


Research Article

Genetic diversity analysis of some *Coriandrum sativum* cultivars exposed to laser using some molecular indicators

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Coriander, *Coriandrum sativum* L. is an herbal crop from the family Apiaceae. It has a wide range of uses, including as a flavouring agent and in medical applications. Therefore, physical factors have recently been given attention, including laser radiation applications used in agriculture for biostimulating seeds and seedlings, thereby leading to changes in the DNA sequence. The present study aimed to identify genetic changes in Coriander plants exposed to laser radiation. Coriander seeds were obtained from local markets in Mosul city in different varieties (local, Syrian, Italian). The study found that there were differences at the molecular level between the three types of coriander used in the study, the difference in the total number of bands/variety of coriander for the total of the five primers used (OPA1, OPA2, OPD1, OPK, POD3) according to the difference in the period of exposure to laser radiation three different times 5, 10, 20 minutes. Depending on the results of the RAPD-PCS analysis, the total number of amplification bands was 182, comprising 180 polymorphic bands and 2 unique bands, which accounted for 98.9% and 1.10%, respectively. There were differences at the molecular level between the three types of coriander according to the different treatments with laser radiation and this primer. Genetic similarity was calculated, with a maximum similarity value of 0.9 and a minimum similarity value of 0.2. The cluster tree analysis showed that genotypes that are close to each other are more similar than those that are far apart. This study used diversity genetic information and different types of coriander seeds with different primers compared with other study.

Keywords: *Coriandrum sativum*, RAPD-PCS, Laser radiation, Mutation**INTRODUCTION**

Coriander *Coriandrum sativum* L. It is an aromatic herbal crop from the family Apiaceae (Lin *et al.*, 2022). This plant is characterised by its fast growth and branched stem, as well as small pink or white flowers (Scandar *et al.*, 2023). Coriander is known for its wide range of uses; for example, it is used as a flavouring agent in food (Bhat *et al.*, 2014). In addition to their use in medical applications (Lin *et al.*, 2022), which is expected to have anti-inflammatory and anticancer effects, they work to relax the nerves (Mahleyuddin *et al.*, 2021; Shoaib *et al.*, 2023). Important in biological activities responsible for antimicrobial activity against pathogenic bacteria, such as *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis* (Hajlaoui *et al.*, 2021; Scandar

et al., 2023), it is considered a natural antioxidant and antifungal (Mahleyuddin *et al.*, 2021). Good preparation of seeds, which aims to increase their ability to germinate and thus improve the strength of seedlings, is one of the essential productivity factors, as it enables young plants to have greater strength, develop better, and tolerate unfavourable environmental stress factors. Therefore, they become more resistant to diseases and need less protection using chemical materials (Podlešný *et al.*, 2012). Recently, attention has been drawn to physical factors, including laser radiation, due to its properties in terms of applications and its use in agriculture as a stimulator for seeds and seedlings. Studies have shown that treating seeds with laser radiation before planting can positively affect germination, development, and grain production. (Chen *et al.*, 2005).

The basis of the laser stimulation mechanism in any plant's physiological stage is the synergy between polarized monochromatic laser beams and photoreceptors. Numerous studies suggest that laser radiation has a bio stimulatory effect on various tissues and organs in plants. Plants absorb light through their photoreceptors, which enables them to control all stages of plant development (Abou-Dahab *et al.*, 2019). Mutations allow for an increase in the possibilities of variability, creating high ornamentation (Tomar-Rukam *et al.*, 2014). Mutations are the natural process that changes the genetic information of the organism. It does not create the pattern of natural genetics. Nevertheless, chemical and physical factors influence it, leading to changes in the DNA sequence. Genetic variation is beneficial because it enables organisms to survive and adapt over time (Roychowdhury and Tah, 2011). The present study aimed to identify genetic changes in Coriander plants exposed to laser radiation

MATERIALS AND METHODS

Experiment of the greenhouse

Coriander seeds were obtained from local markets in Mosul city in three different varieties (local, Syrian, Italian) and their productivity was tested. The seeds were treated with laser rays from a semiconductor laser device (UK-Scientific LU) with a 650 nm wavelength, 50 mW/cm² power, and the seeds were at a distance of 15 cm from the radiation source. And at three different times: 5, 10, and 20 minutes (Osman *et al.*, 2009), in addition to the control sample is the coriandrum plant seeds without treatment with laser rays. All the seeds were planted in pots, and one month after germination, the plants were harvested. Subsequent studies were then conducted on them. After crushing the plant tissue from the leaves, it was mixed with 1 mL of Trizol, which is used for cell lysis and to protect RNA from lysis.

DNA extraction

Total genomic DNA was extracted from the fresh leaves of coriander using the Geneaid Genomic Plant DNA Extraction Kit (Geneaid, Taiwan) following the method described by Michiels *et al.* (2003). The DNA concentration of the samples was measured using a nanodrop device (the concentration ranged between 50 and 90ng/μL).

Molecular diversity

Amplification of RAPD fragments was performed according to Tomar-Rukam *et al.* (2014). PCR conditions were as follows: 1 cycle at 95°C for 5 min, followed by 47 cycles with a denaturation step of 1 min at 95°C, 1 min at 40°C, and 2 min at 72 °C, followed by 1 cycle at 72 °C for 5 min. The primers used for RAPD analysis are listed in Table 1. PCR products were run on a 2% agarose gel for 45 min at 80 V. The PCR product bands were visualised under UV light using a transilluminator and photographed (Ibrahim and Faisal, 2024).

Similarity and genetic distance by statistical analysis

The different present in DNA by using the RAPD (Random Amplified Polymorphic DNA) marker to determine the degree of similarity and genetic variation between different strains and cultivated varieties, as well as at the following :

Based on the RAPD results, it was observed that the analysis depends on the presence or absence of specific DNA bands in different samples. The value "0" is assigned when the band is absent, and "1" when the band is present.

To find the genetic relationships between genetic groups, the characterization data were converted into similarity values using a computer within an NTSYS program, which depends on the following formula:

$$\text{Genetic Similarity (GS)} = 2 \text{ nxy} / \text{nx} + \text{ny} \quad \text{Eq.1}$$

Estimated the percentage of genetic distance (GD) between genetic groups in the study that depends on the results of genetic similarity using the following formula:

$$\text{Genetic Distance (GD)} = - \ln (\text{GS})$$

nxy = Represents the number of bands shared between the two lines.

x,y = Represents the genetic groups, the traits between which the similarity and genetic distance between it.

nx : Represent the total number of bands in the model x.

ny : Represent the total number of bands in the model x (Ezzulddin *et al.*, 2020) .

Dendrogram

Dendrogram shows the genetic relationships between the pure genetic groups and the hybridization and cross -breeding groups under study, which depends on the genetic values Obtained in the previous paragraph and the program used (NTSYS2.10e software) after enter-

Table 1. List of RAPD primers used in the current study

Primer name	Primers sequence 5'-3'	Reference
OPA1	CAGGCCCTTC	(Tomar-Rukam <i>et al.</i> , 2014)
OPA2	TGCCGAGCTG	
OPD1	ACCGCGAAGG	
OPD3	GTCGCCGTCA	
OPK	CACCTTTCCC	

ing the values into the computer to obtain the cluster analysis chart (Dendrogram) and the genetic similarity table.

RESULTS AND DISCUSSION

The present study, as observed in Table 2, revealed that the differences at the molecular level between the three types of coriander used in the study were related to the total number of bands per type. It was observed that the total number of bands is 182. Based on the results of the RAPD-PCS analysis, the lowest number of bands was observed for the OPA1 primer, which yielded 29 bands, and the highest number of bands was observed for the OPK primer, which yielded 43 bands.

As for the treatments, the local variety showed the lowest number of bands when the seeds were treated with laser radiation for 5 minutes, with 16 bands, compared to the control sample, which showed 17 bands. However, the Syrian variety decreased the number of bands with increased exposure to the laser rays over a longer period. As for the Italian variety, the number of bands was the least when the seeds were treated with laser radiation for 20 minutes, compared with a control sample. It was also shown that there are no significant differences between the primers at a probability level ≤ 0.01 , as determined by the Chi2 test (Table 3), indicating that these primers have the same ability to distinguish between different bands when used individually.

As for the product of the electrophoresis process related to the random duplication of the OPA1 primer bands, the results showed the presence of different bands at molecular weights 701, 699, 610, 600, and 400, some of which were similar to the control sample

(0) and others were different, while this primer was not able to distinguish any type of unique bands at all molecular weights and for all types of coriander varieties used. Thus, the primer reveals differences in the distinguishing bands among the three varieties (local, Syrian, and Italian) and across all treatments. This indicates that the three varieties differ at the molecular level due to the varying treatments with laser radiation, allowing this primer to distinguish polymorphic bands at a molecular weight of 701. Their number was six, distributed in the local variety across all treatments, while in the Syrian variety, polymorphic bands appeared when the seeds were treated with radiation for 5 minutes. As for the Italian variety, it did not show any polymorphic bands and therefore, did not differ from the control sample.

At a molecular weight of 699, the primer could distinguish the polymorphic bands, and there were 5 bands in the Syrian variety when the seeds were treated with laser radiation for 20 minutes. The primer could also distinguish the polymorphic bands in all treatments of the Italian variety. At a molecular weight of 610, the primer was able to distinguish six bands, distributed as at a molecular weight of 701, and at a molecular weight of 600, the primer was able to distinguish 5 polymorphic bands distributed at a molecular weight of 699, and 400, the primer was able to identify 7 polymorphic bands that appeared in the local variety in all treatments. In contrast, in the Syrian variety, the bands appeared at 5 and 10 minutes with laser treatments, respectively, as shown in Table 4 and Fig. 1.

On the other hand, random replication of the OPA2 primer, as observed in the electrophoresis process, revealed the presence of polymorphic bands at various molecular weights (2200, 2100, 1900, 1250, 950, 705,

Table 2. Showing the results of the amplification/ primer depending on the result of the RAPD-PCS analysis

Varieties of Coriander seeds	Treated of Coriander seeds with laser rays (minute)	OPA1	OPA2	OPD1	OPK	POD3	Total number of bands
Local	*0	3	4	3	4	3	17
	5	3	3	3	4	3	16
	10	3	4	3	4	3	17
	20	3	4	3	4	3	17
Syrian	*0	3	4	3	4	3	17
	5	3	3	3	4	3	16
	10	1	2	3	4	4	14
	20	2	4	2	2	3	13
Italian	*0	2	4	3	3	3	15
	5	2	4	2	4	3	15
	10	2	4	3	4	3	16
	20	2	1	1	2	3	9
Total number of bands/Primer		29	41	32	43	37	182
Number of samples with mono morphic bands / primer		0	0	0	0	0	
Number of samples with poly morphic bands / primer		12	11	12	12	11	

*Control

Table 3. Total number of amplified bands, polymorphic, and Unique/Primer

Primers	Total number of the amplified bands	Unique bands	Polymorphic bands	Efficiency%	Discriminatory Ability %	Value Chi ²
OPA1	29	0	29	15.93	16.11	3.61 g.m.
OPA2	41	1	40	22.53	22.22	
OPD1	32	0	32	17.58	17.78	
OPK	43	0	43	23.63	23.89	
POD3	37	1	36	20.33	20.00	
Total	182	2 (1.10%)	180 (98.90%)	100.00	100	

* g.m. It indicates that there are no significant differences at the probability level ($p \leq 0.01$).

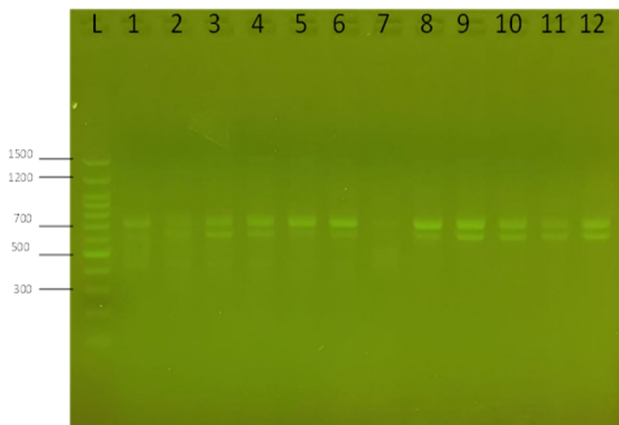


Fig. 1. Products of random amplification of the primer OPA1 in all laser treatments for the three varieties of coriander seeds

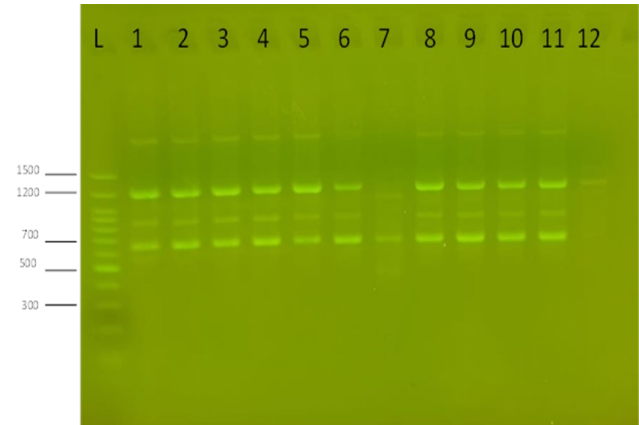


Fig. 2. Products of random amplification of the primer OPA2 in all laser treatments for the three varieties of coriander seeds

and 680), which were either identical to or different from those in the control sample. Their number varied according to the in duration of exposure to laser radiation and all types used in the study. As for the discrimination of unique bands by the primer, it was able to distinguish a unique band at a molecular weight of 1800, belonging to the Syrian variety, and when treated with laser radiation for 10 minutes, the result of the electrophoresis of the bands resulting from the primer OPA2, as this primer was able to distinguish different bands at a molecular weight of 2200. The number of bands was 4, of which the Syrian variety appeared when the seeds were treated with laser radiation for 20 minutes, thus differing from the control sample. As for the Italian array, it appeared when the seeds were treated with laser for 5 and 10 minutes, thus resembling the control sample. However, when treated for 20 minutes, they were not distinguished by any band. At a molecular weight of 2100, three polymorphic bands appeared, including one in the local variety, when the seeds were treated for 10 and 20 minutes. Thus, it differed from the control sample. In the Syrian variety, the band appeared only in the control sample.

At a molecular weight of 1900, two polymorphic bands were distinguished in the local variety, the control sam-

ple, and when treated for 5 minutes. The bands were also distinguished at 1250, that is, 10 bands, of which 3 were of the local variety when treated for 10 and 20 minutes, respectively, and thus did not differ from the control sample. As well as in the Syrian variety at 5 and 20 minutes, respectively, and the rest of the bands in the Italian variety in all treatments, as shown in Table 5 and Fig. 2.

As a result, electrophoresis of the primer OPD1, random duplication showed that this primer was not able to distinguish any unique band, but it distinguished many polymorphic bands at all its molecular weights (850, 825, 615, 605, 315, and 310), which differed according to the type and duration of exposure to laser radiation. At a molecular weight of 850, two bands were observed in the Italian variety: one for the control sample and the other for the treatment of the seeds with laser radiation for 20 minutes. In the Italian variety, when the seeds were treated with laser radiation for 5 and 10 minutes, respectively, two bands were also observed. In addition to the other molecular weights, 615, 605, 315, and 310, numerous polymorphic bands were observed, distributed differently among the three varieties and various treatments, as shown in Table 6 and Figure 3.

Regarding the bands resulting from the electrophoresis

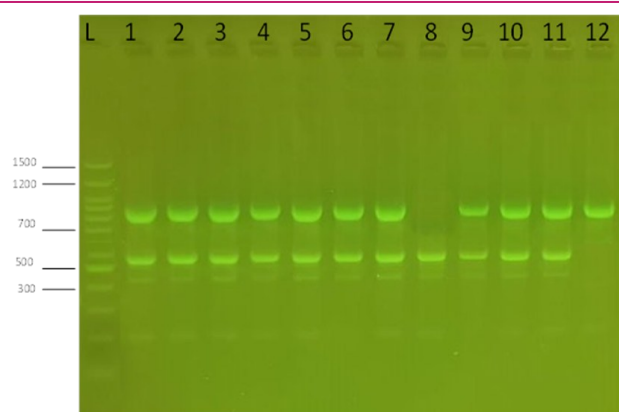


Fig. 3. Products of random amplification of the primer OPD1 in all laser treatments for the three varieties of coriander seeds

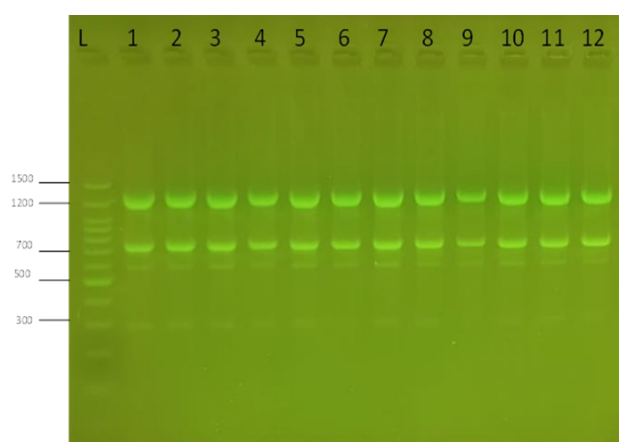


Fig. 5. Products of random amplification of the primer POD3 in all laser treatments for the three varieties of coriander seeds

process of random replication of the primer OPK, as shown in Table 7 and Fig. 4, this primer was able to distinguish many polymorphic bands; it reached 43, distributed differently among the varieties according to the different treatments of the seeds with laser radiation. On the other hand, this primer could not distinguish any unique types of bands.

Regarding the result of random replication of primer POD3 during the electrophoresis process, this primer was able to distinguish 36 bands distributed at molecular weights 580, 590, 600, 698, 705, 710, 1180, 1190, and 1200, respectively. However, it distinguished one unique band in the Italian variety at molecular weight 1200, which belonged to the control sample, as shown in Fig. 5 and Table 8. Thus, it was observed that there were differences among the genotypes of the three varieties of coriander seeds, in addition to the difference in one variety when treated with laser rays at different times. This is evidence of genetic mutations due to the treatment of coriander seeds at various times; this statement agrees with Abou-Dahab *et al.* (2019) in their study of coriander plants and the treatment of their seeds with laser rays before planting.

There are many studies agree with the current study,

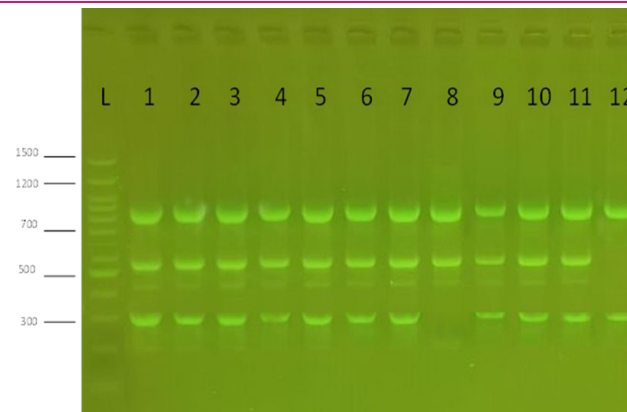


Fig. 4. Products of random amplification of the primer OPK in all laser treatments for the three varieties of coriander seeds

such as (Sharma *et al.*, 2019; Singh *et al.*, 2012; Melo *et al.*, 2010), that show the use of 14 primers and 23 genotypes of the *C.sativum* L. in India, as the amplification products were 72 bands, including 48 polymorphic bands 25% for OPB-50 primer and 100% for OPA-60 and OPB-01 primer. As for the monomorphic bands, they appeared in 3 primers, at a rate of 7 bands per primer, in addition to the appearance of 3 unique bands in three primers, OPA-05, OPB-01, and OPB-07, for three genotypes of coriander, and the size of these bands ranged from 300 to 1400 bp. And agree with the study of (Osman *et al.*, 2009) that showed the effect of treatments with a helium-neon laser for 5, 10, and 20 minutes at a power density of 95 mW/cm³ on the productivity of the coriander plant from Cairo, *Coriandrum sativum* L., obtaining the tallest plants, the highest number of branches/plant, and the highest percentage of essential oil after 20 minutes of laser treatment due to the genetic mutations resulting from exposure to laser rays.

Other studies, such as Hassan *et al.* (2024), utilise laser rays from Corms of *Gladiolus grandiflorus* cv. with lower power and shorter irradiation times. 112 bands 22 SSR primers, ranging between (130–540 bp), with 32 bands having polymorphism ranging from 17–100%. Out of the 22 SSR primers, 3 primers exhibited a high polymorphism percentage (SSR6, SSR16 and SSR22) which exhibited 7 positive markers. These findings revealed the efficiency of SSR primers for differentiating *Gladiolus* plants and revealed that some alleles were affected by laser rays and study Hasan *et al.* (2020) it showing the effects of different laser wavelengths, different times, and low-power-intensity laser irradiation on maize seeds had a positive impact on the growth and development of maize plants and had a positive influence on the seed yield of maize.

Genetic similarity was calculated based on RAPD analysis using the Jaccard similarity coefficient analysis method. A maximum similarity value of 0.9 was observed between the genotypes belonging to the local

Table 4. Bands resulting from random amplified of the primer OPA1 during the electrophoresis process.

Varieties of Coriander seeds	Treated of Coriander seeds with laser rays (minute)	Polymorphic bands					Unique
		701	699	610	600	400	0
Local	*0	1	0	1	0	1	0
	5	1	0	1	0	1	0
	10	1	0	1	0	1	0
	20	1	0	1	0	1	0
Syrian	*0	1	0	1	0	1	0
	5	1	0	1	0	1	0
	10	0	0	0	0	1	0
	20	0	1	0	1	0	0
Italian	*0	0	1	0	1	0	0
	5	0	1	0	1	0	0
	10	0	1	0	1	0	0
	20	0	1	0	1	0	0

*Control

Table 5. Bands resulting from random amplified of the primer OPA2 during the electrophoresis process

Varieties of Coriander seeds	Treated of Coriander seeds with laser rays (minute)	Polymorphic bands							Unique
		2200	2100	1900	1250	950	705	680	1800
Local	*0	0	0	1	1	1	0	1	0
	5	0	0	1	0	1	0	1	0
	10	0	1	0	1	1	0	1	0
	20	0	1	0	1	1	0	1	0
Syrian	*0	0	1	0	1	1	1	0	0
	5	0	0	0	1	1	1	0	0
	10	0	0	0	0	0	1	0	1
	20	1	0	0	1	1	1	0	0
Italian	*0	1	0	0	1	1	1	0	0
	5	1	0	0	1	1	1	0	0
	10	1	0	0	1	1	1	0	0
	20	0	0	0	1	0	0	0	0

*Control

Table 6. Bands resulting from random amplified of the primer OPD1 during the electrophoresis process

Varieties of Coriander seeds	Treated of Coriander seeds with laser rays (minute)	Polymorphic bands						Unique
		850	825	615	605	315	310	
Local	*0	0	1	0	1	1	0	0
	5	0	1	0	1	1	0	0
	10	0	1	0	1	1	0	0
	20	0	1	0	1	1	0	0
Syrian	*0	0	1	1	0	1	0	0
	5	0	1	1	0	1	0	0
	10	0	1	1	0	0	1	0
	20	0	0	1	0	0	1	0
Italian	*0	1	0	1	0	0	1	0
	5	0	1	0	0	0	1	0
	10	0	1	1	0	0	1	0
	20	1	0	0	0	0	0	0

*Control

Table 7. Bands resulting from random amplification of the primer OPK during the electrophoresis process

Varieties of Coriander seeds	Treated of Coriander seeds with laser rays (minute)	Polymorphic bands										Unique
		735	725	720	710	550	515	435	410	305	290	
Local	*0	0	0	0	1	0	1	0	1	0	1	0
	5	0	0	0	1	0	1	0	1	0	1	0
	10	0	0	0	1	0	1	0	1	0	1	0
	20	0	0	1	0	0	1	0	1	0	1	0
Syrian	*0	0	0	1	0	0	1	0	1	0	1	0
	5	0	0	1	0	0	1	0	1	1	0	0
	10	0	0	1	0	0	1	0	1	1	0	0
	20	0	0	1	0	1	0	0	0	0	0	0
Italian	*0	1	0	0	0	1	0	1	0	0	0	0
	5	0	1	0	0	1	0	1	0	1	0	0
	10	0	1	0	0	1	0	1	0	1	0	0
	20	1	0	0	0	0	0	0	0	1	0	0

*Control

Table 8. Bands resulting from random amplified of the primer POD3 during the electrophoresis process.

Varieties of Coriander seeds	Treated of Coriander seeds with laser rays (minute)	Polymorphic bands										Unique
		1200	1190	1180	710	705	698	600	590	580	1210	
Local	*0	0	0	1	0	0	1	0	0	1	0	0
	5	0	1	0	0	0	1	0	0	1	0	0
	10	0	0	1	0	0	1	0	0	1	0	0
	20	0	1	0	0	1	0	0	1	0	0	0
Syrian	*0	0	0	1	0	1	0	0	1	0	0	0
	5	0	1	0	0	1	0	0	1	0	0	0
	10	0	1	1	0	1	0	0	1	0	0	0
	20	0	1	0	0	1	0	0	1	0	0	0
Italian	*0	0	0	0	1	0	0	0	1	0	1	0
	5	1	0	0	1	0	0	1	0	0	0	0
	10	1	0	0	1	0	0	1	0	0	0	0
	20	1	0	0	1	0	0	1	0	0	0	0

*Control

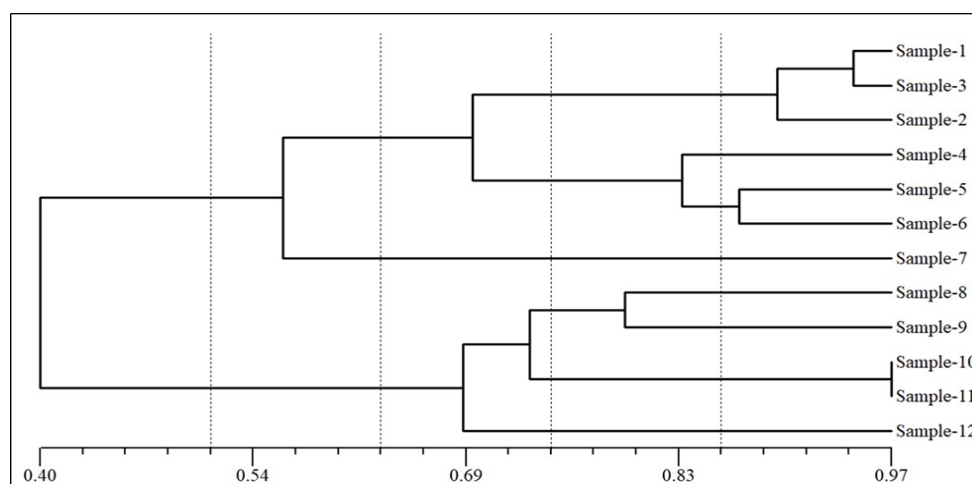
**Fig. 6.** Genetic similarity diagram (Dendrogram) between the samples under study based on RAPD indicators

Table 9. Genetic similarity between the samples under study

Sample-12	Sample-11	Sample-10	Sample-9	Sample-8	Sample-7	Sample-6	Sample-5	Sample-4	Sample-3	Sample-2	Sample-1
											Sample-1
											Sample-2
											Sample-3
											Sample-4
											Sample-5
											Sample-6
											Sample-7
											Sample-8
											Sample-9
											Sample-10
											Sample-11
											Sample-12
1											1
	1										0.9231
		1									0.9487
			1								0.7436
				1							0.6923
					1						0.6154
						1					0.4359
							1				0.3333
								1			0.2821
									1		0.3333
										1	0.3077
											0.3846
											0.359

variety of coriander seeds treated with laser radiation for 5 and 10 minutes, respectively, with the control sample of the same variety, as well as in the Italian variety of the seed sample treated with the laser irradiation for 10 minutes, as well as a seed sample treated for 5 minutes for the same variety.

The minimum similarity value was 0.2 between the genotypes of the Italian variety for the control sample with the local variety for the seed sample treated with laser irradiation for 5 and 10 minutes, respectively, as well as the Italian variety for the seed sample treated with laser irradiation for 10 minutes, as well as the seed sample treated with laser radiation for 5 minutes for the local variety, and thus the genetic tree diagram that was created based on the Jaccard similarity coefficient as shown in Table 9 and Fig. 6. The resulting diagram was divided into a leading group at a similarity level of 0.40, which comprised two main groups at similarity levels of 0.54 and 0.69, respectively. The first leading group comprised a sample of coriander seeds treated with laser radiation for 10 minutes for the Syrian variety, with another subgroup at a similarity level of 0.69, which included two subgroups with similarity levels of 0.83 and 0.97, respectively. The second leading group has a similarity level 0.69 for the seed samples treated with a laser for 20 minutes. The Italian variety was included with a subgroup that included the seed samples treated with a laser for 5 and 10 minutes, respectively, for the Italian variety, with a secondary subgroup at a similarity level of 0.83 that included the control sample of the Italian variety and the seed sample treated with a laser for 20 minutes to the Syrian variety. Thus, the cluster tree analysis revealed that close genotypes were more similar than distant genotypes, based on the similarity coefficient value. This finding is consistent with the study conducted in India by Sharma *et al.* (2019), when they used different genotypes of coriander seeds, as well as the study by Othman *et al.* (2009) in Cairo, when they studied the effect of helium-neon laser exposure for 5, 10, and 20 minutes at an energy density of 95 mW/cm³ on coriander yield (dry and wet). It is worth noting that the current study used three different types of coriander seeds (Iraqi, Syrian, and Italian) that differed from the genotypes used in previous studies with different primers.

Conclusion

This study used different types of coriander seeds with different primers compared with other studies. It was found from the present study differences between types of Coriander seeds from different sources at the molecular level, the difference in the total number of bands/variety of coriander for the total of the five primers used according to the difference in the period of exposure to laser radiation three times 5 ,10,20 minutes depending

on the results of the RAPD-PCS analysis. These results may be due to the genetic mutations resulting from exposure of coriander seeds to laser radiation.

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Conflict of interest

The authors declare that they have no conflict of interest.

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