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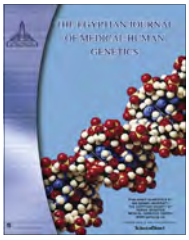

ORIGINAL ARTICLE

Molecular fingerprinting of the Egyptian medicinal plant *Cocculus pendulus*

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Abstract *Background and aims:* The genus *Cocculus* belongs to the family *Menispermaceae* which comprises about 35 species of shrubs or woody climbers. Only one species, *Cocculus pendulus* is found in Egypt. This plant is reported to have good medicinal values in traditional system of medicine. Despite the wide occurrence of *C. pendulus* in the Egyptian deserts, attention was paid only to its distribution and morphological description ignoring the biochemical constitution, the genome makeup and environmental aspects which are not given due consideration. Since no information about the genome of *C. pendulus* is available, the current study deals with molecular investigation of *C. pendulus* expressed by DNA fingerprinting of the young leaves of this plant using amplified fragment length polymorphism (AFLP) technique with four primer combinations.

The obtained results revealed a total of 228 bands with an average of 57 bands for each primer combination, of which 61 bands were polymorphic (26.8%) ranging in size from 59 to 570 bp. The number of amplicons/primer pairs ranged from 48 (E-AGG/M-CAC) to 72 (E-AAC/M-CAG) while the number of polymorphic amplicons varied from 13 to 21 with polymorphism percentage of 22.03–29.17%. Thus the average number of polymorphic fragments/combinations was 15. In this regard, the combination E-AGG/M-CAC was more efficient as confirmed by computing the discriminating power (D) of all primer combinations. Also, the AFLP marker gives a complete informative and highly discriminative picture about *C. pendulus* as shown by (PIC = 0.99).

1. Introduction

The genus *Cocculus* belongs to the family *Menispermaceae* which comprises about 35 species of shrubs or woody climbers. *Cocculus pendulus* is perennial, non succulent glycophyte. The members of this genus are used in the traditional system of medicine for various ailments Kirtikar and Basu [1]. Only one species, *C. pendulus* is found in Egypt. This plant is a

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scandent shrub, growing particularly along rocks and in the dry mountainous areas of the Eastern desert. Despite the wide occurrence of *C. pendulus* in the Egyptian deserts, attention was paid only to its distribution and morphological description ignoring the biochemical constitution, the genome makeup and environmental aspects which are not given due consideration.

The leaves and stems of the plant are possibly used in the indigenous system of medicine for the treatment of intermittent fever and biliousness Oliver-Bever [2], febrifuge, vermifuge, antipyretic, diuretic, cholagogue, menstrual cycle troubles, internal parasites antiblennorrhagic and rheumatic pain. Hypotensive and anticancer activity have been attributed to the alkaloid fractions from leaves and stems. Furthermore, other information demonstrated the effectiveness of this folk medicine to diminish the symptoms of Alzheimer's disease and Atta-Ur-Rahman et al. [3] and Atia-tul-Wahab et al. [4] the acquired immunodeficiency syndrome (AIDS) Singla et al. [5].

Due to the lack of pertinent information concerning the molecular aspects of this plant species in Egypt and the stress of studies abroad on the active ingredients, the current investigation was planned after the acceptance of the Desert Research Center to give an insight on the molecular fingerprint of this plant species and provide information about its genome.

2. Materials and methods

2.1. The study area

In the Eastern desert, Gebel Elba near the Red Sea is among the notable mountains because of its unique attributes and is considered the most important area for flora and fauna in Egypt. It lies in the extreme South-East corner of Egypt, known as the Halaib Triangle situated between Egypt and Sudan. Therefore, the study area includes two of the most relevant wadis, Yahmeib and Izab, Fig. 1 in which *C. pendulus* is grown intensively.

2.2. Field work

The concerned plant *C. pendulus* was identified (Fig. 2) and authenticated in February, 2009, then the fresh young leaves were collected and transferred into liquid nitrogen for molecular analysis.

2.3. Laboratory analyses

2.3.1. DNA fingerprinting

Two plant samples (young leaves) of *C. pendulus* grown in two wadis (Yahmeib and Izab) in Gebel Elba were collected and used to construct a DNA fingerprint using AFLP technique. DNA was successfully isolated using a DNeasy Plant Mini Kit (Qiagen, Santa Clarita, CA). The AFLP analysis was conducted using the AFLP® Analysis System II (Invitrogen, USA) (Cat. No. 10483-022), according to the manufacture's protocol. Selective amplification was carried out using four primer combinations between EcoRI + 3 extension bases and MseI + 3 extension bases (Table 1).

3. Results and discussion

The land attributes usually correspond to three main factors: soil site, climate and natural vegetation. The climatic data revealed that the area under study is characterised by long hot rainless summer and short rainless winter. According to the aridity index (<0.05), United Nations Environment Programme (UNEP) [6], the study area is hyper arid. Soil analysis indicates that soils beneath *C. pendulus* grown in the studied wadis are sandy, slightly to moderately saline, impoverished in nutritive elements and non-polluted, El-Shaimaa [7].

A number of molecular tools and procedures are employed to establish DNA fingerprinting profiles, of which amplified fragment length polymorphic DNA analysis (AFLP) is a useful procedure especially when very little information is known about the genome of the plant under study, Meudt and Clarke [8].

AFLP described by Vos and Zabeau [9] is a highly sensitive and reproducible method for detecting polymorphisms in DNA of different genomic regions simultaneously.

AFLP not only has higher reproducibility, resolution, and sensitivity at the whole genome level compared to other techniques, Mueller and Wolfenbarger [10] but also has the capability to amplify between 50 and 100 fragments at one time. The variations in DNA sequences are exploited by the AFLP technology such that "fingerprints" of particular genotypes can be routinely generated. As a result, AFLP has become extremely beneficial in the study of taxa where much is still unknown about their genomic makeup, Vos et al. [11], Lin et al. [12], Majer et al. [13], Buhariwalla et al. [14] and Laurentin and Karlovsky [15].

The obtained AFLP fingerprint results, Fig. 3 and Table 2, reveal a total of 228, bands with an average of 57 bands for each primer combination, of which 61 bands were polymorphic (26.8%) ranging in size from 59 to 570 bp. The number of amplicons/primer pairs ranged from 48 (E-AGG/M-CAC) to 72 (E-AAC/M-CAG) while, the number of polymorphic amplicons varied from 13 to 21 with polymorphism percentage (22.03–29.17%). Thus, the average number of polymorphic fragments per combination was 15.

Also, there are remarkable differences in the size range of amplicons between primer combinations, where the primer combination E-AAC/M-CAG produced large amplified fragments up to 570 bp while the amplified fragments obtained from the other combinations were lower than this size (462–483 bp). An account on the primer combinations including variations within and between combinations encountered is given hereafter (Table 3).

3.1. Combination of E-AAC/M-CAG (1/3)

In both plant samples, this primer combination produced a total of 72 bands (monomorphic and polymorphic) ranging in size from 60 to 570 bp with the polymorphism percentage of 29.2% (Table 3). For convenience, a number of 51 common bands (monomorphic) were shared between the two plant samples from 65 to 553 bp. In addition, 21 polymorphic bands appear. Plant sample No. 1 representing *C. pendulus* grown in wadi Yahmeib displayed a total number of 62 bands. Likewise; the plant sample No. 2 from wadi Izab had a number of 61 bands per primer pair.

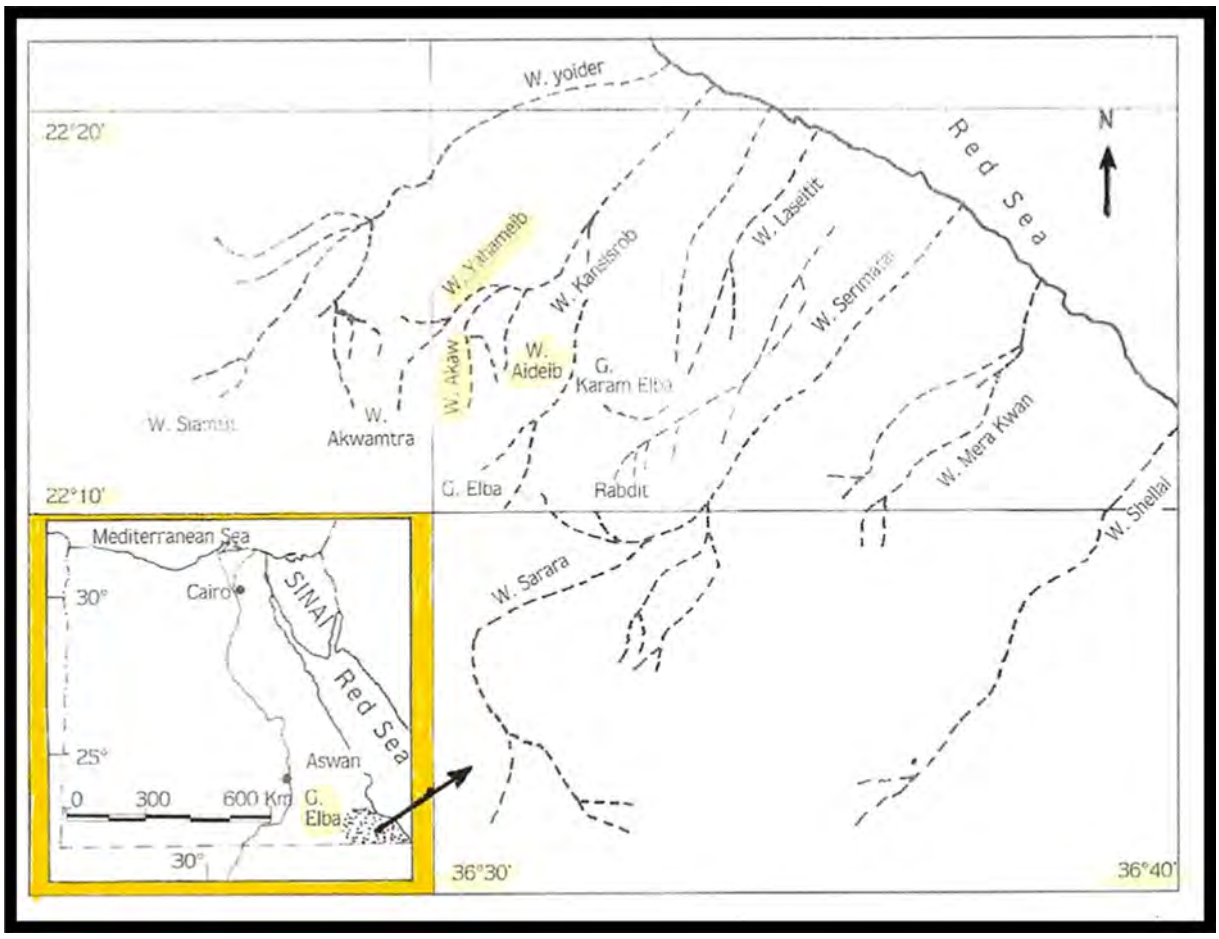

Figure 1 A map showing the sampling sites (wadi Yahmeib and wadi Izab) in Gebel Elba area, South Egypt.

Figure 2 Collection of *Cocculus pendulus* from two wadis in Gebel Elba area.

3.2. Combination of E-AGG/M-CAC (8/2)

Using this primer combination, a total of 48 bands ranging from 60 to 483 bp were detected. Apart from these bands, only 14 polymorphic bands were detected, thus leading to a polymorphism percentage of 29.2% (Table 3). A number of 34 unique bands (monomorphic) were observed with different sizes from 60 to 483 bp. Here it is worth to note that the two plant samples revealed a great resemblance in the same number of amplified fragments (41) with this primer combination regardless of polymorphism percentage.

3.3. Combination of E-AGG/M-CAG (8/3)

This primer combination exhibited a total of 49 bands ranging between 59 and 462 bp (Table 3). Out of which, only 13 polymorphic bands were recorded, thus polymorphism percentage is 26.5% while 36 common (monomorphic) bands

Table 1 A list of the AFLP primers and adapter sequences.

Table 1 A list of the AFLP primers and adapter sequences.

Name	Sequence (5′–3′)
*Mse*I forward adapter	GACGATGAGTCCTGAG
*Mse*I reverse adapter	TACTCAGGACTCAT
*Eco*RI forward adapter	CTCGTAGACTGCGTACC
*Eco*RI reverse adapter	AATTGGTACGCAGTCTAC
*Mse*I primer core region (M)	GATGAGTCCTGAGTAA
*Mse*I pre-selective primer (M + 1)	M + C
*Mse*I selective primers (M + 3)	
M + CAG	GATGAGTCCTGAGTAA**CAG**
M + CAC	GATGAGTCCTGAGTAA**CAC**
M + CAG	GATGAGTCCTGAGTAA**CAT**
M + CTC	GATGAGTCCTGAGTAA**CTC**
*Eco*RI primer core region (E)	GACTGCGTACCAATTC
*Eco*RI pre-selective primer (E + 1)	E + A
*Eco*RI selective primers (E + 3)	
E + AAC	GACTGCGTACCAATTC**AAC**
E + AGG	GACTGCGTACCAATTC**AGG**
E + AGG	GACTGCGTACCAATTC**AGG**
E + AGG	GACTGCGTACCAATTC**AGG**

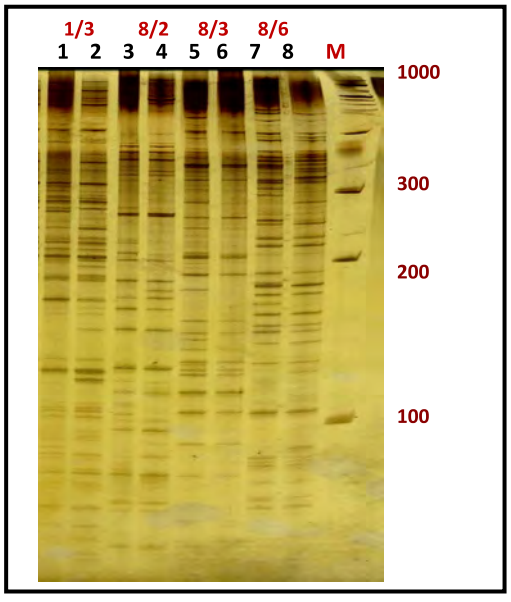

Figure 3 The AFLP fingerprint of *Cocculus pendulus* plant species collected from two sites in Gebel Elba region using four primer combinations (1/3 – 8/2 – 8/3 – 8/6).

were detected in the two plant samples. Aligned with these results, wide variations were observed in the number of amplified fragments obtained among the two plant samples when compared with other primer combination results, where sample No. 1 revealed 48 bands while sample No. 2 showed 37 amplified fragments.

3.4. Combination of E-AGG/M-CTC (8/6)

A total number of 59 amplified fragments were recorded on using this primer pair with a fragment size ranging from 59 to 476 bp (Table 3). Thirteen polymorphic bands were obtained representing 22.03%. Common (monomorphic) bands resulted were 46 amplified fragments. A great similarity was observed in plant sample Nos. 1 and 2 which produced 52 and 53 amplified fragments, respectively.

The previous results revealed that the combination E-AAC/M-CAG exhibited the largest number of amplified fragments (62) in sample No. 1 collected from Wadi Yahmeib. In contrast, combination E-AGG/M-CAG produced the smallest number of amplified fragments (37) in sample No. 2 collected from Wadi Izab. This discrepancy may be rendered to the C and G composition of the selective nucleotides. In general, the more Cs and Gs employed as selective nucleotides in the

amplification primers, the fewer DNA fragments amplified. Also, the former combination produced the largest number of monomorphic bands (51) in both plant samples while combination E-AGG/M-CAC had the smallest number of unique bands (34). In general, the total number of bands as well as the number of polymorphisms depend on the crop, variety, complexity of genome and primer pair used.

Based on the foregoing results, one can conclude that the monomorphic bands resulting from each primer pair are considered as species specific marker for the *C. pendulus* plant while the polymorphic bands may be rendered to the nature of the plant as a dioecious where cross pollination may affect the genetic characters and information in plant especially in open environment. Also, one can conclude that the

Table 2 The AFLP–PCR fragments of the two individuals of *Cocculus pendulus* from two wadis in Gebel Elba using four primer combinations.

Band No.	Primer combination											
	AAC /CAG			AGG /CAC			AGG /CAG			AGG / CTC		
	Size (bp)	Lane 1	Lane 2	Size (bp)	Lane 3	Lane 4	Size (bp)	Lane 5	Lane 6	Size (bp)	Lane 7	Lane 8
1	570	0	1	483	1	1	462	1	1	476	1	0
2	561	1	0	416	0	1	448	1	1	469	1	1
3	553	1	1	398	1	1	442	1	0	448	1	1
4	537	1	1	386	1	1	435	1	1	442	1	1
5	513	1	1	375	1	1	422	1	1	416	1	1
6	505	1	1	364	1	0	410	1	1	410	1	1
7	498	1	1	358	1	1	380	1	1	404	1	1
8	476	1	1	342	1	0	375	1	1	375	1	1
9	462	1	1	337	1	1	369	1	0	369	0	1
10	448	1	0	327	1	1	358	1	1	364	1	1
11	442	1	1	318	1	1	348	1	1	358	1	1
12	429	1	1	295	1	1	342	1	1	348	1	1
13	404	0	1	290	1	1	332	1	1	337	1	1
14	398	1	1	282	1	1	323	1	1	332	1	1
15	375	1	1	270	1	0	318	1	1	323	0	1
16	369	1	0	258	1	1	308	1	0	318	1	1
17	358	1	0	222	1	1	299	1	1	313	1	1
18	353	1	1	219	1	1	278	1	1	308	1	1
19	337	1	1	215	1	0	254	1	1	304	1	1
20	332	1	0	212	0	1	239	1	1	295	1	1
21	313	1	1	206	1	0	229	1	0	290	1	1
22	308	1	0	203	1	1	222	1	0	286	0	1
23	295	1	1	197	1	1	215	1	0	278	1	1
24	290	1	1	175	1	1	206	1	1	270	1	1
25	286	1	1	164	0	1	203	1	1	266	1	0
26	282	1	1	160	1	1	200	1	1	258	1	1
27	278	1	1	148	1	1	194	1	1	250	1	1
28	270	1	1	108	1	1	188	1	0	246	1	1
29	266	1	0	99	1	1	183	1	1	225	1	1
30	254	1	1	96	1	1	167	1	0	215	1	1

31	250	1	0	92	1	1	162	1	0	206	1	1
32	243	0	1	91	1	1	150	1	0	183	1	1
33	239	1	1	90	1	1	140	1	1	175	1	0
34	225	1	1	89	1	1	106	0	1	172	0	1
35	219	1	1	85	1	1	101	1	1	169	1	0
36	212	1	1	82	0	1	89	1	1	162	1	1
37	203	1	1	80	1	1	82	1	1	153	1	0
38	200	1	1	79	1	1	79	1	1	144	1	1
39	197	1	0	77	1	1	78	1	1	97	1	1
40	191	0	1	76	1	0	75	1	1	95	1	1
41	183	1	1	75	0	1	71	1	1	91	1	1
42	180	1	1	74	1	0	68	1	1	90	1	1
43	177	1	1	72	0	1	67	1	1	88	1	1
44	157	1	1	71	1	1	66	1	0	87	1	1
45	150	1	1	70	0	1	65	1	1	86	1	1
46	146	1	1	69	1	1	63	1	1	85	1	1
47	114	0	1	67	1	1	61	1	0	82	0	1
48	112	0	1	60	1	1	60	1	1	76	1	1
49	111	0	1				59	1	1	75	1	1
50	106	0	1							74	0	1
51	102	1	1							73	1	1
52	97	1	1							72	0	1
53	92	1	1							71	1	1
54	91	1	1							69	1	1
55	89	1	1							68	1	0
56	88	1	1							66	1	1
57	85	1	1							62	1	1
58	84	1	1							60	1	1
59	82	1	0							59	1	1
60	81	1	1									
61	80	1	1									
62	79	1	1									
63	78	1	0									
64	76	1	1									
65	75	1	1									
66	74	1	1									
67	73	1	1									
68	69	1	1									
69	68	0	1									
70	67	1	1									
71	65	1	1									
72	60	0	1									

Table 3 Selective nucleotides of AFLP primer combinations, total number of bands and polymorphic bands.

Primer comb.	Selective nucleotides		Number of bands			% of polymorphism
	EcoRI	MseI	Total	Polymorphic	Monomorphic	
1/3	AAC	CAG	72	21	51	29.17
8/2	AGG	CAC	48	14	34	29.17
8/3	AGG	CAG	49	13	36	26.5
8/6	AGG	CTC	59	13	46	22.03
Total			228	61	167	
Average			57	15	41	26.72

combination E-AAC/M-CAG was more efficient and informative in producing large numbers of amplicons and high levels of polymorphisms compared to the other combinations. Furthermore, one can recommend this combination since it gives reliable information about the genome of *C. pendulus* (Table 3). The large number of fragments gives an estimate of variation across the entire genome, thus gives a good general picture of the level of genetic variation of the studied

Table 4 The genetic similarity between *Cocculus pendulus* samples using two different similarity coefficients.

Primer combination	Genetic similarity (Dice)%	Genetic similarity (Jaccard) %
AAC/CAG	**82.9**	**70.8**
AGG/CAC	**82.9**	**70.8**
AGG/CAG	**84.7**	**73.5**
AGG/CTC	**87.6**	**78.00**

Table 5 Information obtained from AFLP using four primer combinations.

Parameters and their abbreviations		AFLP
Number of primer combinations	U	4
Number of non polymorphic bands	n_{np}	167
Number of polymorphic bands	n_p	61
Average number of polymorphic bands/assay unit	n_p/U	0.023952
Number of loci	L	228
Number of loci/assay unit	n_u	114
Total number of effective alleles	N_e	285.4522
Total banding pattern	Bp	12
Effective number of patterns/assay unit	P	3
Min. range of PIC value	PIC	0.983653
Max. range of PIC value	PIC	0.989149
Average of PIC	PIC	0.994444
Fraction of polymorphic loci	β	0.267544
Assay efficiency index	A_i	71.36305
Effective multiplex ratio	E	30.5
Marker index	MI	30.33053

organism [10]. In other words, the superiority of AFLP over RFLP, RAPD, or ISSR analyses is clearly demonstrated since AFLP promotes assessing of the genetic background and produces a much higher percentage of polymorphic bands per analysis as confirmed by Vos et al. [11], Lin et al. [12] and Archak et al. [16].

In fact, most AFLP fragments correspond to unique positions on the genome, and, hence can be exploited as landmarks in genetic and physical maps, each fragment being characterised by its size and its primers required for amplification. Consequently, the AFLP technique is not simply a fingerprinting technique, it is an enabling technology in genome research, because it can bridge the gap between genetic and physical maps.

3.5. Data analysis

Establishing accurate genetic similarity and dissimilarity between individuals is an essential and decisive point for clustering and analysing inter and intra population diversity Dalirsefat et al. [17]. The choice of an appropriate coefficient of similarity is very important to evaluate clustering, true genetic similarity between individuals, analysing diversity within populations and studying relationships between populations, because different similarity coefficients may yield conflicting results Kosman and Leonard [18].

In the current study, two similarity coefficients were used to calculate the genetic similarity between each pair of individuals (Sorensen-Dice and Jaccard coefficients) via the SPSS (Statistical Package for Social Science) computer program ver.17.0. It was found that there are big differences in similarity estimates with the two coefficients where Dice Coefficient presented 82.9% between the two individuals with both primer combinations AAC/CAG and AGG/CAC (Table 3) while the combination AGG/CAG exhibited a similarity of 84.7%. The combination AGG/CTC recorded the highest similarity between the two plant samples (87.6%). On the other hand, the similarities between individuals by using Jaccard coefficient were lower than those obtained by using Dice coefficient. The genetic similarities ranged from 70.8% to 78.00% (Table 4) where the primer combination AGG/CTC recorded the highest similarity between the two individuals while both the primer combinations AAC/CAG and AGG/CAC resulted in the smallest similarity 70.8%. The combination AGG/CAG was between those extremes (73.5%).

These results are in agreement with Dalirsefat et al. [17] where they concluded that the Sorensen-Dice coefficient of similarity is frequently referred to as the measure of genetic similarity of Nei and Li [19]. The related values of Jaccard's similarity are always smaller than those of the Sorensen-Dice similarity and the simple matching coefficient. In contrast,

Table 6 The calculated discrimination power of the four primer combinations.

Primer combination	Discriminating power (D)
E-AAC/M-CAG	0.45
E-AGG/M-CAC	0.47
E-AGG/M-CAG	0.41
E-AGG/M-CTC	0.36
AFLP technique	0.42

values of the Sorensen-Dice similarity may be greater or smaller than the related values of the simple matching coefficient. These authors added that the coefficients which exclude negative co-occurrences have more justification for being used when closely related organisms are being compared. Thus, it should be possible to use Jaccard or Sorensen-Dice to obtain satisfactory results when the organisms are closely related.

Also, the polymorphism information content (PIC) values were calculated according to Smith et al. [20] (Table 5) using the following formula as follows:

$$\mathrm{PIC} = 1 - \Sigma \mathrm{fi}^2$$

where fi^2 is the frequency of the allele. PIC provides an estimate of the discriminatory power of a locus by taking into account, not only the number of alleles but also the relative frequencies of those alleles. PIC values vary from 0 (monomorphic) to 1 (very highly discriminative, with many alleles in equal frequencies). To compare the efficiency of the four primer combinations and also the informativeness of the AFLP marker to give a complete picture about *C. pendulus* plant samples, the parameters estimated for each assay unit are depicted in Table 5.

The previous results revealed that the PIC values are very high, ranging from 0.98 to 0.99 for the primer combinations AGG/CAG and AAC/CAG, respectively with an average of 0.99 for the 4 primer combinations. This proves the efficiency of the primer combination AAC/CAG to clarify the minute variations among *C. pendulus* plant samples and also show the highly discriminative power of AFLP marker for *C. pendulus* plant samples.

Further investigation of data was based on developing a parameter by which the efficiency of a primer (used alone or in combination with others) can be evaluated for the purpose of identification of varieties. To achieve this goal, the discrimination power (D) of each primer was estimated as shown by Tessier et al. [21] where C is the confusion probability, i.e., the probability that two randomly chosen individuals from the sample of a number of varieties have identical banding patterns, then $D = 1-C$ represents the probability that two randomly chosen individuals have different patterns, and thus are distinguishable from one another. For the ith pattern of the given jth primer, present at frequency pi in this set of varieties, the confusion probability Ci is:

$$Ci = \frac{(N_{\mathrm{p}i} - 1)}{N - 1}$$

Thus the discriminating power of the jth primer is equal to:

$$D = 1 - \Sigma_{i=1}^{I} \mathrm{p}i \frac{(N_{\mathrm{p}i} - 1)}{N - 1}$$

These authors also reported that the efficiency of a given primer does not depend only on the number of patterns it generates. For example, even if two primers produce the same number of patterns, they can have very different discriminating powers. On the contrary, two primers with quite a different number of patterns can have similar discrimination powers.

Computing the above equation has resulted in a more efficient use of the primer combination of E-AGG/M-CAC rather than E-AAC/M-CAG in discriminating the individuals of *C. pendulus* plant species (Table 6) which does not coincide well with the previous anticipation in this regard.

References

[1] Kirtikar KR, Basu BD. Indian medicinal plants. 2nd ed, 2nd ed., 4. Rajpur road, Dehradun, India: International Book Distributors; 1993, 2435–2438.

[2] Oliver-Bever B. Medicinal plants in tropical West Africa. Cambridge, U.K.: Cambridge University press; 1986.

[3] Atta-ur-Rahman, Atia-tul-Wahab, Nawaz SA, Choundhary MI. New cholinesterase inhibiting bisbenzylisoquinoline alkaloids from *Cocculus pendulus*. Chem Pharm Bull 2004;52(7):802–6.

[4] Atia-tul-Wahab, Atta-ur Rahman, Choundhary MI. Cholinesterase inhibiting bisbenzylisoquinoline alkaloids from *Cocculus pendulus*. Planta Med 2008;74:1–338.

[5] Singla D, Sharma A, Kaur J, Panwar B, Raghava GPS. BIAdb: a curated database of benzylisoquinoline alkaloids. BMC Pharmacol 2010;10:4.

[6] United Nations Environment Programme (UNEP). World Atlas of Desertification, UNEP Prog., UN, Rome, 1992.

[7] El-Shaimaa S. El-Demerdash. Molecular and biochemical studies on *Cocculus pendulus* from the Eastern Desert. Ph.D. Thesis, Faculty of Sci., Ain Shams Univ., Cairo, Egypt, 2013.

[8] Meudt HM, Clarke AC. Almost forgotten or latest practice? AFLP applications, analyses and advances. Trends Plant Sci 2007;12(3):106–17.

[9] Vos P, Zabeau M. Selective restriction fragment amplification: a general method for DNA fingerprinting. Berlin: Springer; 1993.

[10] Mueller UG, Wolfenbarger LL. AFLP genotyping and fingerprinting. Trends Ecol Evol Amst 1999;14(10):389–94, The Netherlands.

[11] Vos P, Hogers MB, Reijans MR, Vandelee T, Hornes M, Frijters A, et al. AFLP: a new technique for DNA-fingerprinting. Nucleic Acids Res 1995;23(21):4407–14.

[12] Lin JJ, Kuo J, Jin M, Saunders JA, Beard SH, McDonald H, et al. Identification of molecular markers in soybean; comparing RFLP, RAPD and AFLP DNA mapping techniques. Plant Mol Biol Rep 1996;14:156–69.
[13] Majer D, Lewis BG, Mithen R. Genetic variation among field isolates of *Pyrenopeziza brassicae*. Plant Pathol 1998;47:22–8.
[14] Buhariwalla HK, Srilakshmi P, Kannan S, Kanchi RS, Chandra S, Satyaprasad K, et al. AFLP analysis of *Trichoderma* spp. from India compared with sequence and morphological-based diagnostics. J Phytopathol 2005;153:389–400.
[15] Laurentin EH, Karlovsky P. Genetic relationship and diversity in a sesame (*Sesamum indicum* L.) germplasm collection using amplified fragment length polymorphism (AFLP). BMC Genet 2006;7:10.
[16] Archak S, Gaikwad B, Gautam D, Rao V, Swamy M, Karihaloo L. Comparative assessment of DNA fingerprinting techniques (RAPD, ISSR and AFLP) for genetic analysis of cashew (*Anacardium occidentale* L.) accessions of India. Genome 2003;46:362–9.
[17] Dalirsefat SB, Meyer Ads, Mirhoseini SZ. Comparison of similarity coefficients used for cluster analysis with amplified fragment length polymorphism markers in the silkworm, *Bombyx mori*. J Insect Sci 2009:9. Art.71.
[18] Kosman E, Leonard KJ. Similarity coefficients for molecular markers in studies of genetic relationships between individuals for haploid, diploid, and polyploid species. Mol Ecol 2005;14:415–24.
[19] Nei M, Li WH. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 1979;76:5269–73.
[20] Smith JSC, Chin ECL, Shu H, Smith OS, Wall SJ. An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparisons with data from RFLPs and pedigree. Theor Appl Genet 1997;95:163–73.
[21] Tessier C, David J, This P, Boursiquot JM, Charrier A. Optimisation of the choice of molecular markers for varietal identification in *Vitis vinifera* L. Theor Appl Genet 1999;98:171–7.