Morphological, agronomical and molecular characterization in irradiated Cowpea (Vigna unguiculata (L.) Walp.) and detection by start codon target markers

Asmaa Ezzat, Mohamed Adly and Ayman El-Fiki

*Horticulture Department, Faculty of Agriculture, Minia University, Minia, Egypt; †National Centre for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt

ABSTRACT
Cowpea (Vigna unguiculata (L.) Walp.) is one of the most important foods and economic vegetable crops in the world. Plant breeders resorted to using mutagenesis, especially gamma radiation to improve the yield production and protein content. Two Egyptian cowpea varieties Dokii 331 and Kaha 1 were exposed to different doses of gamma rays (0, 50, 100, 150, 200, 250 and 300 Gy) to obtain mutations of economic value and to identify genetic variation by SCoT markers. The exposure of cowpea CVs. Dokii 331 and Kaha 1 to these gamma radiation doses had a positive effect on the early flowering, increase of the number of primary branches number of flowers per plant, pods per plant and the number of seeds, and consequently crop quantity. The dose of 150 Gy was effective in obtaining a dwarf mutation in cv. Kaha 1 where the length of the plant reached 33.4 cm. While in cv. Dokki 331, the dose of 250 Gy induced a change in size and pod color and increase number of pods per plant. While the effect of dose 300 Gy caused to change the color of flower from white to violet and plant length until it reached to 277.4 cm. However, the dose 300 Gy caused changing in the flower color and plant length reached 277.4 cm. Genetic variation in both irradiated cultivars was determined by SCoT markers analysis. The isolated DNAs from irradiated plants were amplified by fifteen SCoT primers. It was obtained 219 bands identified in both cultivars. Dakii 331 (105) Band was given by Polymorphic band number 46 (43.81%). Kaha 1 was 114 Band by Polymorphic band number 55 (48.25%). Some SCoT markers were able to generate with a total of 26 specific markers. The cluster analysis based on Jacqaud’s similarity coefficients and UPGMA algorithms were calculated.

1. Introduction
Cowpea (Vigna unguiculata (L.) Walp.) is one of the most important vegetable legume crops in the world in general and particularly in Egypt. The world’s cultivated area is about 16 million hectares, so the cultivated area in Egypt is about 2832.8 hectares with a productivity 36,772 hg/ha (www.faostat.fao.org/faostat). Millions of people depend on cowpea as an important food source because of its high nutritional value. The grains contain a high protein content of 20-30%. This protein is rich in amino acids lysine and tryptophan (Steele, 1976; Yasmin, Arulbalachandran, Soundarya, & Vanmathi, 2019).

The genetic improvement of crops is one of the main factors for solving food shortage problems worldwide (Ronald, 2011), to meet the needs of continuous population growth (FAO, 2009; Ray, Mueller, West, & Foley, 2013; Tester & Langridge, 2010). In the absence of sufficient natural variations, in this case, these variations can be induced through chemical or physical mutagens.

Mutation breeding is one of the oldest breeding programs. Due to the limitations of some biotechnologies such as hybridization, cross-breeding, and genetically modified plants, breeding mutations program has become more common and widespread among plant breeders Oladosu et al. (2016). Physical mutagens (X-rays, UV light, neutrons-alpha-beta particles, fast and thermal neutrons, gamma rays) are more widely used than chemical mutagens ethyl methanesulfonate (EMS) because physical mutagens are more precise, safer, and cheaper than chemical mutagens (Jain, 2010; Singh & Krishna, 2006). Plant breeders turned to the use of mutagenic agents either chemical or physical to cause an increase in the rate of genetic variation. Gamma rays are the most common and used radiation in the mutation breeding program Beyaz and Yildiz (2017). Numerous studies have been carried out on radiation and have an impact on genetic, morphological and biological changes and the consequent different applications in many fields including agriculture, pharmacy, and medicine. These variations help breeders to crop improvements and acquire new varieties, as it is difficult to rely on spontaneous mutations in improvements to slow their occurrence (Tester & Langridge, 2010; Olasupo, Ilori, Forster, & Bado, 2016, 2018).

The induced mutations have played a large and effective role in the improvement of plants worldwide and
their effect has been clear and noticeable on the increase in the productivity of some crops, although most of these mutations are recessive and their impact harmful. The effect of mutation methods has already been evaluated to crop improvement through several publications (Jain, 2010; Maluszynski, Ahlloowalia, & Sigurbjornsson, 1995; Micke, Donini, & Maluszynski, 1990; Rutgers, 1992).

The mutation induced in plants has used a lot of functional genomics in model organisms and crops. There is a new method of plant genetic marker developed where it depends on the short conserved region flanking the ATG start codon in plant genes. Start codon targeted (SCot) markers polymorphism is a simple technique for generating gene-targeted markers in plants (Collard & Mackill, 2008; Xiong et al., 2011). The amplification profile of SCot-PCR indicated that the SCot marker is a dominant marker as RAPD and ISSR. The objective of this study is induced genetic variation in cowpea (Vigna unguiculata (L.) Walp.) by using gamma irradiation and the selection of some crop characteristics of economic value. As well as the identification and evaluation of these genetic changes using the SCot genetic marker.

2. Materials and methods

2.1. Seed material

Cowpea Seeds, Dokii 331 and Kaha 1 cultivars obtained from the Faculty of Agricultural, Horticulture Department, University of Minia.

2.2. Gamma radiation treatments

Irradiation was carried out with the ^{137}Cs source at the dose rate 1 Gy/2 min 14 sec, at National Center for Radiation Research and Technology, Cairo, Egypt.

Both cowpea cultivars Dokii 331 and Kaha 1 are exposed to different gamma radiation doses (50, 100, 150, 200, 250 and 300 Gy). The un-irradiated (control) and irradiated seeds in both cultivars were cultivated in a Randomized Complete Block Design (RCBD) at Experimental Farm of Horticulture Department, Faculty of Agriculture, Minia University, Minia, Egypt. The experiments were done in two seasons in April of 2017 and 2018 to get the first (M₁) and second (M₂) mutated generation. Morphological measurements for both mutated generations M₁ and M₂ were recorded and included % Emergence, number of branches, peduncle number per plant, plant heights (cm), fresh weight (g), pods number per plant, pod length (cm), seeds number per pod, hundred seed weight (g), and leaf length and width (cm).

2.2.1. Statistical analysis

To compare the varieties and their irradiated treatments, data were analyzed using ANOVA as described by Gomez and Gomez (1984) using MSTAT-C software version 4.

2.3. Genomic DNA extraction

Weighed about 1.5 g from irradiated plant leaves samples with different gamma radiation doses (0, 50, 100, 150, 200, 250 or 300 Gy) and ground by liquid nitrogen to obtain a fine powder. Total genomic DNA was isolated from leaves of each the two cowpea varieties and their irradiated treatments according to the protocol described by Anderson, Oghara, Sorrells, and Tanksley (1992) with a few modifications intended to improve the quality of DNA: two consecutive extractions with phenol: chloroform (1:1) were carried out by an additional wash of 97% (left at −20°C for one hour) an 70% pre-cooled ethanol, respectively. The yield and quality of DNA were assessed by spectrophotometer and gel electrophoreses.

2.4. SCot—PCR amplification

Fifteen (SCot) primers were selected according to Collard and Mackill (2009), Table 1. Amplification reactions were carried out in a total volume of 25 µl, which contained 250 µM of each primer, 0.2 mM of each deoxynucleotide, 1.5 mM MgCl₂, 1 unit Taq polymerase, and 50–100 ng of template DNA. All reaction volumes were 25 µl overlaid with a drop of mineral oil. The thermostrizing program used was: one cycle at 94°C for 3 min, 35 cycles at 94°C for 50 sec, 1 min at 50°C, 2 min at 72°C, and the final extension step of 7 min at 72°C. Electrophoresis was done to visualize the PCR amplified product. It was carried out on 1.0% agarose gel and amplified fragments were visualized by staining with ethidium bromide.

2.5. Data analysis

Fragment sizes of both SCot and ISSR were determined with PyElph 1.4 software Pavel and Vasile (2012) comparison with the marker. Amplified products were scored as present (1) or absent (0) to form a binary matrix.

In order to measure the informativeness of the markers to differentiate between genotypes, polymorphism information content (PIC) and marker index (MI) were calculated. PIC was calculated according to the formula of Anderson et al. (1992), as $\text{PIC} = 1 - \sum p_i^2$.

Table 1. SCot primers code and nucleotide sequences.

| No | Primer name | Sequence | %GC |
|----|-------------|----------|-----|
| 1  | SCoT –1     | 5'-CACAATGGCTACCCACA-3' | 50  |
| 2  | SCoT –2     | 5'-CACAATGGCTACCCCA-3' | 56  |
| 3  | SCoT –3     | 5'-CACAATGGCTACCCCG-3' | 56  |
| 4  | SCoT –4     | 5'-CACAATGGCTACCAAA-3' | 50  |
| 5  | SCoT –5     | 5'-CACAATGGCTACCCGCA-3' | 50  |
| 6  | SCoT –11    | 5'-AAGCAATGGCTACCAAA-3' | 50  |
| 7  | SCoT –12    | 5'-ACGACATGGCCGACACCCG-3' | 61  |
| 8  | SCoT –13    | 5'-ACGACATGGCCGACACACG-3' | 61  |
| 9  | SCoT –14    | 5'-ACGACATGGCCGACACGC-3' | 67  |
| 10 | SCoT –16    | 5'-ACGACATGGCCGACACACG-3' | 56  |
| 11 | SCoT –22    | 5'-ACGACATGGCTACCCACA-3' | 56  |
| 12 | SCoT –28    | 5'-CAGACATGGCTACCCACA-3' | 67  |
| 13 | SCoT –35    | 5'-CAGACATGGCTACCCGCCG-3' | 72  |
| 14 | SCoT –33    | 5'-CAGACATGGCTACCCGCCG-3' | 67  |
| 15 | SCoT –36    | 5'-CAGACATGGCTACCCGCCG-3' | 56  |
where $pi$ is the frequency of the $i$th allele of the locus in six gamma radiation treatments. MI was determined according to Varshney et al. (2007), as the product of PIC and effective multiplex ratio. To characterize genetic variation, some parameters, such as the effective number of alleles ($Ne$), Nei’s gene diversity ($H$) and Shannon’s information index ($I$) were calculated using PopGen 1.3.1 software, Yeh, Yang, Boyle, Ye, and Mao (1999). Jaccard’s similarity coefficient was calculated to construct a similarity matrix and the UPGMA algorithm was used to perform hierarchical cluster analysis and to construct a dendrogram by using MVSP, Ver 3.1 Kovach (1998).

3. Results

Two Egyptian cowpea varieties Dokii 331 and Kaha 1 were exposed to different gamma radiation doses 50, 100, 150, 200, 250 or 300 Gy. The morphological and agronomical alterations were characterized.

3.1. Morphological characterization

Gamma radiation doses have caused numerous morphological changes in both cultivars as shown in Figure 1. For example, the dose of 150 Gy in Kaha 1 cultivar caused changes in the form of leaf (Figure 1(b)), as well as an increase in the number of peduncles (Figure 1(c)), and also resulted in dwarfing in the plant, where the length of the plant reached to 33.4 cm (Figure 1(d)). On the other hand, in Dokii 331 cultivar, the irradiated dose 300 Gy resulted in a change in the color of the flower from white to violet (Figure 1(f)), the dose of 250 Gy also increased the number of pods per plant (Figure 1(g)), as well as all gamma radiation doses that were used caused changes in the size, form and color pods (Figure 1(h)), and the dose of 300 Gy had an effect on the plant height, where the length of the plant reached to 277.4 cm (Figure 1(i)).

3.2. Agronomical characterization

Radiation had a major role in producing agronomical changes that can be summarized in Table 2. The doses of radiation used in this experiment did not have a significant effect on emergence percentage. While the plant height was increased by increasing gamma radiation dose until the plant heights to 277.4 cm in the Dokii 331 cultivar with dose 300 Gy. While these doses had a different effect in the Kaha 1 cultivar, some of these doses had an effect on plant height increase, such as dose 50, 200, 250 and 300 Gy, while the doses of 100 and 150 Gy had a negative effect on plant height and it results dwarf plant, where the length of the plant reached to 33.4 cm (Figure 1(d)).

Figure 1. Illustrated gamma radiation doses 50, 100, 150, 200, 250 and 300 Gy impact on plant morphology. (a): control Kaha 1, (b) Dwarfism and change of leaf morphology, (c): Number of peduncles, (d): Intact dwarfism plant, (e): Control Dokii 331, (f): change in flower color, (g): Number of pods, (h): changes of pods color and size, (i): Intact giant plant.
Table 2. The effect of gamma irradiation on morphological and agronomical characterization in both Dokii 331 and Kaha 1 cultivars.

| Season          | Parameters                          | Cultivars | Gamma radiation doses/Gy | LSD | 0   | 50  | 100 | 150 | 200 | 250 | 300 | 0  | 50  | 100 | 150 | 200 | 250 | 300 | LSD |
|-----------------|-------------------------------------|-----------|--------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| season 2017 (M₁) | % Emergence                         | Dokii 331 |                          |     | 100 | 100 | 100 | 80  | 80  | 90  | 80  | 100 | 90  | 90  | 80  | 80  | 90  |     |     |
|                 | Plant heights (cm)                  |           |                          |     | 105.4 | E 142.9 D | 178.9 C | 192.1 BC | 198.3 BC | 216.3 B | 277.4 A | 26.86 | 74.73 D | 95.60 BC | 70.27 D | 33.40 E | 87.67 C | 101.2 B | 111.5 A | 8.505 |
|                 | No. of Branches                     |           |                          |     | 4.200 | 4.733 CD | 6.333 A | 4.600 CD | 6.067 B | 4.467 CD | 4.133 D | 0.9073 | 7.067 A | 5.200C | 5.133 C | 4.600CD | 6.067 B | 4.467 CD | 4.133 D | 0.7789 |
|                 | No. of peduncles/Plant              |           |                          |     | 8.600 | A 9.200 A | 5.733 C | 4.400 D | 5.200 CD | 5.600 C | 7.200 B | 0.8713 | 9.600 D | 9.600 D | 6.333 D | 46.60 A | 19.60 C | 21.20BC | 23.73 B | 3.704 |
|                 | Fresh weight (g)                    |           |                          |     | 0.348 | 0.372 D | 0.519 C | 0.451 CD | 0.671 B | 0.728 AB | 0.808 A | 0.1056 | 0.436 B | 0.402 B | 0.110 D | 0.321 C | 0.404 B | 0.609 A | 0.07594 |
|                 | Number of pods/plant                |           |                          |     | 18.53 | D 18.66 | 18.80 D | 19.80 D | 25.86 B | 23.93 C | 41.46 A | 1.894  | 21.40 BC | 21.808 | 21.33 BC | 35.40 A | 19.73 C | 19.80 D | 21.06 B | 1.566 |
|                 | Pod length (cm)                     |           |                          |     | 19.91 CD | 10.17 CD | 11.29 C | 13.01 D | 10.03 D | 12.39 B | 15.59 A | 1.071  | 11.39 B | 14.11 A | 11.20 BC | 9.97 D | 10.81 CD | 9.89 D | 12.05 B | 0.9886 |
|                 | Number of seeds/pod                 |           |                          |     | 6.33C | 7.66 B | 7.93 B | 9.26 A | 7.86 B | 7.86 B | 9.60 A | 0.3944 | 6.73 C | 6.73 C | 8.00 B | 9.26 A | 7.66 B | 7.00 C | 6.60 C | 0.4302 |
|                 | Weight of 100 seed (g)              |           |                          |     | 19.47 | AB 19.87 A | 19.30AB | 19.50AB | 19.57 AB | 19.92 A | 19.178 B | 0.6188 | 17.80C | 15.57D | 15.87 D | 21.07 A | 18.97 B | 18.93 B | 19.23 B | 0.3413 |
| season 2018 (M₂) | Plant heights (cm)                  | Kaha 1    |                          |     | 79.00 E | 98.00 D | 157.00 C | 112.00 D | 209.00 B | 235.00 A | 5.357 | 69.50 E | 95.70 A | 81.30 C | 30.5 F | 75.50 D | 95.70 A | 87.00 B | 5.357 |
|                 | No. of Branches                     |           |                          |     | 3.60 C | 3.70 C | 4.30 B | 5.10 A | 4.18BC | 4.40 B | 4.60 AB | 0.5258 | 5.30 ABC | 6.10 AB | 4.50 C | 6.60 A | 5.30 ABC | 4.20 C | 4.80 BC | 1.403 |
|                 | No. of peduncles/Plant              |           |                          |     | 9.80 A | 9.90 A | 7.30 B | 9.40 A | 9.50 A | 9.90 A | 9.40 A | 9.00 C | 7.00 C | 52.70 A | 19.60 B | 21.50 B | 22.10 B | 4.211 |
|                 | Fresh weight (g)                    |           |                          |     | 0.384 D | 0.372 D | 0.519 C | 0.451 CD | 0.671 B | 0.728 AB | 0.808 A | 0.1056 | 0.432 B | 0.436 B | 0.128 C | 0.415 B | 0.481 B | 0.561 C | 0.07285 |
|                 | Number of pods/plant                |           |                          |     | 19.60 C | 21.60 B | 21.70 B | 18.70 C | 19.40 C | 18.80 C | 29.20 A | 1.223  | 19.60 CD | 21.60 B | 21.70 B | 38.70 A | 19.40 CD | 18.80 B | 20.20 B | 0.7737 |
|                 | Pod length (cm)                     |           |                          |     | 10.68 B | 12.93 A | 9.75 C | 11.50 B | 11.27 B | 12.50 A | 11.00 | 8.64 BC | 12.58 A | 9.17 BC | 8.48 C | 8.84 BC | 9.49 B | 8.92 BC | 0.8919 |
|                 | Number of seeds/pod                 |           |                          |     | 6.20 D | 8.20 B | 7.60 BC | 7.20 BCD | 6.60 CD | 8.30 B | 10.60 A | 1.152  | 6.50 C | 9.50 A | 8.20 B | 6.50 C | 8.30 B | 8.80 B | 8.60 B | 0.2230 |
|                 | Weight of 100 seed (g)              |           |                          |     | 21.20 A | 20.77 AB | 18.57 C | 20.23 B | 18.47 C | 18.23 C | 18.03 C | 0.2608 | 15.83 D | 15.90 D | 15.80 D | 21.17 A | 20.03 B | 19.00 C | 18.83 C | 0.2049 |

Note: In each column mean of each treatment followed by the same letter (s) are not significant at 0.05 level of probability by Duncan’s Multiple Range Test (DMRT)
Plant height was 33.40 cm with dose 150 Gy. The doses of irradiation used had a positive effect on the number of branches in the Dokii 331 cultivar where the maximum increase was observed with the dose 200 Gy, while it had a negative effect in Kaha 1 cultivar. Peduncles number per plant increased with dose 50 Gy by 9.200 and decreased with other gamma radiation doses in Dokii 331 cultivar, but in Kaha 1 cultivar were decreased with dose 100 Gy while other doses 150, 200, 250 and 300 Gy induced significant increasing and the highest value was 46.60 shown with the dose 150 Gy. Gamma radiation doses had a positive effect on fresh weight in Dokii 331 cultivar, while in Kaha 1 cultivar had a negative effect except dose 300 Gy caused an increase in fresh weight. The number of pod per plant was increased with all gamma

Figure 2. Representative of fifteen SCoT primers profile of irradiated two Egyptian cowpea cvs Dokii 331 and Kaha 1 plants. Lane (1) Control (0 Gy); Lane (2) 50 Gy; Lane (3) 100 Gy; Lane (4) 150 Gy; Lane (5) 200 Gy; Lane (6) 250 Gy; Lane (7) 300 Gy for cv. Dokii 331; and from Lane (8) to (14) for the same doses cv. Kaha 1.
radiation doses in Dokki 331 cultivar and the maximum number 41.46 observed with dose 300 Gy, however in Kaha 1 cultivar observed significant increasing value 35.40 with dose 150 Gy, whereas the first two doses 50 and 100 Gy values were similar to control and the other doses 200, 250 and 300 Gy were a negative impact. The radiation doses had a negative effect on the pod length in both cultivars, except doses 50 and 300 Gy in the Kaha 1 cultivar where it had a positive effect. Numbers of seeds per pod were increased with gamma radiation doses and maximum number observed by dose 300 Gy in Dokki 331 cultivar, while in Kaha 1 cultivar gamma radiation doses had no significant effect except the dose of 150 Gy, which had a significant effect on increasing the number of seeds per pod. There were no significant differences in weight of 100 seed in Dokki 331 cultivar, but there was a decrease in doses 50 and 100 Gy, while the rest of the doses 150, 200, 250 and 300 Gy resulted in an increase and the highest value 21.07 g at the dose 150 Gy in Kaha 1 cultivar. The $M_2$ generation results of the second season were in the same trend as the $M_1$ generation of the first season with different data due to the high temperature from 7–9°C during the period of germination until flowering.

4. SCoT analysis

All fifteen SCoT primers used in the analysis of the cowpea varieties and the six radiation treatments for each were able to form polymorphic fingerprint patterns Figure 2. Fifteen primers produced 105 DNA fragments with an average of 7 bands per primer in Dokki 331 cultivar, however in the Kaha 1 cultivar; the primers produced 114 DNA fragments with an average 7.6 per primer (Table 3). The polymorphism number in the Dokki 331 cultivar was 46 (43.81%) with an average of 3.06 polymorphic bands per primer. The highest polymorphic band observed was with the SCoT-22 primer (7), while the lowest polymorphic band was one observed with the SCoT-33 primer. In the Kaha 1 cultivar, out of the total 114 amplified fragments, 55 (48.25%) were polymorphic with an average of 3.6 polymorphic bands per primer. The SCoT-12 primer had the ability to produce higher polymeric bands (6), however, SCoT-2 and SCoT-3 gave the lowest number of polymorphic band (1). The highest size of the band was 6239 bp and 4445 bp observed with SCoT-22 primer in Dokki 331 and Kaha 1, respectively. However, the lowest band size was 103 bp and 144 bp observed with SCoT-3 and SCoT-2 primer in Dokki 331 and Kaha 1 cultivars, respectively. The polymorphic information content (PIC) values varied from 0.235 (SCoT-3) to 0.497 (SCoT-22) with an average of 0.39 in the Dokki 331 cultivar, while were 0.109 (SCoT-3) to 0.584 (SCoT-4) with an average 0.37 in the Kaha 1 cultivar. Marker index (MI) values were calculated also for both cultivars. The lowest MI value was 0.04 with the SCoT-33 primer, while the highest MI value observed was with the SCoT-22 (0.34) primer. However, in Kaha 1 cultivar, the lowest and highest MI values observed were with the SCoT-2 and SCoT-3 primers (0.02) and the SCoT-4 primers, respectively.

5. Genetic diversity assessment by SCoT markers

Genetic diversity summarizes in Table 4 between the two cultivars of cowpea Dokki 331 and Kaha 1 and among their radiation treatments. In Dokki 331 cultivar, the effective number of alleles (Ne) value was ranged from 1.5455 ± 0.1472 (200 Gy) to 1.8188 ± 0.1583 (300 Gy). Nei’s genetic diversity ($H$) varied from 0.3481 ± 0.0553 (200 Gy) to

| Table 3. Amplification results generated by SCoT primers in irradiated Dokki 331 and Kaha 1 cowpea plants. |
|---------------------------------------------------------------|
| **Primer No.** | **Dokki 331** | | | **Kaha 1** | | |
| | **Total No.of Band** | **No. of Polymorphic band** | **Band size/ bp** | **MI** | **PIC** | **Total No.of Band** | **% of Polymorphism** | **Band size/ bp** | **MI** | **PIC** |
| SCoT-1 | 8 | 2 (25%) | 224-1718 | 0.06 | 0.245 | 9 | 3 (33.3%) | 400-1466 | 0.10 | 0.303 |
| SCoT-2 | 7 | 3 (42.8%) | 144-774 | 0.10 | 0.235 | 7 | 1 (14.3%) | 144-912 | 0.02 | 0.151 |
| SCoT-3 | 6 | 2 (33.3%) | 103-793 | 0.12 | 0.368 | 5 | 1 (20%) | 176-794 | 0.02 | 0.109 |
| SCoT-4 | 6 | 3 (50%) | 775-1630 | 0.20 | 0.409 | 8 | 5 (62.5%) | 333-1049 | 0.36 | 0.584 |
| SCoT-5 | 8 | 5 (62.5%) | 321-1465 | 0.25 | 0.489 | 7 | 4 (57.1%) | 385-2614 | 0.24 | 0.425 |
| SCoT-11 | 6 | 2 (33.3%) | 414-1581 | 0.12 | 0.387 | 6 | 3 (50%) | 414-1162 | 0.19 | 0.387 |
| SCoT-12 | 6 | 2 (33.3%) | 387-838 | 0.13 | 0.409 | 10 | 6 (60%) | 478-2357 | 0.28 | 0.481 |
| SCoT-13 | 8 | 3 (37.5%) | 336-3067 | 0.16 | 0.449 | 8 | 4 (50%) | 478-2228 | 0.19 | 0.393 |
| SCoT-14 | 7 | 4 (57.1%) | 481-1640 | 0.24 | 0.425 | 7 | 4 (57.1%) | 558-1822 | 0.13 | 0.235 |
| SCoT-16 | 8 | 4 (50%) | 211-2697 | 0.22 | 0.453 | 8 | 5 (62.5%) | 227-3665 | 0.27 | 0.437 |
| SCoT-22 | 10 | 7 (70%) | 174-6239 | 0.34 | 0.497 | 8 | 4 (50%) | 428-4445 | 0.23 | 0.477 |
| SCoT-28 | 8 | 4 (50%) | 330-3221 | 0.23 | 0.469 | 8 | 4 (50%) | 453-3089 | 0.14 | 0.293 |
| SCoT-35 | 6 | 2 (33.3%) | 373-1672 | 0.13 | 0.409 | 8 | 4 (50%) | 508-2971 | 0.16 | 0.337 |
| SCoT-33 | 5 | 1 (20%) | 342-1590 | 0.04 | 0.245 | 9 | 5 (56.5%) | 343-2560 | 0.24 | 0.441 |
| SCoT-36 | 6 | 2 (33.3%) | 294-1408 | 0.12 | 0.368 | 6 | 2 (33.3%) | 400-1466 | 0.18 | 0.544 |
| Total | 105 | 46 (43.8%) | | 2.46 | 5.87 | 114 | 55 (48.25%) | | 2.75 | 5.59 |
| Mean | 7 | 0.16 | 0.39 | 7.6 | 0.18 | 0.37 |
0.4461 ± 0.0509 (300 Gy). Shannon’s information index (I) ranged from 0.5310 ± 0.0612 (200 Gy) to 0.6372 ± 0.0537 (300 Gy). On the other hand, in the Kaha 1 cultivar the effective number of alleles (Ne) value varied from 1.5791 ± 0.1726 (50 Gy) to 1.8564 ± 0.2560 (100 Gy). Nei’s genetic diversity (H) ranged from 0.3602 ± 0.0648 (50 Gy) to 0.4453 ± 0.0489 (250 Gy). Shannon’s information index (I) ranged from 0.5440 ± 0.0714 (50 Gy) to 0.6365 ± 0.0516 (250 Gy).

6. Treatments specific markers

Some of the fifteen SCoT primers did not obtain specific markers, two primers with the Dokki 331 cultivar and eight primers with the Kaha 1 cultivar. However, the other SCoT primers succeeded to generate a total of 26 specific markers which ranged from one to two specific markers. There were some similarities in specific markers in both cultivars with primers SCoT-1, SCoT-5, SCoT-13, SCoT-14 and SCoT-33 Table 5.

7. Genetic relationships

The genetic identity and genetic distance of 12 gamma radiation treatments in both cowpea cultivars Dokii331 and Kaha 1 are presented in Table 6. The Nei’s genetic identity was the highest (0.9958) in treatments pairs 50 Gy in cv. Kaha 1. As well, the lowest genetic identity was (0.8263) in treatments pairs 100 Gy in the same cultivar. On the other hand, the highest Nei’s genetic distance was (0.1555) between the un-irradiated cv. Dokii 331 and 100 Gy in cv. Kaha 1. The lowest Nei’s genetic distance was (0.0043) within irradiated Dokii 331 with dose 200 Gy and irradiated Kaha 1 with dose 50 Gy.

Principle component analysis was used to illustrate genetic relationships between two cowpea genotypes and their gamma radiation treatments as shown in Figure 3. Of the total polymorphism, the first two components accounted for only 69.599%, this proves that the SCoT markers used in this study have an appropriate dispersion of markers in the genome. The two cowpea cultivars Dokii 331 and Kaha 1 and their gamma radiation treatments were clustered into four groups. The first group includes Kaha 1 cultivar and its radiation treatment of 100, 250 and 300 Gy, as well as radiation treatments of the Dokii 331 cultivar 150, 250 and 300 Gy. The second group includes the irradiation treatment of 150 Gy for the Kaha 1 cultivar and 50 Gy for the Dokii 331 cultivar. The third group includes irradiation of 50 and 200 Gy of the Kaha 1 cultivar. The fourth group includes both Dokii 331 and its radiation treatments 100 and 200 Gy. The analysis of principle components thus is largely compatible.
with those from cluster analysis obtained from UPGMA.

7.1. Discussion

Of the results obtained, it is clear that the gamma radiation treatments used in this experiment, 50, 100, 150, 200, 250 and 300 Gy have led to many morphological and agronomical changes. It was noted that of the doses of gamma radiation that made the most change, dose 150 Gy in the Kaha 1 cultivar was the most significant, as this dose led to changes in leaf size and shape, increase peduncles number and dwarf plants. The increase of peduncles due to the increase in the numbers of pods resulted in an increase in the yield. The irradiated plant with gamma-ray induced genetic variation and mutation leading to qualitative and quantitative alterations depend on the strength and duration of the gamma irradiation dose exposure, (Gnanamurthy, Mariyammal, Dhanavel, & Bharathi, 2012; Kim, Kim, Lee, Baek, & Kim, 1998; Wi et al., 2005). Gamma Radiation had a positive effect on the early flowering, increase the number of primary branches number of flowers per plant, pods per plant and the number of seeds, and consequently crop quantity, (Jan, Parween, Siddiqi, & Mahmooduzzafar, 2010; Khan et al., 2000) in legumes. However in French beans (Phaseolus vulgaris L.), gamma radiation had a negative impact on seed maturity, flowering, plant height, seed yield per plant, prolonged the growth period and retarded plant height (Svetleva & Petkova, 1992; Yousaf, Raziuddin, & Ahmad, 1991).

Table 5. The specific SCoT markers generated in both cultivars Dokii 331 and Kaha1 and their treatments.

| Primer's name | Varieties |
|---------------|-----------|
|               | Dokii 331 | Kaha1 |
| SCoT −1       | 1466 bp (50, 150, 250, 300 Gy); 1718 bp (150, 250, 300 Gy) | 1466 bp (50, 100, 200, 250, 300 Gy) |
| SCoT −2       | 774 bp (50, 150, 250, 300 Gy) | 0 |
| SCoT −3       | 793 bp (50, 250, 300 Gy) | 0 |
| SCoT −4       | 629 bp (150, 250, 300 Gy); 1049 bp (150, 200, 250 Gy); 757 bp (100 Gy) | 0 |
| SCoT −5       | 1465 bp (150 Gy); 1173 bp (150 Gy) | 1465 pb (50 Gy); 1173 pb (100 Gy) |
| SCoT −11      | 0 | 0 |
| SCoT −12      | 670 bp (100 Gy) | 0 |
| SCoT −13      | 2228 bp (150, 250 Gy); 1033 bp (150, 250 Gy); 2280 bp (300 Gy); 2228 bp (100, 300 Gy) | 0 |
| SCoT −14      | 1640 bp (50 Gy); 1018 bp (50, 150, 250, 300 Gy) | 1640 bp (250, 300 Gy) |
| SCoT −16      | 2697 bp (150, 250, 300 Gy); 1651 bp (15, 25, 30 Gy) | 0 |
| SCoT −22      | 4445 bp (150 Gy); 3231 bp (150, 300 Gy) | 0 |
| SCoT −28      | 1627 bp (250 Gy); 1377 bp (250 Gy) | 0 |
| SCoT −35      | 1590 bp (250, 300 Gy) | 1590 bp (100, 300 Gy) |
| SCoT −33      | 1408 bp (200, 250 Gy); 973 bp (100, 300 Gy); 2560 bp (100 Gy); 2522 bp (300 Gy) | 0 |

Table 6. Genetic identity (above diagonal) and genetic distance (below diagonal) values between two Egyptian cowpea cv. Dokii 331 and Kaha1 and their irradiated treatments.

| Cont. | Dokii 331 | 50 Gy | 100 Gy | 150 Gy | 200 Gy | 250 Gy | 300 Gy |
|-------|-----------|------|-------|-------|-------|-------|-------|
| Cont. | 0.9718 | 0.9519 | 0.9472 | 0.9379 | 0.9276 | 0.9173 | 0.9070 |
| 50 Gy | 0.0255 | 0.9827 | 0.9856 | 0.9894 | 0.9870 | 0.9846 | 0.9812 |
| 100 Gy | 0.0091 | 0.9876 | 0.9944 | 0.9972 | 0.9939 | 0.9908 | 0.9864 |
| 150 Gy | 0.0559 | 0.9912 | 0.9940 | 0.9968 | 0.9990 | 0.9990 | 0.9990 |
| 200 Gy | 0.0146 | 0.9944 | 0.9972 | 0.9990 | 0.9990 | 0.9990 | 0.9990 |
| 250 Gy | 0.0286 | 0.9974 | 0.9990 | 0.9990 | 0.9990 | 0.9990 | 0.9990 |
| 300 Gy | 0.0493 | 0.9990 | 0.9990 | 0.9990 | 0.9990 | 0.9990 | 0.9990 |

| Cont. | Kaha1 | 50 Gy | 100 Gy | 150 Gy | 200 Gy | 250 Gy | 300 Gy |
|-------|------|------|-------|-------|-------|-------|-------|
| 50 Gy | 0.1407 | 0.8888 | 0.8827 | 0.8869 | 0.8919 | 0.8969 | 0.8969 |
| 100 Gy | 0.0138 | 0.8560 | 0.8571 | 0.8597 | 0.8637 | 0.8697 | 0.8697 |
| 150 Gy | 0.0091 | 0.9827 | 0.9856 | 0.9894 | 0.9939 | 0.9908 | 0.9908 |
| 200 Gy | 0.0559 | 0.9912 | 0.9940 | 0.9968 | 0.9990 | 0.9990 | 0.9990 |
| 250 Gy | 0.0146 | 0.9944 | 0.9972 | 0.9990 | 0.9990 | 0.9990 | 0.9990 |
| 300 Gy | 0.0286 | 0.9974 | 0.9990 | 0.9990 | 0.9990 | 0.9990 | 0.9990 |
These results are in agreement with the results of Ogidi, Omosun, Markson, & Kalu, 2010; Horn & Shimelis, 2013; Badr et al., 2014; Olasupo et al., 2016, 2018), where they found that doses more than 100 Gy lead to a reduction of cowpea leaf size and plant height. On the other hand, in cv. Dokii 331, the highest doses 200 Gy showed the morphological and agronomical alterations. It was observed that the number of branches increased with the dose of 200 Gy. The plant height, number of pods per plant, pods length, and the number of seeds per pod, as well as the fresh weight, were increased with the dose 300 Gy. These results are agreement with (Horn, Ghebrehiwot, & Shimelis, 2016; Singh & Krishna, 2006), this dose also caused changes in the flower color and the color of the pods. These results are consistent with (Horn et al., 2016; Olasupo et al., 2018).

The Start codon-targeted markers (SCoT) technique has had a major role in the identification, characterization and genetic comparison between many plant varieties. These results were in accordance with Huang et al. (2014) for Hemarthria, Que et al. (2014) for sugarcane, Gajera, Bambharolia, Domadiya, Patel, and Golakiya (2014) for mango, Gao et al. (2014) for Lycomis, Fang-Yonga and Ji-Honga (2014) for Myrica rubra, Jiang et al. (2014) for orchard grass, Satya et al. (2015) for ramie (Boehmeria nivea L. Gaudich.), Zhang, Xie, Wang, and Zhao (2015) for Elymus sibiricus, Vivodík, Gálová, Balážová, and Petrovičová (2016) for maize.

Radiation is one of the best and most successful mutagens used in plant breeding programs. It has the ability to make large genetic changes in a safe and conclusive manner in a short time. Thus, the plant breeder has a large area of selection and improvement of plants. This has had a significant impact on the cowpea, where high-value mutations such as the dwarf mutant, highly productive obtained in kaha 1 cultivar resulting from irradiation treatment 150 Gy. The use of SCoT marker has been of great importance in the characterization and identification of genetic variation between varieties and radiation treatments with high accuracy.

Acknowledgments

This research was done in collaboration with National Centre for Radiation Research and Technology, Egyptian Atomic Energy Authority and Horticulture Department, Faculty of Agriculture, Minia University, Minia, Egypt.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Anderson, J. A., Ogihara, Y., Sorrells, M. E., & Tanksley, S. D. (1992). Development of a chromosomal arm map for wheat based on RFLP markers. *Theoretical and Applied Genetics*, 83(8), 1035–1043.

Badr, A., Sayed Ahmed, H. I., Hamouda, M., Halawa, M., & Elhiti, M. A. (2014). Variation in growth, yield and molecular genetic diversity of M2 plants of cowpea following exposure to gamma radiation. *Life Sciences - Journal*, 11((8)), 10–19. http://www.lifesciencesite.com

Beyaz, R., & Yildiz, M. (2017). The use of gamma irradiation in plant mutation breeding. *ntechopen*, 33–46. doi:10.5772/intechopen.69974

Collard, B. C. Y., & Mackill, D. J. (2008). Start Codon Targeted (SCoT) polymorphism: A simple, novel DNA marker technique for generating gene-targeted markers in plants. *Plant Molecular Biology Reporter / ISPMB*, 27, 86–93.

Fang-Yonga, C., & Ji-Honga, L. (2014). Germplasm genetic diversity of Myrica rubra in Zhejiang Province studied using inter-primer binding site and start codon-targeted polymorphism markers. *Scientia Horticulturae*, 170, 169–175.

FAO. (2009). FAO’s director-general on how to feed the world in 2050. *Population and Development Review*, 35(4), 837–839.
A software tool for gel mutation breeding revolutions

Gao, Y. H., Zhu, Y. Q., Tong, Z. K., Xu, Z. Y., Jiang, X. F., & Huang, C. H. H. (2014). Analysis of genetic diversity and relationships among genus Lycoris based on start codon targeted (SCot) marker. Biochemical Systematics and Ecology, 57, 221–226.

Gnanamurthy, S., Mariyammal, S., Dhanavel, D., & Bharathi, T. (2012). Effect of gamma rays on yield and yield components characters R3 generation in cowpea (Vigna unguiculata (L.) Walp.). International Journal of Plant Sciences, 2, 39–42.

Gomez, K. A., & Gomez, A. A. (1984). Statistical procedures for agriculture research (pp. 680). New York: John Wiley&Sons.

Horn, L., & Shimelis, H. (2013). Radio-sensitivity of selected cowpea (Vigna unguiculata) genotypes to varying gamma irradiation doses. Scientific Research and Essays, 8, 1991–1997.

Horn, L. N., Ghebrehiwot, H. M., & Shimelis, H. A. (2016). Selection of novel cowpea genotypes derived through gamma irradiation. Frontiers in Plant Science, 7, 262–272.

Huang, L., Huang, X., Yan, H., Yin, G., Zhang, X., Tian, Y., … Nie, G. (2014). Constructing DNA fingerprinting of Heterothria cultivars using EST-SSR and SCot markers. Genetic Resources and Crop Evolution, 61(6), 1047–1055.

Jain, S. M. (2010). Mutagenesis in crop improvement under the climate change. Romanian Biotechnological Letters, 15(2), 88–106.

Jan, S., Parween, T., Siddiqi, T. O., & Mahmooduzzafar, X. (2010). Gamma radiation effects on growth and yield attributes of Psoralea corylifolia L. with reference to enhanced production of psoralen. Plant Growth Regulation, 64(2), 163–171.

Jiang, L. F., Qi, X., Zhang, X. Q., Huang, L. K., Ma, X., & Xie, W. G. (2014). Analysis of diversity and relationships among orchardgrass (Dactylis glomerata L.) accessions using start codon-targeted markers. Genetics and Molecular Research: GMR, 13(2), 4406–4418.

Kim, J. S., Kim, J. K., Lee, Y. K., Baek, M. W., & Kim, J. G. (1998). Effects of low dose gamma radiation on the germination and yield components of Chinese cabbage. Korean Journal of Environmental Agriculture, 17, 274–278.

Kovach, W. L. (1998). MVSP: A multivariate statistical package for windows, ver. 3.1. Pentraeth, Wales: Kovach computing services.

Maluszynski, M., Ashloovalia, B. S., & Sigurbjornsson, B. (1995). Application of in vivo and in vitro mutation techniques for crop improvement. Euphytica, 85(30), 3–315.

Micka, A., Donini, B., & Maluszynski, M. (1990). Induced mutations for crop improvement. Mutation Breeding Revolutions, 7, 1–41.

Ogidi, E. G. O., Omosun, G., Markson, A. A., & Kalu, M. (2010). Effects of gamma ray irradiation on the genetic traits of vegetable cowpea (Vigna unguiculata (L.) walp) in Umudike Southern Nigeria. Asian Journal of Science and Technology, 5, 86–90.

Oladosu, Y., Rafiu, M. Y., Abdullah, N., Hussin, G., Ramli, A., Rahim, H. A., … Usman, M. (2016). Principle and application of plant mutagenesis in crop improvement: A review. Biotechnology & Biotechnological Equipment, 30(1), 1–16.

Olasupo, F. O., Ilori, C. O., Forster, B. P., & Bado, S. (2016). Mutagenic effects of gamma radiation on eight accessions of cowpea (Vigna unguiculata [L.] Walp.). American Journal of Plant Sciences, 7, 339–351.

Olasupo, F. O., Ilori, C. O., Forster, B. P., & Bado, S. (2018). Selection for novel mutations induced by gamma irradiation in cowpea (Vigna unguiculata [L.] Walp.). International Journal of Plant Breeding and Genetics, 12(1), 1–12.

Pavel, A. B., & Vasile, C. I. (2012). PyElph – A software tool for gel images analysis and phylogenetics. BMC Bioinformatics, (13), 13–19.

Que, Y., Pan, Y., Lu, Y., Yang, C., Yang, Y., Huang, N., & Xu, L. (2014). Genetic analysis of diversity within a chinese local sugarcane germplasm based on start codon targeted polymorphism. BioMed Research International, 5, 1–10.

Ray, D. K., Mueller, N. D., West, P. C., & Foley, J. A. (2013). Yield trends are insufficient to double global crop production by 2050. PLoS One, 8(6), e66428.

Ronald, P. (2011). Plant genetics, sustainable agriculture and global food security. Genetics, 188(1), 11–20.

Rutger, J. N. (1992). Impact of mutation breeding in rice - a review. Mutation Breeding Revolutions, 8, 1–24.

Satya, P., Karana, M., Jana, S., Mitra, S., Sharma, A., Karmakar, P. G., & Rayb, D. P. (2015). Start codon targeted (SCot) polymorphism reveals genetic diversity in wild and domesticated populations of ramie (Boehmeria nivea L. Gaudich.), a premium textile fiber producing species. Meta Gene, 3, 62–70.

Singh, V. V., & Krishna, K. R. (2006). Induced chemical mutagenesis in cowpea (Vigna unguiculata L. Walp). Indian Journal of Genetics, 66(4), 312–315.

Steele, W. M. (1976). Evaluation of crop plant (pp. 339–343. (N. W. Simmonds, ed.). London: Longman.

Svetleva, D., & Petkova, S. (1992). Association between changes in the M1 and mutability in the M2 in the French bean variety 564 after combine treatment with γ radiation and N-allyl-N-nitrosourea. Genetika-i-Selektsiva, 25, 254–260.

Tester, M., & Langridge, P. (2010). Breeding technologies to increase crop production in a changing world. Science, 327 ((5967)), 818–822.

Varshney, R. K., Marcel, T. C., Ramsay, L., Russell, J., Röder, M. S., Stein, N., … Graner, A. (2007). A high density barley microsatellite consensus map with 775 SSR loci. Theoretical and Applied Genetics, 114(6), 1091–1103.

Vivodik, M., Gálová, Z., Balážová, Z., & Petrovičová, L. (2016). Start codon targeted (SCOT) polymorphism reveals genetic diversity in European old maize (Zea mays L.) genotypes. Potravinarstvo Slovak Journal of Food Sciences, 10(1), 563–569.

Xiong, F., Zhong, R., Han, Z., Jing, J., He, L., Zhuang, W., & Tang, R. (2011). Start codon targeted polymorphism for evaluation of functional genetic variation and relationships in cultivated peanut (Arachis hypogaea L.) genotypes. Molecular Biology Reports, 38, 3487–3494.

Yasmin, K., Arulbalachandran, D., Soundarya, V., & Vannamathi, S. (2019). Effects of gamma radiation (y) on biochemical and antioxidant properties in black gram (Vigna mungo L. Hepper). International Journal of Radiation Biology, 95(8), 1–20.

Yeh, F. C., Yang, R. C., Boyle, T. B. J., Ye, Z. H., & Mao, J. X. (1999). POPGENE. The user friendly software for population genetic analysis.

Yousaf, H., Raziiuddin & Ahmad, H. (1991). Morphology and chemical studies of irradiated lentil (Lens culinaris Med.). Sarhad Journal of Agriculture, 7, 361–368.

Zhang, J., Xie, W., Wang, Y., & Zhao, X. (2015). Potential of Start Codon Targeted (SCOT) markers to estimate genetic diversity and relationships among Chinese Elymus sibiricus accessions. Molecules, 20(4), 5987–6001.