International Journal of Agriculture, Environment and Biotechnology
Citation: IJAEB: 9(4): 513-523 August 2016
DOI Number: 10.5958/2230-732X.2016.00068.1

Molecular diversity study on dessert banana genotypes (*Musa* spp.) from Odisha using ISSR markers

S. Swain[1], S. Das[2]*, P.S. Munsi[4], P.C. Lenka[1] , G.R. Rout[3] and D. Swain[3]

[1]Department of Fruit Science, Orissa University of Agriculture & Technology, Bhubaneswar, Odisha, India
[2]Department of Plant Breeding & Genetics, Orissa University of Agriculture & Technology, Bhubaneswar, Odisha, India
[3]Department of Agril. Biotechnology, Orissa University of Agriculture & Technology, Bhubaneswar, Odisha, India
[4]Department of Horticulture, Visva-Bharati, West Bengal, India

*Corresponding author: swarnalata1967@rediffmail.com

Paper No. 471 Received: 23-1-2016 Accepted: 14-8-2016

Abstract

Banana is an important fruit of India and in Odisha it is also a highly demanded fruit. Banana improvement programme mainly depends on genetic variability present in the population. 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. Similarity index were estimated using the Dice coefficient of similarity (Nei and Li 1979). The genetic similarity values ranged from 0.71 to 0.96. At 90 % phenon level 22 genotypes were distributed in fourteen clusters. The results revealed twenty five percent variability at genetic level based on ten ISSR markers which could be utilised for further banana improvement programme.

Highlights

- ISSR markers were used to characterize and compare the local dessert banana genotypes of Odisha with the national released cultivars showing that this technique could be successfully adopted to identify the local banana germplasm of Odisha.

Keywords: ISSR analysis, molecular diversity, dessert banana, Odisha.

Banana (*Musa spp*.), is the 2nd most important fruit crop in India next to mango. World banana production is around 103 million tonnes annually, of which bananas cultivation for the export trade account for only 10% (Aurore *et al.* 2009). India is the largest producer of banana in the world producing about 30.0 million metric tonnes from 8.3 lakh hectares (NHB 2010). Hence, fruit harvested from bananas are important components of food security in the tropical world and provide income to the farming community through local and international trade. Banana is a very popular fruit due to its low price and high nutritive value. It is a rich source of carbohydrate and rich in vitamins particularly vitamin B. It is also a good source of potassium, phosphorus, calcium and magnesium. The fruit is easy to digest, free from fat and cholesterol. Banana plants also provide useful by-products, such as fiber, vegetables, beer, wine and vinegar (Aurore *et al.* 2009).

In order to evaluate genetic variability, following phylogenetic origin and extent of ecologically distinguished species or subspecies and to develop efficient crop breeding systems, plant breeders need to have a definitive identification both of cultivars and selections of crop plants. Reliable methods of identification are also required for the establishment of plant variety rights (Kjeldgaad *et al.* 1994). Unambiguous identification is especially important in a clonally-propagated crop such as banana

(Kester 1983). The bananas are natural triploid (2n=3x=33) hybrids of two diploid species, *Musa acuminate* Colla and *Musa balbisiana* Colla, which contributed the A and B genomes, respectively (Swennen *et al.* 1995).

Banana production in Odisha is very low, contributing only 2% to the total banana production in the country (NHM year book). But banana cultivation is an important source of income to the farming community. Odisha is abundant with indigenous dessert banana land races having some peculiar characters that could be utilised for banana improvenment. The land races of banana in Odisha need to be characterised for use in breeding programs and plant variety rights. Germplasm characterization and classification provide useful information for the genetic improvement of crops (Ortiz 1997). Morphological description has proven very useful for the identification of the large diversity of dessert banana cultivars. However, close genetic relationships among cultivars as well as frequent somatic mutations and morphological changes due to environment which have resulted in large number of cultivars, are major obstacles that limit the use of this technique. Under such circumstances DNA- based molecular marker techniques could be used efficiently. The present investigation aims to study the genetic relationships between dessert banana land races from Odisha and released banana cultivars of the nation using inter simple sequence repeats (ISSR) markers system for use in banana improvement programs.

Materials and Methods

Plant materials

Fresh leaves of 11 local banana genotypes (Fig.1) of Odisha namely Champa, Chini Champa, Deshi Patkapura, Ranital Patkapura, Chandanpur Patkapura, Red Green banana, Satsankha Patkapura, Balipatna Champa, Sakhigopal Patkapura, Champapatia, Gangatulsi, and Harianta Chini Champa and 11 national released cultivars H-531, Grand Naine, Robusta, Red banana, Chakrakeli, BCB-1, Ney Poovan, Martman, Amrutpani, Manjeri Nendran were collected from individual plants in an ice box from the Horticulture Research Station maintained by the Orissa University of Agriculture and Technology, Bhubaneswar, Odisha, India. Leaf materials were collected from four individuals of each genotype.

Molecular Studies

DNA extraction

Semi-matured leaves (2.5g) were ground to a fine powder in liquid nitrogen in a mortar and pestle. The fine powder was resuspended in preheated 10 ml DNA extraction buffer[(1M Boric acid (pH 8.0), 2 mM EDTA, 1.4 M Nacl, 1.5% hexadecyltrimethylammonium bromide (CTAB), 0.2% β-mercaptoethanol (v/v)].The mixtures were subsequently heated at 60^0 C for 3 hours. This was followed by addition of 10 ml of chloroform and spin at 8,000 g. DNA was precipitated with 5 M Nacl and Ethanol. The crude DNA pellet was resuspended in 1 ml of Tris-EDTA (10Mm-1Mm). Subsequently, it was treated with 3 µl RNase (10 mg/ml) and incubated for one hour at 37^0C .DNA purification was made through clean Genei kit (M/S Bangalore Genei,Bangalore). The DNA was washed in 70% ethanol and resuspended in 0.05 ml sterile distilled water. DNA quantification's were performed by visualizing DNA bands under UV light, after electrophoresis on 1.0% (w/v) agarose gel. The resuspended DNA was then diluted in sterile distilled water to 5ng/µl concentration for use in amplification reactions.

Primer screening

Forty 10-mer primers, corresponding to kits A, B, D and N from Operon Technologies (Alameda, California) were initially screened using five varieties to determine the suitability of each primer for the study. Primers were selected for further analysis based on their ability to detect distinct, clearly resolved and polymorphic amplified products within the cultivars of banana. To ensure reproducibility, the primers generating no, weak, or complex patterns were discarded.

PCR amplifications

A set of forty random decamer oligonucleotides obtained from Operon Technologies Inc. (Alameda,California,USA) was used as single primers for the amplification of ISSR fragments. Polymerase Chain Reactions (PCR) were carried out in a final volume of 25 µl containing 20 ng template

DNA, 100 µM each deoxynucleotidetriphosphate, 20 ng of decanucleotide primers (M/S operon Technology, Inc., Alameda, CA 94501, USA), 1.5 mM $MgCl_2$, 1x Taq buffer (10 mM Tris-HCl (pH-9.0), 50 mM KCl , 0.01% gelatin] and 0.5 U Taq DNA Polymerase (M/S Bangalore Genei, India). Amplification was achieved in a PTC 100 thermal cycler (MJ Rrsearch, USA) programmed for a 4 min denaturation step at 94^0 C, followed by 45 cycles of denaturation at 94^0 C for 1 min, annealing at 37^0 C for 1 min and extension at 72^0 C for 2 min, finally at 72^0C for 10 min. Amplification products were separated alongside a molecular weight marker (1 kb ladder, MBI Fermentas,USA) by electrophoresis on 1.2% agarose gels run in o.5X TAE (Tris Acetate EDTA) buffer, stained with ethidium bromide and visualized under UV light. Gel photographs were scanned through Gel Doc System (Gel Doc. 2000, BioRad USA) and the amplification product sizes were evaluated using the software Quantity one (BioRad USA).

Data Analysis

Data were recorded as presence (1) or absence (0) of band from the examination of photographic negatives. Each amplification fragment was named by the source of the primer (Operon, Advanced Biotechnologies), the kit letter or number, the primer number and its approximate size in base pairs. Bands with similar mobility to those detected in the negative control, if any, were not scored. Similarity index were estimated using the Dice coefficient of similarity (Nei and Li 1979). Cluster analyses were carried out on similarity estimates using UPGMA (Unweighted pair-group method using arithmetic average) in the NTSYS-PC, version 1.80 programs (Rohlf 1995).

Results and Discussion

The description of *Musa* genotypes used in this study is presented in Table1. Twenty two genotypes used for molecular characterization were collected from Odisha, West Bengal, Andhra Pradesh, Tamil Nadu and Kerala. All the genotypes are triploid in nature having genomic constitution of either AAA (autotriploid) or AAB or ABB (allotriploid) except Ney Poovan which is diploid (AB) in nature. The skin colour of ripen fruit of Champa, Robusta, Ranital Patkapura, Chandanpur Patkapura, Chakrakeli, Satsankha Patkapura, Balipatna Champa, Ney Poovan, Sakhigopal Patkapura, Martman, Amrutpani, Red Green banana and Champapatia were observed to be yellow whereas the skin colour of H-531 was bright yellow; Chini Champa, BCB-1 and Gangatulsi had ashy yellow skin; Manjeri Nendran and Harianta Chini Champa had dull yellow skin; Grand Naine had green; and Red banana had dull red colour skin.

Molecular characterization of the 22 dessert banana genotypes was done by using ISSR markers. Forty primers were tried for initial screening of which ten primers (USB-810, USB-811, USB-815, USB-835, USB-836, USB-838, USB-841, USB-842, UBC-818, UBC-864) were found suitable to form polymorphic banding pattern for the genotypes. With these ten primers, the 22 banana genotypes produced a total of 76 amplification products (bands) out of which 40 were monomorphic and 36 were polymorphic (Table 2). The primers USB-835 and USB-841 produced the highest number of polymorphic bands each (6), while USB-836 produced only one polymorphic band. Different levels of polymorphisms were detected since as the percentage of polymorphic bands (% PB) ranged from 14.3 for USB-836 to 66.6 for UBC primers, with an average of 57.78%. All ten primers were effective in bringing out differences among the 22 genotypes in terms of presence or absence of the polymorphic bands. The size of DNA bands varied from 100 bp to 3000 bp. The lowest band size (100 bp) was produced by the ISSR markers UBC-818 and UBC-864 whereas the highest band size (3000 bp) was generated by USB-811 followed by USB-842 (Table 2).

The genetic similarity values of 22 dessert banana genotypes ranged from 0.71 to 0.96 (Table 3). The lowest genetic similarity (0.71) was observed between Chini Champa (V4) & Robusta (V5); Amrutpani (V18) & Robusta (V5) while the highest genetic similarity was detected between Ranital Patakpura (V8) & Chandanpur Patakpura (V9); Sakhigopal Patkapura (V16), Martman (V17) & Amrutpani (V18).

Cluster analysis at 75% phenon level

The dendrogram depicted in Fig. 3 indicated that the genotypes used for the study appeared as a single cluster at 75% phenon level i.e. all genotypes were alike and their distinction was not possible below 75% phenon level. This indicated that at molecular level the genotypes were 75% similar

to each other and only 25% variation was existed among the genotypes based on the ISSR markers used. With the increase in phenon level from 75 % to 78 % the genotypes were classified into two major clusters i.e. cluster I (Cl-I) and cluster II (Cl-II). The larger cluster, Cl- I comprised of 20 genotypes having approximately 79 % similarity at molecular level. The smaller cluster, Cl- II had two genotypes namely Chini Champa and BCB-1 having approximately 88 % similarity. Chini Champa, the local one and BCB-1, the national released cultivar had genomic constitution of AAB and ABB but they formed a single cluster. Therefore, it was suspected that they were somewhat unique as compared to others.

Cluster analysis at 80% phenon level

At 80% phenon level the larger cluster i.e. Cl-I was found to be divided into three sub clusters namely A, B and C. Among these three sub clusters, cluster A was bigger containing 14 genotypes (V6, V8, V9, V10, V13, V14, V15, V16, V17, V18, V19, V20, V21 & V22). All the genotypes of cluster A had two sets of "A" genome and one set of "B" genome except V15 (Ney Poovan) which was diploid having one set of "A" and one set of "B"genome. Sub cluster – B consisted of two genotypes namely Red Banana (V7) and Red Green Banana (V11) the mutant of Red Banana and these two were found to be autotriploid having three sets of "A" genome. Sub cluster – C comprised of four genotypes of which two genotypes Champa (V1) and H-531 (V2) had genomic constitution of "AAB" and other two genotypes Grand Naine (V3) & Robusta (V5) had genomic constitution of "AAA".

Cluster analysis at 85% phenon level

At 85 % similarity level sub cluster A was divided into 2 groups i.e. A1 and A2; sub cluster B remained as such and sub cluster C divided into 2 groups such as C1 and C2. A1 had two genotypes Gangatulsi (V20) and Harianta Chini Champa (V22) whereas A2 being larger consisted of twelve genotypes (V6, V8, V9, V10, V13, V14, V15, V16, V17, V18, V19, V21). C1 contained two autotriploid (AAA) genotypes namely Grand Naine (V3) and Robusta (V5) with different skin colour i.e. green and yellow respectively during ripening stage. C2 consisted of two genotypes i.e. Champa (V1) and H-531(V2) having genotypic constitution "AAB". The total numbers of clusters were six at 85 % phenon level.

Cluster analysis at 90% phenon level

With the increase in phenon level from 85 % to 90 %, the number of clusters increased from 6 to 14. V1, V2, V6, V13, V19, V21, V4 and V12 formed 8 distinct monoclusters. Other 6 clusters were V3 & V5; V7 & V11; V8, V9 & V10; V14 & V15; V16, V17 & V18; V20 & V22. At 96 % phenon level all the genotypes were found to separate from each other and formed 22 clusters. This results showed that among the local genotypes, Red Green banana was genetically similar to the national released cultivar Red banana of Kearala; Balipatna Champa the local one was similar to the national released cultivar Ney Poovan of Kerala; two local genotypes Ranital Patkapura and Chandanpur Patkapura were identical to Chakrakeli of Andhra Pradesh; the local genotype Sakhigopal Patkapura had more than 90% genetic similarity with both the national cultivars Martman of West Bengal & Amrutpani of Odisha. The local genotypes Chini Champa, Deshi Patkapura, Satsankha Patkapura and Champapatia did not show any genetic similarity either with each other or with any national cultivars at 90 % phenon level. Two local genotypes Gangatulsi and Harianta Chini Champa were found to be closely related to each other at molecular level.

The present study indicated that each ISSR primer could generate polymorphisms among the 22 banana genotypes. The polymorphism may be due to mutation at priming sites and/or insertion/ deletion event within the SSR region; and the extent of polymorphism also varies with the nature and the sequence repeat of the primer used (Reddy *et al*. 2002). Inter Simple Sequence Repeats (ISSR) markers, on the other hand, require only small amount of DNA sample without involving radioactivity tests and are simpler as well as faster. ISSR technique is also very simple, fast, cost-effective, highly discriminative and reliable (Reddy *et al*. 2002). At present, ISSR markers have been successfully applied to detect the genetic similarities or dissimilarities in various crop plants (Carvalho *et al*. 2004; Martins *et al*. 2004; Ramage *et al*. 2004; Modgil *et al*. 2005). Brown *et al*. (2009) studied genetic diversity among 21 cultivars (Musa spp.) in Mauritius using RAPD markers. The ISSR technique

developed by Zietkiewicz *et al*. (1994) does not require the knowledge of flanking sequences and has wide applications for all organisms, regardless of the availability of information about their genome sequence. They have also proved to be simple, fast, cost effective and versatile sets of markers for repeatable amplification of DNA sequences using single primers.

The Inter Simple Sequence Repeats (ISSR) markers has been successfully used to determine genetic diversity and relationships in Musa germplasm (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). ISSR markers can generate unlimited number of polymorphic bands with relative ease from very small amounts of genomic DNA (Reddy *et al*. 2002) allowing simultaneous screening of a large number of accessions.

Ray *et al*. (2006) reported that ISSR markers detected more polymorphism than RAPD while studying the genetic stability of three economically important micropropagated banana (*Musa* spp.) cultivars. Similarly, ISSR were used for detection of genetic uniformity of micropropagated plantlets (Rout *et al.* 2009) and for screening *in vitro* mutagenesis and variance (Khatri *et al*. 2011). Another study reported the use of ISSR to assess the genetic diversity and classification of 27 wild banana accessions collected in Guangxi, China. The results showed that the collected germplasm was derived from diverse origins and evolutionary paths of banana in Guangxi (Qin *et al*. 2011). ISSR were employed for molecular assessment of genetic identity and genetic stability in banana cultivars (Lu *et al.* 2011). Recently, ISSR were used to analyze the pattern of genetic variation and differentiation in 32 individuals along with two reference samples of wild *Musa*, which corresponded to three populations across the biodiversity-rich hot-spot of the southern Western Ghats of India (Padmesh *et al*. 2012).

UPGMA cluster analysis in the present investigation could not group the genotypes according to their genome constitution (AB, AAA, AAB and ABB) or geographical origin. The genotypes were mixed up in all the clusters irrespective of their genomic constitution or geographical origin. Lack of clear clustering based on geographical origins among the banana cultivars can be due to introductions followed by migration of cultivars in areas away from their initial origins.

The present investigation was primarily focused on the study of genetic variation among the dessert banana genotypes locally available in Odisha. Using ISSR molecular markers it was revealed that significant amount of genetic diversity existed in the samples studied. Therefore, understanding genetic variation between and within the genomic groups is very important for the evaluation of plants with superior traits. The findings derived out of this study will provide useful information for future breeding and conservation strategies of dessert banana genetic resources in Odisha and India.

Table 1: List and description of *Musa* genotypes used in present study

Code	Cultivar	Genome	**Place of collection**	**Skin colour (ripe fruit)**
V1	Champa	AAB	Athgarh, Odisha, N	Yellow
V2	H-531	AAB	Coimbatore, Tamil Nadu, N	Bright yellow
V3	Grand Naine	AAA	Tissue culture banana, N	Green
V4	Chini Champa	AAB	Bhadrak, Odisha, L	Ashy yellow
V5	Robusta	AAA	Tissue culture banana, N	Yellow
V6	Deshi Patkapura	AAB	Aadashpur, Odisha, L	Yellow

V7	Red banana	AAA	Kannar, Kerala, N	Dull red
V8	Ranital Patkapura	AAB	Ranital, Odisha, L	Yellow
V9	Chandanpur Patkapura	AAB	Chandanpur, Odisha, L	Yellow
V10	Chakrakeli	AAB	Kovvur, AP, N	Yellow
V11	Red Green banana	AAA	Bhubaneswar , Odisha, L	Yellow
V12	BCB-1	ABB	Mohanpur, West Bengal, N	Ashy yellow
V13	Satsankha Patkapura	AAB	Satsankha, Odisha, L	Yellow
V14	Balipatna Champa	AAB	Balipatna, Odisha, L	Yellow
V15	Ney Poovan	AB	Kannar, Keral, N	Yellow
V16	Sakhigopal Patkapura	AAB	Sakhigopal, Odisha, L	Yellow
V17	Martman	AAB	BCKV, West Bengal, N	Yellow
V18	Amrutpani	AAB	Balipatna, Odisha, N	Yellow
V19	Champapatia	AAB	Baliapal, Odisha, L	Yellow
V20	Gangatulsi	AAB	Baliapal, Odisha, L	Ashy yellow
V21	Manjeri Nendran	AAB	Kannar, Kerala, N	Dull yellow
V22	Harianta Chini Champa	AAB	Harianta, Odisha, L	Dull yellow

L: Local genotype; N: National cultivar

Table 2. List of primers, their sequences and size of the amplified fragments generated by 10 ISSR primers

ISSR code	Primer (5′ – 3′)	Sequence	Band range (bp)	Total number of band	No. of Polymorphic bands	No of monomorphic bands	% of ploymorphism
ISSR-3	USB-810	$(GA)_8T$	600-1100	06	04	02	66.6
ISSR-4	USB-811	$(GA)_8C$	600-3000	09	03	06	33.3
ISSR-5	USB-815	$(CT)_8G$	450-1500	07	04	03	57.1
ISSR-6	USB-835	$(AG)_8YC$	200-1200	09	06	03	66.6
ISSR-7	USB-836	$(AG)_8T$	200-1500	07	01	06	14.3
ISSR-9	USB-838	$(GA)_8T$	500-1000	06	02	04	33.3
ISSR-12	USB-841	$(AG)_8YC$	350-2000	09	06	03	66.6
ISSR-13	USB-842	$(AG)_8YA$	550-2200	08	03	06	37.5
ISSR-20	UBC-818	$(CA)_8G$	100-1000	06	04	02	66.6
ISSR-27	UBC-864	$(ATG)_6$	100-1200	08	03	05	37.5
				76	36	40	

Table 3: Similarity coefficient matrix based on 10 ISSR markers

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20	V21	V22
V1	1.00																					
V2	0.87	1.00																				
V3	0.88	0.83	1.00																			
V4	0.73	0.78	0.72	1.00																		
V5	0.84	0.78	0.92	0.71	1.00																	
V6	0.80	0.82	0.83	0.78	0.87	1.00																
V7	0.79	0.83	0.80	0.77	0.83	0.86	1.00															
V8	0.82	0.87	0.83	0.80	0.84	0.91	0.88	1.00														
V9	0.82	0.87	0.86	0.80	0.84	0.89	0.86	0.96	1.00													
V10	0.93	0.80	0.86	0.76	0.89	0.91	0.83	0.91	0.78	1.00												
V11	0.78	0.78	0.77	0.76	0.82	0.80	0.92	0.80	0.78	0.80	1.00											
V12	0.81	0.79	0.82	0.88	0.77	0.79	0.76	0.83	0.79	0.77	0.72	1.00										
V13	0.90	0.81	0.82	0.77	0.79	0.88	0.80	0.90	0.79	0.90	0.72	0.84	1.00									
V14	0.88	0.81	0.84	0.74	0.81	0.86	0.80	0.90	0.81	0.88	0.77	0.82	0.91	1.00								
V15	0.84	0.73	0.81	0.73	0.80	0.82	0.83	0.87	0.73	0.84	0.82	0.79	0.86	0.92	1.00							
V16	0.89	0.80	0.79	0.76	0.76	0.84	0.79	0.91	0.78	0.87	0.78	0.79	0.90	0.92	0.89	1.00						
V17	0.89	0.78	0.79	0.76	0.78	0.84	0.79	0.91	0.73	0.87	0.78	0.79	0.88	0.90	0.91	0.96	1.00					
V18	0.84	0.80	0.74	0.76	0.71	0.84	0.77	0.87	0.78	0.82	0.76	0.74	0.88	0.90	0.84	0.96	0.93	1.00				
V19	0.82	0.78	0.79	0.71	0.76	0.84	0.83	0.84	0.78	0.80	0.82	0.77	0.86	0.90	0.89	0.89	0.89	0.91	1.00			
V20	0.80	0.80	0.74	0.82	0.73	0.78	0.79	0.82	0.76	0.78	0.84	0.79	0.86	0.83	0.80	0.84	0.84	0.87	0.76	1.00		
V21	0.81	0.79	0.82	0.74	0.81	0.81	0.82	0.83	0.77	0.81	0.90	0.78	0.80	0.84	0.83	0.86	0.88	0.86	0.81	0.86	1.00	
V22	0.79	0.77	0.76	0.83	0.84	0.74	0.78	0.79	0.74	0.77	0.83	0.80	0.82	0.80	0.79	0.81	0.81	0.81	0.77	0.94	0.89	1.00

Fig. 1 : Land Races of Dessert Banana from Odisha

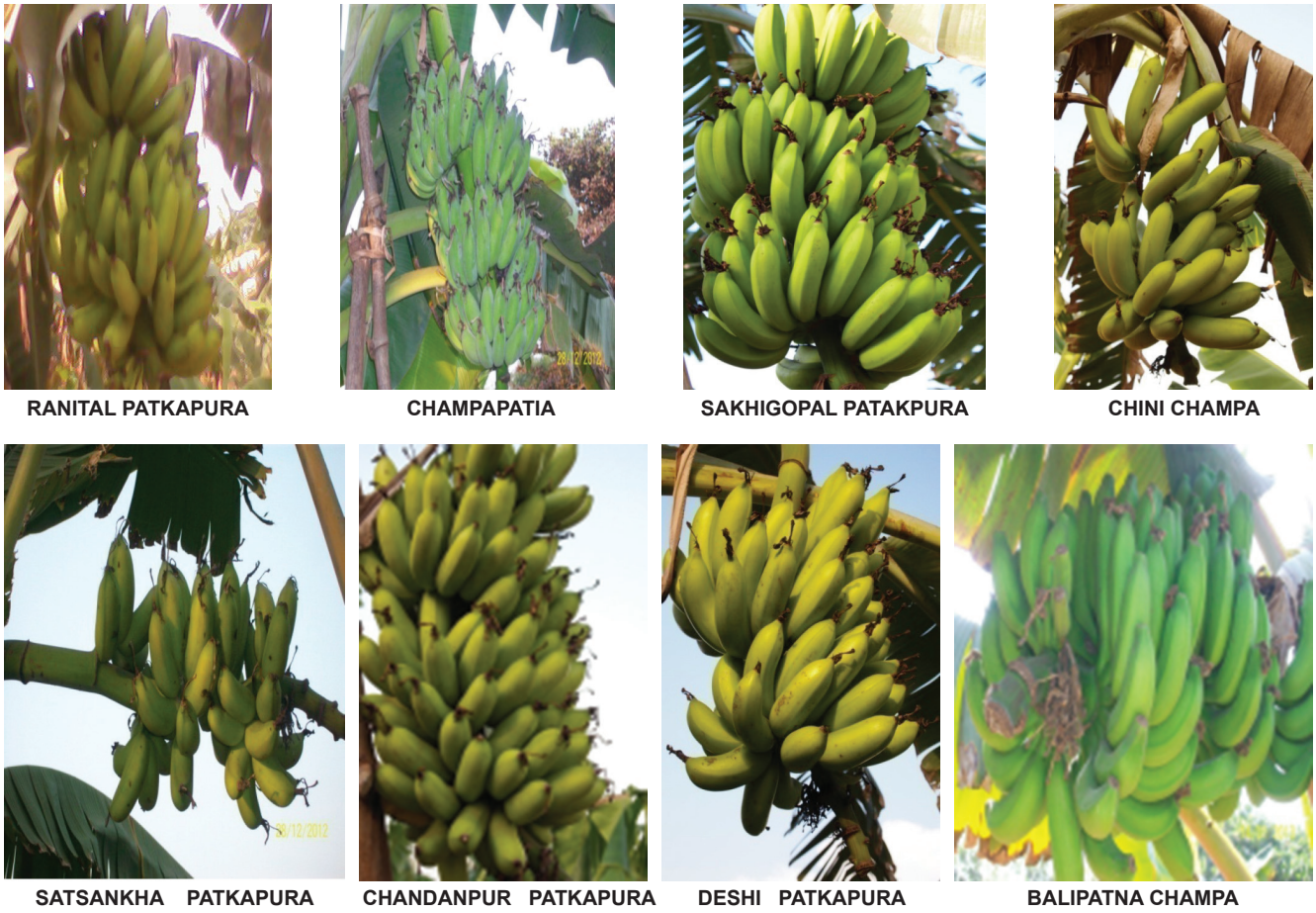

Fig. 1

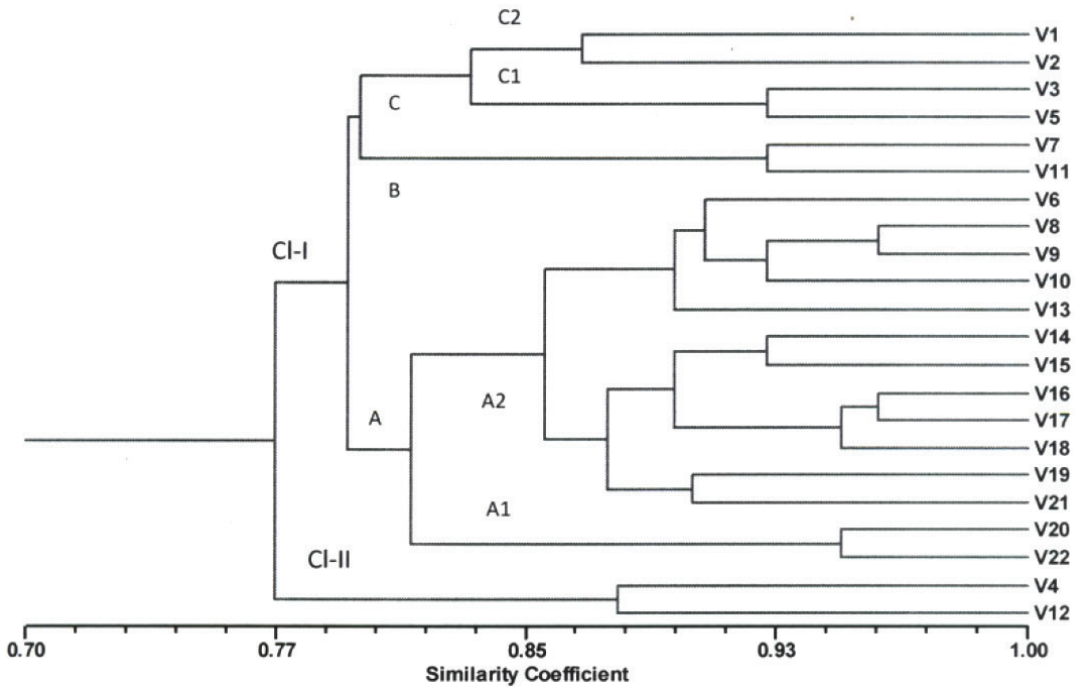

Fig. 2: UPGMA dendrogram based on genetic similarity among 22 banana genotypes

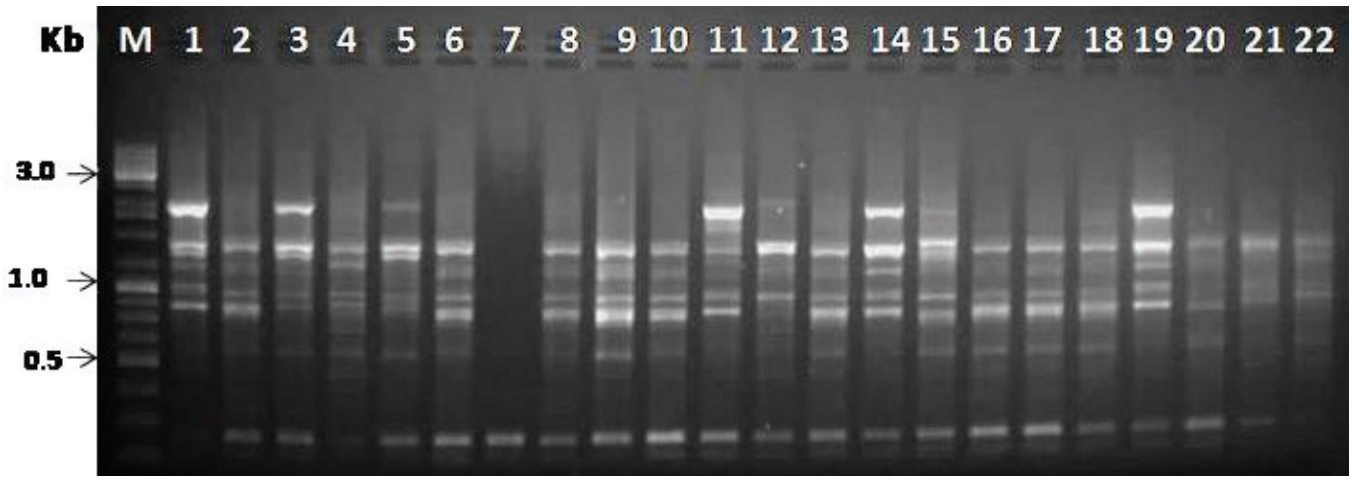

Fig. 3: Polymorphic banding pattern of 22 dessert banana genotypes produced by ISSR marker USB-836

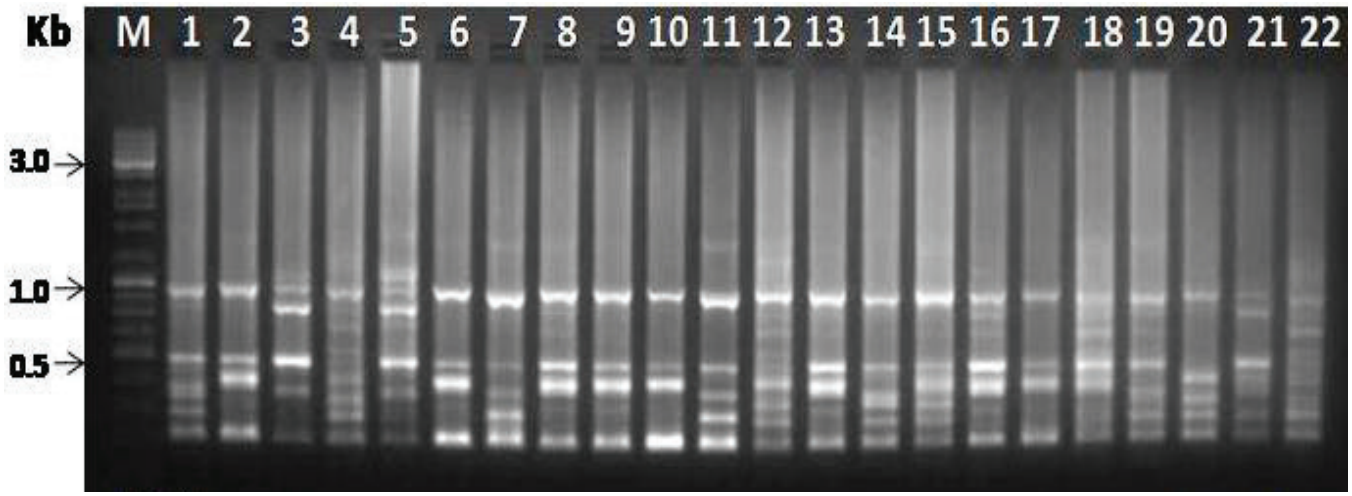

Fig. 4: Polymorphic banding pattern of 22 dessert banana genotypes produced by ISSR marker UBC-818

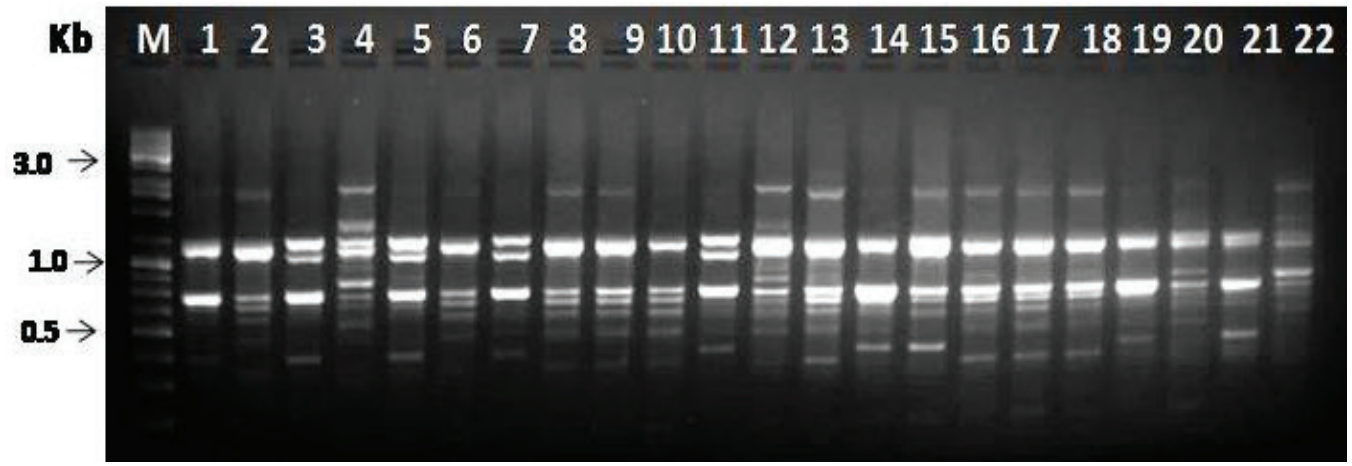

Fig. 5 : Polymorphic banding pattern of 22 dessert banana genotypes produced by ISSR marker USB-841

References

Agoreyo, B.O., Golden, K.D., Brown, S.E. 2008. Analysis of genetic variability among plantain cultivars (*Musa paradisiaca* L.) using arbitrarily primed PCR technique, *African J Biotech* **7**: 1041-1045.

Aurore, G., Parfait, B., Fahrasmane, L. 2009. Bananas raw materials for making processed food products, *Trends in Food Science and Technology* **20**:78-91

Brown, N., Venkatasamy, S., Khittoo, G., Bahorun, T., Jawaheer, S. 2009. Evaluation of genetic diversity between 27 banana cultivars (*Musa* spp.) in Mauritius using RAPD markers, *African J Biotech* **8**: 1834-1840.

Carvalho, L.C., Goulao, L., Oliveira, C., Gonçalves, J.C. Amancio, S. 2004. RAPD assessment for identification of clonal identity and genetic stability of in vitro propagated chestnut hybrids, *Plant Cell, Tissue and Organ Culture* **77:** 23-27.

Crouch, J.H., Crouch, H.K., Constandt, H., Van Gysel, A., Bretne, P., Van Montagu, M., Jarret, R.I., Ortiz, R. 1999. Comparison of PCT-based molecular marker analyses of *Musa* breeding populations, *Mol Breeding* **5**: 233-244.

Grajal-Martin, M., Siverio-Grillo, G., Marrero-Dominguez, A.1998. The use of randomly amplified polymorphic DNA (RAPD) for the study of genetic diversity and somaclonal variation in *Musa*, *Acta Horticulturae* **490**: 445-454.

Harirah, A.A. and Khalid, N. 2006. Direct regeneration and RAPD assessment of male inflorescence derived plants of *Musa acuminata* cv. Berangan, *Asia Pacific J Mol Biol Biotech* **1**: 11-17.

Howell, E.C., Newbury, H.J., Swennen, R.L., Withers, L.A., Ford–Lloyd, B.V. 1994. The use of RAPD for identifying and classifying *Musa* germplasm, *Genome* **37**: 328-332.

Jain, P.K., Saini, M.K., Pathak, H., Gupta, V.K. 2007. Analysis of genetic variation in different banana (*Musa* species) variety using random amplified polymorphic DNAs (RAPDs), *Afri J Biotechnol* **6**: 1987-1989.

Kester, D.E. 1983. The clone in horticulture, *Hort Science* **75**:831-837.

Khatri, A., Dahot, M.U., Khan, I., Raza, S., Bibi. S., Yasmin, S., Nizamani DGS. 2011. *In vitro* mutagenesis in banana and variant screening through ISSR. *Pak J Bot* **43**(5): 2427-2431.

Kjeldgaad, R.H., Marsh, D.R. 1994. Intellectual property rights for plants, *Plant Cell* **6**: 1524-1528

Lakshmanan, V., Venkataramareddy, S.R., Neelwarne, B. 2007. Molecular analysis of genetic stability in long-term micropropagated shoots of banana using RAPD and ISSR marker, *Electronic J Biotech* **10**

Lu, Y., Xu, W.H., Xie, Y.X., Zhang, Z., Pu, J.J., Qj, Y.X., Li, H.P. 2011. Isolation and characterization of nucleotide-binding site and C-terminal leucine-rich repeat-resistance gene candidates in bananas, *Genet Mol Research* **10**: 3098-3108.

Martins, M., Sarmento, D., Oliveira, M.M. 2004.Genetic stability of micropropagated almond plantlets as assessed by RAPD and ISSR markers. *Plant Cell Reports* **23**: 492-496.

Modgil, M., Mahajan, K., Chakrabarti, S.K., Sharma, D.R., Sobti, R.C. 2005. Molecular analysis of genetic stability in micropropagated apple rootstock MM106, *Scientia Horticulturae* **104**: 151-160.

Nei, M., Li, W.H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Science U.S.* **76**: 5269-5373.

National Horticulture Mission Year book, 2013. Novak FJ, 1992. *Musa* (*Banana, Plantain*). In: Hammerschlag FA, Litz R (eds.).

Ortiz, R. 1997. Morphological variation in *Musa* germplasm, *Gen Res Crop Evol* **44**: 393-404.

Padmesh, P., Mukunthakumar, S., Vineesh, P.S., Hari Kumar, K., Krishnan, P.N. 2012. Exploring wild genetic resources of *Musa acuminata* Colla distributed in the humid forests of southern Western Ghats of peninsular India using ISSR markers, *Plant Cell Reports* **31**: 1591-1601.

Pillay, M., Nwakanma, D.C., Tenkouano, A. 2000. Identification of RAPD markers linked to A and B genome sequences in *Musa, Genome* **43**: 763-767.

Poerba, Y.S., Ahmad, F. 2010. Genetic variability among 18 cultivars of cooking bananas and plantains by RAPD and ISSR markers, *Biodiversitas* **11**: 118-123.

Qin, X.Q., Peng, H.X., Long, X., Yao, J.Y. 2011. Preliminary study on ISSR analysis and classification of wild *Musa* germplasm in Guangxi, China, *Acta Hort* **897**: 259-262.

Racharak, P., Eiadthong, W. 2007. Genetic relationship among subspecies of *Musa acuminata* Colla and A-genome consisting edible cultivared bananas assayed with ISSR markers, *Songklanakarin J Sci Tech* **29**: 1479-1489.

Ramage, C.M., Borda, A.M., Hamill, S.D., Smith, M.K. 2004. A simplified PCR test for early detection of dwarf off-types in micropropagated Cavendish banana (Musa spp. AAA), *Scientia Horticulturae* **103**: 145-151.

Ray, T., Dutta, I., Saha, P., Das, S. and Roy, S.C. 2006. Genetic stability of three economically important micropropagated banana (*Musa* spp.) cultivars of lower Indo-Gangetic plains, as assessed by RAPD and ISSR markers, *Plant Cell Tiss Org Cult* **85**: 11-21.

Reddy, M.P., Sarla, N. and Siddiq, E.A. 2002. Inter simple sequence repeats (ISSR) polymorphism and its application in plant breeding, *Euphytica* **128**: 9-17.

Rohlf, F.J. 1992. NTSYS-PC. Numerical taxonomy and multivariate analysis. Version 2.0. Exeter Software, New York.

Rout, G.R., Senapati, S.K., Aparajita, S., Palai, S.K. 2009. Studies on genetic identification and genetic fidelity of cultivars banana using ISSR marker, *Plant Omics Journal* **2**: 250-258.

Ruangsuttapha, S., Eimert, K., Schoder, M.B., Silayoi, B., Denduangboripant, J., Kanchanapoom, K. 2007. Molecular phylogeny of banana cultivars from Thailand based on HAT-RAPD marker, *Gen Res Crop Evol* **54**: 1565-1572.

Swennen, R., Vuylsteke, D., Ortiz, R. 1995. Phenotypic diversity and patterns of variation in West and Central African plantains (*Musa* spp., AAB Group, Musaceae), *Econ Bot* **49**: 320-327.

Venkatachalam, L., Sreedhar,V., Bhagyalakshmi. 2007. Genetic analyses of micropropagated and regenerated of banana as assessed by RAPD and ISSR markers, *In Vitro Cellular Dev Biol Plant* **43**: 267-274.

Zietkiewicz, E., Rafalski, J.A., Labuda, D. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification, *Genomics* **20**:176-183.