Journal of Genetic Engineering and Biotechnology 16 (2018) 683–692

HOSTED BY

Contents lists available at ScienceDirect

Journal of Genetic Engineering and Biotechnology

journal homepage: www.elsevier.com/locate/jgeb

Original Article

Physiological and molecular studies on the effect of gamma radiation in fenugreek (*Trigonella foenum-graecum* L.) plants

Rania Samy Hanafy *, Samia Ageeb Akladious

Biological and Geological Sciences Department, Faculty of Education, Ain Shams University, 11341 Cairo, Egypt

ARTICLE INFO

Article history:
Received 13 October 2017
Received in revised form 14 January 2018
Accepted 28 February 2018
Available online 6 March 2018

Keywords:
Fenugreek
Gamma ray
Phenolic
RAPD-PCR
Vitamin

ABSTRACT

This experiment assessed the biochemical changes in fenugreek plants exposed to gamma radiation. Two pot experiments were carried out during two growing seasons of 2015 and 2016. Seeds were subjected to five doses of gamma irradiation (25, 50, 100, 200 and 400 Gy) and were immediately planted into soil pots in a greenhouse. The experimental analysis was performed in M_1 and M_2 generations. Significant differences between irradiated and control plants were detected for most studied characters in M_1 and M_2 generations. It was demonstrated that low doses of gamma irradiation led to gradually increases in growth, yield characters, leaf soluble protein concomitantly with increases in the contents of phenolic and flavonoids compounds particularly at 100 Gy. These changes were accompanied by a substantial increase in ascorbic acid, α-tocopherol and retinol contents. Proline content was increased under all doses of gamma rays in M_1 generation and the highest amount of proline was obtained at 200 Gy with visible decrease in M_2 generation under the same dose. Meanwhile, the highest dose of gamma radiation (400 Gy) decreased all the studied parameters in both mutagenic generations as compared with control plants. In addition, gamma irradiation doses induced changes in DNA profile on using five primers and caused the appearance and disappearance of DNA polymorphic bands with variation in their intensity. These findings confirm the effectiveness of relatively low doses of gamma rays on improving the physiological and biochemical criteria of fenugreek plants.

1. Introduction

Gamma radiation has been recognized as a fast and reliable means for the alteration of physiological and biochemical processes in plants. It is one of the important physical agents used to improve the characters and productivity of many plants. Using of gamma radiation technique represented a significant role in plant breeding programs and genetic studies aimed to improve yield and produce desirable traits in many crops under both normal and stress conditions [3]. Many studies showed that the relatively low doses of ionizing irradiation could be useful for acceleration of cell proliferation, germination rate, cell growth, enzyme activity, stress resistance, and crop yields [5]. It solves many of agricultural crop problems such as reducing of post-harvest losses resulted from contamination, eradication of insect pests and food-borne diseases [24]. On the other hand, the irradiation of seeds with high doses of gamma rays caused adverse effects on important components of plant cells. Such damage effects of GR come from its interact with atoms and molecules, thus producing free radicals in cells which affect the synthesis of protein, enzyme activity, hormone balance, leaf gas exchange and water exchange depending on the irradiation dosage [55]. Furthermore, irradiation by gamma rays leads to increasing the level of DNA break formation that can be mitigated through direct identification of genotypes with DNA based assays [6]. One such method is detected by changes in random amplification of polymorphic DNA (RAPD) profiles which amplifies random genomic DNA sequences using single, short arbitrary primers, and these can be effectively used as genetic markers.

Fenugreek (*Trigonella foenum-graecum* L.) is an annual herb that belongs to the family *Leguminosae*, and has been commonly known as medicinal and economical plants. It is considered as rich source of protein (25%), lysine (5.7 g/116 g N), soluble (20%) and insoluble (28%) dietary fiber, alkaloid (trigoneline) (36%), flavonoids like ornithine, viticsine and quercetin that have anti-cancer properties. In addition, the seeds of fenugreek contain fix oil, essential nutrients (calcium, iron and beta-carotene), as well as different steroid

Peer review under responsibility of National Research Center, Egypt.
* Corresponding author.
E-mail address: raniaamin91@yahoo.com (R.S. Hanafy).

https://doi.org/10.1016/j.jgeb.2018.02.012
1687-157X/

saponins such as diosgenin, ticogenine and neoticogenine [43]. The present work aimed to assess the effect of different doses of gamma irradiation on some physiological and biochemical attributes of fenugreek (*Trigonella foenum-graecum* L.) plants and to find out the potential role of these parameters to determine the appropriate radiation dose for plant.

2. Material and methods

2.1. Plant materials and mutagenic treatment gamma irradiation treatment

Seeds of fenugreek (*Trigonella foenum-graecum* L.) were obtained from the Crop Institute, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt. The seeds were washed with tap water for one hour, then sterilized in 10% sodium hypochlorite for 10 min and then rinsed with sterile deionized water. A pot experiment was performed during the two successive seasons of 2015 and 2016 at the Faculty of Education, Ain Shams University, Cairo, Egypt. Seeds were sown in loamy clay soil on the 2nd December for both seasons. Dry seeds were subjected to different doses (25, 50, 100, 200, 400 Gy) of gamma radiation using a Co^{60} gamma cell source at the National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt (NCRRT). The Gamma cell 220 Excel Co^{60} irradiation facility (manufactured by MDS Nordion, Canada) is a compact and self-contained irradiation unit offering an irradiation volume of approximately 6 L. The activity of this irradiation facility was 11994.8 Ci at the time of installation (18 Jan. 2002). The gamma cell was calibrated using Fricke reference standard dosimetry system according to [11]. Seeds of the control were not irradiated. The irradiated seeds were immediately planted in soil in a greenhouse. Dose selection was based on our preliminary experiment on the fenugreek seeds, where a decrease in germination rate and growth parameters was observed above the dose of 400 Gy. Each treatment was replicated five times with 10 seeds in each replicate and the seeds allowed to germinate in pots (25 cm in diameter) containing equal amounts of homogeneous loamy clay soil. The seeds were sown at 3–4 cm depth in each pot and after the emergence was complete (after 6 days) the density was reduced to ten plants per pot. After 10 weeks from sowing 5 plants for M_1 generation were collected to determine some growth parameters (shoot length, root length, fresh and dry weights of shoots and roots) in addition to some physiological and biochemical criteria. At harvest, plant height, number of pods/plant, and number of seeds/plant and 100-seed weight were measured. To determine M_2 generation; which raised from M_1 generation; observations on various quantitative traits were recorded on plants of each treatment in the same above mentioned criteria as in the following:

2.2. Determination of total soluble protein

Total soluble protein was done by the method of Lowry et al. [40]. Alkaline tartarate reagent (20 g sodium carbonate and 0.5 g tartarate) were dissolved in 1000 ml of (0.1 N) NaOH. 10 μL of the protein sample were added to 5 mL of the alkaline copper reagent, and was allowed to stand for 15 min. at room temperature. Immediately, the dilution Folin reagent (0.5 ml) was then mixed with the mixture and allowed to stand at room temperature for 30 min. The resulting color of samples was measured at 750 nm.

2.3. Estimation of proline content

Fresh weight of leaves (0.5 g) were blended in 3% sulfosalycylic acid then allowed to settle. The filtrate (2 ml) was mixed with (2 ml) ninhydrin and (2 ml) of glacial acetic acid. The mixture was boiled for 1 h. Then the reaction was terminated in an ice bath, 4 ml of toluene was added to the mixture. The organic phase was collected and the absorbance was read at 520 nm using proline as standard [31].

2.4. Determination of total phenolic content (TPC)

Total phenolic contents were determined as a tannic acid equivalent (TAE) based on Folins-Ciocalteu method [4]. Known weight of fresh leaves (1g) was mixed for extraction with 50 ml of 80% cold methanol (v/v) for 3 times at 90 °C. Combined extract was collected and filtrated then made up to a known volume using methanol. 1 ml of methanolic extract was added to 1 ml of 10% Folins-Ciocalteu reagent. 2 ml of Na_2CO_3 solution (25%) and extract were mixed well and left for 60 min in dark. The absorbance was measured at 750 nm using a UV–Vis spectrophotometer and expressed as mg tannic acid g^{-1} FW.

2.5. Determination of total flavonoids (TF)

0.5 g of fresh plant leaves were mixed with 10 ml of 80% aqueous methanol and filtered. 1 ml of each extract, 4 ml distilled water, 0.3 ml of 5% $NaNO_2$, and after 5 min 0.3 ml of 10% $AlCl_3$ was added and mixed, and then the samples incubated for 6 min followed by the addition of 2 ml of NaOH (1 M). The solution was diluted to a final volume of 10 ml with H_2O and mixed well. Absorbance was measured at 430 nm with a spectrophotometer using quercetin as the standard. Total flavonoids were expressed as mg quercetin g^{-1} FW [49].

2.6. Determination of ascorbic acid

A known weight (2 g) of fresh leaves was ground in 6% trichloroacetic acid (TCA) and the extract filtered and centrifuged at 1000 g for 20 min. The filtrate was made up to a known volume (10 ml) with TCA. 4 ml of the extract and 2 ml of 2% dinitrophenyl hydrazine (in acidic medium) were mixed followed by the addition of drop of 10% thiourea (mixed with70% ethanol). The mixture was boiled for 15 min in a water bath then cooling. 5 ml of 80% (v/v) H_2SO_4 and the mixture were mixed at 0 °C (in ice-bath). The absorbance was measured at 530 nm using U-Vis spectrophotometer. The content of ascorbic acid was calculated from a standard curve using a known concentration of ascorbic acid and expressed as mg g^{-1} FW [51].

2.7. Determination of retinol (vitamin A)

A known weight (0.5 g) of fresh leaves was ground with 2.5 ml alcoholic potassium hydroxide (KOH) (12%) in a water bath (60 °C, 30 min). The extract was transferred to the separating funnel; 10 ml of petroleum ether was added to the extract and mixed well. The lower aqueous layer was then transferred to another separating funnel and the upper petroleum ether layer containing the carotenoids was collected. The extraction was repeated until the aqueous layer became colorless. A small amount of anhydrous sodium sulphate was added to the petroleum ether extract to remove excess moisture. The final volume of the petroleum ether extract was noted. The absorbance of the yellow color was read in a visible Spectrophotometer at 450 nm using petroleum ether as blank [10].

2.8. Determination of α-tocopherol

500 mg of fresh tissue was homogenized with 10 ml mixture of petroleum ether and ethanol (2:1.6 v/v) and the mixture was

centrifuged at 10.000 g for 20 min. The supernatant was collected. 1 ml of extract was added to 0.2 ml of 2% 2.2-dipyridyl in ethanol and mixed well and kept in the dark for 5 min. The resulting red color was diluted with 4 ml of distilled H_2O_2. The absorbance of α-tocopherol was recorded at 520 nm. α-tocopherol content in the extracts was calculated from the regression equation of the standard curve made with a known amount of α-tocopherol. The results were expressed in μg/g FW [13].

2.9. RAPD-PCR of genomic DNA

Leaf tissue of fenugreek plants (100 mg) was ground under liquid nitrogen to a fine powder, and then bulk DNA extraction was performed using the DNA easy plant Mini Kit (Qiagen). RAPD-PCR reaction was conducted using five 10-mer arbitrary primers with the sequences shown in Table 4. The amplification reaction was carried out in 25 μl reaction volume containing 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 μM primer, 1 U Go *Taq* DNA polymerase (Promega, USA) and 25 ng templates DNA. Amplification was carried out in a programmed PCR for one cycle at 94 °C for 4 min followed by 45 cycles of 1 min at 94 °C, 1 min at 36 °C, and 2 min at 72 °C. The reaction was finally stored at 72 °C for 10 min. The amplified products were size-fractioned using a ladder marker (100 bp) by electrophoresis in 1.5% agarose gels in TBE buffer at 120 V for 1 h. The bands were visualized with ethidium bromide under UV florescence and photographed [58]. RAPD patterns were scored and genetic distances were calculated according to Sokal and Snetath [48] by using RAPD distance software package, version 1.04 [29].

2.10. Statistical analysis

The data was statistically analyzed using Least Significant Difference (LSD) at 5% level of probability according to SAS-program [21]. The results were subjected to one-way analysis of variance (ANOVA) and the mean differences were compared by the Duncan test. Vertical bars in figures and values in tables indicate ±SE.

3. Results

3.1. Growth and yield parameters

The changes in growth characters of fenugreek plants of both M_1 and M_2 generations produced from seeds irradiated with different doses of gamma radiation are illustrated in (Table 1). The obtained results showed that there was a significant increase in growth characters of plants treated with gamma rays compared to the control. The stimulating effect of radiation has positive correlation with dose. The enhancement was progressively increased with gamma doses from 25 to 200 Gy. The dose 100 Gy was the most effective. On the other hand, the highest dose of 400 Gy of both mutagenic generations resulted in greater growth inhibition, decreasing shoot length, root length, and the fresh and dry weights of shoots and roots below that of the control. The increase in radiation intensity (25–200 Gy) was associated with the increase in number of pods/plant and seeds weight/plant in the first mutagenic generation as compared with control. On the other hand, pod length and No. of seeds /pod showed no noticeable increases in these traits (Table 2).

Table 1
Gamma radiation effect on growth parameters of fenugreek plants produced from seeds irradiated with different doses of gamma rays. Control represented un-irradiated seeds.

Gamma radiation	Shoot length (cm)	Root length (cm)	Fresh weight of shoot (g)	Dry weight of shoot g)	Fresh weight of root (g)	Dry weight of root (g)
M_1 Generation						
Control	24.43 ± 0.55	15.27 ± 0.37	0.60 ± 0.07	0.11 ± 0.02	0.12 ± 0.01	0.024 ± 0.003
25 Gy	29.17 ± 0.60[c]	17.67 ± 0.46[c]	0.73 ± 0.03[d]	0.15 ± 0.02[d]	0.14 ± 0.02[d]	0.027 ± 0.003[d]
50 Gy	33.83 ± 0.44[a]	19.2 ± 0.53[c]	1.04 ± 0.03[a]	0.19 ± 0.017[c]	0.15 ± 0.03[d]	0.028 ± 0.002[d]
100 Gy	35.43 ± 0.29[a]	22.8 ± 0.40[a]	1.35 ± 0.03[a]	0.24 ± 0.02[a]	0.18 ± 0.01[c]	0.028 ± 0.002[d]
200 Gy	26.63 ± 0.40[c]	16.73 ± 0.28[c]	0.67 ± 0.04[d]	0.13 ± 0.02[d]	0.13 ± 0.02[d]	0.026 ± 0.002[d]
400 Gy	20.73 ± 0.37[b]	12.60 ± 0.38[b]	0.73 ± 0.05[d]	0.10 ± 0.01[d]	0.09 ± 0.02[d]	0.02 ± 0.001[d]
M_2 Generation						
Control	19.0 ± 4.05	15.7 ± 1.23	0.70 ± 0.11	0.08 ± 0.20	0.10 ± 0.003	0.012 ± 0.002
25 Gy	24.0 ± 1.53[d]	16.5 ± 0.76[d]	1.00 ± 0.11[d]	0.13 ± 0.29[d]	0.13 ± 0.031[d]	0.016 ± 0.003[d]
50 Gy	26.6 ± 3.84[d]	18.4 ± 1.40[d]	1.11 ± 0.03[c]	0.16 ± 0.40[d]	0.14 ± 0.03[d]	0.018 ± 0.002[d]
100 Gy	28.3 ± 2.03[a]	19.82 ± 1.64[d]	1.22 ± 0.04[a]	0.19 ± 0.13[d]	0.17 ± 0.03[d]	0.02 ± 0.001[d]
200 Gy	25.0 ± 1.15[d]	15.33 ± 2.17[d]	1.00 ± 0.17[d]	0.11 ± 0.30[d]	0.12 ± 0.032[d]	0.015 ± 0.003[d]
400 Gy	17.0 ± 2.89[d]	14.1 ± 1.58[d]	0.60 ± 0.09[d]	0.06 ± 0.17[d]	0.07 ± 0.017[d]	0.009 ± 0.011[b]

Data presented as means of 5 replicates ±SE.
[a] Highly significant increase.
[b] Highly significant decrease.
[c] Significant.
[d] Non-significant change.

Table 2
Gamma radiation effect on yield parameters of fenugreek plants produced from seeds irradiated with different doses of gamma rays. Control represented un-irradiated seeds.

Gamma radiation	Pod length	Pods No./plant	Seeds no/pod	Seeds wt/plant
Control	5.5 ± 0.68	4.33 ± 0.88	5.83 ± 0.92	1.25 ± 0.34
25 Gy	6.5 ± 0.66[b]	6.2 ± 0.82[b]	7.40 ± 0.88[b]	2.18 ± 0.33[b]
50 Gy	6.74 ± 0.66[b]	7.5 ± 0.76[a]	7.40 ± 1.22[b]	2.4 ± 0.40[a]
100 Gy	6.88 ± 0.75[b]	8.21 ± 0.64[a]	7.63 ± 1.24[b]	2.45 ± 0.40[a]
200 Gy	5.92 ± 0.95[b]	5.5 ± 0.44[b]	6.25 ± 0.37[b]	1.5 ± 0.35[b]
400 Gy	5.38 ± 0.54[b]	4.0 ± 0.69[b]	4.50 ± 0.76[b]	0.83 ± 0.14[b]

Data presented as means of 5 replicates ±SE.
[a] Highly significant increase.
[b] Non-significant change.

3.2. Total protein content

Effect of different doses of gamma irradiation on total protein content is shown in Table 3. In M_1 and M_2 generations, it was an increase in total protein content of the leaves generated from irradiated seeds with lower doses of gamma rays. This content was recorded to be in a higher value at M_1 generation. Highest dose of gamma radiation (400 Gy) led to a significant decrease in the total protein contents by (11.65%) and (29.29%) in M_1 and M_2 generations respectively as compared to untreated control plants. The maximum amount of total protein contents was recorded at 100 Gy of γ-radiation and the minimum amounts were recorded at 400 Gy of gamma radiation.

3.3. Proline content

Different doses of gamma radiation increased proline content of fenugreek plants of both generations as indicated in Table 3. Exposure the seeds to different doses of gamma radiation (25, 50, 100, 200 and 400 Gy) caused significant increase in the total proline contents by compared to untreated control plants during M_1 generation. The maximum amount of total proline contents was recorded at 200 Gy of gamma radiation. Meanwhile, exposure the seeds to the highest dose (400 Gy) showed no noticeable increases in this content in M_2 generation.

3.4. Changes in total phenols and flavonoids contents

As can be seen from Table 3, gamma irradiation significantly affected the total phenolic and flavonoid contents of fenugreek plants in both generations when compared with control. Exposing fenugreek seeds to lower doses of radiation (25, 50, 100 & 200 Gy) caused a significant increase in total phenols and flavonoids contents of the produced plants as compared to control. The magnitude of induction was much more pronounced at 100 Gy. In contrast, total phenol and flavonoids contents were decreased in plants raised from seeds irradiated with the highest dose (400 Gy) of radiation.

3.5. Changes in ascorbic acid content

The effects of gamma irradiation on vitamin C (ascorbic acid) at M_1 and M_2 generations are shown in Fig. 1. The data demonstrated that plants produced from the irradiated seeds up to 25 Gy had higher ascorbic acid content in their leaves than control plants, parallel to increasing irradiation dose, whereas this content was lowered at 400 Gy.

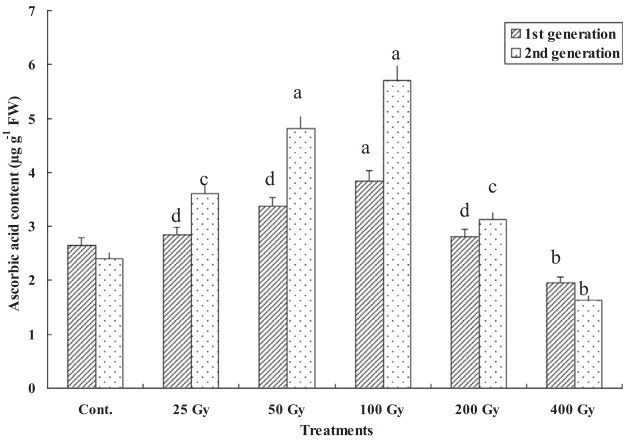

Fig. 1. Gamma radiation effect on ascorbic acid (vitamin C) content of fenugreek plants produced from seeds irradiated with different doses of gamma rays at 1st & 2nd generations. Error bars represent the SE (n = 3).

3.6. Changes in retinol and α-tocopherol contents

The present results showed significant increase in retinol and α-tocopherol contents (vitamins A and E) for the doses at 25, 50, 100 and 200 Gy of both generations as compared to control (Figs. 2 and 3). On the other hand, exposure the seeds to the highest dose of gamma rays (400 Gy) caused a significant reduction in α-tocopherol and retinol contents in the leaves of the produced plants as compared to control.

3.7. Random amplified polymorphic DNA (RAPD) analysis

In the present experiment, RAPD analysis has been performed to evaluate the variability and molecular changes in fenugreek genomes challenged due to treatments with different doses of gamma rays at both growth seasons. In the present work, five-mer primers were used. The results of RAPD-PCR indicated the existence of differences in RAPD fragments. RAPD analysis

Table 3
Gamma radiation effect on total protein, proline, total phenolic and total flavonoid contents of fenugreek plants produced from seeds irradiated with different doses of gamma rays. Control represented un-irradiated seeds.

Gamma radiation	Total protein (mg/ g FW)	Proline content (μg g^{-1} FW)	Total phenol (mg gallic acid /100 g FW)	Total flavonoid (mg quercetin/100 g FW)
M_1 Generation				
Control	30.71 ± 0.39	18.63 ± 0.28	161.75 ± 1.63	18.30 ± 0.35
25 Gy	33.53 ± 0.29[c]	28.17 ± 0.73[a]	180.44 ± 0.80[c]	21.11 ± 0.55[c]
50 Gy	34.5 ± 0.17[c]	32.53 ± 0.29[a]	186.10 ± 1.06[a]	26.42 ± 0.58[a]
100 Gy	38.64 ± 0.32[a]	36.77 ± 0.46[a]	192.21 ± 0.21[a]	27.18 ± 0.43[a]
200 Gy	32.08 ± 0.11[c]	43.80 ± 0.14[a]	175.43 ± 0.29[c]	19.63 ± 0.29[d]
400 Gy	27.13 ± 0.18[b]	25.50 ± 0.29[c]	159.58 ± 0.67[d]	16.10 ± 0.66[c]
M_2 Generation				
Control	8.26 ± 0.19	11.67 ± 0.81	70.3 ± 5.91	10.17 ± 0.73
25 Gy	16.26 ± 2.03[a]	18.4 ± 1.40[c]	94.9 ± 5.77[d]	14.76 ± 0.28[c]
50 Gy	20.24 ± 2.03[a]	26.12 ± 0.05[a]	105 ± 3.15[d]	18.09 ± 0.13[a]
100 Gy	22.62 ± 0.67[a]	28.55 ± 0.48[a]	126.0 ± 3.84[a]	20.71 ± 0.68[a]
200 Gy	13.24 ± 1.01[c]	17.6 ± 1.44[c]	85.3 ± 2.90[d]	12.5 ± 0.07[c]
400 Gy	5.84 ± 0.44[d]	8.43 ± 2.83[d]	59.84 ± 4.28[d]	8.69 ± 0.07[b]

Data presented as means of 5 replicates ±SE.
[a] Highly significant increase.
[b] Highly significant decrease.
[c] Significant.
[d] Non-significant change.

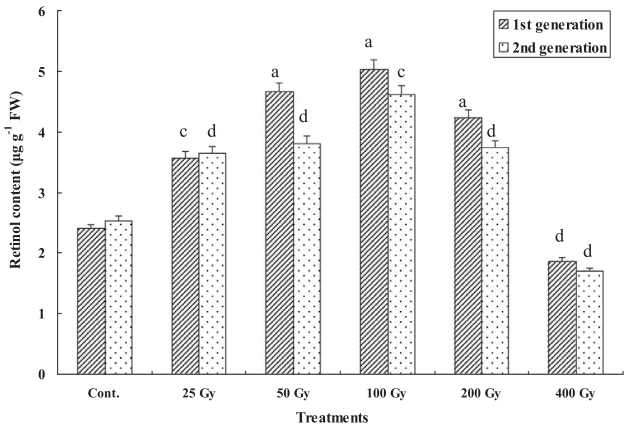

Fig. 2. Gamma radiation effect on retinol (vitamin A) content of fenugreek plants produced from seeds irradiated with different doses of gamma rays at 1st & 2nd generations. Error bars represent the SE (n = 3).

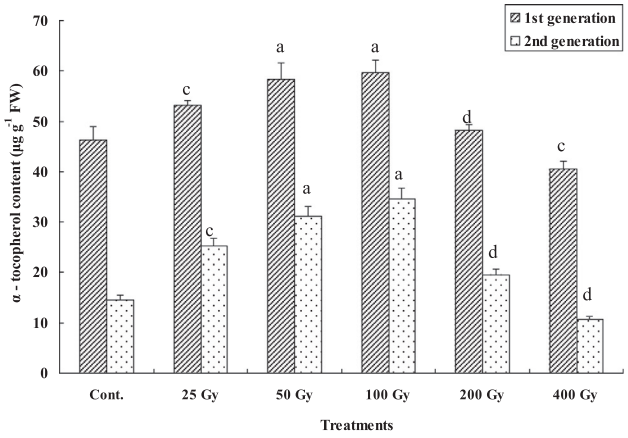

Fig. 3. Gamma radiation effect on α -tocopherol (vitamin E) content of fenugreek plants produced from seeds irradiated with different doses of gamma rays at 1st & 2nd generations. Error bars represent the SE (n = 3).

indicated that all five primers used resulted in the appearance and disappearance of PCR products with a variable number of bands (Table 4) at various gamma doses during both generations (Table 6). At 1st generation (M_1), the data show that 37 DNA bands were detected among all treatments in fenugreek leaves, of which 23 bands were polymorphic (62%) (Table 4 and Fig. 4). The primer OP-B07 was most successful and produces a highest number of RAPD bands (12 bands), while the primer OP-A10 gave poor reproducibility (5 bands). Table 4 shows the polymorphic bands generated from each primer. The data showed that polymorphism levels differed from one primer to the other. Four primers gave nine molecular markers (nine positive) associated with radiation stress (Table 5) which could be considered for marker assisted selection. At 2nd generation, the total number of DNA bands was increased to 41 bands, of which 38 bands were polymorphic (93%) as compared with the 1st generation (23 bands with polymorphism 62%). The highest number of RAPD bands was detected for primer OP-A10 (12 bands), while the lowest was scored for OP-B07 (4 bands) (Table 4 and Fig. 5). The five primers gave ten molecular markers (ten positive) associated with radiation stress (Table 5). The highest numbers of unique positive markers at 440, 344, 304 and 205 were detected using primer OP-B11. These positive markers could be used to identify genes conferring stress tolerance and facilitate marker-assisted breeding for radiation tolerance. Furthermore, polymorphism percentage recorded high levels (100%) with the RAPD primers of OP-A01, OP-B02, OP-B07 and OP-B11 at the second generation as compared with the first generation.

4. Discussion

Gamma radiation is one of the powerful agents that can alter physiological and biochemical properties of plants depending on the absorbed doses [5]. Several studies showed that higher doses of gamma rays have an inhibitory effect on plants, it resulted in the production of free radicals which have destructive effects on physiological, morphological and anatomical aspects according to the irradiation level.

In the present work, increasing gamma irradiation from 25 to 200 Gy increased all studied growth parameters. These results are consistent with Akshatha et al. [30] who reported that seeds of *Terminalia arjuna* Roxb irradiated with 25, 50, 100 and 200 Gy showed a slight increase in the root and shoot lengths, number of plant leaves and dry weights of the plants. Singh and Datta [14] studied the effect of low dose of gamma ray on wheat plant; they found improvement in plant growth, yield, flag leaf area and photosynthesis. Furthermore, Hamideldin and Eliwaa [37] found that exposure of mustard seeds to 25 and 50 Gy caused a significant increase in the dry weights. The induction of plant growth may be attributed to RNA activation and protein synthesis during

Table 4
List of primers, their sequence, numbers and size of the amplified fragments (bands) generated with RAPD primers in fenugreek leaves at two mutagenic generations (M_1 & M_2).

Primer code	Sequence (5′ to 3′)	Monomorphic bands	Polymorphic bands		Total bands	% of polymorphism	Size range (bp)
			Shared bands	Unique bands			
1st generation (M_1)							
OP-A01	5′-CCTTGACGCA-3′	2	1	3	6	67	261–810
OP-A10	5′-CAATCGCCGT-3′	2	3	–	5	60	373–601
OP-B02	5′-CAT CCC CCT G-3′	2	3	3	8	75	230–810
OP-B07	5′-GGT GAC GCA G -3′	6	4	2	12	50	134–1177
OP-B11	5′-GTA GAC CCG T-3′	2	3	1	6	67	420–833
Total		14	14	9	37	62	
2nd generation (M_2)							
OP-A01	5′-CCTTGACGCA-3′	0	8	1	9	100	304–1014
OP-A10	5′-CAATCGCCGT-3′	3	8	1	12	75	181–1194
OP-B02	5′-CAT CCC CCT G-3′	0	4	2	6	100	205–582
OP-B07	5′-GGT GAC GCA G -3′	0	2	2	4	100	488–1014
OP-B11	5′-GTA GAC CCG T-3′	0	6	4	10	100	205–1112
Total		3	28	10	41	93	

Monomorphic Bands → Same Bands (similar Bands).
Polymorphic Bands → Different Bands (present in few but absent in others /not present in all).

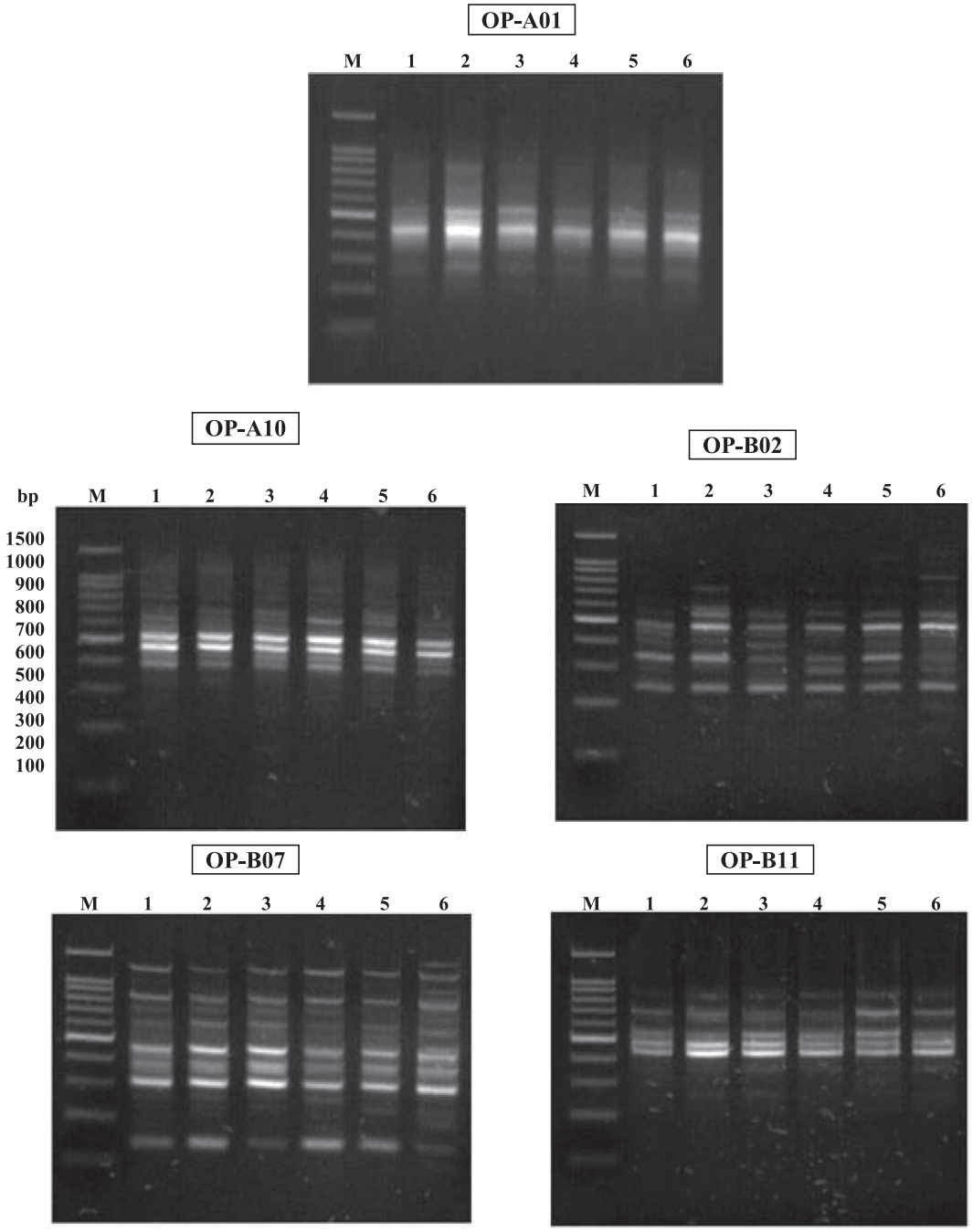

Fig. 4. DNA polymorphism using randomly amplified DNA (RAPD) procedure of fenugreek plants produced from irradiated seeds with different doses of gamma rays at 1st generation. *1 = control (0) Gy, 2 = 25 Gy, 3 = 50 Gy, 4 = 100 Gy, 5 = 200 Gy, 6 = 400 Gy.*

the early stages of germination [7]. In contrast, the highest implement gamma ray dosage (400 Gy) had negative and hazardous effects on fenugreek morphology and growth compared to control plant at both mutagenic generations. In this concern, Sarduie-Nasab et al. [52] reported that high doses of gamma irradiation reduce emergence index, stem height and width of barley as compared with control plants. Moreover, Preussa and Britta [54] stated that the high dose of gamma radiations contributes in cell cycle arrest during G2/M phase caused decrease in growth rate during cell division and (or) varying damage to the entire genome. The reduction in fresh and dry weights of shoot might be attributed to the decrease in shoot moisture contents due to radiation stress [1]. Results showed that gamma rays applied at doses from 25 to 200 Gy had a positive effect on number of pods /plant and seeds weight/plant in M_1 generation. It indicates that the improvement in quantitative traits could be possible though selection in mutated generation produced from gamma rays irradiation. These results are in accordance with those reported by Abdel-Hady and Ahmed [36] who showed that low doses gamma rays seemed to have a stimulatory effect on four wheat cultivars in M_1 generation. Several researchers stated that gamma rays used at low doses have positive effect on the plants [27]. On the other hand, previous results reported that gamma rays applied at doses of 50, 100 and 150 Gy reduced plant height, number of productive tillers, spike length and grain yield/plant in M_1 and M_2 generations of three wheat cultivars [22].

Table 5
RAPD markers for the five primers for radiation tolerance assessment.

Primer code	No. of marker/primer	M. size (bp)	Marker type
1st generation (M_1)			
OP-A01	3	810, 635, 280	Positive
	1	261	Negative
OP-A10	2	420, 373	Negative
OP-B02	3	810, 717, 373	Positive
	2	520, 358	Negative
OP-B07	2	1031, 230	Positive
	1	768	Negative
OP-B11	1	635	Positive
	2	689, 520	Negative
2nd generation (M_2)			
OP-A01	1	344	Positive
	3	660, 510, 440	Negative
OP-A10	1	205	Positive
	2	1194, 344	Negative
OP-B02	2	582, 440	Positive
OP-B07	2	660, 488	Positive
OP-B11	4	440, 344, 304, 205	Positive
	1	750	Negative

The production of defense systems is one of the important protective reactions of plant cells to gamma irradiation stress [50]. One of the protective mechanisms of plant against gamma irradiation damages is the increase in the content of soluble protein [35]. In this study, exposure of the seeds to low doses of gamma rays increased the content of total soluble protein in the produced plants as compared with control plants in M_1 generation and the same result obtained in M_2 generation (Table 3). In contrast, fenugreek plants that exposed to higher dose of irradiation (400 Gy) showed a significant decrease in soluble protein content. Data obtained by other authors also showed that total proteins reduced with increasing gamma ray dosage caused by higher metabolic and hydrolyzing enzyme activities in germinating seeds [26]. Protein degradation and recycling are essential response of the plants to stresses since the breakdown of proteins generate free amino acids which required for the de novo synthesis of new proteins [12]. The usage of high dose of irradiation can also lead to high compound extractability. This could explain the lower values of protein content observed at the dose of 400 Gy compared with that found in lower doses and control plants. Moreover, gamma radiation formed di-sulphide bridge between polypeptide chain that may be effect on the aggregation and conformation of the low molecular weight protein [42].

Proline is one of the important solutes that act as osmoregulator via the tolerance, protection against various stresses [61]. It has compatible properties which interact with enzymes to preserve its activities and reduce its denaturation. In addition, it can scavenge the hydroxyl radical and helps in regulating and stabilizing numerous structures such as DNA, proteins and membranes [41]. The results obtained demonstrated that various doses of gamma rays increased proline content of the wheat leaves particularly at 200 Gy in M_1 generation. These results are in harmony with [2] who found that proline content was enhanced when wheat seedlings exposed to 100, 200, and 300 Gy. Akshatha et al. [30] reported that the increase in the dosage of radiation caused increase in the proline concentration when the seeds of *Terminalia arjuna* were irradiated with 100–200 Gy.

Plants provide a defense system against irradiation via accumulation of phenolic and flavonoids compounds due to their antioxidant properties. Phenolic compounds have antioxidant defense properties by donating hydrogen atoms or electrons and they can also stable intermediary radicals. The obtained results (Table 3) indicated that the phenolic content increased at the lower irradiation dose levels in both mutagenic generations while the highest dose level of 400 Gy gave the lowest phenolic content. The effect of gamma-irradiation on increasing of phenolic content was noticed in soybean plants treated with γ-irradiation at levels ranging from 50 to 150 Gy compounds [44].

Gamma-rays interact with some atoms and molecules in the cell, particularly water molecules and produce free radicals that can modify important components of plant cells depending on the irradiation dose [56]. Indeed, free radicals generation acts as stress signals and trigger stress responses that may increase polyphenol acid content which had notable antioxidant properties [62]. As mentioned earlier, phenolic compounds play a crucial role in plants defense against radiation [46], which indicates that phenolic compounds are important factors in fenugreek defense against gamma radiation. This could be due to radiolysis of phenolics (eg. Gallic acid, Caffeic acid, etc.) which led to degradation and hydroxylation effect of pheolics [20]. The increase in total phenol can be attributed to the phenylalanine ammonia lyase (PAL) activity, which is one of the synthesis enzymes of phenolic compounds [19]. In this respect, it was found that irradiation can increase PAL activity [15], resulting in phenolic accumulation in plant tissues. On the other hand, the lower content of phenolic content at high levels of radiation may be due to the degradation or insolubilization of phenolic compounds. Ahn et al. [23] found that when Chinese cabbage exposed to high dose of gamma-irradiation, the phenolic contents significantly reduced.

Flavonoids are one of secondary metabolites which broadly distributed in plants. They alleviate the damages induced by irradiation stress. The results of this study showed that there was a significant increase in total flavonoids content in both mutagenic generations by using the lower doses of irradiation, whereas the highest dose (400 Gy) caused a reverse pattern of change. The decrease in flavonoids content was attributed to their counteracting role to the oxidative stress system induced by gamma irradiation. Peng and Zhou [45] showed similar results with soybean seedlings, they reported that flavonoid content of soybean seedlings increased in response to UV-B radiation but prolonged stress resulted in a decrease in the efficiency of the secondary metabolism biosynthesis system. Hence, biosynthesis and accumulation of flavonoids decline. Taheri et al. [53] stated that radiation dose up to 20 Gy can induce the accumulation of bioactive compounds, including phenolic and flavonoid leading to the improvement of scavenging activity in *Curcuma alismatifolia* leaves. Also, Said et al. [8] reported that using of lower doses of gamma radiation (2, 4, 8, 16, 32 and 64 Gy) significantly induce total flavonoids content of dill herb. Several studies found good correlations between antioxidant capacities and phenolic synthesis as well as flavonoid levels [60], indicating that the phenolic and flavonoid compounds are one of the major components responsible for the antioxidant activity of fenugreek plants.

In order to repair the damage initiated by the ROS, plants have evolved highly complex antioxidant defense strategy that included enzymatic and non-enzymatic scavengers, which plays an effective role in the cellular defense system against radiation stress depends on the absorbed doses [18]. Non-enzymatic antioxidants display an important role in metabolism and in scavenging reactive oxygen species (ROS) in biological systems [53].

Ascorbic acid (vitamin C), is an important antioxidant, is involved in several metabolic processes and in control of cell division and expansion of the cell wall during growth.

Several investigators have pointed out that vitamin E (α-tocopherol) is one of the hydrogen donor and the best singlet oxygen quenchers, and can act as a chain-breaking non-enzymatic antioxidant. Also, alfa-tocopherol is consider the major form found in green parts of plants, which protects lipids and other components of cell membrane by physical quenching and chemical reacting with singlet oxygen.

Table 6
RAPD-PCR fragments and their molecular sizes in base pairs generated by five decamer primers in fenugreek leaves as influenced by gamma rays treatments at two mutagenic generations (M_1 & M_2).

No.	Size (bp)	OP - A01						OP- A10						OP- B02						OP- B07						OP- B11					
		Cont. (0)	25 Gy	50 Gy	100 Gy	200 Gy	400 Gy	Cont. (0)	25 Gy	50 Gy	100 Gy	200 Gy	400 Gy	Cont. (0)	25 Gy	50 Gy	100 Gy	200 Gy	400 Gy	Cont. (0)	25 Gy	50 Gy	100 Gy	200 Gy	400 Gy	Cont. (0)	25 Gy	50 Gy	100 Gy	200 Gy	400 Gy
1	1177	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	–	–	–	–	–	–
2	1031	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–
3	833	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	+	+	
4	810	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
5	768	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	–	–	–	–	–	–
6	717	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7	689	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	–	–	+	+		+	+	+
8	635	––	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–
9	601	–	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
10	580	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	–	–	–	–	–	–
11	520	+	+	+	+	+	+	–	–	–	–	–	–	–	+	+	+	+	+	–	–	–	–	+	+	+	–	+	+	+	+
12	485	–	–	–	–	–	–	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+
13	459	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
14	420	+	+	+	+	+	+	–	+	+	+	+	+	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+
15	373	–	–	–	–	–	–	+	–	+	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
16	358	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	–	+	+	+	+	+	+	–	–	–	–	–	–
17	280	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	+	+	+	+	+	+	–	–	–	–	–	–
18	261	+	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
19	230	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	–	–	–	+	–	–	–	–	–	–	–	–
20	134	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	–	–	–	–	–	–
Total		3	5	3	3	3	3	4	3	4	5	4	4	3	5	5	5	5	4	8	8	8	9	9	11	4	3	4	5	5	4
2nd generation (M2)																															
1	1194	–	–	–	–	–	–	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2	1112	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	–	–
3	1014	+	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–
4	930	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	+	–	+	–	–	–	+
5	750	–	+	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	+	+	+
6	660	+	+	–	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	+	–	–	+	+	+	–	+	–
7	582	+	–	+	–	–	–	+	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	+
8	510	+	+	–	+	+	+	+	+	+	+	+	+	+	–	–	–	–	+	–	–	–	–	–	–	–	–	+	+	–	+
9	488	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–
10	440	+	+	+	+	–	+	+	+	+	+	+	+	–	–	–	–	–	+	–	–	–	–	–	–	–	+	–	–	–	–
11	390	–	–	–	–	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
12	344	–	–	–	–	–	+	+	+	+	+	+	–	–	+	+	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–
13	304	–	+	+	–	–	–	–	+	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–
14	255	–	–	–	–	–	–	–	–	+	–	–	+	–	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–
15	229	–	–	–	–	–	–	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
16	205	–	–	–	–	–	–	–	–	–	–	+	–	–	+	–	–	+	+	–	–	–	–	–	–	+	–	–	–	–	–
17	181	–	–	–	–	–	–	–	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Total		6	6	4	5	2	6	8	7	7	8	7	7	1	2	2	2	1	4	2	0	0	3	1	1	5	5	4	4	2	4

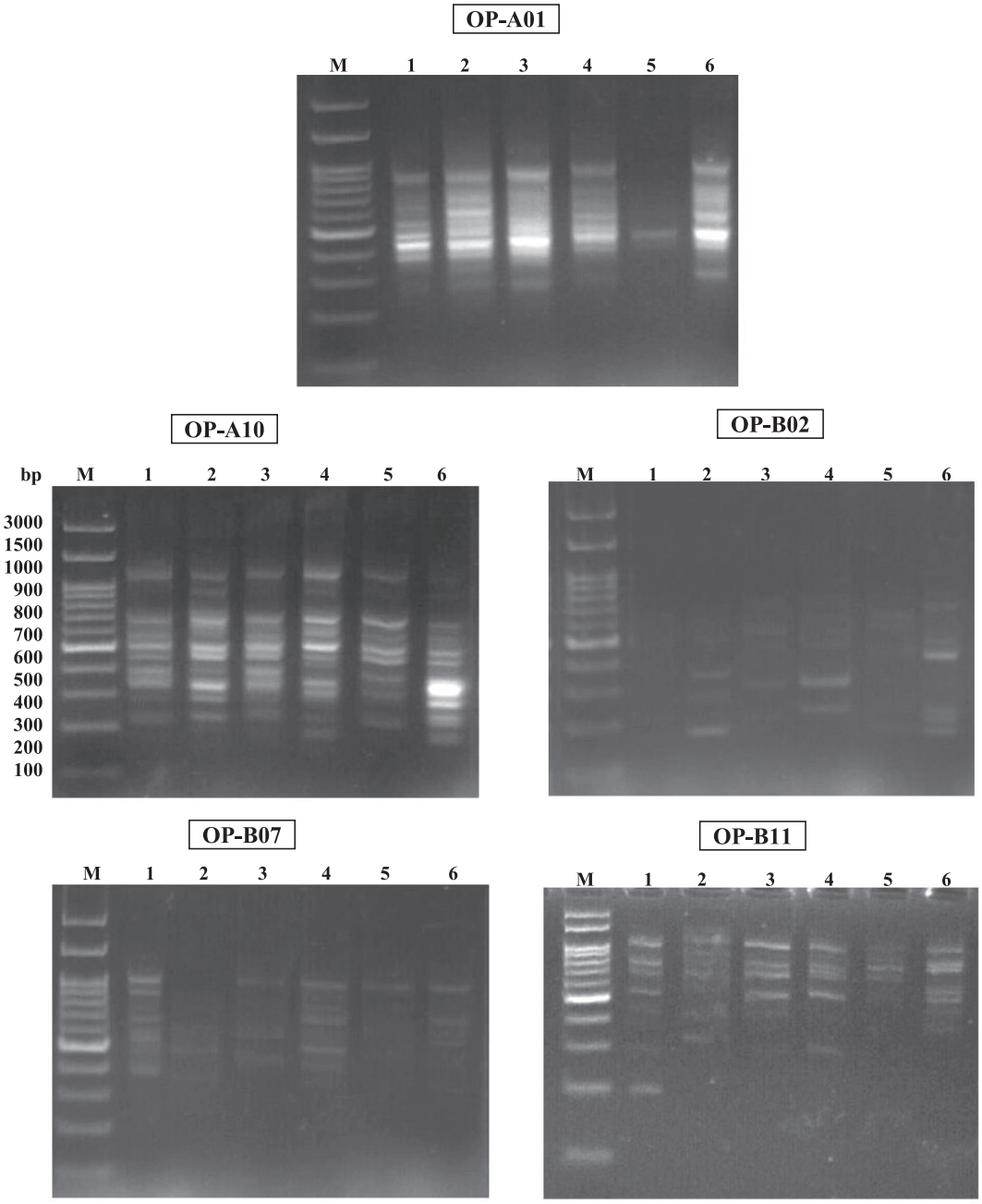

Fig. 5. DNA polymorphism using randomly amplified DNA (RAPD) procedure of fenugreek plants produced from irradiated seeds with different doses of gamma rays at 2nd generation. *1 = control (0) Gy, 2 = 25 Gy, 3 = 50 Gy, 4 = 100 Gy, 5 = 200 Gy, 6 = 400 Gy.*

In addition, α-tocopherol may regulate the intracellular signaling, concentrations of ROS and hormones in plant cells, such as jasmonic acid, which control the growth and development of plants, and response of plant to stress [57]. In this study, irradiation significantly enhanced all non-enzymatic antioxidant contents (Ascorbic acid, retinol and α-tocopherol) particularly at 200 Gy, whereas these contents were lowered at 400 Gy in both mutagenic generations (Table 4). These results agree with those of Sanni et al. [59] who found that low doses of gamma irradiation (below 20 kGy) led to an increase in vitamin A (retinol) content of sorrel seeds. On the other hand, Patil et al. [16] reported a marked reduction in ascorbic acid content of early season grapefruit induced by irradiation above 200 Gy. Vitamin C is rated to be one of the most sensitive soluble vitamins to irradiation after thiamine (vitamin B) [59]. The possible reason for accelerated decrease of ascorbic acid at higher doses of irradiation which observed in this study might be due to the increase in respiration value resulting in enhance the activity of enzymes causing rapid degradation of ascorbate or due to a partial conversion of ascorbate to dehydroascorbic which could account for the loss of ascorbic acid level in plant [28]. Low vitamins level may be correlated with the neutralization of ROS produced by irradiation [34].

RAPD-PCR method is considered as an important tool for gamma-rays to induce growth alterations and bring about genetic variability in breeding purposes [32]. Hegazi and Hamideldin [9] used RAPD analysis for detection of DNA profile and structural changes in okra (*Abelmoschus esculentus* L.) due to treatments with different doses of gamma irradiation. They observed changes in the DNA bands. The quantitative polymorphism obtained in this study might be due to the changes of some regions of the nucleotide

sequences aligned by arbitrary primers as a result of the promotive effects of low doses of GR or due to the enhancement of annealing efficiency between primers and DNA templates by activating the recognition of sequences and/or activation of Tag polymerase activity by the steroidal hormones. In this regard, Esmer et al. [25] reported that the variation of band intensities and disappearance of bands linked with the existence of DNA photoproducts produced by radiation. Also, the free radicals associated with radiation stress are suspected of assault on chromosomal DNA [47]. Thus, low doses of GR via enhancing the activity level of non enzymatic antioxidants could reduce the incidence of DNA damage, explaining the appearance of new DNA in GR treatment. In this connection, numerous studies have demonstrated that priming is associated with an increase in protein synthesis as well as in nucleic acid synthesis and repair [17].

Furthermore, appearance of new bands (unique bands) and disappearance of some bands (polymorphic bands) are usually resulting from some DNA structural changes such as Breaks, transpositions, deletion etc. [39]. These results agreed with El-Khateeb et al. [33] who investigate the effect of gamma irradiation on strawflower growth in two generations using RAPD and ISSR DNA analysis. They revealed that irradiation with gamma doses caused induction of new bands and the absence of others in the obtained mutants as compared with the control individuals. The positive markers observed in this study could be used to identify genes conferring radiation stress and facilitate marker assisted breeding for radiation tolerance. Any changes in structure of DNA lead to functional changes, which are result from DNA damage mostly after exposure to radiations. For survival all the environmental fluctuations certain ionizing radiations can help to enhance the plant germplasm [50]. Thus, it can be concluded that DNA polymorphism detected by RAPD analysis offers a useful molecular marker for the identification of changes in gamma radiation treated plants. This finding was also supported by Kamaruddin et al. [38].

5. Conclusion

In conclusion, the present data suggest that relatively low doses of gamma rays increase growth, yield characters and some biochemical constituents of fenugreek plants concomitant with induction of non-enzymatic antioxidants compounds. Gamma irradiation at 100 Gy was superior in enhancement of these parameters, whereas, high dose of gamma irradiation (400 Gy) caused decrease in these contents during both generations. In addition, RAPD technique could be considered as an alternative molecular marker tool for rapid evaluation of genetic variability obtained by radiation. Band sequence of the positive and negative markers can be used to detect various types of DNA damage and mutation in plants induced by radiation, which may be beneficial for crop improvement.

Conflict of interest

Authors declare that they have no conflict of interest.

References

[1] Abdul Majeed A, Khan R, Ahmad H, Muhammad Z. ARPN J Agric Biol Sci 2010;5:39–42.
[2] Borzouei A, Kafi M, Khazaei H, Naseriyan B, Majdabadi A. Pak J Bot 2010;42:2281–90.
[3] Borzouei A, Kafi M, Sayahi R, Rabiei E, Sayad Amin P, Pak. J. Bot. 2013;45:473–77.
[4] Dihazi A, Jaiti F, Zouine J, Hasni ME, Hadrami IE. Phytopathol Mediterr 2003;42:9–16.
[5] Kiong A, Ling Pick A, Grace Lai SH, Harun AR. Am.-Eurasian. J. Sustain Agric. 2008;2(2):135–49.
[6] Mengoni A, Gori A, Bazzicalupo M. Plant Breed 2000;119:311–7.
[7] Aly AA. Analele Universităţii din Oradea – Fascicula Biologie, Tom XVII 2010:356–61.
[8] Said AHA, Sarhan AMZ, Abou Dahab ADM, Abou-Zeid EN, Ali MS, Naguib NY. Int J Life Sci Eng 2015;1:145–9.
[9] Hegazi AZ, Hamideldin N. J Horti Forest 2010;2:38–51.
[10] AOAC, Official method of analysis of association of analytical chemists international, 17th. ed., Horowitz, Maryland; 2000.
[11] ASTM E1026–13, standard practice for using the Fricke Dosimetry System, ASTM International, West Conshohocken, PA; 2013.
[12] Hieng B, Ugrinovic K, Sustar-vozlic J, Kidric M. J Plant Physiol 2004;161:519–30.
[13] Philip B, Bernard L, William H. Vitamins and deficiency diseases. In: practical physiological chemistry, McGraw-Hill company, INC. N.Y., Toronto, London; 1954: 1272–74.
[14] Singh B, Datta PS. Radiat Phys Chem 2010;79:139–43.
[15] Tomás B, Espín CJ. J Sci Food Agric 2001;81:853–76.
[16] Patil BS, Vanamala J, Hallmanc G. Postharvest Biol Technol 2004;34:53–64.
[17] Bray D. Nature 1995;376:307–12.
[18] Marcu D, Damian G, Cosma C, Cristea V. J Biol Phys 2013;39:625–34.
[19] Gitz DC, Lui GL, McClure JW, Huerta AJ. J Exp Bot 2004;55:919–27.
[20] Breitfellner F, Solar S, Sontag G. J Food Sci 2002;67:517–21.
[21] G. Der, B.S. Everittt, J. R. Stat. Soc. Ser. A Stat. Soc. 172(2). (2009).
[22] Rachovska G, Dimova D. Rasteniev dni Nauki 2000;37:413–9.
[23] Ahn HJ, Kim JH, Kim JK, Kim DH, Yook HS, Byun MW. Food Chem 2005;89:589–97.
[24] Moussa HR. Russ J Plant Physiol 2006;53:193–7.
[25] Esmer I, Tüney I, Özakça DU, Sukatar A. Botanica Serbica 2017;41(1):17–24.
[26] Maity JP, Chakraborty A, Saha A, Santra SC, Chanda S. Radiat Phys Chem 2004;71:1065–72.
[27] Maity JP, Mishra D, Chakraborty A, Saha A, Santra SC, Chanda S. Radiat Phys Chem 2005;74:391–4.
[28] Bandekar JR, Dhokane VS, Shashidhar R, Hajare S, Saroj S, Sharma A. Proceedings of a final research coordination meeting organized by the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture and held in Islamabad, Pakistan; 2006.
[29] Armstrong JS, Gibbs AJ, Peakall R, Weiller G. The RAPD instance Package; 1994.
[30] Akshatha Chandrashekar KR, Somashekarappa HM, Souframanien J. Radiat Prot Environ 2013;36:38–44.
[31] Bates LS, Waldren RP, Teare LD. Plant Soil 1973;39:205–7.
[32] El-Khateeb MA, Abdel-Ati KEA, Khalifa MAS. Middle East. J Agric Res 2016;5:6–13.
[33] El-Khateeb MA, Eid RA, Mahfouze HA, Ashor HA, Mabrouk RMS. Middle East. J Agric Res 2017;6(2):282–93.
[34] Sajilata MG, Singhal RS. Radiat Phys Chem 2006;75:297–300.
[35] Al-Rumaih MM, Al-Rumaih MM. American. J Environ Sci 2008;4:151–6.
[36] Abdel-Hady MS, Ahmed MF. Egypt J Agron 2004;26:77–87.
[37] Hamideldin N, Eliwaa NE. Am J Agric Biol Sci 2015;2:164–70.
[38] Kamaruddin NY, Abdullah S, Pertanika. J Sci Technol 2017:25 (S);325–34.
[39] Danylchenko O, Sorochinsky B. Biology 2005;5(1):59.
[40] Lowry OH, Rosembrough NJ, Farr AL. J Biol Chem 1951;193:267–75.
[41] Kishor PK, Sangam S, Amrutha R, et al. Curr Sci 2005;88:424–38.
[42] Singh PK, Singh D. Scholars Acad J Bio Sci 2015;3:104–7.
[43] Kailash PP, Kalpesh MP. J Life Sci Technol 2013;1:10–3.
[44] Variyar PS, Limaye A, Sharma A. J Agric Food Chem 2004;52:3385–8.
[45] Peng Q, Zhou Q. J Agro-Environ Sci 2008;27:462–6.
[46] Ulm R, Heijde M. Trends Plant Sci 2012;17:230–7.
[47] Larson RA. CRC Press LLC. Boca Raton; 1997. p. 9.
[48] Sokal RR, Sneath PHA. Principles of numerical taxonomy. San Francisco: W.H. Freeman; 1963.
[49] Bushra S, Farooq A, Muhammad A. Molecules 2009;14:2167–80.
[50] Jan S, Parween T, Siddiqi TO, Zafar M. Environ Rev 2012;20(1):17–39.
[51] Mukherjee S, Choudhuri M. Physiol Planta 1983;58:166–70.
[52] Sarduie-Nasab S, Sharifi SGR, Torabi SMH. Global. J Agric Sci 2013;1:45–9.
[53] Taheri S, Abdullah TL, Karimi E, Oskoueian E, Ebrahimi M. Int J Mol Sci 2014;15:13077–90.
[54] Preussa SB, Britta AB. Genetics 2003;164:323–34.
[55] Wi SG, Chung BY, Kim JH, Baek MH, Yang DH, Lee JW. J Plant Biol 2005;48:195–200.
[56] Wi SG, Chung BY, Kim JS, Kim JH, Baek MH, Lee JW, Kim YS. Micron 2006;38:553–64.
[57] Abbas SM, Akladious SA. Fresenius Environ Bull 2012;21(3):563–77.
[58] Maniatis T, Frictsch EF. J.A. laboratory manual. NY: Cold Spring Harbor; 1982.
[59] Sanni TA, Ogunbusola EM, Oladimeji O. 2nd International Conference on Chemical, Biological, and Environmental Sciences, Dubai (UAE), 2015.
[60] Baltrusaityte V, Venskutonis PR, Ceksteryt V. Food Chem 2007;101:502–14.
[61] Kuznetsov VV, Shevyakova NI. Plant Stress 2007;1:50–71.
[62] Fan X, Toivonen PMA, Rajkowski KT, Sokorai KJB. J Agric Food Chem 2003;51:1231–6.