

## DETERMINATION OF DNA FINGERPRINTING OF NINE *ARGEMONE* PLANT SAMPLES USING RAPD-PCR TOOL

**Bazaid, S.A.<sup>1</sup>; Mohamed Muneera, A.S.<sup>1</sup>; Hussein Sonya, H.M.<sup>1,2</sup> and Sadik, A.S.<sup>1,3</sup>**

<sup>1</sup>Department of Biology, Faculty of Sciences, Taif University, P.O. Box 888, Taif, Kingdom of Saudi Arabia, <sup>2</sup>Department of Agricultural Microbiology, Institute of Soil Water and Environment Research Institute, Agricultural Research Center (ARC), P.O. Box 12619, Giza, Egypt, <sup>3</sup>Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, P.O. Box 68 Hadayek Shubra, 11241, Cairo, Egypt

### ABSTRACT

We are herein focusing on molecular analysis of *Argemone* plants collected from open area (Al-Shafa, Jabajeb and Al-Arafah), dams (Gadeer Albanat and Ekrima) and valleys (Thumalah, Wadi-Sa'b, Saysid and Wadi-Jaleel) at Taif Governorate in Kingdom of Saudi Arabia. At the level of molecular analyses, DNA fingerprinting of nine *Argemone* plant samples (1-9) via RAPD-PCR using 10 random primers was determined. Data showed that total fragments of 108 (28 polymorphic fragments+80 monomorphic fragments) were amplified. Out of the 108 fragments, PS21 samples showed 84, 82, 84, 84, 83, 87, 85, 85 and 83, respectively. A number of 14 unique fragments out of the 108 were obtained using the ten RAPD-PCR primers, and could be used as DNA markers. The dendrogram based on RAPD-PCR analysis of the nine PS21 samples showed the presence of four clusters (A, B, C and D) with highest and lowest similarities of 98 and 89 %, respectively. Results of SDS-PAGE analysis of the applied PS21 samples was in harmony with that of RAPD-PCR analysis. Results paid an attention to the availability of RAPD-PCR technique as one of the molecular tool for determining the DNA fingerprinting of such important plants.

**Key words:** Taif, *Argemone* weed, RAPD-PCR, DNA fingerprinting, molecular analysis.

### INTRODUCTION

Spread of *Agremone* is thought to be related to human activities such as site disturbance and overgrazing. The genus *Argemone* (Papaveraceae) comprises nearly 28 species (Mabberley, 1997, Smith, 2002, Ownby, 2007 and Stevens, 2007). The economic importance of *Argemone* weed plant could be concluded as sources of oil, alkaloids and renewable energy (Augustus *et al.*, 2003, Yuh Chwen *et al.*, 2003, Novizar *et al.* 2009; Bhalke *et al.*, 2009; Singh and Singh, 2010 and Singh *et al.*, 2010). It was also, reported as a medicinal plant (Deka and Deka, 2007) and a weed plant having antioxidant effect (Gacche *et al.*, 2008 and Perumal *et al.*, 2010) as well as

antagonistic activities (Singh and Tripathi, 2005, Saadabi, 2006, Tripathi and Sharma, 2007, Abou-Zeid and El-Fattah, 2007, Murugesan and Daniel, 2008, Elbadri *et al.*, 2009 and Osho and Adetunji, 2010). The first documentation of the genus in Saudi Arabia was given by Migahid (1974). Currently, two species are already identified in Saudi Arabia; *A. mexicana* L. and *A. ochroleuca* Sweet (Collenete, 1985 and Chaudhary and Al-Jowaid, 1999).

Introducing species into new environment can have devastating effects on ecosystem quality (Van Groenendael *et al.*, 1998). *Argemone* spp. is an example of a noxious weed that has been introduced to Saudi Arabia (Migahid,

1974). This relatively newly introduced noxious weed accounts for the loss of **rangeland** value in valleys around Taif mountains area. The history of introduction of *A. mexicana*, now occupying large tracts of deteriorated rangelands in Asir region, is not traceable. *A. ochroleuca* was most widespread in Taif area (Shorbaji and Abidiain, 1999).

The aim of this study was determination of the DNA fingerprinting of *Argemone* plants collected from different climatic locations in Taif Governorate.

## MATERIALS AND METHODS

**DNA extraction, electrophoresis and estimation:** DNA was extracted from Argemone plant materials according to the method described by Dellaporta *et al.* (1983). On electrophoresis of DNA extracts using 1% agarose gel in TAE buffer as described by Sambrook *et al.*, (1989), the concentration and purity of DNA extracts of the Argemone plant samples were determined as recommended by Brown (1990). Samples of DNA with a ratio of 1.8 the absorbance at  $A_{260}/A_{280}$  nm were adjusted to 10ng/ $\mu$ l.

**RAPD-PCR:** Amplification reaction was carried out in a total volume of 25 $\mu$ l as described by Bagheri *et al.* (1995). Each reaction mixture contained: 2.5 $\mu$ l 10X PCR buffer (500mM KCl, 100mM Tris-HCl (pH=9.0) and 1% Triton-100), 1.5 $\mu$ l MgCl<sub>2</sub> (1.5 mM), 0.5 $\mu$ l dNTPs mix (0.2 mM dATP, dCTP, dTTP, dGTP), 1 $\mu$ l Template DNA (100 ng genomic DNA), 1.5 $\mu$ l (0.4 $\mu$ M decamer oligonucleotide primer Table-1), 0.5 $\mu$ l *Taq* DNA polymerase (two units of *Taq* DNA polymerase (Promega Crop, Madison, WI, USA)) and 17.5 $\mu$ l d.H<sub>2</sub>O. PCR ampli-

fication was performed in a GeneAmp 2400 PCR machine using the following program:

**Denaturation:** 5 min at 94°C (1 cycle); 35 cycles each of denaturation for 1 min at 94°C, annealing for 1 min at 36°C and extension for 2 min at 72°C. The primer extension segment was extended to 5 min at 72°C in the final cycle. The amplification products were resolved by electrophoresis in a 1.0% agarose gel at 60 volts for 3.5 hr with 1X TAE buffer (Sambrook *et al.*, 1989).

**Table-1:** RAPD-PCR primers used for determination of DNA fingerprinting of nine Argemone plant samples.

Operon technologies, Alameda CA kits	primer code	Sequences (5' ----- 3')
OPA	05	AGGGGTCTTG
	06	GGTCCCTGAC
	10	GTGATCGCAG
	16	AGCCAGCGAA
OPB	03	CATCCCCCTG
OPC	01	TTCGAGCCAG
OPE	04	GTGACATGCC
OPG	10	CACCAAGTGA
	03	GAGCCCTCCA
	18	GGCTCATGTG

**RAPD-PCR analysis:** Amplified products were visually examined under UV trans-illuminator and the presence or absence of each sizes class was scored as 1 (present) or – 0 (absent), respectively. Bands of the same mobility were scored as identical. The similarity coefficient (F) between isolates was defined by the formula of Nei and Li (1979). A dendrogram was derived from the distance by unweighted paired group method, arithmetic mean (UPGMA) algorithm contained in the computer program package NTSYS 1.5 (Rohlf, 1990).

## RESULTS AND DISCUSSION

In this experiment, a number of nine Argemone plant samples (1, 2, 3, 4, 5, 6, 7, 8 and 9) were collected from the nine locations, i.e., Thumalah, Gadeer-Abanat, Saysid, Wadi-Jaleel, Al-Arafah, Wadi-Sa'b, Ekrima, Jabajeb and Al-Shafa, respectively. These samples were subjected to a molecular study aimed to determine the DNA fingerprinting of these plant samples, in a trail to demonstrate the genetic relationship between these plant samples using RAPD-PCR molecular tool.

Results in Tables (2, 3, 4, 5, 6, 7, 8, 9, 10 and 11) and Figures (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10) showed that a number of ten RAPD-PCR primers OPA05, OPA06, OPA10, OPA16, OPB03, OPC01, OPE04, OPE10, OPG03 and OPG18, respectively, were used.

Data in Tables-12 showed that total amplified fragments of 108 were obtained using the 10 RAPD-PCR primes distributed as follows: 12,7,15,15,10,13, 9,8,7 and 12 fragments using the RAPD-PCR primers OPA05, OPA06, OPA10, OPA16, OPB03, OPC01, OPE04, OPE10, OPG03 and OPG18, respectively. Concerning the unique fragments produced by the ten primers 14 fragments (2, 1, 1, 7 and 3) were obtained using OPA05, OPA06, OPB03, OPC01, OPG03 and OPG18, primers, respectively.

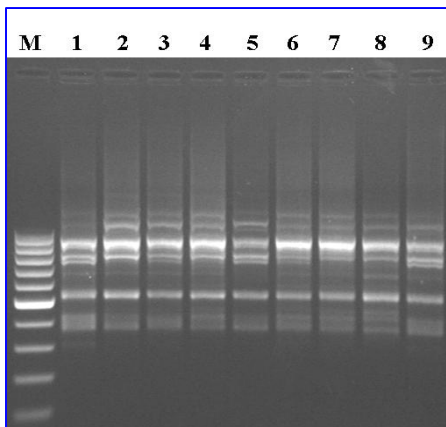
At the level of total amplified fragments amplified from the DNA of Argemone plant samples 84, 82, 84, 84, 83, 87, 85, 85 and 83 out of 108 fragments were produced from the DNA of 1, 2, 3, 4, 5, 6, 7, 8 and 9 Argemone plant samples, respectively.

Results in **Table-13** showed that numbers of 28 polymorphic fragments (7, 4, 1, 11 and 5) were amplified using only five RAPD-PCR primers (OPA05,

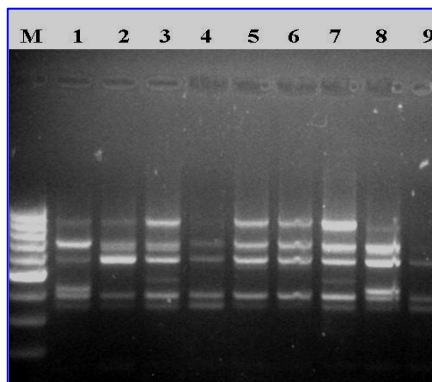
OPA06, OPB05, OPC01 and OPG03, respectively). The remaining 80 monomorphic fragments (5, 3, 15, 15, 9, 2, 9, 8, 2 and 12) were produced by RAPD-PCR analysis of nine *Argemone* plant samples using 10 RAPD-PCR primers, respectively. Only 1,1,1,5,2,3 and 1 unique fragments were amplified via a, 1,2,3,5,6,8 and 9 Argemone plant samples, respectively.

**Table-2:** Total amplified fragments (TAFs), unique fragments (UF), polymorphic fragments (PMF) and monomorphic fragments (MMFs) produced by RAPD-PCR analysis of nine *Argemone* plant samples using OPA05 RAPD-PCR primer.

Fragments	<i>Argemone</i> samples								
	1	2	3	4	5	6	7	8	9
F1	1	1	1	1	0	1	1	1	0
F2	0	0	0	0	1	0	0	0	0
F3	0	1	1	1	0	1	1	1	1
F4	1	1	1	1	1	1	1	1	1
F5	1	1	1	1	1	1	1	1	1
F6	1	0	1	0	1	1	0	0	1
F7	1	1	1	1	0	1	0	1	0
F8	1	1	1	1	1	1	1	1	1
F9	0	0	0	0	0	0	1	1	0
F10	1	1	1	1	1	1	1	1	1
F11	1	1	1	1	1	1	1	1	1
F12	1	0	0	0	0	0	0	0	0
<b>TAFs (12)</b>	<b>9</b>	<b>8</b>	<b>9</b>	<b>8</b>	<b>7</b>	<b>9</b>	<b>8</b>	<b>9</b>	<b>7</b>
<b>UFs (2)</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
<b>PMFs</b>	7								
<b>MMFs</b>	5								



**Figure-1:** Agarose gel electrophoresis (1%) stained with ethidium bromide shows RAPD-PCR variation of DNA genome among nine samples of *Argemone* plant using OPA05 RAPD-PCR primer. M: DNA marker.



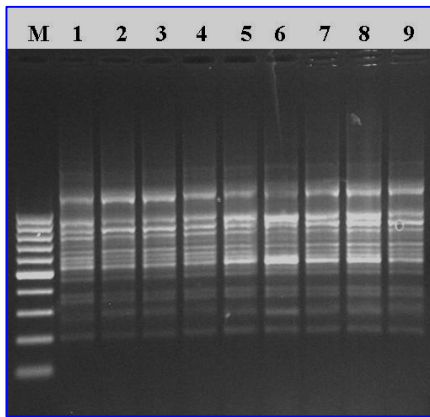
**Figure-2:** Agarose gel electrophoresis (1%) stained with ethidium bromide shows RAPD-PCR variation of DNA genome among nine samples of *Argemone* plant using OPA06 RAPD-PCR primer. M: DNA marker.

**Table-3:** Total amplified fragments (TAFs), unique fragments (UF), polymorphic fragments (PMF) and monomorphic fragments (MMFs) produced by RAPD-PCR analysis of nine *Argemone* plant samples using OPA06 RAPD-PCR primer.

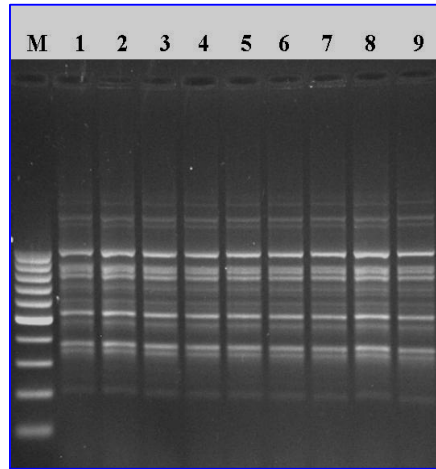
Fragments	Argemone samples								
	1	2	3	4	5	6	7	8	9
F13	1	1	1	0	1	1	1	0	0
F14	1	1	1	1	1	1	1	1	0
F15	1	1	1	1	1	1	1	1	1
F16	0	0	1	0	0	0	1	0	0
F17	1	0	0	0	0	0	1	0	0
F18	1	1	1	1	1	1	1	1	1
F19	1	1	1	1	1	1	1	1	1
<b>TAFs (7)</b>	<b>6</b>	<b>5</b>	<b>6</b>	<b>4</b>	<b>5</b>	<b>5</b>	<b>7</b>	<b>5</b>	<b>4</b>
<b>UFs (1)</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>1</b>
<b>PMF</b>	<b>4</b>								
<b>MMFs</b>	<b>3</b>								

**Table-4:** Total amplified fragments (TAFs), unique fragments (UF), polymorphic fragments (PMF) and monomorphic fragments (MMFs) produced by RAPD-PCR analysis of nine *Argemone* plant samples using OPA10 RAPD-PCR primer.

Fragments	Argemone samples								
	1	2	3	4	5	6	7	8	9
F20	1	1	1	1	1	1	1	1	1
F21	1	1	1	1	1	1	1	1	1
F22	1	1	1	1	1	1	1	1	1
F23	1	1	1	1	1	1	1	1	1
F24	1	1	1	1	1	1	1	1	1
F25	1	1	1	1	1	1	1	1	1
F26	1	1	1	1	1	1	1	1	1
F27	1	1	1	1	1	1	1	1	1
F28	1	1	1	1	1	1	1	1	1
F29	1	1	1	1	1	1	1	1	1
F30	1	1	1	1	1	1	1	1	1
F31	1	1	1	1	1	1	1	1	1
F32	1	1	1	1	1	1	1	1	1
F33	1	1	1	1	1	1	1	1	1
F34	1	1	1	1	1	1	1	1	1
<b>TAFs (15)</b>	<b>15</b>	<b>15</b>	<b>15</b>	<b>15</b>	<b>15</b>	<b>15</b>	<b>15</b>	<b>15</b>	<b>15</b>
<b>UFs (0)</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
<b>PMFs</b>	<b>0</b>								
<b>MMFs</b>	<b>15</b>								



**Figure-3:** Agarose gel electrophoresis (1%) stained with ethidium bromide shows RAPD-PCR variation of DNA genome among nine samples of *Argemone* plant using OPA10 RAPD-PCR primer. M: DNA marker.



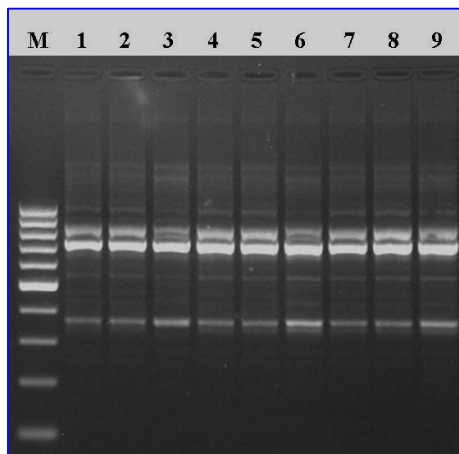
**Figure-4:** Agarose gel electrophoresis (1%) stained with ethidium bromide shows RAPD-PCR variation of DNA genome among nine samples of *Argemone* plant using OPA16 RAPD-PCR primer. M: DNA marker.

**Table-5:** Total amplified fragments (TAFs), unique fragments (UFs), polymorphic fragments (PMF) and monomorphic fragments (MMFs) produced by RAPD-PCR analysis of nine *Argemone* plant samples using OPA16 RAPD-PCR primer.

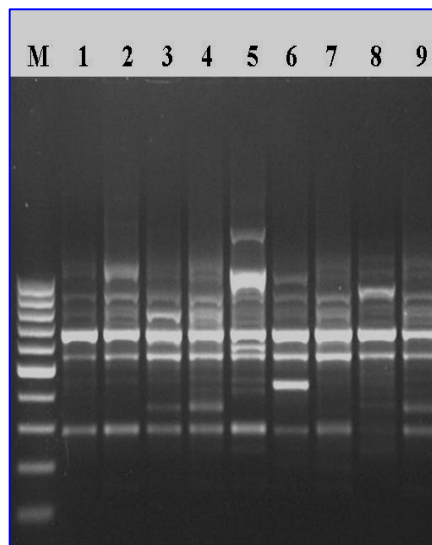
Fragments	<i>Argemone</i> samples								
	1	2	3	4	5	6	7	8	9
F35	1	1	1	1	1	1	1	1	1
F36	1	1	1	1	1	1	1	1	1
F37	1	1	1	1	1	1	1	1	1
F38	1	1	1	1	1	1	1	1	1
F39	1	1	1	1	1	1	1	1	1
F40	1	1	1	1	1	1	1	1	1
F41	1	1	1	1	1	1	1	1	1
F42	1	1	1	1	1	1	1	1	1
F43	1	1	1	1	1	1	1	1	1
F44	1	1	1	1	1	1	1	1	1
F45	1	1	1	1	1	1	1	1	1
F46	1	1	1	1	1	1	1	1	1
F47	1	1	1	1	1	1	1	1	1
F48	1	1	1	1	1	1	1	1	1
F49	1	1	1	1	1	1	1	1	1
<b>TAFs (15)</b>	<b>15</b>	<b>15</b>	<b>15</b>	<b>15</b>	<b>15</b>	<b>15</b>	<b>15</b>	<b>15</b>	<b>15</b>
<b>UFs (0)</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
<b>PMFs</b>	<b>0</b>								
<b>MMFs</b>	<b>15</b>								

**Table-6:** Total amplified fragments (TAFs), unique fragments (UF), polymorphic fragments (PMF) and monomorphic fragments (MMFs) produced by RAPD-PCR analysis of nine *Argemone* plant samples using OPB03 RAPD-PCR primer.

Fragments	<i>Argemone</i> samples								
	1	2	3	4	5	6	7	8	9
F50	1	1	1	1	1	1	1	1	1
F51	1	1	1	1	1	1	1	1	1
F52	1	1	1	1	1	1	1	1	1
F53	1	1	1	1	1	1	1	1	1
F54	1	1	1	1	1	1	1	1	1
F55	1	1	1	1	1	1	1	1	1
F56	1	1	1	1	1	1	1	1	1
F57	1	1	1	1	1	1	1	1	1
F58	1	1	1	1	1	1	1	1	1
F59	1	1	0	1	1	1	1	1	1
<b>TAFs (10)</b>	<b>10</b>	<b>10</b>	<b>9</b>	<b>10</b>	<b>10</b>	<b>10</b>	<b>10</b>	<b>10</b>	<b>10</b>
<b>UFs (1)</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
<b>PMFs</b>	<b>1</b>								
<b>MMFs</b>	<b>9</b>								



**Figure-5:** Agarose gel electrophoresis (1%) stained with ethidium bromide shows RAPD-PCR variation of DNA genome among nine samples of *Argemone* plant using OPB03 RAPD-PCR primer. M: DNA marker.



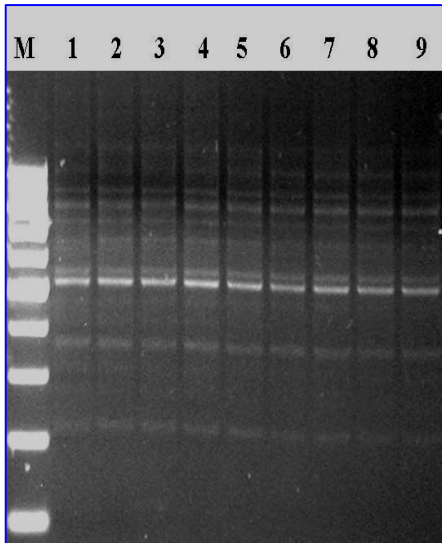
**Figure-6:** Agarose gel electrophoresis (1%) stained with ethidium bromide shows RAPD-PCR variation of DNA genome among nine samples of *Argemone* plant using OPC01 RAPD-PCR primer. M: DNA marker.

**Table-7:** Total amplified fragments (TAFs), unique fragments (UF), polymorphic fragments (PMF) and monomorphic fragments (MMFs) produced by RAPD-PCR analysis of nine *Argemone* plant samples using OPC01 RAPD-PCR primer.

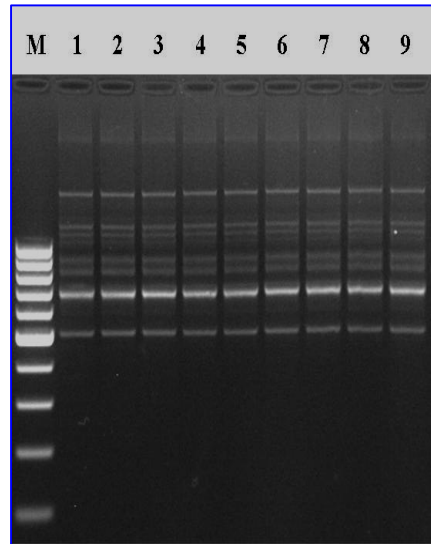
Fragments	<i>Argemone</i> samples								
	1	2	3	4	5	6	7	8	9
F60	0	0	0	0	1	0	0	0	0
F61	0	0	0	0	0	0	1	1	1
F62	1	0	1	1	1	1	1	1	1
F63	0	0	0	0	0	0	0	1	0
F64	1	1	1	1	1	1	1	0	1
F65	1	1	1	1	1	1	1	1	1
F66	0	0	0	0	1	0	0	0	0
F67	1	1	1	1	1	1	1	1	1
F68	1	1	1	1	1	0	0	0	0
F69	0	0	0	0	0	1	0	0	0
F70	0	0	0	1	1	0	0	0	0
F71	0	0	1	1	0	0	0	1	1
F72	1	1	1	1	1	1	1	0	1
<b>TAFs (13)</b>	<b>6</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>6</b>	<b>6</b>	<b>7</b>	<b>7</b>
<b>UFs (7)</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>2</b>	<b>1</b>	<b>0</b>	<b>3</b>	<b>0</b>
<b>PMFs</b>	<b>11</b>								
<b>MMFs</b>	<b>2</b>								

**Table-8:** Total amplified fragments (TAFs), unique fragments (UF), polymorphic fragments (PMF) and monomorphic fragments (MMFs) produced by RAPD-PCR analysis of nine *Argemone* plant samples using OPE04 RAPD-PCR primer.

Fragments	<i>Argemone</i> samples								
	1	2	3	4	5	6	7	8	9
F73	1	1	1	1	1	1	1	1	1
F74	1	1	1	1	1	1	1	1	1
F75	1	1	1	1	1	1	1	1	1
F76	1	1	1	1	1	1	1	1	1
F77	1	1	1	1	1	1	1	1	1
F78	1	1	1	1	1	1	1	1	1
F79	1	1	1	1	1	1	1	1	1
F80	1	1	1	1	1	1	1	1	1
F81	1	1	1	1	1	1	1	1	1
<b>TAFs (9)</b>	<b>9</b>	<b>9</b>	<b>9</b>	<b>9</b>	<b>9</b>	<b>9</b>	<b>9</b>	<b>9</b>	<b>9</b>
<b>UFs (0)</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
<b>PMFs</b>	<b>0</b>								
<b>MMFs</b>	<b>9</b>								



**Figure-7:** Agarose gel electrophoresis (1%) stained with ethidium bromide shows RAPD-PCR variation of DNA genome among nine samples of *Argemone* plant using OPE04 RAPD-PCR primer. M: DNA marker.



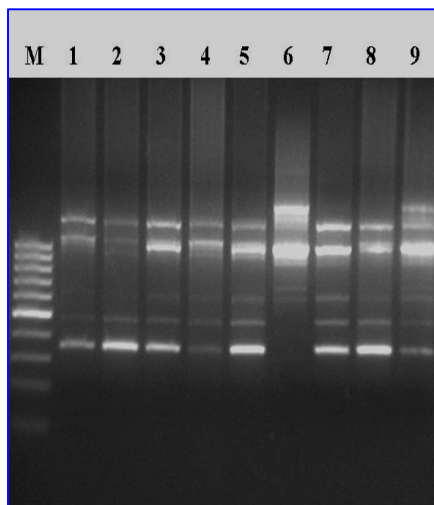
**Figure-8:** Agarose gel electrophoresis (1%) stained with ethidium bromide shows RAPD-PCR variation of DNA genome among nine samples of *Argemone* plant using OPE10 RAPD-PCR primer. M: DNA marker.

**Table-9:** Total amplified fragments (TAFs), unique fragments (UF), polymorphic fragments (PMF) and monomorphic fragments (MMFs) produced by RAPD-PCR analysis of nine *Argemone* plant samples using OPE10 RAPD-PCR primer.

Fragments	<i>Argemone</i> samples								
	1	2	3	4	5	6	7	8	9
F82	1	1	1	1	1	1	1	1	1
F83	1	1	1	1	1	1	1	1	1
F84	1	1	1	1	1	1	1	1	1
F85	1	1	1	1	1	1	1	1	1
F86	1	1	1	1	1	1	1	1	1
F87	1	1	1	1	1	1	1	1	1
F88	1	1	1	1	1	1	1	1	1
F89	1	1	1	1	1	1	1	1	1
TAFs (8)	8	8	8	8	8	8	8	8	8
UFs (0)	0	0	0	0	0	0	0	0	0
PMFs	0								
MMFs	8								

**Table-10:** Total amplified fragments (TAFs), unique fragments (UF), polymorphic fragments (PMF) and monomorphic fragments (MMFs) produced by RAPD-PCR analysis of nine *Argemone* plant samples using OPG03 RAPD-PCR primer.

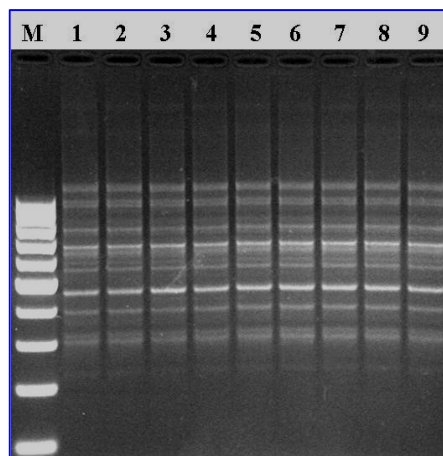
Fragments	<i>Argemone</i> samples								
	1	2	3	4	5	6	7	8	9
F90	0	0	0	0	0	1	0	0	1
F91	1	1	1	1	1	1	1	1	1
F92	1	1	1	1	1	1	1	1	1
F93	0	0	0	0	0	1	0	0	0
F94	0	0	1	1	1	1	1	1	1
F95	1	1	1	1	0	1	1	1	1
F96	1	1	1	1	0	1	1	1	1
TAFs (7)	4	4	5	5	3	8	5	5	6
UFs (3)	0	0	0	0	2	1	0	0	0
PMFs	5								
MMFs	2								



**Figure-9:** Agarose gel electrophoresis (1%) stained with ethidium bromide shows RAPD-PCR variation of DNA genome among nine samples of *Argemone* plant using OPG03 RAPD-PCR primer. M: DNA marker.

**Table-11:** Total amplified fragments (TAFs), unique fragments (UF), polymorphic fragments (PMF) and monomorphic fragments (MMFs) produced by RAPD-PCR analysis of nine *Argemone* plant samples using OPG18 RAPD-PCR primer.

Fragments	<i>Argemone</i> samples								
	1	2	3	4	5	6	7	8	9
F97	1	1	1	1	1	1	1	1	1
F98	1	1	1	1	1	1	1	1	1
F99	1	1	1	1	1	1	1	1	1
F100	1	1	1	1	1	1	1	1	1
F101	1	1	1	1	1	1	1	1	1
F102	1	1	1	1	1	1	1	1	1
F103	1	1	1	1	1	1	1	1	1
F104	1	1	1	1	1	1	1	1	1
F105	1	1	1	1	1	1	1	1	1
F106	1	1	1	1	1	1	1	1	1
F107	1	1	1	1	1	1	1	1	1
F108	1	1	1	1	1	1	1	1	1
<b>TAFs (12)</b>	<b>12</b>	<b>12</b>	<b>12</b>	<b>12</b>	<b>12</b>	<b>12</b>	<b>12</b>	<b>12</b>	<b>12</b>
<b>UFs (0)</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
<b>PMFs</b>	<b>0</b>								
<b>MMFs</b>	<b>12</b>								



**Figure-10:** Agarose gel electrophoresis (1%) stained with ethidium bromide shows RAPD-PCR variation of DNA genome among nine samples of *Argemone* plant using OPG18 RAPD-PCR primer. M: DNA marker.

**Table-12:** Total amplified fragments (TAFs), unique fragments (UF), polymorphic fragments (PMF) and monomorphic fragments (MMFs) produced by RAPD-PCR analysis of nine *Argemone* plant samples using 10 RAPD-PCR primers.

Primers	No. Fragments	<i>Argemone</i> samples									
		1	2	3	4	5	6	7	8	9	
OPA05	TAFs	12	9	8	9	8	7	9	8	9	7
	UFs	2	1	0	0	0	1	0	0	0	0
OPA06	TAFs	7	6	5	6	4	5	5	7	5	4
	UFs	1	0	0	0	0	0	0	0	0	1
OPA10	TAFs	15	15	15	15	15	15	15	15	15	15
	UFs	0	0	0	0	0	0	0	0	0	0
OPA16	TAFs	15	15	15	15	15	15	15	15	15	15
	UFs	0	0	0	0	0	0	0	0	0	0
OPB03	TAFs	10	10	10	9	10	10	10	10	10	10
	UFs	1	0	0	1	0	0	0	0	0	0
OPC01	TAFs	13	6	6	7	8	9	6	6	7	7
	UFs	7	0	1	0	0	2	1	0	3	0
OPE04	TAFs	9	9	9	9	9	9	9	9	9	9
	UFs	0	0	0	0	0	0	0	0	0	0
OPE10	TAFs	8	8	8	8	8	8	8	8	8	8
	UFs	0	0	0	0	0	0	0	0	0	0
OPG03	TAFs	7	4	4	5	5	3	8	5	5	6
	UFs	3	0	0	0	0	2	1	0	0	0
OPG18	TAFs	12	12	12	12	12	12	12	12	12	12
	UFs	0	0	0	0	0	0	0	0	0	0
Total	TAFs	<b>108</b>	<b>84</b>	<b>82</b>	<b>84</b>	<b>84</b>	<b>83</b>	<b>87</b>	<b>85</b>	<b>85</b>	<b>83</b>
	UFs	<b>14</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>0</b>	<b>5</b>	<b>2</b>	<b>0</b>	<b>3</b>	<b>1</b>



**Table-13:** Total amplified fragments (TAFs), polymorphic fragments (PMFs) and monomorphic fragments (MMFs) produced by RAPD-PCR analysis using 10 RAPD-PCR primers.

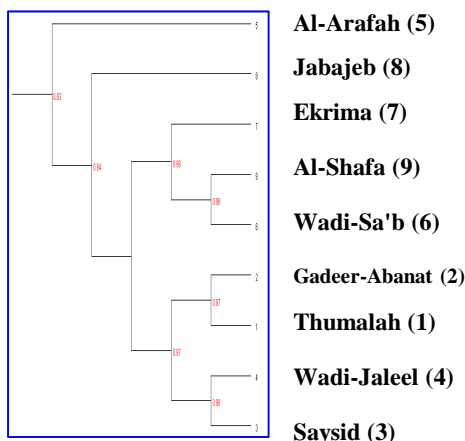
Primers	TAFs	PMFs	MMFs
OPA05	12	7	5
OPA06	7	4	3
OPA10	15	0	15
OPA16	15	0	15
OPB03	10	1	9
OPC01	13	11	2
OPE04	9	0	9
OPE10	8	0	8
OPG03	7	5	2
OPG18	12	0	12
<b>Total</b>	<b>108</b>	<b>28</b>	<b>80</b>

Results in Table-14 showed that the highest similarities of the nine *Argemone* plant DNA samples based on RAPD-PCR analysis using 10 RAPD-PCR primers was 98 % between Argemone samples 1 (Thumalah location) and 2 (Gadeer-Albanat location), while, the lowest similarity was 89% between Argemone samples 5 (Al-Arafah location) and 8 (Jabajeb location).

Based on the dendrogram (Figure 12) produced by RAPD-PCR analysis of nine *Argemone* plant DNA samples of nine *Argemone* plants collected from nine locations of this study four clusters (A, B, C and D) were obtained. Cluster A contains Argemone samples 3, 4, 1 and 2 and Cluster B contains Argemone samples 6, 9 and 7. On the other hand both of Cluster C and D contain Argemone samples 8 and 5, respectively.

As a conclusion, one can recommend the use of RAPD-PCR technique as one of the molecular tool for determining the DNA fingerprinting of such important plants. This conclusion could be supported by studies of Ali *et al.* (2007), Mandal *et al.* (2007), Nazari and Pakniyat (2008), Heikal *et al.* (2008), Alghamdi (2009), Moktaduzzaman and

Rahman (2009), Sesli and Yegenoglu (2010).



**Figure-11:** Dendrogram based on RAPD-PCR analysis of nine *Argemone* plant DNA samples of nine *Argemone* plant DNA samples from nine locations of this study.

**Table-14:** Similarities between RAPD-PCR analyses of nine *Argemone* plant DNA samples using 10 RAPD-PCR primers.

Argemone samples	% Similarities								
	1	2	3	4	5	6	7	8	9
1	100								
2	98.0	100							
3	97.0	97.0	100						
4	96.0	98.0	98.0	100					
5	94.0	93.0	94.0	94.0	100				
6	96.0	96.0	96.0	96.0	94.0	100			
7	95.0	96.0	96.0	95.0	93.0	96.0	100		
8	93.0	94.0	94.0	96.0	89.0	93.0	95.0	100	
9	94.0	94.0	95.0	96.0	93.0	96.0	96.0	94.0	100

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