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RAPD Analysis for Detection and Fingerprinting of Sunflower Mutants Induced by Sodium Azide

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ABSTRACT

The aim of this study was to identify and fingerprint the mutants of sunflower plants using RAPD analysis. Ten mutants of two cultivars Giza 1 and Giza 102 of *Helianthus annuus* produced by sodium azide treatments and the control were evaluated by RAPD markers. The mutants of cultivar Giza 102 were as follows: mutant 1 which changes in the form of inflorescence, mutant 2 is a dwarfed plant, mutant 3 is a plant with large leaves, mutant 4 is a plant with many inflorescences, mutant 5 is a dwarfed plant also. The mutants of cultivar Giza 1 were as follows: mutant 6 with reddish leaf petiole, mutant 7 is a dwarfed plant, mutant 8 with changes in the leaf form, mutant 9 is a plant with dark green inflorescence calyx, mutant 10 is a plant with stripped ray florets. Ten random primers were used; eight out of ten primers used showed variations among the mutants. The 98 amplified fragments were yielded, 83 bands were polymorphic and the total polymorphic rate was 84.6%. Some fragments were common for both genotypes and others were absent as a result of treatments. A dendrogram based on the genetic distance grouped the mutants and the two control cultivars to three clusters A, B and C. The polymorphism detected in all the mutants with both genotypes suggested that sodium azide could be used as effective mutagenic agents in sunflower. RAPD marker is a powerful technique enables fingerprinting of selected mutants in breeding of sunflower plants.

Key words: RAPD analysis, fingerprinting, sunflower, mutants, sodium azide

INTRODUCTION

There are about 70-80 species of *Helianthus*, some of them are perennials, many are popular garden ornamentals. *H. annuus* is one of the most important species belongs to the family Asteraceae (Compositae) and is known as the sunflower .All sunflowers are good and long lasting as cut flowers. Sunflowers are widely grown commercially for the oil that is extracted from the seeds which contain 35-40% oil. They are high in polyunsaturated fat and contain no cholesterol. Seeds also are used to feed the birds (Gvozdenovic *et al*., 2009).

Induced mutation using physical and chemical mutagen is a method to create genetic variation resulting in new varieties with better characteristic (Wongpiyasatid *et al*., 2000; Arulbalachandran *et al*., 2009).

Sodium azide (NaN3) is a chemical mutagen and has been one of the most powerful mutagens in plants. Its application on plant is easy and inexpensive and creates mutation to improve their traits. The efficiency of mutant production depends on the conditions such as pH, soaking into water, azide solution, temperature, concentration of azide and treatment duration. It creates point mutation, damages the chromosomes and thus produces tolerance in the plants for numerous adverse conditions (Al-Qurainy and Khan, 2009).

The identification and analysis of mutants using molecular technique of DNA fingerprinting such as RAPD allow a direct comparison of the effect on genotypes at the DNA level as reported by Arulbalachandran *et al.* (2009). The RAPD method does not require DNA probes or prior sequence information. The method is simple, requires only small amounts of DNA and can be performed without the use of radioactivity (Karp *et al.*, 1996).

Sodium azide as used in many studies to induce mutation as found by Akhaury *et al.* (1996) on *Hordium vulgare*, Bhate (1999) on *Ipomea purpurea*, El-Nashar (2006) on *Amaranthus caudatus* and Al-Gawwad and Makka (2009) on *Mirabilis jalapa*.

Traditional methods for mutant plant identification based on morphological and biochemical markers but these markers are less reproducible due to being influenced by environmental conditions. The mutation detection based on Polymerase Chain Reaction (PCR) techniques are reliable and reproducible and have been used in various mutants as obtained by Jayakumar and Selvaraj (2003), Erdem and Oldacay (2004) and Gowda and Bhat (2009).

The aim of this study was to identify and fingerprint obtained mutants of sunflower plants using RAPD analysis.

MATERIALS AND METHODS

Plant materials: Ten mutants of two cultivars of *Helianthus annuus* Giza 1 and Giza 102 were used in this study to identify them from the control. The mutants were obtained after sodium azide treatments (Mostafa, 2011). Table 1 illustrates the mutants. The identification and fingerprinting of the mutants was carried out at the Department of Nucleic Acid Research, Mubarak City for Scientific Research and Technology Application, New Borg El- Arab, Egypt.

Table 1: Description of the mutants

Cultivar	Mutant No.	Variation describe
Giza 102	Control	Normal plant as a control
	Mutant 1	Changes the form of inflorescence
	Mutant 2	Dwarfed plant
	Mutant 3	Plant with biggest leaves
	Mutant 4	Plant with many inflorescence
	Mutant 5	Dwarfed plant
Giza 1	Control	Normal plant as control
	Mutant 6	Plant with reddish leaf petiol
	Mutant 7	Dwarfed plants
	Mutant 8	Chances the form of leaves
	Mutant 9	Plant with inflorescence calyx having green dark colour
	Mutant 10	Plant with stripped ray florets

Table 2: Primers sequences employed in the RAPD-PCR

Primers	Sequence 5′→3′
OPA 01	CAGGCCCTTC
OPA 02	TGCCGAGCTG
OPA 07	GAAACGGGTG
OPA 13	CAGCACCCAC
OPR 14	CCAGCCGAAC
R5	ATG CCC CTGT
R6	AAA GCT GCGG
R9	GGTCTACACC
R10	GAGAGCCAAC
T1	GTTGCGATCC

DNA isolation and RAPD analysis: Genomic DNA was extracted from the fresh leaves of the plants using Dneasy mini plant kit according to manufacturer's instructions (QIAGEN). Ten random primers (10 mer) were used for RAPD (Table 2). The reaction mixture of 25 µL contained 10 ng sunflower DNA, 10x promega PCR buffer, 50 mM $MgCl_2$, 0.2 mM each of dATP, dTTP, dCTP, dGTP, 25 pmol primer and 0.5 unit of promega taq polymerase. Amplification was performed on thermocycler (Eppendorf 2231, Hamburg, Germany) using The following PCR program, 95°C for 5 min; 40 cycles of 95°C for 1 min., annealing at 53°C for 1 min and extension at 72°C for 1 min. A final extension step at 72°C for 10 min. PCR products were separated on agarose gel electrophoresis using 1.5% and then visualized on gel documentation system. The amplified fragments were scored as 1 for presence and 0 for absence of band and the data was analyzed for clustering using the formula of Nei and Li (1979). A similarity coefficient was used for cluster analysis following the UPGMA (unweighted pair grouping method of averages method).

RESULTS AND DISCUSSION

Ten random primers were used to evaluate the genetic variation of the two genotypes of sunflower which obtained from sodium azide treatment. RAPD analysis revealed that eight out of ten primers used in this study showed variations among the mutant plants (Fig. 1-8). The eight primers yielded 98 amplified fragments, 83 bands were polymorphic and the total polymorphic rate was 84.6% (Table 3). The primers R5, OPA07 and R9 showed the highest percentage polymorphism (100, 93.3 and 92.3%, respectively), while, primer R10 presented the lowest percentage polymorphism (57.1%). The other primers (OPA01, R6, OPA14 and T1) presented 88.8, 78.6, 76.9 and 71.4% polymorphism, respectively (Table 3). The primers OPA01, OPA07 and OPA14 showed high number of bands ranged from (13-18) and produced unique patterns with sample 9 (mutant 7) as shown in Fig. 1-3. Low molecular weight bands (250-380) were obtained with primer R10 (Fig. 5). The amplification profiles with the primers R10 and R5 showed a highly polymorphic profile in all mutants of Giza 1, and by both cultivars for the two primers, respectively (Fig. 3, 6). It was found that the primer OPA01 showed polymorphic bands in the mutant 1, 2,6,7,8 and 10 (Fig. 1). The amplification profile produced using the primer R6 showed polymorphic profile in the mutants 6, 7 and 10 (Fig. 7).Major differences were found among the treated plants and controls of two genotypes with the primers R5, R6, R9 and T1 (Fig. 4-8). Some bands were

Table 3: RAPD marker produced by eight primers among ten mutants of the two cultivars Giza 102 and Giza 1 of Helianthus annuus produced by sodium azide treatment and the control. Total number of bands, number of mono morphic band, number of polymorphic bands and polymorphism %

Primers	Total No. of bands	No. of monomorphic bands	No. of polymorphic bands	% polymorphism
OPA 01	18	2	16	88.8
OPA 07	15	1	14	93.3
OPR 14	13	3	10	76.9
R5	11	0	11	100
R6	14	3	11	78.6
R9	13	1	12	92.3
R10	7	3	4	57.1
T1	7	2	5	71.4
Total	98	14	83	84.6
Average	12.3	1.7	10.4	

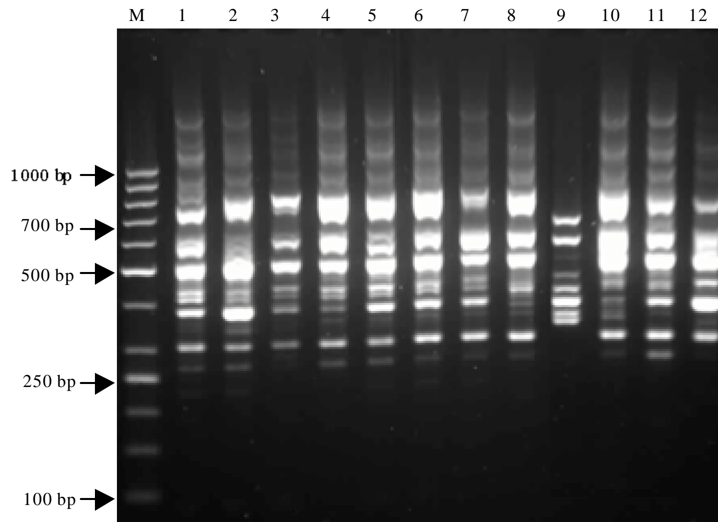

Fig. 1: RAPD-PCR using the primer OPA01; M: 50 bp marker, Lane 1-12: Giza 102 cultivar (control), mutant 1, 2, 3, 4, 5, Giza 1 cultivar (control), 6, 7, 8, 9 and 10 of *Helianthus annuus*, respectively

common for both genotypes and others were absent as a results of treatments. These polymorphic bands could be very valuable for DNA fingerprinting and its identification between mutants and the control plants of the both cultivars.

A dendrogram was constructed using data from UPGMA cluster analysis. Based on the Genetic Distance (GD) and coefficient matrices of the two genotypes were analyzed using eight primers. The similarity matrix presented in Table 4. The dendrogram grouped the 12 samples into three clusters A, B and C. The genotype Giza 102 (control and mutants 1, 2 and 3) grouped into cluster A. Cluster B grouped the mutants 4, 5, 6 of genotype Giza 102 with genotype Giza 1 (control and treated plants except sample 9 was grouped into individual group as C (Fig. 9). The high percentage of polymorphism (84.6%) with all mutants obtained from sodium azide treatment with

Table 4: Genetic similarity of DNA among all mutants of the two cultivars Giza 102 and Giza 1 of Helianthus annuus produced by sodium azide treatments and the control

	1	2	3	4	5	6	7	8	9	10	11	12
1	100											
2	83	100										
3	85	82	100									
4	70	81	71	100								
5	70	79	69	84	100							
6	70	85	73	84	90	100						
7	63	76	68	71	73	77	100					
8	59	72	64	79	79	83	80	100				
9	46	45	49	40	44	42	47	53	100			
10	60	75	67	68	70	76	73	77	50	100		
11	59	72	68	65	71	73	76	76	53	87	100	
12	55	62	64	59	57	63	66	66	57	71	74	100

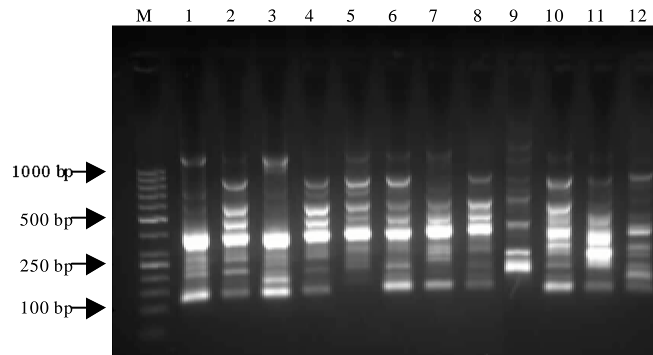

Fig. 2: RAPD-PCR using the primer R9; M: 50 bp marker, Lane 1-12: Giza 102 cultivar (control), 1, 2, 3, 4, 5, Giza 1 cultivar (control), 6, 7, 8, 9 and 10 of Helianthus annuus, respectively

the two genotypes may be due to mutations which led to base pair substitution, resulting in amino acid change which change the function of protiens (Van der Veen 1966). Sodium azide was known to induce chromosome damage and chromosome aberrations (lagging chromosome, translocation, chromosome stikness) which increased with the increase of sodium azide dose (Al-Qurainy and Khan, 2009).

The main changes in the RAPD profiles of the present investigation were the appearance or disappearance of different bands with variation in their intensity. These effects might be due to the structural rearrangements in DNA caused by different types of DNA damages. RAPD method is applicable for the detection of changes in the DNA structure after different treatments (sodium azide).

However, present findings are in agreement with the results were previously found by Erdem and Oldcay (2004) who used RAPD marker to study genetic variation in sunflower after exposing with different doses of gamma and magnetic field. Also, the same results were found by

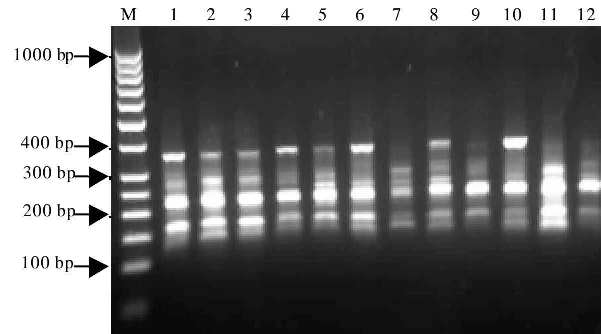

Fig. 3: RAPD-PCR using the primer R10; M: 50 bp marker, Lane 1-12: Giza 102 cultivar (control), 1, 2, 3, 4, 5, Giza 1 cultivar (control), 6, 7, 8, 9 and 10 of *Helianthus annuus*, respectively

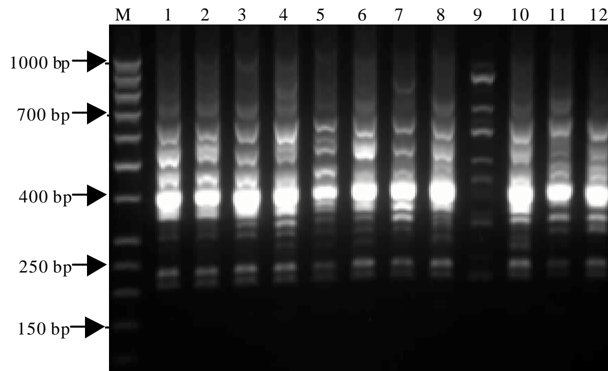

Fig. 4: RAPD-PCR using the primer OPR14; M: 50 bp marker, Lane 1-12: Giza 102 cultivar (control), 1, 2, 3, 4, 5, Giza 1 cultivar (control), 6, 7, 8, 9 and 10 of *Helianthus annuus*, respectively

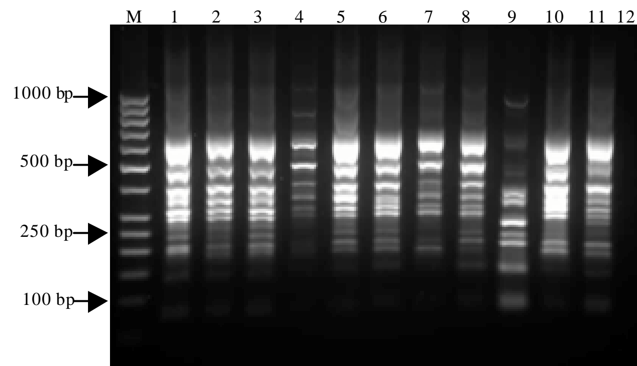

Fig. 5: RAPD-PCR using the primer OP?A07; M: 50 bp marker, Lane 1-12: Giza 102 cultivar (control), 1, 2, 3, 4, 5, Giza 1 cultivar (control), 6, 7, 8, 9 and 10 of *Helianthus annuus*, respectively

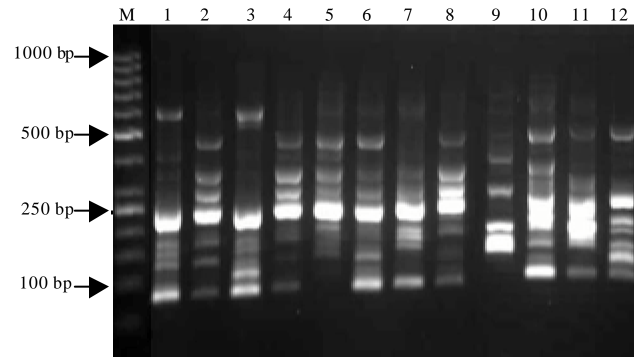

Fig. 6: RAPD-PCR using the primer R5; M: 50 bp marker, Lane 1-12: Giza 102 cultivar (control), mutant 1, 2, 3, 4, 5, Giza 1 cultivar (control), 6, 7, 8, 9 and 10 of *Helianthus annuus*,respectively

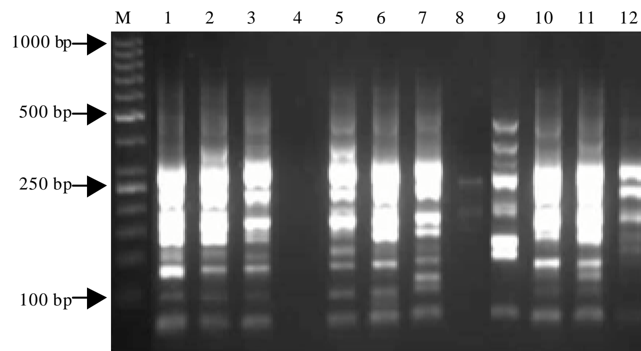

Fig. 7: RAPD-PCR using the primer R6; M: 50 bp marker, Lane 1-12: Giza 102 cultivar (control), 1, 2, 3, 4, 5, Giza 1 cultivar (control), 6, 7, 8, 9 and 10 of *Helianthus annuus*, respectively

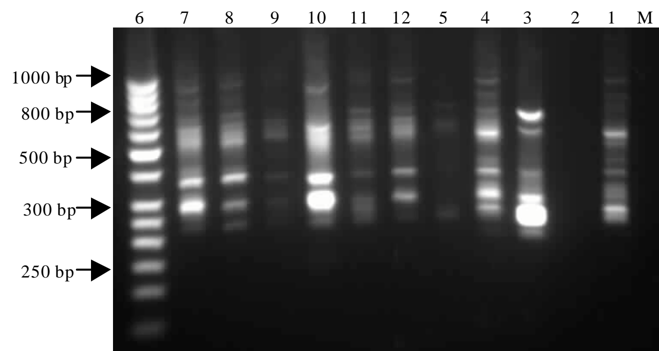

Fig. 8: RAPD-PCR using the primer T1; M: 50 bp marker, Lane 1-12: Giza 102 cultivar (control), mutant 1, mutant 2, nutant 3, mutant 4, mutant 5, Giza 1 cultivar (control), mutant 6, mutant 7, mutant 8, mutant 9 and mutant 10 of *Helianthus annuus*, respectively

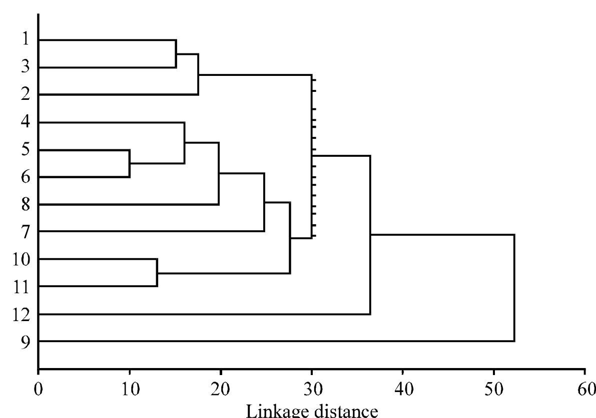

Fig. 9: Tree diagram for 12 variables unweighted pair-group average squared Euclidean distances

Abid Hussain *et al.* (2008) who detected rapidly evolving markers (deletions and duplications in genome) using RAPD technique. Gowda and Bhat (2009) found that RAPD able to differentiate groundnut parents and its mutants after different treatments of EMS. Babaei (2010) who found that RAPD molecular markers are useful tools to evaluate genetic diversity for 15 selected genotypes of rice mutants. And the same were found by Tawfik *et al.* (2011) on *Senna occidentalis*.

Eventually, in this study, polymorphisms were detected in all the treatments with two genotypes, suggesting that sodium azide is one of the most powerful mutagens in sunflower plants, as consequence, could be used in breeding and crop improvement. RAPD marker is a powerful technique also enables fingerprinting of selected mutants in breeding of sunflower plants.

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