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MB Kadam
Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, Maharashtra, India

RS Patil
Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, Maharashtra, India

VS Supe
Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, Maharashtra, India

Correspondence
RS Patil
Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, Maharashtra, India

Morphological and molecular characterization of pomegramate (*Punica granatum* L.) Cultivars (Maharashtra)

MB Kadam, RS Patil and VS Supe

Abstract
The morphological and RAPD markers were successfully applied to distinguish eleven commercially grown pomegranate cultivars of Maharashtra state. The unique morphological marker (yellow colour of style) in cv. Ganesh was first time reported and helpful to distinguish cv. G-137 (Clonal selection from Ganesh). While for molecular characterization, random amplified polymorphic DNA (RAPD) markers were used to investigate the genetic diversity among 11 cultivars of pomegranate cultivated in Maharashtra state. Unweighed pair-group method average clustering divided the 11 cultivars into two main groups. In RAPD analysis, six out of 30 employed random primers showed good amplification and polymorphism on pomegranate samples with a total of 49 amplicons of which 12 were monomorphic (24.49 %) and 37 were polymorphic (75.51%). Similarity co-efficient ranged from 0.278 to 0.880 for 11 pomegranate cultivars under study indicating the genetic diversity among them. Maximum similarity coefficient (0.880) was observed between cv. Bhagawa and cv. Phule Arakta while minimum (0.278) was observed in between cv. Mridula and cv. Bhagawa. In spite of the relatively low number of primers and cultivars, RAPD constitutes an appropriate procedure to assess the genetic diversity and to survey the phylogenetic relationships in this crop.

Keywords: Characterization, morphological, RAPD, pomegranate, cultivars

Introduction
Pomegranate (*Punica granatum* L.) one of the important table fruit grown in dry land/ arid zone of tropical and subtropical region and believed to be native to the region between Iran to northern India (Stover and Mercure, 2007) [21]. It belongs to family Punicaceae (2n = 16 or 18) with two species, *Punica granatum* L. and *Punica protopunica* Balf. It is important commercially grown fruit in India, Iran, USA, Greece, Spain and Tunisia. India is the largest pomegranate producer (91.42 lakh ha area with 20.95 lakh tonnes production in 2014-2015) in the world sharing about 36 % of the world's production. The fruits can be processed into juice, syrup, jams and wine (Poyrazo_lu *et al*., 2002) [18]; and its popularity is increasing worldwide due to the rich dietary source of antioxidant, phenolics and anthocyanins (Ozgen *et al*., 2007, 2008) [14].

Large variability of fruit and plant characteristics has been noticed in *punica* germplasm due to cross pollination, seed propagation and heterozygous nature. Mars and Marrakchi (1999) [8] and Zamani *et al.* (2013) [25] reported that the fruit morphological characteristics are useful for pomegranate identification. Correct identification of genotypes of pomegranate is necessary for breeding purposes and for the protection of the plant breeder's rights. Use of molecular markers is a reliable alternative for such studies as these markers are stable and detectable in all plant tissues, regardless of environmental conditions and developmental stage. Main advantage of molecular markers is reduced time required for the genetic study of individuals and the possibility of evaluation during seed or seedling stages. There are some reports using RAPDs markers (Talebi Bodaff *et al.*, 2003; Sarkhosh *et al.*, 2006) [22, 20], to analyze the genotypic characteristics and genetic relationships of pomegranate cultivars (Zahra *et al.*, (2012) [27].

Pomegranate cultivars have been studied by various workers using different morphological and molecular markers (Mars and Marrakchi, 1999; Talebi *et al.,* 2003; Sarkhosh *et al.,* 2006; Zamani *et al.,* 2007 and Jbir *et al.,* 2008) [8, 22, 20, 26, 7]. However, there are no reports available on the assessment of genetic relationship among pomegranate cultivars in India. This study reported first morphological marker to distinguish Ganesh and G-137 (Clonal selection from Ganesh) cultivars of pomegranate and RAPD markers to assess the genetic relationship in commercially grown pomegranate cultivars in India.

Materials and methods

The pomegranate cultivars *viz; Alandi, Ganesh, G-137, Gul-e-Shah Red, Mridula, Muscat, P-23, P-26, Ruby, Phule Arakta* and *Phule Bhagwa* (Table 1) were used for morphological and molecular investigation.

Table 1: Pomegranate cultivars used for morphological and molecular characterization

Sr. No	Cultivars	Pedigree of cultivar
1	*Alandi*	Local Collection from *Alandi* (Pune) region
2	*Ganesh*	Seedling selection from open pollinated fruits of cultivar *Alandi*
3	*G-137*	Clonal selection from *Ganesh*
4	*Gul-e-Shah Red*	Introduction from USSR
5	*Mridula*	F_2 selection from the cross of *Ganesh* x *Gul-e-Shah Red*
6	*Muscat*	Introduction from Iran
7	*P-23*	Seedling selections from *Muscat*
8	*P-26*	Seedling selections from *Muscat*
9	*Ruby*	Multiple hybrid derivative from three way cross between *Ganesh* x *Kabul* x *Yercaud* and *Gulsha Rose Pink*
10	*Phule Arakta*	F_2 selection from the cross of *Ganesh* x *Gul-e-Shah Red*
11	*Bhagawa*	F_2 selection from the cross of *Ganesh* x*Gul-e-Shah Red* observed in farmer's field

Morphological characterization: Traits were described by rating based on guidelines of descriptor. Quantitative variables were measured and weighed adopting a manual caliper and a precision (0.01 g) electronic balance, respectively. Colour parameters were visually determined and other qualitative characteristics were attributed by using illustrated charts.

Data of qualitative characteristics (nature of growth, nature of foliage, leaf shape, colour of ventral and dorsal surface of leaf, colour of petiole, colour of petals, inner and outer side colour of sepals, colour of style, colour of stigma, colour of fruits, fruit surface, fruit shape, aril colour,), biometrical variables (fruit weight, length and breadth of fruit, aril size, aril weight and rind thickness) and biochemical parameters (TSS and acidity) (Table 3 and 4) were collected for all the studied accessions as per descriptor in three cropping seasons *viz*., *mrig bahar* (June), *hasta bahar* (Oct) and *ambia bahar* (Jan) 2007-08.

Table 2: List of characters, range for characterization and character state

Sr. No.	Character	Range for characterization	Character state
1	Nature of growth (Spread: Height ratio)	< 0.80 0.81 - 1.00 > 1.00	Erect Semi-spreading Spreading
3	Nature of foliage	-	Evergreen Deciduous
4	Colour of the new flush	-	- brown foliage with brown shoots -brown foliage with light brown shoots -light brown foliage with brown shoots -light brown foliage with light green shoots
12	Shape of leaf Leaf apex Leaf base Leaf margin	- - - -	Broadly lanceolate Nearly lanceolate Oblong Rounded Acute Cuneate Obtuse Entire Serrated
13	Colour of leaf (ventral side)	-	Dark green Green
14	Colour of leaf (dorsal side)	-	Green Light green
15	Colour of petiole (ventral and dorsal side)	-	Green, Green with red tinge and Dark red
17	Colour of petals	-	Orange, Orange red,Red and Brownish red
18	Outer colour of sepals	-	Orange, Red,Dark red
19	Inner colour of sepals	-	Orange, Red and Dark red
20	Colour of style	-	Yellow, Yellowish red and Red
21	Colour of stigma	-	Green, Greenish brown and Brown
22	Fruit colour	-	Orange red, Red, Yellowish red Reddish yellow and Dark red
23	Fruit surface smoothness	-	Rough, Semi-smooth and Smooth
24	Shape of fruit (Length: Breadth ratio)	< 0.95 0.96 - 1.05 > 1.05	Flat round Round Oval
24	Weight of fruit (g)	< 150 150 - 250 > 250	Small Medium Big
25	Length of fruit (cm)	< 7.5 7.5 - 9.0	Short Medium

		> 9.0	Long
26	Breadth of fruit (cm)	< 6.0 6.0 - 7.5 > 7.5	Narrow Medium Broad
27	Colour of the aril	-	Creamy white, Light pink, Pink Red and Dark red
28	Aril size by volume Aril length (cm) Aril breadth (cm)	< 1.0 > 1.0 < 0.6 > 0.6	Small Long Narrow Broad
29	Aril size by weight (g) (100 arils weight)	< 25 25-35 > 35	Small Medium Large
30	Rind thickness (cm)	< 0.30 0.30 - 0.40 > 0.40	Thin Medium Think
31	Total soluble solids (%)	< 15.0 > 15.0	Low High
32	Acidity (%)	< 0.50 > 0.50	Low High

Molecular characterization

In this study attempts were made to standardize the DNA extraction protocol and PCR amplification condition in pomegranate genotypes and to fingerprint and estimate the genetic diversity among the pomegranate genotypes. Fresh young leaf samples were collected from the selected genotypes and genomic DNA was extracted using slight modification in Porebeski *et al*. (1997*)* [17]. The genomic DNA was quantified on 0.8% agarose gel and diluted to a uniform concentration of 50 ng/ul for RAPD analysis. The PCR procedure described by Williams *et al*. (1990) [23] was followed with minor modifications. The amplification of RAPD fragments was performed with 100 ng template DNA, 40 pmoles of primer, 2.5 mM Mg2+ions, 200 µM each dNTP, 1.0 U Taq DNA polymerase in 1x assay buffer in a final volume of 25µL. The chemicals for PCR mastermix used were of Bangalore Genei Pvt. Ltd., make. Thirty random primers (OPA, OPB, OPC, OPD, OPE, OPF, OPG, OPI, OPJ, OPK and OPO series) from Operon Technologies Inc, Alameda, USA were used in this study.

The DNA amplification reaction were carried out in a thermal cycler (Eppendorf, Mastercycle gradient, Germany), by following cycle profile: 1 cycle of initial denaturation at 95^0C for 3 minutes, followed by 45 cycles of denaturation at 94^0C for 1 minute, annealing at 35^0C for 2 minute and primer extension 72^0C for 2 minute. A final extension at 72^0C for 5 minutes was given at the end of the cycles and the samples were held at 4^0C in the thermal cycler till electrophoresis. Electrophoresis was performed on 1.5% agarose gel. The amplified PCR products were observed under UV transilluminater in the gel documentation system (Fluor Chem^{TM} Alpha Innotech, USA).

The molecular weight of each fragment amplified by RAPD primers was estimated by using ladder plus marker (Lamda DNA/*Eco*R1/*Hind* III Double Digest). All bands obtained for cultivars under study were scored for their presence or absence (1 or 0). Only clear and strong bands were scored. Data was analyzed and similarity matrix was constructed from binary data with Dice Similarity Coefficient calculated as per model suggested by Nei and Li (1979) [12]. Unweighted Pair Group Method using Arithmetic Averages (UPGMA) was employed for cluster analysis. Bootstrap support for the branches of the dendrogram was generated with 1000 boot strapped samples in the WINBOOT programme as described by Yap and Nelson (1996) [24].

Results and discussion

Morphological characterization

The morphological characters like plant nature, growth habit, leaf shape, leaf colour, petiole colour, flower bud colour, colour of petals, sepals, style, stigma and fruit characters like fruit colour, aril colour were useful for characterization of cultivars. On the basis of spread: height ratio, plants were classified into three types *viz.*, erect, semi-spreading and spreading. The cv. *Gul-e-Shah Red* had a spread: height ratio less than 0.80 and are of erect type. While all the other cv. *viz.*, *Alandi, Ganesh*, *G-137*, *Mridula, Muscat*, *P-23, P-26*, *Ruby, Phule Arakta* and *Bhagawa* were spreading type with a spread: height ratio more than 1.00.

Patil and Sanghavi (1980) [16] and Jagtap (1989) [5] also observed that temperate zone cultivars (Gul-e-Shah Red) were erect in growth habit while cv. *Ganesh, Alandi and Muscat* was of spreading habit with evergreen foliage under Maharashtra condition.

The leaf shape was broadly lanceolate in cv. *Alandi* and *P-23* whereas it was nearly lanceolate in cv. *Gul-e-Shah Red, Ganesh*, G-137, *Mridula, Muscat*, *P-26*, *Phule Arakta* and *Bhagawa*. While cv. *Ruby* had oblong leaf shape. The leaf tip was acute in cv. *Ganesh*, *G-137*, *Mridula, Muscat*, *P-26*, *Phule Arakta* and rounded in cvs. *Alandi, Gul-e-Shah Red*, *P-23*, Ruby and *Bhagawa*. The colour of ventral and dorsal side of leaf surface was dark green and green, respectively, for all cultivars under study except cv. *P-23* and *G-137*. Cultivar *P-23* had green and light green colour to ventral and dorsal surface of leaf respectively and cv. *G-137* had light green colour to dorsal surface of leaf. Nath and Randhawa (1959) [10] also studied the leaf colour for classification of pomegranate cultivars and observed cultivar differences.

Cv. *Gul-e-Shah Red, Ganesh*, *P-26*, *Phule Arakta* and *Bhagawa* had green with red tinge colour on both the sides of the petiole whereas cv. *Alandi* had green with red tinge and green, *G-137* had dark red and green with red tinge, cv. *Muscat* and *P-23* had dark red and green, cv. *Mridula* had green with red tinge and dark red and cv. *Ruby* had green on ventral surface and dorsal surface petiole colour respectively. The colour of the petals was orange red in cv. *Alandi, Ganesh*, *G-137 Muscat*, red in cv. *Gul-e-Shah Red*, *Ruby, Phule Arakta* while characteristic brownish red colour was observed in cv. *Mridula*. cv. *P-23, P-26* and *Bhagawa* had orange colour petals. The outer colour of sepals was red in cv. *Alandi, Gul-e-Shah Red*, *G-137*, *Muscat, Ruby, Phule Arakta* while orange colour noticed in cv. *Ganesh*, *P-23, P-26* and

Bhagawa but distinguishing dark red colour of sepal was observed in cv. *Mridula*. The inner colour of sepals was red in cv. *Alandi, Gul-e-Shah-Red, Muscat, Ruby*, *Phule Arakta* while orange in *Ganesh*, *G-137, P-23, P-26*, *Bhagawa* and Dark red in cv. *Mridula*.

The colour of the style was yellowish red in cv. *Alandi*, *G-137*, *Muscat*, *P-23, P-26*; red in cv. *Gul-e-Shah Red, Mridula, Ruby, Phule Arakta, Bhagawa*; cv. *Ganesh* had yellow colour of style which is emerged as an unique character which distinguished it from two clonal and phenotypically similar cultivars, Ganesh and *G-137*. The colour of the stigma was green for all pomegranate cultivars under study except cv. *G-137*, *Gul-e-Shah Red, Mridula, Muscat* and *Phule Arakta* where greenish brown stigma was observed.

Especially five fruit colours were observed in different pomegranate cultivars is as follows, orange red in cv. *Bhagawa*, dark red in cv. *Mridula, Ruby* and *Phule Arakta*; Red in cv. *Alandi* and *Gul-e-Shah Red*; yellowish red in cv. *Ganesh* and *G-137*; reddish yellow in cv. *Muscat*, *P-23* and *P-26*. The colour of the aril was observed as red in cv. *Alandi* and *Gul-e-Shah Red*; pink in cv. *Ganesh* and *G-137*; creamy white in *Muscat* and *P-26*; light pink in *P-23*; dark red in cv. *Mridula, Ruby, Phule Arakta* and *Bhagawa*.

The cultivars G*ul-e-shah Red, Mridula, Ruby, Phule Arakta and Bhagawa* were recorded short fruit length. The cv. *Alandi, Ganesh*, *G-137*, *Muscat*, *P-23* and *P-26* had medium fruit length.While the cv. *Gul-e-shah Red* had narrow fruit breadth. The cultivars *Alandi*, *P-23*, *Mridula, Ruby, Phule Arakta* and *Bhagawa* were recorded medium fruit breadth. The cv. *Ganesh*, *G-137*, *Muscat*, *P-26* had broad fruits. On the basis of fruit weight cv. *Alandi, Ganesh*, *G-137*, *Muscat*, *P-23, P-26*, *Phule Arakta* and *Bhagawa* had large sized fruits (more than 250 g) while cv. *Mridula* and *Ruby* had medium sized fruits (150 to 250 g). Cv. *Gul-e-Shah Red* had small sized fruits (less than 150 g). The cv. *Alandi* and *Gul-e-Shah Red* were recorded short aril length. All other cultivars recorded long aril length. All the cultivars had broad arils except cv. *Gul-e-Shah Red*. Av. wt of 100 arils were studied and they were grouped into small, medium and large. The cv. *Alandi, Ganesh*, *G-137, P-23, P-26, Mridula, Ruby, Phule Arakta, Bhagawa* and all the synonyms of cv. *Bhagawa* showed large aril weight (more than 35 g), cv. *Muscat* had medium aril weight (25-35 g) and cv. *Gul-e-Shah Red* showed less (less than 25 g) aril weight.

The cv. *Alandi, Ganesh*, *G-137*, *Muscat*, *P-23, P-26* and *Bhagawa* had thick rind (more than 0.40 cm), cv. *Gul-e-Shah Red, Ruby* and *Phule Arakta* had medium thick rind (0.30 to 0.40 cm) whereas the cv. *Mridula* had thin (less than 0.30 cm) rind. Bailey (1917) [2] and Hodgson (1917) [4] were the first to recognize the use of morphological characters and incorporated such important features, as colour of the rind, colour of petals and size of the tree in their descriptions. No single character could be depended upon to establish the identity of any pomegranate cultivar, but a combination of several characters was more useful in this direction.

Biochemical parameters

T.S.S. (%)

The cv. *Alandi, Gul-e-Shah Red P-26* and *Ruby* had low T.S.S. content (less than 15.0%) where as the cv *Ganesh*, *G-137*, *Mridula, Muscat*, P-*23*, *Phule Arakta,* and *Bhagawa* had high (more than 15.0%) T.S.S. content.

Acidity (%)

All the cultivars except *Alandi* and *Gul-e-Shah Red* under study recorded low acid content (less than 0.50%). Cv *Alandi* (0.60%) and *Gul-e-Shah Red* (3.18%) recorded high (more than 0.50%) acid content. Patil and Sanghavi (1980) [16] and Jagtap (1989) [5] also observed similar results for qualitative characteristics.

It is well-known fact that the environment has a great effect of expression of quantitative traits. However, several characteristics of these cultivars (style colour, fruit and aril color, biochemical characteristics) are stable across environments.

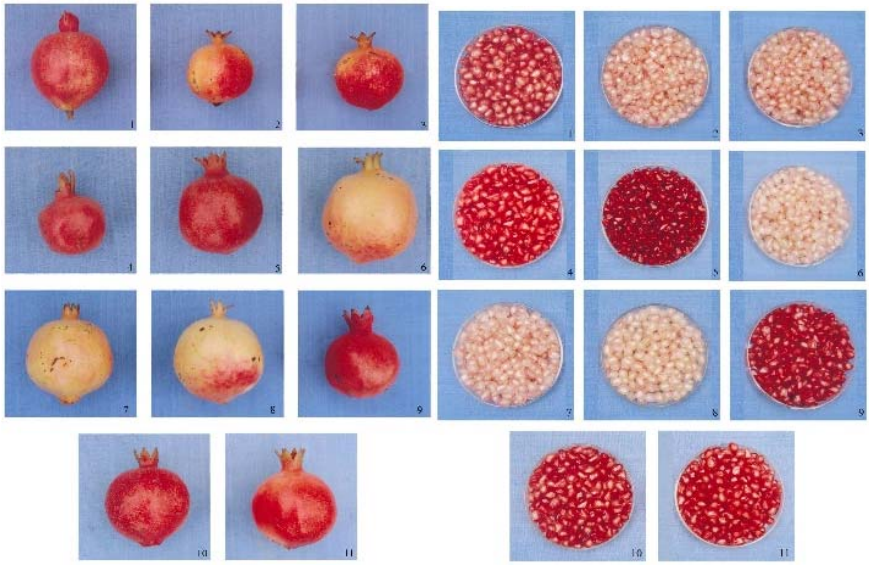
Table 3: Morphological characterization in 11 pomegranate cultivars for plant growth and flower characters

Sr. No.	Cultivars	Nature of growth	Nature of foliage	Leaf shape	Leaf Colour		Petiole Colour		Colour of the petals	Colour of the sepals		Colour of the style	Colour of the stigma
					Ventral	Dorsal	Ventral	Dorsal		Outer	Inner		
A. Red colour (Traditional) cultivars													
1	Alandi	(1.03) Spreading	Evergreen	Broadly Lanceolate	Dark green	Green	Green with red tinge	Green	Orange red	Red	Red	Yellowish red	Green
4	Gul-e-Shah Red	(0.78) Erect	Deciduous	Nearly lanceolate	Dark green	Green	Green with red tinge	Green with red tinge	Red	Red	Red	Red	Greenish brown
B. Pink colour aril cultivars													
2	Ganesh	(1.08) Spreading	Evergreen	Nearly lanceolate	Dark green	Green	Green with red tinge	Green with red tinge	Orange red	Orange	Orange	Yellow	Green
3	G-137	(1.20) Spreading	Evergreen	Nearly lanceolate	Dark green	Light green	Dark red	Green with red tinge	Orange red	Red	Orange	Yellowish red	Greenish brown
C. White colour aril cultivars													
6	Muscat	(1.12) Spreading	Evergreen	Nearly lanceolate	Dark green	Green	Dark red	Green	Orange red	Red	Red	Yellowish red	Greenish brown
7	P-23	(1.20) Spreading	Evergreen	Broadly lanceolate	Green	Light green	Dark red	Green	Orange	Orange	Orange	Yellowish red	Green
8	P-26	(1.26) Spreading	Evergreen	Nearly lanceolate	Dark green	Green	Green with red tinge	Green with red tinge	Orange	Orange	Orange	Yellowish red	Green
D. Dark red aril colour cultivars													
5	Mridula	(1.20)	Evergreen	Nearly	Dark	Green	Green	Dark red	Brownish	Dark	Dark	Red	Greenish

		Spreading		lanceolate	green		with red tinge		red	red	red		brown
9	Ruby	(1.00) Spreading	Evergreen	Oblong	Dark green	Green	Green	Green	Red	Red	Red	Red	Green
10	Phule Arakta	(1.04) Spreading	Evergreen	Nearly lanceolate	Dark green	Green	Green with red tinge	Green with red tinge	Red	Red	Red	Red	Greenish brown
11	Bhagawa	(1.02) Spreading	Evergreen	Nearly lanceolate	Dark green	Green	Green with red tinge	Green with red tinge	Orange	Orange	Orange	Red	Green
	SE +	0.02											

Table 4: Morphological characterization in 11 pomegranate cultivars for fruit characters and biochemical parameters

Sr No	Cultivars	Fruit colour	Fruit surface	Av. fruit length (cm)	Av. fruit breadth (cm)	Fruit shape	Fruit wt. (g)	Aril colour	Av. aril size			Rind Thick-ness (cm)	T.S.S (%)	Acidity (%)
									By volume		By wt. (g)			
									L (cm)	B (cm)	100 arils			
A. Red colour (Traditional) cultivars														
1	Alandi	Red	Smooth glossy	8.70 (Medium)	7.50 (Medium)	Oval, Nipple	285.00 (Big)	Red	0.96 (Short)	0.70 (Broad)	40.790 (Large)	0.44 (Thick)	14.6 (Low)	0.60 (High)
4	Gul-e-Shah red	Red	Smooth glossy	4.50 (Short)	4.68 (Narrow)	Round	107.67 (Small)	Light red	0.90 (Short)	0.60 (Broad)	20.995 (Small)	0.35 (Medium)	11.8 (Low)	3.18 (High)
B. Pink colour aril cultivars														
2	Ganesh	Yellowish red	Smooth glossy	8.37 (Medium)	8.06 (Broad)	Round	302.33 (Big)	Pink	1.00 (Long)	0.74 (Broad)	37.991 (Large)	0.42 (Thick)	16.2 (High)	0.49 (Low)
3	G-137	Yellowish red	Smooth glossy	8.00 (Medium)	8.30 (Broad)	Round	318.00 (Big)	Pink	1.10 (Long)	0.78 (Broad)	35.398 (Large)	0.41 (Thick)	16.6 (High)	0.41 (Low)
C. White colour aril cultivars														
6	Muscat	Reddish yellow	Smooth glossy	8.30 (Medium)	8.20 (Broad)	Round	322.33 (Big)	C. White	1.00 (Long)	0.70 (Broad)	33.734	0.41 (Thick)	15.8 (High)	0.41 (Low)
7	P-23	Reddish yellow	Smooth glossy	7.70 (Medium)	7.50 (Medium)	Round	332.00 (Big)	Light pink	1.01 (Long)	0.68 (Broad)	40.440 (Large)	0.45 (Thick)	16.6 (High)	0.47 (Low)
8	P-26	Reddish yellow	Smooth glossy	7.90 (Medium)	7.90 (Broad)	Round	312.00 (Big)	C. White	1.10 (Long)	0.70 (Broad)	41.492 (Large)	0.41 (Thick)	14.8 (Low)	0.43 (Low)
D. Dark red colour aril cultivars														
5	Mridula	Dark red	Smooth glossy	6.70 (Short)	6.70 (Medium)	Round	215.67 (Medium)	Dark red	1.01 (Long)	0.66 (Broad)	35.44 (Large)	0.29 (Thin)	15.3 (High)	0.41 (Low)
9	Ruby	Dark red	Smooth glossy	5.90 (Short)	6.50 (Medium)	Round	213.33 (Medium)	Dark red	1.09 (Long)	0.65 (Broad)	36.542 (Large)	0.35 (Medium)	14.8 (Low)	0.41 (Low)
10	Phule Arakta	Dark Red	Smooth glossy	6.00 (Short)	6.10 (Medium)	Round	252.67 (Medium)	red	1.10 (Long)	0.68 (Broad)	39.760 (Large)	0.32 (Medium)	15.6 (High)	0.41 (Low)
11	Bhagwa	Orange red	Smooth, V.glossy	7.00 (Short)	7.10 (Medium)	Round	268.50 (Big)	red	1.10 (Long)	0.65 (Broad)	40.145 (Large)	0.44 (Thick)	15.4 (High)	0.43 (Low)
	SE +			0.23	0.13		11.89		0.03	0.10	2.11	0.02	0.19	0.05



1 2

Fig 1: Fruit colour (1) and aril colour (2) of pomegranate cultivars

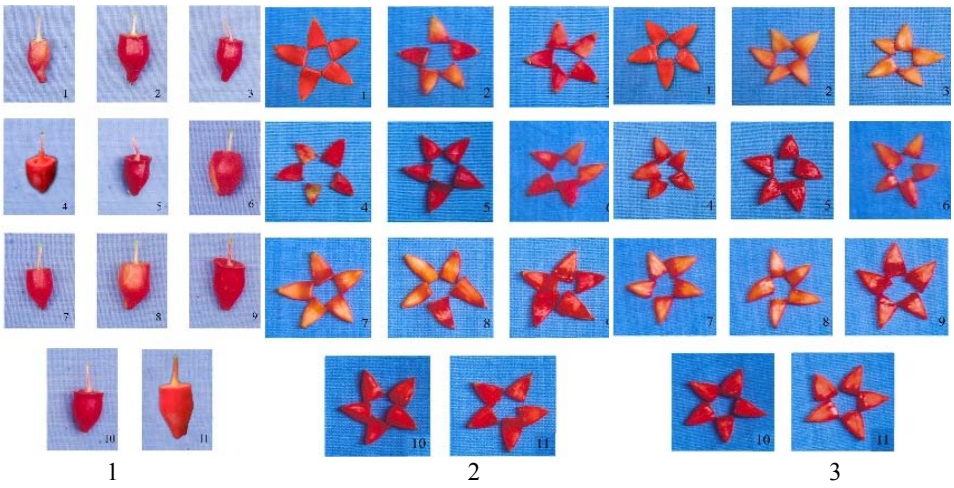


Fig 2: Colour of style (1) and outer (2) and inner (3) surface of sepals of pomegranate cultivars

1. Alandi 2. Ganesh 3. G-137 4. Gul-e.Shah Red 5. Mridula 6. Muscat
7. P-23 8. P-26 9. Ruby 10. Phule Arakta 11. Phule Bhagwa

RAPD analysis of genomic DNA of 11 pomegranate cultivars

The genomic DNAs of the 11 pomegranate cultivars *viz*., *Alandi*, *Ganesh*, G-137, *Gul-e-Shah Red*, *Mridula*, *Muscat*, P-23, P-26, *Ruby*, *Phule Arakta*, *Bhagawa*, were subjected to PCR amplification using 30 random primers. Out of 30 primers screened (Table 5), six showed maximum polymorphism and were involved in characterization of pomegranate cultivars.

Table 5: RAPD analysis of genomic DNA of 11 pomegranate cultivars.

Sr. No.	Details	
1	Total No. of primers used	30
2	No. of polymorphic primers	06
3	Total No. of bands	49
4	Total No. of polymorphic bands	37
5	Total No. of unique bands	06
6	Percentage polymorphism	75.51 %
7	Size of amplified products	187 to 3614 bp

The amplification profile of 11 cultivars of pomegranate with six primers summarized in Table 5. It was observed that 49 fragments were generated in all the cultivars with six primers, of which 37 fragments were polymorphic with fragment size 187 to 3614 bp. Maximum number of bands were observed in OPB 07 primer, whereas least banding pattern was generated by OPD 15. The primer OPB 08 showed the maximum percentage of polymorphism (87.50 %) while the least (57.14 %) by OPD 03. Six primers produced six unique bands which were variety specific in the present study (Table 6).

The primer OPA 11 recorded 77.78% polymorphism. It gave amplification profile of nine bands out of which seven were polymorphic, two were unique (Table 6, Fig 3). The variety specific unique bands were present in cv. *Mridula* (506 bp) and cv. P-23 (1266 bp) with primer OPA 11. It also differentiates morphologically similar cultivars Mridula and Phule Arakta. Cultivars *Gul-e-Shah Red* and *Phule Arakta* are differentiated from each other with unique band of size 1567 bp with primer OPA 11 So, this marker is useful for distinguishing cv. *Phule Arakta* from cv. *Mridula, Bhagawa* and *Ruby.*

Table 6: Total number of RAPD markers and polymorphic markers produced by random primers in 11 pomegranate cultivars.

Sr. No.	Random primers	No. of bands generate	Polymor-phic bands	Monomor-phic bands	Unique bands	% polymor-phic bands	Fragment size (bp)
1.	OPA 11	9	7	-	2	77.78	190 to 1981
2.	OPB 07	10	8	2	-	80.00	362 to 2040
3.	OPB 08	8	7	1	-	87.50	187 to 1175
4.	OPD 03	9	6	2	1	66.67	453 to 1877
5.	OPD 05	7	4	-	3	57.14	650 to 3614
6.	OPD 15	6	5	1	-	80.00	406 to 1905

Genetic diversity analysis of genomic DNA of 11 pomegranate cultivars

The diversity observed in 11 pomegranate cultivars is mainly attributed to the genetic dissimilarity. The Dice similarity coefficient values among the 11 pomegranate cultivars are presented in Table 7. It was observed that similarity coefficient ranged from 0.278 to 0.880 implying that a part of the genome is similar among the cultivars.Thus, these cultivars are genetically divergent. Minimum similarity coefficient about 0.278 indicated maximum divergence between cv. *Mridula* and *Bhagawa*. Maximum similarity coefficient about 0.880 indicated that cv. *Phule Arakta* is less divergent from cv. *Bhagawa*.

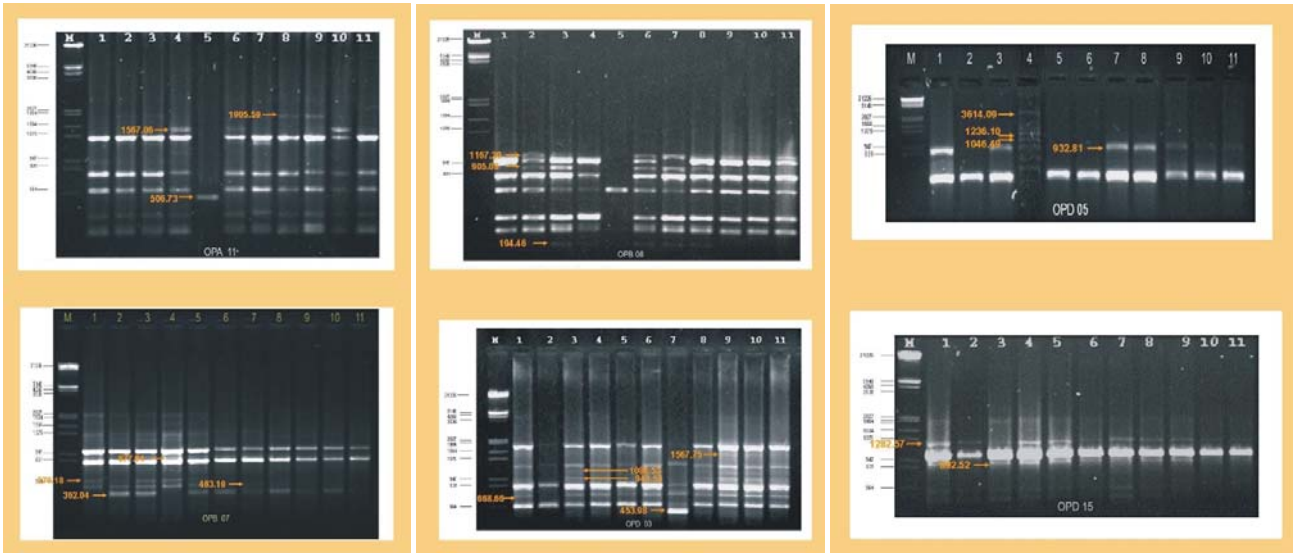


Fig 3: Molecular characterization of pomegranate cultivars by using RAPD markers

1. Alandi 2. Ganesh 3. G-137 4. Gul-e.Shah Red 5. Mridula 6. Muscat 7. P-23
8. P-26 9. Ruby 10. Phule Arakta 11. Phule Bhagwa

Table 7: Dice similarity coefficient values based on RAPD marker data of 11 pomegranate cultivars

Cultivars	Alandi	Ganesh	G-137	Gul-e- Shah Red	Mridula	Muscat	P-23	P-26	Ruby	Arakta	Bhagawa
Alandi	1.000										
Ganesh	0.568	1.000									
G-137	0.750	0.622	1.000								
Gul-e-Shah Red	0.825	0.465	0.704	1.000							
Mridula	0.583	0.382	0.513	0.476	1.000						
Muscat	0.575	0.710	0.806	0.545	0.405	1.000					
P-23	0.575	0.559	0.625	0.478	0.368	0.611	1.000				
P-26	0.550	0.576	0.684	0.523	0.342	0.781	0.676	1.000			
Ruby	0.579	0.562	0.632	0.548	0.324	0.719	0.617	0.800	1.000		
Arakta	0.450	0.500	0.538	0.465	0.306	0.656	0.432	0.625	0.724	1.000	
Bhagawa	0.500	0.516	0.553	0.476	0.278	0.677	0.486	0.645	0.815	0.880	1.000

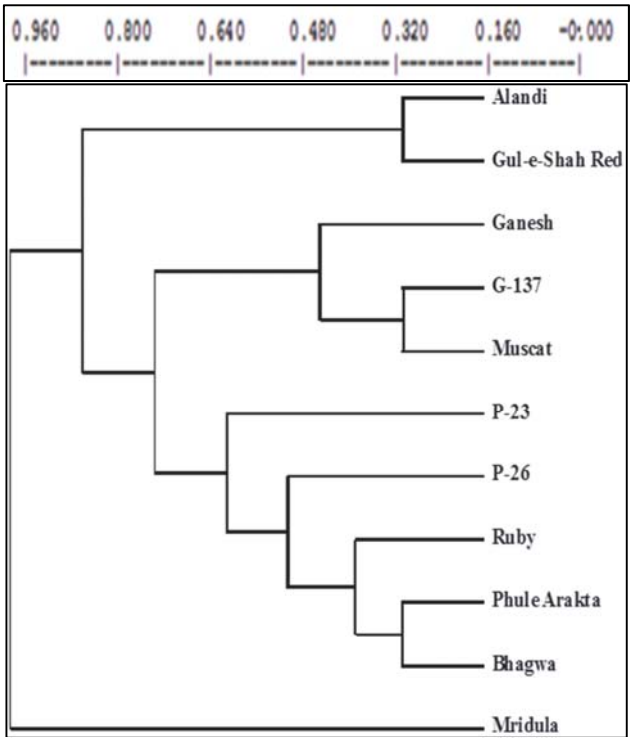


Fig 4: Consensus tree showing clustering of 11 pomegranate cultivars using RAPD analysis

Conclusion

This study concludes that morphological and molecular markers can be used together to identify and develop pomegranate genotypes. RAPD markers were found to be effective in studying genetic relationship among pomegranate cultivars and distinguishing morphologically similar cultivars.

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