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EFFECT OF INDUCED MUTATION FOR VARIETAL IMPROVEMENT IN SOME LOCAL GRAPEVINE CULTIVARS

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ABSTRACT

Grape is important crop with high nutritional value, however there is a great need for improving the quality of the crop. Cultures were maintained *in vitro* on MS medium supplemented with 2.5 mg/L BAP+ TDZ 3.0 mg/ L. The effect of different doses of gamma irradiation as well as chemical mutagenesis by Sodium azide was studied. It was noted that doses up to 5 Gy helped to increase plant height from irradiated nodes. At 5 Gy, there was an increase of 4.7, 5 and 4.5 cm in plant height in Desi, Sundar Khani and Chinese varieties of grapes respectively. On further increase in concentration from 6 to 10 Gy, there was a gradual decrease in plant height. Sodium azide was selected to induce mutations in the nodal explants. Nodal explants of the varieties were subjected to different sodium azide concentrations ranging from 0.1 to 0.5%. It was noted that the height of plantlet decreased with an increase in concentration of sodium azide In explants treated with 0.2% sodium azide the plant height was 4 cm which increased to 4.2cm when the explants were treated with 0.3% sodium azide. Further increase in concentration not only caused decreases in length but also led to complete necrosis. In the present study, DNA analysis by RAPD markers was used to analyze mutagenesis. The RAPD analyses indicated that the plantlets subjected to gamma radiation had a great genetic diversity as compared to the control.

Key words: Grapevine, Induced mutation, varietal improvement

INTRODUCTION

Grape is one of the oldest and best vital perennial crops in the world, which has about 1000 species, assemblage in seventeen genera Arroyo *et al*. (2006). It is effectively cultivated only in stable and temperate climate regions with plenty rain, warm and dry summers and comparatively mild winters (Vivier and Pretorius, 2000). Grapes are very nutritious and contains 288 kJ (69 kcal) of energy per 100 g -dry or fresh produce. Chemical composition of grapes is highly complex containing different compounds but water is present in plentiful quantity, along with different type of sugars (glucose and fructose), acids (tartaric and malic), amino acids, proteins, phenolics, anthocynanics and flavonols (Zhang *et al*. 2005). Hence, it is widely used in the production of table and wine grapes, raisins, and juices. It is available in both fresh and dried forms (Sajid *et al*. 2003). About 71% of world grape production is used for wine, 27% as fresh fruit, and 2% as dried fruit (Patrice *et al*. 2006). Diseases like heart attack and cancer are foiled by grapes, it also aids to fortify bones and sustain immunity. Grapes contain special kind of acid named ellagic acid; that has an ability to block the production of enzyme that cancer cell needs to grow (Ren *et al*. 2000). All parts of grapes are widely used in herbal medications. In the past, grapes as a diet ingredient have been given to the cancer patients as an alternative means of treatment. Grapes extract contains proanthocyanidins and its skin has resveratrol, both are in use for possible prevention and treatment of cancer and other illness (Athar *et al*. 2007). Among animals, it provide shield to the heart of rat from ischaemia, amplifies nitric oxide synthesis, provide relaxation in supplying blood to the heart and inhibits the factor expression in vascular cells (Sato *et al*. 2000).

Variability among organisms is due to mutations. Mutation causes an important and desired improvement to plant breeding (Breider*et al*. 1956). Tissue culture techniques, when shared a mutual relationship with a mutagenesis treatment, pace up the breeding plan (Novak, 1991).

The exploit of physical mutagens, like X-rays, gamma rays and neutrons and chemical mutagens for inducing variation is well recognized and has been used in agriculture. These mutagens unbind the chromosomes and can cause modifications at the cellular level (Giosanu *et al*. 1993).These induced mutations aid to develop many agronomic important traits such as shorter growing period, suitable for rotation, increased tolerance and resistance to biotic and a biotic stress (Oudat, 1990, Kuksova *et al*. 1997, Maluszynski *et al*. 2000).

Chemical mutagenesis is another means to cause mutations in plants in order to improve their agronomic traits. Sodium azide (NaN_3) is a chemical mutagen and has been one of the most powerful mutagens in crop plants. Being a strong mutagen in plant, it affects the different parts of the plants and their growth developmental phenomena by disturbing the metabolic

activities. It maintains resistance and improves yield and quality (Salim *et al*. 2009).

RAPD is suitable to perform with good polymorphism and can be used in examining genetic diversity and the relation between species at molecular level (Lanying *et al*. 2008, Arya *et al*. 2010). It consists of fragments having 10 nucleotide in length which are amplified through PCR of random segments of genomic DNA with one primer of random nucleotide sequence. RAPDs have been used consistently as molecular markers for classification of different grapes cultivars (Buscher *et al*. 1993).

Keeping in view the importance of grapevine, the present work highlights the effect of induced mutagenesis in three grapevine cultivars. The effect of different doses of gamma irradiation as well as chemical mutagenesis by Sodium azide was studied in term of various growth parameters. The comparison of mutants with the control plants was made by using RAPD analysis.

MATERIALS AND METHODS

Collection of plant material: The plant material, *Vitis vinifera* L. of the three selected grape varieties Desi, Sundar Khani and Chinese grapes (named as V1, V2 and V3 during the present study) was collected from the Botanical garden of Lahore College for Women University.

Preparation and Inoculation of explants: The explants of about 1cm were cut from young branch of grapevine containing the nodal portion without any leaf. These explants were surface sterilized by rinsing with tap water for three times to remove dirt particles. Then surface sterilized by washing with detergent and soaked in a chemical sterilizing agent i.e sodium hypochlorite (5%) for 15 minutes. The explants were then rinsed with autoclaved water and cultured on Murashige and Skoog's (1962) medium supplemented with 2.5 mg/L BAP+ TDZ 3.0 mg/ L

Treatment with gamma Radiation: Gamma radiation was given to the cultures at PARAS using cobalt 60 as a source of gamma rays. Explants were treated with various doses of gamma radiation ranging from 1 to 10 Gy. For each treatment, experiments were carried out with 10 replicates. The data were recorded for plant height, number of nodes and number of leaves at day 30.

Treatment with sodium azide: For chemical mutagenesis, a chemical mutagen sodium azide was used. After sterilization, nodal explants of grapes were treated with different concentrations (0.1, 0.2, 0.3, 0.4 and 0.5%) of sodium azide. Various time durations (1, 3, 6, and 24 hours) of sodium azide treatment were also optimized. Continuous shaking was applied during each treatment. The explants were then inoculated in MS medium supplemented with 1mg/L BAP.

For each treatment experiments were carried out with 10 replicates. The data were recorded for plant height, number of nodes and number of leaves at day 30.

RAPD Analysis: The plants regenerated after physical and chemical mutagenesis was compared using RAPD analysis. DNA was extracted following a CTAB-based method and then was amplified using 15 RAPD primers designed for grapes. The PCR reactions were accomplished in 134.5 µl reaction mixture containing 78.0 µl distilled water, 6.0 µl Buffer (which buffer, for example TE, TBE, etc) without $MgCl_2$, 21.6 µl Buffer with $MgCl_2$, 14.4µl dNTPs, 2.4µl Taq polymerase and primer along with DNA sample was carried out in thermal cycler for 36 cycles. PCR products were visualized on 0.8% agarose gel. The resulting bands were analyzed using DNA man software (version 5.2.2).

For RAPD analysis, genomic DNA from *in vitro*, and induced variants were extracted from all the three varieties.

Total of 15 random primers were used to amplify PCR products and to define genomic and hereditary resemblances and likeness.

Following 9 samples were compared with RAPD marker using 15 primers:

- DNA samples from *in vitro* (V_1,V_2,V_3) grown cultivars.
- DNA sample from plants of three grape varieties subjected to 0.2% concentration of sodium azide. (S_1,S_2, S_3)
- DNA sample from plants of three varieties subjected to 5Gy dose of Gamma radiation. (G_1,G_2, G_3)

RESULTS AND DISCUSSION

When nodal explants were treated with different doses of gamma radiation (1- 10 Gy) and cultured on MS medium supplemented with 2.5 mg/L BAP+ TDZ 3.0 mg/ L, regeneration potential of explants was observed in varying levels.

Effect of gamma irradiation on nodal explants: The explants (nodes) were irradiated with different levels of gamma radiation (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 Gy). After irradiation, the cultures were shifted to the growth room to examine the effect of gamma radiation on the growth of explant. It was observed that explant response showed variation in terms of plant height with reference to the control (3.1cm). Fig. 1 indicates that with a rise in radiation up to 5 Gy increase in plant height and number of shoot was observed. At 1 Gy, 3.2 cm height was recorded in V_1 while 3.5 and 3 cm was observed in V_2 and V_3.

Up to 5 Gy, height of plantlet was increased with gradual pace and 4.7, 5 and 4.5cm long shoots were observed when 5 Gy dose of gamma radiation was given to V_1, V_2 and V_3 respectively.

It was also observed that with an increase in radiation dose above 5 Gy a decrease in plant height was recorded for all the three grape varieties used during the present study. From 6 Gy, reduction in height was observed 1.5 cm in V_1 while 1.8 and 1.5 in V_2 and V_3 respectively. The same trend was recorded for the higher doses up to 10 Gy (Fig 4)

Charbaji and Nabulsi (1999) also recorded the effect of low doses (0, 2, 5 and 7 Gy) of gamma rays on grapes. The dose of 7 Gy enhanced shoot length while 5 Gy augmented root length. In fig plants Ferreira *et al*. (2009) also found the dose of gamma radiation as an important factor. These researchers found that 30 Gy was an optimum dose for Fig plants because root formation was repressed in doses larger than 30 Gy. In another study Gawad *et al*. (2011) suggested that 5 Gray proved to be a promoter for survival of plantlets; however growth rate was inhibited with 30 Gray.

Effect of different concentrations of sodium azide on shoot tips: After treatment with sodium azide, shoot tips had differential response on all the varieties with reference to the concentration of mutagen used. It was found that when nodal explants were treated with different concentrations of sodium azide, a variation in response was recorded for different concentrations. At 0.1% sodium azide concentration, greater callus mass was obtained in V_1, V_2 and V_3, respectively. However, in explants treated with 0.1% no shoot formation was recorded. While all the varieties produced callus and became swollen from their lower portions. The weight of callus increased from control (Fig 5).

At 0.2% less whitish mass was obtained and the plant showed leaf induction along with swollen from the bottom. Furthermore multiple leaf formation was recorded at this concentration. At 0.3% shoot length was greater at day 25 as compared to 0.2% sodium azide treated explants but with less number of leaves. When explants were treated with 0.4% sodium azide less shoot length with mean value of 1, 1.3 and 0.9 in V_1, V_2 and V_3 respectively was recorded. By elevating the concentration of mutagen shoot length was stunned with decrease height (Table 1).

Plant height was elevated at the 0.3% in all three varieties which was decreased to less shoot length by further decreasing the sodium azide concentration. V_2 was at apex followed by V_1 and V_3 respectively (Fig 6) while no. of nodes decreased as compared to control but here after 0.2% concentration showed highest number of nodes i.e 2, 4 and 3 in V_1, V_2 and V_3 respectively. (Fig 7) whereas V_2 also exceeded with five number of leaves; however number of leaves decreased with increasing sodium azide concentration. (Fig 8).Hence, 0.2 and 0.3% sodium azide treated explants were found to give better response regarding shoot formation.

The results of the present work indicated that by increase in concentration of mutagen beyond a certain limit not only length decreases but also complete necrosis was observed. Mensah and Akomeah, (1992) have reported an increase the mutagenic dose lower the survival percentage of the explants in cowpea. Hence present results of the study regarding this aspect are in agreement with such earlier reports. However lower concentrations resulted an increase in plant height and number of nodes. This chemical mutagen has already been reported for its effectiveness by a number of researchers (Kleinhofs and Sander, 1975, Mashenkov, 1986) who described the role of sodium azide in improving genetic variability in higher plants because it is the major feature to get successful breeding plans. Hence it can be utilized successfully to generate genetic variability in plants (Ricardo and Ando, 1998).

Identification of polymorphic bands: Initially, 15 RAPD primers were screened among 9 selected samples (Fig 9) and were used to reveal the genetic diversity. It was found that Primer 11 resulted in the highest number of bands (10) and clearly separated all the mutants among there varieties. Primer 6 yielded best results among all the primers and all the samples showed amplification. Karatas and Agaoglu (2010) also illustrated that maximum number of polymorphic bands was shown by OPA-18, OPO-07 and P-123 (Fig 10).

Table 1 shows total number of primers with total number of bands generated by each primer in all the genome of grapes samples. It also explicits sequences of primers, polymorphic bands, size range of generated fragments along with polymorphic percentage in the band profile of each primer. Maximum number of loci produced was 10 in primer 11, having range of band size 600-1600bp with 6 polymorphic bands showing 60% polymorphism, whereas minimum number of loci was produced by primer 4 and 10, with range of band size 300-1530bp and 550-900bp respectively. The rate of polymorphism was 80% with primer 10 while 60% with primer 4.

It is also evident from Table 1 that maximum polymorphic band percentage was 83.3 with primer 12 and minimum polymorphic band percentage was responded by primer 6. It is also clear from the Table 1 that the highest band size range was 300-1550bp which was observed in primer 12 while minimum range of band size 300-900bp was obtained in primer 9. The maximum number of bands (53) was recorded with V_3. Comparison of different grape varieties using RAPD markers has been reported by many workers. In one such study Maia *et al*. (2009) found that highest number of fragments was observed in OPP-08.

Multiple sequence alignment by DNAMAN software: Data presented in Figure 10 explicates the multiple alignment of sequence produced by 0-1 matrix through gel scoring. Software alignment multiple sequences with a percentage of 76.94 similarity index in the DNA of nine grape varieties. With the help of scoring method DNAMAN helps to align similar sequences were highlighted with different colours. Each lane in the figure shows its similarity index with digit value at its end.

Phylogenetic tree: Phylogenetic tree was made by DNAMAN software between 9 grape samples in the present study (Fig 11).

In the present study DNAMAN software clustered 9 grape samples into 2 major groups (I and II). The first single group (I) ontained three clones V_1, V_2, V_3 having the sequence weights 0.02, 0.02 and 0.013, respectively

The second group (II) was further divided into 5 subgroups IIA, IIB, IIC, IID, IIE, and IIF IIA contains two clones V4 and V_6 of sequence weights 0.119 and 0.318 respectively.

IIB has single clone of V_9 (sqwt: 0.182) while IIC, IID, IIE and IIF contains single clone of such as V_8, V_7, V_5 respectively.

Genetic distance matrix among the grape samples: A number of workers have used RAPD markers to study genetic variability (Lanying*et al*. 2008, Arya*et al*. 2010). Genetic distance between 9 selected grape samples used during the present study was evaluated by the use of DNAMAN software that supports the whole sequence and then created distance matrix. Table 2 shows genetic distance among all grape samples with maximum genetic distance of 0.370 in G_1 whereas minimum genetic distance 0.151 was found in V_3.

Table 2 also explains that V_3 and G_1 are genetically much dissimilar as compared to other grape samples studied in present report. Much similar and less diverse clones were V_3 and G_2. While G_3 and G_1 also show similarity genetic matrix.

Hence our results are in line with the previous reports that RAPD markers are a useful marker in studying the genetic diversity (Kim *et al.* 2002).

Fig 1. Shoot formation in nodal explants treated with 5 Gy dose of gamma radiation at day 15 for a) V1 b) V2 c)

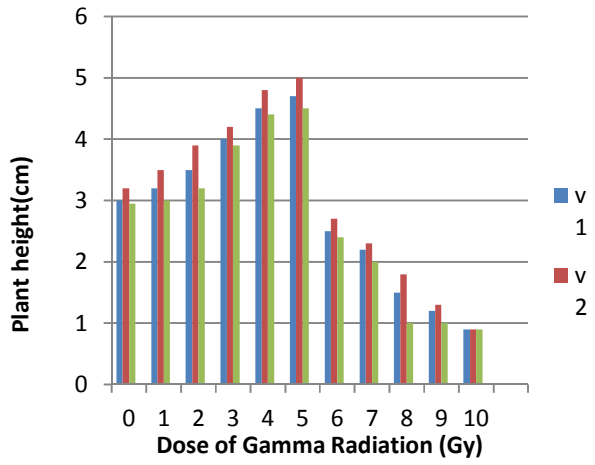

Fig 2. Effect of gamma radiations on plant height of the selected grapes cultivars (V1-V3)

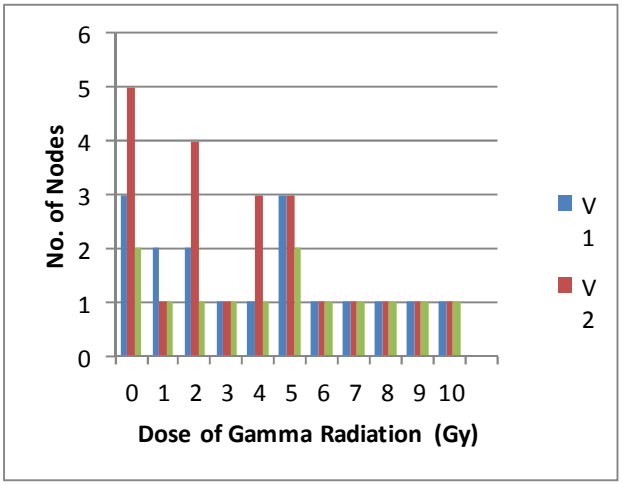

Fig 3: Effect of gamma radiation on numbe of nodes of the selected grapes cultivars (V1-V3)

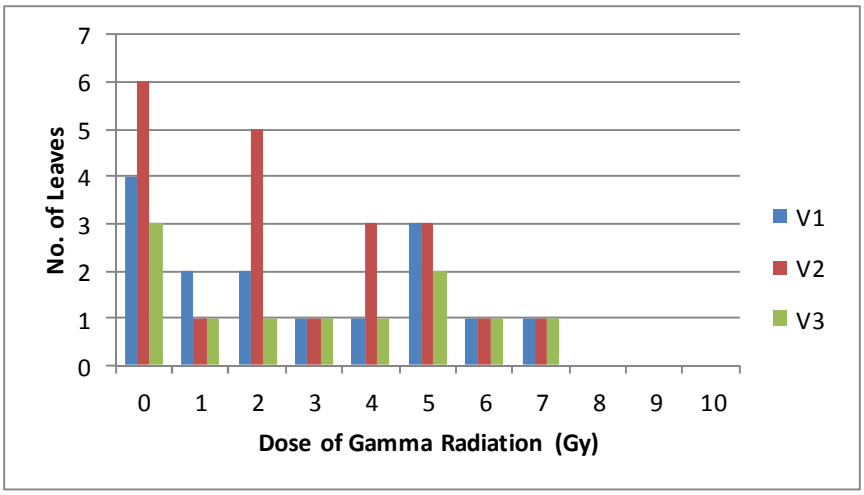

Fig 4. Effect of Gamma radiation on No. of Leaves of the selected grapes cultivars (V1-V3)

a b C

Fig 5. Nodal explants treated with 0.1% sodium azide for a) V_1; b) V_2; c) V_3, respectively

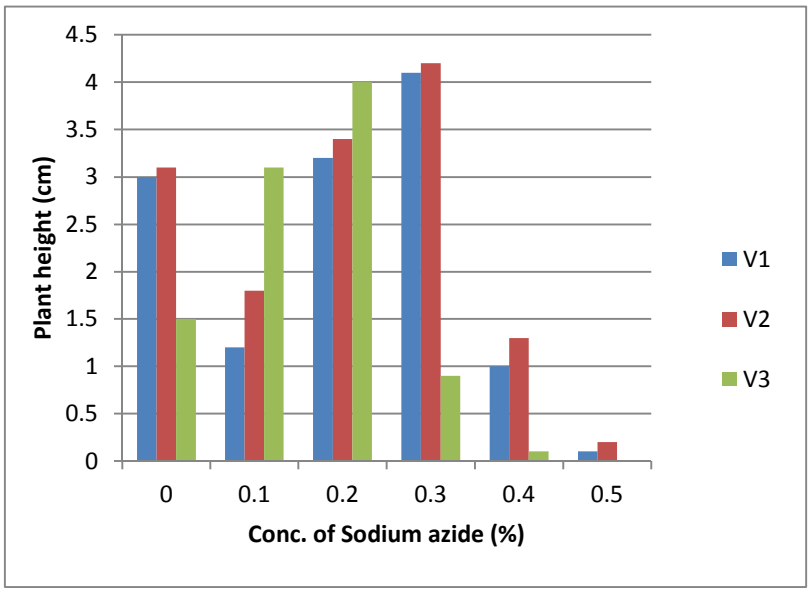

Fig 6.Effect of Sodium azide on plant height of the selected grapes cultivars (V1-V3)

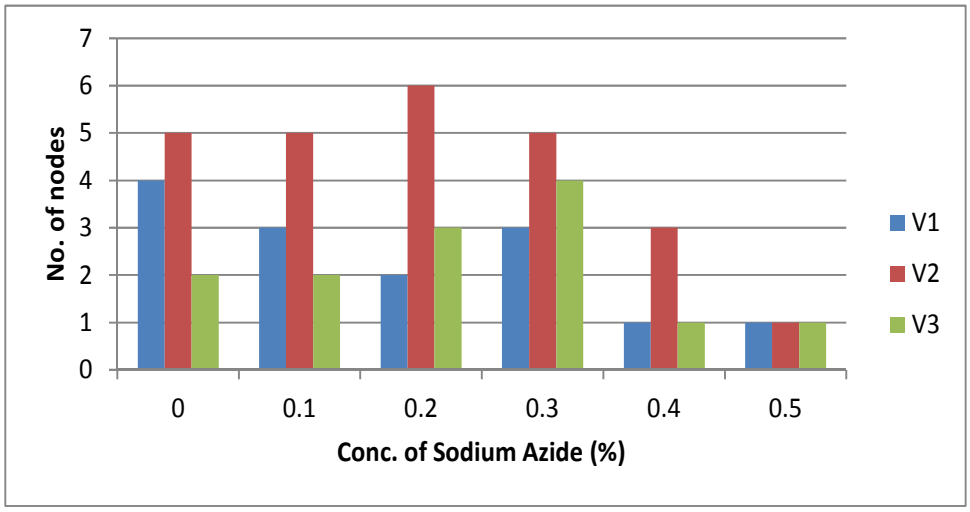

Fig 7. Effect of Sodium azide on No. of nodes of the selected grapes cultivars (V1-V3)

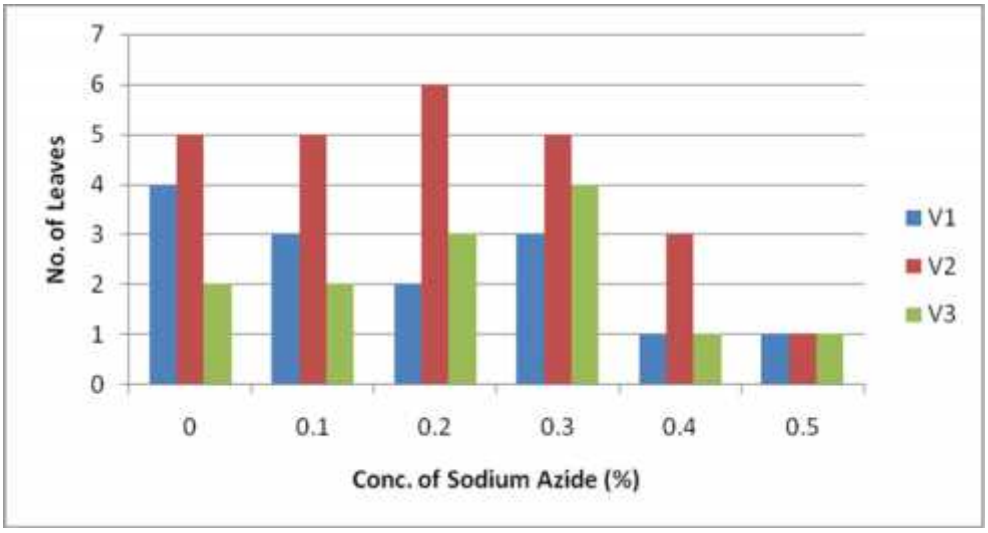

Fig 8. Effect of Sodium azide on No. of leaves of the selected grapes cultivars (V1-V3)

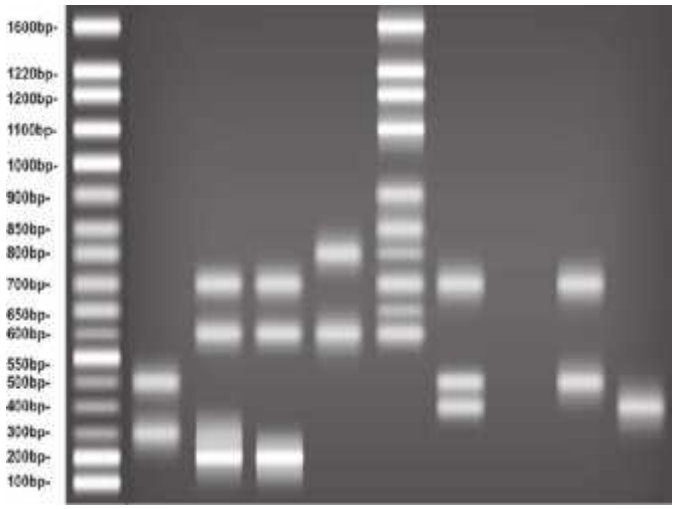

Fig 9. Illustration for the electrophoresis result of Primer 6 (V1, V2, V3, S1, S2, S3, G1, G2 and G3)

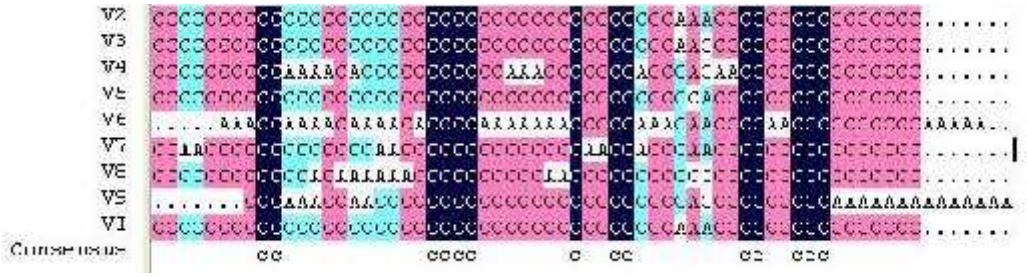

Fig 10. Multiple sequence alignment of 9 samples of grapes by DNAMAN

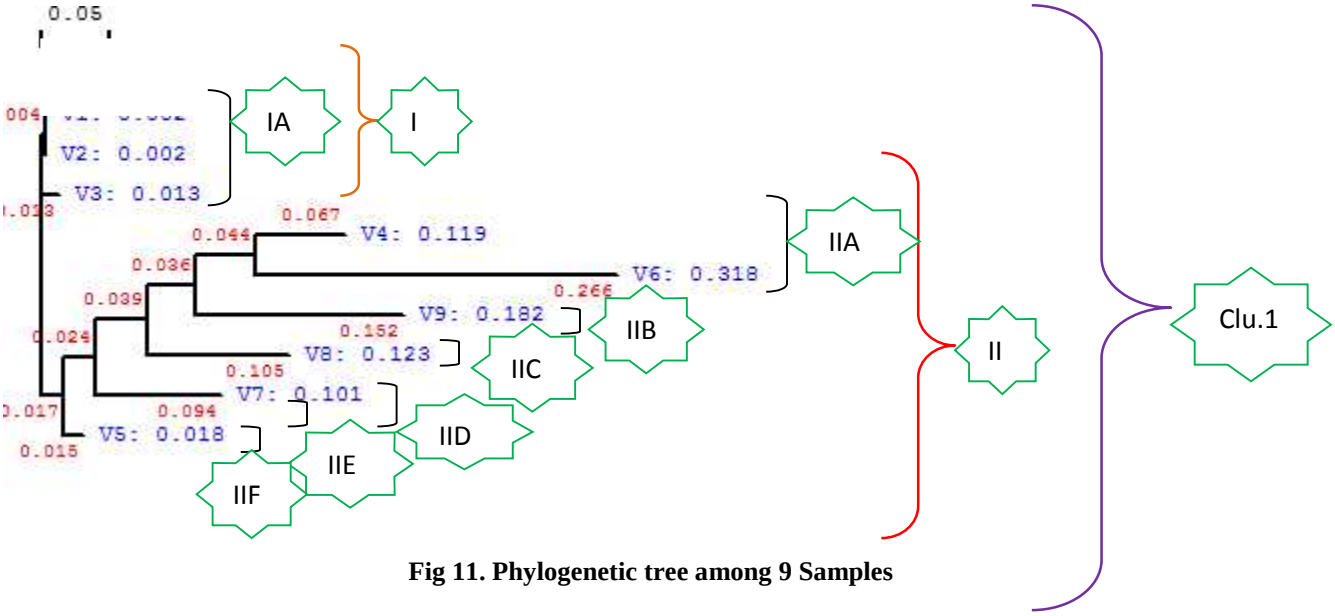

Fig 11. Phylogenetic tree among 9 Samples

Table 1. Total number of loci, range of band size, and polymorphism observed with different primers

primer	Primer sequence	Total no. of loci produced	Range of band size (bp)	Total no. of polymorphic bands	Rate of polymorphism
3	GACCGCTTGT	7	300-1200	5	71.4%
4	CTGTCTGTGG	5	300-1530	3	60%
6	AATCGGGCTG	6	700-1250	3	50%
9	CTGGGCACGA	7	300-900	5	71.4%
10	GGCACTGAGG	5	550-900	4	80%
11	AAGCGACCTG	10	600-1600	6	60%
12	CTGCTGGGAC	6	600-1550	5	83.3%

Table 2. Genetic distance matrix among the grapes

V1	0
V2	0.219 0
V3	0.151 0.203 0
S1	0.222 0.324 0.268 0
S2	0.365 0.370 0.411 0.319 0
S3	0.209 0.265 0.221 0.215 0.343 0
G1	0.219 0.306 0.264 0.260 0.370 0.258 0
G2	0.194 0.258 0.182 0.319 0.418 0.233 0.324 0
G3	0.292 0.258 0.303 0.270 0.246 0.306 0.297 0.328 0

Conclusion: Callus formation depends upon the explant used, nature, concentrations of hormones and culture conditions. Chemical as well as gamma radiation are important source of mutations. In the present study

Gamma irradiated mutants showed better response in terms of growth parameters as compared to chemical mutagesis. Treatmnet with sodium azide gave better response at low concentrations but with an increase in sodium azide concentration resulted in browning of the explant. The results of the RAPD analysis are also indicative of the fact markers are a useful marker in studying the genetic diversity and hence characterization of molecular differences as a result of *in vitro* mutagenesis.

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