Genetical studies on some genotypes in chickpea (*Cicer arietinum* L.) in Egypt

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ABSTRACT:

The present investigation was conducted during the two seasons 2017-2018 and 2018-2019. Two field experiments were conducted with Egyptian soil cooperation between Genetic section, Agric-Botany Department, Faculty of Agriculture, Cairo, Al-Azhar University and Genetic Resources Research Department Bahteem, Field Crop Research Institute (FCRI), Agricultural Research Center (ARC), Bahteem Research Station, Egypt, on four chickpea genotypes (*Cicer arietinum*). The work was done to study genetic improvement of four chickpea genotypes (*Cicer arietinum*) using three different doses of gamma rays (200, 300 and 400Gy) and Comparison of results through the morphological and molecular studies by ISSR-PCR primers. The results also indicate that the different doses of gamma rays (Except for the deadly ones) positively affected early rate with genotypes L1, L2, L3 and L4 compared to the control from each genotype, looking at the results of the second season. The doses of 400Gy affected the number of pods per plant and consequently on the number of seeds per plant in the genotype L4 by decrease it in the first season only, while it affected it by increase it in the second season. It also affected the number of seeds per pod by increasing it in both seasons. It was also found that the ISSR technique was effective in this study, especially the primer ISSR-16, where the polymorphism rate was 95.7%, while the lowest percentage of Polymorphism was in the primer R-04, where the polymorphism rate was 40%.

Keywords: chickpea; Gamma Rays; Mutation; ISSR Marker.

INTRODUCTION:

Chickpea (*Cicer arietinum* L.) is an old world pulse and one of the seven Neolithic founder crops in the Fertile Crescent of the Near East (Jukanti *et al*., 2012), and is an important grain legume of the semi-arid tropics and warm temperate zones, chickpea is the third most important cool season food legume in the world after Dry beans and Peas, It has been cultivated mainly in the Indian subcontinent, West Asia, and North Africa, but recently large acreages have been introduced in the Americas and Australia (Singh *et al*., 2008; Kumar *et al*., 2015). Currently, chickpea is grown in over 50 countries across the Indian subcontinent, North Africa, the Middle East, Southern Europe, the Americas and Australia. India is the largest chickpea producing country, accounting for (66%) of global chickpea production. The other major chickpea producing countries include Pakistan, Turkey, Australia, Myanmar, Ethiopia, Iran, Mexico, Canada and USA (Jukanti *et al*., 2012). Despite its agronomical importance the seed productivity of Chickpea is quite low (Kumar *et al*., 2015).

Chickpea is known as Gram, Bengal gram or Chola (Karim *et al*., 2008), also called Garbanzo bean (Jukanti *et al*., 2012, Kumar *et al*., 2015), also called Chana (Hindi) (Singh *et al*., 2008). Two main types of Chickpea cultivars are grown globally Kabuli or (macrosperma) and Desi or (microsperma), representing two diverse gene pools (Singh *et al*., 2008), In addition, an intermediate type with pea-shaped seeds of local importance is recognized in India. The seed weight ranges from 0.1 to 0.3g and 0.2 to 0.6g in the Desi and Kabuli types, respectively. The Desi types account for about 80 - 85 % of the total chickpea area and are mostly grown in Asia and Africa, The Kabuli types are grown in West Asia, North Africa, North America and Europe (Jukanti *et al*., 2012).

Chickpea is a member of the legume family (Fabaceae or Leguminosea). These families constitute the third largest family among the higher plants and are second only to cereals in agricultural and economic importance. This family contains about 750 genera and 20,000 species, and includes major grain legumes, oilseed, forage, medicinal, ornamental crops and agroforestry species (Gupta and Gopalakrishna, 2012).

Chickpea is a member of the genus *Cicer* L. that consists of 44 species including 35 perennials and eight annual wild species and

one the domesticated chickpea, (*Cicer arietinum* L.) (Toker, 2008). Chickpea is a diploid with 2n = 2x = 16, having a genome size of approximately 931 Mbp, it is a highly self-pollinated crop with an out crossing rate of less than 1%. and plays an important role in the enrichment of soil fertility (Singh *et al*., 2008; Basu *et al*., 2018).

Chickpea is an important grain legume, and forms one of the major components of the human diet. However, a narrow genetic base of cultivated chickpea (*Cicer arietinum* L.) has hindered the progress in realizing high yield gains in breeding programs (Singh *et al*., 2008). Lately, irradiation technology is widely used to produce changes in the product characteristics leading to the development of new products (Piri *et al*., 2011). Variability in the population creates the chance of selection for Desirable improvement. Induced mutagenesis can be used to create variability as the rate of spontaneous mutation is extremely low. The use of induced mutation has been widely accepted by plant breeders as a tool in crop improvement. The induction of mutation in plant materials can be achieved either through physical or chemical mutagens. Many workers have attempted to exploit somaclonal variation for crop improvement through physical mutagens particularly treated by gamma rays. Mutation breeding can play an efficient role in developing an ideal plant type having high yield potential, mutation breeding may be an alternative and supplement to hybridization as a source of variability. Through effective selection, varieties of better types can be developed out of the mutated population, the development of varieties using conventional breeding usually takes a longer time but if conventional as well as mutation breeding program can be taken simultaneously the development of new variety may take a shorter time (Karim *et al*., 2008; Dawood *et al*., 2022). In this study it was used gamma irradiation to create genetic variability among four Chickpea genotypes. Chickpea has been recognized as a crop with minimal genetic variation; However, recently ample genetic diversity has been reported using short sequence tandem repeats (Kumar *et al*., 2015).

The aim of study: This study aims to the possibility of producing new genotypes by producing genetic variations by treating Chickpea's seeds with one of the chemical or physical mutagen at the appropriate concentrations and doses, and then studying some morphological traits, yield and its components under Egyptian conditions.

MATERIALS AND METHODS

The present investigation was conducted during the two seasons 2017-2018 and 2018-2019. Two field experiments were conducted with Egyptian soil cooperation between Genetic section, Agric-Botany Department, Faculty of Agriculture, Cairo, Al-Azhar University and Genetic Resources Research Department Bahteem, Field Crop Research Institute (FCRI), Agricultural Research Center (ARC), Bahteem Research Station, Egypt, on (*Cicer arietinum*). The work was done to study genetic improvement of four chickpea genotypes (*Cicer arietinum*) using gamma rays and Comparison of results through the morphological and molecular studies.

The investigation materials and methods can be presented in two main parts as following:

Part I. Mutation induction:

The genotypes that was used:

Four chickpea genotypes (*Cicer arietinum*) 1-(L.1), 2-(Giza531), 3-(Giza195) and 4-(F-06-74-C) differ in morphological properties and origin in addition to the local variety (Giza3) as general control for all genotypes used in the study were studied and evaluated under Egyptian conditions (Table 1).

Gamma rays:

Seeds radiation of four chickpea genotypes under the study that subjected to three different doses of gamma rays (200, 300 and 400 Gy) as well as zero Gy as control treatment for each genotype (Raina *et al*., 2017; Eissa *et al*., 2021). Gamma rays were conducted at Genetic Resources Research Department, Field Crop Research Institute (FCRI), Agricultural Research Center (ARC), Bahteem Station, Egypt during 2017-2018 and 2018-2019 growing seasons.

***Planting method*:**

All chickpea genotypes and one local variety were sown according to the recommended seeding rate (Each dose planted in plots on one side of the ridge of three meters length and 60 (cm) width. Hills were spaced by 20 (cm) with one seed per hill).

Fertilization:

Regarding seed inoculation and chemical fertilizers (nitrogen, phosphate, potassium)

nothing was applied during the chickpea plant stages in both seasons.

Irrigation:

All plots were irrigated by surface irrigation system. Mohaya irrigation was done after 15 days after the sowing date. After that, irrigation was applied every 15-17day intervals for all chickpea genotypes and the local variety according to region conditions.

Experimental design:

Season 2017-2018:

The irradiated plants were cultivated, as well as non-irradiated plants of each genotype as control for each genotype, in addition to cultivar (Giza3) in plots (according to the recommended seeding rate), Each plot was divided into three

lines and the selection was done inside each line and between lines. Four plants were selected from each treatment, so that every plant represents its repetition.

Season 2018-2019: All the (M1) generation seeds were sown (selected plants and Bulk), in the same way for the previous season, as well as non-irradiated treatments (Control) and cultivar (Giza 3).

***Studied attributes*:**

In the 2017-2018 season: Early (days to 50 % flowering and days to maturity), Germination rate, the yield and its components of chickpea plants were studied, and four single plants were selected from each treatment (Gautam *et al*., 2021).

Yield and Yield components of check pea plants: Number of pods per plant, Number of seeds per plant, Number of seeds per pod, seeds weight per plant (g) and 100-seeds weight (g).

In the 2018-2019 season: The same traits studied in the previous season were studied, in addition to some morphological qualities which are: Length of leaf and length of pinnule and Color of flower.

Part II. Molecular Studies:

Molecular analysis of M3 seeds produced from selected M2 plants, as well as for the non-irradiated treatments (control) and the cultivar (Giza 3) was conducted using ISSR method by selecting five primers.

ISSR-PCR Reactions:

five ISSR primers were used in the detection of polymorphism (Table 2). The amplification reaction was conducted in 25µl reaction volume containing 12.5µl Master Mix (sigma), 2.5µl primer (10pcmol), 3µl template DNA (10ng) and 7µl dH_2O, according to (Ibrahim *et al*., 2019).

Thermocycling Profile PCR:

PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (*PE* Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94ºC. Each cycle consisted of a denaturation step at 94ºC for 1 min, an annealing step at 45ºC for 1 min, and an elongation step at 72ºC for 1.5 min. The primer extension segment was extended to 7min at 72ºC in the final cycle.

Detection of the PCR Products:

The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts. PCR products were visualized on UV light and photographed using a Gel Documentation System (BIO-RAD 2000).

Data analysis:

ISSR analysis, only clear and unambiguous bands were visually scored as either present (1) or absent (0) for all samples and final data sets included both polymorphic and monomorphic bands. Then, a binary statistic matrix was constructed. Dice's similarity matrix coefficients were then calculated between genotypes using the unweighted pair group method with arithmetic averages (UPGMA). This matrix was used to construct a phylogenetic tree (dendrogram) was performed according to Euclidean similarity index using the PAST software Version 1.91 (Hammer *et al*., 2001).

Statistical analysis:

The experiments were subjected to completely randomized design. Each treatment was replicated three times. Values from triplicate determinations of each sample were averaged and represented as mean± standard deviation (SD). The data were analyzed statistically by analysis of variance (ANOVA), and the difference between the mean of samples were analyzed by least significant difference (LSD) test at a probability level of 5% (Sokal and Rohlf, 1995). Data analysis was performed by Minitab and statistics 8 computer statistically analysis program.

The observed values of seed germination rate, callus induction and regeneration rate characters were estimated as percentages of callus number for each (PGRs) concentration and each explant (Cattanceo and Qiao, 1991). One-way analysis of variance was also performed to examine the significant differences between means of treatments.

RESULTS AND DISCUSSION

PART (A): Mutation induction of chickpea plant:

Germination rate / Early rate (days to 50% flowering and days to maturity):

Variances between the genotypes used for chickpea and cultivar (Giza3).

(Table 3) shows the percentage of germination in the used genotypes compared to the Giza3 variety, through season 2017-2018 & season 2018-2019. The highest percentage of germination was in the Giza3 variety, with a germination rate of 100.00%, followed by the genotype L1 with a germination rate of 90.00%, followed by the genotypes L2 and L4. with a germination rate of 80.00% for genotype L2 in both seasons. and 78.00% in the first season and 82.50% in the second season for the genotype L4. and the lowest percentage of germination was in the genotype L3 with a germination rate of 58.25% in the first season and 60.00% in the second season.

As for the Early rate, (Table 4) show the number of days from the date of planting to the flowering of plants by 50%, and the number of days from the date of planting to maturity. Through which they we can know the early. The earliest plants were those of the genotype L1, the number of days until flowering was 46.20 days in the first season, and 35.00 days in the second season. The number of days to maturity was 158.50 days in the first season, and 143.00 days in the second season. This was followed in the earliest by plants of the Giza3 variety, where the number of days until flowering was 53.70 days in the first season, and 44.00 days in the second season. The number of days to maturity was 160.50 days in the first season, and 150.00 days in the second season. The most delayed plants were those of the genotype L4, where the number of days until flowering was 60.00 days in the first season, and 47.00 days in the second season. The number of days to maturity was 169.00 days in the first season, and 156.25 days in the second season.

Effect of the different doses of gamma radiation.

Data given in (Table 3) indicate Effect of different gamma ray doses on germination rate in genotypes of chickpea through season 2017-2018 & season 2018-2019, With a comparison of the effect of different doses in each genotype with the non-irradiated plants of the same genotype and Giza 3 variety. In the genotypes L1 and L2, the percentage of germination was extremely low in the first season with the dose of 400 Gy (2.00%), while its effect was fatal for plants in the second season. The doses 200 Gy and 300 Gy, respectively, affected the percentage of germination in both seasons by decrease, but in the second season, it was higher than the first (32.00% in the first season, 80.00% in the second season) for L1 D200. (12.00% in the first season, 42.50% in the second season) for L1 D300. (52.00% in the first season, 72.00% in the second season) for L2 D200. (36.00% in the first season, 53.75% in the second season) for L2 D300. In the genotype L3, the percentage of germination was higher than control with dose 200 Gy in both seasons (60.50% in the first season, 93.70% in the second season). and with dose 300 Gy it was less than control in the first season (54.00%), while in the second season the percentage of germination was 100.00% as the general control (Giza3). and with dose 400 Gy it was low in the first season (24.00%), deadly for plants in the second season. In the genotype L4, the percentage of germination was higher than control with dose 200 Gy in both seasons (80.00% in the first season, 90.00% in the second season), and with dose 300 Gy it was low in the first season (29.00%) and equal to control in the second season (82.50%), and with dose 400 it was low in the first season (28.25%) and higher than control in the second season (87.50%). From the above it is clear that the best doses affected the germination rate is the dose 200 Gy in the genotype L3, looking at the results of both seasons, and the dose 300 Gy, looking at the results of the second season. and with the genotype L4, the dose 200 Gy, in both seasons, and the dose 400 Gy in the second season.

Data given in (Table 4) indicate effect of the different doses of gamma rays on early rate in genotypes of chickpea through season 2017-2018 & season 2018-219, With a comparison of the effect of different doses in each genotype with the non-irradiated plants of the same genotype and Giza 3 variety. The results show that the earliest plants are the genotype L1, even with the different doses of gamma rays.

The results also indicate that the different doses of gamma rays (Except for the deadly ones) positively affected early rate with genotypes L2, L3 and L4 compared to the control from each genotype, looking at the results of the second season.

Number of pods per plant / Number of seeds per pod / Number of seeds per plant:

Variances between the genotypes used for chickpea and cultivar (Giza 3).

The data in (Table 5) show the number of pods per plant and number of seeds per pod of four genotypes for chickpea compared to Giza 3, during the two growing seasons 2017-2018 & 2018-2019. It appears from the table that the most plants in the number of pods are genotype L1 (70.00 pods in the first season and 60.50 pods in the second season). It is followed by the Giza 3 variety (48.75 pods in the first season and 38.25 pods in the second season). It is followed by genotype L2 (23.50 pods in the first season and 25.25 pods in the second season). The number of pods/plant for genotype L3 was (18.75 pods in the first season and 20.75 pods in the second season), and for genotype L4 (20.75 pods in the first season and 17.50 pods in the second season). The number of seeds per pod ranges from 1 to 2 seeds/pod. The lowest percentage was in genotype L4, where the average number of seeds per pod was (1 seed) in both seasons, also, in genotype L3, where the average number of seeds per pod was (1.25 seed) in both seasons. While the percentage in genotype L2 and Giza 3 ranged between (1.50 seed) and (1.75 seed) in both seasons. and in genotype L1 it was (1.50 seed) in both seasons. From the above and through (Table 6) it appears that the most number of seeds per plant is in genotype L1, where the average number of seeds was 106.00 seeds in the first season and 93.00 seeds in the second season, followed by Giza 3, (the average number of seeds was 71.75 seeds in the first season and 68.00 seeds in the second season), followed by genotype L2 (42.25 seeds in the first season, 37.75 seeds in the second season). The number of seeds for genotype L3 was (25.50 seeds in the first season, 25.75 seeds in the second season), and for genotype L4 was, 20.75 seeds in the first season, 17.50 seeds in the second season.

Effect of the different doses of gamma radiation.

From (Table 5 and 6) the following is noted: The dose of 200 affected the number of pods per plant and consequently on the number of seeds per plant in the genotypes L1 and L2 by decreasing it in both seasons. While it affected genotypes L3 and L4 by increasing it in both seasons, it did not affect the number of seeds per pod in the genotypes L1, L4 in the second season, and L3 in the first season. While it affected the genotypes L2, L3 in the second season, L1 and L4 in the first season by increase it. And it affected the genotype L2 in the first season by decreasing it. The dose of 300 affected the number of pods per plant and consequently on the number of seeds per plant in the genotypes L1 and L2 by decrease it in both seasons. And it affected the genotypes L3 and L4 by decreasing it in the first season, while it affected them by increase it in the second season. It also affected the number of seeds per pod in the genotypes L1 in both seasons, and L2 in the first season only by decrease it, and it did not affect the genotypes L3 and L4 in both seasons. The doses of 400 Gy affected the number of pods per plant and consequently on the number of seeds per plant in the genotype L4 by decrease it in the first season only, while it affected it by increase it in the second season. It also affected the number of seeds per pod by increasing it in both seasons.

Seeds weight per plant (g) / 100-seeds weight (g):

Variances between the genotypes used for chickpea and cultivar (Giza 3).

(Table 7) indicates the weight of seeds per plant of the germplasm used in the study compared to Giza 3 cultivar, during the 2017-2018 and 2018-2019 planting seasons. Through which it is possible to calculate the weight of 100 seeds through the equation ($\frac{\text{Seeds weight per plant (g)}}{\text{Number of seeds per plant}} x100$), through which it is infers the seed size, seed atrophy and its percentage. As the varieties and lines of chickpea seeds are divided into two categories, large seeds and small seeds. The weight of 100 seeds in varieties and lines large seed size ranges from 10 to 30 g/100 seeds, and in varieties and lines small seed size from 30 to 60 g/100 seeds. It appears from the table that both genotypes L1 and L4, as well as the cultivar Giza 3 have small seed size. Where weigh of 100 seeds in genotype L1 in the first season was 26.73g, and in the second season 25.29g. and in the genotype L4 it was 25.22g in the first

season, 24.97g in the second season. and in Giza 3 it was 24.91g in the first season, 26.02g in the second season. While genotypes L2 and L3 have large seed size, the weight of 100 seeds in genotype L2 in the first season was 51.68g, and in the second season is 54.42g. and in the genotype L3 it was 50.00g in the first season, 53.06g in the second season.

Effect of the different doses of gamma radiation.

From (Table 7) noticed that through the average weight of 100 seeds for the different genotypes used with the different doses, the different doses did not have a significant effect on seed size, as the average weight of 100 seeds in the genotype L1 with the different doses ranged between 24.21g and 27.20g in both seasons, in the genotype L2 it ranges between 50.00g and 53.45g in both seasons, in the genotype L3 it ranges between 50.94g and 54.58g in both seasons, and in the genotype L4 it ranges between 23.13g and 23.68g in both seasons.

PART (B): Molecular studies on chickpea plant:

Amplification of ISSR marker and polymorphism in Chickpea genotypes:

ISSR analysis was used to detect the variation in plant tissue with four different doses of gamma radiation (0, 200, 300 and 400 Gy) on five samples of random primer (ISSR-11, ISSR-12, ISSR-16, R-04 and R-07)

ISSR-11 primer:

Dealing with results in (Table 8) and (Figure 1) explain the total of bands, molecular weight and percentage of polymorphism according to ISSR analysis of ISSR-11 primer. The results obtained indicated that the amplified fragment of ISSR-11 primer was four bands with molecular weight (MW) ranging between 371 to 642 bp. In total, this primer showed 53 bands. No unique observed.

ISSR-12 primer:

Data in (Table 9) and (Figure 2) illustrated the total of Amplified Fragment (AF), percentage of polymorphism and range of size of ISSR-12 primer. The result obtained was 21 Amplified Fragment with molecular weight (MW) ranging between 169 to 906 bp. In addition, the total bands of ISSR-12 primer were 82 bands. 11 unique bands were observed. First of it was at (AF2) in L1 D0 with molecular weight (MW) of 700 bp. The second one was at (AF6) in L4 D400 with molecular weight (MW) of 528 bp. The Third one was at (AF7) in L3 D0 with molecular weight (MW) of 473 bp. The 4th one was at (AF10) in L3 D0 with molecular weight (MW) of 423 bp. The 5th one was at (AF11) in L1 D200 with molecular weight (MW) of 415 bp. The 6th one was at (AF13) in L3 D0 with molecular weight (MW) of 381 bp. The 7th one was at (AF14) in L3 D300 with molecular weight (MW) of 371 bp. The 8th one was at (AF15) in L2 D300 with molecular weight (MW) of 355 bp. The 9th one was at (AF18) in L1 D300 with molecular weight (MW) of 312 bp. The 10th one was at (AF19) in L3 D0 with molecular weight (MW) of 305 bp. The 11th one was at (AF20) in Giza3 with molecular weight (MW) of 292 bp.

***ISSR-16 primer*:**

Data in (Table 10) and (Figure 3) illustrated the total of Amplified Fragment (AF), percentage of polymorphism and range of size of ISSR-16 primer. The result obtained was 24 Amplified Fragment with molecular weight (MW) ranging between 132 to 1505 bp. In addition, the total bands of ISSR-16 primer were 93 bands. Thirteen unique bands were observed. First of it was at (AF1) in L4 D300 with molecular weight (MW) of 1505 bp. The second one was at (AF2) in L4 D400 with molecular weight (MW) of 1418 bp. The Third one was at (AF4) in L1 D0 with molecular weight (MW) of 1034 bp. The 4th one was at (AF5) in L1 D200 with molecular weight (MW) of 918 bp. The 5th one was at (AF12) in Giza3 with molecular weight (MW) of 527 bp. The 6th one was at (AF13) in L1 D0 with molecular weight (MW) of 517 bp. The 7th one was at (AF14) in L2 D0 with molecular weight (MW) of 487 bp. The 8th one was at (AF15) in Giza3 with molecular weight (MW) of 459 bp. The 9th one was at (AF17) in Giza3 with molecular weight (MW) of 400 bp. The 10th one was at (AF20) in L4 D0 with molecular weight (MW) of 335 bp. The 11th one was at (AF21) in L2 D0 with molecular weight (MW) of 315 bp. The 12th one was at (AF22) in L2 D0 with molecular weight (MW) of 259 bp. The 13th one was at (AF24) in Giza3 with molecular weight (MW) of 132 bp.

ISSR-R-04 primer:

Dealing with results in (Table 11) and (Figure 4) explain the total of bands, molecular weight and percentage of polymorphism according to ISSR analysis of ISSR-R-04 primer. The results obtained indicated that the amplified fragment of ISSR-R-04 primer was

five bands with molecular weight (MW) ranging between 189 to 491 bp. In total, this primer showed 65 bands. No unique observed.

ISSR-R-07 primer:

Data in (Table 12) and (Figure 5) showed Results indicated that the Amplified Fragment (AF) of ISSR-R-07 primer was four bands with molecular weight ranging between 151 to 314 bp. This primer made the total of bands (29). It is obvious that there were two unique bands; The first was observed at (AF1) at L4 that radiated with D400 Gy with molecular weight (MW) of 314 bp, while the second was at (AF4) in L4 that radiated with D400 Gy with molecular weight 151 bp.

Total polymorphism (%) among ten primers using ISSR analysis.

Data in (Table 13) showed that the ISSR analysis was used to detect the variation of *Cicer arietinum* maintained on Murashige and Skoog (MS) medium with different doses of gamma rays. The polymorphism percentage among ten random primers was recorded using ISSR analysis. It is evident the present results expose that the total 322 scorable bands were produced with five primers ranging between 4 to 24 band, while the total of bands among all primers were ranged between 29 to 93 bands. The average of polymorphism scored 70.8 % ranging between 40% to 95.7%. In addition, in ISSR-12 primer the unique was represented by 52.3%, in ISSR-16 primer the unique was represented by 54.1%, in R-07 primer the unique was represented by 50%, while the primers ISSR-11 and R-04 showed no unique bands. Moreover, Polymorphic (without Unique) was represented by 50%, 38%, 41.6%, 40%, and 25% in primers ISSR-11, ISSR-12, ISSR-16, R-04 and R-07respectively. On the other case, Polymorphic (with Unique) was represented by 50%, 90.4%, 95.7%, 40% and 75% for the previous primers, respectively.

Finally, the highest percentage of Polymorphism was in the primer ISSR-16, while the lowest percentage of Polymorphism was in the primer R-04.

Cluster analysis and percentage of similarities and differences indices between 14 accessions of chickpea plant by ten primers ISSR.

DNA was isolated from control and other treatments and using ISSR-PCR analysis and the cluster obtained analysis to determine the degree of kinship between the different genotypes of chickpea plant. Results shown in dendrogram (Figure 6) revealed that, Cluster analysis grouped the five-chickpea genotypes into two clusters at distance 5.00. Cluster I consisted of two different sub-clusters (A.1 and A.2) at distance of 4.5. Sub-cluster (A.1) consisted of single genotype (L2) which irradiated by (D0). Sub-cluster (A.2) consisted of two different sub-sub-clusters (A.2.1 and A.2.2). sub-sub-clusters (A.2.1) consisted of single genotype (Giza3). while sub-sub-clusters (A.2.2) divided into two sub-sub-sub-cluster at distance 4.3. First, Sub-sub-sub-clusters (A.2.2.1) consisted of single genotype (L1 D0). Second, sub-sub-sub-clusters (A.2.2.2) consisted of two genotypes (L1 D200 and L1 D300) in two groups at distance 4.00. Whereas Cluster II consisted of two different sub-clusters (B.1 and B.2) at distance 4.4. sub-cluster (B.1) consisted of two different sub-sub-clusters (B.1.1 and B.1.2). sub-sub-clusters (B.1.1) consisted of single genotype (L3 D300). while sub-sub-clusters (B.1.2) consisted of two genotypes (L2 D200 and L2 D300) in two groups at distance 3.5. As for, sub-cluster (B.2) consisted of two different sub-sub-clusters (B.2.1 and B.2.2). sub-sub-clusters (B.2.1) consisted of two genotypes (L3 D0 and L3 D200) in two groups at distance 4.0.

Regarding, sub-sub-clusters (B.2.2) consisted of two different sub-sub-sub-clusters (B.2.2.1 and B.2.2.2). sub-sub-sub-clusters (B.2.2.1) consisted of single genotype (L4 D400). while sub-sub-sub-clusters (B.2.2.2) divided into two sub-sub-sub-sub-cluster at distance 3.4. First, sub-sub-sub-sub-clusters (B.2.2.2.1) consisted of single genotype (L4 D200). Second, sub-sub-sub-sub-clusters (B.2.2.2.2) consisted of two genotypes (L4 D0 and L4 D300) in two groups at distance 3.00. Through the cluster diagram in (Figure 6) as well as (Table 13); It is noted that the highest kinship ratio was between L4 D0 and L4 D300 with a rate of 89%, while the lowest kinship ratio was between L4 D0 and L2 D0 at 58%. Cluster analysis resulted in the distribution of gamma radiation and susceptible genotypes in separate groups, which revealed the presence of inherent variations (Sehrawat *et al*., 2014). These variations were effectively explored for ISSR markers studies. The developed ISSR markers may help along with already available markers to execute further research. The markers coupled with specific loci linked with gamma irradiation. The developed markers will help to identify the QTLs (quantitative trait loci) or other important genes. These markers can also be utilized for testing the purity of hybrids or diversity assessment for important agronomic traits.

CONCLUSION:

Found that the different doses of gamma rays on the different genotypes of chickpea plant had a positive effect, especially for strain L1 under doses of 200 and 300Gy for most of the traits in both seasons. While the dose of 400Gy was fatal for most strains. It was also found that the ISSR technique was effective in this study, especially the primer ISSR-16, where the polymorphism rate was 95.7%, while the lowest percentage of Polymorphism was in the primer R-04, where the polymorphism rate was 40%.

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Table 1: Pedigree of Four chickpea genotypes (*Cicer arietinum*) and one local variety according to Genetic Resources Research Department Bahteem, Field Crop Research Institute (FCRI), Agricultural Research Center (ARC), Bahteem Research Station, Egypt.

No	Genotype	Origin	Pedigree	Testa color	Testa shape	Seed size	100-seed/weight (g)
1	L1	Landraces in Egypt	AL-minya	Beige	Rough	Small	28 g
2	L2	Varieties in Egypt	Varieties in Egypt	Beige	Rough	Big	50 g
3	L3	Varieties in Egypt	Varieties in Egypt	Beige	Rough	Big	50 g
4	L4	ICARDA Syria	Individual plant selection-Syria	Beige	Smooth	Small	25 g
5	Giza 3	Varieties in Egypt	Varieties in Egypt	Yellow	Smooth	Small	25 g

Table 2: List of the primer names and their nucleotide sequences used in the study for ISSR procedure.

Primer Name	Sequence
ISSR- 11	5'-ACACACACACACACACYA-3'
ISSR- 12	5'-ACACACACACACACACYC-3'
ISSR- 16	5'-TCTCTCTCTCTCTCTCA-3'
R-04	5'-GTGTGTGTGTGTYG-3'
R-07	5'-CTCTCTCTCTCTCTCTT-3'

A: Adenine, T: Thymine, G: Guanine, C: Cytosine, Y: (C or T)

Table 3: Effect of different gamma rays doses on germination rate in chickpea through season 1 & season 2.

Treatments	M. of germination rate	
	(Season 1)	(Season 2)
L1D0	90.00	90.00
L1D200	32.00	80.00
L1D300	12.00	42.50
L1D400	2.00	0.00
L2D0	80.00	80.00
L2D200	52.00	72.00
L2D300	36.00	53.75
L2D400	2.00	0.00
L3D0	58.25	60.00
L3D200	60.50	93.70
L3D300	54.00	100.00
L3D400	24.00	0.00
L4D0	78.00	82.50
L4D200	80.00	90.00
L4D300	29.00	82.50
L4D400	28.25	87.50
GIZA 3	100.00	100.00
T. Mean	48.12	65.56
ANOVA	1.0686 NS	34.539 NS
LSD 5%	1.4674788	8.342

Table 4: Effect of different gamma rays doses on early rate (days to 50% flowering and days to maturing) in chickpea through season1 & season2.

Treatments	M. of days to 50% flowering		M. of days to maturing	
	(Season 1)	(Season 2)	(Season 1)	(Season 2)
L1D0	46.20	35.00	158.50	143.00
L1D200	50.20	33.00	159.20	141.00
L1D300	50.00	35.00	157.50	141.50
L1D400	0.00	0.00	0.00	0.00
L2D0	56.20	45.00	163.50	152.75
L2D200	53.50	43.20	161.20	150.25
L2D300	62.70	45.00	170.70	151.75
L2D400	0.00	0.00	0.00	0.00
L3D0	54.50	45.00	161.50	154.75
L3D200	49.20	40.00	156.20	150.00
L3D300	50.20	44.50	159.70	151.00
L3D400	0.00	0.00	0.00	0.00
L4D0	60.00	47.00	169.00	156.25
L4D200	64.50	44.70	171.50	152.00
L4D300	67.00	45.00	174.50	152.25
L4D400	61.00	43.00	171.20	150.00
GIZA 3	53.70	44.00	160.50	150.00
T. Mean	45.83823	34.676	134.98	123.32
ANOVA	3.69 NS	0.28 NS	2.14 NS	0.22 NS
LSD 5%	2.72735	0.756	2.08008	0.674

Table 5: Effect of different gamma rays doses on number of pods per plant and number of seeds per pod in chickpea through season 1 & season 2.

Treatments	M. Number of pods per plant		M. Number of seeds per pod	
	(Season 1)	(Season 2)	(Season 1)	(Season 2)
L1D0	70.00	60.50	1.50	1.50
L1D200	25.50	43.25	1.75	1.50
L1D300	17.00	52.75	1.25	1.25
L1D400	0.00	0.00	0.00	0.00
L2D0	23.50	25.25	1.75	1.50
L2D200	12.00	14.50	1.50	1.75
L2D300	15.50	14.75	1.00	1.50
L2D400	0.00	0.00	0.00	0.00
L3D0	18.75	20.75	1.25	1.25
L3D200	28.25	30.25	1.25	1.50
L3D300	14.50	29.25	1.25	1.25
L3D400	0.00	0.00	0.00	0.00
L4D0	20.75	17.50	1.00	1.00
L4D200	23.50	26.50	1.50	1.00
L4D300	18.25	20.25	1.00	1.00
L4D400	11.00	32.75	1.25	1.50
GIZA 3	48.75	38.25	1.50	1.75
T. Mean	20.43	25.09	1.10294	1.13
ANOVA	72.0 NS	14.8 NS	0.18 NS	0.17 NS
LSD 5%	12.0532	5.470	0.60456	0.588

Table 6: Effect of different gamma rays doses on number of seeds per plant in chickpea through season 1 & season 2.

Treatments	M. Number of seeds per plant	
	(Season 1)	(Season 2)
L1D0	106.00	93.00
L1D200	47.00	64.50
L1D300	19.50	67.75
L1D400	0.00	0.00
L2D0	42.25	37.75
L2D200	18.00	25.50
L2D300	15.50	22.25
L2D400	0.00	0.00
L3D0	25.50	25.75
L3D200	32.00	44.50
L3D300	17.75	36.75
L3D400	0.00	0.00
L4D0	20.75	17.50
L4D200	35.75	26.50
L4D300	18.25	20.25
L4D400	14.00	49.00
GIZA 3	71.75	68.00
T. Mean	28.47	35.24
ANOVA	65.6 NS	13.7 NS
LSD 5%	11.50181	5.269

Table 7: Effect of different gamma rays doses on seeds weight per plant (g) and 100 seed weight (g) in chickpea through season1 & season2.

Treatments	M. of. Seeds weight per plant (g)		M. of 100 Seed weight (g)	
	(Season 1)	(Season 2)	(Season 1)	(Season 2)
L1D0	28.10	22.70	26.73	25.29
L1D200	12.73	15.35	27.20	24.21
L1D300	5.25	16.25	26.59	25.01
L1D400	0.00	0.00	0.00	0.00
L2D0	21.75	20.25	51.68	54.42
L2D200	9.08	12.75	50.54	50.00
L2D300	7.75	11.75	50.00	53.45
L2D400	0.00	0.00	0.00	0.00
L3D0	12.75	13.50	50.00	53.06
L3D200	16.38	23.78	50.94	53.03
L3D300	9.00	19.95	50.96	54.58
L3D400	0.00	0.00	0.00	0.00
L4D0	5.25	4.38	25.22	24.97
L4D200	8.45	6.25	23.60	23.51
L4D300	4.55	4.75	23.68	23.44
L4D400	3.13	11.50	23.13	23.29
GIZA 3	17.80	18.00	24.91	26.02
T. Mean	9.53	11.83	29.72	30.25
ANOVA	4.4 NS	3.4 NS	10.4 NS	5.38 NS
LSD 5%	3.0059	2.639	4.57852	3.294

Table 8: Distribution and size of mono-morphic and polymorphic bands that obtained by ISSR analysis of the fourteen accessions of chickpea plants generated by primer ISSR-11.

ISSR-11 primer																
AF*	MW*	L1			L2			L3			L4				Giza3	Polymorphism
	bp	D0	D200	D300	D0	D200	D300	D0	D200	D300	D0	D200	D300	D400		
1	642	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
2	543	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
3	459	1	1	1	0	1	1	1	0	1	1	1	1	1	1	Polymorphic
4	371	1	1	1	0	1	1	1	1	1	1	1	1	1	1	Polymorphic
Total		4	4	4	2	4	4	4	3	4	4	4	4	4	4	53

* AF= Amplified Fragment, MW=Molecular Weight, 1=presence of band and 0= absence of band

Table 9: Distribution and size of mono-morphic and polymorphic bands that obtained by ISSR analysis of the fourteen accessions of chickpea plants generated by primer ISSR-12.

ISSR-12 primer																
AF*	MW*	L1			L2			L3			L4				Giza3	Polymorphism
	bp	D0	D200	D300	D0	D200	D300	D0	D200	D300	D0	D200	D300	D400		
1	906	0	1	1	1	1	1	1	1	1	1	1	1	1	1	Polymorphic
2	700	1	0	0	0	0	0	0	0	0	0	0	0	0	0	Unique
3	695	0	1	1	0	1	1	0	1	1	1	1	1	1	1	Polymorphic
4	614	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
5	539	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
6	528	0	0	0	0	0	0	0	0	0	0	0	0	1	0	Unique
7	473	0	0	0	0	0	0	1	0	0	0	0	0	0	0	Unique
8	443	0	0	0	0	0	0	0	0	0	1	0	1	0	0	Polymorphic
9	434	0	0	0	0	0	1	0	0	1	0	0	0	0	0	Polymorphic
10	423	0	0	0	0	0	0	1	0	0	0	0	0	0	0	Unique
11	415	0	1	0	0	0	0	0	0	0	0	0	0	0	0	Unique
12	389	0	0	1	0	0	1	0	0	0	0	0	0	0	0	Polymorphic
13	381	0	0	0	0	0	0	1	0	0	0	0	0	0	0	Unique
14	371	0	0	0	0	0	0	0	0	1	0	0	0	0	0	Unique
15	355	0	0	0	0	0	1	0	0	0	0	0	0	0	0	Unique
16	333	0	0	0	0	0	0	0	0	0	0	1	0	1	0	Polymorphic
17	326	0	0	0	0	0	0	0	0	0	1	0	1	0	0	Polymorphic
18	312	0	0	1	0	0	0	0	0	0	0	0	0	0	0	Unique
19	305	0	0	0	0	0	0	1	0	0	0	0	0	0	0	Unique
20	292	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Unique

21	169	0	0	1	0	0	0	1	1	1	1	1	1	1	Polymorphic
Total		3	5	7	3	4	7	8	5	7	7	6	7	7	6	82

* AF= Amplified Fragment, MW=Molecular Weight, 1=presence of band and 0= absence of band

Table 10: Distribution and size of mono-morphic and polymorphic bands that obtained by ISSR analysis of the fourteen accessions of chickpea plants generated by primer ISSR-16.

ISSR-16 primer																
AF*	MW* bp	L1			L2			L3			L4				Giza3	Polymorphism
		D0	D200	D300	D0	D200	D300	D0	D200	D300	D0	D200	D300	D400		
1	1505	0	0	0	0	0	0	0	0	0	0	0	1	0	0	Unique
2	1418	0	0	0	0	0	0	0	0	0	0	0	0	1	0	Unique
3	1317	1	0	1	0	1	0	0	1	1	1	1	1	0	1	Polymorphic
4	1034	1	0	0	0	0	0	0	0	0	0	0	0	0	0	Unique
5	918	0	1	0	0	0	0	0	0	0	0	0	0	0	0	Unique
6	868	0	0	0	0	1	1	1	1	1	1	1	1	1	0	Polymorphic
7	865	1	0	1	0	0	0	0	0	0	0	0	0	0	1	Polymorphic
8	723	1	0	1	0	0	0	0	0	0	0	0	0	0	0	Polymorphic
9	612	1	1	1	0	0	0	0	0	0	0	0	0	0	1	Polymorphic
10	609	0	0	0	0	1	1	1	1	1	1	1	1	0	0	Polymorphic
11	535	0	0	1	0	0	1	0	0	0	0	0	1	1	0	Polymorphic
12	527	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Unique
13	517	1	0	0	0	0	0	0	0	0	0	0	0	0	0	Unique
14	487	0	0	0	1	0	0	0	0	0	0	0	0	0	0	Unique
15	459	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Unique
16	451	0	1	1	0	1	1	1	1	1	1	1	1	1	0	Polymorphic
17	400	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Unique
18	391	1	1	1	1	1	1	1	1	1	1	1	1	1	0	Polymorphic
19	344	0	0	0	0	1	1	1	0	0	0	0	0	0	0	Polymorphic
20	335	0	0	0	0	0	0	0	0	0	1	0	0	0	0	Unique
21	315	0	0	0	1	0	0	0	0	0	0	0	0	0	0	Unique
22	259	0	0	0	1	0	0	0	0	0	0	0	0	0	0	Unique
23	221	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
24	132	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Unique
Total		8	5	8	5	7	7	6	6	6	7	6	8	6	8	93

*AF= Amplified Fragment, MW=Molecular Weight, 1=presence of band and 0= absence of band

Table 11: Distribution and size of mono-morphic and polymorphic bands that obtained by ISSR analysis of the fourteen accessions of chickpea plants generated by primer ISSR-R-04.

ISSR-R-04 primer																
AF*	MW* bp	L1			L2			L3			L4				Giza3	Polymorphism
		D0	D200	D300	D0	D200	D300	D0	D200	D300	D0	D200	D300	D400		
1	491	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
2	402	1	1	1	1	1	1	1	1	1	0	1	0	1	1	Polymorphic
3	287	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
4	236	1	1	1	1	1	1	1	1	1	1	0	0	1	0	Polymorphic
5	189	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
Total		5	5	5	5	5	5	5	5	5	4	4	3	5	4	65

* AF= Amplified Fragment, MW=Molecular Weight, 1=presence of band and 0= absence of band

Table 12: Distribution and size of mono-morphic and polymorphic bands that obtained by ISSR analysis of the fourteen accessions of chickpea plants generated by primer ISSR-R-07.

ISSR-R-07 primer																
AF*	MW* bp	L1			L2			L3			L4				Giza3	Polymorphism
		D0	D200	D300	D0	D200	D300	D0	D200	D300	D0	D200	D300	D400		
1	314	0	0	0	0	0	0	0	0	0	0	0	0	1	0	Unique
2	235	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Monomorphic
3	203	1	1	1	0	1	1	1	1	1	1	1	1	1	1	Polymorphic
4	151	0	0	0	0	0	0	0	0	0	0	0	0	1	0	Unique
Total		2	2	2	1	2	2	2	2	2	2	2	2	4	2	29

* AF= Amplified Fragment, MW=Molecular Weight, 1=presence of band and 0= absence of band

Table 13: Polymorphism, monomorphic bands, polymorphic without unique, unique bands, polymorphic with unique bands, total number of bands and their percentage as detected by ISSR marker for chickpea.

Primer Name	Molecular Weight (bp)	Total (AF)	Monomorphic Bands	Polymorphic (without unique)	Unique Bands	Polymorphic (with unique)	Total number of bands	Unique (%)	Polymorphism without unique (%)	Polymorphism with unique (%)	Mean of band frequency
ISSR- 11	371-642	4	2	2	0	2	53	0%	50%	50%	0.947
ISSR- 12	169-906	21	2	8	11	19	82	52.3%	38%	90.3%	0.279
ISSR- 16	132-1505	24	1	10	13	23	93	54.1%	41.6%	95.7%	0.277
R-04	189-491	5	3	2	0	2	65	0%	40%	40%	0.929
R-07	151-314	4	1	1	2	3	29	50%	25%	75%	0.518
Total	132-1505	58	9	23	26	49	322	M. 31.28%	M. 38.92%	M. 70.8%	2.95

Percentage (%) of polymorphism = (No. of polymorphic bands ÷ Total bands) X 100.

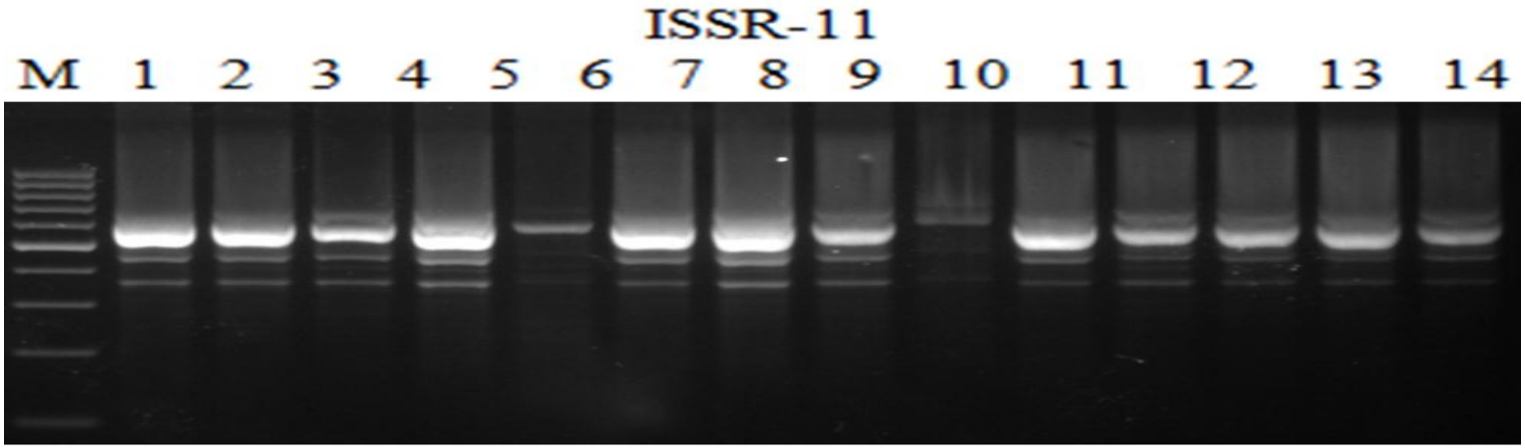

Figure 1: ISSR analysis for the fourteen accessions of chickpea plants generated by primer ISSR-11

M = master bands, 1 = Giza3, 2, 3, 4 = L1 (D0, D200, D300), 5, 6, 7 = L2 (D0, D200, D300), 8,9,10 = L3 (D0, D200, D300), 11,12,13,14 = L4 (D0, D200, D300, D400)

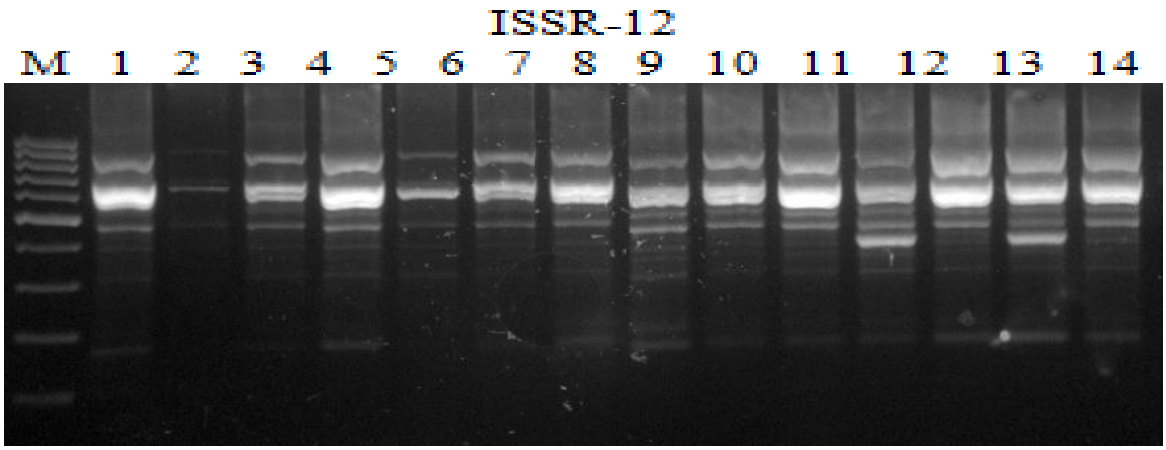

Figure 2: ISSR analysis for the fourteen accessions of chickpea plants generated by primer ISSR-12

M = master bands, 1 = Giza3, 2, 3, 4 = L1 (D0, D200, D300), 5, 6, 7 = L2 (D0, D200, D300), 8,9,10 = L3 (D0, D200, D300), 11,12,13,14 = L4 (D0, D200, D300, D400)

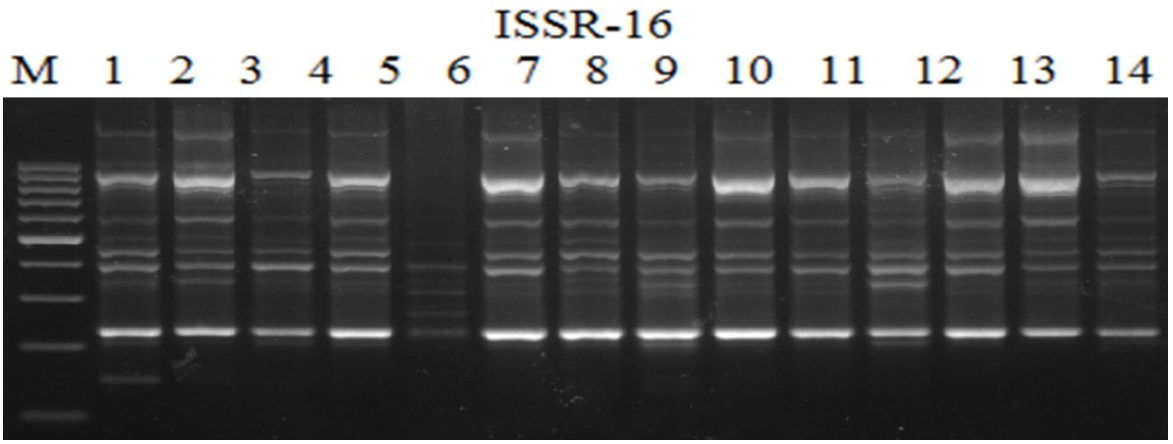

Figure 3: ISSR analysis for the fourteen accessions of chickpea plants generated by primer ISSR-16

M = master bands, 1 = Giza3, 2, 3, 4 = L1 (D0, D200, D300), 5, 6, 7 = L2 (D0, D200, D300), 8,9,10 = L3 (D0, D200, D300), 11,12,13,14 = L4 (D0, D200, D300, D400)

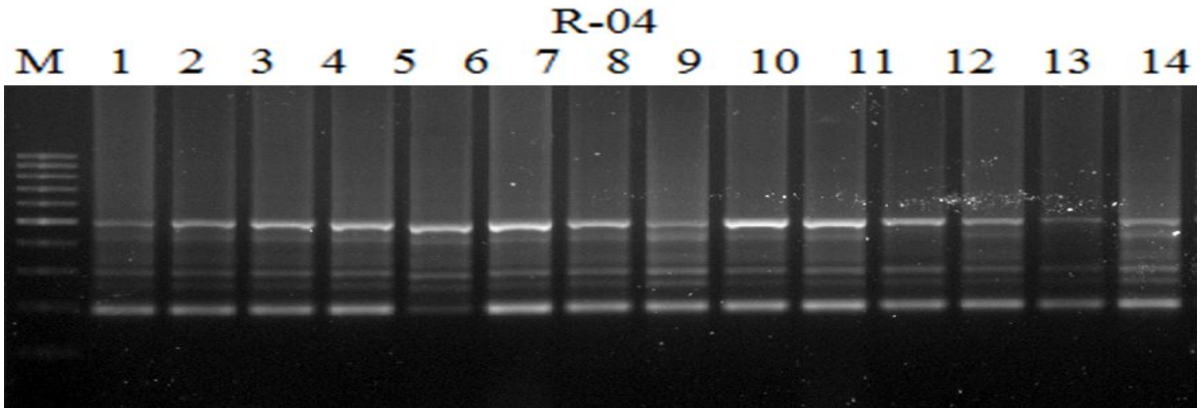

Figure 4: ISSR analysis for the fourteen accessions of chickpea plants generated by primer ISSR-R-04

M = master bands, 1 = Giza3, 2, 3, 4 = L1 (D0, D200, D300), 5, 6, 7 = L2 (D0, D200, D300), 8,9,10 = L3 (D0, D200, D300), 11,12,13,14 = L4 (D0, D200, D300, D400)

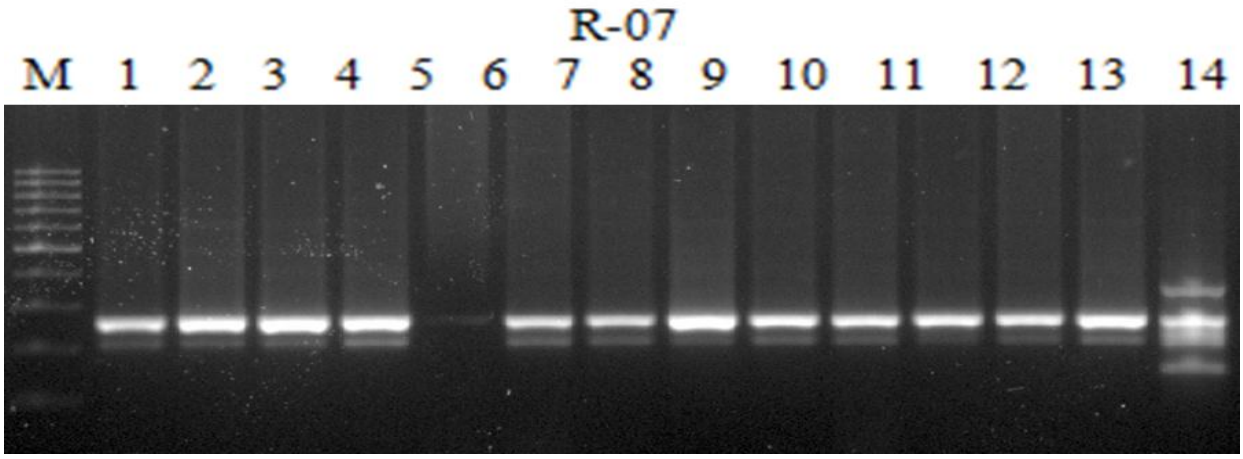

Figure 5: ISSR analysis for the fourteen accessions of chickpea plants generated by primer ISSR-R-07

M = master bands, 1 = Giza3, 2, 3, 4 = L1 (D0, D200, D300), 5, 6, 7 = L2 (D0, D200, D300), 8,9,10 = L3 (D0, D200, D300), 11,12,13,14 = L4 (D0, D200, D300, D400)

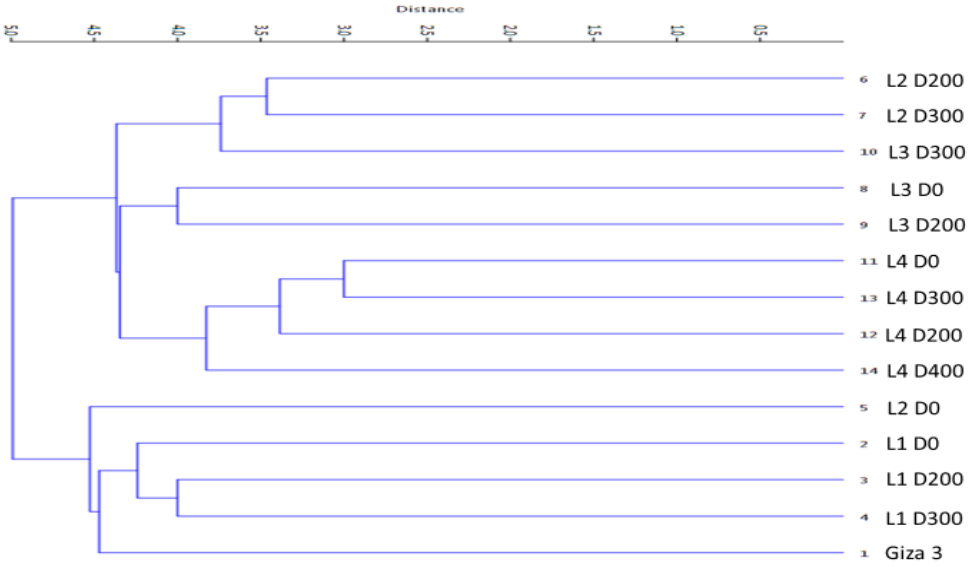

Figure 6: Dendrogram for 9 accessions of chickpea plant induced via using three doses of gamma radiation 200, 300, 400Gy and comparison them by control for each accessions and cultivar (Giza3) by ten primers ISSR.

"دراسات وراثية على بعض التراكيب الوراثية في الحمص بمصر"

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الملخص العربى

تم إجراء هذه التجربة خلال موسمي 2017-2018 و2018-2019؛ حيث تم إجراء تجربتين ميدانيتين تحت ظروف التربة المصرية بالتعاون بين شعبة الوراثة، قسم النبات الزراعي، كلية الزراعة بالقاهرة، جامعة الأزهر، وقسم بحوث الأصول الوراثية ببهتيم، التابع لمعهد بحوث المحاصيل الحقلية بمركز البحوث الزراعية، وذلك على نبات الحمص *Cicer arietinum*، حيث تم العمل على دراسة التحسين الوراثي لأربعة طرز وراثية من الحمص *Cicer arietinum* باستخدام أشعة جاما ومقارنة النتائج من خلال الدراسات المورفولوجية والجزيئية بواسطة تقنية ISSR-PCR. وأشارت النتائج إلى أن الجرعات المختلفة من أشعة جاما (ما عدا المميتة) أثرت إيجابياً على المعدل المبكر للتراكيب الوراثية محل الدراسة مقارنةً بالكنترول من كل تركيب وراثي بالنظر إلى نتائج الموسم الثاني. وقد أثرت الجرعات 400جراى على عدد القرون لكل نبات وبالتالي على عدد البذور لكل نبات في التركيب الوراثي L4 عن طريق إنقاصها في الموسم الأول فقط. بينما أثرت عليها بزيادتها في الموسم الثاني كما أثرت على عدد البذور لكل قرن من خلال زيادتها في الموسمين. كما تبين أن الجرعات المختلفة لأشعة جاما على التراكيب الوراثية المختلفة لنبات الحمص كان له تأثير إيجابى خاصة السلالة L1 تحت الجرعات 200 و300 جراى لأغلب الصفات فى كلا الموسمين. فى حين أن الجرعة 400 جراى كانت مميتة للسلالات L1 وL2 وL3.ووجد أن تقنية ISSR كانت فعالة في هذه الدراسة وخاصة البادئ ISSR-16 حيث بلغ معدل تعدد الأشكال 95.7٪ بينما كانت أقل نسبة تعدد الأشكال في التمهيدي R-04 حيث كان معدل تعدد الأشكال 40٪.

الكلمات الاسترشادية: الحمص وأشعة جاما والطفرات والواسم الجزيئي ISSR .