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Determination of the Genetic Variations of Chickpea (*Cicer Arietinum* L.) Genotypes Preserved in the Jordanian Seed Genbank Using ISSR and SCoT Molecular Markers

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Abstract

This study was the first report on the genetic diversity among 18 local chickpea (*Cicer arietinum*) genotypes presented in the Jordanian National Agricultural Research Center (NARC) Seed Genbank collected from different Jordanian regions. Inter-Simple Sequence Repeat (ISSR) and Start Codon Target (SCoT) Polymorphism molecular markers were used to investigate their relationship. 13 different ISSR primers amplified 135 bands, among them 110 were polymorphic. In comparison, the 10 SCoT primers amplified 166 bands, among them 129 were polymorphic. The polymorphism average and the PIC values in ISSR and SCoT primers were 72%, 75%, 0.17, and 0.26, respectively. The genetic similarity values were moderate, for ISSR-PCR analyses between 0.769-0.452, while for SCoT-PCR analyses ranged from 0.744 to 0.468. The UPGMA analysis grouped the 18 *Cicer arietinum* genotypes into two main clusters divided into sub-clusters and smaller groups in both ISSR and SCoT analyses. STRUCTURE analyses produced three populations mixed within each other in ISSR and SCoT analyses. Current results indicated that SCoT molecular marker proved to be more informative in distinguishing closely related genotypes than ISSR molecular marker. The 18 *C. arietinum* genotypes are genetically related to each other, even though they were geographically distant landraces. Our findings provided significant data for characterizing the genotypes within the seed genebank.; however, there is no duplication among the stored *Cicer arietinum* genotypes.

Keywords: Genetic diversity, Chickpea, Seed Genbank, ISSR, SCoT, PIC.

1. Introductıon

Chickpea (*Cicer arietinum* L.) is a self-pollinated legume species from the family Fabaceae (2n = 16), a semi-arid region growing type (De Giovanni *et al*., 2017), containing a high nutritive content and being a cheap source of protein, in addition to improve land fertility (Saeed *et al*., 2011) and soil health through symbiotic nitrogen fixation (Thudi *et al*., 2016). Chickpeas were domesticated as a crop around 10,000 years ago in Southwest Asia (Anatolia). From there, they spread throughout the Fertile Crescent, reaching South Asia, East Africa (Ethiopia), Australia, and North America (Sani *et al*., 2018). Cultivated Chickpea (*C. arietinum*) is part of a group of annual chickpea species native to Mediterranean regions, while the majority of the remaining species in the genus are perennial species native to colder climates in Anatolia, the Caucasus, and Central Asia (Coyne *et al*., 2020). Chickpea has little genetic diversity due to obligatory self-pollination and an extensively monotonous genome (Aggarwal *et al*., 2015).

Genetic diversity assessment is important for crop improvement and efficient management and conservation of germplasm resources (Pakseresht *et al*., 2013). The concept of genetic resources frameworks breeding, and conservation involved a genocentric perspective with their erosion and the danger of losing biodiversity (Aubry, 2019). Genbank widely reflects the available sources of genetic diversity in landraces, The National Plant Germplasm System (a collaborative program that preserves the genetic diversity of plants) contains over 4000 accessions of chickpeas that originated from almost 50 countries, which made them available to scientists worldwide to support plant breeding and other research program on chickpea (Redden and Berger, 2007). The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has the largest collection with 19,959 accessions of cultivated chickpeas and 308 accessions of 18 wild Cicer species from 60 countries (Plekhanova *et al*., 2017).

Genetic techniques and biotechnology tools enable us to leverage the information stored in genebanks more efficiently and rapidly. Thus, genotyping of the collections will allow us to identify the duplication, establish varietal limits, and estimate the population variability (Díez *et al*., 2018). ISSR markers can be targeted towards specific sequences, which are reported to be abundant in the genome and can overcome the technical difficulties of RFLP and RAPD. The previous two markers could not address the reliable genetic variation within chickpeas (Pakseresht *et al*., 2013).

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ISSR markers were reported to be more compatible than the RAPD markers, as they were highly reproducible polymorphic DNA markers able to expose various informative loci from a single amplification and had been successfully used to study diversity and phylogenetic relationships for the last decade (Aggarwal *et al*., 2015). For a long time, genetic fingerprinting in chickpeas was inhibited by the little genome variability, which can be facilitated by highly polymorphic functional markers such as SCoT marker. ISSR and SCoT techniques were more informative than prior biochemical such as isozymes and storage proteins, in addition to other molecular methods as RAPD markers, which are used to study variation and genetic relationships in *Cicer* species (Pakseresht *et al*., 2013). The genetic variation detected by ISSR and SCoT markers within and between *Cicer* species was higher than the amount computed from RAPD and AFLP markers (Amirmoradi *et al*., 2012).

Pakseresht *et al*. (2013) mentioned in their study that 40 Chickpea landraces were collected from different geographical locations in northwest Iran and were amplified using ISSR and SCoT markers. The accessions from the same geographical regions showed more genetic similarities than those from different or isolated places. According to the observed results, SCoT marker was more informative than ISSR marker, and both of them were revealed to be better than previous markers for *Cicer* genotypes diversity assessment. Another study done on 48 Iranian chickpea genotypes was identified using 9 SCoT primers; the average PIC value was 0.45 and showed an elevated level of polymorphism and diversity (Hajibarat *et al*., 2015).

In other research, a genetic diversity study on cultivated chickpea *C. arietinum* and its wild progenitor *C. reticulatum* using RAPD and ISSR markers concluded that ISSR analysis was a more reliable reference for genetic diversity estimation than RAPD marker (Gautam et al., 2016). Ahmad and Talebi (2017) examined the genetic diversity of 35 chickpea breeding lines using 14 SCoT primers, with an average PIC value of 0.36 per primer. Cluster analysis grouped the 35 breeding lines into three major clusters.

Jordan has four distinct biogeographical regions, the cultivated areas for *C. arietinum* were found in the Mediterranean and Irano-Turanian regions. This is the first report of studying genetic variability and the relationship of *C. arietinum* genotypes at a DNA level. Therefore, this study aimed to determine the genetic variability of different *C. arietinum* genotypes, and to find any duplication presented in the National Agricultural Research Center (NARC) seed genbank using ISSR and SCoT molecular markers.

2. Methodology

2.1. Plant Material and DNA Extraction

The genetic material used in this study were seeds of 18 local genotypes of *Cicer arietinum* (Kabuli type), obtained from the seed genbank of the NATIONAL AGRICULTURAL RESEARCH CENTER (NARC), Jordan, shown in Table 1. Total genomic DNA was extracted from the young leaf tissues of 2-week-old *C. arietinum* seedlings according to the 2X CTAB protocol (Doyle and Doyle, 1990). DNA quality was assessed using electrophoresis in a 0.8% agarose gel with a 1kb DNA ladder, while DNA purity and quantification were measured using a Nanodrop Spectrophotometer.

Table 1: List of locally cultivated genotypes of *Cicer arietinum* studied, working code, original serial number, and province.

Working code	Serial No.	Taxonomic Name	Province
C1	2775	*Cicer arietinum*	Amman
C2	2777	*Cicer arietinum*	Balqa'
C3	2779	*Cicer arietinum*	Mafraq
C4	2780	*Cicer arietinum*	Amman
C5	2787	*Cicer arietinum*	Ma'an
C6	2788	*Cicer arietinum*	Unknown
C7	2791	*Cicer arietinum*	Jerash
C8	2793	*Cicer arietinum*	Ma'an
C9	2794	*Cicer arietinum*	Karak
C10	2795	*Cicer arietinum*	Zarqa
C11	2796	*Cicer arietinum*	Ma'an
C12	2798	*Cicer arietinum*	Madaba
C13	2799	*Cicer arietinum*	Irbid
C14	2801	*Cicer arietinum*	Unknown
C15	3588	*Cicer arietinum*	Unknown
C16	3590	*Cicer arietinum*	Unknown
C17	4361	*Cicer arietinum*	Ma'an
C18	4537	*Cicer arietinum*	Ma'an

2.2. ISSR-PCR Analysis

26 ISSR primers (designed by the UNIVERSITY OF BRITISH COLUMBIA BIOTECHNOLOGY LABORATORIES) were used in this study. ISSR amplifications were performed with a volume of 15 µl including 10X Taq Buffer (1.5 µl), 25 mM $MgCl_2$ (1.2 µl), 2.5 mM dNTP (1.2 µl), 2.5 µM primer (1 µl), 5 ng/ µl of templet DNA (3 µl), 1U of *Taq* Polymerase (0.15 µl) (FERMENTAS). Amplifications were performed in the thermal cycler with the following program: initial denaturation at 94 °C 4 min, 45 cycles at 94 °C 45 sec, annealing at 50-61 °C 90 sec, extension at 72 °C 2 min, and final extension at 72 °C 5 min. PCR products were electrophoresed in 1.8 % agarose gel containing 5µl Red Safe run for 1 h and 30 min at 85V in the gel tank, then the records were photographed and preserved.

2.3. SCoT-PCR Analysis

19 SCoT primers (synthesized by OLIGOMER BIOTECHNOLOGY COMPANY, ANKARA, TURKEY) were used in this study. SCoT amplifications were performed with a volume of 25 µl including 10X Taq Buffer KCL (2.5 µl), 25 mM $MgCl_2$ (2 µl), 2.5 mM dNTP (2 µl), 2.5 µM primer (5 µl), 10 ng/ µl of templet DNA (5 µl), 1U of *Taq* Polymerase (0.2 µl) (FERMENTAS). Amplifications were performed in the thermal cycler with the following program: initial denaturation at 94°C 3 min, 40 cycles at 94 °C 1 min, annealing at 50-61 °C 1 min, extension at 72 °C 2 min, and final extension at 72 °C 5 min. PCR products were electrophoresed in 1.8 % agarose gel containing 5µl Red Safe run for 1 h and 30 min at 85V in the gel tank, photographed with a UV documentation system and the records were preserved.

2.4. Data Analysis

The amplified products of each ISSR and SCoT PCR reaction were analyzed using the TOTALLAB CLIQS (1D gels) software program. A binary matrix was prepared based on the results obtained, as present (1) or absent (0). The PIC (polymorphism information content) was calculated as a formula suggested by De Riek *et al*. (2001). Genetic similarity values were calculated by the Jaccard coefficient using the DendroUPGMA program. Dendrograms were constructed using the MEGA software version 11. The population structure analysis was performed by the STRUCTURE software program v. 2.3.4 (Pritchard *et al*. 2000). STRUCTURE HARVESTER (Earl and VonHoldt, 2012) was used to calculate the K values, delta K graph and to extract the suitable bar plot graph using the binary matrix.

3. Results

3.1. Assessment of ISSR-PCR Analysis

Out of 26 ISSR primers, 13 ISSR primers produced clear and reproducible band profiles (Fig. 1) which amplified a total of 135 bands from 18 *C. arietinum* genotypes; among these bands 110 were polymorphic. The number of amplified bands varied between 2-18 with an average of 10.4 bands per primer. The band sizes ranged from 333 bp to 3919 bp. PPB% ranged from 0 to 100% with an average of 72%. The PIC values ranged from 0 to 0.3 with an average of 0.17 (Table 2).

Table 2. Primers, primer sequences, annealing temperatures, band profiles, polymorphism percentages, and PIC values of *C. arietinum* genotypes using ISSR molecular marker.

Primer	Sequences (5'-3')	An.T	Band Size (bp)	TB	PB	PPB%	PIC
ISSR 807	$(AG)_8$-T	52	566-1790	10	7	70	0.17
ISSR 810	$(GA)_8$-T	50	467-2231	8	7	87.5	0.2
ISSR 811	$(GA)_8$-C	50	333-3919	18	17	94	0.26
ISSR 812	$(GA)_8$-A	50	560-1700	9	6	67	0.23
ISSR 813	$(CT)_8$-T	50	406-1560	4	2	50	0.13
ISSR 817	$(CA)_8$-A	50	949-1320	2	0	0	0
ISSR 818	$(CA)_8$-G	53	609-2381	14	14	100	0.3
ISSR 819	$(TC)_8$-C	50	712-1423	5	3	60	0.05
ISSR 829	$(TG)_8$-C	53	510-3000	16	15	94	0.27
ISSR 834	$(AG)_8$-YT	53	364-1122	13	12	92	0.17
ISSR 847	$(CA)_8$-RC	55	582-2368	13	10	77	0.14
ISSR 853	$(TC)_8$-RT	53	400-1433	13	10	77	0.18
ISSR 855	$(AC)_8$-YT	53	470-1887	10	7	70	0.1
SUM				135	110	-	-
AVG				10.4	-	72	0.17

Where Y= (C, T), R= (A, G), An.T: annealing temp., TB: total no. of bands, PB: polymorphic bands, PPB%: The polymorphism percentage, and PIC: polymorphism information content.

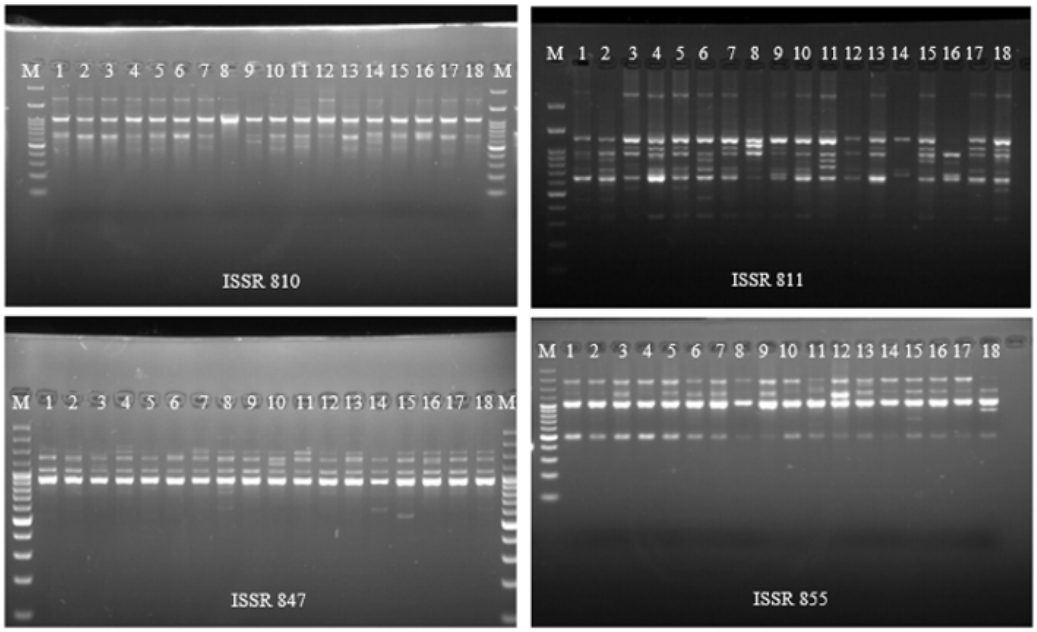

Figure 1. ISSR band profiles of *C. arietinum* genotypes with ISSR primers 810, 811, 847and 855. M: 100 bp plus DNA marker (FERMENTAS), (1:C1, 2:C2, 3:C3, 4:C4, 5:C5, 6:C6, 7:C7, 8:C8, 9:C9, 10:C10, 11:C11, 12:C12, 13:C13, 14:C14, 15:C15, 16:C16, 17:C17, and 18:C18).

The genetic similarity among the 18 *C. arietinum* genotypes ranged from 0.769 to 0.452. The highest similarity (0.769) was observed between 4537 (C18) and 4361 (C17) genotypes both from Maan, and the lowest genetic similarity (0.452) was found between 2798 from Madaba (C12) and 2795 from Zarqa (C10) (Table 3). UPGMA dendrogram grouped the 18 *C. arietinum* genotypes into two main clusters (Fig. 2). Cluster I included only one variety 2795 from Zarqa (C10), and cluster II divided into two sub-clusters; the first sub-cluster included 2801 Unknown (C14), and the second sub-cluster included all the other 16 *C. arietinum* genotypes divided into smaller groups. For more genetic examination within the 18 *C. arietinum* genotypes population structures, STRUCTURE analysis was used and according to Delta K, they were divided into three clusters (Fig. 3 and Fig. 4).

Table 3. ISSR genetic Jaccard similarity matrix between *C. arietinum* genotypes.

	C1 Amman	C4 Amman	C2 Balqa	C3 Mafraq	C5 Maan	C8 Maan	C11 Maan	C17 Maan	C18 Maan	C7 Jerash	C9 Karak	C10 Zarqa	C13 Irbid	C14 Unknown	C15 Unknown	C16 Unknown	C6 Unknown	C12 Madaba
C1 Amman	1	0.634	0.592	0.56	0.62	0.533	0.646	0.556	0.539	0.541	0.592	0.507	0.527	0.521	0.545	0.5	0.566	0.551
C4 Amman		1	0.643	0.676	0.671	0.603	0.603	0.676	0.587	0.634	0.618	0.533	0.62	0.548	0.613	0.569	0.635	0.603
C2 Balqa			1	0.634	0.629	0.629	0.631	0.657	0.657	0.638	0.6	0.514	0.623	0.571	0.662	0.618	0.662	0.656
C3 Mafraq				1	0.686	0.573	0.571	0.62	0.558	0.581	0.55	0.547	0.547	0.541	0.605	0.52	0.584	0.618
C5 Maan					1	0.681	0.662	0.766	0.639	0.643	0.671	0.562	0.606	0.556	0.69	0.556	0.714	0.688
C8 Maan						1	0.612	0.687	0.662	0.62	0.584	0.481	0.606	0.514	0.644	0.556	0.667	0.714
C11 Maan							1	0.694	0.692	0.646	0.52	0.582	0.606	0.486	0.623	0.552	0.623	0.587
C17 Maan								1	0.769	0.697	0.63	0.542	0.632	0.557	0.721	0.627	0.671	0.694
C18 Maan									1	0.603	0.55	0.506	0.568	0.52	0.649	0.629	0.605	0.618
C7 Jerash										1	0.551	0.527	0.614	0.5	0.63	0.563	0.653	0.621
C9 Karak											1	0.538	0.644	0.616	0.595	0.513	0.556	0.562
C10 Zarqa												1	0.577	0.467	0.513	0.467	0.494	0.452
C13 Irbid													1	0.549	0.639	0.507	0.573	0.559
C14 Unknown														1	0.526	0.479	0.526	0.552
C15 Unknown															1	0.589	0.653	0.672
C16 Unknown																1	0.547	0.576
C6 Unknown																	1	0.6
C12 Madaba																		1

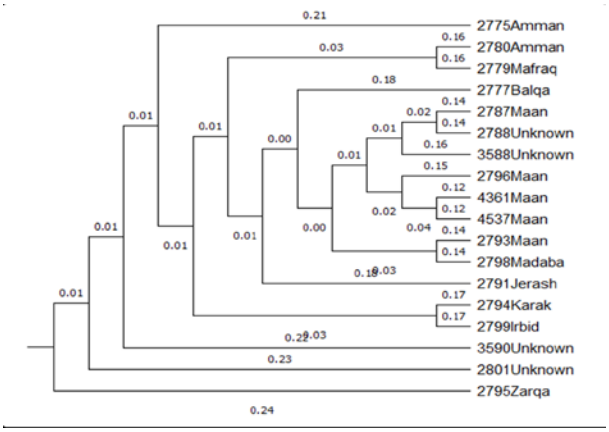

Figure 2. UPGMA clustering pattern of 18 *C. arietinum* genotypes based on the genetic similarity values of ISSR analyses.

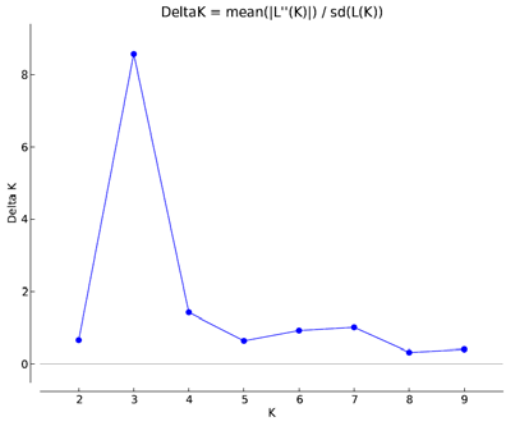

Figure 3. STRUCTURE analysis for the number of clusters (K = 3) of *C. arietinum* genotypes based on ISSR analyses.

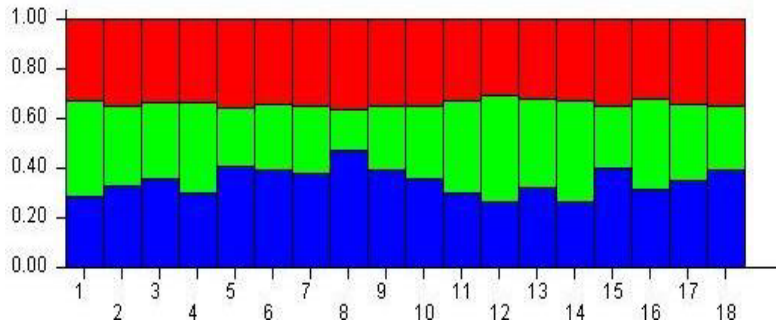

Figure 4. STRUCTURE analysis of the 18 *C. arietinum* genotypes based on ISSR analyses. The same color indicates the same group.

3.2. Assessment of SCoT-PCR Analysis

Of 19 SCoT primers, 10 primers produced clear and reproducible band profiles (Fig. 5) which amplified a total of 166 bands from 18 *C. arietinum* genotypes; among these bands, 129 were polymorphic. The number of amplified bands varied between 8-25 with an average of 16.6 bands per primer. The band sizes ranged from 185 bp to 3825 bp. PPB% ranged from 50 to 92% with an average of 75%. The PIC values ranged from 0.13 to 0.35 with an average of 0.26 (Table 4).

Table 4. Primers, primer sequences, annealing temperatures, band profiles, polymorphism percentages, and PIC values of *C. arietinum* genotypes using SCoT molecular marker.

Primer	Sequences (5'- 3')	An.T	Band Size (bp)	TB	PB	PPB%	PIC
SCoT 3	CAACAATGGCTACCACCG	52	588-2650	13	12	92	0.35
SCoT 9	CAACAATGGCTACCAGCA	54	800-2935	8	4	50	0.19
SCoT 12	ACGACATGGCGACCAACG	56	185-2729	20	16	80	0.26
SCoT 15	ACGACATGGCGACCGCGA	54	225-2911	21	18	86	0.31
SCoT 16	ACCATGGCTACCACCGAC	52	288-3404	20	18	90	0.30
SCoT 18	ACCATGGCTACCACCGCC	54	227-2927	13	7	54	0.13
SCoT 19	ACCATGGCTACCACCGGC	54	688-3825	17	15	88	0.31
SCoT 21	ACGACATGGCGACCCACA	52	230-1733	14	10	71	0.21
SCoT 28	CCATGGCTACCACCGCCA	50	295-3048	15	9	60	0.24
SCoT 32	CCATGGCTACCACCGCAC	50	240-3281	25	20	80	0.26
SUM				166	129	-	-
AVG				16.6		75	0.26

An.T: annealing temp., TB: total no. of bands, PB: polymorphic bands, PPB%: The polymorphism percentage, and PIC: polymorphism information content.

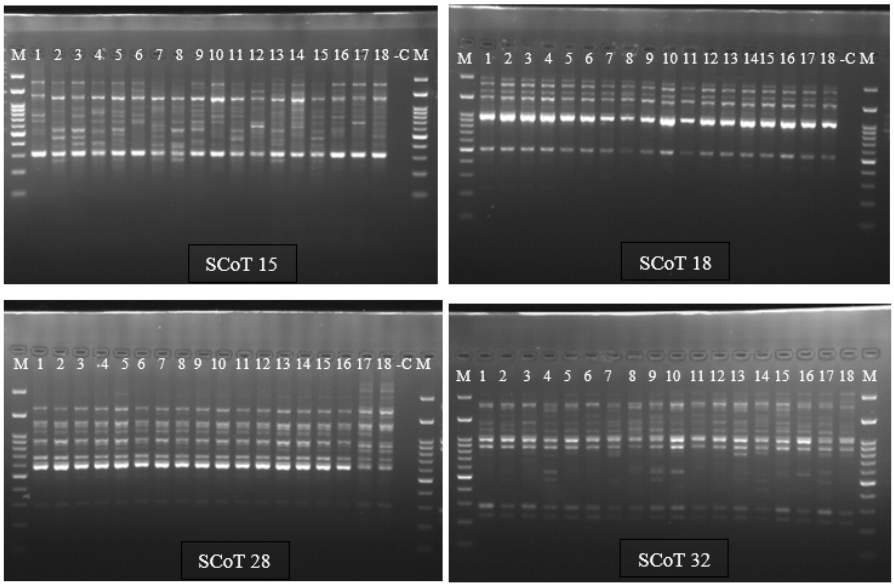

Figure 5. SCoT band profiles of *C. arietinum* genotypes with SCoT primers 15, 18, 28 and 32. M: 100 bp plus DNA marker (FERMENTAS), -C: negative control, (1:C1, 2:C2, 3:C3, 4:C4, 5:C5, 6:C6, 7:C7, 8:C8, 9:C9, 10:C10, 11:C11, 12:C12, 13:C13, 14:C14, 15:C15, 16:C16, 17:C17, and 18:C18).

The genetic similarity among the 18 *C. arietinum* genotypes ranged from 0.744 to 0.468. The highest similarity (0.744) was observed between 2779 from Mafraq (C3) and 2777 from Balqa (C2) genotypes, and the lowest genetic similarity (0.468) was found between 3590 Unknown (C16) and 2779 from Mafraq (C3) (Table 5). UPGMA dendrogram grouped the 18 *C. arietinum* genotypes into two main clusters (Fig. 6). Cluster I included genotypes 3590 Unknown (C16) and 3588 Unknown (C15), and cluster II divided into two sub-clusters; sub-cluster I included genotypes 2801 Unknown (C14), 2799 from Irbid (C14), 2798 from Madaba (C12), 2795 from Zarqa (C10), 2794 from Karak (C9), 2788 Unknown (C6), 2791 from Jerash (C7), with 2793 (C8), 4537 (C18), 4361 (C17), 2796 (C11) and 2787 (C5) from Maan divided in much smaller groups. Sub-cluster II included 2779 from Mafraq (C3), 2777 from Balqa (C2), 2780 (C4), and 2775 (C1) from Amman genotypes. For more genetic examination within the 18 *C. arietinum* genotypes population structures, STRUCTURE analysis was used and according to Delta K, they were divided into three clusters (Fig. 7 and Fig. 8).

Table 5. SCoT genetic Jaccard similarity matrix between *C. arietinum* genotypes.

	2775Amman	2780Amman	2777Balqa	2779Mafraq	2787Maan	2793Maan	2796Maan	4361Maan	4537Maan	2791Jerash	2794Karak	2795Zarqa	2798Madaba	2799Irbid	2801Unknown	3588Unknown	3590Unknown	2788Unknown
2775Amman	1	0.698	0.646	0.653	0.59	0.518	0.643	0.56	0.518	0.533	0.559	0.625	0.545	0.523	0.589	0.496	0.519	0.571
2780Amman		1	0.63	0.67	0.654	0.549	0.66	0.636	0.633	0.565	0.604	0.611	0.562	0.569	0.606	0.5	0.495	0.59
2777Balqa			1	0.744	0.59	0.518	0.66	0.518	0.491	0.505	0.504	0.565	0.532	0.551	0.56	0.565	0.519	0.54
2779Mafraq				1	0.644	0.579	0.649	0.536	0.564	0.552	0.564	0.615	0.565	0.514	0.565	0.527	0.468	0.561
2787Maan					1	0.627	0.735	0.642	0.655	0.617	0.596	0.604	0.613	0.606	0.642	0.575	0.532	0.583
2793Maan						1	0.648	0.625	0.595	0.6	0.568	0.56	0.583	0.508	0.517	0.574	0.491	0.551
2796Maan							1	0.713	0.676	0.59	0.615	0.654	0.663	0.625	0.587	0.578	0.577	0.635
4361Maan								1	0.682	0.571	0.581	0.56	0.556	0.575	0.611	0.484	0.587	0.596
4537Maan									1	0.627	0.593	0.628	0.529	0.602	0.581	0.508	0.615	0.641
2791Jerash										1	0.598	0.62	0.558	0.593	0.558	0.562	0.532	0.684
2794Karak											1	0.688	0.623	0.616	0.623	0.559	0.492	0.579
2795Zarqa												1	0.574	0.609	0.534	0.593	0.55	0.618
2798Madaba													1	0.618	0.556	0.574	0.504	0.551
2799Irbid														1	0.575	0.595	0.61	0.588
2801Unknown															1	0.56	0.545	0.596
3588Unknown																1	0.55	0.528
3590Unknown																	1	0.602
2788Unknown																		1

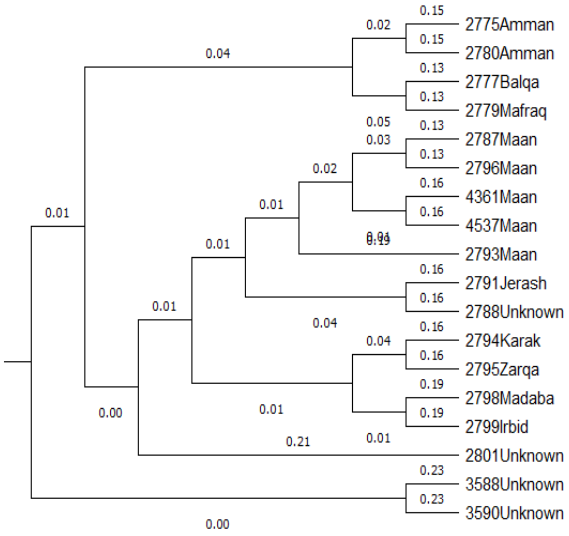

Figure 6. UPGMA clustering pattern of 18 *C. arietinum* genotypes based on the genetic similarity values of SCoT analyses.

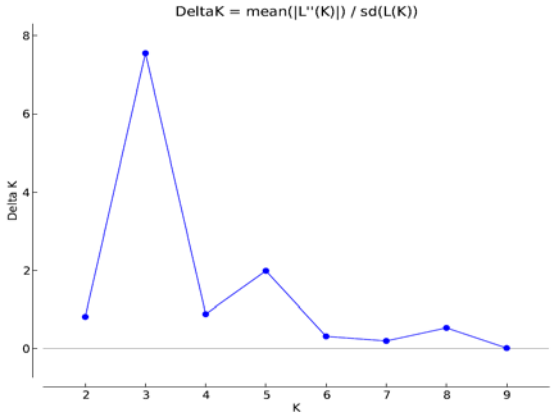

Figure 7. STRUCTURE analysis for the number of clusters (K = 3) of *C. arietinum* genotypes based on SCoT analyses.

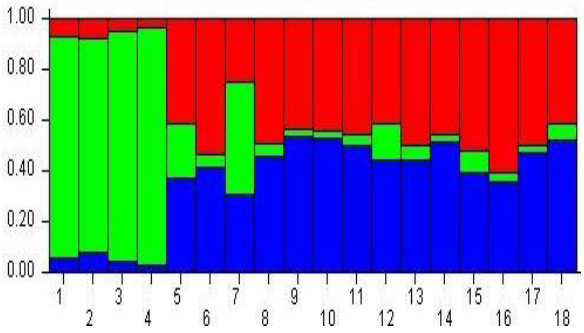

Figure 8. STRUCTURE analysis of the 18 *C. arietinum* genotypes based on SCoT analyses. The same color indicates the same group.

4. Discussion

The collection of *C. arietinum* genotypes obtained from the Jordanian seed genebank (NARC) contains 18 genotypes collected from 10 different regions of Jordan presented in Table 1. In this study, genetic diversity was assessed among 18 Jordanian genotypes of *C. arietinum* using 13 ISSR and 10 SCoT primers. The ISSR analyses yielded a total of 135 bands of which 110 were polymorphic, with an average of 72 % polymorphism. The number of amplified bands varied between 2 -18 with an average of 10.4 bands per primer. The average PIC value of ISSR primers was calculated as 0.17. On the other hand, 10 SCoT primers amplified reproducible 166 bands, among these 129 were polymorphic (PPB: 75%). The number of amplified bands varied between 8 - 25 with an average of 16.6 bands per primer. The average PIC value of SCoT primers was calculated as 0.26. The number of bands and polymorphism levels of SCoT primers were higher than ISSR.

The average PIC value of SCoT primers (0.26) was higher than that of ISSR (0.17), although the polymorphism rate for these two primers was high, their informativeness (PIC) was not the highest value for a dominant marker, which is said to be 0.5 according to De Riek *et al.* (2001). These results showed that SCoT primers were more efficient and informative than ISSR in estimating genetic diversity in studied Jordanian *C. arietinum* genotypes. *C. arietinum* is known to have a narrow genetic base and minimal polymorphism as previously demonstrated by molecular markers, including seed protein, isozyme, and RFLP analyses (Iruela *et al*., 2018). Using RAPD and ISSR markers, a molecular analysis was conducted to determine the genetic diversity and relationships among chickpea cultivars; 33 bands overall were produced by the 5 polymorphic primers with 63.6 % polymorphism (Tahir and Karim, 2011). In another study, 10 SCoT primers were used to fingerprint 10 genotypes of *Cicer arietinum*, 33 polymorphic bands out of 94 amplified bands were recorded, and the polymorphism percentage was 35 % (Serag, 2021).

On the other hand, some studies obtained higher polymorphism levels than the present study. For example, ISSR and SCoT markers were used for the analysis of genetic relationships among 38 accessions of 8 annual *Cicer* species, including the cultivated *C. arietinum* which detected an elevated level of polymorphism about 97 % (Amirmoradi *et al*., 2012). Results obtained from the same study confirmed that *C. arietinum* has the narrowest genetic base among all the annual species and the wild *C. reticulatum* genetically is the closest species to C. *arietinum.* Aggarwal *et al*. (2015) reported that 26 ISSR primers yielded a total of 232 bands representing the genetic diversity of 125 cultivars of chickpeas of Indian origin, 213 of which were polymorphic (91.8 %) with an average of 9 bands per primer.

In addition, Hajibarat *et al.* (2015) showed that 9 SCoT primers amplified a total of 145 bands from 48 Iranian chickpea accessions; among these bands 133 were polymorphic. The average PIC value was 0.45 and showed a prominent level of polymorphism and diversity. The three prior studies reported higher polymorphism levels than this study due to the use of different *Cicer* species and a greater number of genotypes. However, Pakseresht *et al.* (2013) studied 40 Iranian chickpea genotypes using 7 ISSR and 10 SCoT primers. The average PIC values were 0.216 for ISSR markers and 0.232 for the SCoT markers, which was close to the results of our study. They concluded similarly that SCoT marker was more informative than ISSR markers.

The genetic similarity matrix between *C. arietinum* genotypes was calculated based on Jaccard's similarity coefficient. For ISSR molecular marker, the genetic

similarity values were moderate (0.769 - 0.452). The genetically closest genotypes were 4537 from Maan and 4361 from Maan (0.769), and the most distant genotypes were 2798 from Madaba and 2795 from Zarqa (0.452). For SCoT molecular marker, the genetic similarity values were between 0.744 and 0.468; the genetically closest genotypes were 2779 from Mafraq and 2777 from Balqa (0.744), and genetically the lowest genotypes were 3590 Unknown and 2779 from Mafraq (0.468). The similarity coefficient values for the two molecular markers were similar, but they did not produce identical clusters in the UPGMA dendrograms. The difference between the two markers is due to the different DNA sequences presented in each primer, which tracked different readings among the PCR analyses, producing different band profiles.

According to UPGMA clustering pattern obtained from ISSR analyses, the 18 *C. arietinum* genotypes were grouped into 2 main clusters: cluster I included only one variety 2795 from Zarqa, and cluster II was divided into two sub-clusters, the first sub-cluster included 2801 Unknown, and the second sub-cluster included the rest 16 *C. arietinum* genotypes divided into smaller groups. 3590 Unknown placed closest to 2801 Unknown in the first sub-cluster, followed by 2799 from Irbid, 2794 from Karak, and 2791 from Jerash were grouped close with each other. After that, 2798 from Madaba, 2793 from Maan, 4537 from Maan, 4361 from Maan, 2796 from Maan, 3588 Unknown, 2788 Unknown, 2787 from Maan, and 2777 from Balqa were genetically grouped. 2779 from Mafraq, 2780 from Amman, and 2775 from Amman were placed close altogether.

On the other hand, the UPGMA dendrogram produced using SCoT analyses grouped the 18 *C. arietinum* into 2 main clusters: cluster I included 3590 Unknown and 3588 Unknown, and cluster II contained the remaining 16 genotypes divided into two main sub-clusters. Sub-cluster I was split into more small groups; group I with only 2801 Unknown (the closest to cluster I), group II contained 2799 from Irbid, 2798 from Madaba, 2795 from Zarqa, and 2794 from Karak. Group III had 2788 Unknown and 2791 from Jerash, while group IV included all genotypes from the same collected provinces 2793, 4537, 4361, 2796, and 2787 from Maan. Sub-cluster II included 2779 from Mafraq, 2777 from Balqa, 2780 from Amman, and 2775 from Amman. In both UPGMA dendrograms, the *C. arietinum* genotypes collected from Amman and Maan provinces were placed in the same group "genetically close to each other." The four genotypes with Unknown localities were placed differently each time. However, 2801 Unknown and 3590 Unknown lay close in both analyses.

The ISSR genetic STRUCTURE analyses grouped the 18 Jordanian *C. arietinum* genotypes into 3 clusters, which were presented and mixed in all the 18 genotypes; the red cluster presented somehow in equal balance. However, the green and blue clusters fluctuate all over the 18 genotypes. Likewise, the SCoT genetic structure grouped them also into 3 clusters, with different arrangements all over the 18 genotypes. The green cluster presented the most in 2775 from Amman, 2780 from Amman, 2779 from Mafraq, 2777 from Balqa, and 2791 from Jerash genotypes, while the red and blue clusters ranged in a balance between the remaining genotypes. SCoT molecular marker proved to be more informative in distinguishing closely related genotypes than ISSR molecular marker. These findings implied that the 3 clusters (populations) were mixed over the years as they had been cultivated throughout the country.

5. Conclusion

To our knowledge, this is the first report estimating the genetic variability of the cultivated *C. arietinum* genotypes using ISSR and SCoT molecular marker techniques. Molecular markers are stable, quick, and reliable techniques that can be applied in laboratory conditions. ISSR and SCoT molecular markers are dominant, highly reproducible, require a small amount of DNA, simple to work with, making them a valuable tool for studying plant genetic populations and diversity. In this study, we cannot prove any differences between the cultivated *C. arietinum* genotypes according to the biogeographical areas via ISSR analyses. However, SCoT analysis showed that the genotypes from Amman, Mafraq, and Balqa were the genotypes genetically closest to each other according to UPGMA dendrogram and Structure software clustering model.

Amman, Mafraq, and Balqa provinces presented in the Middle to North of Jordan and considered near each other in the same biogeographical regions mixed between the Mediterranean and the Irano-Turanian, have the same genetic structure. The genotypes from Maan province were all clustered in the same group and genetically the same. All of the 18 *C. arietinum* genotypes are genetically related to each other, even though they were geographically distant landraces. According to our ISSR-PCR and SCoT-PCR results, most of the genotypes were the same in some of the primers, but they were not identical. Therefore, there is no duplication among the stored genotypes; however, more studies can be done to ensure the previous results.

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