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Full Length Research Paper

Phylogenetic relationship among Mango (*Mangifera indica* L.) Landraces of Saurashtra based on DNA fingerprinting

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Twenty mango cultivars (*Mangifera indica* Linn) collected from Gir region of Saurashtra were examined by ISSR markers. Of the 50 primers screened, 21 primers gave reproducible, polymorphic DNA amplification patterns, and were selected to construct a DNA fingerprinting map to distinguish the genotypes of mango. According to the banding patterns obtained with 21 selected primers, all cultivars tested in this study except Jamadar and Kesar were distinguished from each other and showed ample genetic diversity, indicating that ISSR-PCR was an effective method for cultivar identification of mangoes. Based on 125 selected bands, all Gir mango landraces tested were clustered into a three big groups with 'Kaju' and 'Khodi' in first group, Dudh Pendo, Sopari, Jamadar, Kesar and Ashadhiya in second group, while the third cluster was composed of Agargato, Amir Pasand, Pethal, Gajariyo, Chhappaniyo, Alphanso, Neelum, Jamrukhiyo, Kavasji Patel, Giriraj, Amrutiyo, Dasheri and Deshi based on UPGMA analysis, indicating that some Gir landraces had a close relationship with each other, while some were drastically dissimilar from other landraces.

Key words: *Mangifera indica* L., Genetic diversity, ISSR markers, Mango landraces.

INTRODUCTION

Mango (*Mangifera indica* L.) a diploid fruit tree with 2n=40 chromosomes (Mukherjee, 1950) originated in the Indo-Burma region during the earlier period of the Cretaceous era (Yonemori et al., 2002) and gradually spread to the tropical and subtropical regions of the world. India is thought to be the primary centre of diversity along with its status as the centre of origin for mango. Presently, India harbours more than 1000 mango cultivars and represents the biggest mango germ pool in the world. Australia, China, USA, Israel and Thailand are the other regions that maintain such healthy mango germ pool.

In the last decade molecular methods have been implemented in mango and these included isozymes (Degani et al., 1990), variable number tandem repeats (VNTR) (Adato et al. 1995), random amplified polymorphic DNA (RAPDs) (Schnell et al. 1995; Bally et al.1996; Lopez-Valenzuela et al., 1997; Ravishankar et al.2000; Karihaloo et al. 2003); inter-simple-sequence-repeats (ISSRs) (Eiadthong et al., 1999; Gonzalez et al., 2002) and amplified fragment length polymorphism (AFLPs) (Fang et al., 1999; Eiadthong et al., 2000). RAPDs and AFLPs have also been applied in discrimination of mango cultivars in China (Xu et al., 1998; Fang et al., 1999, 2000, 2001), while ISSRs in mango have not been reported in India. Among these, Inter Simple Sequence Repeat, ISSR (Zietkiewicz et al., 1994) is a reproducible semi-arbitrary primed PCR method that uses simple sequence repeats as primers, combining most of the advantages of micro-satellites and Amplified Fragment Length Polymorphism (AFLP), to the universality of Randomly Amplified Polymorphic DNA. 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. This is also the attribute of ISSRs, which renders them useful asa supple-mentary system to any of the random dominant marker systems. Automated PCR base makes ISSRs the markers of choice for screening genotypes.

In Gujarat, during the Mugal empire lots of mango cultivars were planted in the surrounding of Gir region. Among these

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

S/No.	Name of Primer	Primer sequence 5'-3'	Tm (^{0}C)	% GC
1	UBC-807	$(AG)_8T$	42.4	47.1
2	UBC-808	$(AG)_8C$	46.7	52.9
3	UBC-809	$(AG)_8G$	46.5	52.9
4	UBC-811	$(GA)_8C$	43.2	52.9
5	UBC-812	$(GA)_8A$	44.3	47.1
6	UBC-817	$(CA)_8A$	52.7	47.1
7	UBC-825	$(AC)_8T$	49.2	47.1
8	UBC-834	$(AG)_8YT$	45.3	44.4
9	UBC-835	$(AG)_8YC$	45.5	50.0
10	UBC-836	$(AG)_8YA$	43.3	44.4
11	UBC-840	$(GA)_8YT$	45.7	44.4
12	UBC-844	$(CT)_8RC$	46.5	50.0
13	UBC-845	$(CT)_8RG$	47.7	50.0
14	UBC-848	$(CA)_8RG$	55.5	50.0
15	UBC-855	$(AC)_8YT$	51.9	44.4
16	UBC-856	$(AC)_8YA$	49.8	44.4
17	UBC-857	$(AC)_8YG$	43.7	50.0
18	UBC-864	$(ATG)_6$	51.2	33.3
19	UBC-884	HBH $(AG)_7$	41.9	41.2
20	UBC-889	DBD $(AC)_7$	47.0	41.2
21	UBC-891	HVH $(TG)_7$	51.7	41.2

cultivars Kesar variety is famous throughout the world for its taste and flavor. Along with Kesar there are many other landraces which has the equal potential. Precise information on the genetic relationships within such germplasm diversity is needed for carrying out efficient breeding programmes. In view of this, the present experiment was conducted during December 2009 with 20 mango landraces of Gir region. These 20 mango landraces were analyzed for phylogenetic relationship with ISSR's in order to assess the genetic diversity.

MATERIALS AND METHODS

Plant material

For the present experiment, 20 landraces of Gir region were selected on the basis of their consistency in behavior for the last 30 years at their growing region. Their fruit size, sweetness and yielding behavior were also considered for selection.

DNA isolation

DNA was extracted from mango leaves using the hexadecyltrimethylammonium bromide (CTAB) method described by Doyle and Doyle (1990) with a little modification. Sample of 0.5 g leaves were ground to fine power in liquid nitrogen and added into a 10 ml tube with 4 ml preheated (65ºC) extraction buffer consisting of 2% CTAB, 1.4 mol L^{-1} NaCl, 0.1% (v/v) β-mercaptoethanol, 20 mmol L^{-1} EDTA, 100 mmol L^{-1} Tris-HCl (pH 8.0), and 1% (w/v) PVP-40. The homogenates were incubated at 65ºC for 1 h and extracted with 4 ml chloroform: isoamyl alcohol (24:1) solution. The tubes were mixed for about 5 min, and spun at 12000 rpm for 10 min. The supernatant was transferred to a new tube containing twice volume of 100% ethanol and 1/10 volume 3 mol L^{-1} NaAc, mixed gently, left at -20ºC for 1 h and spun at 12000 rpm for 15 min. The supernatant was then discarded and the pellet washed twice with 70% ethanol. The pellet was dried at room temperature, and resuspended into 200 µl 0.1 × TE with RNase. The DNA was precipitated with 400 µl 100% ethanol at 20ºC for 1 h and spun at 12000 rpm for 15 min. The pellet was resuspended into 100 µl 0.1 × TE. DNA was quantified by picodrop.

ISSR markers

In this study, initially 50 ISSR primers were used for screening of mango landraces. But only the polymorphic ones were used for further analyses and estimating of genetic diversity among mango landraces. ISSR primers synthesized by Bangalore Genei were used for PCR. The polymorphic ISSR markers are given in Table 1 along with Tm (°C) and %GC content. An amplification reaction was performed as follows:

25 ng of template DNA, 200 µM of each dNTPs (Bangalore Genei), 20 ng of primer (Bangalore Genei), 0.5 U of Taq DNA polymerase (Bangalore Genei, India) and 1× reaction buffer (Banglore Genei, India) 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 in a Bio-Rad thermal cycler.

Agarose gel electrophoresis

Amplified products were separated in 1.5% agarose gel containing ethidium bromide using 1× TAE buffer. A constant voltage of 55 was provided for 3 to 4 h. DNA fragments were visualized under

Table 2. Polymorphism obtained with different ISSR primers generated from 20 *M. indica* genotypes.

S/No.	Name of primer	Polymorphic bands			Monomorphic bands	Total bands	Polymorphic (%)	Average No. of loci produced in 20 genotypes	Resolution power
		S	U	T					
1	UBC-807	2	2	4	1	5	80.0	1.40	2.80
2	UBC-808	4	2	6	0	6	100.0	1.55	3.10
3	UBC-809	4	0	4	1	5	80.0	3.40	6.80
4	UBC-811	2	1	3	2	5	60.0	3.55	7.10
5	UBC-812	5	1	6	0	6	100.0	1.65	3.30
6	UBC-817	4	0	4	0	4	100.0	1.10	2.20
7	UBC-825	4	0	4	0	4	100.0	1.60	3.20
8	UBC-834	4	2	6	0	6	100.0	2.15	4.30
9	UBC-835	7	1	8	0	8	100.0	4.40	8.80
10	UBC-836	5	0	5	1	6	83.3	3.05	6.10
11	UBC-840	5	6	11	0	11	100.0	2.75	5.50
12	UBC-844	2	2	4	0	4	100.0	0.40	0.80
13	UBC-845	3	2	5	0	5	100.0	0.60	1.20
14	UBC-848	4	4	8	0	8	100.0	0.90	1.80
15	UBC-855	4	5	9	0	9	100.0	1.50	3.00
16	UBC-856	5	2	7	0	7	100.0	0.70	1.40
17	UBC-857	5	2	7	0	7	100.0	1.05	2.10
18	UBC-864	6	0	6	0	6	100.0	1.20	2.40
19	UBC-884	3	1	4	0	4	100.0	1.70	3.40
20	UBC-889	3	1	4	1	5	80.0	2.25	4.50
21	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.

UV light. The patterns were photographed using Geldoc system(Bio-Rad) and stored as digital pictures. The reproducibility of the the amplification was confirmed by repeating each experiment three times.

Data collection and analysis

Since ISSR primers are dominant markers, amplified bands were scored for presence (1) or absence (0) of bands. Statistical analysis of data was performed by means of NTSYS-pc software (Rohlf, 2000). Cluster analysis demonstrating genetic relationships of accessions were generated using unweighted pair-group method using arithmetic averages (UPGMA) and simple matching coefficient. Resolution power was calculated according to Prevost and Wilkinson (Prevost and Wilkinson, 1999), which states that the Resolving power (Rp) of a primer is:

$Rp = \sum IB$

where IB (band informativeness) takes the value of: $1 – [2 \times (0.5 – p)]$, where p being the proportion of the 20 landraces (Mango) containing the band.

RESULTS AND DICUSSION

A set of 50 ISSR primers were used for initial screening of mango landraces, of which 21 primers (Table 1) were selected for further analysis of all the landraces depending on the amplification of the DNA. The information obtained on banding pattern by the analysis of twenty landraces of Gir region with 21 ISSR primers is summarized in Table 2. Twenty one ISSR primers used in this study yielded a total 125 bands and 119 scorable polymorphic

Table 3. Jaccard's similarity matrix for twenty mango landrace of Gir region.

	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. Dasheri; 17. Neelum; 18. Alphanso; 19. Jamadar; 20. Sopari.

markers, accounting for 95.2% of total reproducible amplification products in the range 42 to 2522 bp after amplification of total genomic DNA of twenty landraces of mango. Each primer could amplify 4 to 11 DNA bands, of which primer UBC-840 generated the highest number of bands followed by UBC 855, UBC 835, and UBC 848. While primer UBC 817, UBC 825, UBC 844, UBC 884 and UBC 891 showed lowest number 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 twenty genotypes by the primers 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 2). The clearest 125 amplified bands were treated as dominant genetic markers. For each sample ISSR bands were scored as 1 (present) or 0 (absent) and these binary data were used to assemble a rectangular matrix. The use of UPGMA algorithm permitted to cluster the data and to draw the relationships between the tested landraces. Jaccard's similarity between landraces ranged from 0.21 to 0.59 (Table 3), with maximum genetic similarity between 'Jamadar' and 'Kesar' and lowest genetic similarity between Khodi and Agargato. The landraces having more than 50% similarity are as follow:

Giriraj and KavasjiPatel; KavasjiPatel and Neelum; AmirPasand and Agargato; Jamrukhiyo and Chhappaniyo; Jamrukhiyo and Neelum; Jamrukhiyo and Kesar; Amrutiyo and Neelum; Neelum and Alphanso.

In addition to this, relationships among the different landraces was distributed among three

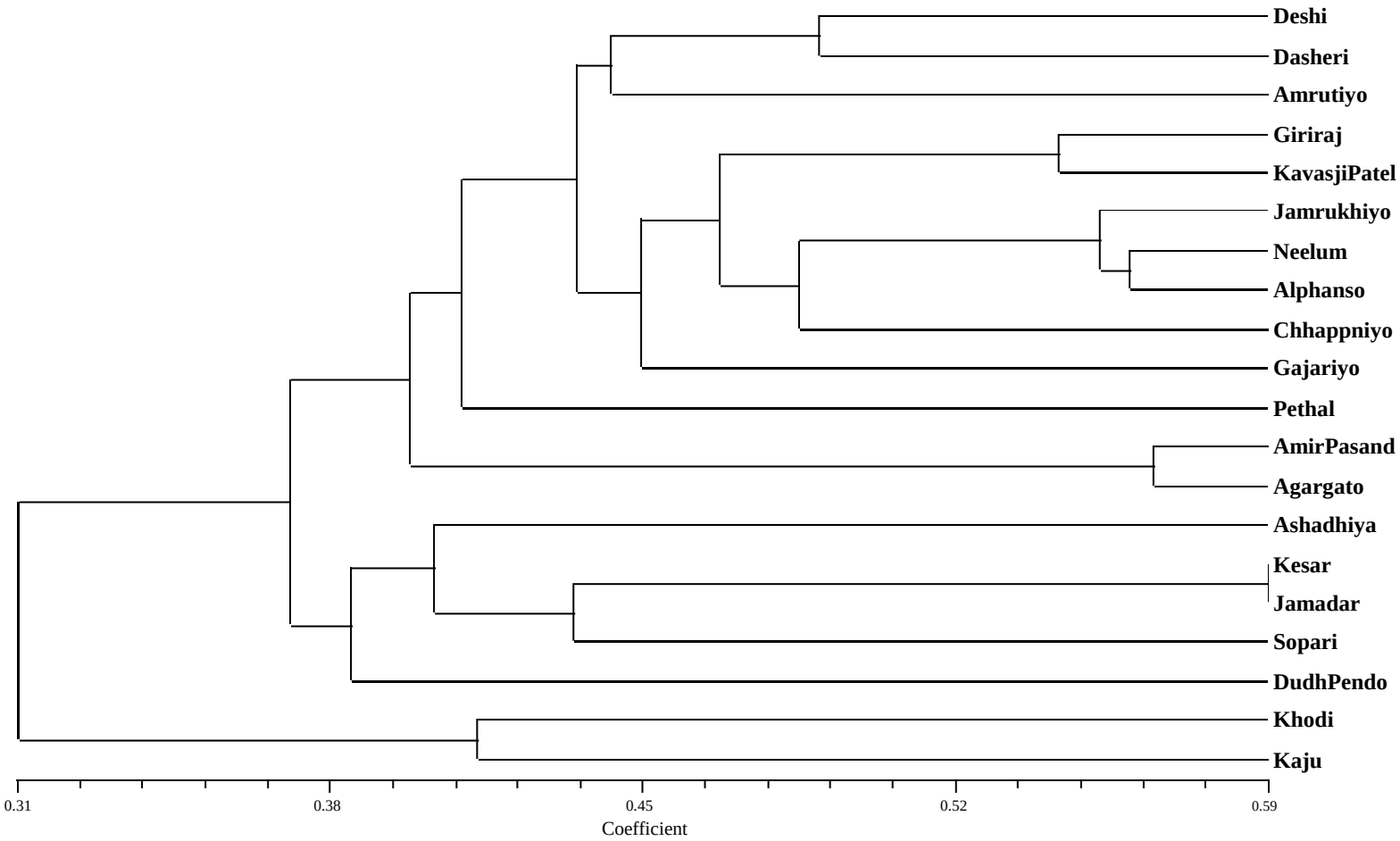

Figure 1. Dendrogram of phylogenetic relationship among 20 mango landraces by ISSR markers based on UPGMA analysis.

main divergent clusters significantly (Figure 1). The first cluster consists of 'Kaju' and 'Khodi'. The second cluster was composed of two subclusters (A and B): the subcluster A consists only of'Dudh Pendo', while the subcluster B consists of 'Sopari', 'Jamadar', 'Kesar' and 'Ashadhiya'. Again the third cluster was composed of two subclusters (C and D). Landraces Agargato and Amir Pasand were in subcluster C, while Pethal, Gajariyo, Chhappaniyo, Alphanso, Neelum, Jamrukhiyo, Kavasji Patel, Giriraj, Amrutiyo, Dasheri and Deshi were in cluster D.

In Figure 2 first two eigenvectors were plotted indicating the separation of population in four clear groups. These groups indicate the genotypic similarity among the groups. The first group consists of only Khodi, indicating genotypic diversity present from other genotypes. The second group consists of Agargato, Kaju, DudhPendo and Kesar, while group three consist of maximum genotypes indicating similarity among these

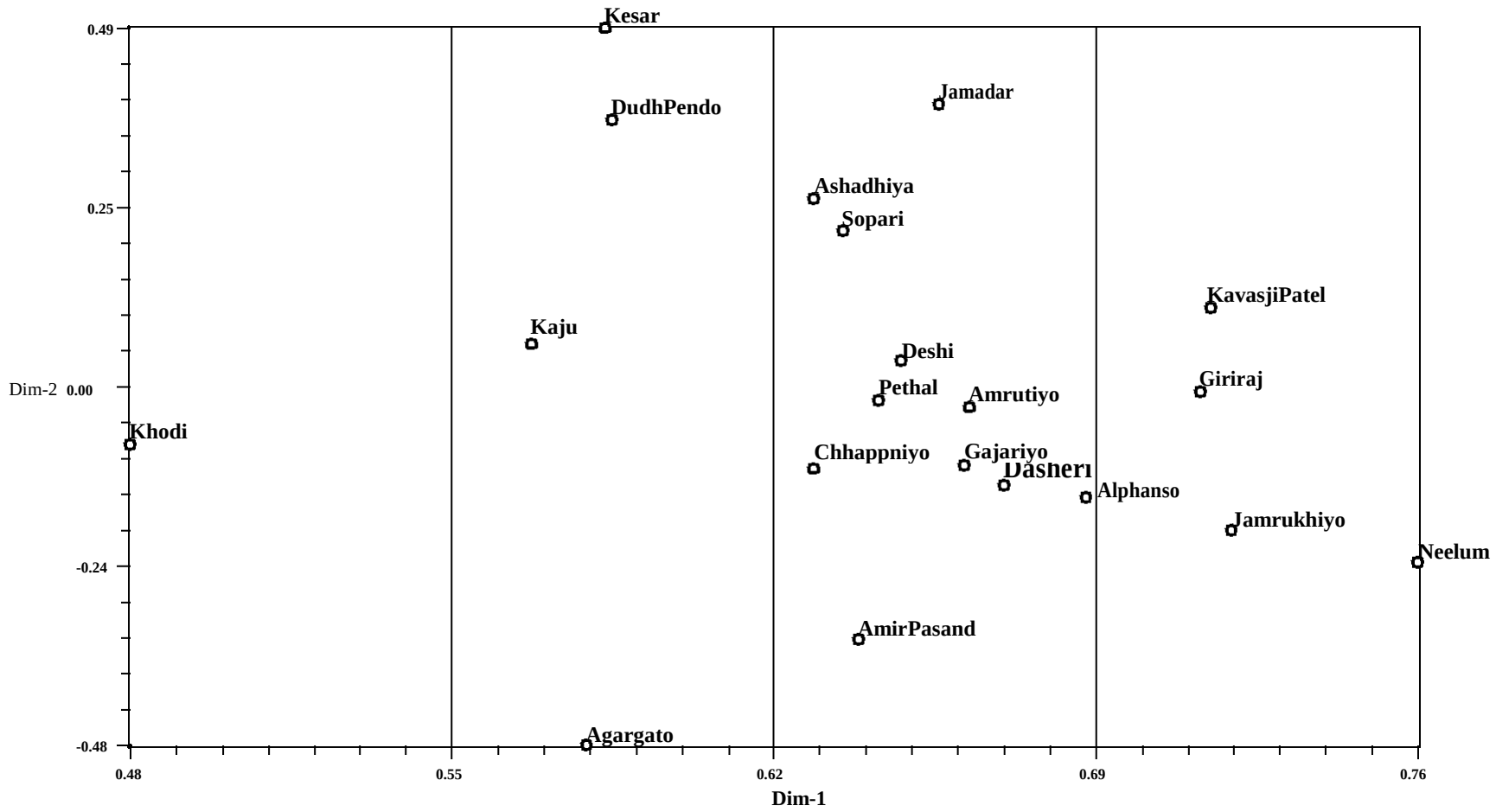

Figure 2. Principal coordinate analysis of 20 mango landraces using ISSR markers.

genotypes. The fourth group consists of Jamrukhiyo, Giriraj, KavasjiPatel and Neelum.

In this study, ISSR profiling technology was used in order to enlarge the number of molecular markers that are suitable in the molecular characterization and examination of the genetic relationships between the landraces of mango. The present work 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 assessment, it was demonstrated that most of the cultivars can be easily distinguished. Moreover, some fragments were uniquely amplified or absent in some of the landraces. These fragments are of great interest in optical management and genetic identification of *M. indica* accessions in the germplasm collection. Over all these data extends the knowledge of ISSR application as a molecular tool in mango as reported by Yonemori et al. (2002), Adato et al. (1995), Schnell et al. (1995), Lopez-Valenzuela et al. (1997), Karihaloo et al. (2003), Eiadthong et al. (1999), Eiadthong et al. (2000), Gonzalez et al.

(2002), Fang et al. (1999), Fang et al. (2000); Fang et al. (2001) and Xu et al. (1998), who have used ISSR and other markers for molecular characterization of mango.

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