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Research Article
Applicability of Different Molecular Markers Techniques for Genetic Distinguish Between Two Genera *Cressa* Linn. and *Cuscuta* Yunck. Family Convolvulaceae

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Abstract

Background and Objective: The DNA fingerprinting is used to determine the relationship between species in the same genus or between genera related to the same family. The aim of this study was to determine the relationships between two samples related to the same family Convolvulaceae, representing two genera, *Cressa* Linn. and *Cuscuta* Yunck. by RAPD, ISSR and SCoT molecular techniques (PCR based DNA fingerprint). **Materials and Methods:** The RAPD, ISSR and SCoT based DNA fingerprinting techniques were implemented to identify the fingerprint diversity between two genera, *Cressa* Linn. and *Cuscuta* Yunck-belonging to the family Convolvulaceae. **Results:** Applying of RAPD technique revealed that using OP-A02, OP-A09, OP-A10, OP-C04 and OP-M01 primers recorded 60, 83.33, 100, 50 and 70.66% polymorphism, respectively. On the other hand, ISSR technique recorded 40, 50, 100, 66.67, 33.33 and 37.5% polymorphism with 44B, HB-08, HB-09, HB-10, HB-11 and HB-12 primers, respectively. However, amplification of SCoT technique, SCoT 1, SCoT 2, SCoT 3, SCoT 4, SCoT 6, SCoT 8, SCoT 10 and SCoT 12 primers recorded 33.33, 28.57, 14.28, 66.66, 25, 40, 42.85 and 50%, respectively . The total polymorphism recorded 73.33, 54.58 and 37.7% for RAPD, ISSR and SCoT techniques, respectively. **Conclusion:** The result of this study indicated that SCoT technique was more efficient and sustainable for distinguish between two genera under investigation.

Key words: *Cressa*, *Cuscuta*, DNA fingerprinting, DNA polymorphism, RAPD, ISSR, SCoT

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Deoxyribonucleic acid (DNA) fingerprinting is considered as one of the most important tools for genetic identification in plant breeding and germplasm management of cultivar or varietal identification[1,2]. It is also used to study and characterize relationships between different plant genera in the same family or different species and to measure genetic diversity among genotypes of the same species, based on the inherent DNA polymorphism present among those genotypes[3,4]. Hybridization-based fingerprinting and polymerase chain reaction (PCR) were developed by Williams *et al.*[5].

Random amplified polymorphic DNA (RAPD) marker is easy technique, quick and not requires information about the sequence. The method depends on the amplification of random genomic DNA fragments by arbitrarily selected primers. The generated patterns depend on the sequence of the PCR primers and the nature of the template[6,7]. Amplification product derived from a region of the genome having two short DNA segments with some homology to the primer, must be present on opposite DNA strands and sufficiently close to each other to allow DNA amplification[8-10]. Polymorphism is then observed and scored as the presence or absence of a fragment and relates to sequence variation due to nucleotide insertion, deletion or substitution[11,12]. The homozygous presence of fragment is not distinguishable from its heterozygote and hence, RAPDs are dominant markers. RAPD polymorphism results from mutation or rearrangements at or between oligonucleotide primer binding sites in the genome were indicated. Such polymorphism behave as dominant genetic markers[13-15]. Inter Simple Sequence Repeat (ISSR) amplification is another method, which a marker system now referred to. This makes the usage of anchored primers to amplify simple sequence repeats without the requirement for prior sequence information[16,17]. This technique is more reliable than the RAPD technique and generates larger numbers of polymorphisms per primer[18]. Theoretically, polymorphisms should be easier to detect because variable regions in the genome are targeted[16,19,20].

Start codon targeted (SCoT) polymorphism is recommended as a new, simple and reliable gene-targeted marker technique based on the translation start codon[21,22]. Their markers are more reproducible than RAPD and ISSR. It is reported that this primer has been used in genetic diversity analysis in a number of plant species[23-27]. The SCoT markers were successfully utilized to analyze the genetic diversity of rice[21], peanut[28], mango[29-31], potato[22], grape[25], Lycoris[32] and *Dendrobium nobile*[33] for their highly polymorphism and efficiency.

The genus *Cressa* is perennial herbaceous plant[34-36]. *Cressa cretica* Linn. is a remarkable salt tolerant plant, common in coastal areas and represent the genus[37]. It is used in folk medicine as tonic, stomachic, anthelmintic and in the treatment of hepatic disorders, urinary discharges, diabetes, asthma, ulcers, constipation and also in aphrodisiac purposes[38]. Traditionally, the plant is used as expectorant, emetic and antibilious agent in Bahrain and Sudan[39].

The genus *Cuscuta* is a widespread parasitic weeds, occurring in both crop- cultivated and non-crop wild areas[40]. *Cuscuta* seeds have been used as a Chinese medicine for many years. However, there has been little scientific investigation into the actual effectiveness of its use[41]. Among this genus *Cuscuta campestris* Yunck.[42]. which is known by its Arabic name Al-hamol[34]. It is used in traditional Chinese medicine and in popular medicine for the treatment of fresh wounds, hepatic, stomach and urinary tract disorders[43].

This study aimed to determine the relationships between two samples related to the same family Convolvulaceae, representing two genera, *Cressa* Linn. and *Cuscuta* Yunck. Three molecular techniques (PCR based DNA fingerprint), RAPD (5 primers), ISSR (6 primers) and SCoT (8 primers) were adopted to identify the molecular basis between the two genera under investigation.

MATERIAL AND METHODS

This investigation was carried out in Plant Biotechnology Department, National Research Centre, Cairo, Egypt during the period from January, 2017-March, 2018.

Plant materials: *Cressa cretica* Linn. aerial parts was collected from the road to Qaron lake, El-Faiyum, Egypt. *Cuscuta campestris* Yunck. aerial parts was collected in flowering stage as a parasite on *Ipomoea cairica* L. sweet from Banha, Minia el kamh road, Kaliobeya Governorate, Egypt. These plants were kindly authenticated by Dr. Abd Elhalim Abd El Motgali, Professor of flora and phyto taxonomy researches, Horticultural Research Institute, Agriculture Research Centre, Dokki, Cairo (Egypt). They have voucher specimens numbers 23-2-2015 A and B, respectively. These plants are kept in the herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University.

DNA extraction: Samples of DNA were prepared, extracted and performed according to the described method by Dellaporta *et al.*[44].

Polymerase chain reaction (PCR)

RAPD-PCR analysis: In this process, various factors were optimized and performed according to Williams *et al.*[5]. However, only five primers were succeeded to generate reproducible polymorphic DNA products.

ISSR and SCoT-PCR analysis: The PCR amplification was completed and performed using random 10 mer arbitrary primers synthesized by Operon biotechnologies, Inc. (Germany). In order to obtain clear reproducible amplification products, different preliminary experiments were carried out in which a number of factors were optimized as followed method by Williams *et al.*[5]. In PCR reaction, only six and eight primers were succeeded to generate reproducible polymorphic DNA products for ISSR and SCoT techniques, respectively.

Statistical analysis: The DNA bands generated by each primer were counted and their molecular sizes were compared with those of the DNA markers. The bands scored from DNA profiles generated by each primer were pooled together. Then the presence or absence of each DNA band was treated as a binary character in a data matrix (coded 1 and 0, respectively) to calculate genetic similarity among the studied samples[45].

RESULTS

The RAPD, ISSR and SCoT banding profiles produced by using primers in the two examined samples of plants, *Cressa cretica* Linn. and *Cuscuta campestris* Yunck. were illustrated in Fig. 1 and Table 1-3.

The RAPD banding profiles produced by the five primers OP-A02, OP- A09, OP-A10, OP-C04 and OP-M01. Illustrated data in Table 1 showed that using OP-C04 primer presented 8 bands giving zero monomorphic and 8 polymorphic bands with 100% polymorphism between genera *Cressa* and *Cuscuta*. While, OP-A10 showed 6 bands illustrated 1 monomorphic and 5 polymorphic bands producing 83.33% polymorphism. On the other hand, OP-M01 showed 6 bands resulted into 3 monomorphic and 3 polymorphic bands with 50% polymorphism. The total polymorphism after applying the RAPD method was found 73.33% between the two genera under study.

Table 1: Primers sequence, total band, monomorphic and polymorphic percentage of polymorphism revealed by the five 10-mer primers using RAPD technique

Primers	Sequence (5'→3')	Number of bands	Monomorphic bands	Polymorphic bands	Polymorphism (%)
OP-A02	GAA AGG GGT G	5	2	3	60.00
OP-A09	GGG TAA CGC C	5	2	3	60.00
OP-A10	GAC GGA TCA G	6	1	5	83.33
OP-C04	CCG CAT CTA C	8	0	8	100.00
OP-M01	GGA CCC AAC C	6	3	3	50.00
Total		30	8	22	73.33

Table 2: Percentage of polymorphism revealed by the five 10-mer primers using ISSR technique

Primers	Sequence (5'→3')	Number of bands	Monomorphic bands	Polymorphic bands	Polymorphism (%)
44B	CTC TCT CTC TCT CTC TTG	5	3	2	40.00
HB-08	GAG AGA GAG AGA GG	8	4	4	50.00
HB-09	GTG TGT GTG TGT GC	8	0	8	100.00
HB-10	GAG AGA GAG AGA CC	6	2	4	66.67
HB-11	GTGTGTGTGTGTCC	9	6	3	33.33
HB-12	CAC CAC CAC GC	8	5	3	37.50
Total		44	20	24	54.58

Table 3: Percentage of polymorphism revealed by the five 10-mer primers using SCoT technique

Primers	Sequence (5'→3')	Number of bands	Monomorphic bands	Polymorphic bands	Polymorphism (%)
SCoT1	ACG ACA TGG CGA CCA CGC	9	6	3	33.33
SCoT2	ACC ATG GCT ACC ACC GGC	14	10	4	28.57
SCoT 3	ACG ACA TGG CGA CCC ACA	7	6	1	14.28
SCoT 4	ACC ATG GCT ACC ACC GCA	9	3	6	66.66
SCoT 6	CAA TGG CTA CCA CTA CAG	4	3	1	25.00
SCoT 8	CAA TGG CTA CCA CTA CAG	5	3	2	40.00
SCoT 10	ACA ATG GCT ACC ACC AGC	7	4	3	42.85
SCoT 12	CAA CAA TGG CTA CCA CCG	6	3	3	50.00
Total		61	38	23	37.70

Regarding ISSR banding profiles produced by the six primers, 44B, HB-08, HB-09, HB-10, HB-11 and HB-12, in the two examined samples of *Cressa* Linn. and *Cuscuta* Yunck were illustrated in Table 2.

From Table 2 primer, HB-09 showed 8 bands giving zero monomorphic and 8 polymorphic bands with 100% polymorphism between genera *Cressa* and *Cuscuta*, while HB-10 showed 6 bands giving 2 monomorphic and 4 polymorphic bands resulting in 66.67% polymorphism. However, HB-11 showed 9 bands giving 6 monomorphic and 3 polymorphic bands indicating 33.33% polymorphism. The total polymorphism after applying the ISSR method was found 54.58%.

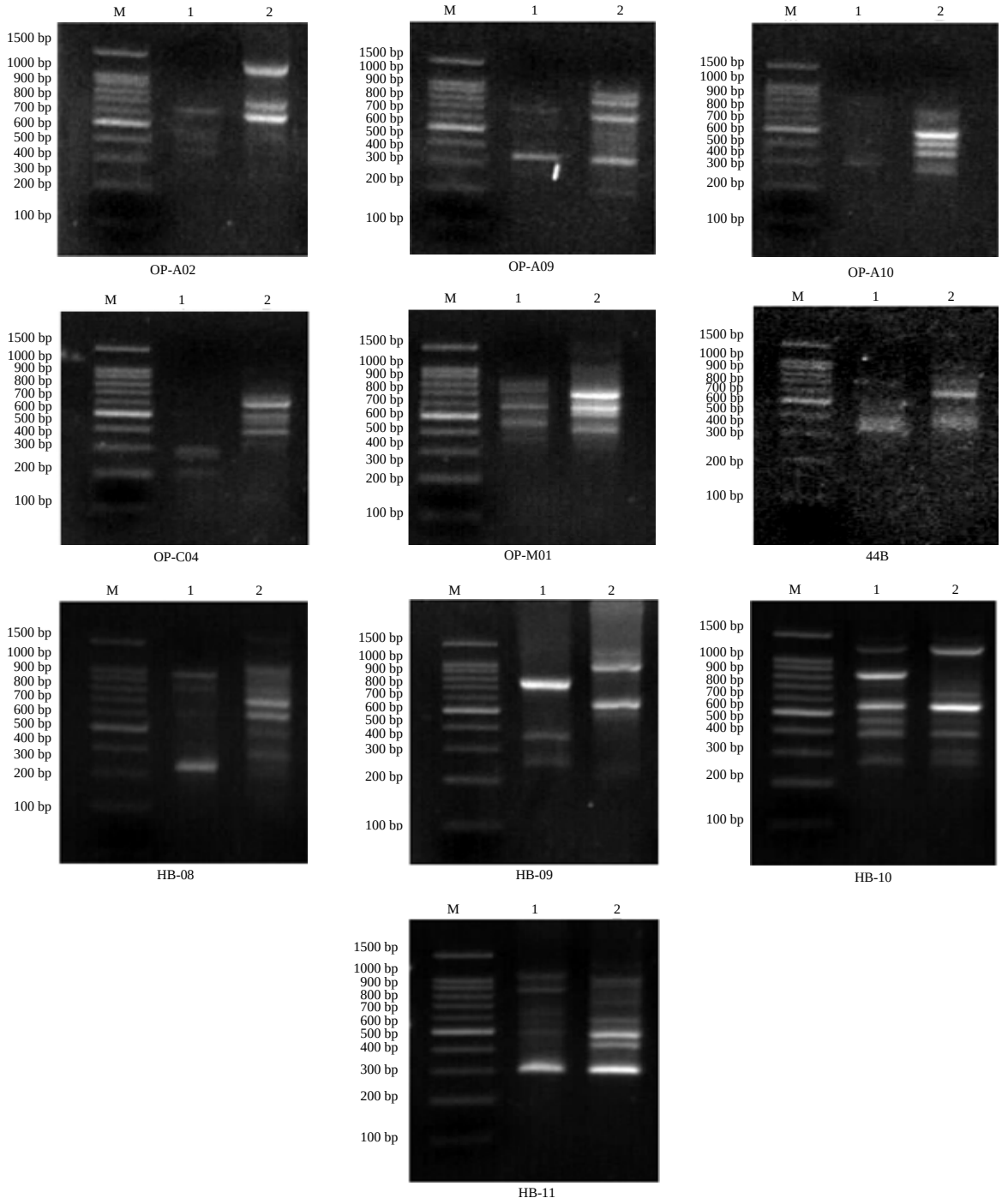

Fig. 1: Continue

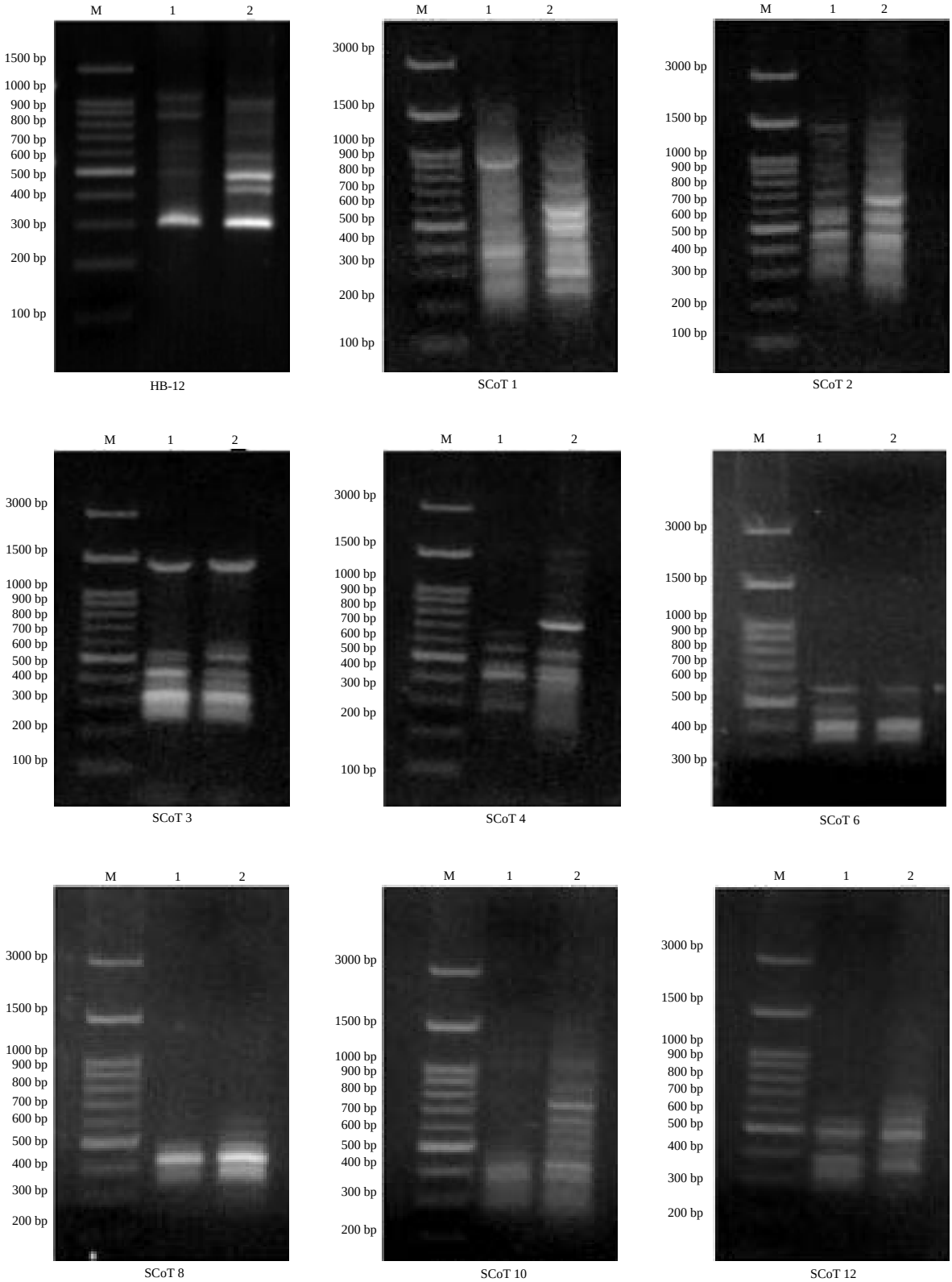

Fig. 1: RAPD, ISSR and SCoT amplification electrophoretic profile of *Cressa cretica* Linn. and *Cuscuta campestris* Yunck
M: DNA marker (1 Kb ladder) 1: *Cressa cretica* Linn., 2: *Cuscuta campestris* Yunck

Table 4: Summary of the generated data by RAPD, ISSR and SCoT techniques

Profile techniques	Number of bands	Monomorphic bands	Polymorphic bands	Polymorphism (%)
RAPD	30	8	22	73.33
ISSR	44	20	24	54.58
SCoT	61	38	23	37.70

The SCoT amplification Technique resulting in banding profiles produced by the eight primers were illustrated in Table 3.

From Table 3 primers, SCoT 4 showed 9 bands giving 3 monomorphic and 6 polymorphic bands with 66.66% polymorphism between genera *Cressa* and *Cuscuta*. While SCoT 1 showed 9 bands giving 6 monomorphic and 3 polymorphic bands with 33.33% polymorphism. On the otherwise, SCoT 6 producing 4 bands with 3 monomorphic and 1 polymorphic bands indicating 25% polymorphism. The summary of the amplification of the three techniques for making DNA analysis to assess the genetic diversity between the two genera under investigation were presented in Table 4.

DISCUSSION

The obtained results of this study revealed that SCoT technique shows 37.7% polymorphism between the two genera under investigation, *Cressa* Linn. and *Cuscuta* Yunck. belong to the same family, Convolvulaceae recording more efficient than that of other techniques, RAPD and ISSR (73.33 and 54.58%), respectively. The extracted results were firstly done, since no reports could be traced concerning this study based on molecular markers. These techniques; RAPD, ISSR and ScoT were previously used to investigate the polymorphism between cultivars of other plants.

The RAPD and ISSR markers[46] have been used extensively in genetic analysis of prokaryotes and eukaryotes. Identification of many crops including potato can be achieved using RAPD technique[47-50]. In this respect and in agreement of current extracted results, RAPD, ISSR, Simple Sequence Repeat (SSR) and Amplified Fragment Length Polymorphism (AFLP) were reported to be successfully distinguished between 39 potato cultivars, respectively[51].

Moreover, primers of ISSR technique have high efficiency power in fingerprinting and diversity analysis for many crops being easy to generate, inexpensive and powerful in detecting polymorphisms[52,53]. In close of present obtained results ISSR was reported[54,55] to be useful in detecting genetic diversity and population structure of coffee, teff, lentils and barley than RAPD technique.

In similarity, SCoT markers were proved to be useful in evaluating the genetic relationship among different cultivars and showed high level of polymorphism[21,22,25,31,33,56,57]. Moreover, Genetic diversity had been assessed through molecular data provided by application of SCoT markers as being an efficient and inexpensive way in DNA analysis.

CONCLUSION

This study is the first record to distinguish between the two genera under investigation related to the same family. Furthermore, it assessed the ability of the RAPD, potentially useful ISSR marker and efficiency of SCoT systems to distinguish the studied samples and make comparison based on fragment polymorphism. The obtained results of RAPD technique showed a high percentage of polymorphism (73.33%), while ISSR and SCoT techniques resulted in lower percentage (54.58 and 37.7%) than the other, respectively.

Finally, it can be recommended that SCoT technique is the most efficiency to distinguish between two genera, *Cressa* Linn. and *Cuscuta* Yunck. based molecular analysis than ISSR and RAPD techniques, consequently.

SIGNIFICANT STATEMENT

This study discovered the applying of SCoT as molecular technique that can be beneficial for genetic diversity between two genera; *Cressa* Linn. and *Cuscuta* Yunk. belong to the same family, Convolvulaceae rather than RADP and ISSR techniques. Further, this study will help the researchers to uncover the critical areas of genetic bands which related to the same family that many researchers were not able to explore. Thus a new theory on genetic distinguish between different genera belong to the same family may be arrived at.

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