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Molecular and Morphological Variations Induced by Gamma Rays in Yardlong Bean

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THIS study was carried out to identify the genetic variations in yardlong bean plants irradiated by various doses (75, 150, 300, 450 and 600Gy) of gamma rays using RAPD and ISSR markers techniques as well as, their effect on some vegetative traits were studied. ten RAPD and 10 ISSR markers were used in this investigation. The obtained results showed that the ten RAPD primers produced 117 bands only 94 of them were polymorphic while 23 bands were monomorphic. The percentages of polymorphism among primers ranged from 0.0% to 100%. All of the RAPD primers produced unique bands except OPK-06 primer which did not produced any unique bands. Meanwhile, 62 amplified bands including 26 polymorphic and 36 monomorphic bands were generated by ten ISSR primers. All of the ISSR primers did not produce any unique bands except ISSR4 primer which produced only one unique band, these results demonstrated that this unique sequence could be used as a molecular marker associated with gamma irradiation. The field results showed that the emergence percentage decreased with increasing doses up to 600Gy treatment which gave the lowest emergence percentage. Inconstant values of vegetative characteristics were obtained after each treatment with gamma ray doses; 300Gy treatment gave the highest value of plant height, number of branches, pod length and number of seeds per pod compared to control and other treatments. The values of coefficients of phenotypic and genotypic variation, heritability and expect genetic advance did not follow regular increase or decrease with the radiation doses in both seasons. The present data demonstrate that using gamma rays to induce DNA polymorphism in this plant genome which might be lead to appearance of a desired phenotype and genetic characteristics which could be used in yardlong bean improvement programs.

Keywords: Yardlong bean, Gamma ray, Mutations, Heritability, Genetic advance, RAPD, ISSR.

Introduction

Yardlong bean (*Vigna unguiculata* (L.) Walp. sub. sp. *sesquipedalis*) is a climber annual Vigna crop of Leguminosae family with chromosome number 2n = 2x = 22. It's known as long-podded cowpea, asparagus bean, pea bean, snake bean, garter bean and Chinese long bean. It has ability to fix atmospheric nitrogen with its symbiotic bacteria (Rhizobia) and increases productivity of the soil (Pandey et al., 1989). It is much more a climbing and trailing plant than the cowpea, often reaching 9-12 feet in height and it is characterized by its very long pods 30-90 cm in length with seeds usually 8–12 mm long. It is commonly cultivated

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in China, and South, South-East Asia and West Africa (Kongjaimun et al., 2012). Yardlong bean is very important food for nutrition. The green tender pods are rich in digestible protein (23.52 – 26.27 %) as well as, vitamin A (941 IU) and C (13 mg), iron (2.5 mg), calcium (80 mg), phosphorus (74 mg) in addition to zinc, manganese, cobalt, thiamin, riboflavin and dietary fiber (2 g). Fresh green pods fiber is very useful for health, in particular helps the digestive system and gives long sense felling of satiety, reducing blood cholesterol and to enhance kidney's and spleen's function (Rubatzky and Yamaguchi 1997, Singh et al., 2001 and Huque et al., 2012**)**.

It is mainly a warm-season crop and able to survive in extreme humidity as well as, well acclimatization of crop to warm weather (Hall, 2004), poor soil conditions (Hamidou et al. 2007) and phytostabilization capacity (Deivanai and Thulasyammal 2014) may give belief in the role of phytoremediation.

Induced mutation is considered one of the best alternatives for improving crops. It can help to regenerate and restore the variations which, often lost in the adaptation process to various stresses. Genetic variations are the main source of plant breeders to produce new and important cultivars. Recombination and independent assortment of favorable alleles helps breeders to produce new and unique superior individuals from which to select and produce the lines that could be serving as new cultivars (Joseph et al., 2015).

Using breeding mutants as a valuable supplement in conventional breeding to crop improvement has been least applied in grain legumes. For example, only eight out of over 1000 improved mutant varieties of different crops released up to 1989 in over 48 countries were cowpeas **(**Micke et al., 1990**)**.

Mutations can be induced through various ways, such as exposure of plant propagules (seeds, tissues and organs) to physical and chemical mutagens (Mba et al., 2010). Physical mutagens are mostly electromagnetic radiations such as gamma ray, X-ray and UV light.

Gamma radiation is often used to develop cultivars that are agriculturally and economically important and have high productivity potential (Muthusamy et al. 2003). Low doses of gamma irradiation have been used for mutant isolation in conventional plant breeding (Albokari et al., 2012). Several studies have shown that exposure to gamma rays have stimulatory effects on specific morphological parameters and can increase the yield of plants and their resistance to drought (Hanafiah et al., 2010, Badr et al., 2014a and b, Ariraman et al., 2016, Gaafar et al., 2016 and Ezzat et al., 2019).

Molecular markers are highly heritable and polymorphic enough to enable the discrimination of closely related genotypes. O'Neill et al. (2003) reported that molecular genetic techniques using DNA polymorphism have been increasingly used to characterize and identify a novel germplasm for use in the crop breeding process. Mohamed (2011) reported that toxic element and gamma rays induced changes in the genomic pattern of DNA represented in appearance or disappearance of DNA morphic bands.

Several DNA marker systems are now commonly used in diversity studies of plants. The most commonly used marker systems are restriction fragment length polymorphism (RFLP) (Soller and Beckmann, 1983), random amplified polymorphic DNA (RAPD) (Williams et al. 1990), amplified fragment length polymorphism (AFLP) (Vos et al., 1995), microsatellites or simple sequence repeats (SSRs) (Becker and Heun, 1994) and inter-simple sequence repeats (ISSRs) (Zietkiewicz et al., 1994).

Among of these molecular markers, RAPD and ISSRs have been widely used for assessing the alteration in DNA sequences induced by mutagenic agents such as gamma radiations (Mudibu et al. 2011, Mejri et al., 2012, Hamideldin & Eliwa, 2015 and Gaafar et al., 2016).

RAPD and ISSR markers are advantageous over other markers because they are easier to use, less expensive, faster and involve non -radioactive substances (Adnan & Katsuhiko, 2011 and Malviya et al., 2012). So, this investigation was carried out to determine the DNA polymorphism and its reflection on vegetative traits of yardlong bean plants irradiated with different doses of gamma ray as well as, detecting mutants which could be useful the for ongoing yardlong bean improvement programs at Minia University.

Materials and Methods

Plant materials

Asian yardlong bean intermediate climber genotype of cowpea cultivar were kindly obtained from the Horticulture Dept., Fac. of Agric. Minia

Univ. Irradiation was carried out with the Cs^{137} source at the dose rat 1 Gy /2min.14sec at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. Yardlong bean seeds are exposed to different gamma ray doses (75, 150, 300, 450 and 600Gy). Untreated seeds as well as treatments were grown in the Vegetable Greenhouse in the Experimental Farm of Horticulture Dept. Faculty of Agric, Minia Univ., Minia, Egypt. The study was done in two generations, the first mutant generation (M_1) was in 2017 and the second mutant generation (M_2) was in 2018 with complete randomized design (CRD).

Field experiment

The following data were recorded

All recoded data for all plants from all treatments, seeds collected from each plant separately.

Emergence percentage: The emerged seeds above soil surface in the M1 generation were recorded daily up to 15 days from planting and the percentage was calculated by dividing emerged seeds by the total planted seeds.

Plant height (cm): All plants in each treatment were taken for the determination of plant height average.

Number of branches/plant: Average of number of branches for all plants in each treatment was calculated.

Number of days to flowering: Number of days from planting date until 50% flowering

Pod length (cm): Normal and fully developed pods from all plants in each treatment were taken to determine pod length.

Number of pods/plants: Average of all pods in all plants was estimated.

Number of seeds /pod: 20 pods from each treatment were taken to determine their average.

Hundred seed weight: Average weight of ten samples for each treatment was determined.

Statistical analysis and genetic parameters estimation:

The genetic parameters were estimated using the proper equations as reported by Singh and Chaudhary (1979) as follow:

Genotypic Variance $\sigma^2 g = \frac{(MSt - MSe)}{r}$

Whereas, MSt = Mean sum of squares for irradiation treatments

MSe = Mean sum of squares for error

r = Number of replications

Phenotypic Variance "$\sigma^2 p$" = $\sigma^2 g + \sigma^2 e$

$\sigma^2 p$ = Phenotypic Variance for each treatment

$\sigma^2 g$ = Genotypic Variance for each treatment

Whereas, $\sigma^2 e$ = environmental variation among the tested treatments

Phenotypic Coefficient of Variation (PCV)

$$= \frac{\sqrt{Phenotypic\ Variance}}{Mean} \times 100$$

Genotypic Coefficient of Variance (GCV)

$$= \frac{\sqrt{Genotypic\ Variance}}{Mean} \times 100$$

The PCV and GCV expressed as percentages as suggested by Burton (1946) and were classified according to Sivsubramanian and Menon (1973) to:

Less than 10% = Low

10 – 20 % = Moderate

More than 20% = High

Heritability (broad sense)

It is the ratio of genetic variance to the phenotypic variance as reported by Allard (1970)

Heritability (H) = $\frac{Genotypic\ variance}{Phenotypic\ variance} X\ 100$

It is categorized according to Robinson et al. (1949) to:

0 -30 % = Low
31 – 60 = Medium
Above 60 % = High

Genetic advance as a percent of mean "GAM %"

It was estimated and categorized as reported by Johnson et al. (1955) by the following formula:

GAM % = $\frac{K * H * p}{Mean} X\ 100$

Whereas k = 2.06 at 5 % selection
H = Heritability
P = Phenotypic stander deviation
GAM less than 10 % = Low
GAM equal to 10 – 20 % = Moderate

GAM more than 20 % = High

Correlation coefficient (r) the association between gamma rays doses and total abnormalities were estimated using the following formula as reported by Singh (1993).

$r = Cov_{X*Y} / (Vx * Vy)^{1/2}$
wheare, Cov_{x*y} = Covariance X and Y
V_x = Variance "x" (Gamma doses)
V_y = Variance y (% abnormalities)

Laboratory experiment

This study was carried out at Molecular Genetics Lab., Faculty of Agric., Minia Univ. Twenty primers (10 RAPD and 10 ISSR) were used to determine the genetic variability induced by different doses of gamma ray in yardlong bean plants.

DNA isolation

Genomic DNA was extracted from freshly young leaves of yardlong bean plants (as a bulk) using Cornell extraction buffer (Kiruthika and Padmanabha, 2018**)**. Subsequently, the DNA extracts were purified using the phenol-chloroform-isoamyl alcohol extraction method and precipitated by ice cold absolute ethanol. The DNA quantity and quality were determined using spectrophotometer based on absorbency at 260 and 280 nm, respectively.

RAPD procedure

The RAPD analysis was carried out using ten RAPD primers (Table 1). The amplified PCR products were in a final volume of 20 µl. Each PCR reaction contains (4 µl of DNA template (25 ng/ µl), 2 µl of primer and 10 µl (10 pmol), of master mix *taq*. DNA polymerase, Sigma Scientific Services Co., Egypt). The reaction volume was completed to 20 µl with sterilized deionized water. The reactions were preheated at one step of 5 min at 94° C followed by 40 cycles of 3 steps (DNA denaturation at 94° C for 1 min, primer annealing at 36° C for 1min and primer extension at 72° C for 2 min, respectively) in each and a final subsequent cycle of post extension at 72° C for 5 min.

ISSR procedure

Ten ISSR primers (Table 2) were used in the present experiment, to amplify the genomic DNA samples. Amplification reactions were carried out in 20µl volumes, containing (4 µl of DNA template (25 ng/ µl), 2 µl of primer (10 pmol) and 10 µl of master mix taq. DNA polymerase, Sigma Scientific Services Co., Egypt). The reaction volume was completed to 20 µl with sterilized deionized water. The Thermal cycler was programmed for initial denaturation at 94 ºC 5 min, 1 min denaturation at 94 ºC, 1 min annealing at 40 ºC and 2 min extension at 72 ºC followed by final extension for 7 min at 72 ºC. DNA denaturation, annealing temperature and number of cycles of each RAPD and ISSR primers are shown in Table (1 and 2).

The amplification of RAPD and ISSR primers were carried out in Multigene Thermal Cycler (Labnet). PCR products were resolved by gel electrophoresis on 2% agarose gels in Tris-acetate EDTA (TAE) buffer at 100 volts. Subsequently, gels were stained with Ethidium bromide (0.1g in 10 ml 1X TAE buffer) for 30 min., visualized on UV light and photo-documentation was

TABLE 1. RAPD-Primers, sequences, melting, annealing temperatures and number of cycles used for amplification in yardlong bean plants irradiated with different doses of gamma ray.

Primer Name	Sequence (5′-3′)	Tm ˚C	Annealing temperature (ºC)	Number of cycles
OPK-02	GTC TCC GCA A	34	36	40
OPK-03	CCA GCT TAG G	32	36	40
OPK-04	CCG CCC AAA C	34	36	40
OPK-06	CAC CTT TCC C	32	36	40
OPK-07	AGC GAG CAA G	34	36	40
OPK-08	GAA CAC TGG G	32	36	40
OPK-10	GTG CAA CGT G	34	36	40
OPL-01	GGC ATG ACC T	32	36	40
OPM-01	GTT GGT GGC T	32	36	40
OPM-08	TCT GTT CCC C	31	36	40

TABLE 2. ISSR-Primers, sequences, melting, annealing temperatures and number of cycles used for amplification in yardlong bean plants irradiated with different doses of gamma ray.

Primer Name	Sequence (5′-3′)	Tm ˚C	Annealing temperature (ºC)	Number of cycles
ISSR1	(GA)9 T	55	53	40
ISSR2	(TG)8 A	50	52	35
ISSR3	(CA)8 GC	56	52	35
ISSR4	(GACA)4	48	52	35
M2	(AC)8(C/T)G	54	50	40
M3	(GA)8(C/T)C	54	50	40
M7	(CAG)5	52	50	40
M8	(GTG)5	52	50	40
M12	(CA)6(A/G)(C/T)	38	50	40
A1	(GAA)7	54	54	40

performed. The size of amplified fragments was estimated according to the standared ladder of 100 bp.

Statistical analysis

The statistical analysis of field data was done for all recorded data and all means were compared. Gel images detected via PCR-based methods were analyzed using (GelAnalyzer version 3, 2007). Molecular sizes of the amplified fragments, presence (1) or absence (0) through samples, frequencies through samples, and polymorphism type (either monomorphic or polymorphic) as well as the polymorphism percentage for each primer were determined. Dice (1945) genetic similarity coefficient values (*S*) within yardlong bean plants, expressed as band sharing frequency (BS), were calculated for all possible pairs or operational taxonomic units (OUT) by using SPSS software (version 12.0.1, 2004) based upon coding of the amplified bands numbers related to their presence or absence. Hierarchical cluster analysis was conducted with the PAST software version 1.88 (Hammer et al. 2009) based on Dice (1945) similarity coefficient matrix within different yardlong bean treated plants.

Results and Discussion

Field experiment

Results of seed emergence are presents in Fig. (1). Data showed that seed emergence percentage decreased with increasing of gamma doses to 300, 450 and 600Gy. The lowest value was found with 600Gy while, high emergence percentage values were obtained by the control, 75Gy and 150Gy. Treated seeds with gamma rays may produce immediate effects such as physiological disturbance on the organism and induced mutations which can be transmitted to subsequence generations (Sparrow & Evans, 1961 and Girija & Dhanavel 2009). Low emergence percentage in M_1 generation of irradiated cowpea plants have been attributed to genetic and physiological damage to embryo cells or tissues (Lagoda, 2012 and Mudibu et al., 2012).

Different growth and yield components traits of irradiated M_1 and M_2 generations of yardlong bean plants were shown in Tables (3) and (4). The values of plant height in M1 generation ranged from 160.5 cm (control) to 294 cm (300Gy) while, in M_2 generation ranged from 157cm (control) to 305cm (300Gy). The highest values of the phenotypic coefficient variation (PCV%) of plant height were observed in control (10.3%) followed by 600Gy and 150Gy treatments (6.7 and 6.0%, respectively) in M_1 while, data of the M_2 showed that 450Gy treatment had the highest value of PCV% (15.2%) while, the lowest value was given by 600Gy with (3.4%).

Heritability (H%) estimate in broad sense of the M_1 generation ranged from 32.8% for number of branches to 97.7% for plant height, and ranged from 34.3 to 99.2% for the same characters in M_2 generation. The high value of genetic advance regarding the start of flowering (8.1%) was observed with 150Gy treatment while, the lowest one was recorded at control and 300Gy treatments (4.2%). Treatment 300Gy scored the highest value

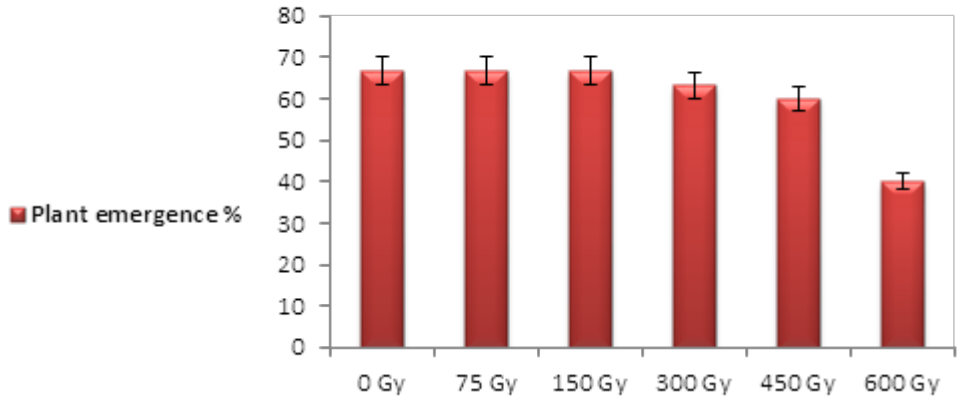

Fig. 1. Effects of different doses (0, 75, 150, 300, 450 and 600Gy) of gamma ray on the emergence percentages of yardlong bean in M_1 generation.

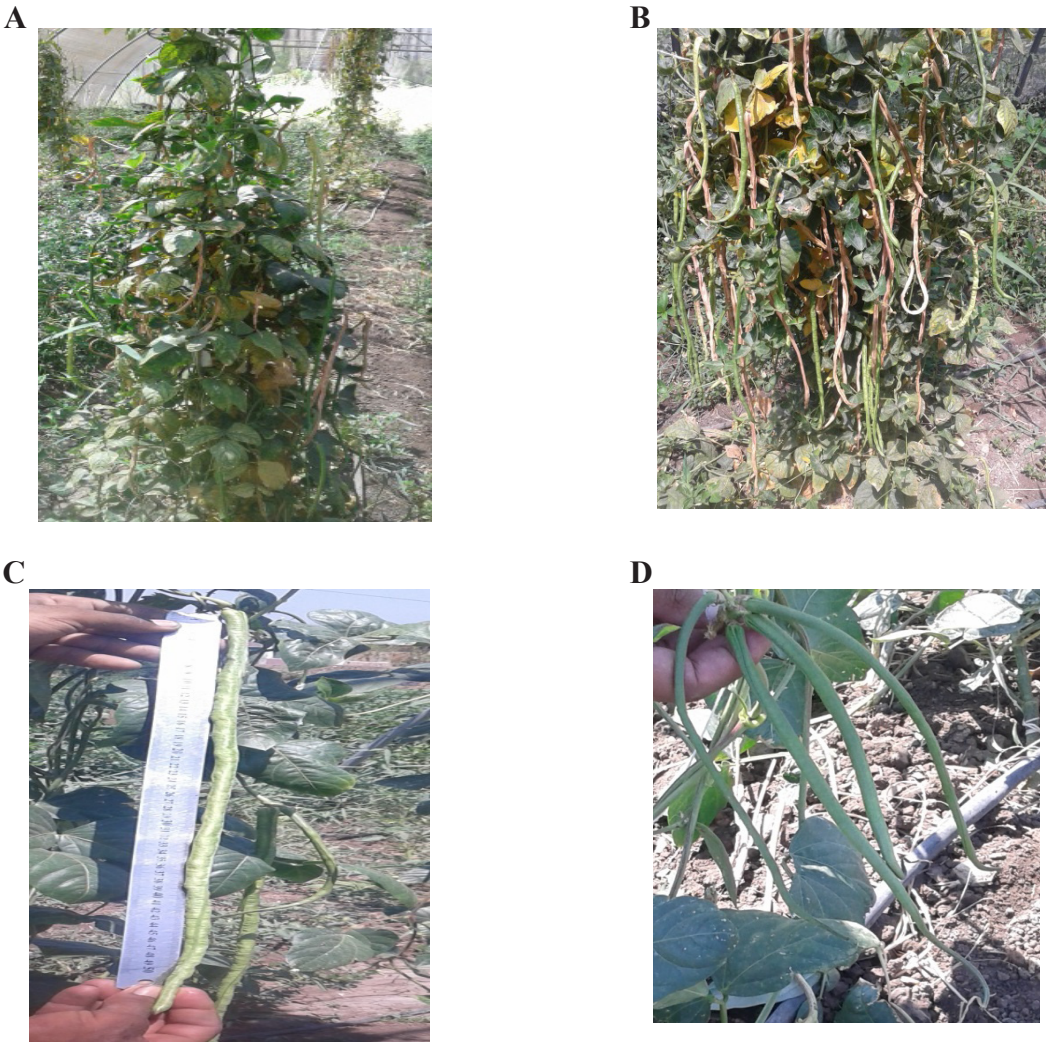

Fig. 2. The effect of 300Gy of gamma irradiation on yardlong bean plants morphology, (A): control, (B): changes in number of pods, (C): changes in pod length and (D): changes in number of pods/peduncle.

TABLE 3. Mean, phenotypic variance (PV), genotypic variance (GV), phenotypic coefficient of variation (PCV %), genotypic coefficient of variation (GCV %), broad sense heritability (H %) and genetic advance as a percent of mean (GAM %) for morphological traits in the first mutated generation (M_1) in yardlong bean plants grown during 2017 season.

Doses of gamma ray (Gy)	Mean	Range	P. V.	G.V.	PCV %	GCV %	H %	GAM %
First mutant generation (M1)								
Plant height (cm)								
0.0	160.5 D	150-200	200.0	195.5	10.3	10.0	97.7	28.5
75	194.5 C	190-200	25.3	23.7	2.6	2.5	93.7	9.7
150	208.0 B	195-230	156.7	153.1	6.0	5.9	97.7	25.2
300	294.0 A	285-308	46.3	44.2	2.3	2.3	95.3	13.4
450	282.8 A	260-295	156.2	152.2	4.4	4.4	97.4	25.1
600	215.0 B	190-230	205.6	201.0	6.7	6.6	97.7	28.8
No. branches								
0.0	1.0 B	1.0-2.0	0.100	0.002	29.0	4.1	1.9	0.013
75	1.6 B	1.0-2.0	0.267	0.104	32.3	20.2	38.9	0.414
150	1.4 B	1.0-3.0	0.489	0.268	49.94	36.9	54.8	0.789
300	3.0 A	2.0-4.0	0.222	0.073	15.7	9.0	32.8	0.319
450	1.5 B	1.0-3.0	0.500	0.276	47.1	35.0	55.2	0.804
600	1.6 B	1.0-3.0	0.933	0.627	60.34	49.5	67.2	1.337
Days to flowering								
0.0	56.1 B	52-60	5.4	4.7	4.2	3.9	86.4	4.2
75	57.0 AB	55-60	6.7	5.9	4.5	4.2	87.8	4.7
150	59.6 AB	55-64	18.0	16.7	7.1	6.9	92.6	8.1
300	43.3 C	40-45	5.6	4.8	5.4	5.1	86.6	4.2
450	57.8 AB	55-62	8.6	7.7	5.1	4.8	89.2	5.4
600	60.1 A	57-67	7.4	6.6	4.5	4.3	88.4	4.9
No. pods /plant								
0.0	12.4 C	10-18	7.6	6.7	22.2	20.9	88.5	0.15
75	18.0 B	10-18	4.0	3.4	11.1	10.2	84.2	3.5
150	19.8 B	15-20	0.4	0.2	3.2	2.3	50.0	0.65
300	27.9A	22-35	23.8	22.2	16.4	15.9	93.5	9.4
450	21.4 B	18-25	6.3	5.5	11.7	10.9	88.4	4.6
600	19.6 B	15-22	5.8	5.1	12.3	11.5	86.9	4.4
Pod length (cm)								
0.0	38.8 B	35-42	4.6	3.9	5.5	5.1	85.3	3.8
75	37.1 B	32-42	18.3	16.9	11.5	11.1	92.6	8.2
150	29.6 C	22-35	22.9	21.4	16.2	15.6	93.4	9.2
300	55.9 A	52-68	28.3	26.6	9.5	9.2	94.1	10.3
450	38.1 B	31-42	18.9	17.6	11.4	11.0	92.7	8.3
600	33.6 BC	30-41	15.2	13.9	11.6	11.1	91.9	7.4
No. seeds/pod								
0.0	16.1 C	11-20	8.3	7.4	17.9	16.9	89.0	5.3
75	21.5 B	18-25	8.5	7.6	13.6	12.8	89.2	5.4
150	19.8 B	18-20	0.4	0.2	3.2	2.3	50.0	0.7
300	32.6 A	30-36	7.6	6.7	8.6	7.9	88.5	5.11
450	22.8 B	18-25	5.1	4.4	9.9	9.2	85.9	3.9
600	20.9 B	20-25	0.8	0.2	4.2	2.4	31.4	1.8
100 seed weight (g)								
0.0	112.0 C	110-120	12.2	11.1	3.1	2.9	90.9	6.5
75	119.2 AB	112-130	28.6	26.9	4.5	4.4	94.1	10.4
150	119.4 AB	112-130	28.8	27.3	4.5	4.3	94.6	9.5
300	117.9 B	110-125	32.3	30.5	4.8	4.7	94.4	b
450	123.1 A	118-125	5.4	4.7	1.9	1.8	86.4	4.2
600	123.8 A	122-125	2.4	1.9	11.2	10.0	79.6	2.5

In each column mean of each treatment followed by the same letter (s) are not significant at 0.05 level of probability using Duncan's Multiple Range Test (DMRT)

TABLE 4. Mean, phenotypic variance (PV), genotypic variance (GV), phenotypic coefficient of variation (PCV %), genotypic coefficient of variation (GCV %), broad sense heritability (H %) and genetic advance as a percent of mean (GAM %) of morphological traits in the second mutated generation (M_2) in yardlong bean plants grown during 2018 season.

Doses of gamma rays(Gy)	Mean	Range	P. V.	G.V.	PCV %	GCV %	H %	GAM %
Second mutant generation (M2)								
Plant height (cm)								
0.0	157.0 D	150-168	34.0	32.2	3.7	3.6	94.6	11.7
75	212.8 C	190-250	429.3	422.7	9.8	9.7	98.5	42.1
150	210.8 C	200-250	250.8	245.8	7.5	7.4	98	31.9
300	305.3 A	300-333	134.2	130.6	3.8	3.7	97.3	23.2
450	254.0 B	200-290	1493.0	1480.8	15.2	15.1	99.2	78.9
600	201.0 C	190-210	48.0	45.8	3.4	3.4	95.4	13.6
No. branches								
0.0	1.6 BC	1.0-2.0	0.3	0.1	32.3	20.2	38.9	0.4
75	2.1 B	2.0-3.0	0.1	0.1	15.1	14.3	90.0	0.6
150	1.7 BC	1.0-2.0	0.2	0.1	28.4	16.6	34.3	0.3
300	2.7 A	2.0-3.0	0.2	0.1	17.9	10.5	34.3	0.3
450	1.1 C	1.0-2.0	0.1	0.1	28.7	27.3	90.0	0.6
600	1.7 BC	1.0-3.0	0.5	0.2	39.7	29.0	53.3	0.7
Days to flowering								
0.0	55.7 B	54-58	2.7	2.2	2.9	2.6	80.7	2.8
75	57.3 AB	50-59	7.1	6.3	4.7	4.4	88.2	4.8
150	52.2 C	57-60	1.1	0.7	1.8	1.5	69.4	1.5
300	44.1 D	43-45	1.0	0.7	2.3	1.9	68.3	1.4
450	57.8 A	55-59	1.1	0.7	1.8	1.5	69.4	1.5
600	57.7 AB	54-60	4.5	3.8	3.7	3.4	85.0	3.7
No. pods /plant								
0.0	21.0 B	18-22	12.7	11.5	16.9	16.2	91.1	6.7
75	20.3 B	18-25	6.6	5.8	12.6	11.8	87.6	4.6
150	22.9 B	19-25	6.1	5.3	10.8	10.1	87.2	4.4
300	33.8 A	30-35	3.1	2.5	5.2	4.7	81.9	2.9
450	22.3 B	18-25	6.5	5.7	11.4	10.7	87.6	4.6
600	19.8 B	16-22	5.1	4.4	11.4	10.5	85.9	3.9
Pod length (cm)								
0.0	43.1 C	40-46	3.9	3.3	4.6	4.2	83.9	3.4
75	54.0 A	42-49	5.8	5.0	5.3	4.9	86.8	4.3
150	43.7 BC	42-45	1.6	1.2	2.9	2.5	74.7	1.9
300	55.7 A	52-60	8.5	7.5	5.2	4.9	89.1	5.3
450	44.2 BC	42-48	3.7	3.1	4.4	3.9	83.6	3.3
600	46.0 B	44-48	3.1	2.6	3.8	3.5	82.1	2.9
No. seeds/pod								
0.0	19.6 C	18-22	2.3	1.8	7.7	6.8	79.0	2.5
75	22.6 B	22-25	1.6	1.2	5.6	4.8	75.0	1.9
150	22.4 B	22-23	0.3	0.1	2.3	1.4	38.9	0.4
300	30.9 A	30-35	2.8	2.2	5.4	4.8	80.9	2.8
450	23.4 B	18-25	5.6	4.9	10.1	9.4	86.6	4.2
600	19.3 C	18-20	0.7	0.4	4.3	3.3	61.7	1.1
100 seed weight(g)								
0.0	119.4 A	118-15	4.7	4.0	1.8	1.7	85.4	3.8
75	120.2 A	118-15	5.1	4.4	1.9	1.7	85.9	3.9
150	120.6 A	118-125	23.4	21.9	4.0	3.9	93.5	9.3
300	123.2 A	122-126	2.4	1.9	1.3	1.1	79.6	2.5
450	119.1 A	115-125	13.9	12.7	3.1	2.9	91.5	7.0
600	120.0 A	117-125	8.4	7.5	2.4	2.3	89.1	5.3

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)

of pod length, number of pods per plant and number of seeds per pod in both M_1 and M_2 generations compared with other treatments Fig. (2).

The genetic coefficient of variation (GCV) values ranged from 1.8% (450Gy) for 100 seeds weight character to 49.5% (600Gy) for number of branches in M_1 generation. While, in M_2 generation ranged from 1.1% (300Gy) for 100 seed weight to 29.0% (600Gy) for number of branches character. In general, the gamma radiation treatment 300Gy affected positively the number of branches, number of pods / plant, pod length and number of seeds per pod (Fig. 2).

Inducing mutations and genetic variability by gamma ray were observed in many previous studies (Vandana et al. 1992, Vandana and Dubky 1995 and Kumer and Ratnam 2010**).** Physiological mutants such as, early flowering and early maturity were observed in all mutagenic treatments (Giriga and Dhanavel 2009).

The obtained results are in agreement with the previous studies which indicated that low doses of gamma irradiation stimulated cell division, growth and development in various organisms, and this might be due to hormonal signaling network in cells of plant in different plants species **(**Luckey, 1980, Planel et al. 1987 and Hassan et al. 2015).

RAPD-PCR analysis

Molecular studies were conducted on first mutated generation (M_1) seeds. All RAPD primers produced scorable amplified bands in all irradiated and non-irradiated yardlong bean plants except the two primers OPK-03 and OPM-01 which, did not produced any amplicons at control treatments (Fig. 3). A total of 117 amplified bands at size ranged from 207 to 2300 bp were produced by ten RAPD primers with an overall mean of 11.70±1.34 and ranged from 4 bands of OPK-06 primer to 17 bands in OPK-03 primer (Table 5). From 117 generated bands 94 were polymorphic with an overall mean of 9.40±1.56 and 23 bands were monomorphic with an overall mean of 2.30±0.54. The percentages of polymorphism among primers ranged from 0.0% to 100% with an overall mean of 73.77±9.20% as shown in Table 5.

RAPD amplicons produced by OPK-03 and OPM-01 primers exhibited 100% polymorphism between all treatments, while OPK-06 primer showed 0.0% polymorphism. Data in Table 5 revealed that, all the RAPD primers produced unique bands except OPK-06 primer which did not produced any unique bands.

The number of amplicons detected by any of the RAPD primers depends on primer sequence and the extent of variation of the examined genotype(s). From the above-mentioned results, it can be concluded that the ten utilized primers generated relatively high polymorphism within the studied yardlong bean treated plants. The primers of OPL-01, OPM-08, OPK-03 and OPK-02 were the highest and a more successful in proofing identity of the present Yardlong bean

TABLE 5. Fragment size, total number of polymorphic and unique bands and polymorphism % obtained using ten RAPD primers in yardlong bean plants treated with different doses of gamma ray.

Primers	Fragment size (bp)	Monomorphic bands	Unique bands	Polymorphic bands		Total number of bands	Polymorphism (%)
				Without unique	With unique		
OPK-02	327-1909	4	4	8	12	16	75
OPK-03	347-2172	0	6	11	17	17	100
OPK-04	223-2300	4	3	7	10	14	71.43
OPK-06	264-778	4	0	0	0	4	0
OPK-07	243-1322	3	2	5	7	10	70
OPK-08	352-931	1	3	2	5	6	83.33
OPK-10	245-1186	4	3	3	6	10	60
OPL-01	296-2272	2	7	4	11	13	84.62
OPM-01	207-1548	0	3	9	12	12	100
OPM-08	245-1757	1	7	7	14	15	93.33
Total		23	38	56	94	117	
Mean ± SE		2.30±0.54	3.80±0.71	5.60±1.08	9.40±1.56	11.70±1.34	73.77±9.20

treated plant. It was generated (7, 7, 6 and 4 unique bands, respectively) then it can be used as a marker to distinguish among them. Generally, the present data of yardlong bean treated plants are in agreement with those reported by Mohamed (2011), Badr et al. (2014b), Saleh and Salama (2015) and Gaafar et al. (2016).

The resulted data from RAPD analysis were used in the estimation of genetic relationships among yardlong bean plants irradiated with different doses of gamma ray and its control through a UPGMA cluster analysis of genetic similarity matrices. Cluster analysis was achieved based on Dice's similarity coefficient matrix. The results revealed that the highest similarity value (0.874) was found between yardlong bean plants irradiated with 450Gy and 600Gy. On the contrast, control plants and 600Gy exhibited the lowest value (0.414), as shown in Table 6.

In order to determine the genetic variability among treated yardlong bean plants, matrix of Dice (1945) similarity coefficient (S) was calculated based upon band sharing frequency (BS) as shown in Table 6. The dendrogram was constructed using the hierarchical cluster analysis method with the average linkage between pairs

TABLE 6. Dice's similarity coefficient matrix within yardlong bean plants irradiated with different doses (75, 150, 300, 450, and 600Gy) of gamma rays based on polymorphism bands of RAPD primers.

Treatments	Control	75 GY	150 GY	300 GY	450 GY	600 GY
Control (0Gy)	-					
75 GY	0.608	-				
150 GY	0.500	0.800	-			
300 GY	0.464	0.714	0.871	-		
450 Gy	0.449	0.595	0.770	0.809	-	
600 GY	0.414	0.554	0.722	0.757	0.874	-

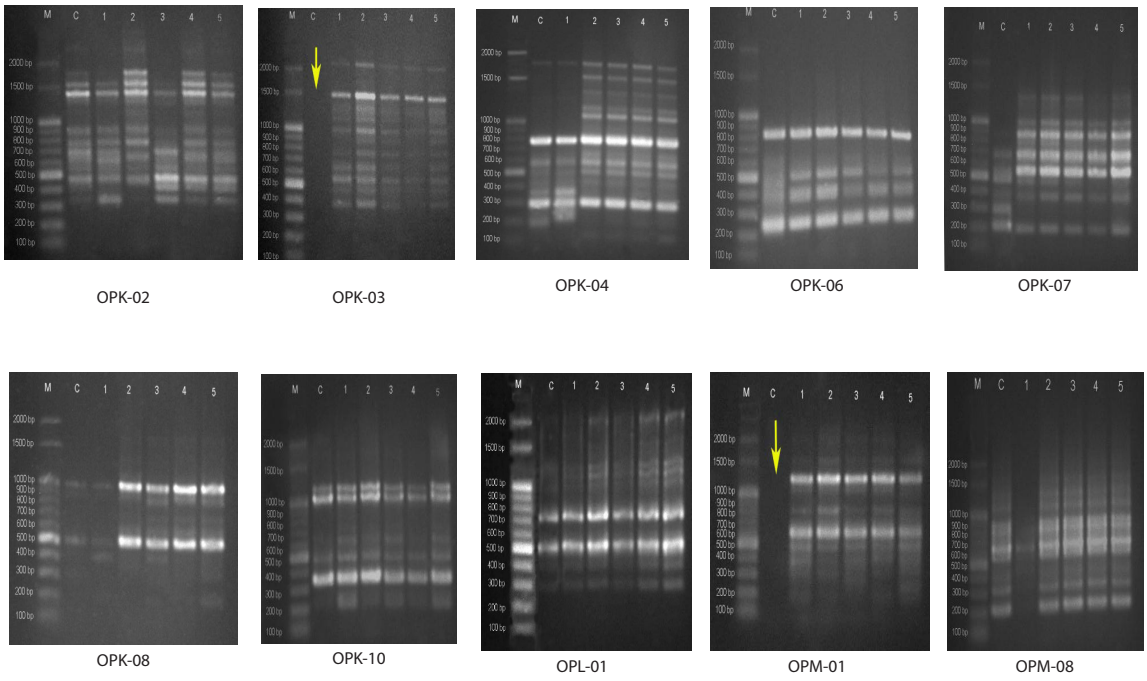

Fig. 3. Electrophoretic gel patterns of RAPD DNA products generated by OPK-02, OPK-03, OPK-04, OPK-06, OPK-07, OPK-08, OPK-10, OPL-01, OPM-01 and OPM-08 primers. Lane C, Control; lane 1, 75Gy; lane 2, 150Gy; lane 3, 300Gy; lane 4, 450Gy and lane 5, 600Gy

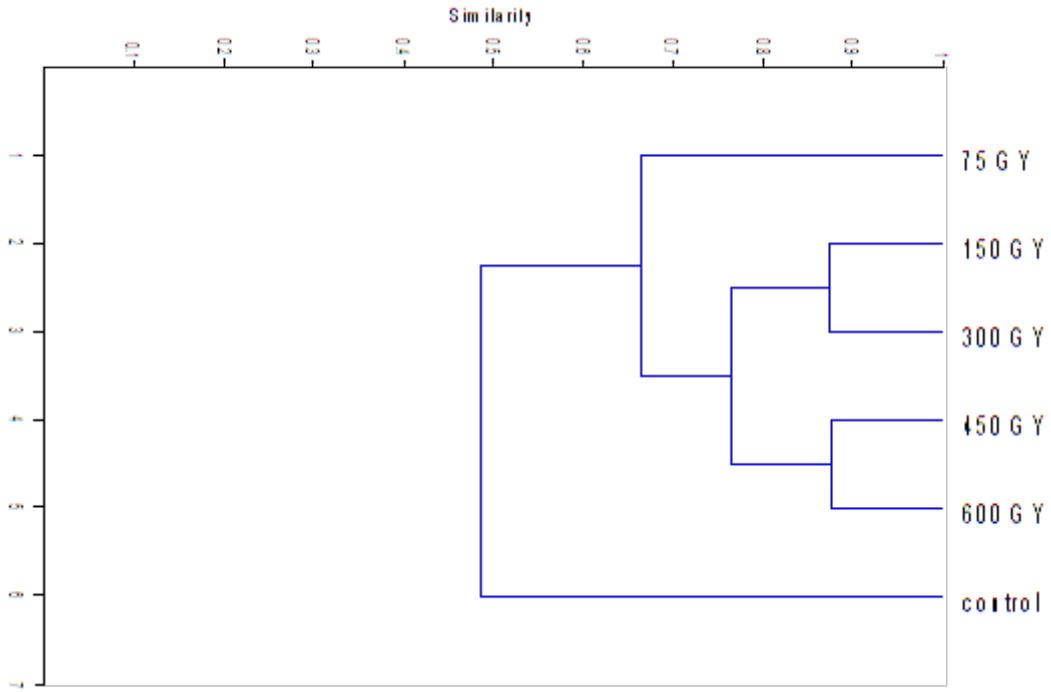

Fig. 4. The dendrogram of genetic relationships among yardlong bean irradiated plants and its control based on RAPDs markers.

from the matrix of Dice (1945) and similarity coefficient values (S) within irradiated yardlong bean plants (Fig. 4).

All the tested yardlong bean treated plants were distributed by the dendrogram into two main clusters; the first one was related to untreated control. while, the second cluster splitted into two sub-clusters, the first sub cluster was for plants treated with 75Gy while the second sub-cluster subdivided into two main groups, the first one splitted to two sub-clusters the first one was for 150Gy and the other one was for 300Gy treatment. On the other side, the second group was splitted to two sub–cluster the first one was for plants treated with 450Gy however, the second sub-cluster was related to plants irradiated with 600Gy.

ISSR- PCR ANALYSIS

Although, ISSR technique was commonly used to determine genetic diversity, phylogeny, gene tagging, genome mapping and evolutionary biology studies (Reddy *et al.* 2002), it has been also used to detect DNA polymorphism induced by gamma rays (Labajova´ et al. 2011 and Mejri et al. 2012). All the ten ISSR primers used in the present work produced scorable amplified fragments in all irradiated and non-irradiated yardlong bean plants (Control) except M2 primer which, did not produced any amplicons at control plants (Fig. 5).

As shown in Table (7), 62 amplified fragments at size ranged from 206 to 1973 bp were produced by the ten ISSR primers with an overall mean of 6.2 ± 0.36 and ranged from 4 bands of A1 primer to 8 bands in ISSR4 primer. From 62 generated bands only 26 were polymorphic with an overall mean of2.6 ± 0.82. The percentages of polymorphism among primers ranged from 0.0% to 100% with an overall mean of 48.04 ± 12.48% as shown in Table 7. The primer M2 did not produce any monomorphic fragments and generate 6 polymorphic with 100% polymorphism. ISSR amplicons generated by M2 primer only exhibited 100% polymorphism among all treated plants, while the four primers (ISSR1, M8, M12 and A1) revealed 0.0% polymorphism.

Data in Table 7 revealed that, all the ISSR primers did not produce any unique bands except ISSR4 primer which produced only one unique band. These results demonstrated that this unique sequence could be used as a molecular marker associated with gamma irradiation. Abdelfattah et al. (2014) exposed five cowpea seed varieties to different doses of gamma rays at 50, 100, 200 and 300Gy and scored the variation in seed protein electrophoretic pattern, RAPD and ISSR fingerprinting to assess genetic variation among

the M2 genotypes. The gamma dose of 50Gy resulted in an increase of growth parameters and enhanced yield components in most of varieties. Gamma rays induced more genetic variation in the genotypes of cv. Kaha 1 and cv. Dokki 331 compared to other cultivars as estimated by the cluster analysis of seed protein, RAPD and ISSR markers. On the same side, Mudibu et al. (2011) found an increase in DNA polymorphism at soybean plants irradiated with gamma rays. The effects might be due to changes in DNA structure caused by different types of DNA damages **(**Sonia et al. 2012**)**.

Genetic similarity and cluster analysis among yardlong bean irradiated plants and its control were determined based on ISSR markers. The results revealed that the highest similarity index (0.939) was found between yardlong bean plants irradiated with 450Gy and 150Gy. However, the lowest similarity index (0.824) was observed between plants irradiated with 300Gy and 75Gy as shown in Table 8.

The obtained dendrogram showed that all irradiated yardlong bean plants and its control were distributed into two main clusters. The first one was related to control plant (0GY), while the second cluster splitted into two sub-clusters, the first one was for plants irradiated with 75Gy

TABLE 7. Fragment size, total number of polymorphic and unique bands and polymorphism % obtained by using ten ISSR primers in yardlong bean plants irradiated with different doses of gamma ray.

Primers	Fragment size (bp)	Monomorphic bands	Unique bands	Polymorphic bands		Total number of bands	Polymorphism (%)
				Without unique	With unique		
ISSR1	264-625	5	0	0	0	5	0.00
ISSR2	291-1617	4	0	3	3	7	42.86
ISSR3	291-1343	3	0	4	4	7	57.14
ISSR4	206-946	1	1	6	7	8	87.50
M2	209-1474	0	0	6	6	6	100
M3	242-1973	3	0	3	3	6	50
M7	187-1468	4	0	3	3	7	42.86
M8	209-1149	6	0	0	0	6	0.00
M12	222-975	6	0	0	0	6	0.00
A1	282-494	4	0	0	0	4	0.00
Total		36	1	25	26	62	
Mean ± SE		3.6 ± 0.62	0.1 ± 0.10	2.5 ± 0.76	2.6 ± 0.82	6.2 ± 0.36	48.04 ± 12.48

TABLE 8 . Dice's similarity coefficient matrix within yardlong bean plants irradiated with different doses (75, 150, 300, 450, and 600 Gy) of gamma rays based on bands polymorphism of ISSR primers.

Treatment	Control	75 GY	150 GY	300 GY	450 GY	600 GY
Control (0Gy)	-					
75 GY	0.857	-				
150 GY	0.887	0.927	-			
300 GY	0.837	0.824	0.909	-		
450 GY	0.874	0.879	0.939	0.935	-	
600 GY	0.893	0.916	0.922	0.879	0.929	-

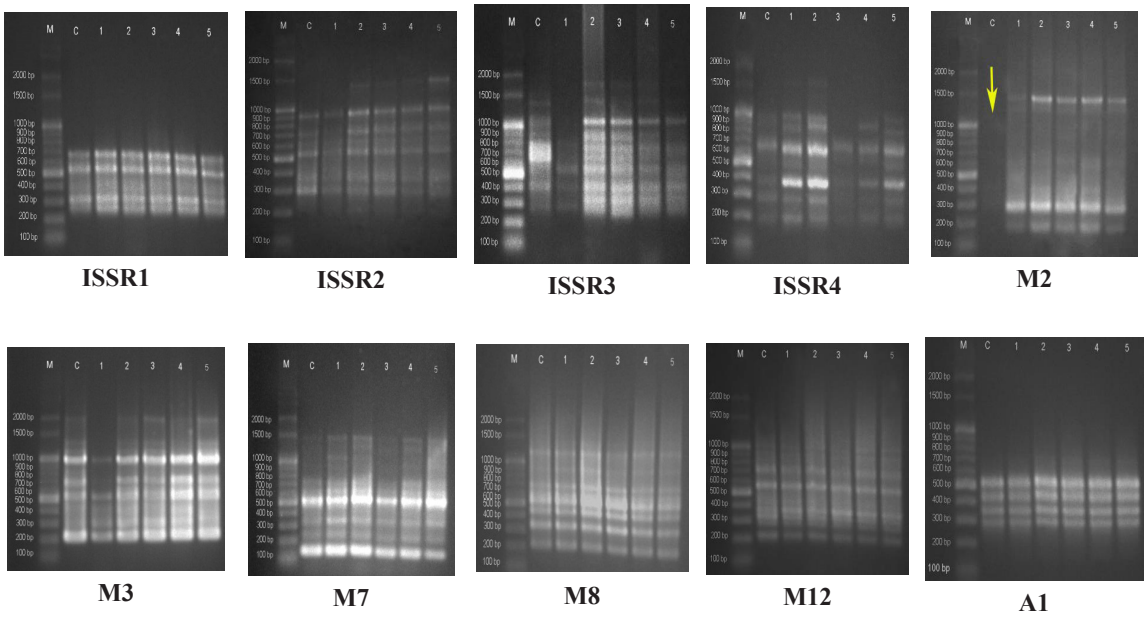

Fig. 5. Electrophoretic gel patterns of ISSR DNA products of ISSR1, ISSR2, ISSR3, ISSR4, M2, M3, M7, M8, M12 and A1 primers, Lane C, Control; lane 1, 75Gy; lane 2, 150Gy; lane 3, 300Gy; lane 4, 450Gy and lane 5, 600Gy.

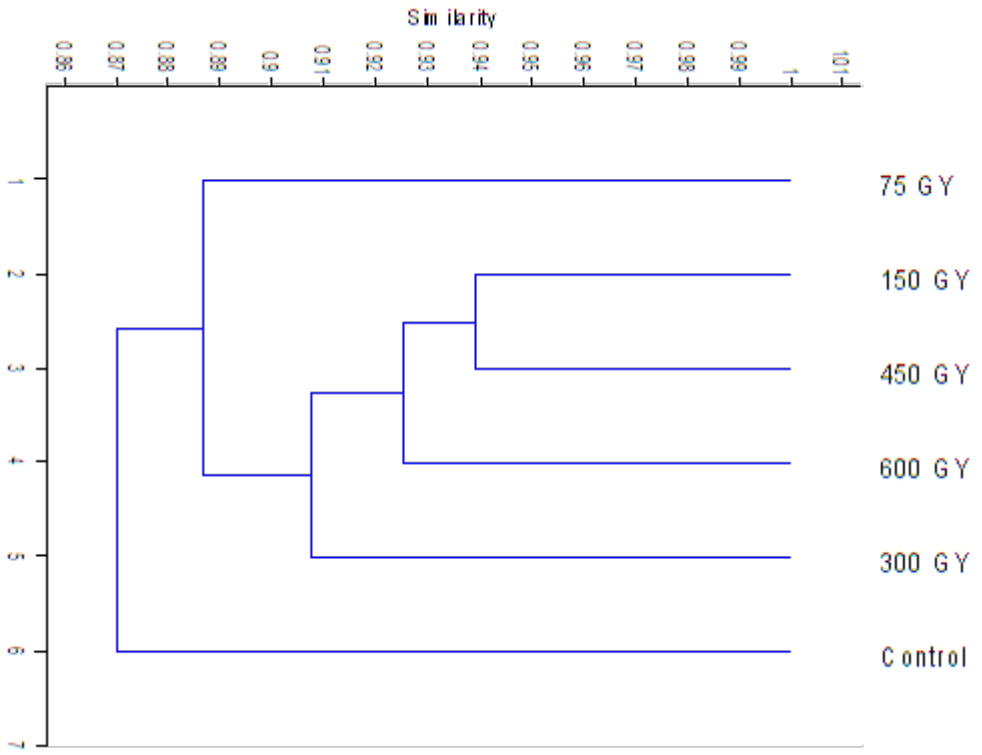

Fig. 6. The dendrogram of genetic relationships among yardlong bean irradiated plants and its control based on ISSR markers.

whereas the second sub-cluster contained all of the other irradiated plants. That cluster, in turn, was subdivided into two main groups (Fig.6).

In conclusion, the present data demonstrated that treatment of yardlong bean seeds with different gamma ray doses induced variability at both molecular and whole plant levels which could be used as good materials in yardlong bean breeding programs.

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Conflict of Interest

We wish to confirm that there are no known conflicts of interest associated with this publication

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التباينات الوراثية والصفات الخضرية المستحدثة بواسطة أشعة جاما فى نباتات اللوبيا الطويلة

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أجريت هذه الدراسة لدراسة التباينات الوراثية والخضرية الناتجة عن معاملة بذور نباتات اللوبيا الطويلة بأشعة جاما بإستخدام جرعات إشعاعية مختلفة (٧٥، ١٥٠، ٣٠٠، ٤٥٠، ٦٠٠ جراي) وقد استخدمت تقنية الـ RAPD و الـ ISSR لدراسة هذه الاختلافات حيث إستخدام ١٠ بادئات للـ RAPD و ١٠ بادئات للـISSR وهذه البادئات انتجت ١١٧ حزمة مع RAPD منها ٩٤ حزمة مختلفة و ٢٣ حزمة متشابهة وتراوحت النسبة المؤية للـ للحزم المختلفة من صفر الي ١٠٠٪. جميع البادئات التي أستخدمت مع تقنية الـ RAPD أنتجت حزم منفردة أو متخصصة بإستثناء OPK—06 والذي لم ينتج عنه أى حزم متخصصة. وعلى صعيد أخر، الـ ٦٢ حزمه المتضاعفة بإستخدام العشرة بادئات فى تقنية الـ ISSR أشتملت على ٢٦ حزمه متعددة و ٣٦ حزمه متشابهة. جميع بادئات الـ ISSR لم تعطى حزم متخصصة ماعدا بادئ الـ ISSR4 الذى أعطى حزمة متخصصة واحدة فقط. هذة النتائج أوضحت أن مثل هذة التتابعات المتخصصة يمكن إستخدامها كواسمات جزيئية مرتبطة عند استخدام أشعة جاما. وأظهرت النتائج الحقلية أن نسبة الإنبات إنخفضت مع زيادة الجرعات الإشعاعية حتى ٦٠٠ جراي و التي أعطت اقل نسبة انبات. تم الحصول على قيم غير ثابتة للصفات الخضرية بعد كل معاملة إشعاعية، حيث أعطت معاملة ٣٠٠ Gy أعلى قيمة لارتفاع النبات وعدد الأفرع وطول القرن وعدد البذور لكل قرن مقارنة بمعاملة االكنترول والمعاملات الأخرى. لم تأخذ قيم معاملات التباين المظهرى والوراثى مسارا منتظما سواءا مع زيادة أو نقصان الجرعات الإشعاعية في كلا الموسمين. بناء على النتائج المتحصل عليها فى هذة التجربة يمكن القول بأنه يمكن استخدام أشعة جاما للحث على استحداث تباينات وراثية والتي يمكن استخدامها في برامج تحسين نبات اللوبيا الطويلة.