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RESEARCH ARTICLE

Analysis of Genetic Distance and Similarity in Some Radish Cultivars (*Raphanus sativus* L.) by Random Amplified Polymorphic DNA (RAPD) Markers

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Abstract

The random amplified polymorphic DNA (RAPD) assay is a useful method for to Detection Genetic Distance and Similarity among cultivars. In the present study, The DNA of the five Radish genotypes were screened with 5 primers generated reproducible and easily storable RAPD profiles with a number of amplified DNA fragments ranging from 9 to 12. The number of polymorphic ranged from 5 to 9 with an average reached 7.4 fragments / primers with the polymorphic percentage ranged from 41.66%to 90%. The number of monomorphic ranged from 0 to 5 and was total of the monomorphic 10 with an average reached 2 fragments / primers with the monomorphic percentage was 0% to 41.66%. A maximum genetic distance value was obtained in Local white radish and white radish Hybrid F1 reached 0.662. While a minimum genetic distance value was seen between Local red radish and Local white radish reached 0.458. The least genetic similarity was between the two cultivars Local red radish and Local white radish 40%. This study shows that the genetic diversity among radish cultivars can be used to improve plant growth, especially medical active compounds production in addition to increasing the plant yield and improve the quality.

Keywords: *Radish, Genetic Diversity, Molecular marker, RAPD.*

Introduction

Radish Raphanus sativus L. is an edible root vegetable plant from the family Brassicaceae, and follows Raphanus genus. The original home of radish plant in central and western China and India [1].Radish is important source for vitamins A and B, and minerals such as calcium, potassium, iron and phosphor, the edible part is the root, although the whole plant is edible, and it can be considered leafy vegetables [2].Radish also contain carbohydrates, sugars, Dietary fiber, proteins and some fat and fluoride [3].

Radish has many medicinal properties such as Increases appetite, prevents constipation, useful for patients those suffer from hemorrhoids, treatment liver problems, Splenomegaly, Jaundice, gallbladder and Urinary disorders [2]. There are many cultivars of radish in the world and these cultivars differ from each other in in shape, size and color of root [4], and also in root length, diameter and weight [5]. Radish was thought to be derived from hybridization between Raphanus maritimus and Raphanus landra and which exhibiting an RR genom of 468-662 Mb [6]. Raphanus sativus genomic research has not progressed as far as for members of the Brassica, possibly because the genus Raphanus is less species and less economically important. Several genetic maps of R. sativus have been constructed using RFLP, AFLP, and RAPD markers, and these have been applied to QTL identification of disease and pest resistance, the shape and pigmentation of roots, and the flowering time [6, 7, 8].

RAPD markers used because it is an effective technique in discrimination Rapahnus sativus L. from other brassicae [9] and from other species of the same genus [10,11] in the dentification and analysis of genetic variation in broccoli (Brassica oleracea L.), cauliflower (Brassica oleracea L. var. botrytis) and rice (Oryza sativa L.) [9, 12].To obtains genetic linkage maps, different types of molecular markers have been used such as random amplified polymorphic DNA (RAPD) markers. Random amplified polymorphic DNA (RAPD) so far is the most common marker because it is inexpensive, genomic

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DNA required is low, easy to do it, and the produces markers that represent the whole genome are highly polymorphic [13, 14].In order to detect Random amplified polymorphic DNA (RAPD) markers, polymerase chain reaction (PCR) can be used by the random amplification of genomic DNA fragments of different sizes [15]. To estimate the relatedness between different accessions and following inheritance of economically important characters, the development of molecular markers based on DNA sequences has provided an ideal means for identifying genotypes.

Materials and Methods

Plant Material

A total of five radish genotypes were collected from the agricultural offices in AL-Najaf, used in this study; White Radishes Hybrid F1, Syrian red radish, White icicle, Local red radish and Local white radish.

DNA Isolation

Total genomic DNA was isolated from fully expanded leaves using the Kit, leave samples (300 mg) were ground to a fine powder in liquid nitrogen. DNA was extracted by using Genomic DNA Mini Kit (Gene aid\UK). The extracted DNA (200 µl) was stored at -20°C until use .Concentration, quality and quantity of DNA were determined by Nano drop-spectrophotometric at 260 nm. The analysis was conducted in the laboratory of Molecular Genetic in the university of Baghdad, genetic engineering and biotechnology institution.

PCR Procedure

The RAPD primers (Table 1) were purchased from BIONEER\South Korea. A total of 6 decamer oligonucleotides of arbitrary sequence were tested for PCR amplification. Accup Power Gold Multiplex PCR premix (BIONEER \South Korea) was used to DNA amplification with RAPD primers and the thermal cycler conditions for PCR reactions were an initial denaturation cycle of 1 min and 30 s at 94 °C was followed by 45 cycles comprising 1 min at 94 °C, 1 min at 36 °C and 2 min at 72 °C. An additional cycle of 7 min at 72 °C was used for final extension.

DNA Electrophoresis

Amplification products were separated by electrophoresis (100 V) for (30 minutes) in 1.5% agarose gels and stained in ethidium bromide. A photographic record was taken under UV illumination.

Data Analysis

Only clear and repeatable application products were scored as 1 for present bands and 0 for absent ones. The specific bands useful for identifying species and cultivar were named with primer number followed by the approximate size of the amplified fragment in base pairs. Amplified products were analyzed by pairwise comparisons of the genotypes based on the percentage of common fragments, and a similarity matrix was calculated [16]. The 0 or 1data matrix was created and used to calculate the genetic distance and similarity using 'Simqual', a subprogram of the NTSYS-PC program (numerical taxonomy and multivariate analysis system program) [17]. A dendrogram was constructed based on the genetic distance matrix by applying an unweighted pair group method with arithmetic averages (UPGMA) cluster analysis using the MEGA (Molecular Evolutionary Genetics Analysis) version 2.0 [18].

Results

The results obtained in Table (1) show of isolated total DNA of the leaves of the studied radish cultivars manner filters and then migrated to agar gel 1.5 %, voltage 100 V for 30 minutes noting the success of the method to isolate DNA from this verity of radish.

Figure 1: Represents the isolated total DNA of the Radish leaves 1: White Radishes Hybrid F1 2: Syrian red radish 3: White icicle 4: Local red radish 5: Local white radish on agarose gel (1.5%) and voltage (100 V) for (30 minutes)

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Polymorphisms and Monomorphisms Detected by RAPAD Markers

In this study the RAPD technique was used to detecting of high levels of polymorphism (Figure 2).

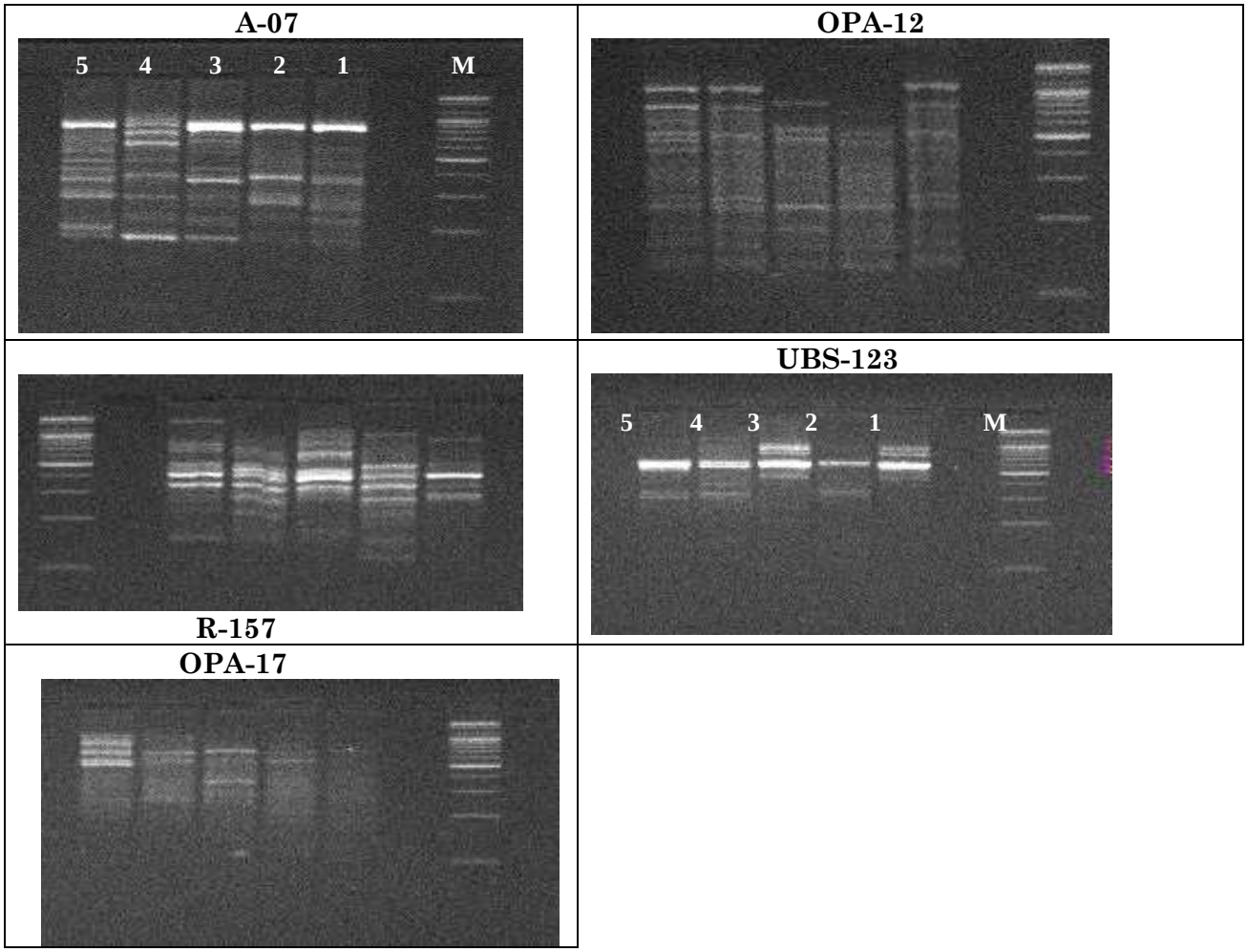

Figure 2: RAPD profiles of the Radish genotypes amplified with RAPD primers, M: molecular weight marker, radish cultivar: 1: White Radishes Hybrid F1. 2: Syrian red radish. 3: White icicle. 4: Local red radish. 5: Local white radish on agarose gel (1.5%) and voltage (100 V) for (30 minutes)

The DNA of the five Radish genotypes were screened with 5 primers generated reproducible and easily storable RAPD profiles with a number of amplified DNA fragments ranging from 9 to 12 (Table 1). The total numbers of fragments produced by 5 primers were 61 with an average of 12.2 fragments / primers. While the number of polymorphic ranged from 5 to 9 with an average reached 7.4 fragments / primers with the polymorphic percentage ranged from 41.66% to 90%. While the number of monomorphic ranged from 0 to 5 and was total of the monomorphic 10 with an average reached 2 fragments / primers with the monomorphic percentage was 0% to 41.66%. As shown in Table (1) a maximum numbers of amplicons was amplified with primer OPA-12 reached 15 while the minimum number of fragments was amplified with primers A-07 reached 10. The highest number of polymorphic bands reached 9 was obtained with primer A-07, while the highest number of monomorphic bands reached 5 was obtained with primer R-157.The highest number of Unique bands reached 5was obtained with primer UBC-123.

Table 1: Total number of amplicons, polymorphic, monomorphic amplicons, and percentage of monomorphism, polymorphism as revealed by RAPD markers among the five Radish cultivars

RAPD Primers	Primer sequences 5' to 3 '	Number of Total amplified fragments	Number of Unique Fragments Bands	Unique Fragments Bands Percentage (%)	Number of Polymorphic Fragments Bands	Polymorphism Fragments Percentage (%)	Number of Monomorphic Fragments Bands	Monomorphic Fragments Percentage (%)
A-07	GAAACGGGTG	10	1	10	9	90	0	0
OPA-12	TCGGCGATAG	15	3	20	8	53.33	4	26.66

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R-157	GTCGGTTCCT	12	2	16.66	5	41.66	5	41.66
UBC-123	GTCTTTCAGG	13	5	38.46	7	53.84	1	7.69
PA-17	GACCGCTTGT	11	3	27.27	8	72.72	0	0
TOTEL		61	14		37		10	
Average		12.2	2.8		7.4		2	

Radish Cultivars Fragments Numbers RAPD Markers

When the comparison was made among five Radish cultivars shown from RAPD marker data that high fragments number were obtained in Local white radish reached 34 , while the less fragments number was observed in Syrian red radish reached 21 fragments band (Table 2).

Table2: Radish cultivars fragments numbers RAPD markers

Genotype	Number of total fragment
White Radishes Hybrid F1	23
Syrian red radish	21
White icicle	31
Local red radish	28
Local white radish	34

Genetic Distance among Radish Cultivars

Data of RAPD markers scanned from five Radish cultivars with reproducible primers were used to genetic distance and similarity value co-efficient. A maximum genetic distance value was obtained in Local white radish and White Radishes Hybrid F1 reached 0.662.While a minimum genetic distance value was seen between Local red radish and Local white radish reached 0.458 (Table 3).

Table 3: genetic distance among Radish cultivars

	White Radishes Hybrid F1	Syrian red radish	White icicle	Local red radish	Local white radish
White Radishes Hybrid F1	**0**	0.592	0.561	0.577	0.662
Syrian red radish	0.592	**0**	0.592	0.512	0.606
White icicle	0.561	0.592	**0**	0.512	0.512
Local red radish	0.577	0.512	0.512	**0**	0.458
Local white radish	0.662	0.606	0.512	0.458	**0**

The dendrogram shows the radish as the items divided into two groups, the first group included the cultivar White Radishes Hybrid F1, while the second group included the remaining four cultivars, which were divided into two sub-groups (3 and 4). The sub group (3) included the cultivar Syrian red radish, while the sub group (4) was divided into two other groups, the first group included White icicle cultivar, while the second group included both cultivars Local red radish and Local white radish. The least genetic similarity was between the two cultivars Local red radish and Local white radish 40%.

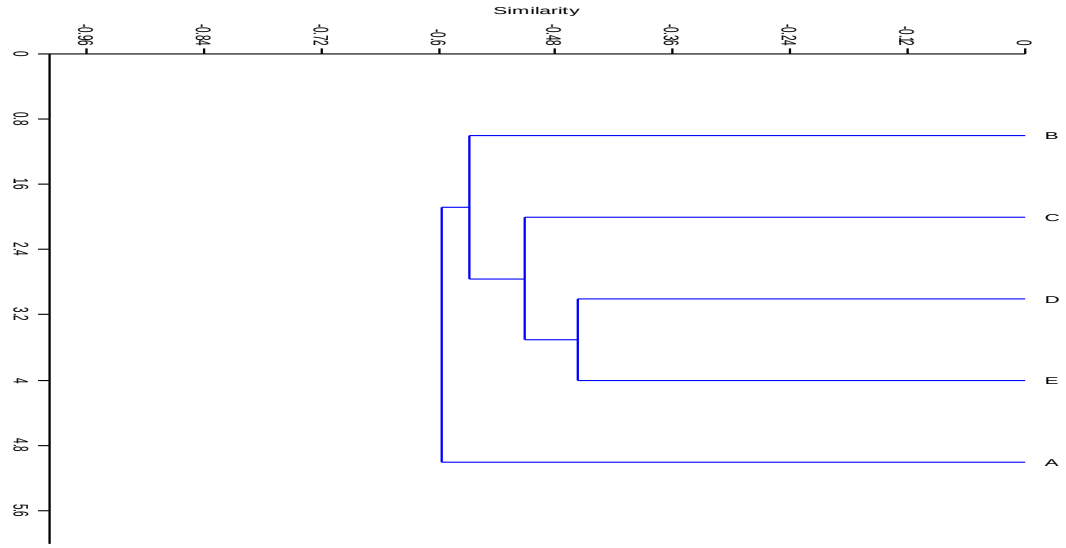

Figure 3: Dendrogram for the 5 Radish cultivars genotypes was pointed from RAPDs data using Unweighted Pair-group Arithmetic Average (UPGMA) and similarity matrices computed according to coefficients. Radish cultivars 1: White Radishes Hybrid F1 2: Syrian red radish 3: White icicle 4: Local red radish 5: Local white radish

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Discussion

There are many of molecular markers like random amplified polymorphic DNA (RAPD) used to estimate the genetic differences of radish [19, 20, 3, 21].RAPD markers used because it is an effective technique in discrimination *Rapahnus sativus* from other brassicae [22].This agree with [23], who explained that RAPD and ISSR biochemical and molecular markers are efficient to show differences among cultivars of the radish.

Where this technique has been used (RAPD) in many plant species greatly, for varieties analysis, population studies and genetic linkage mapping [15, 24, 25].

The results obtained were showed that their differences among the studied cultivars, where the high fragments number were obtained in Local white radish compared with the lowest fragments number was in Syrian red radish (Table 2), and high genetic distance was obtained in Local white radish and white radish Hybrid F1, while the low genetic distance was seen between Local red radish and Local white radish (Table 3), and by observing the dendrogram (Figure 3) shows the radish divided into two groups, the first group included the cultivar white radish Hybrid F1, while the second group included the remaining four cultivars.

The genetic convergence or divergence among studied cultivars may be due to many reasons: origin home, different environmental conditions and breeding or hybridization for these cultivars.

This was observed between the two local varieties (Local red radish and Local white radish), where genetic distance between them was low, as a result of similar environmental growth conditions, compare with high genetic distance was obtained between Local white radish and white radishes Hybrid F1. This agree with [26, 28] through his study on 56 radish accessions by using RAPD and AFLP, where he found the diversity may due to the different in regions and countries to which the radish belong that can be a high diversity among them, or as observe [29].

That the out-crossing and open-pollination system used by radish might also help to conserve genetic diversity, and the variation of cultivated plant species depend on mutation and hybridization, range of spread, agricultural processes and domestication [30, 31] or depends on other different factors, such as ecological, geographical, breeding system & anthropogenic effects [32, 33].

Explained that a large portion of the genetic variation among plant species due to the differences in their life history and ecological characteristics. This agree with [11] find a high genetic variation by his studying the diversity of 30 radishes in Pakistan, and compatible with [34], which found that there were differences in genetic distance between tomato (*Lycopersicon esculentum* Mill.) cultivars studied, and also agree with [35, 37] through his study on twenty three genotypes of radish, where he found highly significant differences among genotypes for all the traits studied.

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