Variation in Growth, Yield and Molecular Genetic Diversity of M2 Plants of Cowpea Following Exposure to Gamma Radiation

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Abstract: Seeds of five cowpea varieties, Kaha 1, Dokki 331, Azmerly, Cream 7 and Giza 6, were exposed to different doses of gamma radiation at50, 100, 200 and 300Gy. Some growth parameters and yield components were measured in 22 M2 genotypes. Variation in seed protein electrophoretic pattern, RAPD and ISSR fingerprinting was scored to assess genetic variation among the M2 genotypes. T he gamma dose of 50 Gy resulted in an increase of growth parameters and enhanced yield components in the three varieties Dokki 331, Azmerly and Cream 7; while the dose of 100 Gy resulted in higher growth rate and yield in var. Kaha 1 and var. Giza 6. Analysis of seed protein profile indicated specific bands for each variety; two bands appeared only in control plants and two other bands appeared inM2 plants of exposed seeds. Seven RAPD primers produced 30 polymorphic and 30 monomorphic bands. Meanwhile, 54 markers including 45 polymorphic bands were produced by the nine ISSR primers. Gamma radiation induced more genetic variation in the genotypes of var. Kaha 1 and var. Dokki 331 compared to other varieties, as estimated by the cluster analysis of seed protein, RAPD and ISSR markers.
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Introduction

Cowpea [*Vignaunguiculata* (L.) Walp.] is one of the most important food and forage legumes in the semi-arid tropics of Asia, Africa, Southern Europe, Southern United States, and Central and South America (Singh 2005; Timko *et al.,* 2007).It is a major source of protein as unripe pods and dry seeds (Singh, 2002) and as fodder (Pasquet and Baudoin, 2001; Tarawali *et al.,*2002). Cowpea is therefore regarded as a truly a multifunctional crop, providing food for man and feed for livestock and serving as a valuable and dependable revenue-generating commodity for farmers and grain traders (Timko and Singh, 2008).

Gamma rays are ionizing radiation with low linear energy transfer (LET), generating non-transmissible mutations via complicated chromosomal alterations at high doses (Sachs *et al.,*2000). However, low doses of gamma ray irradiation have been used for mutant isolation in conventional plant breeding (Chopra, 2005; Albokari *et al.,*2012). It is often used to develop varieties that are agriculturally and economically important and have high productivity potential (Jamiland Khan, 2002; Muthusamy *et al.,*2003). Many mutant crop varieties including resistant to diseases, cold, salt and with desired qualities have been developed using gamma rays (Jain *et al.,*1998; Chopra, 2005; Gnanamurthy *et al.,*2012; Mehlo *et al.,*2013; Tshilenge-Lukanda *et al.,*2013).

Changes in seed storage protein markers as revealed by electrophoresis have been successfully used to resolve and characterize species and varieties in several legumes such as *Trifolium* (Badr *et al.,*1995), *Lathyrus* (Badr *et al.,*2002) *Lupinus* (El-Shazly *et al.,*2006) and*Vigna* (Ghafoor& Ahmad, 2005; Ghafoor *et al.,*2002).Two cowpea cultivars exposed to three mutagens; sodium azide (NaN_3), ethyl methane sulphonate (EMS) and gamma rays showed differences in number and intensity of bands, (Osanyinpeju and Odeigah, 1998).

The random amplified polymorphic DNA (RAPD)amplified by arbitrary primers (Williams *et al.,*1990) andthe inter-simple sequence repeats (ISSRs)whichreveal regions that lie within the microsatellite repeats (Zietkiewicz *et al.,*1994; Goodwin *et al.,*1997; Reddy *et al.,*2002) determine intra-genomic and inter-genomic diversity and has been commonly applied in legume diversity analysis and breeding. Ba *et al.,*(2004) used RAPD to characterize genetic variation in domesticated cowpea and its wild progenitor, as well as their relationships. RAPD markers were also used to evaluate the genetic diversityin a representative population of cowpea from different eco-geographical regions of India (Prasanthi *et al.,*(2012). Diouf and Hilu (2005) applied RAPD and SSR markers in determining genetic diversity among cowpea breeding lines and local varieties in Senegal.

Opportunities for improving cowpea exist by the use of breeding more productive cultivars and by using marker-assisted selection that will likely increase the overall efficiency and effectiveness of cowpea improvement programs. The main objective of the present study was to determine the effect of low doses of gamma radiation on some varieties of cowpea at morphological and molecular levels in the M2 generation. Seed Protein banding patterns were studied using sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE) technique. In addition, molecular fingerprinting was achieved using the RAPD and ISSR markers. Variations in molecular markers have been related to changes in growth parameters and yield components and used to assess genetic variation among the M2 genotypes.

2. Material and Methods

Plant material

Seeds of five cowpea varieties;Kaha1,Dokki 331, Azmerly, Cream 7 and Giza 6 were kindly provided by the Agriculture Research Center, Giza, Egypt and exposed to four different doses of gamma radiation i.e. 50, 100, 200 and 300 Gy at the Atomic Energy Center, Nasr City, Cairo; Egypt using cobalt 60 as a source. Exposed and control seeds of the five cowpea varieties were grown to maturity in 50 cm wide pots. Seeds were collected from all M1 plants except for the plants of the varieties Dokki 331, Azmerly, and Cream 7 following exposure to 300 Gy which did not produce seeds in the first generation. The seeds of the M1 generation were cultivated in the field and grown for the present study under the recommended conditions for growing cowpea, plants were irrigated every 10 to 14 days from sowing until maturity and phosphate fertilizer was applied at 9kg/acre as a side band at the seedling stage and fruiting stage. Morphological measurements were recorded for six weeks old M2 plants and included shoot length, root length, and shoot fresh and dry weight. Four yield components were determined at maturity; these are the number of pods/ plant, pod length, number of seeds/ pod and weight of 100 seeds. Data were statistically analyzed using ANOVA to compare treatments and cultivars using the software SPSS V17.

Protein extraction and electrophoreses

For protein extraction, seeds of M2 plants were ground to fine powder and proteins were extracted in Tris-HCl buffer-pH 8 containing 5% glycerol and 0.1% β-mercaptoethanol. The extracted protein solutions were resolved in12.5% polyacrylamide gel using a Pharmacia low molecular weight standard molecular weight marker in a Cole Parmer vertical gel electrophoresis apparatus (Model SE400). At the end of electrophoresis, protein bands were revealed by Comassie Brilliant Blue R-250 staining and destained by methanol and acetic acid solution for overnight. The gel was then photographed with Kodak digital camera ModelAF3X optical aspheric lens,9.2 mega bixel, and molecular weight for protein bands was calculated using the Lab Image software version 2.7 produced by Kapelan GmbH, Germany.

DNA extraction

DNAwas extracted from leaves of two weeks old M2 seedlings, grown in pots in the laboratory at 20ºC,using the DNA-easy Plant Mini kit (Qiagen, USA, Cat. # 69104) as described in the instruction manual. The quality and quantity of the extracted DNA were measured using nano-drop 2000C (Thermo Scientific) and its integrity was tested in1%agarose gel. DNA concentration in all samples was adjusted to 25 ng/µl for PCR reactions.

RAPD protocol

For RAPD finger-printing,as outlined by Williams *et al.*(1990) a total of 16 random primers were tested for production of RAPD fragments, only seven of them revealed clear readable and constant profiles (Table 2).The RAPD-PCR procedure was performed as described in Sambrook and Russell (2001) in a total of 25 µl reaction mixture as the following; 1 µlDNA (25 ng/),1 µl MgCl2 (50 mM), 2 µl primer (10 nM),12.5 µl Bio-Mix Red (GE Healthcare UK Limited, Amersham, UK) and 8.5 µl of nuclease free water.The applied amplification condition was as follows: 95ºC for 5 min as initial denaturation followed by 40 cycles of 95ºC for 30sec., 38ºC for 45sec., and 72ºC for 1 min. The PCR products were left at 72ºC for 15 min for final extension. The PCR products were screened using 1.5% agarose gel.

ISSR protocol

The ISSR fingerprinting was performed usinga protocol,developed by Sigma (Germany), and does not involve DNA extraction. The procedures recommended by the manufacturer have been followed. In brief, a small disc of fresh leaves taken from actively growing seedlings using a 50 mm Harris Uni-core puncher supported by cutting mat. The disc was added directly into 25 µl PCR reaction mix containing 25 mM MgCl2, 1X PCR buffer, 200 µMdNTPs (Applied Biosystems), 1U of Taq DNA polymerase (Applied Biosystems, Ampli-Taq Gold), 2 pmole of each primer, and the leaf disc. Polymerase chain reaction was made for amplification of ISSR fingerprinting. The PCR amplification was performed using Bio-Rad thermo-cycler according to the following cycle profile: initial denaturation at 95°C for 10 min, followed by 35 cycles of 30 sec at 94ºC, 30 sec at 60°C and 30 sec at 72°C,and5 min at 72°C for final product extension. A total of ten ISSR

primers were used and only nine primers produced a clear readable profile (Table 2).

The PCR products of RAPD were separated in 1.5% agarose gel containing 0.5 µg/ml ethidium bromide using a submarine EC370 Minicell and an EC105 power supply (EC Apparatus corporation, USA). The DNA size was calibrated against Hyper-Ladder I (GE Health care UK Limited, Amersham, UK. Meanwhile, the ISSR PCR products were separated in 1.8% agarosegel. The DNA size was calibrated against 100 bp ladder (Thermo scientefic). The Lab image program version 2.7 produced by Kapelan Bio-Imaging GmbH (2003) was used for DNA size determination.

The presence or absence of protein, RAPD and ISSR bands was scored as 1 for presence or 0 for absence of markers respectively for estimating genetic variation.Euclidian distance (Romesburg, 1990) was calculated and used for measuring the similarity between the parent varieties and the M2 genotypes using the software program, Community Analysis Package 4.0 (CAP) developed and produced by Seabyand Henderson (2007). The CAP software was used for to clustering the M2 genotypesbased on distance estimates using the agglomerative clustering analysis and the average linkage tree building method.

3.Results

Most doses of Gamma-irradiation induced increment of the measured morphological criteria and yield components compared to the control plants (Table 3). Shoot length of control varieties ranged from 20.03±0.21 cm in var. Cream 7 to 24.27±0.65 cm in var. Kaha1; the latter variety showed the highest increase in shoot length (38.67±0.78 at the dose of 100 Gy. The root length ranged from 6.93±0.06 cm in var. Cream 7 to 10.93±0.15 cm in var.Dokki331 which showed the highest value for root length (18.03±0.32) at the dose of 50 Gy of gamma rays). Fresh and dry weight on control varieties were highest in var.Azmerely (5.67±0.12 g & 0.69±0.02 g), however the highest increase in fresh weight was recorded in var. Dokki331 (12.60±0.27) at the dose of 50 Gy while the highest increase in dry weight showed the highest score (0.97±0.02 g) in var. Kaha 1 at the dose of 100Gy.

The number of pods/plant in control plants ranged from 13.67±1.53 in var. Kaha1 to 23.67±2.08 in var. Azmerly, however, the highest increase in pod number/plant was scored at the dose of 100 Gy in var. Giza6. On the other hand pod length ranged between 10.63±0.32 cm in var. Kaha1 and 12.60±0.36 cm in var. Cream7 and the most evident increase was observed at the dose of 100 Gy in the two varieties Kaha1 (17.27±0.25 cm) and Giza6 (17.73±0.15 cm). The number of seeds/pod ranged between6.00±0.00 in var. Kaha1 and 7.33±0.58 in var. Azmerly, however two most significant increases were scored at the dose of 100 Gy in the var. Kaha1 (12.67±0.58) and var. Giza 6 (12.33±0.58). The weight of 100 seeds in control plants ranged between 15.88±0.01g in var. Giza 7 to 18.76±0.30 g in var.Azmerly, the most prominent increase was found in var. Giza6 (18.73±0.19 g) but the highest weight of 100 seeds/plant was recorded for var. Azmerly (20.29±0.28 g) following exposure to 50 Gy of g-radiation.

In brief, the 50Gy treatments resulted in prominent increases in all measured traits of the three varieties Dokki, Azmerly and Cream 7, while the 100 Gy dose resulted in most prominent increase invar. Kaha 1 and var. Giza 6. The var. Kaha 1showedthe highest increase in shoot length, dry weight, pod length and number of pods/plant at the dose of 100 Gy as compared to the control. Variety Dokki however, showed the highest increase in fresh weight and var. Cream 7 showed the highest increase in root length and weight of 100 seeds compared to the control. On the other hand, var. Azmerly, showed the least increase in most studied yield parameters; number of seeds per pod, number of pods per plant and weight of 100 seeds (19.35±0.15g), as compared to the control values.

Protein polymorphism in M2 plants

Figure 1illustrates the SDS-PAGE electrophoretic banding pattern of seed protein polypeptides for the M2 generation of cowpea following seeds exposure to the applied doses of g-radiation. In total, 30 protein bands ranging from 9 kDa to 151 kDawere observed; 15 bands are polymorphic and 15 are monomorphic. A band with molecular weight of 146 kDa appeared only in control plants but not in M2 genotypes from seeds exposed to g-radiation. On the other hand, specific bands for each variety were observed such as a 87 kDain var. Dokki 331, 75 kDa in var. Kaha1, 59 kDa in var. Azmerly, 50 kDa in var. Giza and 35. kDa in var.. Cream7.New bands of 27 kDa appear only at the dose of 300 Gy in the M2 plants of both var. Kaha 1 and var. Giza 6, while a 44 kDa band appeared in var.Kaha1 and var.Dokki331 and a 65 kDa appeared in the rest of varieties. Finally one 18 kDaband was absent in control plants but was evident in all varieties following exposure to all of the applied doses of g-radiation.

RAPD and ISSR fingerprinting in M2 plants

Seven primers revealed stable and reproducible RAPD polymorphism in the generated DNA of M2 plants of cowpeaM2 genotypes; the number of bands, number of polymorphic bands and percentage of polymorphism and the size range of markers for each primer are given in Table 1. A total

of 60 bands including 30 polymorphic and 30 monomorphic markers were recorded. RAPD fingerprinting for the primers numbered 1, 2, and 3 (Table 1) are shown in Figure 2 A, B and C respectively; polymorphic markers are arrowed. The highest number of bands (10) was produced by primer 3 and the lowest number (6 bands) was produced by primer 6; primer 2 showed the highest percentage of polymorphism (75.0%).Relatively high percentages of polymorphism were scored by the primers 1, 2 and 3 compared to the four primers 4,5,6 and 7; the percentage of polymorphism revealed by the latter primers ranged between 12.5% for primer 4 and 22.5% for primer 7. The variation in markers as revealed by primers 1 and 2 is due to exposure to low doses of g-radiation, while markers revealed by primer 3 showed variety specific markers with little variation as a result of seed exposure to g-radiation.

The RAPD polymorphic markers varied between primers and varieties. However, a number of bands were specific for certain varieties; these bands are particularly evident in the profile of primer 3 (Figure 2C). Other markers were scored in the M2 genotypes following seed exposure to g-radiation. The most prominent were the polymorphism revealed by primer for var. Dokki 331 at 50 Gy and Kaha 1 following most of the doses applied. More specific markers were found for the varieties Dokki 331, Azmerly and Giza 6 by primer 2 following seed exposure to 100 Gy and in all genotypes by primer 3 following seeds exposure to 50 Gy. Additional specific bands were observed in var. Giza 6 by primer 5 following exposure to 100 Gy and var. Kaha 1 by primer 7 after exposure to 50 Gy and also in var. Dokki 331 after exposure to 200 Gy and var. Cream 7 following exposure to 50 Gy and 100 Gy.

ISSR fingerprinting in M2 plants

Out of the ten ISSR primers used in this study, nine gave rise to reproducible markers (Table 2). A total of 54markers including45 polymorphic markers were revealed in the 22 genotypes. In general, the size of the markers in DNA bp, produced by the ISSR primers, is much smaller than that of the RAPD markers. The highest number of ISSR markers was 7 and was revealed by four of the nine primers; 17898B, 17899A, HB8 and HB14 bands; three primers (HB11, HB12 and HB13) revealed six markers and the remaining two primers (HB9 and HB10 revealed only four markers. Relatively high percentages of polymorphism were scored by all primers in the 22 genotypes of cowpea; it ranged from 75% by primers HB 9 and HB 10 to 85.6% by four primers (Table 2); the smallest marker revealed by all nine markers was monomorpic to all genotypes. New markers appeared after all treatments but few markers were found only in control plants; some markers disappeared at high doses of g-radiation. The fingerprinting patterns revealed by three ISSR primers are shown in Figure 3; polymorphic bands are arrowed.

Table 1 Sequence of seven RAPD primers that revealed polymorphism in cowpea M2 genotypes and the number of total and polymorphic bands, and percentage of polymorphism for each primer.

Primer number	Primer sequence	Number of bands	Number of polymorphic bands	Percentage of polymorphism	Markers size range in DNA bps
1	5'CCTGGGCTT C3'	9	6	66.67%	1500-350
2	5'CCTGGGTTC C3'	9	6	66.67%	2000-400
3	5'CCTGGGCTT A3'	10	7	70.0%	1500-300
4	5'GAGGGCGGGA3'	8	1	12.5%	1500-350
5	5'GAGGGCGTGA3'	9	2	22.2%	2100-400
6	5'GGGCCCGAGG3'	6	1	16.7%	1250-300
7	5'GGGGGCTTGG3'	9	2	22.2%	2000-400
Total number		60	30		

Table 2 Sequence of nine ISSR primers that produced polymorphism in cowpea M2 genotypes, number of total and polymorphic bands and the percentage of polymorphism.

Primer code	Sequence of primer	Number of bands	Number of polymorphicbands	Percentage of polymorphism	Markers size range in DNA bps
17898 B	5'CACACACACACAGT3'	7	6	85.6%	577-240
17899 A	5'CACACACACACAAG3'	7	6	85.6%	875-332
HB-8	5'GAGAGAGAGAGAGG3'	7	6	85.6%	557-214
HB-9	5'GTGTGTGTGTGTGG3'	4	3	75.0%	421-156
HB-10	5'GAGAGAGAGAGACC3'	4	3	75.0%	333-197
HB-11	5'GTGTGTGTGTGTCC3'	6	5	80.0%	534-204
HB-12	5'CACCACCACGC3'	6	5	83.3%	774-124
HB-13	5'GAGGAGGAGGC3'	6	5	85.6%	532-157
HB-14	5'CTCCTCCTCGC3'	7	6	85.6%	741-58
Total number		54	45		

Table 3 The values of eight growth and yield traits in the M2 genotypes of cowpea produced following seed exposure to the applied doses of g-radiation

Variety	Gamma Dose (Gy)	Plant height	Root length	Fresh weight	Dry weight	Pod length	No of seeds per pod	No of pods per plant	Weigh of 100 seeds
Kaha 1	0.0	24.27±0.65	8.07±0.35	4.77±0.12	0.47±0.03	10.63±0.32	6.00±0.00	13.67±1.53	15.99±0.46
	50	28.17±0.61**	10.17±0.57**	5.97±0.25**	0.72±0.03**	15.50±0.46**	11.67±0.58**	24.67±1.53**	15.99±0.20
	100	38.67±0.78**	14.03±0.15**	9.57±0.25**	0.97±0.02**	17.27±0.25**	12.67±0.58**	32.33±2.52**	17.73±0.20**
	200	33.37±0.42**	12.77±0.15**	7.67±0.15**	0.77±0.03**	13.03±0.42**	8.67±0.58**	23.33±1.53**	15.15±0.28
	300	22.90±0.78	7.13±0.32	5.14±0.38	0.45±0.02	11.77±0.15*	5.00±1.00	17.00±1.00	14.27±0.45**
Dokki 331	0.0	23.37±0.52	10.93±0.15	5.27±0.21	0.62±0.03	11.87±0.47	7.00±0.00	21.33±2.65	17.79±0.13
	50	34.77±0.58**	18.03±0.32**	12.60±0.27**	0.92±0.01**	15.10±0.46**	11.33±0.58**	37.00±2.65**	19.38±0.16**
	100	31.83±0.85**	15.67±0.15**	9.03±0.23**	0.80±0.02**	13.17±0.32*	8.67±0.58*	25.33±1.53**	18.00±0.11
	200	28.33±0.47**	14.27±0.32**	7.33±0.15**	0.80±0.03**	11.90±0.40	6.67±0.58	20.67±1.53	16.71±0.46**
Azmerly	0.0	23.10±0.26	9.97±0.21	5.67±0.12	0.69±0.02	11.97±0.42	7.33±0.58	23.67±2.08	18.76±0.30
	50	29.30±0.46**	12.87±0.40**	7.73±0.06**	0.81±0.02**	16.40±0.17**	11.67±0.58**	37.00±2.00**	19.35±0.15
	100	26.50±1.00**	11.97±0.67**	7.07±0.15**	0.80±0.03**	14.67±0.15**	11.00±0.00**	29.67±3.06**	16.63±0.35**
	200	25.00±0.44**	9.90±0.00	6.13±0.21*	0.69±0.01	12.23±0.38	7.00±0.00	20.00±1.00	15.68±0.19**
Cream 7	0.0	20.03±0.21	6.93±0.06	4.63±0.15	0.46±0.01	12.60±0.36	6.67±0.58	15.00±2.00	16.99±0.21
	50	24.47±0.31**	12.40±0.61**	6.10±0.27**	0.64±0.01**	15.63±0.23**	10.67±0.58**	31.67±2.52**	20.29±0.28**
	100	23.77±0.15**	9.17±0.21**	4.87±0.06	0.58±0.01**	13.50±0.20**	8.33±0.58*	21.67±1.53**	15.73±0.17**
	200	22.47±0.95**	7070±0.17	4.93±0.15	0.57±0.02**	12.47±0.12	6.68±0.58	15.67±1.16	15.38±0.28**
Gisa 6	0.0	22.60±0.26	10.77±0.15	1.97±0.12	0.58±0.02	11.70±0.20	6.67±0.58	22.33±0.58	15.88±0.01
	50	25.77±0.15**	12.73±0.21**	6.07±0.15**	0.66±0.02**	15.70±0.10**	11.00±0.00**	33.00±2.00**	18.73±0.19**
	100	28.17±0.58**	13.23±0.39**	6.67±0.15**	0.80±0.02**	17.73±0.15**	12.33±0.58**	41.67±1.53**	15.58±0.27
	200	26.80±0.50**	13.80±0.44**	6.20±0.17**	0.72±0.03**	12.70±0.10**	6.67±0.58	28.33±0.58**	14.99±0.12**
	300	25.37±0.15**	13.10±0.36**	5.87±0.60**	0.65±0.03*	11.00±0.50*	5.33±0.588	23.33±1.53	14.44±0.35**

*statistically significant at the 0.05 level; **highly significant at the 0.01 level

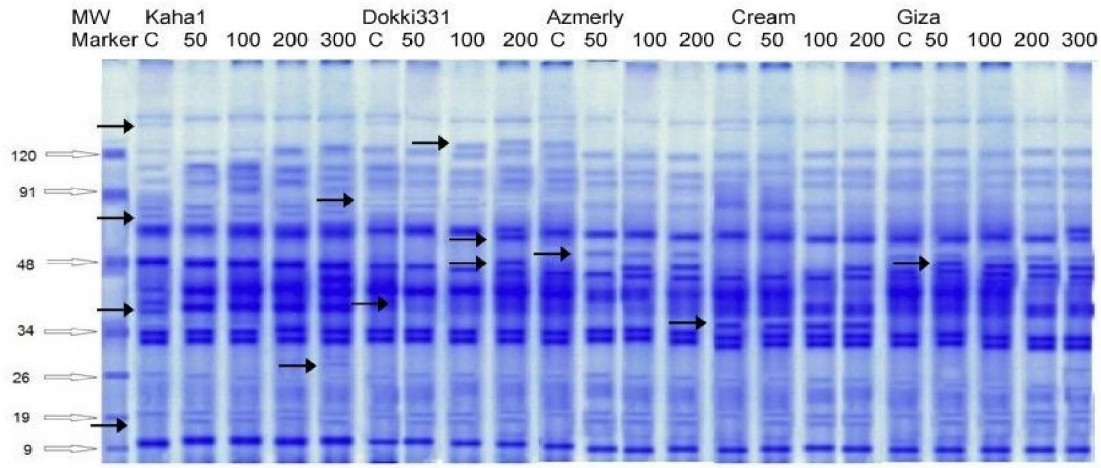

Figure 1: Seed protein SDS-PAGE profile of M2 plants of the five cowpea varieties following exposure to different doses of gamma radiation

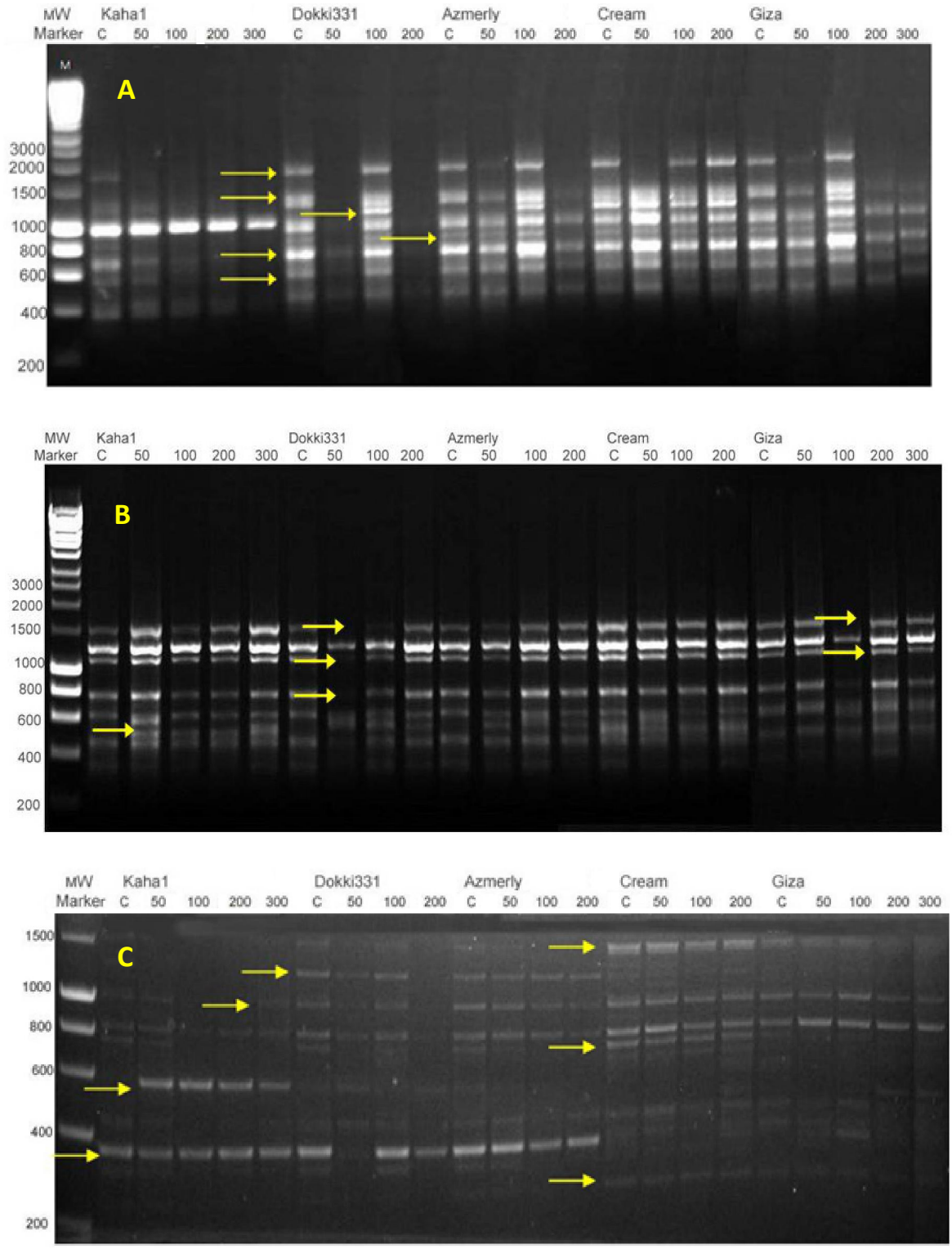

Figure 2: Examples of RAPD profiles produced in M2 plants of the five varieties of cowpea following seed exposure to different doses of gamma radiation, by three primers Pr. 1 (A), Pr. 2 (B) and Pr. 3 (C).

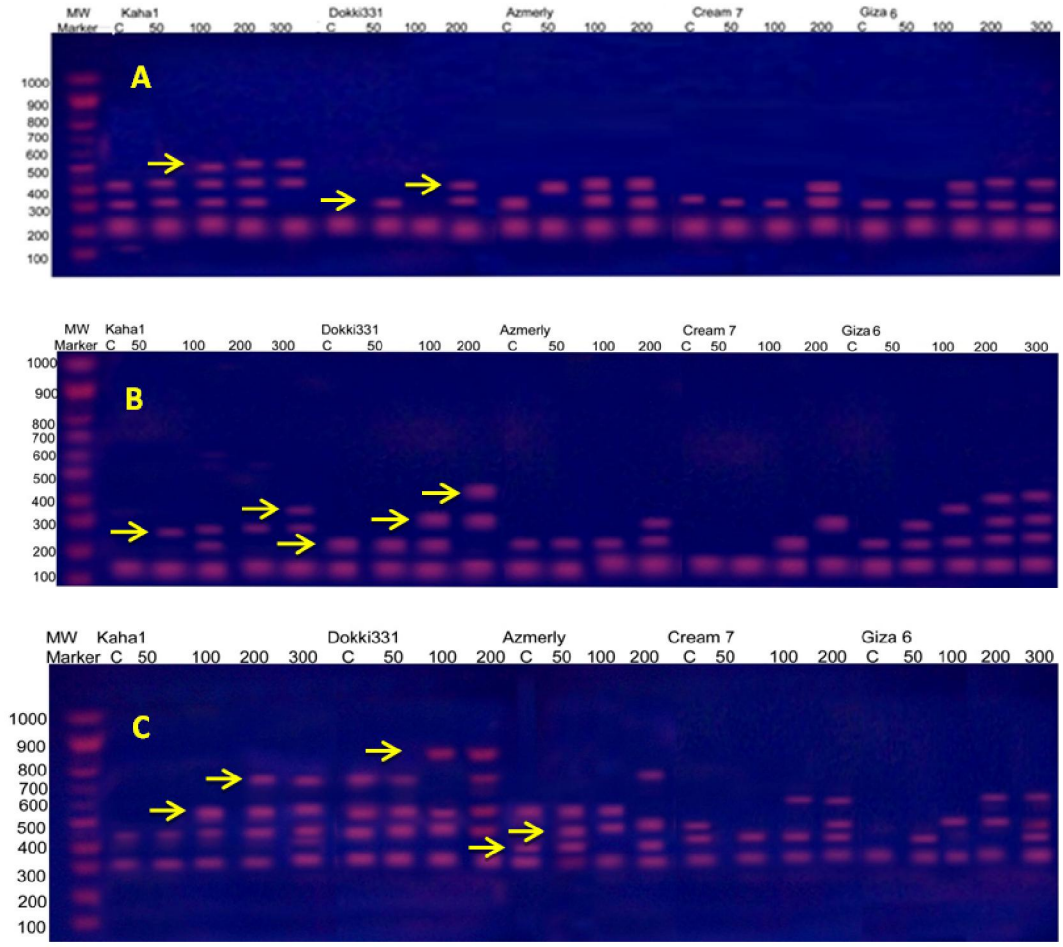

Figure 3: Examples of the ISSR profiles produced in M2 plants of the 5 varieties of cowpea following seed exposure to different doses of gamma radiaton, by three primers, HB-9, HB-12 and HB-13

Average linkage distance

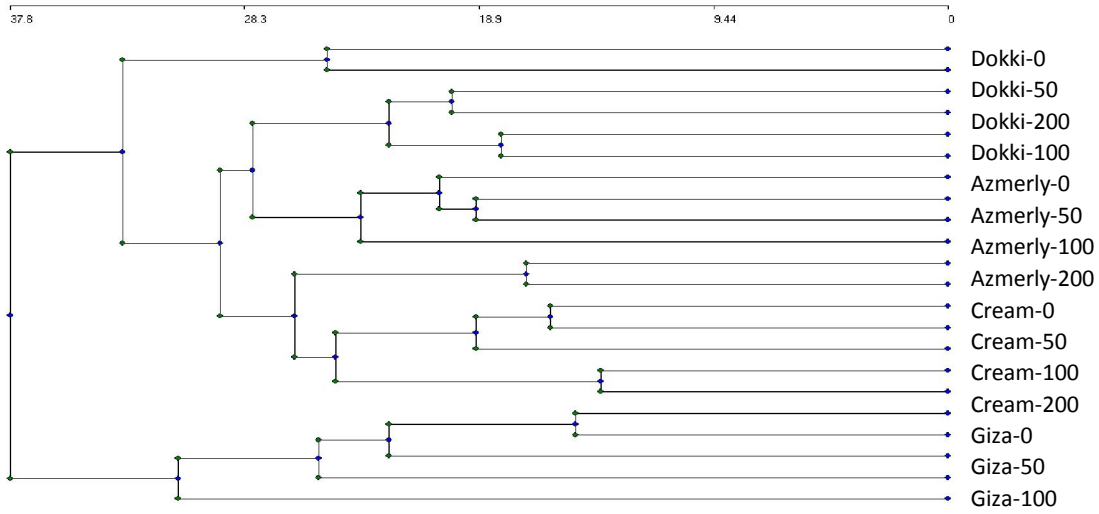

Figure 4: Agglomerative clustering dendrogram constructed using average linkage method illustrating the variation among the 22 M2 genotypes of cow pea.

Genetic variation among the M2 genotypes

The polymorphic markers, in the protein and DNA fingerprinting profiles of the 22 M2 genotypes, produced a number of similar dendrograms. A dendrogram constructed using the agglomerative clustering and the average linkage method of the CAP software is illustrated in Figure 4. This dendrogram clearly show that the two varieties Kaha 1 and Dokki331 are clearly distinguished from the varieties Giza 6, Azmerly and Cream 7. It is apparent that the M2 genotypes of these two varieties showed much variation following exposure to g-radiation compared to the other three varieties as judged by the distance scale.

4.Discussion

The results of the current study showed that the dose of 50 Gy of g-radiation was the most effective in increasing shoot length, root length, fresh weight and dry weight in varieties Dokki331, Azmerly and Cream7, while, the 100 Gy dose, was more effective in varieties Kaha1 and Giza6. These results are in agreement with results reported by Sakin (2002) who found that low g-radiation treatment increased the average plant height of durum wheat. Similar results were also reported by Chaudhuri (2002) and Borzouei *et al.* (2010) on the two wheat genotypes Roshan and T-65-58-8,by increasing g-radiation dose to 200, 300 and 400 Gy shoot length declined in both genotypes and significant difference in root length was observed; the minimum length of the root was found at the dose of 400 Gy. However, Melki and Marouani (2009)reported an improvement by 18% and 32% in root number and root length of hard wheat at a 20 Gy of g-radiation associated with highly significant effect on the root and shoot weight.

Increasing the g-radiation dose to 300 Gy dose, resulted in inhibition of shoot and root length and severely reduced the measured vegetative traits and yield components. These results are in agreement with the results obtained on mungbean by Shakoor *et al.*(1978),who observed that g-radiation in the range of 10-30 kR doses was notsignificantly different but at the dose of 40 kR the plants became stunted. Singh *et al.*(2001) and Tah (2006), also, observed that mungbean M1 plant height decreased at high doses of g-radiation due to the treatment of g-radiation with the highest decline occurring at a dose of 40 kR. Meanwhile, Wi *et al.* (2007) reported no significant morphological aberrations in the phenotype of plants irradiated with relatively low doses of g-radiation, while high-doses of g-radiation inhibited seedling growth remarkably.

In the present study, the measured productivity criteria i.e. number of pods/plant, pod length, number of seeds/pod, and 100 seeds weight were increased following exposure to the 100 Gy dose in varieties Kaha 1 and Giza 6 and at the 50 Gy dose in the other three varieties. The highest 100 seeds weight was produced by 50 Gy dose in varieties Cream 7 and Giza 6 compared to control. The increase in the number of pods per plant following exposure to low doses of 50 and 100 Gy is supported by previous reports by Swaminathan (1973) who found that an increase in the yield of pulses. These results are also in agreement with the results of Gnanamurthy *et al.*(2012) who reported an increase in most of yield component of cowpea following g-radiation.

Gamma rays have been also reported to have beneficial effects on many crops. via induction of mutations.In cowpea,Badiane *et al.*(2012) reported potential mutations in flower and pod color, pod length, and seed color after exposing the seeds to g-radiation. In the current study, two important mutations have been scored; one is concerned with whole seed color or the color of the seed eye. In variety Kaha 1, the 50 Gy produced black seed coat in the M2 generation while in other varieties different helium colors were scored compared to control. In a similar situation, Wani and Anis (2001) reported that bold seeded mutant of *Vignamungo*was associated was significant level of yield attributes.

At the molecular level, new protein bands appeared in the seed protein while other bands disappeared after exposure to the g-radiation; these changes may be due to denaturation of protein or to protein association or deamination (Abu *et al.*, 2005). The appearance and disappearance of protein band may refer to environmental stresses that affect causes changes in gene expression (Struh and Tjiian, 1995). On the other hand, Kiong *et al.* (2008) reported no significant change in protein constituents after g-irradiation. For most prominent mechanism, the production of different proteins entails a vast array of DNA binding proteins that act in various combinations to either activate or repress gene expression (Freeman *et al.*, 2003).

The RAPD markers provided some markers that are potentially useful in studies on genetic diversity and breeding of cowpea through mutations using the g-radiation. In this respect, Diouf and Hilu (2005) studied the potential application of RAPD and SSR techniques in determining genetic diversity among cowpea breeding lines and local varieties in Senegal. Among the 61 RAPD primers used, 12 showed polymorphism; a much lower proportion compared to the proportion of RAPD polymorphism found in the 22 M2 genotypes produced by exposure to different doses of g-radiation used in the current investigation. Primer 3 however, produced a number of makers that are specific for different varieties and may also be important markers for the identification of

these varieties in future studies on the genetic diversity and breeding of new cowpea lines.

Yoko *et al.*(1996) studied the effect of gamma irradiation on the genomic DNA of corn, soybeans and wheat. They concluded that, large DNA strands were broken into small strands at low irradiation dose but small and large DNA strands were broken at higher irradiation doses. The RAPD method was also used by Raisheed *et al.* (2001) to detect the genetic variation induced by gamma rays. These results are in agreement with the results of Mudibu, *et al.*(2011) who indicated an increase in polymorphism after irradiation with gamma rays of soybean. The effects on ISSR finger printing might be connected to structural rearrangements in DNA caused by different types of DNA damages (Sonia *et al.*, 2012)

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