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Original Research Paper

Influence of gamma radiation on morpho-physiological and biochemical traits of *in vitro* raised wine grape (*Vitis vinifera* L.) genotypes

Dev R.[1#], Singh Sanjay K.[2*], Arora A.[3], Singh A.K.[1], Asrey R.[4], Alizadeh M.[5] and Dayal V.[1,$]

[1]Division of Fruits & Horticultural Technology, [3]Division of Plant Physiology,
[4]Division of Post-harvest Technology, ICAR-Indian Agricultural Research Institute, New Delhi - 110 012, India
[2]Deputy Director General (Horticultural Science), Division of Horticultural Science, Krishi Anusandhan Bhawan - II, New Delhi - 110 012, India
[5]Gorgon University of Agricultural Sciences & Natural Resources, Golestan, Iran
[$]Presently: ICAR-Central Institute for Subtropical Horticulture, Lucknow - 227 107, India
[#]Presently: ICAR-NBPGR, Regional Station, Bhowali - 263 132, Uttarakhand, India
[*]Corresponding author Email: sanjaydr2@gmail.com

ABSTRACT

Grapes are a worldwide significant fruit, important for the wine industry, trade, and health benefits. Despite its significance, grape cultivation in India faces numerous biotic and abiotic challenges. Traditional breeding in this crop is very expensive, time-consuming and presents many technical obstacles. Since the plant has reproductive sterility, creating variability for these characteristics is possible only through induced mutation. The present study was undertaken to investigate the effect of gamma irradiation (0, 5, 15, 20 & 25 Gy) on morpho-physiological and biochemical traits of four *in vitro* raised wine grape genotypes (Pusa Navrang, H-76-1, Pearl of Csaba and Julesky Muscat). The gamma-irradiated shoot cultures were evaluated under *in vitro* and *ex vitro* conditions. All irradiation treatments affected growth traits. However, response was found to be genotypic and dose-dependent. The growth of irradiated cultures was stimulated at dose (5 Gy) less than LD_{50} dose, whereas, treatment of cultures with a dose higher than 15 Gy, reduced the growth and caused deformation in cultures. Based on plant growth and survival traits, 10 Gy was identified as LD_{50}. Among the genotypes tested, Pusa Navrang exhibited good final recovery (10.43%) at high dose (20 Gy). However, genotype H-76-1 was found to be the most sensitive genotype to the higher dose (>15 Gy) of gamma irradiation. Amount of proline, total phenols and total sugars increased, while chlorophylls and carotenoids decreased irrespective of genotypes in response to gamma irradiation (>15 Gy). The results of present study will be very useful for future improvement of grapevine scion cultivars.

Keywords: Gamma irradiation, genotype, grape, growth responses, plant survival

INTRODUCTION

Grapevine (*Vitis vinifera* L.) is generally considered to have the most desirable fruit quality. It fruits utilized globally for a wide range of products, including fresh fruit, juice, jams, jellies, wine, raisins and other processed products. Grapes are a worldwide significant fruit, important for the wine industry, trade and health benefits. China, Italy and the United States are among the largest manufacturers. India is among the top ten grape-producing countries, with approximately 162 thousand hectares under cultivation and an annual production of around 3.47 million metric tons (Department of Agriculture & Farmers Welfare, 2022). Grapes are largely cultivated in Maharashtra, Karnataka and Tamil Nadu and they contribute significantly to agricultural GDP and exports. Despite obstacles such as climate fluctuation and pest management, they are critical for both domestic consumption and worldwide trade. India is a major exporter of fresh grapes around the world. During the fiscal year 2023-24, the country exported 343,982.34 MT of grapes to the world, valued at Rs. 3,460.70 crores/417.07 USD million (APEDA, 2024). Despite its significance, grape cultivation in India faces numerous challenges, including diseases such as downy mildew, powdery mildew and anthracnose, as well as pests like mealybugs and flea beetles etc. *Vitis* species are highly polymorphic, highly heterozygous, and exhibit pronounced inbreeding depression during conventional hybridization (Einset & Pratt, 1975). Additionally, grape genotypes carry

an excessively heavy load of deleterious recessive alleles and inbreeding depression is so severe that it leads to second or third-generation sterility. Due to such factors, the traditional breeding of *Vitis* sp. is very expensive, time-consuming and presents many technical obstacles. Since the plant has reproductive sterility, creating variability for these traits is possible only through induced mutation. In this regard, mutagenesis has various benefits for overcoming these constraints and improving grapevine performance. Previously, improved clonal selections were identified from natural spontaneous mutated grape buds (Einset & Pratt, 1975). However, the occurrence of natural, random mutation events is rare and accidental, hence cannot be used for the directed genetic improvement of the grape. *In vitro* mutagenesis speeds up trait improvement by directly triggering mutations, allowing for focused improvements in specific qualities such as fruit quality and yield. The approach also minimizes linkage drag, providing a more effective path to attaining breeding objectives. It complements traditional breeding and allows for the development of new cultivars with increased disease resistance, insect tolerance, and environmental adaptability. Induced mutagenesis through the physical mutagens (*i.e.* gamma rays) is very effective as it can penetrate deeper into the tissue and cause nuclear DNA breakage, leading to new random and heritable mutants. The direct use of gamma rays is a very useful supplementary approach when it is desired to improve one or two easily identifiable traits in an otherwise well-adapted variety. In many vegetatively propagated crops, induction of mutation in combination with *in vitro* culture technique is the only effective method for crop improvement (Ahloowalia, 1998). *In vitro* culture techniques for induction of mutagenesis have certain advantages over conventional mutagenesis, *i.e.* a controlled environment providing ideal conditions for the survival of mutated cells or tissues, higher recovery of induced mutants and better method for isolation and validation of desired solid mutants. Moreover, applying mutagen in the *in vitro* developed plantlets is relatively easy, efficient and cost-effective (Ahloowalia, 1998). The above background indicated that this valuable tool was not yet fully exploited in the improvement of locally adapted grape cultivars. Hence, an attempt was made to examine the effects of gamma rays on *in vitro* and *ex vitro* growth and physiological and biochemical attributes of the mutant's grape plantlets with objectives of creating novel mutants with early maturity, seedlessness, enhanced berry quality etc. Therefore, the current study investigated the effect of 0-25 Gy gamma irradiation (^{60}Co source) on the morpho-physiological and biochemical traits in *in vitro* raised grape genotypes.

MATERIALS AND METHODS

In vitro culture establishment

The newly sprouted and one season matured nodal segments (single node) of four wine grape genotypes, *i.e.* Pusa Navrang, Pearl of Csaba, H-76-1 (Hur × Cardinal), and Julesky Muscat were used as explant for *in vitro* culture initiation. The explant (single node segments) from just matured vines were procured from the fruit orchard of the Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India. For culture initiation, Murashige and Skoog's (MS) medium was used as basal medium upon supplemented with different concentrations of growth regulators, *i.e.* 2.0 BAP, and 4.0 mg L^{-1} kinetin individually and in combination with 0.2 mg L^{-1} NAA were used. While for shoot multiplication and rooting, MS basal medium was supplemented with different concentrations of IBA (2.0 and 4.0 mg L^{-1}) with or without activated charcoal (200 mg L^{-1}).

Irradiation treatment and *in vitro* morphological growth evaluation

In vitro raised plantlets with 4-5 grown leaves were treated with five levels of gamma irradiation (0, 5, 15, 20 and 25 Gy). The gamma irradiation treatment was carried out on the plants established in the conical flasks at a dose rate of 7030 Gy/h with ^{60}Co source at the Nuclear Research Laboratory, ICAR-IARI, Pusa, New Delhi. Three samples were evaluated for each irradiation dose. Further, irradiated cultures were aseptically excised into two-node micro-cuttings and sub-cultured under culture multiplication cum rooting medium. The irradiated cultures were multiplied and maintained upto vM5 generations and characterized using morphological parameters, *i.e.* plantlet survival (%), shoot length (cm), internodal length (cm), number of leaves per sprout, rooting (%), root length (cm), number of roots per shoot. Explant survival (%) of gamma-irradiated plantlets was recorded at every 15 day interval, and LD_{50} dose for each genotype was

estimated based on the survival percentage and culture growth. Survived cultures of gamma rays induced mutants of each generation (vM_{1-5}) were initially hardened under *in vitro* conditions.

Ex vitro evaluation of physiological and biochemical traits

The hardened *in vitro* raised plantlets with roots were transferred to glasshouse conditions at the age of 45 days of irradiation, and leaf samples were collected for determining biochemical changes, *i.e.* total chlorophyll content, total carotenoids content, total soluble sugars, proline, and total phenols after 120 days of irradiation treatment. The chlorophyll content of the leaves was estimated as per Barnes et al. (1992). The carotenoids content of the fresh leaves was assessed using the method of Lichtenthaler & Wellburn (1983). The total soluble sugars content of the sample was quantified using a method standardized by Anthony & Mutton (2006). The colorimetric method suggested by Bates et al. (1973) was employed to determine the total proline content in mature leaves. The leaf total phenols content was quantified through the method suggested by Malik & Singh (1980). The experiment was conducted in factorial complete randomized design with three replications. Each treatment consisted of 25 *in vitro* established cultures. The percentage data were subjected to angular transformation before analysis. ANOVA was calculated as a portion of variance, as reported by Gomez & Gomez (1984). The P values ≤ 0.05 were considered significant.

RESULTS AND DISCUSSION

In vitro plantlet survival (%) and standardization of LD_{50} dose

In vitro plantlet survival was both dose and genotype-dependent, with survival decreasing as gamma irradiation increased. Non-irradiated plantlets showed the highest survival, while 52.63% survived at 10 Gy. Doses above the LD_{50} significantly reduced survival, with 20–25 Gy causing severe (~90%) plantlets mortality, though some plantlets (10.69%) survived at 20 Gy. Plantlets treated with the highest dose (25 Gy) initially showed 6.03% survival. Still, without any growth activity, the death of plantlets was finally noted after 45 days of culture (data not shown). Among four genotypes, the maximum *in vitro* plantlet survival was observed in the Pusa Navrang, followed by Julesky Muscat. Conversely, the highest plantlet mortality was found in the H-76-1 genotype at 30 days of irradiation treatment.

Number of leaves per sprout

Gamma irradiation stimulated leaf production at lower doses, with a 9.5% increase in leaves per sprout at 5 Gy compared to control. However, leaf number declined by 21.5% at 10 Gy, with the highest reduction of 65.16% observed at 25 Gy (Table 1).

The effect of irradiation dose on the leaf number concerning genotype was more pronounced, Julesky Muscat plants produced the highest number of leaves (6.22%), followed by Pearl of Csaba (6.07%) and the minimum in the H-76-1 (5.35%) genotype. The stimulation of morphological growth under low gamma irradiation is a general phenomenon known as 'Hormesis' and supported by various workers (Islam et al., 2015).

Shoot and internode length

The response of genotypes to different gamma irradiation doses varied considerably. The drastic reduction in the shoot length of plantlets received irradiation exposure above the LD_{50} dose especially at 25 Gy dose (Table 1). Julesky Muscat showed more stunted growth among treated genotypes with the highest irradiation dose (25 Gy). While, the lower irradiation dose (5 Gy) stimulated the shoot growth over the control. Similar to shoot growth, the assessment for increment for internodal length stated that a lower dose has increased internodal length by 17.09% over the non-irradiated plantlets. If irradiation doses increased to 20 and 25 Gy dose, the length of the internode was reduced by 23.07 and 42.73%, respectively. The longest internode was noted in Pearl of Csaba at 10 Gy irradiation dose; on the contrary, a higher gamma irradiation dose (25 Gy) yielded the shortest internode length in H-76-1. Up to the LD_{50} dose (10 Gy), the internode length remained comparable to control plants, supporting Islam et al. (2015), who noted stimulatory effects at lower gamma doses. However, doses above 10 Gy caused a marked reduction in internode length (Islam et al., 2015), likely due to gamma-induced cell cycle arrest, genomic damage, or reduced mitotic activity (Wi et al., 2007b).

Root length and number of roots/plantlet

As the dose of irradiation increases from 5 to 25 Gy root traits like root length and number of roots per

Table 1 : Effect of gamma irradiation dosages on plantlet growth parameters of grape genotypes under *in vitro* conditions

Treatment	Genotype	Shoot length (cm)	Internode length (cm)	*In vitro* plantlet survival (%)	No. of leaves sprout^{-1}	Root length (cm)	No. of roots shoot^{-1}
0 Gy	PN	5.07±0.30#	1.17±0.07	89.37 (70.95)	7.47±0.09#	7.07±0.30#	7.03±0.27
	H-76-1	4.70±0.26	1.17±0.03	89.20 (70.80)	7.40±0.12	7.13±0.59	6.10±0.21
	POC	4.93±0.15	1.13±0.03	86.47 (68.39)	8.00±0.12	6.10±0.31	5.47±0.09
	JM	5.13±0.12	1.20±0.06	87.13 (68.97)	8.00±0.29	6.50±0.38	6.20±0.36
5 Gy	PN	6.53±0.15	1.10±0.06	85.03 (67.21)	7.60±0.17	7.20±0.17	8.30±0.25
	H-76-1	6.03±0.12	1.30±0.06	82.33 (65.12)	8.40±0.26	6.73±0.34	8.53±0.38
	POC	9.33±0.44	1.57±0.12	87.43 (69.22)	9.00±0.29	8.07±0.43	8.60±0.31
	JM	6.47±0.15	1.50±0.06	85.93 (67.95)	8.80±0.30	6.80±0.15	8.17±0.20
10 Gy	PN	5.33±0.13	1.10±0.06	33.23 (35.19)	5.17±0.20	6.63±0.13	6.20±0.27
	H-76-1	5.53±0.18	1.10±0.06	28.47 (32.16)	5.43±0.20	5.43±0.52	5.77±0.21
	POC	6.30±0.10	1.40±0.06	22.90 (28.57)	6.90±0.23	5.93±0.22	4.23±0.09
	JM	6.00±0.29	1.23±0.03	23.47 (28.96)	6.73±0.28	6.40±0.49	5.47±0.36
15 Gy	PN	5.10±0.15	1.13±0.03	33.23 (35.19)	7.52±0.38	6.45±0.40	4.43±0.35
	H-76-1	4.83±0.20	0.93±0.09	28.47 (32.16)	4.83±0.24	5.53±0.52	4.70±0.18
	POC	4.73±0.27	0.90±0.06	22.90 (28.57)	5.27±0.20	5.10±0.23	5.17±0.37
	JM	5.27±0.03	1.03±0.09	23.47 (28.96)	6.30±0.06	5.33±0.52	6.20±0.18
20 Gy	PN	4.60±0.06	0.83±0.03	10.43 (18.83)	4.28±0.08	6.03±0.59	4.03±0.27
	H-76-1	5.63±0.26	0.90±0.06	11.20 (19.53)	3.63±0.07	4.07±0.30	3.77±0.18
	POC	5.03±0.32	0.90±0.06	11.20 (19.53)	4.30±0.12	4.07±0.38	4.57±0.18
	JM	4.77±0.22	0.97±0.09	9.93 (18.36)	4.37±0.22	3.60±0.31	3.80±0.25
25 Gy	PN	2.50±0.23	0.67±0.03	8.00 (16.42)	2.30±0.06	2.40±0.36	3.20±0.21
	H-76-1	3.53±0.18	0.57±0.03	5.60 (13.68)	2.40±0.06	1.93±0.22	2.30±0.06
	POC	3.57±0.12	0.70±0.06	4.80 (12.64)	2.97±0.23	1.77±0.37	2.33±0.12
	JM	2.30±0.06	0.73±0.03	5.73 (13.84)	3.10±0.12	1.87±0.20	1.63±0.09
Treatment mean	0	4.96	1.17	88.04 (69.78)	7.72	6.7	6.2
	5 Gy	7.09	1.37	85.18 (67.38)	8.45	7.2	8.4
	10 Gy	5.79	1.21	52.63 (46.49)	6.06	6.1	5.42
	15 Gy	4.98	1	27.02 (31.22)	5.98	5.6	5.13
	20 Gy	5.01	0.9	10.69 (19.07)	4.15	4.44	4.04
	25Gy	2.98	0.67	6.03 (14.15)	2.69	1.99	2.37
Genotype mean	PN	4.86	1	46.19 (42.37)	5.72	5.96	5.53
	H-76-1	5.04	1	44.36 (40.98)	5.35	5.14	5.19
	POC	5.65	1.1	44.50 (40.96)	6.07	5.17	5.06
	JM	4.99	1.11	44.68 (41.08)	6.22	5.08	5.24
CD at 5%	Treatment (T)	0.3	0.09	1.24	0.29	0.54	0.37
	Genotype (G)	0.24	0.07	1.01	0.24	0.44	0.3
	T x G	0.6	0.17	2.48	0.58	1.07	0.75

#Data represent the mean ± standard error of three independent determinates. *Arc Sin $\sqrt{}$ % transformed values; PN: Pusa Navrang; H-76-1: Hybrid 76-1; POC: Pearl of Csaba; JM: Julesky Muscat; DAI: days after irradiation

plantlet, were affected negatively (Table 1). Root length of irradiated plants decreased by 8.96, 16.42, and 33.73% at 10, 15 and 20 Gy, respectively, with the highest reduction (70.3%) at 25 Gy. In contrast, low doses slightly stimulated root growth, showing a 7.46% increase over the control across genotypes.

Among the tested genotypes, good number of roots with better length was recorded in Pusa Navrang (5.96 cm) and shortest in Julesky Muscat (5.08 cm). At 25 Gy dose of irradiation, in addition to shortening of roots, thinning and distortion of roots were also commonly observed in all the genotypes. The number of roots per plant varied in between 2.37 and 8.40 with highest (25 Gy) and lowest (5 Gy) irradiation doses, respectively. Thus, it was inferred from the data that the root number increased under lower (5 Gy) irradiation dose, which thereafter reduced. Other reports, such as grape (Islam et al., 2015) and strawberry (Gupta et al., 2018), showed a comparable rise in the root number after exposure to low dose (5 Gy) of irradiation.

Evaluation of mutants under *ex vitro* conditions

The genotypes showed better survival percentage upto 15 Gy irradiation treatments. A further increase in irradiation dose caused significant mortality of plantlets irrespective of genotypes. Plantlets of different genotypes irradiated with 25 Gy dose though survived initially but died between five to six weeks of *in vitro* hardening and could not be transferred to *ex vitro* conditions. Only a few Pusa Navrang plantlets survived at 20 Gy, while other genotypes that initially survived *in vitro* failed during *ex vitro* hardening. Similarly, Surakshitha et al. (2018) reported low regeneration in grape cuttings at 21 Gy, with maximum regeneration at 3 Gy. This finding aligns with Islam et al. (2015), suggesting that reduced plant survival at higher gamma doses may be due to decreased auxin levels, auxin inactivation and chromosome damage (Banerji & Datta, 2002).

Vine and internode length (cm)

Under *ex vitro* conditions, 5 Gy-irradiated mutant plantlets showed shoot length comparable to the control, whereas *in vitro* conditions stimulated shoot length growth (Table 2). While, 38.49% reduction in vine length of genotype was recorded under high (20 Gy) dose. Among the genotypes, Pusa Navrang plantlets had the longest vine length (27.7 cm), while, Julesky Muscat had the shortest (23.65 cm). Genotypes showed significant variation in vine length due to irradiation; Pusa Navrang had the highest reduction at 20 Gy, while Pearl of Csaba maintained vine length comparable to the control at 5 Gy. Generally, the internode length of all the treated genotypes was lower than that of the non-treated control plantlets (Table 2). However, maximum decline (51.95 and 53.52%) in internode length was observed with the 15 and 20 Gy irradiation doses, respectively. Regardless of treatment, Pusa Navrang (3.38 cm) genotype recorded higher internodal length and minimum in H-76-1 (2.55 cm). Higher irradiation doses caused shoot stunting, leaf variegation and eventual explant mortality. The observed reduction in plant height was primarily due to the apical meristem damage or shortened internodes, as observed under the present investigation. Whereas, Surakshitha et al. (2018) reported a 6% reduction in shoot length at 3 Gy dose, with further decline of 33.06 and 46.69 % at 18 and 21 Gy, respectively, accompanied by stunted shoots and abnormal leaves. This reduction may result from inhibited cell division and low meristematic activity at higher doses. Additionally, higher doses could have damaged the apical meristems, disrupt enzyme synthesis, reduce amylase activity and temporarily suspend cell division (Avinash, 2013). In contrast, growth enhancement at lower doses may result from the stimulatory effects of low-dose gamma radiation, which can alter hormonal signaling or boost cellular antioxidative capacity to better cope with environmental stressors like light and temperature fluctuations (Kim et al., 2004; Wi et al., 2007b).

Number of leaves per plant and leaf area

The number of leaves per plantlet was higher in plantlets treated with 5 Gy over untreated plants (Table 2). However, at subsequent doses (15 and 20 Gy), the number of leaves declined by 30.42 and 71.62%, respectively. The genotypic difference was also observed in different irradiation, and the maximum number of leaves was recorded in genotype Julesky Muscat (21.77), followed by Pearl of Csaba (20.61) and minimum in Pusa Navrang (18.31). This study showed a significant decline in leaf area of grape genotypes treated with gamma irradiation compared to non-irradiated plantlets. The leaf area declined with increase in the treatment dose (>10 Gy) and reached a minimum level (47.75% reduction) with 20 Gy

Table 2 : Effect of gamma irradiation dosages on the plantlet growth parameters under *ex vitro* conditions

Treatment	Genotype	Vine length (cm)	Internode length (cm)	No. of leaves plant^{-1}	Leaf area (cm^2)	Root length (cm)	No. of roots shoot^{-1}
0 Gy (control)	PN	33.67±0.84#	4.90±0.21	22.03±0.66#	24.00±0.87	14.40±0.49#	19.37±0.55
	H-76-1	35.40± 0.52	4.90±0.15	27.20±0.92	22.97±0.30	15.03±0.73	21.90±0.79
	POC	35.80± 0.85	4.77±0.15	25.50±0.28	25.87±0.49	17.50±0.68	21.37±0.58
	JM	34.73± 0.28	5.90±0.21	29.13±1.00	21.57±0.56	18.43±0.39	20.57±0.74
5 Gy	PN	32.27±1.16	3.73±0.19	25.73±0.35	21.40±0.47	14.27±0.58	19.43±0.88
	H-76-1	33.80±1.12	2.80±0.26	29.83±0.85	24.50±0.56	15.23±0.17	20.13±0.46
	POC	35.83±0.92	3.30±0.10	26.73±0.62	21.17±0.33	18.03±0.33	23.60±0.26
	JM	32.70±0.79	3.33±0.15	31.93±0.97	23.33±0.52	18.47±0.52	20.47±0.48
10 Gy	PN	27.67±1.11	2.93±0.27	21.67±0.70	20.60±0.44	11.43±0.41	14.27±0.55
	H-76-1	29.37±0.49	2.73±0.39	24.00±0.78	23.33±0.55	13.13±0.32	14.07±0.57
	POC	30.07±0.85	2.60±0.30	29.80±0.60	20.33±0.49	11.47±0.49	12.00±0.73
	JM	26.73±0.86	2.50±0.15	29.27±0.67	21.37±0.55	15.27±0.52	13.37±0.84
15 Gy	PN	23.40±0.51	2.97±0.12	14.77±1.00	18.40±0.52	11.00±0.40	7.40±0.49
	H-76-1	25.80±0.89	2.33±0.13	18.00±0.40	21.17±0.24	13.17±1.20	5.50±0.91
	POC	26.03±0.82	2.60±0.35	21.00±0.35	17.67±0.41	11.13±0.32	6.30±0.61
	JM	24.07±0.81	1.93±0.09	18.50±0.61	19.13±0.58	12.57±0.29	7.30±0.79
20 Gy	PN	21.50±0.58	2.38±0.19	7.37±0.44	12.23±0.47	5.63±0.32	5.37±0.55
	H-76-1	-	-	-	-	-	-
	POC	-	-	-	-	-	-
	JM	-	-	-	-	-	-
Treatment mean	0 Gy	34.9	5.12	25.97	23.6	16.34	20.8
	5 Gy	33.65	3.29	28.56	22.6	16.5	20.91
	10 Gy	28.46	2.69	26.18	21.41	12.83	13.43
	15 Gy	24.83	2.46	18.07	19.09	11.97	6.63
	20 Gy	21.5	2.38	7.37	12.23	5.63	5.37
Genotype mean	PN	27.7	3.38	18.31	19.33	11.35	13.17
	H-76-1	24.87	2.55	19.81	18.39	11.31	12.32
	POC	25.55	2.65	20.61	17.01	11.63	12.65
	JM	23.65	2.73	21.77	17.08	12.95	12.34
CD at 5%	Treatment	1.03	0.28	1.07	0.59	0.62	0.55
	Genotype	0.92	0.25	0.95	0.53	0.56	0.49
	T x G	2.06	0.57	2.13	1.18	1.25	1.1

#Data represent the mean ± standard error of three independent determinates. *Arc Sin $\sqrt{}$ % transformed values (PN: Pusa Navrang; H-76-1: Hybrid 76-1; POC: Pearl of Csaba; JM: Julesky Muscat)

irradiation dose. The maximum leaf area was observed in 5 Gy irradiation dose among irradiation treatment. Among genotypes, the mean leaf area of Pusa Navrang (19.33 cm^2) was highest followed by H-76-1 (18.39 cm^2) genotype and the lowest leaf area was registered in Pearl of Csaba (17.01 cm^2). Similar effects of higher gamma irradiation doses were reported by Islam et al. (2015) and Surakshitha et al. (2018), who observed smaller and narrower leaves with reduced leaf area in grape cultivars.

Root length and number of roots per plant

These traits were decreased gradually with an increase in radiation dose from 10 to 20 Gy (Table 2). Significant root stunting (65.54%) and reduced, deformed roots were observed at the highest dose (20 Gy). The genotype Julesky Muscat showed the least reduction in root length among genotypes. The highest number of roots was recorded at 5 Gy, while, the lowest was at 20 Gy. In comparison, many roots with higher length were noted in the Pusa Navrang and Pearl of Csaba. At the same time, the lowest number of roots were recorded in H-76-1. The interaction between the irradiation dose and genotype indicated that the genotypes showed a decreasing trend for root number as the irradiation dose increased. Under the present study, a higher dose of gamma rays' exposure induces more pronounced effect on root parameters than shoot growth parameters. Roots become thin, short and distorted in the plantlets treated with the 20 and 25 Gy irradiation, and these roots could not support the plants. In the present study, grape plantlets treated with 5 Gy showed higher root length and number, aligning with findings of Islam et al. (2015) in grape and Gupta et al. (2018) in strawberry, indicating that low-dose irradiation promotes rhizogenesis up to a certain level; however, doses beyond the LD_{50} (10 Gy) were detrimental to root growth, as also reported earlier in both studies.

Total chlorophyll and total carotenoids content (mg g^{-1} FW)

Plantlets treated with 5, 10 and 15 Gy showed increased chlorophyll content by 10.53, 7.52, and 13.91%, respectively, and a 9.68% rise in total carotenoids at low doses (Fig. 1 & 2). In contrast, 20 Gy reduced chlorophyll by 14.66% and carotenoids by 56.80%. Pusa Navrang showed the highest total chlorophyll and carotenoid contents at 15 and 5 Gy, respectively. Overall, the lower doses enhanced pigment content, while, higher doses led to a decline, similar to the trends observed in gamma-irradiated strawberry plants (Gupta et al., 2018). Similarly, a decrease in chlorophylls and carotenoids content at higher irradiation exposure (2.5 kGy) was noted in *Arthrospira platensis* plants (Abomohra et al., 2016), while a gradual increase at low irradiation dose recorded in the red paper (Kim et al., 2004) and Lady finger banana (Sasipriya et al., 2023).

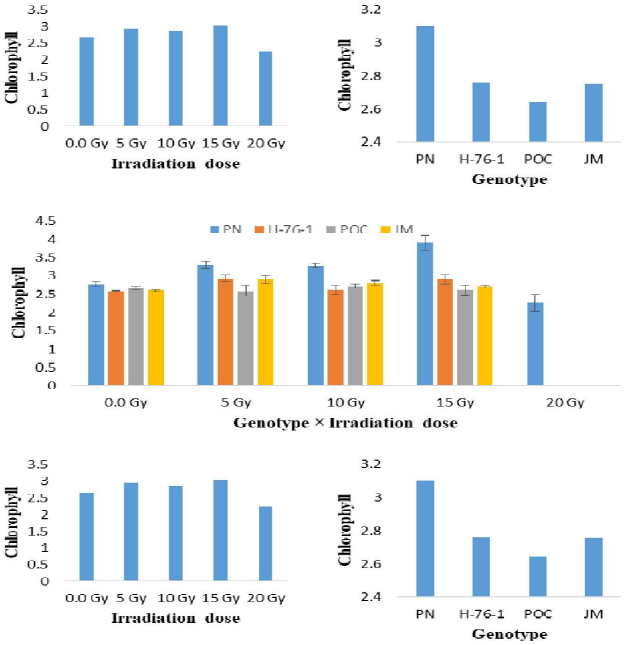

Fig. 1 : Effect of gamma irradiation dosages on the total chlorophylls content under *ex vitro* conditions

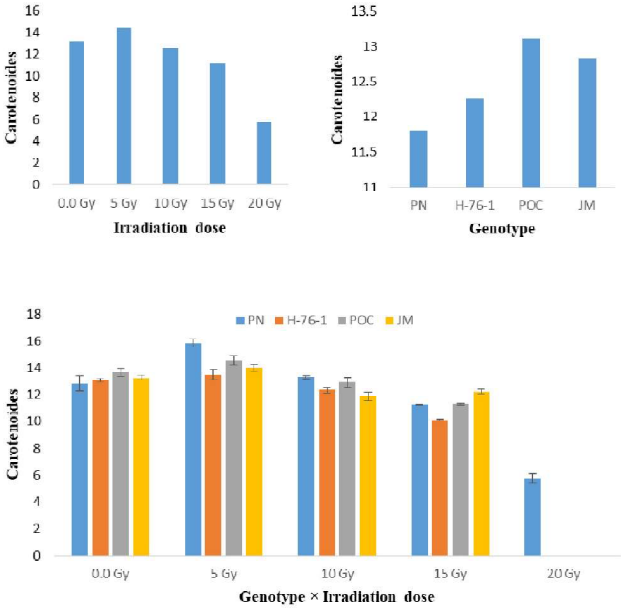

Fig. 2 : Effect of gamma irradiation dosages on the total carotenoids content under *ex vitro* conditions

Proline and total phenols content (µg g^{-1} FW)

Irradiation doses significantly increased proline and phenol content, with levels rising progressively with higher doses compared to the control (Fig. 3 & 4). A significant increase in proline (18.84%) and total phenol (8.37%) content was observed at 20 Gy compared to untreated plantlets. Among genotypes, Pusa Navrang showed the highest levels, followed by H-76-1, with the lowest in Pearl of Csaba. Similarly, Shabana et al. (2017) reported a two-fold increase in proline and elevated total phenols content in *Arthrospira platensis* under high gamma irradiation (2/kGy). Gamma irradiation generates reactive oxygen species, leading to oxidative stress that stimulates proline and phenolic compound synthesis as a plant defense mechanism (El-Beltagi et al., 2013). Proline aids in osmotic balance and cellular protection (Kaur & Asthir, 2015), while, both proline and phenolics help stabilize cells and reduce oxidative damage (Gill & Tuteja, 2010).

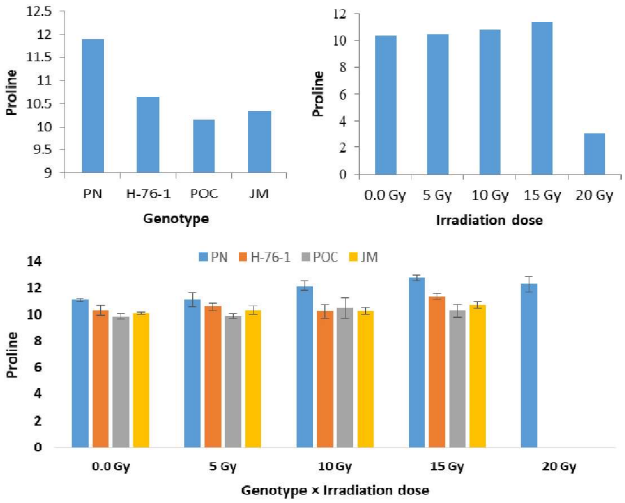

Fig. 3 : Effect of gamma irradiation dosages on the total proline content under *ex-vitro* conditions

Total soluble sugars (mg g^{-1} DW)

Non-irradiated plants generally showed lower total sugar content compared to all irradiated genotypes (Fig. 5). Total soluble sugars content increased with rising irradiation doses, *i.e.* 0.3% at 5 Gy, 0.54% at 10 Gy, 0.72% at 15 Gy and 2.78% at 20 Gy. Pusa Navrang showed the highest increase (1.23%) at 15 Gy compared to its control, while, the lowest sugars content was observed in H-76-1 at 10 Gy. The study found that gamma irradiation positively influenced total soluble sugars content in grape plants, likely due to enhanced metabolism (Bhatia & Mandaokar, 2020),

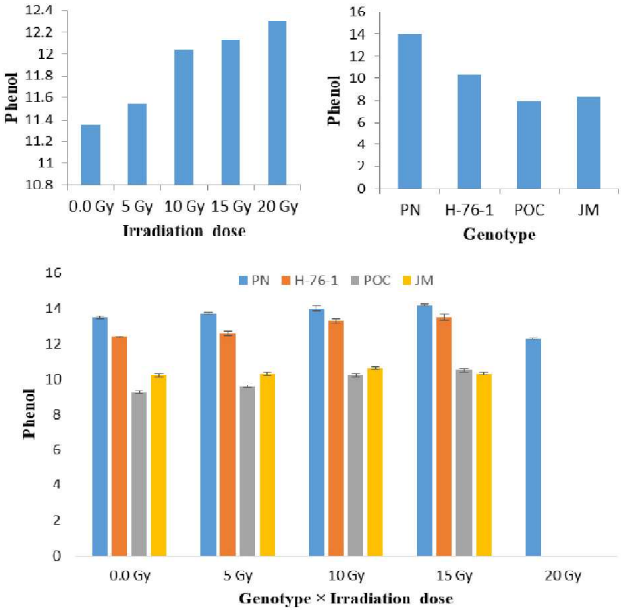

Fig. 4 : Effect of gamma irradiation dosages on the total phenols content under *ex-vitro* conditions

stress responses (Kim et al., 2004), increased photosynthesis (Ashraf et al., 2003), and genetic changes (Wi et al., 2007a), consistent with findings of Islam et al. (2015).

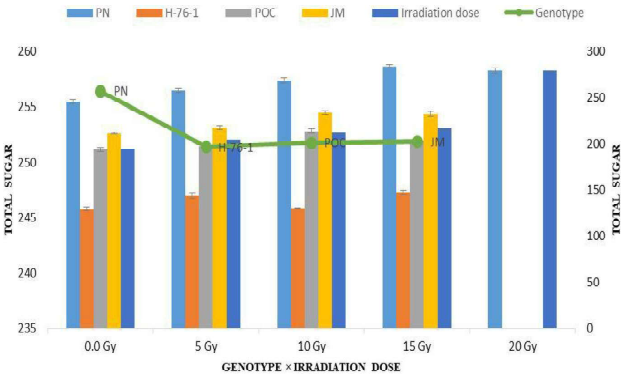

Fig. 5 : Effect of gamma irradiation dosages on the total sugars content under *ex vitro* conditions

Fron the results obtained it can be concluded that *in vitro* and *ex vitro* radiosensitivity was both genotype and dose dependent. Genotype Pusa Navrang was the least, and H-76-1 the most sensitive to high doses (>15 Gy). Pusa Navrang shows potential as a parent genotype in future grape breeding after genetic base enhancement *via* induced mutation. Lower gamma irradiation dose (5 Gy) positively influenced most of the morphological and biochemical traits, while, slightly higher doses (around 15 Gy) may be effective for generating stable mutants.

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