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# Assessment of genetic diversity of grape mutants based on RAPD and SSR markers

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## ABSTRACT

**The present investigation was undertaken to assess the genetic diversity in gamma rays irradiated mutants of four grape genotypes based on RAPD and SSR markers. There were 36 mutants selected from in vitro mutated four grape genotypes, namely Pusa Navrang, H-76-1, Pearl of Csaba and Julesky Muscat, based on morpho-physiological and biochemical traits. These mutants and non-irradiated individuals were subjected to RAPD and SSR marker analysis to detect the genetic variation amongst them. Six RAPD and eleven SSR primers were employed for PCR amplification, and 63 distinct and easily scoreable bands were generated, ranging between 110 (SSR) and 2500 (RAPD) bp. All the bands were found to be polymorphic and identified 13 solid mutants among the initially selected 36 putative mutants. The distribution of mutants by cluster analysis suggested the existence of a high degree of difference at the genetic level in the mutants and non-irradiated control individuals. The mutants were primarily grouped into the four major clusters based on the doses of gamma irradiation, except for some mutants from Julesky Muscat and Pearl of Csaba genotypes. A minimum similarity coefficient (0.37) was noted in mutants of Pusa Navrang and a maximum (0.70) in Hybrid-76-1. Unique banding patterns were amplified by both markers (RAPD and SSR). It was suggested that Julesky Muscat was amplified uniquely by RAPD-OPA01, OPP02, SSR-VVMD14, VMCIB11, VMC8G9 primers, H-76-1 by RAPD- OPA01 and SSR-VVMD 14 primers, Pusa Navrang by RAPD-OPP02 and SSR-VVMD-21 primers and Pearl of Csaba by RAPD-OPA01, OPP04, SSR-VMC8G9 primers. The result from this study suggests that OPA01 (RAPD) and VVMD14 (SSR) were the most informative primers and generated maximum numbers of reproducible bands. The combination of these primers will be beneficial for identifying solid mutants which could be evaluated in the field.**

**Keywords:** Genetic diversity, gamma rays, grape mutants, RAPD and SSR markers.

## INTRODUCTION

Grapevine (*Vitis vinifera*) is grown worldwide for a variety of purposes including fresh fruit, juice, jams, jellies, wine, raisins and other processed products. India is in the top ten grape producing countries with an area of 0.139 million hectares and 2.92 million MT production. Although in India, grape productivity is high 21.0 tonnes per hectare (Anonymous, 2). However, its cultivation faces many biotic and abiotic problems *i.e.* Downy mildew, powdery mildew, anthracnose, bacterial leaf spot, black rot, mealybugs, flea beetle, grape cane girdler *etc*. Its hybridization with resistant species has been the only method available to produce resistant genotypes. However, due to polymorphic and heterozygous nature as well as an excessively heavy load of detrimental recessive alleles, it exhibits pronounced inbreeding depression or sterility during traditional hybridization and breeding become tedious and time-consuming operation in *Vitis* (Olmo, 13). Although, the creation of genetic variation at the DNA level through mutagenesis is a very important tool to ameliorate one or two economical traits in an already well-adapted cultivar.

Induced mutagenesis through the gamma rays can produce good numbers of heritable mutants possibly due to its high penetrating capacity in plant tissue (Jain, 4). In mutation breeding identification of new mutant lines at an early growth stage is very advantageous. Genetic markers are can be used as an effective and more reliable selection tool for identification of desired traits in breeding. Marker-assisted selection increases the selection efficiency by allowing for early selection and reducing the mutants for field evaluation. In such a situation, *in vitro* mutagenesis coupled with the characterization of putative mutants using morphological traits and molecular marker techniques would prove to be a practical alternative. Traditionally, identification has

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been based on morphological traits but new technique called random amplified polymorphic DNA (RAPD) and modification thereof, has become a useful tool in the disciplines of Genetics, Taxonomy, and Plant breeding since their inception a few years ago. These new techniques based on DNA information have made quicker and precise identification of the new genotypes. The SSRs are molecular markers characterized by their highly polymorphic nature, abundance in the genome, reproducibility and simple to use. These are ideal genetic markers for detecting differences between and within species of eukaryotes (Kumar *et al.*, 6). These markers are the most potent for screening the genotypes for genetic variation because they are polymorphic, co-dominant, high reproducible, widely distributed throughout the genomes, amenable to automation, shows high levels of genetic variation in the number of tandemly repeating units at a locus and required low quantities of template DNA (Kumar *et al.*, 6). The PCR based technique; RAPD and SSR (Simple sequence repeat) are two very popular and widely used for characterization and identification of genetic variation in plant germplasm. In the present investigation, RAPD and SSR markers were employed to detect the genetic polymorphism in the gamma rays induced mutants in four grape genotypes.

## MATERIALS & METHODS

The *in vitro* developed plantlets of four grape genotypes *viz*. Pusa Navrang, H-76-1, Pearl of Casba and Julesky Muscat were used as plant material for exposing to five levels of gamma rays irradiation treatments (0, 5, 15, 20, and 25 Gy). The irradiation treatment was done at the Nuclear Research Laboratory, ICAR-IARI, Pusa, New Delhi with $^{60}$Co source and the dose rate of 7030 Gy/h. Before actual irradiation treatment, micropropagation protocol using single-node segments (1.5 to 2 cm long) was used for culture initiation (Singh *et al.*, 15). Cultures were initiated on MS medium containing growth regulators BAP (2 mg $l^{-1}$) + NAA (0.2 mg $l^{-1}$). Well proliferated cultures of these genotypes were raised on standardized proliferation cum rooting medium (MS basal medium + 2.0 mg $l^{-1}$ IBA + 250 mg $l^{-1}$ activated charcoal). Thereafter, the irradiated cultures were aseptically excised into two-node micro-cuttings and sub-cultured to raise a vM1 generation. After complete growth of the sub-cultured micro-cuttings, the vines were again excised into the two-node micro-cuttings and sub-cultured. The irradiated cultures were multiplied and maintained up to the *vM5* generations. The physical induced mutants of each generation (*vM*1-5) were hardened in the pots filled with cocopeat + vermiculite + perlite (2:1:1) in the plastic pots with polyethene cover and transferred to glasshouse for their further screening and observations on the growth characteristics.

Molecular marker-based characterization of *in vitro* mutated grape plantlets was performed at Division of Genomic Resources, National Bureau of Plant Genetic Resources, New Delhi. Total genomic DNA was extracted from young fresh leaves by cetyltrimethyl ammonium bromide (CTAB) method (Lodhi, 8) with minor modifications. Thereafter, DNA purification was carried out using 1μl RNase and sodium acetate (3M). The DNA concentration for each sample was checked using VersaFluor™ Fluorometer (BIO-RAD, USA). The isolated DNA was diluted in TE buffer having a concentration up to 20 - 30 ng and kept under the -20°C refrigerator for further use. For DNA quantification, the isolated DNA was run in 0.8% agarose gel. Electrophoresis was carried out in 1x TAE buffer at 100 V for 1 hour, *i.e.,* till the bromophenol blue dye travelled less than 2/3rd of the gel length. The gel visualized under UV light on a UV-trans illuminator and photographed by using Polaroid Gelcam and Digital DC-40. The extract was incubated with 1 µl of RNase A (per 100 µl DNA) solution at 37 ºC for 1 h, in order to remove contaminating RNA. Part of each DNA sample was diluted with sterilized double-distilled water to yield a working concentration of 10-20 ng/µl. PCR conditions were standardized earlier for the grape genotype characterization. Twenty-six oligonucleotides (10-mer) (Sigma-Aldrich Chem., St. Louis, USA) were selected for screening.. These primers were then dissolved in double distilled water and used as a single primer for PCR. The primer was provided by the manufacturer in a lyophilized form. Based on the molecular weight of a given primer, a stock solution of 100 µM was prepared by adding the required amount of TE buffer and the stock was stored at -20°C. PCR amplification was carried out with 50 ng of genomic DNA, 2 µl $MgCl_2$, 1 U Taq DNA polymerase, 10x PCR buffer without $MgCl_2$, 0.2 nmol of each of primers (Operon Technologies Inc.) and 10 mM of dNTPs. The volume was made up to 25 µl with sterile distilled water. PCR tubes containing the above components were capped and centrifuged at 10,000 rpm for 2 minutes to allow proper settling of the reaction mixture. Amplification was performed in three different types of PCR machines, *viz*., Perkin Elmer GeneAmp PCR System 2400®, Biometra® and Flexigene® thermocycler. The different PCR machines were used to check the consistency of the results. According to the following programme: denaturation at 94°C for 6 min; then 40 cycles of denaturation at 94°C for 1 min; primer annealing at 37°C for 2 min, and primer extension at 72°C for 2 min.; final extension step at 72°C for 10 min, the PCR was

conducted. Amplified DNA fragments were separated on 1.4% agarose gel stained with ethidium bromide (10 mg/ml). Wells were loaded with 25 µl of reaction volume of the amplified PCR product and 5 µl loading buffer (sucrose and bromocresol green dye) together. Electrophoresis was conducted at 50 - 75 V for 4 - 5 h and the gel photographed under UV light using a gel dock system (Herolab laboratories, USA). Molecular sizes of the amplification product were estimated using marker λ DNA digested with EcoR-I and Hind-III run along with the amplified PCR product. SSR primers are synthesized from Imperial Bio-Medical (P) Ltd., Chandigarh, India (Table 1). The 11 microsatellite markers used for the study were earlier reported by several workers in grape (Khawale *et al.*, 5; Lal *et al.*, 7; Nagaty and Assal, 9). Extracted grape genomic DNA was PCR-amplified using 8 pair primers flanking SSR sequences previously cloned and sequenced in grape. The Eleven EST-derived SSRs also screened to amplify grape DNA. PCR was carried out in Perkin Elmer 9,600 Thermocycler (USA) using the standard protocol. PCR amplification was performed using the following programme: denaturation at 95°C for 5 min.; 35 cycles at 94°C for 1:30 min., 1 min. at 56.7-62.1°C and 1:30 min. at 72°C for with a final 7 min. extension at 72°C. For SSR analysis, the primer annealing temperature was standardized for each SSR primers with the help of gradient PCR. The annealing temperature ranges from 53.3 to 65.5°C.

The PCR products were loaded onto a 2% agarose gel, stained with ethidium bromide (0.05%) and visualized on a UV transilluminator. PCR amplified products were run onto a 4% high-resolution agarose (Metaphor™) gel, 2.5 µl loading dye was added to each PCR tube containing amplified products and loaded in the slots of the metaphor agarose gel. The 2.5 µl GeneRulerTM (100 bp ladder) was loaded in the first lane of each gel to determine sizes of identified bands. Electrophoresis was carried out at 10 V/cm for 2.5 h. The gels were on a UV transilluminator using a polaroid camera. Each band was treated as one marker. Scoring of bands was done from the photographs. Homology of bands was based on their migration distance in the gel. The presence of a band was scored as "1", the absence of a band as "0" and missing datum was denoted by

**Table 1.** Details of SSR primers sequence.

| Primer No. | SSR primer | Primer sequence (5' → 3') |
|---|---|---|
| 1 | VVMD-5F | CTAGAGCTACGCCAATCCAA |
| | VVMD-5R | TATACCAAAAATCATATTCCTAAA |
| 2 | VVMD-6F | ATCTCTAACCCTAAAACCAT |
| | VVMD-6R | CTGTGCTAAGACGAAGAAGA |
| 3 | VVMD-7F | AGAGTTGCGGAGAACAGGAT |
| | VVMD-7R | CGAACCTTCACACGCTTGAT |
| 4 | VVMD-14F | CATGAAAAAATCAACATAAAAGGGC |
| | VVMD-14R | TTGTTACCCAAACACTTCACTAATGC |
| 5 | VVMD-21F | GGTTGTCTATGGAGTTGATGTTGC |
| | VVMD-21R | GCTTCAGTAAAAAGGGATTGCG |
| 6 | VVMD-24F | GTGGATGATGGAGTAGTCACGC |
| | VVMD-24R | GATTTTAGGTTCATGTTGGTGAAGG |
| 7 | VVMD-25F | TTCCGTTAAAGCAAAAGAAAAAGG |
| | VVMD-25R | TTGGATTTGAAATTTATTGAGGGG |
| 8 | VVMD-27F | GTACCAGATCTGAATACATCCGTAAGT |
| | VVMD-27R | ACGGGTATAGAGCAAACGGTGT |
| 9 | VMC1A2F | TAAAATGTAGGGCGGCCACC |
| | VMC1A2R | AACATAAATGGCCACCAGGG |
| 10 | VMC1B11F | CTTTGAAAATTCCTTCCGGGTT |
| | VMC1B11R | TATTCAAAGCCACCCGTTCTCT |
| 11 | VMC8G9F | AACATTATCAACAACATGGTTTTA |
| | VMC8G9R | ATATTCATCCTTCCCATCACTA |

"g". Similarity index was applied to determine the mutagenic change in the treated grape explants. Cluster analysis was carried out using the SHAN module in NTSYS pc 2.21 software (Rohlf, 12). An unweighted pair group method of arithmetic mean (UPGMA) dendrogram was generated from Jaccard's similarity values individually for RAPD, SSR (data not shown) and the pooled data.

## RESULTS AND DISCUSSION

The total 36 putative mutants were developed in four genotypes *i.e.* Pusa Navrang and Pearl of Csaba, H-76-1 and Julesky Muscat through gamma irradiation treatment. Thence, developed putative mutants were characterized using the polymorphic RAPD and SSR primers for confirmation of solid mutants. On the basis of molecular characterization with RAPD and SSR markers, the 13 solid mutants were selected from initially morphologically characterized 36 mutants of 4 grape genotypes. The 23 mutants which showed similar banding pattern with parent genotype were discarded from the population. The 26 RAPD primers were screened for polymorphism and of which six primers were able to identify solid mutants and selected for the further screening of the *in vitro* developed mutant population. The RAPD primers revealed a total of 45 clear and easily scorable bands. The size of the bands that were produced in the PCR reactions ranged from 250-2000 bp, but most of the bands were between 300 and 2,000 bp. The primers were able to amplify the genomic DNA giving both monomorphic and polymorphic bands. The level of polymorphism was different with different primers among different cultivars and mutants. Maximum numbers (11) of polymorphic bands were produced by primer OPA 01, whereas, minimum (02) by OPA 02 in the four parents and their 13 mutants population. Unique bands were amplified with OPA 01, OPA 02 and OPP 04 in all the identified mutants of four grape genotypes. The size of the SSR amplification products ranged from the 110 to 290 bp. The primers, which showed the polymorphism among them and with the respective parent genotype were analysed further. Initially, 11 SSR primers were used for screening out of which six SSR were selected for further analysis for gamma irradiation mutagens. The SSR primers were able to generate 18 distinct and clearly scorable bands. Among SSR primers, VVMD 14 primer was most informative which was able to detect variation between all parents and their mutant population.

The results obtained from the pooled analysis of RAPD and SSR markers are given in the Table 2. Six RAPD and 6 SSR primers were selected for detecting genetic variation due to gamma irradiation in the putative mutants. The total 63 distinct and easily scorable bands were generated. The ranges of bands were varied from the 110 to 2500 bp. All the bands were found to be polymorphic in nature. Each individual RAPD marker did not amplify in all the mutants. However, the generated RAPD markers showed the presence or absence of band(s) in the mutant population, which was dependent on the genotype and the mutagen dose, while SSR marker showed variation due to the mutation in the form of the difference of change in band distance.

Earlier ten potential mutants were suspected on the basis of morphological markers in the Pusa Navrang genotype from the *υM5* population of the different doses. When the *υM5* generation of this mutant was subjected to RAPD and SSR markers analysis, four mutants were confirmed and found genetically dissimilar to the mother plant. The overall mutation efficiency was 40%. Maximum two solid mutants were detected with $LD_{50}$ dose (10 Gy). Out of sixteen RAPD and eleven SSR markers screened for mutant population, four RAPD and six SSR markers were found suitable in differentiating

**Table 2.** Comparative data obtained by the RAPD, SSR and pooled data analysis of mutants and non-irradiated grape individuals.

| Particulars | RAPD | SSR | Pooled analysis |
|---|---|---|---|
| No. of primers used | 06 | 11 | 18 |
| Total no. of bands obtained | 45 | 18 | 63 |
| Av. bands/ marker | 7.5 | 1.6 | 3.5 |
| Band size range (bp) | 250-2500 | 110-290 | 110-2500 |
| Unique primer | | | |
| Pusa Navrang | OPP02 | VVMD-21 | |
| H-76-1 | OPA01, OPP04 | VVMD-14, VVMD-21 | |
| Pearl of Csaba | OPP04 | VMC8G9 | |
| Julesky Muscat | OPA01,OPP02 | VVMD-14, VMC8G9, VMC1B11 | |

the *in vitro* gamma irradiated mutant population of Pusa Navrang in which two primers OPP02 (RAPD) and VVMD-21 (SSR) differentiated uniquely (Fig. 1-2). The genetic similarity values and dendrogram developed clearly showed that all the mutants developed were genetically distinct from the mother plant. Basically, three major clusters were generated in which similarity diversity ranged from 48 - 63%. The highest similarity coefficient (0.52) with respect to the mother plant was noted in the PN-P4.c vegetative mutants. While the lowest similarity coefficient compared to the mother plant (0.37) was noted in the PN-P2.a mutant followed by PN-P1.b and PN-P3.b. Mutant identification in H-76-1 was initially, based on morpho-physiological and biochemical parameters resulted nine putative mutants. Of which three

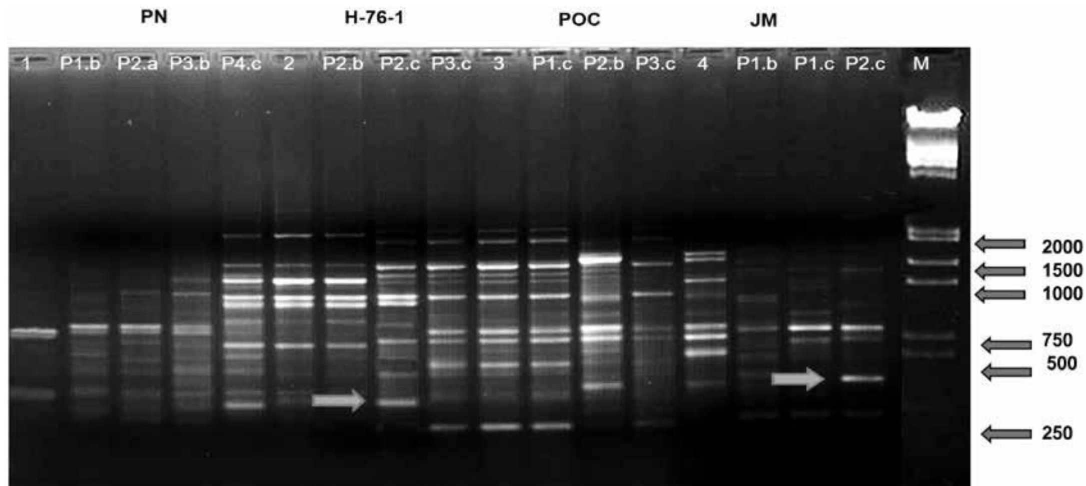


**Fig. 1.** RAPD patterns of mother plant and *in vitro* raised grape plantlets with primer (A) OPA01. *Note:* Lane number 1,2,3,4 corresponds to the control (field-grown mother plant); lane 2-5, 7-9, 11-13 represents *in vitro* raised mutated genotype; lane M- is the DNA size marker (Lambda DNA digest with/*Eco*R I/*Hin*d III); PN: Pusa Navrang; H-76-1: Hybrid-76-1; PoC: Pearl of Csaba and JM: Julesky Muscat; A: 5 Gy; B: 10 Gy; C: 15 Gy. Arrow indicates solid mutants.

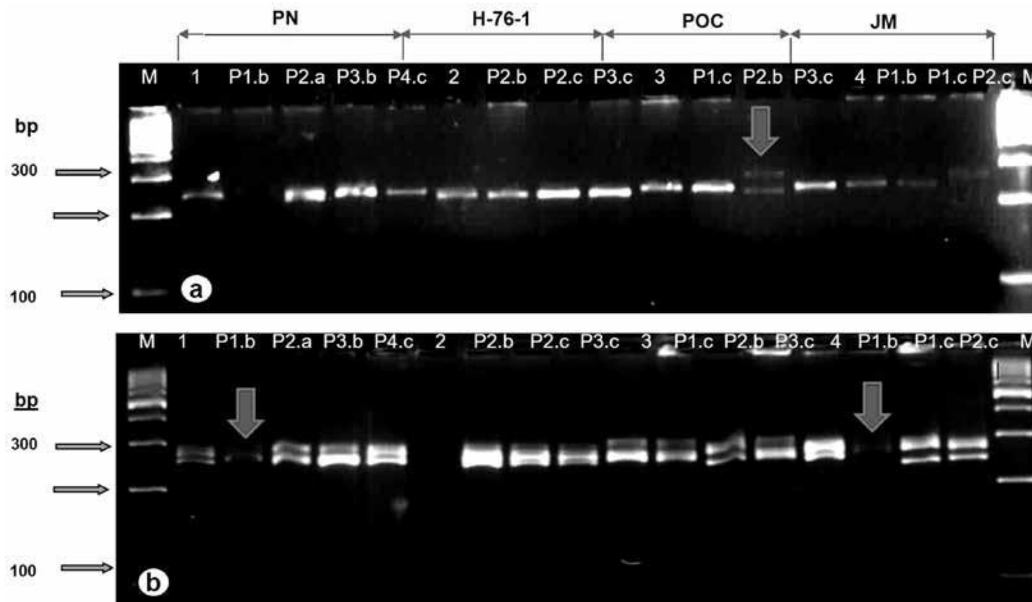


**Fig. 2.** SSR patterns of mother plant and *in vitro* raised grape plantlets with primer (a) P11 and (b) P5 *Note:* Lane number 1,6,10,14 corresponds to the control (field-grown mother plant); lane 2-5, 7-9, 11-13 represents *in vitro* raised mutated genotype ; lane M- is the DNA size marker (Lambda DNA digest with/*Eco*R I/*Hin*d III); PN: Pusa Navrang; H-76-1: Hybrid-76-1; POC: Pearl of Csaba and JM: Julesky Muscat, A:5 Gy, B: 10Gy, C:15 Gy.

solid mutants were confirmed with the help of four RAPD and six SSR primers. The primers including OPA01, OPP04 (RAPD) and VVMD-14 (SSR) were generated well-defined segments (Fig. 1-2). The $LD_{50}$ dose (10 Gy) of gamma rays induced maximum (2) solid mutants followed by 15 Gy dose. While, plotting the dendrogram based on mutagenic dose and disimilarity of the mutants to parent, the mutant H-P3.a developed by the 15 Gy was found to be most dissimilar. Dendrogram also suggests that all the mutants were genetically diverse from the control (Fig. 3). The nine potential mutants were initially screened in Pearl of Csaba but after molecular analysis, three solid mutants were recognized by three RAPD and six SSR markers giving the mutagenic efficiency of 33.33%. Out of three mutants, two most dissimilar (similarity coefficient 0.21) mutants were identified with the 15 Gy (PoC-P3.a and PoC-P1.a), which were distinct to the parent genotype and other mutants (PoC-P2.b) having 0.54 similarity coefficient to the mother plant. These mutants (PoC-P3.a and PoC-P1.a) of same dose (15 Gy) were grouped together in the separate group. For characterization of PoC genotype, OPA01 (RAPD) and VMC8G9 (SSR) found to be unique primers which yielded distinct band (Fig. 1-2). Primer OPA01, OPP02 (RAPD), VVMD14, VMC8G9 and VMC1B11 (SSR) are the unique primer gave unique band for Julesky Muscat (Fig. 1-2). These markers confirmed three mutants in Julesky Muscat among the eight putative mutants. Six RAPD and six SSR markers were identified to discriminate the mutant population and Julesky Muscat parent genotype. The identified mutants were found to be the most distinct from the mother plant. The lowest similarity coefficient was noted in JM-P1.a and JM-P2.a (0.38), to the mother plant. Although, these two mutants were found most dissimilar to mother plant they were more similar to each other (0.46). It was also evident from the cluster analysis; mutant JM-P1.b was more similar to a mutant of Julesky Muscat (0.52) than its mother plant (0.44). based on the overall observation, the maximum RAPD and SSR markers were identified in the genotype Julesky Muscat (11) followed by Pusa Navrang (10) to discriminate the gamma rays induced mutants from the parent genotype. Whereas, the minimum number of RAPD and SSR markers (9) were able to discriminate the mutants and parent from each other in Pearl of Casba genotype. Concurrent results were reported by Rodrigues *et al*. (11) while, characterizing the gamma rays induced genetic variability in fig using molecular markers. They confirmed the genetic variation through RAPD and AFLP molecular markers. Additionally, the plant which is identical to their parents could be easily identified through RAPD markers (Alizadeh and Singh, 1; Nagaty and Assal, 9). The genetic alterations produced by ionizing radiation due to ionization and excitations of the DNA molecule which can be revealed using molecular markers. SSR markers are the ideal genetic markers

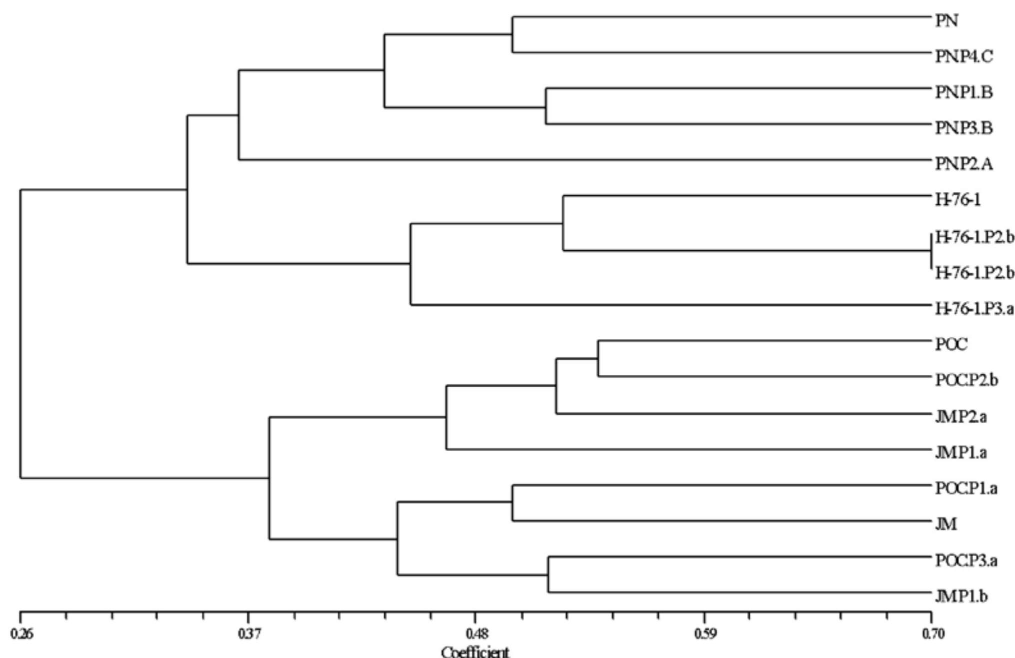


**Fig. 3.** Jaccard similarity coefficient based on pooled data (PN: Pusa Navrang; H-76-1: Hybrid-76-1; PoC: Pearl of Csaba and JM: Julesky Muscat).

for detecting differences between and within species of all Eukaryotes (Kumar *et al.*, 6). Similar results were reported by Khawale *et al.* (5) where variation created through gamma irradiation and the developed mutants could effectively be identified by RAPD technique. Similarly, Yang and Schmidt (17) used the RAPD analysis to differentiate mutants developed by X-rays treated cherry leaf plants. RAPD pattern obtained with the primer OPP02 in gamma-ray induced mutants in the Pusa Navrang and H-76-1 indicate the addition and deletion of bands due to irradiation dose respectively. Similarly, the RAPD pattern was developed with OPP01 primers in Pearl of Csaba and Julesky Muscat genotype, due to gamma irradiation treatment (Fig. 1). Primer VMC8G9 (P11) showed unique SSR banding pattern in cultivars Pearl of Csaba and Julesky Muscat mutants (Fig. 2). A similar pattern was also noticed in primer VVMD-21 (P5) in Pusa Navrang and H-76-1 genotype and their mutants (Fig. 2). The various past studies made concurrent report on SSR markers (Lal *et al.*, 7; Singh *et al.*, 13; Singh and Singh, 14). They reported that SSR analysis can be used for analysis of genetic relationship among the grape cultivars. In general, the RAPD and ISSR technique allowed discriminating phenotypically similar grape cultivars as well as confirm the true to type genotype (Alizadeh and Singh, 1). RAPD assay performed for the identification of fourteen *in vivo* and one *in vitro* lemon mutants in comparison with a known zygotic origin genotype (Deng *et al.*, 3).

Efforts were made to remove the escape mutants, which were genetically similar to the parental plants thus resulting in the reduction of the mutant population. The Genetic similarity values were developed from the identified markers using the program NTSYSpc 2.1 for Windows 7. Furthermore, the similarity matrix was subjected to the cluster analysis by unweighted pair group method for the arithmetic mean (UPGMA) and a dendrogram generated based on the pooled data (Fig. 3). The generated dendrogram showed the genetic dissimilarity among mutants and its mother plant. The Jaccard's similarity coefficient varied between 0.26 and 0.70 among non-treated and gamma rays treated plants. Based on pooled data analysis, individuals were grouped into 4 major clusters. Overall clustering patterns of individuals divulge that the grouping of mutants was largely based on the doses of gamma irradiation except for some Julsky Muscat and Pearl of Csaba mutants. The results *in vitro* developed mutant population are presented as follows. This indicated that, high dose of gamma irradiation was responsible for creating more genetic variation among these genotypes. The dendrogram clearly illustrating clustering of amplification pattern of *in vitro* gamma rays induced grape mutants based on RAPD and SSR results. It gave clear picture of solid mutants and their relationships with their parent genotypes. The genetic similarity coefficient decreased as irradiation doses increased as compared to their respective no irradiated mother plants. Similarly, high genetic variation in between non-treated and gamma rays induced mutants were reported by Dice's genetic similarity coefficient in *Curcuma alismatifolia* (Taheri, 16). Gamma irradiation assisted mutants were found more dissimilar with each other and also to their respective parent genotypes. RAPD, which can quickly detect a large number of genetic polymorphism, have led to the creation of genetic maps, to detect mutations and DNA damage in a number of fruit crops (Yang and Schmidt, 17).

The results of the present study indicated that use of DNA markers (RAPD and SSR) can effectively be employed for identifying the closely related grape mutants. In total, six RAPD and SSR markers were identified to discriminate the thirty-six mutants with their respective parents. Gamma irradiation developed thirteen solid mutants, maximum, of which the maximum mutants were detected in Pusa Navrang (4) followed by in the H-76-1 (3) and Julesky Muscat (3). The highest number RAPD and SSR markers were identified in the Julesky Muscat (11) followed by Pusa Navrang (10) to discriminate the gamma rays induced mutants. Whereas, the lowest (9) RAPD and SSR markers were found to discriminate the mutants and parent with each other in Pearl of Casba genotype. Combination of RAPD and SSR markers is the most effective strategy to detect the genetic variation created in grape genotypes through irradiation. Using these DNA markers potential solid mutants can be easily identified which would be further evaluated.

## AUTHORS' CONTRIBUTION

Conceptualization of research (Singh, S.K. and Dev, R.); Designing of the experiments (Dev, R., Singh, S.K., Singh, A.K. and Patel, V.B.); Contribution of experimental materials (Montha, K. and Alizadeh, M.); Execution of field/lab experiments and data collection (Dev, R., Singh, S.K., Rakesh, K. and Kumar, K.); Analysis of data and interpretation (Dev, R., Singh, S.K.); Preparation of the manuscript (Dev, R., Singh, S.K. and Montha, K. and Alizadeh, M.).

## DECLARATION

The authors declare no conflict of interest.

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