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Research Article

Physiological variation of irradiated red radish plants and their phylogenic relationship using SCoT and CDDP markers

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Abstract

Greenhouse experiment is carried out to explore the outcome of γ-radiation on physiological and genetic variation in red radish (*Raphanus sativus*) for two generations. Gamma rays from ^{60}Co were used to penetrate red radish seeds with different dose levels (0.0, 10, 20, 40 and 80 Gy). Plants generated from irradiated seeds and from self-pollination of these plants, called M1 and M2 generations, respectively. Some morphological and physiological traits were then determined, and the genetic diversity of both generations was studied using Start Codon Targeted (SCoT) and Conserved DNA-Derived Polymorphism (CDDP) molecular markers. All studied morphological traits (number of leaves/plants, leave height, root diameter, and root weight) were steadily improved by raising irradiation dose rate, reaching a cumulative raise at the irradiation doe level 40 Gy and decreased at dose level 80 Gy. Photosynthetic pigments of red radish plants released a notable increase by increasing gamma rays dose level for chlorophyll (a), chlorophyll (b) and carotenoids for 40 Gy dose rate. Proline content was elevated proportionally to the irradiation dose level, with the greatest increase seen at dose level of 80 Gy. Moreover, phytochemical screening was detected for the both two generations. Fourteen SCoT primers generated a total number of banding patterns of 194 with average 13.86 and the primer SCoT-33 released the highest number banding patterns (21). The percentage mean of polymorphism for all the SCoT primers was 74.66% and was 66.49 and 63.74% for M1 and M2 respectively. Furthermore, fifteen CDDP primers generated a total number of banding patterns of 186 and the primer CDDP-5 relieved the highest number of banding patterns (20). The percentage mean of polymorphism for all the CDDP primers was 73.41% and was 64.38 and 65.91% for M1 and M2 respectively. It could be concluded that gamma irradiation exhibited an appropriate variation in red radish M1 and M2 which was detected by SCoT and CDDP molecular markers.

Keywords: gamma radiation; *Raphanus sativus*; phytochemical screening; pigments; proline; SCoT; CDDP

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Introduction

Radish (*Raphanus sativus)* is a root vegetable that belongs to the family Brassicacea (Cruciferae), and it's cultivated and consumed worldwide and considered to be part of the human diet even though it's not widespread in certain cultures. There are various skin colours for radishes (red, purple, black, yellow, and white to pink), though usually the flesh is white. Moreover, the radish edible roots differ in size, taste, and length around the world (Banihani, 2017). Glucosinolates, anthocyanins, isothiocyanates and phenolic acids, are among the phytochemicals found in radish extracts make radishes have significant health benefits, as it's viewed as protecting vascular smooth muscle cells, being anti-cancer, anti-inflammatory, and antidiabetic (Shin *et al.*, 2015; Manivannan *et al.*, 2019). Induced mutagenesis has been widely used as an effective tool to create desirable variations in traits of some plant species. Plant breeding uses many approaches, among them are induced mutagenesis to create new plant variants. This method is seen as a fast, low-cost, and reproducible technique to speed up the process of generating and viewing crop genotypes with recovered genomics (FAO/IAEA 2017). One of the most widely used physical mutagen is gamma radiation, which has been viewed as a reproducible, reliable, and quick tool to generate morphological and biochemical variations in plant (Jan *et al.*, 2012), hence, gamma radiation has gained an important role in plant breeding and genetic studies intended to progress yield and other advantageous characters in numerous crops (Aly *et al.*, 2019a). Moreover, Gamma radiation can cause variation in the molecular basis of the plants by causing DNA breaks, this can be analyzed by using DNA-based assays to directly identified genotypes (Mengoni *et al.*, 2000). Possibilities in mutation breeding have been increased enormously with the advancements made in the field of genomics and with the developments made in molecular markers techniques. Also, the use of molecular markers in mutation breeding can give us a better insight into the molecular variations that are caused by irradiation with gamma rays, thereby making them ideal for efficient crop breeding, as molecular markers pose as a quick, reproducible, and cost- efficient tool for high throughput screening of mutations in plants (Gaafar *et al.*, 2016). Because of the advancement in molecular systematic approaches, molecular markers appear to be effective methods in assessment of genetic diversity compared to morpho-chemical characterizations (Agarwal *et al.*, 2019). The SCoT and CDDP molecular markers have been developed by Collard and Mackill (2009a and b), those two molecular markers have become one of the most effective gene targeting marker systems in plants. Collard and Mackill (2009a) established SCoT polymorphism as a major marker to identify mutant genotypes as it is relying on the short-conserved regions flanking the ATG start codon of plant genes are needed. Therefore, they can be useful for many applications in plant genotyping and QTL mapping as they are able to screen for polymorphism that might be directly related to gene functions since they based on transcribed regions of the plant genome (Collard and Mackill, 2009c; Tiwari *et al.*, 2016). Marker as SCoT has many advantages over the other commonly used molecular markers for instance AFLP, RAPD and ISSR, as it has been shown to have more reproducibility, stability, as well as producing dependable bands. In addition, it's easy to design, highly polymorphic, and provides ample genetic information of the plant (Satya *et al.*, 2015; Gupta *et al.*, 2019). Such advantages are validated in the latest researches made for the mango genetic diversity, (Zhou *et al.*, 2020), *Manilkara* (Vanijajiva, 2020), *Vitis vinifera* (Yue *et al.*, 2019), kiwifruit (Chen *et al.*, 2018). Furthermore, SCoT markers have shown great importance in the identification of genetic variation and characterization of irradiated grape mutant materials (Yue *et al.*, 2019). Otherwise, the CDDP method for generating plants DNA markers based on the information mined for small conserved amino acid sequences present in plant proteins (Mokhtar and Atia, 2019). Such gene-targeted techniques via the use of a gene or promoter in their primers, SCoT and CDDP were created to merge the achievement of marker procedures with modern practical originality, also give greater replication and declaration, by the instantaneous happening of dominant and co-dominant markers (Abouseadaa *et al.*, 2020). Therefore, in the current study an effort was made to study the genetic diversity using gene targeted (SCoT and CDDP) markers and compare their effectiveness in genetic variation in red radish seeds which were exposed to four levels of γ-rays doses (10, 20, 40 and 80 Gy) then subjected to field trials for two seasons. As well as some physiological and phytochemical characteristics were determined to understand the irradiation effects on plant biochemistry characteristics were concerned.

Materials and Methods

Gamma irradiation

Healthy and dry radish seeds (*Raphanus sativus* L.) which obtained from Gaara and Parteners Company, Bab El-Khalk, Cairo, Egypt. were packed in polypropylene bags and then irradiated by using ^{60}Co as a source for irradiation with gamma doses (0.0, 10, 20, 40 and 80 Gy) at a dose rate of (0.30 Gy/Sec) using the research irradiator (^{60}Co Gamma cell 220 Canada), EAEA, National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt.

The research was conducted at the NCRRT greenhouse during fall seasons of 2019/2020 to investigate the response of red radish to gamma irradiation and the physiological and genetic variation. The M1 radish seeds were planted in the experimental farm, they placed 70 cm apart and 30 cm between seeds on both ends of the rows and M1 plants were self-pollinated to produce the M2.

Plant vegetative growth characters

A random sample size of six for both two generations plants from each treatment was gathered at the horticultural ripeness stage 45 days from sowing. The radish was then deracinated carefully and washed with tap water to remove any residue soil from the deracinated roots. The gathered radish samples were utilized to measure certain vegetative growth parameters, those being, number of leaves, leaves length, root diameter and root weight.

Determination of photosynthetic pigments

Radish leaves chlorophyll a, b, and carotenoids were measured following a spectrophotometric technique Vernon and Seely (1966). Pigments concentration was evaluated in milligrams per gram of FW.

Proline content

Concentration of proline was measured according to Bates *et al.* (1973) method. The findings were calculated in mg of proline per gram fresh weight.

Preparation of plant extract

Briefly, 2.0 g of the fresh root samples were grinded with liquid nitrogen after 25 ml of 80% ethanol and shaking for 24 h at room temperature. Whatman filter paper number one was used to filter the extracts then the extraction was repeated twice (Sobhy *et al.*, 2009). The resulting ethanolic extracts volume were adjusted and used for the analysis of the phytochemical screening.

Phytochemical screening analysis for the fresh radish roots

Various plant compounds were screened using standard analysis for the ethanolic extract. Secondary metabolites like phenols, flavonoids, tannins, coumarins, xanthoproteins, quinones, phlobatannins, saponins, amino acids, alkaloids, carbohydrates, phytosterol, and cholesterol were screened in the extracts for various irradiation dose levels as suggested before by (Trease and Evans 1989; Harborne, 1973; Idu and Igeleke, 2012).

DNA extraction

Genomic DNA was isolated from the received fresh leaves (~100 mg) sample using a DNeasy Plant Mini Kit (QIAGEN, Santa Clarita, CA), following the producer's procedure. Purified DNA was then quantified using Nanodrop 8000 (Thermo Fisher Scientific Inc.).

SCoT and CDDP PCR data analysis

The SCoT PCR amplification analysis was done according to Bhawna *et al.* (2017), as well as CDDP PCR amplification analysis was done according to Collard and Mackill (2009b). Ten DNA samples corresponding to each treatment from each generation were analyzed for genetic diversity using a set of 14 SCoT primers (Table 1) and 15 CDDP primers. The PCR amplification reaction was performed in reaction mixture included one X Taq polymerase PCR buffer, one U TaKaRa Taq™ DNA Polymerase (Takara Bio Inc.), 2.0 µM of the SCoT and CDDP primer respectively, 2.5 mM $MgCl_2$, 0.2 µM of each dNTPs and 50-100 ng of DNA gnomic. Nuclease free water was added to maintain a final reaction mixture volume of 25 µl. The PCR conditions for SCoT was adjusted with a preliminary denaturation at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 95 °C for one minute, annealing for one minute at 50 °C, and extension at 70 °C for 90 seconds. This was finalized with a final extension at 70 °C for 7 minutes. Also, the PCR conditions for CDDP at the standard typical PCR cycle was used, which included a 3-minute denaturation stage at 94 °C, followed by 35 cycles of 94 °C for one minute, 50 °C for one minute, and 72 °C for two minutes, with a 5-minute last extension. The augmented PCR products were then electrophoresed on 1.5 and 1.2% agarose gel for SCoT and CDDP primers, respectively comprising of ethidium bromide in one X TAE buffer. Amplified PCR products size was measured using a 100 - 1500 bp DNA ladder-plus. The PCR for both primers SCoT and CDDP samples ordered in ascending way (sample-1 to sample-10) during the loading on the agarose gel. Visualization of the PCR products were carried out under UV light and gel images analysis and phylogenetic were achieved using the Python-based tool called PyElph. The base sequences of the DNA primers that generated revealing polymorphic amplicons are listed in Table 1. Used primers sequences that produced polymorphic amplicons are displayed in Tables 4 and 5.

Table 1. SCoT and CDDP primers used in the PCR and their nucleotide sequences

No.	SCoT primers	Primer's sequence	CDDP primers	Primer's sequence
1	SCoT-31	CCATGGCTACCACCGCCT	CDDP-1	TGGCGSAAGTACGGCCAG
2	SCoT-33	CCATGGCTACCACCGCAG	CDDP-2	GTGGTTGTGCTTGCC
3	SCoT-34	ACCATGGCTACCACCGCA	CDDP-3	GCCCTCGTASGTSGT
4	SCoT-35	CATGGCTACCACCGGCCC	CDDP-4	GCASGTGTGCTCGCC
5	SCoT-36	GCAACAATGGCTACCACC	CDDP-5	TGSTGSATGCTCCCG
6	SCoT -43	CAATGGCTACCACCGCAG	CDDP-6	CCGCTCGTGTGSACG
7	SCoT -51	ACAATGGCTACCACTGTC	CDDP-7	GGCAAGGGCTGCCGC
8	SCoT -52	ACAATGGCTACCACTGCA	CDDP-8	GGCAAGGGCTGCCGG
9	SCoT -69	ACCATGGCTACCAGCGCA	CDDP-9	CACTACCGCGGSCTSCG
10	SCoT -71	CCATGGCTACCACCGCCG	CDDP-10	GCSGAGATCCGSGACCC
11	SCoT -77	CCATGGCTACCACTACCC	CDDP-11	TGGCTSGGCACSTTCGA
12	SCoT-84	CAACAATGGCTACCACGA	CDDP-12	AAGGGSAAGCTSCCSAAG
13	SCoT-86	CAACAATGGCTACCACGC	CDDP-13	CACTGGTGGGAGCTSCAC
14	SCoT-89	CAACAATGGCTACCACGG	CDDP-14	AAGCGSCACTGGAAGCC
15	-	-	CDDP-15	ATGGGCCGSGGCAAGGTGC

Statistical analysis

Three replicates were used in a complete randomized design and the data were displayed as mean ± standard error. Statistical analysis was carried out using one-way ANOVA, and the differences in means were compared using Duncan's multiple range tests (1955) at $p \leq 0.05$.

Molecular analysis

GelAnalyser3 software was used to analyze the DNA banding patterns produced by each primer. Obvious amplicons were scored as present (1) or absent (0) in a binary matrix for every primer. From this matrix, resolving power (R_p) was computed, in accordance with Prevost and Wilkinson (1999), and was estimated following the equation $R_p = \Sigma I_b$, where $I_b = 1 - (2 \times |0.5 - p|)$, and p is the genotypes proportion that contains an amplicon. Diversity index (DI) and Polymorphic Information Content (PIC) were determined in accordance with Gorji et *al.* (2011). Similarity estimates were analyzed using UPGMA (Unweighted pair group method using arithmetic averages). Dice coefficient was then used to calculate the molecular distance (Dissimilarity) (Nei and Li, 1979). A dendrogram was then created based on the similarity data using XLSTAT.7 software.

Results and Discussion

Growth parameters

The response of gamma irradiation at different dose levels (0.0, 10, 20, 40 and 80 Gy) on growth parameters in both M1 and M2 generations of radish plant was studied. The obtained results as outlined in Table 2 showed that a remarkable increase of growth parameters by raising exposure dose where the greatest raise was observed at the dose level of 40 Gy and decreased at dose level 80 Gy. The obtained results as outlined in Table 2 showed that irradiation dose level increased the number of leaves/plants, leaves length, root diameter and root weight by increasing irradiation dose level till the dose level 40 Gy (12.87, 54.51 cm 4.87 cm, and 112.49 g), respectively for M1 generation and a similar trend was obtained in M2. Irradiating plants with gamma rays thus show a great effect on the growth parameters of the supposed plant, as it can have stimulatory effects of such morphological aspects and increase yield or height of the plant. This stimulation depends on the dosage of the γ-rays used and the irradiation rate. Generally, it's seen that irradiated plants with low doses of γ-rays can enhance growth, cell division, and development in many organisms. Plant growth and development, as well as their adaptation to force challenges, are managed by a number of mechanisms that rely on proper mobilization of growth hormones, cell cycle control, enhancement of relevant enzymes, and adequate nutrient supply as well as the timely appropriate expression of genes that control development phenomena (Salehin *et al.*, 2015). Plants may have their growth traits improved by increasing growth influencing factors and related characteristics. Gamma rays have long been known to improve the growth characteristics of many efficiently significant plants, particularly at low levels (Majeed *et al.*, 2018). Otherwise, Ilyas and Naz (2014) detected enhanced leaf, root, and shoot development in *Curcuma longa* treated by 60 Gy. Low dose levels of irradiation have been shown to improve plant germination and development, whereas high dose levels result in growth defects, delay in germination, or death of irradiated plants (Majeed *et al.*, 2016; Hong *et al.*, 2017). Growth promoting characteristics and germination of plants subjected to low doses of γ-irradiation can be due to beneficial mutational effect on genes controlling such characteristics, such as fast DNA repair, and stimulation of hormones and enzymes associated with germination and growth mechanisms (Majeed *et al.*, 2018). Recently, Ahumada-Flores *et al.* (2021) declared that gamma irradiation induced changes at morphological, physiological, and agronomical levels in wheat.

Table 2. The mean morphological traits for M1 and M2 generations of red radish (*Raphanus sativus*) affected by gamma irradiation (Gy)

Irradiation dose level (Gy)	Leaves number	Leaves length (cm)	Root diameter (cm)	Root weight (g)
	First generation (M1)			
0	6.45C±0.605	39.37D±0.507	2.96C±0.191	69.42D±1.045
10	6.86C±0.613	43.737C±0.144	3.67B±0.243	78.61C±1.239
20	9.49B±0.487	47.653B±0.461	3.803B±0.026	95.13B±1.365
40	12.87A±0.517	54.517A±0.598	4.87A±0.338	112.49A±1.96
80	6.31C±0.22	36.81E±0.507	2.597C±0.178	73.55D±1.274
Second generation (M2)				
0	7.04C±0.499	42.66C±0.662	2.95B±0.219	69.08D±1.653
10	6.5C±0.318	45.58B±0.846	3.54B±0.219	78.94C±1.323
20	9.94B±0.454	47.53B±0.478	3.78B±0.338	95.80B±1.726
40	13.69A±0.504	52.90A±0.901	5.24A±0.618	116.49A±1.238
80	6.783C±0.372	37.37D±1.013	3.01B±0.143	72.89D±1.349

Data are given as mean ± SE and various letters within the same column are significantly differences ($p \leq 0.05$). M1; First generation, M2; Second generation

Photosynthetic pigments

The combined graphs in Figure 1 illustrate the effect of gamma irradiation on photosynthetic pigments of radish plants leaves. It is cleared that increasing gamma rays increased the photosynthetic pigment especially at 20 and 40 Gy dose levels. A notable decrease was observed with increasing dose to 80 Gy for both generations. Moreover, an increase in chlorophyll a content was noticed to be higher than the amount of chlorophyll b in un-irradiated and irradiated plants. However, the chlorophyll content was seen to significantly increase with the increase of irradiation dose compared to the control. At the vegetative stage, the results showed that under all gamma irradiation dose levels chlorophyll a was relatively higher than chlorophyll b and the increase in gamma doses increased the difference between them may be due to the more inhibition in chlorophyll b synthesis. The results of the current study concordance with past studies made on the effects of irradiation on pigment content. The current results are in a harmony with Akshatha *et al.* (2020) who found significantly higher chlorophyll contents have been observed in seedlings treated with radiation compared to the control. This is in concordance with Mohajer *et al.* (2014), who reported an increase in photosynthetic pigments of the plant *Onobrychis viciifolia* when exposed to different doses of gamma irradiation compared to un-irradiated plants. According to Majeed *et al.* (2010), enhanced leaf chlorophyll concentrations and grain yield of *Lepidium sativum L.* at low doses may be the product of improved water and mineral absorption attributable to cell division and enzyme activity enhancement through seed growth. In addition, Zabalza *et al.* (2006) have suggested that the enhancing effect of radiation on chlorophyll concentration is due to the stabilization of the energetic spot of the enzyme and photosynthetic responses. Marcu *et al.* (2013) reported a change in pigment concentration and structure in lettuce (*Lactuca sastiva*) seeds that were irradiated with a gamma radiation at doses ranged from 2 to 70 Gy. This irradiation was detailed to enhance the photosynthetic pigment content at lower doses (2-30 Gy) while at higher doses (70 Gy), the photosynthetic pigments decreased significantly. It was hypothesized that elevated levels of γ-irradiation have the ability to break the photosynthetic pigments, with the accompanying crippling of the photosynthetic capability. The enhanced levels of chlorophyll a and b might be attributed to the enhanced biosynthesis of such pigments in response to the low doses of gamma rays, and/or due to a delay in its degradation process (Aly *et al.*, 2018). Moreover, Saha *et al.* (2010) assumed that the observed lower levels in chlorophyll b compared to chlorophyll a, in response to higher levels of gamma radiation, might be attributed to deprivation to its biochemical pioneers or due to disruptions in its biosynthesis. Gaafar *et al.* (2016) have planned that such mutation achieved might have established variations in the structure of the plant cells and its metabolites such as dilation of the thylakoid membranes, modification of photosynthesis and accumulation of pigments which would afterward cause a modify of color and textures of the plant.

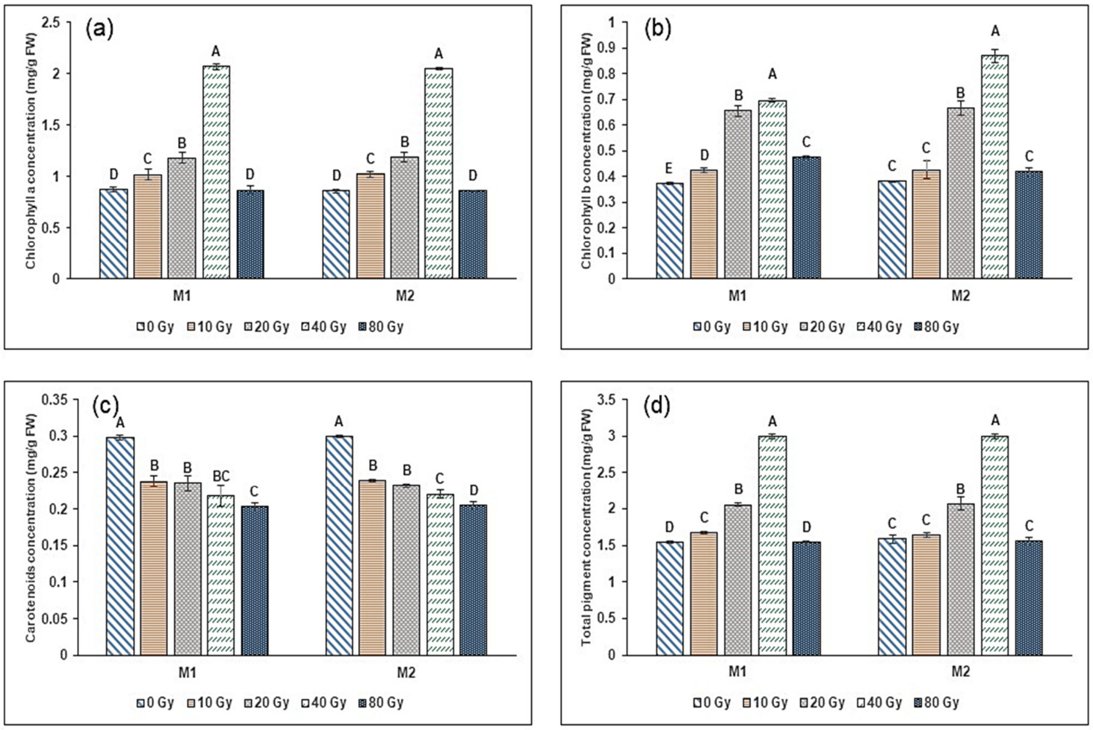

Figure 1. Chlorophyll a (a), chlorophyll b (b), carotenoids (c) and total pigments (d) as affected by gamma irradiation for M1 (First generation) and M2 (Second generation)

Data are given as mean (n = 3). Vertical bars show ±SE and various letters on the bars indicate significantly differences at $p \leq 0.05$.

Proline content

Proline content expanded as the irradiation dose rate increased and come to the most extreme increment within the gamma ray level 80 Gy as well as proline content was more pronounced in M2 than M1 (Figure 2). The current study confirms the function of proline as a compatible solute by increasing proline concentration with increasing radiation level, indicating that proline participates in an important role in tolerating the effects of gamma ray. The findings of the current research are in agreements by Akshatha *et al.* (2013) who reported that proline content increased with increasing irradiation doses in *Terminalia arjuna* Roxb. Furthermore, Aly *et al.* (2018 and 2019a) detected that exposure to gamma rays significantly increased proline accumulation in wheat leaves. Radiation stimulates formation of reactive oxygen species (ROS), which is extremely toxic to plant cells. Proline can also be accumulated in a wide variety of organisms as a cytosolic osmoticum, which keeps cells from different environmental stresses with adjusting the osmotic power of the cytosol with that of the vacuole and the exterior atmosphere, in the meantime, as a no enzymatic antioxidant. Proline can scavenge some reactive oxygen species, therefore can stabilizing the structure and role of macromolecules like proteins, lipids and DNA (Wang *et al.*, 2017). The greater proline substances established in plants subjected to extreme and moderate stress might have an important function in plant mending (Khaleghi *et al.*, 2019).

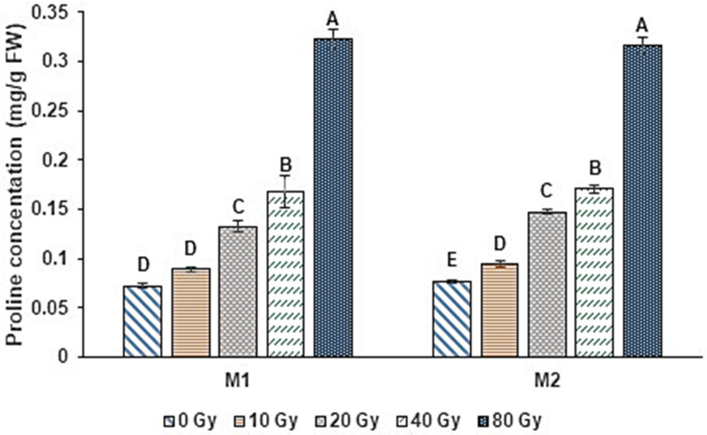

Figure 2. Proline content for M1 and M2 generations of red radish (*Rapha sativus*) as affected by gamma irradiation (Gy)

Data are given as mean ± SE (n = 3). Various letters on the bars indicate significantly differences at $p < 0.05$.

Phytochemical screening of the ethanolic extract

Phytochemical screening results of the ethanolic extract of the fresh red radish roots are given in Table 3. The qualitative results were expressed and ranged from presence/positive reaction (+) weakly positive to strong positive reaction (+++) with higher intensity (more concentrated reactions) of phytochemicals. The results showed the presence of phytochemically active compounds such as phenols, flavonoids, tannins, coumarins, xanthoproteins, quinones, phlobatannins, saponins, amino acids, alkaloids, carbohydrates, phytosterol and cholesterol. In the present, study the findings indicated that γ-rays increased gradually the concentrations of the most phytochemical constituents by increasing irradiation dose level. This is in concordance with Vardhan and Shukla (2017) and El-Beltagi *et al.* (2013) who detected that low dose of γ-irradiation may be successful in increasing the development of secondary metabolites. And this is hypothesized to be due to enhance activity of certain biosynthetic enzymes. In the same manner, the effects of various γ-rays doses (ranging from 5 to 40 Gy) on the synthesis of alkaloids and with increasing irradiation doses, the alkaloids content increased as explored by Mohajer *et al.* (2014). Meanwhile, El-Garhy *et al.* (2016) proved that there was a connection between the enhancement of flavonoid substances and upregulation of chalcone synthase (CHS) genes in response to γ-rays. Also, demonstrating that the upgrade in flavonoids substance is went with an increment in CHS expression. Further, the increase in different phenolics and flavonoids can help to mitigate the radiation stress-induced damage. Each phytochemical has a distinct biological action, that may lead to the development of new composites as anti-microbials combat pathogens or to help improve human health (Hassan *et al.*, 2019).

Molecular variation assessment of SCoT and CDDP DNA Markers

The genetic diversity and phylogenetic relationships of the irradiated M1 and M2 red radish were analyzed using SCoT and CDDP markers. Irradiated *Rapha sativus* M1 and M2 generations using SCoT marker compared with CDDP marker, fourteen SCoT and fifteen CDDP of the tested primers on red radish sample gave prominent and reproducible amplicons. These primers were selected to perform PCR and obtained banding patterns of these techniques were shown in (Figures 3-5).

The molecular data obtained from banding patterns analysis of SCoT and CDDP (Tables 4 and 5) showed that the SCoT-primers targeted 194 scorable amplicons. Where, a number of amplicons per SCoT-primer ranged from 6 for SCoT-86 to 21 for SCoT-33, as shown in Table 4. The proportion mean of polymorphism for all the used SCoT-primers was 74.66% and were 66.49 and 63.74% for M1 and M2, respectively (Table 4). Also, the molecular size of all targeted ranged from 163 to 2572 bp and the polymorphic percentage ranged from 33.3 to 85.7% in M1 while in M2 ranged from 16.7 to 82.4%.

Table 3. Phytochemical screening of M1 and M2 fresh red radish roots as affected by gamma irradiation

Chemical constituents	Irradiation dose levels (Gy)									
	M1					M2				
	Control	10	20	40	80	Control	10	20	40	80
Phenol	++	++	+++	++++	++++	++	++	+++	++++	++++
Flavonoids	++	+++	+++	++++	++++	++	+++	+++	++++	++++
Tannins	+	+	+	++	++	+	+	+	++	++
Coumarins	+++	+++	+++	++++	++++	+++	+++	+++	++++	++++
Xanthoproteins	+++	+++	++++	++++	++++	+++	+++	++++	++++	+++
Quinones	+++	+++	++++	++++	+++	+++	+++	++++	++++	+++
Phlobatannin	+++	+++	++	++++	+++	+++	+++	++	++++	+++
Saponins	++	+++	+++	++++	++++	++	+++	+++	++++	++++
Amino acids	++	+++	+++	++++	+++	++	+++	+++	++++	+++
Alkaloids	++	++	+++	++++	++++	++	++	+++	++++	++++
Carbohydrates	++	++	+++	++++	++++	++	++	+++	++++	++++
Phytosterol	++	++	++	+++	+++	++	++	++	+++	+++
Cholesterol	+++	+++	++	++	++++	+++	+++	+++	++	++

+++ Abundant, ++ moderately presence, + present

Table 4. Molecular data estimated from banding patterns of SCoT technique

Primer	**Molecular size range bp**			**All TA**	**Pol%**	**PIC**	**Rp**	**M1**						**M2**					
								TA	**A+**		**A-**		**Poly**	**TA**	**A+**		**A-**		**Poly**
									PA -	**UA -**	**PA +**	**UA +**			**PA -**	**UA -**	**PA +**	**UA +**	
SCoT-31	345	:	2549	17	82.4	0.296	7.6	14	2	4	2	2	71.4	16	4	4	4	1	81.3
SCoT-33	223	:	2572	21	85.7	0.314	10.0	17	5	2	4	3	82.4	19	6	1	5	3	78.9
SCoT-34	363	:	1994	12	66.7	0.228	4.2	11	1	0	4	1	54.5	11	3	0	2	1	54.5
SCoT-35	321	:	2515	17	82.4	0.251	5.8	9	2	0	1	3	66.7	17	2	1	3	8	82.4
SCoT-36	163	:	1913	16	87.5	0.379	10.2	14	5	0	7	0	85.7	16	7	1	4	0	75.0
SCoT-43	244	:	2040	20	80.0	0.285	8.6	18	4	3	4	2	72.2	17	4	2	4	3	76.5
SCoT-51	266	:	2045	13	92.3	0.32	6.0	12	3	2	5	0	83.3	13	2	2	3	2	69.2
SCoT-52	309	:	1486	12	83.3	0.297	5.2	9	0	2	3	1	66.7	12	2	0	6	1	75.0
SCoT-69	219	:	1275	12	66.7	0.258	5.0	12	2	0	4	1	58.3	11	2	3	2	0	63.6
SCoT-71	298	:	1739	15	66.7	0.195	4.0	13	3	2	1	2	61.5	15	2	0	1	4	46.7
SCoT-77	254	:	1292	10	70.0	0.25	3.8	9	2	1	1	1	55.6	10	1	1	5	0	70.0
SCoT-84	170	:	960	15	73.3	0.288	6.4	14	2	2	4	1	64.3	13	2	3	3	1	69.2
SCoT-86	199	:	489	6	33.3	0.153	1.6	6	2	0	0	0	33.3	6	0	0	1	0	16.7
SCoT-89	464	:	1242	8	75.0	0.21	2.4	8	0	0	4	2	75.0	6	2	0	0	0	33.3
	Means			**13.86**	**74.66**	**0.266**	**5.77**	**11.86**	**2.36**	**1.29**	**3.14**	**1.36**	**66.49**	**13.00**	**2.79**	**1.29**	**3.07**	**1.71**	**63.74**
All in one	**170**	**:**	**2572**	**194**				**166**	**33**	**18**	**44**	**19**	**68.67**	**182**	**39**	**18**	**43**	**24**	**68.13**
	%							**85.57**	**19.88**	**10.84**	**26.51**	**11.45**		**93.81**	**21.43**	**9.89**	**23.63**	**13.19**	

T.A: Total amplicons; A (–): Amplicons, appear in control and absent in one or more of the treatments; PA (–): Polymorphic A (-); UA (–): Unique negative amplicon, absent in one treatment; A (+): Amplicons, absent in control and appear in one or more of the treatments; PA (+): Polymorphic A (+); UA (+): Unique positive amplicon appears in one treatment; Poly. %: Polymorphism percent.

Table 5. Molecular data estimated from banding patterns of CCDP technique

Primer	Molecular size range bp			All TA	Pol %	PIC	Rp	M1						M2					
								TA	A+		A-		Poly	TA	A+		A-		Poly
									PA-	UA-	PA+	UA +			PA -	UA -	PA +	UA +	
CDDP-1	217	:	1484	12	91.7	0.280	4.40	10	0	0	4	5	90.0	12	3	2	1	3	75.0
CDDP-2	242	:	1984	16	68.8	0.210	4.40	14	3	1	2	2	57.1	13	4	0	1	2	53.8
CDDP-3	257	:	1939	12	83.3	0.295	5.40	9	2	1	3	1	77.8	12	2	1	3	2	66.7
CDDP-4	277	:	1934	15	80.0	0.256	5.200	9	3	0	1	3	77.8	15	3	0	4	5	80.0
CDDP-5	154	:	1870	20	75.0	0.319	10.600	19	5	3	2	3	68.4	20	4	1	5	4	70.0
CDDP-6	395	:	1727	12	91.7	0.313	5.60	10	2	1	3	1	70.0	12	6	2	1	3	100.0
CDDP-7	278	:	2030	16	87.5	0.366	7.200	12	4	1	2	2	75.0	15	5	2	5	1	86.7
CDDP-8	153	:	1956	16	81.3	0.238	4.200	13	8	1	0	0	69.2	12	4	0	2	3	75.0
CDDP-9	313	:	1476	12	66.7	0.207	3.60	10	2	1	2	0	50.0	11	3	2	0	0	45.5
CDDP-10	312	:	1895	11	72.7	0.238	3.800	11	0	1	7	0	72.7	11	0	0	4	1	45.5
CDDP-11	202	:	1675	14	78.6	0.270	5.400	10	1	0	2	3	60.0	13	2	2	1	3	61.5
CDDP-12	461	:	1978	13	100.0	0.345	6.800	10	4	2	1	3	100.0	11	5	0	4	1	90.9
CDDP-13	231	:	512	7	42.9	0.146	1.400	6	1	0	0	0	16.7	7	2	1	0	1	57.1
CDDP-14	349	:	888	7	14.3	0.026	0.200	7	1	0	0	0	14.3	7	0	1	0	0	14.3
CDDP-15	644	:	709	3	66.7	0.300	1.400	3	1	0	1	0	66.7	3	1	0	0	1	66.7
	Means			**12.40**	**73.41**	**0.254**	**4.64**	**10.20**	**2.47**	**0.80**	**2.00**	**1.53**	**64.38**	**11.60**	**2.93**	**0.93**	**2.07**	**2.00**	**65.91**
All in one	**154**	**:**	**2030**	**186**				**153**	**37**	**12**	**30**	**23**	66.67	**174**	**44**	**14**	**31**	**30**	**68.39**
	%							**82.26**	**24.18**	**7.84**	**19.61**	**15.03**		**93.55**	**25.29**	**8.05**	**17.82**	**17.24**	

T.A: Total amplicons; A (−): Amplicons, appear in control and absent in one or more of the treatments; PA (−): Polymorphic A (-); UA (−): Unique negative amplicon, absent in one treatment; A (+): Amplicons, absent in control and appear in one or more of the treatments; PA (+): Polymorphic A (+); UA (+): Unique positive amplicon appears in one treatment; Poly. %: Polymorphism percent

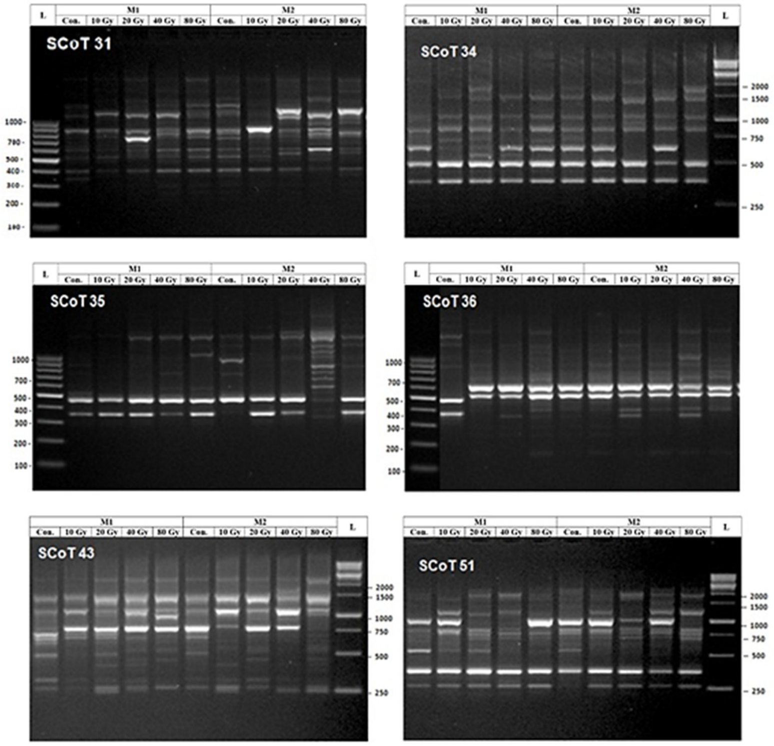

Figure 3. Agarose gel electrophoresis of SCoT amplifications example of the red radish First (M1) and second generations (M2) with 14 primers as affected by gamma irradiation. L, DNA ladder (100–1500 bp) and lanes from 2 to 10 represent the irradiation dose levels for M1 and M2

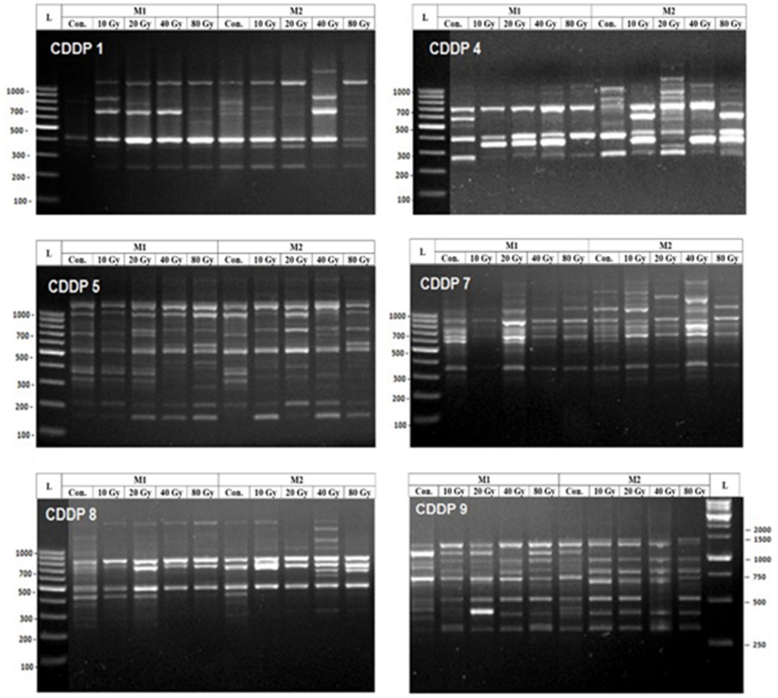

Figure 4. Agarose gel electrophoresis of CDDP amplifications examples of the red radish First (M1) and second generations (M2) with 15 primers as affected by gamma irradiation. L, DNA ladder (100–1500 bp) and lanes from 2 to 10 represent the irradiation dose levels for M1 and M2

Moreover, the molecular data showed that the CDDP-primers targeted 186 scorable amplicons. Where, a number of amplicons per CDDP-primer ranged from 3 for CDDP-15 to 20 for CDDP-5, as shown in Table 5. The proportion mean of polymorphism for all the used CDDP-primers was 73.41% and were 64.38 and 65.91% for M1 and M2, respectively (Table 5). In addition to, the molecular size of all targeted ranged from 153 to 2030 bp and the polymorphic percentage ranged from 14.3 to 100.0% in both M1 and M2 generations.

The resolving power (Rp) of the SCoT-primers extended from 1.6 for SCoT-86 to 10.2 for SCoT-36, whereas Rp of the CDDP-primers extended from 0.2 for CDDP-14 to 10.60 for CDDP-5.

The polymorphic information content (PIC) is a method to measure the allelic diversity by each primer. The PIC of SCoT-primers ranging from 0.153 for SCoT-86 to 0.379 for SCoT-36. The PIC of CDDP-primers ranging from 0.026 for CDDP-14 to 0.366 for CDDP-7. Both techniques are proved to be efficient to assess genetic diversity even with different levels of polymorphism as they exhibited 74.66 and 73.41 for SCoT and CDDP, respectively (Table 6). Where, the diversity index (DI) and Rp values for SCoT technique (0.266 and 5.77, respectively were higher than that of CDDP technique (0.254 and 4.64, respectively). Moreover, they were 0.26 and 5.21, respectively when the two markers combined.

This indicates that the SCoT technique has superior to CDDP technique to distinguish between the studied irradiated red radishes two generations and the assessment for genetic diversity among them.

Cluster analysis was performed to appear the effects of γ-rays for the molecular levels as well as the relationship between them (Figure 5). The UPGMA dendrograms based on molecular distances (MD) representing the cluster analysis for control and irradiation treatments. The dissimilarity coefficient was 0.20 and 0.25 for M1 and M2 respectively, with a total mean of 0.240 when M1 and M2 combined, where the MD is ranged from 0.185 to 0.320. From these results, it could be concluded that DNA changes occurred in response to irradiation of red radish seeds. The dendrogram, showing 5 main mutant groups, illustrated the effects that take place in plant genetics and diversity when subjected to various γ-rays doses. It's illustrated that with increasing the doses of radiation, the genetic similarity coefficient of plants that had been irradiated decreased (MD increased), which shows that genetic variation in the irradiated radish increased with the increase of the radiation dose. Nevertheless, the dissimilarity coefficients within M1 and M2 were different at the same irradiation doses. As shown in Table 2, irradiation treatments up to 40 Gy were led to significant economic improvement in several morphological traits that could be linked to the molecular changes.

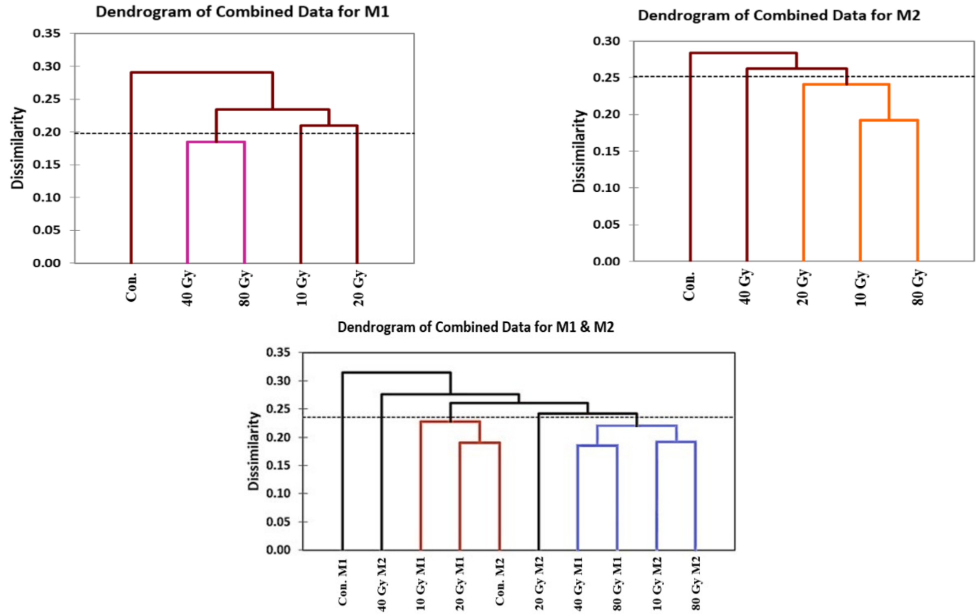

Figure 5. Dendrograms derived by UPGMA method using Dice-dissimilarity coefficient for combined binary data of SCoT and CDDP techniques for M1, M2 and two generations together

Table 6. Comparison of genetic diversity assessment by SCoT and CDDP technique

Molecular marker technique	Molecular size range bp	Average of TA	Poly. %	DI	Rp	UA-in each generation			UA+ in each generation			Poly % in each generation	
						M1	M2	M	M1	M2	M	M1	M2
SCoT	163:2572	13.86	74.66	0.266	5.77	18	19	2.64	18	24	3.00	68.67	68.13
CCDP	153:2030	12.40	73.41	0.254	4.64	12	23	2.33	14	30	2.93	66.67	68.39
Combined	153:2572	13.10	74.04	0.26	5.21	30	42	2.48	32	54	2.97	67.67	68.26

TA: Total amplicons; Poly. %: Polymorphism Percent; DI: Diversity Index; Rp: Resolving power; M: mean

The cluster analyses, showed notable variation among control and irradiation treatments for each generation. This variation proved that irradiation treatments applied in this study was succeeded for inducing genetic variations among molecular and phenotypic levels of the both two generations. The distance relation between the irradiation different treatments for M1 and M2 is given in Figure 5.

Results obtained from Table 2 and molecular assessment indicated that, most of irradiation treatments were led to molecular variation with desirable improvement of many studied morphological traits. As no significant decrease in vegetative traits was observed in the current research, this method could be encouraging for breeding efforts, notably for doses 20 Gy and 40 Gy. It's good to note that this is the first study showcasing the genetic diversity and polygenic relationships of irradiated M1 and M2 red radish by utilizing the SCoT and CDDP molecular markers techniques. The use of SCoT and CDDP markers for studying genetic diversity are reported here for the first time for irradiated red radish. This confirms that the combined data of SCoT and CDDP techniques were suitable for evaluating the genetic relationships among the red radish two generations because they accurate the information about genetic diversity.

The findings of the existing study are in accordance by means of Abd El-Aziz *et al.* (2017) who indicated that using molecular markers to detect γ-irradiation mutations associated with desirable traits could be used as marker-assisted selection for improving yield and its components in breeding programs and improvement of okra. In the same concern, Singh and Datta (2009) demonstrated that gamma irradiation encouraged phenotypic changes in wheat, whereas total leaf mass, plant mass and the number of tillers increased 3 times more than control as a result of gamma irradiation. Some past studies made on the effects of γ-radiation in plants reported that gamma irradiation can cause variations in vegetative characteristics, flowering development, rhizome traits, as well as in maturity in both a negative or positive manner, depending on the applied dose and the radio-sensitivity of the plant being irradiated, where at low doses the plant shows a progression in growth while at high doses of radiation the opposite is seen (Taheri *et al.*, 2014; Ambavane *et al.*, 2015; Aly *et al.*, 2019a). Otherwise, Taheri *et al.* (2014) observed comparable declines in plant length in the mutant populaces of *Curcuma alismatifolia Gagne*, and these tendencies are believed to occur as a result of chromosomal aberrations as well as genetic mutations.

It has previously been shown that γ-irradiation is an important mean of induction mutations in plants (Orazem *et al.*, 2013; Taheri *et al.*, 2014; Aly *et al.*, 2019b). Thus, we can conclude that irradiation with gamma rays was an efficient tool for induction of mutation and reorganization in red radish. These mutants can be efficiently identified using molecular markers such as SCoT and CDDP markers, as seen in the previous results. The SCoT and CDDP markers have been previously used to study genetic diversity in broad series of plant species and have greater benefits in using such markers in the assessment of genetic diversity compared to conventional markers like ISSR (Hamidi *et al.*, 2014; Hajibarat *et al.*, 2015; Saidi *et al.*, 2017). This was confirmed on a study made on potato by Gorji *et al.* (2011), and in another study made on durum wheat by Etminan *et al.* (2016). Others have suggested using combination with more than DNA markers for distinctive fingerprinting (Baghizadeha and Dehghan, 2018). Moreover, Wang *et al.* (2017) confirmed in their study in *S. davidii* that irradiation by gamma-irradiated seeds can produce an adequate number of mutations and the use of molecular markers can be a great tool for identifying such mutations in plants. In the same context, Hajibarat *et al.* (2015) reported that CDDP and SCoT marker strategies could be used to identify novel genes found by plant breeders as a result of *in-vitro* mutagenesis management. It could be summarized that using these advanced molecular techniques showed changes in non-irradiated and irradiated treatments, based on SCoT and CDDP polymorphism.

Conclusions

In conclusion, gamma rays were successful in inducing desirable changes in the red radish at the morphological and the molecular levels for both two generations. The SCoT and CDDP molecular markers which could be good methods in generating high number of polymorphism and in detecting genetic diversity in red radish, which might be used for the selection of improved traits induced by gamma irradiation treatments in programs of breeding of red radish. Whereas, the DI and Rp values for SCoT technique (0.266 and 5.77, respectively were higher than that of CDDP technique (0.254 and 4.64, respectively). The maximum number of amplicons (21) was scored by primer SCoT-33 while it was (20) for CDDP-5 primer. Treating red radish with radiation as a mutagenesis resulted in a number of plant morphological and molecular variations in the red radish. The red radish responded to γ-rays was explored for morphological features, and γ-rays dose level 40 Gy was found to be most effective as compared to other treatments. This research offers scientific support for the usefulness for considering γ-rays as an appropriate method for inducing genetic diversity in red radish and SCoT and CDDP markers were effectively in detecting the genetic diversity for radish as seen from the average percentage of polymorphism produced by each marker.

Authors' Contributions

A.A.A. - Conceptualization, designed the study, supervision, project administration, curation, editing and reviewed the initial and final draft; N.E.E. - Contributed in the collection of data, prepared the initial draft, the methodology, visualization, review; Z.M.B. - Literature review and contributed in mathematical processing, design, analyzed the data, mathematical processing and genetic analysis; G.S. - Contributed to the conceptualization of ideas, contributed in study design and reviewed the initial draft the manuscripts' review writing and curation, and editing. All authors have read and agreed to the published version of the manuscript. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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