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Genetic Polymorphisms of Four Egyptian Plant Species using some Molecular Markers Techniques RAPD, ISSR, and SCoT

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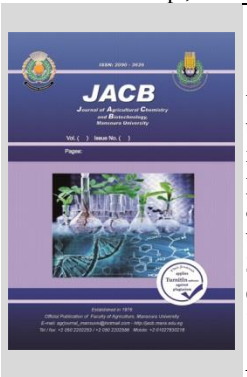

ABSTRACT

Four plant species belonging to the family Lamiaceae were assessed with different molecular markers using five RAPD, six inter simple sequence repeats (ISSR), and six start codon targeted (SCoT) primers to detect their levels of genetic diversity. The RAPD primers identified a total of 41 amplified bands, while all primers inducing five unique markers among the four species used in this study, furthermore the polymorphism percentage reached to 73.71%. ISSR primers generated 31 amplified bands including eight unique markers with a polymorphism percentage reached to 54.83%. SCoT primers exhibited a total of 43 amplified bands. Four of these primers revealed 14 unique genotype specific markers with a polymorphism percentage reached to 39.53%. The four Lamiaceae species were separated into two major groups using cluster analysis: the first group comprised *Phlomis floccosa* and *Salvia officinals*, while the second group included *Teucrium polium* and *Thymus capitatus*.

Keywords: Lamiaceae, genetic diversity, genetic polymorphism, RAPD, ISSR, SCoT

INTRODUCTION

Lamiaceae, popularly known as the mint family, is a family of flowering plants that contains 236 genera and 7000 species (Nousiba *et al* 2021). The largest genera include *Salvia* (900 species), *Scutellaria* (360 species), *Stachys* (300 species), *Plectranthus* (300 speicies), *Hyptis* (280 species), *Teucrium* (250 species), *Vitex* (250 species), *Thymus* (220 species), and *Nepeta* (200 species). Species within this family display incredible variety. A cosmopolitan appearance are found in distinct natural ecosystems. Once thought to be closely linked to the Verbenaceae family (Harley *et al* 2004), Lamiaceae has a long history of successful cultivation and traditional uses including, seasoning, food preservation, most prominently and medicinal applications. Lamiaceae are perennial or annual herbs, shrubs, and rarely trees (Migahid and Hammouda 1974, Watson and Dallwitz 1992). They have taproots and fibrous adventitious roots (Kotb 1985 and Bakr 2017), with erect stems or rhizomes that are branched, quadrangular, hairy and woody or herbaceous (Kotb 1985 and Boulos 2002).

The natural flora of Egypt is comprised of 2094 local and naturalized species, as well as 151 infra-specific taxa from 725 genera from 121 families. Six species of gymnosperms and 435 species of monocots are found in Egypt, with dicots making up the remaining 1637 species. Egyptian flora is particularly interesting given its unique combination of local African and Asiatic species. In total, only 61 flora species are endemic to Egypt (Boulos 1995) with no endemic families (Wickens 1976). Most endemic species belong to Lamiaceae, Liliaceae, Scrophulariaceae or Asteraceae. On the Sinai Peninsula, 33 endemic species (60.7 %) are found, 24 of which are recorded in South Sinai (Hegazy and Amer 2002).

Genetic diversity assessments of plant species have been performed using random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats, inter simple sequence repeats (ISSR), sequence-related amplified polymorphisms (Abd El-Maboud and Khalil 2013, Abd El-Maboud and El-Zayat 2020) and start codon targeted (SCoT) polymorphisms (Collard and Mackill 2009, Rayan and Osman 2019). For example, RAPD and ISSR markers were utilized to measure the hereditary relationships between 12 Lamiaceae species in Saudi Arabia (Ahmed and Al-Sodany 2019). The advancement of molecular markers recent years has created new opportunities for genetic characterization and biodiversity studies in plants.

In this context, the purpose of this study was to investigate the genetic polymorphism using three molecular markers in four Lamiaceae species found in Egypt.

MATERIALS AND METHODS

Genetic materials:

Four plants species belonging to the family Lamiaceae as *Phlomis floccosa*, *Salvia efficinalis*, *Teucrium polium* and *Thymus capitatus* obtained from Wadi Habis ecosystem of Matrouh in Egypt were used in this study. Fresh leaves and sources of four species were collected by staff members of the Desert Research Center, EL Matariya, Cairo.

Methods

DNA extraction:

For DNA extraction, the collected leaf samples were refrigerated at −20 °C until further use. The DNeasy Plant Mini Kit was used to extract DNA (QIAGEN Germantown, Maryland USA.) according to the manufacturer instructions.

RAPD-PCR amplification:

Five RAPD primer sequences were used to assess the polymorphisms between the four plant species (Table 1).

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PCR were performed using an automated thermal cycle (model Techno 512) programmed with a 30 µl mixture containing 3 µl 10x buffer, 3 µl MgCl2 25mM, 3 µl dNTP's 2.5 mM, 2 µl ISSR or RAPD primers 10 pmol, 2 µl diluted preheated DNA (2 µl DNA in 16.80 µl H2O then heated for 3 mins at 70 °C) and 0.20 µl Taq polymerase enzyme 5u. The PCR products were amplified via ISSR or RAPD under the following thermal cycle conditions: 4 min at 94 °C, followed by 45 cycles at 1 min at 94 °C, 1 min at 57 °C and 2 min at 37 °C. Afterwards, the reaction was kept at 72 °C for 10 min.

Table 1. Molecular genetic data derived from the RAPD technique's amplified banding patterns.

Primer name	Sequence (5´→ 3`)	Range of molecular sizes	Total amplified band	Monomorphic band	Polymorphic band	Unique band	Polymorphic %
OP-A1	CAG GCC CTT C	235:800	11	3	8	7	72.72%
OP-A3	CAG CAC CCA C	200:760	8	3	5	4	62.50%
OP-C9	CTC ACC GTC C	300:1560	9	3	6	4	66.66%
OP-K3	CCC TAC CGA C	300:840	7	2	5	1	71.42%
OP-O11	GAC AGG AGG T	380:1480	6	-	6	1	100%
Total			41	11	30	17	73.17

ISSR and SCoT-PCR amplification

Six ISSR primer sequences previously reported by Yousefi *et al* (2015) and Tapeh *et al* (2018) (Table 2), and six SCoT primer sequences (Table 3) reported by Alqahtani M.M. *et al* (2020) were used to identify the level of genetic variability in the four investigated species.

Table 2. Molecular genetic data derived from the ISSR technique's amplified banding patterns.

Primer name	Sequence (5´→ 3`)	Range of molecular sizes	Total amplified band	Monomorphic band	Polymorphic band	Unique band	Polymorphic %
14A	CTC TCT CTC TCT CTC TTG	400:865	4	2	2	1	50%
44B	CTC TCT CTC TCT CTC TGC	285:545	4	3	1	1	25%
HB-8	GAG AGA GAG AGA GG	385:875	6	3	3	2	50%
HB-10	GAG AGA GAG AGA CC	185:570	4	2	2	2	50%
HB-11	GTG TGT GTG TGT TGT CC	170:645	7	4	3	1	42.85%
HB-13	GAG GAGGAG C	370:1480	6	-	6	1	100%
Total			31	14	17	8	54.83

Table 3. Molecular genetic data derived from the SCoT technique's amplified banding patterns.

Primer name	Sequence (5´→ 3`)	Range of molecular sizes	Total amplified band	Monomorphic band	Polymorphic band	Unique band	Polymorphic %
SCoT 1	CAA CAATGGCTACCACCC	170:2975	12	7	5	4	41.66%
SCoT 2	CAACAATGGCTACCACCC	165:680	6	4	2	2	33.33%
SCoT 3	ACG ACA TGG CGA CCC ACA	235:415	3	3	-	-	-
SCoT4	ACC ATG GCT ACC ACC GCA	260:1385	8	4	4	4	50%
SCoT 6	CAACAATGGCTACCACGC	330:1080	6	4	2	-	33.33%
SCoT 8	CAACAATGGCTACCACGT	315:750	8	4	4	4	50%
Total			43	26	17	14	39.53

PCR product separation and photography

The amplified products were then loaded and sorted on a 1.5 % agarose gel using ethidium bromide. The run lasted about 30 min at 100 volts by Yousefi V *et al* (2015).

Molecular data analysis

Each primer pair was graded on whether DNA bands were present (1) or absent (0). A dendrogram was created using IBM SPSS windows software version 10 (New York, USA). Genetic similarities were estimated using Dice software (Bio-Rad, Hercules, CA, USA), which was also used to calculate the pairwise difference matrix and depict the phenogram among the species (Yang and Quiros 1993).

RESULTS AND DISSCUSSION

RAPD analysis:

An equal sum of 41 bands were visualized crossways (Table 1 and Plate 1). The four species exhibited 5 random primers. The maximum polymorphism percentage was 100 % created by primer OP-O11, while the lowest polymorphic percentage was 62.50 %, produced by primer OP-A3. The five primers revealed a total of 11 monomorphic bands and 17 distinct marker bands. Primer OP-A1 had the most amplified bands (11), whereas primer OP-O11 had the least (6 bands).

ISSR analysis:

ISSR banding patterns were used to access the molecular diversity among the four species. The DNA polymorphisms are shown in Table (2) and Plate (2). There were 31 bands in total, out of them 17 were polymorphic (54.83%). The highest polymorphism percentage (100%) was created with primer HB-13, while the lowest polymorphic percentage (25%) was produced with primer 44B. The six primers produced a total of 14 monomorphic bands and 8 unique marker bands. Primer HB-11r had the most amplified bands (7 bands), whereas primers 14A, 44B and HB-10 had the least (4 bands each).

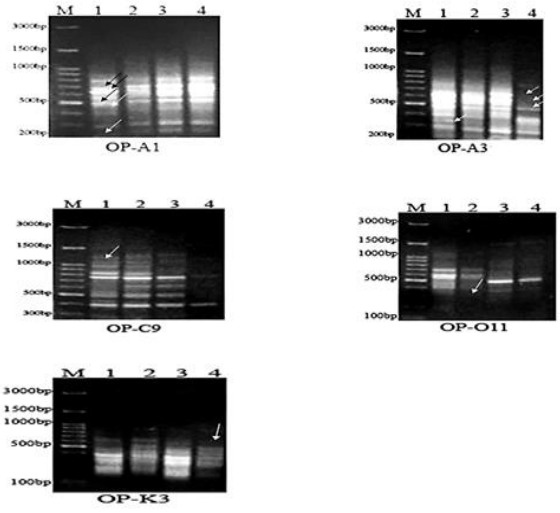

Plate 1. RAPD-PCR profiles of the four amplified plant species using five primers used for each analysis.

M: 3000 bp. 1 = *Phlomis floccose*; 2 = *Salvia officinalis*; 3 = *Teucrium polium*; 4 = *Thymus capitatus.*

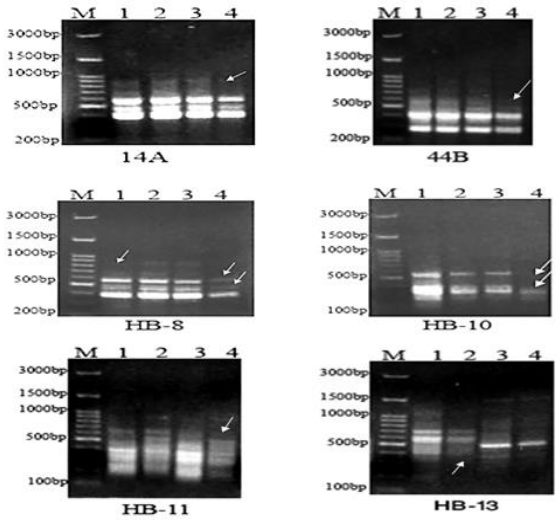

Plate 2. ISSR-PCR profiles of the four amplified plant species using six primers used for each analysis.

M: 3000 bp. 1 = *Phlomis floccose*; 2 = *Salvia officinalis*; 3 = *Teucrium polium*; 4 = *Thymus capitatus*.

SCoT analysis:

SCoT analysis showed that there was a total of 43 amplified bands out of which 17 were polymorphic (39.53%) (Table 3 and Plate 3).The maximum polymorphism percentage was 50 %, while the lowest polymorphic percentage was 33.33%. All six primers produced a total of 26 monomorphic bands and 14 distinct marker bands. SCoT 1 had the most amplified bands (12), whereas SCoT 3 had the fewest (3 bands).

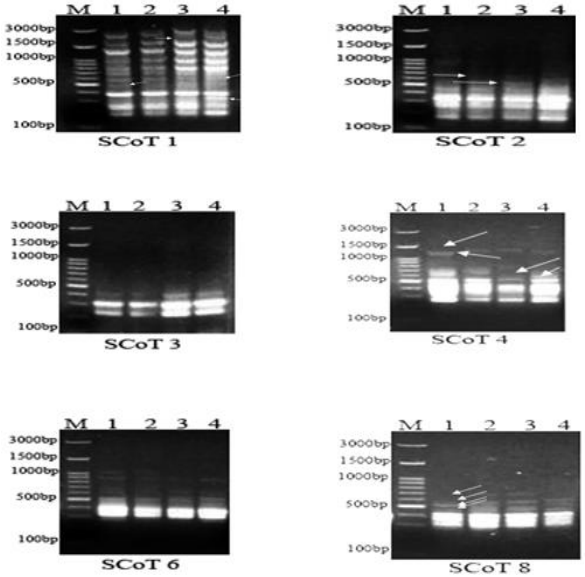

Plate 3. SCoT-PCR profiles of the four amplified plant species using six primers used for each analysis.

M: 3000 bp. 1 = Phlomis floccose; 2 = Salvia officinalis; 3 = Teucrium polium; 4 = Thymus capitatus

RAPD, ISSR and SCoT combination analysis:

The combined results (Table 4) showed that four plant species described by five RAPD primers, six ISSR primers and six SCoT primers resulted in 64 polymorphic bands from a total of 115 amplified bands and a total polymorphism average reached to 55.65%. These results show that the RAPD-PCR, ISSR-PCR, and SCoT-PCR procedures were successful in distinguishing between the four plant species examined.

Table 4. RAPD, ISSR and SCoT analyses yielded polymorphic, monomorphic, unique bands, and polymorphic percentages.

Primer name	Total amplified band	Monomorphic band	Polymorphic band	Unique band	Polymorphic %
RAPD	41	11	30	17	73.17 %
ISSR	31	14	17	8	54.83 %
SCoT	43	26	17	14	39.53 %
combined	115	51	64	39	55.65 %

Molecular distance of phylogenetic relationships based on RAPD, ISSR and SCoT analyses.

Table (5) shows the molecular distance (MD) matrix between the four plant species based on the RAPD, ISSR and SCoT combined results. Salvia officinals and Thymus capitatus had a MD of 0.71, whereas Phlomis floccosa and Salvia officinals had a MD of 0.87. Data obtained from the RAPD, ISSR and SCoT trees are illustrated in Figure (1). The four Lamiaceae species were split into two major groups using UPGMA's dendrogram: the first group included Phlomis floccosa and Salvia officinalis, while the second group included Teucrium polium and Thymus capitatus.

Table 5. Dice similarity coefficient of 4 plant species based on RAPD, ISSR, and SCoT data analysis

Plant species	*Phlomis floccosa*	*Salvia officinals*	*Teucrium polium*	*Thymus capitatus*
Salvia officinals	0.87			
Teucrium polium	0.75	0.80		
Thymus capitatus	0.63	0.719	0.82	1

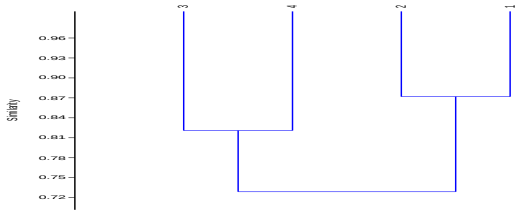

Fig. 1. Dendrogram derived by UPGMA method using Dice-dissimilarity coefficient for combined binary data of RAPD, ISSR and SCoT analyses of four species.

1 = *Phlomis floccose*; 2 = *Salvia officinalis*; 3 = *Teucrium polium*; 4 = *Thymus capitatus*

The RAPD method revealed the highest polymorphism (73.17%) among the three studied markers followed by ISSR and SCoT. These results agreed with those obtained by Abd El-Maboud and Khalil (2013), who observed 73% polymorphisms among seven *Suaeda* genotypes using five RAPD primers. The highest polymorphism with primer OP-O11 agrees with the results of Ahmed and Al-Sodany (2019), who was able to distinguish between 12 Lamiaceae species. The eight unique bands obtained from the five ISSR primers could be used as specific markers for the studied Lamiaceae species. El-Senosy *et al* (2015) studied the genetic diversity in five genotypes of *Halocnemum strobilaceum* using five ISSR primers and found 12 polymorphic bands from a total of 34 bands (35% polymorphism). Abd El-Maboud and El-Zayat (2020) investigated seven ISSR primers in seven

populations of *Thymelaea hirsuta* collected from the north western coast of Egypt, which ranged from 25 % to 94 %, with an average polymorphism of 73.7 %.SCoT markers have been used to study the genetic diversity among six genotypes of the Egyptian soybean *Glycine max* L (Rayan and Osman 2019). The same authors used 11 SCoT markers that produced 106 amplicons with 49.11 % polymorphism.

CONCLUSION

In conclusion, DNA fingerprinting database was produced using three different PCR-based molecular marker systems as RAPD, ISSR, and SCoT). The results obtained in this study could serve as a reasonable source of information to help breeders determine genetic variability among different genotypes. RAPD and ISSR proved to be accurate and efficient tool for assessing genetic polymorphisms and the relationships between plant species. In addition, SCoT markers were useful in detecting high levels of genetic diversity in plant species.

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تعدد الأشكال الجينية في أربعة أنواع نباتية في مصر باستخدام علامات متعدد الاشكال المخم عشوائيا والتكرار المتسلسل البسيط وتكرار الكودون المستهدف

أمينة رزق أحمد[1] ، خالد عبد العاطى سليمان[1] ، نعمة قطب السنوسي[1] و سيد عبد السلام عمر[2]

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تم تقييم أربعة أنواع نباتية تابعة للعائلة الشفوية وهم (ضرس الشايب والجعدة والمريمية والزعتر) والتي تم الحصول عليها من وادي حابس - مطروح - مصر ، وذلك باستخدام علامات جزيئية مختلفة شملت خمسة من علامات متعدد الاشكال المختارة عشوائيا RAPD وستة من التكرار المتسلسل البسيط ISSR وستة من تكرار الكودون المستهدف لتحديد الاختلافات الجينية SCoT بين الانواع . وقد اظهرت نتائج علامات متعدد الاشكال المضخمة عشوائيا مجموعة من 41 حزمة من جميع البادئات وخمسة حزم احادية بين الانواع الاربعة ونسبة تعدد اشكال 73.71%. والتي أنتجت بادئات التكرار المتسلسل البسيط 31 حزمة مضخمة مع 8 حزم احادية ونسبة تعدد اشكال 54.83% وعرضت بادئات تكرار الكودون المستهدف مجموعة من 43 حزمة مضخمة كشفت أربعة من هذه البادئاتعن 14حزمة احادية مع نسبة تعدد اشكال 39.53% . تم فصل الاربعة انواع من نباتات العائلة الشفوية الى مجموعتين رئيسيتين باستخدام التحليل العنقودي هما المجموعة الأولى تضمنت ضرس الشايب والجعدة بينما شملت المجموعة الثانية المريمية والزعتر.