COMPARATIVE ANALYSIS OF RAPD MARKER SEQUENCES IN RELATION TO GAMMA IRRADIATION 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 agro-morphological 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 has been 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).

RAPD analysis has been 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). RAPD analysis
was 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 was 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 possible occurrence 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 were 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). RAPD technique was used 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 of 25 µl 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 µg/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**

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 algorithm at http://www.ncbi.nlm.

**RESULTS AND DISCUSSION**

**Identification of RAPD marker**

RAPD-PCR amplified several different regions of sesame genome and exhibited substantial 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 & 2011). In this study, six random primers were used to identify irradiation-induced molecular marker/s. The data obtained from RAPD analysis showed 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’) amplified a single, intense band of 762 bp from all genotypes except Taka-3 which was absent in this genotype (Fig. 1). 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 associated with gamma irradiation.

The absence of this molecular marker 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 was 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 marker in Taka-3 irradiated with 100 Gy γ-rays indicates that repair of DNA damage has occurred. The number of lost bands in Jatropha curcas plants exposed to gamma irradiation was found higher than those 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.

**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. Figure (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 point mutations, namely, base substitution which was observed in Giza-24 (I) (Table 2). This table showed that 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 raw 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 $R = \frac{\text{A}*\text{G}*K_1 + \text{T}*\text{C}*K_2}{(\text{A+G})*(\text{T+C})}$. All calculations were conducted using MEGA 4 software.

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 $2.5 \times 10^{-8}$ (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).

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 acids 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 acids 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 OsJ020780 and microtubule associated protein.

These results indicated base pair alterations of DNA sequence and changes of its protein function after gamma irradiation. Culligan et al. (2006) reported that 163 genes were induced in Arabidopsis after irradiation at 100 Gy; 17% of the genes were related to DNA metabolism, chromosomal structure and cell-cycle checkpoints and 11% were related to transcription factors. 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.

**SUMMARY**

The use of molecular markers for the 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. The results showed that an 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 acids 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 nucleotide 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 impact of ionizing radiation in the induction of genetic variation at the molecular level in plants.

ACKNOWLEDGEMENT

The author is grateful to the Department of Agronomy, Faculty of Agriculture, Cairo University, Egypt and the Atomic Energy Establishment at Inshas, Egypt, for providing the sesame genotypes.

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

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5' GGTGCGGGAA 3'</td>
</tr>
<tr>
<td>2</td>
<td>5' GTTTCGCTCC 3'</td>
</tr>
<tr>
<td>3</td>
<td>5' GTAGACCCGT 3'</td>
</tr>
<tr>
<td>4</td>
<td>5' AAGAGCCCGT 3'</td>
</tr>
<tr>
<td>5</td>
<td>5' AACGCGCAAC 3'</td>
</tr>
<tr>
<td>6</td>
<td>5' CCCGTAGCA 3'</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Base Pairs</th>
<th>Nucleotide</th>
<th>Number of Base</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identical</td>
<td>TT</td>
<td>192</td>
<td>0.252</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>214</td>
<td>0.280</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>118</td>
<td>0.155</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>156</td>
<td>0.205</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>680</td>
<td>0.892</td>
</tr>
<tr>
<td>Transition (si)</td>
<td>T↔C</td>
<td>13</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>A↔G</td>
<td>15</td>
<td>0.020</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>28</td>
<td>0.037</td>
</tr>
<tr>
<td>Transversion</td>
<td>T↔A</td>
<td>12</td>
<td>0.016</td>
</tr>
<tr>
<td>(sv)</td>
<td>T↔G</td>
<td>11</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>C↔A</td>
<td>8</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>C↔G</td>
<td>23</td>
<td>0.030</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>54</td>
<td>0.071</td>
</tr>
<tr>
<td>(si) / (sv)</td>
<td></td>
<td>28/54</td>
<td>0.5</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>T</th>
<th>C</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-</td>
<td>8.95</td>
<td>10.06</td>
<td>12.81</td>
</tr>
<tr>
<td>T</td>
<td>5.78</td>
<td>-</td>
<td>6.68</td>
<td>7.69</td>
</tr>
<tr>
<td>C</td>
<td>5.78</td>
<td>5.94</td>
<td>-</td>
<td>7.69</td>
</tr>
<tr>
<td>G</td>
<td>9.62</td>
<td>8.95</td>
<td>10.06</td>
<td>-</td>
</tr>
</tbody>
</table>
Table (4): The effect of gamma ray treatment (100 Gy γ-rays) on amino acid compositions and frequencies (%) of Giza 24 sesame genotype.

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

Fig. (1): RAPD profiles of sesame genotypes amplified with Ready-To-Go primer 5 (5' AACGCGCAAC 3') on 1.5% agarose gel. M: molecular size 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.
COMPARATIVE ANALYSIS OF RAPD MARKER IN SESAME

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 \( \gamma \)-rays.