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A Comparative of Molecular Characteristics of some Genera of the Brassiceae Tribe (Brassicaceae) Growing in Northern Iraq

M I AAl-Hat Hout, and N M M Al-Abide

Department of Biology, College of Education for Pure Sciences, Tikrit University, Salahuddin, Iraq

Corresponding Author Email: naglaa.mustafa@tu.edu.iq

Abstract. The current study dealt with the study of species of the Brassicaceae family, and the study showed (12) species belonging to (11) genera belonging to the Brassiceae tribe in northern of Iraq, which are the species: *Aethionema cordifolium* L. *Capsella bursa-pastoris* L. *Erucaria macrocarpa* L. *Erysimum repandum* L. *Hirschfeldia incana* L. *Isatis tinctoria* L. *Neslia paniculata* L. *Rapistrum rugosum* L. *Rhamphospermum arvense* L. *Sisymbrium irio* L. *Sisymbrium orientale* L. *Strigosellea Africana* L. Fresh leaves were collected on 1/3/2024_30/3/2024 from various areas of northern of Iraq: Sulaymaniyah: Bazian, Jabal Azmar, Sharbaghir, and Penjwen, Erbil: mountain of Safin, Rawanduz, and Haj Omran, Duhok: Rovia, Akre , and Gale Zanta. The principles used in this study for the SSR technique are: Mstg 008, Mstg 012, Mstg 013, Mstg 025, Mstg 027, Mstg 028, Mstg 034, Mstg 039, Mstg 052, and Mstg 055. As a result, the study identifies 10 principles used in the actual study of the SSR index on expression methods. The total number of these methods was 212 bands, across 79 sites, including 209 heterogeneous bands and 3 heterogeneous bands. The Belgian SSR index discrimination score recorded by Mstg027 was 16.666, while the Mstg028 principle recorded the lowest discrimination score of 1.754. However, the highest dynamic ratio of the characteristics was recorded by Mstg027, at 16.239%, and the lowest dynamic ratio was recorded by Mstg028, at 1.909. The distinction between *Erysimum repandum* L. and *Hirschfeldia incana* L. was not yet clear (0.20851), while the most complete distinction between *Rhamphospermum arvense* L. and *Sisymbrium orientale* L. was recorded (0.65938).

1. Introduction

The Brassicaceae family, previously known as Cruciferae, includes a wide range of plants commonly referred to as the mustard or cabbage family. It is globally widespread and diverse. The former name "Cruciferae" originates from the cross-shaped arrangement of the flower's four petals and four sepals. One of the keys to identifying features of this family is the number and relative length of the stamens. The pistil typically has a single ovary, which is divided by a false septum. The fruit is either a slender silique or a rounded silicle, and it contains seeds in two compartments separated by the false septum. Each side of the fruit is enclosed by a valve. The leaves are generally simple and alternate, often covered with unbranched or forked hair, as well as unicellular stellate hair [1]. The Brassicaceae (formerly Cruciferae) family is a large family, comprising 328 genera and 3,709 species [2]. most of which are found in temperate regions of the world. The mustard family gains its importance from the fact that it includes economic species as food sources, such as mustard (*Brassica nigra*) and turnip (*Brassica rapa*), and



spices, in addition to the use of some of them as ornamental plants [3]. This family has characteristics that other plant families lack, the most important of which is mustard fruit and mustard [4]. This family has received great attention from researchers worldwide, including the study by [5]. on the anatomical and morphological characteristics of pollen grains of the species *Alyssum obtusifolium* Stev. In Iraq, it has been studied by many researchers, including the study by [6]. on the genus *Brassica* L., the study by [7]. on the morphology and anatomical characteristics of certain species of the Brassicaceae genera in Iraq, the study by [8]. on the anatomical and chemical characteristics of different species of the Brassicaceae family in Anbar Governorate, and the study by [9]. on the characteristics of the anatomical and chemical characteristics of fourteen wild-growing Brassicaceae species in Diyala Governorate. The comparative molecular and biochemical studies of two Brassicaceae genera by [10]. and the morphological and anatomical studies of *Raphanus* and *Lepidium* by [11]. were conducted. The most recent study, by [12]. investigated the molecular characterization of the radish plant *Raphanus sativus* cultivated in Iraq and its relationship to the concentrations of some active ingredients. Genetic studies play an important role in plant classification by examining the genetic similarities and differences between genera and species, as well as identifying distinct varieties of species [13]. A review of the literature and research revealed that several studies have focused on morphological and anatomical features [14,15,16,17,18]. Therefore, the current study aimed to:

1. Determine the genetic distance between the species under study by using molecular markers using PCR and SSR techniques to separate species genetically through the genetic tree.
2. Identify genetic differences between plant species, which helps understand genetic diversity within or between groups and determine the genetic origin or relationship between species.

2. Material and Methods

2.1 Molecular Study

2.2 Collection of specimens

specimens were collected through field trips, which totaled (3) trips, confined to the northern regions of Iraq: Sulaymaniyah (Bazian, Mountain of Azmar, Sharbaghir, Penjwen), Dohuk (Rovia, Akrae, Gale Zanta), and Erbil (Mountain of Safin, Rawanduz, Haji Omran). The aim was to obtain fresh samples for use in molecular studies. Leaf samples were collected from the middle of the plant, and all plants were mature in the flowering and fruiting stages. Fresh samples were preserved, and important information was recorded on a special identification (label), which included the species name, collection location, collection date, collector's name, in addition to other environmental observations.

2.3 Procedure Genomic DNA Isolation

DNA was isolated from the plant's fresh leaves using CTAB and the method mentioned by [19]. and based on the principles by [20].

2.4 DNA extraction

Genomic DNA was isolated from fresh plant vegetative parts (leaves) and some dry samples using the CTAB method described by [21]. The extraction process was carried out according to the genome protocol (Promega, kits). The kit prepared by Gene aid was used for DNA extraction.

2.5 Agarose of DNA Gel Electrophoresis

1- Prepare agarose gel at a concentration of 1% to transfer the results of DNA extraction and detection, and at a concentration of 2% to transfer the results of PCR reactions, as it is an appropriate concentration for the size of the DNA fragment for the genes studied.

2- Dissolve 0.5 grams of agarose powder in 50 ml of SB 1X solution to obtain agarose gel at a concentration of 1%, and dissolve 1 gram of agarose powder in 50 ml of SB 1X solution to obtain agarose gel at a concentration of 2% using a Microwave device with circular stirring until it is ensured that there are no undissolved particles and leave to cool slightly.

3- Add 3 microliters of safe red dye Red save 50 ml of agarose with gentle and circular stirring to mix the dye with the agarose gel and leave until the temperature reaches between 60-50 degrees Celsius.

4- Prepare the agarose gel casting mold, ensuring it is properly balanced. Place the combs to form wells for loading the samples. Carefully pour the agarose into the mold to avoid the formation of air bubbles and allow it to set completely for approximately 20 minutes.

5. Once the gel has solidified, carefully remove the plastic frames from the mold. Then, place the mold containing the gel into the electrophoresis tank filled with 1X SB buffer. Gently lift the combs, ensuring the agarose gel is fully submerged in the 1X SB solution.

6- Mix 2 microliters of the loading buffer with 8 microliters of the DNA sample, The PCR reaction product is transferred directly by taking 5 microliters and carefully transferring to the well holes then connect the electrophoresis tank to the power supply. After the transfer time is over, the gel is examined using ultraviolet rays to see and estimate the DNA bands.

7- After migration time has ended, the gel is examined using ultraviolet light to see and estimate the DNA bands [22].

2.6 Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction PCR technique was used to detect genetic variation among plant species using the primers shown in the following table 1.

Table 1. Atrial natriuretic peptide gene sequences.

No.	SR Marker	Primer Sequences	Bases No.	Annealing
1	Mstg 008	F- TCT. CTT. TGA. AAT. CTC. AAC. CCA. R- AGA. TGG. CAT. GTT. AAA. CTG. AAC.	21 21	47 C
2	Mstg 012	F- TGA. TAC. ATA. GAC. TTG. GTG. GTG. R- CGG. CAT. TAT. CTT. GAA. CAC. GTT.	21 21	48 C
3	Mstg 013	F- AGA. TTT. GGC. TTA. CAC. GAC. GAC. R- ATA. TAC. CAG. GTA. CCG. TCA. CTC.	21 21	50 C
4	Mstg 025	F- AGA. GGC. AGT. TAC. GTT. CAC. GTC. R- CAT. CGC. ACT. CGT. GCT. TCT. TTC.	21 21	52 C
5	Mstg 027	F- CTC. TTT. TGG. TCA. GCT. TCC. TCA. R- TTG. TTA. GTT. AGA. TCC. TCG. TAG.	21 21	48 C
6	Mstg 028	F- GCC. AAG. AAG. ACG. AAG. ATT. CTC. R- AGG. TTC. TCG. ATT. TAG. GAA. CCG.	21 21	49 C
7	Mstg 034	F- CGA. CTG. GTA. ATA. TTC. TGA. CTC. R- CAT. GAA. AGA. CTC. TCA. AAT. CCC.	21 21	46 C
8	Mstg 039	F- GAA. TGG. TGG. TTC. TTG. TGT. GTC. R-GAC. AAT. CTT. GGA. GTC. ATC. TCG.	21 21	50 C
9	Mstg 052	F: AGT. AAC. ATG. TTT. TCT. TTT. GTG. R CAT. CAG. ATG. CTC. AAG. GAA. CTT.	21 21	46 C
10	Mstg 055	F- ACA. CGC. GCC. TAT. GCA. GAA. TAC. R- CTT. AGC. GAT. TAC. GGT. GAA. GCC.	21 21	52 C
Total			420 bases	

2.7 polymerase chain reaction

1- Primers were prepared from the Korean manufacturer Macro gene in powder form at a concentration of 100 picomoles/microliter. The primers were dissolved by adding 300 microliters of deionized water (Nuclease Free Water) according to the instructions of the manufacturer.

2- By mixing (10 microliters) of the stock solution with (90 microliters) of deionized water the primer was diluted to a concentration of 10 picomoles/microliter.

- 3- Transfer the 2 microliters of primer for each sample (10 microliters for each sample) to a clean, sterile tube.
- 4- Add 10 microliters of the reaction mixture prepared by the company (50 microliters for each sample) to the same tube containing the primer.
- 5- Add 3 microliters of deionized water to the tube containing the reaction mixture and mix well.
- 6- Distribute the mixture into new sterile tubes of 0.2 ml volume at a rate of 15 microliters for each sample.
- 7- Finally add 5 microliters of DNA sample and mix all the components well.
- 8- Spin the samples "briefly" in the microcentrifuge.
- 9- Place the reaction tubes in the Thermocycler using the following program.
- 10- Remove the samples from the container polymerize and perform electrophoresis of the PCR product at a concentration of 1.5% and visualize the results. Table 2. [23].

Table 2. Thermal polymerization program for the atrial natriuretic peptide gene.

No.	Phase	°C	Time	No. of cycle
1	Initial Denaturation	95 °C	4 min	1 cycle
2	Denaturation -2	95 °C	30 sec.	
3	Annealing	59 °C	30 sec.	35 cycles
4	Extension-1	72 °C	45 sec.	
5	Extension -2	72 °C	4 min	1 cycle

2.8 Statistical Analysis

The outcomes of the PCR amplifications using SSR primers were organized into tables that recorded the presence (denoted by 1) or absence (denoted by 0) of DNA fragments across various samples. Based on this binary data, both genetic similarity and genetic distance coefficients were computed among the species studied. A cluster analysis was then carried out using the unweighted pair group method with arithmetic mean (UPGMA), following the approach of [24]. to construct a dendrogram illustrating genetic relationships. All statistical procedures were performed using the NTSYS-PC software (version 2.02e). This software generated a matrix summarizing the banding patterns for all primers and samples, and genetic similarity was determined using the formula: $GD = 1 - [2 \times (N_{ij} / (N_i + N_j))]$, where N_{ij} represents the number of shared bands between sample i and sample j . Additionally, key parameters such as polymorphism rate, primer efficiency, and discriminatory power were calculated using the following formulas:

- Polymorphism (%) = (Number of polymorphic bands per primer / Total number of bands per primer) $\times$ 100
- Primer Efficiency (%) = (Total number of bands per primer / Total number of bands across all primers) $\times$ 100
- Discriminatory Power (%) = (Number of polymorphic bands per primer / Total number of polymorphic bands for all primers) $\times$ 100 [25].

3. Results and Discussion

3.1 Molecular Study

By mixing 3 microliters of DNA sample with 2 microliters of loading buffer as shown in (Figure 1), DNA bands were found on agarose gels of the species that were being studied and plant leaves.

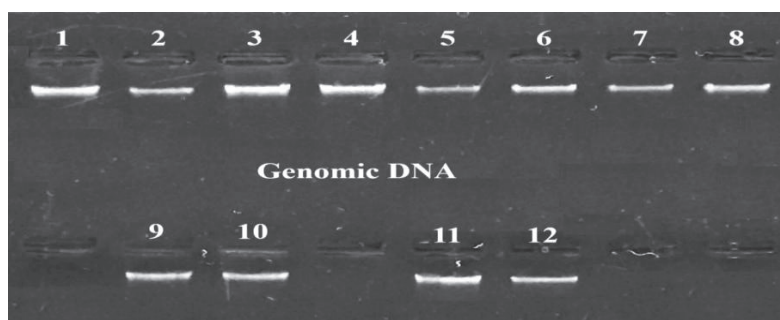


Fig 1. DNA bands in the agarose gel phase of the studied species.

1-Aethionema cordifolium L. 2-Capsella bursa-postoris L. 3-Erucaria macrocarpa L. 4-Erysimum repandum L. 5- Hirschfeldia incana L. 6-Isatis tinctoria L. 7-Neslia paniculata L. 8-Rapistrum rugosum L. 9-Rhamphospermum arvense L. 10-Sisymbrium irio L. 11-Sisymbrium orientale L. 12-Strigosellea Africana L.

3.2 Purity and concentration of DNA extracted from the studied species

The purity of DNA extracted from the studied species ranged between 1.72 and 1.94 ng/μL when using fresh plant samples. Additionally, the DNA concentration was found to range from 51 to 92 ng/μL. The extraction was performed using a commercial kit produced by the Korean company FAVORGEN BIOTECH CORP, and all procedures were carried out in the scientific research laboratory. This method yielded satisfactory results, demonstrating the effectiveness of the extraction protocol, as presented in Table 3.

Table 3. DNA purity and concentration

NO.	Species	Purity	Concentration
1	<i>Aethionema cordifolium L.</i>	1.76 ng/μL	92 ng/μL
2	<i>Capsella bursa-postoris L.</i>	1.87 ng/μL	55 ng/μL
3	<i>Erucaria microcarpa Boiss</i>	1.72 ng/μL	84 ng/μL
4	<i>Erysimum repandum L.</i>	1.83 ng/μL	89 ng/μL
5	<i>Hirschfeldia incana L.</i>	1.90 ng/μL	51 ng/μL
6	<i>Isatis tinctoria L.</i>	1.79 ng/μL	63 ng/μL
7	<i>Neslia paniculata L.</i>	1.92 ng/μL	59 ng/μL
8	<i>Rapistrum rugosum L.</i>	1.84 ng/μL	71 ng/μL
9	<i>Rhamphospermum arvense L.</i>	1.77 ng/μL	60 ng/μL
10	<i>Sisymbrium irio L.</i>	1.94 ng/μL	58 ng/μL
11	<i>Sisymbrium orientale L.</i>	1.87 ng/μL	56 ng/μL
12	<i>Strigosellea africana L.</i>	1.85 ng/μL	62 ng/μL

3.3 Polymerase chain reaction for SSR-PCR:

In the present study, ten SSR marker primers were employed. All primers produced positive amplification results, and the transcription process was successfully carried out across all examined species. Each primer demonstrated the ability to differentiate among the studied species through the distinct appearance of polymorphic, monomorphic, and unique bands. The banding patterns varied among species, and in some cases, no bands were observed, as detailed below.

3.3.1 Primer *mstg008*

The primer revealed 11 loci producing polymorphic bands, with all bands exhibiting varying molecular sizes across the examined species. These band sizes ranged from 150 to 1000 base pairs. In total, 19 polymorphic bands were detected. The species *Sisymbrium orientale* L. exhibited the highest number of productive bands with five, while no productive bands were observed in *Aethionema cordifolium* L., *Erucaria microcarpa* Boiss., and *Hirschfeldia incana* L. as shown in Figure 2. My study did not agree with [26,27,28,29,30,31]. on this principle in terms of locations and number of bundles.

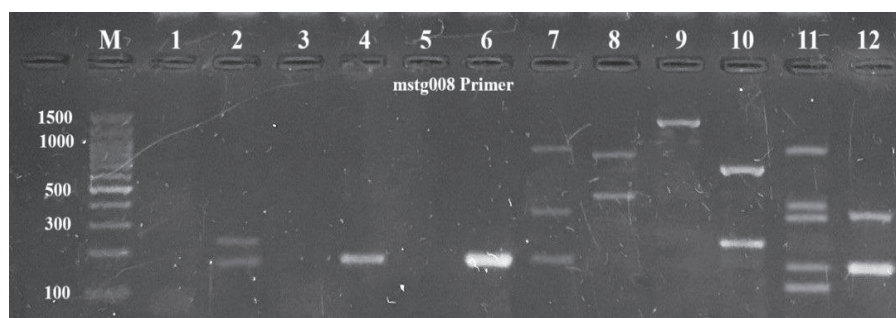


Fig 2. The results of DNA analysis using the primer *mstg008* after electrophoresis on an agarose gel for the studied species, with [m] indicating the molecular size marker of [1500bp].

3.3.2 Primer *Mstg012*

This primer recorded 10 sites for the productive bands and all the bands were of different molecular size in all the studied species. The molecular sizes varied between 150 and 1000 base pairs. A total of 24 productive bands were detected. The species *Sisymbrium irio* L. exhibited the highest number of productive bands, with four bands recorded. In contrast, only a single productive band was observed in *Aethionema cordifolium* L., *Erucaria microcarpa* Boiss., *Erysimum repandum* L., and *Hirschfeldia incana* L. as shown in Figure 3. My study did not agree with [26,27,28,29,30,31]. on this principle in terms of locations and number of bundles.

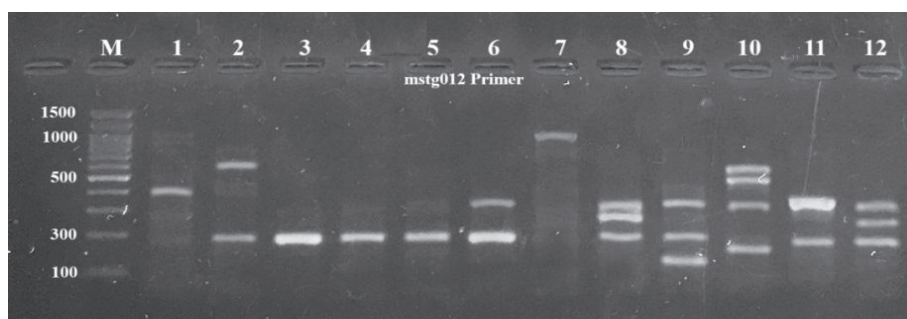


Fig 3. The results of DNA analysis using the primer *mstg012* after electrophoresis on an agarose gel for the studied species, with [m] indicating the molecular size marker of [1500bp].

3.3.3 Primer *mstg013*

The primer detected ten loci associated with productive bands. Among these, one consistent band of 200 base pairs was observed across all species, while the remaining bands showed variability in molecular size, ranging from 50 to 1300 base pairs. In total, 35 productive bands were identified. The maximum number of productive bands-five-was recorded in *Capsella bursa-pastoris* L., *Neslia paniculata* L., and *Rapistrum rugosum* L. Conversely, the minimum number, just one band, was found in *Erucaria microcarpa* Boiss, *Erysimum repandum* L., and *Hirschfeldia incana* L. as shown in Figure 4. My study did not agree with [26,27,28,29,30,31]. on this principle in terms of locations and number of bundles.

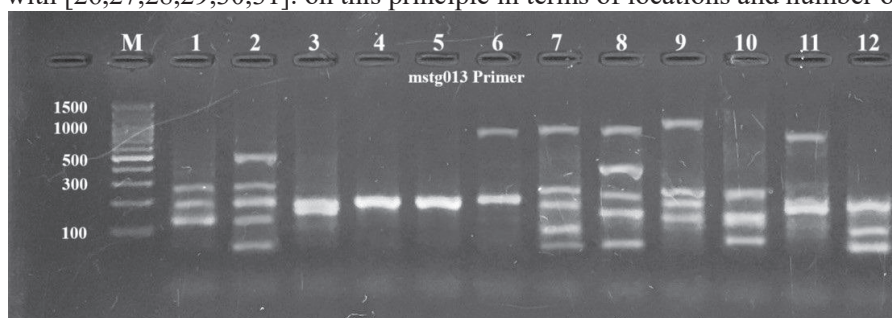


Fig 4. The results of DNA analysis using the primer *mstg013* after electrophoresis on an agarose gel for the studied species, with [m] indicating the molecular size marker of [1500bp].

3.3.4 Primer *mstg025*

This primer identified seven loci generating productive bands, with one site consistently showing a molecular size of 250 base pairs. The remaining loci produced bands of varying sizes, ranging from 100 to 900 base pairs. Altogether, 24 productive bands were observed. The highest count of productive bands-three-was recorded in the species *Erysimum repandum* L., *Neslia paniculata* L., *Rhamphospermum arvense* L., and *Sisymbrium orientale* L. In contrast, the lowest count, with only one productive band, was noted in *Aethionema cordifolium* L., *Erucaria microcarpa* Boiss, *Rapistrum rugosum* L., and *Strigosellea africana* L. as shown in Figure 5. My study did not agree with [26,27,28,29,30,31]. on this principle in terms of locations and number of bundles.

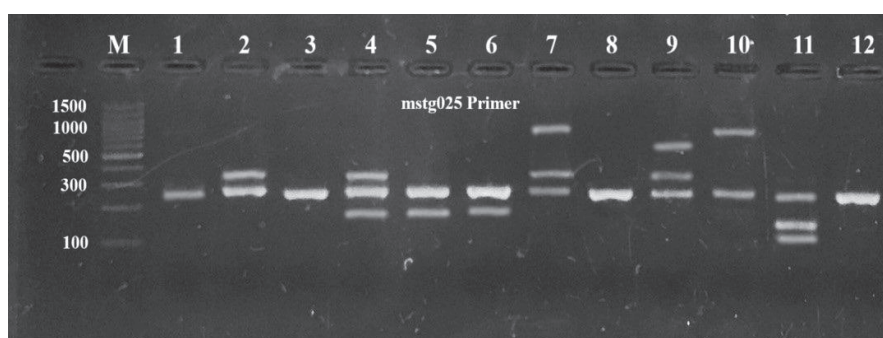


Fig 5. The results of DNA analysis using the primer *mstg025* after electrophoresis on an agarose gel for the studied species, with [m] indicating the molecular size marker of [1500bp].

3.3.5 Primer *mstg027*

This primer detected 11 loci producing distinct bands, each exhibiting unique molecular sizes across all the species examined. The fragment sizes varied from 150 to 1500 base pairs. A total of 38 productive bands were identified. The greatest number of bands (5) was observed in *Capsella bursa-pastoris* L. and *Rapistrum rugosum* L., whereas *Hirschfeldia incana* L. showed the fewest, with only one productive band. As shown in Figure 6. My study did not agree with [26,27,28,29,30,31]. on this principle in terms of locations and number of bundles.

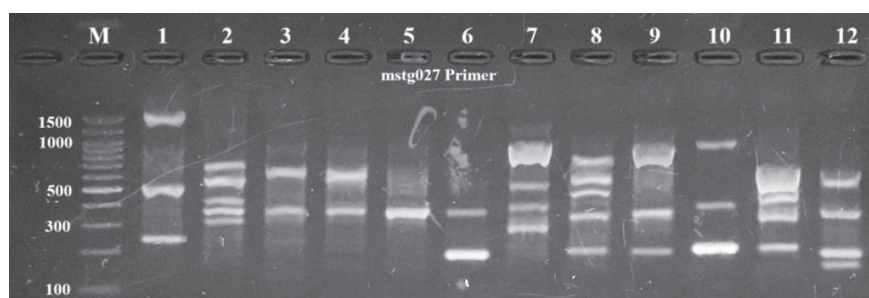


Fig 6. The results of DNA analysis using the primer *mstg027* after electrophoresis on an agarose gel for the studied species, with [m] indicating the molecular size marker of [1500bp].

3.3.6 Primer *mstg028*

This primer amplified three distinct loci, generating bands of varying molecular sizes across all examined species. The band sizes ranged from 500 to 600 base pairs. In total, four bands were detected. The maximum number of bands observed per species was one, specifically in *Isatis tinctoria* L., *Rapistrum rugosum* L., *Rhamphospermum arvense* L., and *Strigosellea africana* L. In contrast, no bands were detected in the other species, as illustrated in Figure 7. My study did not agree with [26,27,28,29,30,31]. on this principle in terms of locations and number of bundles.

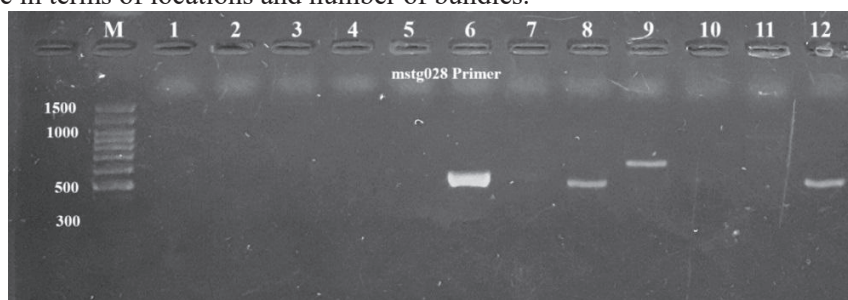


Fig 7. The results of DNA analysis using the primer *mstg028* after electrophoresis on an agarose gel for the studied species, with [m] indicating the molecular size marker of [1500bp].

3.3.7 Primer *mstg034*

This primer detected four loci of productive bands, one of which corresponded to identical bands with a molecular size of 300 base pairs. The remaining location exhibited bands of varying molecular sizes, ranging from 200 to 700 base pairs. A total of 21 productive bands were recorded. The highest number of productive bands (3) was observed in *Strigosellea africana* L., while the lowest number (1 band) was found in *Aethionema cordifolium* L., *Erysimum repandum* L., *Hirschfeldia incana* L., and *Sisymbrium orientale* L. as shown in Figure 8. My study did not agree with [26,27,28,29,30,31]. on this principle in terms of locations and number of bundles.

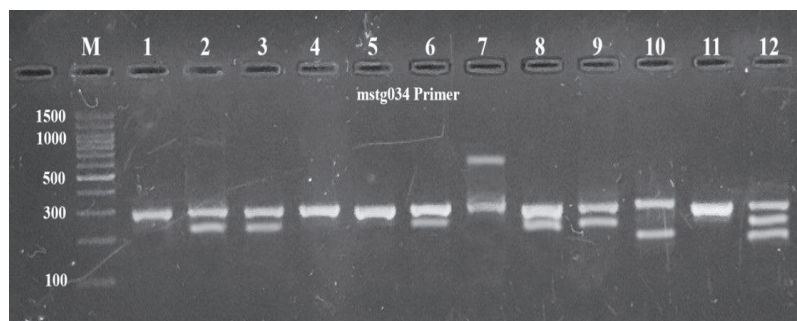


Fig 8. The results of DNA analysis using the primer mstg034 after electrophoresis on an agarose gel for the studied species, with [m] indicating the molecular size marker of [1500bp].

3.3.8 Primer mstg039

Using this primer, seven loci were detected, and the resulting bands exhibited varying molecular sizes across all examined species. The molecular weights of the bands ranged from 100 to 400 base pairs. In total, 14 bands were generated. The highest number of bands (3) was observed in *Sisymbrium orientale* L., while no bands were detected in *Capsella bursa-pastoris* L., *Erucaria microcarpa* Boiss., and *Hirschfeldia incana* L. as in Figure 9. My study did not agree with [26,27,28,29,30,31]. on this principle in terms of locations and number of bundles.

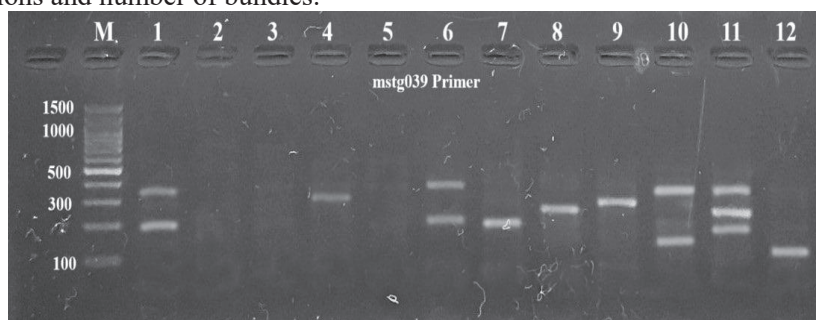


Fig 9. The results of DNA analysis using the primer mstg039 after electrophoresis on an agarose gel for the studied species, with [m] indicating the molecular size marker of [1500bp].

3.3.9 Primer mstg052

This primer recorded 8 sites for the productive bands and all the bands were of different molecular sizes in all the studied species. The fragment sizes ranged from 100 to 1500 base pairs. In total, 15 productive bands were identified across the studied species. *Sisymbrium orientale* L. exhibited the highest number of productive bands with three, whereas no productive bands were detected in *Capsella bursa-pastoris* L., *Erucaria microcarpa* Boiss., and *Hirschfeldia incana* L. as in Figure 10. My study did not agree with [26,27,28,29,30,31]. on this principle in terms of locations and number of bundles.

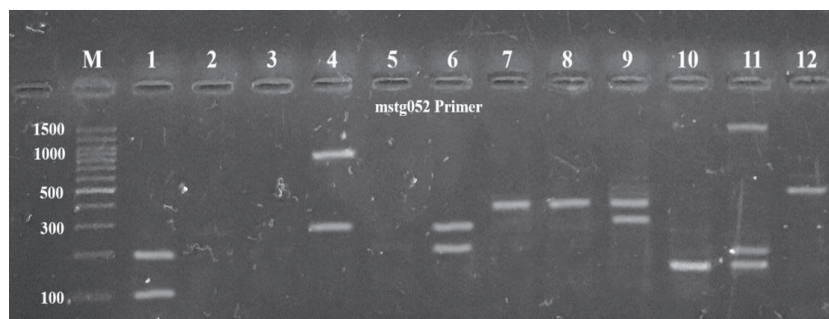


Fig 10. The results of DNA analysis using the primer mstg052 after electrophoresis on an agarose gel for the studied species, with [m] indicating the molecular size marker of [1500bp].

3.3.10 Primer mstg055

This primer generated bands at eight distinct loci, with each band displaying a unique molecular size across all the species examined. The band sizes varied from 100 to 1000 base pairs. In total, 18 bands were observed. The species *Rhamphospermum arvense* L., *Sisymbrium irio* L., and *Sisymbrium orientale* L. exhibited the highest number of bands, each producing three. Conversely, no bands were detected in *Rapistrum rugosum* L., marking it as the species with the fewest. as in Figure 11. My study did not agree with [26,27,28,29,30,31]. on this principle in terms of locations and number of bundles.

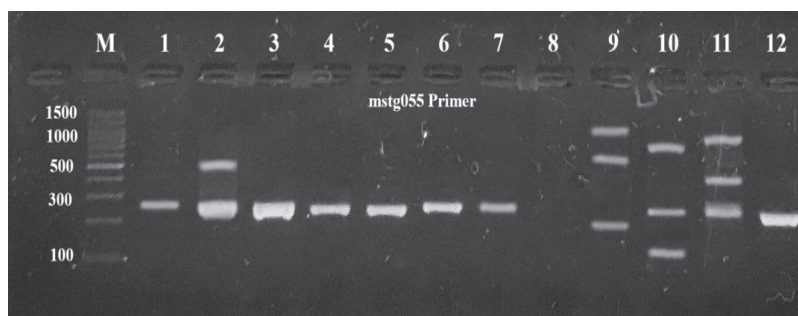


Figure 11. The results of DNA analysis using the primer mstg055 after electrophoresis on an agarose gel for the studied species, with [m] indicating the molecular size marker of [1500bp].

3.4 Polymorphism, primer efficiency, and discrimination ability of the SSR marker.

The results of the SSR-PCR reactions varied depending on the primers used in the SSR marker. The total number of divergent bands was 212, while primer mstg027 recorded the highest number of divergent bands, with 38, and primer mstg028 recorded the lowest number of divergent bands, with 4. The polymorphism, efficiency, and discrimination ability of each primer were calculated. Primers Mstg008, Mstg012, Mstg027, Mstg028, Mstg039, Mstg052, and Mstg055 recorded the highest percentage of polymorphism, reaching 100%. Primers Mstg034, Mstg025, and Mstg013 showed the lowest percentage of polymorphism, reaching 95%-97%. The current study showed a clear variation in the efficiency of the primers used, as the highest efficiency rate was recorded for the primer Mstg027, which reached 16.239%, and the lowest efficiency rate was recorded for the primer Mstg028, which reached 1.909. as shown in Table 4. My study did not agree with [26].

Table 4. Total and differentiated packages with their efficiency ratios and discriminating ability for the studied species.

NO:	Primer	Total of Bands	Number of Polymorphic Bands	Polymorphism Percentage of the Primer(%)	Primer Efficiency (%)	Discriminating Power of the Primer(%)
1	Mstg008	19	19	100%	8.119	8.333
2	Mstg012	24	24	100%	10.256	10.256
3	Mstg013	35	34	97.14	14.957	14.912
4	Mstg025	24	23	95.83	10.256	10.087
5	Mstg027	38	38	100%	16.239	16.666
6	Mstg028	4	4	100%	1.909	1.754
7	Mstg034	21	20	95.23	8.974	8.771
8	Mstg039	14	14	100%	5.982	6.140
9	Mstg052	15	15	100%	6.410	6.578
10	Mstg055	18	18	100%	7.692	7.894
Total		212	209			

3.5 Determining the genetic distance between the species studied using the SSR marker

Based on DNA fragments obtained from the amplification of random primers and by the SSR-PCR technique, as well as the number of bands and their sizes determined by analyzing the results obtained from the studied species using the program (NTSYS pc v.2.02e), the genetic distance between the genetic structures of the studied species was calculated. and the similarity percentage ranged between (0.20851 - 0.65938) and the shortest distance was between the two species *Erysimum repandum* L. and *Hirschfeldia incana* L. as it reached (0.20851) The green color indicates the closest genetic distance between species. While the farthest distance was recorded between the two species *Rhamphospermum arvense* L. and *Sisymbrium orientale* L. as it reached (0.65938) the blue color represents the farthest genetic distance.as in Table 5. My study did not agree with [26].

Table 5. Convergence and divergence between the studied species using the SSR marker

	1	2	3	4	5	6	7	8	9	10	11	12
1	0.000 00											
2	0.521 28	0.000 00										
3	0.417 02	0.375 91	0.000 00									
4	0.442 32	0.402 71	0.329 69	0.000 00								
5	0.390 09	0.403 70	0.208 51	0.255 37	0.000 00							
6	0.466 25	0.477 76	0.361 15	0.361 15	0.329 69	0.000 00						
7	0.531 60	0.501 00	0.510 75	0.510 75	0.489 00	0.532 30	0.000 00					
8	0.551 67	0.521 28	0.466 25	0.531 60	0.478 25	0.530 50	0.604 23	0.000 00				
9	0.571 04	0.541 73	0.489 00	0.510 75	0.487 01	0.530 61	0.607 91	0.551 67	0.000 00			
10	0.551 67	0.580 47	0.532 40	0.551 67	0.512 63	0.571 04	0.589 76	0.607 91	0.625 54	0.000 00		

1	0.531	0.616	0.531	0.532	0.514	0.551	0.625	0.605	0.659	0.625	0.000	
1	60	79	60	57	75	67	54	72	38	54	00	
1	0.561	0.531	0.454	0.501	0.454	0.502	0.616	0.477	0.561	0.580	0.541	0.000
2	44	60	44	28	4	06	79	76	44	47	73	00

3.6 Phylogenetic free tree analysis between species for SSR markers

The cluster analysis of the SSR index of the studied species included five main groups A. B. C. D. and E. The first group A. included two groups A1 and A2. Group A1 included the species *Aethionema cordifolium* L. Group A2 was also divided into two groups AB1 and included the species *Capsella bursa-pastoris* L. and AB2 was divided into two groups ABC1 and included the species *Erucaria microcarpa* Boiss, *Hirschfeldia incana* L. and *Erysimum repandum* L. Group ABC2 included the species *Isatis tinctoria* L. Group A2 included the species *Rapistrum rugosum* L. and *Strigosellea africana* L. Group B included the species *Rhamphospermum arvense* L. Group C included the species *Neslia paniculata* L. Group D included the species *Sisymbrium irio* L. Group E included the species *Sisymbrium orientale* L. The closest two species are *Erucaria microcarpa* Boiss and *Hirschfeldia incana* L., where the convergence ratio between them was 0.21, while the furthest distance between the two species is *Sisymbrium irio* L. and *Sisymbrium orientale* L., where the ratio was 0.58. The convergence and divergence of this genetic index SSR is based on the presence, number, and molecular size of the bands or their absence, which indicates that the studied species are genetically close, but there is variation between them in phenotypic, anatomical, and physiological traits. Therefore, this genetic index SSR showed which species were genetically close and which were distant, as shown in Figure 12. My study did not agree with [26].

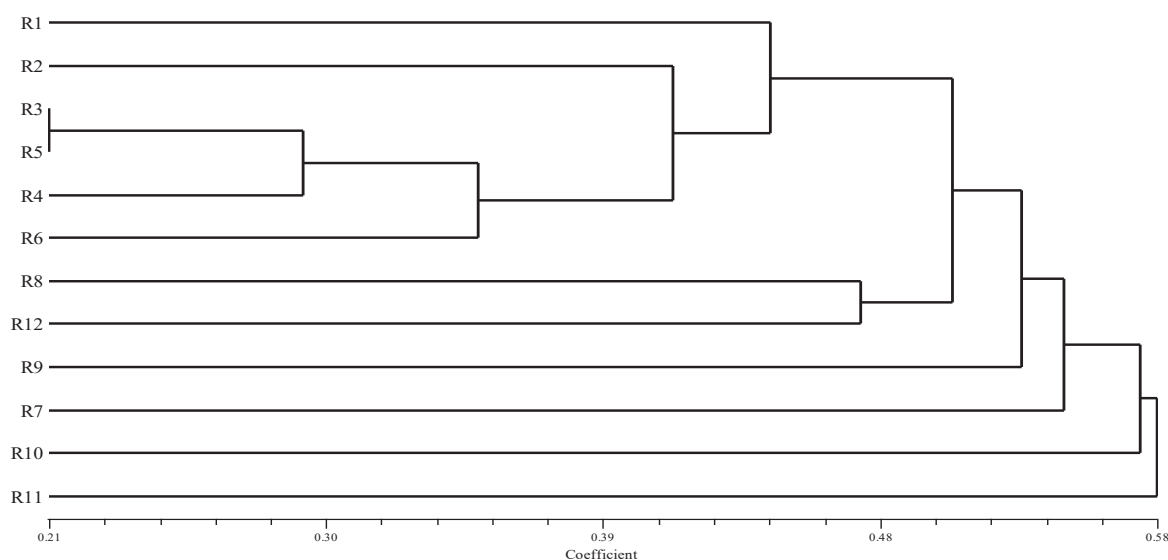


Fig 12. The three diagram shows the genetic dimension of the SSR marker in the studied species.

4. Conclusions

The highest discriminatory ability of the SSR index was recorded by primer Mstg027, at 16.666, while primer Mstg028 recorded the lowest discriminatory ability, at 1.754. The highest efficiency rate was recorded by primer Mstg027, at 16.239%, and the lowest was recorded by primer Mstg028, at 1.909. The highest polymorphism rate was recorded by primers Mstg008, Mstg012, Mstg027, Mstg028, Mstg039,

Mstg052, and Mstg055, at 100%. The lowest percentage was recorded by primers Mstg013, Mstg025, and Mstg034, at 95%. The shortest genetic distance was between *Erysimum repandum* L. and *Hirschfeldia incana* L., reaching 0.20851, while the longest genetic distance was between *Rhaphospermum arvense* L. and *Sisymbrium orientale* L., reaching 0.65938. The green color indicates the closest genetic distance between species, while the blue color represents the farthest genetic distance. Conduct a comprehensive survey of the Mustard family in all Iraqi provinces to determine the true number of genera and species within this family. I recommend using the SSR marker with DNA from other plant species, given its importance in studying genetic diversity. Use more accurate molecular genetics techniques, such as ITS, SCOT, and ARPS, to support phenotypic and anatomical traits.

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