Molecular Biology: Open Access
ISSN: 2168-9547

Thesis Open Access

C-Banding Karyotype and Molecular Characterization on Cumin, Caraway and Coriander

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Abstract

Our goal for this study to characterize three species germplasm of family Apiaceae, namely (cumin, caraway and coriander), to make a chromosome characterization and molecular fingerprint for the mentioned species. Results performed on Karyomorphological showed that; *Cuminum cyminum* L. (cumin: 2n=14), *Carum carvi* (caraway: 2n=20) and *Coriandrum sativum* (coriander: 2n=22). The total Chromatin Relative Length percentage (RL%) showed cumin ± 10.69 µm ± 19.70 µm, caraway ± 7.30 µm ± 13.40 µm and coriander ± 5.58 µm ± 12.07 µm. Satellites in all the cases were associated to short arms. The molecular characterization for the three species (caraway, cumin, and coriander) was conducted using 5 AFLP combinations and 15 anchored-ISSR primers. The total amplified bands were 330 (162 ISSR+168 AFLP), with an average 83.75% (89.5 ISSR+78 AFLP) per primer. The combined dendrogram based on both AFLP and SSR markers for the three accessions was divided into 2 main clusters; the first cluster has 2 accessions (caraway and cumin) with 60% similarity, while coriander falls in a distinct cluster.

Keywords: Karyotype; Cytogenetic; Cytology; C-banding; Chromosome; Cumin; Caraway; Coriander; AFLP; ISSR molecular marker

Abbreviations: ISSR: Inter Simple Sequence Repeat; AFLP: Amplified Fragment Length Polymorphism; RAPD: Random Amplified Polymorphic DNA; SSR: Simple Sequence Repeat.

Introduction

The Apiaceae family include of a plants with species that distributed in most parts of the world, although more commonly found in temperate region. The botanists of the 16th century was classifying plants and Apiaceae was the first family to been recognized and scientifically studies and stated that it including cumin (*Cuminum cyminum*), caraway (*Carum carvi*) and coriander (*Coriandrum sativum*) [1].

Cumin (*Cuminum cyminum*), caraway (*Carum carvi*) and coriander (*Coriandrum sativum*) are important seed species for their significant as potential medicinal herbs. Exhibited a board range of pharmacological properties including antibacterial, antifungal, antioxidant, hypoglycaemic and anti-carcinogenic [2].

The cytological studies are important in plant species and their population specifically wild and endemic species is significant, because it could be explained genetically variation, variation in their shapes and the size of chromosome in the mitosis division and the behaviour of chromosomes in the meiotic division. Therefore, studies on karyotype have a well-known importance for species characterization, which has a great value because the chromosomal formula is useful to prove taxonomic place of each species, to choose the best way for choice and to understand speciation process [1].

Also, Karyotype describe the phenotypic features, the chromosomes complement of species in their terms of number, size, arm ratio and centromere index and other landmark. Karyotype dynamic structure evolving through numerical and structural changes. The difference in the number of chromosomes cause of characteristic creation and separation and therefore, karyotype evolution is significant important. In addition, for several decades, Karyotype diversity has been a crux of plant evolution studies for the main reasons [3].

Recently, through efficient breeding methodologies, karyotype used to describe cumin, caraway and coriander to gathering cytological information for effective exploration of the species for further researches on cytogenetics aspects and later crop improvement. Recently, molecular methods had been use for the identification and classification of different species of herbs and medicinal plants [4]. Also, used for determining genetic differences. DNA-based markers give the best assessment of genetic variation because they are plentiful and are not dependent on environ-mental effects. This made this evaluation more efficient and reliable [5,6]. In addition, the development of polymerase chain reaction (PCR) technology [7], based markers were using to apply to assess the genetic variation among populations and genetic resources. They had been show the powerful tool in genetic analysis due to simplicity of the technique, easy handling. The ISSR markers (Inter-Simple Sequence Repeat), are considering useful for cultivar identification, contemplate an action. Phylogenetic relationships, genome mapping and population studies. Techniques such as ISSR based on amplification of regions between 100 and 300 bp [8], between two opposing microsatellites of the same type. They show a high degree of polymorphism, a low-cost [9,10], simplicity and they do not must previous knowledge of the genome sequence, which will be clone [11].

Amplified fragment length polymorphism (AFLP) markers is a PCR-based molecular marker class [12], considered within the group of dominant sequence polymorphism [13]. In addition, this technique has been an option for high throughput with multiplexing primer pairs and automated fragment detection and scoring [14,15].

Therefore, dominant markers like RAPD, ISSR and AFLP have used in cumin, caraway and coriander for genetic analysis of germ plasm as well as genetic diversity investigation. However, only a few molecular markers had reported in coriander like AFLP [16,17], ISSR [18-20]. Also, for cumin [21] used RAPD markers. While, in caraway [19] used RAPD markers for genetic distance among caraway genotypes.

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Received May 09, 2019; **Accepted** June 05, 2019; **Published** June 12, 2019

Citation: Ibrahim MA, Bekhit M, Hassan N, Refaat M, El-Akkad T (2019) C-Banding Karyotype and Molecular Characterization on Cumin, Caraway and Coriander. Mol Biol 8: 229.

Materials and Methods

Karyotype study

The seeds of cumin, caraway and coriander species collected from different regions of Egypt (Upper Egypt) at National Gene Bank (NGB) of Egypt at Agricultural Research Center (ARC).

Collection of root tips

Seeds were germinated on moistened filter paper in petri dishes at 25-30°C in Cooling incubator [22-24]. The lateral roots were collected of about 1.5-2.0 cm length.

Pre treatment of root tips

Root tips of germinated seeds were treat with 0.05% colchicine solution for 2 h at room temperature and Ice for overnight [25,26].

Fixation

The root tips were transfer to freshly prepared fixation solution [27,28] (3 ethanol: 1 glacial acetic acid).

Preservation

Experimental material preserved in 70% ethanol and placed in cool place for further studies.

C-banding and karyotype

After wash, seeds were in 45% glacial Acetic Acid for 5 min at 4°C. Then Squash preparations in 45% glacial Acetic Acid before squashing softens the tissue and makes squashing easer, the phase contrast has to checked of preparation. Slides had to soak in ethanol for overnight after removing the cover using liquid nitrogen immediately. Slides were dried in room temperature for 3-7 days, then treated the slides with 0.2 N HCL at 60°C in water bath for 3-5 min. Washed the slides carefully with distal water then incubated in saturated $Ba(HO)_2$ for 10 min at room temperature, then washed the slides carefully several times to make sure all the Ba has been removed, placed the slides in 2XSSC solution for 1 h at 60°C, and after wash carefully with distal water and placed the slide at Giemsa staining solution for 10-30 min, slides must check every few min for best contrast a staining time of about 30 min.

Microscope examination, karyotype and idiograming

Chromosomes examination has to do using a verticals fluorescence microscope (Leica DM2500) equipped with a cooled monochrome digital camera (Leica DFC340FX). Twenty cells with clearly observed and well spread where check and photographed at 100X magnification under oil immersion. Chromosome counting and karyotype has performed using the automated Karyotype and FISH software processing (Leica CW4000) system. Ideograms were constructed from complete chromosomes which showed the greatest possible banding pattern in at least ten different metaphase plates.

Molecular study

High quality genomic DNA was extract using Qiagen DNeasy kit. DNA quality was determine visually on 0.8% agarose gel The DNA concentration was quantitatively measured on Biophotometer (Eppendorph, Germany) and adjusted to 50 ng/µl.

ISSR-PCR marker

PCR reaction was perform in 25 µl reaction volume containing 2X ready mix (EmeraldAmp Max PCR master mix) 20 pM oligonucleotide primer and 50 ng genomic DNA [20]. This reaction was perform on Eppendorph Master Cycler planned to 35 cycles as follows: an initial denaturation step at 95°C for 5 min, followed by 35 cycles of denaturation step at 94°C for 1 minute, annealing temperature (Ta) for 1 minute, and an extension step at 72°C for 1 minute, and last extension step at 72°C for 10 min. HVD Corporation, Germany, synthesized a set of 15 ISSR primers were used in this study. Ladder DNA used Thermo 100 bp plus.

AFLP-PCR marker

AFLP are on the selective amplification of restriction fragments from total genomic DNA with different primer pairs [12]. AFLP had performed using Invitrogen kit (Cat No. 10483-014), according to manufacturer protocol. The products were analysed on 6% (w/v) denaturing polyacrylamide gels in 1X TBE electrophoresis run buffer. Electrophoresis was performing at 60 W constant power for 2 h. The gel was fixing for 30 min in 10% glacial acetic acid and stained using silver staining kit (Promega Company) according to manufacturer`s manual.

The banding patterns generated by AFLP marker was comparing to figure the genetic relationship of the 3 species. Clear and distinct amplification products have scored as (1) for present and (0) for absent bands. Bands of the same mobility were scoring as same. All scored bands ranged in size from 100 bp to 800 bp. UPGMA (Unweight Pair Group Method Arithmetic Mean) used to measure the genetic similarity resulted from the analysis software of non-Linear dynamics corporation (UK).

Results

Measurements of fifteen cells of well-spread mitotic metaphase plates have been using. Fundamentally, the basic measures used in every method proposed so far, are those about the length of long arm (L=q) and short arm (S=p) of each chromosome in a complement (For instance those with holocentric chromosomes or those with very small chromosomes, 1 µm or less). The arm ratio (r) and length of chromosome where calculate from the long and short arm according to the formula (r=(q/p)), (TL=(p+q)). While the relative length of each chromosome was estimating percent of total length of complement according to the formula (RL=(TL/sum TL) × 100). While, the formula used for the Centromeric Index [29] (CI%) was (CI%=(p/TL) × 100). Chromosomes count for the species is consistent with that reported literature, the analysis of several metaphase plates made it possible to propose the chromosomal formula in the result as shown.

Cuminum cyminum L. (Apiaceae)

Results shows that the somatic chromosome number (2n) of cumin: 2n=14=6Bsm++2Bsm-+6Csa- (Figure 1a-1c) metaphase, karyotype and ideogram for the chromosomes arms ratio. As scored the relative length between 19.70 in the largest and 10.69 in smallest length (Table 1). While El Alaoui-Faris [30] reported that Indians cumin populations at 1991 showed remarkable dominance submetacentric and one to two

	Average						
Chr. No.	p+q (TL)	q	p	RL%	r	CI%	
1	5.11	2.87	2.24	19.70	1.28	43.80	SM+
2	4.29	3.26	1.03	16.54	3.16	24.02	SA-
3	3.92	2.20	1.72	15.11	1.28	43.79	SM+
4	3.56	2.03	1.53	13.73	1.33	42.95	SM+
5	3.28	2.04	1.25	12.66	1.63	37.99	SM-
6	3.00	2.32	0.68	11.56	0.00	22.69	SA-
7	2.77	2.04	0.73	10.69	0.00	26.34	SA-

Table 1: Mean average of Karyomorphological for Cumin species.

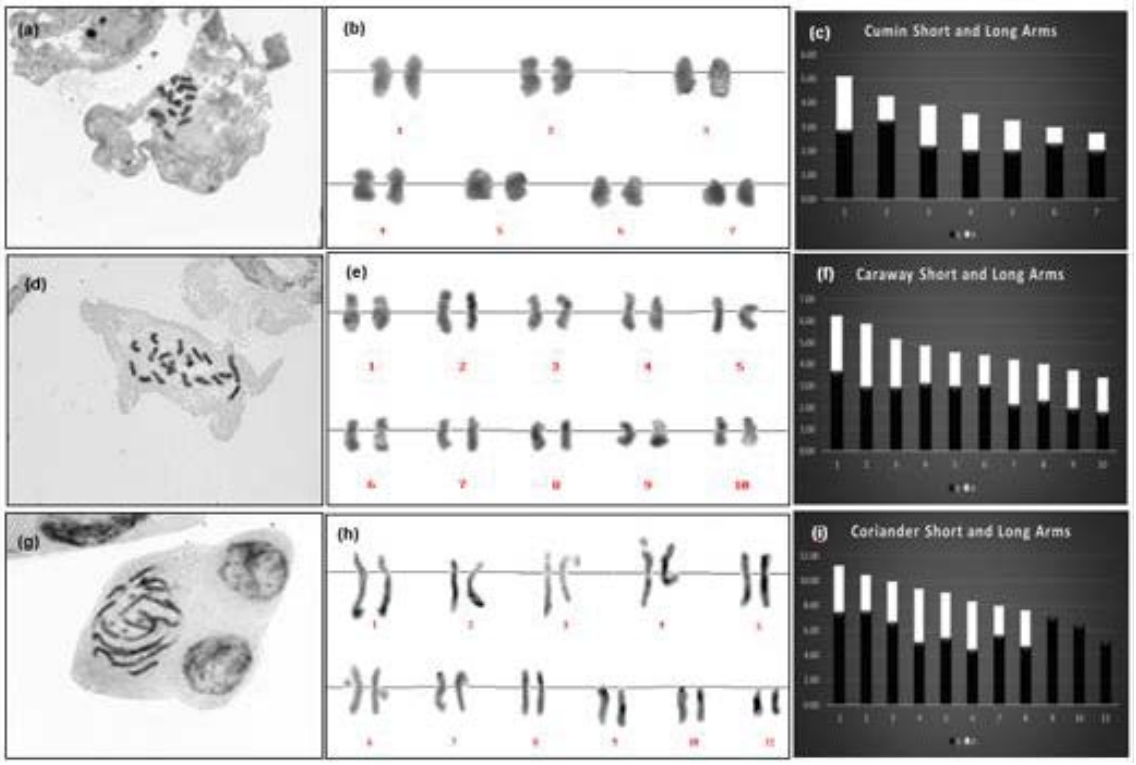

Figure 1: Metaphase Chromosome (a), (d) and (g) of *Cuminum cyminum* (2n=2X=14), *Carum carvi* (2n=2X=20) and *Coriandrum sativum* (2n=2X=22); Karyotype shown in (b), (e) and (h) and ideogram of chromosomes (c), (f) and (i).

pair submetacentric, then he stated that the Iranian cumin. Showed different type of cumin chromosomal karyotype, which has only one pair of the chromosomes (submetacentric) and had a secondary construction (satellite) and lastly remarking the formula by 2008 as 2n=14=6Bsm+8Csa and does not mention any satellite as remarkable. Also, mentioned that the cumin chromosomes [31] number were 2n=14 chromosome [32], and reported the following chromosomal formula 2n=14=4Bsm+2Csasac+6Csa+2Dt as telomeric pair have relocated in metaphase.

Carum carvi L. (Apiaceae)

Results revealed that the somatic chromosome number (2n) of caraway: 2n=20; 2Am+sac+4Am++2Am-+6Bsm++2Bsm-sac+4Csa+ (Figure 1d-1f) metaphase, karyotype and ideogram for the chromosomes arms ratio. Due to the relative length shows, the largest scored 13.40 and 7.30 is smallest length (Table 2), these results in the same line with the results of El Alaoui-Faris [30]. He also exhibited that the chromosomal formula was 2n=20=6Am+10Bsm+4Csa and in different at Romania populations was 2n=20=16Am+4Bsm, at Iranian populations 2n=20=4Am+8Bsm+8Csa and the last US populations was 2n=20=10Am+6Bsm+4Csa.

Coriandrum sativum L. (Apiaceae)

Results displayed that the somatic chromosome number (2n) of coriander: 2n=22=4Am-sac+2Bsm++2Bsm-+2Csa+sac+6Csa++6Dt (Figure 1g-1i) metaphase, karyotype and ideogram for the chromosomes arms ratio. The total relative length been between 12.07 in largest length and 5.58 is shortest length (Table 3), and characterized by percentage a satellite pair among the four of metacentric

Average							
Chr. No.	**p+q (TL)**	**q**	**p**	**RL%**	**r**	**CI%**	
1	6.24	3.69	2.55	13.40	1.45	40.84	SM+
2	5.88	2.97	2.91	12.63	1.02	49.48	M+
3	5.19	2.94	2.26	11.15	1.30	43.45	SM+
4	4.88	3.12	1.76	10.49	1.77	36.12	SM-
5	4.57	3.00	1.57	9.81	1.90	34.44	SA+
6	4.43	3.05	1.39	9.52	2.20	31.28	SA+
7	4.22	2.14	2.08	9.06	1.03	49.19	M+
8	4.02	2.32	1.69	8.63	1.37	42.16	SM+
9	3.73	1.96	1.77	8.01	1.10	47.54	M+
10	3.40	1.80	1.60	7.30	1.13	46.96	M-

Table 2: Mean average of Karyomorphological for Caraway species.

Average							
Chr. No.	**p+q (TL)**	**q**	**p**	**RL%**	**r**	**CI%**	
1	11.21	7.46	3.75	12.07	1.99	33.47	SA+
2	10.49	7.53	2.96	11.30	2.55	28.20	SA-
3	9.94	6.67	3.27	10.70	2.04	32.92	SA+
4	9.39	5.06	4.33	10.11	1.17	46.11	M-
5	9.07	5.41	3.66	9.77	1.48	40.34	SM+
6	8.37	4.48	3.88	9.01	1.15	46.41	M-
7	8.00	5.63	2.37	8.61	2.37	29.63	SA-
8	7.65	4.72	2.92	8.23	1.62	38.23	SM-
9	7.10	7.10	0.00	7.64	0.00	0.00	T
10	6.48	6.48	0.00	6.97	0.00	0.00	T
11	5.18	5.18	0.00	5.58	0.00	0.00	T

Table 3: Mean average of Karyomorphological for Coriander species.

chromosomes and two of sub-acrocentric chromosomes. El Alaoui-Faris [30] reported that the Libyan coriander, chromosomal metaphase showed the following chromosomes formula 2n=22=6Cst+16Dt from two different populations and characterized by presence of a satellite pair among the sub acrocentric chromosomes, in Hungary showed in two different populations to 2n=22=8Cst+14Dt, which is similar to his result as the population at Argentina and Poland too while it showed 2n=22=6Cst+2Cst-t+14Dt as they had doubt about two of centromeric place and last population were in United States and Japan which have another different formula 2n=22=2Bsm+14Cst+6Dt. While Ahmed [1] reported that the coriander formula was 2n=1Bsm+12Csa+8Dt, and Pramanik [33] showed a different chromosomes formula 2n=12Am+10Bsm.

C-banding analysis

Results showed standard for banding on haploid chromosome for the three species individually, in cumin showed 17 banding distributed on haploid chromosome as shown in Table 4, 23 bands on caraway as shown in Table 5 and 43 bands on coriander as shown in Table 6 and Figure 2. Also, cumin had two centromeric bands (3p11.4 and 6q11.5) and two sub-centromeric bands (3q12.4 and 7q12.4) and 3 sub-telomeric bands (2p14.4, 6q15.4 and 7q16.3) (Figure 2). Caraway showed five sub-centromeric bands (1q12.2, 2q12.3, 3q12.3, 6q12.4 and 7q12.3) and three sub-telomeric bands (2p14.3, 4p14.2 and 6q15.3) (Figure 2). Coriander displayed two centromeric bands (9q11.2 and 10q11.2),

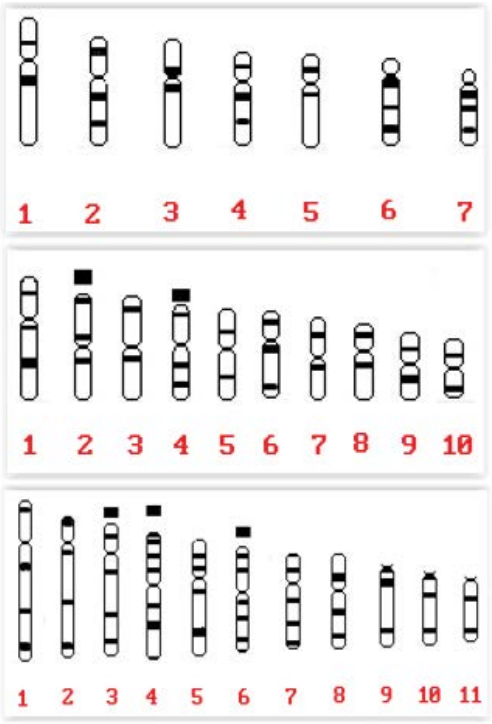

Figure 2: Haploid metaphases, the ideogram of C-banding for cumin-7 chromosomes, caraway-10 chromosomes and coriander 11 chromosomes.

Species	Chr. No.	Bands
Cumin	Chr. 1	1p12.2 1q13.5
	Chr. 2	2p14.4 2q13.4, 2q16.3
	Chr. 3	3p11.4 3q12.4
	Chr. 4	4p13.2 4q12.4, 4q15.3
	Chr. 5	5p12.3 5q12.2
	Chr. 6	6q11.5, 6q13.2 6q15.4
	Chr. 7	7q12.4, 7q14.3 7q16.3

Table 4: Chromosomes number and bands of Cumin species.

Species	Chr. No.	Bands
Caraway	Chr. 1	1p21.1 1q12.2, 2q21.5
	Chr. 2	2p12.3, 2p14.3 2q12.3
	Chr. 3	3p13.3 3q12.3
	Chr. 4	4p14.2 4q13.3, 4q15.3
	Chr. 5	5p13.2 5q14.2
	Chr. 6	6p13.3 6q12.4, 6q15.3
	Chr. 7	7p13.3 7q12.3
	Chr. 8	8p13.3 8q12.3
	Chr. 9	9q13.2, 9q13.4
	Chr. 10	10p12.2 10q13.3

Table 5: Chromosomes number and bands of Caraway species.

Species	Chr. No.	Bands
Caraway	Chr.1	1p14.3 1q14.5, 1q23.3, 1q32.5
	Chr. 2	2p13.5 2q12.3,2q22.3, 2q32.4
	Chr. 3	3p14.3 3q13.3, 3q22.3, 3q32.3
	Chr. 4	4p13.3, 4p16.3, 4p22.3, 4p23.2 4q13.3, 4q22.5
	Chr. 5	5p12.3, 5p14.3 5q12.3, 5q15.5
	Chr. 6	6p13.3, 6p15.3 6q12.3, 6q14.3, 6q17.3
	Chr. 7	7p12.3, 7p14.1 7q13.3, 7q15.3, 7q17.3
	Chr. 8	8p12.5 8q13.4, 8q15.3
	Chr. 9	9q11.2, 9q13.5, 9q15.3
	Chr. 10	10q11.2, 10q13.3, 10q15.3
	Chr.11	11q13.3, 11q16.3

Table 6: Chromosomes number and bands of Coriander species.

four sub-centromeric bands (2q12.3, 5p12.3, 6q12.3 and 9q13.5), two telomeric bands (2p14.3 and 4p23.2), and six sub-telomeric bands (1p14.3, 2q32.4, 4p22.3, 6p15.3, 6q17.3 and 7q17.3). The total bands for cumin, caraway and coriander individually as haploid chromosome distributed loci on the chromatin arms (Tables 4-6) [34].

ISSR analysis

15 ISSR primers (Table 7) were tested for DNA analysis of the three species and were of good quality and all of them yielding polymorphic amplification products and based on the clear scored band pattern

Primers	Size of amplified fragments (bp)	Monomorphic bands	Polymorphic bands	Poly %	Negative Unique bands	Positive Unique bands	Total
P2	496 -1764	1	12	92.3	3	9	13
P5	605 -1414	2	9	81.8	0	9	11
P12	461- 2229	2	12	85.7	4	8	14
P22	395 -1036	1	8	88.9	2	6	9
P24	607-1832	0	7	100	3	4	7
P27	738-2579	1	12	92.3	4	8	13
P29	461-1389	1	12	92.3	6	6	13
P43	618-1472	0	9	100	2	7	9
P47	681- 2014	3	8	72.7	2	6	11
P48	650-2579	1	14	93.3	1	13	15
P52	726-1414	1	6	85.7	2	4	7
P54	605-1414	1	7	87.5	2	5	8
P60	408-2555	1	15	93.8	4	11	16
P61	585-1414	2	8	80	3	5	10
P62	933-1984	0	6	100	3	3	6
Total Avenge	**395-2579**	**17**	**145**	**1346.4**	**41**	**104**	**162**
		1.1	**9.7**	**89.8**	**2.7**	**6.9**	**10.8**

Table 7: ISSR Primers name, size range of amplified fragments, monomorphic and polymorphic amplicons, polymorphic percentage and unique negative and positive bands for the three species of Apiaceae- cumin, caraway and coriander.

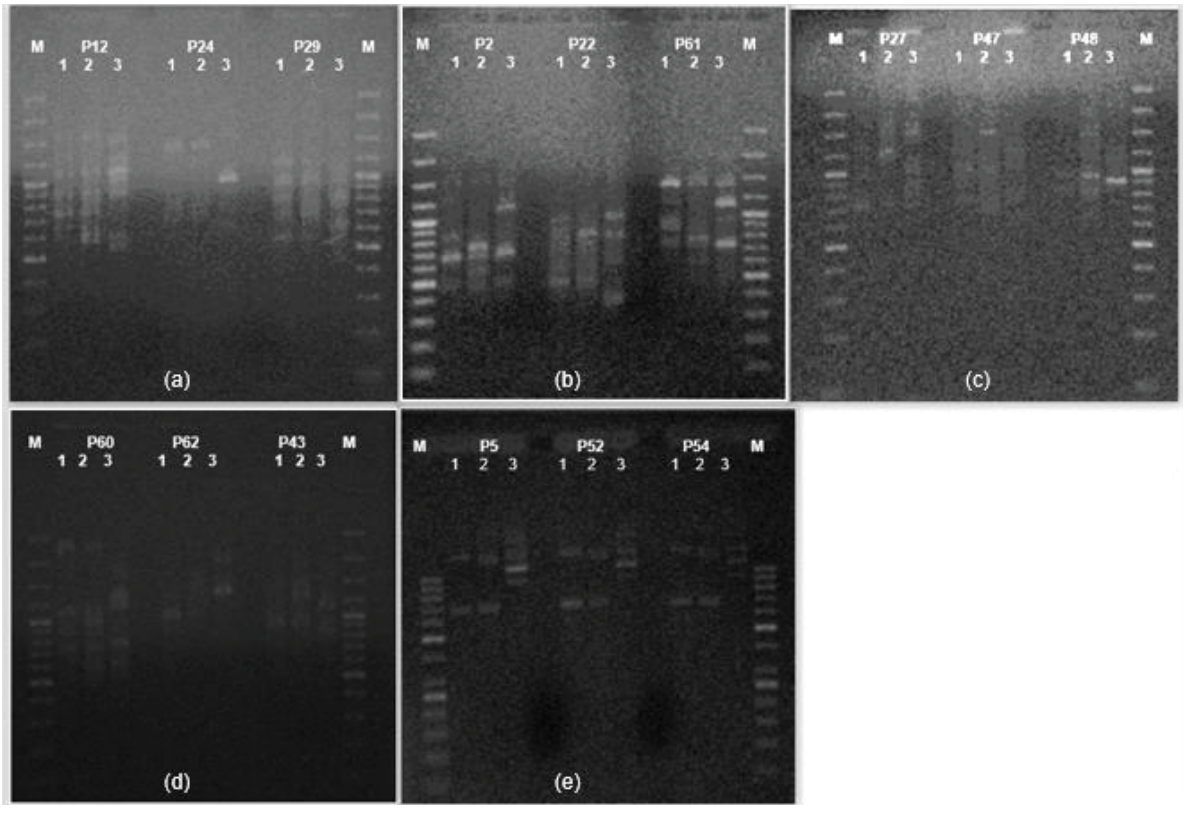

Figure 3: ISSR profiles of cumin, caraway and coriander species as detected by different ISSR Primers.

(Figure 3). The 15 primers chosen for the present study yielded a total of 162 amplified bands, 146 (90%) of which were polymorphic (Table 7). The number of polymorphic bands per primer ranged from 6 to 15 with an average of 10.8 and the percentage of polymorphism per primer ranged from 80% to 100% (Table 7). Taking into consideration all of the 15 primers, the three species had negative bands (8, 5 and 27) for cumin, caraway and coriander respectively and cumin, caraway and coriander had positive unique bands for each one as following 15, 31 and 58 (Table 7). The size of the amplification products ranged from 395 to 2579 bp. The total numbers of scored bands were 162. The highest number of polymorphic bands was obtained with primers, P60 and P48 generated 16 and 15 polymorphic bands with 93% polymorphis. While primer P61 showed low level of polymorphism (80%) [19], stated that using ISSR is efficient for identifying DNA polymorphism in coriander [21], studied the genetic diversity of forty nine cumin ecotypes, belong to nine Iranian regional sub-populations were assessed using RAPD

markers, and twenty three RAPD markers were used for diversity assessment, in which 21 showed polymorphism [35]. Also, estimated genetic diversity of caraway using RAPD-PCR among starting materials and breeding strains, a collection of 17 accessions from botanical gardens in Europe, two cultivars and four own breeding strains. Newly studied genetic diversity of 30 cumin genotypes and five other species of Apiaceae through SSRs markers.

The scored data were analyzed using Dice coefficient to estimate the genetic relationships. Results are in the similarity matrix (Table 7). From the similarity matrix, the highest genetic similarity coefficient was 0.61 between cumin and caraway species. The lowest genetic similarity coefficient was 0.29 between coriander and cumin and caraway. The similarity matrix was used to construct a dendrogram using UPGMA method. The dendrogram (Figure 3) was divided into 2 main clusters at 61% similarity, with 2 species falling in one cluster and the other 1 accession in the first cluster. The second cluster was further sub divided into 2 sub-groups. Both of researchers [21,35] estimated value of genetic distance of cumin using RAPD markers and the value of similarity level of 0.43 and ranged from 0.22 to 0.67 respectively. Recently reported genetic distance of 30 cumin genotypes and five other species of Apiaceae through SSRs markers [36].

AFLP analysis

Using 5 combination primers to perform the results of amplified bands on this study, showed total number of amplified fragments 168 bands (Table 8). Meanwhile the total number of polymorphic bands were 129 with 78 percentage and 37 monomorphic bands with 22 percentage. The average number of bands per primer were 33.6, therefore the average number of polymorphic bands per primer were 25.8. The combination had a wide range of size of fragments DNA ranged between (100-777) with polymorphic bands average 26.2 given 77.2% in percentage; grouped to 69 positive unique bands, in average per primer 13.8 and 62 as negative unique bands in average number per primer 12.4 and 37 bands in monomorphic bands with 22.8%. The results showed that the highest fragment wide bands in size were the E-AAG/M-CAC which generated the highest number of amplicons 59 bands in total sizing ranged from (192-777), the polymorphic bands were 50 (29 positive unique bands and 21 negative unique bands) with percentage of polymorphism reaching as high 84.7% and 9 monomorphic bands; While the lowest number of amplicons were achieved by primer combinations E0AAC/M-CAC and E-AAG/M-CAA were given the total number of bands 23 ranged from 100-628 and 117-628, with total number of polymorphic bands 16 (7 positive unique bands and 9 negative unique bands) with polymorphism percentage of 69.6% and 7 monomorphic bands with 30.4% and 20 polymorphic bands (12 positive unique bands and 8 negative unique bands) with 87% and 3 monomorphic bands with 13%) while the other combinations showed that the total number of bands 37 in combination E-AAC/M-CAA dividing to polymorphic bands 25 bands (12 positive unique bands and 13 negative unique bands) with polymorphism percentage of 67.6% and 12 monomorphic bands with 32.4%. Last combination E-AGC/M-CAT showed the total number of bands were 26 dividing polymorphic bands were 20 (9 positive unique bands and 11 negative unique bands) with polymorphism percentage of 76.9% and 6 monomorphic bands with 23.1% (Figure 4). The average of mean total showed 33.6 in total bands, polymorphic bands per primer were 26.2 with 77.2%, monomorphic bands were 7.4 with 22.8%, positive bands were 13.8 and negative bands were 12.4 (Table 8). Although only limited support was, generated by biochemical data for an earlier phenotypic

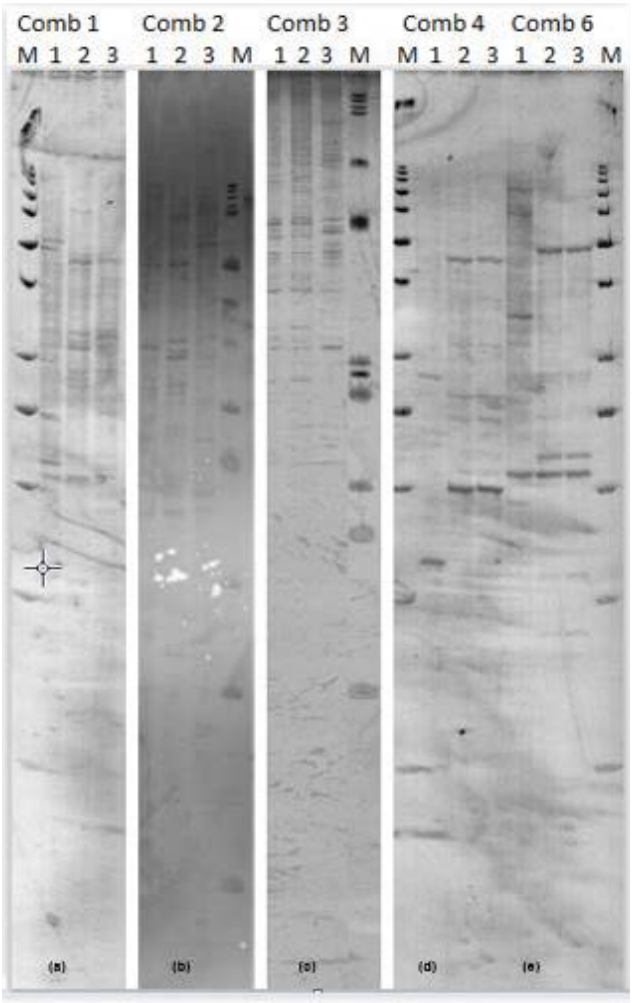

Figure 4: AFLP profiles of cumin, caraway and coriander species as detected by different AFLP primers.

Combination Primers	Size of Amplified Fragments (bp)	Monomorphic bands	Polymorphic bands	Poly %	Unique Negative	Unique Positive	Total
E-AAC/M-CAA	106-545	12	25	67.6	13	12	37
E-AAC/M-CAC	100-628	7	16	69.6	9	7	23
E-AAG/M-CAA	117-628	3	20	87.0	8	12	23
E-AAG/M-CAC	192-777	9	50	84.7	21	29	59
E-AGC/M-CAT	138-728	6	20	76.9	11	9	26
Total	**-**	**37**	**131**	**385.8**	**62**	**69**	**168**
Average	**100-777**	**7.4**	**26.2**	**77.2**	**12.4**	**13.8**	**33.6**

Table 8: AFLP Primers name, size range of amplified fragments, monomorphic and polymorphic amplicons, polymorphic percentage and unique negative and positive bands for the three species of Apiaceae-cumin, caraway and coriander.

intraspecific classification, it should be possible to combine the results from these phenotypic, biochemical, and molecular characterizations and use them to refine the current botanical classification in order to develop a utilitarian classification for coriander populations by using the ICNCP's Group Concept [18].

Combined analysis for ISSR and AFLP

Combined analysis using 20 primers (15 ISSR+5 AFLP) displayed total bands generated 330 bands (162 ISSR and 168 AFLP), while the total number of polymorphic bands were 274 bands with percentage 83.75% and monomorphic bands were 54. In addition, the average number of bands per primer scored 2.2 and average number of polymorphic bands per primer scored 17.75 (Table 9), which is to confirm that the similarity cluster between cumin and caraway is more close to each other more than the coriander cluster with the C-banding karyotype results.

S No	Parameter	ISSR	AFLP	ISSR+AFLP
1	Number of primers used	15	5	20
2	Total number of polymorphic bands	145	129	274
3	Total number of monomorphic bands	17	37	54
4	Total number of bands	162	168	330
5	Percentage polymorphism (%)	89.5	78	83.75
6	Average number of bands/primer	10.8	33.6	22.2
7	Average number of polymorphic bands/primer	9.7	25.8	17.75

Table 9: Comparative result showing different markers (ISSR, AFLP and ISSR+AFLP) obtained from three species.

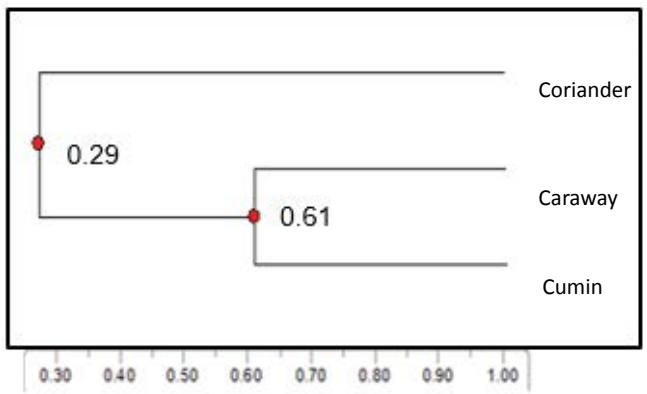

Figure 5: UPGMA dendrogram showing the genetic relatedness of cumin, caraway and coriander species based on ISSR data.

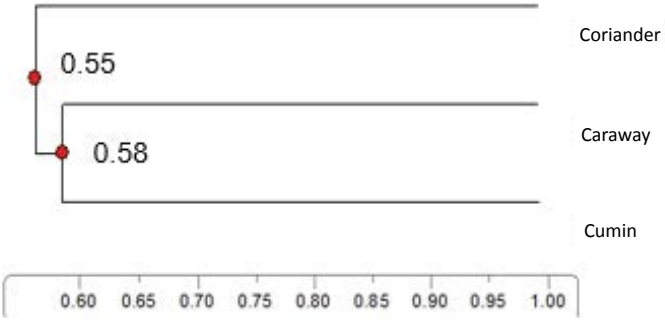

Figure 6: UPGMA dendrogram showing the genetic relatedness of cumin, caraway and coriander species based on AFLP data.

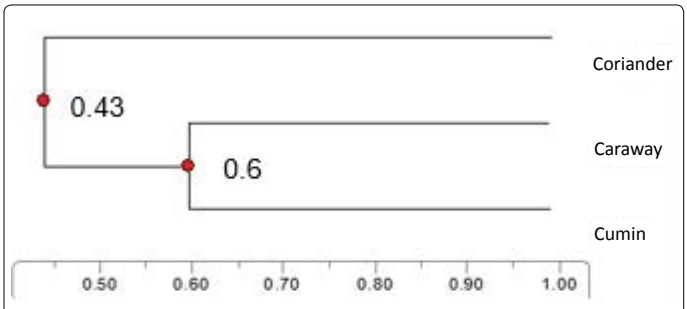

Figure 7: UPGMA dendrogram displaying the genetic similarity estimates according to combined data (ISSRs and AFLPs) within the three species cumin, caraway and coriander.

Conclusion

Through the cytogenetic and molecular level for characterization on this three species genetics resources approved the relative distance between then regarding to C-banding Karyotype, ISSR, AFLP and the combination between ISSR and AFLP markers. The similarity between cumin and caraway were more closely to each other more than coriander while its shows due to ISSR markers 0.61, AFLP markers 0.58 and the combination 0.60, when the result of C-banding shows 17 bands in cumin and 23 in caraway but the result in coriander were 43 bands and the distance between the group of cumin and caraway to the coriander were 0.29 in ISSR, 0.55 AFLP and for the combination shows 0.43 (Figures 5-7).

Acknowledgments

I am thankful for the team of National Gene Bank at Agriculture Research Center of Egypt for the support given to this study through affording all the facilities and the supplies and laboratories.

Significance Statements

This study is helping for making a full Genetics profile markers for the three species genetics resources and a conservation concerning to the National Gene Bank of Egypt concerning its belonging to Egypt and under the full authority of the Egyptian government through making a characterization of DNA level through Cytogenetics C-banding Karyotype and Molecular level by ISSR and AFLP markers.

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