ANTI-POLLUTION CAUSED BY GENETIC VARIATION OF PLANTS ASSOCIATED WITH SOIL CONTAMINATED OF PETROLEUM HYDROCARBONS

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Abstract: The treatment of polluted sites has become a priority for the community due to the increase in quality of life standards, the development of technology and awareness of environmental issues. Biological methods were chosen to treat these pollutants. The samples were collected from the central oil company, which is a polluted site with petroleum hydrocarbons,The analysis focused based on Polymerase Chain Reaction PCR technology before and after treatment and with ten primers to detect oil mutations due to contamination. The plants gave different genetic variations confirmed by the percentage of the genetic distance resulting from the analysis of the results of the RAPD indicators, which reached the highest 40% between the polluted and treated *Alhagi graecorum* plants. The current study proved the efficiency of activated carbon in the treatment of petroleum hydrocarbons .

Keywords: RAPD PCR in plant , Anti-Pollution, Crude oil

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INTRODUCTION

Untreated crude oil residues pose an environmental risk to the population and are a greater source of environmental liability due to their ability to accumulate and release large amounts of contaminants generated by pollutant decomposition. Effluents can reach organisms through soil resources. This is something that researchers have discovered (Oliveira and Pampulha ,2006 and Karlapudi *et al*., 2018). Pollution from a variety of sources harms plants. It could be water pollution, air pollution, land and soil pollution, or even crude oil residues affecting soil and plants, all of which are harmful to plants (Abd el-alkhoris *et al*., 2020) .

Activated carbons are commonly used in anti-pollution processes to remove odors, heavy metals and organic compounds. Plants polluted by crude oil, organic matter, and other causing compounds, in particular, can be effectively removed using this method. Powdered activated carbon is commonly used in the final stage of treatment. Because the filtration process effectively removes pollutants, it should be directly available in polluted areas (Luo *et al*., 2014and Thellmann *et al*., 2017)

MATERIALS AND METHODS:

Kits:

Table 2-1. Kits used in the present work.

The kits	Company	Origin
ZR Plant/Seed DNA MiniPrep™ Kit.	Zymo	USA
Maxime PCR PreMix kit:for PCR reaction and consist of -Taq DNA Polymerase -DNTPs. -Reaction buffer (10X). -Gel loading buffer.	Intron	Korea
Primers	Integrated DNA technologies	USA
Ladder(10,000)bp	Intron	Korea

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Primers:

Table 2-2. Primer sequence used in the study:

NO.	Primer	Sequence
1	OP-E20	AACGGTGACC
2	OP-L20	TGGTGGACCA
3	OP-M14	AGGGTCGTTC
4	OP-M05	GGGAACGTGT
5	OP-M20	AGGTCTTGGG
6	OP-M06	CTGGGCAACT
7	OP-P04	GTGTCTCAGG
8	OP-L05	ACGCAGGCAC
9	OP-V19	GGGTGTGCAG
10	OP-V14	AGATCCCGCC

Sampling:

Three polluted leaf plant samples were collected with three replicates of each sample, dried outdoors for 3-5 days at room temperature, ground with a grinder, and sieved with a 1 mm diameter strainer to be ready for analysis. The total samples were 9 plant samples .

Extraction of DNA:

Plant DNA extraction

Three plants leaves (polluted and treated) by activated carbon carbonization were chosen to extraction DNA (*Phoenix dactylifera ,Typha domingensis , Alhagi graecorum*) It was done according to Sharma *et al* ., (2020).

- The collected plant was first chocked in to small pieces and dried in oven under $50c^0$ for 3 hours.
- Add up to 150 mg of finely cut plant to a ZR BashingBead™ and Lysis Tube. Add 750 μl Lysis Solution to the tube.
- Secure in a bead beater fitted with a 2 ml tube holder assembly and process at maximum speed for 10 minutes and centrifuge the ZR BashingBead™ Lysis tube in a microcentrifuge at 10,000rpm for 1 minute
- Four hundred μl supernatant was transferred up to a Zymo-Spin™ III-F Filter in a collection tube and centrifuged at 8,000 rpm for 1 minute
- 1,200 μl of Genomic Lysis Buffer was add to the filtrate in the collection Tube from step 4 and then mixed will
- Eight hundred μl of the mixture was transferred from Step 5 to a Zymo-Spin™ IIC column2 in a collection Tube and centrifuge at 10,000 rpm for 1 minute
- Repeat Step 6 after removing the flow through from the collection Tube.
- 200 μl DNA Pre-Wash Buffer was added to the Zymo-Spin™ IIC column in a new collection Tube and centrifuge for 1 minute at 10,000 rpm.
- Five hundred μl plant DNA Wash Buffer was add to the Zymo-Spin™ IIC column and centrifuge at 10,000rpm for 1 minute
- The Zymo-Spin™ IIC column was Transfer to a clean 1.5 ml microcentrifuge tube and add 100 μl (50 μl minimum) DNA Elution Buffer directly to

the column matrix. Centrifuge at 10,000 rpm for 30 seconds to elute the DNA

- Zymo-Spin™ III-HRC filter was placed in a clean collection tube and added 600 μl prep solution, centrifuge at 8,000 rpm for 3 minutes.
- The DNA was eluted to be prepare for Zymo-Spin™ III-HRC spin filter was transferred in a clean 1.5 ml microcentrifuge tube and centrifugeed for 3 minutes at exactly 16,000rpm. The final filtered DNA is now ready to be used in PCR and other downstream applications. Beta-mercaptoethanol was added to the Genomic Lysis buffer at a final dilution 0.5%(v/v) or 500 μl per 100 ml.

Primer Solution:

The primers were lyophilized and dissolved in the free nuclease water to give a final concentration of 100 pmol/μl as stock solution which was kept at -20 to prepare 10 pmol/μl concentration as work primer suspended, 10 μl of the stock solution in 90 μl of the free nuclease water to reach a final volume 100 μl, was investigated by IDT (Integrated DNA Technologies company, Canada).

PCR master mix reaction preparation:

PCR mixture was prepared according to the current study to get final volume 25μl as shown in table 2-3

Table 2-3. The necessary components of PCR Master mix:

Components	Concentration
Taq PCR PreMix	5μl
Primer	10 picomols/μl(2 μl)
DNA template	1.5μl
Free nuclease water	16.5 μl
Final volume	25μl

The above mixture was transferred to a PCR eppendorf tube and vortexed for a few seconds.

PCR Thermo cycler Conditions:

All samples were subjected to PCR thermo cycler conditions using the RAPD-PCR thermo cycler system.

Table 2-4. The optimum condition of detection gene.

No.	Phase	Tm (ºC)	Time	No. of cycle
1-	Initial Denaturation	94ºC	5 min.	1 cycle
2-	Denaturation -2	94ºC	30 sec	
3-	Annealing	36ºC	45 sec	
4-	Extension-1	72ºC	45 sec	40 cycle
5-	Extension -2	72ºC	7 min.	1 cycle

Prepare of the Agarose gel

According to Sambrook *et al.* (1989), the agarose gel 2%was prepared in using 100 ml TBE buffer. Agarose has been heated to boil then left to cool down at (45-50ºC) and mixed with a 3 μl Red safe Nucleic acid staining solution in agarose gel with Stirring, the gel has been poured in the pour plate in which the plate of agarose support has been prepared after fixing the comb to make holes that would hold the samples. The gel has been poured gently not to make air bubbles and left 30 minutes to cool down. The comb has been removed gently of the solid agarose. The plate has been fixed to

its stand in the Electrophoresis horizontal unit represented by the tank used in the Electrophoresis.

Gel electrophoresis:

3 µl of the (6x loading dye) has been mixed with 5 µl of the ladder DNA to be electrophoresis . After the mixing process, the process of loading is now to the holes of the gel. An Electric current of 5 v\cm2 has been exposed for 1:30 h till the tincture has reached to the other side of the gel. The gel has been tested by a source of the UV with 336 nm and photos were taken using digital camera.

Determination of the molecular sizes of pieces

Photo capt program was used to calculate the molecular size of the detection bands from PCR reactions and compare them to the size of the DNA Ladder marker (Cerasela *et al.*, 2011).

The results of (RAPD or ISSR)

That appeared in the gel were analyzed after converting the descriptive results into numerical data by placing 1 on the agarose gel when the bandle was present and 0 when it was absent.

The data is presented in a table format, with the results of all prefixes for the samples under for the samples under consideration. The genetic distance coefficient between the samples was calculated using the 72 s'Nie coefficient (Nie and Li, 1979) as follows:

Genetic Distance=1-($\frac{2*N_{xy}}{N_y+N_x}$)

Since: G. D. represents the genetic dimension, Nxy represents the number of bundles shared between the two models x and y that represent two samples, Nx represents the number of total bundles in the sample x and Ny represents the number of total bundles in the sample y, The cluster analysis scheme was drawn according to the UPGMA method (Sneath and Sokal, 1973), using the ready-made program NTSYS-pc (Numerical Taxonomy System) to obtain the genetic distance tree.

The cluster analysis scheme was drawn according to the UPGMA method (Sneath and Sokal, 1973), using the ready-made program NTSYS-pc (Numerical Taxonomy System) to obtain a genetic distance. The polymorphism percentage of the initiator was also calculated through the following equation:

Percent (%) modal multiplicity per initiator = (number of dissimilar packets in the initiator / total number of initiator packets) x 100. The percentage of discriminatory ability for each initiator was calculated according to the following equation:

Discriminative power of each prefix (%) = (Number of variant packets of the initiator / Number of variant packets of all prefixes) x 100. The percentage of efficiency of each starter was calculated according to the following equation:

Efficiency of each initiator (%) = (total number of initiator packets / total number of packets of all prefixes) x 100

Percentage (%) polymorphism of all primers = (number of differentiated packets in all primers / total number of primer packets) x 100. Grundmann *et al* .,1995).

RESULTS

Identification genetic variation by PCR

he results of (RAPD -PCR) that appeared in the gel were analyzed after converting the descriptive results into numerical data by placing 1 when the bundles was present and 0 when it was absent on the agarose gel. and the data is arranged in a tables form that includes the results of all the primers for the studied samples, ten primers gave positive results by successfully amplifying the DNA bundles for all studied samples by 159 bundles, the highest was OP-V14 (19 bundles) and the lowest was for OP-V19 (14 bundles).
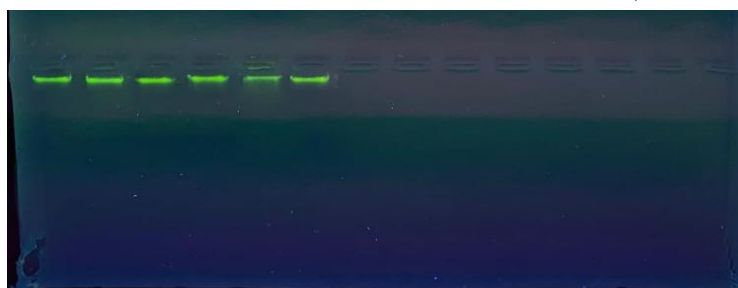

Figure 3-1. Gel electrophoresis of genomic DNA extraction from Plant, 1% agarose gel at 5vol /cm2 for 1:15 houre.

Table 3-1. The molecular weight to detect the bands of leaf plant samples by use Primer OP-E20

(Mw) bp	*Phoenix dactylifera* polluted	*Typha domingensis* polluted	*Alhagi graecorum* polluted	*Phoenix dactylifera* treated	*Typha domingens* treated	*Alhagi graecorum* treated	
	1	2	3	4	5	6	
1800	1	0	0	1	0	1	OP-E20
1500	0	0	0	0	0	1	
1300	1	1	1	1	0	1	
1200	0	0	1	1	0	1	
1000	1	0	0	0	0	1	
900	1	1	1	1	0	0	

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825	1	1	1	0	1	1
725	1	1	0	1	1	0
700	0	1	0	0	1	0
675	1	1	1	1	1	1
600	1	1	1	0	1	1
550	1	1	1	1	1	1
500	1	1	1	1	1	1
425	1	1	1	1	1	1
400	1	1	1	1	1	0
350	1	1	1	0	1	1
300	0	0	1	0	1	1
225	1	1	0	1	0	1

The number of bands recorded in this primer OP-E20 was 18 bands whose sizes ranged between 225-1800 base pairs.
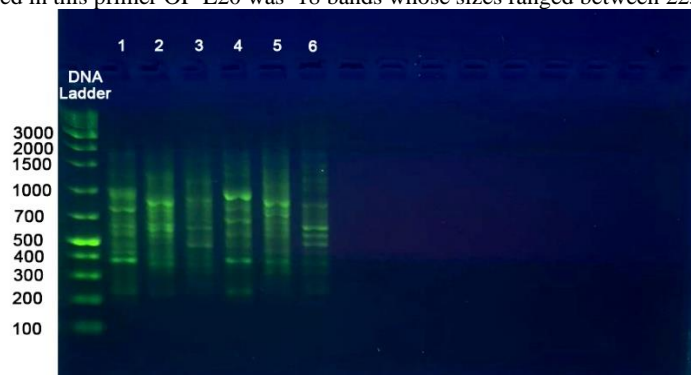
Figure 3-2. PCR product of primer OP-E20 The product was electrophoresis on 2% agarose at 5 volt/cm2. 1x TBE buffer for 1:30 hours. N: DNA ladder 1000 bp

Table 3-2. The molecular weight to detect the bands of leaf plant samples by use Primer OP-L20

(Mw) bp	*Phoenix dactylifera* Polluted	*Typha domingensis* polluted	*Alhagi graecorum* polluted	*Phoenix dactylifera* treated	*Typha domingensis* treated	*Alhagi graecorum* treated	
3000	1	0	0	0	0	0	OP-L20
2000	1	0	0	0	0	1	
1500	1	1	0	0	0	1	
1250	1	1	0	0	0	1	
1000	0	1	1	1	1	1	
950	1	1	1	0	1	1	
800	0	1	1	0	1	1	
750	0	1	1	0	1	1	
700	1	1	0	1	1	1	
625	1	1	0	0	1	1	
525	1	1	1	0	1	1	
500	0	1	1	1	1	1	
400	1	0	1	1	1	1	
375	1	0	1	0	1	1	
300	0	0	0	1	0	0	
250	1	0	0	0	0	1	

The number of bands recorded in this primer OP-L20 was   16 bands whose sizes ranged between 250-3000 base pairs.
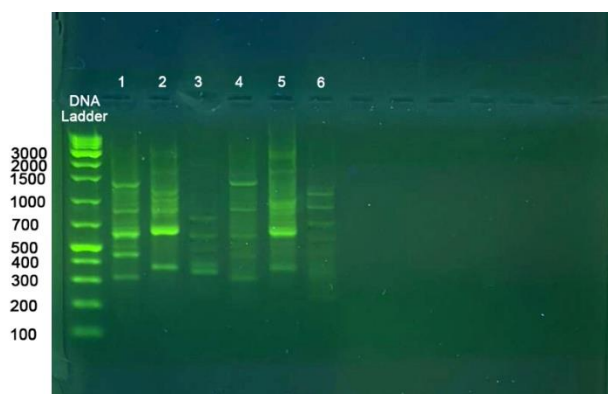
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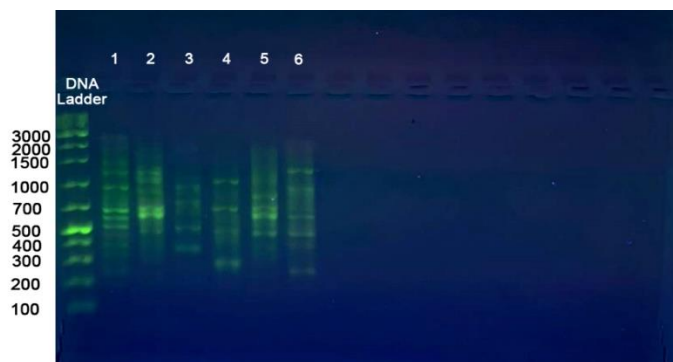
Figure 3-3. PCR product of primer OP-L20 The product was electrophoresis on 2% agarose at 5 volt/cm2. 1x TBE buffer for 1:30 hours. N: DNA ladder1000 bp

Table 3-3. The molecular weight to detect the bands of leaf plant samples by use Primer OP-M14

(Mw) bp	*Phoenix dactylifera* polluted	*Typha domingensis* polluted	*Alhagi graecorum* polluted	*Phoenix dactylifera* treated	*Typha domingens* treated	*Alhagi graecorum* treated	
3000	0	1	0	0	1	0	OP-M14
1750	0	1	0	0	1	0	
1400	1	0	0	1	1	1	
1300	0	1	0	0	1	1	
1000	1	1	0	0	1	0	
850	1	1	0	1	1	1	
725	1	0	1	0	1	1	
700	1	1	0	0	1	0	
600	1	1	1	1	1	1	
500	1	0	1	0	1	1	
450	1	0	0	1	0	1	
375	0	1	1	0	1	0	
325	1	0	1	1	0	0	
300	0	0	0	1	0	0	
225	0	0	0	0	0	1	

The number of bands recorded in this primer OP-M14was   15 bands whose sizes ranged between 225-3000 base pairs.
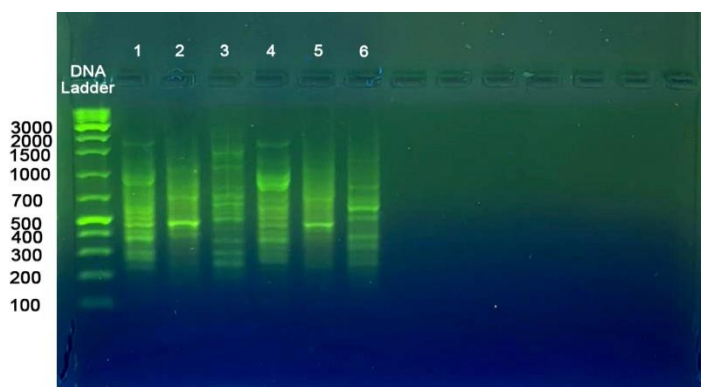

Figure 3-4. PCR product of primer OP-M14 The product was electrophoresis on 2% agarose at 5 volt/cm2. 1x TBE buffer for 1:30 hours.

Table 3-4. The molecular weight to detect the bands of leaf plant samples by use Primer OP-M05

(Mw) bp	*Phoenix dactylifera* polluted	*Typha domingensis* polluted	*Alhagi graecorum* polluted	*Phoenix dactylifera* treated	*Typha domingens* treated	*Alhagi graecorum* treated	
1750	1	0	1	1	0	0	OP-M05
1500	0	0	1	0	0	0	

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(Mw) bp							
1250	1	1	1	1	0	1	
1000	1	0	0	1	0	1	
900	1	1	1	1	1	1	
800	0	1	1	1	1	1	
700	1	1	1	1	1	1	
650	1	1	1	1	1	1	
600	1	1	1	1	1	1	
500	1	1	1	1	1	1	
450	1	1	1	1	1	1	
400	1	1	1	1	1	1	
375	1	1	0	1	1	1	
350	1	1	1	1	1	1	
300	1	1	1	1	0	1	
225	1	0	1	1	0	1	

The number of bands recorded in this primer OP-M05 was 16 bands whose sizes ranged between 225-1750 base pairs.
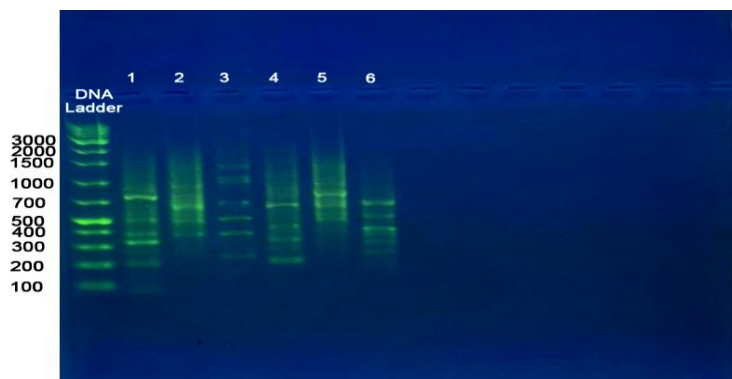

Figure 3-5. PCR product of primer OP-M05 The product was electrophoresis on 2% agarose at 5 volt/cm2. 1x TBE buffer for 1:30 hours.

Table 3-5. The molecular weight to detect the bands of leaf plant samples by use Primer OP-M20

(Mw) bp	Phoenix dactylifera polluted	Typha domingensis polluted	Alhagi graecorum polluted	Phoenix dactylifera treated	Typha domingens treated	Alhagi graecorum treated	
1500	0	0	1	0	0	0	OP-M20
1400	0	0	1	0	0	0	
1100	0	0	1	1	0	0	
1000	0	0	1	1	1	0	
950	1	1	0	1	1	0	
825	1	1	0	1	1	1	
700	0	1	1	1	1	1	
650	0	1	0	0	1	1	
550	1	1	1	0	1	1	
500	1	1	0	1	1	1	
450	0	1	0	1	0	1	
400	1	1	1	1	0	1	
325	1	0	0	1	0	1	
275	1	0	0	1	0	1	
200	1	0	1	1	0	0	

The number of bands recorded in this primer OP-M20 was 15 bands whose sizes ranged between 200-1500 base pairs.
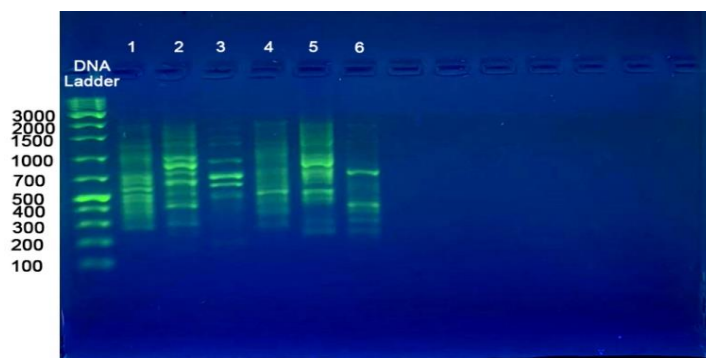
Figure 3-6. PCR product of primer OP-M20 The product was electrophoresis on 2% agarose at 5 volt/cm2. 1x TBE buffer for 1:30 hours.

Table 3-6. The molecular weight to detect the bands of leaf plant samples by use Primer OP-M06

(Mw) bp	*Phoenix dactylifera* polluted	*Typha domingensis* polluted	*Alhagi graecorum* polluted	*Phoenix dactylifera* treated	*Typha domingens* treated	*Alhagi graecorum* treated	
1500	1	1	1	0	1	0	OP-M06
1250	1	1	1	1	1	0	
1000	1	1	0	1	1	0	
950	1	1	1	1	1	0	
850	0	1	0	1	1	0	
750	1	1	1	1	1	0	
700	1	0	1	1	1	1	
600	1	1	1	0	1	0	
550	1	1	1	1	0	1	
500	1	1	1	1	1	0	
450	1	1	0	1	1	1	
400	1	1	0	1	0	1	
350	1	1	0	1	0	1	
300	1	1	0	1	1	1	
225	0	1	0	0	1	1	

The number of bands recorded in this primer OP-M06 was 15 bands whose sizes ranged between 225-1500 base pairs.
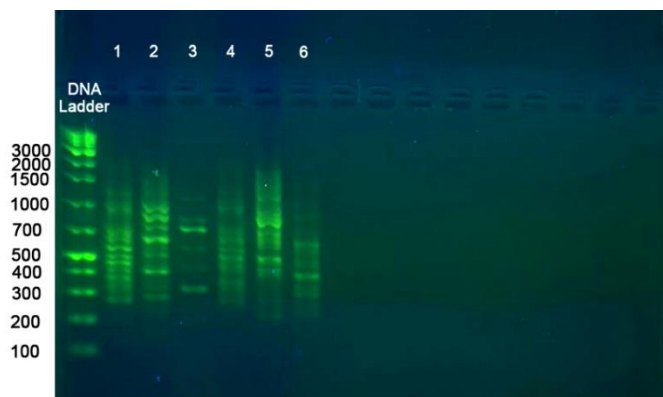

Figure 3-7. PCR product of primer OP-M06 The product was electrophoresis on 2% agarose at 5 volt/cm2. 1x TBE buffer for 1:30 hours.

Table 3-7. The molecular weight to detect the bands of leaf plant samples by use Primer OP-P04

(Mw) bp	*Phoenix dactylifera* polluted	*Typha domingensis* polluted	*Alhagi graecorum* polluted	*Phoenix dactylifera* treated	*Typha domingens* treated	*Alhagi graecorum* treated	
1100	1	1	1	1	1	0	OP-P04
950	1	1	0	1	1	1	

800	0	1	1	0	1	1
700	1	1	1	1	1	0
675	1	0	0	1	1	1
600	1	1	0	1	1	0
550	1	1	1	1	1	1
500	1	1	0	1	1	1
450	1	1	0	1	1	1
400	1	1	1	1	1	0
375	1	1	0	1	1	1
325	1	1	0	1	0	1
300	0	0	1	1	0	1
250	1	1	0	1	1	0
200	0	0	0	0	1	1

The number of bands recorded in this primer OP-P04 was 15 bands whose sizes ranged between 200-1100 base pairs.
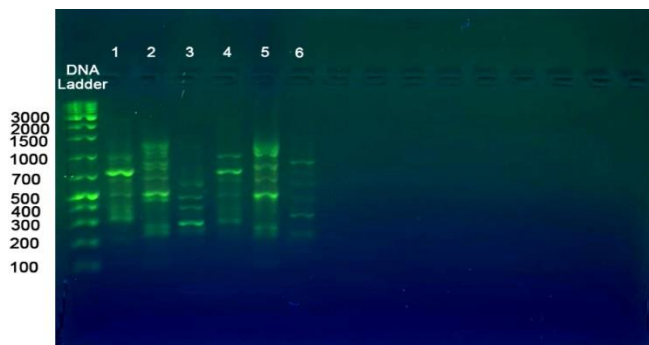

Figure 3-8. PCR product of primer OP-P04The product was electrophoresis on 2% agarose at 5 volt/cm2. 1x TBE buffer for 1:30 hours.

Table 3-8. The molecular weight to detect the bands of leaf plant samples by use Primer OP-L05

(Mw) bp	*Phoenix dactylifera* polluted	*Typha domingensis* polluted	*Alhagi graecorum* polluted	*Phoenix dactylifera* treated	*Typha domingens* treated	*Alhagi graecorum* treated	
1200	0	1	0	0	1	0	OP-L05
1100	0	1	0	0	1	0	
1000	1	1	0	1	1	0	
900	0	1	0	0	1	1	
800	0	1	0	1	1	0	
700	1	1	0	0	1	1	
675	1	0	0	1	0	1	
600	0	1	1	0	1	0	
500	1	1	1	1	1	0	
450	0	0	0	0	0	1	
400	1	0	1	0	0	0	
350	1	0	0	0	0	1	
325	1	0	0	0	0	0	
300	0	0	1	1	0	0	
275	1	1	1	1	1	0	
250	1	1	1	1	1	1	

The number of bands recorded in this primer OP-L05 was 16 bands whose sizes ranged between 250-1200 base pairs.
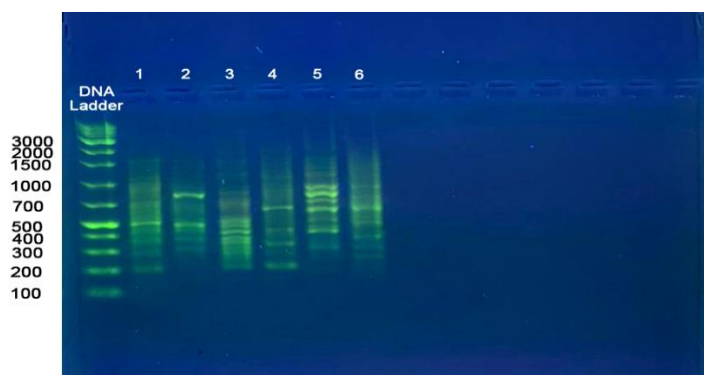
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Figure 3-9. PCR product of primer OP-L05 The product was electrophoresis on 2% agarose at 5 volt/cm2. 1x TBE buffer for 1:30 hours.

Table 3-9. The molecular weight to detect the bands of leaf plant samples by use Primer OP-V19

(Mw) bp	*Phoenix dactylifera* polluted	*Typha domingensis* polluted	*Alhagi graecorum* polluted	*Phoenix dactylifera* treated	*Typha domingens* treated	*Alhagi graecorum* treated	
1500	1	0	0	0	0	0	
1250	0	0	0	0	1	0	
1100	1	0	0	1	1	0	
900	1	0	1	0	1	1	
800	0	1	1	1	1	1	
700	1	0	1	1	1	1	OP-V19
600	1	0	1	1	1	1	
500	1	1	1	0	1	1	
450	1	1	1	0	1	1	
400	0	0	1	1	1	0	
350	1	1	1	1	1	1	
300	1	1	1	1	1	1	
250	0	0	1	1	0	1	
200	1	0	1	1	0	0	

The number of bands recorded in this primer OP-V19 was   14 bands whose sizes ranged between 200-1500 base pairs.
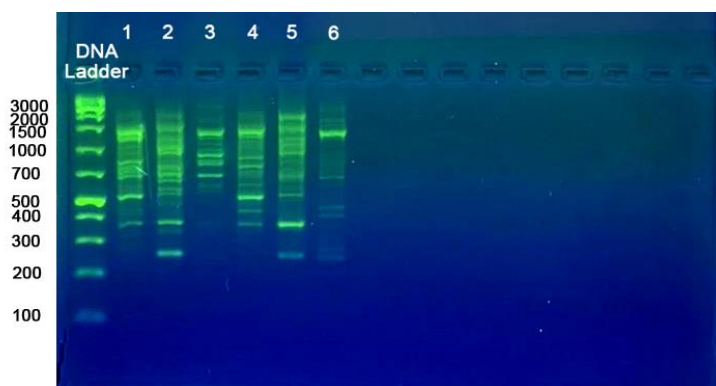

Figure 3-10. PCR product of primer OP-V19The product was electrophoresis on 2% agarose at 5 volt/cm2. 1x TBE buffer for 1:30 hours.

Table 3-10. The molecular weight to detect the bands of leaf plant samples by use Primer OP-V14

(Mw) bp	*Phoenix dactylifera* polluted	*Typha domingensis* polluted	*Alhagi graecorum* polluted	*Phoenix dactylifera* treated	*Typha domingens* treated	*Alhagi graecorum* treated	
2000	0	1	0	0	1	0	
1750	0	1	1	0	0	1	OP-V14
1500	1	1	1	1	1	1	
1200	1	1	1	1	1	1	

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1100	0	1	1	0	1	0	
1000	1	1	1	0	1	0	
800	1	1	1	1	1	0	
725	1	1	1	1	1	0	
700	1	1	0	1	1	0	
675	1	1	1	0	1	1	
600	1	0	0	1	0	0	
575	0	1	0	0	0	0	
525	1	1	0	1	1	0	
475	0	1	0	1	0	1	
400	0	0	0	0	0	1	
375	1	1	0	1	1	0	
350	0	1	0	0	0	0	
257	0	0	0	0	0	1	
250	0	1	0	0	1	1	

The number of bands recorded in this primer OP-V14 was 19 bands whose sizes ranged between 250-2000 base pairs.

Figure 3-11. PCR product of primer OP-V14The product was electrophoresis on 2% agarose at 5 volt/cm2. 1x TBE buffer for 1:30 hours.

The products of the primers from the total and polymorphic band with their efficiency ratios and their differential ability in Table (3-11)

Table 3-11. The products of the primers from the total and polymorphic band with their efficiency ratios and their differential ability for the studied samples.

%The discriminating ability of the primer	Primer efficiency %	Polymorphism %	Number of total polymorphic band	Number of Total band	rimer
8.805031	30.50847	77.77778	14	18	**OP-E20**
9.433962	27.11864	93.75	15	16	**OP-L20**
8.805031	25.42373	93.33333	14	15	**OP-M14**
4.402516	27.11864	43.75	7	16	**OP-M05**
9.433962	25.42373	100	15	15	**OP-M20**
9.433962	25.42373	100	15	15	**OP-M06**
8.176101	25.42373	86.66667	13	15	**OP-P04**
10.06289	27.11864	100	16	16	**OP-L05**
7.54717	23.72881	85.71429	12	14	**OP-V19**
10.69182	32.20339	89.47368	17	19	**OP-V14**
			138	159	

Determine the genetic distance among the studied samples

The results based on the RAPD-PCR, showed the extent of the distance between the tested samples, as it was the highest genetic distance (0.40541) (40%) between the sample3 and 6
It is the largest calculated value and indicates that these two samples have the greatest genetic distance showed figures such as figure (3-15) while it was observed that the lowest genetic distance between the samples 2 and 5 and its value is (0.15349) (15%) showed in figure such as (3-21) which is the smallest value calculated and this indicate that these two samples have the greatest genetic similarity. These results are consistent with results obtained by Haider *et al.*(2012), observed higher polymorphism in 23 date palm

cultivars from Syria representing 18 female and 5 male cultivars. The average polymorphism detected by the RAPD assay (58.5%).

The genetic distance between samples depends on the number of different bundles. The more different bundles, the higher the genetic distance. This indicates a difference in genetic material in that region of the genome.

Table 3-12. The genetic distance among the 6 studied samples before and after teatment.

	1	2	3	4	5	6
1	0					
2	0.27523	0				
3	0.35714	0.37113	0			
4	**0.23077**	0.32039	0.36957	0		
5	0.28111	**0.15349**	0.32642	0.34634	0	
6	0.30144	0.343	**0.40541**	0.37056	0.35922	0

1= polluted *Phoenix dactylifera* ,4= treated *Phoenix dactylifera*
2=polluted *Typha domingensis* ,5=treated *Typha domingensis*
3= polluted *Alhagi graecorum* ,6= treated *Alhagi graecorum*
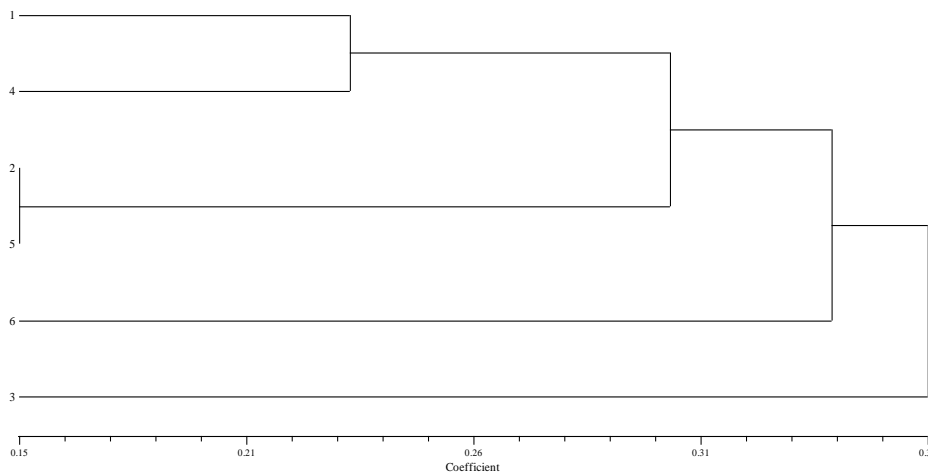
Scheme 3-1. of cluster analysis (Dendrogram) for the samples under study based on RAPD-PCR.

The results from the table(3-12) indicated that there is a genetic distance among the studied samples, as it was the highest in *Alhagi graecorum* plant (polluted and treated) in the sample 3&6 by comparing the results obtained with the results of other researchers, it was found that there is agreement with these results In the study of genetic distance of sunflower using Indicators of RAPD, with the highest genetic distance recorded, reached 0.7778 and the lowest was 0.5159 and the study conducted by Mahmoud and Abdel- Fatah (2012) in which he scored the highest the genetic distance was0. 976 among the breeds and in the *mintha sp*. Plant, Chandrashekar and Nguyen(1993) and Caissard *et al*.(1996), this agree with Pierce(2016), the change in the nucleotide sequence may be due to the role of the EMS mutagen in the induction of genetic variations in the Dawoodi plants , and the lowest in *Typha domingensis* plants, the current study confirmed that the RAPD-PCR technique is effective in detecting genetic variations and helps as tools in facilitating the breeding and improvement programs for plants. The specific surface area and pores of leaf plants are efficiently regenerated, the ash treatments increased the surface area of ash when compared to the initial surface area of untreated spent ash this agrees with San Miguel *et al.*(2001) This is due to metals accumulating in pores and on the plant's surface during the purification process, which could act as catalysts when the plant is exposed to ash , the metal accumulation catalyzes the reaction between surface carbon and oxidizing agents, resulting in pore formation and increased surface area. In addition to the ability to break the bonds caused by an increase in mineral accumulation in the plant, it is a good treatment that is low in cost and does not take a long time to indicate this agrees with Moona *et al.*(2018). And the result agree with Basheer-Salimia *et al.* (2012), the genetic distance matrix in *Ficus carica* L. showed an average distance range from 0.186 to 0.559 with a mean of 0.373. Thus, the cultivars tested are characterized by large divergence at the DNA level.

4-Conclusion: Ten primers gave positive results by successfully amplifying the DNA bundles for all studied samples by 159 bundles, the highest was OP-V14 (19 bundles) and the lowest was for OP-V19 (14 bundles). The lowest genetic distance between the samples 2 and 5 (*Typha domingensis* polluted and trated)and its value

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is (0.15349) (15%) which is the smallest value calculated and this indicate that these two samples have the greatest genetic similarity ,The results of the primers depended on estimating the genetic distance ratio among three types of plants used before and after being treated with activated carbon, which gave the highest percentage of 40% between the polluted and treated *Alhagi graecorum* plants.

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