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Research Article

Mutagenic effect of gamma rays on biochemical and yield-related traits in Chickpea

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Abstract

Chickpea, *Cicer arietinum* L., is a bisexual full self-pollinated crop legume belonging to the family Fabaceae, it has a narrow genetic variability base. Thus, insight into the magnitude of genetic variations in this crop is important to plant breeders when designing breeding techniques. Therefore, the objective of this investigation was to increase genetic variations in chickpeas using gamma rays for isolating new genotypes with better-expressed growth traits and yield components. Chickpea seeds were subjected to different doses of gamma rays. Chlorophyll concentration was measured spectrophotometrically. The results of M_1 generation reflected that the doses of 100 and 200 radiuses had increased the number of primary branches per plant, as well as produced a significant increase in pod weight per plant. Meanwhile, the dose of 200 radius displayed higher plant height, dry weight and the number of primary branches per plant. The doses of 25 and 400 radiuses induced a significant increase in 50 seed weight above the control. Moreover, the dose of 200 radius exhibited the highest number of developed pods per plant, as well as pods weight per plant. The maximum weight of seed yield per plant was produced by the dose of 100 radiuses. The weight of 50 seeds indicates high heterogeneity. In contrast, the weight of seed yield per plant reflected high homogeneity. The results indicated that homogeneity differed between the doses of gamma irradiation within the same trait, as well as from one trait to another in the same genotype. Therefore, gamma ray was recommended to get desirable mutations in economic crops, as well as, to increase the rate of genetic variations in chickpeas has been well documented.

1. Introduction

Spontaneous mutations occur with a very low frequency (10^{-7} to 10^{-9}) and cannot be expected to be used in crop improvement effectively. Therefore, induced mutation is considered as rapid and effective technique in plant breeding for inducing variability. Artificial mutations can be used using physical and chemical mutagens. Mutation offers the availability of inducing desired attributes that cannot be found in nature or losing during evolution [1]. Thus, [2] proposed the hypothesis of induced quantitative variability via mutagenesis. Therefore, induced mutations have played an important role in increasing world food security. Chickpea, *Cicerarietinum* L., is the only cultivated species of the genus *Cicer* [3]. Chickpea belongs to the family Fabaceae, which is considered an important crop legume in the world [4]. It is harbors cleistogamic flowers leading this crop to full self-pollinated. It has a diploid chromosome number (2n=2x=16). Its genome size is about 738 Mb [5]. Chickpea is the third most important food crop legume in the world, cultivated in 40 countries over an area of about 11.2 million hectares adding 9.2 million tons annually to the global food basket, as well as having an average yield of 818 kg/hectar [6]. In general, legumes give a lower yield than cereals. This led to the assumption that legumes may have a lower genetic potential for yield than cereals. The major chickpea-growing countries are India, Turkey, Pakistan, Iran, Australia, Mexico, Ethiopia, Myanmar, Spain, and Bangladesh [7]. It is the earliest domesticated plant in the Mediterranean and Middle Eastern areas [8]. It is valued for its nutritive seed content that is higher in protein composition as a consequence and is increasingly used for human and animal protein feeding [9]. There are two types of chickpeas, Desi (microsperma) and Kabuli (macrosperma). These types are characterized by differences in the color and size of the seed [10]. Kabuli type has large rounded seeds that are cream, beige or white in their seed coat color. It was green aerial parts that lacked anthocyanin pigmentation with developing white flowers. Meanwhile, the Desi type has small rough seeds with an angular appearance that varies from light to black color with all gradations in between, a reticulated surface with anthocyanin-pigmented aerial parts and pink or purple flowers [11]. Kabuli has been grown traditionally in the Mediterranean and Central Asia. However, the Desi type has mainly been produced in India, East Africa and Central Asia [12]. Increasing crop productivity in recent years to ensure food security is a major challenge. Therefore, there is a requirement to produce an additional increase in food security via increasing resources from improved crop varieties or hybrids. This is the main goal for plant breeders to increase the yield of crops coupled with desirable grain quality through the availability of genetic diversity in the crop population, which improves the efficiency of the selection technique. Thus, mutagens such as gamma rays are used to generate the magnitude of genetic variability, which is important for plant breeders to develop superior plant genotypes through breeding techniques in a shorter duration of time. Therefore, mutations bring genetic variations in the genetic resources of the organisms either at the chromosomal level or at the gene level. Mutation also plays an important key role in species evolution, as well as in the development of superior crop genotypes. Thus, it is possible to apply ionizing radiations to increase the frequency of mutation to enable plant breeders to introduce specific improvements in chickpeas without affecting the attributes of crop varieties. Induced mutations create variations leading to plant improvement through selection. Increasing local production of chickpeas will have an important positive economic impact. Mutation breeding is an effective technique and an important approach to legume improvement. To date, more than 3274 varieties from more than 224 plant species has been released from mutagenesis techniques, which have been officially derived as recorded in the FAO/IAEA Mutant Varieties Database. From these, 21 improved chickpea mutants were released for cultivation [13]. Mutations can be induced by chemical or physical mutagens. On the level of physical mutagens, gamma rays are one of the most frequently used [14]. Induced mutations have a great potential technique for enhancing genetic variability and improving yield in chickpeas via effective handling of mutagenized populations [15]. Therefore, the efficiency of selection for improving quantitative traits as yield should preferably be recorded in the early generation (M_2). This is because almost of desired combinations of favorable alleles are likely lost in generations progress due to intensive or even no selection for other traits [16]. Therefore, genetic variability is essential for genotypic improvement, which can be achieved by hybridization and breeding techniques. Mutation breeding techniques have been used to induce new genetic diversity to improve economic traits. Mutational breeding in chickpeas was done to enhance the yield of seed production, as well as nutritional components [17].

Mutation breeding was used to induce mutations in the loci controlling important traits or eliminate undesirable genes from elite breeding lines [18]. The significance of induced mutations is one of the most effective and efficient techniques to generate and restore the genetic variability in chickpeas. Some studies focused on mutation breeding in chickpeas using gamma rays which damage DNA molecules by strand breaks and destruction of the sugar bases [19]. Mutational breeding techniques were significant in chickpea improvement via incorporating beneficial genetic alteration in target loci. The application of mutagens increased genetic diversity for selecting favorable genotypes and thereby speeded up

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Table 1. List of quantitative traits and their techniques of evaluation

Trait	Method of evaluation
Plant height	Height from the base of the plant to the root tip of the last leaf (cm).
Number of primary branches	Total number of primary branches per plant.
Total number of pods/ plant	A total number of pods were counted and recorded per plant.
Seeds yield	Weighting the total number of seeds produced per plant after sun-dried for three weeks and expressed as g/ plant.
Fifty seeds weight	Fifty healthy seeds after sun dried for three weeks were randomly collected, counted, and then weighted by gram.
Plant dry weight	Weighting all the plant growth when the plants became to bloom after sun-drying for three weeks and then oven-dried at 70°C till reached to the constant weight and then the weight was expressed by the gram.

breeding techniques [20]. Chickpea is an important crop for sustainable agriculture since fixing atmospheric nitrogen via symbiotic bacteria improves the growth characteristics and yield components of chickpeas [21]. Induced mutations played a key role in the release of superior genotypes. The present study was undertaken to induce genetic variability in chickpeas via physical mutagens such as gamma rays, not only to improve selection technique in the next generations but also to select the superior genotypes that appeared agronomic improvement at a very early generation.

2. Materials and methods

2.1 Genetic material

One variety of chickpeas from the Kabuli type named Giza 531 was used in this study. It was kindly obtained from the Agronomy Research Institute, currently hosted by the Agriculture Research Center, Giza, Egypt.

2.2 Gamma irradiation

Air-dried seeds of chickpea variety (12-13% moisture content) were gamma irradiated with the following doses; 00, 25, 50, 100, 200, 400 and 800 radiuses (dose rate 1.249 radius h^{-1}). Where Gy is the unit of gamma ionizing radiation dose in the international system of units defined as the absorption of one joule of radiation energy per kilogram of the matter. In mutation breeding experiments, the radiation dose was assessed by the intensity and length of the explosive to radiation. The dose of irradiation is generally expressed as kR or Gray (Gy), where one Gy = 100 radius and one kR = 10 Gy. Where the unit of absorbed dose was radius which means radiation absorbed dose. The seeds were irradiated at the Atomic Energy Center, Nasr City, Cairo, Egypt using the irradiation device GSR D_1 (Germany) by the source of cobalt 60. Unirradiated seeds served as controls.

2.3 Experimental set-up

In this study, the effect of gamma irradiation was investigated to increase genetic diversity in the chickpea genotype. The effect of gamma irradiation was investigated via achieving growth parameters, alteration in some physiological traits such as chlorophyll concentration, as well as yield and its components. Observations of various quantitative traits are listed in Table 1. For this purpose, the field experiment was performed in the Agri-Farm of the Genetic Department during the winter season of 2022/2023, inside the campus of Mansoura University. The irradiated seeds along with the control were sown in a randomized complete block design to raise the M_1 generation with three replicates [22]. About 96 seeds were irradiated with each dose of gamma rays. A similar number of unirradiated seeds were served as a control. The seeds were sown in the field in rows three meters long, each row containing ten holes. Four seeds were sown in each hole. The plants were thinned to two plants per hill after three weeks of germination. The seed-to-seed and row-to-row distances were applied at 30 cm and 60 cm apart, respectively. Data of physiological parameters such as chlorophyll concentration in leaves were recorded at 60 days of sowing. Meanwhile, the data on quantitative traits were recorded at the end of the growth period when the plants began to bloom. Plant height (cm) was measured from the ground level to

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Table 2. Mean values of morphological measurements of chickpea following seeds exposure with gamma irradiation.

Gamma irradiation doses(radius)		Primary branches number per plant	Plant dry weight (g)	Plant height (cm)	Chlorophylls concentration (mg/g fresh weight)		
					Chl a	Chl b	Total Chl
00		25.60	47.63	25.66	1.46	0.21	1.67
25		17.30	38.83	17.33	1.08	0.94	2.02
50		19.30	35.92	19.33	1.02	1.18	2.19
100		29.30	45.41	29.33	1.52	0.25	1.70
200		30.30	51.91	30.33	1.54	0.16	1.70
400		26.60	38.82	26.66	1.21	0.23	1.54
800		23.30	45.52	23.33	1.62	0.20	1.82
F. test		*	Is	Is	Is	**	*
L.S.D.	0.05	05.83	09.39	05.91	1.45	0.19	0.25
	0.01	07.04	13.17	08.29	2.50	0.27	0.35

*, ** Means significance at 0.05 and 0.01 levels of probability, respectively. Is = Insignificant differences.

the tip of the seedling. The number of primary branches/plants, as well as, plant dry weight were measured when the plants began to bloom. Chlorophyll concentration in leaves is one factor of the major sign of successful photosynthesis and dry matter accumulation. Chlorophyll concentrations were measured and expressed as mg/g fresh weight according to [23].

2.4 Statistical analysis

This study was done in triplicate and the data presented are means of three independent measurements. The data were subjected to one-way analysis of variance (ANOVA) and the LSD test was used to compare between two means if the differences between doses were significant [24]. In addition, the coefficient of variation was determined using standard statistical procedures according to [25].

3. Results and discussion

3.1 Vegetative growth affected by irradiation

Mean values of phenotypic traits in chickpeas affected by different doses of gamma rays are summarized in Table 2. The results of the analysis of variance showed that there were significant differences between treatments of gamma rays for the number of primary branches developed per plant, chlorophyll b concentration, as well as total chlorophyll in leaves. These differences indicated that the tested chickpea genotype became genetically diverse. This diversity induced by could be used to improve growth-related traits in chickpeas. These results are in harmony with [26], who found that highly effective mutagenic agents played a significant role in mutagenesis-based breeding techniques. On the other hand, each dose of gamma irradiation affected differently on the different traits from the same genotype of chickpeas throughout the M_1 generation. These findings revealed a significant increase in the concentration of chlorophyll b, as well as total chlorophyll which responded to 50 radius from the ^{60}C source. In addition, the dose of 25 radiuses increased significantly the concentration of total chlorophyll in leaves.

The results of primary branches per plant were impacted by an increase in dosages of 100 and 200 radiuses if compared with the general mean of control plants. These results indicated that the doses of 100 and 200 radiuses increased significantly the number of primary branches per plant. This may probably be due to rapid cell division in the point from which the branch was initiated, as well as elongation of the cells in this point to develop new branches, with stimulation of plant growth hormones related to differentiating primary branches [27]. These results are in line with [28], as well as being similar to the earlier investigations obtained by [26], who revealed that plant genotype exhibited influences on the sensitivity of populations to chemical mutagens. In this criteria, [29] stated that gamma irradiation-induced nucleotide substitutions and deletions in a small number of nucleotides ranged from 2–16 base pairs. Therefore, crop improvement through mutation breeding techniques is the major concern for developing countries like Egypt to increase crop

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Table 3. The variation coefficient of various growth traits in M_1 generation of chickpea treated with gamma irradiation.

Gamma irradiation doses(radius)	Plant dry weight (g)	Plant height (cm)	Primary branches, number plant	Chlorophylls concentration (mg/g fresh weight)		
				Chl a	Chl b	Total Chl
00	**0.13**	**0.04**	**0.13**	**0.07**	**0.21**	**0.02**
25	0.14	0.02	0.06	0.06	0.03	0.03
50	0.04	0.03	0.08	0.04	0.08	0.03
100	0.06	0.04	0.01	0.10	0.17	0.11
200	0.03	0.07	0.19	0.10	0.01	0.13
400	0.14	0.04	0.12	0.08	0.01	0.07
800	0.15	0.03	0.10	0.21	0.21	0.13

Chl = Chlorophyll

productivity to ensure food security. In line with this goal, [30] reported that the lower doses of ionizing radiation proved an effective tool for improving overall nutritional attributes. However, [31] stated that gamma irradiation-induced a dose-dependent decrease in the total quantity of seed proteins at the higher dose of irradiation. This may probably due to de-polymerization and denaturation of amino acids, which affected some physicochemical properties of seed flour proteins like protein solubility, water absorption, as well as foaming properties. Meanwhile, [32] found that higher exposure to gamma irradiation reduced the rate of mitotic activity, meanwhile, the lower doses bring genetic variations in agronomic importance traits. In addition, [31] suggested that the dose of about 5 Gy of gamma rays was significant for exhibiting antioxidant activity in chickpeas.

The consequence effects of gamma irradiation on plant dry weight, plant height and chlorophyll concentration in leaves reflected insignificant differences between the different doses of gamma irradiation. This finding indicates that gamma irradiation does not significantly affect the gene expression related to these traits. Meanwhile, the dose of 200 radius displayed a high value of plant height, as well as plant dry weight and the number of primary branches developed per plant if compared with the control plants. This may be due to the effect of irradiation on the stimulation of plant growth hormones, leading to an increase in the rate of cell cycle, as well as elongation of the cells from which the branches were initiated at these points. These results are in line with [33], who found that plant height was positively influenced by the increased doses of sodium azide as a chemical mutagenic agent.

In this respect, [34] decided that the maximum safe dose of gamma rays in chickpeas is 100 radius which exhibited maximum gross mutations, absence of mitotic cycle inhibition, and occasional membrane damage. Therefore, [35] stated that plant breeding through genetic diversity induced by radiation is a rapid method for improving plant breeding techniques. The results reflected that the different doses of irradiation had different effects on the different morphological traits. In the same criteria, [36] found two types of chlorophyll mutation in local sorghum, namely albino and viridis. The observations of the same authors on the M_2 population showed that gamma rays induced an expansion of the range value for all agronomic traits, in addition to high heritability for panicle length, chlorophyll content, panicle weight and seed yield.

3.2 Analysis of genetic variability in growth traits

As shown in Table 3, the estimated values of the coefficient variation for plant dry weight ranged from 0.03 to 0.15 in comparison with the corresponding check value (0.134). This indicated high homogeneity at the doses of 50, 100, 200 radiuses and high heterogeneity at the doses of 25, 400 and 800 radiuses. Meanwhile, the degree of homogeneity for plant height and the number of primary branches per plant ranged from 0.02 to 0.07 and 0.01 to 0.19, respectively, this indicated homogeneity at all doses of gamma irradiation, except for the dose of 200 radius which recorded the highest heterogeneous values for plant height (0.07) and number of primary branches developed per plant (0.19), where it produced the higher variations within their irradiated plants. Similar findings were shown by [37], who found that the number of primary branches per plant was higher than the control in grass peas treated with the

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Table 4. Mean values of yield components in chickpea treated with gamma irradiation

Doses of gamma irradiation (radius)		Weight of 50 seeds (g)	Pods weight per plant (g)	Pods number per plant	Seed weight per plant (g)
00		22.00	092.76	252	61.59
25		26.46	093.16	253	76.57
50		22.36	093.16	230	69.48
100		19.46	107.97	257	83.20
200		23.26	125.33	321	77.28
400		25.72	077.23	216	57.14
800		22.54	074.54	256	42.31
F. test		**	**	**	**
L.S.D.	0.05	1.34	07.54	11.24	7.09
	0.01	1.88	10.57	15.76	9.94

** Significance at 0.01 level of probability.

following doses; 15, 20, and 25 kR in M_1 and M_2 generations. Therefore, the increase in branching number leads to an increase in the number of pods per plant in mutant genotypes. Meanwhile, the reduction in plant height as shown in this study may be due to the reduction in cell length and cell division, cell elongation, or the reduction in cell number [38]. On the other hand, [39] decided that the major factor in growth inhibition is a chromosomal disorder that leads to inhibiting the mitotic cycle. Thus, the development of superior varieties or genotypes is known as mutation breeding which results from artificially induced mutations via mutagenic agents. Concerning total chlorophyll, the degree of heterogeneity ranged from 0.03 to 0.13 which reflected higher values than the check control (0.02). This heterogeneity indicates high variations in the gene expression of chlorophyll formation which affects the formation of chlorophyll pigment.

In general, the degree of homogeneity varied among the doses of gamma rays in the same trait, as well as from one trait to another in the same genotype. The heterogeneous obtained in chlorophyll formation can be used for improvement of this trait through a simple selection program. Mutation breeding has played an important role in the development of varieties or genotypes harboring varied alleles. Therefore, mutation breeding has become a successful tool in plant breeding techniques. Ionizing physical mutagens as gamma rays produced highly reactive free radicals, which take place between the radicals and DNA molecules, lipids and proteins in the cellular membranes and organelles thus causing genetic alterations. If these radicals interact with DNA molecules, it causes deletions, rearrangements and loss of nitrogen bases that alter protein structures and their function, forming mutant proteins. As a consequently inducing phenotypic change. Meanwhile, non–ionizing radiation such as ultraviolet rays lead to the development of thymine dimmers, causing the strand to buckle, disrupting normal base pairing in DNA molecules, leading to inhibit DNA replication and transcription [40].

3.3 Yield components

As shown from the results tabulated in Table 4, the differences between doses of gamma irradiation were significant concerning the weight of 50 seeds, the weight of pods developed per plant, the number of pods yielding per plant, as well as seed weight per plant. These traits revealed significant variations between the doses of gamma rays. The maximum weight of 50 seeds was 25.72 grams produced by the dose of 400 radius compared with the control (22.0 g) and the minimum (19.46 g) was obtained by the dose of 100 radius. Therefore, the doses of 25 and 400 radiuses produced a significant increase in 50 seeds weight above the check value in the control plants.

The maximum weight of pods yielding per plant was 125.33 grams produced by 200 radius. Meanwhile, the minimum weight (74.54 g) was produced by 800 radius, if compared with the control, which recorded 92.76 g. Therefore, the doses of 100 and 200 rad produced a significant increase in the weight of pods developed per plant if compared with the control check value. These results are in line with [41], who stated that mutation in many agronomic and yield traits of chickpea genotypes may be attributed to the point mutations induced in pleiotropic genes or

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cluster genes or induced chromosomal rearrangement in the plant genome. In addition, Van [42] found early-flowering types in chickpeas by treating seeds with gamma rays with small and large leaf sizes in addition to other variations in M_2 generation. Meanwhile, [43] isolated mutants from chickpea suitable for machinery harvest through different doses of gamma irradiation. Therefore, induced mutation is considered as a rapid technique in plant breeding for creating variability. Mutations induced are of two types, one type is macro-mutations which include high changes in the genetic structure of an organism. The other type of mutation is micro-mutations which involve changes in quantitative parameters that can be measured by various statistical parameters. These micro-mutations are of great importance for plant breeders. It is useful for improving quantitatively inherited traits as yield without disturbing the genotype, as well as, the phenotypic traits of the crop. Mutation breeding plays a great role in increasing food security all over the world [42].

The number of pods developed per plant revealed a maximum number reached to 321 at the dose of 200 radius and the minimum was equal to 252 in the control. These results indicated that the dose of 200 radius exhibited the highest mean values for the total number of pods developed per plant as a consequence of increasing pod weight per plant, as well as seed weight per plant. This indicates the importance of genetic variations induced in these traits for improving and determining seed yielding. This is in harmony with [45], who stated that the maximum genetic variability was found in yield components of chickpeas including the number of seeds per plant, the number of pods per plant, and seed yielding. Similar results in chickpeas were reported before by [46]. In addition, [44] isolated mutants in chickpeas suitable for mechanical harvesting in addition to the early flowering mutants which can be used as genetic stocks in the future to be used in crop improvement programs. The maximum seed weight per plant (83.2 g) was produced by 100 radius. Meanwhile, the minimum weight (42.31 g) was produced by 800 radius, whereas the control recorded 61.57 g. The significant weight of seed yield per plant was shown by the dose of 100 radius. (83.20 g) if compared with the check value in un-irradiated plants (61.59 g). Hence, a significant increase in seed weight per plant was coupled with the number of pods developed per plant, as well as pod weight per plant. These findings agreed with [47] in mutant lines of chickpeas, who found the indirect effect of hundred seed weight on the total number of seeds yielded per plant. In addition, [48] found a direct positive effect of hundred seed weight on seed yield per plant.

The number of seeds yielded per plant had the highest positive effect on seed yielding per plant in chickpeas as reported by [49]. The findings of this study suggested that improvement of seed yield depends upon the selection based on the number of pods developed per plant, as well as the weight of seeds yielding per plant, which will provide positive results in chickpea improving technique. According to [45], the development of chickpea genotypes for improving their productivity is based on seed yielding, plant height, first pods and seed mass. This study indicates genetic variations induced in chickpeas via gamma irradiation may be referred to as small nucleotide variations induced in the pleiotropic gene or the cluster gene. The genetic variability is a pre-requisite to selecting superior genotypes from chickpea populations, which can dramatically improve the efficiency of the breeding process. The success of this approach was based upon the application of irradiation doses that produced sufficient genetic variations without fertility and viability affecting. These variations could be assessed phenotypically and genotypically. Genotypic assessment is based upon estimations of DNA sequence variations, while phenotypic analysis is based on the expression of important agronomic and yield traits, as well as their heritability. The selection of quantitative traits as yield should be preferably done in the early generations because most of the desired combinations of favorable alleles may be lost in the future generations due to intensive or no selection for the other traits [50].

In addition, [7] found that the coefficient of variance had maximum values for all the traits and treatments of chickpea treated with EMS if compared with the control, expect for days to 50 percent flowering and plant height treated with EMS, in addition to the

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Table 5. Variation coefficient of yield components in chickpea treated with gamma irradiation

Doses of gamma irradiation (radius)	Weight of 50 seeds (g)	Pods weight per plant (g)	Pods number per plant	Seed weight per plant (g)
00	0.001	0.013	0.025	0.120
25	0.019	0.030	0.019	0.030
50	0.065	0.030	0.029	0.099
100	0.040	0.010	0.011	0.080
200	0.020	0.014	0.015	0.040
400	0.010	0.020	0.027	0.060
800	0.050	0.110	0.039	0.050

combination between gamma rays + EMS in M_2 generation.

Thus, this work provides important information about induced mutations in chickpea genotype via gamma irradiation, which induced a spectrum of genetic variations, to be more diverse for efficient selection. Using similar observations obtained by [29], they assessed the rate of mutation frequency generated by gamma rays to be one mutation in rice per 6,190 Kb. Meanwhile, [51] found that the average number of mutations per gene in M_2 rice was 1/942 Kb in irradiated lines, as well as, the percentage of mutation sites per the total sequence was equal to 0.67. Likely, mutations induced in the essential genes for important traits will quickly knock out lethality. This makes the evolution of gamma-irradiated plants more challenging if compared with chemically mutagenized populations. The goal of this evaluation is to determine genotypes expressing high growth parameters, seed-yielding, as well as agronomic traits. This is ongoing with the link between genotypic diversity induced with the observed phenotypes expressed. This will provide important information about gene function and genetic biomarkers that facilitate plant breeding techniques. The mutant genotype is highly encouraged, especially on topics surrounding food security and technology, which are major challenges in crop productivity. Chickpea genetic variability is narrow due to its self-pollinating nature [11]. On the other hand, [52] obtained through conventional breeding in chickpeas large grain size, early maturity, as well as pest and disease resistance. Farmers prefer early maturing crops under the effects of severe droughts [53]. Therefore, early-maturity mutants were preferred under severe droughts. However, genetic variability was inadequate in most self-pollinated crops. Therefore, induced mutation via irradiation techniques has been an effective method for generating significant genetic variability because mutagenesis is a faster and more effective tool if compared with conventional breeding [54].

3.4 Analysis of genetic variability of yield

The data tabulated in Table 5 reflected the degree of homogeneity determined based upon the coefficient of variability which is used to assess the magnitude of genetic variations induced within the same genotype of chickpea used in this study. For the weight of 50 seeds, the estimated coefficient of variation was ranged between 0.010 to 0.065 higher than the check value (0.001). This indicated high heterogeneity in this trait, whereas the different doses of gamma irradiation gave higher variations within the same genotype. In contrast, the weight of seeds produced per plant reflected coefficient of variation ranged between 0.030 to 0.099 lower than the check value (0.120). This indicated high homogeneity in this trait, whereas the different genotypes produced by the different doses of gamma irradiation which recorded their values lower than the check value. These genotypes exhibited high uniformity in their plants for the weight of seeds produced per plant.

The estimated coefficient of variance for the weight of pods developed per plant ranged between 0.010 to 0.030 compared with the check value (0.013). Therefore, the degree of homogeneity for pod weight per plant differed among different doses of gamma irradiation in the same genotype. The doses of 25, 50, 200 and 400 radiuses induced heterogeneity in this trait. This is because they recorded higher values in the coefficient of variation ranging between 0.014 to 0.030 above the check value (0.013), then it could be considered as new variations. In general, the degree of homogeneity was varied among the doses of gamma irradiation in the weight of pods yield per plant compared with the check genotype. High

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heterogeneity was obtained for the weight of pods developed per plant. This indicated high genetic variability, which is available for improvement of the genotype through a simple selection technique.

As regards the number of pods yield per plant, only three doses of gamma irradiation including 50, 400, and 800 radiuses recorded higher values in the coefficient of variation ranged between 0.027 to 0.039 higher than the check value (0.025). High heterogeneity obtained herein reflected high genetic variations induced by gamma irradiation within the chickpea genotype. This indicated that they were more phenotypically variant than the un-irradiated genotype, since they showed a variation coefficient higher than the check genotype. However, the remaining doses of gamma irradiation at 25, 100, and 200 radiuses induced the magnitude of genetic variation ranged between 0.011 to 0.019 lower than the check genotype (0.025). This indicated high homogeneity in the number of pods produced per plant because they recorded the lowest values to the check. Therefore, these doses exhibited high uniformity in the number of pods produced per plant. The results obtained herein agreed with [55], who stated that the estimated variation coefficient differed among tomato genotypes in the same trait and from one trait to another in the same genotype. Therefore, the doses of gamma irradiation were affected on the different genes responsible for yield components, not only increasing their activity but also affecting gene expression, leading to new genes coupled with the activated genes creating new phenotypes. By investigating the effect of gamma irradiation on the same genotype. The different irradiation doses had different effects on the genes related to yield components producing a new phenomenon genotype in seed yielding.

Ionizing radiation such as gamma rays used in this study, can cause DNA breaks in the double-strand. The radioactive isotope of cobalt 60 caused point mutations, as well as a small number of nucleotide deletions, leading to induced genetic variations between the doses if compared with the check genotype. Gamma rays are the most popular physical mutagenic agent used in plant breeding techniques because of their ability to penetrate deeply into plant tissues. Hence, irradiation doses should be selected by examining different doses and screening irradiated genotypes for desired mutant phenotypes carrying the desired importance traits such as; viability rate, seed survival, germination rate, chlorophyll mutation, growth rate and so on. These traits are the early biomarkers for the occurrence of mutations which can be used by plant breeders [56]. The low doses of gamma irradiation could generate mutant genotypes in higher plants with a broad spectrum of phenotypes without damage to other plant traits. Gamma rays have been effectively used for inducing chickpea mutant genotypes [57]. In this respect, gamma-irradiated chickpeas reported to found that 20 kR was an effective dose in producing heritable diversity genotypes without loss in total phenotypic variability [57]. Thus, the doses of gamma rays induced heterogeneous phenomena in yield components were attributed to the mode of action of this physical mutagenic agent. Gamma rays affected growth parameters and yield components related genes, not only enhanced their activity but also activated the expression of new genes coupled with the activated genes to be creating new superior phenotypes. The popular strategy in reverse genetics named TILLING, targets induced local lesions in genomes, meaning to assess the extent of natural genetic diversity in selected genotype crops. The approach of reverse genetics combined wild-type and mutant DNA, including a high frequency of point mutations induced by physical mutagenic agents. This work highlights the efficacy of employing new genetic diversity induced in chickpeas via treatment of the seeds of chickpeas with gamma irradiation. Therefore, the plant population is suitable for mutation breeding.

4. Conclusions

In conclusion, induced mutations are necessary to increase the rate of genetic variability in chickpea populations. The importance of genetic variations induced in chickpeas was to isolate high-yield genotypes to overcome the low productivity. Different doses of gamma rays had different effects on growth parameters and yield components. This should be applied in future breeding techniques on chickpeas to get desirable mutations for seed yielding with associated growth traits. The promising

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genotypic mutants must be examined to evaluate their stability in phenotypic expression. Therefore, this evaluation was to assess chickpea genotypes expressing high agronomic traits and yield components. Evaluation of phenotypic expression will provide important information on gene function and gene biomarkers that facilitate plant breeding techniques.

Ethical approval

This study does not indicate any human or animal testing or feeding on irradiated products.

Authors' contributions

It is not applicable because a single author constructed this manuscript.

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Conflicts of interest

The author declares that this manuscript was done in the absence of any commercial or financial relationships that could be conducted as a potential conflict of interest.

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