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Genetic Diversity of some Basil Varieties Estimated using RAPD, SCoT and ISSR Techniques

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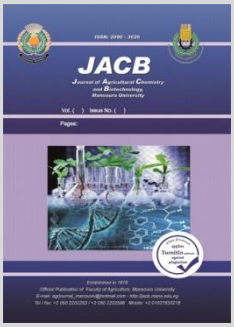

ABSTRACT

Basil *(Ocimum basilicum L.)* belongs to *Lamiaceae* family considered as important medicinal plant, showing several variations in morphology. RAPD, SCoT and ISSR analyses for herb growth among four varieties (Baldy, French, Thai, and Lemon basil) were done. In the present study, the four varieties were characterized using RAPD, SCoT and ISSR markers. A total of 84 major RAPD amplified fragments were produced, out of them 55 (65.48%) were polymorphic and the average of polymorphism percentage was 60.21 %. In SCoT analysis, a total of 79 major SCoT amplified fragments were produced, out of them 51 (94.56%) were polymorphic. . A total of 59 major ISSR amplified fragments were produced, out of them 44 (74.58%) were polymorphic. The polymorphism percentage ranged from 0.00 % (HB14) to 91.6 % (809B). It could be concluded that RAPD, SCoT and ISSR used as effective tools to identify similarity and relationship between the genotypes which are potentially helpful in breeding programs. While this discrimination capability for the RAPD technique confirmed by P%, and Rp values which were 60.21 and 4.55 respectively, compared with the same values for SCoT and ISSR-technique which were 54.83, 38.30, 3.15 and 3.90 respectively. This indicates the high discriminatory potential of using RAPD primers compared with SCoT and ISSR primers. Where, RAPD markers were more discriminating, provided more informative data.

Keywords: sweet basil, DNA fingerprinting, molecular markers (RAPD, SCoT and ISSR).

INTRODUCTION

Basil *(Ocimum basilicum* L.) exists under the Lamiaceae family and grows in tropical and sub-tropical climates (Makri and Kintzios, 2008). The *Ocimum* genus includes about 65 different species of herbs (Pushpangadan and Bardu, 1995). Basil is an important edible herb and essential oil crop that is cultivated and used worldwide (Dube. *et al*., 1989; and Hossain. *et al.*, 2010) and basil essential oil has been widely used in the food industry as a food flavor, in medicine (Simon *et al*., 1990) as an ingredient in oral hygiene and dental preparations, and in perfumes (Kathirvel and Ravi, 2012). Moreover, basil has been claimed to be effective in several medical problems such as anxiety, stomach aches, fever, kidney failure, arthropod bites, sickness, infections, headache, cough, and constipation [(Kathirvel and Ravi., 2012, Lee *et al*., 2005 and Murugan *et al.*, 2007)]. Basil has been used for enhancing milk production in nursing mothers and is used as medication for gastrointestinal disorders (Vieira and Simon, 2000). The morphology, growth habits, color of flowers, leaves, and stems, as well as chemical composition, are all highly variable among the species that make up the genus *Ocimum* (Svecova and Neugebauerov, 2010). Many varieties of sweet basil are found in the world, but French/sweet basil (*Ocimum basilicum* L.) is the most widely grown essential oil crop, grown in many countries. Basil is used as medicinal herbs and spicy in food, while the aromatic properties are determined by the genotype and depend on the main chemical components of essential oil (Telci *et al*., 2006 and Koba *et al*., 2009). Additionally, sweet basil is frequently used in perfumery, cosmetics, and fragrances (Pushpangadan and George, 2012). The medicinal benefits of basil genotype (*O. sanctum* L. and O *tenuifolium* L.) include the treatment of diarrhea, migraines, stress, nausea,

abdominal cramps, insomnia, influenza, low digestive function, and colds and coughs. *O. africanum* Sims. (Lemon basil/tulsi) plant has stimulant, diaphoretic, and carminative effects (Cohen, 2014). Which is a reliable source of citral content as well. Camphor basil (*O. kilim and scharicum* Guerk/*O. americanum* L.) contains camphor, so it is used for antifungal treatment, colds and coughs, flu, chronic diarrhea, dysentery, eczema, indigestion and snakebite wounds. Clove basil (*O. gratissimum L.)* contains eugenol. Due to the independence, extreme polymorphism, and stability of the genus *Ocimum*, several novel molecular markers have recently been developed to assess genetic diversity in medicinal plants (Pourmohammad, 2013). The most used molecular techniques are RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), RFLP (Restriction Fragment Length Polymorphism), ISSR (Inter Simple Sequence Repeats), SSRs (Simple Sequence Repeats) (Patel *et al*., 2015). RAPD is the most used technique for studying of genetic variation in plants particularly in medicinal and aromatic plants such as the genus *Ocimum* due to its low cost, time saving, ease of use, lack of sequence information, rapid comparative analysis, with large coverage of genome and high level of

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polymorphism (Singh *et al*., 2004). SCoT (Start codon-targeted polymorphism) markers are reproducible and present as short-conserved regions in plant genes near the ATG sequence of plant genes. (Thakur *et al.*, 2016). Information on genetic diversity is important for germplasm management and development of conservation strategies. Thus, according to morphological and molecular markers, the current study examined the genetic diversity of four genotypes of *Ocimum basilicum*: Sweet Basil (*Ocimum basilicum* var. *basilicum*), Baldy and French, Thai Basil (*O.basilicum* var. *thyrsiflorum*), and Lemon Basil (*O. basilicum citriodorum*).

MATERIALS AND METHODS

Plant materials:

In the present study four varieties genus *Ocimum*, Sweet Basil (*Ocimum basilicum* var. *basilicum*) Balady and French, Thai Basil Thai (*O.basilicum var. thyrsiflorum*) and lemon basil (*O. citriodorum*) were used. The Genetic diversity was estimated among basil varieties using RAPD, SCoT and ISSR markers.

Isolation of DNA

Genomic DNA was isolated from fresh leaves by DNeasy plant mini kit (biobasic). DNA quality was checked using spectrophotometer and agarose gel electrophoresis.

RAPD -PCR Analysis:

In this study, 15 random DNA nucleotide primers (RAPD) were used independently. PCR amplification was done in 25 µl of reaction solution which containing: 1.5 µl Mg Cl2 (25 mM),

2.5 µl dNTPs (2.5 mM), , 2.5 µl 10x buffer, 2.0 µl primers (2.5 µM), 2.0 µl template DNA (50 ng/µl), 0.3 µl Taq polymerase (5 U/µl), 14.7 µl sterile ddH2O. The obtained mixture was covered with one drop of light mineral oil/sample. Amplification was performed on a Techni TC-512 PCR System. Reactions were subjected to one cycle of 95 ºC for 5 min, 35 cycles of 96 ºC for 30 s, 37 ºC for 30 s, 72 ºC for 30 s, and a final cycle of 72 ºC for 5 min. PCR bands were reacted on a 1.5

% agarose gel at 100 V for 1 h to reveal polymorphism among Ocimum varieties under study. The polymorphisms among the Ocimum varieties under study were detected. Only six primers were able to obtained polymorphic DNA bands reproducibly. PCR products were separated on 1.5% agarose gels and fragment sizes were rated using 100 bp ladder markers (3000, 1500, 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp).

SCoT-PCR Analysis:

Genomic DNA was utilized as a template for PCR amplification with using 10 SCoT primers which were designed by Collard and Mackill. (2009). Amplification reactions for SCoT technique were carried out in Techni TC-512 Thermal Cycler according to Abd El-Aziz *et al*. (2019). The PCR cycles were carried out according to Rehab *et al.* (2020).

Inter Simple Sequence Repeat –PCR (ISSR –PCR) Analysis:

Genomic DNA was used as a template for Polymerase Chain Reaction using six ISSR primers in molecular evaluation for four varieties genus *Ocimum.* Amplification reactions for ISSR techniques were performed as described by Xiong *et al.* (2011). The DNA amplifications were performed in an automated thermal cycle (model Techni 512) programmed for one cycle at 94º C for 4 min followed by 45 cycles of 1 min at 94º C, 1 min at 57º C, and 2 min at 72º C. the reaction was finally stored at 72º C for 10 min.

DNA-profiles were done for all techniques according to Adhikari *et al.* (2015). Polymorphic Information Content (PIC) and Diversity Index (DI) were calculated according to, PIC = $1-p^2-q^2$ (Gorji *et al.*, 2011). In addition, the capability of each primer to distinguish between lines was assessed according to resolving power value (Rp) calculated as described in Prevost and Wilkinson (1999).

RESULS AND DISCUSSION

Molecular genetic identification

Randomly amplified polymorphic DNA (RAPD) markers:

To study the genetic differences and relationships among the four varieties of basil, 15 RAPD primers were used. Only 8 primers were able to obtain polymorphic DNA products reproducibly as shown in Fig 1 and Table 1.

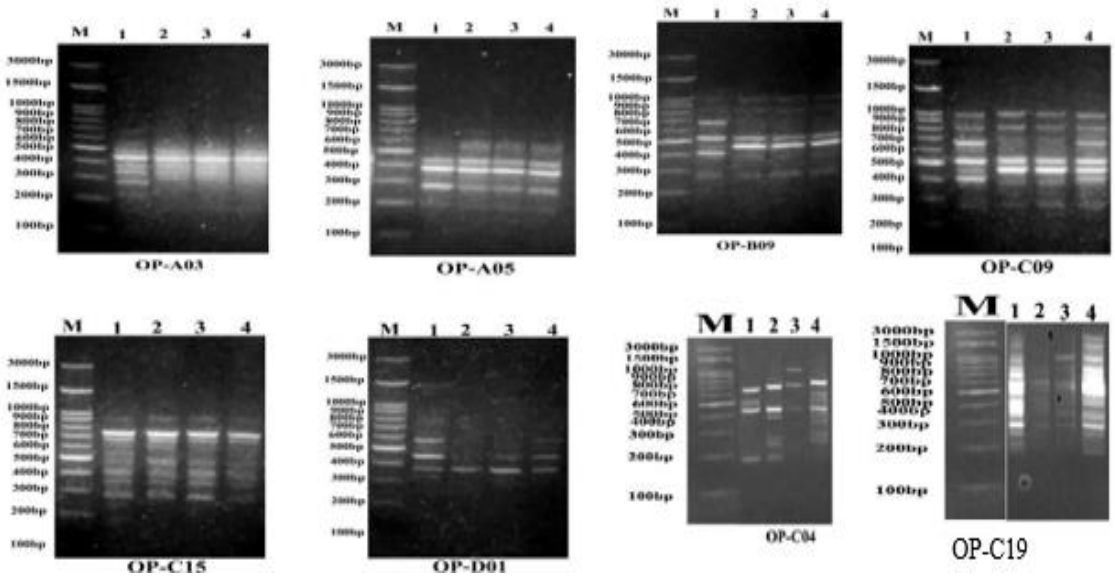

Fig. 1. RAPD-PCR analysis of different basil genotypes cultivated under Egyptian conditions.
1: *Ocimum Basilicum* (Balady), 2: *Ocimum Basilicum* (Frensh), 3: *O.basilicum var. thyrsiflorum* and 4: *O. citriodorum*

Table 1. Molecular data estimated from banding patterns of RAPD marker technique.

Primer			Amplicons							
				Polymorphic						
Name	Sequence (5´→ 3`)	Molecular size range	Monomorphic	Without unique	Unique +	Unique -	Total	Polymorphism %	Polymorphic index content (PIC)	Resolving power Rp
OP-A03	5` AGTCAGCCAC 3`	142 - 673 bp	2	0	7	0	9	63.64	2.63	3.50
OP-A05	5` AGGGGTCTTG 3`	171 – 553 bp	4	0	0	2	6	33.33	1.50	3.00
OP-B09	5` TGGGGGACTC 3`	250 - 1103 bp	4	0	2	2	8	50.00	1.50	2.00
OP-C09	5` CTCACCGTCC 3`	248 - 1096 bp	4	2	3	1	10	60.00	2.49	4.00
OP-C15	5` GACGGATCAG 3`	227 – 980 bp	8	0	4	0	12	33.33	1.48	1.87
OP-D01	5` ACCGCGAAGG 3`	337 – 1477 bp	1	1	1	1	4	75.00	1.25	2.00
C04	5`CCGCATCTAC 3`	172-1191 bp	2	4	7	0	13	84.60	4.60	7.50
C19	5` GACGGATCAG 3`	164-1675 bp	4	7	10	1	22	81.80	7.60	12.50
	Overall	142 - 1675	29	14	34	7	84	60.21	2.88	4.55

The molecular sizes of the amplified bands varied with the different RAPD primers and ranged from 142 bp to1675 bp. A total of 84 major RAPD amplified fragments were produced, out of them 55 (65.48%) were polymorphic and the polymorphism percentage ranged from 33.33 % (OP-A05 and OP-C15) to 84.60 (C04). However, the primers OP-B09, OP-C09 and OP-A03 exhibited the moderate polymorphism (50.00, 60.00 and 63.64 %, respectively). Otherwise, Singh *et al.*, 2004 and Chen *et al.*, 2013 found that high level of polymorphism ranged from 95 and 98.28 % among various species of *Ocimum* using RAPD marker. The total number of polymorphic fragments of DNA ranged from low scored by the primer OP-D01 (3), to high scored by the primer C19-12 (19). All these primers generated good banding patterns that illustrated the rule of RAPD marker in fingerprinting and diversity analyses.The polymorphism index content (PIC) analysis was carried out to conclude the efficiency of each primer RAPD to express polymorphic loci in basil. The calculated PIC values for primers RAPD ranged from 1.25 (OP-D01) to 2.63 (OP-A03). RP values of the ten RAPD primers ranged from 1.87 to 12.50 characteristic the different genotypes whereas the average was 4.55 per RAPD primer. The highest RP values were detected with the primer C19 (12.50) and the lowest with the primer OP-C15 (1.87). Similar findings on basil were also declared by Vierira *et al.* (2003), Lal *et al.* (2012), and Radhika *et al.* (2012). They found that RAPD technique is a precise, effective and sensitive tool for genome analysis of basil speciesThe genetic fingerprints for all four varieties of basil were performed as DNA-profile diagram (Figure 2) based on 84 amplicons obtained using 8 RAPD primers. This profile showed that the amplicons per varieties were variously ranged from 40 (for *O.basilicum var. thyrsiflorum*) to 59 (for *Ocimum Basilicum* (Balady)). In addition, the four varieties were categorized by 41 unique bands (markers) (34 positive and 7 negative). *Ocimum Basilicum* (Balady) had the highest number of positive bands (17). These markers were spread over these lines variously differentiate each variety from the other.

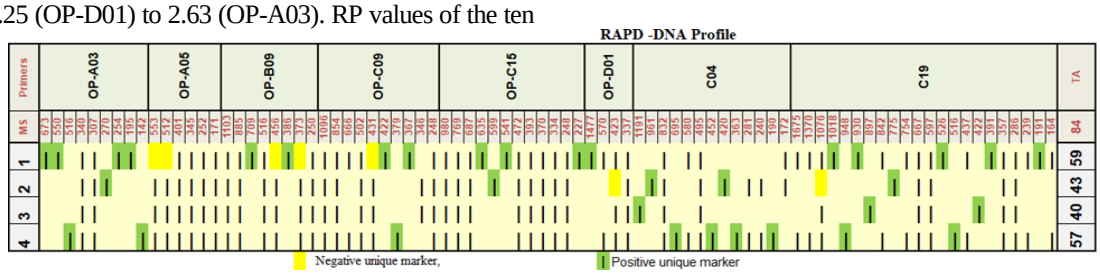

Fig. 2. DNA-profile representation of RAPD fingerprints of four genotypes of basil based on 84 amplicons, 41 of them were marker loci.

1: *Ocimum Basilicum* (Balady), 2: *Ocimum Basilicum* (Frensh), 3: *O.basilicum var. thyrsiflorum* and 4: *O. citriodorum*

According to Table 2, the molecular distance (MD) among all studied four varieties of basil based on RAPD data ranged from 0.588 to 0.843. The highest molecular distance (MD) was among

Table 2. The molecular distance (MD) between all four varieties of basil based on RAPD data.

	Ocimum Basilicum (Balady)	Ocimum Basilicum (Frensh)	O.basilicum var. thyrsiflorum
Ocimum Basilicum (Frensh)	0.588		
O.basilicum var. thyrsiflorum	0.626	0.843	
O. citriodorum	0.707	0.720	0.742

Frensh and *O.basilicum var. thyrsiflorum* (0.843) followed by Frensh and *O. citriodorum* (0.720). While the lowest MD was between Balady and Frensh (0.588) followed by Balady and *O.basilicum var. thyrsiflorum* (0.626). This means that Frensh and *O.basilicum var. thyrsiflorum* were the best genotypes that can be used in breeding programs to obtain the genetic variability and/or hybrid vigor from hybridization between them.

According to the UPGMA clustering algorithm from RAPD markers, four varieties of basil were divided into two major clusters (Fig. 3). The first cluster consisted of *Ocimum Basilicum* (Balady). Meanwhile the second one contained two sub-clusters, the first sub-clusters obtained *Ocimum Basilicum* (French) and O. *basilicum var. thyrsiflorum* and the other sub-clusters contained *O. citriodorum*. The current study's findings demonstrated that RAPD markers successfully distinguished between 4 varieties of *O. basilicum.*

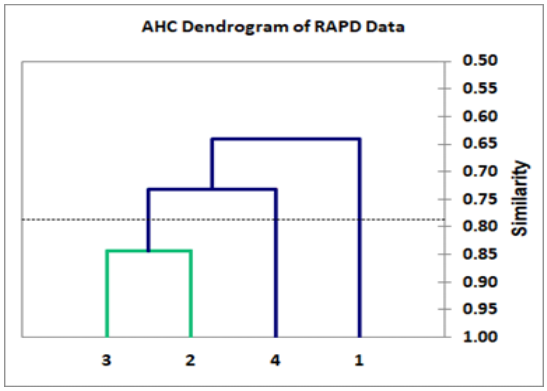

Fig. 3. Agglomerative hierarchical clustering (AHC) dendrograms derived by UPGMA method using RAPD molecular data in four genotypes of basil 1: *Ocimum Basilicum* (Balady), 2: *Ocimum Basilicum* (Frensh), 3: *O.basilicum var. thyrsiflorum* and 4: *O. citriodorum*

The presence of bands was polymorphic within *O. basilicum* indicated genetic diversity, demonstrating the genetic basis of the observed morphological variations. The size of amplified fragments in the present study varied depending on the primers employed this finding is agree with Ibrahim *et al*. (2013).

Start Codon Targeted (SCoT) Technique:

Ten SCoT primers were used to study the genetic diversity among the four varieties of basil as shown in Fig. 4 and Table 3. The molecular sizes of the amplified bands varied with the different SCoT primers and ranged from 167- 2623 bp. All these SCoT primers generated good banding patterns that indicated the influence of SCoT marker in fingerprinting and genetic diversity. A total of 79 major SCoT amplified fragments were produced, out of them 51 (94.56%) were polymorphic. The total number of polymorphic fragments of DNA ranged from one (SCoT 3 and SCoT 5), to 12 scored by the primer SCoT 8. The polymorphism percentage ranged from 25% (SCoT 3) to 100% (SCoT 8). All these primers generated good banding patterns that illustrated the rule of SCoT marker in fingerprinting and diversity analyses.

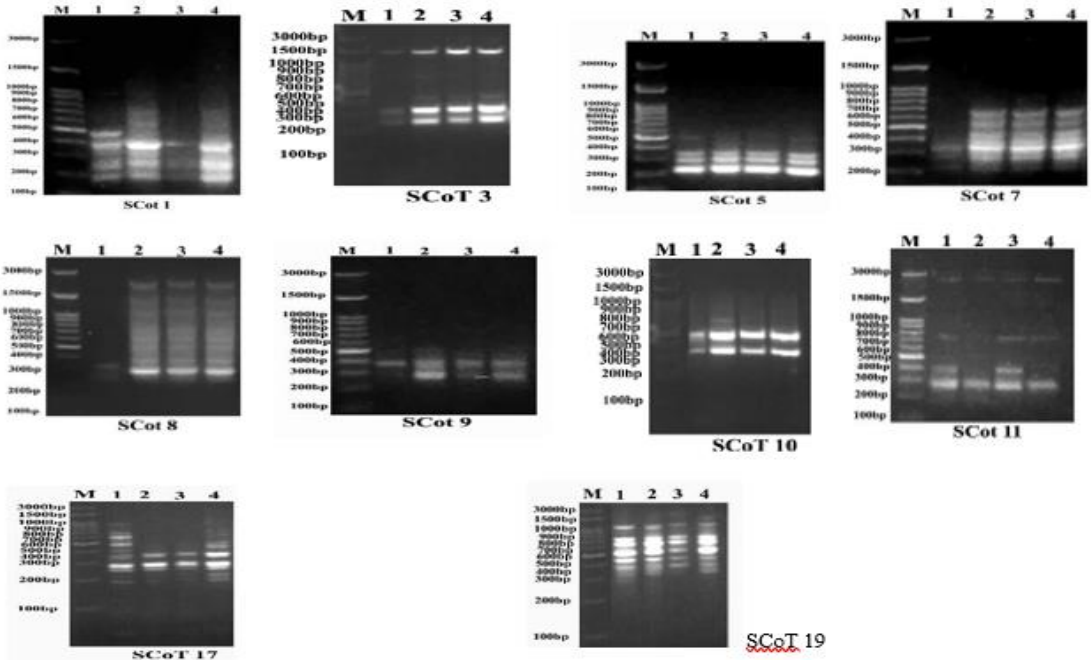

Fig. 4. SCoT-PCR analysis of different basil genotypes cultivated under Egyptian condition.
1: Balady 2: Frensh 3:*O.basilicum var. thyrsiflorum* 4: *O. Citriodorum*.

Table 3. Molecular data estimated from banding patterns of SCoT molecular marker technique.

Primer			Amplicons							
				Polymorphic						
Name	Sequence (5´→ 3`)	Molecular size range	Monomorphic	Without unique	Unique +	Unique -	Total	Polymorphism %	Polymorphic index content (PIC)	Resolving power Rp
SCoT 1	5` ACGACATGGCGACCACGC 3`	167-671	3	3	1	1	8	62.5	2.25	4.0
SCoT 3	5`ACGACATGGCGACCCACA 3`	327-1523	3	0	0	1	4	25.0	0.75	0.5
SCoT 5	5`CAATGGCTACCACTAGCG 3`	203-468	3	0	1	0	4	40.0	0.75	0.5
SCoT 7	5`ACAATGGCTACCACTGAC 3`	215-641	2	0	1	5	8	75.0	2.25	3.0
SCoT 8	5`CAACAATGGCTACCACGT 3`	248-2129	0	0	4	8	12	100	4.50	6.0
SCoT 9	5`ACAATGGCTACCACTGCC 3`	291-505	1	0	2	2	5	80.0	1.50	2.0
SCoT 10	5`ACAATGGCTACCACTGCC 3`	341-811	3	0	4	0	7	57.0	1.87	2.5
SCoT 11	5`ACAATGGCTACCACTACC 3`	243-2623	2	1	0	1	4	50.0	0.88	1.5
SCoT 17	5`ACCATGGCTACCACCGAG3`	193-1000	5	1	7	0	12	61.5	3.12	4.5
SCoT 19	5`CCATGGCTACCACCGGCG 3`	375-1539	6	4	3	1	14	57.1	4	7.0
	Overall	167- 2623	28	9	23	19	79	54.38	2.19	3.15

The minimum Polymorphic information content (PIC) value recorded by SCoT 3 and SCoT 5 (0.75) and the highest PIC value was recorded by SCoT 8 (4.50). The resolving power (Rp) provides a SCoT primers ability to distinguish among genotypes (Rehab *et al*., 2020). Thus, the Rp of each primer was estimated to determine the most informative ones for the ten primers. Rp ranged from 0.5 (for SCoT 3 and SCoT 5) to 7.0 (for SCoT 19) with a mean value 3.15. Two of the SCoT primers (SCoT 8 and SCoT 19) produced high Rp values (6.0 and 7.0 respectively) which mean that they are most effective in surveying genetic diversity.

The genetic fingerprints for all four varieties of basil were performed as DNA-profile diagram (Figure 5) based on 79 amplicons obtained using ten SCoT primers. This profile showed that the amplicons per variety were variously ranged from 74 (for Balady) to 60 (for Frensh). In addition, the four varieties were categorized by 51 unique bands (markers) (23 positive and 19 negative). Balady had the highest numbers of positive bands (ten). These unique bands may be suitable as unique markers as clarified by Rehab *et al*. (2020) in tomato and Rehab *et al*. (2021) in barley.

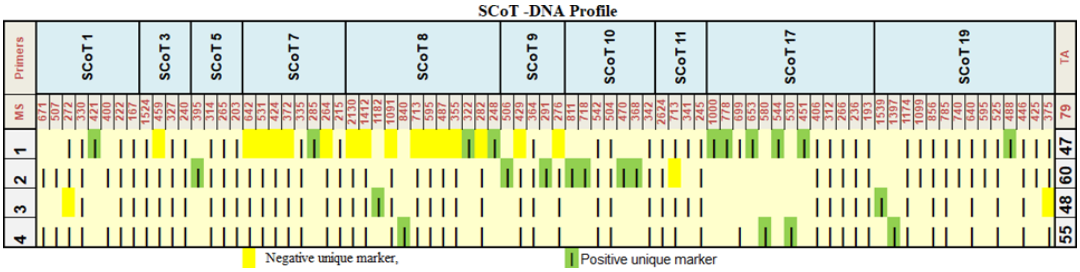

Fig. 5. DNA-profile representation of SCoT fingerprints of four genotypes of basil based on 79 amplicons, 51 of them were marker loci

By utilizing UPGMA computer analysis, the SCoT data were used to determine the values of similarity between the four varieties of Basil (Table 4and Fig.6).

Table 4. The molecular distance (MD) between all four varieties of basil based on SCoT data.

	Ocimum Basilicum (Balady)	Ocimum Basilicum (Frensh)	O.basilicum var. thyrsiflorum
Ocimum Basilicum (Frensh)	0.636		
O.basilicum var. thyrsiflorum	0.632	0.815	
O. citriodorum	0.627	0.852	0.874

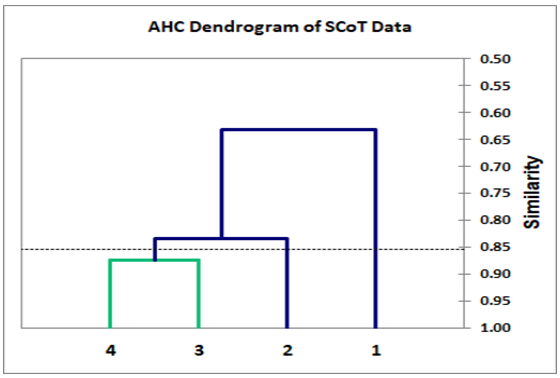

Fig. 6. Agglomerative hierarchical clustering (AHC) dendrograms derived by UPGMA method using SCoT molecular data in four varieties of basil

1: *Ocimum Basilicum* (Balady), 2: *Ocimum Basilicum* (Frensh), 3: *O.basilicum var. thyrsiflorum* and 4: *O. citriodorum*

The molecular distance (MD) among all studied four varieties of basil based on SCoT data ranged from 0.627 to 0.874. The highest molecular distance (MD) was among *O.basilicum var. thyrsiflorum* and *O. citriodorum* (0.874) followed by Frensh and *O. citriodorum* (0.852). While the lowest MD was between Balady and *O. citriodorum* (0.627) followed by Balady and Frensh (0.636). This means *O.basilicum var. thyrsiflorum* and *O. citriodorum* were the best genotypes that can be used in breeding programs to obtain the genetic variability and/or hybrid vigor from hybridization between them. The four varieties of basil were divided into two main groups based on their genetic relationship. The *Ocimum Basilicum* (Balady) was the only member of the first group, while the second one contained two sub-groups, one each of *Ocimum Basilicum var. thyrsiflorum* and *O. citriodorum* and the other subgroup contained *O.basilicum* (French).

Inter Simple Sequence Repeat (ISSR):

The ISSR banding patterns among the four varieties of basil using six selected ISSR primers were obtained the molecular data were estimated (Fig 7 and Table 5). The molecular sizes of the amplified bands varied with the different SCoT primers and ranged from 101- 1410 bp. A total of 59 major ISSR amplified fragments were produced, out of them 44 (74.58%) were polymorphic. Primers 49B, 809B and 844B generated the maximum number of bands (10, 12 and 18, respectively), whereas the primer HB-14 generated the minimum number of bands (2).

The polymorphism percentage ranged from 0.00 % (HB14) to 91.6 % (809B) and varied from each ISSR primers. These results do not agree with Patel *et al*. (2015) they found that the highest polymorphism level (98.17 %) among *Ocimum* specie.

The polymorphism index content (PIC) analysis was carried out to conclude the efficiency of each primer ISSR to express polymorphic loci in basil. The calculated PIC values for primers ISSR ranged from 0.00 (HB-14) to 6.70 (844B). RP values of the ten ISSR primers ranged from

0.00 to 10.50 characteristic the different genotypes whereas the average was 3.9 per ISSR primer. The highest RP values were detected with the primer 844B (10.50) and the lowest with the primer HB-14 (0.00). Mahgoub *et al.* (2020) found that ISSR markers may be more successful in generating reliable markers in basil. Also, Zahraa and Attyaf (2023) showed that ISSR markers were effective in generating polymorphism in basil germplasm reached to 100% and giving unique fingerprint to three cultivars by eight primers.

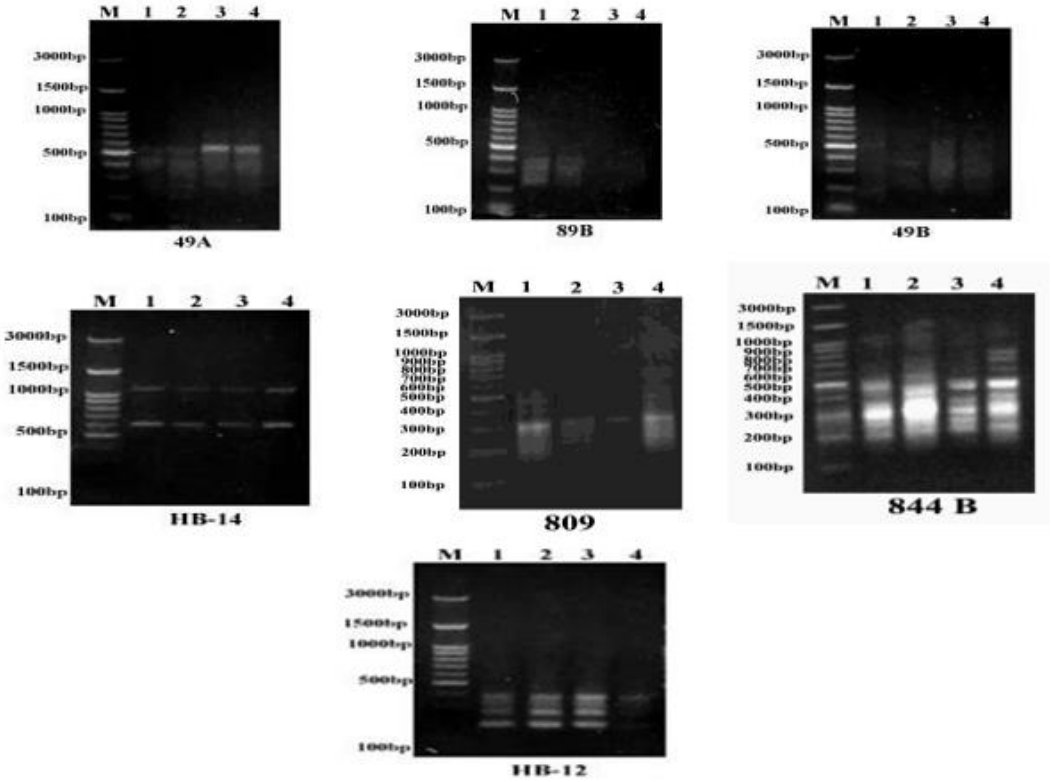

Fig. 7. ISSR-PCR analysis of different basil genotypes cultivated under Egyptian condition.
1: Balady 2: Frensh 3:*O.basilicum var. thyrsiflorum* 4: *O. Citriodorum.*

Table 5. Molecular data estimated from banding patterns of ISSR marker technique.

Primer		Amplicons								
				Polymorphic						
Name	Sequence (5′→3`)	Molecular size range	Monomorphic	Without unique	Unique +	Unique -	Total	Polymorphism %	Polymorphic index content (PIC)	Resolving power Rp
49A	5`CAC ACA CAC ACA AG 3`	101:688	2	2	4	0	8	75.0	2.50	4.0
49B	5`CAC ACA CAC ACA GG 3`	176:731	1	0	8	1	10	0.9	3.73	4.5
89B	5` ACA CAC ACA CAC AC 3`	103:346	2	0	2	0	4	0.5	0.75	1.0
844B	5`CTC TCT CTC TCT CTC TGC3`	190:974	3	6	8	1	18	83.3	6.70	10.5
HB-12	5`CAC CAC CAC GC 3`	207:433	4	0	1	0	5	20.0	0.38	0.5
HB-14	5` CTC CTC CTC GC 3`	586:1193	2	0	0	0	2	0.0	0.00	0.0
809B	5` AGA GAG AGA GAG AGA GG3`	185:1410	1	3	8	0	12	91.6	4.50	7.0
Overall		101- 1410	15	11	31	2	59	38.8	2.65	3.9

The genetic fingerprints for all four varieties of basil were performed as DNA-profile diagram (Figure 8) based on 59 amplicons obtained using ten ISSR primers. This profile showed that the amplicons per variety were variously ranged from 23 (for *O.basilicum var. thyrsiflorum*) to 37 (for *O. Citriodorum*). In addition, the four varieties were categorized by 33 unique bands (markers) (310. positive and 2 negative). *O. Citriodorum* had the highest numbers of positive bands (13).

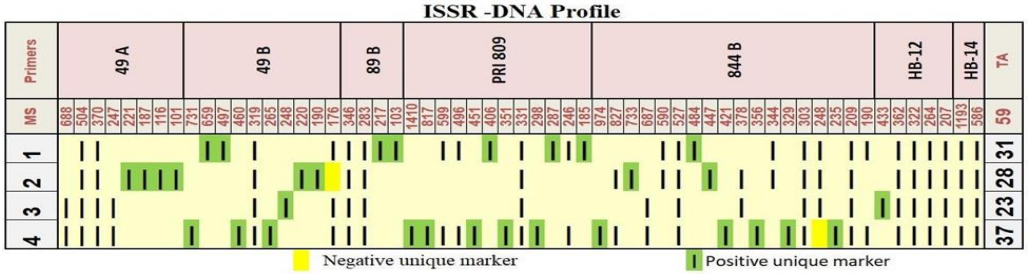

Fig. 8. DNA-profile representation of ISSR fingerprints of four genotypes of basil based on 60 amplicons, 34 of them were marker loci.
1: Balady 2: Frensh 3:*O.basilicum var. thyrsiflorum* 4: *O. Citriodorum.*

According to Table 6, the molecular distance (MD) among all studied four varieties of basil based on ISSR data ranged from 0.507 to 0.679. The highest molecular distance (MD) was among Frensh and *O.basilicum var. thyrsiflorum* (0.679) followed by Balady and *O.basilicum var. thyrsiflorum* (0.643). While the lowest MD was between Frensh and *O. citriodorum* (0.507) followed by Balady and *O.basilicum var. thyrsiflorum* (0.626). This means that Frensh and *O.basilicum var. thyrsiflorum* were the best genotypes that can be used in breeding programs to obtain the genetic variability and/or hybrid vigor from hybridization between them.

Table 6. The molecular distance (MD) between all four varieties of basil based on ISSR data

	Ocimum Basilicum (Balady)	Ocimum Basilicum (Frensh)	O.basilicum var. thyrsiflorum
Ocimum Basilicum (Frensh)	0.623		
O.basilicum var. thyrsiflorum	0.643	0.679	
O. citriodorum	0.600	0.507	0.645

According to the UPGMA clustering algorithm from ISSR markers, four varieties of basil were divided into two major clusters (Fig. 9). The first cluster consisted of *O. citriodorum*. Meanwhile the second one contained two sub-clusters, the first sub-clusters obtained *Ocimum Basilicum* (Balady) and the other sub-clusters contained *Ocimum Basilicum* (Frensh) and *O. basilicum var. thyrsiflorum*

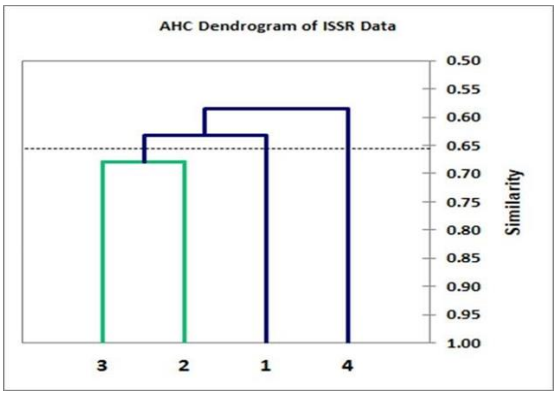

Fig. 9. Agglomerative hierarchical clustering (AHC) dendrograms derived by UPGMA method using ISSR molecular data in four varieties of basil

1: *Ocimum Basilicum* (Balady), 2: *Ocimum Basilicum* (Frensh), 3: *O.basilicum var. thyrsiflorum* and 4: *O. citriodorum*

Combined identification based on RAPD, SCoT and ISSR analysis:

UPGMA computer programmer using to determine the similarity and the relationships between basil genotypes based on combined data from RAPD, SCoT and ISSR markers. As can be seen from the Table 7, the molecular distance (MD) among all studied four varieties of basil based on combined data ranged from 0.612 to 0.793. The lowest molecular distance (MD) was among Balady and Frensh (0.612) followed by Balady and *O.basilicum var. thyrsiflorum* (0.629). While the highest MD was between Frensh and *O.basilicum var. thyrsiflorum* (0.793). This means that Frensh and *O.basilicum var. thyrsiflorum* were the best genotypes that can be used in breeding programs to obtain the genetic variability and/or hybrid vigor from hybridization between them.

Table 7. The molecular distance (MD) between all four varieties of basil based on RAPD, SCoT and ISSR combined data

	Ocimum Basilicum (Balady)	Ocimum Basilicum (Frensh)	O.basilicum var. thyrsiflorum
Ocimum Basilicum (Frensh)	0.612		
O.basilicum var. thyrsiflorum	0.629	0.793	
O. citriodorum	0.650	0.721	0.769

According to the UPGMA clustering algorithm from combined data, four varieties of basil were divided into two clusters based on their genetic relationship. the first cluster contained the *Ocimum Basilicum* (Balady), while the other three genotypes were included in the second group which divided into two sub-clusters. The first one contain *O.citriodorum* and the second sub-cluster contain *Ocimum basilicum* (Frensh) *and Ocimum basilicum var. thyrsiflorum*

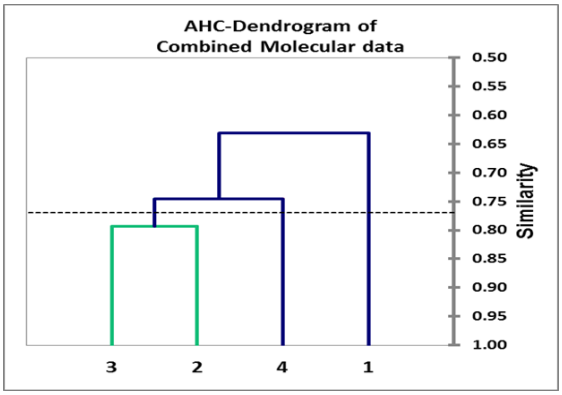

Fig. 10. Agglomerative hierarchical clustering (AHC) dendrograms derived by UPGMA method with combined molecular data in four genotypes of basil

1: *Ocimum Basilicum* (Balady), 2: *Ocimum Basilicum* (Frensh), 3: *O.basilicum var. thyrsiflorum* and 4: *O. citriodorum*

In comparison between combined molecular data for RAPD, SCoT and ISSR primers, it is evident from Table 8 that the total number of scorable RAPD amplicons was 84 with an average of 10.5 amplicons/primer, with a product size ranged from 142 to 1675 bp. While the total number of scorable SCoT amplicons was 79 with an average of 7.9 amplicons/primer, with a product size ranged from 167 to 2623. In addition, the total number of scorable ISSR amplicons was 59 with an average of 8.5 amplicons/primer, with a product size ranged from 101 to 1410. Also, through better discrimination capabilities compared with RAPD, SCoT and ISSR, RAPD primers targeted generating 55 polymorphic amplicons with an average of 6.88/primer and 41 unique markers with an average of 5.13/primer. While SCoT primers targeted generating 55 polymorphic amplicons with an average of 5.1 /primer and 42 unique markers with an average of 4.2 /primer. ISSR primers targeted generating 44 polymorphic amplicons with an average of 6.3/primer and 33 unique markers with an average of 4.7/primer. These results agree with Amina *et al.* (2022) they found that the RAPD-PCR, ISSR-PCR, and SCoT-PCR procedures were successful in distinguishing between the four plant species belonging to the family *Lamiaceae* examined.

This discrimination capability for the RAPD technique confirmed by P%, and Rp values which were 60.21 and 4.55 respectively, compared with the same values for SCoT and ISSR- technique which were 54.83, 38.30, 3.15 and 3.90 respectively. This indicates the high discriminatory potential of using RAPD primers compared with SCoT and ISSR primers. Where,

RAPD markers were more discriminating, provided more informative data. Also, confirms that it can be relying on the RAPD technique to evaluate the genetic diversity among the basil varsities better than SCoT and ISSR markers. These results are not consistent with Hajibarat *et al.* (2015), they found that SCoT marker is generated from the functional region of the genome, so genetic analyses such as genetic diversity, genotype identification, construction of linkage maps and QTL mapping using this marker would be more useful. While Esraa *et al*. (2019) found that the RAPD marker is the best choice for the evaluation of diversity and the genetic relationships between two wild Thymus species.

Table 8. Comparison of discriminating capacity between RAPD, SCoT and ISSR Molecular markers techniques.

Technique	MS	SA		PA		UA		P %	Rp
		Total	Mea n	Total	Mea n	Total	Mean		
RAPD	142:1675	84	10.5	55	6.88	41	5.13	60.21	4.55
SCoT	167:2623	79	7.9	51	5.1	42	4.2	54.38	3.15
ISSR	101:1410	59	8.5	44	6.3	33	4.7	38.80	3.90

MS: Molecular size; SA: Scorable Amplicons; PA: Polymorphic Amplicons; UA: Unique Amplicons; P%: Polymorphism % ; Rp: Resolving power.

In conclusion, the examined four *Ocimum* varieties exhibited genetic variations by RAPD, SCoT and ISSR markers showed high levels of polymorphism, RAPD markers revealed more specific markers than the other techniques and evaluated the genetic diversity among the basil genotypes. So, we recommend extending in these genotypes under Egyptian conditions and benefit from the results of the molecular techniques applied in this study to benefit from molecular markers characterizing desirable traits in these genotypes to use in Egyptian improving programs. This study made a strong case for the complementary use of molecular assays in characterizing the variety of *Ocimum* genotypes, their specific identification, and taxonomic classification.

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تقدير التنوع الوراثي في بعض اصناف الريحان باستخدام تقنيات RAPD و SCoT و ISSR

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الملخص

ينتمي الريحان الى العائلة الشفوية و يعتبر نباتا طبيا هاما ويظهر العديد من الاختلافات في الشكل. تم إجراء تحليلات RAPD و SCoTو ISSR على اربعة اصناف (البلدي و الفرنسي و القدسي و الاحمر) لتحديد صفات النمو. في هذه الدراسة تم تشخيص الاربعة انواع باستخدام ISSR , SCoT , RAPD . ومن خلال تقنية RAPD أظهرت النتائج 84 شظية ، كان 55 منها (65.4%) متباينة وراثيا وكان متوسط النسبة المئوية للتباين الوراثي 60.21 %. وفي تحليل SCOT، تم إنتاج 79 شظية رئيسية من SCOT، منها 51 (94.56 %) كانت متباينة وراثيا. بينما انتج ISSR 59 شظية ، منها 44 شظية متباينة وراثيا (74.58 %). وتراوحت النسبة المئوية للتباين الوراثي بين 0.00 في المائة (HB14) و91.6 في المائة. (809B).و يمكن استنتاج ان RAPD,SCoT,ISSR يتم استخدامهم كوسائل فعالة لتحديد العلاقة و درجة القرابة بين الاصناف و الذي يعتبر هاما في برامج التربية. هذه القدرة على التمييز بالنسبة لتقنية RAPD التي أكدتها P%، وقيم Rp التي كانت 60.21 % و4.55 على التوالي، مقارنة بالقيم نفسها بالنسبة لتقنية ISSR , SCOT التي كانت 54.83% و38.30% و3.15 و3.90 على التوالي. وهذا يدل على وجود إمكانية تمييزية عالية لاستخدام بادئات RAPD مقارنة ببادئات SCoT و ISSR. ولذلك كانت بادئات RAPD أكثر تمييزاً، قدمت المزيد من البيانات المفيدة.