of banana genotypes

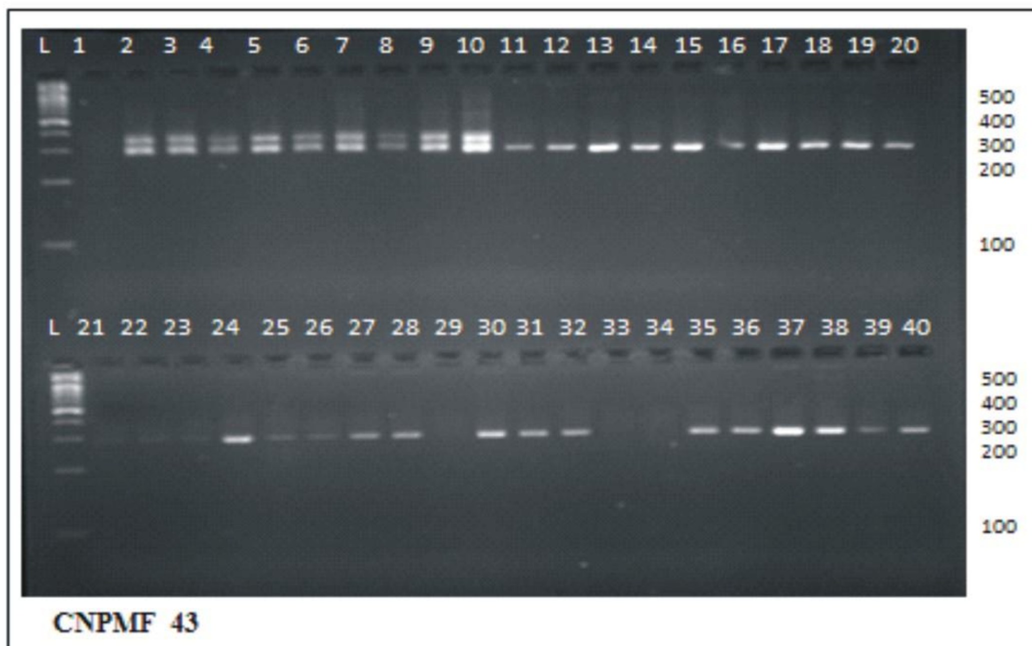


Plate 3c. Banding profile of CNPMF 43 obtained on agarose gel of banana genotypes

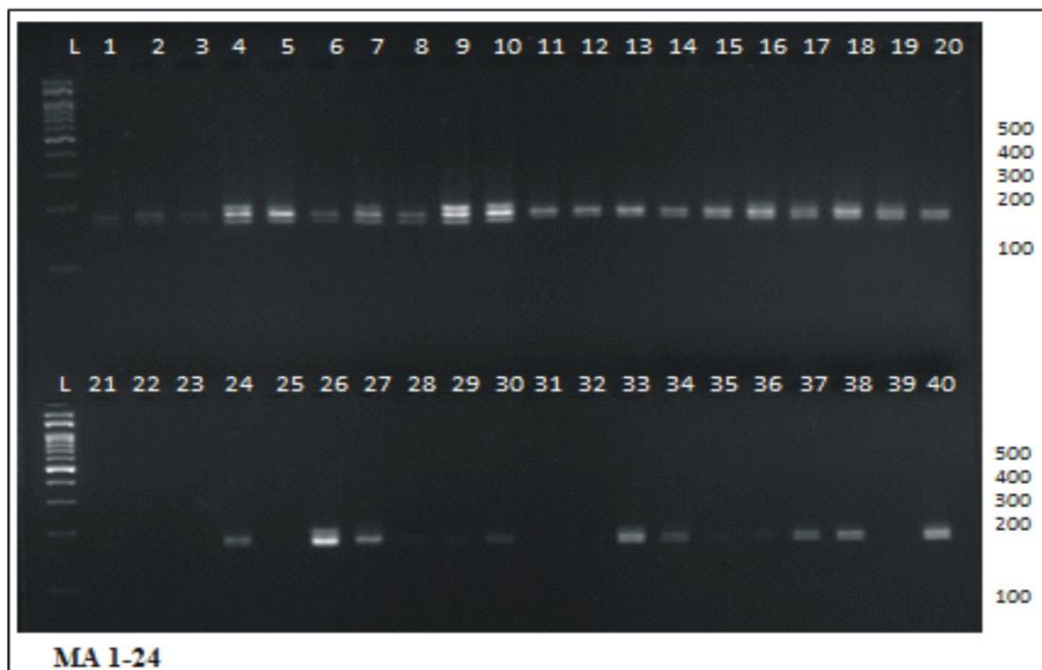


Plate 3d. Banding profile of MA 1-24 obtained on agarose gel of banana genotypes

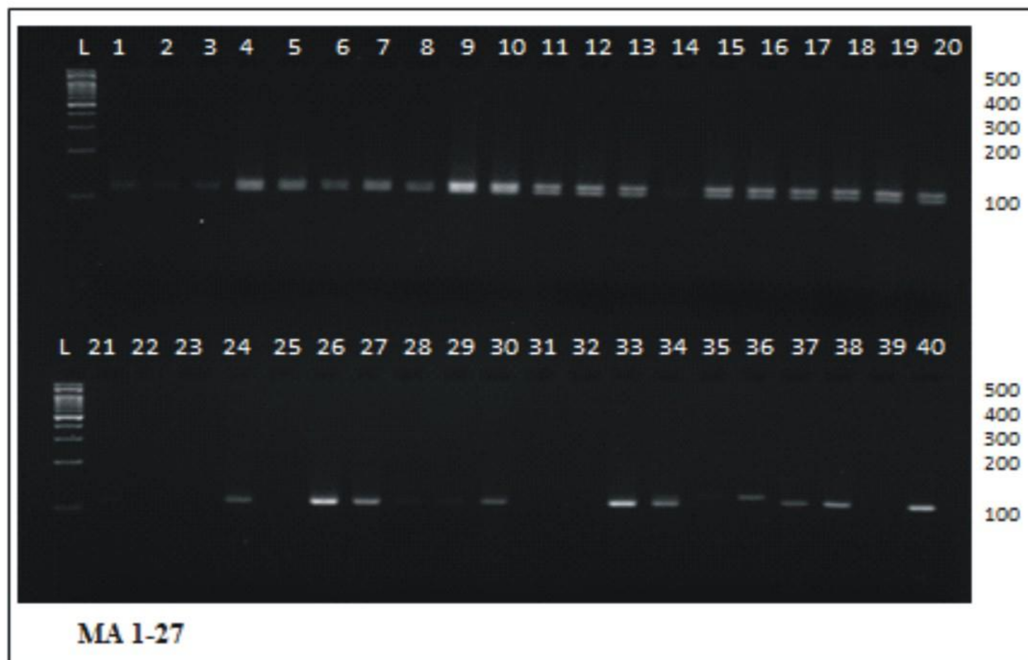


Plate 3e. Banding profile of MA 1-27 obtained on agarose gel of banana genotypes

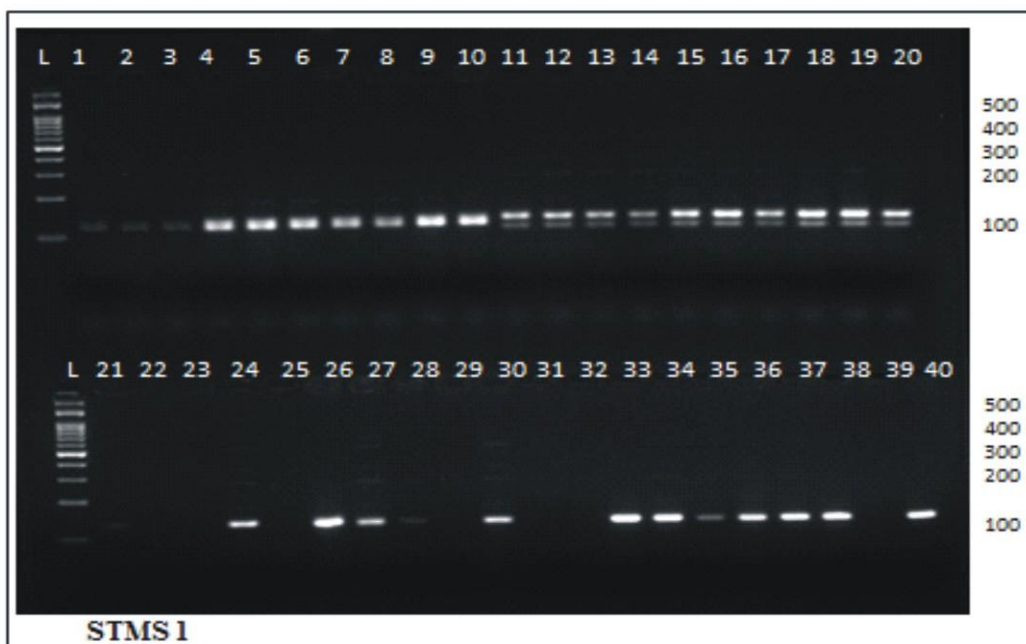


Plate 3f. Banding profile of STMS 1 obtained on agarose gel of banana genotypes

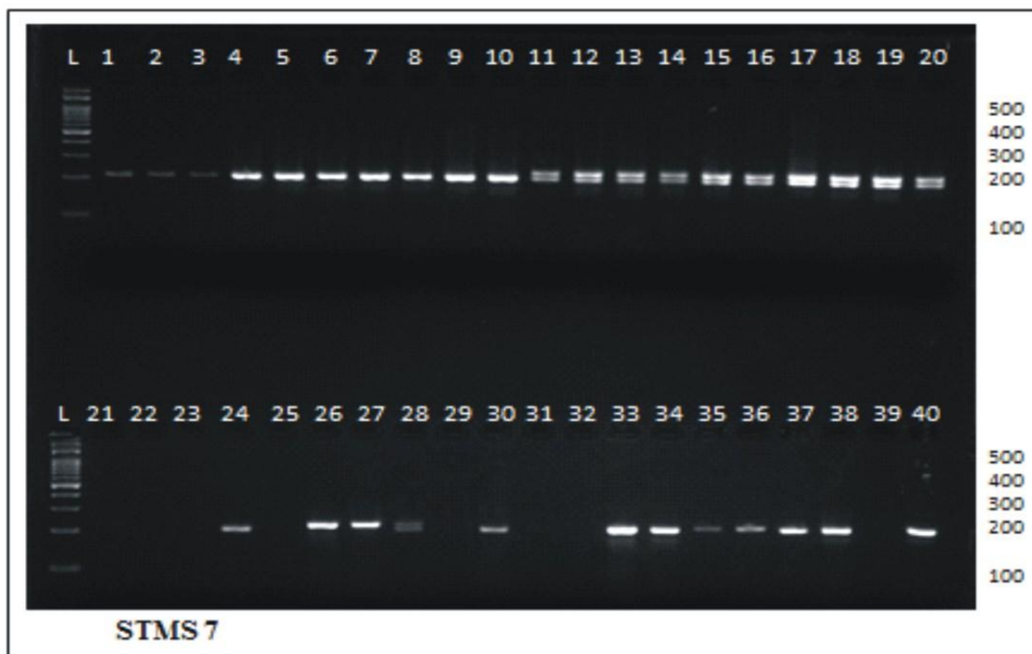


Plate 3g. Banding profile of STMS 7 obtained on agarose gel of banana genotypes

L= 100bp ladder

1 Elakki-1	11 Rajapuri-1	21 Red banana-1	31 Rasabale-1
2 Elakki-2	12 Rajapuri-2	22 Red banana-2	32 Rasabale-2
3 Elakki-3	13 Rajapuri-3	23 Red banana-3	33 Rasabale-3
4 Elakki-4	14 Rajapuri-4	24 Red banana-4	34 Rasabale-4
5 Elakki-5	15 Rajapuri-5	25 Red banana-5	35 Rasabale-5
6 Elakki-6	16 Rajapuri-6	26 Red banana-6	36 Rasabale-6
7 Elakki-7	17 Rajapuri-7	27 Red banana-7	37 Rasabale-7
8 Elakki-8	18 Rajapuri-8	28 Red banana-8	38 Rasabale-8
9 Elakki-9	19 Rajapuri-9	29 Red banana-9	39 Rasabale-9
10 Elakki-10	20 Rajapuri-10	30 Red banana-10	40 Rasabale-10

Table 7. Estimation of mineral nutrients in four genotypes of banana

Mineral Nutrient (in mg/100 g fruit)	Rajapuri	Elakki	Red Banana	Rasabale
Phosphorous (P)	82.5	92.3	105	149
Sulphur (S)	118.6	82.3	73.1	92.9
Potassium (K)	3374	1859	1759	2908
Calcium (Ca)	123	87.5	128.6	272.4
Copper (Cu)	131.2	119.2	113.8	100.7
Iron (Fe)	89.8	59.7	63.8	52.5
Magnesium (Mg)	303.24	202.59	182.14	213.38
Zinc (Zn)	9.78	6.4	11.79	6.99
Manganese (Mn)	5.75	4.22	3.72	3.61
Boron (B)	2.9	1.91	3.53	2.24

done to assess the nutritional differences between them. It consists of nutrients, including sugars, minerals, vitamins and other components.

In this study, four main genotypes, were used for estimation of minerals (phosphorous, sulphur, potassium, calcium, copper, iron, magnesium, zinc, manganese and boron), TSS, reducing sugars, non-reducing, total sugars, β-carotene and also estimation of color differences of pulp and skin during different ripening stages of banana. The results of estimation of above mentioned nutritional parameters are as follows.

4.4.1 Mineral nutrients

Phosphorous content was highest in genotype Rasabale (149), followed by Red banana (105) and Elakki (92.3) and it was lowest in Rajapuri (82.5). Rajapuri was having higher concentration of sulphur *i.e.*, 118.6, compared to Rasabale (92.9) and Elakki (82.3), however Red banana showed lower sulphur content with 73.1 mg/100 g fruit. Among four genotypes, Rajapuri was having highest concentration of potassium with 3374 mg/100g, Rasabale stood second with 2908 mg/100g, followed by Elakki (1859) and minimum potassium content was found in Red banana.

Calcium content among four genotypes was highest in Rasabale (272.4), followed by Red banana and Rajapuri *i.e.*, 128.6 and 123 mg/100g respectively. Lowest content of calcium was observed in Elakki with 87.5 mg/100g. Rajapuri was having higher concentration of copper with 131.2 mg/100g. Next to Rajapuri, Elakki and Red banana were having 119.2 and 113.8 mg/100g, respectively and Rasabale contained minimum content of copper with 100.7 mg/100g. Iron concentration among the genotypes ranged from 89.8 mg/100g, 63.8 mg/100g, 59.7 mg/100g in Rajapuri, Red banana and Elakki respectively. Lowest concentration was found in Rasabale with 52.5 mg/100g.

Rajapuri was having higher concentration of magnesium with 303.24 mg/100g, followed by Rasabale and Elakki having 213.38 and 202.59 mg/100g. Red banana was having lower concentration of magnesium compared to others *i.e*, 182.14 mg/100g. Zinc content was higher in Red banana (11.79 mg/100g), followed by Rajapuri and Rasabale having 9.78 and 6.99 mg/100g respectively. Elakki contained the minimum amount of

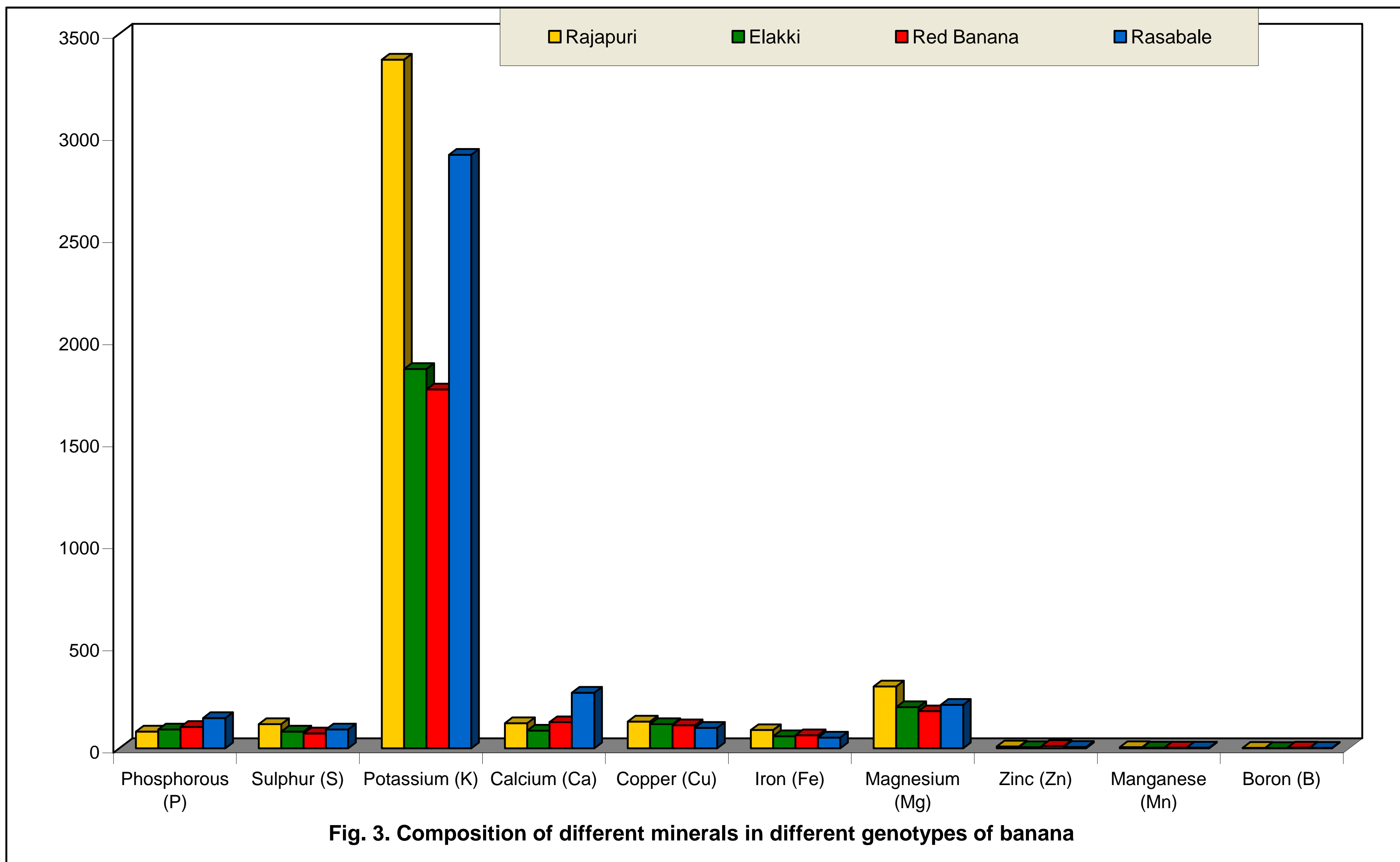


Fig. 3. Composition of different minerals in different genotypes of banana

Table 8. Estimation of TSS, sugars and β-carotene in four genotypes of banana

Parameters	Rajapuri	Elakki	Red banana	Rasabale
TSS (^{0}brix)	20.33	24.12	21.2	21.67
Reducing sugars (mg/100g fruit)	6710	8720	7280	8010
Total sugars (mg/100g fruit)	10650	12940	11060	11980
β-Carotene (mg/100g fruit)	1.9	1.7	2.34	1.76

zinc with 6.4 mg/100g concentration. Manganese content was higher in Rajapuri with 5.75 mg/100g concentration. Next came Elakki, followed by Red banana having 4.22 and 3.72 mg/100g zinc content respectively. Last was the Rasabale with lower concentration of 3.61 mg/100g. Red banana was having highest concentration of boron (3.53 mg/100g), followed by Rasabale (2.24 mg/100g) and Rajapuri (2.9 mg/100g). Lowest concentration of boron was found in Elakki (1.91 mg/100g) (table 7).

4.4.2 TSS, sugars and β-carotene

Elakki was having high TSS of 24.12^0 brix, Rasabale was having 21.67^0 brix, Red banana was having 21.2 ^{0}brix and Rajapuri was having lowest TSS *i.e*, 20.33^0 brix compared to other genotypes. Elakki contained higher concentration of reducing and total sugars *i.e.*, 8720 and 12940 mg/100g respectively. Next to Elakki, Rasabale contained 8010 and 11980 mg/100g of reducing and total sugars respectively. Red banana contained 7280 and 11060 mg/100g of reducing and total sugars respectively and the lowest concentrations were observed in Rajapuri with 6710 and 10650 mg/100g reducing and total sugars respectively.

Red banana contained large amount of β-carotene concentration with 2.34 mg/100g, followed by Rajapuri and Rasabale with 1.9 and 1.76 mg/100g and the lowest concentration was observed in Elakki with 1.7 mg/100g β-carotene.

4.4.3 Estimation of color differences of skin and pulp at different ripening stages of banana using *L**, *a** and *b** coordinates

The LAB color space (also known as CIE L*a*b* or sometimes abbreviated as simply "Lab" color space) is a color_space defined by the International_Commission on Illumination (CIE) in 1976. It expresses color as three numerical values, *L** for the lightness and *a** and *b** for the green–red and blue–yellow color components.

The space itself is a three-dimensional real-number space, allowing an infinite number of possible representations of colors. In practice, the space is usually mapped onto a three-dimensional integer space for digital representation, and thus the *L**, *a**, and *b** values are usually absolute, with a pre-defined range.

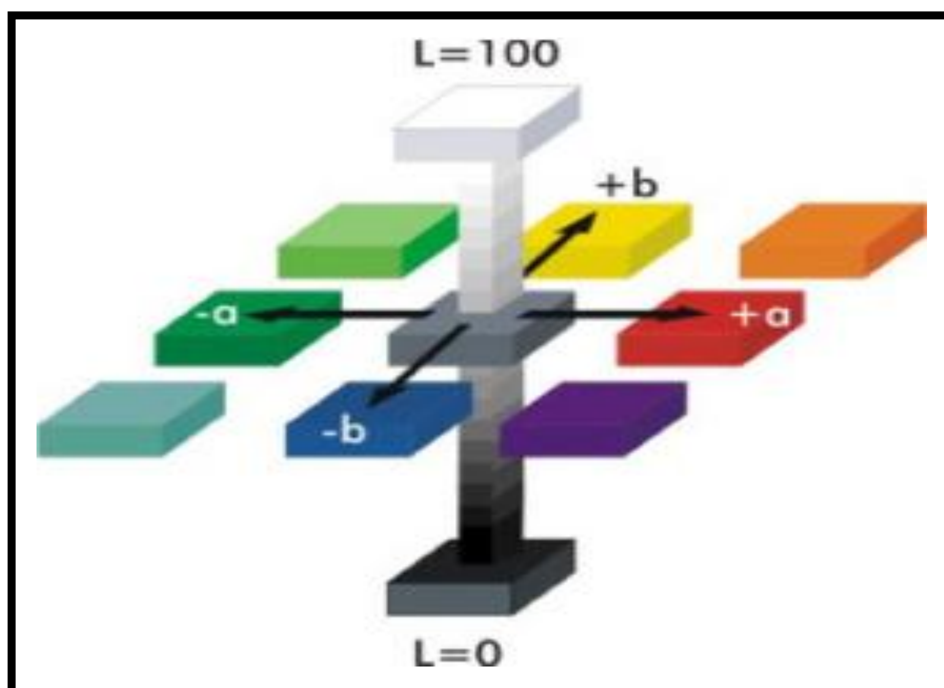
Table 9. Estimation of color differences of skin and pulp of banana genotypes at different ripening stages using L*a*b* coordinates

Genotype	First Day			Second Day			Third Day			Fourth Day		
	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*
Rajapuri Peel	55.82	-9.76	38.16	65.49	-0.28	46.86	65.01	7.47	53.86	59.71	8.5	65.10
Pulp	77.63	3.53	22.66	76.34	3.65	26.28	75.88	3.47	28.64	72.41	5.14	34.71
Elakki Peel	62.49	-9.6	33.17	67.19	-3.63	38.64	69.28	5.24	40.11	72.32	8.67	45.50
Pulp	83.24	1.52	18.89	79.96	1.88	20.46	77.89	2.08	29.92	76.9	3.96	32.63
Rasabale Peel	65.69	-5.96	41.02	67.14	1.72	55.3	75.85	4.81	64.66	86.36	5.52	68.67
Pulp	82.39	2.69	20.91	80.18	2.93	23.93	77.36	3.86	25.22	73.3	4.06	27.52
Red banana Peel	30.91	11.38	16.7	37.19	16.58	18.03	39.99	27.30	23.52	43.81	29.94	25.83
Pulp	83.83	3.18	24.73	81.32	4.43	27.36	80.91	5.08	29.92	79.98	5.65	32.37

L*= Lightness

a*= Red/green coordinate

b*= Yellow/blue coordinate



Hunter lab color space for L*a*b* coordinates

The lightness value, *L**, represents the darkest black at *L** = 0, and the brightest white at *L** = 100. The color channels, *a** and *b**, represent true neutral gray values at *a** = 0 and *b** = 0. The *a** axis represents the green–red component, with green in the negative direction and red in the positive direction. The *b** axis represents the blue–yellow component, with blue in the negative direction and yellow in the positive direction. The asterisk (*) after *L*, *a* and *b* are pronounced *star* and are part of the full name, since they represent *L**, *a** and *b**, to distinguish them from Hunter's *L*, *a*, and *b*.

In our experiment, four genotypes (Elakki, Rajapuri, Red banana and Rasabale) were analyzed for color changes from unripe to ripe stages for both peel and pulp upto four days. In Rajapuri, the peel lightness was increased from day 1(55.82) to day 4(71.71), but in case of pulp, the lightness gradually reduced (77.63, 76.34, 75.88 and 72.41), because the ripened fruits were less bright compared to unripe fruits. Greenness of peel ranged from -9.76 to 8.5, *viz*., as the fruit ripened the greenness decreased and values shifted to positive axis towards red but the peel was not red, it decreased the greenness. While we saw the pulp, there were no negative values (3.53, 3.65, 3.87 and 5.14), which showed green component in pulp was absent. b* value for both peel and pulp was showing positive values showing the yellowness. For peel, the values ranged from 38.16, 46.86, 53.86 and 65.10 and for pulp it ranged from 22.66, 26.28, 28.64 and 34.71.

In Elakki, the peel lightness was increased from day 1(62.49) to day 4(72.32), but in case of pulp, the lightness gradually reduced, because the ripened fruits were less bright compared to unripe fruits and the values ranged from 83.24, 79.96, 77.89 and 76.9. Greenness of peel ranged from -9.6 to 8.67, *viz*., as the fruit ripend the greenness decreased and values shifted to positive axis towards red but the peel was not red, it decreased the greenness. While we saw the pulp, there were no negative values (1.52, 1.88, 2.08 and 3.96), which showed green component in pulp was absent. ‘b*’ value for both peel and pulp were showing positive values showing the yellowness. For peel, the values ranged from 33.17, 38.64, 40.11 and 45.50 and for pulp it ranged from 18.89, 20.46, 29.92 and 32.63.

In Rasabale, the peel lightness was increased from day 1(65.69) to day 4(86.36), but in case of pulp, the lightness gradually reduced, because the ripened fruits were less

bright compared to unripe fruits and the values ranged from 82.39, 80.18, 77.36 and 73.3. Greenness of peel ranged from -5.96 to 5.52, *viz*., as the fruit ripened the greenness decreased and values shifted to positive axis towards red but the peel was not red, it decreased the greenness. While we saw the pulp, there were no negative values (2.69., 2.93, 3.86 and 4.06), which showed green component in pulp was absent. ‘b*’ value for both peel and pulp were showing positive values showing the yellowness. For peel, the values ranged from 41.02, 55.3, 64.66 and 68.67 and for pulp it ranged from 20.91, 23.93, 25.22 and 27.52.

In Red banana, the peel lightness was less compared to other genotypes because of red peel and increased from day 1(30.91) to day 4(43.81), but in case of pulp, the lightness gradually reduced, because the ripened fruits were less bright compared to unripe fruits and the values ranged from 83.83, 81.32, 80.91 and 79.98. Here redness of peel was measured instead of greenness, because the values of a* were higher compared to other genotypes, *viz*. greenness was absent and the values ranged from 11.38 to 29.6, *viz*., as the fruit ripened the redness increased. While we saw the pulp, there were no negative values (3.18, 4.43, 5.08 and 5.65), which showed green component in pulp was absent. ‘b*’ value for peel was less compared to other genotypes due to redness of peel (16.7, 18.03, 23.52 and 25.83) and pulp was showing highest b* value compared to other genotypes, showing the yellowness and the values ranged from 24.73, 27.36, 29.92 and 32.37.

5. DISCUSSION

The experimental findings of the present investigation "DNA fingerprinting and nutritional profiling of different cultivars of banana *Musa sp.* L" have been discussed on the following heads in light of the available literature.

5.1 Morphological variation among banana genotypes

5.2 Analysis of variance

5.3 Range and mean performance

5.4 DNA fingerprinting

5.4.1. SSR analysis

5.5 Nutritional profiling

5.5.1. Mineral nutrients

5.5.2. TSS, sugars and beta carotene

5.5.3. Color differences of skin and pulp at different ripening stages

5.1 Morphological variation among banana genotypes

Presence of genetic variability is a prime requirement in any crop improvement programme. Mean performance of the four banana genotypes indicated that Red banana was promising with respect to yield performance associated with high pseudostem length and pseudostem girth, fruit length, fruit weight and bunch weight. It is evident from the data presented in Table 4 that there were significant varietal differences in respect of growth and yield attributes. Significantly moderate pseudostem length and pseudostem girth was recorded by Elakki (233.6 cm) and Rajapuri (57.76 cm) respectively however, least pseudostem length and girth was recorded by Rajapuri (195.5 cm) and Elakki (53.87 cm) respectively. Maximum total number of leaves per plant (18.4) was noticed under Rasabale, however minimum was with Elakki (15.5). Such type of varietal variation in 73 genotypes of banana has been reported by Jamuna *et al.,* 2011.

Significantly lowest fruit length was observed with Elakki (10.6 cm) while Red banana (17.2 cm) recorded maximum fruit length. Similar results were also reported by, Jamuna *et al.* (1989), Ge *et al.* (1993) and Ning *et al.* (1994).

Maximum number of hands per bunch was recorded in Rasabale (12.2) which was significantly superior over all the varieties followed by Elakki (12.13). Significantly maximum, number of fruits per hand was recorded in Elakki (14.3) however it was least with Rajapuri (12.6). Significantly higher bunch weight was noticed with Red banana (23.73 kg) followed by Rasabale with bunch weight of 20.33 kg. The results in respect of higher bunch weight reported in Red banana may be due to maximum fruit length and fruit weight. The results obtained in respect of yield and yield attributes are in agreement with the results of Jamuna *et al.* (1989), Ge *et al.* (1993) and Ning *et al.* (1994), Ortiz (1997), Karamura (1999), Mohamad (2006), Pillay *et al.* (2004) and Padmesh *et al.* (2012).

5.2 Analysis of variance

Presence of genetic variability is a prime requirement in any crop improvement programme. The set of genotypes used in the present study indicated existence of significant differences among themselves of all the character studied, as evidenced from the analysis of variance (Table 3).

The main objective of the present investigation was to study the diversity present in four banana genotypes. The estimates of mean sum of square due to genotypes were highly significant for all the characters, indicating the presence of genetic diversity in the existing material. Similar observation has been noted by Ssali *et al.* (2016), reported the high estimates of variability for selection of important banana genotypes to achieve the good breeding material.

5.3 Range and mean performance

The mean performance of the genotypes revealed a wide range of variability for all the traits. The variation was highest for pseudostem length (195.5 – 239.43 cm.) followed by pseudostem girth (53.86 – 62.13 cm), leaf length (106.1 – 113.97 cm), leaf breadth (43.2 – 48.5 cm), number of leaves (15.5 – 18.43), fruit length (10.6 – 17.2 cm),

fruit weight (65.3 – 118.5 g), number of fruits per hand (12.6 – 14.3), number of hands per bunch (7.53 – 12.2) and bunch weight (17.33 – 23.73 kg). The findings were quite similar to as reported by Ssali *et al.* (2016).

5.4 DNA fingerprinting

Assessing the genetic diversity of germplasm based on morphological traits is the common method, which results in overlapping complex results. These morphological methods depend on the environmental conditions hence there is a chance of occurrence of variation at phenotypic expression level. Some genotypes diverse at morphological level may not at molecular level and vice-versa. Therefore, there is a need to support morphological diversity with molecular diversity in selecting genotypes from germplasm for breeding programme.

Molecular marker techniques have various applications in almost all the fields of life sciences and thus have great importance in biology. Molecular characterization of plants helps us in differentiating a particular organism from others and is useful in defining individuality of that particular organism. Characterization of cultivars through DNA fingerprinting by using different markers like RAPD, ISSR, SSR, RFLP, AFLP have used successfully in several crop species (Karamura (1999).Although a wide array of molecular marker techniques are available for the detection of the genetic variability for fruit crops. SSR technique is of choice because of its simplicity and is fastest to perform.

The present investigation demonstrated the potential use of SSR markers for assessment of genetic diversity and relationship among forty ecotypes of four genotypes of banana. The results obtained in the present study are briefly discussed after taking into consideration of the previous information available in this subject. A critical analysis of data is attempted to exhibit possible trends and draw definite conclusions with supporting evidences.

About 41 polymorphic alleles have produced by sixteen polymorphic markers for 40 banana ecotypes of four genotypes used. It indicates that, very less per cent of polymorphism present in the population it may be due to all the banana ecotypes have smaller genetic diversity. This is confirmed with the work of Ray *et al.* (2006) and

discussed the smaller genetic diversity in native bananas. In contrast to this study, Rosales *et al.* (1999) observed 130 alleles using 48 SSR markers with high level of polymorphism for 44 banana genotypes.

The use of SSR molecular markers to study the genetic diversity and relationships among the different cultivars has been previously reported in banana by Santos *et al.* (2010), Saraswati *et al.* (2011), Ray *et al.* (2006) and Rosales *et al.* (1999).

The dendrogram separated the genotypes into four clusters in such a way that each cluster having ten ecotypes of single genotype separately. It indicates that the genotypes are efficiently separated with large distinctiveness but not within the ecotypes. This may be due to the close relatedness between the ecotypes irrespective of their geographical area from where they have originated, less number of primers were used for screening to check the polymorphism within genotype and also it may be due to lack of use of different gel compositions like SDS (Sodium Dodecyl Sulphate), PAGE (Poly Acrylamide Gel Electrophoresis) etc. So this study reveals that there is polymorphism between genotypes but not within genotype.

5.4.1. SSR analysis

The number of alleles per locus ranged from 2 to 6 with an average of 2.35. The highest number of alleles (6) was detected in primers CNPMF 32. The polymorphic information content (PIC) value ranged from 0.19 to 0.79 with mean value of 0.31. On the basis of PIC value obtained in this study, the further use of CNPMF 32 SSR markers for genetic diversity analysis in banana might be advocated. Similar study was conducted by Wong *et al.,* 2001, with PIC value of 0.65 for 72 genotypes of banana using 40 markers.

5.5 Nutritional profiling

Nutritional profiling is the science of classifying the foods according to their nutritional composition to prevent diseases and to promote health (Silayoi and Chomchalow, 1987). Nutritional profiling of the four elite cultivars (Elakki, Rajapuri, Red banana and Nanjangud Rasabale) was done to assess the nutritional differences

between them. It consists of nutrients, including sugars, minerals, vitamins and other components.

In this study, above mentioned four genotypes were used for estimation of minerals (phosphorous, sulphur, potassium, calcium, copper, iron, magnesium, zinc, manganese and boron), TSS, reducing sugars, total sugars, β-carotene and also estimation of color differences of pulp and skin during different ripening stages of banana. The experimental findings have been discussed below in light of the available literature.

5.5.1 Mineral nutrients

Phosphorous content was highest in genotype Rasabale, followed by Red banana and Elakki and it was lowest in Rajapuri. The findings were similar to that of Simmonds (1962), Singh and Uma (2000) and Babu *et al.* (2012). Rajapuri was having higher concentration of sulphur, compared to Rasabale and Elakki, however Red banana was showing lower sulphur content. Among four genotypes, Rajapuri was having highest concentration of potassium, Rasabale stood second, followed by Elakki and minimum potassium content was found in Red banana. The same study was conducted by Saraswathi *et al.* (2011) in genotypes, Nendran, Monthan, and Poovan.

Calcium content among four genotypes was highest in Rasabale, followed by Red banana and Rajapuri. Lowest content of calcium was observed in Elakki. Rajapuri was having higher concentration of copper. Iron concentration among the genotypes ranged from 89.8 mg/100g, 63.8 mg/100g, 59.7 mg/100g in Rajapuri, Red banana and Elakki respectively. Lowest concentration was found in Rasabale with 52.5 mg/100g. The findings were similar to that of Swennen and Rosales (1994) in genotypes YKM-5, FHIA-3 and Cavendish.

Rajapuri was having higher concentration of magnesium with, followed by Rasabale and Elakki. Red banana was having lower concentration of magnesium compared to others. Zinc content was higher in Red banana, followed by Rajapuri and Rasabale. Elakki contains the minimum amount of zinc. Manganese content was higher in Rajapuri, next comes Elakki, followed by Red banana. Red banana was having highest concentration of boron, followed by Rasabale and Rajapuri, lowest

concentration of boron was found in Elakki. Similar findings were discussed by Singh and Uma (2000) in genotype Pisang Mas, Simmonds (1962) in cultivar Grand Naine, Jordan *et al.* (2001) in Canendish and Karpuravalli. Arora *et al.* (2008) in Kokopo.

5.5.2 TSS, sugars and beta carotene

Elakki was having high TSS compared to other genotypes, Rasabale, Red banana and Rajapuri. These findings matched the findings of Sheng *et al* (2010). Elakki contained higher concentration of reducing and total sugars *i.e.*, 8720 and 12940 mg/100g respectively. Next to Elakki, Rasabale contains 8010 and 11980 mg/100g of reducing and total sugars respectively. Red banana contained 7280 and 11060 mg/100g of reducing and total sugars respectively and the lowest concentrations were observed in Rajapuri with 6710 and 10650 mg/100g reducing and total sugars respectively. Similar study were carried out by Valasquez *et al*. (2009) in genotypes, Williams, GCTV 215, Dwarf Cavendish, Grand Naine and IC 2.

Red banana contained large amount of β-carotene concentration with 2.34 mg/100g, followed by Rajapuri and Rasabale with 1.9 and 1.76 mg/100g and the lowest concentration was observed in Elakki with 1.7 mg/100g β-carotene. The findings were in agreement to the findings of Venkatachalam *et al*. (2008), Uma *et al.* (2008), Turner *et al.* (2007) and Qin *et al.* (2011).

5.5.3 Color differences of skin and pulp at different ripening stages

The three coordinates of LAB represent the lightness of the color ($L^* = 0$ yields black and $L^* = 100$ indicates diffuse white), its position between red/magenta and green (a^*, negative values indicate green while positive values indicate magenta) and its position between yellow and blue (b^*, negative values indicate blue and positive values indicate yellow). The asterisk (*) after *L*, *a* and *b* are pronounced *star* and are part of the full name, since they represent L^*, a^* and b^*.

In our experiment, Rasabale was having higher range of peel lightness, followed by Elakki and Rajapuri and it was minimum in Red banana. However, pulp lightness was highest in Red banana followed by Elakki, Rasabale and it was minimum in Rajapuri. Coming to green to red *viz.*, a* values, greenness was observed in first two

days and as the fruit ripens the values increase. Rasabale was showing higher a* value, compare to others. But in case of Red banana the redness was seen from the first day onwards and gradually increased as the fruit ripens. a* value was higher in Rajapuri for pulp. b* values *viz*., yellowness of peel was maximum in Rasabale, followed by Rajapuri and Elakki and it was minimum in Red banana due to its redness of peel. Yellowness of pulp was highest in Red banana and it was followed by Rajapuri and Rasabale, however it was minimum in case of Elakki. The findings were in agreement to the findings of Rout *et al.* (1999) for color changes of banana skin during ripening during storage studies of banana in genotypes, Pogatau, Pongani, Wambo and Galeo.

6. SUMMARY AND CONCLUSION

The present investigation entitled "DNA Fingerprinting and Nutritional Profiling of different cultivars of banana *Musa sp.* L" carreid out during 2017-18 in College of Horticulture, UHS, Bagalkot (Karnataka). The experimental material for the present investigation is divided into two components, for DNA fingerprinting, the 40 local ecotypes of four gennotypes of banana (Rajapuri, Elakki, Red banana and Rasabale) were used. On the other hand for morphological diversity and nutritional profiling only four main genotypes (Rajapuri, Elakki, Red banana and Rasabale) were used as treatments These genotypes were planted in randomized block design with six replications. Observations were recorded on the basis of five random competitive plants selected from each genotype separately for morphological, yield and quality parameters for evaluation as per standard procedures.

The analysis of variance revealed significant difference among the genotypes and the mean performance of the genotypes revealed a wide range of variability for all the traits. The variation was highest for fruit weight followed by number of hands per bunch and individual fruit length. Genotype Red banana performed best in terms of yield, fruit length and fruit weight, followed by genotype Rasabale and Rajapuri. So these genotypes can be further exploited in selection programmes for quality and quantity.

Based on SSR marker assay, 40 banana ecotypes of four genotypes were analyzed. PIC values ranged from 0.19 to 0.79 with an average of 0.31. The number of alleles per locus ranged from 2 to 6 with an average of 2.35. The highest number of alleles (6) was detected in primers CNPMF 32. The observed heterozygosity (H_0) based on polymorphic data is varied from 0.00 to 1.00 with a mean of 0.31. It may be concluded that marker CNPMF 32 can be used as efficient marker for genetic diversity analysis in banana genotypes.

In order to see the inter-relationship among the banana ecotypes used in this study, a phylogenetic tree was constructed from the pairwise distance matrices. Cluster analyses of 40 ecotypes were finger printed with 20 SSR markers. Genetic diversity of banana genotypes were analyzed using Darwin's software with 10,000 boot straps.

The dendrogram based on UPGMA cluster analysis separated the genotypes into four major clusters but the distinctiveness between the ecotypes was absent. The cluster I consisted of all the Elakki ecotypes, whereas, Rajapuri ecotypes were located in cluster II, and Red banana ecotypes were located in cluster III and cluster IV consisted of Rasabale ecotypes. This may be due to the close relation between ecotypes of particular genotype, irrespective of their origin and utilization of less number of primers for screening the ecotypes.

Nutritional parameters like minerals, sugars, TSS and beta carotene were analyzed in four main genotypes of banana and also the difference in color changes of peel and pulp of banana fruits during different ripening stages was analyzed. Every genotype showed significant differences for their nutritional composition for different parameters.

As per the estimation of minerals, Rajapuri is having higher concentrations of sulphur, potassium, copper, iron, magnesium and manganese, compared to other genotypes. Red banana contains higher concentrations of zinc and boron, whereas, Rasabale contains maximum of phosphorous and calcium. Overall performance of genotypes in mineral nutrient estimation, suggests that genotype Rajapuri is performing better compared to other genotypes with respect to mineral nutrient composition.

During estimation of TSS, sugars and beta carotene, Elakki is having higher concentrations of reducing and total sugar and also higher TSS content. Coming to beta carotene, Red banana stands first compared to other genotypes.

When we see the differences in color changes of peel and pulp of banana during different ripening stages, lightness of peel is highest in Rasabale, whereas in pulp, it is Red banana. Greenness *viz.*, a* values are higher in Rasabale for peel and Rajapuri for pulp. Yellowness or b* values are more in case of Rasabale in peel and Red banana in pulp. We can conclude that the color change due to ripening is highly noticed in Rasabale than other genotypes.

The genetic diversity analysis has shown that variation exists between the genotypes and genotypes could be used as novel germplasm sources for further breeding and crop improvement programmes. SSR markers used in the study have

shown moderate PIC with average number of allele, so further improvement in selection of markers should be done to potentially utilize the marker assisted breeding in banana genotypes. There is a need to develop higher polymorphic markers to distinguish more closely related banana genotypes. As the banana ecotypes were highly similar, they can be further subjected to SNPs (Single Nucleotide Polymorphism) as SNPs can detect the variation at nucleotide level.

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Appendix-I: Meteorological data recorded during 2017-2018 at MHREC, UHS, Bagalkot

Month-2017	Temperature (°C)				Relative Humidity (%)				Rainy days		Rainfall (mm)	
	Maximum		Minimum		RH (%) Morning		RH (%) After Noon		Normal	2017	Normal	2017
	Normal	2017	Normal	2017	Normal	2017	Normal	2017				
January	30.0	30.0	16.0	17.0	46.0	60.0	30.0	26.5	0	0	0.0	0.0
February	33.0	33.0	18. 0	20.0	43.0	44.8	23.0	15.8	2	0	2.0	0.0
March	36.0	37.0	19.0	23.0	37.0	48.8	25.0	16.1	3	1	5.0	23.0
April	37.0	39.0	23.0	25.0	43.0	51.8	27.0	14.5	1	0	21.0	0.0
May	37.0	38.4	23.0	26.7	55.0	62.2	31.0	26.2	1	3	51.0	50.0
June	30.0	32.3	22.0	24.8	84.0	81.3	65.0	48.8	4	4	69.0	85.1
July	28.0	30.3	21.0	23.9	89.0	86.0	66.0	55.0	5	9	64.0	74.10
August	29.0	30.63	21.0	23.68	86.00	87.7	68.00	52.4	7	7	70.0	59.5
September	28.0	30.42	21.0	23.33	85.00	94.01	61.00	59.21	12	13	139.0	286.50
October	30.9	31.57	18.0	22.62	84.00	96.83	69.00	49.47	04	06	93.0	68.20
November	28.0	32.0	17.0	17.0	70.00	NA	42.00	NA	4	0	29.0	0.0
December	28.4	30.0	15.2	14.0	62.00	NA	33.00	NA	2	0	9.0	0.0
Total									**39**	**43**	**552.00**	**646.40**

Source: Automatic weather station, (Climate Resilient Hort. Promotion Centre Project), MHREC, UHS, Bagalkot

DNA FINGERPRINTING AND NUTRITIONAL PROFILING OF DIFFERENT CULTIVARS OF BANANA *MUSA sp* L.

B. R. CHAITRA **2018** **Dr. S. R. MULLA**
Major Advisor

ABSTRACT

A study entitled "DNA fingerprinting and nutritional profiling of different cultivars of banana *Musa sp* L." was conducted at the Department of Biotechnology and Crop Improvement, College of Horticulture, Bagalkot during the year 2017-18. Analysis of genetic diversity of different banana genotypes was carried out based on morphological traits and molecular markers. Around 40 ecotypes of four genotypes (Elakki, Rajapuri, Red banana and Rasabale) were collected from different geographical regions of Karnataka and some parts of Kerala and Tamil Nadu. Growth parameters like pseudostem length, girth, leaf length, breadth, number of leaves, fruit length, weight, number of fruits per hand, hands per bunch and bunch weight were observed at different growth stages. Nearly 20 SSR primers were screened, out of which 14 were polymorphic between genotypes. From the cluster analysis, dendrogram was obtained and it showed that 40 ecotypes were grouped into four clusters, each containing 10 ecotypes of same genotype. This revealed that there was close relatedness between ecotypes, which could not differentiate them irrespective of their different places of origin. Apart from genetic diversity analysis, nutritional composition of different genotypes was analyzed. Nutritional parameters like sugars, beta-carotene, TSS, minerals (P, K, Ca, Mg, S, Cu, Mn, Fe, Zn and B) and colour changes of peel and pulp during different ripening stages were carried out using L*a*b* coordinates. Elakki ranked first in sugars and TSS, Red banana in beta-carotene, boron and zinc, Rajapuri in Sulphur, Potassium, Copper, Iron, Magnesium and Manganese and Rasabale in Phosphorous and Calcium. Peel lightness was highest in Rasabale as the fruit ripened whereas, pulp lightness was highest in Red banana as the fruit ripened.

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