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ANALYSIS OF GENETIC DIVERSITY AMONGST BANANA CULTIVARS PREVALENT IN GUJARAT REGION OF INDIA USING ISSR MARKERS

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ABSTRACT

Banana is very important fruit for India as it is amongst top most producers in the world and also for the economic reasons. The aim of this study was to identify genetic variation amongst local hybrids to main banana cultivars by means of ISSR-based genetic analysis. For the present study seven ISSR primers were used to differentiate the six banana cultivars commonly grown in Gujarat region of India. Total fifty-six scorable fragments were obtained, out of which 39 (69.64%) were polymorphic and only 17 (30.36%) were monomorphic. Genetic similarity and genetic distance calculations were based on Nei's coefficient (1972). Maximum genetic similarity was observed between Mahalaxmi and Robusta (94.64%) and minimum genetic similarity was observed between Hill banana and Sona (50%). Based on the analytical results, a dendrogram of the banana cultivars is presented which shows close relationships among Mahalaxmi, Robusta, Williams, Sona and Grand naine cultivars as all belongs to same genome group AAA and Hill banana was placed in a separate group, as it belongs to genome group AAB.

Key words: Banana, Genetic similarity, Genetic variation, ISSR markers.

INTRODUCTION

Banana is one of the widely grown fruit in the world, both as a staple food as well as a major export commodity for many tropical and sub-tropical countries. The bananas originated in South-east Asia (Ude *et al.,* 2003; Simmonds and Shepherd, 1955). The banana includes dessert types whereas "Plantain" refers to those types that are standing on ripening and are usually cooked before consumption. Banana is an important member of the kingdom *Plantae*, family *Musaceae* and belongs to genus *Musa,* order *Zingiberales*. The global production of banana is around 76,436 thousand tons of which India contributes 24.5%. India has first position in the world in banana production; other major banana producing countries are Brazil, China, Philippines, Costa Rica and Ecuador. Banana (*Musa spp.*), is the 2nd most important fruit crop in India next to mango. In India, Gujarat ranks 3rd in banana production (Source: National Horticulture Board; http://nhb.gov.in/index.html). Its year round availability, affordability, varietals range, test, nutritive and medicinal value makes it the favorite fruit amongst all classes of people. Banana is the rich source of carbohydrates and vitamins, particularly vitamin B; it is also a good source of potassium, phosphorus, calcium and magnesium and provides good amount of energy. The fruit is easy to digest free from fat and cholesterol. Banana powder is used as the first baby food. It helps in reducing risk of heart disease when used regularly and is recommended for patients suffering from high blood pressure, arthritis, ulcer, gastroenteritis, and kidney disorders.

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Beside these it has great medicinal value. The central core of pseudo stem is used as vegetable in South India which was believed to neutralize the ill effect of stones, hair pieces etc., taken in with the food (Madhava Rao, 1984; Amalraj *et al.,* 1993).

One of the common sources for inducing genetic variability in banana plants is somaclonal variation. Somaclonal variation has been considered as a rapid and reliable approach for improvement of plants as the somaclonal variation can be used either directly or indirectly in a breeding program for crop improvement (Jain, 2000). On the basis of phenotypic characters, it is always not possible for a molecular breeder to identify the correct banana species, true hybrids or clonally propagated plants. So, a reliable and more authentic system is required by using the current DNA marker technology. The Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR) are DNA-based marker techniques that has been successfully used to determine genetic diversity and relationships *Musa* germplasm (Kaemmer *et al.,* 1992; Howell *et al.,* 1994; Bhat and Jarret, 1995; Crouch *et al.,* 2000; Jain *et al.,* 2007; Racharak and Eidathong, 2007; Ruangsuttapha*et al.,* 2007; Agoreyo*et al.,* 2008; Brown *et al.,* 2009) and for genome identification (Howell *et al.,* 1994; Pillay*et al.,* 2000), analysis of *Musa* breeding populations (Crouch *et al.,* 1999), detection of somaclonal variants (Grajal-Martin *et al.,* 1998), and genetic stability (Harirah and Khalid, 2006; Ray *et al.,* 2006; Lakshmanan *et al.,* 2007; Venkatachalam *et al.,* 2007; Ying et al., 2011). Among the molecular markers, Inter Simple Sequence Repeats (ISSR) marker have proven to be simple, fast, cost effective, and versatile set of markers that repeatable amplification of DNA sequences using single primers (Zietkiewicz *et al.,*1994). ISSR (Inter Simple Sequence Repeat) is a PCR (Polymerase Chain Reaction) based DNA marker in which DNA fragments get amplified using polymerase chain reaction. In ISSR markers, the microsatellite repeats used as primers can be di-, tri-, tetra- or penta- nucleotides. The primers used can be either unanchored (Gupta *et al.*, 1994; Meyer *et al.*, 1993; Wu *et al.*, 1994) or more usually anchored at 3' or 5' end with 1 to 4 degenerate bases extended into the flanking sequences.

In Arbitrary primed PCR techniques like ISSRs use longer primers (15–30 bp) as compared to RAPD primers (10 bp), which permit the subsequent use of high annealing temperature leading to higher stringency. Each band corresponds to a DNA sequence delimited by two inverted microsatellites. Like RAPDs, ISSRs markers are quick and easy to handle, but they seem to have the reproducibility of SSR markers because of the longer length of their primers. In the present study, the primary objectives were to use ISSR markers to examine the genetic diversity amongst banana cultivars prevalent in Gujarat region of India.

MATERIALS AND METHODS

Plant materials

For the present study a total of 6 banana cultivars of AAA and AAB genome subgroup were collected from Sun Agrigenetics Pvt Ltd. Vadodara and are popular banana cultivars grown in Gujarat state. Plants were maintained at Xcelris Labs Ltd, Ahmedabad **(Table-1)**.

Table-1: List of plant materials used in the present study.

S. No.	Banana cultivars Name	Cultivar group
1	Mahalaxmi	AAA group
2	Robusta	AAA group
3	Hill banana	AAB group
4	Williams	AAA group
5	Sona	AAA group
6	Grand Naine	AAA group

DNA extraction

For the DNA extraction leaf samples were washed and surface sterilized. Total genomic DNA was extracted by modified CTAB (Cetyl trimethyl ammonium bromide) method described by Saghai-Maroof et al., (1984). DNA quantity was determined by Nanodrop 8000 Spectrophotometer (Thermo Scientific) using ND-8000 V2.0.0 software. Integrity of DNA was evaluated by electrophoresis on

0.8% agarose gel and observed in gel-documentation system (Bio-Rad) using Quantity One software. DNA samples were diluted with appropriate amount of Tris-EDTA (10:1) buffer to yield a working concentration of 50 ng/µl.

ISSR assay

ISSR assay was performed with screened di- and tri- nucleotide repeats ISSR primers. Polymerase chain reaction (PCR) was carried out in a volume of 25 µl containing 1 X Reaction buffer with 2.0 mM $MgCl_2$, 10 pM primer, 200 µM each of deoxynucleotides (dNTPs), 1 unit of *Taq* polymerase, and 50 ng of genomic DNA. The PCR amplification was carried out as described in **table-2**.

Table 2: PCR conditions used for ISSR amplification.

Step	Temperature (°C)		Duration
Initial denaturation	94		5 minutes
Denaturation	94		30 seconds
Annealing	44	X 35 cycles	45 seconds
Extension	72		90 seconds
Final extension	72		20 minutes
Hold	4		Infinite

Amplified products along with external size standard were separated in a horizontal gel electrophoresis unit using 2.0 % agarose gel in 1 X TAE (Tris base acetic acid EDTA) buffer at 110 volt for one and half hours (run 2/3 of gel) and stained with ethidium bromide (10 mg/ml). The banding pattern was visualized under UV (Ultraviolet) light and photographed using a Gel Documentation System (Bio-Rad). The number and intensity of monomorphic and polymorphic bands were recorded. The PCR was repeated at least twice in order to check reproducibility. The amplification profiles of those primers which produced amplification with all samples only were used in the final analysis.

Data analysis

The amplification products were scored across the lanes comparing their respective molecular weights. Each band was treated as one ISSR marker. Scoring of bands was done from gel photographs. Homology of bands was based on distance of migration in the gel. The ISSR bands were scored as 1 for present or 0 for absent across the genotypes and only those bands which were well defined and consistently reproducible in two independent amplifications were included in the final analysis. All clear and intense bands were scored for the construction of the data matrix. The data were scored in a excel sheet and was converted manually in a text format for POPGENE software. POPGENE version 1.32 (Yeh *et al.*, 1999) was used to calculate all genetic parameters including genetic distances (GD) and genetic identity (GI) for all possible population pairs as per Nei's co-efficient (1972). Cluster analysis was performed using the Unweighted Pair Group Method with Arithmetic mean (UPGMA). Dendrograms were constructed using the UPGMA algorithms in the MEGA 4.0 software (Tamura *et al.*, 2007).

RESULTS AND DISCUSSIONS

Banana is the most important fruit for India. The most important banana cultivars belongs to the genome group AAA of *Musa acuminate,* commonly refer to 'Cavendish' group. Mahalaxmi, Robusta, Williams, Sona and Grand naine cultivars belongs to this group and developed through somaclonal variation. Somaclonal variation has been used to improve the horticultural traits of banana cultivars for last more than 20 years, such as plant stature, bunch size and the length of growing cycle etc. (Hwang and Tang, 1999). Other cultivar used in present study, Hill banana belongs to genome group AAB. DNA-based markers provide precise information on genetic diversity and identification of variety-specific markers because of the independence of the confounding effects of environmental factors (Powell *et al.*, 1995). In the non-availability of sequence based markers, random primers such as RAPD, ISSR and AFLP markers are the marker of choice. ISSR primers are semi-arbitrary, has more reproducibility than RAPD, which screens various regions of the genomic DNA.

Genetic Diversity analysis

The selected 7 primers generated 56 bands and the size of amplification products ranged from 100bp to 2Kb (**Figure 1 and 2**). The number of bands generated per primer were ranged from 4 (primer 17898B) to 12 (primer HB14). Out of 56 bands generated, 39 bands (69.64%) were polymorphic and 17 bands (30.35%) were monomorphic**.** The percentage of polymorphism per primer ranged from 42.85% to 85.7%. The primer which showed the maximum number of polymorphic bands was primer 834 (6 bands out of 7) and the minimum number of polymorphic bands was primer 814 (3 bands out of 7). In our study the polymorphic bands per primer and total number of bands per primer were 5.57 and 8 bands per primer, respectively with 7 primers which was very close to Rout *et al.*, 2009 as they found 4 polymorphic bands per primer and 9 total numbers of bands per primer with 15 ISSR primers.

Figure 1: ISSR marker profiles of six banana cultivars generated by primer 841. L-External DNA Ladder, 1-Mahalaxmi, 2-Robusta, 3-Hill banana, 4-Williams, 5-Sona, 6-Grand Naine.

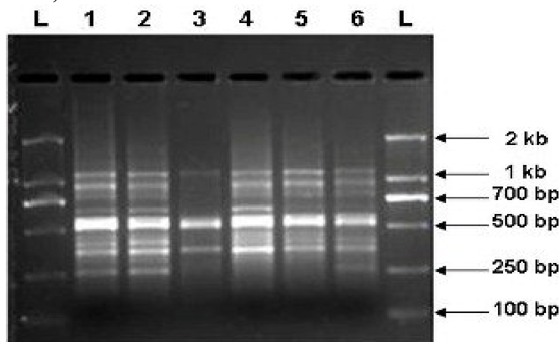
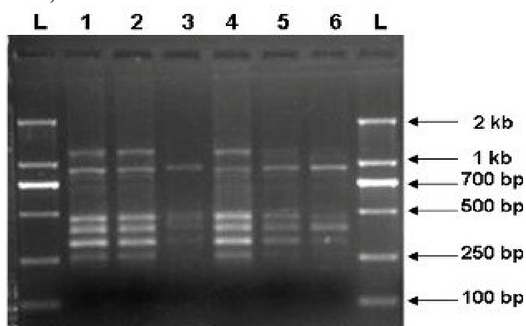


Figure 2: ISSR marker profiles of six banana cultivars generated by primer 814. L-External DNA Ladder, 1-Mahalaxmi, 2-Robusta, 3-Hill banana, 4-Williams, 5-Sona, 6-Grand Naine.



The mean Nei's genetic diversity (1973) across all loci was found low to moderate (25.4%) and Shannon Information Index was about 38.1%. This is mainly due to selection of less number of primers used in the present study. The amplification profiles are given in **Table-3**.

Table 3: Genetic diversity parameters for ISSR markers.

Primers	Primer sequence	Total No of bands	No of monomorphic bands	No of polymorphic bands	% Polymorphism
814	$(CT)_8TG$	7	4	3	42.85
834	$(AG)_8CTT$	7	1	6	85.71
841	$(GA)_8CTC$	10	3	7	70
HB14	$(CTC)_3GC$	12	3	9	75
HB10	$(GA)_6CC$	10	2	8	80
ISSR5	$(AC)_8TG$	6	3	3	50
17898B	$(CA)_6GT$	4	1	3	75
Total		**56**	**17**	**39**	**69.64**
Average		**8**	**2.43**	**5.57**	**68.36**

Similarity analysis

Similarity analysis was calculated using POPGENE version 1.32 using Nei's coefficient **(Table-4).** Banana cultivars Mahalaxmi and Robusta shows maximum genetic identity and minimum genetic distance whereas cultivars Hill banana and Sona shows minimum genetic identity and maximum genetic distance.

Table 4. Nei's Original Measures of genetic similarity (above diagonal) and genetic distance (below diagonal) as per Nei 1972 for ISSR analysis.

Population ID	Mahalaxmi	Robusta	Hill banana	Williams	Sona	Grand naine
Mahalaxmi	*****	0.9464	0.5714	0.8750	0.7143	0.6429
Robusta	0.0551	*****	0.5179	0.9286	0.7321	0.6607
Hill banana	0.5596	0.6581	*****	0.5536	0.5000	0.5714
Williams	0.1335	0.0741	0.5914	*****	0.8036	0.6607
Sona	0.3365	0.3118	0.6931	0.2187	*****	0.7500
Grand naine	0.4418	0.4144	0.5596	0.4144	0.2877	*****

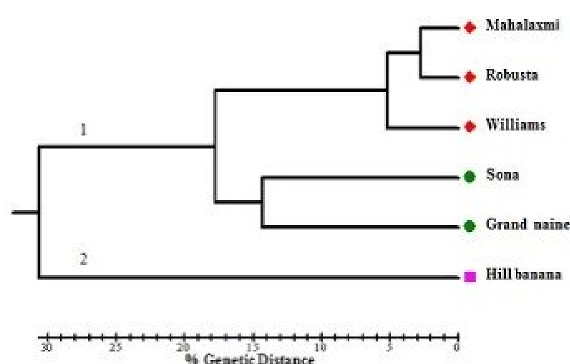
ISSR analysis carried out by Rout *et al.,* 2009 for banana showed genetic similarity of 0.67 between Grand naine and Robusta which was almost similar to the results of ISSR analysis carried out by us for the same species that is 0.6607. In another study carried out by Abdullah *et al.,* 2009 for different cultivars of banana using RAPD markers showed genetic similarity of 0.58 between Grand naine and Willliams hybrid which was found close to the our result of 0.6607 with the same pure cultivars of Grand naine and Williams done by ISSR markers. In both the cases genetic

similarity was calculated by Nei's similarity co-efficient.

Clustering analysis

Phylogenetic tree among 6 banana plant cultivars were constructed based on Nei's co-efficient (1972) and standard genetic distances using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method (**Figure 3**). The genetic distance values for 6 banana samples ranged from 0.0551 to 0.6931. The lowest genetic distance (0.0551) was observed between Mahalaxmi and Robusta, while the highest genetic distance (0.6931) was observed between Hill banana and Sona. From ISSR data the phylogenetic tree generated shows about 69.5% similarity in all cultivars of bananas. At 69.5% genetic similarity, the phylogenetic tree was divided into 2 major groups. Mahalaxmi, Robusta, Williams, Sona and Grand naine were clustered into same group (group-1) as all belongs to same genome constitution AAA and are clones of 'Cavendish'. The variation among all five cultivars is mainly due to somatic mutation. Hill banana was placed in a separate group (group-2), as it belongs to genome group AAB.

Figure: 3. Phylogenetic tree based on Nei's (1972) standard genetic distances using UPGMA method generated for ISSR data.



In conclusion, the research demonstrated ISSR markers to be useful tool to detect DNA polymorphisms to examine genetic relationships on the basis of genome constitution.

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