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# Genetic diversity analysis of indigenous pomegranate (*Punica granatum* L.) germplasm lines

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**Abstract**

Pomegranate (*Punica granatum* L.) is an ancient edible fruit crop well recognized for its immense nutraceutical and medicinal properties. Precise morphological characterization of wild, exotic and commercial (cultivar) varieties and understanding the molecular variability in perennial crops like pomegranate is essential for developing varietal DNA fingerprints, identifying true-to-type of genotypes, to avoid duplication and overcome the problem of clonal admixture. It has become a challenge to identify varieties based solely on observable phenotypic traits due to dynamic environmental factors that influence physical traits and increasing number of varieties. Hence, the implementation of stable molecular markers is the best complementary biotechnological tool, which fulfil the above requirements. In our first objective, we identified 24 polymorphic SSR markers out of 40. These SSRs screened on 13 different varieties of pomegranate. The PIC value ranged from 0.13 to 0.37 (mean 0.25) and heterozygosity percent ranged from 0.14% to 0.50% (mean 0.35%). Highest PIC value and heterozygosity observed in PgSSR78 marker. Similarity coefficient matrix showed the genetic distance between the twenty-four varieties, ranged from 0.58 to 0.99. Maximum similarity was observed between Alah and Khandari. Minimum similarity was found between IC318743 and 1185 followed by Maha and 1185. PgSSR16, PgSSR23, PgSSR49, PgSSR60, PgSSR32, PgSSR40, PgSSR78 markers were found with high PIC value and heterozygosity and these primers could be useful for pomegranate cultivar identification. From this study, microsatellite markers have proven to be very useful marker for developing varietal identification marker at early growth stage due to its high polymorphism, abundance, multi-allelic and co-dominant inheritance.

**Key words:** pomegranate; SSR; characterization; genetic; dendrogram; alleles; polymorphism

## INTRODUCTION

The pomegranate (*Punica granatum* L.) is the predominant member of Lythraceae family. It has two species *P. protopunica Balf.* and *P. granatum* L., of which *P. granatum* is the cultivated species for fruit production. The scientific name of Pomegranate is derived from words: Pomum (apple) and granatus (grainy) or seeded apple. It's a diploid (2n=2x-16) perennial shrub from the family Lythraceae (Nath & Randhawa, 1956; Smith, 1976). It is one of the oldest edible fruits known to mankind. It is a small shrub, growing best in semi-arid and mild-temperature to subtropical climates. It has been propagated by seeds, but vegetative propagation by cuttings is the most commercial method of propagation. Demand is on the rise, both at domestic level and in the export market. India is the only country able to deliver pomegranates around the year. In recent year's pomegranate has acquired central place

in the fruit basket of the semi-arid regions of India because of its versatile adaptability, high market demand, more returns and less water requirement. India is the world's largest producer of pomegranate covering 2.22 lakh ha area with an annual production of 26.34 lakh tons and productivity of 11.86 tons/ha. In spite of this, India exports only 1.79% of its total production due to biotic and abiotic stresses (Saroj & Kumar, 2019). Efforts to improve pomegranate through standard breeding practices have led to the development and release of few improved varieties in India (Jalikop et al., 2005).

The determination of novel genotypes based on their morphological characteristics can be achieved, yet their manifestation may be subject to the impact of environmental factors. Hence, with the advent of molecular markers the effect of environment can be reduced and selection can be more precise. Among the different marker systems co-dominant markers will help in extracting more information than dominant with respect to selection. Among different codominant markers short tandem repeats (SSRs) of simple (1–6) nucleotide motifs and their value for genetic analysis lies in their multi-allelism, codominant inheritance, they are relatively abundant, cover the entire genome, and are highly suitable for high throughput Polymerase Chain Reaction (PCR)-based platforms (Powel et al., 1996; Zietkiewicz et al., 1994). It was assumed that SSRs were primarily associated with noncoding DNA, but it has now become clear that they are also abundant in the single and low-copy fraction in coding regions (Morgante et al., 2002; Toth et al., 2000). The advantages of microsatellite markers for plant germplasm characterization relative to other PCR-based markers have been demonstrated in many fruit crop species and found to be better (Ergül et al., 2002; Sánchez-Pérez et al., 2005; Boz et al., 2011; Caliskan & Bayazit, 2012). Diverse SSR primer pairs have been published for pomegranate (Curró et al., 2010; Hasnaoui et al., 2010; Soriano et al., 2011). Due to the limitations of morphological markers, DNA markers are being used to evaluate germplasm diversity (Soriano et al., 2011). As DNA markers are independent of environmental conditions, are potentially unlimited in number, and can show a high level of polymorphism. Therefore, they are invaluable tools for determining genetic relationships, evaluating diversity, performing selection during plant breeding and genome mapping (Currò et al., 2010; Zaouay & Mars, 2011).

## MATERIAL AND METHODS

### Plant material

Plant material used in this study consists of 13 genotypes of pomegranate, which includes both cultivated and wild types (Table 1). All genotypes are being maintained at the field Gene Bank of ICAR-National Research Center on Pomegranate, Solapur, India.

**Table 1.** Details of 13 pomegranate genotypes with its features

| Sr.No. | Genotypes | Type | Origin | Fruit size | Fruit colour | Aril colour |
|---|---|---|---|---|---|---|
| 1 | 1252 | Wild | India, Uttarakhand. | Small | Yellow with pink | Pink |
| 2 | 1185 | Wild | India, Uttarakhand. | Small | Yellow with pink | Pink |
| 3 | Khandhari | Exotic | Afghanistan | Medium | Yellow with pink | Light Pink |
| 4 | IC 318706 | Wild | India (Himachal Pradesh) | Small | Yellow with pink | Pink |
| 5 | IC 318712 | Wild | India (Himachal Pradesh) | Small | Yellow with pink | Pink |
| 6 | IC 318705 | Wild | India (Himachal Pradesh) | Small | Yellow with pink | Pink |
| 7 | IC 318743 | Wild | India (Himachal Pradesh) | Small | Yellow with pink | Pink |
| 8 | Bhagawa | Cultivated | India (Maharashtra) | Medium | Red | Red |
| 9 | Mridula | Cultivated | India (Maharashtra) | Medium | Deep red | Dark red |
| 10 | Alah | Cultivated | Iran | Small | Pinkish | Red |
| 11 | Maha | Cultivated | Iran | Small | Pinkish | Red |
| 12 | Patna 5 | Cultivated | India (MPKV, Rahuri) | Medium | Yellow with pink | Light pink |
| 13 | 1180 | Wild | India, Uttarakhand. | Small | Yellow with pink | Pink |

**DNA Isolation**

The fresh leaf samples of all the genotypes were collected and subjected to DNA extraction using modified CTAB method as described by (Ravishankar et al., 2000). Quality and concentration of DNA was determined on 0.8 % agarose gel (Fig. 1). Final dilutions of 10 ng/µl were made for subsequent PCR reactions.

**PCR amplification and genotyping**

PCR amplification was carried out in 10µl reaction volume containing 1.0 µl (10 ng) of Template DNA, 0.7 µl each of forward and reverse primers (10 pmol) 4 µl of 2X *Taq* PCR mixture. The PCR was carried out using Prime-96™ (Himedia, India) thermal cycler, for 5 min at 94ºC, followed by 36 cycle at 94ºC for 1 min, 55ºC for 1 min at, 72ºC for 2min with final extension time of 7min at 72ºC. PCR products were separated on 3% agarose gel containing 0.5 µg/ml ethidium bromide and 1X TAE running buffer for 4h at 120 V, visualized and photographed in gel documentation system (Fig. 2). Twenty four SSR markers were used and listed in Table 2.

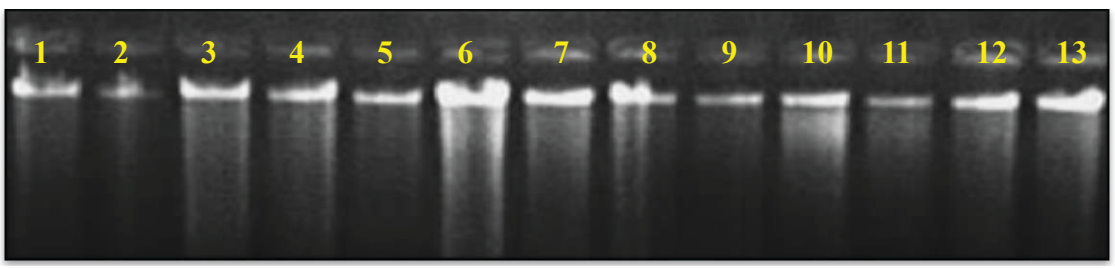


**Figure 1**. Gel image of 13 pomegranate DNA samples on 0.8% agarose gel

**Table 2.** Details of polymorphic SSR markers identified for diversity analysis

| Sr. No. | Name | Forward sequence | Reverse sequence |
|---|---|---|---|
| 1 | PgSSR 5 | ACTCTGATTGAGCCCTACTG | GACTACCTTACACACCTCTCTC |
| 2 | PgSSR 6 | ATTCAGCAGATTTTCAGGTC | GATGAGGTGTGAGTTTGATG |
| 3 | PgSSR 7 | TTTACTTTACCCTCTTCCCC | TAAACCAAAGCTACCAAGGA |
| 4 | PgSSR 10 | TCTCTCCCTTCCACAAAAG | GGGAGGTGCACAGGATATAGAA |
| 5 | PgSSR 11 | TCTCACACACACACGCAGAA | GAGAAAGAGGAAACCGCAGA |
| 6 | PgSSR 13 | GACGCCTTTAGTTTGCTCCA | CTCGGGACAGGACTTGAAT |
| 7 | PgSSR 14 | CCCCTAGTAAAGTCCCACCT | AGAGGTATTCGCAGGTTTTG |
| 8 | PgSSR 16 | TTCCTTTCGCTTTCACTCATC | CCCGATCATTTAAATCCACAAA |
| 9 | PgSSR 17 | GATGGCGAAGTGTGTCCTCT | TTGGGACTGTGTTCGACTGCT |
| 10 | PgSSR 19 | ATCTCTCATCTCTGCTTCCC | GCACACTTTCCTCCCTATGT |
| 11 | PgSSR 23 | AGTTGATCGACTGAGGAATG | CACTCGAGAAGCTCTGTG |
| 12 | PgSSR 26 | ATTTCGTGCTCTGTGCCTCT | GTGTTGGGAAGAACGGAAAA |
| 13 | PgSSR 32 | TGACACGGAACAGAGCTGAA | GGGGAAGAAACGAAGAAGAA |
| 14 | PgSSR 37 | CTAATGGCTTCCAGTGAAGT | TTTCACCGAAATTCCCAAAC |
| 15 | PgSSR 38 | CCTTCACCTCCCCACATAGA | TCGACCGGTTCATCTCTTTC |
| 16 | PgSSR 40 | CAACAGAACACCACCCACAC | CCCCCTGGAAGAAAATTGTA |
| 17 | PgSSR 49 | TAACAACCATGCCCCTTAAT | CCAATTAAAACGCCTCATCT |
| 18 | PgSSR 50 | AAACCCAGAAGAAGAACGAG | AAGAGAGAAACAGAGGAGGAAG |
| 19 | PgSSR 59 | TGCATCCTTCCCCTACTCTC | AGCTCATGTAATGCGTCGTG |
| 20 | PgSSR 60 | TACAGGCTACCACAGGTTGA | ATTGCCACCACATCACTG |
| 21 | PgSSR 63 | GTAGCCACTTTAGGGCGAGA | CGTCTAAAAGCGACAGCAAG |
| 22 | PgSSR 76 | TATCTGTCGCAGGAAGGATG | GAAGCCAATTCCTCAAAGATG |
| 23 | PgSSR 78 | GGTCTGACTGGACCGTTGC | GAGAACGAAGATCCCGGTTT |
| 24 | PgSSR 80 | GCCACCTCTGCAATTCTCTC | GCAAAGGTTAGGCTCCGAAT |

**Data analysis**

The scored data was subjected to molecular diversity analysis using NTSys software (Rohf, 2000). The similarity matrix was calculated by using NTSys. In that software, dendrogram clustering obtained by using the Unweighted Pair-Group with Arithmetic Average (UPGMA) method. The Polymorphism information content (PIC) and Heterozygosity (He) was calculated using PIC online calculator https://gene-calc.pl/pic.

The genetic distance was obtained by Unweighted Neighbour Joining (NJ) method based on similarity indices. Principal Component Analysis (PCA) plot was constructed to identify the major variance proportion to total proportion.

## RESULTS AND DISCUSSION

The molecular marker data was subjected to statistical analysis. As a result, a total of 64 alleles were found, with an average of 1.73 alleles per primer. The polymorphic information contents (PIC) values of 24 primers ranged from 0 to 0.37 (Table 3). The highest PIC value is 0.37 was exhibited by PgSSR 78 markers followed by PgSSR 32, PgSSR 60, PgSSR 49, PgSSR 23, PgSSR 78 and PgSSR 32, whereas lowest PIC value is 0.14 shown by PgSSR 14. Earlier studies on pomegranate diversity assessment re-

**Table 3.** The number of alleles (Na), the Polymorphic Information content (PIC) and heterozygosity value obtained by SSR markers

| Sr no. | Name of markers | No. of alleles (Na) | Heterozygosity (He) | PIC |
|---|---|---|---|---|
| 1 | PgSSR 5 | 2 | 0.32 | 0.26 |
| 2 | PgSSR 6 | 2 | 0.37 | 0.30 |
| 3 | PgSSR 7 | 2 | 0.34 | 0.28 |
| 4 | PgSSR 10 | 2 | 0.37 | 0.30 |
| 5 | PgSSR 11 | 2 | 0.24 | 0.21 |
| 6 | PgSSR 13 | 2 | 0.48 | 0.36 |
| 7 | PgSSR 14 | 2 | 0.14 | 0.13 |
| 8 | PgSSR 16 | 2 | 0.49 | 0.37 |
| 9 | PgSSR 17 | 2 | 0.16 | 0.15 |
| 10 | PgSSR 19 | 2 | 0.34 | 0.28 |
| 11 | PgSSR 23 | 2 | 0.49 | 0.37 |
| 12 | PgSSR 26 | 2 | 0.16 | 0.15 |
| 13 | PgSSR 32 | 2 | 0.49 | 0.37 |
| 14 | PgSSR 37 | 2 | 0.15 | 0.14 |
| 15 | PgSSR 38 | 2 | 0.32 | 0.26 |
| 16 | PgSSR 40 | 2 | 0.50 | 0.37 |
| 17 | PgSSR 49 | 2 | 0.49 | 0.37 |
| 18 | PgSSR 50 | 2 | 0.42 | 0.33 |
| 19 | PgSSR 59 | 2 | 0.16 | 0.15 |
| 20 | PgSSR 60 | 2 | 0.49 | 0.37 |
| 21 | PgSSR 73 | 2 | 0.44 | 0.34 |
| 22 | PgSSR 76 | 2 | 0.32 | 0.26 |
| 23 | PgSSR 78 | 2 | 0.49 | 0.37 |
| 24 | PgSSR 80 | 2 | 0.33 | 0.28 |
| | | **Average** | **0.35** | **0.28** |
| | | **min** | **0.14** | **0.13** |

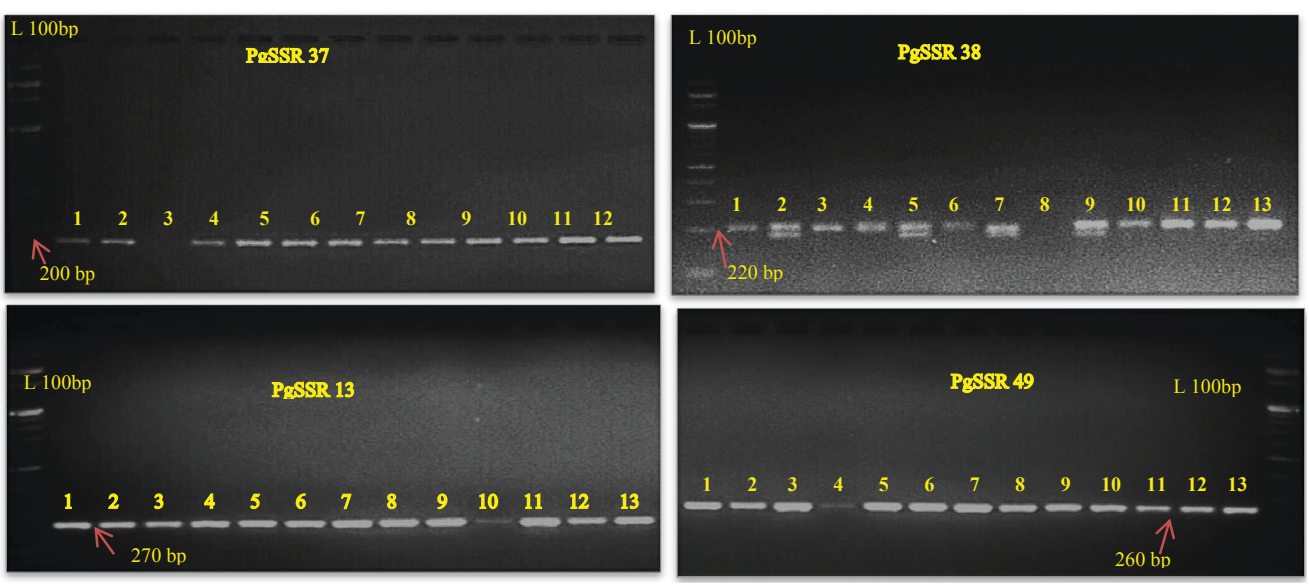


**Figure 2.** Amplification pattern of SSRs (PgSSR37, PgSSR38, PgSSR13 & PgSSR49) on 3% agarose gel (1-13: Pomegranate germplasm and L: 100bp DNA ladder)

ported comparable PIC values using SSR markers (Hasnaoui et al., 2010). The heterozygosity values ranged from 0 to 0.50. Likewise Patil et al. (2020) also reported heterozygosity in the range from 0.03 to 0.48. The value of PIC and He clearly indicated the diversity among the genotypes is very low due to existence of only two species in the *Punica* genus hence it clearly indicates lower diversity.

In order to reveal the relationship between varieties and genotypes cluster analysis was performed by using NTSys software opting UPGMA method. This program creates a dendrogram from a similarity matrix. The software calculates a similarity matrix into distance and makes a clustering using the UPGMA method. Cluster analysis between pomegranate genotypes revealed two main clusters (Fig. 3). The first main group consists of 4 sub clusters. The first sub cluster consists of 8 genotypes namely Maha, Patna 5, 1180, Khandari, 1252, IC 318706, Alah and IC 318705. The second sub cluster consisted of 3 genotypes were *i.e.* IC 318712, Bhagawa and Mridula revealing that cultivated types are evolved from its wild relatives. Second major cluster was consisting of 2 genotypes namely 1185 and IC 318743. It was observed that there was a close correlation between IC 318743 and 1185 belongs to wild types. In this study, wild and domestic pomegranate genotypes were divided into different clusters according to their geographical origins. Similar results were also observed in previous studies of pomegranate diversity by (Sarkhosh et al., 2006; Bedaf et al., 2003; Rahimi et al., 2006). It is known that some mutations and genetic changes that are easily recognizable phenotypically may be detectable by application of molecular markers (Bedaf et al., 2003).

The genetic distance between the 13 genotypes were calculated, which ranged from 0 to 1 whereas the lowest genetic distance recorded was 0.58 between IC 318743 and 1185 and 0.99 is highest genetic distance showed both are closely related between Alah and Khandari cultivated types. (Table.4). SSR markers would enable to distinguish the genotypes and help in advancing breeding procedures through marker assisted selection, variety protection, and conservation of the germplasm.

Principal Component Analysis (PCA) also performed to know the variance proportion as it is one

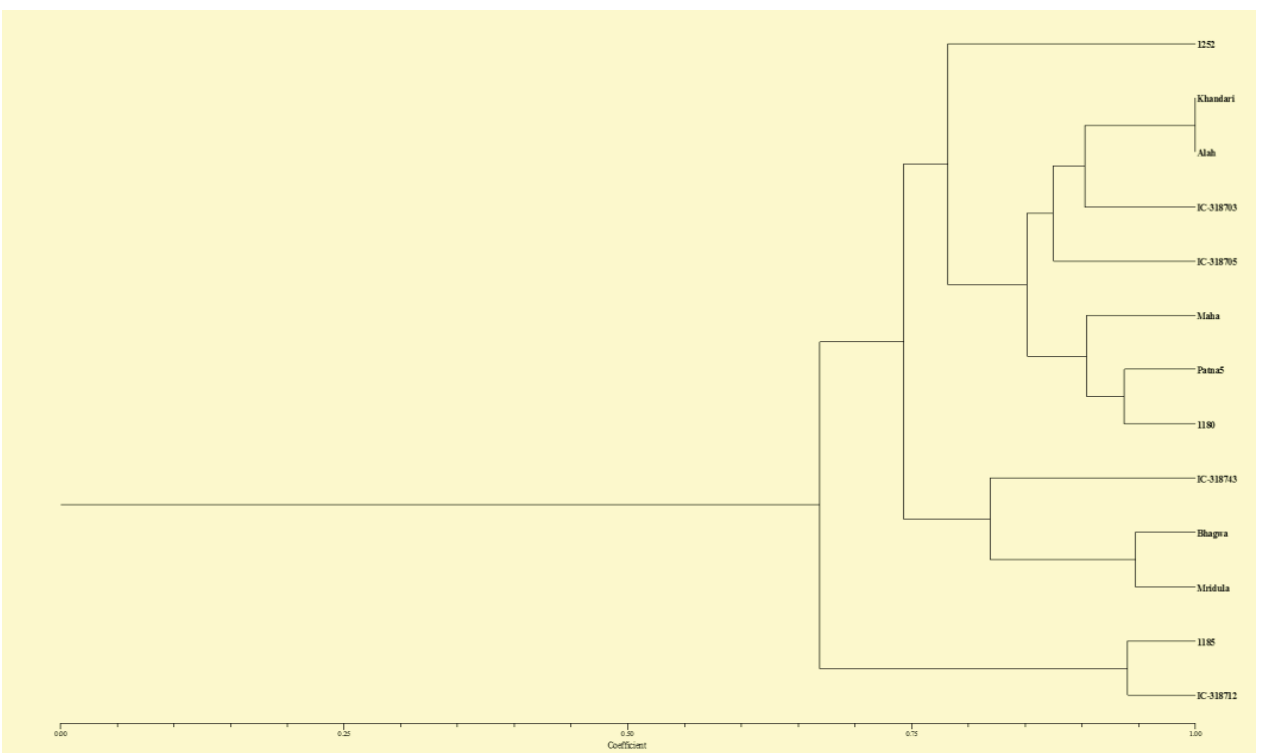


**Figure 3.** Dendrogram showing genetic relationship among 13 pomegranate genotypes based on UPGMA (Unweighted pair group method) method with SSR markers data

**Table 4.** Genetic distance among 13 pomegranate genotypes based on SSR markers

| | 1252 | 1185 | Khandari | IC 318706 | IC 318712 | IC 318705 | IC 318743 | Bhagawa | Mridula | Alah | Maha | Patna 5 | 1180 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1252** | 1 | | | | | | | | | | | | |
| **1185** | 0.69 | 1 | | | | | | | | | | | |
| **Khandari** | 0.96 | 0.66 | 1 | | | | | | | | | | |
| **IC 318706** | 0.8 | 0.625 | 0.92 | 1 | | | | | | | | | |
| **IC 318712** | 0.67 | 0.94 | 0.66 | 0.68 | 1 | | | | | | | | |
| **IC 318705** | 0.76 | 0.58 | 0.86 | 0.88 | 0.63 | 1 | | | | | | | |
| **IC 318743** | 0.65 | 0.67 | 0.59 | 0.66 | 0.69 | 0.71 | 1 | | | | | | |
| **Bhagawa** | 0.79 | 0.65 | 0.71 | 0.82 | 0.73 | 0.85 | 0.86 | 1 | | | | | |
| **Mridula** | 0.67 | 0.75 | 0.67 | 0.74 | 0.83 | 0.74 | 0.78 | 0.95 | 1 | | | | |
| **Alah** | 0.79 | 0.67 | 0.99 | 0.89 | 0.71 | 0.89 | 0.69 | 0.9 | 0.79 | 1 | | | |
| **Maha** | 0.74 | 0.59 | 0.79 | 0.89 | 0.63 | 0.85 | 0.73 | 0.89 | 0.80 | 0.90 | 1 | | |
| **Patna 5** | 0.74 | 0.64 | 0.89 | 0.93 | 0.69 | 0.87 | 0.67 | 0.83 | 0.78 | 0.93 | 0.91 | 1 | |
| **1180** | 0.67 | 0.63 | 0.76 | 0.81 | 0.67 | 0.79 | 0.68 | 0.75 | 0.72 | 0.81 | 0.9 | 0.93 | 1 |

of the most useful techniques for the data reduction. In this study, the first two PCA components are able to explain the total variance. PCA1 explained 0.609% and PCA2 0.391% variation. Hence, the two components are sufficient to explain the maximum variance than other components (Table 5 & 6). On the other hand, Biplot analysis describes two-dimension representation of variables in the graph. The biplot analysis depicted variance proportion with respect to all the genotypes variation in the biplot (Fig. 4) indicating the variance effects between the genotypes.

Compared to previous works, the results from this experiment showed better polymorphism among pomegranate genotypes. The polymorphic microsatellites presented here, function as efficient genetic markers, and will assist in pomegranate genotype identification and assessment of genetic diversity.

**Table 5.** Eigen value of principal component analysis

| Sr. No. | Eigen-value | Per cent (%) | Cumulative | Expected |
|---|---|---|---|---|
| **1** | 10.26 | 78.9 | 78.9 | 24.46 |
| **2** | 0.97 | 7.5 | 86.39 | 16.77 |
| **3** | 0.64 | 4.93 | 91.32 | 12.92 |
| **4** | 0.42 | 3.24 | 94.56 | 10.36 |
| **5** | 0.26 | 2 | 96.56 | 8.44 |
| **6** | 0.18 | 1.42 | 97.98 | 6.9 |
| **7** | 0.13 | 1 | 98.98 | 5.62 |
| **8** | 0.11 | 0.88 | 99.85 | 4.52 |
| **9** | 0.08 | 0.6 | > 100% | 3.56 |
| **10** | 0.04 | 0.34 | > 100% | 2.7 |
| **11** | 0.02 | 0.17 | > 100% | 1.93 |
| **12** | -0.01 | -0.04 | > 100% | 1.23 |
| **13** | -0.12 | -0.93 | 100 | 0.59 |

**Table 6.** Proportion variance components of PCA

| Component | Variance | Proportion | Cumulative proportion |
|---|---|---|---|
| 1 | 6.696 | 0.609 | 0.609 |
| 2 | 4.304 | 0.391 | 1.000 |

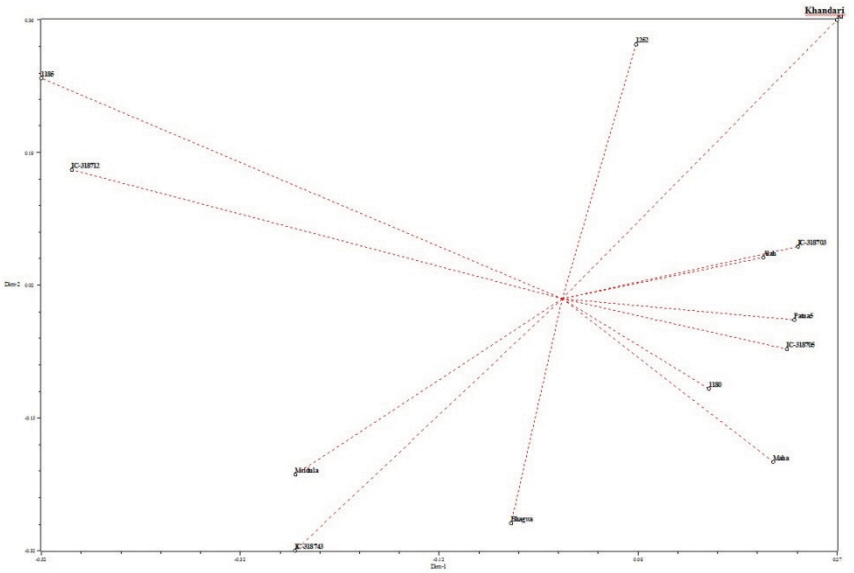


**Figure 4.** Principal Component Analysis (PCA) of 13 pomegranate genotypes using 24 SSR markers

## CONCLUSION

The genetic distance between the genotypes indicated diversity level between the lines. Hence one can identify the diversified lines based on their genetic dissimilarity. The group of markers with high PIC can be used in future selection of lines i.e. marker assisted selection can be done by using those markers. This study shows that SSR markers can be used to successfully detect genetic diversity in pomegranate. The use of SSR markers provided an early detection method to select and screen out plants even at an early stage of development.

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## REFERENCES

**Baddaf, T, Sharifi, M. & Bahar, N. B**. (2003). Analysis of genetic diversity in pomegranate cultivars of Iran, using Random Amplified Polymorphic DNA (RAPD) markers. In: *Proceedings of the Third National Congress of Biotechnology,* Iran. 2, 343–345.

**Boz, Y., Bakir, M., Çelikkol, B., Kazan, P., Yilmaz, K., Çakir, F. & Özer, S**. (2011). Genetic characterization of grape (*Vitis vinifera* L.) germplasm from Southeast Anatolia by SSR markers. *Vitis*, 50, 99–106.

**Caliskan, O., & Bayazit, S.** (2012). Phytochemical and antioxidant attributes of autochthonous Turkish pomegranates. *Scientia Horticulturae,* 147, 81–88.

**Currò, S., Caruso, M., Distefano, G., Gentile, A. & La Malfa, S.** (2010). New microsatellite loci for pomegranate, *Punica granatum* (Lythraceae). *American Journal of Botany,* 97(7), 58-60.

**Ergül, A., Marasali, B. & Agaoglu, Y. S.** (2002). Molecular discrimination and identification of some Turkish grape cultivars (*Vitis vinifera* L.) by RAPD markers. *Vitis,* 41, 159–160.

**Hasnaoui, N., Buonamici, A., Sebastiani, F., Mars, M., Trifi, M., & Vendramin, G. G.** (2010). Development and characterization of SSR markers for pomegranate (*Punica granatum* L.) using an enriched library. *Conservation Genetics Resources*, 2, 283–285.

**Jalikop, S. H., Rawal, R. D. & Kumar, R.** (2005). Exploitation of sub-temperate pomegranate Daru in breeding tropical varieties. *Acta Horticulturae*, 696, 107–112.

**Morgante, M., Hanafey, M. & Powell, W.** (2002). Microsatellites are preferentially associated with nonrepetitive DNA in plant genomes. *Nature Genetics,* 30(2), 194–200.

**Nath, N., & Randhawa, G. S.** (1959). Classification and description of some varieties of *Punica granatum* L. *Indian Journal of Horticulture,* 16, 189–201.

**Patil, P. G., Jamma, S. M., Singh, N. V., Bohra, A., Parashuram, S., Injal, A. S., Gargade, V. A., Chakranarayan, M. G., Salutgi, U. D., Dhinesh Babu, K. & Sharma, J.** (2020). Assessment of genetic diversity and population structure in pomegranate (*Punica grana-*

*tum* L.) using hypervariable SSR markers. *Physiology and Molecular Biology of Plants*, 26, 1249-1261.

**Powel, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S. & Rafalski, A.** (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding,* 2(3), 225–238.

**Rahimi, T., Sayed Tabatabaei, B. E., Sharifnabi, B. & Ghobadi, C.** (2006). Genetic relationships between Iranian pomegranate (*Punica granatum* L.) cultivars, using Amplified Fragment Length Polymorphism (AFLP) marker. *Iranian Journal of Agricultural Sciences*, 36, 1373–1379.

**Ravishankar, K. V., Anand, L. & Dinesh, M. R.** (2000) Assessment of genetic relatedness among mango cultivars of India using RAPD markers, *The Journal of Horticultural Science and Biotechnology,* 75:2, 198-201,

**Rohlf, F. J.** (2000). NTSYS-PC, Numerical taxonomy and multivariate analysis system - Version 2.1. New York, Applied Biostatistic Inc., 44 p.

**Sánchez-Pérez, R., Ruiz, D., Dicenta, F., Egea, J. & Martínez-Gómez, P.** (2005). Application of Simple Sequence Repeat (SSR) markers in apricot breeding: molecular characterization, protection, and genetic relationships. *Scientia Horticulturae*, 103(3), 305-315.

**Sarkhosh, A., Zamani, Z., Fatahi, R. & Ebadi, A.** (2006). RAPD markers reveal polymorphism among some Iranian pomegranate (*Punica granatum* L.) genotypes. *Scientia Horticulturae*, 111(1), 24–29.

**Saroj, P. L. & Kumar, R.** (2019). Recent advances in pomegranate production in India-a review. *Annals of Horticulture,* 12(1), 1-10.

**Smith, P. M.** (1976). Minor crops. In: Simmonds NW (Ed) Evolution of Crop Plants, Longman, London, pp 301-324.

**Soriano, J. M., Zuriaga, E., Rubio, P., Llácer, G., Infante, R. & Badenes, M. L.** (2011). Development and characterization of microsatellite markers in pomegranate (*Punica granatum* L.). *Molecular Breeding*, 27(1), 119–128.

**Toth, G., Gáspári, Z. & Jurka, J.** (2000). Microsatellites in different eukaryotic genomes: survey and analysis. *Genome Research*, 10(7), 967–981.

**Zaouay, F. & Mars, M.** (2011). Diversity among Tunisian pomegranate (*Punica granatum* L.) cultivars as assessed by pomological and chemical traits. *International Journal of Fruit Science*, 11(2), 151–166.

**Zietkiewicz, E., Rafalski, A. & Labuda, D.** (1994). Genome fingerprinting by Simple Sequence Repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics,* 20(2), 176–183.

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