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Genetic diversity and DNA fingerprinting analysis among commercial pomegranate (*Punica granatum* L.) using RAPD and SSR markers

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Abstract: Pomegranate (*Punica granatum* L.) is one of important horticultural plant in India with about several genotypes cultivated in several regions of country. Molecular markers including RAPD, and SSR were used to evaluate genetic diversity of twenty four pomegranate cultivars. Genetic parameters consisted of effective alleles (Ne), Nei genetic diversity (H), and polymorphic information content (PIC) was calculated based on molecular data. RAPD markers with their dominant nature showed the highest value of genetic parameters aforementioned with PIC value among all markers. Combined data of RAPD markers showed higher genetic diversity than SSR markers. UPGMA tree obtained from combined molecular data (total 1017 amplicons) discriminated pomegranate genotypes in two major groups. Principle Component Analysis (PCA) based on the first two components confirmed clustering. The homonymous, synonymous and/or mislabelled genotypes were identified using random decamer DNA markers. The matrix analysis showing calculated genetic similarity values indicated significant similarity (p= 0.94) between pomegranate genotypes in different localities. Only 2% of overall genetic variation was due to among locality groups differencewhile 98% of variation was due to within group differences.

Keywords: Combined molecular markers, Homonym, PIC, *Punica granatum* L. Genetic diversity DNA Fingerprinting; RAPD; SSR; PCR; Decamer primers; Dendrogram

Abbreviations: Simple Sequence Repeat (SSR), Random Amplified Polymorphic DNA (RAPD), principle Component analysis (PCA), Polymorphic Information Content (PIC), Unweighted Paired Group Mean using Arithmetic Average (UPGMA), Neighbor Joining (NJ).

Introduction

Pomegranate (*Punica granatum* L.) belongs to family Punicaceae (2n=16) is a monoecieous, grown in tropical and subtropical regions of the world including India, Iran, Mediterranean, Spain, Egypt, Afghanistan, Arabia, Baluchistan, Burma, China, Japan, USA, USSR, Bulgaria and Southern Italy as Patil and Karle (1990). Pomegranate production has attracted several farmers in India for its wider adaptability, relatively low cost of cultivation, drought tolerance, good yields and export potential. The pomegranate fruit is symbolic for its cool, refreshing juice and valued for its medicinal properties as Pirseyedi *et al.* (2010). A wide genetic base of crop varieties / cultivars helps to overcome sudden outbreak of pests and diseases. High genetic diversity among the cultivated varieties of crop plants is expected to overcome effects of climate change and for genetic improvement of crop. Sub- Himalayan region of India is known to have very high diversity of pomegranate. Very recently, efforts are being made in terms of collection and utilization of genetic diversity present in the wild for the important of pomegranate is being undertaken by national institutes in India. Morphometric and phytochemical criteria have recently been attended to determine the degree of polymorphism among pomegranate collections (Wunsch and Hormaza, 2002). Isozymes and molecular markers have been used to a limited extent in genetic diversity characterization. Development of highly reliable and discriminatory methods has become increasingly important for identifying varieties / cultivars.

The introduction of PCR-based methods constituted a new milestone in the field of DNA ûngerprinting and assessment of genetic variation. Precise, fast, cost-effective and reliable identiûcation of varieties / cultivars is needed. Unlike morphological characters, molecular markers are net influenced by the environmental factors & the estimate of genetic diversity is generally precise therefore in present study objective is to access genetic diversity and DNA fingerprinting using DNA markers such as Random Amplified Polymorphic DNA (RAPD) and simple sequence repeat (SSR) in pomegranate.

Materials and Methods

Plant materials: Leaf samples were collected from Indian Institute of Horticultural Research (IIHR), Bangalore, Karnataka and National Research Centre on Pomegranate (NRCP), Solapur, Maharashtra. The leaf samples were detached from three to four plants of same (NRCP) varieties / cultivars and washed three times in sterile distilled water. Further, the leaf samples were frozen in liquid nitrogen and kept at -80 °C until used. A set of twenty four pomegranate varieties / cultivars were used in present study (Table-1).

DNA extraction and diversity analysis: The genomic DNA was extracted from leaf samples collected from 24 varieties / cultivars of pomegranate method given by Krishna and Jawali (1997). The quantity and quality of isolated genomic DNA was determined using agarose gel [0.5% (w/v)] electrophoresis and a nano drop spectropho-tometer (ND 1000, USA).

Random decamer DNA marker analysis: One hundred twenty random decamer RAPD primers were used from Operon Technologies, Calif., USA and their combination were used. RAPD reactions were conducted in 20 μL containing 50 ng of template DNA solution; 1X PCR buffer (10 mM Tris–HCl buffer at pH 8; 50 mM KCl); 1.5 mM Mg2+; 200μM dNTP mix ; 0.4 μM primer and 0.4 unit *Taq* polymerase (Bioron, Germany). Thermal program was carried out in thermocycler (Techne, UK). The profile used consisted of an initial denaturation for 3 min at 94°C, followed by 35 cycles in three segments: 1 min at 92°C, 1 min at 36°C, 1 min at 72°C and final extension for 10 min at 72°C. Amplification products were visualized by running on 1.5% agarose gel in 0.5 X TBE buffer systems, followed by ethidium bromide (0.5 μg mL^{-1}) staining. Fragment size was estimated by using a 100 base pairs (bp) molecular size ladder (Fermentas, Germany).

Microsatellite amplification: Eighteen SSR primers specifically produced for pomegranate were used (Hasnaoui, 2010; Pirseyedi, 2010). Amplification of microsatellites were performed in PCR reactions in a total volume 20μl, containing 20 ng genomic DNA, 1X supplied PCR buffer (Bioron, Germany), 2 mM $MgCl_2$, 200iM of each dNTP (Bioron, Germany), 1 unit of *Taq* DNA polymerase (Bioron, Germany) and 0.2 μM of forward and reverse primers. PCRs were carried out on a thermal cycler (Techne, UK) programmed with a denaturation at 94ºC for 5 min, 35 cycles of 94ºC for 1 min, the annealing temperature 54 ºC for 1 min and 72ºC for 1.5 min and final extension at 72ºC for 15 min. Separation and visualization of PCR products was done on both agarose (2.5 per cent) as well as polyacrylamide gels (6 per cent). Agarose gels were used to check amplification and polymorphism between the genotypes, if any, could be detected. The markers which did not show any detectable polymorphism between genotypes on 2.5 per cent agarose gels with ethidium bromide staining were carried forward to 6 per cent polyacrylamide gels with silver staining procedure. The products denaturing gel was casted in Sequi-Gen GT nucleic acid electrophoresis cell (Bio-Rad Pvt. Ltd, USA) as Sambrook (2001). Several modifications to the original protocol were done to suit the bench level operations.

Statistical analysis: Required numbers of replication were maintained for all experiments. The experimental design used for statistical analysis was simple randomized block design and means were evaluated. The genetic diversity analysis among 24 varieties / cultivars of pomegranate was performed using NTSYS statistical algorithm.

Results and Discussion

Among the 120 random decamer DNA markers, 16 random markers produced maximum number of amplicons that were consistent, clear and also detected polymorphism when repeated for three times. Essentially, these 16 random DNA markers were selected for further analysis of genetic diversity and fingerprinting of 24 pomegranate varieties / cultivars. Details on sequence of 16 random decamer DNA primers and sequence of primer pairs of SSRs are presented in table 2. Among the shortlisted 16 random DNA marker, seven markers *viz*., N-18 (240bp), I-12 (1300bp), L-13 (800bp), L-14 (400bp), N-15 (200bp), N-15 (200bp) and N-7 (450bp), could effectively detect the polymorphism between a set of seven pomegranate varieties *viz*., Mana, Daru, Amlidana, Bhagwa, Kandahari, Bassein Seedless and Arakta respectively. The unique amplicon of size 200bp from marker OPB-14, 880bp from marker N-18, 1400bp from marker L-13 and of size 1100bp from marker AT-12 were noticed with KY variety. Similarly, the random DNA markers, OPAC-4 (500bp), N-15 (700bp and 390bp) recorded amplicons in Ruby variety. Further, OPB-14 (500bp and 250bp), were noticed with Daru and Nana variety respectively. On other hand, random DNA markers OPAC-4, OPK-3 and I-12 showed unique amplicons for Jodhpur collection variety with the amplification of specific size amplicons *viz*., 190bp, 280bp, 250bp and 600bp, respectively. The set of two decamer markers, N-7 (200bp) and N-18 (480bp), could successfully detect and fingerprint P-23 variety. The markers OPK-3 (50bp), AC-9 (400bp) and N-7 (510bp) detected polymorphism in Yercaud variety. In case of pomegranate variety "Ganesh", the RAPD markers *viz*., OPK-3 (210bp), N-15 (210bp), L-13 (400bp) and L-14 (500bp) produced polymorphic and fingerprinting amplicons. A set of two markers *viz*., AC-3 (600bp) and AT-7 (400bp) fingerprinted Jyothi variety, whereas, Gulesha Red variety was uniquely detected by I-14 and I-10 marker with its 900bp and 410bp amplicons (Table-3, Fig. 1,2,3).

Using the 16 random DNA markers data, dendrogram was generated with the neighbor-joining clustering method that clearly separated varieties / cultivars into two major clusters- labeled, 1 and 2 respectively (Fig. 4). The observation of dendrogram indicated that, cluster 1 comprised of only KY. The cluster 2 was subdivided into two sub-clusters, sub-cluster 1 (2.1) and Sub-cluster 2 (2.2). The sub-cluster 2.1 comprised of only Nana and the sub-cluster 2:2 divided into two groups, (2.2.1) and (2.2.2). The sub-cluster 2.2.1 consist of Ganesh and Geps genotypes, were also found close to each other, while sub-cluster 2.2.2 was subdivided two sub-clusters, sub-cluster 1 (2.2.2.1) and Sub-cluster 2 (2.2.2.2). The sub-cluster 2.2.2.1 was subdivided again two sub-clusters, sub-cluster 1 (2.2.2.1.1) and Sub-cluster 2 (2.2.2.1.2). The sub-cluster 2.2.2.1.1 comprised of Yercaud and Daye, the sub-cluster 2.2.2.1.2 divided into two groups, (2.2.2.1.2.1) and (2.2.2.1.2.1.2). The sub-cluster 2.2.2.1.2.1 again divided into two groups (2.2.2.1.2.1.1) and (2.2.2.1.2.1.2) each consisted of the genotype *viz*. Kandahari, Arakta, Gulesha Pink, Gulesha Red, Ganesh Selection, Mrudula and the genotype Jyothi and Bassein Seedless respectively (Fig. 4).

There was a strong and positive similarity correlation between the tested pomegranate varieties / cultivars as revealed

Table-1: Pomegranate varieties / cultivars used in the present study

Sl. No.	Varieties / cultivars	Sl. No.	Varieties / cultivars
1	Amlidana	13	Jodhpur Red
2	Arakta	14	Jodhpur Collection
3	Bassein Seedless	15	Jyothi
4	Bhagwa	16	Kandahari
5	Daru	17	KY
6	Daye	18	Mana
7	Ganesh	19	Mridula
8	Ganesh Selection	20	Musket
9	Geps	21	Nana
10	Gulesha Pink	22	P -23
11	Gulesha Red	23	Ruby
12	Jallore Seedless	24	Yercaud

Table-2: Random decamer and SSR markers successfully amplified and the number of total and polymorphic amplicons amplified in pomegranate varieties / cultivars used in the present study

Primer name	Sequence (5'-3')	Total amplicons	No. of polymorphic amplicons	Polymorphic amplicons (%)
RAPD				
OPB-14	AAGTGCGACC	69	47	68.11
OPK-3	GAGAACGCTG	86	46	53.48
AC-9	AGAGCGTACC	78	48	61.53
AC-3	CACTGGCCCA	96	47	48.95
I-10	ACAACGCGAG	67	17	25.37
I-14	GTGACAGGCT	57	25	43.85
L-12	GGGCGGTACT	31	20	64.51
M-11	GTTGGTGGCT	21	2	09.52
N-7	CAGCCCAGAG	113	65	57.52
N-15	CAGCGACTGT	67	29	43.28
N-18	GGTGAGGTCA	25	13	52.00
L-13	ACCGCCTGCT	48	21	43.75
L-14	GTGACAGGCT	57	49	85.96
AT-7	ACTGCGACCA	81	6	07.40
AT-12	CTGCCTAGCC	52	7	13.46
OPAC-4	ACGGGACCTG	72	62	86.11
SSR				
FBPG-MP07	F:5-GATTAACAGCA AAGCCTAGAGG-3 R:5-AGTAGCTGCAA CAAGATAAGG-3	1	0	0
FBPg10	F:5-CCTCATTGCTG ATGAATCTT-3 R:5-ACTCGAGAAG CTCTGTGAAG-3	1	0	0
FBPg14	F:5-CGCATTTGGTTG TAGAAGAC-3 R:5-AGGAGCGTCTG TTTTAATCTT-3	1	0	0
FBPg46	F:5-CTTCCTCCTACC GAACTATG-3 R:5-CCCACTTTGAC ACTTCTACC-3	1	0	0

Table-3: Summary of PCR-RAPD fragments distinctly present in the different pomegranate varieties / cultivars to serve as DNA fingerprints

varieties/ cultivars	Marker	Base pair(bp)	Finger prints
Mana	N-18	240	$N18_{240}$
KY	OPB-14	200	$OPB14_{200}$
	N-18	880	$N18_{880}$
	L-13	1400	$L13_{1400}$
	AT-12	1100	$AT12_{1100}$
Daru	I-12	1300	$I12_{1300}$
Ruby	OPAC-14	500	$OPAC14_{500}$
	N-15	700	$N15_{700}$
	N-15	390	$N15_{390}$
	AC-9	450	$AC9_{450}$
Bhagva	L-13	800	$L13_{800}$
Amlidana	L-14	400	$L14_{400}$
Jodhpur Collection	OPAC-4	190	$OPAC4_{190}$
	OPK-3	280	$OPK3_{280}$
	I-12	250	$I12_{250}$
	I-12	600	$I12_{600}$
	OPB-14	200	$OPB14_{200}$
P-23	N-7	200	$N7_{200}$
	N-18	480	$N18_{480}$
Yercaud	OPK-3	50	$OPK3_{50}$
	AC-9	400	$AC9_{400}$
	N-7	510	$N7_{510}$
Ganesh	OPK-3	210	$OPK3_{210}$
	N-15	210	$N15_{210}$
	L-13	400	$L13_{400}$
Geps	OPB-14	250	$OPB14_{250}$
	L-14	500	$L14_{500}$
	OPK-3	210	$OPK3_{210}$
Jyothi	AC-3	600	$AC3_{600}$
	AT-7	400	$AT7_{400}$
Kandahari	N-15	200	$N15_{200}$
Bassein Seedless	N-15	200	$N15_{200}$
Arakta	N-7	450	$N7_{450}$
	AC-3	1000	$AC3_{1000}$
Gulesha Red	I-14	900	$I14_{900}$
	I-14	410	$I14_{410}$

through genetic similarity coefficient matrix analysis. it showed that, the genetic similarity between Mana and KY was 0.85. Further, Daru (0.91), Ruby (0.88), Bhagwa (0.90), Amlidana (0.89), Jodhpur Red (0.85), Jodhapur Pink (0.87), Musket (0.88), P-23 (0.86), Jallore Seedless (0.86), Yrecaud (0.85), Daye (0.87), Nana (0.85), Ganesh (0.86), Geps (0.87), Jyothi (0.88), Kandahari (0.90), Bassein Seedless (0.86), Arakta (0.90), Mridula (0.89), Ganesh Solapur (0.88), Gulesha Red (0.89) and Gulesha Pink (0.89) was assayed. The genetic similarity between the KY and Daru, Musket, Jallore Seedless and Geps was 0.84. Cultivar Ruby, Kandahari, Mridula, Ganesh Solapur and Gulesha Pink were notice 0.85. Similarly, cultivar Bhagwa, Jodhpur Pink, Arkata and Gulesha Red were (0.86), P-23 (0.81), Yercaud and Nana (0.82), (Table 4).

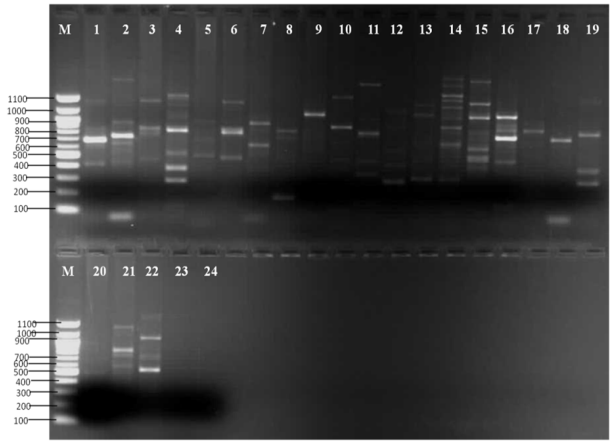
Genetic similarity between the pomegranate genotype Daru and Ruby, Mridula were recorded (0.89), Bhagwa (0.90), Amlidana (0.91), Jodhpur Red, Jallore seedless, Daye, Nana, Ganesh and Jyothi were comprise 0.85, Jodhpur Pink, Arkata, Ganesh solapur and Ganesh Red (0.87), Musket and Kandahari (0.88), Geps and Bassein Seedless have been observed 0.84, and Gulesha Pink (0.86). Genetic similarity between Ruby and Bhagwa was 0.90, Amlidana was 0.88, Jodhpur Red, Daye, Geps and Gulesha Pink were 0.85, and Jodhpur Pink, Musket, Jallore Seedless, Kandahari, Arkata, Ganesh Solapur and Ganesh Red were 0.86. The genetic similarity between the pomegranate varieties / cultivars P-23, Nana, Ganesh, Jyothi and Bassein Seedless were 0.84, Yercaud (0.83) and Mridula was 0.87. The genetic similarity between the pomegranate varieties / cultivars Bhagwa and Mana, Daru and Ruby was (0.90), KY (0.86). The maximum genetic similarity between the pomegranate genotype Gulesha Red and Gulesha Pink observed 0.94 (Table-4).

Screening a set of 120 random DNA markers and a set of 18 SSR markers using randomly selected DNA samples from 24 different pomegranate varieties / cultivars was done to identify the markers for further detailed analysis of genetic diversity and to identify the DNA fingerprints. Among the 120 markers, 16 random markers produced consistent amplicon pattern across all the 24 pomegranate varieties / cultivars. Further, Initially we used 18 SSR,

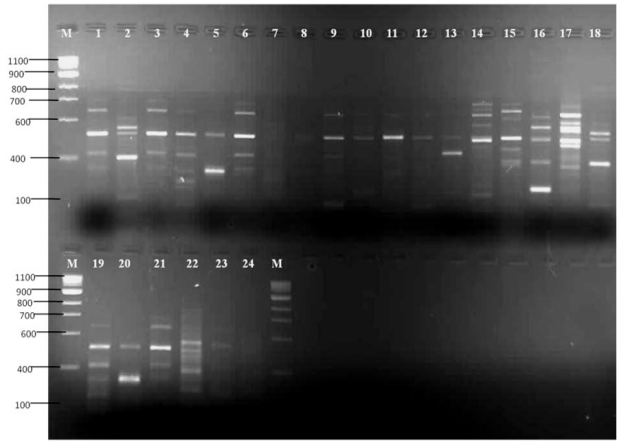
all of which were amplified monomorphic. Hence, RAPD OPB-14, OPK-3, AC-9, AC-3, I-10, I-14, L-12, M-11, N-7, N-15, N-18, L-13, L-14, AT-7, AT-12, OPAC-4 were used to assess the genetic diversity and fingerprints of pomegranate varieties / genotypes. Sheidai *et al.* (2008) studied RAPD and cytogenetic study of eleven pomegranate varieties / cultivars for their genetic genuineness and genetic diversity. The present study result consisted with the study of Ercisli *et al*. (2011); random DNA markers owing to arbitrary nature and short length of primer find amplification patterns in the same genotype vary which is undesirable for genetic diversity analysis.

The summary statistics of PCR-RAPD analysis of pomegranate varieties / cultivars reveled that the size of the amplicons ranged from 50 to 1400 bp. Only the consistent and major/brighter PCR products were accounted to 1017 levels across all the 24 pomegranate varieties / cultivars studied. Among the 1017 total amplicon levels 504 were polymorphic. On an average 31.5% polymorphic amplicon levels /marker was recorded. The maximum number of amplicon levels observed was 113 and minimum level of amplicons observed was 21. The 19 promising pomegranate genotypes have been characterized from AFLP markers and capillary electrophoresis. Four AFLP marker combinations were used, generating a total of 297 fragments, 213 of which were polymorphic 73.0% (Zahra, 2012). Resolving power of the AFLP markers ranged from 0.70 to 1.01, with a total of 3.440, while polymorphism information contents ranged from 0.70 to 0.83 with an average of 0.76. UPGMA clustering of the genotypes showed two major groups. Most of the fruit characteristics of the genotypes within the same group were variable (Zahra *et al*., 2012). Zamani *et al*. (2007) stated that there is a poor correlation between fruit characteristics and RAPD data in pomegranate, and they found that the correlation coefficient between morphological and RAPD data was only 23%.

Identification of DNA fingerprints for selected commercially important pomegranate varieties / cultivars: A total of 36 fingerprints were obtained on screening the 24 selected

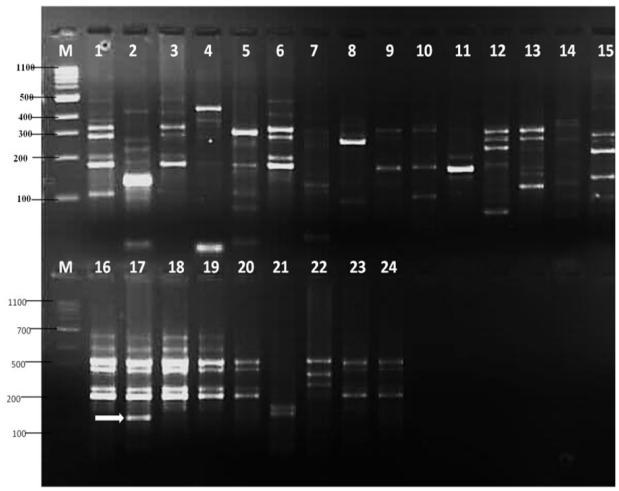


1a) PCR-RAPD amplification profile resulting from OPB-14 primer

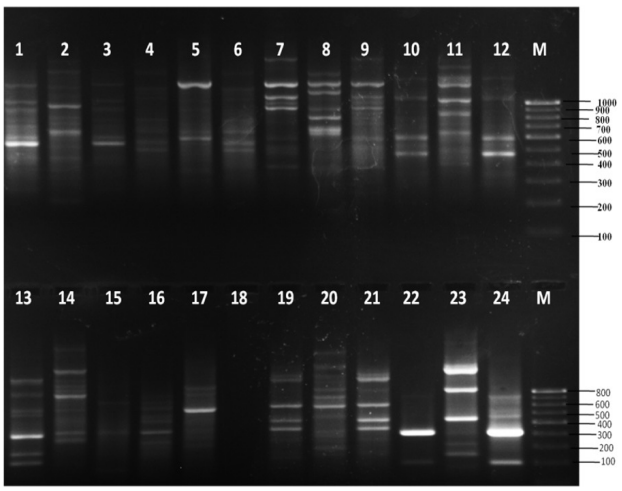


1b) PCR-RAPD amplification profile resulting from OPK-3 primer

Fig. 1: PCR-RAPD amplicons separated on 1.5% agarose gel with ethidium bromde staining [M – 100bp ladder; 1. Mana; 2. KY; 3. Daru; 4. Ruby; 5. Bhagwa; 6. Amlidana; 7. Jodhpur Red; 8. Jodhpur Collection; 9. Musket; 10. P-23; 11. Jallore Seedless; 12. Yercaud; 13. Daye; 14. Nana; 15. Ganesh; 16. Geps; 17. Jyothi; 18. Kandahari; 19. Bassein Seedless; 20. Arakta; 21. Mridula; 22. Ganesh Selection; 23. Gulesha Red; 24. Gulesha Pink]

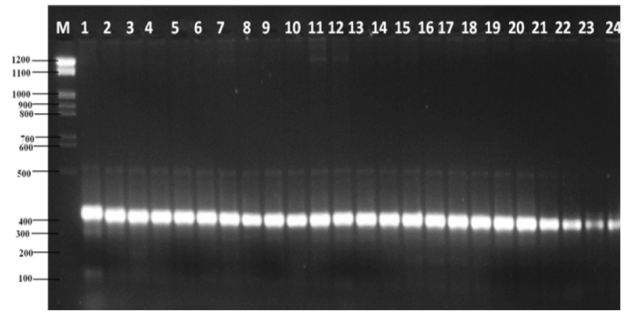


2a) PCR-RAPD amplification profile resulting fromAC-9 primer

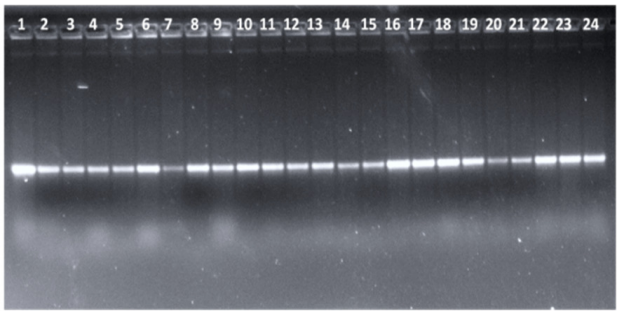


2b) PCR-RAPD amplification profile resulting from AC-3 primer

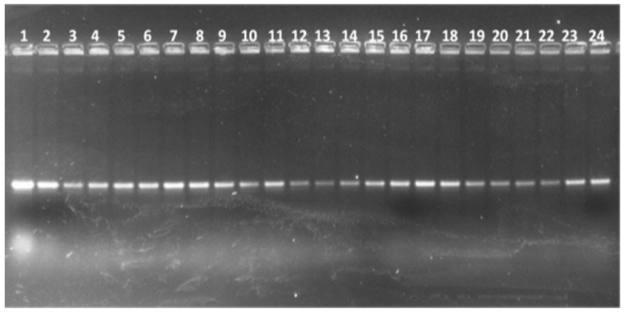
Fig. 2: PCR-RAPD amplicons separated on 1.5% agarose gel with ethidiumbromde staining [M – 100bp ladder; 1. Mana; 2. KY; 3. Daru; 4. Ruby; 5. Bhagwa; 6. Amlidana; 7. Jodhpur Red; 8. Jodhpur Collection; 9. Musket; 10. P-23; 11. Jallore Seedless; 12. Yercaud; 13. Daye; 14. Nana; 15. Ganesh; 16. Geps; 17. Jyothi; 18. Kandahari; 19. Bassein Seedless; 20. Arakta; 21. Mridula; 22. Ganesh Selection; 23. Gulesha Red; 24. Gulesha Pink]



a) MP07



b) FBPg 14



c) FBPg 46

3a) Amplified SSR loci a) MP07 b) FBPg 14 c) FBPg 46 markers

3b) SSR amplicons separated on 6% PAGE with silver staining to resolving the FBPg 10 marker

Fig. 3: Nuclear SSR amplicons separated on agarose and PAGE with silver staining to resolving marker [M – 100bp ladder; 1. Mana; 2. KY; 3. Daru; 4. Ruby; 5. Bhagwa; 6. Amlidana; 7. Jodhpur Red; 8. Jodhpur Collection; 9. Musket; 10. P-23; 11. Jallore Seedless; 12. Yercaud; 13. Daye; 14. Nana; 15. Ganesh; 16. Geps; 17. Jyothi; 18. Kandahari; 19. Bassein Seedless; 20. Arakta; 21. Mridula; 22. Ganesh Selection; 23. Gulesha Red; 24. Gulesha Pink]

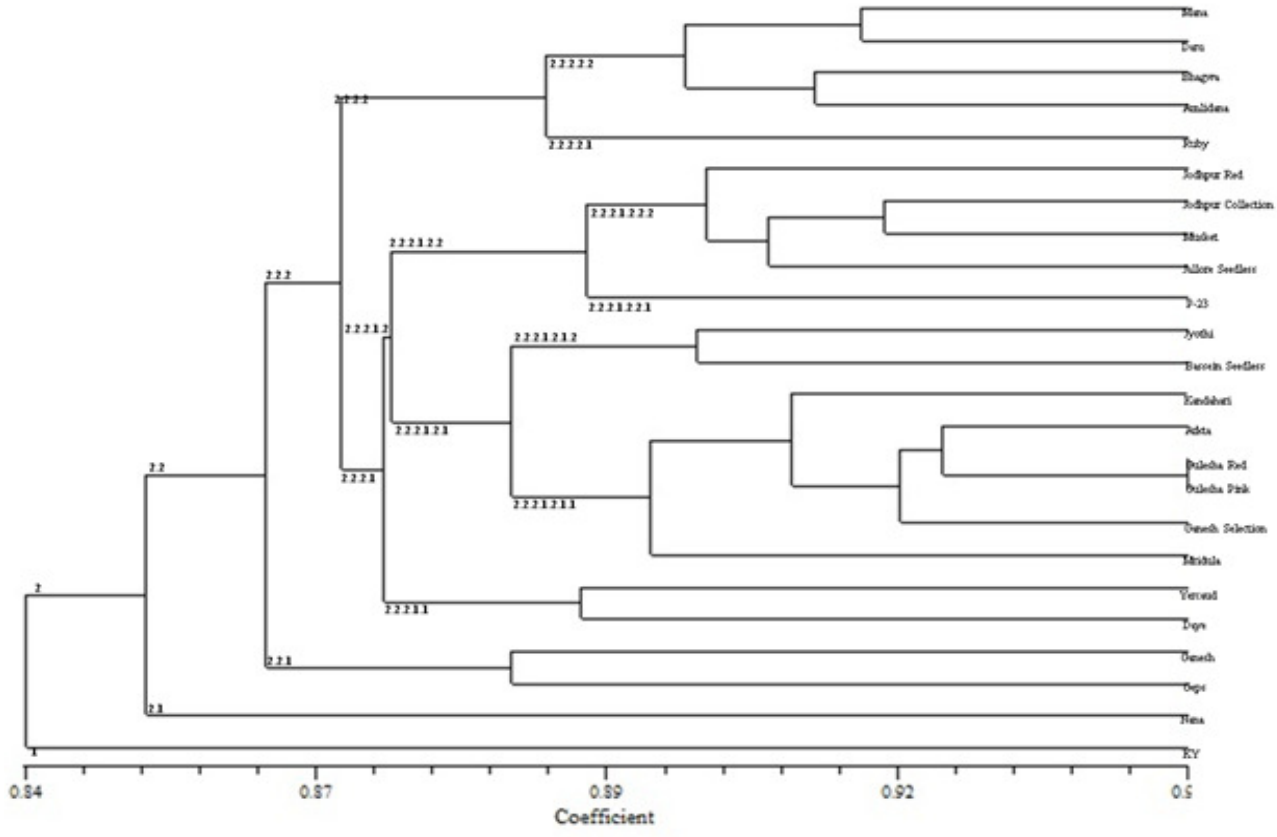


Fig. 4: UPGMA phenogram of 24 pomegranate varieties / cultivars based on average distance of PCR-RAPD fragment frequencies

Table-4: Matrix showing calculated genetic similarity values for all pairs of accessions in the study

	Mana	KY	Daru	Ruby	Bhagwa	Amlidana	Jodhpur Red	Jodhpur Collection	Musket	P-23	Jallore Seedless	Yercaud	Daye	Nana	Ganesh	Geps	Jyothi	Kandahari	Bassein Seedless	Arakta	Mridula	Ganesh Solapur	Gulesha Red	Gulesha Pink
Mana	1																							
KY	0.85	1																						
Daru	0.91	0.84	1																					
Ruby	0.88	0.85	0.89	1																				
Bhagwa	0.90	0.86	0.90	0.90	1																			
Amlidana	0.89	0.83	0.91	0.88	0.91	1																		
JodhpurRed	0.85	0.83	0.85	0.85	0.88	0.85	1																	
Jodhpur collection	0.87	0.86	0.87	0.86	0.90	0.88	0.90	1																
Musket	0.88	0.84	0.88	0.86	0.89	0.89	0.90	0.92	1															
P - 23	0.86	0.81	0.86	0.84	0.86	0.86	0.89	0.88	0.91	1														
Jallore Seedless	0.86	0.84	0.85	0.86	0.89	0.87	0.90	0.90	0.91	0.88	1													
Yercaud	0.85	0.82	0.84	0.83	0.85	0.84	0.86	0.86	0.88	0.88	0.88	1												
Daye	0.87	0.83	0.85	0.85	0.88	0.85	0.86	0.88	0.89	0.86	0.89	0.89	1											
Nana	0.85	0.82	0.85	0.84	0.86	0.84	0.84	0.86	0.87	0.85	0.86	0.83	0.88	1										
Ganesh	0.86	0.83	0.85	0.84	0.87	0.86	0.85	0.87	0.88	0.86	0.88	0.87	0.89	0.86	1									
Geps	0.87	0.84	0.84	0.85	0.86	0.85	0.85	0.85	0.85	0.83	0.85	0.85	0.85	0.84	0.88	1								
Jyothi	0.88	0.83	0.85	0.84	0.86	0.86	0.83	0.86	0.86	0.84	0.85	0.84	0.87	0.85	0.85	0.87	1							
Kandahari	0.90	0.85	0.88	0.86	0.90	0.88	0.86	0.89	0.88	0.87	0.88	0.86	0.86	0.84	0.87	0.89	0.89	1						
BasseinSeedless	0.86	0.83	0.84	0.84	0.87	0.87	0.84	0.85	0.86	0.84	0.86	0.85	0.86	0.82	0.85	0.86	0.90	0.89	1					
Arakta	0.90	0.86	0.87	0.86	0.88	0.88	0.87	0.89	0.88	0.87	0.88	0.87	0.89	0.85	0.86	0.87	0.89	0.90	0.90	1				
Mridula	0.89	0.85	0.89	0.87	0.89	0.88	0.86	0.88	0.89	0.87	0.88	0.86	0.89	0.87	0.86	0.85	0.87	0.89	0.89	0.91	1			
Ganesh Solapur	0.88	0.85	0.87	0.86	0.88	0.86	0.87	0.88	0.89	0.88	0.88	0.86	0.88	0.85	0.88	0.86	0.87	0.90	0.88	0.91	0.88	1		
GuleshaRed	0.89	0.86	0.87	0.86	0.90	0.87	0.87	0.89	0.90	0.87	0.90	0.88	0.89	0.86	0.88	0.88	0.89	0.92	0.88	0.92	0.90	0.91	1	
GuleshaPink	0.89	0.85	0.86	0.85	0.89	0.88	0.88	0.89	0.89	0.89	0.90	0.89	0.90	0.86	0.88	0.88	0.89	0.91	0.88	0.93	0.90	0.93	0.94	1

pomegranate varieties / cultivars with 16 RAPD random markers. A total of 16 RAPD random markers amplified 504 polymorphic amplicons in 24 genotypes. The size of specific amplicon ranged from 50 to 1400 bases. Marker N-15 amplified five different amplicons of variesd sizes (200 to 700 bases) in four different genotypes. The random decamer OPK-3 amplified 4 specific amplicons, in all four different varieties / genotypes. From this it can be said that, the marker N-15 had a better capacity to distinguish twenty-four varieties / cultivars. Thus, the study provided a detailed first analysis and quantification of genetic diversity in selected varieties / cultivars of India. The data also reaffirms the power of RAPD marker to distinctly group closely related Landraces. Among markers, RAPD and SSRs are simple and provide a quick screen for DNA polymorphism. A very small amount of DNA is required for actual analysis. In addition, information on template DNA sequence is not necessary. However, with respect to RAPD markers problems of reproducibility are reported (Muthusamy *et al.* 2008). In order to assure reproducibility, optimization of PCR reaction and also its repetition is essential. In general, among the set of accessions investigated, the efficiency of a molecular marker technique depends on the amount of polymorphism it can detect. In our study, RAPD fingerprinting was more efficient than the SSRs assay. Results of present study clearly demonstrated the ability of random DNA markers to amplify differential amplicons that could fingerprint the commercial varieties / cultivars of pomegranate. A similar effect in related crops such as mango by Srivastava *et al.* (2007) have been reported where a total of 158 amplicons ranging between 250 to 2,500bp, of which 134 (84%) have been polymorphic.

Kumar *et al*. (2013) reported a similar as our study low polymorphism situation using SSR markers where only five markers were polymoprhic out of 35 SSR markers used. Generally, SSR loci from transcribed regions had a low level of polymorphism compared to genomic SSRs (Kumpatla and Mukhopadhyay, 2005). However, the allelic number and allelic diversity detected by the EST-SSR markers in study were nearly the same as those reported for genomic SSRs (Hasnaoui*,* 2010, Pirseyedi, 2010), where a number of pomegranate cultivars from several countries were tested. Most probably, this is due to the application of many pomegranate landraces with abundant genetic variation in our study. Similarly, four SSR primers specifically produced polymorphic amplicons for pomegranate were used *via* POM0-21, POM-AGC5 and POM-AGC11 (Hasnaoui *et al*. 2010) and ABRII-MP30 (Pirseyedi *et al*. 2010). Basaki *et al.* (2013) evaluate the relationships between SSR markers and important traits using the multiple stepwise linear regressions in pomegranate. The DNA fingerprints / polymorphic amplicons for varieties *viz*., Mana, Daru, Bhagwa, Amlidan, Kandahari, Bassein Seedless and Arakta were identified with arbitrary primed marker, N-18, I-12, L-13, L-14, N-15 and N-7 respectively. In case of KY variety polymorphic DNA fragment was identified with OPB-14, N-18, L-13 and AT-12. Similarly, for Ruby variety DNA fingerprint was identified with OPAC-14, N-15 random DNA markers. On other hand, OPAC-4, OPK-3 and I-12 could develop distinct polymorphic amplicon for Jodhpur variety. In case of P-23 variety, the DNA fingerprint was identified using N-7 and N-18 arbitrary markers. The OPK-3, AC-9 and N-7 detected DNA fingerprints for pomegranate genotype 'yercaud'. Random DNA markers *viz*., OPK-3, N-15 and L-13 detected polymorphic amplicons for the Ganesh variety. Whereas, in case of Jyothi variety; AC-3 and AT-7, and Gulesha Red variety I-14 decamer marker reported the polymorphic loci. In the present study, the RAPD revealed high variation at the molecular level, indicating the suitability of the RAPD for genetic clustering. Narzary *et al*. (2009) analyzed of genetic diversity among 49 wild pomegranate accessions using PCR-DAMD and RAPD has been reported.

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