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USE OF RAPD FOR THE ASSESSMENT OF GENETIC DIVERSITY AMONG EXOTIC AND COMMERCIAL BANANA CLONES

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Abstract

Banana production in Pakistan is confined to subtropical regions. Most orchards are based on the cultivar Basrai. This monoculture facilitates the diseases spread and causing epidemics resulting in the simultaneous decimation of the crop in the entire region. The aim of this study was to identify different features of exotic banana clones by means of RAPD-based genetic analysis and to compare them with 'Basrai'. Fourteen different banana clones were assessed, 12 of which were exotic and 2 local cultivars. Fifteen primers were used to amplify the fragments from banana genome. Ninety-six scorable fragments were obtained, out of which 78 (81.25%) were polymorphic and only 18 (18.75%) were monomorphic. Similarities among cultivars were calculated according to Nei & Li method. Maximum similarity was observed between GCTCV 247 and GCTCV 215 (0.89) and minimum between FHIA 18 and FHIA 23 (0.38).

Introduction

Banana (*Musa* spp.) is one of the major food crop contributing significantly to the national economy. It is grown in 122 countries, with a cultivated area of 3.8 million hectares and a total production of 56.4 million metric tonnes (Chai *et al*., 2004; Hamide *et al*., 2004). In Pakistan it is grown extensively in lower part of Sindh province covering about 32200 hectare with a production of 126300 tonnes (Anon., 2007). Basrai is the only dominating variety covering almost 98% area under cultivation (Baloch & Siddique, 1991; Khatri *et al*., 1997). This has created monoculture, which facilitated the disease (BBTD) spread in 1992 (Khushk *et al*., 1993).

Genetic improvement of banana is the main solution to overcome this disease problem. Conventional breeding efforts have been rather slow in developing new clones with improved characteristics due to the complex and polyploidy nature of *Musa* genome. Sterility, low seed set and long crop cycle are other obstacles that hinder conventional breeding in banana (Novak *et al*., 1992). In most of the countries where banana production has been improved it is either by importing promising cultivars/selection from distinct geographical areas or through identification of superior and stable local selections (Eckstein *et al*., 1998; Khayat *et al*., 1998; Smith *et al*., 1998; Hamide *et al*., 2004).

Morphological markers had been used for the characterization of *Musa* germplasm (Ortiz, 1997; Ortiz *et al*., 1998). Molecular markers are currently being used to study the genetic diversity and to aid in identification and selection of plants and animals. The employment of molecular markers in genetic assessment provides opportunities for elucidation of genome organization. In our study we used RAPD technique, which is based on Polymerase Chain Reaction (PCR) developed by Williams *et al*., (1990) and Welsh & McClelland (1990). RAPD markers are inexpensive and relatively easier to apply than other molecular techniques. Moreover, it does not require large amounts of DNA or prior knowledge of the target genome (Thu *et al*., 2002; Ford-Lloyd *et al*., 1996). High annealing temperature ranges 50-55°C can be helpful in RAPD study to avoid the spurious formation of amplification products and to increase reproducibility (Atienzer *et al*., 2000).

The purpose of this study was to determine the genetic relationships between some exotic banana clones and cultivar Basrai by means of RAPD-based genetic analysis. The selections identified could be used to increase the genetic diversity of cultivated bananas in Sindh and diversify the current monoculture. This information may also be fruitful in other part of the world where monoculture dominated by dwarf cavandish is cultivated.

Materials and Methods

Fourteen different banana clones were analysed, 12 of which were from exotic material, provided by International Network for Improvement of Banana and Plantain (INIBAP) and two indigenous (Table 1).

Isolation of DNA: DNA was extracted from fresh leaves of banana using GENTRA Kit. Two hundred milligrams of fresh leaves were grounded in liquid nitrogen. The ground sample was placed in 15ml tube, 3 ml of the cell lysis solution (Tris [hydroxymethyl] aminomethane, ethylenediaminetetraacetic acid & sodiumdodecyl sulfate) was added and tube was incubated at 65°C for 60 minutes. Fifteen micro-litters of RNase solution was then added to the cell lysate and incubated at 37°C. After 30 minutes the protein precipitation solution was added and tube vortexed for 20 seconds and placed on ice for 30 minutes. The mixture was centrifuged at 2000 x g for 10 minutes. The supernatant containing DNA was poured into separate 15ml centrifuge tube. Three milliter of isopropanol was added and DNA was then precipitated by centrifuging at 2000 x g. The pellet was washed with 70 % ethanol and the DNA samples were then hydrated with TE buffer. DNA was quantified on ®BIOMATE 3 (spectrophotometer).

DNA amplification: Fifteen primers from Gene Link (U.S.A), each ten bases in length, were used to amplify the DNA (Table 2). PCR reaction was carried out in 25µl reaction mixture volume containing 13ng of template (genomic DNA), 2.5mM MgCl, 0.33mM of each dNTPs, 2.5U of Taq polymerase and 1µM of primer in a 1xPCR reaction buffer (Eppendorf, Hamburg, Germany). The amplification reaction was performed in the Eppendorf Master cycler with an initial denaturation for 5 min., at 94°C, then 32 cycles:1 min denaturation at 94°C; 1 min annealing at 52°C and 2min extension at 72°C. Final extension was carried out at 72°C for 10 min. Amplified products were analyzed through electrophoresis on 1.5% agrose gel containing 0.5XTBE (Tris Borate EDTA) at 90 Volts for 2 hours, the gel contained 0.5µg/ml ethidium bromide to stain the DNA and photograph was taken under UV light.

Data analysis: Presence of band on gel was scored as (1) and absence of band is (0) for the analyses. The similarity Coefficient was calculated according to Nei & Li (1979).

Result and Discussion

Thirteen of the 15 primers produced multiple fragments (Fig. 1). The total number of scorable bands were 96, out of which 78 (81.25%) were polymorphic and only 18 (18.75%) were monomorphic. The number of fragments produced by the primers ranged from 2- 16, with an average of 7 fragments.The size of fragments ranged from 200 bp – 9.0 kbp. Primer A-10 produced 16 fragments and primer A-12 produced only 2 fragments.

Table 1. Clones and genotypes employed in the study.

S. No.	Clones	Genotype	S. No.	Clones	Genotype
1.	GCTCV247 (Exotic)	AAA	8.	SH3634 (Exotic)	-
2.	GCTCV215 (Exotic)	AAA	9.	Basrai (local)	AAA
3.	GCTCV106 (Exotic)	AAA	10.	FHIA01 (Exotic)	AAAB
4.	Amrat Sagar (Exotic)	AAA	11.	FHIA17 (Exotic)	AAAA
5.	Grand Naine (Exotic)	AAA	12.	FHIA18 (Exotic)	AAAB
6.	GN60A (Exotic)	AAA	13.	William hybrid (local)	AAA
7.	Km5 (Exotic)	AAA	14.	FHIA23 (Exotic)	AAAA

Table 2. Sequence of the Primers.

Primer	Sequence	Primer	Sequence
A-01	CAGGCCCTTC	A-14	TCTGTGCTGG
A-02	TGCCGAGCTG	A-15	TTCCGAACCC
A-03	AGTCAGCCAC	A-16	AGCCAGCGAA
A-04	AATCGGGCTG	A-17	GACCGCTTGT
A-10	GTGATCGCAG	A-18	AGGTGACCGT
A-11	CAATCGCCGT	A-19	CAAACGTCGG
A-12	TCGGCGATAG	A-20	GTTGCGATCC
A-13	CAGCACCCAC		

Table 3. Similarity coefficient among the banana cultivars calculated according to Nei & Li (1979).

	L-2*	L-3	L-4	L-5	L-6	L-7	L-8	L-9	L-10	L-11	L-12	L-13	L-14	L-15
L-2	1													
L-3	0.58	1												
L-4	0.55	0.89	1											
L-5	0.61	0.73	0.6	1										
L-6	0.64	0.84	0.76	0.73	1									
L-7	0.65	0.73	0.73	0.64	0.77	1								
L-8	0.57	0.58	0.64	0.6	0.59	0.63	1							
L-9	0.63	0.76	0.71	0.69	0.8	0.65	0.61	1						
L-10	0.63	0.85	0.77	0.64	0.78	0.78	0.68	0.76	1					
L11	0.61	0.67	0.64	0.69	0.67	0.68	0.6	0.69	0.71	1				
L-12	0.56	0.66	0.61	0.62	0.64	0.66	0.57	0.63	0.64	0.62	1			
L-13	0.56	0.62	0.57	0.65	0.67	0.63	0.58	0.6	0.7	0.65	0.55	1		
L-14	0.4	0.68	0.63	0.56	0.68	0.61	0.42	0.58	0.68	0.59	0.46	0.65	1	
L-15	0.38	0.56	0.51	0.46	0.53	0.56	0.38	0.56	0.58	0.49	0.39	0.58	0.66	1

*L2=FHIA01, L3=GCTCV247, L4=GCTCV215, L5=Km5, L6=Amrat Sagar, L7=GN60A, L8=FHIA18, L9= Grand Naine; L10=GCTCV106; L11=SH3634, L12=FHIA17, L13=Basrai, L14=William Hybrid, L15=FHIA23.

Genetic similarity: The similarities reflected the genetic relationship between the clones. The greatest similarity was observed between GCTCV- 247 and GCTCV -215 (0.89%) and the least similarity between FHIA 18 and FHIA 23 (0.38%) (Table 3). The clones with genome 'AAAA' (FHIA 23, FHIA 17) and 'AAAB'(FHIA 18, FHIA 01) were remarkably distinct with the other clones. Fourteen banana clones were classified into nine subclasses based on their similarity matrices. Three clones fall in the first subclass i.e., GCTCV-247, GCTCV-215, and GCTCV-106. Amrat sagar and Grand Naine grouped in the second subclass and GN60A latterly joined the main group. There were two individuals in the 4th sub class i.e., Km5 and SH3634. The other four clones (Basrai, FHIA 18, FHIA 01and FHIA17) not forming any group with other individuals. Only William Hybrid showed 66% similarity to FHIA 23.

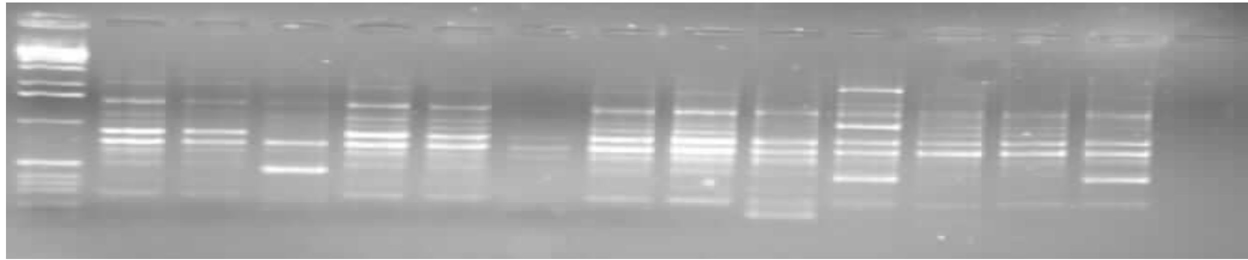

Fig. 1. Result of RAPD –PCR with primer A-10. 1, ladder; 2, FHIA-01; 3,GCTCV-247; 4,GCTCV-215; 5, Km5; 6, Amrat Sagar; 7,GN60A; 8, FHIA-18; 9, Grand naine; 10,GCTCV-106; 11, SH-3634; 12, FHIA-17; 13,Basrai; 14,William hybrid and 15, FHIA-23.

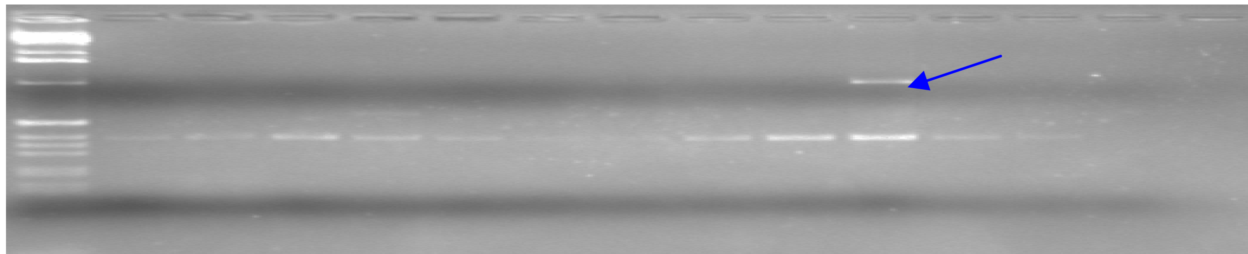

Fig. 2. Result of RAPD –PCR with primer A-12. 1, ladder; 2, FHIA-01; 3,GCTCV-247; 4,GCTCV-215; 5,Km5; 6, Amrat Sagar; 7,GN60A; 8, FHIA-18; 9, Grand naine; 10,GCTCV-106; 11, SH-3634; 12, FHIA-17; 13,Basrai; 14,William hybrid and 15, FHIA-23.

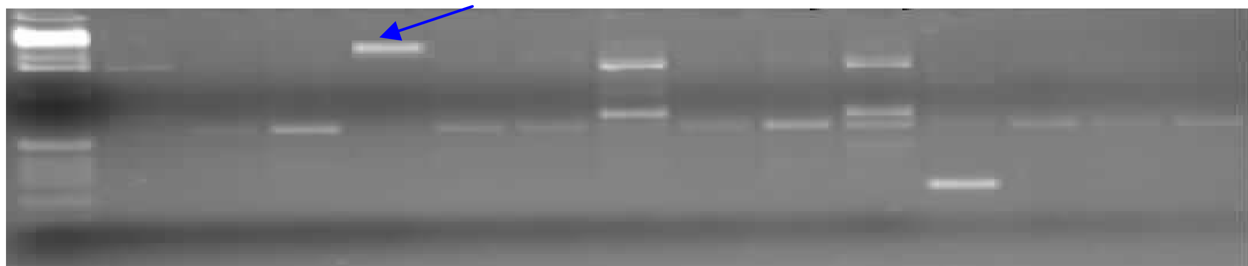

Fig. 3. Result of RAPD –PCR with primer A-17. 1, ladder; 2, FHIA-01; 3,GCTCV-247; 4,GCTCV-215; 5,Km5; 6, Amrat Sagar; 7,GN60A; 8, FHIA-18; 9, Grand naine; 10,GCTCV-106; 11, SH-3634; 12, FHIA-17; 13,Basrai; 14,William hybrid and 15, FHIA-23.

Some specific RAPD bands have also been identified, reflecting the application of RAPDs for the identification of banana cultivar. We found that clone SH3634 contain a specific DNA segment of 3.1kb amplified by primer A-12 (Fig. 2) and clone Km5 gives a specific band of 5.4kb amplified by primer A-17 (Fig 3). Such fragments may subsequently be sequenced *via* SCAR (Sequence Characterized Amplified Region) approach enabling design of specific primers.

It was observed that the 'Basrai'was more similar to GCTCV-106, followed by Km5 and distant from FHIA 17, FHIA 01 and FHIA 23. Therefore, it is concluded that cultivation of FHIA clones which are high yielding endowed with diseases resistance would be recommended for general cultivation alongwith 'Basrai' in the province of Sindh. Furthermore, our results confirmed that RAPD markers could be applied for detection of variation among banana clones.

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