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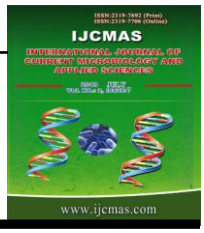

Original Research Article

Detection of Genetic Diversity of Commercial Banana Varieties Using RAPD Markers

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A B S T R A C T

The present investigation revealed that the Banana plants are dioceious and cross pollinated with each other to produce fertile hybrid revealed a closer genetic relationship. This cannot be noticed at the species level. Usually traditional methods like morphological characters/traits are not very successful in establishing the diversity and relationship among different Banana varieties because of environment influence. PCR based molecular marker method; RAPD was employed to study the genetic diversity and inter-relationships among 20 Banana varieties. On an average, RAPD analysis generated 43 discrete bands/varieties with 12 arbitrary primers. The size of the amplified products ranged from 300-5000 bp with an average of 2-4 bands per primer. Of 73 amplified fragments, 62 were polymorphic (94%) with at least one pair-wise comparison between 20 varieties. RAPD analysis identified varieties specific amplification products, which will be useful in germplasm classification and introgression studies. These results suggest that RAPD based markers are useful for genetic characterization of Banana species/ varieties.

Keywords

Genetic Diversity, Banana Varieties, RAPD Markers

Introduction

The banana is one of the oldest, the commonest and the cheapest of Indian fruits except perhaps, the Mango. In ancient literature it has been called "Fruit of the wise man". Its easy digestible qualities have made it very popular even among the working class people in recent years.Edible bananas is believed to have originated in the hot tropical regions of South East Asia (Spiden, 1926, Sauer, 1952). An examination of publication shows that the earliest cultivation was recorded from India (Reynolds, 1951). Stretching from India to Papua New Guinea and including Malaysia and Indonesia. India has the second largest diversity of indigenous banana in the world. The existence of seeded varieties such as *Musa kattuvazhana* K. C. Jacob *sp. nov.*, which occurs wild in the evergreen forests of the Western Ghats of the Madras province and *Musa balbisiana* colla, which according to Roxburgh also occurs wild in the forests of Chittagong, leads to support to the view that India is the ancestral home of the banana. Bananas are well suited for intercropping system and to mixed farming with livestocks and are also popular as a backyard crop. In mixed farming system,

banana is often used as a ground shade and nurse crop for a range of shade loving plants including Cocoa, Coffee, black pepper and nutmeg.

In India, banana is cultivated in 444.4 thousand ha with a total production of 11.83 million tones, contributing 31% of the total fruit production. India ranked second after China with a production of about 33.23 million tones. Our share in the world production of fruits is about 8% and India produces about 11% of the world bananas. Although banana occupies less than 12% of the area under fruits, it contributes nearly 32% of the total fruit production in the country. In Karnataka banana is growing in area of 39,760 ha with the total production of 11,770 tones.

The banana is one of the oldest, the commonest and the cheapest of Indian fruits except perhaps, the Mango. In ancient literature it has been called "Fruit of the wise man". Its easy digestible qualities have made it very popular even among the working class people in recent years. India is the lending producer of Banana and plantains in the world, producing 16.91 million tones from 4.97 lakhs ha. the increased production of banana is due to the adoption of many improved technologies including use of tissue culture plants, drip irrigation and fertigation. Even though our country is leading in production, to meet the requirement of the ever-growing population, we have to produce more bananas in coming years. Edible bananas originated in the Indo-Malaysian region reaching to northern Australia. They were known only by hearsay in the Mediterranean region in the 3rd Century B.C., and are believed to have been first carried to Europe in the 10th Century A.D. early in the 16th Century, Portuguese mariners transported the plant from the West African coast to South America. The types found in cultivation in the Pacific have been traced to eastern Indonesia from where they spread to the Marquesas and by stages to Hawaii. The existence of seeded varieties such as *Musa kattuvazhana* K. C. Jacob *sp. nov.*, which occurs wild in the evergreen forests of the Western Ghats of the Madras province and *Musa balbisiana* colla, which according to Roxburgh also occurs wild in the forests of Chittagong, leads to support to the view that India is the ancestral home of the banana.Banana is a major fruit crop of Karnataka. It is considered as very good source of vitamins and minerals. Contains more than 25% of sugar, which is main source of energy. It is one of the most delicious table varieties.

Differentiation of cultivars through morphological features is inefficient and inaccurate. This problem is further compounded by the medicinal value of the crop. The use of biochemical and genetic markers for identification of varieties offer a viable alternative method. Fingerprinting a vast number of banana cultivars is a significant contribution to banana cultivation, as presently several banana cultivars have many synonyms in different regions, which makes identification difficult.

As the efficiency of a selection scheme or genetic analysis based on phenotype is a function of the heritabillity of the trait, factors like environment, traits of multigenic and quantitative inheritance, or partial and complete dominance often confounded the expression of genetic traits. Many of these complications of phenotype-based assay can be overcome trough direct identification of genotypes with DNA based diagnostic assay. For this reason, DNA based genetic markers are being integrated into several plant systems and are expected to play an important role in the future plant improvement programmes.

Polymerase chain reaction (PCR) technology has led to the development of several novel genetic assays based on selective DNA amplification. A genetic assay was developed independently by two laboratories. RAPD (Random Amplified Polymorphic DNA) assay detects nucleotides sequence of polymorphisms in DNA using only a single primer of arbitrary nucleotide sequence. The protocol is also relatively quick and easy to perform and uses fluorescence instead of radioactivity. Because the RAPD technique is an amplification-based assay, only nanogram quantities of DNA are required. One of the strengths of these new assays is that they are more amenable to automation than conventional techniques. It is simple to perform and is preferable to experiments where the genotypes of a large number of individual are to be determined at a few genetic loci.

Materials and Methods

Plant Material

Leaf samples of the banana cultivars studied in this research were obtained from the orchard of Biotechnology Centre, Hulimavu, Bangalore. Genotypes were chosen arbitrarily to represent wide geographic sources, while a few cultivars were chosen on the basis of their historic and economic significance. The recently matured leaves were collected, sealed in brown paper covers and, later in the laboratory oven dried and sealed in polybags.

Sample Preparation

The leaf samples for DNA extraction were prepared according to Tai and Tanksley (1990) with some modifications. The matured leaves from trees were collected , dried at 40ºC for 48h and ground to powder by using a 'Remi' mixer for 45 to 60 sec. Powdering 5 g of leaf tissue yielded 1.5g of fine powder when passed through a 60-mesh sieve.

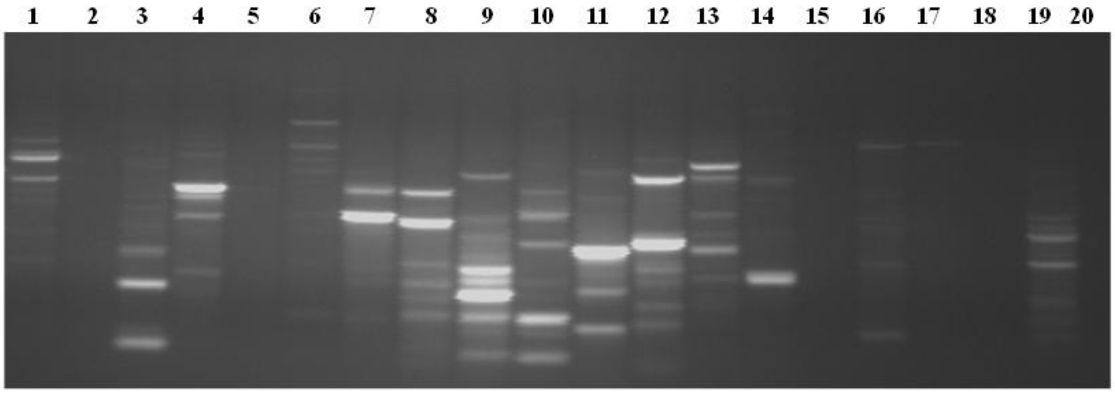
Extraction of DNA

The basis DNA extraction protocol (Dellaporta et al., 1983) was slightly modified following Porebski et al., 1997. The 500mg powdered leaf tissue was transferred to a tube containing 20ml of extraction buffer (3% CTAB, 100mM Tris, 20mM ECTA, 1.4M NaCl, 2% PVP and 1% b-mercaptoethanol) preheated to 65 ºC and maintained at this temperature for 1 h with intermittent shaking. The centrifuge tube was brought to room temperature and 6ml of Chloroform and isoamyl alcohol (24:1) were added. The contents were mixed well by inverting the tube gently 25-30 times, then spun at 6,000rpm for 15 min. the supernatant was transferred to a fresh tube and this clean-up step was repeated until a clear supernatant was obtained. Supernatant was kept overnight at 4ºC to precipitate DNA by adding half a volume of 5M NaCl and one volume of isopropanol. The DNA was pelleted by centrifuging at 10,000rpm for 20 min and the pellet was washed with 70% ethanol. The dried DNA pellet was resuspended in 1 ml TE (Tris-EDTA) buffer. Contaminating RNA was removed by digestion with 10µg of Rnase for 30 min at 37ºC. The DNA was further purified by extracting twice with an equal volume of phenol followed by an equal volume of phenol: chloroform (1:1) and finally with an equal volume of chloroform. The DNA was precipitated by the addition of one volume of isopropanol and spun at 5,000rpm for 5min. the final pellet was dissolved in 0.5ml TE. The DNA concentration was determined by using "UV-Visible spectrophotometer" and the quality verified by electrophoresis on a 1% Agarose gel.

Table Synthetic Deoxynucleotides used as Primers for Amplification of Davana DNA

Primer	Sequence	Total no. of fragments amplified
OPA-02	TGCCGAGCTG	9
OPB-08	GTCCACACGG	8
OPB-15	GGAGGGTGTT	9
OPC-08	TGGACCGGTG	7
OPC-13	AAGCCTCGTC	10
OPD-07	TTGGCACGGG	8
OPD-09	CTCTGGAGAC	10
OPD-18	GAGAGCCAAC	9
OPD-20	ACCCGGTCAC	12
OPE-04	GTGACATGCC	11
OPF-08	GGGATATCGG	10
OPJ-04	CCGAACACGG	9

+



Gel Profile of Banana varieties screened using selected primers like OPB-01 to OPB-20 series

DNA Amplification

The basic protocol reported by Williams et al., (1990) for PCR was followed with slight modifications. A single decamer of arbitrary sequence was used in each PCR reaction. With the PCR reaction conditions optimized, informative and reproducible fingerprint profiles were obtained in mango. Amplification reactions were carried out in 25µl reaction mixture containing template DNA (25ng), 5pmol of primer (Operon USA, Inc.), 2mM MgCl2, 50mM KCl, 10mM Tris-HCl and 0.1% Triton X-100. One unit of Taq DNA polymerase and 200uM of each dNTP. The mixture was overlaid with one drop of mineral oil to prevent evaporation of the reaction mixture. Amplification was performed in a thermal cycler for 42 cycles after an initial denaturation at 94ºC for 2 min. in each cycle, denaturation for 1 min at 94ºC, annealing for 1 min at 35ºC and extension for 2 min at 72ºC was programmed with a final extension step at 72ºC for 8 min after the 42nd cycle. Negative control was used initially to check the fidelity of the PCR reaction. Negative control with no template

sometimes resulted in nonspecific bands that disappeared after adding the template. For further reactions negative controls were not used.

DNA Electrophoresis

Amplified DNA fragments were separated out on 2% agarose gel stained with ethidium bromide. Running buffer containing Tris-base, boric acid and EDTA (pH 8.0) was used for electrophoresis and for preparing gels. Wells were loaded with 25ul of reaction volume and 5ul of loading buffer (sucrose and bromo-phenol blue dye) together. Electrophoresis was conducted at 50-60 V for 3 h and the gel photographed under UV light using a gel dock system.

DNA Analysis

Statistica, Ver 5.0 a computer application, was used to generate dendrogram by Wards method and genetic distances by Squared Eucledean distances. A dendrogram revealed that a maximum similarity between commercial dwarf cawendish and robusta varieties, similarly yelakki bale and Nanjanagud Rasabale coming under one group.

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