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***RESEARCH PAPER***

# RAPD based molecular diversity analysis of different varieties of pomegranate (*Punica granatum* L.)

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**Abstract :** Genetic diversity of eight pomegranate varieties was carried out using five RAPD primers. The DNA was extracted from young leaves using CTAB method. The PCR for RAPD was performed with two primers from OPA series, and four primers from OPB series. The RAPD analysis with five arbitrary oligonucleotide primers amplified a total of 28 DNA bands out of which 25 were found to be polymorphic. The average polymorphism recorded by the RAPD loci was 89.28 per cent. The number of DNA fragment varied from four to seven. The mean number of polymorphic bands per primer among eight pomegranate varieties was 5.6 and per cent polymorphism ranged from 75 to 100. The size of PCR amplified DNA fragment ranged from 88.49 to 1430.11bp and PIC value varied from 0.70 to 0.83. The dendrogram constructed using pooled RAPD loci data clearly showed two varieties (Ganesh and Mrudula) were highly similar and different from other genotypes. The genetic similarities ranged from 0.32 to 0.72 and mean similarity co-efficient was 0.61.

**Key Words :** RAPD, *Punica granatum* L., PCR, Primer, Molecular marker

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## INTRODUCTION

Pomegranate (*Punica granatum* L.) belongs to Punicaceae family and is an important fruit tree of tropical and subtropical regions of the world which is valued highly for its delicious edible fruits. The cultivated varieties of pomegranate, *Punica granatum* is to contain 2n=2x=16, 18 chromosomes. It belongs to the subclass Rosidae and believed to be native to the region between Iran to northern India (Stover and Mercure, 2007). In India, pomegranate grows wild in Western Himalayan regions that include states like Himachal Pradesh, Jammu and Kashmir and Uttarakhand (Misra *et al*., 1983; Pandey *et al*. (2008). Pomegranate may be classified according to the acidity of its fruit into sour, sour-sweet or sweet.

The total area under cultivation of pomegranate in India is 113.240 thousand ha and production is around 744.950 MT tonnes in 2012-13. Maharashtra is the leading producer of pomegranate having 55.76 per cent production followed by Karnataka, Andhra Pradesh, Gujarat and Tamil Nadu. The total area under cultivation of pomegranate in Maharashtra is 78 thousand ha and production is around 408 thousand MT in 2012-13. Ganesh, Bhagwa, Ruby, Arakta and Mridula are the different varieties of pomegranates produced in Maharashtra. Currently, it is an important fruit species for India, Iran, USA and Mediterranean countries like Greece, Spain, Tunisia. Pomegranate fruit juice makes an excellent drink which contains potassium, phosphorus and calcium as well as micronutrients like iron, manganese, zinc and copper. The antioxidant, immune-boosting, and anti-carcinogenic properties of the pomegranate offers its multiple potential

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medical applications (Kaplan *et al.*, 2001). These have been demonstrated to be beneficial in combating high blood pressure and other serious diseases such as diabetes and various cancers (Shishodia *et al.*, 2006). Although the chemical composition of the fruit is affected from cultivar, growing region, climate, maturity, cultural practice and storage (Melgarejo *et al*., 2000). In addition, the tree is also cultivated for its pharmaceutical and ornamental usages (Levin, 1994).

DNA markers are independent from environmental interactions and show high level of polymorphism therefore, they are considered as useful tools for determining genetic relationships and diversity. Polymorphisms are detected from differences in the length of the amplified fragments by polyacrylamide gel electrophoresis (PAGE) (Matthes *et al.*, 1998) or by capillary electrophoresis. They also have been used to investigate relationships of closely related taxa (Miller and Tanksley, 1990; Lanner *et al*., 1997), as fingerprinting tools (Fang *et al*., 1997), for diversity studies (Debreuil *et al.*, 1996). Although a wide range of morphological and physiological characters show variability's in the pomegranate, molecular studies of the pomegranate have been restricted to examinations of RAPD (Durgac *et al.*, 2008; Zamani *et al.*, 2007 and Sarkhosh *et al.*, 2009), to investigate the population dynamics of economically important cultivars.

## MATERIAL AND METHODS

The present investigation entitled RAPD based molecular diversity analysis of different varieties of pomegranate (*Punica granatum* L.) was carried out at Lokmangal Agricultural Biotechnology College, Wadala, Solapur, Maharashtra.

**Plant material :**

Experimental material comprised of eight cultivated varieties of pomegranate namely Ganesh, Bhagwa, Arakta, Supper Bhagwa, Mrudulla, Rubby, G-137 and Tissue culture Bhagwa were collected from different pomegranate cultivating area of Maharashtra.

**DNA isolation :**

Genomic DNA was isolated from juvenile fresh leaves of 8 different varieties of pomegranate collected from different pomegranate cultivation area of Maharashtra following CTAB (CetylTrimethyl Ammonium Bromide) extraction method given by Gawel and Jarret (1991) with some modifications.

**RAPD analysis :**

Five of the available decamer random oligonucleotide primers were used to ascertain polymorphism among eight different varieties of pomegranate. The polymerase chain reaction (PCR) as adopted by Mathews *et al.* (2007) with minor modifications was carried out in 25 µl of reaction mix (1X Taq buffer, 17µl sterile $DDH_2O$, 25mM $MgCl_2$, 2.5mM dNTP, 20pmol primer, 1U Taq DNA polymerase, 50 ng DNA). Amplification reactions were carried out for 40 cycles. Each cycle comprised of 1 min at 94ºC, 1:30 min at 37ºC and 2 min at 72ºC. Amplified product were separated on 1.6 per cent agarose gel, stained with ethidium bromide and photographed under UV light.

**Data analysis :**

Data were scored for computer analysis on the basis of the presence or absence of the PCR products. If a product was present in a genotype, it was designated as '1' and if absent; it was designated as '0'. The data generated by RAPD and SSR loci were analyzed with the software NTSYSpc version 2.02 (Rohlf, 1994). The PIC values were calculated with formula $PIC=1-\Sigma pi^2$ (where pi is the frequency of the i th allele) given by Smith *et al.* (1997).

## RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under following heads :

**Random amplified polymorphic DNA (RAPD) :**

The genomic DNA extracted from each genotype was subjected to polymerase chain reaction using fourteen random decamer primers from OPA, OPB and OPP series. Molecular characterization of eight different pomegranate varieties carried out using RAPD primer. In RAPD assay total five primers were selected for pooled analysis from fourteen primers. PCR amplification of DNA, using five primers from OPA and OPB series for RAPD analysis, produced 28 DNA fragments. All the selected 5 primers amplified DNA fragments across 8 genotypes. The total number of amplified fragments was varying from four (OPB-18) to seven (OPA-07), with size ranged from 88.49 to 1430.11bp (Table 1). Same result was found by Hasnaoui *et al.* (2010) in their study on molecular polymorphisms in Tunisian pomegranate. Out of 28 DNA fragments 25 were polymorphic giving 89.28 per cent average polymorphism for total OPA and OPB series. The average polymorphic band per primer was 5.6 and per cent polymorphism ranged from 75 (OPB-18) to 100 *i.e.* OPA-07and OPB-15, respectively (Plate 1). Near about same results were revealed by Jambhale *et al.* (2007) in their study on molecular characterization of pomegranate cultivars with RAPD markers. The PIC value varied from 0.70 (OPB-18) to 0.83(OPA-07) (Fig. 1).

Dendrogram (Fig. 1) constructed with the data generated by all five OPA and OPB primers and their amplicons grouped the all pomegranate varieties in to one cluster *i.e.* cluster A. The cluster A grouped all seven varieties of pomegranate *i.e.* T.C. Bhagwa, S. Bhagwa, Arakta, Mrudulla, Ganeh, Rubby and Bhagava except one variety *i.e.* G-137. Cluster A further divided into sub cluster A1 and sub-cluster A2. Sub-cluster A1 grouped total five different varieties *i.e.* T.C. Bhagwa, Mrudulla, Ganeh, Arakta and Rubby while sub-cluster A2

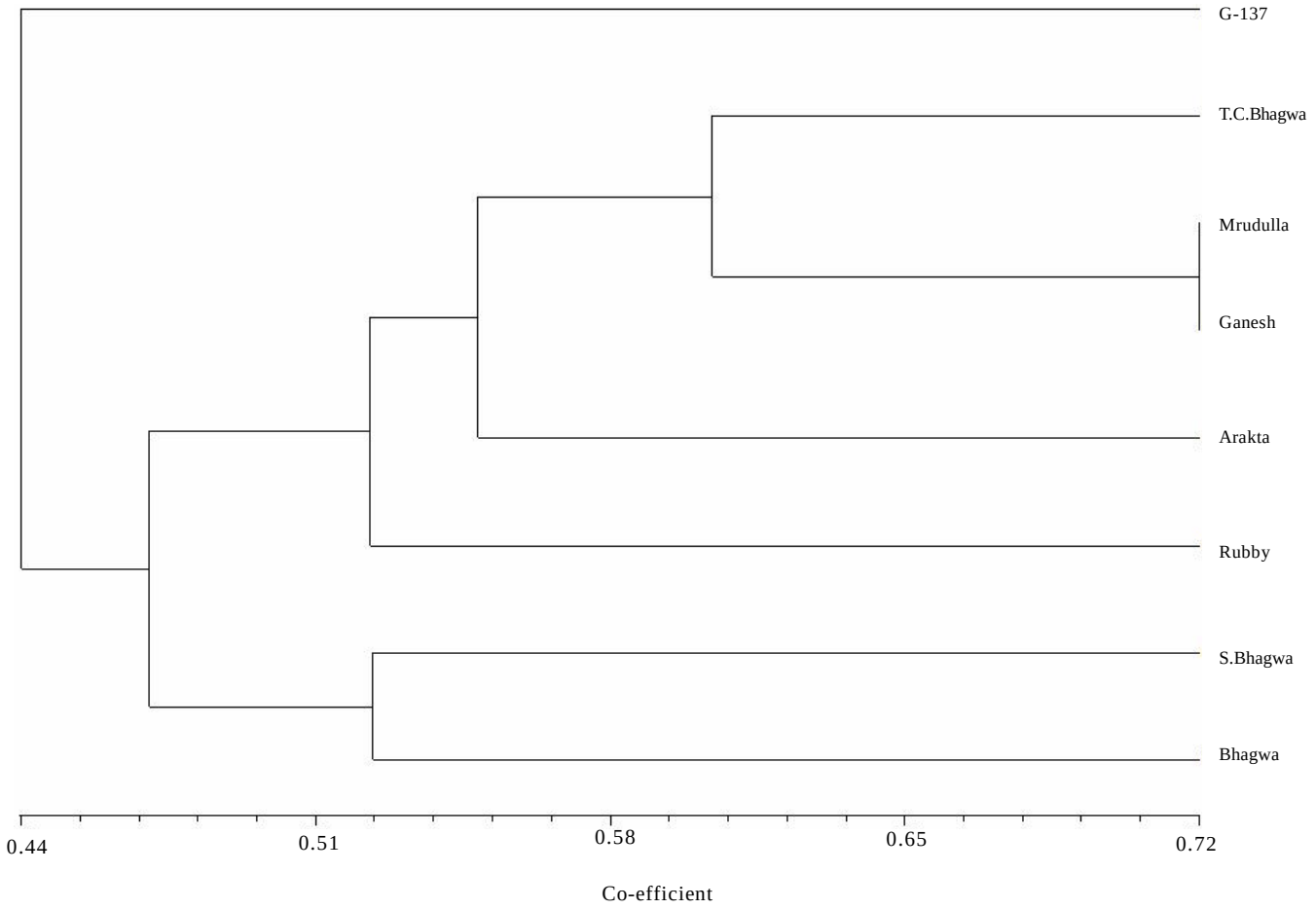


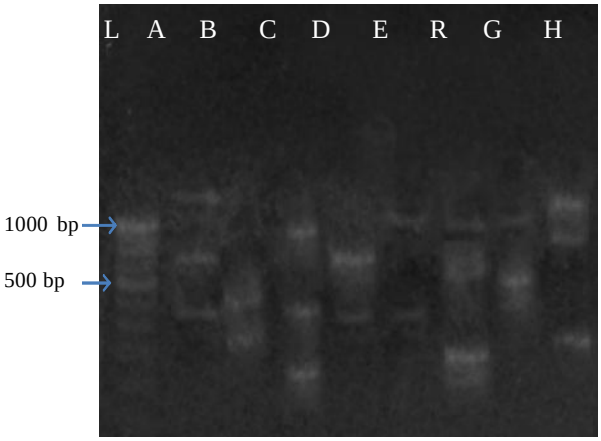

**Fig. 1 : Dendrogram showing clustering of eight pomegranate varieties obtained from RAPD marker**

**Table 1 : Result of RAPD primers**

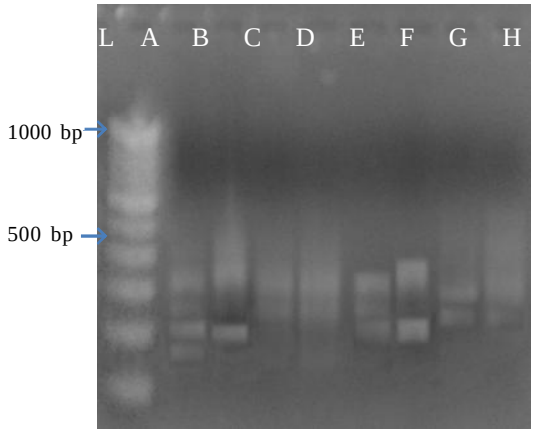
| Sr. No. | Primer code | Mole wt. (bp) | | Total no. of band | No. of polymorphic band | Per cent polymorphism | PIC value |
|---|---|---|---|---|---|---|---|
| | | High | Low | | | | |
| 1. | OPB-15 | 1430.11 | 174.81 | 6 | 6 | 100 | 0.79 |
| 2. | OPB-18 | 304.84 | 134.9 | 4 | 3 | 75 | 0.70 |
| 3. | OPA-19 | 1013.16 | 308.19 | 5 | 4 | 80 | 0.74 |
| 4. | OPB-07 | 451.72 | 88.49 | 6 | 5 | 83.33 | 0.77 |
| 5 | OPA-07 | 458.95 | 90.42 | 7 | 7 | 100 | 0.83 |
| | Total | | | 28 | 25 | Average | 89.28 |

**Table 2 : Jaccard's similarity co-efficient for eight pomegranate varieties based on RAPD data analysis**

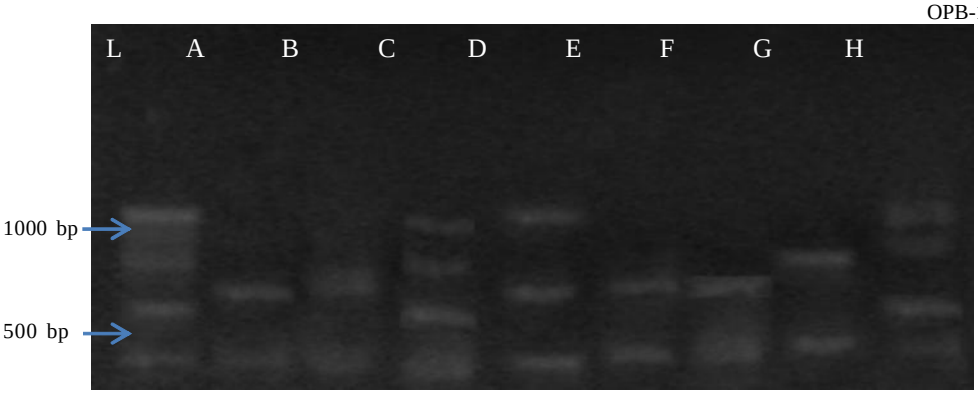
| Varieties | G-137 | T.C. Bhagwa | S. Bhagwa | Arakta | Mrudulla | Ganesh | Rubby | Bhagwa |
|---|---|---|---|---|---|---|---|---|
| G-137 | 1.0000 | | | | | | | |
| T.C. Bhagwa | 0.5500 | 1.0000 | | | | | | |
| S. Bhagwa | 0.3200 | 0.3913 | 1.0000 | | | | | |
| Arakta | 0.4500 | 0.4736 | 0.5000 | 1.0000 | | | | |
| Mrudulla | 0.4761 | 0.5789 | 0.6842 | 0.6470 | 1.0000 | | | |
| Ganesh | 0.4545 | 0.6315 | 0.5000 | 0.5263 | 0.7222 | 1.0000 | | |
| Rubby | 0.4166 | 0.5000 | 0.5217 | 0.4761 | 0.5714 | 0.5454 | 1.0000 | |
| Bhagwa | 0.4090 | 0.3636 | 0.5238 | 0.4000 | 0.4285 | 0.4761 | 0.4347 | 1.0000 |



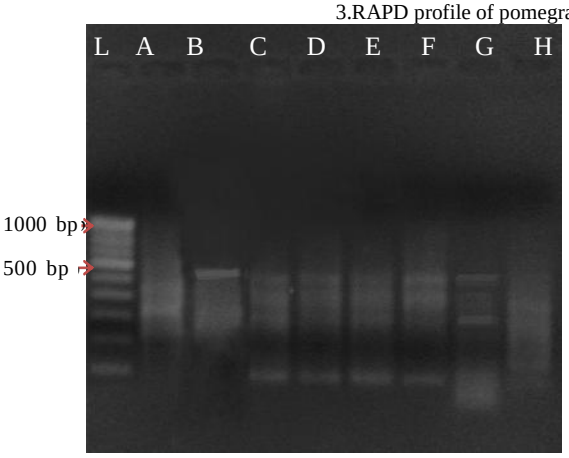

1.RAPD profile of pomegranate (*Punica grantum* L.) with primer OPB-15



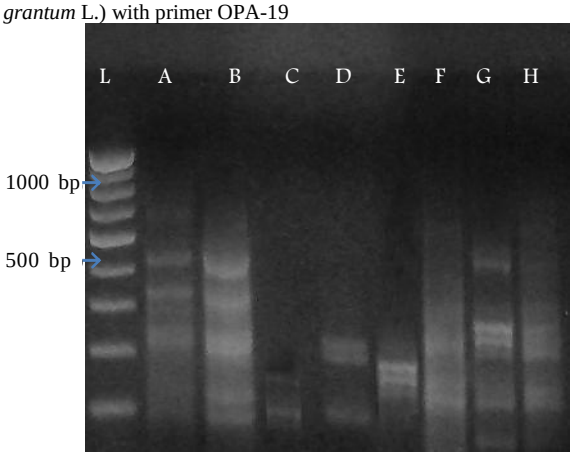

2.RAPD profile of pomegranate (P*unica grantum* L.) with primer OPB-18




3.RAPD profile of pomegranate (*Punica grantum* L.) with primer OPA-19




4. RAPD profile of pomegranate (*Punica grantum* L.) with primer OPB-07




5. RAPD profile of pomegranate (*Punica grantum* L.) with primer OPA-07

L – Ladder 100 bp, A -G-137, B -T.C.Bhagwa, C -S. Bhagwa, D –Arakta, E – Mrudula, F-Ganesh, G-Rubby, H-Bhagwa.

**Plate 1 : RAPD pattern of different pomegranate varieties produced by OPB-15, 18, 07, OPA- 19, 07.**

grouped remaining two varieties *i.e.* S. Bhagwa and Bhagwa.

Jaccard's pair-wise similarity co-efficient values for eight genotypes were calculated and are presented in Table 2. The genetic similarities ranged from 0.32 to 0.72.The present finding is near about similar with result obtained by Sheidai *et al.* (2008) studied on pomegranate cultivars. The average genetic similarity among these 8 genotypes was 0.61 (Table 2). The highest similarity index value of 0.72 was found between

Ganesh and Mrudula (Table 2) and lowest similarity index value of 0.32 was found between S. Bhagwa and G-137 (Table 2).

**Conclusion :**

The result indicates the OPB-15 and OPA-07 primers were more informative as compared to other primers for pomegranate genotyping. The dendrogram constructed using molecular data generated by five RAPD primers showed higher similarity in between Mrudula and Ganesh while lowest similarity found in S Bhagava and G-137. RAPD markers were found to be highly polymorphic and can be utilized for genetic diversity analysis of pomegranate genotypes.

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