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Mutation induced by gamma irradiation in coriander seeds and their identification by RAPD makers

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***Abstract*---**This study was accomplished to detect variation generated through exposure of Coriander (Coriandrum sativum L.) seeds to five gamma irradiation doses (0,25,50,100 and150) GY by using eighteen RAPDs primers as molecular marker ,results indicated that primers OPC-05 ,OPC-19, OPA-10,OPA-15 and OPB-06 success in giving three treatments a unique fingerprint .Molecular size of amplified ranged between 1860 bp produced by primer OPA-04 and 115 bp produced by primer OPW-04 . Highest degree for polymorphism was 91.66 % produced by primer OPA-04. Monomorphic bands appeared in their higher value in primer OPC-09 . Primer OPA-10 gave highest number unique bands (reached to seven bands) Primer OPC-19 gave highest value for amplified bands , primer OPA-10 gave highest value for main bands, Discrimination, in addition to primer efficiency value produced in their high value by primer OPA-04.This study revealed RAPD markers in detection mutation induced by gamma irradiation.

***Keywords*---**coriander, RAPD, gamma ray, mutation.

Introduction

Coriander (*Coriandrum sativum* L.) is an important medicinal plant belongs to family Apiaceae ,It is believed that coriander originated from around the Mediterranean and cultivated widely, mainly in the tropics ,India has the prime position in the cultivation and production of coriander In addition to India, coriander is also cultivated in Morocco, Rumania, France, Spain, Italy ,the Netherlands, Myanmar, Pakistan, Turkey, Mexico Argentina and, to some extent, in the UK and the USA (Sahib *et al.*,2013 ;khan *et al.*, 2014) , it is cultivated

commercially in Europe, Asia and Africa (Bhat *et al.*,2014; Ahmed *et al.*,2018). Its matured fruits and fresh leaves are used as spices and traditional medicine.(Shahwar *et al.*, 2012; Izgi ,2020), this due to its fruits content of essential oil,the major component of the oil is linalool, accounts for about two-thirds of the oil (Shahwar *et al.*, 2012; Izgi ,2020). Agents causing mutation induction are called mutagens. The main mutagens used in plant breeding may be categorized as physical (e.g., gamma rays, X-rays) or chemical (e.g., alkylating agents such as *N*-methyl-*N*-nitrosourea). Mutation techniques are also powerful tool in functional genomics studies.(Maluszynski *et al.*,2017).

Mutation induction can be done on the plants by mutagenic treatment of certain materials of plant reproductive organs such as seeds, stem cuttings, pollen, root rhizome, tissue culture and others. If a natural mutation process is very slow, the acceleration, frequency and spectrum of mutations can be induced by mutagen treatment of certain materials (Hanafiah *et al.*, 2010).Mutation induction with radiation is the most frequently used method to develop direct mutant varieties, where improvement by acclimatization, selection, hybridization and also laborious have proven to be time consuming and also with limited genetic variation. Mutation breeding of plants is useful to improve the character if the character you want is not located in a plant germplasm of a species, and also for generating variability in the existing varieties (Van Harten 1998; Yaqoob and Rashid 2001; Khan and Goyal 2009).

Random amplified polymorphic DNA (RAPD) markers represent amplification products from a polymerase chain reaction (PCR) utilizing arbitrary primers and genomic DNA ,standard RAPD technology proposed by Williams *et al.*, (1990) utilizes short synthetic oligonucleotides (10 bases long) of random sequences as primers to amplify nanogram amounts of total genomic DNA under low annealing temperatures by PCR. Amplification products are generally separated on Agarose gels and stained with ethidium bromide. In coriander ,RAPD markers used in detection molecular diversity (Choudhary *et al.*,2019),molecular characterization of genotypes(Sharma *et al.*,2019) , detection of phytotoxic and genotoxic effects of copper nanoparticles (AlQuraidi *et al.*,2019).

Material and Methods

Seed treatment pre DNA extraction

This study was conducted at laboratories of Biology Department, College of Sciences, Kufa University. Grains of Coriander were irradiated with gamma rays at dose levels (25,50, 100 and 150 Gy) in addition to control treatment (not irradiated) using Cobalt ^{60} as irradiation source. Fresh plant material at age of 30 days (fresh fully expanded and undamaged leaves), was separated from whole plants and grinded by liquid nitrogen pre DNA extraction for molecular genetic characteristics studies.

DNA extraction

DNA was extracted from fresh apical leaves at age of thirty days using DNA Mini Kit, Taiwan. The isolated DNA concentration and purity were evaluated using Bio-

drop apparatus Agarose gel electrophoresis for total genomic DNA accomplished using 1% Agarose at 90 volt for about one hour following the procedure of Sambrook and Russell (2001).

PCR amplification

RAPD amplification were performed in 20 µl volume of amplification reaction of Maxime PCR Pre Mix Kit (i-Taq) including 7 µl of template DNA, 5 µl of reaction mixture containing (i-Taq DNA polymerase (5U/µl):2.5U, dNTPs 2.5 mM each, reaction buffer (10x):1x, gel loading buffer:1x), 5 µl of primer and final reaction volume of 20 µl completed by deionized distilled water and amplification were performed in a programmed Thermo Cycler, Agilent Technology Sure Cycler 8800/Malaysia)

PCR programmed

PCR programmed as mentioned by Carelli et al., 2006; Abd El-Hady et al., 2010; Ezekiel et al., 2011 and El-Assal and Gaber,2012 (Initial denaturation at 94 for 3min,40 cycles , denaturation at 94 for 1min, annealing variable as in table (1) for 1min, extension for 1min at 72 and final extension at 72 for 5min

RAPD markers amplification product electrophoresis

Product electrophoresis accomplished at 80 V using 1.2 % agarose for about two hours. The Primers were provided by Bioneer Corporation in lyophilized form, dissolved in TE buffer to obtain 100 pmol/µl as a final concentration (stock solutions). Working solutions 10 pmole/µl were prepared from stock solutions, eighteen primers were used in application of eighteen RAPDs markers in tables (1) with their nucleotide sequences and names of each primer.

Table 1
Primers used as RAPDs markers

Primer name	Sequence 5' ⟶ 3'	Temperature
OPA-01	CAGGCCCTTC	40 C°
OPA-02	TGCCGAGCTG	40 C°
OPA-03	AGTCAGCCAC	40 C°
OPA-04	AATCGGGCTG	40 C°
OPA-10	GTGATCGCAG	40 C°
OPA-14	TCTGTGCTGG	37 C°
OPA-15	TTCCGAACCC	37 C°
OPB-06	TGCTCTGCCC	37 C°
OPB-17	AGGGAACGAG	37 C°
OPC-05	GATGACCGCC	37 C°
OPC-08	TGGACCGGTG	37 C°

OPC-09	CTCACCGTCC	37 C°
OPC-19	GTTGCCAGCC	40 C°
OPH-01	GGTCGGAGAA	37 C°
OPW-04	CAGAAGCGGA	40 C°
OPX-03	TGGCGCAGTG	40 C°
OPX-17	GACACGGACC	40 C°
OPA-17	GACCGCTTGT	37 C°

PCR Pre Mix master mix. Bioneer Corporation USA, (0.2ml) thin-wall 8-strip tubes with attached cup / 96 tubes were used .PCR amplification programmed according to Carelli *et al.*, 2006; Abd El-Hady *et al.*, 2010; Ezekiel *et al.,* 2011 and El-Assal and Gaber,2012 (initial denaturation 94 C° for 3min, 40 cycles including denaturation 94 C° for 1min , annealing for 1min and temperature extention for 1min at 72 C° and final extention at 72 C° for 10 min ,later agarose gel electrophoresis using 1.5% agarose ,70 volt for two hours (Sambrook and Russel (2001), The photographs resulted from agarose gel electrophoresis was used to score data, presence of a product was identified as (1) and absence was identified as (0), data then entered into PAST statistic vital program, Version 62.1 (Hammer *et al.,* 2001)

Results and Discussion

Evaluation of genomic

DNA was performed using Bio drop apparatus. The concentration was 78.91µg/ml with purity 1.8 figure(1)

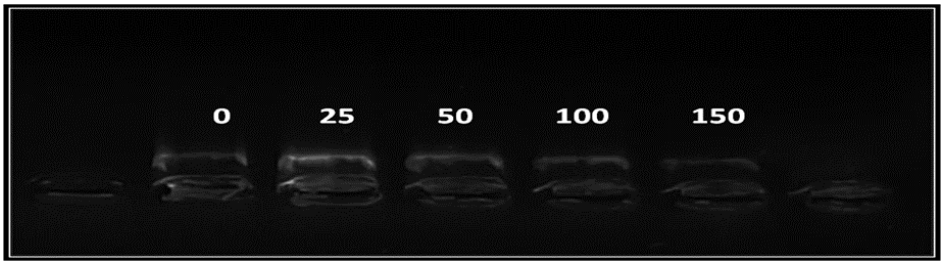

Figure 1. Agarose gel electrophoresis of coriander total genomic DNA: control (0 : not irradiated) and irradiation treatment : 25 GY , 50 GY ,100 GY and 150 GY

Results in table (2) show that primers OPC-05 ,OPC-19, OPA-10,OPA-15 and OPB-06 success in giving three treatments a unique fingerprint ,the rest primer gave lower number for treatment fingerprinting , when primer possess ability to produce polymorphic and unique bands especially with diverse doses , both polymorphic and unique alleles inside genotypes increase chance for producing unique fingerprint (Kachapur *et al.*, 2009 , Idris, *et al.*, 2012,Fadoul *et al.*, 2013 ,AL-Tamimi, 2014 and AL- Ghufaili,2017 , AL-Tamimi,2021).

Table 2
Coriander treatment fingerprinting (DNA profile) using 16 RAPD marker

No.	Primer	Treatments	No. of Fingerprint
1	OPC-05	0,25,50	3
2	OPB-17	None	0
3	OPC-09	None	0
4	OPC-08	0	1
5	OPA-04	50	1
6	OPA-10	0,25,50	3
7	OPC-19	0,25,50	3
8	OPA-15	50,100,150	3
9	OPA-01	0,25	2
10	OPA-02	50	1
11	OPA-03	None	0
12	OPA-14	None	0
13	OPA-17	None	0
14	OPB-06	0,25,50	0
15	OPH-01	None	0
16	OPX-03	None	0
17	OPX-17	None	0
18	OPW-04	None	0

Table (3) and figures (2-7) illustrates total data for analysis of eighteen RAPD primers , it indicates that the higher molecular size was 1860 bp produced by primer OPA-04 and lower one was 115 bp produced by primer OPW-04. Since irradiation was able to change DNA profile in some treatments , this appeared clearly through arising of polymorphic and unique bands, its associated with mutation by insertions or deletions mutations which cause a change in primer annealing sites consequently change size of amplified fragment (product), because it could change distance between two annealing sites of primer on target DNA (Powell *et al.*, 1996 ,Fadoul *et al.*,2013 and AL-Saadi.,2018,AL-Tamimi.,2021),this later will affect both amplified and main bands .

Highest degree for polymorphism was 91.66 % produced by primer OPA-04 ,this due to that primer when recognize a high number of annealing sites due to its structure ,this increase possibility of detecting DNA polymorphisms among individuals , polymorphism always related to increasing number of polymorphic bands (eleven from twelve were polymorphic in OPA-04) (Hunter and Gaston 1988 and Graham and McNicol 1995), thus, RAPD could be used for the detection of DNA alteration after the influence of mutagenic agent (Al-Tamimi,2021) ,since , formation of new variation that could be detected by changes in RAPD profiles (Selvi *et al.*, 2007). The appearance of new bands could be explained as a result of DNA structural changes.

Monomorphic bands appeared in their higher value in primer OPC-09. ,since all treatments belong to coriander , appearance related to this relationship (Al-Judy.,2004) because genome contains conserved sequence retained in all treatments including those arises from irradiation treatments) ,these shared

sequences appeared in form of monomorphic bands (Russel *et al.*, 1997; ,AL-Badeiry, 2013 and Al-Tamimi,2020). Primer OPA-10 gave highest number unique bands (reached to seven bands) ,primer ability of to recognize a unique annealing site on genome success in producing a unique DNA fingerprint for a particular genotype (Fadoul *et al.*,2013 ;AL- Ghufaili, 2017, AL-Saadi.,2018 ,AL-Tamimi.,2021). Primer OPC-19 gave highest value for amplified bands , primer OPA-10 gave highest value for main bands ,these two criteria concerned primer ability to recognize more annealing sites.

Discrimination, in addition to primer efficiency value produced in their high value by primer OPA-04, Both, primer efficiency, discriminatory value concerned with primer ability to produce polymorphic bands Hunter and Gaston (1988) and by Graham and McNicol (1995), these two criteria increase primer ability in giving unique fingerprint and consequently increase polymorphism as produced in primer. Primers OPB-17,OPC-09,OPA-03,OPA-14,OPA-17,OPH-01,OPX-03,OPX-17 and OPW-04 ,this may due to primer ability to bind with many annealing sites which increase its chance to generate polymorphism, it's also concerned with both template and primer nucleotide sequence. The rest set of primers that gave lowest value for calculated data since their parameters value less than the other previous primers.

Table 3
Summarized results of RAPDs amplification product include :Amplified bands molecular size range in bp ; No. of : main , amplified ,monomorphic, polymorphic and unique bands ; primer polymorphism (%) , efficiency and discriminatory value (%)

Primers	molecular size in bp	Main bands	Amplified bands	Monomorphic band	Polymorphic band	Unique bands	Polymorphism (%)	Efficiency	Discriminatory Value (%)
OPC-05	1391-305	12	26	2	4	5	33.33	0.15	7.69
OPB-17	1040	1	5	1	0	0	0	0	0
OPC-09	1208-277	6	30	6	0	0	0	0	0
OPC-08	1294-239	6	29	5	1	0	16.66	0.03	1.92
OPA-04	1860-258	12	24	0	11	1	91.66	0.45	21.15
OPA-10	1350-208	14	27	1	6	7	42.85	0.22	11.53
OPC-19	1803-285	13	33	2	7	4	53.84	0.21	13.46
OPA-15	1133-288	9	18	1	5	3	55.55	0.27	9.61
OPA-01	1413-194	12	24	0	10	2	83.33	0.41	19.23
OPA-02	841-156	5	18	2	3	0	60	0.16	5.76
OPA-03	396-300	2	10	2	0	0	0	0	0
OPA-14	360	1	5	1	0	0	0	0	0
OPA-17	296-194	2	10	2	0	0	0	0	0
OPB-06	2635-249	6	13	0	5	1	83.33	0.38	9.61
OPH-01	633-154	4	20	4	0	0	0	0	0
OPX-03	886-205	4	20	4	0	0	0	0	0

OPX-17	852-115	5	25	5	0	0	0	0	0
OPW-04	479	1	5	1	0	0	0	0	0

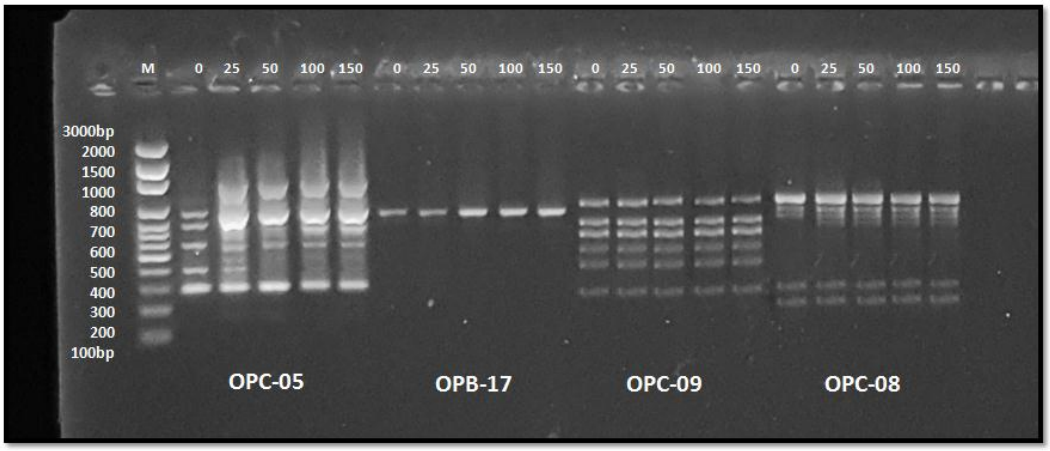

Figure 2. Agarose gel electrophoresis for PCR amplification product of primers OPC-05,OPB-17,OPC-09,OPC-08 . Coriander treatment : 1. control (0: not irradiated) and irradiation treatment 2- 25 GY 3 -50 GY 4-100 GY 5-150 GY,lane M for 100 bp DNA ladder.

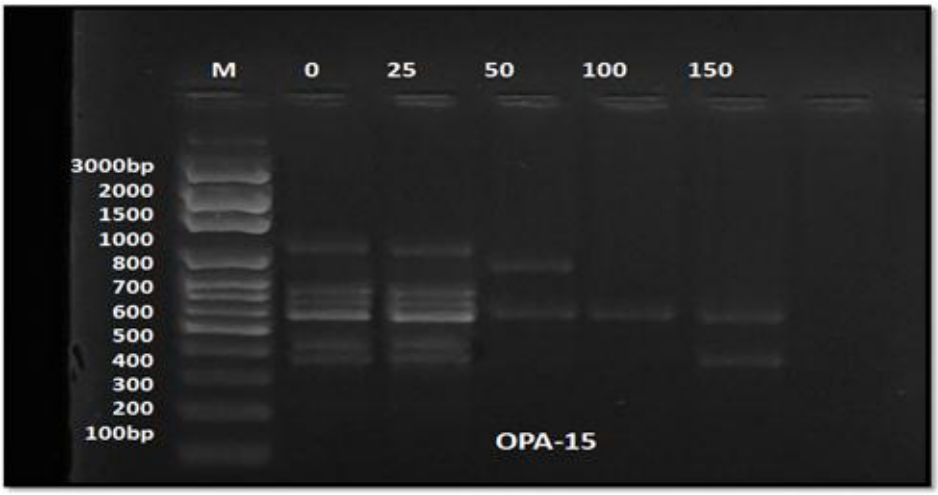

Figure 3. Agarose gel electrophoresis for PCR amplification product of primers OPA-15 . Coriander treatment : 1. control (0: not irradiated) and irradiation treatment 2- 25 GY 3 -50 GY 4-100 GY 5-150 GY,lane M for 100 bp DNA ladder

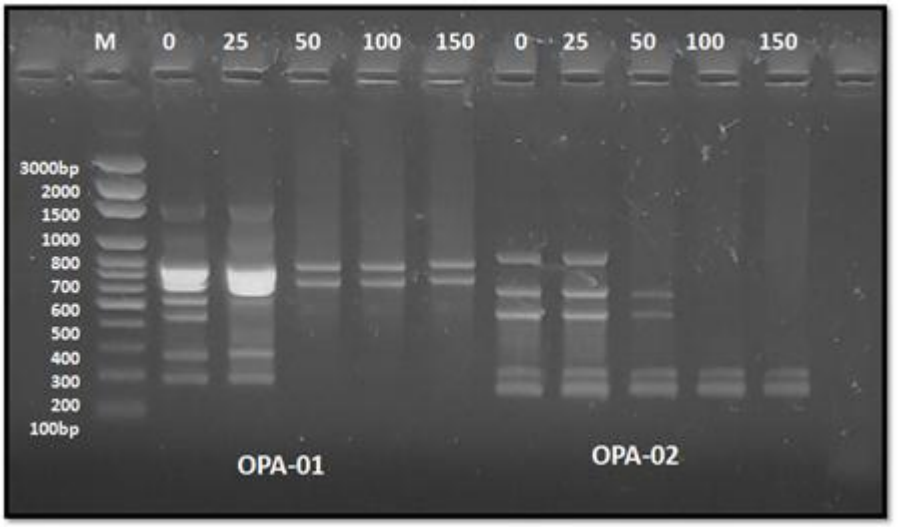

Figure 4. Agarose gel electrophoresis for PCR amplification product of primers OPA-02 and primers OPA-01. Coriander treatment: 1. control (0: not irradiated) and irradiation treatment 2- 25 GY 3 -50 GY 4-100 GY 5-150 GY,lane M for 100 bp DNA ladder

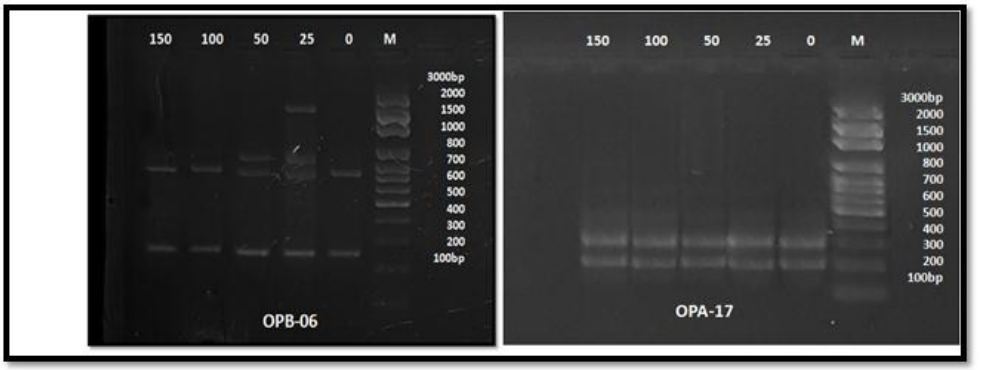

Figure 5. Agarose gel electrophoresis for PCR amplification product of primers OPA-17 and primers OPB-06. Coriander treatment: 1. control (0: not irradiated) and irradiation treatment 2- 25 GY 3 -50 GY 4-100 GY 5-150 GY,lane M for 100 bp DNA ladder

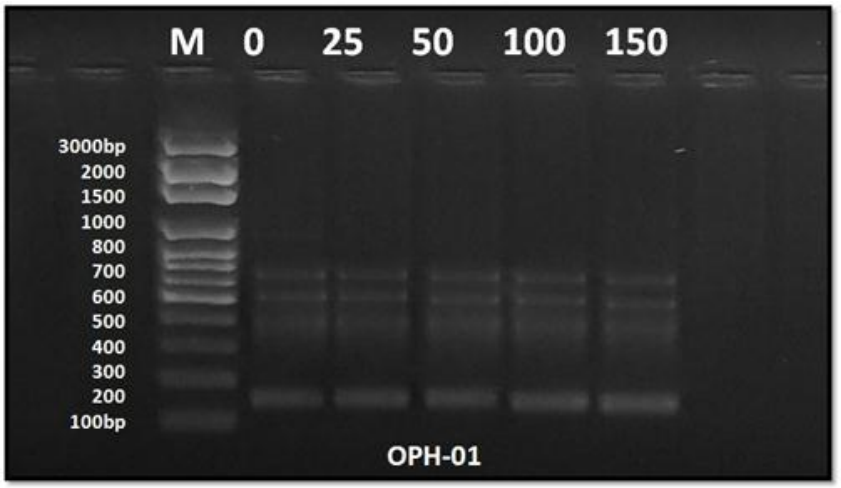

Figure 6. Agarose gel electrophoresis for PCR amplification product of primers OPH-01. Coriander treatment: 1. control (0: not irradiated) and irradiation treatment 2- 25 GY 3 -50 GY 4-100 GY 5-150 GY,lane M for 100 bp DNA ladder

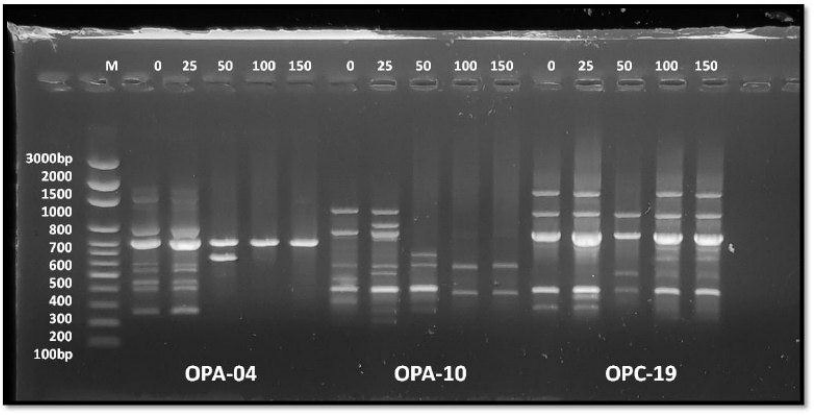

Figure 7. Agarose gel electrophoresis for PCR amplification product of primers OPA-04, OPA-10 and OPC-19. Coriander treatment: 1. control (0: not irradiated) and irradiation treatment 2- 25 GY 3 -50 GY 4-100 GY 5-150 GY, lane M for 100 bp DNA ladder

Conclusion

Gamma irradiation able to generates genetic variation through polymorphism detected by RAPD markers.

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Conflict of interest

There is no conflict of interest in this work.

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