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Characterization of mutation induced in Fenugreek by Gamma Ray using rapid marker

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***Abstract*---**This study was accomplished to detect variation generated through eposure of fenugreek (trigonella foenum-graecum L.) seeds to seven gamma irradiation doses (0,25,50,100,150,300 and 400) GY by using twenty RAPDs primers as molecular marker ,results indicated that primers OPA-14 ,OPC-19 and OPH-01success in giving five treatments a unique fingerprint. Molecular size of amplicon ranged between 2913 bp produced by primer OPB-06 and 118 bp produced by primer OPW-04.Highest degree for polymorphism was 90.92% produced by primer OPC-05. Monomorphic bands appeared in their higher value in primer OPC-08. Primer OPA-15 gave highest number unique bands Primer OPH-01 gave highest value for (amplified, main) bands, discrimination, in addition to primer efficiency value. Silght variation produce among primers OPE-02, OPC-05, OPH-01 and OPA-14. This study revealed RAPD markers in detection mutation induced by gamma irradiation.

***Keywords*---**Fenugreek, Rapd, Gamma Ray, Mutation.

Introduction

Nutritional and bioactive substances found in fenugreek are essential to human health and the functioning of biological systems. This seed contains carbs, protein, fat, and fiber. Fenugreek leaves, on the other hand, contain carbs, protein, and fiber. According to Wani and coworkers (2018)

There has been a surge in interest in the use of medicinal and aromatic plants (MAPs), because of their role as key sources of plant secondary metabolites

crucial to human health and well-being (Chaudhary et al.,2018). Fenugreek has been found to be an important medicinal plant with a significant variety of therapeutic qualities such as anti-diabetic, hypercholesterolemic, antileukemic, anti-nociceptive, antipyretic and antibacterial (Acharya et al. 2007). Since ancient times, fenugreek has been used as an important spice in many cuisines around the world, with around 70 to 97 different species being grown. In a study by Syed et al., 2020).Mutagenic agents, such as physical, chemical, and insertional mutagen treatments, fall under the umbrella term "mutagens" (Chaudhary et al., 2019).

In plant mutation breeding programs, ionizing radiation such as alpha, beta, protons, X rays, cosmic rays, gamma rays, and high-energy UV rays has been used to select for novel genotypes with enhanced crop traits. Thus, radiation-induced mutation (particularly gamma rays) became the most extensively utilized way to enhance direct mutant genotypes (Beyaz and Yildiz, 2017). As electromagnetic radiation, gamma radiation ionizes the molecules within the cells, causing mutagenesis and physiological alterations at the plant level. This may lead to the creation of free radicals, which may readily modify or damage the cell components. It's been reported that (Hasbullah and colleagues, 2012).

For genetic variation, the molecular markers (DNA based markers) are used as tools to represent the different segments of DNA whose sequence is related to genomes which is responsible for a specific character (Bagali et al., 2010). Wheat possesses large size of genome and a wide range of uses with agronomic and nutritional values and thus, it an important crop among other cereal crops (Motawei et al., 2007).

Random Amplified Polymorphic DNA (RAPD) depends upon amplifying a random segment of DNA template using identical pair of primers about 8 to 10 bp in length with low annealing temperatures usually (36 to 40 °C) resulting a number of amplifying products representing different loci (Liu and Cordes, 2004). Polymorphism resulted from different mutations and causes changing in primer annealing site (Welsh and McClelland, 1990). The main advantage of this technique is that it is very simple and cheap procedure, and the prior acknowledgement of template sequence is also not required (Weising et al., 2005; Edwards and Mccouch, 2007). In wheat, RAPDs are used to study many aspects including genetic relationships among cultivars (Al-Ghufaili and Al-Tamimi, 2018), genetic diversity and fingerprinting (Fadoul et al., 2013), identification of drought tolerant genotypes (Deshmukh et al., 2012) and detection of genotypes resistance to different pathogens (Patil and Hanchinal, 2011).

Using RAPDS markers in mutation detection induced by gamma ray established in several crops including characterization induced rose mutants and and polymorphism of induced mutants (M1 generation) (Dhillon et al.,2014 z), genetic variation using RAPD markers among M1V2 and M1V3 generations of irradiated ginger (Sharim and Shamsiah .,2021), detection of mutation in torch ginger (YUNUS et al.,2013), variation among banana cultivar (Ganapathi , et al.,2008), genetic variability detection of mutant soybean (Wahyudi et al.,2020), polymorphism and mutation detection in wheat (AL-Tamimi .,2021),

Material and Method
Seed treatment pre DNA extraction

Seeds of Fenugreek *(Trigonella foenum-graecum* L) were provided from General company of Mabayn Alnahrayn for seed certification, later, 40 gm of seeds were put in petridishes for each individual irradiation treatment .Irradiated at seven doses (0,25 Gy, 50 Gy ,100 Gy,150 Gy, 300 Gy,and 400 Gy) as proposed by Hanafy and Akladious (2018) at average of 18 Gy/hour using Cobalt 60 as irradiation source.

DNA extraction

DNA was extracted from fresh apical leaves at age of three weeks 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.

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°

OPA-17	GACCGCTTGT	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°
OPD-13	GGGGTGACGA	40 C°
OPE-02	GGTGCGGGAA	38C°
OPH-01	GGTCGGAGAA	37 C°
OPW-04	CAGAAGCGGA	40 C°
OPX-03	TGGCGCAGTG	40 C°
OPX-17	GACACGGACC	40 C°
Carelli *et al.*, 2006; Abd El-Hady *et al.*, 2010; Ezekiel *et al.,* 2011 and El-Assal and Gaber,2012		

Table (1)
Fenugreek treatment fingerprinting (DNA profile) using 20 RAPD marker

No.	Primer	Treatments	No. of Fingerprint
1	OPA-14	0,25,50,100,400	5
2	OPA-17	100,150,300	3
3	OPC-08	100,150	2
4	OPC-19	0,100,150,300,400	5
5	OPH-01	0,25,50,100,150	5
6	OPX-03	50	1
7	OPW-04	50,150,400	3
8	OPB-17	150, 400	2
9	OPC-05	150	1
10	OPC-09	150,0	2
11	OPD-13	0,50,300	3
12	OPX-17	None	0
13	OPE-02	100,150,300,400	4
14	OPB-06	25,50,400	3
15	OPA-01	0,150,400	3
16	OPA-02	50,150,400	3
17	OPA-03	25	1
18	OPA-04	400	1
19	OPA-10	0	1
20	OPA-15	50,100,150	3

Result

Results in table (2) show that primers OPA-14, OPC-19 and OPH-01success in giving five treatments a unique fingerprint while primer OPX-17failed to give unique fingerprint

Table (2)
The following are some of the highlights from RAPD's amplification product: Molecular size range of amplified bands in bp; number of major, amplified, monomorphic, polymorphic, and unique bands; primer polymorphism (%), efficiency, and discriminatory value (%)

Primers	Amplified bands molecular size in bp	Number of Main bands	Number of Amplified bands	Number of Monomorphic band	Number of Polymorphic band	Number of Unique bands	Primer Polymorphism (%)	Primer Efficiency	Primer Discriminatory Value (%)
OPA-14	174-1500	11	41	0	9	2	81.81	0.219	11.1
OPA-17	157-1076	8	37	3	4	1	50	0.10	4.9
OPC-08	366-923	7	44	5	2	0	28.57	0.045	2.46
OPC-19	147-712	9	39	2	5	2	55.5	0.128	6.17
OPH-01	140-1159	16	57	2	12	2	75	0.210	14.8
OPX-03	361-984	4	22	3	0	1	0	0	0
OPW-04	118-876	6	34	3	3	0	50	0.069	3.7
OPB-17	158-784	8	40	4	3	1	37.5	0.075	3.7
OPC-05	279-796	11	50	1	10	0	90.9	0.2	12.3
OPC-09	168-761	5	27	3	2	0	40	0.07	2.46
OPD-13	223-1152	11	49	1	7	3	63.63	0.142	8.64
OPX-17	400-635	4	28	4	0	0	0	0	0
OPE-02	229-2224	11	29	1	6	4	54	0.20	7.4
OPB-06	276-2913	10	56	4	6	0	60	0.089	7.4
OPA-01	119-971	8	31	3	1	4	12.5	0.032	1.23
OPA-02	263-1150	8	34	2	4	2	50	0.117	4.93
OPA-03	235-568	5	28	3	1	1	20	0.035	1.23
OPA-04	290-1088	5	29	4	0	1	0	0	0
OPA-10	217-1047	6	35	4	1	1	16.6	0.028	1.23
OPA-15	254-922	12	33	1	5	6	41.6	0.15	6.17

Total RAPD marker analysis

Table (2) illustrates total data for analysis of twenty RAPD primers, it indicates that the higher molecular size was 2913 bp produced by primer OPB-06 and lower one was 118 bp produced by primer OPW-04. Highest degree for polymorphism was 90.92% produced by primer OPC-05. Monomorphic bands appeared in their higher value in primer OPC-08. Primer OPA-15 gave highest number unique bands Primer OPH-01 gave highest value for (amplified, main) bands, discrimination, in addition to primer efficiency value. Silght variation produce among primers OPE-02, OPC-05, OPH-01 and OPA-14. The rest set of primers that gave lowest value for caculated data since their parameters value less than the other previous primers.data illustrated in figures (1-9).

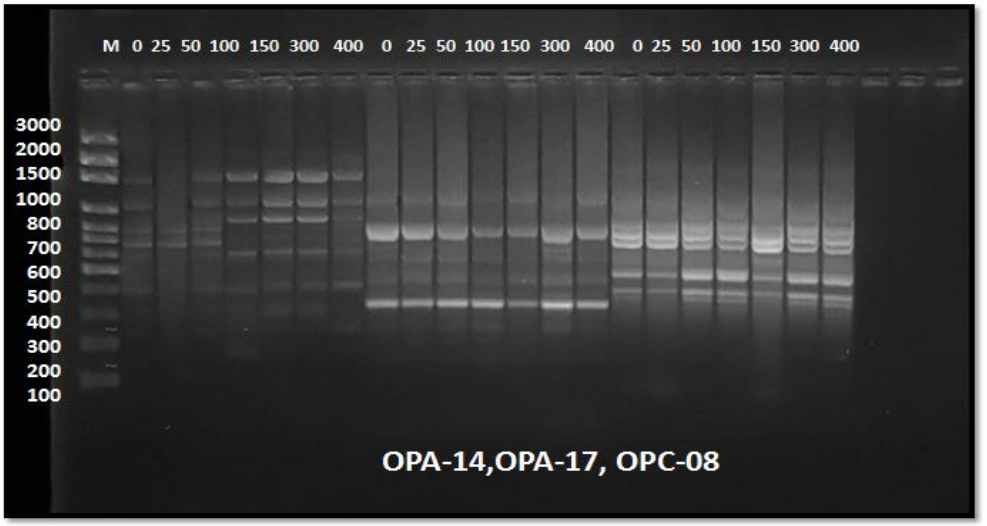

Figure: (1) Agarose gel electrophoresis for PCR amplification product of primers OPA-14, OPA-17 and OPC-08. Fenugreek treatment: 1. control (0: not irradiated) and irradiation treatment 2- 25 GY 3 -50 GY 4-100 GY 5-150 GY 6-300 GY 7-400 GY, lane M for 100 bp DNA ladder.

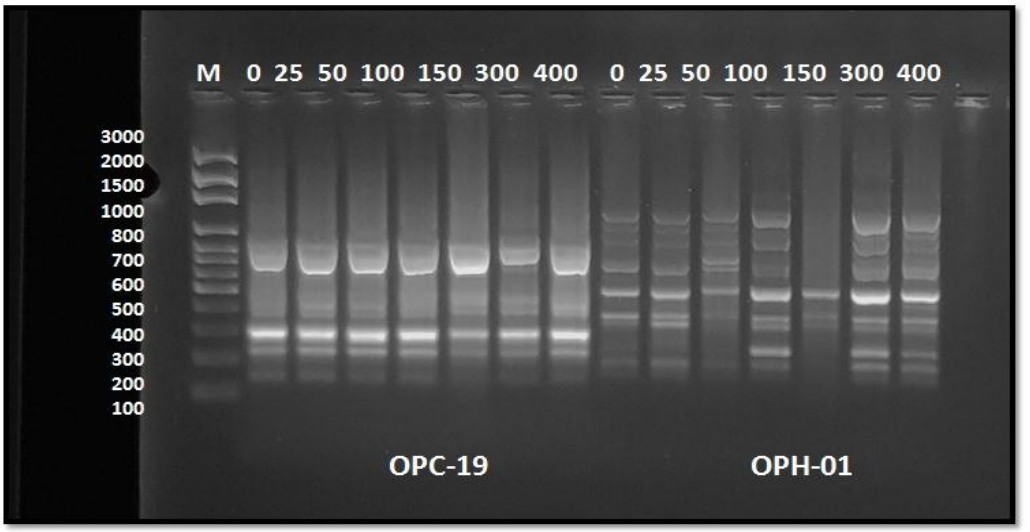

Figure (2) Agarose gel electrophoresis for PCR amplification product of primers OPC-19 and OPH-01. Fenugreek treatment: 1. control (0: not irradiated) and irradiation treatment 2- 25 GY 3 -50 GY 4-100 GY 5-150 GY 6-300 GY 7- 400 GY, lane M for 100 bp DNA ladder.

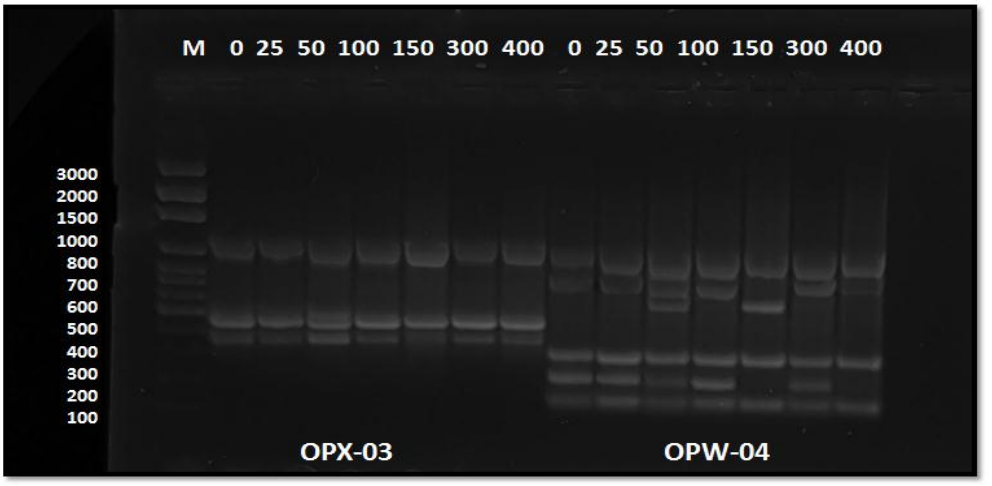

Figure: (3) Agarose gel electrophoresis for PCR amplification product of primers OPX-03 and OPW-04. Fenugreek treatment: 1. control (0: not irradiated) and irradiation treatment 2- 25 GY 3 -50 GY 4-100 GY 5-150 GY 6-300 GY 7- 400 GY, lane M for 100 bp DNA ladder.

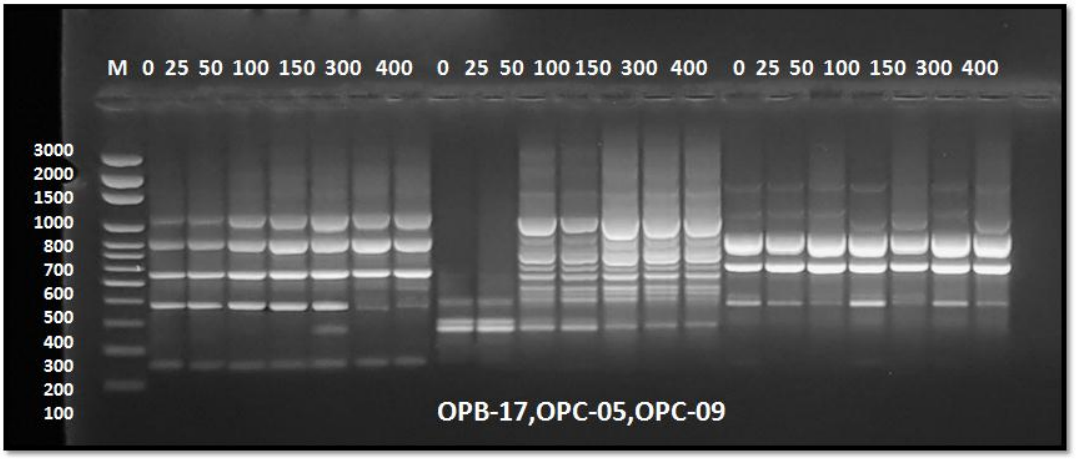

Figure: (4) Agarose gel electrophoresis for PCR amplification product of primers OPB-17, OPC-05 and OPC-09. Fenugreek treatment: 1. control (0: not irradiated) and irradiation treatment 2- 25 GY 3 -50 GY 4-100 GY 5-150 GY 6-300 GY 7- 400 GY, lane M for 100 bp DNA ladder.

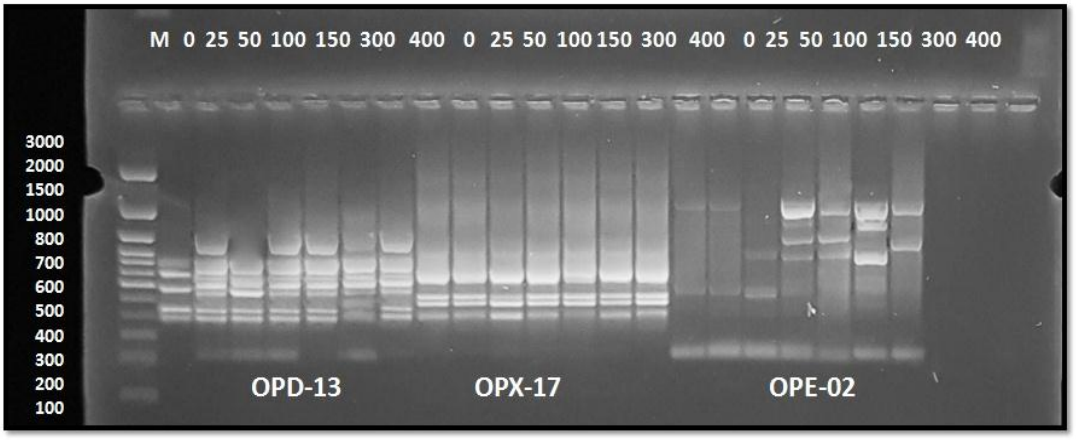

Figure: (5) Agarose gel electrophoresis for PCR amplification product of primers OPD-13, OPX-17 and OPE-02. Fenugreek treatment: 1. control (0: not irradiated) and irradiation treatment 2- 25 GY 3 -50 GY 4-100 GY 5-150 GY 6-300 GY 7-400 GY, lane M for 100 bp DNA ladder.

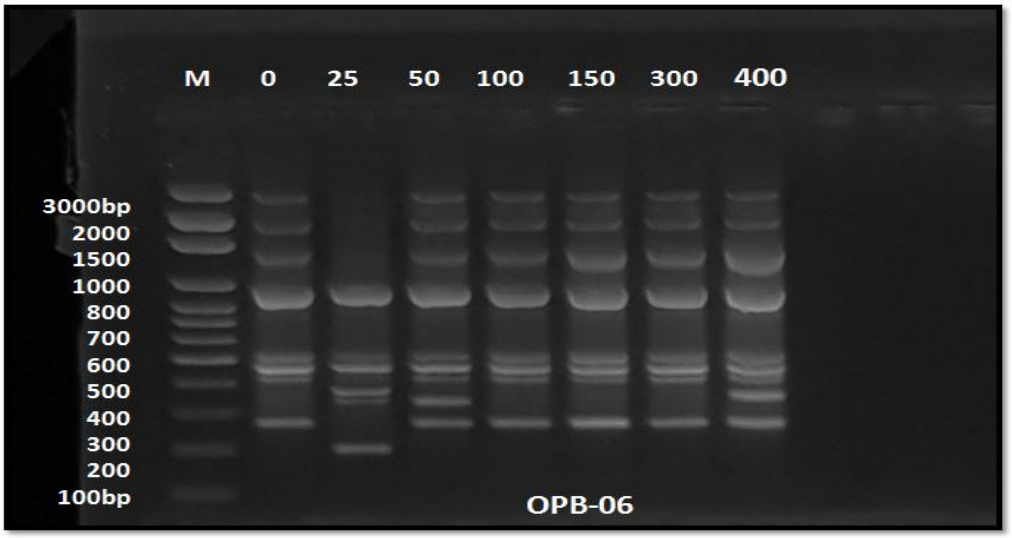

Figure: (6) Agarose gel electrophoresis for PCR amplification product of primer OPB-06 .Fenugreek treatment: 1. control (0: not irradiated) and irradiation treatment 2- 25 GY 3 -50 GY 4-100 GY 5-150 GY 6-300 GY 7- 400 GY, lane M for 100 bp DNA ladder.

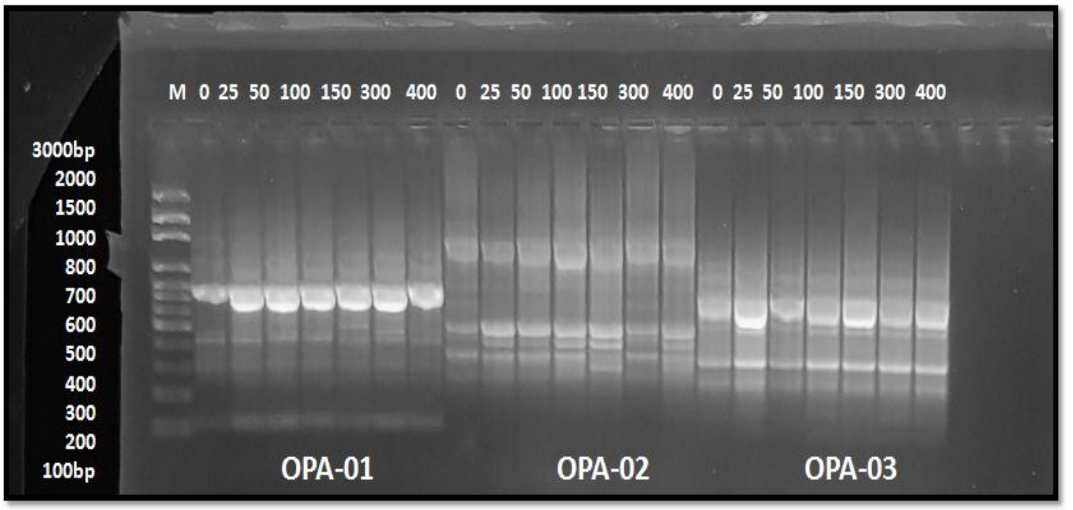

Figure: (7) Agarose gel electrophoresis for PCR amplification product of primers OPA-01, OPA-02 and OPA-03. Fenugreek treatment: 1. control (0: not irradiated) and irradiation treatment 2- 25 GY 3 -50 GY 4-100 GY 5-150 GY 6-300 GY 7-400 GY, lane M for 100 bp DNA ladder.

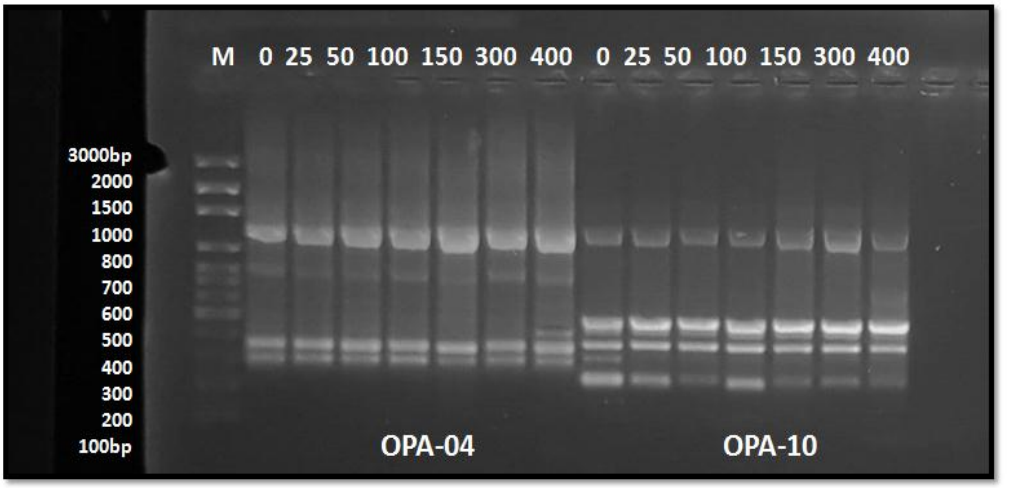

Figure: (8) Agarose gel electrophoresis for PCR amplification product of primers OPA-04 and OPA-10 .Fenugreek treatment: 1. control (0: not irradiated) and irradiation treatment 2- 25 GY 3 -50 GY 4-100 GY 5-150 GY 6-300 GY 7- 400 GY, lane M for 100 bp DNA ladder.

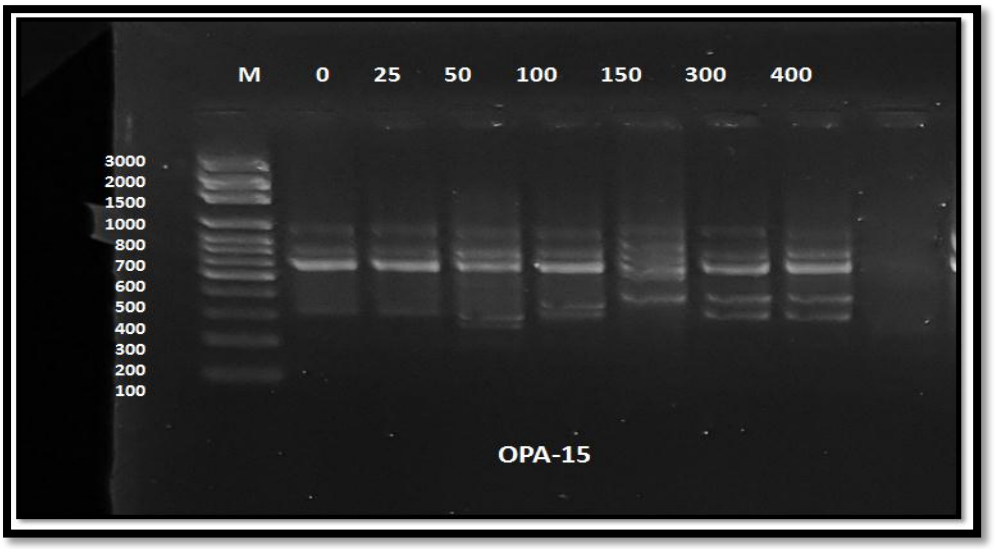

Figure: (9) Agarose gel electrophoresis for PCR amplification product of primer OPA-15. Fenugreek treatment: 1. control (0: not irradiated) and irradiation treatment 2- 25 GY 3 -50 GY 4-100 GY 5-150 GY 6-300 GY 7- 400 GY, lane M for 100 bp DNA ladder.

Due to the fact that the majority of treatments change the DNA profile, this is evident through the appearance of polymorphic and unique bands, and it's often linked to mutations that cause changes in primer annealing sites, which in turn alter the size of an amplified fragment (product). This is because the distance between the two annealing sites of the primer on target DNA can change (Powell et al., 1996 ,Fadoul et al.,2013 and AL-Saadi.,2018,AL-Tamimi.,2021),this later will affect both amplified and main bands .

High polymorphism produced by several primers is dueto that primer when recognize a high number of annealing site due to its structure, this increase posibility of detecting DNA polymorphisms among individuals, Increases in polymorphism are invariably linked to an increase in the number of polymorphic bands (Hunter and Gaston 1988, and Graham and McNicol 1995), hence RAPD might be used to identify DNA modification following the action of a mutagenic agent (Selvi et al., 2007). Changes in the DNA structure might account for the development of novel banding patterns.

Since all treatments belong to fenugreek , apperance of monomorphic bands related to this relationshipe (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).

DNA fingerprinting for a certain genotype may be generated by a primer's capacity to detect a specific genomic location for annealing (Fadoul et al., 2013; AL-Ghufaili, 2017; AL-Saadi., 2018 ; AL-Tamimi.,2021).

There is a correlation between the effectiveness of the primer and its discriminating value in producing polymorphic bands. Both Hunter and Gaston (1988), as well as Graham and McNicol (1995), found that these two requirements boost the primer's capacity to provide a unique fingerprint (Newton and Graham, 1997; Arif et al., 2010).

Conclusion

Radiation changed the shape of DNA in most treatments, and using RAPD technology, OPA-14, OPC-19 and OPH-01 were shown primers polymorphous and unique ranges, in giving five unique imprint treatments

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