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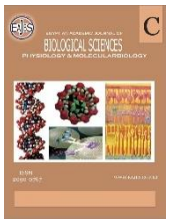
**Citation**: *Egypt.Acad.J.Biolog.Sci. ( C.Physiology and Molecular biology ) Vol. 15(2) pp825-838 (2023)*
**DOI: 10.21608/EAJBSC.2023.329415**

**Egypt. Acad. J. Biolog. Sci., 15(2):825-838 (2023)**

**Egyptian Academic Journal of Biological Sciences**
**C. Physiology & Molecular Biology**
**ISSN 2090-0767**
**www.eajbsc.journals.ekb.eg**



# ISSR Markers Related to Effects of Gamma Irradiation on Growth and Yield Parameters of Fenugreek (*Trigonella foenum-graecum* L.)

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**ARTICLE INFO**
**Article History**
Received:7/11/2023
Accepted:10/12/2023
Available:14/12/2023
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***Keywords***:
Fenugreek, Gamma rays, Growth, Yield, ISSR markers.

**ABSTRACT**

The impact of gamma irradiation on fenugreek (*Trigonella foenum-graecum* L.) for two generations was investigated in this research. The study involved exposing dry seeds of fenugreek to five doses of gamma rays (25, 50, 75, 100, and 200 Gy) using a cobalt 60 gamma radiation source. The irradiated and unirradiated seeds were grown in the field for two consecutive seasons. The results showed significant changes in vegetative growth traits as well as yield parameters in both the $M_1$ and $M_2$ generations. Low doses of 25–100 Gy stimulated growth and increased yield in $M_1$ and $M_2$ plants, while the highest dose of 200 Gy showed an inhibitory effect on the same parameters. The 75 Gy resulted in the highest increases in all traits. Eleven ISSR primers were used to identify molecular differences in ISSR fingerprinting in response to γ-irradiation treatments in the $M_2$ plants. The 11 ISSR primers produced 118 markers, revealed as bands on the agarose gel, including 73 monomorphic bands, 26 polymorphic bands, and 19 unique bands with an average polymorphism of 37%. The cluster analysis of ISSR data differentiated the $M_2$ plants grown from seeds exposed to 75 Gy of gamma radiation, indicating that the highest increase in vegetative and yield traits was associated with polymorphic ISSR markers. This result shows that ISSR profiling is a good way to connect genetic differences that happen in plants of the $M_2$ generation in response to γ-irradiation treatments.

## INTRODUCTION

Gamma irradiation has been proven to be an effective means of altering biochemical and physiological processes in plants, by producing free radicals in the cells (Kim *et al.*, 2004; Wi *et al.*, 2005). Many crops have benefited from gamma radiation's ability to induce favorable characteristics and increase yields under both normal and stressed growing circumstances (Borzouei *et al.*, 2013).

Previous research has shown that the use of low-dose irradiation has the potential to improve a wide variety of biological processes, including the rate of germination, the resistance to stress, and the total crop yields (Ling *et al.,* 2008). However, excessive doses of gamma rays directed at seeds can have deleterious effects on vital components of plant cells. Depending on the dosage of irradiation, such free radicals can interfere with a wide range of biological functions (Wi *et al.,* 2005). It is generally accepted that gamma irradiation is a crucial technology used to develop mutants with desirable agricultural properties (Sato *et al.,* 2006).

Fenugreek (*Trigonella foenum-graecum* L.) is an annual plant in the family Fabaceae that is grown for its medicinal and commercial use. It is considered a valuable aromatic and spice herb native to Asia and southern Europe. Consumption of the entire plant is due to its high nutrients (Badi *et al.,* 2018; Zandi *et al.,* 2017). In folk medicine, fenugreek seeds are used to treat various diseases like diabetes, fever, and paralysis (Eidi *et al.,* 2007). Several chemicals in fenugreek have beneficial effects on the body. These include the alkaloid trigonelline, the steroidal sapogenin diosgenin, and the polysaccharide combination mucilage. (Zandi *et al.,* 2015).

There has been a growing need for effective methods to evaluate genetic diversity (Badr *et al.,* 2012). DNA markers, especially ISSR markers, have become a popular choice due to their ability to identify variations in the inter-microsatellite DNA regions without earlier knowledge of the sequence (Zietkiewicz *et al.,* 1994). ISSR markers employ primers based on a repeat sequence, amplifying the sequence between two microsatellites producing several amplification products per primer, which means they are reproducible and cost-effective. The ISSR markers have been demonstrated to be a rapid, simple, dependable, and highly informative method for DNA fingerprinting, and there is a lack of necessity for prior knowledge regarding the sequences to be amplified (Pradeep Reddy *et al.,* 2002). The utilization of ISSR analysis has proven to be effective in assessing genetic diversity and establishing genetic relationships in various economically significant legume species, including cowpea, soybean (Ajibade *et al.,* 2000; Badr *et al.,* 2014), common bean (Galván *et al.,* 2003; González *et al.,* 2005), chickpea (Sudupak et al., 2004), and faba bean (Terzopoulos *et al.,* 2008). The ISSR fingerprinting was also used to differentiate the M2 of cowpea genotypes derived from parents exposed to gamma radiation (Abdoun *et al.,* 2022; Gaafar *et al.,* 2016).

The objectives of the current investigation are to investigate the response of fenugreek (*Trigonella foenum-graecum* L.) to different doses of gamma irradiation (25, 50, 75, 100, and 200 Gy) to record and evaluate the induced variations in the $M_1$ and $M_2$ generations in morphological traits and yield parameters. In addition, ISSR fingerprinting polymorphism has been investigated in the $M_2$ generation for related molecular markers with growth traits and yield components. The findings may provide valuable insights into the potential of gamma irradiation to induce variations in fenugreek plants and how ISSR markers can be used to assess genetic diversity and relate genetic markers to morphological traits and yield components.

## MATERIALS AND METHODS

### Plant Materials:

Seeds of fenugreek (*Trigonella foenum-graecum* L.) were collected by the Agricultural Research Center (ARC), Giza, Egypt, from the Field Crops Research Institute's (FCRI) Food Legumes Research Department.

### Seed Irradiation with Gamma Rays and Seed Sowing:

The dry fenugreek seeds were irradiated with gamma rays from a cobalt-60 source at Egypt's National Center for Radiation Research and Technology (NCRRT) in Cairo. Irradiation with gamma rays occurred at 25, 50, 75, 100, and 200 Gy.

Control samples of seeds were not exposed to irradiation. Both irradiated and unirradiated seedlings were grown in the field of the Botanical Garden of the Botany Department, Faculty of Science, Suez Canal University in Ismailia, Egypt. This was done over two successive seasons (2018-2019 and 2019-2020) to obtain the $M_1$ and $M_2$ generations. The experiment was conducted using a completely randomized block design (CRBD) at the Botanic Garden of the Botany and Microbiology Department, Suez Canal University.

**Growth and Yield Parameters:**

After 10 weeks of sowing, three randomly selected $M_1$ plants were raised from the irradiated seeds with the applied gamma radiation doses and compared to $M_1$ plants raised from non-irradiated seeds in terms of shoot length (cm), root length (cm), shoot fresh weight (g), shoot dry weight (g), root fresh weight (g), and root dry weight (g). Pod length, pod number per plant, seed number per pod, seed number per plant, seed yield per plant (g), and 1000-seed weight (g) were measured at harvest. To get the $M_2$ generation of plants, the seeds from the $M_1$ plants were preserved and sown the next season. The $M_2$ generation of plants was cultivated in the same environment as the previous generation, and similar growth and yield parameters were recorded.

**Statistical Analysis Of Morphological and Yield Parameters:**

The completely randomized block design (CRBD) was used for both first and second-generation plants and for the treated samples and their corresponding controls. IBM SPSS 26 statistical software (SPSS Inc., Chicago, IL, USA) was used to conduct an analysis of variance (ANOVA) on all the factors under investigation. Data presented as means of 3 replicates ± SE. Least significant differences (LSD) and Duncan's multiple range test (DMRT) were used to compare the means at the 5% probability level ($P \leq 0.05$).

**ISSR-PCR of Genomic DNA:**

**Extraction and Purification of Genomic DNA:**

Liquid nitrogen was used to grind the leaf tissues of $M_2$ fenugreek plants into a fine powder. The DNA Easy Plant Mini Kit (Qiagen, Santa Clarita, CA) was then used to extract DNA in accordance with the manufacturer's instructions. The concentration of DNA was measured by running 2 µl of the parent DNA samples on a 1% agarose gel and 10 µl of a DNA size marker (100 bp DNA ladder). The fluorescence of the DNA sample was compared to the different bands in the DNA size marker to estimate its concentration.

**ISSR Primers and PCR Reaction:**

The $M_2$ genotypes obtained from $M_1$ plants developed from seeds subjected to the five applicable gamma irradiation doses were examined for polymorphism using eleven ISSR primers (Table 1). According to Ibrahim *et al.,* (2019), the amplification reaction was conducted in a 25-µl reaction container with 12.5 µl Master Mix (Sigma), 2.5 µl primer (10 pcmol), 3 µl template DNA (10ng), and 7 µl dH2O. A Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) was used to do the PCR amplification. It was set up to complete 40 cycles following a 5-minute denaturation cycle at 94 ºC. Each cycle consisted of a denaturation step at 94 ºC for 45 seconds, an annealing step at 50 ºC for 50 seconds, and an elongation step at 72 ºC for 1 minute. In the last cycle, the primer extension section was prolonged to 7 minutes at 72 ºC.

**Table 1:** List of eleven ISSR primer sequences used in M2 generation of Trigonella foenum-graecum following the exposure of the parent seeds to different doses of g-radiation.

| Primer | Sequence |
|---|---|
| ISSR-1 | 5'-AGAGAGAGAGAGAGAGYC-3' |
| ISSR-2 | 5'-AGAGAGAGAGAGAGAGYG-3' |
| ISSR-3 | 5'-ACACACACACACACACYT-3' |
| ISSR-4 | 5'-ACACACACACACACACYG-3' |
| ISSR-5 | 5'-GTGTGTGTGTGTGTGTYG-3' |
| ISSR-6 | 5'-CGCGATAGATAGATAGATA-3' |
| ISSR-7 | 5'-GACGATAGATAGATAGATA-3' |
| ISSR-8 | 5'-AGACAGACAGACAGACGC-3' |
| ISSR-9 | 5'-GATAGATAGATAGATAGC-3' |
| ISSR-10 | 5'-GACAGACAGACAGACAAT-3' |
| ISSR-11 | 5'-ACACACACACACACACYA-3' |

**Gel Electrophoresis:**

The amplicons were run on 1.5% agarose gel containing ethidium bromide (0.5 ug/ml) in 1X TBE buffer at 95 volts. Gel Documentation System (BIO-RAD 2000) was used to photograph PCR products under UV light. To determine the size of the DNA bands, a DNA size marker (a 100 bp DNA ladder) was utilized.

**Data Analysis of ISSR Fingerprinting:**

ISSR-PCR marker analysis banding patterns were employed to compare study treatments for genetic similarities. Both polymorphic and monomorphic bands were included in the final data sets since only clear and unambiguous bands were visually evaluated as present (1) or absent (0) for all samples during ISSR analysis. Then, a binary data matrix was constructed. The Dice's similarity matrix coefficients were then calculated between the treatments using the unweighted pair-group method with arithmetic averages (UPGMA). This matrix was used to construct a dendrogram using the PAST software Version 1.91 to express the resemblance between the M2 genotypes based on ISSR polymorphism (Hammer, 2001).

## RESULTS AND DISCUSSION

**Effect of Irradiation on Growth Parameters:**

The positive effects of gamma radiation on plant growth, at low doses of gamma radiation, are linked to improvements in germination patterns and the acceleration of overall plant development. Several studies have shown that gamma irradiation enhances plant growth characteristics across various plant species, such as wheat (Singh *et al.*, 2010) and barley (Wang *et al.*, 2018). The effect of gamma radiation on growth and development can differ depending on the characteristics of the plant material, the radiation dose, and the environmental conditions (De Micco *et al.*, 2011). Higher doses of gamma radiation can have a negative impact on plant growth by generating free radicals that can harm various physiological, morphological, and anatomical components of the plant (Parchin *et al.*, 2019). Despite the beneficial impacts of gamma rays on plant development, high doses of gamma rays may have unfavorable effects on some growth characteristics. Low-dose irradiation has been shown to increase development by enhancing hormonal signaling and antioxidative capacity, allowing the organism to better withstand environmental stress (Hong *et al.*, 2014).

The variation in growth parameters of fenugreek plants of both $M_1$ and $M_2$ generations produced from seeds irradiated with the applied doses of gamma irradiation (shoot and root length and fresh and dry weight) is illustrated in Table 2. Meanwhile, Figure 1 illustrates the differences in the percentage increase or reduction in each trait compared to the same traits in the control plants. Plants exposed to low levels of gamma irradiation showed considerable improvements in all measured growth parameters. The improvement was gradually increased with increasing gamma doses, which ranged from 25 Gy to 100 Gy. The dose of 75 Gy was the most effective in inducing increased growth in treated plants compared to the control (Fig. 1). However, the high dose of 200 Gy resulted in growth inhibition of $M_1$ and $M_2$ generation. The 200 Gy was the most effective in shortening shoot and root lengths and reducing shoot and root fresh and dried weights below control (Fig. 1). These findings are in agreement with Chaudhuri (2002), who observed that root and shoot length decreased after exposure to high levels of gamma rays.

**Table 2:** Effect of gamma irradiation on growth parameters of Trigonella foenum-graecum plants produced from seeds irradiated with different doses of gamma rays. Control means plants produced from unirradiated seeds. PC % (Percentage change in growth parameters compared to the same parameter in the control plants).

| γ-radiation dose (Gy) | Shoot length (cm) | PC % | Root length (cm) | PC % | Shoot fresh weight (g) | PC % | Root fresh weight (g) | PC % | Shoot dry weight (g) | PC % | Root dry weight (g) | PC % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $M_1$ generation | | | | | | | | | | | | |
| control | 43.00 ± 0.58 $^{e}$ | | 12.33 ± 0.33 $^{d}$ | | 7.00± 0.58 $^{cd}$ | | 0.93 ± 0.09 $^{c}$ | | 0.81 ± 0.06 $^{cd}$ | | 0.28 ± 0.03 $^{bc}$ | |
| 25 | 46.33 ± 0.88 $^{d}$ | 8% | 13.17 ± 0.44 $^{cd}$ | 7% | 7.67 ± 0.33 $^{bc}$ | 10% | 1.17 ± 0.03 $^{b}$ | 25% | 0.89 ± 0.05 $^{bc}$ | 9% | 0.30 ± 0.06 $^{bc}$ | 6% |
| 50 | 52.00 ± 1.15 $^{c}$ | 21% | 14.17 ± 0.17 $^{b}$ | 15% | 8.73 ± 0.15 $^{b}$ | 25% | 1.27 ± 0.03 $^{b}$ | 36% | 0.95 ± 0.03 $^{bc}$ | 17% | 0.37 ± 0.03 $^{ab}$ | 29% |
| 75 | 60.67 ± 0.76 $^{a}$ | 41% | 15.23 ± 0.15 $^{a}$ | 24% | 10.17 ± 0.73 $^{a}$ | 45% | 1.60 ± 0.06 $^{a}$ | 71% | 1.22 ± 0.08 $^{a}$ | 50% | 0.42 ± 0.02 $^{a}$ | 47% |
| 100 | 56.67 ± 1.67 $^{b}$ | 32% | 13.33 ± 0.33 $^{bc}$ | 8% | 8.93 ± 0.52 $^{ab}$ | 28% | 1.47 ± 0.09 $^{a}$ | 57% | 1.02 ± 0.09 $^{b}$ | 25% | 0.35 ± 0.03 $^{ab}$ | 24% |
| 200 | 40.33 ± 0.33 $^{e}$ | -6% | 10.13 ± 0.13 $^{e}$ | -18% | 5.83 ± 0.17 $^{d}$ | -17% | 0.80 ± 0.06 $^{c}$ | -14% | 0.63 ± 0.03 $^{d}$ | -22% | 0.22 ± 0.02 $^{c}$ | -24% |
| LSD 0.05 | 3.02 | | 0.87 | | 1.43 | | 0.20 | | 0.19 | | 0.10 | |
| F-ratio | 66.03 | | 37.56 | | 11.04 | | 22.82 | | 10.42 | | 4.31 | |
| P-Value | ≤ 0.001*** | | ≤ 0.001*** | | ≤ 0.001*** | | ≤ 0.001*** | | ≤ 0.001*** | | ≤ 0.001*** | |
| $M_2$ generation | | | | | | | | | | | | |
| control | 41.67 ± 0.88 $^{d}$ | | 11.17 ± 0.44 $^{c}$ | | 5.23 ± 0.15 $^{e}$ | | 0.81 ± 0.05 $^{c}$ | | 0.61 ± 0.05 $^{d}$ | | 0.24 ± 0.02 $^{c}$ | |
| 25 | 44.00 ± 0.58 $^{c}$ | 6% | 12.20 ± 0.20 $^{b}$ | 9% | 6.50 ± 0.17 $^{d}$ | 24% | 0.93 ± 0.04 $^{c}$ | 15% | 0.66 ± 0.06 $^{cd}$ | 8% | 0.26 ± 0.01 $^{c}$ | 10% |
| 50 | 48.33 ± 0.33 $^{b}$ | 16% | 12.97 ± 0.26 $^{b}$ | 16% | 7.23 ± 0.15 $^{c}$ | 38% | 1.10 ± 0.06 $^{b}$ | 36% | 0.75 ± 0.03 $^{bc}$ | 22% | 0.30 ± 0.01 $^{b}$ | 25% |
| 75 | 55.17 ± 0.60 $^{a}$ | 32% | 14.67 ± 0.33 $^{a}$ | 31% | 9.00 ± 0.12 $^{a}$ | 72% | 1.37 ± 0.07 $^{a}$ | 69% | 0.97 ± 0.04 $^{a}$ | 60% | 0.38 ± 0.00 $^{a}$ | 57% |
| 100 | 49.00 ± 0.58 $^{b}$ | 18% | 12.80 ± 0.20 $^{b}$ | 15% | 8.10 ± 0.06 $^{b}$ | 55% | 1.22 ± 0.04 $^{ab}$ | 51% | 0.82 ± 0.04 $^{b}$ | 35% | 0.32 ± 0.01 $^{b}$ | 32% |
| 200 | 37.33 ± 0.33 $^{e}$ | -10% | 9.10 ± 0.21 $^{d}$ | -19% | 4.90 ± 0.21 $^{e}$ | -6% | 0.63 ± 0.03 $^{d}$ | -21% | 0.45 ± 0.03 $^{e}$ | -26% | 0.19 ± 0.00 $^{d}$ | -22% |
| LSD 0.05 | 1.80 | | 0.89 | | 0.46 | | 0.15 | | 0.13 | | 0.03 | |
| F-ratio | 116.18 | | 42.75 | | 116.78 | | 29.85 | | 19.40 | | 47.78 | |
| P-Value | ≤ 0.001*** | | ≤ 0.001*** | | ≤ 0.001*** | | ≤ 0.001*** | | ≤ 0.001*** | | ≤ 0.001*** | |

Data presented as means of 3 replicates ± SE, SE: Standard Error. Different superscript letters (a, b, c, d, e, ab, bc, and cd) denote significant difference at P ≤ 0.05 between different treatments according Duncan's multiple-range test. *: significant at P ≤ 0.05, **: significant at P ≤ 0.01, ***: significant at P ≤ 0.001 according to LSD test. PC % (Percentage change between each treatment and control plants).

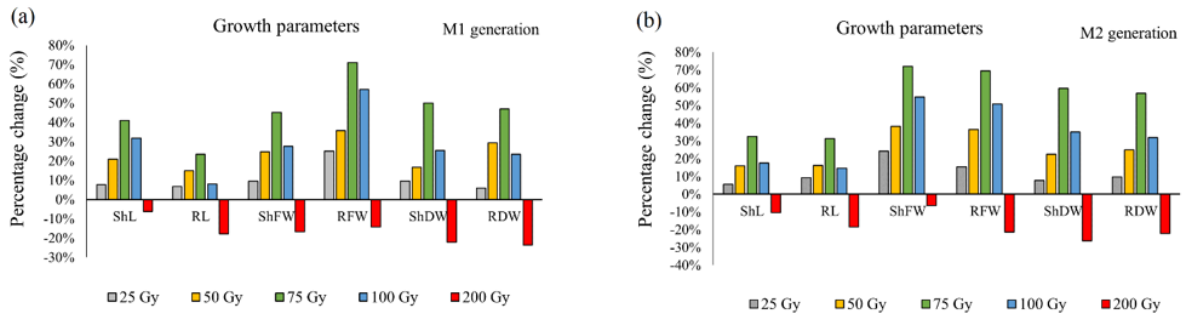


**Fig. 1.** Percentage change (increase or reduction) in growth parameters compared to the same parameter in the control plants of fenugreek plants produced from seeds irradiated with different doses of gamma rays at $M_1$ generation (a) and $M_2$ generation (b). Abbreviations: SHL: Shoot length; RL: Root length; SHFW: Shoot fresh weight; RFW: Root fresh weight; SHDW: Shoot dry weight; RDW: Root dry weight.

The highest value of shoot length (60.67 ± 0.76 cm) for $M_1$ generation represents an increase of 41% over the control plants (43.00 ± 0.58 cm). For the $M_2$ generation, an increase of 32% over the control plants was induced by the dose of 75 Gy. The highest value of root length (15.23 ± 0.15 cm) for the $M_1$ generation showed an increase of 24% over the control plants. For the $M_2$ generation, root length (14.67 ± 0.33 cm) recorded an increase of 31% over the control plants. Shoot fresh weight of $M_1$ generation (10.17 ± 0.73 g) showed an increase of 45% over the control plants (7.00 ± 0.58 g). However, for the $M_2$ generation, an increase of 72% over the control plants was recorded. The highest value of root fresh weight (1.60 ± 0.06 g) for $M_1$ generation showed an increase of 71% over the control plants and the value of 1.37 ± 0.07 g for $M_2$ generation showed an increase of 69 % over the corresponding control plants. The highest value of shoot dry weight (1.22 ± 0.08 g) for the $M_1$ generation showed an increase of 50

% over the control plants, while 0.97 ± 0.04 g for the $M_2$ generation plants showed an increase of 60% over the control plants. On the other hand, the highest value of root dry weight (0.42± 0.02 g) was for $M_1$ generation plants with an increase of 47% over the control plants and a value of 0.38 ± 0.01 g for $M_2$ generation plants with an increase of 57% over the control plants.

**Effect of Irradiation on Yield Parameters:**

Table 3 illustrates the variations in yield parameters of $M_1$ and $M_2$ generation fenugreek plants grown from seeds treated with the applied gamma irradiation dosages. In the meantime, Figure 2 illustrates the differences in the percentage increase or reduction in each yield parameter compared to the same traits in the control plants. The gamma dose of 75 Gy was the most effective in inducing increases in all measured yield parameters. The highest pod length (12.07 ± 0.30 cm) for $M_1$ generation was 23% more than the control pod length and the pod length of 11.33 ± 0.33 cm for $M_2$ generation was 21% over the control plants (Fig. 2). The highest number of pods per plant (39.00 ± 0.58) was recorded for $M_1$ plants with an increase of 30% over the control plants and 36.33 ± 0.67 for $M_2$ plants with an increase of 35% over the control plants. The highest number of seeds per pod was (15.67 ± 0.58) for $M_1$ plants with an increase of 47% over the control plants and (13.50 ± 0.29) for $M_2$ plants with an increase of 31% over the control plants. The highest number of seeds per plant (610.67 ± 7.06) was for $M_1$ plants, with an increase of 91% over the control plants; (490.83 ± 18.70) for the $M_2$ plants, there was an increase of 76% in plant seed number over the control plants. The dose of 75 Gy also induced the highest values of seed yield per plant (16.12 ± 0.21 g) for $M_1$ plants with an increase of 133 % over the control plants and (11.46 ± 0.50 g) for $M_2$ plants with an increase of 105 % over the control plants (Table 3 and Fig. 2). The highest value of 1000-seed weight (26.40 ± 0.15 g) for $M_1$ plants with an increase of 22 % over the control plants and (23.33 ± 0.33 g) for $M_2$ plants with an increase of 16 % over the control plants were also induced by the dose of 75 Gy (Table 3 and Fig. 2).

**Table 3:** Effect of gamma irradiation on yield parameters of Trigonella foenum-graecum plants produced from seeds irradiated with different doses of gamma rays. Control means plants produced from unirradiated seeds. PC % (Percentage change in yield parameters compared to the same parameter in the control plants).

| γ-radiation dose (Gy) | Pod length (cm) | PC % | Number of pods/plant | PC % | Number of seeds/pods | PC % | Number of seeds/plant | PC % | Seed yield/plant (g) | PC % | 1000-seed weight (g) | PC % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $M_1$ generation | | | | | | | | | | | | |
| control | 9.80 ± 0.12 $^{c}$ | | 30.00 ± 0.58 $^{d}$ | | 10.67 ± 0.33 $^{d}$ | | 320.00 ± 11.85 $^{e}$ | | 6.91 ± 0.29 $^{e}$ | | 21.60 ± 0.17 $^{d}$ | |
| 25 | 10.10 ± 0.10 $^{c}$ | 3% | 31.67 ± 0.33 $^{c}$ | 6% | 12.33 ± 0.33 $^{c}$ | 16% | 390.33 ± 6.33 $^{d}$ | 22% | 8.56 ± 0.13 $^{d}$ | 24% | 21.93 ± 0.03 $^{cd}$ | 2% |
| 50 | 11.17 ± 0.20 $^{b}$ | 14% | 33.33 ± 0.33 $^{b}$ | 11% | 13.33 ± 0.58 $^{bc}$ | 25% | 444.33 ± 9.60 $^{c}$ | 39% | 9.91 ± 0.26 $^{c}$ | 43% | 22.30 ± 0.10 $^{c}$ | 3% |
| 75 | 12.07 ± 0.30 $^{a}$ | 23% | 39.00 ± 0.58 $^{a}$ | 30% | 15.67 ± 0.58 $^{a}$ | 47% | 610.67 ± 7.06 $^{a}$ | 91% | 16.12 ± 0.21 $^{a}$ | 133% | 26.40 ± 0.15 $^{a}$ | 22% |
| 100 | 11.43 ± 0.23 $^{ab}$ | 17% | 34.00 ± 0.58 $^{b}$ | 13% | 14.67 ± 0.88 $^{ab}$ | 38% | 497.67 ± 21.96 $^{b}$ | 56% | 11.69 ± 0.48 $^{b}$ | 69% | 23.50 ± 0.12 $^{b}$ | 9% |
| 200 | 8.07 ± 0.35 $^{d}$ | -18% | 26.00 ± 0.58 $^{e}$ | -13% | 9.33 ± 0.58 $^{d}$ | -13% | 243.00 ± 13.75 $^{f}$ | -24% | 5.03 ± 0.34 $^{f}$ | -27% | 20.67 ± 0.33 $^{e}$ | -4% |
| LSD 0.05 | 0.72 | | 1.57 | | 1.45 | | 39.65 | | 0.94 | | 0.55 | |
| F-ratio | 37.78 | | 72.69 | | 25.80 | | 102.86 | | 162.81 | | 129.70 | |
| P-Value | ≤ 0.001*** | | ≤ 0.001*** | | ≤ 0.001*** | | ≤ 0.001*** | | ≤ 0.001*** | | ≤ 0.001*** | |
| $M_2$ generation | | | | | | | | | | | | |
| control | 9.33 ± 0.33 $^{c}$ | | 27.00 ± 0.58 $^{d}$ | | 10.33 ± 0.33 $^{d}$ | | 278.67 ± 4.67 $^{d}$ | | 5.60 ± 0.08 $^{d}$ | | 20.10 ± 0.59 $^{c}$ | |
| 25 | 9.80 ± 0.42 $^{bc}$ | 5% | 30.67 ± 0.67 $^{c}$ | 14% | 11.77 ± 0.39 $^{c}$ | 14% | 361.20 ± 18.37 $^{c}$ | 30% | 7.70 ± 0.03 $^{c}$ | 38% | 21.33 ± 0.33 $^{bc}$ | 6% |
| 50 | 10.17 ± 0.44 $^{abc}$ | 9% | 32.33 ± 0.33 $^{bc}$ | 20% | 12.13 ± 0.22 $^{bc}$ | 17% | 392.37 ± 9.36 $^{bc}$ | 41% | 8.76 ± 0.09 $^{bc}$ | 56% | 22.33 ± 0.33 $^{ab}$ | 11% |
| 75 | 11.33 ± 0.33 $^{a}$ | 21% | 36.33 ± 0.67 $^{a}$ | 35% | 13.50 ± 0.29 $^{a}$ | 31% | 490.83 ± 18.70 $^{a}$ | 76% | 11.46 ± 0.50 $^{a}$ | 105% | 23.33 ± 0.33 $^{a}$ | 16% |
| 100 | 10.83 ± 0.44 $^{ab}$ | 16% | 33.00 ± 0.58 $^{b}$ | 22% | 13.10 ± 0.31 $^{ab}$ | 27% | 432.23 ± 11.46 $^{b}$ | 55% | 9.71 ± 0.59 $^{b}$ | 74% | 22.43 ± 0.79 $^{ab}$ | 12% |
| 200 | 7.33 ± 0.33 $^{d}$ | -21% | 24.00 ± 0.58 $^{e}$ | -11% | 9.33 ± 0.33 $^{e}$ | -10% | 224.00 ± 9.54 $^{e}$ | -20% | 4.10 ± 0.25 $^{e}$ | -27% | 18.27 ± 0.37 $^{d}$ | -9% |
| LSD 0.05 | 1.19 | | 1.78 | | 0.98 | | 40.24 | | 1.09 | | 1.51 | |
| F-ratio | 13.21 | | 58.89 | | 25.67 | | 56.87 | | 57.85 | | 14.32 | |
| P-Value | ≤ 0.001*** | | ≤ 0.001*** | | ≤ 0.001*** | | ≤ 0.001*** | | ≤ 0.001*** | | ≤ 0.001*** | |

Data presented as means of 3 replicates ± SE, SE: Standard Error. Different superscript letters (a, b, c, d, e, f, ab, bc, cd, and abc) denote significant difference at $P \leq 0.05$ between different treatments according to Duncan's multiple-range test. *: significant at $P \leq 0.05$, **: significant at $P \leq 0.01$, ***: significant at $P \leq 0.001$ according to LSD test. PC % (Percentage change between each treatment and control plants).

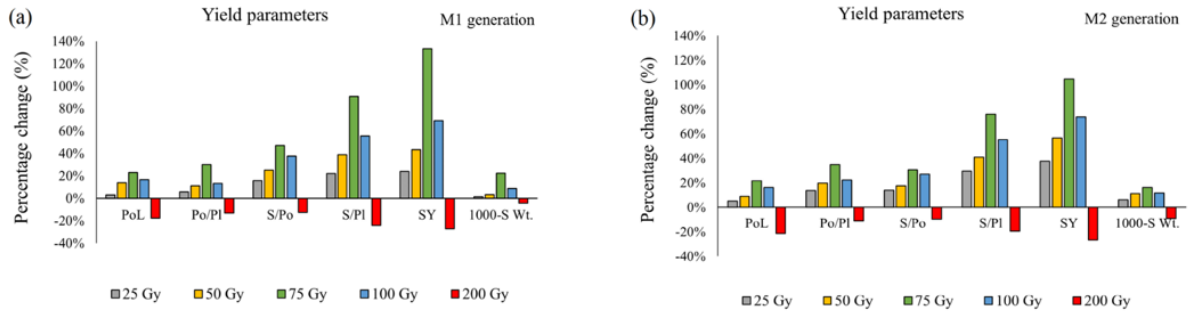


**Fig. 2.** Percentage change (increase or reduction) in yield parameters compared to the same parameter in the control plants of fenugreek plants produced from seeds irradiated with different doses of gamma rays at $M_1$ generation (a) and $M_2$ generation (b). Abbreviations: PoL: Pod length; Po/Pl: Number of pods per plant; S/Po: Number of seeds per pod; S/Pl: Number of seeds per plant; SY: Seed yield per plant; 1000-S Wt.: 1000-seed weight.

**Intersimple Sequence Repeats (ISSR) Marker Analysis:**

Previous studies showed that exposure to low-dose radiation resulted in the activation of a resistance mechanism that mitigates DNA damage (Dimova *et al.*, 2008). However, exposure to high doses of gamma radiation has the potential to cause detrimental effects resulting in different forms of damage across the whole genome (Marcu et al., 2013; Shikazono *et al.*, 2005). Several studies have shown evidence that gamma irradiation stimulates an excessive generation of reactive oxygen species (ROS), resulting in detrimental effects on various organic compounds such as lipids, proteins, and nucleic acids (Hanafy *et al.*, 2018). It has been discovered that irradiating seeds with gamma rays has a significant influence on plant development. Growth (e.g., taller plants), reproductive success (e.g., formed seeds), and drought tolerance are all areas in which g-irradiation exposure has been shown to improve crop yields and morphological anomalies (Maity *et al.*, 2005; Melki *et al.*, 2009; Yu *et al.*, 2007; Zaka *et al.*, 2002). Several authors used these findings to infer that irradiating seeds before planting held tremendous promise for practical use in agriculture. Low-dose gamma rays promote cell division, growth, and development in organisms. Radiation dosage intensity and duration determine changes in morphology, structure, and function. The biological responses of plants to gamma radiation range from stimulation to inhibition of germination and seedling growth (Kim *et al.*, 2004; Wi *et al.*, 2005).

Molecular markers play a crucial role in detecting genetic variability among species and infraspecific genotypes, hence serving as valuable tools for the conservation of germplasm and the identification of cultivars. Several DNA-based markers are now available that can be used to accurately measure genetic diversity in plant species. The ISSR marker exhibits a high degree of polymorphism, which makes it useful for studying biodiversity, genome mapping, and evolutionary genetics (Joshi *et al.*, 2007). Changes in the structure of DNA, such as breaks, transpositions, deletions, etc., can lead to the formation of new bands (unique bands) and the removal of existing bands (polymorphic bands) (Danylchenko *et al.*, 2005). The impact of g-radiation on strawflower growth in two generations using RAPD and ISSR DNA analysis was investigated by El-Khateeb *et al.* (2017). In their research, they found that gamma radiation led to the appearance of new bands and the absence of others in the mutants compared to the control plants.

Eleven primers were used in the study; their names and sequences are given in Table 1. The ISSR analysis was made to compare the $M_2$ genotypes in fenugreek leaves. A total of 118 bands were produced by the 11 primers in all genotypes, of which 26 bands were polymorphic and 19 were unique

(Table 4 and Fig. 3). The primer ISSR-9 produced the highest number of bands (14 bands), while the primer ISSR-6 gave the smallest number of 6 bands. Tables 3 and 5 show the bands generated by each primer (Table 4).

**Table 4:** The polymorphism generated by eleven ISSR primers used in M2 plants of Trigonella foenum-graecum following exposure of parent seeds to the applied doses of g-radiation.

| Primer | Range of band size (bp) | M-bands | P-bands (without unique bands) | U-bands | P-bands (with unique bands) | Total number of bands | Mean of band frequency | PIC | P (%) |
|---|---|---|---|---|---|---|---|---|---|
| ISSR-1 | 154-713 | 8 | 2 | 0 | 2 | 10 | 1.0 | 0.06 | 20 |
| ISSR-2 | 170-506 | 8 | 1 | 2 | 3 | 11 | 0.8 | 0.10 | 27 |
| ISSR-3 | 161-547 | 9 | 3 | 0 | 3 | 12 | 0.9 | 0.12 | 25 |
| ISSR-4 | 192-655 | 7 | 3 | 3 | 6 | 13 | 0.7 | 0.15 | 46 |
| ISSR-5 | 282-931 | 3 | 4 | 1 | 5 | 8 | 0.7 | 0.24 | 63 |
| ISSR-6 | 307-553 | 5 | 0 | 1 | 1 | 6 | 0.9 | 0.05 | 17 |
| ISSR-7 | 361-944 | 2 | 4 | 5 | 9 | 11 | 0.4 | 0.29 | 82 |
| ISSR-8 | 200-911 | 8 | 3 | 1 | 4 | 12 | 0.8 | 0.14 | 33 |
| ISSR-9 | 205-374 | 8 | 4 | 2 | 6 | 14 | 0.8 | 0.16 | 43 |
| ISSR-10 | 175-1231 | 8 | 2 | 3 | 5 | 13 | 0.8 | 0.12 | 38 |
| ISSR-11 | 288-873 | 7 | 0 | 1 | 1 | 8 | 0.9 | 0.03 | 13 |
| **Total** | | 73 | 26 | 19 | 45 | 118 | --- | --- | --- |
| **Mean per primer** | | 6.64 | 2.36 | 1.73 | 4.09 | 10.73 | 0.79 | 0.13 | 37 |

M-bands (monomorphic bands), P-bands (polymorphic bands), U-bands (unique bands), P (%) polymorphism percentage.

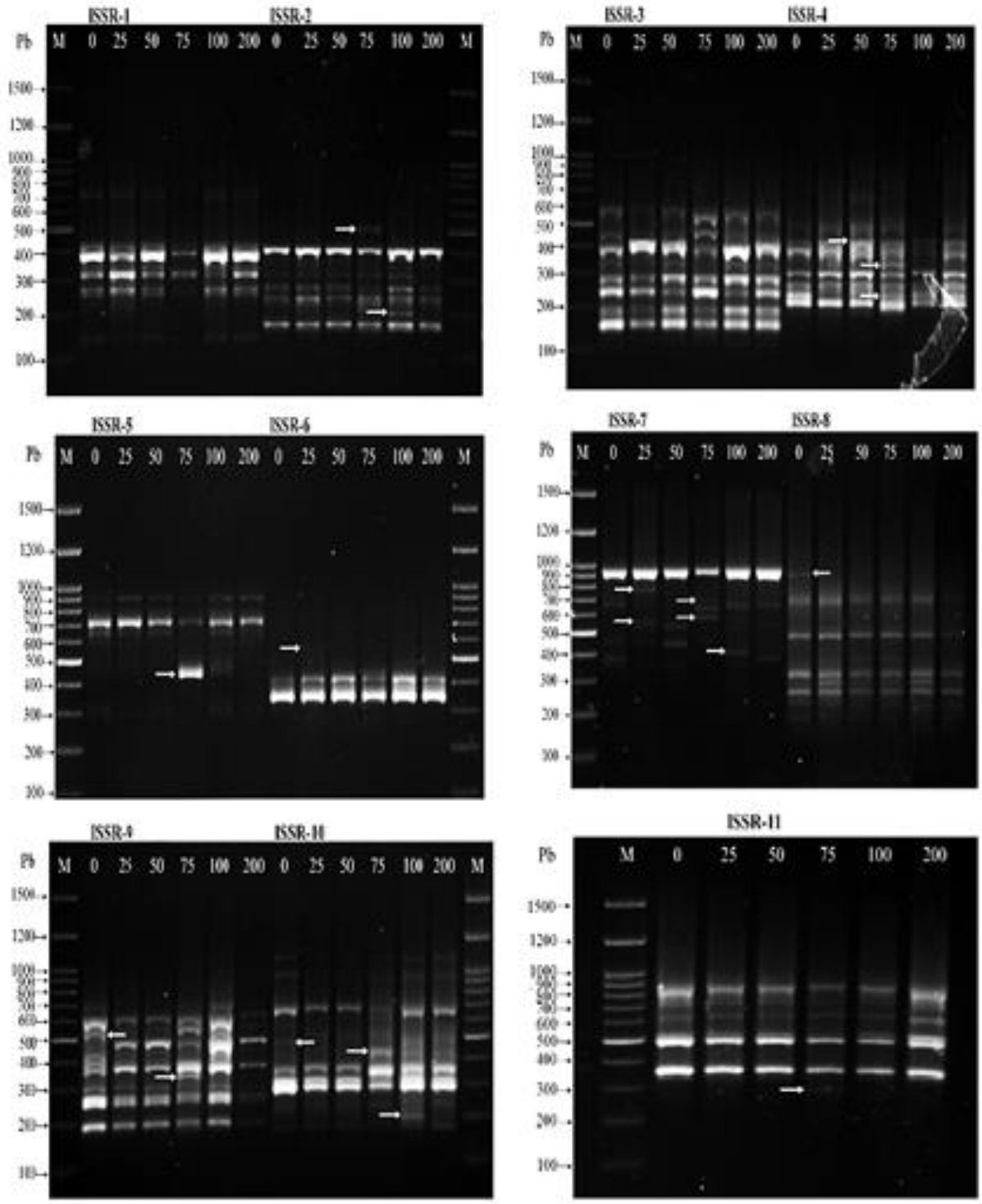


**Fig. 3.** ISSR amplification profiles patterns obtained using eleven primers in $M_2$ generation of *Trigonella foenum-graecum* following exposure of parent seeds to the applied doses of g-radiation, (M) referred to size marker 100 bp DNA ladder.

According to the results, the amounts of polymorphism varied between primers. The highest percentage of polymorphism (82 %) was recorded by the ISSR primer 7 and the lowest (13 %) was produced by the ISSR-11. Nine primers gave 19 molecular markers (three negative and 16 positive) related to radiation treatments (Table 5), which may be a form of marker-assisted selection of traits associated with genetic variation. Two unique positive markers with a molecular size of 204 bp at the dose of 100 Gy and 506 bp at the

dose of 75 Gy were detected using ISSR-2 primer (Table S1). The three unique positive markers at 203 bp, 320 bp at the dose of 75 Gy and 436 bp at the dose of 50 Gy were detected using ISSR-4 primer (Table S1). The details of the unique markers produced by the used primers are illustrated in Fig. 3 and Table 5. These markers have the potential to be utilized for the identification of genes that confer stress tolerance, hence enabling marker-assisted breeding for radiation tolerance and traits induced by gamma irradiation (Table 5). Three negative markers, which were found only in the control plants, were noticed at 911 bp by the ISSR-8 primer, 511 bp by the ISSR-9 primer, and 441 bp by the ISSR-10. The polymorphic information content (PIC) value ranged from 0.03 for ISSR pr-11 primer to 0.29 for ISSR primer-7, with an average of 0.13 (Table 4). The Dice's similarity coefficient along with the treatments ranged from 0.83 (between control plants and 75 Gy treatment) to 0.90 (between 25 Gy treatment and 50 Gy treatment), as revealed in Table 6.

**Table 5:** Positive unique markers (PUM) and negative unique markers (NUM) of the eleven ISSR primers used in M2 generation of Trigonella foenum-graecum following the exposure of the parent seeds to different doses of g-radiation.

| Primer | PUM (bp) | NUM (bp) |
|---|---|---|
| ISSR-1 | -- | -- |
| ISSR-2 | 204-506 | -- |
| ISSR-3 | -- | -- |
| ISSR-4 | 203-320-436 | -- |
| ISSR-5 | 437 | -- |
| ISSR-6 | 553 | -- |
| ISSR-7 | 393-529-554-664-758 | -- |
| ISSR-8 | -- | 911 |
| ISSR-9 | 333 | 511 |
| ISSR-10 | 201- 417 | 441 |
| ISSR-11 | 288 | -- |
| **Total** | 16 | 3 |

**Table 6:** Similarity matrix among M2 plants of Trigonella foenum-graecum following exposure of parent seeds to the applied doses of γ-radiation according to Dice`s coefficient from ISSR pattern generated by eleven ISSR primers.

| Treatment | Control | 25 Gy | 50 Gy | 75 Gy | 100 Gy | 200 Gy |
|---|---|---|---|---|---|---|
| Control | 1.0 | | | | | |
| 25 Gy | 0.90 | 1.0 | | | | |
| 50 Gy | 0.90 | 0.94 | 1.0 | | | |
| 75 Gy | 0.83 | 0.85 | 0.86 | 1.0 | | |
| 100 Gy | 0.89 | 0.89 | 0.92 | 0.85 | 1.0 | |
| 200 Gy | 0.93 | 0.91 | 0.92 | 0.87 | 0.93 | 1.0 |

The dendrogram of the applied treatments with gamma radiation doses and the control plants based on ISSR markers polymorphism using UPGMA and similarity matrix computed according to the Dice coefficient is illustrated in Figure 4. The dendrogram comprised two main clusters; the first cluster contains the treatment with a dose of 75 Gy. The second cluster was divided into two sub-clusters; one sub-cluster contains the genotype induced by the doses of 25 Gy and 50 Gy. The other sub-cluster comprises the

control plants as a branch, and the other two genotypes are induced by 100 Gy and 200 Gy together. The study results indicate that the positive markers found could be useful for finding genes that help the plant manage the harmful effects of radiation. In addition, these markers can help with marker-assisted breeding, especially when it comes to making plants more resistant to radiation. Because of this, it can be concluded that using ISSR analysis to find DNA polymorphism is a useful chemical way to find changes in plants that have been exposed to gamma radiation.

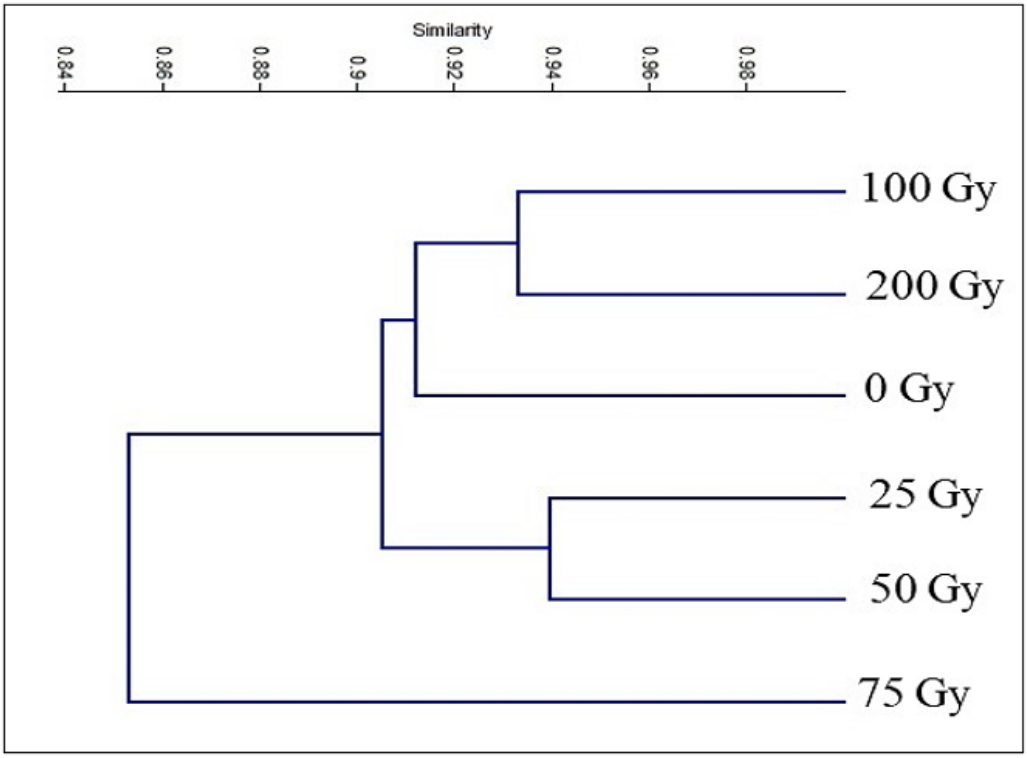


**Fig. 4.** Dendrogram for $M_2$ plants of *Trigonella foenum-graecum* following exposure of parent seeds to the applied doses of γ-radiation constructed from ISSR data using UPGMA and similarity matrix computed according to Dice`s coefficient.

## CONCLUSION

The study concludes that lower doses of gamma rays applied to fenugreek plants resulted in improved growth and yield characteristics across $M_1$ and $M_2$ generations. The dose of 75 Gy was the most effective in increasing growth and yield parameters. Additionally, the ISSR technique, as a molecular marker tool, was used to assess the genetic variability resulting from radiation-induced effects. Furthermore, the detection and analysis of positive and negative marker band sequences can help detect various types of DNA damage and mutations in plants caused by radiation exposure, thereby offering potential advantages for crop improvement.

There are no conflicts of interest, as the authors have stated.

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