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Genetic Diversity Among some Parental Varieties of *Vicia faba* L. Using ISSR and SCoT Molecular Marker Techniques

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ABSTRACT

This study was executed to evaluate molecular variations among 13 genotypes of *Vicia faba* from Egypt and Kurdistan of Iraq for use in crop breeding programs in Kurdistan. The genetic relationships have been detected among these genotypes using ISSR and SCoT markers. Ten and twelve primers had successfully generated reproducible polymorphic amplicons that were suitable for studying the genetic variability between studied genotypes. Out of 225 clear amplicons detected, (77 for ISSR and 92 for SCoT were polymorphic) with 70.71 and 70.03 polymorphism %, respectively. SCoT markers provided more discriminating data and were more informative with high UM%, DI, and Rp values (37.0, 0.23, and 3.81, respectively) compared with ISSR markers (25.7, 0.21, and 3.50, respectively). Cluster analysis and PCo analysis based on ISSR-SCoT combined data succeeded in the description of genetic diversity and heterogeneity within studied genotypes. Hence, setting the studied genotypes into four major groups. The first group included check variety only while the second group included sakha1, sakha3 and sakha4, the third group included only Giza843, and the last group contained all other genotypes, including all studied cultivars from Kurdistan. Crosses between divergent genotypes out of these groups may lead to the development of effective breeding strategies in the future. Therefore, we recommend more studies on the genetic improvement of these cultivars and their inclusion in breeding programs in Kurdistan of Iraq due to the significant genetic differences between them.

Keywords: Genetic diversity among of Vicia faba L. - SCoT and ISSR.

INTRODUCTION

The faba bean (*Vicia faba* L. 2n=12) is considered to be one of the most important pulse crops produced in Iraq. The small-seeded *Vicia faba* major (broad bean), the large-seeded *Vicia faba* minor field or (horse bean), and the medium-seeded type (*Vicia faba*) equine are the three different varieties of faba beans. In actuality, the only difference between the three types of seed size is that they all belong to the same species Hanelt and Mettin (1989). The edible seed of faba beans, a resource with great economic value that complements cereal-based diets with substantial amounts of protein, makes it an ideal crop for rotation with cereals. The ultimate objective of plant breeders in order to sustain high agricultural productivity is the production of varieties with high yield potential. A novel cultivar should not only have a high yield potential but also consistent performance and extensive adaptability to a variety of settings. Given that genotypes reply to environmental change in different ways, the genotype environment (G×E) interaction is crucial for faba bean breeders. Strong G×E interactions for quantitative traits like seed yield can dramatically reduce the benefits of selecting superior genotypes for improved cultivar development (Fathi *et al.*, 2013). Evaluating the stability of performance and breadth of adaptability for cultivars being chosen for a wide range of settings has become more crucial. When genotypes are tested in various conditions, number of stability metrics have been proposed to define yield stability, with varying degrees of success (Dawod and Al-Layla, 2008).

On the other hand, the assessment of genetic variation is a crucial component of genetic studies, biodiversity research, germplasm characterization, and choosing favorable genotypes in plant breeding programs. Where it is becoming known that significant levels of genetic variation are not expressed in the phenotype. Recently, technological developments in molecular genetics have made it possible to quantify genetic diversity at the DNA level by detecting different molecular markers (Abid *et al.*, 2015). One of the most important advantages of molecular markers is that they are not affected by plant developmental stages or environmental factors (Abdel-Razzak *et al.*, 2012).

Therefore, molecular marker techniques are used in order to assess and utilize genetic variation in favor of a breeding program. Where, marker-assisted selection (MAS) is expected to change the strategies used for faba bean breeding, as it has in many other crop species. Already, different types of molecular marker techniques have been employed to assess the genetic variations among V. faba genotypes such as RFLP, RAPD, AFLP, TRAP, and SSAP (Abid *et al.*, 2015). Inter-simple sequence repeat (ISSR) molecular marker technique was also utilized in order to explore the genetic diversity of *V. faba* genotypes (Terzopoulos and Bebeli, 2018; Asfaw *et al.*, 2018). Recently, Collard and Mackill (2009) outlined a quick and innovative DNA marker technology called start codon targeted (SCoT) polymorphism. This marker was developed based on the short-conserved region flanking the Adenine-Thymine-Guanine (ATG) start codon in plant genes. SCoT markers are like RAPD and ISSR molecular marker techniques because a single primer is used as forward and reverse. Moreover, as a PCR-based gene target technique, SCoT analysis has low cost and is effective to use (Bhattacharyya *et al.*, 2013).

Many studies found that SCoT might be more effective than other dominant DNA molecular markers like RAPD and ISSR because it is gene-targeted (Gupta *et al.*, 2018). Also, SCoT is superior to these markers in higher

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polymorphism and better marker resolvability (Gorji *et al.*, 2011). Moreover, SCoT can generate co-dominant markers caused by insertions and deletions, as well as they can generate dominant markers caused by sequence variations like RAPD and ISSR (Aswathy *et al.*, 2017). However, using ISSR and SCoT markers together gives very effective, reliable, and more superior results in genetic diversity study than the use of single markers (Mao *et al.*, 2018).

Therefore, the objective of this study was to assess molecular variations among 13 faba bean genotypes from Egypt and Kurdistan using ISSR and SCoT molecular marker techniques to test the efficiency of these techniques for genotype identification and marker-assisted selection. Hence, then help in managing the germplasm, improving present breeding methods, and introducing new cultivars in breeding programs in Kurdistan.

MATERIALS AND METHODS

This research was conducted during summer season 2021 at a private laboratory in Egypt (Creative Egyptian Biotechnologists; Giza, Egypt) to evaluate molecular variation among 13 genotypes of faba bean.

Genetic materials

The genetic materials used in this study were seeds of thirteen varieties of *Vicia faba* (Table 1) shows the pedigree of these genotypes and contains information such as origin, earliness, foliar diseases reaction, Orobanche reaction, drought-tolerant, seed color and seed size.

Table 1. List of cultivars used in the study.

	Cultivars	Abbreviation	Origin	Pedigree	Earliness	Foliar Diseases reaction	*Orobanche* reaction	Drought tolerant	Seed color	Seed size
1.	Sakha 1	Sa1	FCRI, EGYPT	Giza 716 x 620/283/85	Early	Resistant	Susceptible	Susceptible	light brown	Medium
2.	Sakha 3	Sa3	FCRI, EGYPT	Promising line 716/402/2001 derived from cross716 (Giza461 X 503/453/83)	Late	Resistant	Susceptible	Susceptible	light brown	Medium
3.	Sakha 4	Sa4	FCRI, EGYPT	Sakha 1 X Giza 3	Early sowing	Resistant	Susceptible	Susceptible	light brown	Medium
4.	Nubaria 3	Nu3	FCRI, EGYPT	Selected from Ahnacia line	Late	Resistant	Susceptible	tolerant	light brown	Medium
5.	Nubaria 4	Nu4	FCRI, EGYPT	-	Late	Resistant	Susceptible	tolerant	light brown	Medium
6.	Nubaria 5	Nu5	FCRI, EGYPT	-	Late	Resistant	Susceptible	tolerant	light brown	Medium
7.	Giza 716	Gi716	FCRI, EGYPT	461\842\83x503\453\83	Early	Resistant	Susceptible	tolerant	light brown	large
8.	Giza 843	Gi843	FCRI, EGYPT	561/2076/85 X 461/845/83	Late	Moderately resistant	Resistant	Susceptible	light brown	Medium
9.	Misr3	Mi3	FCRI, EGYPT	L667 x (Cairo 241 x Giza 461)	Moderate	Moderately resistant	Resistant	Susceptible	light brown	Medium
10.	FLIP17-066FB	R117	IRAQ	Selection for heat from shambat	-	-	-	-	-	Medium
11.	FLIP17-071FB	R179	IRAQ	S88135-3-2-1	-	-	-	-	-	Medium
12.	FLIP19-218FB	F19	IRAQ	S88094-8-1	-	-	-	-	-	Medium
13.	Check variety	CV	IRAQ	Local variety	Moderate	Moderately resistant	known	known	brown	Medium

Molecular SCoT and ISSR analysis

DNA Extraction

Thirteen cultivars of *Vicia faba* were collected separately then DNA extraction was performed using DNeasy plant Mini Kit (Bio Basic).

Polymerase chain reaction (PCR)

In the molecular analysis of thirteen types of Vicia faba, genomic DNA was employed as a template for PCR amplification using 10 ISSR primers and 12 SCoT primers. Primers for ISSRs purchased from Operon Technology in Alameda, California, the consensus sequence used in Joshi *et al.* (1997) and Sawant *et al. (*1999). Research, on the other hand, was used to create SCoT primers, which were purchased from Biobasic Com. The ISSR and SCoT methods' amplification reactions were carried out in accordance with the instructions provided by Fathi *et al.* (2013) and Xiong *et al.* (2011), respectively.

1- Inter Simple Sequence Repeat (ISSR)

The DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 94º C for 4 min followed by 40 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 the reaction was finally stored at 4 ºC.

2-Start Codon Target Translation (SCoT).

The DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 94º C for 4 min followed by 40 cycles of 1 min at 94º C, 1 min at 55º C, and 2 min at 72ºC. The reaction was finally stored at 72º C for 10 min the reaction was finally stored at 4 ºC.

Gel electrophoresis

Amplified products on a 1.5% agarose gel with ethidium and 100 bp to 1.5 kb ladder markers, were separated. The run took place in a tiny submarine gel Bio-Rad for about 30 minutes at 100V.

Gel reading and analysis

DNA GelAnalyzer3 software was employed to evaluate banding pattern images after they were taken with the Bio-1D Gel Documentation System. This program rated clear amplicons as present (1) or absent (0) for each primer and input the results as a binary data matrix. According to Adhikari *et al.*, (2015) DNA-profiles for ISSR and SCoT methods were produced from this matrix.

Polymorphic Information Content (PIC) and DI (Diversity Index) were calculated according to, PIC = 1-p2-q2, DI=average PIC value Gorji *et al.*, (2011) where p is frequency of present amplicon and q is frequency of absent amplicon.

The ability of each primer to differentiate among the studied was also evaluated using binary data and the resolving power (Rp) value calculated as described by Prevost and Wilkinson (1999) using the formula:

$$Rp = \sum I_a = \sum 1 - [2 \times (|0.5 - p|)]$$

Where, ^{I}a (amplicon informativeness) was calculated for each amplicon scored individually by the primer, p being the ratio of studied lines containing theI amplicon.

Comparative analysis

According to binary data matrix, similarity coefficients were calculated by Dice coefficient Nei and Li, (1979). Agglomerative hierarchical clustering (AHC) analysis (Cluster analysis) derived from Unweight pair-group average (UPGMA) method and Principal Coordinate (PCo) analysis were performed using XLSTAT 2019.2.2 software according to Hamada *et al.,* (2019). Where PCo analysis is used for classification of genotypes and in fact, it is a supplementary method for cluster analysis Singh and Chaudhary, (1985).

RESULTS AND DISCUSSION

Molecular variation assessment using ISSR technique.

Molecular banding patterns and DNA profiles estimated from ISSR technique were shown in Figs. 1 and 2.

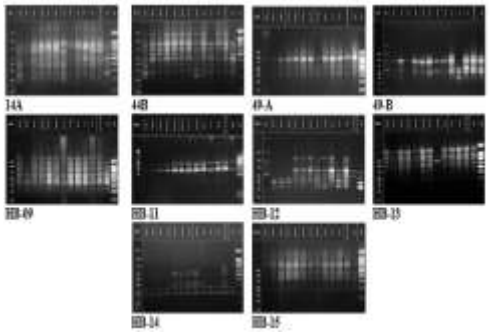

Fig. 1. Banding patterns of ISSR -PCR products for 13 *Vicia faba* L. cultivars produced with ten primers. L, ladder (0.1 :1.5 kb).

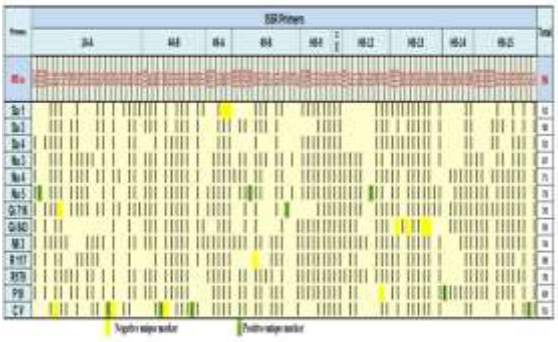

Fig. 2. DNA-profile representation of ISSR fingerprints of 13 *Vicia faba* L. cultivars based on 9666 amplicons 21 of them were marker loci according to Adhikari *et al.* (2015).

These Figures clearly demonstrated that there were differences among patterns of *Vicia faba* genotypes. This was accomplished by using ten primers targeted 96 scoreable amplicons with product sizes ranging between 174 and 1955 bp.

Molecular data obtained from analyzing ISSR banding patterns and recorded in Table 2 showed that polymorphism levels varied from one primer to the other and ranged from 0.0 to 100%. Where the 14A primer targeted the highest number of amplicons (21) with the highest value of Rp (9.394) and polymorphism percentage and PIC were 95.24% and 0.309. Demonstrating that the 14A primer showed a high level of discrimination and informativeness in the identification when compared to other primers.

On the other hand, Table 3 showed the similarity matrix based on ISSR-technique data where the lowest values of similarity coefficient were 0.585 between Giza843 and check variety, followed by 0.628 between Giza716 and check variety. While the highest values of similarity coefficient were 0.931 between Misr3 and FLIP17-071FB followed by 0.914 between Nubaria3 and Nubaria5.

Table 2. Molecular data estimated from banding patterns of ISSR 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
14A	$(CT)_8TG$	174:1867	1	15	2	3	21	95.24	0.309	9.394
44B	$(CT)_8GC$	191:1236	4	5	2	1	12	70.50	0.162	2.618
49-A	$(CT)_6AG$	342:1809	1	2	0	2	5	80.00	0.169	1.694
49-B	$(CA)_6GG$	267:1569	0	10	2	1	13	100.00	0.324	6.006
HB-09	$(GT)_6GC$	359:1955	3	3	0	0	6	50.00	0.152	1.386
HB-11	$(GT)_6CC$	212 :410	2	0	0	0	2	0.00	0.000	0.000
HB-12	$(CAC)_3GC$	237:1190	0	7	1	1	9	100.00	0.318	4.158
HB-13	$(GAG)_3GC$	275:1340	2	4	0	4	10	80.00	0.170	2.310
HB-14	$(CTC)_3GC$	186: 692	2	3	1	0	6	66.70	0.220	2.464
HB-15	$(GTG)_3GC$	283:1601	4	7	1	0	12	66.67	0.268	4.928

Table 3. Similarity matrix for 13 *Vicia faba* L. cultivars based on ISSR-technique data

Cultivars	Sa 1	Sa 3	Sa 4	Nu 3	Nu 4	Nu 5	Gi 716	Gi 843	Mi 3	R 117	R179	F19
Sa 3	0.843											
Sa 4	0.846	0.843										
Nu 3	0.790	0.803	0.807									
Nu 4	0.748	0.793	0.797	0.899								
Nu 5	0.720	0.748	0.752	0.914	0.903							
Gi 716	0.689	0.767	0.770	0.818	0.894	0.867						
Gi 843	0.673	0.648	0.710	0.754	0.794	0.766	0.752					
Mi 3	0.746	0.742	0.762	0.865	0.883	0.871	0.833	0.806				
R 117	0.721	0.697	0.793	0.810	0.800	0.803	0.791	0.772	0.857			
R179	0.721	0.750	0.754	0.891	0.851	0.867	0.814	0.784	0.931	0.853		
F19	0.701	0.730	0.735	0.848	0.853	0.855	0.800	0.783	0.863	0.839	0.889	
C V	0.699	0.752	0.680	0.695	0.672	0.645	0.628	0.585	0.720	0.727	0.694	0.690

Comparative analysis for data of molecular markers techniques enables the determination of proximity or distance between genotypes through displays of genotypes in clusters (Ozturk *et al.,* 2022). For this purpose, cluster analysis was

performed among thirteen cultivars of faba bean, a dendrogram for the genetic relationship among cultivars were carried out as Fig. 3 which separated them into four major groups at a similarity coefficient = 0.799. The first group included (check variety) only while the second group included (sakha3, sakha1 and sakha4), the third group included only (Giza83), the last group divided into (four) subgroups, the first subgroup included (FLIP17-071FB, Misr3) and (FLIP19-218FB) only, the second subgroup (Nubaria5, Nubaria3, Nubaria4), the third one is (Giza716) and the last one is (FLIP17-066FB).

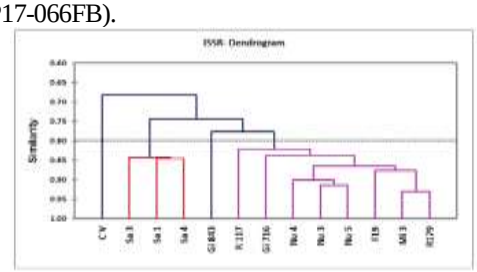

Fig. 3. UPGMA clustering dendrogram for 13 *Vicia faba* L. cultivars based on ISSR molecular data using Nei and Lis similarity coefficient.

Legend: TL represents truncated line at the coefficient of similarity =0.799.

Molecular variation assessment using SCoT technique.

In the same vein, Figs. 4 and 5 displayed various molecular banding patterns and DNA profiles conducted but using the SCoT molecular marker technique. This was accomplished by targeting 129 scoreable amplicons using 12 primers with product sizes between 116 and 1635 bp.

Table 4 shows molecular data obtained from analyzing SCoT banding patterns. Polymorphism levels varied amongst primers, with the SCoT-7 primer targeting the highest number of amplicons (17) with a polymorphism percent of 94.10% and a PIC of 0.357 in comparison to other primers. While the ScoT-5 primer targeted the fewest amplicons and furthermore demonstrated the lowest RP value of 1.386, polymorphism%, and PIC, which were 60.00% and 0.139, respectively. This demonstrates the SCoT-7 primer's high-informative and discriminatory capabilities in the discovery of genetic diversity.

Table 5 showed the similarity matrix based on SCoT-technique the lowest value of similarity coefficient was 0.680 between Sakha3 and check variety, followed by 0.693 between Sakha1 and FLIP17-066FB. While the highest value of similarity coefficient was 0.900 between Giza716 and Misr3 followed by 0.893 between Sakha4 and Nubaria3.

Cluster analysis was performed among the thirteen cultivars of faba bean using SCoT molecular data. A dendrogram obtained from this analysis was illustrated in Fig. 6 which separated all studied cultivars into four groups at a similarity coefficient =0.795. The first group included (Sakha1, Sakha3) while the second group included FLIP17-071FB, FLIP19-218FB, FLIP17-066FB and check variety. The third group included (Giza843) only, while the last included Sakha4, Nubaria3, Misr3, Giza716, Nubaria4 and Nubaria5.

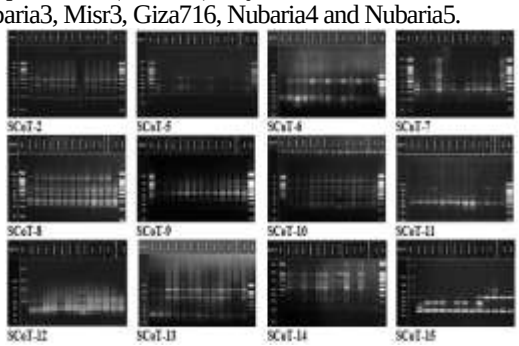

Fig. 4. Banding patterns of SCoT-PCR products for 13 *Vicia faba* L. cultivars produced with 12 primers. L, 1.5 kb

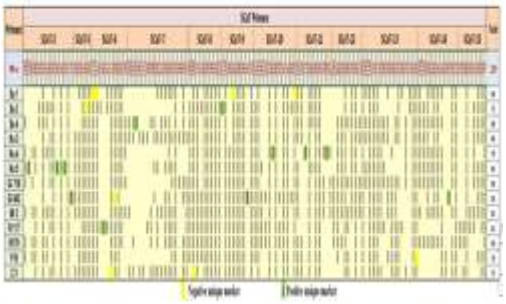

Fig. 5. DNA-profile representation of ScoT fingerprints of 13 *Vicia faba* L. cultivars based on 129 amplicons 21 of them were marker loci according to Adhikari *et al.*, (2015).

Table 4. Molecular data estimated from banding patterns of SCoT 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-2	CAACAATGGCTACCACCC	115:1386	4	6	4	0	14	71.43	0.233	5.236
SCoT-5	CAACAATGGCTACCACGA	202: 624	2	2	0	1	5	60.00	0.139	1.386
SCoT-6	CAACAATGGCTACCACGC	199:1119	1	4	1	4	10	90.00	0.216	2.772
SCoT-7	CAACAATGGCTACCACGG	197:1635	1	15	1	0	17	94.10	0.357	8.932
SCoT-8	CAACAATGGCTACCACGT	214:1283	6	2	0	1	9	33.33	0.100	1.386
SCoT-9	CAACAATGGCTACCAGCA	239:1372	5	1	2	1	9	44.44	0.095	1.078
SCoT-10	CAACAATGGCTACCAGCC	236:1477	5	5	1	1	12	58.33	0.166	2.618
SCoT-11	AAGCAATGGCTACCACCA	277:1107	3	5	2	0	10	70.00	0.232	3.388
SCoT-12	ACGACATGGCTACCAACG	213:1064	2	6	0	0	8	75.00	0.337	4.312
SCoT-13	ACGACATGGCGACCATCG	224:1542	5	10	0	1	16	68.80	0.272	6.776
SCoT-14	ACGACATGGCGACCACGC	214:1438	1	9	1	0	11	99.90	0.319	5.082
SCoT-15	ACGACATGGCGACCGCGA	185:794	2	6	0	0	8	75.00	0.255	2.772

The underlines of ATG codon in the primer sequence were fixed

Table 5. Similarity matrix for 13 *Vicia faba* L. cultivars based on SCoT-technique data.

Cultivars	Sa 1	Sa 3	Sa 4	Nu 3	Nu 4	Nu 5	Gi 716	Gi 843	Mi 3	R 117	R179	F19
Sa 3	0.809											
Sa 4	0.803	0.813										
Nu 3	0.797	0.783	0.893									
Nu 4	0.716	0.768	0.802	0.786								
Nu 5	0.797	0.782	0.849	0.855	0.859							
Gi 716	0.764	0.775	0.864	0.881	0.874	0.860						
Gi 843	0.711	0.737	0.762	0.793	0.767	0.780	0.821					
Mi 3	0.758	0.744	0.844	0.840	0.865	0.852	0.900	0.837				
R 117	0.693	0.719	0.722	0.741	0.725	0.764	0.757	0.758	0.809			
R179	0.761	0.759	0.805	0.800	0.824	0.824	0.828	0.807	0.865	0.826		
F19	0.748	0.775	0.772	0.755	0.765	0.753	0.823	0.773	0.815	0.781	0.833	
C V	0.736	0.680	0.761	0.817	0.714	0.755	0.748	0.723	0.778	0.782	0.795	0.814

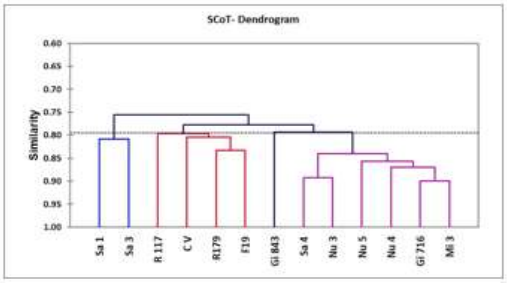

Fig. 6. UPGMA clustering dendrogram for 13 *Vicia faba* L. cultivars based on SCoT molecular data using Nei and Lis similarity coefficient.

Legend: TL represents truncated lineat the coefficient of similarity =0.795.

Resulted data comparison of discriminating capacity between ISSR and SCoT Molecular markers techniques in Table (6) were recorded. The total number of scorable ISSR amplicons was 96 with an average of 9.6 amplicons/primer, with a product size ranged from 174 and 1955 bp. While the total number of scorable SCoT amplicons was 129 with an average of 10.8 amplicons/primer, with a product size ranged from 115 and 1635 bp. Also, through better discrimination capabilities compared with ISSR, SCoT primers targeted generating 92 polymorphic amplicons with an average of 7.7/primer and 21 unique markers with an average of 1.8/primer. While ISSR primers except HB-11 targeted generating 77 polymorphic amplicons with an average of 7.7/primer and 21 unique markers with an average of 2.1/primer. This discrimination capability for the SCoT technique was confirmed by UM%, DI%, and Rp% values which were 37.0, 0.23 and 3.81, respectively compared with ISSR which were 25.7%, 0.21 and 3.50, respectively. This indicates the high discriminatory potential of using SCoT primers compared with ISSR primers. Where SCoT markers were more discriminating, provided more informative data. Also, confirms that it can be relying on the SCoT techique to evaluate the genetic diversity among the faba bean cultivars better than ISSR markers. More importantly 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 Hajibarat *et al.* (2015).

This result agrees with Abdel-Hameed *et al.* (2020); Bashandy *et al.* (2020); Ola-Ahmed and Abd EL-Aziz (2021) in Grape, Gorji *et al*. (2011) in potato and Etminan *et al.* (2016) in durum wheat. They found that the SCoT marker was more informative and effective than the ISSR marker to estimate the genetic diversity and perform fingerprinting in these plants. While this result disagrees with Ramadan *et al*. (2019), who found that the ISSR marker is more discriminating and provides more informative data than SCoT in fennel cultivars. While Baghizadeha and Dehghan (2018) and other researchers recommended that it is preferable to use these molecular marker techniques in combination with each other for distinctive fingerprinting. Also indicated that cluster analysis based on ISSR and SCoT data obviously discriminated among the Iranian pistachio cultivars, this was confirmed by Abd EL-Aziz *et al.* (2019), who showed that the combined data of ISSR and SCoT molecular marker techniques were suitable and more informative for assessing the genetic relationships and genetic diversity among apricot strains.

Table 6. Comparison of discriminating capacity between ISSR and SCoT Molecular markers techniques.

Technique	MS	SA		PA		TUA		P %	UM%	DI	Rp
		Total	Mean	Total	Mean	Total	Mean				
ISSR	174: 1955	96	9.6	77	7.7	21	2.1	70.7	25.7	0.21	3.5
ScoT	115: 1635	129	10.8	92	7.7	21	1.8	70	37	0.23	3.81

MS: Molecular size; SA: Scorable Amplicons; PA: Polymorphic Amplicons; TUA: Total unique amplicons (+&-); P%: Polymorphism %; UM%: Unique Marker %; DI: Diversity Index; Rp: Resolving power,

In Table 7 on combined data of both ISSR and SCoT-technique the lowest value of similarity coefficient was 0.667 between Giza843 and check variety, followed by 0.695 between Sakha1 and Giza843. While the highest value of similarity coefficient was 0.894 between Misr3 and FLIP17-071FB followed by 0.883 between Nubaria4 and Giza716.

Table 7. Similarity matrix for 13 *Vicia faba* L. cultivars based on combined data of ISSR and SCoT technique.

Cultivars	Sa 1	Sa 3	Sa 4	Nu 3	Nu 4	Nu 5	Gi 716	Gi 843	Mi 3	R 117	R179	F19
Sa 3	0.823											
Sa 4	0.820	0.824										
Nu 3	0.794	0.791	0.858									
Nu 4	0.731	0.779	0.800	0.837								
Nu 5	0.763	0.767	0.808	0.882	0.879							
Gi 716	0.731	0.771	0.826	0.854	0.883	0.863						
Gi 843	0.695	0.700	0.742	0.777	0.779	0.774	0.792					
Mi 3	0.753	0.743	0.810	0.851	0.873	0.861	0.870	0.824				
R 117	0.705	0.710	0.750	0.770	0.759	0.781	0.772	0.764	0.830			
R179	0.744	0.755	0.784	0.840	0.837	0.843	0.822	0.797	0.894	0.838		
F19	0.727	0.755	0.756	0.797	0.807	0.801	0.812	0.778	0.837	0.807	0.859	
C V	0.721	0.710	0.729	0.766	0.696	0.707	0.697	0.667	0.753	0.759	0.752	0.759

The results of the-detected and characteristic ISSR and SCoT molecular markers of faba bean genotypes used in this investigation are mentioned in Table (8). All studied genotypes had positive and negative unique amplicons except-Nubaria3, Misr3 and FLIP17-071FB. These markers were 21 positive unique and 21 negative unique amplicons. Where Nubaria5 was characterized by the highest number of positive unique amplicons (6), while Sakha1 was characterized by the highest number of negative unique markers which was also 6 amplicons. However, the Check variety was characterized by the highest number of total unique markers with a total of 9 amplicons (4 positives and 5 negatives).

Table 8. Detected and characteristic ISSR and SCoT molecular markers of the studied genotypes

Inbred lines	Unique markers					
	Primer	Molecular size	Type	Number		
				+	-	All
Sa 1	49-A	342, 486	-	0	6	6
	SCoT-6	1017,1119	-			
	SCoT-9	827	-			
	SCoT-10	236	-			
Sa 3	SCoT-5	263	-	1	1	2
	SCoT-9	1372	+			
Sa 4	SCoT-7	1447	+	1	0	1
Nu 3	-	-		0	0	0
Nu 4	SCoT-10	693	+	3	0	3
	SCoT-11	328,774	+			
Nu 5	14-A	1224	+	6	0	6
	49-B	1095	+			
	HB-12	692	+			
	SCoT-2	242,283,1252	+			
Gi 716	14-A	775	-	1	1	2
	49-B	340	+			
Gi 843	HB-13	409,483,810,1130	-	3	5	8
	SCoT-2	115	+			
	SCoT-6	328	-			
	SCoT-9	301	+			
	SCoT-14	342	+			
Mi 3	-	-		0	0	0
R 117	49-B	942	-	1	1	2
	SCoT-6	494	+			
R179	-	-		0	0	0
F19	HB-12	304	-	1	2	3
	HB-14	692	+			
	SCoT-13	224	-			
C V	14-A	354,876	-	4	5	9
	14-A	368	+			
	44-B	669	-			
	44-B	282,733	+			
	HB-15	373	+			
	SCoT-6	377	-			
	SCoT-8	1090	-			

Comparative analysis based on ISSR and SCoT combined molecular data

Comparative analysis of genotypes based on combined molecular data of more than one molecular technique enables the determination of proximity or distance between genotypes with more accurately and clearly (Abd EL-Aziz *et al.*, 2019). A dendrogram for the genetic relationship among genotypes was carried out as Fig. 7 which separated them into four major groups at a similarity coefficient =0.790. The first group included check variety only while the second group included sakha1, sakha3 and sakha4, the third group included only Giza83. The last group was divided into two subgroups, the first subgroup included (FLIP17-066FB) only, and the second subgroup includes the other genotypes and was divided into two sub-subgroups. The first sub-subgroup included only FLIP19-218FB, and the second sub-subgroup included six genotypes were Misr3, FLIP17-071FB, Nubaria4, Giza716, Nubaria3, and Nubaria5.

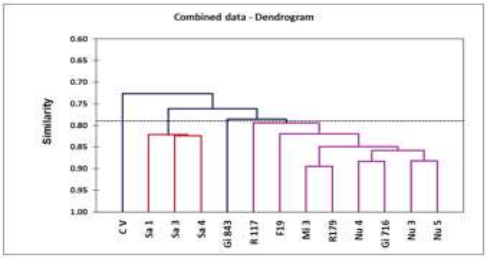

Fig. 7. UPGMA clustering dendrogram for 13 *Vicia faba* L. cultivars based on ISSR-SCoT combined data using Nei and Lis similarity coefficient.

Legend: TL represents truncated line at the coefficient of similarity = 0.790 using ISSR-SCoT combined data.

The PCO in Fig. 8 results reveal that the first three axes represent only 31,47% of the total variability, of these the two first coordinates accumulated 16,54% of variability and the first 14,93%. Moreover, in this analysis four main groups and an independent one, comprising check variety are observed. Scattering diagram ISSR and SCoT data were subjected to a principal component analysis (PCo) in order to obtain an alternative view of the relationships between the varieties in Fig. 7. In the two pco (F1 and F2), in general all the accessions of faba bean were classified into four groups of the plot from pco. The scales of pco scattering diagram constructed from the data, four groups are distinguishable from the dendrogram which was confirmed also by pco diagram, group one included 3 genotypes (Giza843, Misr3 and Nubaria4), group two included 4 genotypes (FLIP17-066FB, FLIP17-071FB, FLIP19-218FB and check variety), group three included 2 genotypes (Giza716 and Nubaria5) finally group four included 4 genotypes (Nubaria3, Sakha4, Sakha1 and Sakha3).

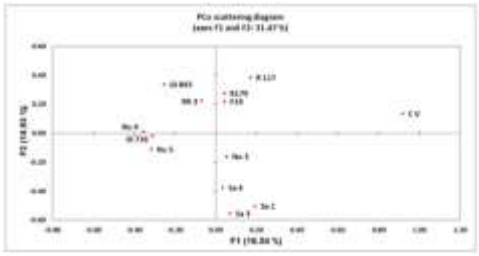

Fig. 8. PCo scattering diagram for 13 *Vicia faba* L. cultivars based on dice dissimilarity index for combined data.

CONCLUSION

Two molecular markers ISSR and SCoT were used to analyze the relationship among thirteen cultivars of *Vicia faba L.* It was discovered that the SCoT markers are effective than ISSR for differentiating and identifying the cultivars of faba bean under study. In summary, SCoT markers successfully evaluated the genetic relationships among the *Vicia faba* genotypes used and generated a high level of RP. The 13 genotypes were clustered into four groups using the UPGMA dendrogram, crosses between divergent genotypes out of these groups may lead to the development of effective breeding strategies in the future. The results of this study will aid in managing the germplasm, improving present breeding methods, and introducing new cultivars.

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التنوع الوراثى بين بعض الأصناف الأبوبة للفول البلدى باستخدام تقنيات العلامات الجزيئية ISSR و SCoT

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الملخص

نفذت هذه الدراسة لتقييم الاختلافات الوراثية الجزيئية بين 13 صنف أو تركيب وراثى من الفول البلدى من مصر وكردستان العراق لاستخدامها في برامج تربية المحاصيل في كردستان. وتم الكشف عن العلاقات الوراثية بين هذه التراكيب باستخدام كل من التقنية الجزيئية ISSR و SCoT حيث نجحت عشرة واثني عشر من البادئات على الترتيب في إنتاج متضخمات (أمبليكونات) متباينة قابلة للتكرار وكانت مناسبة لدراسة التباين الوراثى بين التراكيب الوراثية تحت الدراسة. وتم الكشف عن 225 متضخم (77 لـ ISSR و 92 لـ SCoT) بنسبة تباين 70.71 و 70.03٪ على التوالي. واظهرت النتائج ان تقنية SCoT كانت أكثر تميزًا بنسبة علامات متفردة ودليل تنوع وقدرة تمييزية (0.37 و 0.23 و 3.81 على التوالي) أعلى مقارنة بتقنية ISSR (25.7و 0.21 و 3.50 على التوالي). نجح التحليل العنقودي وتحليل التناسق الأساسى بالإعتماد على البيانات المدمجة لتقنيتي ISSR و SCoT في وصف التنوع الوراثى وعدم التجانس بين التراكيب الوراثية المدروسة. حيث قسمت التراكيب الوراثية المدروسة إلى أربع مجموعات رئيسية المجموعة الأولى تضمنت الصنف المحلي الإختبارى فقط بينما المجموعة الثانية تضمنت سخا 1 ، سخا 3 ، سخا 4 والمجموعة الثالثة تضمنت جيزة 843 فقط ، والمجموعة الأخيرة تضمنت جميع التراكيب الوراثية الأخرى بما في ذلك جميع الأصناف المختبرة من كردستان. ومن ثم فإن عمليات التهجين بين هذه التراكيب الوراثية المتباينة من هذه المجموعات قد تؤدي إلى تطوير استراتيجيات تربية فعالة في المستقبل . لذلك ، نوصي بإجراء المزيد من الدراسات حول التحسين الوراثي لهذه الأصناف وإدراجها في برامج التربية في كردستان العراق بسبب الاختلافات الجينية الكبيرة بينهم.