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Molecular analysis of Mango (*Mangifera indica* L.) Cv. Alphonso from different locations of South Konkan

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Abstract

The present study was carried out with an objectives to establish the molecular profile of Alphonso from different locations of South Konkan and to analyze the variation between them. Five components of the extraction buffer were standardized by using rapid method. The results obtained using 0.09 g ml^{-1} glucose, 0.01g ml^{-1}PVP, 0.004g ml^{-1} sodium bisulphite, 0.005g ml^{-1} sodium lauryl sulphate, 50µl ml^{-1} sarcosyl were most suitable. Modifications in extraction procedure resulted into better and clear banding pattern when subjected to PCR analysis. The average per cent polymorphism between ten locations were 46.62 per cent. In ISSR marker, the overall range of similarity ranged from 0.559 to 0.733. The average PIC value was 0.733 per primer. Substantial average polymorphism was detected by ISSR primers among five samples from Achara (42.83 %), Adivare (42.39 %), Rameshwar-Girye (42.73 %), Nadan-Jamsande (46.77 %), Katta (44.22%), Ambivali-Kelshi (44.68 %), Murud (46.29 %), Padel (30.45 %), Pawas (47.05 %) and Vengurle (42.67 %).This study indicates that genetic variation was found between selected ten locations and also within five samples of each location. Such study is important for detecting the distinctness of the same variety from different geographical locations.

Keywords: ISSR, Polymorphism, Mango, *Mangifera indica*, Molecular markers.

Introduction

Mango (*Mangifera indica* L., Family: Anacardiaceae, Chromosome number: 2n = 40)is one of the oldest and most important tropical fruits worldwide. It is rightly known as 'King of fruits' owing to its nutritional richness, unique taste, and pleasant aroma. It is also considered as 'National fruit of India'. It originated in the South East Asian or Indo-Burma Region and has been cultivated for 4000 years in India (Mukherjee, 1953; Kostermans and Bompard, 1993). It is cross-pollinated and exhibits high levels of genetic variation among cultivars. Genetic variation within a single variety of mango would be detrimental to its economic value.

The molecular analysis is an important for mango improvement programs and management of genetic resources. In mango growing regions, breeding attempts are always in progress for creating better cultivars. The main objectives of mango breeding aimed to improve both plant and fruit characteristics such as dwarf trees, profuse and regular bearing, good fruit size and edible quality, less fibers, attractive peel and pulp color, diseases resistance and long storage life (Usman *et al.*, 2001). Precise information is needed for carrying out efficient breeding programs. In order to analyze molecular makeup in Alphonso, PCR-based DNA markers are among the best tool. Unlike the agronomic and morphological characteristics, the molecular markers are not subjected to the environmental effect.

Inter Simple Sequence Repeats or ISSR have been proven useful for detecting genetic polymorphisms among accessions by generating a large number of markers that target multiple microsatellite loci distributed across the genome. ISSR are often used because of their capability of reproducibility, with no gene sequence information, and prior genetic studies are required for the analysis. ISSR markers have been reported to undertake the assessment of genetic diversity of mango genotypes (Pandit *et al*., 2007).

In Konkan, Deogad Alphonso (Hapus) has signature characteristics like the aroma, no fiber and sweet taste. These characteristics are ascribed to geographical conditions of Deogad and not observed in varieties grown elsewhere. As we move from South Konkan to North Konkan, Alphonso differ in taste. Very few research has been done on phenotypic characters of Alphonso of South Konkan and other region of Konkan. But the genotypic information of Alphonso is lacking.

On account of this, experiment was conducted with the following objectives:

1. To establish Alphonso mango genotype profiles through ISSR markers.
2. To analyze genetic variability of Alphonso mango through ISSR marker.

Materials and Methods

Plant material:

For the present study, the experimental study leaf samples of the mango cultivar Alphonso were collected from the following different locations (5 samples from each location) and mentioned in Table 1.

Table 1: Details of 50 leaf samples used in the study.

Sr. No.	Name of farmer & Location	Sample No.	North Latitude	East Longitude
1.	Dr. Vishwas Ashok Kelkar **Ambivali–Kelshi** Tal. Dapoli Dist. Ratnagiri	K1	17°90'78.12"	73°08'04.91"
		K2	17°90'82.41"	73°08'04.52"
		K3	17°90'78.64"	73°08'18.14"
		K4	17°90'78.65"	73°08'17.74"
		K5	17°90'78.57"	73°08'17.41"

2.	Dr. Makrand Shrinivas Joshi **Murud** Tal. Dapoli Dist. Ratnagiri	M1	17°77'31.62"	73°11'89.24"
		M2	17°77'35.80"	73°11'97.96"
		M3	17°46'60.96"	73°90'52.46"
		M4	17°46'80.51"	73°75'52.62"
		M5	17°46'20.41"	73°71'60.42"
3.	Mr. Sunil Godbole **Pawas** Tal. Ratnagiri Dist. Ratnagiri	P1	16°52'55.74"	73°19'21.52"
		P2	16°53'15.68"	73°19'80.76"
		P3	16°52'56.01"	73°19'23.26"
		P4	16°52'56.51"	73°19'23.66"
		P5	16°52'56.18"	73°19'21.04"
4.	Mr. Surendra Shridhar Karekar **Adivare** Tal. Rajapur Dist. Ratnagiri	A1	16°43'12.43"	73°20'38.56"
		A2	16°43'12.26"	73°20'39.40"
		A3	16°43'12.48"	73°21'80.15"
		A4	16°43'10.95"	73°20'40.33"
		A5	16°43'11.21"	73°20'40.33"
5.	Mango Research Sub Centre, **Rameshwar-Girye** Tal. Deogad Dist. Sindhudurg. Dr. B. S. K. K. V. Dapoli.	G1	16°31'40.51"	73°20'43.10"
		G2	16°31'40.39"	73°20'12.44"
		G3	16°31'39.71"	73°20'44.78"
		G4	16°31'39.48"	73°20'45.44"
		G5	16°31'39.84"	73°20'45.33"
6.	Mr. Kiran Manohar Marathe **Padel** Tal. Deogad Dist. Sindhudurg.	D1	16°28'22.10"	73°23'40.42"
		D2	16°28'41.79"	73°22'34.36"
		D3	16°28'21.98"	73°23'40.08"
		D4	16°28'21.79"	73°23'40.78"
		D5	16°28'21.79"	73°23'40.74"
7.	Mr. Prasanna Gogate **Nadan–Jamsande** Tal. Deogad Dist. Sindhudurg.	J1	16°26'30.54"	73°24'60.80"
		J2	16°26'29.45"	73°26'62.70"
		J3	16°26'30.23"	73°24'61.12"
		J4	16°26'31.56"	73°24'61.43"
		J5	16°26'30.75"	73°24'61.69"
8.	Mr. Mahesh Madhukar Rane **Achara** Tal. Malvan Dist. Sindhudurg.	C1	16°12'44.12"	73°28'51.45"
		C2	16°12'51.46"	73°28'50.23"
		C3	16°12'36.63"	73°28'53.66"
		C4	16°12'51.23"	73°28'53.81"
		C5	16°12'51.79"	73°28'52.92"
9.	Regional Fruit Research Station (RFRS), **Vengurle**. Tal. Vengurle Dist. Sindhudurg. Dr. B. S. K. K. V. Dapoli.	V1	15°52'48.85"	73°38'66.12"
		V2	15°52'60.64"	73°38'59.53"
		V3	15°52'55.19"	73°38'48.87"
		V4	15°52'36.20"	73°38'87.66"
		V5	15°52'72.43"	73°38'59.90"
10.	Mr. Satish Wanjari **Katta** Tal. Malvan Dist. Sindhudurg.	L1	15°52'21.55"	73°39'29.55"
		L2	15°52'20.33"	73°39'33.28"
		L3	15°52'20.45"	73°39'54.92"
		L4	15°52'23.91"	73°39'33.50"
		L5	15°52'20.42"	73°39'31.74"

All leaf samples were frozen in ice bags for transportation to the laboratory and subsequently stored at below 0°C until processed.

DNA Extraction

DNA was isolated following the protocol of Doyle and Doyle (1990) with slight modifications. Three different solutions (T_1, T_2, and T_3) were tested to extract mango leaf DNA (Table 2). The young newly emerged leaf samples (0.2 g each) were collected to extract genomic DNA and sterilized with 70% ethanol to avoid the contamination. RNA was removed by treatment with 100 µg ml^{-1} RNase (Merck Pvt. Ltd., Mumbai, India). The size range and concentration of genomic DNA in each sample was determined after electrophoresis using a standard DNA ladder (100 bp) in 0.8% (w/v) Agarose gel and by comparing the intensity of staining with 10 mg ml^{-1} Ethidium bromide.

Table 2. Chemical composition of the three extraction solutions T_1–T_3 used to isolate genomic DNA from mango leaves.

Sr. No.	Components	T_1	T_2	T_3
1.	Glucose	0.08 g ml^{-1}	0.09g ml^{-1}	0.10 g ml^{-1}
2.	PVP	0.005 g ml^{-1}	0.01 g ml^{-1}	0.015 g ml^{-1}
3.	Sodium Bisulphite	0.003g ml^{-1}	0.004 g ml^{-1}	0.005 g ml^{-1}
4.	Sodium Lauryl Sulphate	0.004g ml^{-1}	0.005 g ml^{-1}	0.006 g ml^{-1}
5.	Sarcosyl (5%)	40 µl ml^{-1}	50 µl ml^{-1}	60µl ml^{-1}
6.	Working buffer (Tris- HCl 200 mM, EDTA 25 mM & NaCl 250 mM)	10 ml	10 ml	10 ml

PCR amplification

The extracted DNA from all the samples was in varying concentrations. While preparing the working DNA for primer amplification all samples were diluted in such a manner as detailed in Table 3, so as it maintains the DNA content in all the samples in similar concentration.

Table 3: Sample wise dilution of the extracted DNA

Sr. No.	Dilution Ratio	Quantity of DNA (µl)	Quantity of D/W (µl)
1.	1:10	1	10
2.	1:20	1	20
3.	1:30	1	30
4.	1:40	1	40
5.	1:50	1	50
6.	1:60	1	60
7.	1:70	1	70
8.	1:80	1	80
9.	1:90	1	90
10.	1:100	1	100

Sixteen inter-simple sequence repeat (ISSR) markers, composed of short, pre-defined tandem repeat sequences with an anchor sequence, and representing different di- and tri-nucleotide microsatellites were used for PCR amplification. The ISSR markers were used for molecular analysis of mango between ten selected locations and within each location also. A PCR protocol was standardized for all 16 ISSR markers. Each 20 µl PCR contained 40 ng template DNA, 2.5 µl of 10X PCR buffer, 0.5 µl of 25 mM $MgCl_2$ (Bangalore Genei Pvt. Ltd., Banglaore.), 1 µl of 10 mM dNTPs (HiMedia Laboratories Pvt. Ltd.), 10 pmol of each ISSR primer (Bioresource Biotech Pvt. Ltd., Pune, India) and 3.0 units of *Taq* polymerase (HiMedia Laboratories Pvt. Ltd.).

Thermal profiles were standardized for each ISSR primer based on its melting temperature using a Master Cycler 2231 gradient-PCR machine (Eppendorf, Hamburg, Germany). The standard annealing temperatures of all ISSR primers are given in Table 4**.**

Table 4: Sequences and annealing temperatures of the 16 ISSR primers used in this study

Primer	Primer sequence (5' – 3')	Temp. Range (^{0}C)	Standardized Annealing temp. (^{0}C)	GC Content(%)
UBC 811	GAG AGA GAG AGA GAG AC	40-50	43.4	52.9
UBC 812	GAG AGA GAG AGA GAG AC	45-55	52.0	52.9
UBC-815	CTC TCT CTC TCT CTC TG	40-50	49.5	52.9
UBC-817	CAC ACA CAC ACA CAC AA	40-50	47.0	47.1
UBC-818	CAC ACA CAC ACA CAC AG	45-55	47.9	52.9
UBC-834	AGA GAG AGA GAG AGA GT	45-55	50.4	50.0
UBC-853	TCT CTC TCT CTC TCT CRT	45-55	54.5	50.0
UBC-854	TCT CTC TCT CTC TCT CRG	45-55	54.8	55.5
UBC-857	ACA CAC ACA CAC ACA CCG	45-55	51.7	55.5
UBC-876	GAT AGA TAG ACA GAC A	40-50	40.0	37.5
UBC-881	GGG TGG GGT GGG GTG	45-55	50.0	66.6
UBC-884	HBH AGA GAG AGA GAG AG	40-50	40.0	47.1
UBC-885	HBH AGA GAG AGA GAG AG	40-50	40.7	52.9
UBC-886	VDV CTC TCT CTC TCT CT	45-55	51.4	52.9
UBC-889	DBD ACA CAC ACA CAC AC	45-55	47.0	47.1
UBC-891	AGA TGT GTG TGT GTG TG	45-55	50.0	47.1

***** B = C, G, T; H = A, C, T; V = A, C, G; and D = A, G, T.

The PCR-amplified products were separated by electrophoresis in 2% (w/v) agarose gels at 110 V. The agarose gel was stained with 10 mg ml^{-1} ethidium bromide and photographed under UV light using Pentax K 312 nm camera. The images of gel were also taken by the documentation systems (Uvi-Tech. Fire reader, Cambridge, England) and the data were stored for further analysis.

Data Analysis

ISSR markers across the 50 samples were scored for their presence (1) or absence (0) of bands for each primer. The binary data so generated was used to estimate the levels of polymorphism by dividing the number of polymorphic bands by the total number of scored bands. Jaccard's similarity coefficients for each pairwise comparison between samples were calculated and similarity co-efficient matrix was generated. This matrix was subjected to unweighted pair group method with arithmetic mean (UPGMA) to construct a dendrogram (Padwale, 2012). The similarity co-efficient analysis and dendrogram construction were carried out by using MVSP-A Multivariate Statistical Package-5785 (Version 3.1). Cluster analysis was performed from the similarity matrix using Jaccard's similarity coefficients. Based on the ISSR banding patterns obtained, polymorphism percentages were calculated for the different primers.

Polymorphic information content (PIC) value were calculated as per formula developed by Powell *et al.*, (1996).

$$PIC = 1-\sum P_{ij}^{2}$$

Results and Discussion

The isolation of good quality deoxyribonucleic acid (DNA) is the pre-requisite for molecular research. Mango leaves contain high levels of polysaccharides, polyphenols, proteins, tannins and other secondary metabolites, whose presence can inhibit advanced molecular research from extracted genomic DNA (Uddin *et al*., 2014). Therefore, there was a need to establish the protocol for DNA extraction from mango leaf samples to yield high concentrations of good quality DNA was suitable for polymerase chain reaction (PCR) applications. Total of 50 samples were selected for extraction of genomic DNA from Alphonso samples which were labeled in order from K1 to L5. DNA was isolated from young tender leaves of each Alphonso sample using the rapid protocol of Doyle and Doyle (1990), with slight modifications in buffer composition and concentration. Various concentrations of glucose, polyvinylpyrrolidone (PVP), and sodium lauryl sulphate (SLS), were tested (Table 2). A combination of 0.09 g ml^{-1} glucose, 0.01 g ml^{-1} PVP, 0.004 g ml^{-1} sodium bisulphite, 0.005 g ml^{-1} SLS, and 50 µl ml^{-1} sarcosyl produced the highest yields and best quantity DNA without any contamination of polysaccharides and phenolic compounds also and showed clear DNA bands.The clear DNA bands observed in gel photograph revealed that the sample size of 0.2-0.3g was suitable, as it reduces the content of mucilage, which hampers the quality of DNA. A similar quantity of leaf sample were used by Uddin *et al.*, (2014).

The clear banding pattern was observed with 1 µl of diluted crude extract. This quantity given 40 ng of template DNA for reaction. Padmalatha and Prasad (2006) observed the clarity of bands with 1 µl of template DNA, and there was the presence of smearing at higher concentrations of the DNA template, which affected the repeatability; there was an absence of amplification with lower concentration.

Marker analysis

Molecular markers for assessment of genetic variation in plant have shown many advantages. They are neutral, not related to age and tissue type, and not influenced by the environmental conditions, have feasibility and lower costs, and are more informative than morphological markers (Ariffin *et al.*, 2015). The molecular markers are capable for detection of differences in the genetic information carried by the different individuals. Therefore, these markers are powerful tools in genotype identification or fingerprinting and the estimation of relatedness between genotypes (Kumar *et al.*, 2013).

Among these, Inter Simple Sequence Repeat (ISSR) 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 as a supplementary system to any of the random, dominant marker systems (Pandit *et al*., 2007).

Polymorphic Information Content (PIC):

Markers that have the ability to detect high number of discernable alleles are the suitable marker for molecular characterization and genetic diversity analysis. The polymorphism information content (PIC) value of each marker, which can be evaluated on the basis of its alleles, varied greatly for all tested ISSR loci. The PIC expresses the discriminating power of the locus taking into account not only the number of alleles that are expressed, but also their relative frequencies and frequency of alleles per locus (Samant *et al*., 2010). The PIC values calculated for the 16 ISSR primers. In this present study the highest PIC value produced by the primer UBC-876 (0.884).The ISSR profile of primer UBC-876 showed that the fragment having size 0.612 Kb was absent in sample C1, C2 and C4. Also sample C3 and A1 from Adivare were not displayed any fragment having size more than 1.9 Kb. Absence of such fragments in particular sample were shown by arrow (Fig. 1). The lowest PIC value given by the primer UBC-891 (0.00). The primer UBC-891 produced monomorphic banding pattern in all samples (Fig. 2). Average polymorphic information content was 0.733 among the all 50 samples of Alphonso. These results are conformation with Gajera *et al*., (2011) that is the PIC value was ranged from 0.138 to 0.398 across 20 mango genotypes. Also the primer UBC-886 showed higher polymorphism percentage. This primer showed absence of two fragments in sample G1 from Rameshwar-Giryeat 1.1 Kb and 2.0 Kb also in sample J1 and J2 from Nadan-Jamsande at 1.8 -1.9 Kb(Fig. 3).

The higher PIC value indicated the informativeness of the primer. Hence the primer UBC-876, UBC-886, UBC-818 and UBC-881 can use in future studies in the field of taxonomical and genetic resource management.

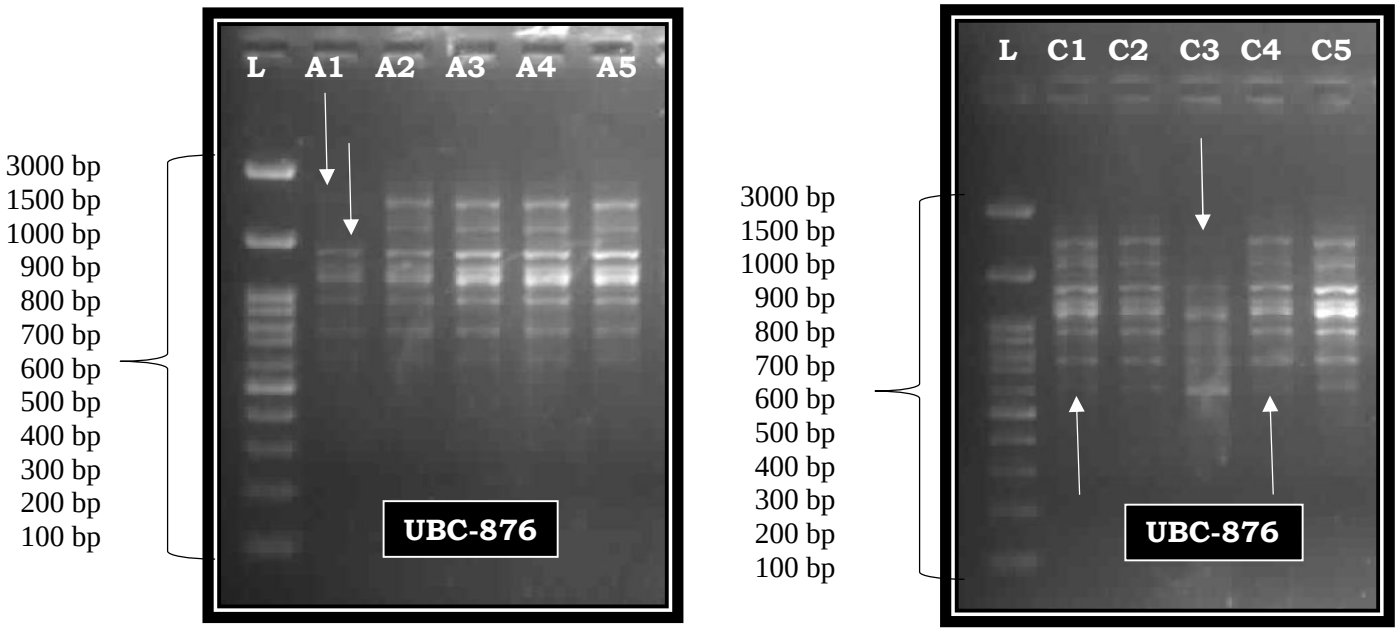


Fig. 1: Primer: UBC-876
Annealing temperature: 40˚C
L: DNA ladder (100-3000 bp)
C: - Samples collected from Achara (C1, C2, C3, C4 and C5)
A:-Samples collected from Adivare (A1, A2, A3, A4 and A5)

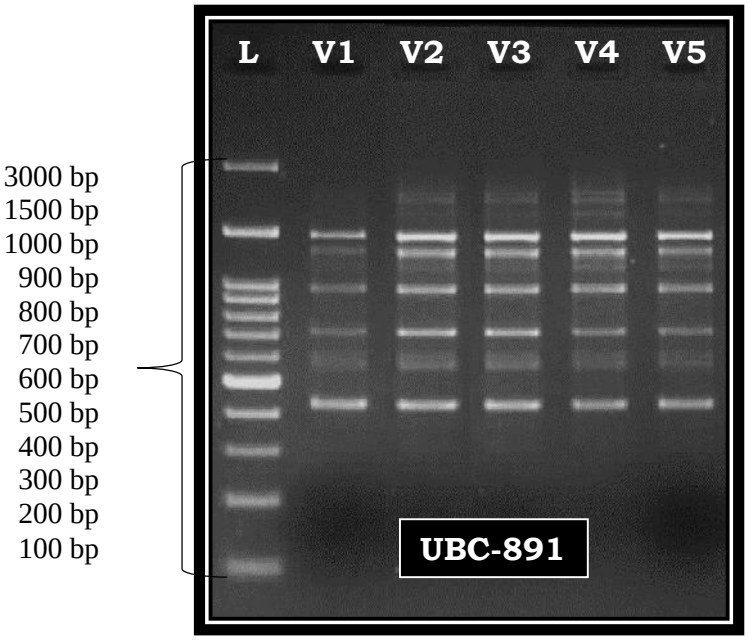


Fig. 2: Primer- UBC 891 (Annealing temperature: 50˚C)
L: DNA ladder (100-3000 bp)
V:-Samples collected from Vengurle (V1, V2, V3, V4 and V5)

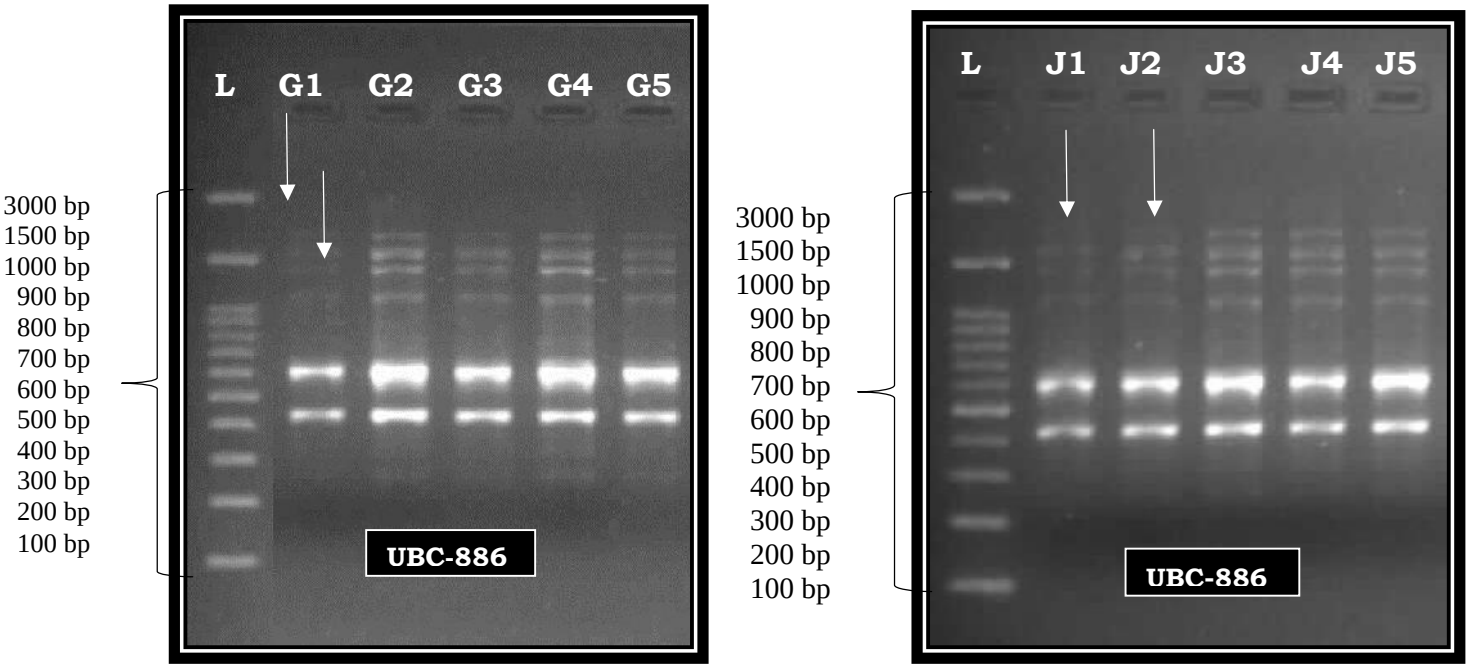


Fig. 3: Primer- UBC 886Annealing temperature: 51.4˚C
L: DNA ladder (100-3000 bp)
G: - Samples collected from Rameshwar-Girye (G1, G2, G3, G4 and G5)
J:-Samples collected from Nadan-Jamsande (J1, J2, J3, J4, and J5)

Marker Analysis between selected 10 locations

Sample number C1, A1, G1, J1, L1, K1, M1, D1, P1 and V1 was taken individually to characterize and to assess the genetic variation present in between selected 10 locations by using 16 ISSR primers. A total of 746 scorable DNA fragments were produced and among them 326 DNA fragments were found to be polymorphic in between these ten samples of Alphonso. Pandit *et al*., (2007) reported the genetic diversity between 71 mango genotypes with 33 ISSR primers yielded total 420 scorable bands on amplification. The average per cent polymorphism across the 16 primers in between ten selected locations found to be 46.62 per cent which is higher than those obtained in between population studies of mango (42.86 % by Kheshin *et al*., 2016). The product size ranged from 320 bp to 2891 bp is somewhat similar with the result of Kheshin *et al*., 2016 (326 bp to 3125 bp). The overall range of the similarity in between ten selected locations of Alphonso was 0.559-0.733 is less than result of Kheshin *et al*., 2016 (0.83-0.95). In the present study some fragments were uniquely identified or absent in some of the locations. These fragments are of great interest in identification of Alphonso of particular location.

Cluster analysis of selected 10 locations of Alphonso

The dendrogram (Fig. 4) separated the selected locations into 2 main clusters, *viz*; I and II. Cluster I is the major cluster showing the most distant location i.e. Pawas. This location is situated at the middle of South Konkan. This region has the latitude 16°50' to 16°55' N and longitude 73°19' E. The cluster II has 2 major clusters, *viz*; IIA and IIB. Cluster IIA with Alphonso samples from 2 locations i.e. Ambivali-Kelshi and Murud from north Ratnagiri being closely related. These two locations comes under same geographical zone i.e. Tahsil- Dapoli and the genetic distance was 0.685. Cluster IIB is further divided into 2 minor clusters IIBa and IIBb. Alphonso sample from Padel and Rameshwar-Girye from same Tahsil-Deogad having the genetic distance 0.723 were included in cluster IIBa. These two locations has the latitude 16°28' N to 16°31' N and longitude 73°20' E. Remaining all locations *viz*; Adivare from Tahsil-Rajapur, Nadan-Jamsande from Tehsil-Deogad, Achara and Katta from Tahsil-Malvan, Vengurle from Tehsil-Vengurle showing close relatedness which are falling under another cluster IIBb. Vasugi *et al.*, (2012) grouped 43 mango genotypes into two major

clusters, 11 varieties in cluster I whereas; 32 varieties were grouped in cluster II. The mango samples does not show a very much marked differences among themselves as the mango samples analyzed belonged to the single region i.e. South Konkan. The vicinity to sea side had a great effect on the mango samples which brought about very minor differences in genetic character among the samples. The similar results were also obtained by Karihaloo *et al.*, (2003) who showed that a high diversity within the regions of India and confirmed that this is not surprising given that the mango is a cross-pollinated plant and selecting superior strains according to the taste among naturally produced seedlings has given birth to the commercial cultivars and the observed appreciable range of variation.

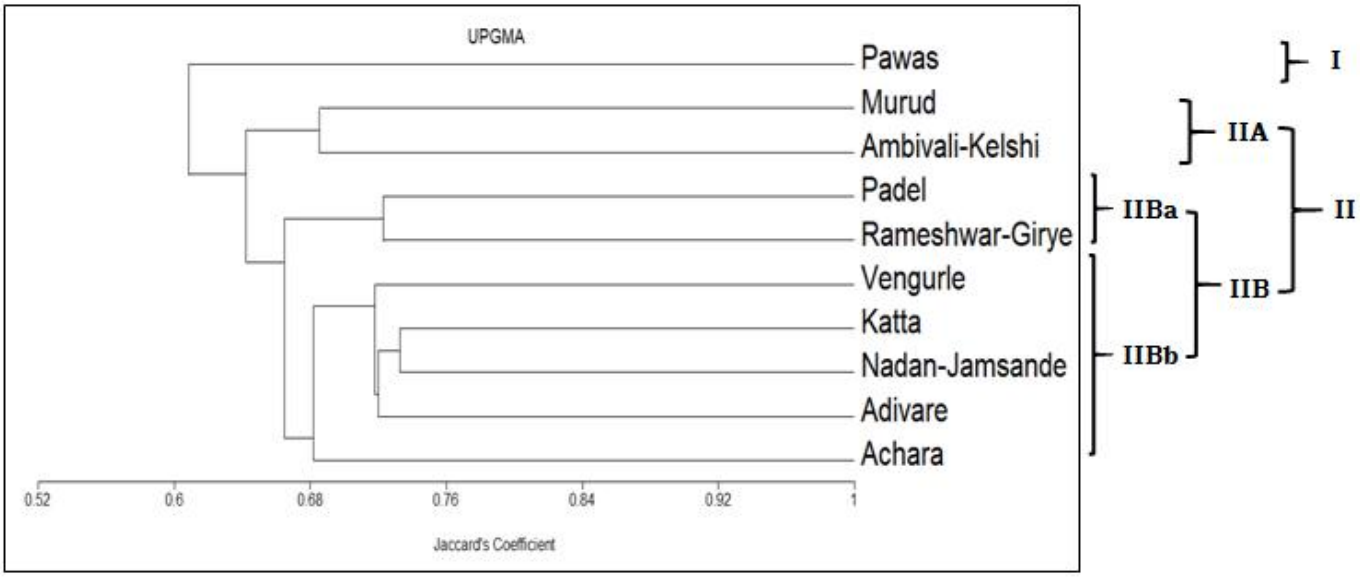
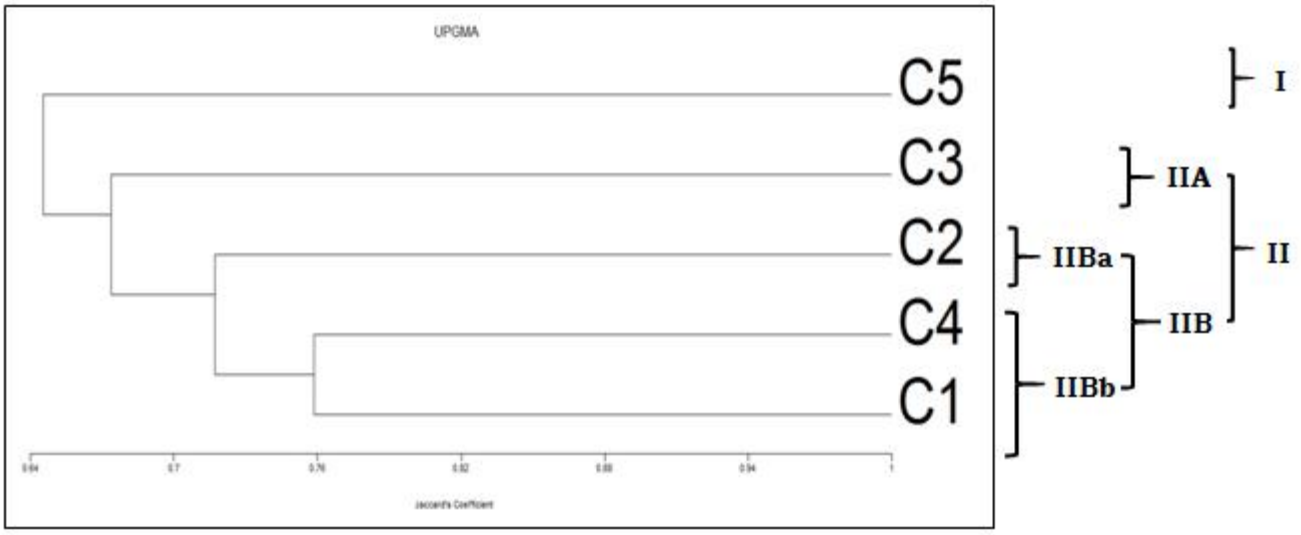


Fig. 4: UPGMA-based dendrogram based on Jaccard's similarity coefficients showing the genetic relationships of ten selected locations using 16 ISSR markers. I, II are clusters; IIA, IIB, IIBa and IIBb are sub-clusters.

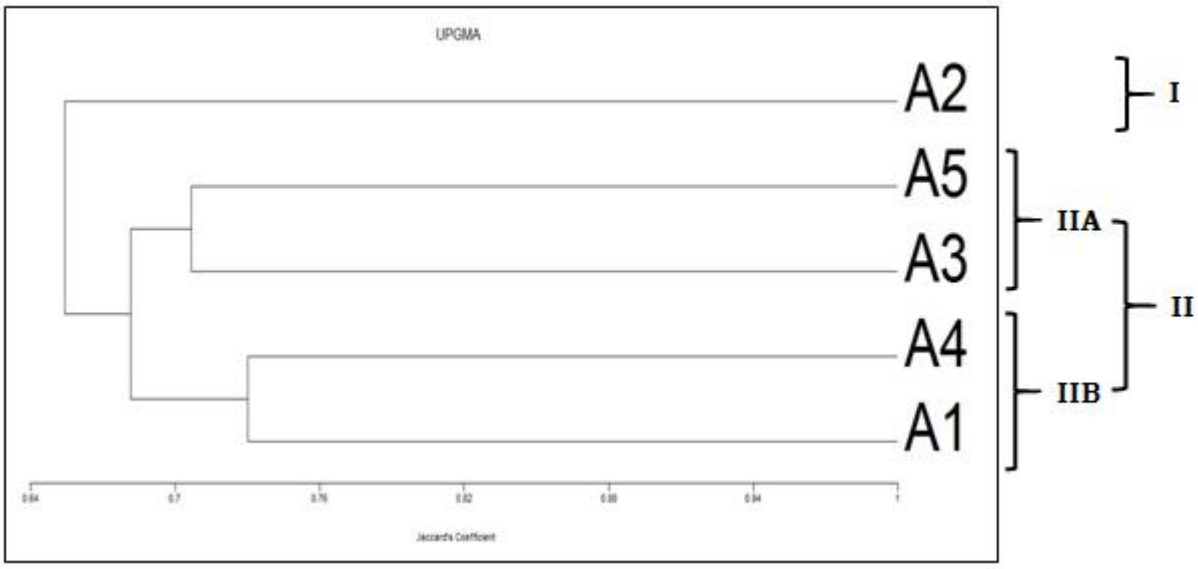
ISSR analysis within each location

Sixteen ISSR primers were used to characterize 50 samples of Alphonso variety. On an average maximum polymorphism percentage was found in Pawas (47.05 %) and minimum polymorphism percentage was observed in Padel (32.18 %). The overall an average maximum similarity found in between five samples of Vengurle (0.648-0.855), whereas minimum in Adivare (0.588-0.730). Bally *et al*., (1996) was observed a genetic dissimilarity of 0.05% among 27 accessions of Kensington Pride using RAPD marker in previous studies. Addition, Singh *et al*., (2009) detected an intracultivar variability in Banganapalli, Dashehri, and Langra cultivars of mango by using ISSRs. The dendrogram (Fig. 5) of each location separated the 5 Alphonso samples into two main clusters, *viz*: I and II. Clustering of cultivar belonging to different geographic region suggests that they might have evolved from the existing mango gene pool from which they were selected by local people to domesticate them in different areas for cultivation (Vasugi *et al*., 2012).

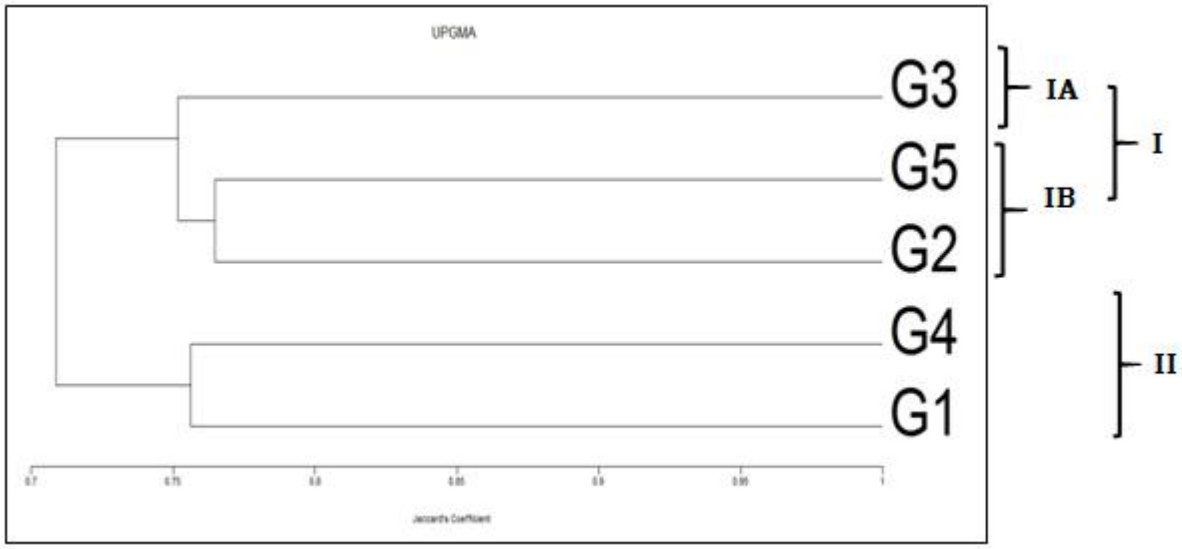
Achara



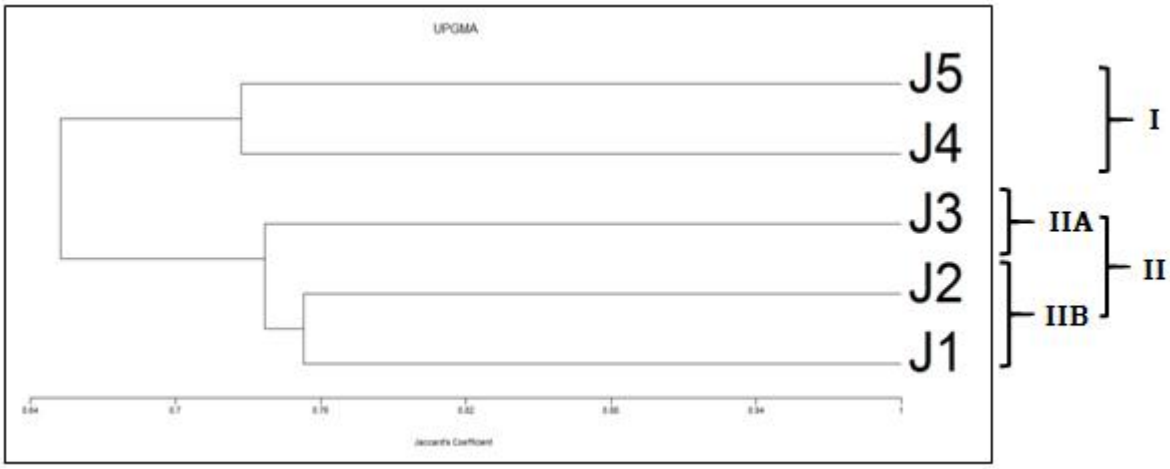
Adivare



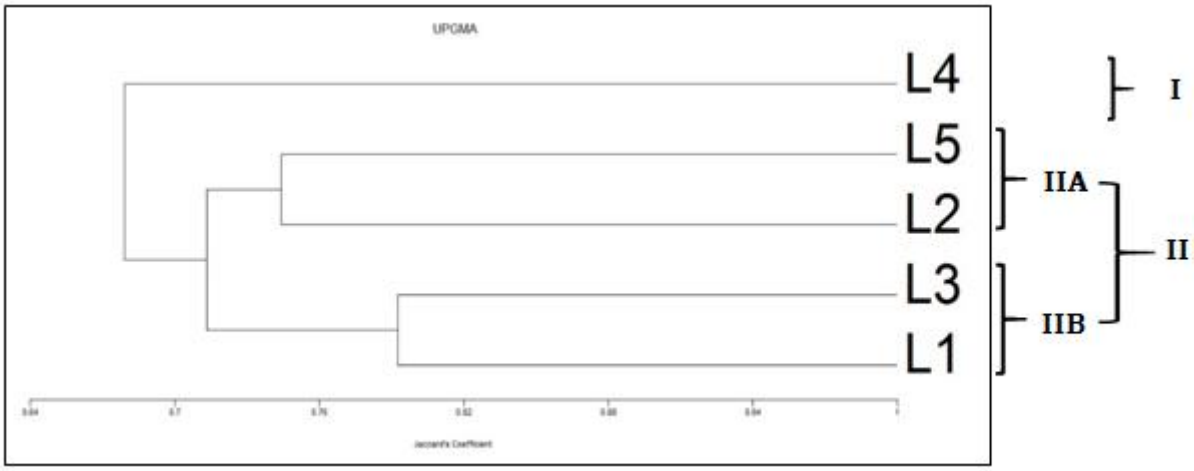
Rameshwar-Girye



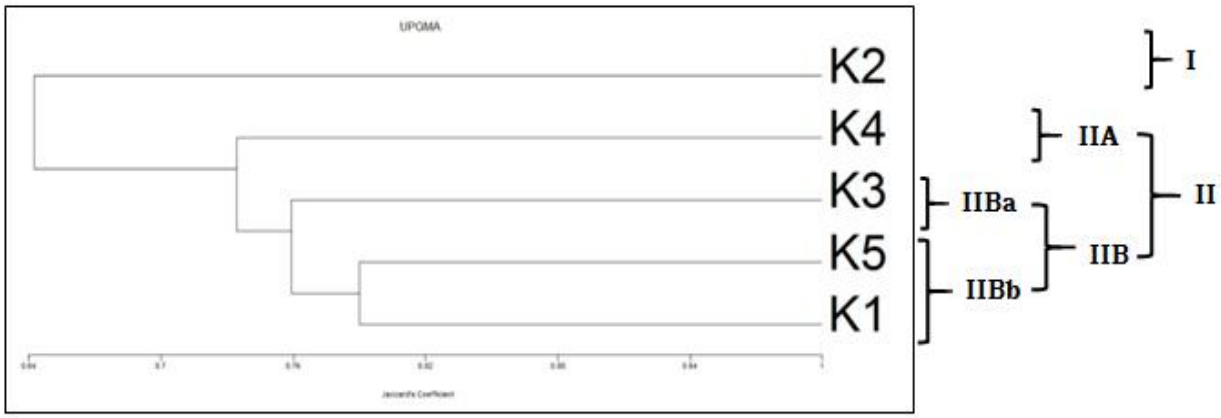
Nadan-Jamsande



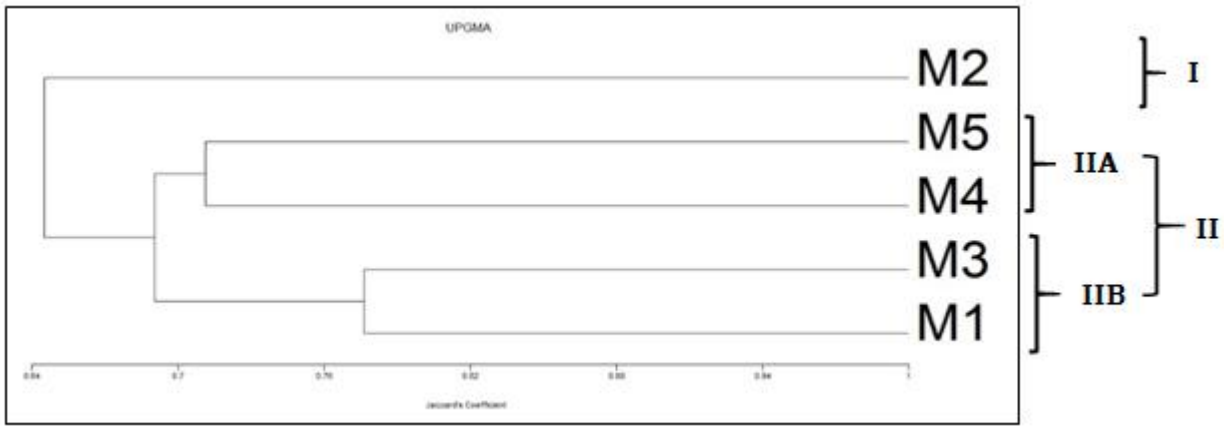
Katta



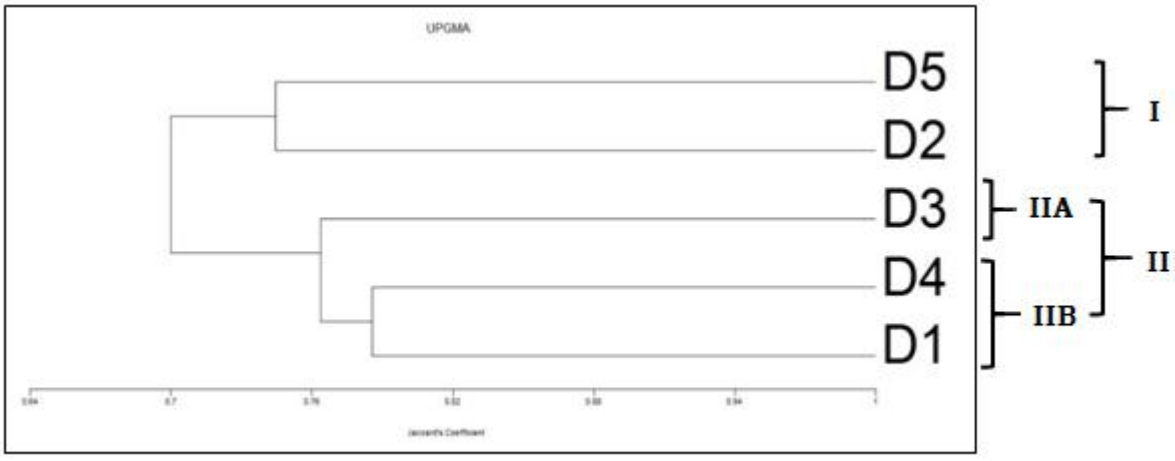
Ambivali-Kelshi



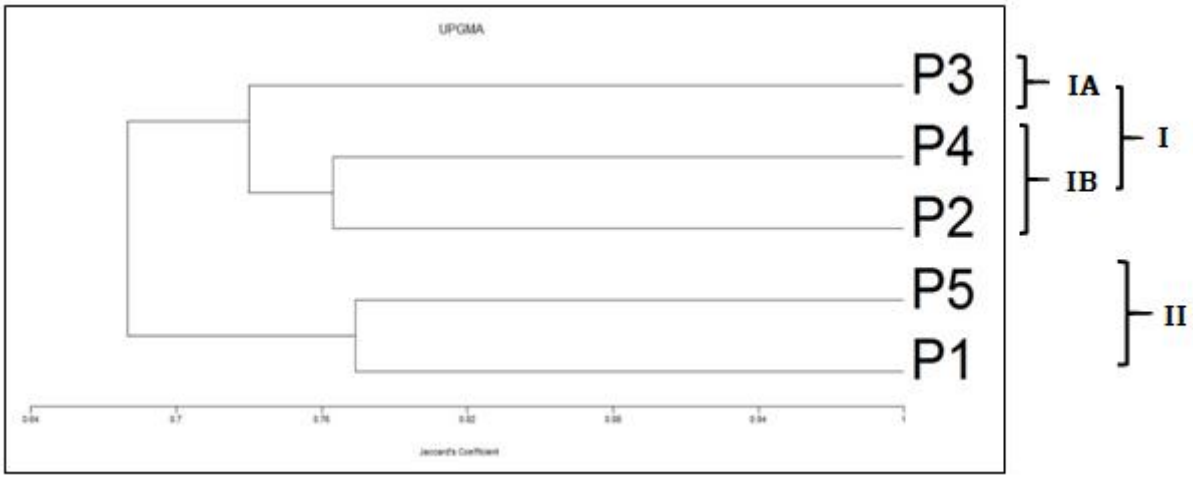
Murud



Padel



Pawas



Vengurle

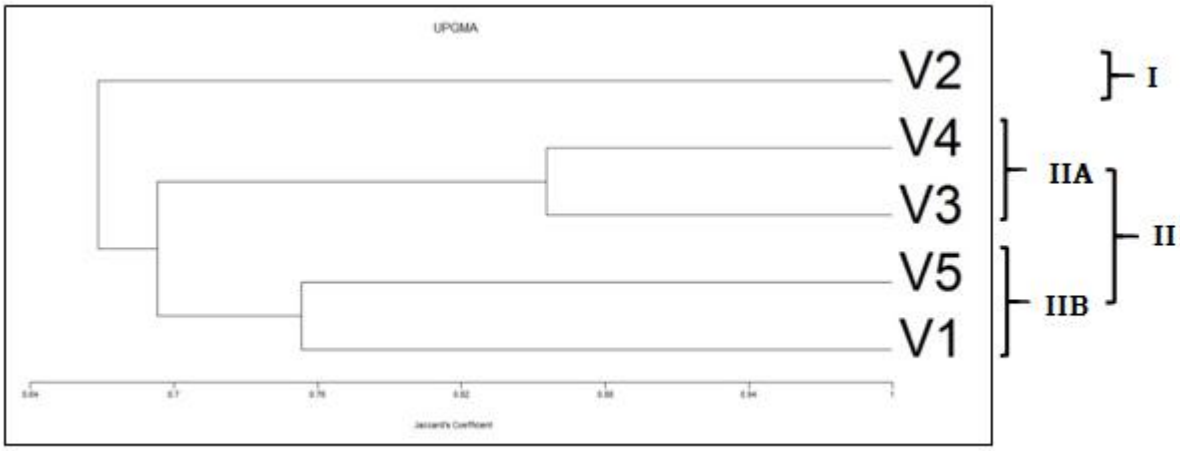


Fig. 5: UPGMA based dendrograms based on Jaccard's similarity coefficients showing the genetic relationships in five samples of selected ten locations (Achara, Adivare, Rameshwar-Girye, Nadan-Jamsande, Katta, Ambivali-Kelshi, Murud, Padel, Pawas, Vengurle) using all16ISSR primer. I,II are clusters; IA, IB, IIA, IIB are sub-clusters.

For every location these Alphonso samples are separated because when cultivars from one region are grown at other region, they vary in bearing characteristics, fruit quality and time of flowering. Superior chance seedlings selected as cultivars lead to fixation of very high degree of heterozygosity, leading to high variation within region (Archak *et al*., 2014). As with other vegetative propagated clonal crops, the differences among mango cultivars can result from epigenic modifications in response to the environment. Somatic and bud mutations also play a minor role in clonal polymorphism in woody plants. The significant variation among the trees of some clones in mango was observed with respect to fruit characteristics and tree performance (Kumar *et al.*, 2013).

The variation observed in each location due to mango may be monoembryonic and polyembryonic, based on their ability to reproduce from seeds. Most of the monoembryonic cultivars are propagated vegetatively by grafting or budding onto monoembryonic or polyembryonic seedling rootstocks. There appears to be a considerable variability reported within certain cultivars grafted onto different rootstocks, which attributed either to somatic mutations or to the influence of non-uniform monoembryonic rootstocks (Venkateswarlu, 2013).

Mango, being highly heterozygous, has a large diversity that has resulted from propagation by its seeds. The selected trees having the age more than 20 years. Since the crop is vegetative propagated, the trees of Alphonso cultivar were subjected to sub-cloning repeatedly over a period of time, across several geographical locations. This would have resulted in the production of bud sprouts or somatic mutants. Intracultivar study of genome from different locations can confirm whether there are any genetic differences among the location specific clones or not (Dinesh *et al*., 2015). Souza *et al.,* (2011) evaluated genetic variability of mango (*Mangifera indica* L.) accessions, of which 35 originated from Brazil, six from USA, and one from India. These accessions were found to have considerable genetic variability, demonstrating the importance of analyzing each genotype in a collection, to efficiently maintain a germplasm collection.

In Konkan region of Maharashtra where Alphonso is largely cultivated, half-sib stones will be invariably used for raising the rootstocks for grafting Alphonso variety. The rootstock effect on scion appeared to be the same, which was evident in the absence of clonal variations in scion material as clones of the Konkan region of Maharashtra (Manchekar, 2008). Due to such problems Alphonso mango has variation within location also.

This study is important for detecting the distinctness of the same variety from different geographical locations and also for identification of desirable samples and it's utilization for further breeding program. But this variation indicates that there is need to maintain original genotype of Alphonso because genetic variation obtained within different plant samples of same location is not expected.

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