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Characterization of mango genotypes of Gir region based on ISSR markers

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ABSTRACT

Present experiment was conducted on 20 mango mango genotypes found in Gir region of Gujarat state using 21 ISSR primers. The primers yielded a total 125 bands and 119 scorable polymorphic markers, accounting for 95.2% of total reproducible amplification products in the range 42 to 2522 bp. Each primer could amplify 4 to 11 DNA bands, of which primer UBC-840 generated the highest number of bands (11) followed by UBC 855, UBC 835, and UBC 848. While primers UBC 817, UBC 825, UBC 844, UBC 884 and UBC 891 showed the lowest number (4) of DNA bands. Out of the total 119 polymorphic DNA bands, 34 were unique indicating of its presence only in one of the landraces. Jaccard's similarity coefficient between genotypes ranged from 0.21 to 0.59. The maximum genetic similarity was found between 'Jamadar' and 'Kesar' and lowest was between 'Khodi' and 'Agargato'.

Key words: Genetic diversity, Gujarat, ISSR markers, mango.

INTRODUCTION

Mango (*Mangifera indica* L.) a diploid fruit tree with 2n = 40 chromosomes originated in the Indo-Myanmar region during the earlier period of the Cretaceous era (Yonemori *et al.*, 10) and gradually spread to the tropical and subtropical regions of the world. India is thought to be the primary centre of diversity. In the last decade, many molecular markers have been implemented in mango, which includes variable number tandem repeats (VNTR) (Adato *et al.*, 1), random amplified polymorphic DNA (RAPD) (Ravishankar *et al.*, 8; Karihaloo *et al.*, 6), inter-simple-sequence-repeats (ISSRs) (Gonzalez *et al.*, 5) and amplified fragment length polymorphism (AFLP) (Eiadthong *et al.*, 3). Among these, ISSR (Zietkiewicz *et al.*, 11) is a reproducible semi-arbitrary primed PCR method that uses simple sequence repeats as primers, combining most of the advantages of microsatellites and AFLP, to the universality of RAPD. ISSRs offer greater probability than any other PCR marker system in the repeat regions of the genome, which are the most potent regions for producing cultivar-specific markers. In Gujarat, during the Mughal Empire lots of mango genotypes were planted in the Gir and surrounding regions. Among these genotypes, 'Kesar' is famous throughout the world for its taste and flavour. Besides 'Kesar', there are many other genotypes which have the equal potential. Precise information on the genetic relationships within such germplasm is needed. In view of this, the present experiment was conducted.

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MATERIALS AND METHODS

Young and tender leaves were collected from 20 mango genotypes found in Gir region, which were selected on the basis of their consistency in behavior for the last 30 years for the traits like fruit size, sweetness and yield. The DNA was extracted from just mature leaves these genotypes following CTAB method described by Doyle and Doyle (2) with a little modification. Initially 50 ISSR primers were screened, of which only 21 polymorphic primes were used for further analyses and estimation of genetic diversity. An amplification reaction was performed (ABI Veriti thermocycler) with reaction tube consisting of 25 ng of template DNA, 200 µM of each dNTPs (Bangalore Genei), 20 ng of IISR primer (Bangalore Genei), 0.5 U of *Taq* DNA polymerase and 1x reaction buffer in a total volume of 25 µl. Amplification was carried out for 45 cycles of 1 min. at 94°C, 1 min. at 45 ± 5°C and 2 min. at 72°C. Amplified products were separated in 1.5% agarose gel containing ethidium bromide using 1x TAE buffer. The band patterns were photographed using geldoc system (Bio-Rad). The reproducibility of the amplification was confirmed by repeating each experiment three times.

The amplified bands were scored for presence (1) or absence (0) of bands. Statistical analysis of data was performed by means of NTSYS-pc software version 2.01 (Rohlf, 9). Cluster analysis demonstrating genetic relationships of accessions were generated using unweighted pair-group method using arithmetic averages (UPGMA) and simple matching coefficient, while resolution power was calculated according to Prevost and Wilkinson (7).

RESULTS AND DISCUSSION

The information obtained on banding pattern by the analysis of 20 mango genotypes from Gir region with 21 ISSR primers is summarized in Table 1. The primers yielded a total 125 bands and 119 scorable polymorphic markers, accounting for 95.2% of total reproducible amplification products in the range 42 to 2522 bp after amplification of total genomic DNA. Each primer could amplify 4 to 11 DNA bands, of which primer UBC-840 generated the highest number of bands (11) followed by UBC 855, UBC 835, and UBC 848. While primers UBC 817, UBC 825, UBC 844, UBC 884 and UBC 891 showed lowest number (4) of DNA bands.

Out of the total 119 polymorphic DNA bands, 34 DNA bands were unique indicating of its presence only in one of the landraces. While 85 polymorphic DNA bands were shared among few landraces. Except UBC 807, UBC 809, UBC 811, UBC 836 and UBC 889 all other primers showed 100% polymorphism. The average number of loci produced in 20 genotypes by the primers used ranged from 0.40 (UBC 844) to 4.40 (UBC 835) with an average of 1.80. The resolution power (Rp) of 21 ISSR primers ranged from 0.80 to 8.80 with an average of 3.58 (Table 1). Jaccard's similarity coefficient between genotypes ranged from 0.21 to 0.59 (Table 3). The maximum genetic similarity was found between 'Jamadar' and 'Kesar' and lowest between 'Khodi' and 'Agargato'. The genotypes having more than 50% similarity were 'Giriraj' and 'Kavasjipatel'; 'Kavasjipatel' and 'Neelum'; 'Amir Pasand' and 'Agargato'; 'Jamrukhiyo' and 'Chhappaniyo'; 'Jamrukhiyo' and 'Neelum'; 'Jamrukhiyo' and 'Kesar'; 'Amrutiyo' and 'Neelum'; and 'Neelum' and 'Alphanso'.

The genetic` relatedness among the different genotypes was distributed among three main divergent clusters (Fig. 1). The first cluster consisted of 'Kaju' and 'Khodi'. The second cluster had two sub-clusters (A & B): the sub-cluster A consists only of 'Dudh

Table 1. Polymorphism obtained with different ISSR primers generated from 20 *M. indica* genotypes.

Name of primer	Polymorphic band (s)			Monomorphic band (s)	Total bands	Percent polymorphism	Av. No. of loci produced	Resolution power
	S	U	T					
UBC-807	2	2	4	1	5	80.0	1.40	2.80
UBC-808	4	2	6	0	6	100.0	1.55	3.10
UBC-809	4	0	4	1	5	80.0	3.40	6.80
UBC-811	2	1	3	2	5	60.0	3.55	7.10
UBC-812	5	1	6	0	6	100.0	1.65	3.30
UBC-817	4	0	4	0	4	100.0	1.10	2.20
UBC-825	4	0	4	0	4	100.0	1.60	3.20
UBC-834	4	2	6	0	6	100.0	2.15	4.30
UBC-835	7	1	8	0	8	100.0	4.40	8.80
UBC-836	5	0	5	1	6	83.3	3.05	6.10
UBC-840	5	6	11	0	11	100.0	2.75	5.50
UBC-844	2	2	4	0	4	100.0	0.40	0.80
UBC-845	3	2	5	0	5	100.0	0.60	1.20
UBC-848	4	4	8	0	8	100.0	0.90	1.80
UBC-855	4	5	9	0	9	100.0	1.50	3.00
UBC-856	5	2	7	0	7	100.0	0.70	1.40
UBC-857	5	2	7	0	7	100.0	1.05	2.10
UBC-864	6	0	6	0	6	100.0	1.20	2.40
UBC-884	3	1	4	0	4	100.0	1.70	3.40
UBC-889	3	1	4	1	5	80.0	2.25	4.50
UBC-891	4	0	4	0	4	100.0	0.70	1.40
Total	85	34	119	6	125	94.4	1.80	3.58

S = Shared; U = Unique; T = Total polymorphic bands

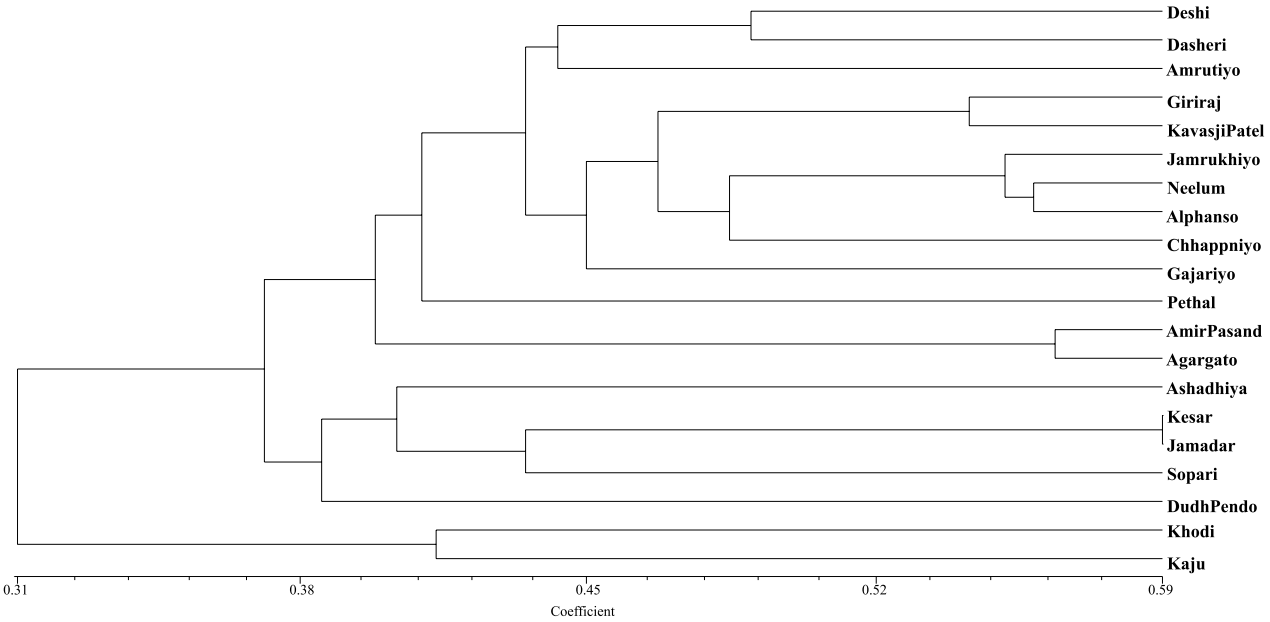

Fig. 1. Dendrogram among 20 mango genotypes by ISSR markers based on UPGMA analysis.

Pendo', while the sub-cluster B consists of 'Sopari', 'Jamadar', 'Kesar' and 'Ashadhiya'. The third cluster was composed of two sub-clusters (C & D). 'Agargato' and 'Amir Pasand' were in sub-cluster C, while 'Pethal', 'Gajariyo', 'Chhappaniyo', 'Alphanso', 'Neelum', 'Jamrukhiyo', 'Kavasji Patel', 'Giriraj', 'Amrutiyo', 'Dashehari' and 'Deshi' were in cluster D.

The first two eigen vectors were plotted indicating the separation of population in four clear clusters indicating the genotypic similarity among the groups. The first group consisted of only 'Khodi', indicating genotypic diversity present from other genotypes. The second group consisted of 'Agargato', 'Kaju', 'DudhPendo' and 'Kesar', while group three consisted

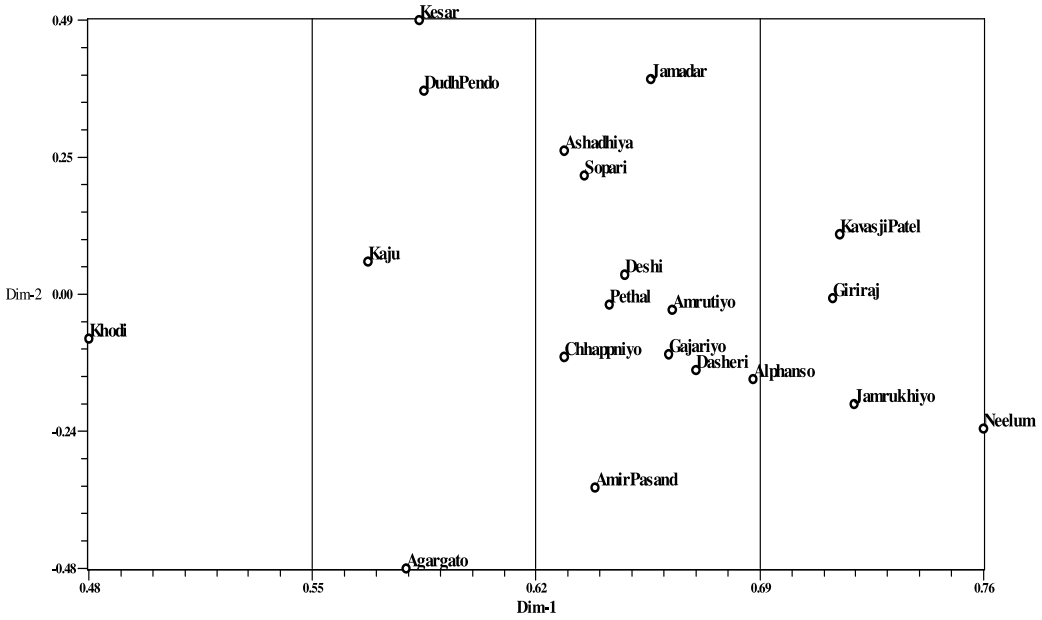

Fig. 2. Principal coordinate analysis of 20 mango genotypes using ISSR markers.

Table 2. Jaccard's similarity matrix for 20 mango genotypes.

Genotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	**17**	**18**	**19**	**20**
1	1.00																			
2	0.45	1.00																		
3	0.40	0.38	1.00																	
4	0.35	0.27	0.27	1.00																
5	0.42	0.38	0.44	0.27	1.00															
6	0.47	0.42	0.40	0.27	**0.54**	1.00														
7	0.37	0.42	0.37	0.24	0.45	0.47	1.00													
8	0.38	0.36	0.24	0.30	0.46	0.45	0.45	1.00												
9	0.38	0.37	0.34	0.29	0.47	0.47	0.46	0.42	1.00											
10	0.34	0.29	0.33	0.24	0.45	0.38	0.44	0.33	**0.54**	1.00										
11	0.40	0.42	0.31	0.33	0.40	0.44	0.36	0.36	0.46	0.38	1.00									
12	0.31	0.36	0.35	0.41	0.40	0.31	0.33	0.33	0.35	0.36	0.36	1.00								
13	0.38	0.39	0.38	0.29	0.47	0.37	0.43	0.37	0.42	0.40	0.41	0.37	1.00							
14	0.31	0.26	0.22	**0.21**	0.43	0.33	0.38	**0.56**	0.47	0.31	0.33	0.23	0.39	1.00						
15	0.34	0.41	0.40	0.20	0.39	0.46	0.36	0.33	0.32	0.28	0.33	0.26	0.34	0.27	1.00					
16	0.48	0.40	0.33	0.26	0.43	0.49	0.48	0.40	0.45	0.38	0.48	0.33	0.34	0.39	0.33	1.00				
17	0.50	0.41	0.37	0.36	0.50	**0.53**	0.46	0.48	**0.55**	0.44	**0.52**	0.41	0.46	0.50	0.33	**0.55**	1.00			
18	0.40	0.36	0.35	0.39	0.44	0.47	0.40	0.41	**0.55**	0.47	0.46	0.33	0.41	0.35	0.33	0.41	**0.56**	1.00		
19	0.36	0.43	0.37	**0.21**	0.45	0.47	0.35	0.36	0.47	0.37	0.42	0.31	0.38	0.31	**0.59**	0.38	0.39	0.39	1.00	
20	0.32	0.36	0.37	0.28	0.41	0.45	0.33	0.31	0.47	0.43	0.42	0.38	0.38	0.27	0.37	0.33	0.47	0.42	0.49	1.00

1= Deshi; 2 = Ashadhiya; 3 = DudhPendo; 4 = Khodi; 5 = Giriraj; 6 = KavasjiPatel; 7 = Gajariyo; 8 = AmirPasand; 9 = Jamrukhiyo; 10 = Chhappniyo; 11 = Amrutiyo; 12 = Kaju; 13 = Pethal; 14 = Agargato; 15 = Kesar; 16 = Dashehari; 17 = Neelum; 18 = Alphanso; 19 = Jamadar; and 20 = Sopari.

of maximum genotypes indicating similarity among these genotypes. The fourth group consists of 'Jamrukhiyo', 'Giriraj', 'Kavasjipatel' and 'Neelum'.

The present study thus provides evidence that the ISSRs appear to be effective to explore the molecular polymorphism and to assess the genetic relationships in the mango. Using ISSR primers, it was demonstrated that most of the genotypes could be easily distinguished. Moreover, some fragments were uniquely amplified or absent in some of the genotypes. These fragments are of great interest in optical management and genetic identification of *M. indica* accessions in a germplasm collection. Over all these data extends the knowledge of ISSR application as a molecular tool in mango as reported by several workers (Yonemori *et al*., 10; Adato *et al*., 1; Karihaloo *et al*., 6; Eiadthong *et al*., 3; Gonzalez *et al*., 5; Fang *et al*., 4), who had used ISSR and other markers for molecular characterization of mango.

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