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Surakshitha NC
Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

M Ganga
Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

K Soorianathasundaram
Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Correspondence
Surakshitha NC
Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Radio-sensitivity of nodal segments of grape cv. 'Red Globe' to gamma rays under *in vitro*

Surakshitha NC, M Ganga and K Soorianathasundaram

Abstract
In order to ascertain the Irradiation sensitivity of grape cv. Red Globe, nodal segments were subjected to Gamma irradiation treatments (0, 3, 6, 9, 12, 15, 18, and 21 Gy) and inoculated on MS medium supplemented with BAP 2mgL^{-1}. The cultures were sub-cultured on the MS modified medium up to three cycles (M_1V_3) and rooting was induced in altered MS medium supplemented with IBA 2mgL^{-1} and activated charcoal 200 mgL^{-1}. The *in vitro* rooted plantlets were transferred to pots in polyhouse for hardening. Morphological observations revealed the mean explant survival was highest in 3 Gy dose (96.5%) among the irradiation treatments. However, as the irradiation dose was increased further, there was significant decrease in explant survival, regeneration rate, days to bud break and multiple shoot induction, number of shoots per explants, days to root initiation, shoot and root length along with significantly enhanced *in vitro* shoot and root abnormalities. The gamma irradiation dose of 18 Gy which resulted in 52.00% survival along with significant *in vitro* shoot abnormalities (33.75%) was adjudged as the lethal dose 50 (LD_{50}).

Keywords: gamma rays; grape; *in vitro* mutagenesis; and red globe

Introduction
Crop improvement efforts involving popular cultivars of fruit crops by conventional hybridization technique very often faces difficulties owing to highly heterozygous nature of parental varieties and many progenies derived from cross-breeding often express a large extent of undesirable attributes. There are also difficulties due to polyploidy, incompatibility, apomixis and long juvenile period, which limit the process of obtaining useful recombinants in perennial fruit kinds (Broertjes, 1977; Hansche and Beres, 1980) [5, 10]. Alternatively, mutation breeding could be resorted to obtain desired variants especially if the crop is clonally propagated as in the case of grapes. Plant mutations are defined as heritable changes in the DNA sequences that are not derived from genetic segregation or recombination (Van Harten, 1998) [23]. In contrast, induced mutations will help to alter and improve one or few specific traits of an established cultivar, without compromising the requirements of the fruit crop industry or the expectations of the consumers.

Tissue culture technique can be employed for improving effectiveness of induced mutations as it offers a broader choice of initial plant materials for treatment (*in vitro* nodal segments, organs, tissues, and cells) which are more suitable for induced mutagenesis as compared to *in vivo* buds. It also allows handling of large number of populations for mutagenic treatments furthering the selection process and cloning of variants. Besides, it shortens the propagation cycles and ultimately offers high phyto-sanitary conditions throughout the mutation process (Ahloowalia, 1998) [1]. Various types of radiations are available for mutagenesis such as ultraviolet radiation (UV) and ionizing radiation (X-rays, gamma-rays, alpha and beta particles, protons and neutrons). These ionizing radiation penetrates deeper into the tissue and can induce chemical changes by muations (Ahnström, 1977) [2]. Britt (1996) [4] outlined complete overview of the changes that can occur at the gene, chromosome, and genome levels, including chromosomal breaks, inversions, duplications, translocations, and point mutations. X-rays and gamma-rays have been the most widely employed ionizing radiations due to effectiveness in fruit breeding.

The foremost step in mutagenic treatment is the estimation of the most appropriate dose of radiation to apply and the procedure usually is based on radio sensitivity of plant material, which is estimated through the physiological response of the irradiated plant material. It involves the determination of the appropriate dose that will not cause more than 50% mortality

by impairing the growth of irradiated plant material (LD_{50}) when compared to non-irradiated plant material in the first vegetative cycle (vM_1) (Gaul, 1977; Broertjes *et al*, 1988) [9, 6]. Radio sensitivity varies with the plant species and the cultivar, the physiological condition of plant and organs and with the manipulation of the irradiated material before and after mutagenic treatments (D'Amato, 1992) [8].

Several studies have been conducted on the radio sensitivity of *in vitro* cultures of fruit crops. In Japanese plum (*Prunus salicina* Lindl.) cv. 'Shiro' micro cuttings irradiated with 10 to 40 Gy of gamma rays and 30 Gy was observed as LD_{50} (Predieri and Gatti, 2000) [20]. Grapevine rootstocks 'Fercal' and 'Gravesac' irradiated with gamma rays at doses of 10 to 60 Gy, the observed LD_{50} was 20 Gy and 30 Gy respectively (Lima da Silva and Doazan, 1995) [14]. Proliferating cultures of grapevine cultivars 'Albana' and 'Trebbiano Romagnolo' differed in their radio sensitivity with only the latter being able to tolerate 40 Gy. Shen *et al*. (1990) [22] irradiated kiwifruit (*Actinidia deliciosa* [A. Chev] Liang and Ferguson) cv. 'Hayward' and 'Clone 4' (*Actinidia chinensis* var. *hispida)* shoots with gamma rays and the LD_{50} was 80–90 and 50–60 Gy respectively.

With the above background indicating that a particular dosage standardized for one variety in a crop species may not be ideal for another variety of the same species, attempts were made to study the radio sensitivity of nodal segments of grape cv. Red Globe and to find out the effect of gamma rays on growth and development of irradiated nodal segments during *in vitro* culture. The grape cv. Red Globe is an introduced variety to India and is gaining popularity amidst consumers for its bold and attractive berries, moderate to high yield levels and fetches premium price in the market.

Materials and Methods

Plant material and irradiation

Actively growing, young shoots of grape cv. Red Globe were collected from a mature and healthy vine without any pest and disease infestation, maintained in the vineyard of college orchard, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. The collected shoots were excised into 1.5–2cm in length by removing off all leaves and separated as nodal segments. For mutation induction, physical mutagen (gamma rays) was used. Collected explants were irradiated with ^{60}CO gamma rays source at different doses (0, 3, 6, 9, 12, 15, 18 and 21 Gy) by wrapping the buds in butter paper at the gamma chamber facility maintained by the Department of Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore.

Explants preparation and In vitro mutagenesis

Irradiated nodal segments were washed thrice thoroughly with teepol followed by 0.5% carbendazim treatment. The nodal segments were then rinsed with 70 % ethanol for 30s followed by rinsing them with 0.5 % sodium hypochlorite for 10 minutes under laminar air flow. Nodal segments were thoroughly rinsed with sterile water in order to remove residues of sterilants. These explants were inoculated on freshly prepared MS basal media + BA 2mgl^{-1} in test tubes. After inoculation, the cultures were incubated in culture room with temperature of 25±2 °C and 16 h photoperiod conditions. The freshly prepared media was used for subculturing till the induction of multiple shoots. The multiple shoots were separated and subcultured to induce shoots of M_1V_1 to M_1V_3 and elongated shoots of 2-3 cm length were transferred to the modified media (½ MS with one and half times of $CaCl_2$ (6.8 gL^{-1}) and BAP 1 mgL^{-1}) with an interval of 3-4 weeks. For *in vitro* rooting, shoots were further subcultured on to fresh ½ MS media supplemented with 2 mgL^{-1} IBA (indole-3-butyric acid) and activated charcoal (200mgL^{-1}). Proliferated cultures of the gamma induced mutants were hardened *ex vitro* and transferred to green house for their further screening. A sequential illustration of *in vitro* mutagenesis undertaken in this present study is depicted in the Plate 1.

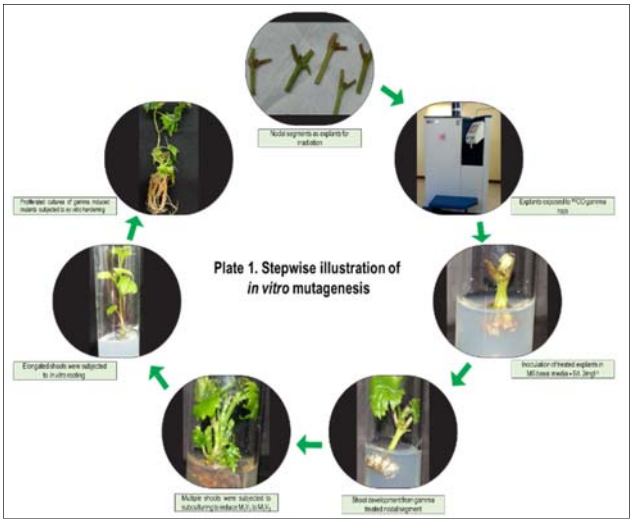

Plate 1

Criteria for optimum mutagenic doses and observations

The criteria of 50 % reduction in survival and regeneration relative to control were used for the estimation of optimum mutagenic doses. The nodal segments turning brown from green after culturing were considered as dead. The observations on per cent of mortality and survival per cent of nodal segments were recorded after inoculation. The days to positive response i.e. days taken for bud break in nodal segments were recorded during culture initiation. Observations on multiple shoot induction, length of shoot (cm), number of new shoots produced per explant and shoot abnormalities (%) were recorded during subsequent subculturing from the shoots subjected to different irradiation dosage Days to rooting, root length per shoot (cm) and root abnormalities were recorded after root initiation from the date of subculturing on to rooting media.

Results

There was concomitant increase in mortality levels and reduction in regeneration rates with the increase in radiation dosages. The highest mortality (53.4%) was observed at higher dosage of 21Gy and the mortality rates were found to be lower at reduced levels of radiation dosages (Fig. 1). The percentage of regeneration was also the lowest at 21Gy (47.9 %) while the highest regeneration rate (97.5 %) was observed at 3Gy and comparable with non-irradiated explants (Fig. 2). The reduction in regeneration rates over non-irradiated explants at 18 and 21 Gy were 39.2 and 49.6 per cent respectively (Fig. 2). As only 46.6% survival was observed at 21 Gy levels, the dosage of 18 Gy with 52 % survival was considered as LD_{50}. A sequential illustration of *in vitro* mutagenesis in the present study is depicted in the Plate 1.

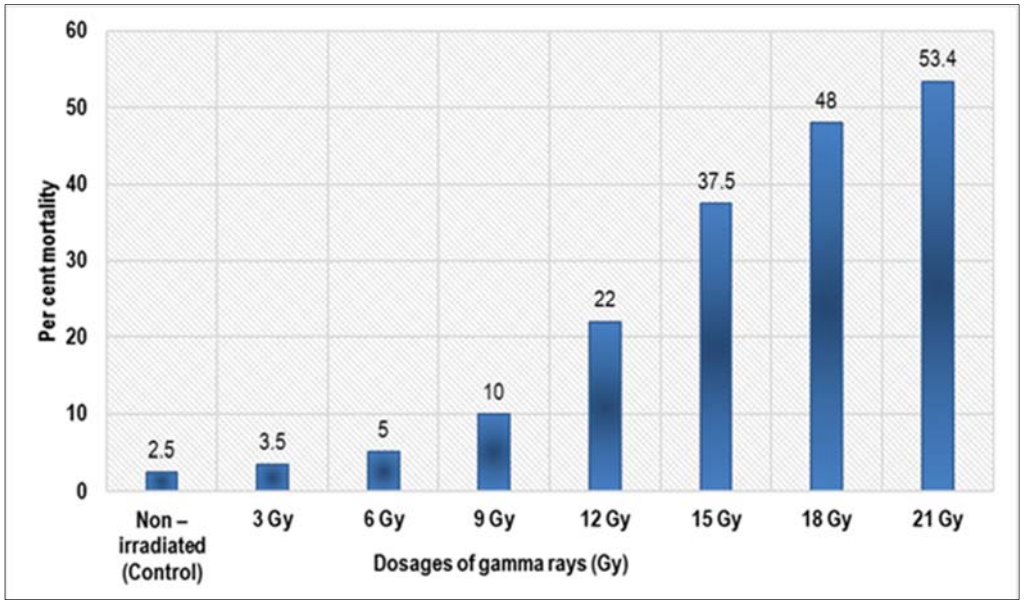

Fig 1: Effect of gamma rays on mortality percentage of nodal segments of grape cv. Red Globe

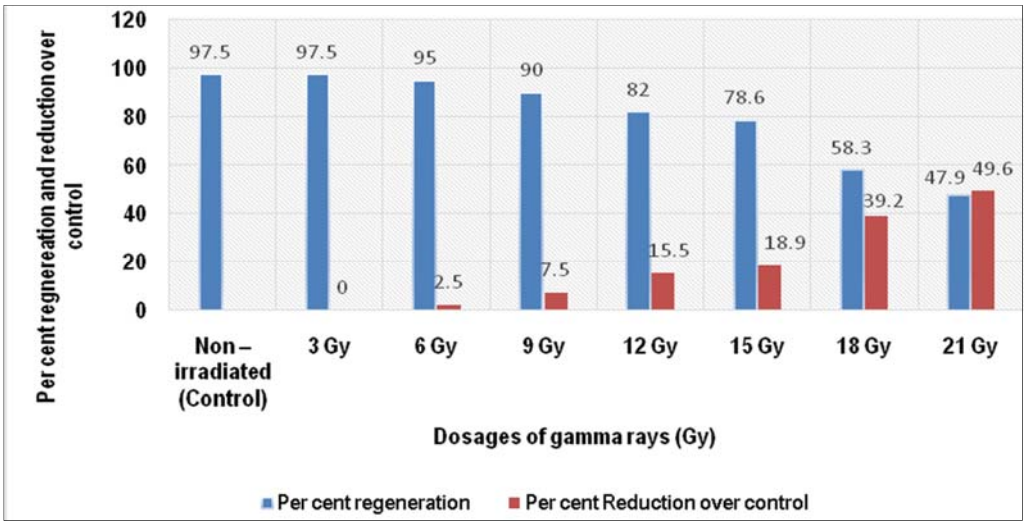

Fig 2: Effect of gamma rays on the regeneration percentage and their per cent reduction over control of nodal segments of grape cv. Red Globe

As the treatment dosage increased the days to bud break and multiple shoot induction also increased (Table 1). The days taken for bud break by non-irradiated control (7.28 days) and 3 Gy (8.59 days) treated explants was lower while 18 and 21 Gy irradiated explants took higher days (16.69 and 18.68 days respectively). The lowest number of days taken for multiple shoot induction was observed with non-irradiated control (37.23 days) followed by the lower dose of 3 Gy (40.15 days) while 18 and 21 Gy dosages took higher number of days for multiple shoot induction (58.19 and 61 days respectively). The length of shoot was found decreased with the increase in dosage (Fig 3). The shoot lengths were higher when no irradiation was given and up to 3 Gy (< 6% reduction). At 18 and 21 Gy the length of the shoots were reduced by 33.06 per cent and 46.69 per cent respectively.

Table 1: Effect of gamma rays on days to bud break and multiple shoot induction in nodal segments of grape cv. Red Globe

Dosage	Days to bud break	Per cent increment over control	Days to multiple shoot induction	Per cent increment over control
Non- irradiated control	7.27	-	37.22	-
3 Gy	8.53	17.33	40.14	7.85
6 Gy	9.02	24.07	45.96	23.48
9 Gy	11.85	63.00	47.81	28.45
12 Gy	12.75	75.38	53.08	42.61
15 Gy	15.44	112.38	54.84	47.34
18 Gy	16.68	129.44	58.18	56.31
21 Gy	18.67	156.81	60.99	63.86
SE.d	0.19		0.64	
CD (P = 0.05)	0.42		1.38	

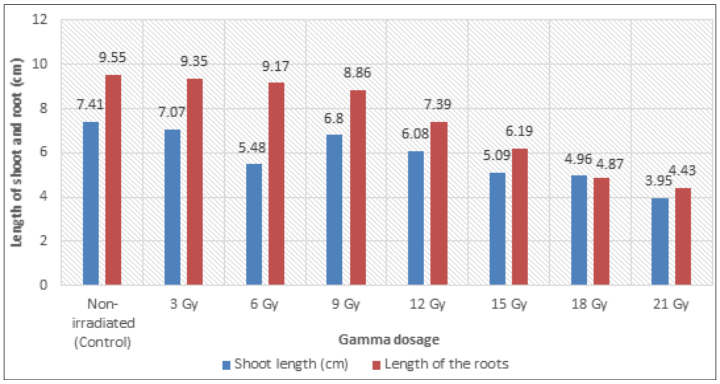

Fig 3: Effect of gamma rays on shoot length and root length of nodal segments of grape cv. Red Globe

The increase in treatment dosage negatively influenced the number of shoots per plant (Table 2). The highest number of shoots were observed in non-irradiated (2.85) explants and lowest was with 21 Gy (2). A higher percentage of abnormal leaves was found at 21 Gy (36.25 %) as compared to non-irradiated (0%) plants and at a dosage of 3 Gy (0%). Shoot abnormalities were to an extent of 28.75 per cent at the LD_{50} dose (18 Gy).

Table 2: Effect of gamma rays on number of shoot per plant and percentage of abnormal leaves in grape cv. Red Globe

Treatment	Number of shoots per plant	Reduction over control (%)	Abnormal leaves percentage (%)
Control	3.44	-	0.00 (0.00)
3 Gy	2.62	23.84	0.00 (0.00)
6 Gy	2.77	19.48	4.99 (12.90)
9 Gy	2.77	19.48	20.00 (26.55)
12 Gy	2.72	20.93	22.49 (28.30)
15 Gy	2.44	29.07	28.74 (32.40)
18 Gy	2.00	41.86	33.74 (35.50)
21 Gy	1.99	42.15	36.24 (37.00)
SE. d	0.28		0.24
CD (P = 0.05)	0.60		0.52

The duration to root initiation was also extended as the irradiation dosage increased (Table 3). The number of days taken for root initiation was lower in non-irradiated control (14.67 days) and at a lower dosage of 3 Gy (17.08 days) whereas it was more than 20 days at dosages higher than 9 Gy. The root lengths were also comparatively higher till 9 Gy dosage and the lowest root length was observed at 21 Gy (4.43 cm) (Fig 3).

Table 3: Effect of gamma rays on days to root initiation on grape cv. Red Globe

Treatment	Days to root initiation	Increment over control (%)
Control	14.66	-
3 Gy	17.07	16.44
6 Gy	17.62	20.19
9 Gy	21.55	47.00
12 Gy	24.67	68.28
15 Gy	27.20	85.54
18 Gy	29.08	98.36
21 Gy	32.22	119.78
SE. d	0.46	
CD (P = 0.05)	0.98	

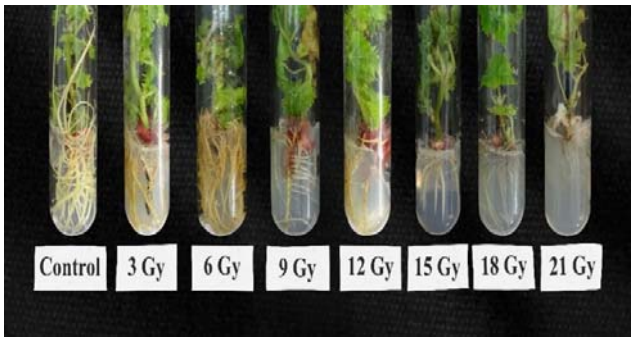

Plate 2: Effect of gamma rays on root parameters of grape cv. Red Globe

Discussion

The higher doses of gamma irradiation lead to an overall reduction in all parameters studied. The mortality percentage was increased with the increase in dosage whereas survival percentage and per cent regeneration were decreased with the increase in dosages. The 18 Gy dose was considered as LD_{50} based on the survival percentage (52%). The sprouted shoots with the higher dose exhibited stunting of shoots and variegated leaves and higher doses resulted in lethality of the explants within a few days of treatment. The inhibitory effect of higher doses of mutagens was earlier reported (Kuksova *et al.*, 1997; Coban *et al.*, 2002; *Mishra et al.* 2007) [12, 7, 15]. The decrease in survival percentage at higher doses of the physical and chemical mutagens may be attributed to disturbances at

cellular level caused either at physiological or physical level including chromosomal damages. It has been demonstrated that gamma irradiation at supra optimal levels may lead to radiation injury can manifest in several forms including a decline in sprouting ability and the number of plant organs (Lamseejan *et al.* 2000; Mwachukwu *et al*., 2009) [13, 18].

As the gamma rays dosage increased the number of days to bud break and multiple shoot induction were also extended. Further the length of shoot was decreased with increase in dosage levels. Other than decrease in shoot length, shoots also showed stunted growth along with the higher abnormal leaves at 18Gy and 21 Gy. Inhibition of cell division and meristematic activities could be the cause for the impaired vegetative parameters at higher dosages. Ndzana *et al*. (2008) [17] and Barakat *et al*. (2010) [3] also reported that increasing exposure to gamma-rays resulted in a significant decrease in plant height of *in vitro*-raised *Xanthosoma*. As the increase in dose there was a decrease in number of shoots per plant. The shoot numbers were higher and more than 20 % variability in the cultures in production of number of shoots in the different dosages up to 12 Gy as compared to 18 or 21 Gy. Mishra *et al.* (2007) [15] and Roongtanakiat (2012) [19] have also reported such detrimental influence of higher gamma dose levels in growth performances of two native Thai vetivers and in banana respectively.

Days to root initiation were extended as the irradiation dosage increased. There was a significant reduction in root length and abnormalities such as thin roots were higher with higher dosages beyond 9 Gy. In lower dose (3 Gy), not much variation in rooting was observed. At higher irradiation dose, the quality of roots was found to be thin and deteriorated and could not support the survival of the plants during hardening phase (Plate 2). Some of the variants observed in the study included dwarf, and rigid-thick-pubescent, mottle leaf and roots. These abnormalities could have resulted due to the disruption in the physiological process and genetic expression (Murti *et al.*, 2013) [16]. Kuksova *et al.* (1997) [12] suggested that mutagen doses also cause change in ploidy levels thus gives rise to abnormalities and increased abnormal vegetative growth. Coban *et al.* (2002) [7] had 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, 1977) [21]. It is opined that variation was probably an expression of the epigenetic activation of DNA elements (Kaeppler *et al.*, 2000) [11] or mutagen that affected the temporary steady state physiology of the plant.

From the observations made during the study, the LD 50 dosage for gamma radiation was standardized at 18 Gy for nodal cuttings of grape cv. Red Globe for further *in vitro* multiplication. Based on higher survival rates and root attributes the dosage levels up to 9 Gy appears to be much safer to recover a large number of plants with possible or potential minor mutations. If the researcher is interested in creating a large extent of variation then the nodal cuttings can be subjected up to 18 Gy.

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