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Molecular Identification of Four *Eruca Sativa L.* Cultivars using RAPD Markers

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ABSTRACT

The current study was conducted at University of Kufa, Faculty of Science, Biology Department for molecular identification of four *Eruca sativa L.* cultivars (1-Turish 2-Syrian 3-Turkish 4-Eygyptian) using eleven Random Amplified Polymorphic DNA. Results indicate primers OPA-04, OPA-10, OPA-02, OPW-04, OPC-09,v and OPD-13 success in giving all cultivars a unique fingerprint .Highest number of main bands , polymorphic bands and discriminatory value were 20 , 18 and 20.22% respectively produced by OPA-02. Highest value for amplified band number and monomorphic bands were 44 and band produced by primers OPX-17 and OPD-13 respectively. Highest value for both polymorphism and Efficiency were 94.11% and 0.48 respectively produced by primer OPA-04. Genetic relationship and genetic distance among *E.sativa* cultivars distribution unrelated to their geographical origin. RAPD markers are efficient tool in studying *E.sativa* germplasm.

1. Introduction

Eruca sativa is commonly known as rocket plant. The local Iraqi name is Jarjeer , it is a member of mustard (Brassicaceae) family originated in the Mediterranean region coast, also grown in the Middle-East, South Asia, and all over the world (Jaafar & Jaafar, 2019). The airy tender fresh parts of plant was used as a medicinal remedy for various diseases (Kishore, et al., 2017). Jarjeer seeds known to have high oil, protein, and glucosinolate content (El Nagar & Mekawi, 2014). Study of genetic diversity(variation in genes and genotypes) using molecular markers offer numerous advantages over conventional phenotype based alternatives as they are stable and detectable in all tissues regardless of growth, differentiation, development, or defense status of the cell (Rao & Hodgkin, 2002; Dhutmal, et al., 2018).

Randomly amplified polymorphic DNA (RAPDs) is used to evaluate genetic diversity in *E. sativa* germplasm (Al-Qurainy, et al., 2010). It is simple, inexpensive, need no knowledge of the target sequence, and it's easy to apply for data analysis (Bahadur, et al., 2015). The selection of genotypes with a high genetic distance in terms of the molecular marker, along with desirable agronomic traits, can be effective in future breeding programs to produce new superior hybrids (Zafar-Pashanezhad, et al., 2020). It is a critical step in plant breeding programs for determining superior hybrid, thus this study aimed to evaluate genetic diversity among *E. sativa* cultivars, examining their antibacterial, antifungal and antioxidant activity and finally determination of seed oil constituents.

Random amplified polymorphic DNA (RAPD) is a PCR based technique for identifying genetic variation. It involves the using of a single arbitrary primer in a PCR reaction, resulting in the amplification of many discrete DNA products. This technique is developed independently by two different laboratories and called as RAPD and APPCR (Arbitrary primed PCR) respectively. No knowledge of the

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DNA sequence for the targeted gene is required, as the primers will bind somewhere in the sequence, but it is not certain exactly where (Williams, et al., 1990; Welsh & McClelland, 1990; Nandani & Thakur, 2014; Rahiman, et al., 2015). It is sensitive in determining the genetic relationships between species or individuals (Deshwal, et al., 2005). Genetic variation among cultivars is an important step in breeding programs to select suitable parent and getting superior hybrid, thus, this study aimed to detect variation among *E.sativa* cultivar at molecular level using RAPD markers.

2. Material and Methods

Seeds of four *Eruca sative* Linn L. cultivars (1-Turkish 2-Syrian 3- Turkish 4- Egyptian) were provided from local market ,seeds sowing was conducted at the orchid of agriculture division at the University of Kufa using plastic pots filled with beat moss to get fresh leaves for DNA extraction and application of RAPD markers. Seeds and leaves illustrates in Figure 1.

Fig. 1. Leaves and seeds of Eruca *sative* cultivars (1-Turkish 2-Syrian 3-Turkish 4- Egyptian

DNA Extraction

Fresh seedling leaves were used to take apical fresh leaves (four weeks age) for genomic DNA extraction using Genomic DNA Mini Kit provided from Geneaid Biotech.

Primers

Bioneer Corporation provided the Primers in lyophilized form, dissolved in TE buffer to obtain 100 pmol/µl as a final concentration (stock solutions). Working solutions 10 pmole/µl were prepared from stock solutions, eleven primers were used in application of RAPD markers (Carelli, et al., 2006; Abd El-Hady, et al., 2010; Ezekiel, et al., 2011; El-Assal & Gaber, 2012) in table (1) with their nucleotide sequences and names of each primer.

Table 1
Primers Used as RAPD Markers

No.	Primer name	Sequence 5' ⟶ 3'	Temperature
1	OPA-04	AATCGGGCTG	40 C°
2	OPA-10	GTGATCGCAG	40 C°
3	OPA-02	TGCCGAGCTG	40 C°
4	OPA-03	AGTCAGCCAC	40 C°
5	OPW-04	CAGAAGCGGA	40 C°
6	OPC-09	CTCACCGTCC	37 C°
7	OPA-01	CAGGCCCTTC	40 C°
8	OPX-03	TGGCGCAGTG	40 C°
9	OPX-17	GACACGGACC	40 C°
10	OPD-13	GGGGTGACGA	40 C°
11	OPA-14	TCTGTGCTGG	37 C°

PCR Content and Amplification Program

PCR Pre Mix master mix. Bioneer Corporation USA, (0.2ml) thin-wall 8-strip tubes with attached cup / 96 tubes were used,(*Top* DNA polymerase(1U), (dATP,dCTP,dGTP,dTTP)(Each 250 µM), Reaction Buffer with 1.5 mM Mgcl2(1X) and Stabilizer and tracking dye, 100 bp DNA ladder used. According to the Experimental Protocol of AccuPower® TLA PCR PreMix (at volume of 5 µl), the PCR reaction mixture was prepared as follows: 5µl template DNA and 5 µl of primer (10 pmole/µl), were added to each AccuPower® TLA PCR Pre Mix tube. Sterilized deionized distilled water was added to AccuPower® TLA PCR PreMix tubes to the final volume of 20 µl.

Performing PCR of samples: the amplified of each primer were done according to annealing temperatures and following program of initial temperature at 94C° for 3 min, 40 Cycles of (denaturation at 94C° for 1min , annealing :variable , extension at 72 C° for 1min and final extension at 72 C° for 5min .

Agarose Gel Electrophoresis

The gel electrophoresis methods were done according to Sambrook and Russel (2001) using 1.2% agarose at 70volt for two hours.

Statistical Analysis

The photographs resulted from agarose gel electrophoresis was used to score data, presence of a product was identified as (1) and absence was identified as (0), data then entered into PAST statistic vital program, Version 62.1 (Hammer, 2001) and analyzed using SIMQUAL (Similarity for Qualitative Data) routine to generate genetic similarity index: GS =2Nij (Ni+Nj) (Nei & Li, 1979). Nij is the number of bands in common between genotypes I and j, and Ni and Nj are the total number of bands observed for genotypes I and j, a dendrogram was constructed based on genetic distance (GD=1-GS) using the Unweighted Pair-

Group Method with Arithmetical Average (UPGMA). Polymorphism, primer efficiency, and discriminatory value were calculated for each primer using the following three

equations as described by Hunter and Gaston (1988) and by Graham and McNicol (1995).

3. Results and Discussion

Determination of concentration of isolated DNA was performed by using Bio drop apparatus. The concentration was 79.81µg/ml with purity 1.9, This accompanied by the locations of bands near wells and their intensity which shows their good quality and high molecular size. The result of electrophoresis of DNA samples using 0.9% agarose gel was shown in Figure (2) (Sambrook & Russell, 2001).

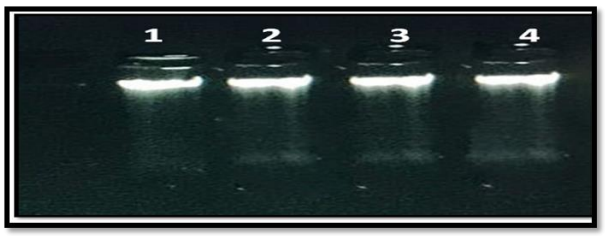

Fig. 2. Genomic DNA agarose gel electrophoresis for *Eruca sativa* cultivars 1-Turkish 2-Syrian 3- Turkish 4- Egyptian

DNA Fingerprint Detected by RAPD Markers

Results in table (2) showed that primers OPA-04, OPA-10, OPA-02, OPW-04, OPC-09,v and OPD-13 success in giving all cultivars a unique fingerprint while primer OPA-03 failed to give any cultivar a unique fingerprint. The presence of unique bands studied RAPD primers reflects their ability to recognize a unique annealing site on template. This affects primers ability to produce a unique DNA fingerprint for a particular *Eruca sativa* genotype.

Table 2
DNA fingerprint of for *Eruca sativa* cultivars 1-Turkish 2-Syrian 3- Turkish 4- Egyptian using RAPD markers

No.	Primer	Treatments	No. of fingerprint
1	OPA-04	1,2,3,4	4
2	OPA-10	1,2,3,4	4
3	OPA-02	1,2,3,4	4
4	OPA-03	non	0
5	OPW-04	1,2,3,4	4
6	OPC-09	1,2,3,4	4
7	OPA-01	3,4	2
8	OPX-03	4	1
9	OPX-17	1,2.3,4	4
10	OPD-13	1,2,3,4	4
11	OPA-14	3,4	2

Total RAPD Marker Analysis

Results in Table (3) illustrate that that higher molecular size was 2400 bp produced by primer OPW-04 while lowest molecular size was 130 bp produced in primer OPA-10. Variation in molecular size of amplified products may be concerned with mutation (insertions and deletions) which may change in annealing sites of primers and result in change in size of amplified fragments and products. Highest number of main bands, polymorphic bands and discriminatory value were 20, 18 and 20.22% respectively produced by OPA-02. Highest value for amplified band number and monomorphic bands were 44 and band produced by primers OPX-17 and OPD-13 respectively. Table (3) summarized the results of RAPDs amplification product include: Amplified bands molecular size range in bp; No. of main, amplified, monomorphic, polymorphic and unique bands; primer polymorphism (%), efficiency and discriminatory value (%).

Table 3
Molecular Size

Primers	Molecular size	Main bands	Amplified bands	Monomorphic band	Polymorphism		Efficiency	Discriminatory
					Polymorphic band	(%)		Value (%)
OPA-04	2069-227	17	33	1	16	94.11	0.48	17.97
OPA-10	1562-130	16	35	5	11	68.75	0.31	12.35
OPA-02	1676-176	20	40	2	18	90	0.45	20.22
OPA-03	772-168	8	30	7	1	12.5	0.03	1.12
OPW-04	2400-168	14	41	7	7	50	0.17	7.86
OPC-09	1393-186	13	27	3	10	76.92	0.37	11.23
OPA-01	1125-211	11	34	6	5	45.45	0.14	5.61
OPX-03	1125-362	8	23	5	3	37.5	0.13	3.37
OPX-17	1857-219	16	44	6	10	62.5	0.22	11.23
OPD-13	1423-148	12	40	8	4	33.33	0.1	4.49
OPA-14	1774-178	10	31	6	4	40	0.12	4.49
Total					89			

Changes in the distance between annealing sites of primers on template DNA, increase and decrease in number of main and amplified bands is directly related to the variation in number of annealing sites recognized by primers which dependent mainly on target DNA sequence in *Eruca*

sativa. Target DNA sequence mostly changed due to mutation that alters the primer-annealing site and affects the number of both main and amplified bands (Williams, et al., 1990).

Highest value for both polymorphism and Efficiency were 94.11% and 0.48 respectively produced by primer OPA-04. Efficiency of primer was related to its ability to produce high polymorphic bands (Hunter & Gaston, 1988; Graham & McNicol, 1995). Highest degree for polymorphism was 94.11% produced by primer OPA-04 ,this due to that primer when recognize a high number of annealing sites due to its structure ,this increase possibility of detecting DNA polymorphisms among individuals, polymorphism always related to increasing number of polymorphic bands (eleven from twelve were polymorphic in OPA-04) (Hunter & Gaston, 1988; Graham & McNicol, 1995). Thus, RAPD could be used for the detection of DNA alteration after the influence of mutagenic agent, since, formation of new variation that could be detected by changes in RAPD profiles.

Other primers gave lowest value for other studied criteria including main bands in primers OPA-03 and OPX-03, amplified bands in OPX-03, monomorphic bands in OPA-04, polymorphic bands, polymorphism, Efficiency and Discriminatory value in primer OPA-03. Agarose gel electrophoresis for PCR amplification product for all previous primers illustrated in figures (4) and (5).

Genetic Relationships

Results in table (4) showed that highest genetic distance was 0.65337 produced between 1-Turkish and 2-Syrian cultivars, while lowest highest genetic distance was 0.28811 produced between 2-Syrian and 3- Turkish cultivars.

Table 4
The genetic distance values among Eruca sativa cultivars 1-Turkish 2-Syrian 3-Turkish 4-Egyptian using RAPD markers

Cultivars	1-Turkish	2-Syrian	3- Turkish	4- Egyptian
1-Turkish	0			
2-Syrian	0.65337	0		
3- Turkish	0.462	0.28811	0	
4- Egyptian	0.6063	0.64423	0.462	0

Phylogenetic Tree

Genetic relationship drowen in figure (3) illustrate that E.sativa cultivars distributed between two main clusters, the first small one included only 1-Turkish cultivar, while other large one included three cultivars divided between two sub cluster ,the first small one included only 4- Egyptian cultivar ,while other large one included both 2-Syrian and 3-Turkish. Genetic distance and arrangement among *Eruca sativa* genotypes in phylogenetic tree were not concerned with genotypes pedigree and collection sites.

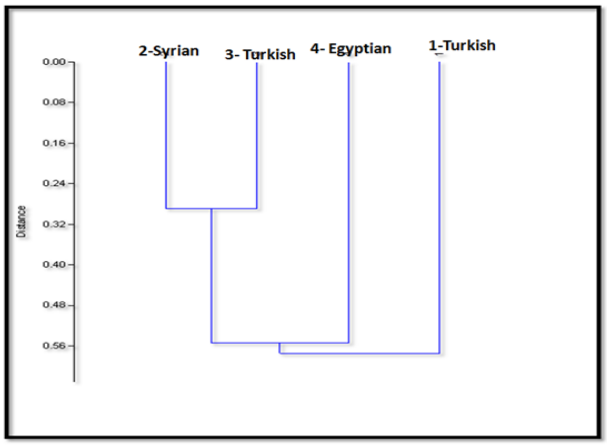

Fig. 3. UPGMA dendrogram illustrating the trees of genetic relationship between *Eruca sativa* cultivars 1-Turkish 2-Syrian 3- Turkish 4- Egyptian using RAPD markers

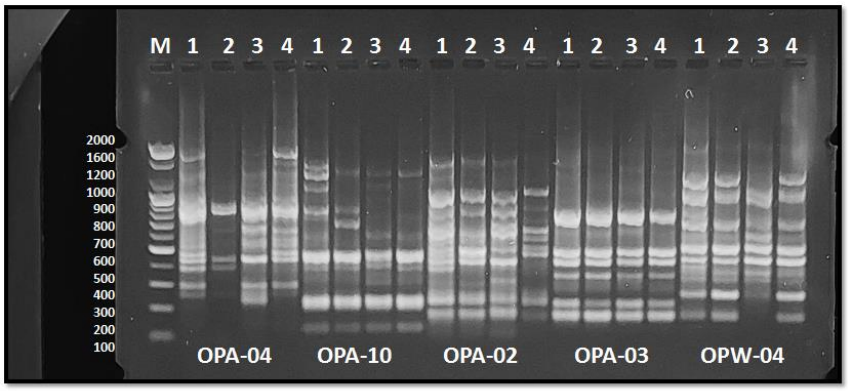

Fig. 4. Amplification product of primers OPA-04, OPA-10, OPA-02, OPA-03 and PW-04, M: DNA ladder, *Eruca sativa* cultivars 1-Turkish 2-Syrian 3- Turkish 4-Egyptian

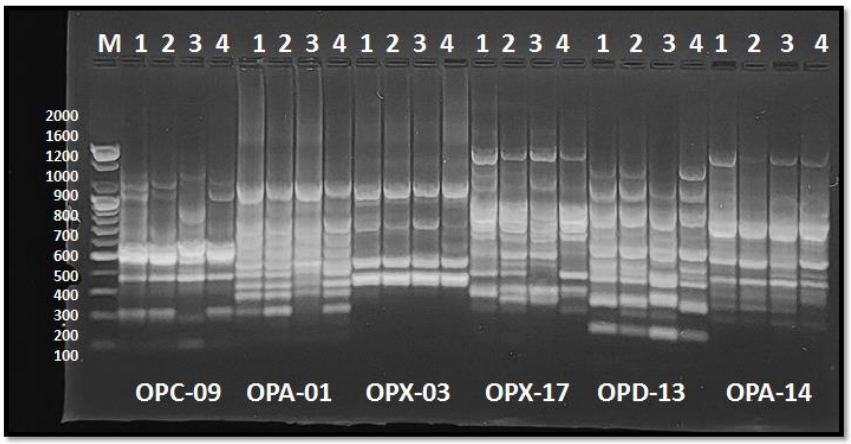

Fig. 5. Amplification product of primers OPX-17, OPD-13and OPA-14 for *Eruca sativa* cultivars 1-Turkish 2-Syrian 3- Turkish 4- Egyptian

4. Conclusion

From previous results, RAPD markers are an excellent tool in discrimination among *E.sativa* cultivars, drawing germplasm relationship, detect polymorphism and cultivar fingerprinting.

Competing Interests

The authors had no competing interests.

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