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GENETIC VARIABILITY AND MOLECULAR MARKERS RELATED TO BROOMRAPE SUSCEPTIBILITY IN CHICKPEA

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ABSTRACT

Chickpea is an important crop legume grown worldwide. There is an increasing concern on narrowing of genetic base in chickpea. Ionizing radiations as gamma rays is one of many tools used for increasing genetic variability. This study aimed to increase genetic variationsin chickpea kabuli genotype to be improvement. Dry seeds were subjected to gamma irradiation at the doses of 0, 25, 50, 100, 200, 400 and 800 radius. M_1 plants were harvested separately and sown in the next season to produce M_2 plants in a row progenies. Variations were recorded in phenotypic expression of qualitative and quantitative parameters. The days needed to first flower appeared was significantly decrease in response to the dose of 25 radius. The dose of 100 radius achieved the greatest number of pods developed per plant and significant increase in seed yielding per plant. Highest heritability value (99.8%) was recorded for the number of pods developed per plant followed by 100-seed weight (98.1%).Genetic diversity between Giza 195 and Giza 531 represent differences at the DNA level.The band with a molecular weight of 500 bp was appeared in the susceptible genotype to broomrape Giza 531 by SCoT-11 and SCoT-36. It is a diagnostic band recommended to fingerprint *Cicer* genotypes for the analysis of susceptible genotypes to broomrape.

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INTRODUCTION

Chickpea, *Cicer arietinum* L. was belonging to the legume family Fabaceae. This family is the third greatest family in the higher plants. It is second to cereals in economic and agricultural importance (Gupta and Gopalakrishna 2012). Chickpea is an old world pulse cultivated mainly in India, North Africa andWest Asia (Kumar *et al*. 2015). Chickpea was originated in the adjoining Syrai and the fertile crescent region of southeastern Turkey (Van der Maesen 1987). Chickpea was diverged from Turkey in two directions. The first direction is the western parts where it is grown in summer and spring. The second direction is the southern and eastern parts where it is grown in the dry cool seasons. The wild *Cicer* species are observed in North Africa and West Asia converging Ethiopia in the south and Turkey in the north (Cubero 1987). Historically, Asia is a major chickpea producer, followed by Australia, Africia, America and Europe. Recently, chickpea production has increased considerably through the period of 1980-2018 with a global values reached about 17.2 million tons in 2018 based on FAOSTAT data (2012). Out of these producers, India is the largest producer of chickpeas with over 11 million tons in 2018 (FAO, 2020). Carbohydrate content in chickpeas was lower than that in wheat. Strach is the main content of carbohydrate (47.4-66.9%) that accounts about of 41.0-50.8% of the total carbohydrates in chickpea (Singh 1985). India contributed with 68% of chickpea worldwide production. Chickpea is an important and cheap source of protein in developing countries as India and in South Asia (Gaur *et al*. 2012). There are two phenotypes of chickpea including "Desi" that referred as microsperma and "kabuli" type that referred as macrosperma. Both types are differing in their geographic distribution. Kabuli types are cultivated in the Mediterranean region, but the desi types are mostly grown in the Indian subcontinent and Central Asia. Desi types possessing predominantly pink-colored flowers, small leaflets, pods and seeds. Meanwhile, kabuli types have a cream seed color or large beige with white flowers and ram's head seeds. Macrosperma or Kabuli types may have originated from the desi phenotypes (Moreno and Cubero 1978). The importing countries of chickpea has increased to 142 in 2009 instead of 64 in 1990 suggesting an increasing global demand of chickpea (FAOSTAT 2012). Chickpea improves the fertility of soil via fixing atmospheric nitrogen. It is covered up around 80% of its nitrogen requirement through nitrogen fixation (Saraf *et al*. 1998). Reductions of atmospheric nitrogen levels (N_2) into biological activities (NH_4) account for 65% of available nitrogen in the biosphere (Lodwig *et al*. 2003). Most of this nitrogen comes from symbiosis reactions mediated by *Rhizobium* that allow the fixation of 65 million tons of atmospheric nitrogen that is released

each year in the biosphere (Graham and Vance 2003). This indicated the importance of legumes in protecting the environment from the chemical pollution of nitrogen fertilizers used in the agro-systems. The genus *Cicer*was belongs to the sub-family papilionoideae. Most species of *Cicer* including chickpea are diploid plants that having a chromosome number of 2n = 2x = 16 chromosomes with a genome size of approximately 931 Mbp. Chickpea is highly self-pollinated plant with an out crossing rate below than 1% (Basu *et al*. 2018). In recent years, mutation breeding particularly by gamma irradiation was used as an effective tool in relation to traditional plant breeding methods for crop improvement. More than 2252 mutant genotypes have been released in more than 50 countries including pulse vegetables, oil seeds, fiber fruits, ornamentals and cereals (Mabrouk *et al*. 2018). Conventional breeding released over than 350 improved chickpea cultivars that have improved in yield, enhanced adaptation to new niches and improved productivity (Gaur *et al*. 2007). About half of 350 improved chickpea varieties have been produced in India which has the higher national chickpea breeding strategy in the world (Guar *et al*. 2007). Diversity in the population support chance of selection for desirable traits. Therefore, induced mutagenesis can be used to generate variability in the population because the rate of spontaneous mutation is extremely low. Create variability by induced mutation is a better tool in crop improvement. Many studies have been used physical mutagens particularly gamma rays for crop improvement. Mutation breeding may be considered as an alternative tool and supplement to hybridization as a source of variability. Effective selection can be developed out from the mutated population especially in chickpea which has been recognized as a minimal genetic variation crop (Kumar *et al*. 2015). Mutation breeding program may take a shorter time than conventional breeding which usually takes a longer time (Kumar *et al*. 2015). Gamma rays is a type of electromagnetic waves that has a good penetration effect in molecules creating material ionization through the penetration of their electrones (UNSC 2000). The ionized cells are categorized by DNA disturbance that led to a notable alteration in the inherited traits.

The physiological genetic effect of low gamma rays doses on plant development and growth might be referred to the interaction between gamma rays and the biological molecules in the cells that leading to produce free radicals. Free radicals released can change the main organelles and molecules in the cells (Kim *et al*. 2006). The doses below 1 KGy of gamma rays are considered as low doses (Iglesias-Andreu *et al*. 2012). The lower doses of gamma irradiation ameliorate photosynthetic pigment content in lettuce. Stimulation impact of gamma rays was referred as the promoting influence of low doses(Calabrese 2019). To data, more than 3274 genotypes in more than 224 plant species were released from mutagenesis programs which have been listed in the FAO/IAEA Mutant Varieties Database (Adhikari and Pandey 1982). From these mutant varieties, 493 mutant genotypes in pulses are registered, including 21 improved chickpea mutants are released for cultivation (Mutant Variety Database 2016). Gamma irradiation are the most frequently used from physical mutagens that accounting for 64% of the radiation-produced mutant genotypes (Jankowicz-Cieslak and Till 2015). Mutants generated exhibited genetic variations and improved the yield in chickpea through mutagenized populations (Wani 2011). Seeds irradiated with higher doses of gamma rays disturbs protein biosynthesis (Xiuzher 1994), leaf gas-exchange, hormonal balance (Rabie *et al*. 1996), water exchange and enzyme activity (Stoeva *et al*. 2001). Mutation breeding induced mutations at specific loci controlling important parameters by eliminating undesirable alleles from elite breeding lines (Lippert *et al*. 1964). The main importance of mutation breeding is the potential for improving one or a few traits without altering the rest of genotypes. Success of mutation breeding based on the production of desirable mutations (Wani and Anis 2008).

Irradiation of chickpea seeds with low doses of gamma rays (< 25 Gy) stimulate root length (Boulbaba *et al*. 2009). The dose of 20 Gy-gamma rays increased plant growth and development by 146.35% if compared with the control plants (Singh 2005). Mutation breeding was used before in rice to develop earliness and semi-dwarfism plants. The rice mutant line *Reimei* referred has originated from gamma-rays was one of the first allele sources used to generate dwarf rice genotypes. In addition, Basmati 370 referred as semi-dwarf mutant of rice genotypes was also utilized from gamma-rays. It was developed through induced mutagenesis. In dwarf mutants defective gibberellin pathway is mostly associated with poor internodal elongation that decreased cell division. The discovery of ionizing radiation and chemical mutagens opened a new field of science referred as "mutation breeding". Chlorophyll mutants segregated in M_2 generation was reflected the mutagenic effect and efficiency of physical mutagens in chickpea. Gamma rays was damage DNA molecules by destruction of sugar bases and strand breaks (de-Winter *et al*. 2000). The available chickpea genotypes does not include enough genetic variations in economical traits. Though, mutation breeding is highly important in chickpea improvement via incorporating beneficial mutations in target loci (Qureshi *et al*. 2014). So, mutation breeding has been used nowadays as a technique in plant breeding to be developed better crop genotypes (Awan 1991). The inheritance of significant parameters as adaptation, quality, yield, stress and pest resistance can be understood via the analysis of a wide range of mutants released. It has been established that the induction of mutation either by chemical and physical mutagens evolving new genotypes (Haq *et al*. 1999). The direct effects of mutagens can occur if ionizing radiation as gamma rays directly damages and ionizing a cell macromolecule as DNA (Kamble and Patil 2014). Although, chickpea does not include enough genetic variations because it is a self pollinating plant, thus mutation strategy could be rewarding to enhancing genetic base for plant yield and yield related traits. Conventional plant breeding approaches used for chickpea improvement has been hampered due to the lack of sufficient genetic variations. Mutagenesis is a common tool efficient in plant breeding to induce new desirable genetic variations in chickpea (Micke 1988).Chickpea is playing an advantage role in food safety through covering proteins deficiency of daily food especially of African and Indian populations. It was used as a common source of protein and carbohydrate (Malunga *et al*. 2014).

Around 6.5 billion people are now live on our planet. This numberof peoples is projected to increase by 2050 with almost to increase than 9 billion people. This required to produce more food for more peoplesbut from fewer resources for this growing demond. Chickpea has a head start in this regard because it is playing an important role in food safety over the world through covering the protein deficiency of daily food ration in human populations (Wood and Grusak 2007). Some reports established that *Kabuli*type are high sensitive than *desi* type of chickpea to mutagens (Kharkwal 1998). There are two institutes at least had a strong programms on mutation breeding for incorporating biotic and abiotic stress in chickpea improvement. These institutes are the Nuclear Institute of Agriculture and Biology, Faisalabad, Pakistan and the Indian Agriculture Research Institute, New Delhi, India (Dua *et al*. 2001).Most molecular marker studies indicated that genetic variability was limited in the cultivated varieties of chickpea. This leading researchers to be use mutation breeding by gamma rays which is the most commonly physical mutagen used in this regard, as well as, interspecific crosses to increase genetic variability in the cultivated species. Bangladesh Institute of Nuclear Agriculture, Mymensingh, released high-protein and high-yielding mutant of chickpea through mutations induced by gamma rays. The released mutant of chickpea had containing 20% higher protein and 20% higher yield than the parental varieties (Oram *et al*. 1987). Hence, this work aimed to increase genetic variability in chickpea via radio sensitivity of gamma rays from Cobalt-60 to select potentially superior individual plants, in addition to molecular differentiation between broomrape resistant and susceptible genotypes based on SCoT molecular markers.

MATERIALS AND METHODS

The present investigation was carried out in the Agri-Farm of Genetic Department inside the campus of Mansoura University through the two Academic years of 2022/2023 and 2023/2024.

Genetic Material: Seeds of chickpea, *Cicer arietinum* L, from macrosperma Kabuli type Giza 531 and Giza 195 with green aerial parts,beige seed coatlacking anthocyanin pigmentation and developing white flowers were used in this study. The selection of these genetic materials was based on its availability and economic importance and. The healthy and viable seeds of Giza 531 (moisture 10%) were selected to be subjected to different doses of gamma rays. The seeds were cleaned before irradiation. The varieties used in this study was kindly provided from Field Crops Research Institute, Agriculture Research Centre, Giza, Egypt. Seeds were obtained from this source to avoid heterogenicity appeared in commercial varieties. Molecular analysis was conducted in this study to differentiate between the resistant (Giza 195) and susceptible (Giza 531) genotypes of chickpea to broomrape based on SCoT molecular markers.

Gamma Irradiation: About 96 seeds of Giza 531 from the healthy, fresh and vigorous seeds of chickpea were acutely irradiated with each dose of gamma rays. Nonirradiated seeds were served as control. The seeds were subjected to six doses of gamma rays included; 25, 50, 100, 200, 400 and 800 Gy (dose rate 1.249 kGy h^{-1}). Gamma irradiatedseeds was conducted at the Atomic Energy Centre, Nasr City, Cairo, Egypt using irradiation device GSR D_1 (Germany) from the Cobalt-60 source radioisotope.

Molecular markers used in SCoT analysis: Thirteen start codon targeted (SCoT) primers (Table 1) were used in this study to detect the molecular variation between two chickpea genotypes resistant (Giza 195) and susceptible (Giza 531) variety to broomrape (*Orobanche crenata*).

Preparation of CTAB Buffer: This buffer was used within 2–3 days after freshly prepared. It was stored in a capped container. Before starting extractions, polyvinylpyrrolidone (PVP-40, molecular weight 40,000) and β-mercaptoethanol were added and stirred until dissolved. The following amounts were used, CTAB 0.5 ml, PVP-40 0.02 g and β-mercaptoethanol 2.5 ml.

Table 1. Primer name and nucleotide sequence of 13 SCoT molecular markers used in this study

Primer name	Nucleoticle sequence
SCOT-24	CACCATGGCTACCACCAT
SCOT-33	CCATGGCTACCACCGCAG
SCOT-34	ACCATGGCTACCACCGCA
SCOT-52	ACAATGGCTACCACTGCA
SCOT-77	CCATGGCTACCACTACCC
SCoT-02	CAACAATGGCTACCACCC
SCoT-04	CAACAATGGCTACCACCT
SCoT-11	CAACAATGGCTACCACCT
SCoT-12	CAACAATGGCTACCACCT
SCOT-31	CCATGGCTACCACCGCCT
SCoT-36	GCAACAATGGCTACCAC
SCoT-A	ACAATGGCTACCACTACC
SCoT-C	CAACAATGGCTACCACGC

SCoT polymerase chain reaction (PCR) reactions: Gently vortex and briefly centrifuge ampR One PCR Master mix (2X)cat. No. SM213-025 Simply kit.Place a thin-walled PCR tube on ice and add the following components for each 25 µl reaction:ampR OnePCR M.M (2X), 12.5 µl;primer, 2 µl; Template DNA (10 ng/ul), 1 µl; Water, nuclease-free, 9.5 µl to be reached a total volume25 µl. Gently vortex the samples and spin down. Perform PCR using the recommended thermal cycling conditions outlined in Table 2.

Table 2. Recommended thermal cycling conditions

Step	Temperature, °C	Time	Number of cycles
Initial denaturation	95	5 min	1
Denaturation	95	1 min	40
Annealing	56	1 min	
Extension	72	2 min	
Final Extension	72	10 min	1

Field trial: The field trial design was conducted in randomize complete block trial consisting of three replicates. Recommended normal agricultural practices released from the Egyptian Ministry of Agriculture for chickpea productivity were applied at the suitable time. The recommended dose of phosphorus chemical fertilizer was used at sowing time by handing on one side of the row at 5 cm depth. After irradiation, the M_1 seeds were planted under normal growing conditions with four seeds that were sown in each port along with untreated controls in three replicates. Each row consists of eight port with 3.0 m length, 30 cm width, 40 cm spacing between the plants within rows, as well as, 20 cm spacing was applied between rows. The M_1 field comprised of six rows for irradiated seeds separated by a spacing of 1.50 m from the three rows of control plants. The individually harvested M_1 plants with early maturity, high seed yielding plants and large seed size were selected to be cultivated in the next season for releasing the M_2 plants. The M_2 seeds were harvested individually. The M_2 seeds were used to establish the M_2 mutant progenies as described in M_1 population. Data were recorded on M_2 generation from ten plants in each progeny row. The M_2 seeds were sown in the next season as a single plant progenies to establish the M_2 generation. After two weeks from the seedling emergence, then the plants were thinned to two plants per hill. Plants were water unirrigated through their growth season until harvest depending on drain water only. Seeds used to produce M_2 plants were obtained from selfing M_1 plants in each treatment.

Biochemical analysis

Concentration of photosynthetic pigments: Total concentration of chlorophyll and carotenoid pigments were measured according to Arnon (1949). In this methodology 0.5 g of fresh leaves was immersed in 4.5 ml methanol for 24 hours in the dark at 4 ^{0}C. The absorption was recorded at 663, 645 and 470 nm against the methanol blank using a Jenway spectrophotometer. The absorption spectrum of different chlorophyll pigments and carotenoids was assessment as described by Lichtenthaler and Wellburn (1983) as follows,

$$\text{Chlorophyll a (µg / mg FW)} = \frac{[12.25\,(A663) - 2.79\,(A645)]\ \text{volume (ml)}}{\text{Weight by mg of leaf tissue}}$$

$$\text{Chlorophyll b (µg / mg FW)} = \frac{[21.5\,(A645) - 5.1\,(A663)]\ \text{volume (ml)}}{\text{Weight by mg of leaf tissue}}$$

$$\text{Carotenoids (µg/mg FW)} = \frac{[1000\,(A470) - 1.82\,(\text{Chl a}) - 85.02\,(\text{Chl b})]\ /\ 198 \times \text{volume (ml)}}{\text{Weight by mg of leaf tissue}}$$

In addition, total chlorophyll was assessed based on Ahmed *et al*. (2020) as follows,

Total Chlorophyll (µg / mg FW)= chlorophyll a + chlorophyll b

This trait was measured after 50 days from sowing. In these equations, V meaning the volume of consumed methanol, as well as W referred as the weight of fresh leaves.

Morphological analysis and seed yielding

Days to 50% flowering: It was registered as the days number from seeding date to the date of 50% of flowering plants in each row of each plot (Abebe *et al*. 2017).

Above ground biomass yield (gram per plant): The above ground biomass yield in each row was measured at harvest time when the plants became to blooming after three weeks of sun dried in the field. Then the plants were transferred to oven dried at 70 ^{0}C for two days and then immediately weighted using electronic balance and then subdivided on the number of plants per each row (Behtash *et al*. 2022).

Plant height (cm): The plant height was estimated started from the base of the main stem to the tip of the plant at the harvesting time. The data recorded by cm for all plants grown in each row per each plot for each treatment and then subdivided on the number of plants

grown in each row (Abebe *et al*. 2017). The observations of different quantitative traits are listed in Table 3.

Seed yield per plant (g): Seed yield per plant was measured by harvesting the chickpea crop from each row of each treatment in each plot, cleaned and adjusted to 14% moisture after three weeks of sun dried. Then the seeds were weighted using an electronic sensitive balance and then subdivided on the number of plants per row (Abebe *et al*. 2017).

Hundred seed weight (g): One hundred seed weight were measured by random taking of 100 seeds after three weeks of sun dried that were well developed and cleaned which were collected from the middle of each row in each plot per each treatment, and weighted using an electronic sensitive balance (Abebe *et al*. 2017).

the gel (presence), while '0' denoted its absence in other sample(s) (absence). The number of monomorphic and polymorphic bands, along with the percentage of polymorphism, was calculated. The similarity index (SI) and similarity matrix (SM) were determined using the SM coefficient. The unweighted pair group method with arithmetic mean (UPGMA) was applied to assess genotype-to-genotype similarity matrix coefficients. Additionally, principal coordinate analysis (PCA) was conducted using the Dice similarity index on this matrix to generate a phylogenetic tree (dendrogram) with the PAST program (Version 1.91) according to Hammer *et al*. (2001).

Statistical analysis: The data obtained in this investigation are subjected to the analysis of variance (ANOVA) according to Snedecor and Cochran (1980).

Table 3. Different quantitative traits assessed and the method of evaluation

Trait	Denotation	Method of evaluation
Days to flowering	DF (days)	Number of days from sowing to the stage when 50% of plants have begun to flowering.
Days to maturity	DM (days)	Number of days from sowing to the stage when the first pod was developed.
Height of first pod	HFP (cm)	Height from the ground level to the first pod developed.
Plant height	HP (cm)	Height from the ground level to the tip of last leaf.
Number of primary branches	NPB	Total number of developing primary branches in each plant.
Total number of pods/plant	TPP	Total number of pods developed per plant.
Seed yield	SY (g/plant)	Weight all the seeds produced by each plant.
Hundred seed weight	HSW (g)	One hundred seeds randomly counted and theyare weighed.

Table 4. Mean performance of vegetative growth traits in M_2 generation of kabuli chickpea irradiated with gamma rays.

Treatments (Radius)		Root length (Cm)	Plant height (Cm)	Number of primary branches per plant	Days needed to first flowering	Days needed to first pod formation	Plant dry weight (g)	Height of first branch above the ground (Cm)
00		11.30	56	17.67	49.17	73.50	51.67	15.00
25		8.30	52.5	13.67	46.53	78.64	44.00	18.03
50		9.77	52.17	22.67	50.42	81.57	43.00	15.50
100		8.47	65.67	27.67	53.83	82.93	57.67	36.00
200		9.83	53.67	33.00	55.30	87.03	48.67	19.00
400		7.80	45.67	14.33	52.47	78.40	38.00	14.83
800		11.40	64.50	13.00	54.40	81.00	25.00	23.17
F-test		**	**	**	**	NS	**	**
LSD	0.05	0.81	4.89	4.15	3.29	8.22	5.60	3.90
	0.01	1.14	6.86	5.82	4.61	11.53	7.86	5.46

NS, **: Non-significant and significant differences at 0.01 probability level, respectively.

Carbohydrate contents: Five grams of seed sample from Giza 531 in each treatment were debrief by ethanol 80% and maintaining overnight at laboratory temperature. The ethanolic solution was used for determining total carbohydrates using anthrone technique. The green color formed was estimated spectrophotometrically at 630 nm based on Sadasivam and Manickam (1996).

Biotoxic impact

Assessment of seed germination: The germination rate was recorded every day from the sowing date till last seedling emerged. Germination ratio was calculated according to Qureshi *et al*. (2014) as follows:

$$\text{Seedling germination ration} = \frac{\text{Number of seedlings emerged}}{\text{Total seed sown}}$$

Genetic viability parameters: To confirm the relative importance of different traits, a set of genetic parameters were calculated as; genotypic and phenotypic coefficient of variations according to Singh and Chaudhary (1985). Heritability in broad sense was assessedbased on Robinson *et al*. (1949). Meanwhile, genetic advance and genetic advance as a percentage of mean were measuredbased on Johnson *et al*. (1955).

DNA isolation: DNA extraction protocol was performed using the CTAB/Chloroform-isoamyl alcohol according to Cullings (1992).

Data analysis: Data analysis for SCoT molecular markers was performed by scoring clear fragments and encoding them as a binary data matrix. A value of '1' indicated the presence of a clear band in

RESULTS AND DISCUSSION

Growth traits: Data obtained in Table 4 were processed significant decrease in root length at the doses of 25, 50, 100, 200 and 400 radius of gamma rays if compared with the control developed from non-irradiated seeds. This indicated that the plants treated with different doses of gamma irradiation were genetically diverse. Whereas, the root length of plants treated with 800 radius is not significantly altered by irradiation. This agrees with Martirena-Ramírez *et al*. (2015), who suggested that germination of irradiated *Phaseolus vulgaris* L. seeds is not altered by irradiation. The results obtained herein are in harmony with Pérez-León *et al*. (2019), who observed that the growth of roots and stems in chickpea were gradually influenced by increasing the doses of gamma irradiation. A drastic reduction in root length (7.8 cm) was obtained at 400 radius if compared with the control (11.3 cm). Meanwhile, the root length at 800 radius (11.4 cm) was increased in relation to the control. The doses of 100 and 800 radius were significantly increased plant height over the control. Plant height was ranged between 52.5 (25 radius) to 64.50 cm (800 radius). The doses of 50 and 200 radius leading to significantly increase the number of primary branches developed per plant. This trait was ranged between 13 at 800 radius to 33 at 200 radius. The dose of 25 radius is the only dose that insignificantly decline the days needed to first flower appeared. Plant dry weight was ranged between 25g at 800 radius to 57.67g at 100 radius. The dose of 100 radius significantly increased plant dry weight over the control. In addition, the dose of 400 radius is the only dose showed insignificantly decrease the height of first branch developed above the

ground level as compared to control. Significant decline in plant dry weight was shown in plants treated with 25, 50, 400 and 800 radius. These results are in line with Sanjeev et al. (2019), who foundthat gamma rays caused physiological and biochemical alteration in chickpea including the inhibition of germination and growth. The same authors observed that root length and stem height are key traits for determining radiosensitivity. In this respect, Pérez-Leónet al. (2019) reported that the importance of radiosensitivity was used in the improvement by mutation technique in every cultivar which showed differentiation in responds to the mutagens. Khah and Verma (2015) stated that seed germination is not a good indicator for radiosensitivity in wheat.

Considering the abovementioned results, the dose of 100 radius induced significant increase in the number of primary branches developed per plant, plant heightand plant dry weight in relation to the control. Thus, the dose of 100 radius can select to gamma irradiate the seeds of chickpea without inducing deleterious effects. This dose should be high enough as to be increase the probability of mutations generatedat below the harmful level caused lethality to tissues and cells. Since mutation induction is a random event and its recovery was based upon plant viability, then this technique increases the probability to obtain a survival mutant. The field evaluation of M2 generation obtained in this study provided enough differentiation as making selection possible. The results obtained herein are in harmony with the findings of Mabrouk et al. (2018), who observed that mutations produced by gamma irradiation are able to generate new and improved variants if compared with the parental cultivars. The plant heights were insignificantly decline at the doses of 25, 50 and 200 radius, but significantly decreases at the dose of 400 radius. This indicated that the high doses gamma rays have exerted an inhibitory impact on plant height. These results are in line with Umavathi and Mullainathan (2014), who decided that the reduction in chickpea germination that treated with gemma rays and EMS may be due to genetic physiological effects resulted in cell maturity. Gamma irradiation induced wider spectrum in growth traits of chickpea due to differential mode of action on different base sequences of different genes.

Since mutation are random events happen in the plant genome, then the chances for isolating superior and novel alleles were still greater (Andrew-Peter-Leon et al. 2021). The decreases in plant height achieved in this study agreed with Andrew-Peter-Leon et al. (2021), who generating rice mutants performing significant decline in plant height and the number of days to flowering which registered yield increased than control.Many investigations in dwarf mutant reflected that defects in gibberellic acid pathway reduced the cell elongation and division which are the major events of internodal elongation that severely affect the plant height. Wang et al. (2009) suggested that the defects in the pathway of gibberellic acid leading to reduced cell division. Therefore, gibberellic acid applied on dwarf mutants can restore the plant height. Thus, gibberellic acid is the main contributor of plant height in the semi-dwarf mutant of rice (Andrew-Peter-Leon et al. 2021). The investigation of Andrew-Peter-Leon et al. (2021) using scanning electronic microscope images appeared that the number of cells was reduced per unit area in the semi-dwarf mutant of rice. This leading to reduction the internode lengths in rice mutants.This hinted that there is a mutant happen in gibberellin pathway leading to gibberellin deficiency in rice mutant caused semi-dwarfism rice (Andrew-Peter-Leon et al. 2021).

The semi-dwarf mutants in rice is an interesting phenotype because of gibberellin oxidase was deficient in these mutants which converts gibberellin intermediates into bioactive forms (Yamaguchi et al. 2008). Hence, the loss of gibberellin oxidase function may resulted dwarfism phenotype. But, reduced the expression in gibberellin oxidase genes produced semi-dwarf plants (Kadambari et al. 2018). Besides, Helliwell et al. (2001) reported that the expression levels of four genes regulate gibberellic acid pathway by converting the intermediary into gibberellin.Therefore, inhibited activity or expression result in dwarfism phenomenon. If the gene is down regulated, this suggested that mutations were occurred in the gene. Mutations in these genes caused dwarfism plants with high tillering (Ishikawa et al. 2005).The results obtained in this study are also in harmony with Kulshreshtha and Singh (1984), who found an increase in plant height at 10 KR of gamma rays in green gram. The same authors observed decrease in plant height at 25 and 30 KR. They also explain the decline in plant height was regarded to the reduction in length of internodes as a consequence of decline in cell length or to the decrease in the cell number. In this criteria, Arumugam et al. (1997) decided that the major factor leading to growth inhibition is the chromosomal damage. Similar findings in the reduction of plant height has been reported in rhodes grass subjected to gamma rays (Khan 1998) and in mungbean treated with MMS (Ansari et al. 1997).

Chlorophyll pigments

A detailed analysis of chlorophyll and carotenoid concentrations is given in Table 5. The spectrum of chlorophyll a concentration was ranged between 0.864 at 50 radius to 1.639 mg/g FW at 25 radius. The doses of 25 and 400 radius produced significant increase in chlorophyll a in relation to the control. Meanwhile, the doses of 50, 100, 200 and 800 radius induced significant decrease in the concentration of chlorophyll a. Furthermore, the doses of 50, 100, 200 ,400 and 800 radius induced significant decrease in chlorophyll b concentration if compared with the control.Total chlorophyll concentration was ranged between 1.432 at 50 radius to 2.447 at 25 radius. The dose of 25 radius is the only dose produced significant increase in total chlorophyll above the control.In addition, the doses of 50, 100, 200 and 800 radius revealed significant decrease in total chlorophyll in relation to the control. Moreover, the doses of 25 and 400 radius achieved significant increase in carotenoid pigment over the control. The concentration of carotenoid pigment was ranged between 8.10 at 50 radius to 12.62 mg/g FW at 25 radius. The doses of 50, 100,200 and 800 radius were significantly decrease carotenoid pigment in relation to the control. These results indicated that the dose of 25 radius is the best dose achieved significant increase in total chlorophyll, chlorophyll aand carotenoid concentrations if compared with the control.

These results are in line with Kharkwal (1998), who found that gamma rays gave the greater frequency of chlorophyll mutants. The doses of 50, 100, 200 and 800 radius appeared significant decline in the concentration of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid concentration in relation to the control.The dose of 400 radius induced significant increase in chlorophyll a and carotenoid concentrations as compared with the control. The results revealed no uniform trend of a dose dependency could be obtained for chlorophyll concentration. Among the doses of gamma rays, the dose of 25 radius was the most effective dose that showed significant increase in chlorophyll and carotenoid concentrations. It is generally indicated that ionising radiations induce a high frequency of significant decrease in chlorophyll and carotenoid concentrations. These results agreed with Sarker and Sharma (1989), who found albina, chlorina and xantha types of chlorophyll mutations using differentmutagens in lentil.

It seems that the strong increase in chlorophyll and carotenoid concentrations reach their saturation point at lower dose of gamma rays as seen by the dose of 25 radius. Further increase in the doses of gamma rays does not added increases in chlorophyll and carotenoid concentrations. This agrees with Fillipeti *et al*. (1977), who decided thatthe strong mutagenic agents become high toxic than that of higher doses from relatively weaker mutagenic agents. Major differences in the doses response were observed in this study concerning a wide spectrum of chlorophyll concentrations in the M_2 population. The results obtained in this study are in harmony with Qureshi *et al*. (2014), who found that the dose of 100 Gy of gamma irradiated chickpea induce best increase in both chlorophyll a, b and total chlorophyllpigments. The results are also supported by Borzouei *et al*. (2010), who stated that 100 Gy could induce high concentration of chlorophyll a, b and total chlorophyll in wheat genotypes. The same authors found that chlorophyll concentration was decline with increased the doses of gamma rays.

Table 5. Mean performance of photosynthetic pigments in leaves of M_2 generation affected by gamma rays

Treatment (Radius)		Photosynthetic pigments (mg/g FW)			
		Chl a	Chl b	Total chlorophyll	Carotenoid content
00		1.424	0.849	2.273	11.83
25		1.639	0.808	2.447	12.62
50		0.864	0.568	1.432	8.10
100		1.105	0.754	1.859	10.58
200		1.214	0.693	1.906	11.31
400		1.479	0.771	2.251	12.04
800		0.980	0.617	1.597	9.33
F-test		**	**	**	**
LSD	0.05	0.042	0.051	0.049	0.082
	0.01	0.058	0.072	0.069	0.115

Table 6. Mean performance of seed characteristic parameters in M_2 generation of kabuli chickpea affected by gamma rays

Treatments (Radius)		Germination ratio	Viability ratio of seeds	Carbohydrates content (ppm)	Hydration coefficient
00		0.81	1.00	51.27	2.19
25		0.95	1.17	42.22	2.20
50		0.68	0.83	37.52	2.17
100		0.76	0.94	54.48	2.18
200		0.84	1.03	55.02	2.17
400		0.69	0.84	55.73	2.13
800		0.67	0.83	60.38	2.21
F-test		*	*	**	**
LSD	0.05	0.15	0.18	0.802	0.044
	0.01	0.22	0.26	1.124	0.061

*, **: Significance at 0.05 and 0.01 levels of probability, respectively.

The results obtained herein agreed with Kharkwal (1998), who found that chemical mutagen produce high frequency of xantha and chlorina types in chickpea, whereas the ionizing radiations induce high frequency of chlorophyll mutation from albina types.Fillipeti *et al*. (1977) reported that the strong ionizing mutagens as gamma rays reach their saturation point at lower doses in the highly mutable germplasm and the increase in mutagen doses does not added to mutation frequency. Increasing the concentration of effective mutagens become high toxic than the higher concentration of weaker mutagens (Fillipeti *et al*. 1977). Kharkwal (1998) found that *desi* genotypes of chickpea are more sensitive towards mutagenic treatment than *kabuli* and green seeded type. The same author also found that chemical mutagens are more efficient agents than physical mutagens for producing chlorophyll mutations. Chlorophyll formation seems to be genetically controlled by different genes located on different chromosomes (Goud 1967). Mutations in these genes may cause chlorophyll mutations. Ramulu (1970) decided that the differences in the mutation rate and spectrum in various genotypes may be due to varied of their location of genes in relationto the centromere. Chlorophyll controlled genes were adjacent to centromeres and proximal segments of the chromosome (Swaminathan 1965). Ramulu (1970) found that there were at least 250-300 loci for chlorophyll formation exist in barley. Mutations in chlorophyll controlled genes bring outs decline in chlorophyll pigments. The frequency of chlorophyll mutants may be regarded to the differences in radio sensitivity. The frequency of chlorophyll mutation is a good indicator factor for evaluating the genetic effects of mutagens. It is useful in the study of specific gene products that are responsible for chlorophyll formation. It is therefore indicated that chlorophyll mutations cause lethal effect that leading it do not have any economic value, but it could have an advantage value for determining the dose of mutagen that would increase the genetic diversity and the number of useful mutants in the segregating progeny (Nagargoje and Kashid 2018). Further, similar conclusions were drawn earlier by Swaminathan *et al*. (1962), who decided that the high frequency of chlorophyll mutation was due to preferential action of chemical mutagen as EMS on chlorophyll development genes pointed near the centromere.

Biochemical traits of seeds: As regarding to Table 6 that shows the seed characteristics influenced by different doses of gamma rays. The results revealed significant differences between the doses of gamma rays among all the biochemical traits of seeds.

Germination ratio of seeds was ranged between 0.67 at 800 radius to 0.95 at 25 radius. Higher insignificant ratio of germination was observed at 25 radius. In addition, the viability ratio of seeds was ranged between 0.83 at 800 and 50 radius to 1.17 at 25 radius. Reduced the viability ratio was due to the doses of 50, 100, 400 and 800 radius pinpointed that gamma rays may induced gross chromosomal breakages affecting the viability of seeds. The viability of seeds was recorded maximum (1.17) at the dose of 25 radius in relation to the control. This agrees with Bharathi *et al*. (2013), who observed that the lower doses of gamma rays showed higher germination percentage and the lowest germination rate was obtained at higher doses of gamma rays.The results obtained herein are supported by the work done by Dhulgande *et al*. (2015), who observed that chickpea mutagenic treatments appeared a gradual decreasing trend in germination from lower to higher doses. It is clearly appeared that gamma rays have clearly exerted an inhibitory effect on seed germination. This are in line with Bhat *et al*. (2012), who found the similar inhibitory effect on seed germination of chickpea affected by gamma rays. Moreover, Roychowdhury (2011) reported that the effect of mutagens leading to decline seed germination due to the disturbed base pair relationship, inhibition of auxin synthesis, disturbance of enzyme formation and decline of assimilation mechanism. Raina *et al*. (2017) reported that isolation of morphological mutants in M_2 progenies determines the genotypic sensitivity and mutagenic mutability. The same authors also stated that every individual gene responsible on agronomic trait of interest can mutate and induce a wide spectrum of viable morphological mutants. Konzak (1965) reported that heritable morphological mutants as dwarf, bushy, semidwarf and bold seeded mutant types were controlled by polygenes. In contrast, Reddy and Gupta (1988) decided that most of the true breeding mutants were controlled by a single recessive genes. Carbohydrates content was ranged between 37.52 at 50 radius to 60.38 at 800 radius. The doses of 100, 200, 400 and 800 radius induced significant increase in carbohydrates content in relation to control. Hydration coefficient was ranged between 2.13 at the dose of 400 radius to 2.21 at the dose of 800 radius. Hydration coefficient have small differences between doses with presenting the highest insignificant value at the dose of 800 radius. The results obtained herein agreed with Ramos *et al*. (2018), who reported that hydration coefficient expressed as a percentage of original sample weight was not based on seed size and presents the better way to express on hydration properties. The same authors found that the soaked seeds of chickpea absorbed a considerable amount of water

and then softened which is referred to volume and weight increases and decrease firmness. Soaking is an important process in several legumes prior to cooking their seeds (Aguirre-Terrazas *et al*. 1992). The results obtained herein are in harmony with Del Valle *et al*. (1992), who established that beans soaked in distilled water exhibited higher absorption of water than that soaked in salt solutions due to the decrease of osmotic pressure across the membranes of cotyledon cells. Therefore, rapid hydration with maximum volume gain or weight is most desirable. It improves profitability in the canning industry. Chickpea that absorbed water faster, declined undesirable risks of prolonged soaking as leaching, reduced soaking times and growth of harmful microorganisms or degradation of nutrients (Wood and Harden 2006).

Considering the abovementioned results, the dose of 100 radius can be selected to irradiate the seeds of chickpea without producing deleterious effects. The field evaluation of the M_2 generation provided genetic variations enough as making selection possible. These results are in harmony with Amri-Tiliouine*et al*. (2018), who found that the selected nine lines induced by gamma irradiation generated the highest mean performance for total number of pods and seeds yielding per plant. The same authors observed the maximal variability for yield components as pods number per plant, number of seeds per plantand yields with a very high coefficient of variation. The results obtained herein are also in line with Pérez-León *et al*. (2019), who found that the total number of seeds developed per plant had the greatest positive direct effect of gamma irradiation, followed

Table 7. Mean performance of various yield related traits in M_2 generation of chickpea affected by gamma irradiation

Treatment (Radius)		Number of pods developed per plant	Weight of dry pods per plant (g)	Seed yield per plant (g)	Hundred seed weight (g)
00		119.00	37.2	201.30	19.81
25		127.67	49.2	126.66	20.20
50		127.00	49.2	163.33	20.74
100		365.33	103.5	265.00	20.26
200		144.67	74.3	220.00	21.75
400		108.67	39.3	113.66	23.52
800		120.67	46.0	105.66	19.43
F-test		**	**	**	**
LSD	0.05	7.15	8.49	18.75	0.34
	0.01	10.03	11.90	26.29	0.48

Table 8. Estimates of genetic variability parameters of different vegetative growth traits in M_2 generation of kabuli chickpea

Traits	Mean ± Sd	GMS	EMS	σ^2e	σ^2g	σ^2p
Root length (Cm)	9.5 ± 0.37	6.21	0.21	0.21	2.00	2.21
Plant height (Cm)	55.7 ± 2.08	152.19	7.57	7.57	48.21	55.77
Number of primary branches per plant	20.3 ± 1.81	180.49	5.44	5.44	58.35	63.80
Days needed to first flowering	51.7 ± 1.54	30.06	3.42	3.42	8.88	12.30
Days needed to first pod formation	80.4 ± 4.30	53.41	21.37	21.37	10.68	32.05
Plant dry weight (g)	44.0 ± 2.37	332.67	9.93	9.93	107.58	117.51
Height of first branch above the ground (Cm)	20.2 ± 1.94	171.25	4.80	4.80	55.49	60.28

Table 8. Continued

Traits	PCV%	GCV%	ECV%	h^2%	EGA	GAM%
Root length (Cm)	15.55	14.80	0.75	91	2.77	29.01
Plant height (Cm)	13.40	12.46	0.95	86	13.30	23.85
Number of primary branches per plant	39.10	37.40	1.71	91	15.05	73.66
Days needed to first flowering	6.78	5.76	1.02	72	5.22	10.08
Days needed to first pod formation	7.04	4.06	2.97	33	3.89	4.83
Plant dry weight (g)	24.64	23.57	1.06	92	20.44	46.46
Height of first branch above the ground (Cm)	38.40	36.84	1.56	92	14.72	72.81

Sd = Standard deviation, GMS = Genotypes mean squares, EMS = Error mean squares, σ^2e = Environmental variance, σ^2g = Genotypic variance, σ^2p = Phenotypic variance, PCV (%) = Phenotypic coefficient of variation, GCV (%) = Genotypic coefficient of variation, ECV (%) = Environmental coefficient of variation, h^2 (%) = heritability in broad sense, EGA = Expected genetic advance, GAM (%) = Genetic advance as a percent of mean at 5% selection intensity.

Yield related traits: The results obtained in Table 7 demonstrated that the number of pods developed per plant was ranged between 108.67 (400 radius) to 365.33 (100 radius). The doses of 25, 50, 100 and 200 radius revealed significant increase in the number of pods developed per plant. The dose of 100 radius exhibited the higher number of pods developed per plant. On the other hand, the dose of 400 radius induced significant decrease in the number of pods developed per plant. The weight of dry pods developer per plant was ranged between 37.2 (control) to 103.5 g (100 radius). The doses of 25, 50, 100,200 and 800 radius appeared significant increase in the weight of dry pods per plant. In addition, the seed yield per plant was ranged between 105.66 (800 radius) to 265g (100 radius). The dose of 100 radius is the only dose observed significantly increase in seed yielding per plant. Hundred seed weight was ranged between 19.43g (800 radius) to 23.52g (400 radius). The doses of 25, 50, 100, 200 and 400 radius observed significant increase in hundred seed weight in relation to the control. The greatest increase in seed yield per plant (265g) obtained at 100 radius was attributed to significant increase in the number of pods developed per plant, weight of dry pods per plant and 100-seed weight. Therefore, the radiosensitivity of chickpea genotype used in this study is going to be used the dose of 100 radius in the improvement of seed yielding by mutations.

by 100 seed weight that indicated as a performance indicators of seed yielding at early breeding phases. Mutation breeding has additional advantages if one or two traits need to improvement in an already well high-yielding genotypes. Mutant variety database has recorded over 3000 mutant varieties in more than 175 plant species. Only 21 mutant varieties were registered in chickpea and have been released for cultivation from which six mutants are released from India (Kozgar 2014). The results obtained in this study revealed that mutation occurred in yield related traits was in both positive and negative direction as compared to the parental genotype in control experiment. This could be due to differential mode of action of different doses on different base sequences in various genes of yield related traits. The results agreed with Barshile *et al*. (2009), who found that the mutant lines obtained in chickpea treated with gamma rays and EMS observed the highest number of seed yield per plant in roundish pod mutant (50.66) if compared with the control (26.5). Significant increase in 100 seed weight obtained from the doses of 25, 50, 100, 200 and 400 radius was attributed to the increased cotyledonary cell volume by maintaining the similar number of cells within unit area (Joshua and Bhatia 1983). This indicated that induced mutations generated variability in yield related traits that offer wide scope in genetic improvement of chickpea through breeding

programme. Seed yield per plant is the most desirable trait in any breeding programme. Regarding to seed yielding per plant, some mutants that are superior in relation to untreated populations can be isolated in the M_2 generation. These mutants were quite distinct morphologically especially in seed shape and size if compared with the control (Wani 2009). According to Wani (2009), who isolated from M_2 progeny of chickpea treated with 200 Gy of gamma rays mutants showed an increase in the size of flowers, pods and seeds. The same author reported that the increase in seed yielding per plant may be due to the mean performance of yield contributing traits as 100-seed weight, seed sizeand number of pods developed per plant.

Genetic parameters of growth traits: According to the result tabulated in Table 8, the assessment of phenotypic coefficient of variation (PCV) were greater than the corresponding values of genotypic coefficient of variation (GCV). The higher values of GCV (> 20%) were registered for the number of primary branches developed per plant, plant dry weight and height of first branch developed above the ground level. Nevertheless, the smallest genotypic coefficient of variation was observed for the days needed to developed the first pod followed by the days required to first flower appeared. The higher PCV values (> 20%) were recorded for the number of primary branches developed per plant followedby the height of first branch above the ground and plant dry weight. The same results of higher PCV and higher GCV were obtained for the genetic advance expressed as a percentage of mean (GAM) for the number of primary branches developed per plant, plant dry weight and height of first branch over the ground level.

Higher values of GAM (> 30%) were recorded for root length, number of primary branches developed per plant, plant dry weight and height of first branch over the ground. The lowest values of GAM were registered for the days required to first pod formation and flowering.Higher heritability values (> 85%) coupled with higher genetic advance (> 20%) were recorded for plant height, root length, number of primary branches developed per plant, plant dry weight and height of first branch developed above the ground. The lowest value (33%) of heritability was recorded for the days needed to first pod formation. These genetic variability parameters is a pre-requisite to the selection of better varieties in any cultivar. Human selection over thousands of years has led to the deletion of potentially important allelic variation. Therefore, this study aimed to increase genetic variation within chickpea gene pool to improve the efficiency of breeding programme.Thus, the application of irradiation dosage that induced sufficient variation with maintaining fertility leading to success this approach. Variation as seen is this study can be measured phenotypically and genotypically. High heritability coupled with high GAM observed in the mutant population reflected that these traits were controlled by additive gene action. The results obtained herein agreed with Raturi *et al*. (2015), who observed higher values of genetic components (h^2, GAM, GCV and PCV) in mungbean for seed yielding, pods developed per plant and seed yielding per plant. Similar results were also obtained by Amri-Tiliouine *et al*. (2018), who found the higher values of GCV and PCV for yield and seed yielding per plant in M_2 generation of chickpea. The same authors also obtained high estimates of GCV (> 20%) for the number of seeds developed per plant and seed yieldingfollowed by the number of primary branches developed per plant and the number of pods developed per plant. The same authors found the smallest genotypic coefficient of variation for the days required to flowering.

Genetic parameters of photosynthetic pigments: As shown from the results presented in Table 9, the values of phenotypic coefficient of variation (PCV) for chlorophyll a, b, total and carotenoid concentration were higher than the corresponding values of genotypic coefficient of variation (GCV). The higher values of GCV (>20%) were registered for chlorophyll a. Nevertheless, the smallest GCVs were recorded for chlorophyll b and carotenoid content. The higher values of PCV (> 20%) were obtained for chlorophyll a. The smallest PCV was obtained for chlorophyll b and carotenoid concentration. The lowest value (0.007%) of ECV was obtained in carotenoid concentration. Similar results were also found for GAM. Higher estimates of GAM (> 30%) were registered for chlorophyll a, total chlorophyll and carotenoid concentration. The lower value for GAM was recorded in chlorophyll b (27.65%). All heritability valves recorded for all chlorophyll and carotenoid pigments were exceeded 90%. This reflected that high heritability estimates were coupled with high genetic advance for chlorophyll a, total chlorophyll and carotenoid content.

Table 9. Estimates of genetic variability parameters of photosynthetic pigments in M_2 generation of kabuli chickpea

Traits (mg/g fresh weight)	Mean ± Sd	GMS	EMS	σ^2e	σ^2g	σ^2p
Chlorophyll a	1.24 ± 0.018	0.239	0.00055	0.00055	0.079	0.080
Chlorophyll b	0.72 ± 0.028	0.031	0.00082	0.00082	0.010	0.011
Total chlorophyll	1.97 ±0.024	0.422	0.00076	0.00076	0.140	0.141
Carotenoid content	10.83 ± 0.055	7.599	0.00213	0.00213	2.532	2.534

Table 9. Continued

Traits (mg/g fresh weight)	PCV%	GCV%	ECV%	h^2%	EGA	GAM%
Chlorophyll a	22.76	22.68	0.078	99.0	0.577	46.42
Chlorophyll b	14.51	13.96	0.550	92.5	0.200	27.65
Total chlorophyll	19.10	19.05	0.051	99.5	0.770	39.14
Carotenoid content	14.69	14.68	0.007	100	3.276	30.22

Sd = Standard deviation, GMS = Genotypes mean squares, EMS = Error mean squares, σ^2e = Environmental variance, σ^2g = Genotypic variance, σ^2p = Phenotypic variance, PCV (%) = Phenotypic coefficient of variation, GCV (%) = Genotypic coefficient of variation, ECV (%) = Environmental coefficient of variation, h^2 (%) = heritability in broad sense, EGA = Expected genetic advance, GAM (%) = Genetic advance as a percent of mean at 5% selection intensity.

This reflected that these parameters were controlled by additive gene interactions.These results are in harmony with the findings of Badigannavar and Murty (2007), who obtained high heritability values coupled with high genetic advance forpods yield per plant, seed yieldingand plant height in M_8 generation of groundnut irradiated with gamma rays. Therefore, selection based on heritability and genetic advance of total chlorophyll, chlorophyll a and carotenoid content in gamma irradiated population of chickpea may be effective for improvement of chickpea. These results are also in line with the findings of Barshile *et al*. (2009), who found high heritability estimatescoupled with high genetic advance for seeds number developed per plant and plant height in chickpea subjected to chemical and physical mutagens, which may be due to additive gene effects.

Genetic parameters of seed characteristics: As shown from the results presented in Table 10, the PCV% was greater than GCV% concerning germination ratio, viability ratio, carbohydrate content and hydration coefficient. PCV% was higher than 10% for germination ratio, viability ratio and carbohydrate content. PCV% was ranged between 1.47% for hydration coefficient to 16.97% for viability ratio. In addition, GCV% was ranged between 0.94% for hydration coefficient to 15.97% for carbohydrates content. The presence of high PCV% greater than GCV% indicated the influence of prevailing

environmental factors that influence on seed characteristics traits, especially for germination and viability ratio of seeds. Environmental coefficient of variation was high for the viability ratio of seeds (4.444%) followed by germination ratio (4.396%). The difference between phenotypic and genotypic variances was ranged between 0.024% for carbohydrates content to 4.444% for viability ratio. germination ratio and viability ratio. Moreover, moderate heritability (30-60%) was coupled with low genetic advance (< 10%) for hydration coefficient.The heritability in broad-sense was remarkably high for carbohydrates content, suggesting a genetic component. This indicated that there is a chance for genetic improvement of carbohydrates content as demonstrated in high heritability coupled

Table 10. Estimates of genetic variability parameters of seed characteristics in M_2 generation of kabuli chickpea affected by gamma rays

Traits	Mean ± Sd	GMS	EMS	σ^2e	σ^2g	σ^2p
Germination ratio	0.77 ± 0.074	0.033	0.0074	0.0074	0.00845	0.016
Viability ratio of seeds	0.95 ± 0.145	0.049	0.0108	0.0108	0.01288	0.024
Carbohydrates content	50.95 ± 0.354	198.858	0.2030	0.2030	66.2183	66.421
Hydration coefficient ratio	2.18 ± 0.017	0.002	0.0006	0.0006	0.00042	0.001

Traits	PCV%	GCV%	ECV%	h^2%	EGA	GAM%
Germination ratio	16.30	11.90	4.396	53.3	0.14	17.91
Viability ratio of seeds	16.97	12.53	4.444	54.5	0.17	19.05
Carbohydrates content	16.00	15.97	0.024	99.7	16.74	32.85
Hydration coefficient ratio	1.47	0.94	0.530	40.9	0.03	1.24

Sd = Standard deviation, GMS = Genotypes mean squares, EMS = Error mean squares, σ^2e = Environmental variance, σ^2g = Genotypic variance, σ^2p = Phenotypic variance, PCV (%) = Phenotypic coefficient of variation, GCV (%) = Genotypic coefficient of variation, ECV (%) = Environmental coefficient of variation, h^2 (%) = heritability in broad sense, EGA = Expected genetic advance, GAM (%) = Genetic advance as a percent of mean at 5% selection intensity.

The difference between phenotypic and genotypic coefficients of variation was < 5% for all seed characteristics. The relatively narrow gap between phenotypic and genotypic variances indicated the lower contribution of environmental factors on the expression of seed characteristic traits. Based on Deshmukh *et al*. (1986) PCV% and GCV% are considered as moderate because they are ranged between 10 and 20 percent forgermination ratio, viability ratio, carbohydrates content. PCV and GCV estimates was lower than 10 percent for hydration coefficient that leading to be considered as low. The correspondence obtained between phenotypic and genotypic expression of seed characteristic traits due to decline influence of environmental factors. This suggested that selection depending on phenotypic expression of genotypes is rewarding to improve seed characteristic traits. On the other hand, low PCV and GCV calculated for hydration coefficient indicated that the improvement of this trait through selection is hardly possible because of the lower variability of the genotypes for this trait. This are in line with Yigezu (2021), who reported that the success of selection greatly based upon the magnitude of genetic variations in the population. The results obtained in this study agreed with Gizaw *et al*. (2019), who obtained high PCV and GCV values in chickpea forthe number of pods developed per plant, grain yieldand number of seeds developed per plant.

In contrast, Ejara *et al*. (2020) found low GCV and PCV for the number days needed to maturity in chickpea. In addition, Tsehaye *et al*. (2020) found low PCV and GCV for seed yielding per pod and the number of primary branches formed on chickpea. Although, Gizaw *et al*. (2019) found moderate PCV and GCV values for plant height, seed weight, days needed to flowering and the number of secondary branches developed per plantin chickpea. In general, PCV was greater than GCV for all seed characteristic traits. The high values of PCV than that of GCV reflected the effect of environmental factors on the expression of seed characteristic traits, especially for germination and viability ratio. Arshad *et al*. (2004) reported that heritability alone is not a very useful value but together coupled with genetic advance, it is valuable. Heritability of seed characteristic traits was ranged between 40.9% for hydration coefficient to 99.7% for carbohydrate contents. Based on Kassa *et al*. (2021), heritability values were high (> 60%) for carbohydrate content and moderate (30-60%) for germination ratio, viability ratio and hydration coefficient.Based on Kassa *et al*. (2021), who decided that genetic advance as a percentage of mean was categorized as high (> 20%) for carbohydrates content (32.85%) and moderate (10-20%) for germination ratio (17.90%) and viability ratio of seeds (19.05%). The results reflected that high heritability value (> 60 %) was coupled with high GAM (> 20%) for carbohydrates concentration. In addition, moderate heritability values (30-60%) were coupled with moderate genetic advance (10-20%) for with high genetic advance which indicated a major genetic effect. The results obtained herein are in harmony with Zali *et al*. (2011), who found high value of heritability coupled with low genetic advance for the number of days required to 50% maturity and the number of days needed to 50% flowering in chickpea which reflecting the influence of dominant and epistatic gene effects on these traits. The same authors found high heritability coupled with high genetic advance for the number of secondary branchesand number of seeds developed per plant, indicated the additive gene effects are important in improving these traits. Therefore, crop improvement was possible via simple selection of the traits expressed high heritability coupled with high genetic advance, as well as, additive gene effects (Noor *et al*. 2003). Hence, heritability estimate indicated the transmissibility of traits controlling genes from generation to another. Mehla *et al*. (2001) stated that swelling capacity, seed volume and hydration coefficient were important traits in chickpea. Similar trend was also observed by Singh (2016), who found that the values of phenotypic coefficient of variation were high for hydration coefficient, density of seed, swelling capacity, seed yield, swelling index and volume of seed in chickpea.The same authors found very low magnitude of variations between PCV and GCV for seed volume, cooking time, protein content, seed density and swelling index which indicates a slight involvement of environmental effects on the gene expression of these traits.

Genetic parameters of yield related traits: Regarding to Table 11, the values of genotypic coefficient of variation (GCV) were lower than the corresponding values of phenotypic coefficient of variations (PCV) in all yield related traits. The higher values of GCV (> 20%) were recorded for the number of pods developed per plant, weight of dry pods per plant and seed yield per plant. Nevertheless, the smallest GCV (6.71%) was recorded for 100-seed weight. Whereas, the classification order obtained in GCV is the same for PCV values. The higher PCV estimates (> 20%) wereobtained for the weight of dry pods per plant, the number of pods developed per plant and seed yield per plant. The smallest value of PCV was observed for 100-seed weight. The same trend was also achieved for the GAM. The values of phenotypic and genotypic variances showed low differences between them for all the traits studied. The relatively narrow gap between these values indicated the smaller contribution of environmental factors on the performance of these traits. Based on Deshmukh *et al*. (1986)**,** GCV and PCV higher than 20% are classified as high.Meanwhile, medium values were ranged from 10-20% and the values less than 10% were classified as low. The results obtained in this study are in harmony with Itana *et al*. (2024), who found that PCV and GCV in chickpea were high for seed yielding (g) per plant, and number of pods developed per plant, in addition

Table 11. Estimates of genetic variability parameters of different yield related traits in M_2 generation of kabuli chickpea affected by gamma rays.

Traits	Mean ± Sd	MSG	MSE	σ^2e	σ^2g	σ^2p
Number of pods developed per plant	159 ± 3.21	25193.77	16.16	16.16	8392.54	8408.69
Weight of dry pods per plant (g)	57 ± 3.83	1706.30	22.76	22.76	561.18	583.93
Seed yield per plant (g)	171 ± 8.41	10868.30	111.06	111.06	3585.74	3696.80
Hundred seed weight (g)	21 ± 0.16	5.89	0.04	0.04	1.95	1.99

Table 11. Continued

Traits	PCV%	GCV%	ECV%	h^2%	EGA	GAM%
Number of pods developed per plant	57.67	57.62	0.054	99.8	188.52	118.50
Weight of dry pods per plant (g)	42.44	41.60	0.835	96.1	47.84	84.02
Seed yield per plant (g)	35.60	35.06	0.539	96.9	121.37	71.05
Hundred seed weight (g)	6.77	6.71	0.064	98.1	2.85	13.69

Sd = Standard deviation, MSG = Genotypes mean squares, MSE = Error mean squares, σ^2e = Environmental variance, σ^2g = Genotypic variance, σ^2p = Phenotypic variance,PCV (%) = Phenotypic coefficient of variation, GCV (%) = Genotypic coefficient of variation, ECV (%) = Environmental coefficient of variation, h^2 (%) = heritability in broad sense, EGA = Expected genetic advance, GAM (%) = Genetic advance as a percent of mean at 5% selection intensity.

to moderate values for hundred seed weight, indicated low influence of environmental factors on the gene expression of these traits, which suggested that selection depending on the phenotypic expression of genotypes is rewarding for improvement of these traits. In this respect, Nwangburuka *et al*. (2012) reported that the high genotypic and phenotypic coefficients of variation are indicated the lower influence of environmental conditions on the gene performance of these traits and higher chance of selection on their improvements. The results obtained by Itana *et al*. (2024) appeared moderate GCV and PCV estimates for plant height,the number of seeds developed per pod and number of primary branches developed on chickpea, while fewer values of GCV and PCV were recorded for maturity and days to flowering. In this case, the improvement of these traits is hardly through selection due to fewer variations in their genotypes. In this criteria, Yigezu (2021) decided that the magnitude of genetic variations in the population determine the success of any selection program. Based on Kassa *et al*. (2021) heritability values are high (> 60%), low (< 30%) and moderate (30-60%). The results obtained herein agreed with Kumar *et al*. (2022), who found high values of heritability for the number of pods developed per plant, number of primary branches developed per plant, seed yielding per plant and thousand seed weight in chickpea. Therefore, the selection based on the phenotypic expression of individual genotypes that had high broad sense heritability indicated that selectionmight be easily because of relatively small contribution of the environment on the phenotype.

Based on Kassa *et al*. (2021) genetic advance as a percentage of mean (GAM) was classified as low (< 10%), high (> 20%)and moderate (10-20%). Higher estimates of GAM (> 30%) were recorded for the number of pods developed per plant, weight of dry pods per plant and seed yield per plant. The smallest value (13.69%) of GAM was obtained in 100-seed weight. The highest value of heritability (99.8%) was recorded for the number of pods developed per plant followed by 100-seed weight (98.1%).High heritability values coupled with high genetic advance expressed as a percentage of mean were recorded for the number of pods developed per plant, weight of dry pods per plant and seed yield per plant. Whereas, high heritability estimate (98.1%) coupled with moderate GAM (13.69%) was achieved for 100-seed weight. These results agreed with Amri-Tiliouine *et al*. (2018), who obtained the highest value of heritability for days to maturity. The same authors recorded higher PCV (> 70%) for seed yielding, number of seeds developed per plant and number of pods developed per plant of chickpea corresponding with the higher estimates (> 50%) of GCV associated with moderate heritability (46-47%) and higher genetic advance (GAM) more than 70%. The increased genetic variations obtained in this study for yield related traits provides great possibility for further selection in chickpea. Thus, genetic variability in yield-related traits is a pre-requisite to the selection of high yielding genotype in chickpea. The success of this trend relies on dosage of gamma irradiation that induced sufficient variation in yield related traits to facilitate the selection of superior genotypes with maintaining fertility. Phenotypic analysis of yield-related traits provides an important data on yield expressions and their heritability.

The results are in line with Amri-Tiliouine *et al*. (2018), who found moderate genetic advance expressed as a percentage of mean in chickpea mutant population coupled with high heritability for seed yielding per plant and number of pods developed per plant.This indicated that these traits arecontrolled by additive gene interaction. Meanwhile, high genetic advance (GAM) was also found by Vaghela *et al*. (2009) in chickpea for seed yielding per plant and number of pods developed per plant. In contrast, Barshile *et al*. (2009) found less heritability coupled with less genetic advance for seed yielding per plant in chickpea subjected to sodium azide, ethylmethane sulphonate (EMS) and gamma irradiation.

Polymorphism profile of PCR products using SCoT primers: Molecular markersas PCR-based dominant markers ISSR and SCoT, are valuable tools for characterizing genetic diversity in plant populations. These markers are highly polymorphic across various varieties, require minimal template DNA information and can be analyzed without the use of radioactivity (Al-Yasi and Al-Qthanin, 2024). In this study, SCoT markers were employed to compare the genetic makeup of two chickpea varieties. All primers induced consistent PCR amplification with distinct banding patterns for each genotype, generating informative and easily interpretable profiles. A total of 13 SCoT primers were used in this study to assess genetic similarities and relationships between the two chickpea genotypes (Figure 1). With an average of 8 bands/primer, 104 bands were amplified by 13 SCoT primers (Table 12). The lower number of bands (3) was generated by SCoT-77, SCoT-02 and SCoT-C. The highest number of bands (15) was obtained by SCoT-31. Among the bands generated by SCoT primers, 47 bands were monomorphic, with an average of 3.6 monomorphic bands per each primer. In addition, the total number of polymorphic bands was reached to 57, with an average of 4.4 polymorphic bands per each primer. The lower number of polymorphic bands (1) was generated by the SCoT-C primer, while the highest number (11) was obtained by SCoT-31. The percentage of polymorphism was ranged between 33.33% (SCoT-33 and C) to 88.89% (SCoT-36), highlighting the variability that reflected genetic diversity obtained by these molecular markers among the two chickpea genotypes including the resistance (Giza 195) and susceptible (Giza 531) genotypes to broomrape. These results indicated that some molecular markers are more informative for detecting genetic differences than others. The high polymorphism observed in certain primers, such as SCoT-36 (88.89%), suggests that this marker may target genomic regions associated with biotic stress-responsive genes.These findings align with the previous studies that used SCoT molecular markers to evaluate genetic relationships among the genotypes. For example, Singh *et al*. (2020) analyzed genetic variations in 13 chickpea genotypes, including both wild and cultivated types, using ISSR markers. Their study revealed high polymorphism, with the UBC-879 primer generating the most informative banding patterns. A total of 150 bands were amplified, averaging 21.4 bands per primer and 1.64 bands per genotype.

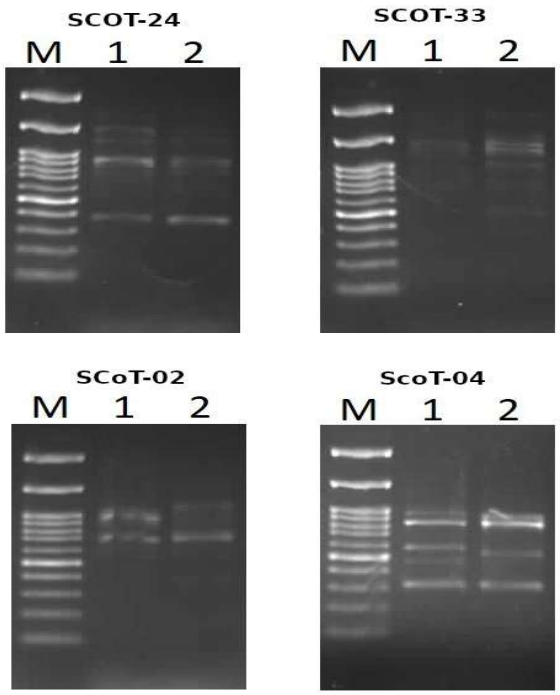

Figure 1. Amplification profile obtained from SCoT primers detected in two Cicer accessions referred as 1 (Giza 195-resistant to broomrape) and 2 (Giza 531-susceptible to broomrape)

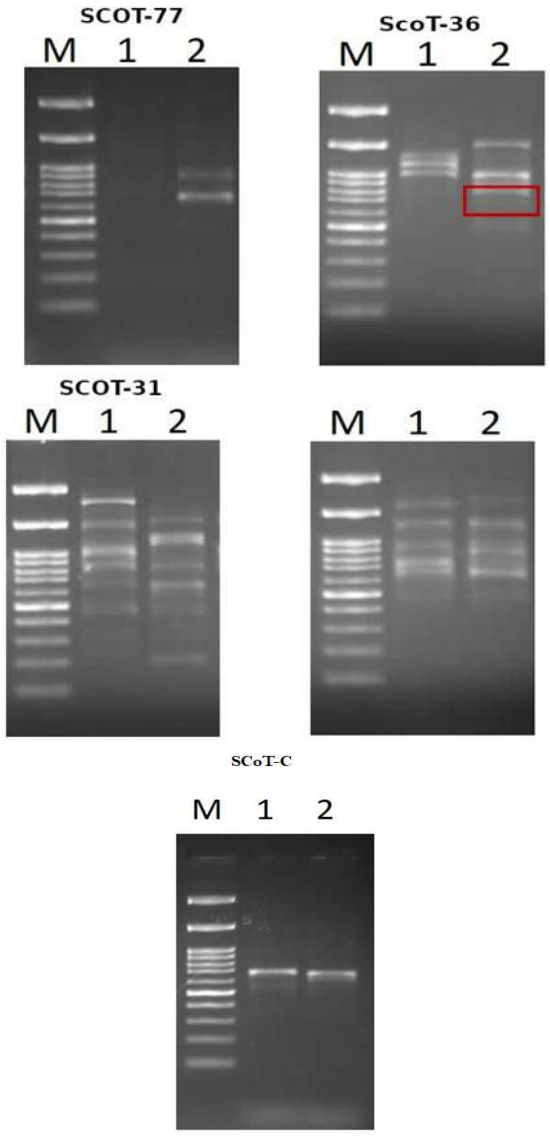

Table 12. Details of amplified bands as revealed by SCoT markers

SCoT primers	T	M	P	% P
SCoT-24	7	4	3	42.86
SCoT-33	6	4	2	33.33
SCoT-34	11	4	7	63.64
SCoT-52	6	2	4	66.67
SCoT-77	3	1	2	66.67
SCoT-02	3	1	2	66.67
SCoT-04	7	4	3	42.86
SCoT-11	11	7	4	36.36
SCoT-12	13	7	6	46.15
SCoT-31	15	4	11	73.33
SCoT-36	9	1	8	88.89
SCoT-A	10	6	4	40
SCoT-C	3	2	1	33.33
Total	104	47	57	-
Average	8	3.6	4.4	54

T: total number of amplified bands, M: monomorphic bands, P: polymorphic bands.

Genetic similarity analysis between resistant and susceptible genotypes to broomrape: Genetic similarity between the two chickpea varieties was measured using the dice similarity index, as illustrated in the similarity dendrogram (Figure 2). The clustering pattern reveals a moderate degree of genetic relatedness, with similarity values ranging from 0.60 to 0.65. This suggests that while the resistant and susceptible genotypes to broomrape share a common genetic background, likely due to their breeding origin, since they also exhibit notable genetic differences. The presence of genetic polymorphisms in the SCoT marker profiles indicates the existence of unique genomic regions that may play a role in susceptible broomrape. These variations could be linked to key stress-responsive genes, including those encoding transcription factors, antioxidant enzymes, and signal transduction components.

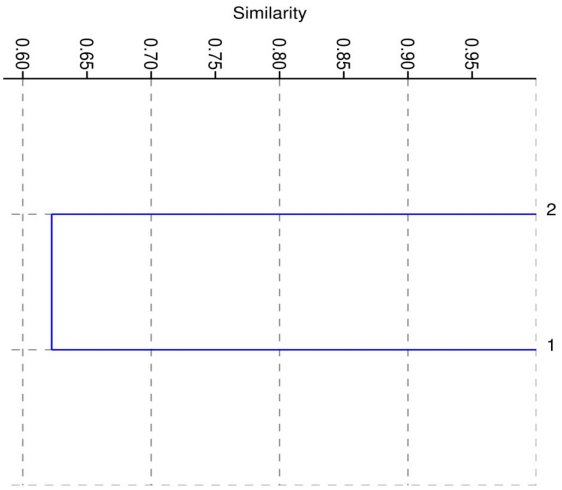

Figure 2. Dendrogram representing the genetic similarity between two chickpea varieties based on SCoT marker data using the Dice similarity coefficient. The X-axis denotes the genetic similarity coefficient, while the U-shaped line indicates the clustering of the two genotypes, with similarity values ranging from 0.60 to 0.65

Such genetic differences may contribute to the distinct physiological and adaptive responses observed in the resistant and susceptible genotypes to broomrape. The results obtained herein promising that Giza 195 was considered as a resistant parental line to broomrape to be used in future chickpea breeding programs. The results obtained in this study agreed with Alotaibi and Abd-Elgawad (2022), who investigated genetic diversity in 29 wild plant species from 15 regions in Al Jubail, Saudi Arabia, using ISSR and SCoT markers. Thestudy of Alotaibi and Abd-Elgawad (2022) generated the amplified bands with ISSR molecular markers reached 142 bands (87% polymorphism) and 163 amplified bands with SCoT molecular markers (84% polymorphism).

Table 13. Comparison between Giza 195 as a resistant genotype and Giza 531 as a susceptible genotype to broomrape based on the number of unique bands

Molecular weight (base pairs)	Primer code	Sample 1	Sample 2	Total number of monomorphic bands	Unique bands		Total number of unique bands
					Sample 1	Sample 2	
3000	SCoT-24	1	0	4	1	2	3
1500		1	1				
1000		1	1				
900		1	1				
800		0	1				
700		1	1				
600		0	1				
3000	SCoT-33	0	1	4	0	2	2
1500		1	1				
1000		1	1				
900		1	1				
800		1	1				
700		0	1				
3000	SCoT-34	1	0	4	5	2	7
1500		1	1				
1000		0	1				
900		1	1				
800		0	1				
700		1	0				
600		1	1				
500		1	1				
400		1	0				
300		1	0				
200		1	0				
3000	SCoT-52	0	1	2	0	4	4
1500		1	1				
1000		0	1				
900		1	1				
800		0	1				
700		0	1				
3000	SCoT-77	0	1	1	0	2	2
1500		1	1				
1000		0	1				
3000	SCoT-02	0	1	1	1	1	2
1500		1	0				
1000		1	1				

The average number of polymorphic bands per ISSR and SCoT primer was reached to 12.4 and 13.7, respectively. The highest genetic similarity values were observed between Zygophyllum qatarense-22 and Juncus rigidus-23 (ISSR: 0.97), as well as, between Zygophyllum qatarense-28 and Zygophyllum qatarense-29 (SCoT: 0.90). Their findings highlighted the effectiveness of ISSR and SCoT markers in assessing genetic variations, supporting conservation and plant breeding strategies. Similarly, the present study using SCoT markers revealed moderate genetic variation between resistant and susceptible chickpea genotypes to broomrape, further supporting the utility of SCoT molecular markers in genetic variations studies and their role in identifying biotic stress-tolerant of genetic resources to broomrapes.

Analysis of polymorphism: The genetic variations based on DNAmolecular markers provide reliable and very powerful tools for genetic variation analysis in chickpea genotypes. Recently, a novel molecular marker methodology named start codon targeted (SCoT) polymorphism was developed by Collard and Mackill (2009). These molecular markers were used herein based on the short conserved region flanking the start codon ATG in chickpea genes. SCoT markers as seen in Table 13 are very reproducible (Amirmoradi *et al*. 2012). A set of 13 SCoT primers were used to genetic variation analysis in two chickpea genotypes as Giza 195 which resistance to broomrape and Giza 531 that suffer from significant levels of infection with broomrape. All SCoT molecular markers produced sharp and distinguished unique bands pattern between two chickpea genotypes. Each of the primers used produced distinct banding patterns. Among the two chickpea accessions, 13 SCoT primers yielded 56 bands, out of them 22 distinct banding patterns were presented in Giza 195 as the resistant genotype and 34 bands were presented in Giza 531 as a genotype suffer from significant levels of broomrape infection. The number of unique bands in Giza 195 was varied from one (SCoT-24, SCoT-02) to 5 (SCoT-31 and SCoT-34). SCoT-31, SCoT-36and SCOT-02 produced unique band in the resistant genotype (Giza 195) having a molecular weight of 1500 bp. SCoT-12 and SCoT-36 produced unique band in the resistance genotype (Giza 195) to broomrape having a molecular weight of 1000 bp. SCoT-04 generated a unique band in Giza 195 genotype having a molecular weight of 900 bp. SCoT-12 and SCoT-31 produced unique band in the resistant genotype (Giza 195) having a molecular weight of 800 bp. SCoT-34 produced unique band in Giza 195 having a molecular weight of 700 bp. SCoT-31 and SCoT-A produced unique band in Giza 195 having a molecular weight of 600 bp. SCoT-34, SCoT-11 and SCoT-A produced unique band in Giza 195 having a molecular weight of 400 bp.SCoT-34 produced two unique bands in Giza 195 having a molecular weights of 300 and 200 bp.

The higher number of unique bands obtained in Giza 195 was achieved by SCoT-31 and SCoT-34 (5 bands), followed by SCoT-04, SCoT-11, SCoT-12, SCoT-36 and SCoT-A (2 bands). Genetic variation analysis using molecular markers information reflect the history of chickpea levels of infection with broomrape. SCoT-31 is the only marker appeared DNA band with a molecular weight of 100 bp in Giza 195. The SCoT-PCR analysis showed high genetic diversity in Giza 195 as a resistant genotype to broomrape. The rateof diversity for broomrape resistance based on SCoT-PCR molecular markers was differed from primer to another. The relationship between molecular markers and broomrape resistance as shown in Giza 195 could be significant if the markers were linked to selected loci (Tale and Abhari 2016). This are in line with the findings of Ahmad and Talebi (2017), who reported that morphological similarities in different plants are not necessarily genetically because the varied gene pools leading to similar phenotypes.

Molecular weight (base pairs)	Primer code	Sample 1	Sample 2	Total number of monomorphic bands	Unique bands		Total number of unique bands
					Sample 1	Sample 2	
3000	SCoT-04	1	0	4	2	1	3
1500		1	1				
1000		1	1				
900		1	0				
800		0	1				
700		1	1				
600		1	1				
3000	SCoT-11	1	0	7	2	2	4
1500		1	1				
1000		1	1				
900		1	1				
800		1	1				
700		1	1				
600		1	1				
500		0	1				
400		1	0				
300		1	1				
200		0	1				
3000	SCoT-12	1	1	7	2	3	5
1500		1	1				
1000		1	0				
900		1	1				
800		1	0				
700		1	1				
600		0	1				
500		1	1				
400		1	1				
300		0	1				
200		1	1				
100		0	1				
3000	SCoT-31	1	0	4	5	3	8
1500		1	0				
1000		1	1				
900		0	1				
800		1	0				
700		0	1				
600		1	0				
500		1	1				
400		1	1				
300		0	1				
200		1	1				
100		1	0				
3000	SCoT-36	0	1	1	2	6	8
1500		1	0				
1000		1	0				
900		1	1				
800		0	1				
700		0	1				
600		0	1				
500		0	1				
400		0	1				
3000	SCoT-A	0	1	6	2	2	4
1500		1	1				
1000		1	1				
900		1	1				
800		1	1				
700		0	1				
600		1	0				
500		1	1				
400		1	0				
300		1	1				
3000	SCoT-C	1	1	2	0	1	1
1500		0	1				
1000		1	1				
Total number of amplified bands				47	22	31	53

Sample 1: Giza-195 resistant to broomrape.
Sample 2: Giza-531 susceptible to broomrape.

Whereas, the number of unique bands in Giza 531 as a susceptible genotype to broomrape was ranged between one (SCoT-02, SCoT-04, SCoT-C) to six (SCoT-36). This indicating that SCoT molecular markers has more potential tool for genetic diversity analysis to broomrape infection levels in chickpea. SCoT-24, SCoT-34, SCoT-11, SCoT-4 and SCoT-31 produced unique band having a molecular weight of 3000 bp in the resistant genotype (Giza 195) to broomrape.

Broomrape (*Orobanche crenata* Forsk) is a root parasitic weed widely located in West Asia and Mediterranean region. Chickpea is an important legume grown in this area as a winter crop which is known to be a host of broomrape. Some genotypes as Giza 195 referred as sample 1 does not suffer from significant values of infection, whereas the other genotype Giza 531 referred as sample 2 was suffer from significant levels of infection. Little information was

known about genetic variation of chickpea resistance to broomrape.Different levels of broomrape infectionin chickpea genotypes were reported by Linke (1992). Thus, broomrape can be a problem in some years and in some chickpea genotypes as Giza 531 that was significantly infected with broomrape. High resistance to broomrape seems to be already available in commercial cultivars and breeding material as Giza 195. Delayed establishment of chickpea varieties infected with broomrape could also be regarded to true genetic resistance. The increased release of phytoalexins (maackiain and medicarpin) has been registered in resistant chickpea genotypes (Wegmann *et al*. 1991). These phytoalexins may play a significant role in the early blocking of broomrape infection in chickpea.The molecular genetic variation detected in this study can be a useful tool for future breeding programs in chickpea. Therefore, the pattern of variations obtained between Giza 195 (resistance to broomrape) and Giza 531 (susceptible to broomrape) based on SCoT-PCR molecular markers will be a useful technique for breeders to choosing genotypes with appropriate resistance to broomrape. The amount of polymorphism and efficiency of SCoT molecular markers demonstrated high efficiency for genetic diversity analysis of chickpea whichappeared resistance to broomrape.

Table 15. Total number of amplified unique bands in the susceptible genotype to broomrape Giza 531 as revealed by SCoT markers

Molecular weight of bands (bp)	SCo T-24	SCo T-33	SCo T-34	SCo T-52	SCo T-77	SCo T-02	SCo T-04	SCo T-11	SCo T-12	SCo T-31	SCo T-36	SCo T-A	SCo T-C	Total
3000	0	1	0	1	1	1	0	0	0	0	1	1	0	6
1500	0	0	0	0	0	0	0	0	0	0	0	0	1	1
1000	0	0	1	1	1	0	0	0	0	0	0	0	0	3
900	0	0	0	0	0	0	0	0	0	1	0	0	0	1
800	1	0	1	1	0	0	1	0	0	0	1	0	0	5
700	0	1	0	1	0	0	0	0	0	1	1	1	0	5
600	1	0	0	0	0	0	0	0	1	0	1	0	0	3
500	0	0	0	0	0	0	0	1	0	0	1	0	0	2
400	0	0	0	0	0	0	0	0	0	0	1	0	0	1
300	0	0	0	0	0	0	0	0	1	1	0	0	0	2
200	0	0	0	0	0	0	0	1	0	0	0	0	0	1
100	0	0	0	0	0	0	0	0	1	0	0	0	0	1
Total number of bands	2	2	2	4	2	1	1	2	3	3	6	2	1	31

produced two amplified bands in the resistant genotype with a molecular weight of 600 and 400 bp. Two primers (SCoT-34 and SCoT-31) out of 13 generated the higher number of amplified bands reached five in the resistant genotype to broomrape (Giza 195). This genetic analysis provide useful information to address the chickpea genotype resistance to broomrape and also confirm the usefulness of diverse genotypes in chickpea breeding programs. The narrow genetic base and lack of genes related to biotic and abiotic stresses in chickpea leads to slow improvement in this crop. Introducing the genes expressed on resistance to biotic and abioticstresses from the population gene pool can leading to increase the yield of chickpea (Choudhary *et al*. 2012). In this study, 13 SCoT primers were used to analysis the genetic diversity among a set of two diverse chickpea genotypes one was resistant and the other was susceptible to broomrape to know if these molecular markers can be effectively used to distinguish between both genotypes to be used in breeding programme. Different molecular marker sets suggested the presence of considerable polymorphism and showed high level of genetic variations among the two genotypes used in this study. This study reflected the importance of SCoT molecular markers in detecting genetic variations among two chickpea genotypes to carry out the resistant genotype to broomrape successfullyas a cheap, fast and informative marker. Therefore, SCoT molecular markers that targets the candidate chickpea genes related to broomrape resistance in comparison with susceptible genotype were used for genetic variations analysis in chickpea. The genes related to broomrape resistance increased the production of phytoalexins which play an important role in the early blocking of broomrape infection in chickpea (Wegmann *et al*. 1991). Genetic differences between Giza 195 and Giza 531 with regard to molecular characteristics are represent differences at the DNA level. Many of genetic strategies were used as an integrated techniques to control broomrapes but all without unequivocal success (Rubiales *et al*. 2009). Germination of broomrape seedsbased on stimulatory signals mainly strigolactones exudated from the roots of the host susceptible plant (Yoneyama *et al*. 2008). The results obtained in this study are in harmony with Brahmi *et al*. (2016), who screening gamma irradiated mutants of susceptible chickpea under artificial infestation in pots experiment, they found that non-irradiated plants were highly parasitized with almost eight broomrape attachments per gram of root dry weight. The same authors found six mutants appeared a significantly lower number of broomrape attachments and five resistant mutants showing fewer number than two broomrape attachments per gram dry weight if compared with the non-irradiated plants. Mutants obtained in the study of Brahmi *et al*. (2016) were classified into three categories, the first category having a number of broomrape attachments with the host equivalent to control plants, the second category showing significantly lower number of broomrape attachment than the control plants, the third composed of most resistant mutants. The same authors found from biochemical analysis that resistant mutant displayed high increases in total phenolic contents and polyphenol oxidase (PPO) at 49 days-plant old in comparison with uninfested roots. Brahmi *et al*. (2016) found that broomrape infection induced a strong overexpression of the *APX* gene (30-fold) in the resistant

The present study reflected the importance of molecular markers in detecting genetic variations among the resistance and susceptible chickpea genotypes to broomrape. 13 SCoT molecular markers used in this study produced clear and reproducible band profiles in chickpea resistance to broomrape which amplified a total of 22 unique bands in the resistant genotype (Giza 195). The number of amplified bands produced by SCoT-34 in the resistance genotype reached to five bands with a molecular weights of 3000, 700, 400, 300 and 200 bp. SCoT-02 generated one amplified molecular band in the resistance genotype have a molecular weight of 1500 bp. The resistance genotype to broomrape appeared two amplified bands with a molecular weight of 3000 and 900 bp. Molecular analysis by SCoT-11 produced two amplified bands in the resistant genotype to broomrape with a molecular weight of 3000 and 400 bp. Therefore, SCoT primers were used in this study to fingerprint two varieties of chickpea one of them was resistant and the other was susceptible to broomrape. This are in line with Hajibarat *et al*. (2015), who found that nine SCoT primers were generated a total of 145 bands from 48 Iranian chickpea varieties, out of them 133 bands were polymorphic. The results obtained herein are also in line with Amirmoradi *et al*. (2012), who found that SCoT primers produce DNA fingerprint in a set of *Cicer* species but the bands were clear,sharpand 100% polymorphic than those produced by RAPD. The genetic analysis used in this study was most importance to show which type of molecular markers represent variation in the entire genomes of chickpea and should be used to derive reliable estimates of diversity related to broomrape resistance. SCoT-12 was successfully amplified resistant genotype which produced two amplified bands with a molecular weight of 1000 and 800 bp. Molecular analysis used SCoT-31 produced the higher number of amplified bands in the resistant genotype to broomrape reached to five bands with a molecular weight of 3000, 1500, 800, 600, and 100 pb. The number of amplified bands produced by SCoT-36 in the resistant genotype reached to two bands with a molecular weight of 1500 and 1000 bp. Meanwhile, SCoT-A

chickpea mutant, whereas four fold increase was shown in the susceptible mutants and control when infested. The same authors found no molecular marker was clearly distinguished the susceptible genotype from the resistant at seven day of infested conditions.The same authors found no complete resistance was generated by gamma irradiation. Therefore, selection of genotype resistance to broomrape under field conditions was highly influenced by genotype by environmental interactions, resulting in the resistance quantitative trait loci (QTL) which is unstable among environments. Screening resistance to broomrape under field conditions have disadvantages of insufficient control of environmental factors and of homogeneity of seed inoculum in the soil (Brahmi *et al*. 2016). This underlines the importance of *in vitro* techniques to study legume-broomrape interaction. Interestingly, Brahmi *et al*. (2016) found that the most resistant mutants to broomrape shared low stimulatory activity of root exudates towardsseed germination of broomrape which are greatly limiting the infection level as expressed by the number of broomrape attachments. The same authors suggested that mutations resistant to broomrape having change in cell-wall structures, oxidant status and production of toxic compounds in roots. The local accumulation of soluble phenolic compounds in roots prevents broomrape development during early stage of infection as in fabe bean challenged with broomrape, *Orobanche crenata*(Pérez-de-Luque *et al*. 2007). Wegmann *et al*. (1991) suggested that some soluble phenolics as maackiain, phytoalexins and medicarpin were involved in the resistance to *Orobanche crenata* in *Medicago* and probably in chickpea.Brahmi *et al*. (2016) found that enhanced increase in polyphenol oxidase (PPO) and gualacol peroxidase (POX) activity in resistant mutants to broomrapes could contribute to changes in cell-wall structures in response to broomrape infection.

Tyrosine residues of cell-wall structural protein and pox oxidases phenolic domains of feruloylated polysaccharides to be producing crosslinking protein and cell wall reinforcement. This mechanism forming a physical barrier to pathogens in the cell wall. The general toxicity of PPO producing quinone and quinones redox cycling leading to indirect generation of reactive oxygen species (ROS) as signal antipathogen agents (Jiang and Miles 1993). The overexpression of PPO and POX geneswas found in broomrape infected pea (Pérez-de-Luque *et al*. 2005) and tomato (Al-Wakeel *et al*. 2013). Brahmi *et al*. (2016) suggested that the overexpression of notably ascorbate-peroxidase and glutathione expressing genes generated higher antioxidant capacity in resistant chickpea mutants if challenged with broomrape. Consequently, the increase in reactive oxygen species (ROS) production may overcome the capacity of ROS detoxification in broomrape generating in parasite necrosis. Therefore, this study remain to be addressed for better characterization of resistant chickpea genotype using the molecular markers SCoT-31 and SCoT-34 which generated total ten unique bands in the resistant chickpea genotype.

These bands may be related to morphological, physiological and yield related traits in the resistant chickpea genotype. Both primers are generated the same DNA band having a molecular weight 3000 bp, but the other eight bands were differed in their molecular weight between both primers. Out of 13 molecular markers used in this study, five primers including SCoT-24, SCoT-34, SCoT-4, SCoT-11 and SCoT-31 are generated the same DNA band having a molecular weight of 3000 bp. The differences between resistant and susceptible genotypes to broomrape may be due to global transcriptomic changes between both genotypes that trigger resistance to broomrape. These changes disturb the parasite life cycle at varied stages through generating attachment necrosis during early infection, limiting seed germination and disturbing subterranean development of surviving broomrapes. Faba bean was suffer from yield losses reached 50 to 80% as medium to high levels of soil infestation by broomrape (Kharrat and Halile 1994). Programs to control broomrape parasitism are limited because of its broad host range and long lasting the viability of their seeds in the fields under different environmental conditions (Rubiales *et al*. 2003). Broomrape are not usually controlled by persistent selective herbicides because these herbicides are not differentiate between the parasitic plants and crops (Rubiales and Fernández-Aparicio 2012). The main biocontrol components management are fungal pathogens, virulent insects and fungal toxins (Fernández-Aparicio *et al*. 2010). Mabrouk *et al*. (2016) found that chickpea inoculated with *Rhizobium* had decreased the number of broomrape, since the dry matter of broomrape per plant was reduced by 95% in chickpea inoculated with rhizobia.These findings are found before by Bouraoui *et al*. (2012), who observed that faba bean preinoculation with *Rhizobium*achieved significant decline in disease severity caused by broomrape.

Indeed, the mechanism of action reducing seed germination of parasite, blocking host tissue penetration and connection to the vascular system, as well as, reducing radical growth. Rhizobia inoculated chickpeas enhanced peroxidase activity and phenylalanine ammonialyase (PAL) activity if compared with non-inoculated chickpeas. Consequently, these enzymes could involved in the resistance of chickpea to broomrape which induced by *Rhizobium* during early and later stages of infection. These enzymes could avoidance the infection of chickpea with broomrape through preventing parasite penetration of chickpea roots or by reducing nutrient fluxes towards broomrape if connection was succeeds (Mabrouk *et al*. 2016). *Rhizobium* inoculation was found to exhibit high level of the defense enzymes as POX and PAL by the host. The high levels of the defense enzymes POX and PAL that may produced by the resistant genotype, Giza 195, were correlated with the decline in seed germination of broomrape and by growth of installed tubercles, as well as, prevention of parasite attachment.Then, the resistant plant to broomrape that produced these defense enzymes was primed for defense (Mabrouk *et al*. 2016). However, Egyptian broomrape (*Phelipanche aegyptiaca* Pers.) have became a major problem in chickpea production (Galili *et al*. 2018). The better broomrape control method including to be cultivated the resistant varieties as Giza 195. Broomrapes are weedy root parasites of dicotyledonous plants, caused severe losses in the yield of crops and their quality (Joel *et al*. 2007). The results obtained herein are in line with Galili *et al*. (2021), who generated mutants in chickpea by EMS mutagenesis that showed high resistance to both broomrapes, *Phelipanche aegyptiaca* and *Orobanche crenata*. The root exudates of these resistant mutants did not stimulate the seed germination of both broomrapes in Petri dishes. Galili *et al*. (2021)making DNA sequence analysis of *CCD7* gene in chickpea mutant referred as CCD7M14, which observed a stop-codon formed from a single nucleotide transition from G- to A- at the position 210. This transition mutant leading to the absence of strigolactones (SLs) in the root exudates of chickpea mutant caused the mutant plant was resistant to broomrape because of no seed germination was happen near the roots of resistant mutant.This mechanism of resistance has reported before in tomato (Bari *et al*. 2021), faba bean (Fernández-Aparicio *et al*. 2012) and pea (Pavan *et al*. 2016).

The results of Galili *et al*. (2021) indicated that one point mutation in the *CCD7* gene resulted in the formation of stop codon at the position 210. All identified *CCD7* gene have single copies as in chickpea, in contrast to two, four and six copies of *CCD8* characterized in maize, rice and sorghum, respectively (Vallabhaneni *et al*. 2010). The resistance mechanism obtained by Galili *et al*. (2021) was based on blockage of SL synthesis due to the point mutation that generating stop codon formation in the *CCD7* gene leading root exudates in the resistant mutant did not contain SLs. The resistant mutant obtained by Galili *et al*. (2021) displayed decline chlorophyll and carotenoid contents, increased branching, decline plant height and increased accumulation of anthocyainin in the leaves if compared with the wild type chickpea.Out of 53 unique bands obtained from the resistant (Giza 195) and susceptible (Giza 531) genotypes, 31 bands were obtained in Giza 531. This indicated that the total number of unique bands appeared in the susceptible genotype are higher than that obtained from the resistant genotype. One of unique bands obtained in susceptible genotype having a molecular weight of 500 bp may be involves in the generation of root-exuded strigolactones (SLs) which have been known to induce broomrape seed germination as the initial step of broomrape-plant recognition (Joel *et al*. 1995). Recently, SLs have been recognized as plant hormones affecting broomrape

development and growth (Xie *et al*. 2010). SLs deficient sorghum and rice mutants also revealed high degrees of resistance to *Striga* spp. (Jamil *et al*. 2011). In addition, resistance to parasitic weeds depend on low concentration of SL exudation exists in faba bean and pea genotypes (Fondevilla *et al*. 2010). The root exudates of resistant genotype to broomrape did not stimulate their seed germination. The results obtained herein indicated that the mechanism of resistance in Giza 195 was referred to its inability to secrete or synthesize SLs into the rhizosphere.

This agrees with Galili *et al*. (2021), who obtained chickpea mutant CCD7M14 resistant to broomrapethrough EMS mutagenesis. These mutant showed high resistance to *O. crenata* and *P. aegyptiaca*. The same authors conducted DNA sequence analysis of *CCD7* gene which appeared stop-codon formation because of single transition mutation transform G- to -A at position 210. This point mutation resulted in the absence of didehydro-robanchol, orobanchyl acetate and SLs in the root exudates of resistant mutant. This leading chickpea mutant resistant to the broomrape parasite due to inhibit seed germination near the roots of the resistant genotype. It had also been indicated that the point mutation in the *CCD7* gene formed stop codon leading to inability of the resistant genotype to synthesize or secrete SLs into the rhizosphere. Genetic resistance to broomrape as seen in Giza 195 consists in stopping parasite progress towards the conducting tissues of host roots by chemical and physical barriers leading to inducing broomrape necrosis. Indeed, mutagenesis is a well-known method used for increasing genetic diversity in crops as chickpea with restricted genetic variability (Parry *et al*. 2009). There are biochemical markers expressed in legume defense against broomrapes (Pérez-de-Luque *et al*. 2006). These markers may be related to the absence of unique bands in the resistant genotype (Giza 195). Themarkers were expressed in the resistant genotypeinclude total soluble phenolic compounds, polyphenol oxidase activities, phenylammonia lyase and gualacol peroxidase (Lozano-Baena *et al*. 2007). These biochemical markers leading to disturb the broomrape life cycle at different stages by disturbing subterranean development of surviving broomrapes, limiting seed germination and inducing attachment necrosis early during infection (Brahmi *et al*. 2016).

The overall size of amplified product appeared in Giza 195 that having a molecular weight of 3000 bp was appeared by five primers. This indicated that this band represent variation by these primers in the entire resistant genome to broomrape and should be used to derive reliable estimates of genetic variation. Therefore, SCoT technique was more informative than biochemical tools to study the genetic diversity in chickpea (Labdi *et al*. 1996). It is interesting to note that SCoT datasets showed high levels of unique bands in the resistant genotype (Giza 195) to broomrape. The absence of DNA marker having a molecular weight of 500 bp in the resistant genotype was known to target repeat or unique sequences in the resistant genotype to broomrape, which may be evolved in the course of natural or human selection to broomrape resistance. The most polymorphic unique band with a molecular weight of 1500 bp was appeared by three primers. In addition, three primers represented a unique band having a molecular weight of 800 bp. This range of genetic diversity appeared in the resistant genotype to broomrape is interesting in genetic studies, as each primer targets different regions of thegenome including resistant and susceptible genotypes. This study provide additional data for identification of *Cicer* species based on molecular characteristics. The results reflected that SCoT molecular markers can be used as a reliable tool for achieving the levels of DNA polymorphism in chickpea. The unique band with a molecular weight of 400 bp appeared in the resistant genotype to broomrape was achieved by three primers. Meanwhile, the unique band with a molecular weight of 1000 bp was achieved in Giza 195 by two primers. In addition, the band with a molecular weight of 600 bpwas achieved in Giza 195 by two primers out of 13 primers. Therefore, molecular genetic techniques enable us to leverage the information stored in the genotype more efficiency and rapidly. Thus, genotyping of *Cicer* collections will allow to identify the resistant genotype to broomrape (Díez *et al*. 2018). Out of 12 molecular bands generated in this study by 13 primers, 11 unique bands were obtained in the resistant genotype to broomrape and 12 were achieved in the susceptible genotype. These bands having the following molecular weights, 3000, 1500, 1000, 900, 800, 700, 600, 400, 300, 200 and 100 bp. The difference between the two genotypes of *Cicer* represent a differences in DNA sequences in each genotype, which tracked different band profiles. All molecular SCoT markers showed sharp and distinguished polymorphic bands pattern between both genotypes of chickpea. Therefore, SCoT molecular markers has more discernible potential for genetic diversity and genotype discrimination in chickpea lines.

As shown from the results tabularized in Tables 14 and 15that the band having a molecular weight of 500 bp was appeared in the susceptible genotype to broomrape Giza 531 by SCoT-11 and SCoT-36. The same band was absent in the resistant genotype to broomrape Giza 195. These results indicated that the relationship between molecular markers and resistance to broomrape was linked to absence of the band having a molecular weight of 500 bp in the resistant genotype. This is a diagnostic band related to susceptibility of chickpea genotypes to broomrape infection. Genetic diversity analysis using SCoT-11 and SCoT-36 provide DNA band with a molecular weight of 500 bp in the susceptible genotype Giza 531.Therefore, the genetic diversity pattern based on SCoT-PCR molecular markers will be a useful tool for choosing chickpea genotypes resistance to broomrape via the lack of DNA band with a molecular weight of 500 bp.Of 13 SCoT primers used in this study, two primers SCoT-11 and SCoT-36 produced clear and reproducible band with a molecular weight of 500 bp in the susceptible genotype. Although the polymorphism rate for these two primers was high in the susceptible genotype to broomrape. Their informative was the highest value as a dominant markers used for distinguish between resistance and susceptible genotypes to broomrape.Therefore, SCoT-11 and SCoT-36 can be used to fingerprint *Cicer* genotypes for the analysis of genetic resistance to broomrape through the lack of DNA band size of 500 bp in the resistant genotype and detected in the susceptible genotype.

This are in line with Aggarwal *et al*. (2015), who detected 26 ISSR primers yielded a total of 232 bands of which 213 were polymorphic bands (91.8%) among 125 cultivars of Indian chickpeas with an average of nine bands per primer.On the other hand, Hajibarat *et al*. (2015) found that nine SCoT primers amplified a total of 145 DNA bands among 48 Iranian *Cicer* accessions, out ofthem 133 were polymorphic. The results reflected that the resistance genotype to broomrape was closest to the lack of DNA band with a molecular weight of 500 bp. Therefore, the difference between two *Cicer* genotypes is due to the different DNA sequences presented in each genotype which producing band size of 500 bp in the susceptible genotype and lacking the same band in the resistant genotype to broomrape. This band was closest to the susceptible genotype. The results confirmed the significance of gene based molecular studies that can be used for detecting genetic variation between chickpea genotypes for resistance or susceptible to broomrape.The molecular band present in the susceptible genotype Giza 531 that having a molecular weight of 500 bp may be based on the ability of susceptible genotype to produce broomrape-plant recognition that including root-exudates strigolactones (SLs) which produce germination of broomrape seeds(Xie *et al*. 2010). Recently, SLs have been recognized as plant hormones affecting on broomrape development and growth (Xie *et al*. 2010). SLs are produced mainly in susceptible plant roots. The SLs biosynthesis pathway is derived from carotenoid pathway, in which beta-carotene was converted into carlactone by three catalytic enzymes, D-27 (trans-beta-carotene isomerase) and two carotenoid cleavage dioxygenases *CCD7* and *CCD8*. (Alder *et al*. 2012, Seto *et al*. 2014). The deletion in one of these genes leading to the inability of the mutant plants to produce SLs resulted in unstimulants of broomrape seed germination. This leading to develop resistant genotype to broomrape. Mutants or genotypes resistant to broomrape are characterized by their defective in SL biosynthesis through the lack of DNA molecular band with a molecular weight of 500 bp which achieved by two SCoT molecular markers as SCoT-11 and SCoT-36. These results agreed with Dor *et al*. (2011), who

obtained a tomato mutant harboring*CCD7*- gene deletion leading to broomrape resistance. However, low SLs exudation was associated to parasitic weeds resistance which exists in faba bean and pea (Fondevilla *et al*. 2010). In addition, SL-deficient mutants of rice and sorghum showing high degrees of resistance to *Striga* spp. (Jamil *et al*. 2011).

Concluding remarks: Gamma rays mutagenesis is an efficient and common tool to generate new desirable genetic variations in chickpea. This study highlights on the employing phenotypic and genotypic screening approaches in gamma irradiated chickpea population. The goal of this screening is to identify the doses expressing high seed yielding per plant, as well as, molecular characterization between susceptible and resistant genotypes to broomrape infection. It is evident that chlorophyll mutations do not have any economical importance due to their lethal effect but it could be useful in identifying mutagen dose that would increase the number of economicaly useful mutants, as well as, the genetic variability in segregating generations.Gamma irradiated plants showed high heterogeneity in chlorophyll a and high homogeneity in chlorophyll b. The dose of 100 radius is the only dose induced a greater number of pods developed per plant, as well as, higher seed yields per plant. The highest heritability estimate was recorded for pods number developed per plant followed by 100-seed weight.Higher values of GCV were associated with the higher values of PCV (> 20%) for plant dry weight, the number of primary branches developed per plant and height of first branch developed above the ground level. The results demonstrated high genetic diversity based on molecular levels exists between the resistance and susceptible genotypes to broomrape. The dendrogram revealing genetic diversity pattern among two chickpea genotypes. SCoT molecular markers generated the functional region of the genome. This investigation provide additional data for characterization of chickpea genotype resistant to broomrape. Out of 13 molecular markers, nine were generated a total number of unique bands reached to 22 in the resistant genotype to broomrape. Some of these bands having the same molecular weight. The diagnostic band having a molecular weight of 500 bp was present in the susceptible genotype (Giza 531) and absent in the resistant genotype (Giza 195) to broomrape. This band can be used for detecting the genotype susceptible to broomrape. The diagnostic band in susceptible genotype may be correlated with strigolactones exudated from the roots of susceptible plants. A huge effort is required to generate chickpea genotypes with satisfactory levels of resistance to broomrape.

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Conflict of interest: The authors declare that this study was conducted in the absence of any commercial or financial relationships that could be construed as a potential with no conflict of interest.

Authors contributions

MIK: Conceptualization, formal analysis, investigation, data availability, data curation, methodology, visualization, planned the experiments, writing original drats. KAZ: revised the manuscript, technical assistance. FFA: methodology, data collection. AHA: technical assistance, revised the manuscript.

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