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In vitro radio-sensitivity (Gamma irradiation) of some juice and wine making grape genotypes

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ABSTRACT

Irradiation sensitivity of four juice and wine genotypes *i.e.*, Pusa Navrang, Pearl of Csaba, Hybrid 76-1 and Julesky Muscat was ascertained. Well proliferated cultures these genotypes were raised on standardized proliferation cum rooting medium (MS basal medium + 2.0 mg/l IBA + 250 mg/l activated charcoal) were subjected to Gamma irradiation treatments (0, 5, 15, 20, and 25 Gy). The cultures after irradiation were sub-cultured following *in vitro* repetitive micro-cutting technique on the same medium upto five cycles (vM_5). Thereafter, the *in vitro* rooted plantlets were hardened and later transferred in pots in glasshouse for survival and morphological analysis. Results showed that the highest mean explant survival was noted in the control (83.78%) over other irradiation dosages except 5 Gy dose (80.95%), which were at par. The gamma irradiation dose of 10 Gy gave the mean survival of 46.66%, shoot sprouting (56.85%) and *in vitro* shoot abnormalities (50.59%), which were significantly different from the other irradiation dosages. The 10 Gy irradiation dose was recorded as a lethal dose 50 (LD_{50}) for all genotypes. However, as the irradiation dose was increased further, the explant survival percentage, shoot sprouting and *in vitro* shoot and root abnormalities significantly enhanced. The highest shoot sprouting was noted in Pusa Navrang (57.59%) followed by Pearl of Csaba (55.59%). Surviving mutants showed altered physiological traits with increasing doses of Gamma irradiation.

Key words: Grape, gamma rays, *in vitro* mutagenesis, physiological changes.

INTRODUCTION

To date, genetic improvement of fruit crops has relied heavily upon vegetative propagation techniques and classical breeding systems. Recent biotechnological techniques have the potential to provide sufficient methods of *in vitro* propagation, genetic improvement through improved mutation and screening techniques and genetic transformation. Genetic variation is an essential component for crop breeding (Jain, 3; Maluszynski and Ahloowalia, 7). Mutation is the alternate source of creating variability in any plant species for some desired traits. It is an effective method to induce variability within a short span of time, especially in fruit crops, which are perennial and vegetatively propagated. Studies on induced mutation in fruit crops have been undertaken particularly in apple, pear, peach, citrus *etc*. though very meagre efforts have been made in grape. Therefore, there is a great potential of *in vitro* induction of mutations for the selection of desirable mutants (Jain, 3; Maluszynski and Ahloowalia, 7).

Physical mutagen post-treatment manipulation is simpler and allows for a more precise determination of exposure time. Maluszynski and Ahloowalia (7) suggested the use of *in vitro* techniques for mutation induction because *in vitro* approach for induction of mutagenesis has certain advantages, *i.e.*, controlled environment providing ideal conditions for survival of mutated cells or tissues, higher recovery of induced mutants and better method for isolation and validation of desired solid mutants. Mutants of horticultural crops have been limited to only ornamentals. This valuable tool not yet fully exploited in grape breeding. Hence keeping the above points in view, the present investigation was undertaken on some grape genotypes with objectives of creating mutants with early maturity, seedlessness, enhanced berry quality *etc*.

MATERIALS AND METHODS

The *in vitro* proliferated cultures (30 days) of the four juice and wine making genotypes, *i.e*. Pusa Navrang, Pearl of Csaba, Hybrid 76-1 (Hur x Cardinal) and Julesky Muscat (25 cultures in 150 ml conical flasks) were subjected to different gamma irradiation doses for *in vitro* induction of desirable mutants. The gamma irradiation facility with ^{60}Co source of the Nuclear Research Laboratory, ICAR-IARI, Pusa, New Delhi was employed. Well grown *in vitro* complete plantlets under the proliferation cum rooting medium (MS basal medium + 2.0 mg/l IBA + 250 mg/l activated charcoal) were subjected to the 0, 5, 15, 20 and 25 Gy dosages of Gamma rays. Thereafter, the irradiated

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cultures were aseptically excised into two-node micro-cuttings and sub-cultured immediately to raise vM_1 generation. After complete growth of the sub-cultured micro-cuttings, the vines were again excised into the two-node micro-cuttings and sub-cultured. Similarly, the irradiated cultures were multiplied and maintained upto the vM_5 generations. Proliferated cultures of the physical induced mutants of each generation (vM_{1-5}) were hardened using the treatment standardized and transferred to glasshouse for their further screening and observations on the growth characteristics.

Explants survival (%) of *in vitro* grown grape plantlets were recorded at 15, 30 and 45 day after inoculation and on the basis of survival percentage LD_{50} dose of mutagen for each genotype were calculated. Another parameters regarding to shoot sprouting (%), days to shoot sprouting, shoot abnormalities (%), rooting (%), root length (cm), and root abnormalities (%) were also recorded. Mutation frequency was calculated as the ratio between such mutants and total number of plants examined under each irradiation treatment. Thereafter, the rooted mutant plantlets (4-week) were shifted for hardening under glasshouse conditions and different observations related to plantlet survival (%), leaf abnormalities (%) *etc*. were made after 4 weeks of hardening. Different physiological parameters like photosynthetic rate, respiration rate and stomatal conductance were recorded with the help of IRGA (ADC Scientific, UK).

RESULTS AND DISCUSSION

The 10 Gy irradiation dose was found as the lethal dose 50 (LD_{50}), which showed the slightly lower survival than the 50%. However, as the irradiation dose was further increased, the explant survival significantly decreased (Fig. 1). Under 25 Gy dose negligible micro-cutting survival was recorded. The survival of irradiated micro-cuttings is given in the Table 1. Amongst all the genotypes, the highest explant survival (57.59%) was noted in Pusa Navrang followed by Pearl of Csaba (55.59%) (Table 2). Whereas, lowest (51.23%) survival was noted in Julesky Muscat. The shoot sprouting was significantly the maximum in control (97.75%) and lower irradiation dose (5 Gy) (94.90%). At LD_{50} dose (10 Gy), the shoot sprouting was noted to be high (59.05%), which was significantly different from other doses. The shoot sprouting was drastically hindered with the increment of irradiation dose from LD_{50} and it was only 11.09% in 25 Gy (Table 2). The highest dose (25 Gy) resulted in lower survival of micro-cuttings though showing symptoms of survival initially but without any growth and finally turned necrotic after two to four weeks of culture. The survival of micro-cuttings with the higher dose besides necrosis of leaves showed other effects like shoot stunting, variegated leaves, *etc*. (Fig. 2). Higher doses caused lethality and the explants died within a few days of treatment. Similar results due to higher doses of mutagens were also reported (Kuksova *et al.*, 6; Coban *et al.*, 1).

The non-irradiated control and 5 Gy dose showed significantly the high rooting compared to the other irradiation treatments (Table 2). However, as the irradiation dose was increased, rooting percentage decreased and abnormalities were noted. Among the genotypes, the highest rooting was noted in the Pusa Navrang and Julesky Muscat, which were non-significant with each other. Whereas, significantly the lowest rooting was noted in H-76-1 (53.72%). The interaction between the genotype to the rooting with

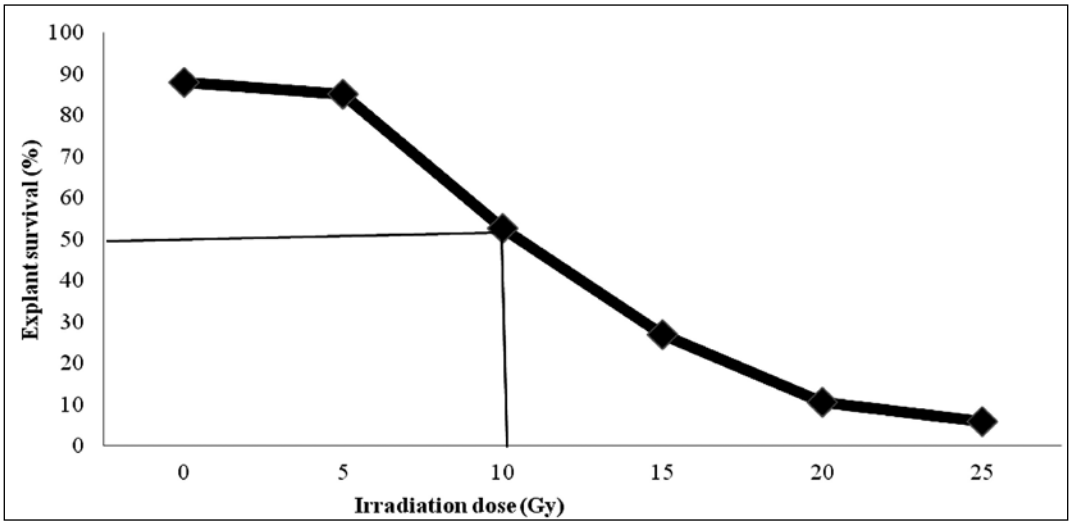

Fig. 1. LD_{50} dose of Gamma rays for the grape genotypes.

Table 1. Effect of gamma irradiation dose on the *in vitro* survival of grape explants.

Dose (Gy)	15 DAI					30 DAI					45 DAI				
	PN	H 76-1	POC	JM	Mean	PN	H 76-1	POC	JM	Mean	PN	H 76-1	POC	JM	Mean
0.0	98.63 (83.31)B*	98.73 (83.64)	96.47 (79.16)	97.17 (80.29)	97.75 (81.60)	89.37 (70.95)	89.20 (70.80)	86.47 (68.39)	87.13 (68.97)	88.04 (69.78)	85.60 (67.68)	83.90 (66.32)	83.50 (66.01)	82.13 (64.97)	83.78 (66.25)
5	94.40 (76.30)	92.00 (73.58)	97.00 (80.25)	96.23 (78.79)	94.9 (77.23)	85.03 (67.21)	82.33 (65.12)	87.43 (69.22)	85.93 (67.95)	85.18 (67.38)	81.30 (64.36)	79.77 (63.25)	81.63 (64.60)	81.10 (64.21)	80.95 (64.10)
10	58.53 (49.89)	54.73 (47.70)	62.77 (52.39)	60.17 (50.85)	59.05 (50.21)	51.07 (45.59)	49.33 (44.60)	54.20 (47.39)	55.90 (48.37)	52.63 (46.49)	48.87 (44.33)	45.80 (42.57)	47.30 (43.44)	44.67 (41.92)	46.66 (43.07)
15	39.43 (38.88)	39.47 (38.90)	45.50 (42.40)	32.70 (34.86)	39.28 (38.76)	33.23 (35.19)	28.47 (32.16)	22.90 (28.57)	23.47 (28.96)	27.02 (31.22)	17.90 (25.02)	17.60 (24.79)	16.60 (24.03)	15.83 (23.44)	16.98 (24.32)
20	27.50 (31.61)	24.57 (29.70)	20.43 (26.86)	12.97 (21.08)	21.37 (27.31)	10.43 (18.83)	11.20 (19.53)	11.20 (19.53)	9.93 (18.36)	10.69 (19.07)	3.13 (10.18)	0.00	0.00	0.00	0.78 (2.55)
25	19.40 (26.12)	10.60 (18.98)	10.53 (18.92)	8.87 (17.32)	12.35 (20.33)	8.00 (16.42)	5.60 (13.68)	4.80 (12.64)	5.73 (13.84)	6.03 (14.15)	0.00	0.00	0.00	0.00	0.00
Mean	56.32 (51.02)	53.35 (48.75)	55.45 (50.00)	51.35 (47.20)		46.19 (42.37)	44.36 (40.98)	44.50 (40.96)	44.68 (41.08)						
CD at 5%															
T					0.86					1.24					0.45
G					0.71					1.01					0.37
T × G					1.73					2.48					0.90

*Data in parenthesis are the Arc Sine √% transformed values of the original data

Table 2. Effect of gamma irradiation dosages on the *in vitro* shoot and root sprouting (%) in grape genotypes.

Dose (Gy)	Shoot sprouting (%)					Root sprouting (%)				
	PN	H-76-1	POC	JM	Mean	PN	H-76-1	POC	JM	Mean
0.0	98.10 (82.09)*	95.33 (77.53)	98.47 (82.99)	99.17 (84.87)	97.77 (81.87)	98.53 (83.18)	97.80 (81.61)	95.77 (78.16)	97.17 (80.32)	97.32 (80.82)
5	92.50 (74.10)	85.50 (67.60)	94.90 (76.93)	86.00 (68.00)	89.73 (71.66)	95.50 (77.77)	94.43 (76.35)	91.40 (72.94)	92.53 (74.19)	93.47 (75.31)
10	60.97 (51.32)	50.50 (45.27)	59.53 (50.48)	56.40 (48.66)	56.85 (48.93)	58.07 (49.65)	54.53 (47.61)	55.27 (48.00)	62.47 (52.20)	57.58 (49.37)
15	50.40 (45.21)	40.57 (39.55)	44.03 (41.56)	40.93 (39.76)	43.98 (41.52)	45.40 (42.34)	41.23 (39.93)	47.37 (43.47)	46.33 (42.88)	45.08 (42.16)
20	31.13 (33.90)	29.73 (33.03)	25.47 (30.29)	15.40 (23.09)	25.43 (30.08)	32.37 (34.66)	26.43 (30.93)	28.50 (32.25)	30.97 (33.80)	29.57 (32.91)
25	12.43 (20.63)	11.27 (19.59)	11.17 (19.50)	9.50 (17.94)	11.09 (19.42)	8.80 (17.24)	7.87 (16.28)	6.47 (14.70)	8.27 (16.70)	7.85 (16.23)
Mean	57.59 (51.21)	52.15 (47.09)	55.59 (50.29)	51.23 (47.05)		55.57 (50.81)	53.72 (48.78)	54.13 (48.25)	56.29 (50.02)	
CD at 5%										
T					0.77					1.35
G					0.63					1.10
T × G					1.55					2.70

*Data in parenthesis are the Arc Sine √% transformed values of the original data

respect to irradiation dose was more pronounced. In lower dose (5 Gy), not much variation in rooting was observed amongst the genotypes. Thereafter, as the irradiation dose was increased, rooting was drastically inhibited and delayed in almost all the genotypes (Table 3). The primary biological effect of physical mutagen was the reduction and delay in seed germination or sprouting of cuttings (Datta *et al.*, 2). The low irradiation dose had the growth promontory effect including rooting and root length, which were increased in some genotypes. However, with the higher irradiation dose, the quality of root was deteriorated, thin and was not useful for the proper growth and further hardening of the plantlets though they survived initially but died afterward.

Mean *in vitro* shoot abnormalities was significantly the highest in H-76-1 (50.29%) followed by Julesky Muscat, which were at par with each other (Table 4). While, the lowest shoot abnormalities were recorded in Pusa Navrang. Among the irradiation doses, 5 Gy gave only about 3.61% shoot abnormalities, which further increased with increase of irradiation doses and the highest *in vitro* shoot abnormalities, *i.e.* LD_{50} dose (10 Gy) was 50.59%, (15 Gy) 67.750% and (20 Gy) 78.56%. The interaction between the irradiation dose and the genotypic response to *in vitro* shoot development showed the minimum abnormalities in Pusa Navrang (1.43%) with 5 Gy dose; while the maximum abnormalities were noted in the H-76-1 (95.43%), suggesting it to be the most sensitive genotype. *In vitro* root abnormalities were found negligible in case of control and 5 Gy dose, while it highest with 25 Gy (97.98%) and were significant among them. The mean *in vitro* root abnormalities was highly significant in case of Julesky Muscat (52.40%) followed by Pearl of Csaba, H-76-1 and lowest in Pusa Navrang (Table 4). Interaction between the irradiation dose with the genotype showed that the minimum *in vitro* root abnormalities were in H-76-1 (2.7%) with 5 Gy dose, while the maximum abnormalities were recorder in Julesky Muscat (99.73%) with 25 Gy dose. The result suggests that the genotype Julesky Muscat was more sensitive to rooting related abnormalities (Table 4). It was found that frequency of *in-vitro* mutation was highest in Pusa Navrang (40%) followed by H-76-1 and Julesky Muskat (37.5%). Variant plants showing dwarf, and rigid-thick-pubescent, mottle leaf and roots (Fig. 3). These abnormalities are the results of gamma ray con secutively causing more disruption in the steady state of the physiological process and genetic expression /rearrangement (Murti *et al.*, 8).

Earlier, Kuksova *et al.* (6) suggested that mutagen doses also cause change in ploidy levels thus gives rise to abnormalities and increased abnormal vegetative growth. Coban *et al.* (1) had

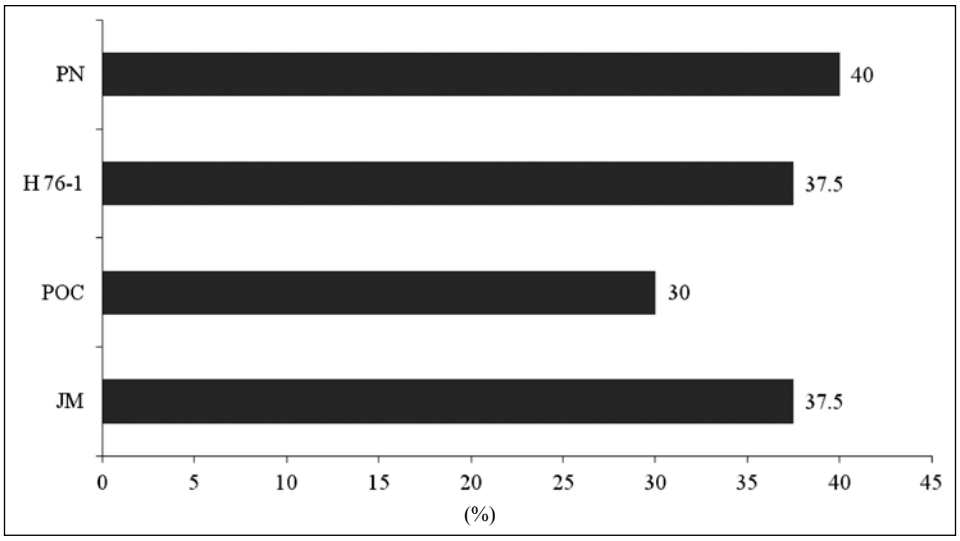

Fig. 2. *In vitro* mutation frequency (%) in grape genotypes.

Table 3. Effect of gamma irradiation dosages on days to root and shoot sprouting in grape genotypes.

Dose (Gy)	Days to root sprouting					Days to shoot sprouting				
	PN	H 76-1	POC	JM	Mean	PN	H 76-1	POC	JM	Mean
Control	18.73 ± 0.34	21.57 ± 0.39	18.47 ± 0.55	17.07 ± 0.30	18.96	18.40 ± 0.44	19.40 ± 0.53	22.30 ± 0.46	21.30 ± 0.47	20.35
5	21.43 ± 0.35	22.87 ± 0.85	22.47 ± 0.61	21.32 ± 0.56	22.02	20.73 ± 0.53	18.57 ± 0.73	21.93 ± 0.88	23.13 ± 0.20	21.09
10	27.17 ± 0.35	27.73 ± 0.23	28.77 ± 0.27	23.47 ± 0.55	26.78	23.63 ± 0.43	24.17 ± 0.49	23.53 ± 0.46	25.50 ± 0.52	24.21
15	29.53 ± 0.54	31.10 ± 0.32	29.83 ± 0.62	31.17 ± 0.33	30.41	26.57 ± 0.41	30.40 ± 0.49	26.87 ± 0.98	28.57 ± 0.59	28.10
20	32.83 ± 0.38	35.13 ± 0.88	33.23 ± 0.90	33.50 ± 0.58	33.68	30.43 ± 0.66	31.40 ± 0.49	29.47 ± 0.52	31.53 ± 0.61	30.71
25	24.14	25.79	25.13	23.99		32.27 ± 0.52	33.33 ± 0.44	31.50 ± 0.58	34.33 ± 0.60	32.86
Mean						25.34	26.21	25.93	27.39	
CD at 5%										
T					0.71					0.80
G					0.58					0.66
T × G					1.43					1.61

observed similar effects in three grape cultivars The primary biological effects of physical mutagens were found to be reduced plant height and various types of leaf abnormalities like multi-lobed, closed petiolar sinus, deep lobes, prominently serrated and small narrow leaves (Sharma and Mukherjee, 9). Furthermore, such variations can also be created due to physiological differences in the tissue plus parts selected for the study. It is opined that variation was probably an expression of the epigenetic activation of DNA elements (Kaeppler *et al*., 4) or mutagen that affected the temporary steady state physiology of the plant.

Four week after rooting, the *in vitro* grown grape plantlets were shifted to glasshouse for hardening and thereafter their survival was recorded. Lower gamma irradiation dose showed maximum plantlet survival as irradiation dose were increased the survival during

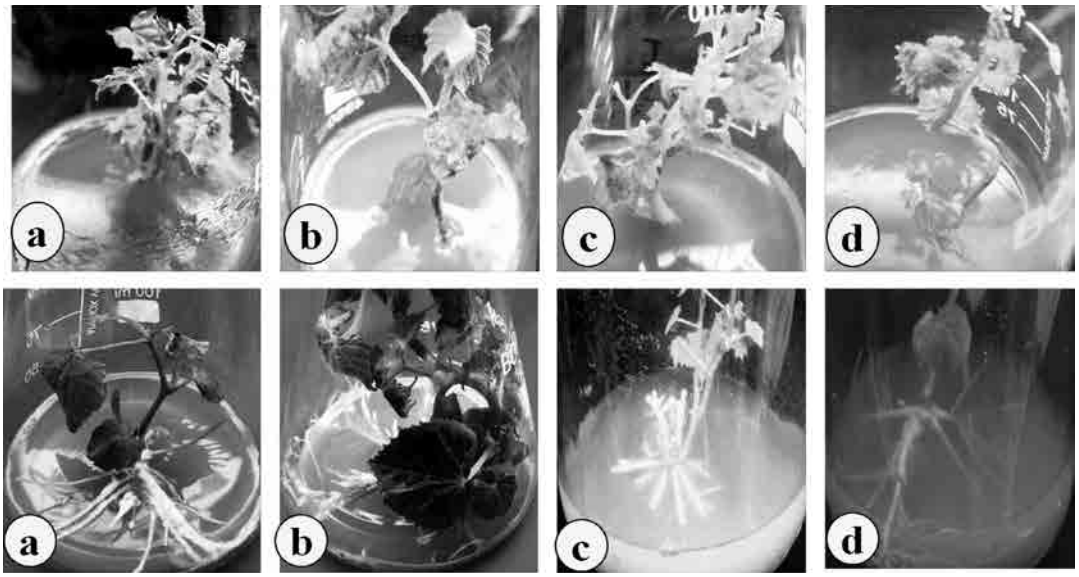

Fig. 3. Effect of gamma irradiation dosages on the *in vitro* shoot and root abnormalities (%) in grape genotypes.
Pusa Navrang, (b) Hybrid-76-1, (c) Pearl of Csaba, and (d) Julesky Muscat.

hardening stage were decreased significantly. Under the 25 Gy dose no plantlet survival was recorded. Significantly, the highest mean plantlet survival was recorded in Pusa Navrang (34.83%) followed Pearl of Csaba (31.67%). Though H-76-1 and Julesky Muscat were non-significantly different to each other except Pusa Navrang.

In glasshouse, the mean leaf area was significantly the highest in Pusa Navrang (16.11 cm^2) significantly followed by H-76-1 (Table 4), while, the lowest was in Pearl of Csaba. The lower irradiation doses were at par with each other with respect to mean leaf area while, significantly, the lowest leaf area was noted in 20 Gy dose (3.06 cm^2). Photosynthesis

Table 4. Effect of gamma irradiation dosages on shoot and root abnormalities (%) in grape genotypes.

Dose (Gy)	Shoot abnormality (%)					Root abnormality (%)				
	PN	H 76-1	POC	JM	Mean	PN	H 76-1	POC	JM	Mean
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	1.43 (6.87)	4.00 (11.48)	4.90 (12.77)	4.10 (11.64)	3.61 (10.69)	3.23 (10.26)	2.70 (9.43)	5.73 (13.84)	5.37 (13.39)	4.26 (11.73)
10	54.67 (47.66)	46.43 (42.94)	45.47 (42.38)	55.80 (48.31)	50.59 (10.69)	44.53 (41.84)	46.40 (42.92)	51.80 (46.01)	54.97 (47.84)	49.43 (44.65)
15	65.35 (53.92)	71.83 (57.93)	67.40 (55.16)	66.40 (54.55)	67.75 (55.39)	64.20 (53.23)	68.77 (56.00)	70.43 (57.05)	69.30 (56.33)	68.18 (55.65)
20	75.43 (60.27)	84.03 (66.43)	79.30 (62.92)	75.47 (60.29)	78.56 (62.48)	81.93 (64.83)	83.07 (65.68)	80.50 (63.77)	85.03 (67.36)	82.63 (65.41)
25	90.87 (72.39)	95.43 (77.69)	91.30 (72.83)	94.50 (76.43)	93.03 (74.83)	98.33 (82.76)	96.80 (79.70)	97.07 63.77)	99.73 (87.05)	97.98 (82.43)
Mean	47.96 (40.18)	50.29 (42.74)	48.06 (41.01)	49.38 (41.87)		48.71 (42.16)	49.62 (42.29)	50.92 (43.48)	52.40 (45.33)	
CD at 5%										
T					0.78					1.23
G					0.63					1.00
T × G					1.55					2.46

Table 5. Effect of gamma irradiation dosages on the physiological parameters of grape mutant genotypes during glasshouse hardening.

Dose (Gy)	Photosynthetic rate (µmol m^{-2} s^{-1})					Respiration rate (µmol m^{-2} s^{-1})					Stomatal conductance (mmol m^{-2} s^{-1})					Leaf relative water content (%)				
	PN	H 76-1	POC	JM	Mean	PN	H 76-1	POC	JM	Mean	PN	H 76-1	POC	JM	Mean	PN	H 76-1	POC	JM	Mean
0.0	12.10	10.45	13.44	9.99	11.49	20.55	22.67	24.77	25.37	23.34	0.26	0.26	0.22	0.20	0.24	67.70	68.17	70.03	71.50	69.35
5	12.30	11.87	11.69	11.53	11.85	25.97	27.50	28.07	28.73	27.57	0.19	0.25	0.08	0.07	0.15	69.30	70.33	72.62	72.83	71.27
10	11.43	8.77	10.81	9.49	10.12	26.83	35.30	33.17	35.47	32.69	0.15	0.06	0.09	0.07	0.09	60.30	58.30	57.53	58.17	58.58
15	10.75	9.01	8.66	9.32	9.43	36.37	40.87	34.50	42.73	38.62	0.09	0.08	0.12	0.04	0.08	57.23	49.87	51.13	50.47	52.18
20	8.75	-	-	-	8.75	38.57	-	-	-	38.57	0.02	-	-	-	0.02	49.67	-	-	-	49.67
Mean	11.07	8.02	8.92	8.07		29.66	25.27	24.10	26.46		0.14	0.13	0.10	0.08		60.84	49.39	50.26	50.59	
CD at 5%																				
T					0.55					0.64					0.02					1.28
G					0.49					0.57					0.02					1.15
T × G					1.09					1.28					0.05					2.56

rate among the different genotypes was recorded at Table 5. The lower irradiation doses gave slight higher photosynthesis rate (11.85 µmol $m^{-2}s^{-1}$) when compared to control (11.49 µmol $m^{-2}s^{-1}$). As the irradiation dose increased, the photosynthetic rate decreased significantly. Lowest photosynthetic rate was noted in highest irradiation dose, *i.e.* 20 Gy followed by 15 and 10 Gy. It was found that photosynthetic rate was significantly the highest in Pusa Navrang (11.07 µmol m^{-2} s^{-1}) followed by Pearl of Csaba, and Julesky Muscat and H-76-1, which was at par with each other except when compared to Pusa Navrang. Murti *et al.* (8) also reported that the chlorophyll content and net photosynthetic rate in leaves of mutants were markedly decreased.

The higher irradiation dose significantly increased the respiration rate, *i.e.*, 15 Gy (38.62 µmol m^{-2} s^{-1}), followed by 20 Gy (38.57), which were at par to each other. However, the lower irradiation doses least affected respiration rate of the mutants, *i.e.* 5 Gy (27.57) when compared to non-irradiated control (23.34). Respiration rate was the highest in the Pusa Navrang (29.66) followed by Julesky Muscat. While lowest respiration rate was observed in Pearl of Csaba (24.10). Kang *et al.* (5) also reported that lower irradiation could reduce the respiration rate of fresh grape and extend the shelf-life of fruit, while higher irradiation dose may increase respiration rate. These findings are in conformity with the findings of Singh and Pal (10).

The irradiation doses significantly affect mean stomatal conductance, *i.e.* control (0.24 µmol m^{-2} s^{-1}), 5 Gy (0.15) and 10 Gy (0.09). Significantly, low mean stomatal conductance was noted in 20 Gy dose (0.02) followed by 15 Gy (0.08). Among the genotypes, the mean stomatal conductance was significantly the highest in Pusa Navrang (0.14 mmol $m^{-2}s^{-1}$) followed by H-76-1 and Pearl of Csaba, which were significant with each other (Table 5). The significantly lowest mean stomatal conductance was registered in Julesky Muscat (0.08 mmol $m^{-2}s^{-1}$). The higher irradiation dose significantly decreased the leaf relative water content, *i.e.*, 10 Gy (58.58%), followed by 15 and 20 Gy, which were significant with each other. However, the lower irradiation doses significantly increased leaf relative water content of the plants, *i.e.*, 5 Gy (71.27%), in comparison to non-irradiation control (69.35%), which was significantly high to other irradiated genotypes. Leaf relative water content was the highest in Pusa Navrang (60.84%), while lowest was observed in H-76-1 (49.39%).

From the above discussion, it is concluded that the Gamma irradiation at LD_{50} dose (10 Gy) gave sufficient variability with development of mutants in table grape genotypes which could

posses the desirable traits. Furthermore, the *in vitro* mutagenesis coupled with clonal micropropagation of putataive mutants would allow application of DNA marker-based diversity analysis to identify solid mutants and escapes, which could be selected culled in the early phase. Besides, beneficial effect of low irradiation dose in creating genetic variability was also noted.

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