Detection of the Genetic Diversity and the DNA of Some Species of the Cruciferous Family (Brassicaceae) using the RAPD-PCR Markers

Qusay Ahmed Olaij[1], Abdul Majeed Abdul Aziz Humadi[2]

[1]Post graduate, [2]Assistant Professor, University of Anbar/ Education College for Pure Sciences /Department of Biology/Iraq

Abstract

The current study aimed to uncover the genetic diversity of eight local species of the Brassicaceae plants by using RAPD markers, where the genetic relationship was determined, the genetic dimension was found between the studied species and the genetic finger print of some of these species was identified. (DNA) was extracted from the young leaves of the cruciferous family plants using the method Ready (Kit). The concentration of the extracted DNA ranged from 148.35-165.3 ng /µl with a purity of 1.53- 1.82. The results obtained from the study showed that 307 band were all differentiated (100%), higher in number than bands (38) with (12.38%) in the primer (MQ10), lower in number than bands (21) and with (6.84%) in the primer (MQ6). The highest molecular volume (pb2500) was characterized by (B1) in the primer (MQ7), while the less Molecular volume (100bp) in the two species (B3 and B5) was distinguished by the primer (MQ8). The results of the genetic dimension showed that the lowest value is for the genetic dimension (0.260) appeared between the two species (B2 and B3), and the highest value of the genetic dimension was (0.975) appeared between the two types (B4 and B5). The phylogenetic tree was drawn between the studied species and the species were divided into two major groups. The first group included the species (B1, B2, B3 and B4) and the second group included the species (B5, B6, B7 and B8).

Key words: *Genetic diversity, Brassicaceae, RAPD-PCR, Cauliflower*

Introduction

The Brassicaceae family is one of the important and large botanical families and is widespread around the world, this family includes about 338 genera and more than 3700 species and 50 tribes, most of the plants of this family are spread in the cool and temperate regions of the Northern Hemisphere(1) . The spread of cruciferous family plants differs from one region to another, as this family includes in Iraq 117 species spread over 83 genera of which 18 are cultivated species of economic importance and 75 wild species and some of these species have medicinal importance and the other section represents ornamental plants(2).

Corresponding author:
Qusay Ahmed Olaij
E-mail: qusayolaij86@gmail.com

Molecular indicators have developed a wide range of applications in the field of molecular biology including genetics, environmental evolution and the study of complex genomic features in plants(3). The distinction between plant varieties has become highly dependent on the technology of polymerase Chains Reaction (PCR), the most prominent and most common of which are RAPD indicators (ISSR, DAF, AFLP and SSR) to distinguish between plant species(4), it is necessary to carry out monitoring and evaluation for Genetic variation as a basis for the conservation and breeding of plant species(5). RAPD markers focused on using single random primers consisting of ten nitrogenous bases paired with complementary sequences on the genome with the presence of DNA Polymerase to work to duplicate the DNA of the genome in some areas of the DNA to show the genetic variation between plant species through the presence or absence of these areas and their

sizes and the number of copies[6]. These indicators are characterized as low cost, easy to use, and have a wide range of applications in many plant studies, for example used in early discrimination Among the plant species, finding the genetic finger print[7]. In view of the lack of extensive studies on the plant of the Brassicaceae family in Iraq using molecular indicators, this study was conducted which aims to uncover the genetic diversity between the DNA of the Brassicaceae family species cultivated in Iraq and to know the genetic dimension between the studied species, as well as determine the genetic fingerprint of some of these species.

Materials and Methods of Work

Plant samples, DNA isolation and PCR interactions

Eight types of Brassicaceae family plants used in the Ramadi district, Anbar, Iraq, (Table 1) were used in this study. DNA isolation of young leaves of plant species studied in a manner reported by (IBI) using a ready kit manufactured by IBI (USA) (www.ibisci.com). DNA samples using the electrophoresis device on the agarose gel revealed a 0.8% concentration for 45 minutes with a difference of 100 mA. In this study, ten prefabricated RAPD prefixes from Korea (IDT) company (Integrated DNA Technologies) (Table 2) were used. The concentration of DNA in all samples studied to conduct RAPD reactions was (50) ng/µl. Prepared the main reaction mixture by mixing the reaction components in a PreMix PCR tube equipped by Bioneer (Korea). DNA was amplified by PCR technology, according to it[8]. Through a primary mutant cycle at 95 ° C for 5 minutes, followed by 35 sessions at 95 ° C. Each session includes one minute of double DNA mutant and 30 seconds at a temperature of (37-50) ° C according to each initiator (Table 2). Then 30 seconds using a temperature of 72 ° C. to elongate the initiator, then a 5-minute final session using 72 ° C. to complete the elongation stage, withdraw 10 µl from the PreMix PCR tube, load the mixture into the pre-prepared agarose gel concentration and concentration 1.2% With DNA Ladder 100bp. The samples were electrically carried over using the Electrophoresis apparatus and at a difference of 100 mA for a period of 40-65 minutes (Table 2). The agarose gel was displayed after electrophoresis on a UV-transilluminator and photographed using a high-resolution digital camera.

Table (1) the species of the Brassicaceae family included in the study.

code	Sample Scientific name
B1	Brassica juncea (Indian Mustard)
B2	Brassica oleracea Var. botrytis
B3	Brassica oleracea Ver. Capitata
B4	Brassica rapa var. rapa
B5	Eruca sativa
B6	Lepidium sativum (cross)
B7	Raphanus sativus (White Radish)
B8	Raphanus sativus (Red Radish)

Table (2) RAPD marker primers used in the study, temperature of each primer and duration of relay for each primer.

NO.	Primer	3 → 5 primer sequence	Tm ˚C	Time Electrophoresis
1	MQ1	GTGAGGCGTC	50˚C	45 min.
2	MQ2	TGGACCGGTG	50˚C	45 min.
3	MQ3	TGCGTGCTTG	37˚C	50 min.
4	MQ4	GTAGACCCGT	37˚C	50 min.
5	MQ5	CCACAGCAGT	37˚C	65 min.
6	MQ6	TCGGCGATAG	48˚C	55 min.
7	MQ7	AGCCAGCGAA	48˚C	40 min.
8	MQ8	AGGTGACCGT	48˚C	45 min.
9	MQ9	TGCGGTGAAC	37˚C	60 min.
10	MQ10	GAGCAGACAC	37˚C	60 min.

Data Analysis

The results of the interactions of the RAPD primers were taken separately in tables based on a comparison of the presence or absence of DNA segments of the various samples as the presence of the DNA bundle is denoted by the number (1) and its absence by the number (0). Genetic dimension coefficient and similarity coefficient between studied species were calculated using Nei coefficient[9]. Conduct Cluster analysis and plot the genetic dimension using the UPGMA method[10]. Statistical analyzes were conducted using the software using the program (NTSYSpc-2.02i)[11]. On the Nei equation[12].

Results and Discussion

Genomic DNA Extraction

DNA was extracted from the young leaves of the Brassicaceae plant species according to the ready-made kit method and carried on a 0.8% agarose gel using the electrophoresis device. Its purity for the studied species ranged between (1.53 - 1.82), and its concentration ranged between (148.35 - 165.3) ng/µl.

Results of RAPD interactions

In this study, ten primers RAPD manufactured by (IDT) Korea were used. Results of their replication with DNA of the studied species showed a difference in the number of replicated sites and their molecular sizes. All primers used showed results with DNA of the studied plant samples, where the results of the replication were appropriate for detection. On genetic relations, as well as creating a genetic finger print and knowledge of the genetic dimension between some cultured plant species (Figure 1). The results of the RAPD 307 band, all of which are varied (100%) that the process of finding genetic variation between species depends on these bands, indicated[13] from the foundations of finding genetic variation is the ratio of varied bands to the total number of correlation sites. The highest primer in terms of the number of correlation sites was (MQ10) with (38) band, while the lowest primer in terms of the number of correlation sites was (MQ6) with (21) band, the

highest molecular size in correlation sites was (2500bp) in the primer (MQ7) and the lowest molecular size it reached (100bp) in the primer (MQ8), the primer (MQ6) recorded the highest number of unique bands sites (7) sites and the primer (MQ10) showed the lowest number of unique bands sites a unique one, the primer (MQ10) showed the highest number of link sites for absent bands for two link sites, while primers (MQ1, MQ2, MQ3, MQ4, MQ5, MQ6, MQ8 and MQ9) did not show results for the multiplication of any link sites for absent bands. The reasons for the discrepancy between the types of Brassicaceae family plants or any other type of plant may be nucleotide substitution within one or both of my sites. The primer correlation, which leads to the presence or absence of variation or a change in the size of the duplicate segment. Thus, the results of the primers used showed different bands in number, location and size and among those bands there are what are known as unique bands and absent bands that have a role in determining the genetic fingerprint of a particular type without the rest of the species included in the study, that the presence of these bands between species reduces the cost and effort because it gives results Distinguished in record time, this is stated by[14] that unique or absent bands distinguish the species in which they appeared from other types.

When studying[15] the eight wild plant species belonging to the Brassicaceae family using (14) primer of the RAPD index, indicated the emergence of (113) band whose molecular sizes ranged between (250bp - 2800bp) and divided the plant species in this study depending on the bands produced by Multiply to two major groups. Also indicated[16] a study on (12) species of the family Brassicaceae using some RAPD index prefixes in determining the genotype and detecting the DNA, it was obtained (241) bands of which (62) Multiforme band (26%). When studying[17] genetic variation and genetic analysis of strains also indicated (13) groups of 6 species belonging to the genus Conringia prevalent in Turkey using seven RAPD primers. A total of (34) bands were detected, of which (30) bands were polymorphic, with (88%).

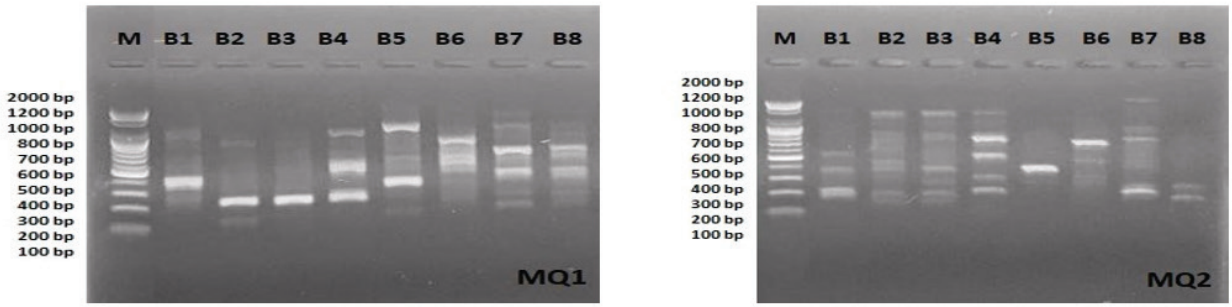

Figure (1) Electrophoresis of PCR reaction products for primers studied by RAPD Markers for Brassicaceae family types studied on agarose gel with a concentration of 1.2% and a difference of 100 mA for a period of 40-65 minutes, M represents the DNA Ladder 100bp, for (MQ1 and MQ2) (B1) *Brassica juncea*, (B2) *Brassica botrytis*, (B3) *Brassica capitata*, (B4) *Brassica rapa*, (B5) *Eruca sativa*, (B6 *Lepidium sativum*, (B7) *Raphanus sativus* (White Radish), (B8) *Raphanus sativus* (Red Radish).

Estimating the genetic dimension based on the results of RAPD

The genetic dimension between the Brassicaceae family types was estimated. Based on the results of the RAPD Markers, the genetic program (NTSYSpc-2.02i). Was used based on the presence of co-bundles between each of the studied species. (Table 3) shows the genetic dimensions between the studied Brassicaceae family types, which ranged between (0.260 - 0.975), when the value of the genetic dimension between two types is equal to zero indicating that there is no difference between the two types and the DNA between them is identical and the genetic similarity is 100%[18].

Table (3) shows the genetic dimension values among the Brassicaceae family according to the results of RAPD.

RAPD	B1	B2	B3	B4	B5	B6	B7	B8
B1	0.000							
B2	0.722	0.000						
B3	0.701	0.260	0.000					
B4	0.657	0.722	0.742	0.000				
B5	0.661	0.826	0.672	0.975	0.000			
B6	0.742	0.734	0.755	0.702	0.755	0.000		
B7	0.761	0.753	0.819	0.684	0.578	0.693	0.000	
B8	0.839	0.663	0.725	0.672	0.603	0.569	0.283	0.000

The results showed that the lowest value of the genetic dimension (0.260) between the two species (B2 and B3), this indicates that they have the largest proportion of similarity in the genetic material (DNA) based on the primers used in this study, and this means the lowest percentage difference between the genome of the two types compared to the types The other, pointed[19] to the reason for the convergence between the two species due to the fact that they are close in proportions and evolutionary history in addition to having the same chromosome group and the differences are minimal in the genome of the two species. The results indicate that the highest genetic dimension was between the two species (B4 and B5) was (0.975). This indicates the presence of the lowest proportion of symmetry in the genetic material (DNA) between these two types, as they participated with the lowest number of bands in relation to other types. This distinction is a result of their great contrast in appearance and physiology.

The genetic dimension values of the studied Brassicaceae family species have been invested in finding the genetic relationship between these species in the form of groups (Figure 2). These species were divided into two main groups:

• First main group

This group consisted of two subgroups, the first of which included the two types (B1 and B4), and the second was represented by the two types (B2 and B3). This first and second group formed as a result of their participation in the largest number of linking sites, and the lowest number of linking sites with the rest of the species (Figure 2).

• Second main group

This group included three secondary groups that included the first group (B6), while (type B5) was in the second group, while the third group included the two types (B7 and B8) which are the closest genetically in this group, where they acquired the largest number From the general bands between them, (Figure 2).

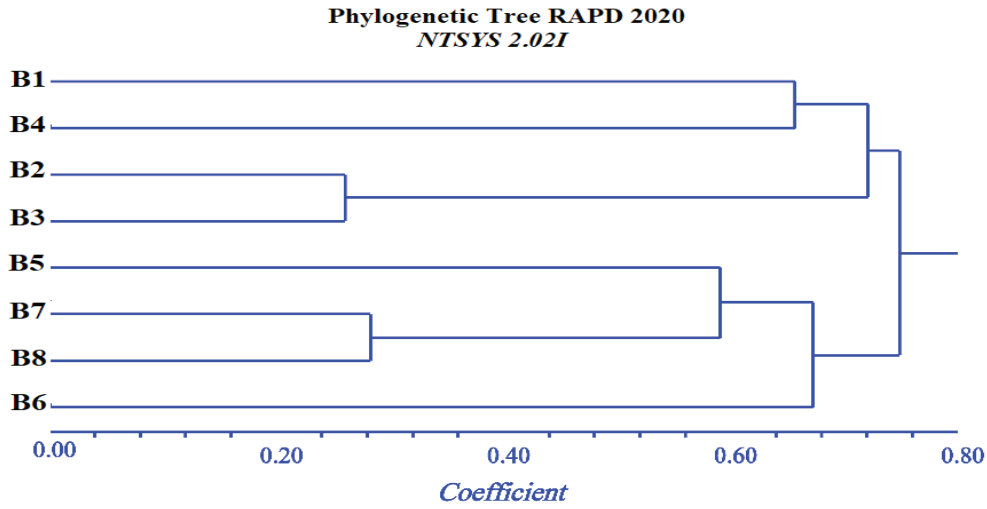

Figure (2) Genetic Relationship between the Brassicaceae Family Types According to RAPD Markers.

Conclusion

The results of this study showed 307 band, all of which are differentiated (100%). The primer (MQ10) showed the highest number of bands (38) and (12.38%) and the lowest number of bands (21) and (6.84%) in the primer (MQ6). The two types (B1 and B4) had the highest number of packages reached (42) packages with a percentage (13.68%) and the lowest number of bands (35) band with (11.40%) in the types (B3 and B5), the results of the genetic dimension showed that the lowest value of the genetic dimension was (0.260) appeared between the two types (B2 and B3), and the highest value for the genetic dimension was (0.975) appeared between the two types (B4 and B5).

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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