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Effect of gamma irradiation on yield components and molecular marker traits in desi and kabuli chickpea

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Chickpeas are a global crop supplying cheap protein and other nutrition. Inducing genetic variability is an essential step in any plant breeding program. This study investigated the direct and indirect effects of various gamma doses (0, 100, 200, 300, and 400 Gy) on seed yield and other quantitative traits in two chickpea genotypes, Kabuli (Giza 531) and Desi (Giza 3) for two subsequent (M_1 and M_2) generations. Non-significant differences were found in the number and percentage of germinations between the irradiated and control seeds. The G.3 variety recorded more pods and seeds, while G.531 excelled in seed yield and pod weight. The most significant response appeared at 200 Gy, with a reduction at 300 Gy and the most severe response at 400 Gy. The number and percentage of survival plants showed highly significant variances in both seasons, which ranked at 60% for 200 Gy, then 56% for control, followed by 100 Gy (48%), 300 Gy (41%), and 400 Gy (24%). Various levels of polymorphism were displayed by molecular markers, with SCoT at 85.05% and SRAP at 70.59%. Polymorphism information, marker index, effective multiplex ratio, and resolved power parameters were computed to assess the effectiveness of the markers. Gamma rays induce wide genetic variability at phenotypic and genotypic levels in chickpeas. The Kabuli genotype (G.531) generally has a more significant response to gamma-used doses than the Desi genotypes (G.3). These findings can guide plant breeders in determining the appropriate gamma dose to enhance chickpea seed yield, warranting further research.

Keywords: Chickpea, Yield performance, Gamma; Genetic diversity, SCoT & SRAP markers

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INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third major legume crop in the world. It is a cheap source and a highly nutritious pulse crop with protein, carbohydrates, unsaturated fatty acids (linolenic and oleic acid), vital minerals and vitamins such as niacin and thiamine, and other nutrients for human needs and animal feed (Sarker *et al.,* 2014 and Kandwal *et al.,* 2022). Chickpea is cultivated in almost 57 countries worldwide in different climates and growing conditions. The cultivated area of this crop is 15 million hectares, with an annual production of 15 million tons. The average yield productivity of this crop is 1000 kg/h (FAO, 2022). In Egypt, over the last few years, there has been a drop in yield production and cultivated area. From 2009 to 2019, the average yield production was 5390 tons with a cultivated area of 6028 feddan, and the seed yield was about 0.91 tons per feddan (Elasraag and Ahmed, 2023). The widely cultivated chickpeas (2n = 16) are divided into Kabuli and Desi types depending on seed size, coat color, and hardness. The two types of chickpeas are a large-seeded Kabuli, adapted to the Middle East and the Mediterranean, including Egypt, and a small-seeded Desi, better adapted to the East Asian region (Saxena and Singh, 1984). Around 80 % of the chickpea varieties are Desi, and the remaining (20%) are Kabuli types (Debnath*et al.,* 2021).

Chickpeas' limited genetic variability is one of the main factors in production breakthroughs. Rapid production enhancement by genetic manipulation can resolve this problem of declining chickpea yields (Wani *et al*., 2014; Umavathi and Mullainathan, 2019). Chickpea genetic improvement can be performed through various crop improvement techniques, including selection, hybridization, mutation, etc. Mutation induction is a powerful tool for inducing genetic variability, crop evolution, and improvement in all adopted breeding strategies (Umavathi and Mullainathan, 2019; El-Lithy et al., 2023). It is a major source of phenotypes and genotypes and a driver of evolutionary diversity (Yasmin and Arulbalachandran, 2022).

Gamma radiation is commonly used as a physical mutagenic agent due to its short wavelength and high energy. Therefore, it can be an alternative strategy to improve plant breeding (Bhoi *et al.,* 2022). According to FAO/IAEA (2019), 92% of the released mutant genotypes of crops were caused by gamma irradiation only. Genetic variability in plants could be generated by applying a wide range of gamma rays, starting from low doses (5–100 Gy) until high doses (>300 Gy), to improve desirable targeting traits such as seed yield, seed quality, and biotic and abiotic stresses. (Viana *et al.,* 2019 and Katiyar *et al.,* 2022).

The molecular marker provides the potential to effectively identify the desired mutants by evaluating the population's genetic variants and helping to detect changes (Abdulhafiz *et al*. 2018). The SCoT markers (Start codon targeted) are one of the molecular markers used to identify genetic variability. This marker depends on polymorphism in the short-conserved area surrounding the start codon ATG (Habiba *et al*., 2021). Insertion and deletion mutations may result in some markers of SCoT being codominant (Samarina *et al*., 2021). In this technique, a single prime is employed as both a forward and a reverse primer to create DNA markers by PCR. Therefore, the SCoT markers are beneficial for generating DNA markers in various plant cultivars and are more trustworthy and efficient because they display a high annealing temperature (Zhao et *al*., 2020; Samarina *et al*., 2021). Sequence-related amplified polymorphism (SRAP) is a PCR-based dominant marker technique. It is superior to common dominant markers because of its high reproducibility, simplicity, inexpensiveness, robustness, efficiency, and versatility (Hassan et *al*., 2020). Its application is based on specifically amplifying genome coding regions using forward primers targeting GC-rich exons and reverse primers targeting AT-rich promoters, introns, and spacers (Yi *et al*., 2021). SRAP has been effectively utilized for marker-based quantitative trait loci, linkage map creation, crop genetic diversity, and gene cloning (Youssef *et al*., 2019).

Increasing seed yield is the primary goal of plant breeding programs. Seed yield is a quantitative trait, meaning it is a complex trait influenced by other simple traits and environmental conditions (Samad *et al.,* 2014). Therefore, the objective of the current study was to determine the direct and indirect effects of different gamma irradiation doses (0, 100, 200, 300, and 400 Gy) on critical characteristics such as germination seeds, survival plants, seed yield, and other yield components. Besides, genetic variability in chickpeas can be evaluated using cluster analyses and molecular markers such as SCoT and SRAP markers.

MATERIAL AND METHODS

Plant material

Two genotypes of chickpea (*Cicer arietinum* L), Kabuli (Giza 531) and Desi (Giza 3) were obtained from the Agriculture Research Center, Giza, Egypt. The two studied varieties were selected based on two different genotypes. Which Giza (531) genotype belongs to the Kabuli type, with larger seeds, lighter colors, and a smoother coat? Meanwhile, the Giza (3) genotype is a smaller Desi type with darker-colored beans and a rougher coat. Dry seeds of the two genotypes were exposed to four doses of gamma irradiations of a Cobalt-60 source, i.e. (100, 200, 300 and 400 Gy) at the National Centre for Radiation Research and Technology (NCRRT), Nuclear Research Centre, Inshas, Egypt, compared to untreated check (zero irradiation).

Germination test

A standard germination test was performed in the germination laboratory of the Faculty of Agriculture, Cairo University, Egypt. For this procedure, Petri dishes with moistened 3 mm blotting paper were used in three replicates; each replicate contained 25 chickpea seeds with a weight of about 3.8 - 4.3g, exposed to the five-gamma treatment for both Giza 531 and Giza 3 genotypes, then incubated at room temperature in the dark. The testing period was two weeks. At the end of the test, seeds containing cotyledons > 1 mm were recorded as germinated seeds on the seventh day. The germination rate was calculated according to the procedure by Kumar *et al.* (2018) and Chakraborty *et al.* (2023). Using this formula: Germination % = (Number of germinated seeds / Total number of sown seeds) x 100} to calculate the number and percentage of germinated seeds (M_1) and (M_2) in the first and second seasons, respectively, as shown in (Fig. 1).

Field Experiment

A two-year field experiment was conducted at the Fac. of Agric., Cairo univ., Egypt during the two seasons of 2020/2021 and 2021/2022. In three replications, the irradiated seeds of two studied chickpea cultivars, M_1 and M_2, were sown using a factorial arrangement treatment randomized completely block design (RCBD). Each replication was a row with dimensions of 0.75 to 4.*0* m containing 80 seeds weighing about 13.5-14.5 g, with two seeds in a hill spaced by 10 cm between hills. The M_1 and M_2 irradiated plants were evaluated, and data collected as individual plants, then the highest seed-yielding plants were shown in the next season, as shown in Fig. 2. The M_1 and M_2 plants were evaluated and considered for the present investigation to elucidate the extent of phenotypic variation compared to molecular characterization. Plant traits data were recorded at maturity stage including biological yield (plant dry weight)/plant (pt), g (BY), plant height, cm (Pt Ht),

number of pods/pt (Pods No.), weight of pods/pt, g (Pods Wt), number of seeds/pt (Seed No.), seed yield/pt, g (SY), seed index: weight of 100 seeds,

*g (SI) and harvest index (HI): (SY/BY)*100, number of surviving plants (Surv. Pt) and percentage of surviving plants (Surv. Pt %): (No. Surv. pt/ No. emerged pt)*100.

Soil composition analysis

The chemical, physical and mechanical soil properties were analyzed at the experimental site during the first season (2020/2021) before the chickpea crop was sown. The soil analysis was conducted at the Soil, Water and Environment Research Institute (SWERI), Agricultural Research Center (ARC), Giza, Egypt. The physical properties of experimental soil were 7.71 pH at (1:2.5) soil: water suspension, 1.3 EC: electrical conductivity (dS m−1), 46.0 SP: saturation percentage, 0.5 K+, 7.2 Na+, 1.5 Mg++, 3.8 Ca++, 3.0 So4-, 9.6 Cl-, 0.4 Hco3-. The chemical analysis classified the soil composition into 33.5 clay, 31.9 sand and 34.6 silt, so the texture class was loamy clay soil with 0.3 total nitrogen and 52.6 humidity fixation at 0.001 level.

Molecular Analysis

DNA Extraction

The most fully expanded leaves of M_2-grown chickpea plants that were 30 days old during the second season (2021-2022) were used for molecular characterization. Genomic DNA was extracted from the treated and controlled leaf samples using the CTAB method, according to Rogers and Bendich (1985). Ethidium bromide was used to stain the DNA, and the quantity and quality of DNA were examined on a 0.8% agarose gel.

SCoT and ISSR-PCR Amplification

SCoT-PCR amplifications were performed in a thermo-cycler (Biometra, Germany) using 10 SCoT primers (Table 1). The 20 µL total volume of the PCR reaction was composed of 1 µL of DNA template (30 ng), 1 µL of primer (10 µM), 10 µL of Master Mix (GeneDireX), and 8 µL of dd.H_2O. The PCR reactions were denaturized to 94 ℃ for 5 min, followed by 35 cycles of 1 min at 94 ℃, 1 min at 50 ℃, and 1 min at 72 ℃. The PCR products were detected by 1.2 % agarose gel electrophoresis stained with ethidium bromide.

Fifteen SRAP primer combinations were produced using switches and combinations of two forward primers named "Me" that target GC-rich exon regions and six reverse primers called "Em" that target AT-rich intron regions (Table 2) . The SRAP-PCR reaction was conducted in 20 µL containing 10 µL Master Mix (GeneDireX), 1 µL of each primer (forward and reverse

Table 1. Name and sequence of SCoT primers.

Primer	Sequence 5'-3'
SCoT-34	ACCATGGCTACCACCGCA
SCoT-52	ACAATGGCTACCACTGCA
SCoT-71	CCATGGCTACCACCGCCG
SCoT-24	CACCATGGCTACCACCAT
SCoT-26	ACCATGGCTACCACCGTC
SCoT-31	CCATGGCTACCACCGCCT
SCoT-13	ACGACATGGCGACCATCG
SCoT-70	ACCATGGCTACCAGCGCG
SCoT-66	ACCATGGCTACCAGCGAG
SCoT-61	CAACAATGGCTACCACCG

Table 2. Name and sequence of SRAP primers.

Forward primer	Sequence (5'–3')	Reverse primer	Sequence (5'–3')
Me1	TGAGTCCAAACCGGATA	Em1	GAC TGC GTA CGA ATT AAT
Me2	TGAGTCCAAACCGGAGC	Em2	GACTGCGTACGAATTTGC
Me5	TGAGTCCAAACCGGAAG	Em3	GACTGCGTACGAATTGAC
Me10	TGAGTCCAAACCGGGAC	Em4	GACTGCGTACGAATTTGA
		Em5	GACTGCGTACGAATTAAC
		Em6	GACTGCGTACGAATTGCA

primers) (10 µM), 1 µL of DNA (30 ng), and 7 µL d.H2O. The SRAP amplification was performed according to Li and Quiros's (2001) program as follows: the initial step of 5 min at 94℃, followed by 5 cycles of 1 min at 94℃, 1 min at 35℃, 1 min at 72℃ after that 35 cycles consist of 1 min at 94℃, 1 min at 50℃ then 2 min at 72℃, and a final extension for 10 min at 72℃. The PCR products were detected by 1.2 % agarose gel electrophoresis stained with ethidium bromide.

Data analysis

A single analysis of variance was done separately for each season's data, according to Snedecor and Cochran (1994)**.** Treatment means were compared using the least significant difference test (LSD) at a 0.05 level of significance, according to Steel *et al***.** (1997). After standardization, the Euclidean distance (ED) was calculated from the quantitative traits studied by subtracting the mean value and then dividing by using standard deviation **(**Sneath and Sokal, 1973**).** The cluster analysis model analyzed the genotype's "r" matrix, which calculates the length of a straight line between two objects to determine similarity/dissimilarity among sets of genotypes as described by Kovach (1995). Minitab v-16 software program was used to automate the clustering of genotypes. The scoreable bands were manually

determined using the presence (1) or absence (0) of bands for SRAP and SCoT amplicons. To establish the informativeness of the tested primers, PIC, EMR, MI, and Rp parameters were acquired for each primer, according to Chesnokov and Artemyeva (2015). Additionally, using the PAST software version 4.03, a cluster analysis was carried out using the Dice similarity coefficient based on the similarity between data sets relative to matches.

RESULTS

Number and percentage of germination of treated chickpea genotypes

The number of germinated seedlings and the percentages of the two chickpea genotypes treated with five gamma-ray treatments are illustrated in (Fig 1). The result was calculated for all the gamma rays applied: 24 and 25 germinated seeds from 25 sowing seeds in a petri dish. By calculating the percentages of the five treatments for each genotype, they were equal to 96% (24 germinated seeds) and 100% (25 germinated seeds). In the first season (2020-2021), our research focused on calculating the number and percentage of germination traits, laying the foundation for our subsequent investigations. The results showed no significant differences between irradiated seeds (M1) and untreated seeds (M_0) in the germination test. The number and percentage of germinations remained nearly like control.

Phenotypic characterization

The recorded traits in two successive seasons (2020/2021 and 2021/2022) showed wide variability, as shown in Figure 2. Results of the analysis of variance (ANOVA) and mean performance of chickpea genotypes, as affected by irradiation treatments and their interactions for seed yield and its components, are shown in Tables 3-7.

Analysis of variance (ANOVA)

As represented in Table 3, the two genotypes revealed a highly significant variance in biological yield and plant height and a significant variance in the number of surviving plants and surviving plants in the first season. In the second season, all studied traits showed high and/or significant variance, except biological yield, number of survived plants, and survival plants. The five treatments demonstrated high and/or significant variations in all studied traits during both seasons. The significant fluctuations in variations in the interaction between the two chickpea genotypes and the five gamma treatments among the two sowing seasons underscore the profound impact of these treatments on the studied traits. The effect of gamma irradiation on the number and percentage of survival plants showed highly and/or significantly different variances in both seasons. Meanwhile, the effect of the interaction between the two genotypes and the five gamma treatments on the number and percentage of survival plants showed highly significant variances in the first season and even insignificant differences in the second season.

Mean performance

Effect of Chickpea Genotypes

According to Tables 4 and 6, the mean performance of both seasons for the G.3 variety recorded a higher number of pods and seeds number and also the harvest index traits than those of the G.531 variety, which was superior in seed yield and pod weight (as compared with the same number of seeds and pods number of both genotypes), in addition to the biological yield, plant height, and seed index. The number of surviving plants and the percentage of surviving plants showed flaunted variance in both seasons. In the first season, the two traits of variety G.3 significantly increased (about 25%) compared to those of G.531. In the opposite direction, in the second season, these two traits of variety G.3 were insignificantly decreased (about 10%) compared to those of G.531.

Effect of treatments of gamma irradiation

Regarding the mean performance of the gamma-ray treatments in both seasons, as shown in Tables 4 and 6, the (200 Gy) treatment was the best recorded in all studied characters compared to the other gamma-ray treatments and the control. This was followed by the treatment (100 Gy), which recorded more superiority than the control except for the number and percentage of surviving plants. The 300 Gy dose started to decline the superiority curve of the mean performance after the 100 and 200 Gy doses. Its mean performance was nearly greater than the mean performance of the control (0 Gy) in plant height, pods wt, seed yield, seed index, and harvest index traits in the first season and recorded higher in all studied traits except in the harvest index, number of surfs. plants and surv. plants% in the second season. Conversely, the last irradiated treatment (400 Gy) had the lowest mean performance of the studied yield traits. Moreover, the number and percentage of survival plants showed highly significant variances in both seasons,

which ranked as 60% for 200 Gy, then 56% for control, followed by 100 Gy (48%), 300 Gy (41%), and 400 Gy (24%) as represented in Figure 3.

Effect of interaction between chickpea genotypes and gamma treatments

Tables 5 and 7 show the mean performance of the interaction between two chickpea genotypes and five treatments with gamma rays. Their interaction could be divided into direct (M_1 plants) and indirect (M_2 plants) effects of gamma doses applied to chickpea genotypes. The direct effect was explored in the first season. It improved the mean performance of all studied traits in the G.531 variety (Kabuli genotype) at the first two gamma doses (100 and 200 Gy). It recorded the top at 200 Gy and started the decline curve at 300 Gy, which kept increasing in some traits: plant height, pod number, pod weight, seed yield, seed index, and harvest index. At the same time, 400 Gy was the least dose of all the characters studied. However, for the G.3 variety (Desi genotype), the best treatment was 200 Gy, followed by 100 Gy as compared to the control, and (300 and 400 Gy) were recorded as less than the control except in the seed index. The 400 Gy treatment was the last one, too.

The indirect effect studied in the second season on the M_2 plants and their interaction between genotypes and five treatments. There were high and/or significant variances in biological yield, plant height, pod number, seed number and seed yield. The G.531 genotype recorded high mean performance in all studied traits at 200 Gy, followed by 100 Gy, then 300 Gy, and the last dose was 400 Gy, exceeding the control, except the harvest index at 300 and 400 Gy (nearly equal to 24) which was less than the control (equal to 28). Meanwhile, the G.3 genotype showed superior mean performance in all studied traits except the number and percentage of surviving plants. The 200 Gy was the highest treatment, followed by the 100 Gy, then the 300 Gy, which increased over the control M_0 except for three traits: plant height, seed index, and harvest index, which were slightly less than the control (M_0 plants).

Regarding gamma's direct and indirect effects on the Desi genotype (G.3), the seed index at 300 and 400 Gy was higher than the control due to their higher yield according to the number of obtained seeds. In the first year, the seed yield was 0.26 g for 2 seeds at 300 Gy, and 9 seeds weighed almost 1.21 g at 400 Gy, while in the M_0 control, 13 seeds weighed 1.45 g. When applying 100 and 200 doses of the G.3 variety, the seed size increases, so the yield increases. It doubled about 60% more in the best treatment (200 Gy) compared to the control treatment from 1.45 to 3.86 g, corresponding to 13 seeds and 23 seeds (in the first season), and from 2.91 to 47.07g, belonging to 20 seeds and 47 seeds (in the second season), respectively. These findings have significant practical implications for the agricultural industry. The mean interaction performance in the number and percentage of survival plants was classified by genotype and gamma treatments. In the G.531 (Kabuli genotype), the highest treatment was 200 Gy, followed by 100 Gy, then 0 Gy (control), then 300 Gy, and then 400 Gy. The G.3 (Desi genotype) showed the best treatment was 100 Gy, 200 Gy followed by control, and 300 Gy followed by 400 Gy. This information can guide farmers and breeders in selecting the most suitable genotypes and gamma treatments for their needs.

Dendrogram cluster analysis of seed yield and other yield components

The present work estimated the similarity levels of ten chickpea genotypes (two genotypes under five gamma irradiations) based on seed yield and its related yield traits. The clustering patterns of these genotypes are listed in Table 8 and schematically shown as a dendrogram in Figure 3.

According to Fig. 3, these genotypes were divided into two main clusters, which were divided into four sub-clusters or groups (A, B, C and D). Each group contained genotypes that exhibited similar phenotypic manifestations, except for the fourth group (D), which consisted of only one genotype (G3-400 Gy). It was clear that the 3rd group (C) included the maximum number of items (4 out of 10 genotypes), followed by the 1st group A (3 genotypes), and finally, the 2nd group (B) had 2 genotypes. The results obtained from Table 8 showed that the first group (A) was considered the best. It recorded superiority in all studied characters of the three genotypes under certain doses of gamma irradiation (G.3-100 Gy, G.3-200 Gy, and G.531-200 Gy), which was in line with the mean performance because it was the best dose of gamma irradiation. The second group (B) had two treatments of the G.531 variety (100 and 300 Gy), which were also superior to both controls in all traits except seed number, harvest index, and survival plant percentage. Meanwhile, the third group (c) grouped the two genotype checks (G3-C and G531-C) with G3-300 Gy and G531-400 Gy. Finally, the fourth

group included the genotype (G.3-400 Gy) with the minimum values of all the studied traits.

Molecular Analysis

The genetic diversity among chickpea genotypes and their treatments

The main goal of plant breeders in breeding is to increase genetic diversity. This variance is a typical result of the interaction between genes and environmental factors. The goal of mutation is to induce genetic variability and finally lead to the emergence of new species. Therefore, gamma radiation treatment to induce genetic diversity is effective for plant breeding, crop improvement, and plant breeding programs. This study used SCoT and SRAP markers to detect genetic variation among gamma-ray-treated chickpea genotypes. DNA samples representative of the studied chickpea varieties was subjected to SCoT analysis using ten different primers. All tested primers produced amplification products with different numbers of bands. The SCoT analysis generated 117 amplified fragments (Table 9 and Figure 5), of which 101 were polymorphic markers with a genetic polymorphism percentage of 85.05 %. The primers used differed in the number of bands they produced. SCoT-24 produced the highest number of bands (16), while the SCoT-71 produced the lowest number (7). However, the primer SCoT-34 has the lowest polymorphism percentage (58.3%), while SCoT-70 showed the highest polymorphism percentage (100%).

Additionally, the genetic diversity parameters of SCoT primers were determined. PIC values ranged from (1.310) obtained by primer SCoT-70 to (7.58) obtained by SCoT-61. The EMR values range from (0.233) by primer SCoT-31 to (0.398) by SCoT-70. Furthermore, the MI values are between (0.522) SCoT-70 and (2.047) SCoT-61. The calculated resolving power (RP) values are between (5.60) of SCoT-71 and (18.40) of SCoT-61.

SRAP markers were generated using fifteen primer combinations, leading to a rich diversity of polymorphic bands. The SRAP analysis resulted in 165 bands detected among the Chickpea genotypes and their treatments (Table 10 and Figure 6). Of these, there are only 118 polymorphic bands (70.59%). The primers used yielded a variety of polymorphic bands, ranging from 4 bands for Me5-Em5 to 13 bands for Me1-Em2. Primers Me10-Em1, Me1-Em2 and Me10-Em2 had the highest polymorphism rate (90, 86.7, 83.3 %). Meanwhile, primers Me5-Em5, Me5-Em3, and Me1-Em4 had the lowest polymorphism rate (50, 55.6, 58.3 %). On the other hand, Me5-Em6 obtained the highest PIC value (0.35), while Me1-Em3 obtained the lowest value (0.18). While the EMR value ranged from (6.82) for Me1-Em4 and (2.03) for Me10-Em1.In addition, the MI value ranged from (1.57) for Me1-Em4 and (0.43) for Me10-Em1. In addition, the RP value ranged from (16.40) for Me1-Em4 and (6.20) for Me10-Em1.

Genetic similarity and cluster analysis as revealed by SCoT and SRAP combined data.

Based on the SCoT and SRAP analysis results, the chickpea genotypes' genetic similarity (GS) and their treatments were determined (Table 11). The degree of genetic similarity value ranged from 0.31 to 0.65. The highest GS (0.65) was achieved between control G.531 and treatment G.531-100 Gy and between treatment G.3-300 Gy and treatment G.3-400 Gy. This suggests that these treatments share a relatively high degree of genetic similarity. Meanwhile, the lowest GS (0.31) was observed between control of G.531 and treatment G.3-200 Gy.

The UPGMA clustering dendrogram, a clear visual representation based on the Dice similarity index, was obtained (Fig. 7). The SCoT and SRAP data effectively separated the controls of two chickpea genotypes and their five treatments (10 genotypes) into two main clusters. The first one contained five genotypes, which were G.3-control and its treatments (G.3-100, G.3-200, G.3-300 and G.3-400 Gy). This cluster was further divided into three sub-clusters, each delineated in the dendrogram; the first group had only one genotype (G.3-control), the second group included G.3-100 Gy and G.3-200 Gy, and the third one had the highest doses of gamma (G.3-300 and G.3-400 Gy).

Meanwhile, the second cluster contained the other five genotypes of the G.531 family, including G.531-C and its treatments (G.531-100, G.531-200, G.531-300, and G.531-400 Gy). It also separated into three subclusters: the first one contained two genotypes (G.531-control and G.531-100 Gy), the second group had only G.531-200 Gy, and the last group also included the highest gamma doses (G.531-300 and G.531-400 Gy). Nevertheless, it is noted that the doses of gamma rays at 300 and 400 Gy were more effective than the other treatments, which led to their separation into two sub-clusters from their controls. Also, the 200 Gy dose has a derived line from the control of both genotypes. In the G.531 variety, 100 Gy was linked with 200 Gy and derived

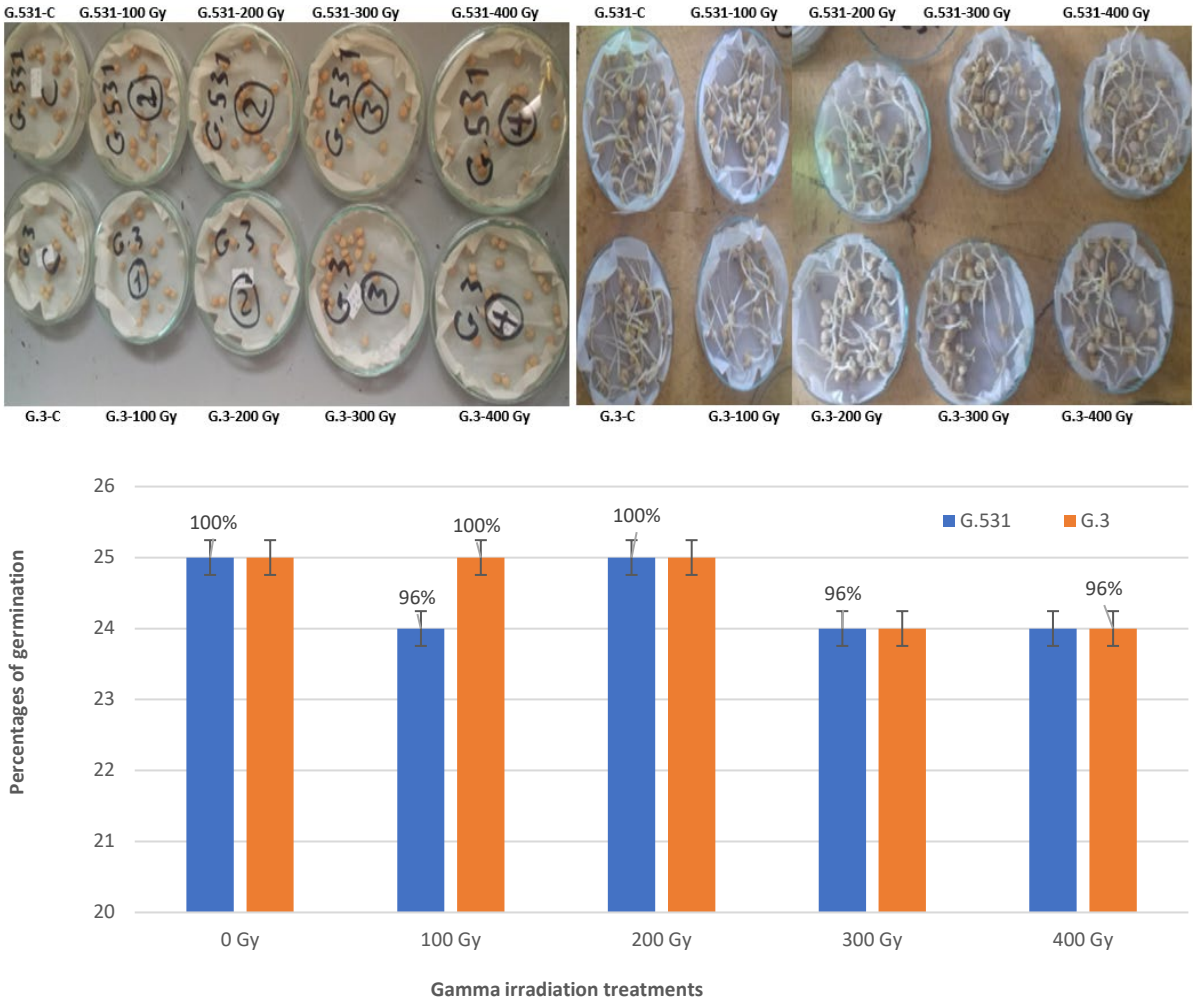

Figure 1. The germination test of two Chickpea genotypes with five gamma treatments in the lab using petri dish in the first season (2020-2021); calculating the number and percentage of germination traits.

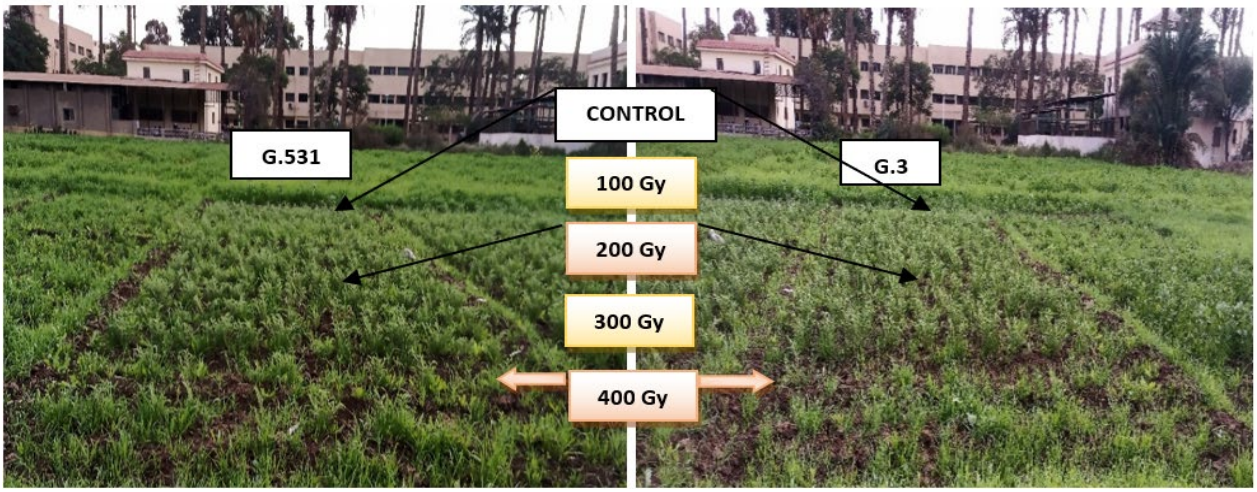

Figure 2. Evaluation of two chickpea genotypes at five gamma doses under field conditions in the second season (2021-2022).

Table 3. Analysis of variance (ANOVA) of the gamma irradiation's effect on two chickpea genotypes under studied traits in both seasons (2020-2022).

Seasons	df	BY		Pt Ht		Pods No.		Pods Wt		Survival Pt	
		2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
Rep		3.078*	0.17^{Ns}	0.71^{Ns}	0.70^{Ns}	0.21^{Ns}	1.02^{Ns}	0.53^{Ns}	1.20^{Ns}	0.75^{Ns}	4.09^{Ns}
Genotypes	1	10.95**	1.48^{Ns}	19.74**	3.01*	0.63^{Ns}	13.60**	0.03^{Ns}	4.58*	3.90*	1.51^{Ns}
Treatments	4	21.14**	8.39**	3.89*	7.39**	10.54**	7.31**	13.69**	11.04**	8.58**	4.57*
Genotype* Treat	4	0.32^{Ns}	4.32*	0.51^{Ns}	4.73**	4.29*	2.99*	2.21^{Ns}	2.24^{Ns}	6.05**	1.53^{Ns}
	df	Seed No.		SY		SI		HI		surv. Pt %	
Seasons		2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
Rep		1.88^{Ns}	1.84^{Ns}	2.49^{Ns}	1.77^{Ns}	3.17*	1.02^{Ns}	0.84^{Ns}	3.46*	0.75^{Ns}	4.09^{Ns}
Genotypes	1	0.25^{Ns}	16.88**	0.62^{Ns}	9.42**	1.07^{Ns}	9.04**	1.33^{Ns}	4.34*	3.90*	1.51^{Ns}
Treatments	4	20.18**	11.89**	23.77**	15.77**	6.38**	2.77*	8.64**	3.20*	8.58**	4.57*
Genotype* Treat	4	1.61^{Ns}	4.42*	1.43^{Ns}	3.56*	0.70^{Ns}	0.15^{Ns}	8.72**	0.21^{Ns}	6.05**	1.53^{Ns}

Ns, *, and ** indicate insignificant, significant, and highly significant at 0.05 and 0.01 levels of probability, respectively.

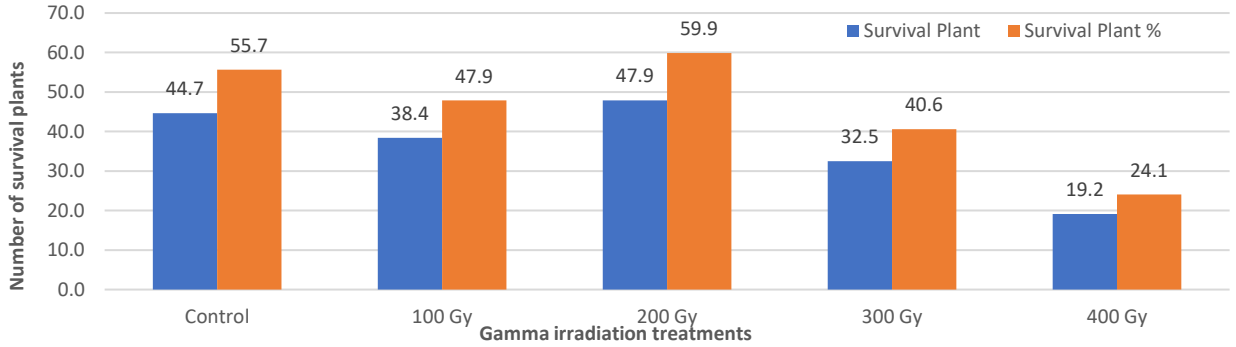

Figure 3. Mean performance of five gamma treatments for number and percentage of survival plants across two seasons (2020-2022).

Table 4. Mean performance of two chickpea genotypes and the five gamma irradiations (as individuals) for seed yield and its yield components across the first season (20/21).

		BY	Pt Ht	Pods No.	Pods Wt	Seed No.	SY	SI	HI	Surv. Pt	Surv. Pt%
CVs.	G.531	27.65	69.57	15.73	3.31	13.26	2.11	15.91	7.78	26.73	33.42
	G.3	21.13	60.04	17.19	3.38	13.93	1.91	13.71	8.58	35.47	44.33
Sig.		**	**	Ns	Ns	Ns	Ns	Ns	Ns	*	*
Treat	Control	26.89	62.80	14.37	2.93	13.61	1.29	9.30	5.55	40.67	50.83
	100 Gy	30.66	68.67	20.37	4.25	17.07	2.43	14.36	8.40	34.67	43.33
	200 Gy	35.45	70.19	25.73	5.61	23.73	4.28	18.17	12.17	46.33	57.92
	300 Gy	19.26	63.96	13.23	2.52	8.50	1.32	15.25	7.96	24.00	30.00
	400 Gy	9.69	58.42	8.60	1.40	5.07	0.72	12.98	6.82	9.83	12.29
$LSD_{0.05}$		6.54	7.13	6.10	1.30	4.83	0.86	3.82	2.40	11.71	16.01

Ns, *, and ** indicate insignificant, significant, and highly significant at 0.05 and 0.01 levels of probability, respectively.

Table 5. Mean performance of the studied characters as affected by the interaction between two chickpea varieties and five doses of gamma irradiations for different studied characters across the first season (20/21).

Cvs.	Treat	BY	Pt Ht	Pods No.	Pods Wt	Seed No.	SY	SI	HI	Surv. Pt	Surv. Pt%
G.531	Control	30.44	68.73	13.80	2.30	14.07	1.18	8.22	3.95	19.33	24.17
	100 Gy	33.71	72.13	18.67	4.33	14.47	2.11	14.75	6.27	30.33	37.92
	200 Gy	39.97	73.27	19.93	5.01	24.87	4.71	19.25	11.71	58.33	72.92
	300 Gy	23.17	71.07	14.87	2.69	8.27	1.43	17.06	10.70	15.67	19.58
	400 Gy	10.95	62.67	11.40	2.21	8.13	1.13	13.75	6.24	10.00	12.50
G.3	Control	23.33	56.87	17.33	3.65	13.16	1.45	10.38	7.15	62.00	77.50
	100 Gy	27.61	65.20	22.07	4.17	19.67	2.76	13.97	10.52	34.33	42.92
	200 Gy	30.93	67.12	31.53	6.20	22.60	3.86	17.09	12.63	39.00	48.75
	300 Gy	15.34	56.85	11.60	2.36	8.73	1.21	13.44	9.69	32.33	40.42
	400 Gy	8.43	54.17	3.40	0.51	2.00	0.26	12.22	2.93	9.67	12.08
$LSD_{0.05}$		Ns	Ns	8.63	Ns	Ns	Ns	Ns	3.40	16.57	22.64

Table 6. Mean performance of the individual two chickpea genotypes and the five gamma irradiations for seed yield and its yield components across the second season (21/22).

		BY	Pt Ht	Pods No.	Pods Wt	Seed No.	SY	SI	HI	Surv. Pt	Surv. Pt%
CVs	G.531	12.03	52.23	16.87	4.33	17.28	2.96	17.12	24.61	44.13	55.09
	G.3	10.78	49.41	25.49	5.56	26.50	4.10	14.91	38.03	39.80	49.64
Sig.		Ns	*	**	*	**	**	**	*	Ns	Ns
Treat	Control	8.85	44.52	17.26	3.93	16.94	2.53	15.06	28.91	48.67	60.51
	100 Gy	12.37	53.97	22.00	5.51	23.33	3.93	17.11	31.32	42.17	52.44
	200 Gy	16.48	55.63	31.83	8.37	35.73	6.19	17.79	37.33	49.50	61.79
	300 Gy	11.19	53.40	21.97	4.03	20.43	3.03	15.44	27.56	41.00	51.27
	400 Gy	8.13	43.57	12.83	2.88	13.00	1.98	14.67	24.62	28.50	35.81
$LSD_{0.05}$		3.40	5.40	7.77	1.91	7.45	1.23	2.43	7.95	12.87	16.01

Ns, *, and ** indicate insignificant, significant, and highly significant at 0.05 and 0.01 levels of probability, respectively.

Table 7. Mean performance of the studies characters as affected by the interaction between two chickpea varieties and five doses of gamma irradiations for different studied characters across the second season (21/22).

Cvs.	Treat	BY	Pt Ht	Pods No.	Pods Wt	Seed No.	SY	SI	HI	Surv. Pt	Surv. Pt%
G.531	Control	8.63	39.27	14.40	3.34	14.07	2.15	15.62	26.15	43.33	53.84
	100 Gy	10.56	55.53	15.67	4.11	16.07	2.95	18.33	27.89	48.33	60.30
	200 Gy	12.73	57.60	22.20	6.91	24.40	4.57	19.06	36.03	56.00	70.06
	300 Gy	11.18	57.60	16.73	3.80	16.00	2.67	16.83	23.71	41.33	51.67
	400 Gy	10.81	51.13	15.33	3.48	15.87	2.47	15.74	23.50	31.67	39.58
G.3	Control	9.07	49.77	20.12	4.52	19.82	2.91	14.49	31.67	54.00	67.18
	100 Gy	14.18	52.40	28.33	6.91	30.60	4.91	15.88	34.75	36.00	44.59
	200 Gy	20.23	53.67	41.47	9.82	47.07	7.80	16.53	38.62	43.00	53.53
	300 Gy	11.21	49.20	27.20	4.59	24.87	3.40	14.06	31.41	40.67	50.87
	400 Gy	5.44	42.00	10.33	1.95	10.13	1.49	13.60	25.75	25.33	32.03
$LSD_{0.05}$		4.81	7.63	10.99	Ns	10.54	1.75	Ns	Ns	Ns	Ns

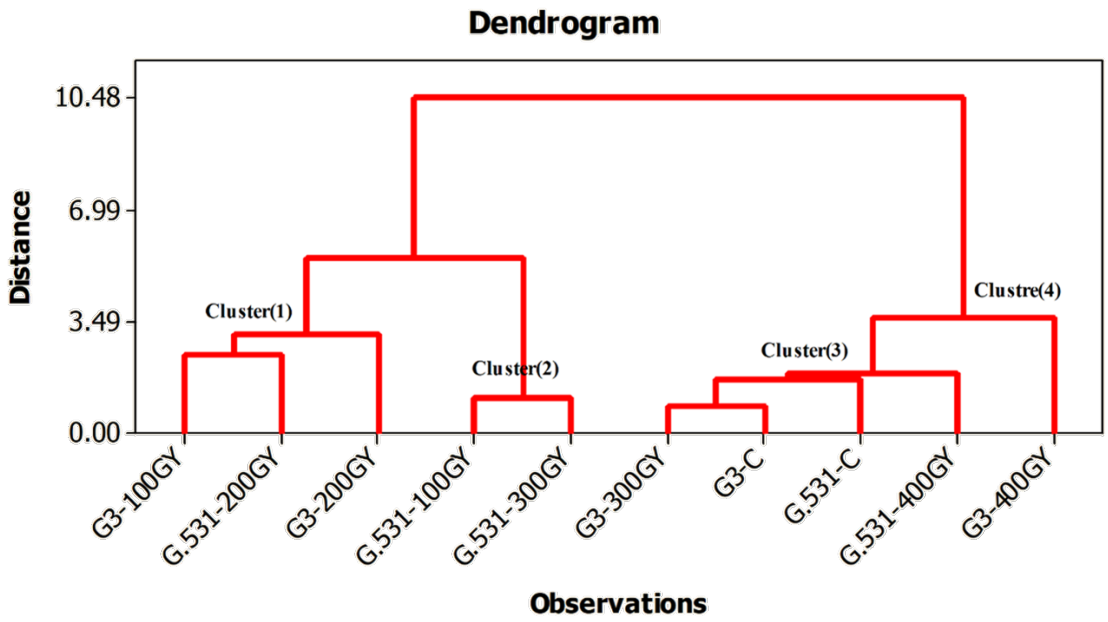

Figure 4. The linkage dendrogram shows the similarity among ten chickpea genotypes based on seed yield and other yield components.

Table 8. The summary of cluster analysis showed the similarity level and cluster mean of the ten chickpea genotypes using the studied yield characters.

Cluster No.	Genotype	BY	Pt Ht	Pods Wt	Pods No.	Seed No.	SY	SI	HI	Survival PT%
Cluster 1	G.3-100Gy, G.3-200Gy, G.531-200Gy	24.27	61.54	6.50	27.59	28.20	4.77	16.96	24.05	55.46
Cluster 2	G.531-100Gy, G.531-300Gy	19.65	61.08	3.73	16.48	13.70	2.29	16.74	16.02	42.37
Cluster 3	G.3-C, G.3-300Gy, G.531-C, G.531-400Gy	14.97	54.31	3.31	16.40	14.84	1.99	13.21	18.03	45.76
Cluster 4	G.3-400Gy	6.94	48.08	1.23	6.87	6.07	0.88	12.91	14.34	22.06

Table 9. PCR amplicons obtained from SCoTs marker in Chickpea genotypes and their treatments, total band number (TBN), polymorphic band number (PBN), polymorphism percentage (P%), polymorphism information content (PIC), effective multiplex ratio (EMR), Marker index (MI) and resolving and resolving power (RP).

No.	Primer name	G.531 with treatments			G.3 with treatments			All genotypes and treatments							Amplicon size range (bp)
		TBN	PBN	P %	TBN	PBN	P %	TBN	PBN	P %	PIC	EMR	MI	RP	
1	SCoT-34	12	7	58.3	11	6	54.5	12	7	58.3	6.350	0.258	1.640	14.80	2000-200
2	SCoT-52	13	11	84.6	11	9	81.8	13	12	92.3	5.290	0.371	1.961	15.40	2000-200
3	SCoT-71	7	6	85.7	4	3	75	7	6	85.7	1.660	0.326	0.541	5.60	1500-400
4	SCoT-24	14	12	85.7	13	12	92.3	16	15	93.8	3.620	0.348	1.258	12.80	2800-200
5	SCoT-26	9	7	77.8	8	7	87.5	10	9	90	2.430	0.334	0.812	8.20	1700-220
6	SCoT-31	7	3	42.9	7	4	57.1	8	5	62.5	3.770	0.233	0.877	9.40	1300-400
7	SCoT-13	14	13	92.9	11	10	90.9	14	13	92.9	2.670	0.376	1.003	10.60	1700-150
8	SCoT-70	11	11	100	12	12	100	13	13	100	1.310	0.398	0.522	7.80	1500-250
9	SCoT-66	12	10	83.3	12	10	83.3	12	11	91.7	3.870	0.372	1.438	12.20	2800-300
10	SCoT-61	12	8	66.7	12	7	58.3	12	10	83.3	7.580	0.270	2.047	18.40	1500-100
	Total	111	88		101	80		117	101						
	Average			77.79			78.07			85.05	3.855	0.3286	1.2099	11.52	

Table 10. PCR amplicons obtained from SRAP markers in Chickpea genotypes and their treatments, total band number (TBN), polymorphic band number (PBN), polymorphism percentage (P%), polymorphism information content (PIC), effective multiplex ratio (EMR), Marker index (MI) and resolving power (RP).

No.	Primer name	G.531 with treatments			G.3 with treatments			All genotypes and treatments							Amplicon size range (bp)
		TBN	PBN	P %	TBN	PBN	P %	TBN	PBN	P %	PIC	EMR	MI	RP	
1	Me1-Em2	12	8	66.7	10	7	70	15	13	86.7	0.32	4.02	1.28	12.80	800-50
2	Me1-Em3	11	4	36.4	7	3	42.9	11	7	63.6	0.18	5.89	1.08	13.80	1000-50
3	Me1-Em4	12	5	41.7	9	4	44.4	12	7	58.3	0.23	6.82	1.57	16.40	1000-50
4	Me2-Em2	9	6	66.7	9	6	66.7	10	7	70	0.27	4.17	1.11	11.00	900-80
5	Me2-Em2	12	6	50	9	5	55.6	12	8	66.7	0.23	6.55	1.47	15.80	1200-80
6	Me5-Em3	9	4	44.4	7	3	42.9	9	5	55.6	0.21	5.35	1.13	12.60	2000-200
7	Me5-Em4	9	5	55.6	8	5	62.5	11	8	72.7	0.27	4.70	1.28	12.40	4000-100
8	Me5-Em5	8	4	50	7	4	57.1	8	4	50	0.24	5.06	1.19	12.00	1500-200
9	Me5-Em6	7	5	71.4	5	3	60	9	7	77.8	0.35	2.93	1.02	9.00	3500-180
10	Me10-Em1	6	4	66.7	7	6	85.7	10	9	90	0.21	2.03	0.43	6.20	1600-400
11	Me10-Em2	10	7	70	7	5	71.4	12	10	83.3	0.32	3.57	1.15	11.00	1400-80
12	Me10- Em3	8	3	37.5	11	6	54.5	11	7	63.6	0.23	6.25	1.42	15.00	2000-400
13	Me10- Em4	9	5	55.6	10	6	60	13	10	76.9	0.29	5.45	1.55	14.60	2000-180
14	Me10- Em5	7	3	42.9	6	3	50	9	6	66.7	0.28	3.72	1.06	10.00	1000-100
15	Me10- Em6	12	7	58.3	9	5	55.6	13	10	76.9	0.22	5.69	1.23	14.20	2000-200
	Total	141	76		121	71		165	118						
	Averge			54.26			58.62			70.59	4.81	0.26	1.20	12.45	

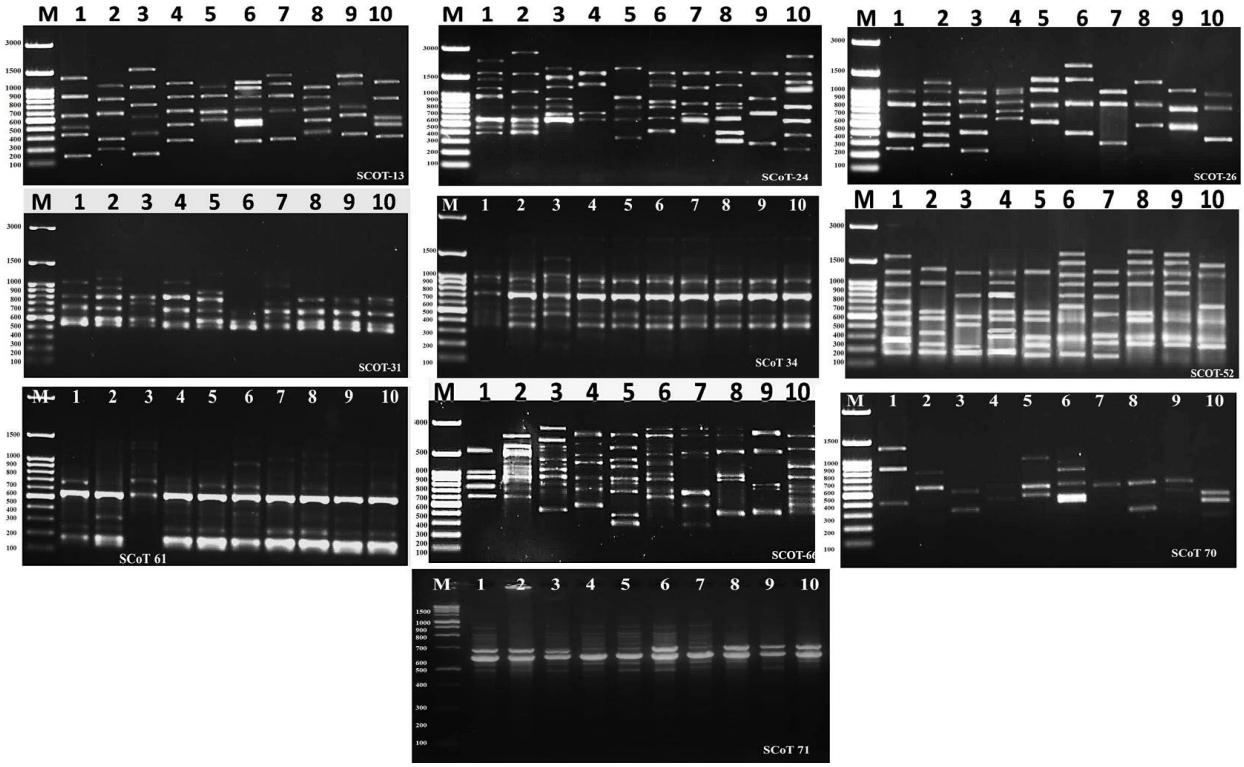

Figure 5. SCoT profile of the chickpea genotypes and their treatments. M refers to DNA marker of 100pb ladder; 1: G53-C, 2: G53-100, 3: G53-200, 4: G53-300,5: G53-400, 6: G3-C, 7: G3-100, 8: G3-200, 9: G3-300, and 10: G3-400.

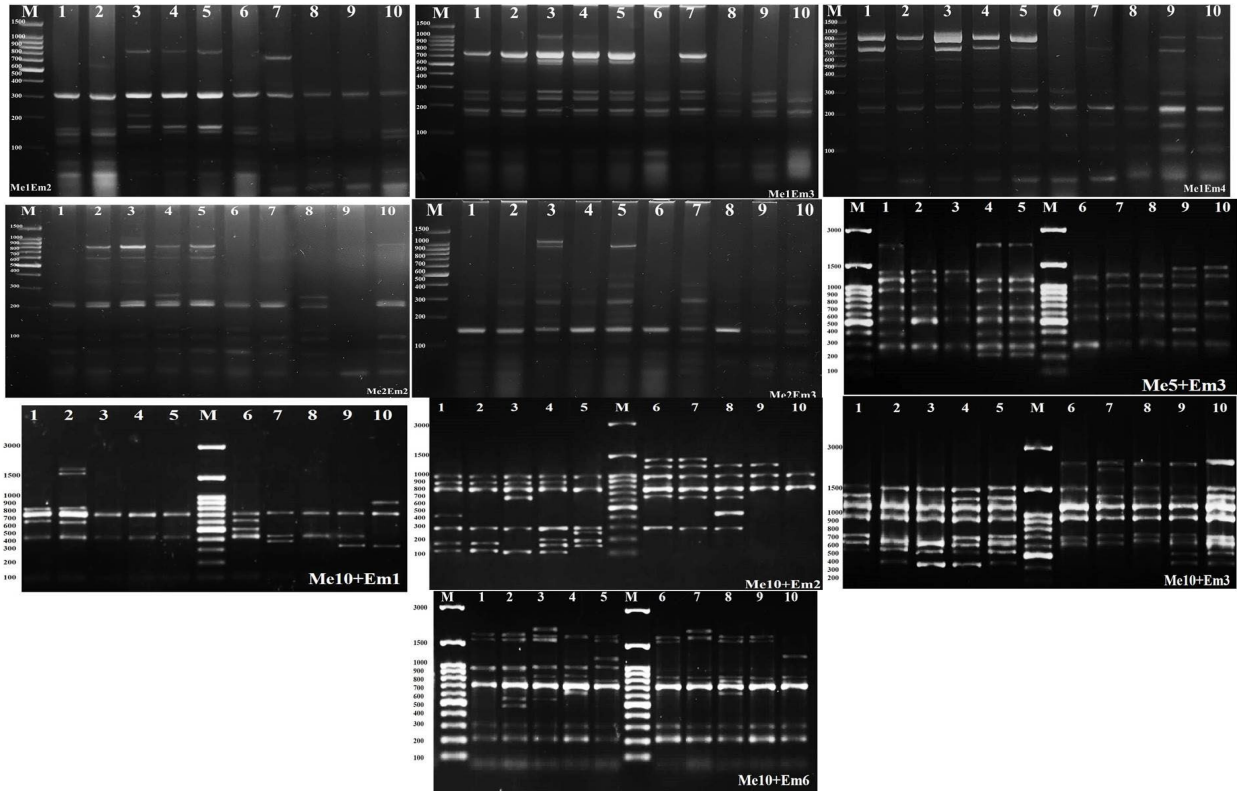

Figure 6. SRAP profile of the chickpea cultivars and their treatments. M refers to DNA marker of 100pb ladder; 1: G53-C, 2: G53-100, 3: G53-200, 4: G53-300, 5: G53-400, 6: G3-C, 7: G3-100, 8: G3-200, 9: G3-300, and 10: G3-400.

Table 11. Similarity matrix based on the combined data analysis.

	G.531-C	G.531-100	G.531-200	G.531-300	G.531-400	G.3- C	G.3-100	G.3-200	G.3-300	G.3-400
G.531-C	1.00									
G.531-100	0.65	1.00								
G.531-200	0.54	0.60	1.00							
G.531-300	0.52	0.53	0.53	1.00						
G.531-400	0.44	0.52	0.48	0.62	1.00					
G.3-C	0.39	0.42	0.41	0.45	0.49	1.00				
G.3-100	0.37	0.41	0.40	0.40	0.43	0.63	1.00			
G.3-200	0.31	0.37	0.39	0.37	0.49	0.62	0.64	1.00		
G.3-300	0.33	0.36	0.36	0.38	0.39	0.49	0.52	0.60	1.00	
G.3-400	0.34	0.39	0.39	0.37	0.45	0.49	0.52	0.50	0.65	1.00

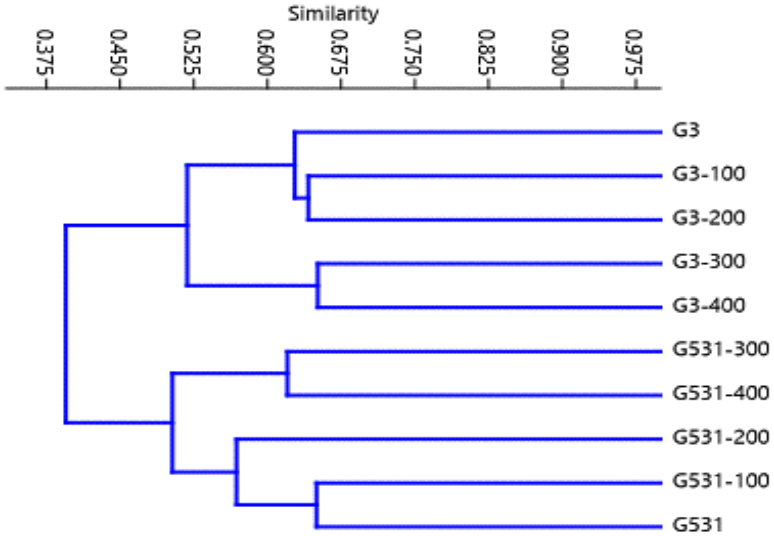

Figure 7. Cluster analysis based on Dice similarity index derived from the combined data of the SCoT and SRAP markers.

from the control. Despite the first gamma dose, 100 Gy was linked with the G.3-control and separated from 200 Gy in the second variety G.3.

DISCUSSION

Seed germination test

No significant variations were revealed between the two genotypes under normal conditions. Also, no significant differences were found between the irradiated doses. Therefore, no significant effect was detected between gamma doses and seedling germination number or percentage. Ahumada-Flores Moreover, this suggests that the lethal dose (LD50) of gamma-treated doses might be above the 400 Gy dose in these chickpea genotypes. However, the LD50 is different from crop to crop. And sometimes from one variety to another for the same crop. So, it may be recorded as a varied range of more or less than 400 Gy according to the used combinations of variety kind, crop type and irradiation doses as mentioned by other researchers Mabrouk *et al.* (2018), Ahumada-Flores *et al.* (2021), Amri-Tiliouine *et al.* (2018) and Saibari *et al.* (2023). It could be concluded that the irradiated seeds could germinate, but that does not mean they would have completed their life cycle as surviving plants in the field. Therefore, it was essential to calculate the numbers of survival-irradiated plants per treatment in both genotypes to judge plant behavior and performance well in field experiments.

et al. (2021) proved that the M_1 seed's germination percentage was non-significantly affected at 100 Gy and 200 Gy compared to M_0 seeds. The percentage of seed germination revealed no significant variations among wheat varieties under normal conditions, and no significant differences were found in the germination percentage for chickpea seeds exposed to less than or equal to 200 Gy, as mentioned by Abdoun et al. (2022). These results were consistent with the findings of Borzouei *et al.* (2010), Melki *et al.* (2010), and Qureshi *et al.* (2014).

Phenotypic characterization

The mean performance of seed yield and its related components showed a wide morphological variability for chickpea genotypes and gamma treatments, which agreed with Qureshi *et al.* 2014, Hajibarate et *al.* 2014 and Tadesse *et al.* 2016. Kassab *et al.* (2012) reported that the Giza 531 variety showed superiority for pod and seed weights per plant, 100-seed weight (SI), total dry matter, seed yield and biological yield per plant. The Giza 3 variety exhibited superiority in several pods and was also higher in plant height. Also, Gul *et al.* (2013) revealed a significant variation among chickpea genotypes for pod number, seed number, 100-seed weight and seed yield. Moreover, the chickpea genotypes recorded significant differences in plant height, pod number, 100-seed weight, harvest index and seed yield (Hajibarat *et al.***,** 2014; Qureshi *et al.***,** 2014**).** The mean performance of investigated characters for gamma treatments and their interaction with chickpea genotypes among both seasons revealed an improvement curve that started with the first irradiated dose of gamma (100 Gy). The top was at the second dose (200 Gy), and then the breakdown began at the third dose (300 Gy), which decreased in many yield traits compared to the control. The most severe damage was recorded in the fourth treatment (400 Gy) as compared to the control. Ahumada-Flores *et al.* (2021) reported that gamma irradiation (100, 200, and 300 Gy) induced changes in wheat's morphological, physiological, and agronomical levels. A decrement in the studied characters was observed by increasing the gamma dose significantly above 200 Gy. Badr *et al.* (2014) revealed that the (100 Gy) dose increased most studied yield parameters: seed number, pod number, growth rate, fresh weight, seed index and seed yield compared to the control in three cowpea genotypes. Increasing the gamma dose to 300 Gy resulted in a severe reduction in yield and component characters.

Singh *et al.* (2001) and Tah (2006) also observed that the plant height of mung bean M_1 decreased at high doses of gamma irradiation, with the highest decline occurring at a dose of 400 Gy. Ahumada-Floreset *et al.* (2021) found that insignificant variance was observed among the survived plant % of M_1 seeds (100 and 200 Gy) as compared to M_0 seeds, even though they showed significant variance compared to 300 Gy, reducing their survived plant% from 92.6% to 44.6%.

Furthermore, the Kabuli genotype (G.531) showed greater tolerance to the gamma dose used than the Desi genotype (G.3). The G.531 variety showed a positive effect until 300 Gy in the first and 400 Gy in the second seasons. Meanwhile, the variety (G.3) showed an improved effect of 200 Gy in the first and 300 Gy in the second season. Arain and Maqbool (2011), Qureshi *et al. (*2014), Abdoun *et al.* (2022) and Pathania *et al.* (2023) concluded that different doses of gamma rays have different effects on quantitative and qualitative phenotypic traits. Gamma doses up to 250 Gy were recommended and should be used in future studies to obtain desirable mutations in seed yield and related traits. After this dose, most parameters exhibit a decline in quantitative characters, such as 300 Gy, which has the maximum reduction in fresh and dry weights and maximum gross mutations. The highest decline occurred at a dose of 400 Gy or above in yielding traits of chickpeas.

Genetic diversity is the key to developing a successful breeding program. Employing multivariate analysis techniques, like cluster applications, is increasingly popular in determining the variance between genotypes and the level of correlation between traits. Cluster analysis has been used as an efficient procedure to determine the structural relationships between the examined genotypes and enable their hierarchical classification (Derbew and Tejada, 2020). Considering previous results of cluster dendrogram, it proved that applied gamma irradiations with higher doses of over 300 Gy in the Kabuli genotype (G.531) and over 200 Gy in the Desi genotypes (G.3) generally reduced the growth rate and seed yield of chickpeas. Moreover, the Kabuli genotype responded more to the used gamma doses than the Desi genotype. It was also concluded that the presence of large genetic diversity among the tested genotypes gave a good opportunity to achieve sufficient scope for the genetic improvement of chickpeas using specific doses of gamma irradiation, as mentioned by Nabati *et al***.,** 2022 and Ningwal *et al.,* 2023**.**

Molecular Analysis

Previous studies have evaluated genetic variation using DNA molecular markers such as SCoT and SRAP (Yousefi *et al***.,** 2018; Gupta *et al***.,** 2021; and Framarzpour *et al*., 2021). In this regard, Khan *et al***.** (2016) stated that molecular characterization of chickpea genotypes using the SRAP marker revealed a high level of polymorphism among chickpea

varieties and was found to have discriminatory power in distinguishing chickpea genotypes. ATG start codons were combined into random primers to generate polymorphic fragments from the genome, as described by Collard and Mackill (2009). Moreover, Surduse et al. (2021) found that the SCoT marker effectively identified genetic variation in chickpeas. Therefore, genetic diversity among radiation treatments and the control (untreated) of chickpea plants was estimated using SCoT and SRAP markers. SCoT and SRAP analyses revealed different levels of polymorphism among chickpea plants and their treatments. Overall, these results highlight different levels of genetic diversity, discriminatory power, and resolution power among the analyzed marker combinations. Markers with higher PIC, EMR, MI and RP values, such as SCoT-61, Me1-Em4 and Me5-Em6, are more informative and influential in detecting genetic variations. Meanwhile, markers with lower values, like SCoT-70, SCoT-31, Me1-Em3 and Me10-Em1, may be of limited utility in studying genetic diversity.

Genetic similarity (GS) and clustering dendrogram analysis results showed distinct differences between chickpea controls and corresponding treatments. The GS values indicate that gamma treatments of both genotypes at doses 100 and 200 Gy are closely related, derived from their controls, suggesting similar genetic effects at these doses. In contrast, gamma-ray doses of 300 and 400 Gy had a stronger effect than the other treatments, as evidenced by their separation into separate sub-clusters from their controls. The two chickpea genotypes showed different responses to five treatments with gamma rays. This finding of the genotypic cluster was practically in line with the results obtained from the phenotypic cluster based on investigated agnomical traits. These findings are consistent with the observations of Umavathi and Mullainathan (2019), who reported that gamma radiation induces changes in DNA structure and leads to genetic variation.

CONCLUSION

It can be inferred from the results of the present study that different doses of gamma radiation have different effects on agronomic characters as well as different genotypes of chickpeas. There were no significant changes between gamma doses and the number of seedling germinations or percentages. The Kabuli genotype (G.531) showed a better effect on gamma doses than the Desi genotype. Treatment (200 Gy) was the best dose observed in all studied characters compared to the other gamma-ray treatments and controls. Then, the treatment (100 Gy) gave superior results to the control treatment except for the number and percentage of surviving plants. Then, the breakdown started at the third dose (300 Gy), and there was a decrease in most yielding traits compared to the control. The greatest effect was observed at the fourth dose (400 Gy) compared to the control. Furthermore, molecular markers analyses indicated that using SCoT and SRAP markers in this study has provided valuable information on genetic diversity and the relationships between chickpea genotypes and their treatments. These results contribute to our understanding of the effects of irradiation treatments on the genetic makeup of chickpeas and have implications for chickpea breeding and improvement.

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COMPETING INTERESTS

The authors declare that they have no conflict of interests.

LIST OF ABBREVIATIONS

Cvs.	Cultivars
G.531	Giza 531 (Kabuli genotype)
G.3	Giza 3 (Desi genotype)
M_0	Control plant/seeds -No gamma irradiation
M_1	First mutant generation
M_2	second mutant generation
Gy	Gray unit (Gray=100 rad)
Pt	plant
BY	biological yield or plant dry weight
Pt Ht	plant height
Pods No	number of pods
Pods Wt	weight of pods
Seed No.	number of seeds
SY	seed yield
SI	seed index
HI	harvest index
Surv. Pt	number of survived plants
Surv. Pt %	percentage of survived plants
RCBD	Randomized complete block design
ANOVA	Analysis of variance
L.S.D	Least significance difference
ED	Euclidean distance
UPGMA	Unweighted pair group method with arithmetic mean
SRAP	Sequence-related amplified polymorphism
SCoT	Start codon targeted polymorphisms
GS	

TBN	Genetic similarity
PBN	Total band number
P %	Polymorphic band number
PIC	Polymorphism percentage
EMR	Polymorphism information content
	Effective multiplex ratio
MI	Marker index
RP	Resolving power

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