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Molecular characterization of pomegranate cultivars with RAPD markers

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ABSTRACT

Molecular characterization was successfully applied to distinguish four popular pomegranate cultivars *viz*; Ganesh, G-137, Mrudulla and Phule Bhagwa in Maharashtra State. RAPD analysis was carried out using 12 oligonucleotide random primers generated 156 amplicons of which 29 were monomorphic (19%) and the rest 127 were polymorphic (81%). All the primers produced typical banding profiles for each of the cultivar suggesting the usefulness of the technique in DNA fingerprinting and cultivar identification. The genetic distance between these four cultivars based on RAPD data was estimated as 1.243, which is high in comparison to the morpho-agronomical difference, suggesting a broad genetic base of the crop.

Key words: RAPD, Pomegranate, Molecular Characterization

Pomegranate (*Punica granatum* L.) is one of the ancient fruit, favourite table fruits of tropical, subtropical region of the world and having therapeutic and keeping quality. Large variability of fruit and plant characteristics has been noticed in *punica* germplasm due to cross pollination, seed propagation and heterozygous nature of the crop. Usually the maintenance and evaluation of the germplasm is based on phenotypic feature such as morphological, physiological or horticultural descriptions. However, description have limited value as the plants grown at different locations; as only specific developmental stages are suitable for screening and detection of hybridization and pedigree determination.

Even though the pomegranate cultivars exhibit distinct horticultural feature. It is cumbersome to delineate then under different agro climatic conditions based on the phenotype. In contrast DNA fingerprinting techniques are quick and accurately revealed the genetic difference among the varieties/cultivars without being influenced by environmental factors. This approach also provides significant advantage in discrimination, reliability, timeliness at reduced cost (Smith, 1997; Koller *et al.*, 1993). DNA fingerprinting studies with RAPD markers have already been conducted for identification of cultivars in many crop plants including fruit plant (Harradda, 1993; Nybom, 1989; Wold and Peter, 1993), ornamentals (Tzuri, 1991; Fukuoka *et al.*, 1992) and cereals (Tinker, 1993; Dewos and Gale, 1992, William *et al.*, 1990). RAPD is an excellent tool to characterize the germplasm, study phylogenetic relationships and gene tagging. Such precise and refined techniques appear to have not been utilized so far the genus *punica* and hence the present study has been attempted. Such technique is useful for claiming Intellectual Property Right (IPR) by the pomegranate breeder.

MATERIALS AND METHODS

Pomegranate cultivars viz; Ganesh, G-137, Mrudulla and Phule Bhagwa were obtained from the germplasm maintained at horticultural department,MPKV,Rahuri and present investigation was carried out at Plant Molecular Biology Laboratory, Biotechnology Centre, MPKV,Rahuri. 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*)*. The genomic DNA was quantified on 0.8% agarose gel and diluted to a uniform concentration of 25 ng/ul for RAPD analysis.PCR reactions were performed according to the protocol of Williams *et al*. (1990). The PCR amplification was carried out in 0.2 ml/tube in TECHNE Genius PCR, 35ul reaction volume containing 0.5ul Taq Polymerase,0.5ul 10mM each dATPs,dTTPs, dCTPs and dGTPs,0.2ul MgCl2, 2ul primer(Genetix) and 2.0 ul template DNA(50ng) and 26.5ul sterile water. The chemicals for cocktail are from Bangalore Genei Pvt. Ltd only.

Twelve random primers (Table1) from Operon Technologies Ines, Alameda,USA were used in the study. Amplification reactions were carried out by following cycle profile: 1 cycle at 92 ^{o}C for 2 min.,34 ^{o}C for 1 min.,72 ^{o}C