

COMPARATIVE ANALYSIS OF RAPD MARKER SEQUENCES IN RELATION TO GAMMA RADIATION IN SESAME

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Sesame (*Sesamum indicum* L.) is one of the most ancient crops (Bedigian *et al.* 1986). It is grown in tropical and subtropical areas (Ashri 1998) on 7.7 million hectares worldwide, producing four million tons of seed (FAO 2009). The seeds contain 50-60% oil, which is highly resistant to oxidative deterioration even though oleic and linoleic acids are the predominant fatty acids of sesame oil, about 80% of its total (Arslan *et al.* 2007, Uzun *et al.* 2007). Sesame crop is well suited to different crop rotations and is mostly grown under moisture stress with low management inputs by small holders (Ashri and van Zanten 1994). In spite of being the first oilseed crop known to man and its long history, sesame is a typically neglected crop. It is not studied by any of the international agricultural research centers (Ashri 1998).

Genetic variation of different sesame collections has been previously reported using agromorphological characters (Bedigian *et al.* 1986; Furat and Uzun 2010; Pham *et al.* 2011). In addition molecular markers, including isozymes (Isshiki and Umezaki 1997), RAPD (Bhat *et al.* 1999; Ercan *et al.* 2004; Pham *et al.* 2009; Pham *et al.* 2011), ISSR (Kim *et al.* 2002), AFLP (Uzun *et al.* 2003; Laurentin and Karlovsky 2006; Ali *et al.* 2007) and SSR (Dixit *et al.* 2005). Some of these techniques, such as RAPD do not require prior knowledge of DNA sequence.

It can be possible to increase the genetic variability in sesame by inducing mutations with ionized radiation. Allowing isolating mutants with desirable characters of economic importance such as increased seed yield, earliness (Wongyai *et al.* 2001), modified plant architecture, closed capsules, disease resistance (Cagirgan 1994, 2001; Ashri 1998; Diouf *et al.* 2010), seed retention, larger seed size, desirable seed color and high oil content (Hoballah 2001).

The RAPD analysis is used to identify the DNA polymorphism induced by gamma rays in groundnut (Bhagwat *et al.* 1997), cypress (Ishii *et al.* 2003), soybean (Atak *et al.* 2004), sunflower (Erdem and Oldacay 2004), Chrysanthemum (Lema-Ruminska *et al.* 2004), sugarcane (Khan *et al.* 2007), amla (Selvi *et al.* 2007), Baby's-breath (Barakat and El-Sammak 2011) and physic nut (Dhakshanamoorthy *et al.* 2011) and detection of mutation in sunflower (Gunhan and Oldacy 2004), grapes (Khawale *et al.* 2007) and banana (Ganapathi *et al.* 2008). The RAPD method can also be used for the detection of DNA damage and mutations in young *Vigna radiate* calli (Roy *et al.* 2006).

However, there are a limited number of reports on the use of RAPD markers for genetic variation studies after gamma irradiation in sesame (Mohamed *et al.* 1999). RAPD and ISSR molecular markers were used for tagging the *dt* gene regulating determinate growth habit (Uzun and Cagirgan 2009). The only observation of DNA sequences of RAPD bands of plant species have been reported by Begum *et al.* (2008) in *Vigna radiata*.

The aim of the present study is to use RAPD technique for the detection of genetic polymorphism among sesame genotypes following gamma irradiation. The produced RAPD bands from control and irradiated samples were cloned and sequenced to determine a presence of base pair alterations.

MATERIALS AND METHODS

Plant materials

Five genotypes of sesame (*Sesamum indicum* L.), Shandaweel-3; Toshka-1; Giza-24 (C); Taka-1; Taka-3, and two irradiated genotypes, Giza-24 (I) and Taka-3 (I), which irradiated with 100 Gy γ -rays were used in this study.

Extraction of genomic DNA and RAPD analysis

DNA was isolated from fresh seedlings of both non-irradiated and irradiated genotypes using modified cetyltrimethyl ammonium bromide (CTAB) extraction method (Laurentin and Karlovsky 2006). The RAPD technique was conducted according to (Williams *et al.* 1990) using six random primers, Amersham Ready-To-Go (Table 1). The PCR amplification mixture was carried out in a total volume 25 μ l containing 25 ng of DNA, 0.6 U of *Taq* DNA polymerase enzyme, 100 mM of each dNTP, 1X *Taq* DNA polymerase buffer with 1.5 mM MgCl₂ and 10 pmol primers. The PCR conditions was as follows: an initial denaturation step at 95°C for 5 min; 40 cycles of 94°C for 1 min, 36°C for 1 min and 72°C for 2 min and a final extension step at 72°C for 10 min, using thermocycler UNO II (Biometra). The PCR products were separated on 1.5% agarose gel, containing ethidium bromide (0.5 μ g/ml) in 1X TBE buffer and photographed under UV light.

Sequencing of RAPD fragments

Cloning and sequencing of the RAPD markers were carried out as described by Zhang and Stommel (2001). The radiation specific molecular marker was separated on a 1% low melting point agarose gel before being excised and purified by means of the QIAquick Gel Extraction Kit (QIAGEN). The purified DNA fragments from both, Giza-24 (C) and Giza-24 (I) genotypes were

ligated and transformed with the pGEM-T Easy Vector System (Promega). The two cloned RAPD fragments were identified via PCR analysis. The sequencing ready reaction big dye terminator kit (Applied Biosystems, USA) in conjunction with ABI-PRISM 310 genetic analyzer was used for sequencing the two cloned fragments using both M13 forward and reverse primers.

Analysis of sequence data

The DNA sequences from both the non-irradiated Giza-24 (C) and irradiated Giza-24 (I) were aligned and analyzed using MEGA software version 4 (Tamura *et al.* 2007). The nucleotide and amino acid composition, nucleotide pair frequencies and the overall transition/transversion bias were accordingly estimated. The maximum composite likelihood estimates of the pattern of nucleotide substitution were calculated following Tamura *et al.* (2004). These two DNA sequences were submitted to GenBank (Accession numbers FJ814732 and FJ814733). Homology searches of these two DNA sequences were performed within GenBank's non-redundant database using the BLASTN 2.2.18 and BLASTP 2.2.18 (Basic Local Alignment Search Tool) algorithm at <http://www.ncbi.nlm>.

RESULTS

Identification of RAPD marker

In this study, six random primers were used to identify a radiation molecular marker/s. The data obtained from RAPD analysis should that five primers amplified several different regions of the sesame genome to observe high polymorphic bands (data unpublished). Only one primer, Ready-To-Go 5 (5' AACGCGCAAC 3') has amplified a single, intense band of 762 bp from all genotypes except Taka-3 which was absent in this genotype (Fig. 1).

Marker sequence analysis

RAPD-PCR molecular marker from non-irradiated Giza-24 (C) and irradiated Giza-24 (I) genotypes were cloned and sequenced. The length of the RAPD marker sequence was 762 bp for each. Fig. (2) shows that the RAPD marker sequence of Giza-24 (I) differed in nucleotide and protein sequences from Giza-24 (C) genotype. The Nucleotide sequence showed a point mutation, namely base substitution which was observed in Giza-24 (I) (Table 2). This table showed there are 680 identical pairs (89.2%), 28 transitional pairs (3.7%) and 54 transversional pairs (7.1%). The maximum composition likelihood estimate of the pattern of nucleotide substitution is demonstrated in Table (3). Each entry shows the probability of substitution from one base (row) to another base

(column) instantaneously. Only entries within a row should be compared. The nucleotide frequencies are 0.178 (A), 0.276 (T), 0.31 (C) and 0.237 (G). The transition/transversion rate ratios are $K_1 = 1.665$ (purines) and $K_2 = 0.664$ (pyrimidines). The overall transition/transversion bias is $R = 0.665$, where $R = [A*G*K_1 + T*C*K_2]/[(A+G)*(T+C)]$. All calculations were conducted using MEGA 4 software.

A total number of 249 amino acids were detected for each of the two protein sequences. As shown in Table (4), the amino acid compositions of these protein sequences are similar. The amino acid Ala, Lys, Met, Thr and Val have the same frequencies of Giza-24 (C) and Giza-24 (I) genotypes and others have different frequencies. The amino acid Leu, Pro, Arg and Ser have higher frequencies in the two protein sequences.

Blast results revealed that the nucleotide sequence of Giza-24 (C) specific marker has partial homology with nucleotide sequences of wine grape. While the nucleotide sequence of Giza-24 (I) has partial homology with nucleotide sequences of wine grape, rice and *Arabidopsis* at different accession numbers. On the other hand, the protein sequence of Giza-24 (C) specific marker has partial homology with protein sequences of aspartyl-tRNA synthetase. While, the protein sequence of Giza-24 (I) have partial homology with protein kinase C, hypothetical protein OsJ 020780 and microtubule associated protein.

DISCUSSION

RAPD-PCR will amplify several different regions of sesame genome to detect a large number of genetic polymorphism in Indian germplasm (Bhat *et al.* 1999), Egyptian genotypes irradiated with gamma rays (Mohamed *et al.* 1999), Turkish populations (Ercan *et al.* 2004) and Vietnam and Cambodia collections (Pham *et al.* 2009 and 2011). In this study, the data obtained from RAPD analysis agree with the previous studies (data unpublished) except the primer Ready-To-Go 5 was amplified single polymorphic band, 762 bp, which present in six genotypes and absent in Taka 3 genotype. Although the two genotypes Taka-1 and Taka-3 were selected from sesame irradiated with 800 Gy γ - rays, this band is present in Taka-1 and absent in Taka-3. Meanwhile this band is present in Giza-24 (I) and Taka-3 (I), which were irradiated with 100 Gy γ - rays. These results demonstrated that this band has a unique sequence and probably used as a molecular marker for gamma irradiation. The absence of this band in Taka-3 indicated that the DNA damage induced by gamma rays (800 Gy γ - rays) occurred in the sequence of this marker. DNA damage can be encountered by RAPD as quickly as 3h after irradiation (200 Gy) in young *Vigna radiate* calli (Roy *et al.* 2006). Meanwhile, the presence of this band in Taka-3 irradiated with 100 Gy γ - rays indicates that repaired of DNA

damage was take place. The number of lost bands in *Jatroha curcas* plants exposed to gamma irradiation was found higher than of extra bands (Dhakshanamoorthy *et al.* 2011). It is suggested that the DNA damage may be serious in the majority of cells in the plant parts exposed to gamma irradiation. The molecular analyses have revealed that gamma rays (Vizir and Mulligan 1999), X-ray and fast neutron irradiation (Shirley *et al.* 1992), T-DNA integration (Laufs *et al.* 1999) and carbon ion (Shikazono *et al.* 2001) could induce DNA rearrangements in the plant genome.

The DNA sequence of Giza-24 (C) has partial homology with nucleotide sequences of wine grape. While the nucleotide sequence of Giza-24 (I) has partial homology with nucleotide sequences of wine grape, rice and *Arabidopsis*. In contrast, the protein sequence of Giza-24 (C) specific marker has partial homology with protein sequences of aspartyl-tRNA synthetase. While, the protein sequence of Giza-24 (I) have partial homology with protein kinase C, hypothetical protein OsJ 020780 and microtubule associated protein. These results indicated base pair alterations of DNA sequence and changes of its protein function after gamma irradiation. After irradiation at 100 Gy, 163 genes were induced in *Arabidopsis*; 17% of the genes were related to DNA metabolism, chromosomal structure and cell-cycle checkpoints and 11% were related to transcription factors (Culligan *et al.* 2006). Protein kinases are enzymes, which catalyze the transfer of phosphate from adenosine 5'-triphosphate (ATP) to certain amino acid residues in certain proteins. Generally, the phosphorylation of a protein changes its functionality, from inactive to active in some cases, and from active to inactive in others. The Nucleotide sequence showed a point mutation, 28 transitional pairs (3.7%) and 54 transversional pairs (7.1%), which was observed in Giza-24 (I). This study established that RAPD marker sequence of the genotype Giza-24, irradiated, with 100 Gy γ - rays sesame plant differ at the 82-nucleotide positions from Giza-24 (C). It gives an estimate of mutation rate 0.11 per nucleotide whereas; the mutation rate per nucleotide in human was 2.5×10^{-8} (Nachman and Crowell, 2000). Begum *et al.* (2008) have reported the only one observation of DNA sequences of RAPD bands of plant species. It is difficult to compare the current data on plant responses to ionizing radiation while models and parameters conditions of previous experiments have varied greatly. In addition, the frequency of mutations varied from one gene to another and results in an alteration of the population genetic structure (Turuspekov *et al.* 2002). The molecular nature of this RAPD fragments was found to be different from the results of *Vigna radiate*, 0.55 recombination induced by gamma radiation (Begum *et al.* 2008). Response variations could be different between studies determined by the type of irradiation (e.g., acute or chronic), the dose applied, the physiological parameters such as the species/variety/cultivar considered, the developmental stage at

the time of irradiation (Boyer *et al.* 2009 ; Kim *et al.* 2009). The present study might help in explaining different responses and complication to ionizing radiation in plants.

SUMMARY

The use of molecular markers for detection of genetic variation and DNA mutagenesis studies after ionizing radiation are limited in sesame. DNA was isolated from fresh seedlings of both non-irradiated and irradiated genotypes using modified cetyltrimethyl ammonium bromide (CTAB) extraction method. To determine alterations in DNA following gamma radiation, random amplified polymorphic DNA (RAPD) molecular marker was used. Results showed that the intensive band of approx. 762 bp was polymorphic, which could be found in all genotypes under investigation except Taka-3 genotype. This specific marker band was cloned and sequenced from Giza-24 (control) and Giza-24 (irradiated) genotypes. The DNA sequence from irradiated samples showed that point mutation (base substitution) when compared to control DNA sequence. A total number of 249 amino acids were detected for each of the two protein sequences and the amino acid Leu, Pro, Arg and Ser have higher frequencies. Blast results revealed that the nucleotide sequence of Giza-24 (C) specific marker has partial homology with nucleotide sequences of wine grape and the protein sequence has partial homology with protein sequences of aspartyl-tRNA synthetase. On the other hand, nucleotide sequence of Giza-24 (I) has partial homology with some DNA sequences from grape, rice and *Arabidopsis* and the protein sequence has partial homology with protein kinase C, hypothetical protein OsJ 020780 and microtubule associated protein. The results of present study might help in explaining the response variations to ionizing radiation in plants.

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Table (1): Ready-To-Go primer sequences were used in RAPD-PCR technique.

Primer	Sequence
1	5' GGTGCGGGAA 3'
2	5' GTTTCGCTCC 3'
3	5' GTAGACCCGT 3'
4	5' AAGAGCCCGT 3'
5	5' AACGCGCAAC 3'
6	5' CCCGTCAGCA 3'

Table (2): The effect of gamma ray treatment (100 Gy γ - rays) on nucleotide sequences of Giza 24 sesame genotype.

Base Pairs	Nucleotide	Number of Base	Frequency
Identical	TT	192	0.252
	CC	214	0.280
	AA	118	0.155
	GG	156	0.205
Transitional (si)	T \leftrightarrow C	13	0.017
	A \leftrightarrow G	15	0.020
Transversional (sv)	T \leftrightarrow A	12	0.016
	T \leftrightarrow G	11	0.014
	C \leftrightarrow A	8	0.011
	C \leftrightarrow G	23	0.030
(si) / (sv)		28/54	0.5

Table (3): Maximum composition likelihood estimate of the pattern of nucleotide substitution.

	A	T	C	G
A	-	8.95	10.06	12.81
T	5.78	-	6.68	7.69
C	5.78	5.94	-	7.69
G	9.62	8.95	10.06	-

Table (4): The effect of gamma ray treatment (100 Gy γ - rays) on amino acid compositions and frequencies (%) of Giza 24 sesame genotype.

Amino Acid Composition	Amino Acid Frequency (%)		Amino Acid Composition	Amino Acid Frequency (%)	
	Giza 24	Giza 24-treated with 100 Gy γ - rays		Giza 24	Giza 24-treated with 100 Gy γ - rays
Ala	0.0602	0.0602	Met	0.0400	0.0400
Cys	0.0482	0.0442	Asn	0.0120	0.0161
Asp	0.0281	0.0321	Pro	0.1165	0.1084
Glu	0.0402	0.0321	Gln	0.0080	0.0120
Phe	0.0522	0.0482	Arg	0.1124	0.1004
Gly	0.0522	0.0602	Ser	0.1165	0.1205
His	0.0361	0.0321	Thr	0.0442	0.0442
Ile	0.0402	0.0361	Val	0.0442	0.0442
Lys	0.0201	0.0201	Trp	0.0161	0.0241
Leu	0.1245	0.1406	Tyr	0.0241	0.0201

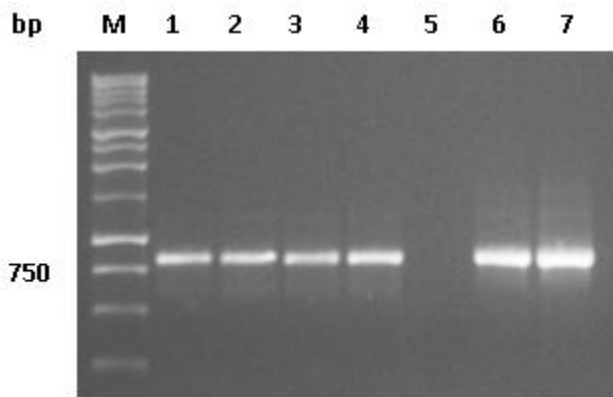


Fig. (1): RAPD profiles of sesame genotypes amplified with Ready-To-Go primer 5 (5' AACGCGCAAC 3') on 1.5% agarose gel. M: molecular weight marker (1 Kb DNA ladder). Lanes from 1 to 7 represent genotypes: Shandaweel-3, Toshka-1, Giza-24 (C), Giza-24 (I), Taka-3, Taka-3 (I) and Taka-1, respectively.

(C): control, non-irradiated.

(I): Irradiated, with 100 Gy γ - rays.

(a)

Giza-24 (C) GCT GGT TCG TAC TTA CCT GGT TTC TGA TGC GAC TTT ATT TGG GGG GAA CCA
Giza-24 (I)C. .G. .GG AA.GT

Giza-24 (C) AGA AAA ACT ACG GCG GAA AGG GAA AAA TTT TGG GTC GAT TTG TTG CCA CTG
Giza-24 (I)C C TT.G. ...

Giza-24 (C) ATA CTT AAC ATC TTG AAG CGG AGT TAT AGT ATT GTA ATT TTT GCA AAA AGG
Giza-24 (I) ..CG. T..

Giza-24 (C) TAA GGC AGC AGC TCC TAG AGC GAG TGG ATA GCT GAA GAT TTC TGC GGC CGG
Giza-24 (I) T.T G.. C.T

Giza-24 (C) ACT TGT ATG GTA GTA TAT TTA TCC TCA ATA ACT ATA TGC GAA GGA CAT CGG
Giza-24 (I)T

Giza-24 (C) TGT TTT TTC TCT ACC TGT AGG GTG GAG GGG AAA ACA CAC ACT ACC TCA CGC
Giza-24 (I)G ..T ... G..G. .G.T .T. ... C.. .G .T. ...

Giza-24 (C) CAC ACC AAT AGA CTC CGG GGT CGG TGT CCT TTT GCG ACC TTG CAC CTG CAT
Giza-24 (I) G.. ..AT ..A ... G.. ...

Giza-24 (C) TAG CCG GCC TTC GCC TGT TAG CAT CCG CTT GCT AGG CCG CAG CAC CGC CCG
Giza-24 (I) C.. .C AG. T.. A.. ... G.A ..C ... G..C

Giza-24 (C) CGC TCA CGG CAG CTG TCG GCC TGG CTC TCG CCA TGT TAT CGT TCA GAC CCT
Giza-24 (I)T. ..A T.. ... A..AC. ... T..A.

Giza-24 (C) GGT GTC CTG CGA CCC CGA GGC CTC GAG CGG TTT CTC AGC GAA CTC GCA CGC
Giza-24 (I) GA.G

Giza-24 (C) GAC GCT CAC AAT CCC CTG ACC GGT GTG CCC CTC CGT AGC CCT GTA CCT CCC
Giza-24 (I) A..C.C ... C.. ... T.. ...

Giza-24 (C) GGG ATA TCT GTG CTC CCT GTA CCC GGT CGT CTC CTC TCC TTG CTC CCG TCC
Giza-24 (I)A. .GA ..TA.G

Giza-24 (C) TCT TGT GTT CCT CCA CCG CCG TTC TGT TTT CCC AGC TCG TTA CCG TAC CTT
Giza-24 (I) C..C ..T ... A..T ...

Giza-24 (C) CTC GAT GCC CTT CGT TCG CTC GAG ATT CCA CCA CAT TCT CGC CCA TAC TGC
Giza-24 (I)G G.. CT.C.

Giza-24 (C) CTG TCC CGA CGT CCC TCC TCC GCT CGC GAC TCC CGT CTC TTC GCC GCG
Giza-24 (I)T A..

(b)

Giza-24 (C) AGSYLPGF*C DFIWGEPRKT TAEREKFWVD LLPLILNILK RSYSIVIFAK R*GSSS*SEW
Giza-24 (I) .AWW...N... ..R... ..D.....F ..R...S... ..F... ..C..

Giza-24 (C) IAEDFCGRTC MVVYLSSITI CEGHRCFFST CRVEGKTHHT SRHTNRLRGR CPFATLHLH*
Giza-24 (I) V...L..... ..L..A ..GG.NI.P. L..... G.....D..Q

Giza-24 (C) PAFAC*HPLA RPQHRPRSQ LSAWLSPCYR SDPGVLRPRG LERFLSELAR DAHNPLTGVP
Giza-24 (I)SSI. G..D....L. .T....S.C ..H.....E. ...L..... N..T...R..

Giza-24 (C) LRSPVPPGIS VLPVPGRLLS LLPSSCVPPP PFCFPSSLPY LLDALRSLEI PPHSRPYCLS
Giza-24 (I) F.....K*H... ..L... ..S..... ..VL.P.....

Giza-24 (C) RRPSSARDSR LFAA
Giza-24 (I)T

Fig. (2): Alignment of RAPD marker amplified from Giza-24 (C) and Giza-24 (I) showing variable sites. **A:** Alignment of nucleotide sequences. **B:** Alignment of protein sequences. '?' represent identical residues. (C): Control, non-irradiated; (I) Irradiated with 100 Gy γ -rays.