Genetic differentiation using ISSR, SCoT and DNA barcoding for Quinoa genotypes

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ABSTRACT

Quinoa is considered a good source of fiber, vitamins and minerals as well as health-benefiting phytochemicals. Molecular markers are essential tools to identify genetic diversity in different species and are useful for germplasm preservation and cultivar identification. The objectives of this study were to use different molecular markers to molecularly characterize seven quinoa genotypes using ISSR, SCoT and the DNA chloroplast markers of rbcL and rpoC1 to reveal genetic polymorphism and identify unique markers for each genotype. Fifteen of ISSR primers were used in this study, a total of 172 amplified bands were generated with an average of 11.7 bands /primer. A total number of amplified polymorphic bands of 90 with an average 6.0 bands /primer; the average level of polymorphism was 49.7%. The efficiency of ISSR markers in discriminating studied genotypes was estimated by obtaining the PIC values, where PIC values varied from 0.21 to 0.84 with an average of 0.69. Twelve SCoT primers were employed to investigate the genetic polymorphism among the seven quinoa genotyping. A total of 157 amplified bands were generated by the 12 primers with an average of 13.1 bands /primer, while a total number of amplified polymorphic bands of 80 with an average of 6.7 band /primer, while the PIC values varied from 0.50 to 0.84 with an average of 0.77. We successfully sequenced barcode genes of rbcL and rpoC1 for the seven quinoa genotypes. These sequences have been submitted to NCBI respiratory and have been assigned to gene bank accession numbers. The sequence alignment revealed that, rbcL retrieved from studied quinoa genotypes with high similarity to other rbcL genes obtained from Chenopodium species by other studies, with similarities ranged from 76% to 80%. In addition, the rbcL gene displayed genetic similarity of high consistency with low genetic evolution and mutation. On the other hand, the sequence alignment revealed that, rpoC1 retrieved from studied quinoa genotypes with high similarity to other rpoC1 genes obtained from other plant species by other studies; similarities ranged from 80% to 81%.

Keywords: *Quinoa, Molecular markers, ISSR, SCoT, DNA barcoding.*

INTRODUCTION

Quinoa (*Chenopodium quinoa*) is a member of the genus *Amaranthaceae* and one of the most common food plants in South America. It is particularly important as a major food stable in the Bolivian and Peruvian Altiplano regions (Wilson, 1988), It is uniquely suited to the high altitudes, arid and sandy soils (Prado *et al*., 2000) with great potential to increase food security in marginal areas due to its outstanding agronomic and nutritional features (Jacobsen and Stølen, 1993).

Quinoa grains are, in addition, especially rich in proteins with a well-balanced and higher content of essential amino acids than common cereals (Bhargava *et al*., 2006). Its grains are also characterized by high contents of isoflavones, which oppose vessel contraction and reduce arterial resistance in humans (Vega-Galvez *et al*. 2010), also, it is considered a good source of fiber, vitamins and minerals as well as health-benefiting phytochemicals (Varli and Sanlier 2016). Unfortunately, many anti-nutritional compounds have been identified in quinoa including saponins, phytic acid, tannins, and protease inhibitors that can adversely affect the performance and longevity of monogastric livestock when used as the primary source of dietary food (Improta and Kellems, 2001). The agronomic promise of quinoa is still underutilized and there are relatively few successful breeding programs available (Zurita-Silva *et al*., 2014). However, no attempts were made to characterize or assess the genetic structure of the introduced germplasm in Egypt and the absence of classification and assessment studies could restrict selection and development as well as expansion of cultivation (Saad-Allah and Youssef, 2018). It has the most qualitative disomic background in its chromosome number and is therefore an allotetraploid species (Ward, 2000). Cultivated quinoa displays a genetic diversity, predominantly expressed in a broad variety of characters such as plant coloration, flowers, protein content, seeds, saponin content, and calcium oxalate content of the leaf, allowing a wide range of adaptability to agroecological conditions (Rodríguez *et al*., 2009). Molecular markers are essential tools to identify genetic diversity in different species and are useful for germplasm preservation and cultivar identification. However, molecular markers enable the implementation of improved breeding programs such as marker-assisted backcross breeding and marker-assisted selection (Maughan *et al*., 2004). For successful quantification of genetic variation in plant species, a variety of DNA-based markers are now accessible. Methods such as amplified fragment length polymorphism (AFLP), inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) are commonly referred to as arbitrarily amplified dominant (AAD) markers (Wolfe and Liston, 1998). Inter-simple sequence repeat (ISSR) markers are random markers which bind several genomic loci and replicate DNA segments in orientation between two identical microsatellite regions (Zietkiewicz *et al*., 1994). ISSR is highly polymorphic and is important in biodiversity, genome mapping and evolutionary genetics studies (Joshi and Dhawan, 2007). This PCR-based approach is used in different plant types and can resolve certain weaknesses in other marker approaches, such as the high costs of AFLP and the poor reproducibility of RAPD (Reddy *et al*., 2002).

Throughout years, in line with the rapid growth of genomic research several new alternative and promising marker approaches have been introduced (Gupta and Rustgi, 2004). A new marker technique named Start

codon targeted (SCoT) was created based on the short conserved region flanking the ATG start codon in the DNA of plant, the use of SCoT markers owing to the longer primer distances and high annealing temperatures would be much more efficient particularly in comparison to other arbitrary markers (Collard and Mackill, 2009). In comparison, the SCoT marker design process does not require any detailed information about the genomic sequence, making it easier to apply to plants without a reference genome (Xiong *et al*., 2009). DNA barcoding is a genetic identification technology that uses a generic genetic region of short DNA sequences, called the DNA barcode (Chen *et al*., 2010). It can be reliably characterized the species with similar morphological characteristics and chemical compositions (Shugen *et al*., 2019). It has two main objectives: identification of organisms, where an unknown sequence matched a sequence of a known species, and exploration of species similar to habitat delimitation and description of species (DeSalle, 2006). Compared to classical PCR-based markers, it enables plant genus and/or species to be classified by acquiring a short DNA sequence from established target regions of the chloroplast genome and linking it to orthologous databases (Viglietti *et al*., 2019). The objectives of this study were to use different molecular markers to molecularly characterize seven quinoa genotypes using: (i) ISSR, (ii) SCoT and (iii) in an attempt to use DNA barcoding markers in quinoa we selected two of candidate DNA chloroplast markers (*rbcL* and *rpoC1*) to reveal genetic polymorphism and identify unique markers for each genotype.

MATERIALS AND METHODS

Plant material

Seven quinoa genotypes introduced to Egyptian market have been collected (Table 1). Fresh leaves have been collected and stored at -80^{o}C for DNA isolation.

DNA Isolation and PCR analysis

Total DNA isolation was conducted using the DNeasy Plant Mini Kit (Qiagen, Germany) in compliance with the manufacturer's protocol. A NanoDrop 2000 Spectrophotometer (Thermo Scientific, Germany) was used to estimate the amount and purity of DNA in samples.

ISSR and SCoT marker analysis

Genetic diversity was investigated using fifteen ISSR and twelve SCoT PCR primers between the studied quinoa genotypes (Table 4 and Table 6) respectively. The PCR amplification of ISSR and SCoT and cycling parameters (Table 3) were carried out as described by Sadek and Ibrahim (2018) and Awad *et al.,* (2019), respectively. The SCoT primers have been developed as Collard and Mackill (2009) have described previously. The PCR amplification products were separated on 1.5% agarose gels in 1xTBE buffer stained with EtBr. The PCR products were visualized on UV light and photographed using a Gel Documentation System (Bio-Rad, USA).

rbcL and *rpoC1* gene barcoding analysis

DNA barcoding analysis was performed with the plastidial *rbcL* and *rpoC1* regions (Tables 2 and 3). The PCR reaction, amplification, band sequencing of *rbcL* and *rpoC1* was conducted as described by (Alshehri *et al.,* 2019). The PCR amplification products were separated on 1.5% agarose gels in 1xTBE buffer stained with EtBr. The PCR products were visualized on UV light and photographed using a Gel Documentation System (Bio-Rad, USA).

Table (1): Origin, source, and seed color of investigated quinoa genotypes.

	Variety Name	Source & Origin	Seed Color
1	Wild Egyptian	Agricultural Research Center (ARC), Egypt	Pale yellow
2	American	Handy Pantry, living whole foods Inc., USA	Yellow
3	Rainbow	Agricultural Research Center (ARC), Egypt	Tri color (mix of Brown, Red and Yellow)
4	Black	NOW Health Group Inc., USA	Black
5	KVL-SRA2	Denmark	Red
6	QL-37	Chile	Light yellow
7	Chipaya	Altiplano Salares, Bolivia	Mixed (White & Paige color)

Table (2): The PCR primers sequence of rbcL and rpoC1 genes and PCR product size.

Primer code	Sequence	Product size
rbcL-F	5'-ATGTCACCACAAACAGAGACTAAAGC-3'	600bp
rbcL-R	5'-TCGCATGTACCTGCAGTAGC-3'	
rpoC1-F	5'-GGCAAAGAGGGAAGATTTCG-3'	550bp
rpoC1-R	5'-CCATAAGCATATCTTGAGTTGG-3'	

Table (3): The PCR reaction parameters of ISSR, SCoT, rbcL and rpoC1.

	Temperature				Time period				Cycles
Step	**ISSR**	**SCoT**	***rbcL***	***rpoC1***	**ISSR**	**SCoT**	***rbcL***	***rpoC1***	
Initial denaturation	94°C	94°C	94°C	94°C	5 min	5 min	5 min	5 min	1
Denaturation	94°C	94°C	94°C	94°C	40 sec	40 sec	30 sec	30 sec	
Annealing	45°C	50°C	48°C	52°C	50 sec	50 sec	30 sec	30 sec	40
Extension	72°C	72°C	72°C	72°C	1 min	1 min	30 sec	30 sec	
Final extension	72°C	72°C	72°C	72°C	7 min	7 min	7 min	7 min	1

Table (4): The list of primers sequence, Total Number of Bands (TB), Monomorphic Bands (MB), Polymorphic Bands (PB), Percentage of Polymorphism (%P), Frequency (F) and Polymorphism Information Content (PIC) as revealed by ISSR analysis of seven quinoa genotypes.

Name	Sequence (5′› 3′)	TB	MB	PB	% P	F	PIC
ISSR-1	AGAGAGAGAGAGAGAGYC	14	9	5	36	0.81	0.70
ISSR-2	AGAGAGAGAGAGAGAGYG	11	6	5	45	0.75	0.64
ISSR-3	ACACACACACACACACYT	10	7	3	30	0.87	0.68
ISSR-4	ACACACACACACACACYG	16	7	9	56	0.71	0.79
ISSR-5	GTGTGTGTGTGTGTGTYG	15	7	8	53	0.71	0.84
ISSR-6	CGCGATAGATAGATAGATA	14	0	14	100	0.28	0.84
ISSR-7	GACGATAGATAGATAGATA	16	6	10	63	0.59	0.84
ISSR-9	GATAGATAGATAGATAGC	10	0	10	100	0.32	0.84
ISSR-10	GACAGACAGACAGACAAT	11	6	5	45	0.75	0.79
ISSR-11	ACACACACACACACACYA	10	6	4	40	0.84	0.79
ISSR-12	ACACACACACACACACYC	7	5	2	29	0.83	0.64
ISSR-16	TCTCTCTCTCTCTCTCA	7	5	2	29	0.86	0.21
ISSR-18	HVHCACACACACACACAT	9	5	4	44	0.78	0.57
ISSR-19	HVHTCCTCCTCCTCCTCC	13	5	8	62	0.68	0.79
ISSR-20	HVHTGTGTGTGTGTGTGT	9	8	1	11	0.94	0.37
Total		**172**	**82**	**90**	**-**	**-**	**-**
Means		**11.7**	**5.5**	**6.0**	**49.7**	**0.66**	**0.69**

Data analysis

For ISSR and SCoT analysis, only clear and unambiguous bands were visually scored as either present (1) or absent (0) for all samples and final data sets included both polymorphic and monomorphic bands. Then, a binary statistic matrix was constructed. Dice's similarity matrix coefficients were then calculated between genotypes using the unweighted pair group method with arithmetic averages (UPGMA). This matrix was used to construct a phylogenetic tree (dendrogram) and Principal coordinate analysis (PCA) was performed according to Euclidean similarity index using the PAST software Version 1.91 (Hammer *et al.,* 2001). The polymorphism information content (PIC) was calculated using the Power Marker software (Liu and Muse, 2005). DNA barcoding of Quinoa genotypes (*Chenopodium quinoa)* was carried out using the Basic Local Alignment Tool (BLAST) available on the National Centre of Biotechnology Information (NCBI) website. All sequences were submitted to GenBank, USA. BLAST searches were applied to all produced sequences using the online databases (GenBank), analyzed using BLASTN 2.9.0 program (http://www.ncbi.nlm.nih.gov/BLAST) and aligned using Align Sequences Nucleotide BLAST. The identification of species was considered successful when the highest similarity percentage included a single species scored more than 97%. Phylogenetic analysis was conducted using MAFFTv6.864, http://www.genome.jp/tools-bin/mafft, and phylogenetic trees were generated.

RESULTS AND DISCUSSION

ISSR polymorphism among the Quinoa genotypes

To investigate the similarity and relationship among the seven quinoa genotypes, 15 of ISSR primers (Fig.1) were used. A total of 172 bands were amplified (Table 4) with average of 11.4 bands/primer. The lowest number of bands (7) was produced by the ISSR-12 and -16, while the highest number of bands (16) was revealed by the ISSR-4 and -7. The number of monomorphic bands reached 82 with an average of 5.5 bands/primer. The maximum number of monomorphic bands (9) was generated by the ISSR-1, while the lowest (0) was produced by the ISSR-6 and -9. The total number of polymorphic bands (90) was averaged as 6.0 bands/primer. The lowest number of polymorphic bands (1) was generated by the ISSR-20, while the highest number of polymorphic bands (14) was revealed by the ISSR-6. The percentage of polymorphism was ranged from 11% (ISSR-20) to 100% (ISSR-6 and -9). The average level of polymorphism was estimated as 49.7%. The frequency ranged from 0.28 to 0.94 for the ISSR-6 and -20; respectively. The PIC values ranged from 0.21 (ISSR-16) to 0.84 (ISSR-5, -6, -7 and -9) with an average of 0.69. In this respect, Saad-Allah and Youssef (2018) have used seven ISSR markers to characterize and genetically compare among five quinoa genotypes. The results showed 85 loci, amongst were 37 with polymorphism (43.53%). The number of amplified loci varied from four (UBC-817) to 26 (UBC-845). The size of amplified fragments was ranged from 139 to 1,111 bp. Also, the overall PIC values were ranged from 0.00 to 0.4. Likewise, Al-Naggar *et al.* (2017) have used 10 ISSR primers to genetically differentiate among five quinoa genotypes. The results produced 53 amplicons, out of which 33 were polymorphic and the average percentage of polymorphism was 61.83%. The range of the number of amplicons/primer was between three and 10 with an average of 5.3 fragments/primer. The number of polymorphic

amplicons varied from one to six with an average of 3.3 fragments/primer. Finally, the size of amplified fragments has been ranged from 130 to 1,456bp among the different used ISSR primers.

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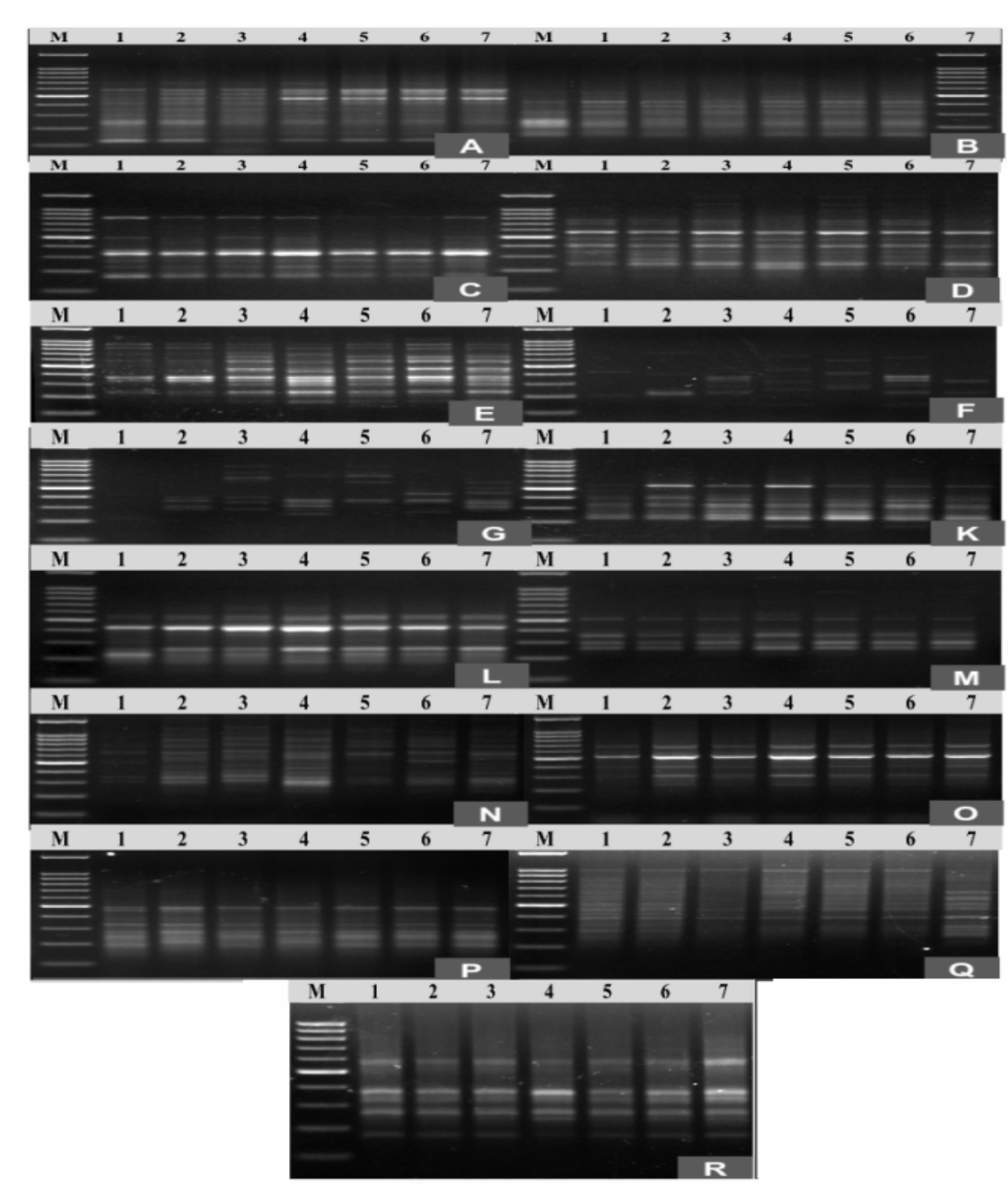

Fig. (1): ISSR profiles , the PCR patterns of the seven quinoa genotypes using the fifteen ISSR Primers; (A) ISSR-1, (B) ISSR-2, (C) ISSR-3, (D) ISSR-4, (E) ISSR-5, (F) ISSR-6, (G) ISSR-7, (K) ISSR-9, (L) ISSR-10, (M) ISSR-11, (N) ISSR-12, (12), (O) ISSR-16, (P) ISSR-18, (Q) ISSR-19 and (R) ISSR-20. M: 100bp DNA ladder (Fermentas, Germany). Lanes 1 to 7 represent: Wild Egyptian, American, Rainbow, Black, KVL-SRA2, QL-37 and Chipaya, respectively.

Genetic similarity and cluster analysis based on ISSR marker

To show the genetic similarity and clustering structure among the seven quinoa genotypes the UPGMA and Dice coefficient (Table 5 and Figure 2) were used. The genetic similarity was estimated between 0.76 and 0.87, revealing a high level of similarity. The frist high genetic similarity (0.87) was detected between the 5 (KVL-SRA2) and 6 (QL-37), while the second high similarity (0.85) was detected between 5 (KVL-SRA2) and 7 (Chipaya), 3 (Rainbow) and 5 (KVL-SRA2). The lowest genetic similarity (0.76) was detected between 2 (American) and 3 (Rainbow), 3 (Rainbow) and 4 (Black). The dendrogram showed two main clusters; the first main cluster has grouped three quinoa genotyping; 2 (American), 4 (Black) and 7 (Chipaya). The second main cluster has divided into two sub clusters; one sub-cluster contained one quinoa genotyping; 1 (Wild Egyptian), while the other sub-cluster contained three quinoa genotypes; 3 (Rainbow), 5 (KVL-SRA2) and 6 (QL-37).

Table (5): Similarity matrix among seven Quinoa genotypes according to Dice coefficient as revealed by ISSR markers.

	Wild Egyptian	American	Rainbow	Black	KVL-SRA2	QL-37	Chipaya
Wild Egyptian	1.00						
American	0.80	1.00					
Rainbow	0.79	0.76	1.00				
Black	0.77	0.81	0.76	1.00			
KVL-SRA2	0.82	0.78	0.85	0.79	1.00		
QL-37	0.78	0.78	0.84	0.79	0.87	1.00	
Chipaya	0.80	0.83	0.80	0.84	0.85	0.83	1.00

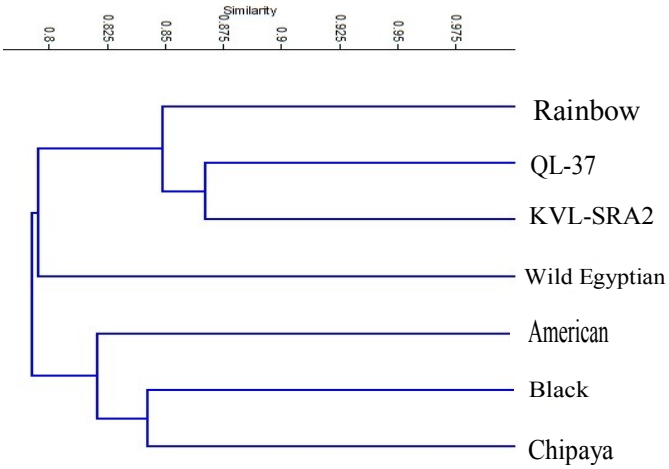

Fig. (2): Dendrogram for the seven Quinoa genotypes constructed from ISSR data using UPGMA and similarity matrix computed according to Dice coefficient.

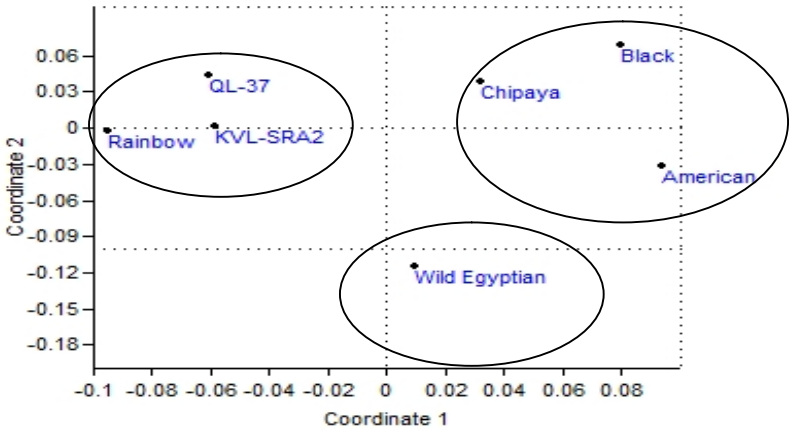

Fig. (3): Principal coordinate analysis based on the calculation of the first three coordinates was performed according to analysis of ISSR markers of the studied seven quinoa genotyping.

To show the genetic relationship among the quinoa genotypes, the principal coordinate analysis (PCA) was directed based on Dice's similarity matrix (Figure 3). The multivariate methodology was used to support the grouping results, since the bunch investigation has showed higher resolution for closely related populations. The first PCA has clarified about 31.1% of all out genotypes, while the second PCA settled 18.3% of all out genotypes. The structure evaluated by the PCA was in concurrence with the bunching analysis.

SCoT polymorphism among the Quinoa genotypes

Twelve SCoT primers were employed to investigate the genetic polymorphism among the seven quinoa genotypes. These SCoT primers produced amplification profile and good reproducible patterns were screened for the presence polymorphism (Table 6 and Figure 4). The total of 157 amplified bands were generated by the 12 primers with an average of 13 band/primer, the lowest number of product bands (8) primer (SCoT-14), while the highest number of product bands (19) primer (SCoT-13). A total number of amplified monomorphic bands 77 average 6.4 band/primer, the highest number of monomorphic bands (13) primer (SCoT-2), while the lowest number of monomorphic bands (3) primer (SCoT-11). A total of number amplified polymorphic bands 80 average 6.7 band/primer, the lowest number of polymorphic bands (2) primer (SCoT-14), while the highest number polymorphic bands (13) primer (ScoT-13). In this study, the polymorphism percentage ranged from 23% (SCoT-2) to 75% (SCoT-11), the average level of polymorphism was 49.5%. The frequency ranged from 0.51 to 0.87 for SCoT-5 and SCoT12, respectively. The efficacy of SCoT markers in discriminating studied genotypes was estimated by obtaining the PIC values of used primers. PIC values varied from 0.50 (SCoT-14) to 0.84 (SCoT-13, SCoT-28, SCoT-3, and SCoT-36) with an average of 0.77. In this respect (Ibrahim *et al*., 2017) used thirty SCoT primers to genetic diversity of Wheat, generated a total of 156 polymorphic loci with an average of 13 amplicons per primer. The PIC values varied from 0.09 to 0.91 with an average of 0.24. Clustering and principal coordinate analyses were performed and revealed useful genetic similarities/relatedness between the studied

cultivars. Lema-Rumińska and Gęsiński (2018) used nine SCoT primers to characterize and genetically compare among quinoa genotypes. These primers have generated 96 loci, 79 of which were polymorphic (75.9 %). The highest number of loci was recorded to the SCoT-3 (17), where the average number of loci was indicated at 8.8. These findings were also coincided with those of Xiong *et al.*, (2011), who stated that SCoT markers are highly polymorphic and therefore are useful for the genetic studies of functional genetic variability and genotypic relationships.

Table (6): The list of primers sequence, Total Number of Bands (TB), Monomorphic Bands (MB), Polymorphic Bands (PB), Percentage of Polymorphism (%P), Frequency (F) and Polymorphism Information Content (PIC) as revealed by SCoT analysis of seven quinoa genotypes.

Name	Sequence (5′› 3′)	TB	MB	PB	% P	F	PIC
SCoT-2	CAACAATGGCTACCACCC	17	13	4	23	0.85	0.68
SCoT-3	CAACAATGGCTACCACCG	18	6	12	67	0.68	0.84
SCoT-4	CAACAATGGCTACCACCT	9	4	5	56	0.75	0.79
SCoT-5	CAACAATGGCTACCACGA	13	4	9	69	0.51	0.79
SCoT-11	AAGCAATGGCTACCACCA	12	3	9	75	0.58	0.74
SCoT-12	ACGACATGGCGACCAACG	12	8	4	33	0.87	0.74
SCoT-13	ACGACATGGCGACCATCG	19	6	13	68	0.60	0.84
SCoT-14	ACGACATGGCGACCACGC	8	6	2	25	0.80	0.50
SCoT-28	CCATGGCTACCACCGCCA	10	6	4	40	0.80	0.84
SCoT-33	CCATGGCTACCACCGCAG	12	7	5	42	0.79	0.79
SCoT-35	CATGGCTACCACCGGCCC	12	6	6	50	0.73	0.79
SCoT-36	GCAACAATGGCTACCACC	15	8	7	47	0.78	0.84
Total		**157**	**77**	**80**	**-**	**-**	**-**
Means		**13.1**	**6.4**	**6.7**	**50.1**	**0.70**	**0.77**

Genetic similarity and cluster analysis based on SCoT marker

To investigate the genetic similarity and cluster analysis among the seven quinoa genotyping based on SCoT markers using UPGMA and similarity matrix computed according to Dice coefficient (Table 7 and Figure 5). The estimated genetic similarities ranged from 0.79 to 0.90 revealing to high levels of genetic similarity among the studied quinoa genotyping. The highest detected genetic similarity (0.90) was between the 2(American) and 7(Chipaya). This was followed by (0.89) between 5(KVL-SRA2) and 6 (QL-37), while the lowest genetic similarity (0.79) was detected between 3(Rainbow) and 4(Black). The dendrogram comprised two main clusters; the first cluster grouped three quinoa genotypes; 2 (American), 7 (Chipaya) and 4 (Black) which derived from the same genetic source (USA and Bolivia). The second cluster divided into two sub clusters; one sub- cluster contains one quinoa variety 1 (Wild Egyptian). While, the other sub-cluster contains three quinoa genotypes; 3 (Rainbow), 5 (KVL-SRA2) and 6 (QL-37).

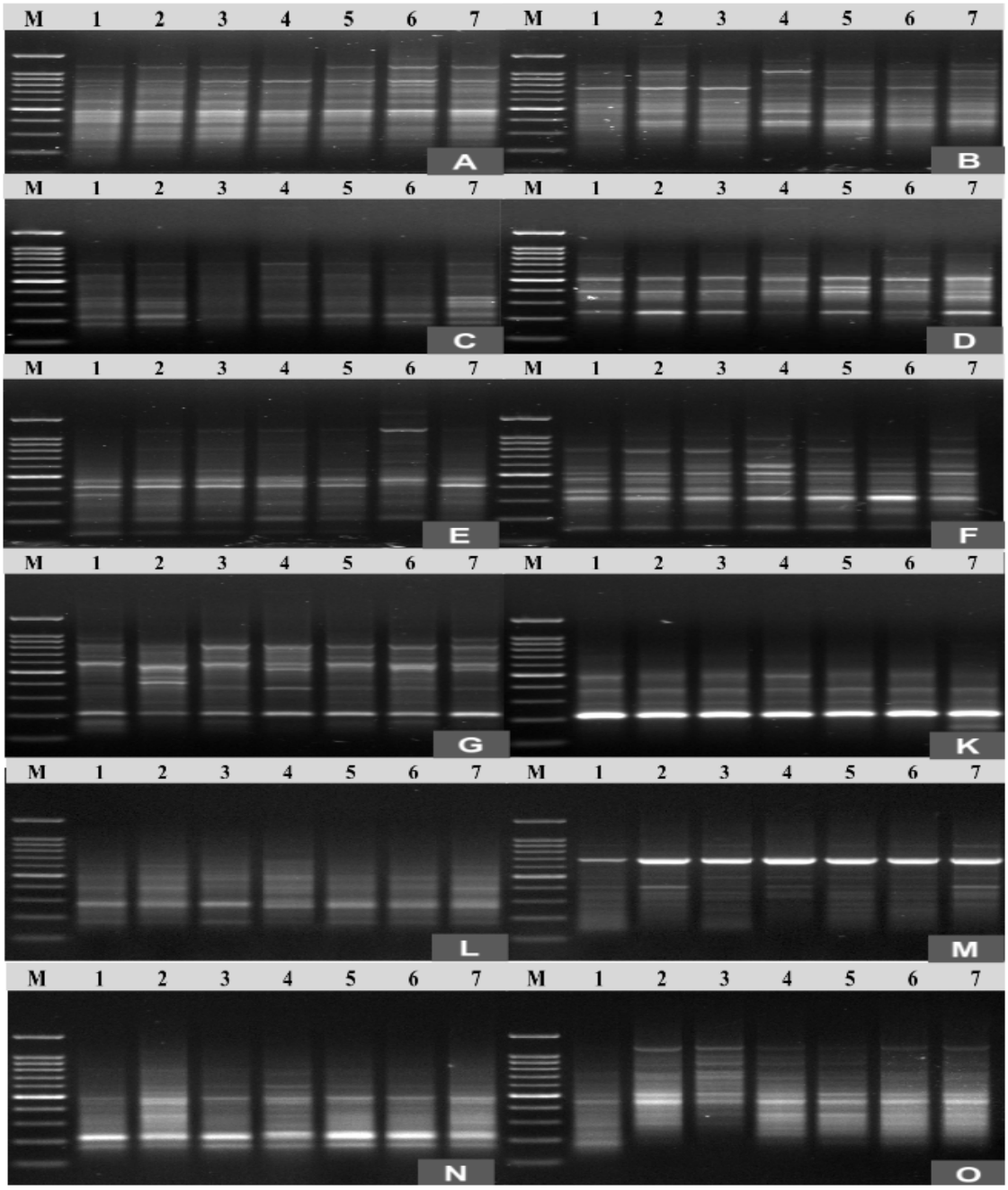

Fig.(4): SCoT profiles of the seven genotypes of quinoa using the twelve SCoT primers; (A) SCoT-2, (B) SCoT-3 , (C) SCoT-4, (D) SCoT-5, (E) SCoT-11, (F) SCoT-12, (G) SCoT-13, (K) SCoT-14, (L) SCoT-28, (M) SCoT-33, (N) SCoT-35 and (O) SCoT-36. M: 100bp DNA ladder (Fermentas, Germany). Lanes 1 to 7 represent Wild Egyptian, American, Rainbow, Black, KVL-SRA2, QL-37 and Chipaya, respectively.

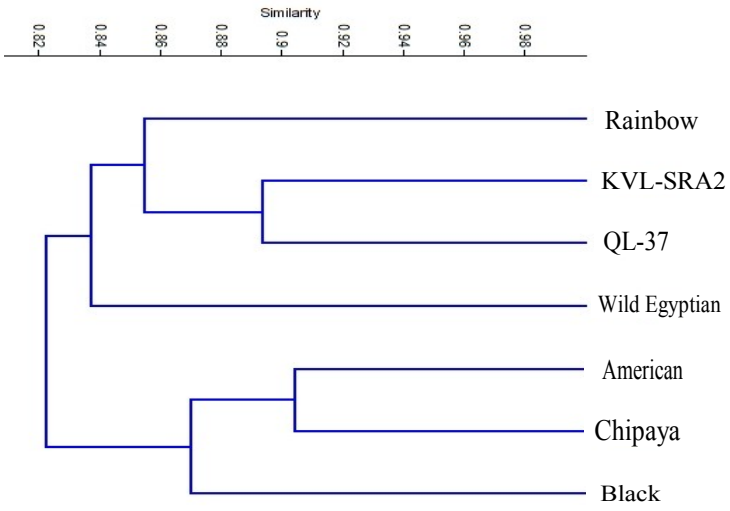

Fig. (5): Dendrogram for the seven Quinoa genotypes constructed from SCoTs data using UPGMA and similarity matrix computed according to Dice coefficient.

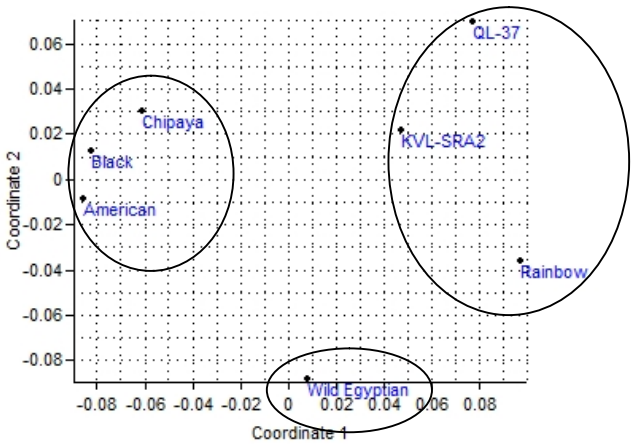

Fig. (6): Principal coordinate analysis based on the calculation of the first three coordinates was performed according to analysis of SCoT markers of the studied seven quinoa genotyping.

To show the genetic relatedness between quinoa genotyping based on SCoT markers, the principal coordinate analysis (PCA) was directed dependent on Dice's similarity matrix (Figure 6). The PCA multivariate methodology was performed to supplement the group examination results, since bunch investigation shows higher resolution for analysis of closely related populations. The first PCA clarified about 44.3% of the all out variety, while the second PCA settled 18.3% of the all out variety. The connections evaluated from PCA were in concurrence with and affirmed the bunching investigation

Table (7): Similarity matrix among seven Quinoa genotypes according to Dice coefficient as revealed by SCoT markers .

	Wild Egyptian	American	Rainbow	Black	KVL-SRA2	QL-37	Chipaya
Wild Egyptian	1.00						
American	0.82	1.00					
Rainbow	0.83	0.81	1.00				
Black	0.83	0.86	0.79	1.00			
KVL-SRA2	0.86	0.82	0.85	0.83	1.00		
QL-37	0.82	0.80	0.85	0.81	0.89	1.00	
Chipaya	0.85	0.90	0.80	0.87	0.88	0.84	1.00

Phylogenetic analysis using *rbcL* and *rpoC1* genes

In this study we successfully sequenced barcode genes of *rbcL* and *rpoC1* for the seven quinoa genotypes. These sequences have been submitted to NCBI respiratory using BankiT tool and have been assigned to gene bank accession numbers (Table 8). The sequence alignment revealed that, *rbcL* retrieved from studied quinoa genotypes with high similar to other rbcL genes obtained from Chenopodium species by other studies, with similarities ranged from 76% to 80%. In addition, the *rbcL* gene displayed genetic similarity of high consistency with low genetic evolution and mutation (Figure 7). Evolutionary divergence in Chenopodium utilizing *rbcL* gene sequences for 19 accessions has reported 0.68% interspecific sequence diversity (Devi and Chrungoo, 2017). A study of the genus Chenopodium stated that the findings demonstrated the ability of a single barcoding gene (*rbcL*) or a combination of these genes to distinguish organisms under the genus Chenopodium (Bafeel *et al.*, 2012). On the other hand, the sequence alignment revealed that, *rpoC1* retrieved from studied quinoa genotypes with high similar to other *rpoC1* genes obtained from other plant species by other studies, with similarities ranged from 80% to 81%. In contrast to the *rbcl* gene, *rpoC1* gene revealed genetic similarity of low consistency with high genetic evolution and mutation (Figure 8). In this respect, the use of *rpoC1* in plant DNA barcodes and species resolution in sedges showed low species resolution *rpoC1* (Starr *et al.*, 2009).

Table (8): NCBI Genbank accession number for rbcL and rpoC1 for the seven quinoa genotypes produced by this study.

S.N	Genotypes name	*rbcL*	*rpoC1*
1	Wild Egyptian	MN862653	MN862646
2	American	MN862654	MN862647
3	Rainbow	MN862655	MN862648
4	Black	MN862656	MN862649
5	KVL-SRA2	MN862657	MN862650
6	QL-37	MN862658	MN862651
7	Chipaya	MN862659	MN862652

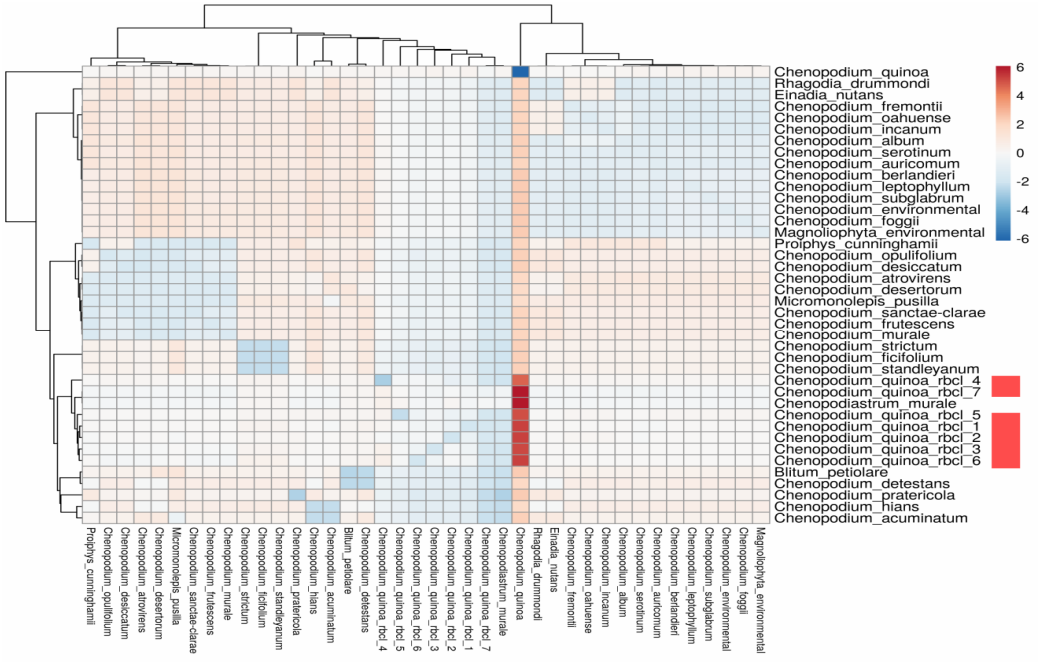

Fig. (7): The matrices of multiple sequence alignment that demonstrate the relationship between distinct rbcL gene in studied quinoa. The scale of similarity varied from highly similar (blue) sequences to strongly divergent (red) sequences.

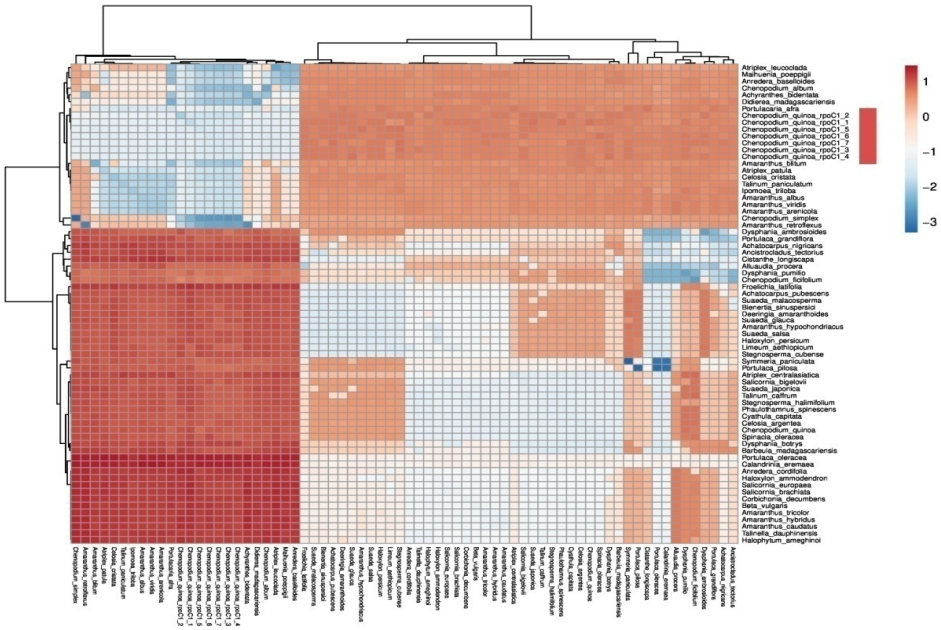

Fig. (8): The matrices of multiple sequence alignment that demonstrate the relationship between distinct rpoC1 gene in studied quinoa. The scale of similarity varied from highly similar (blue) sequences to strongly divergent (red) sequences.

In this study, ISSR and SCoT primers were able to reveal variability between the Quinoa genotypes; the primers used succeeded to produce specific markers that helped in genotyping identification. High levels of polymorphism found in the present study showed that the ISSR and SCoT markers are suitable tools for genetic diversity. These markers could be useful for Quinoa breeding programs and the detection of genetic diversity among the genotypes. Through this study, SCoT marker was found to be better than ISSR marker. So the percentage of polymorphic bands detected by ISSR primer (49%) was lower compared to SCoT (50%). The *rbcL* and *rpoC1* based DNA bar-coding was almost successful to identify different Quinoa genotypes according to genus.

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الملخص العربي

الإختلافات الوراثيه بإستخدام معلمات ISSR، SCoT و DNA Barcoding لأنماط الكينوا

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يعتبر نبات الكينوا مصدرًا جيدًا للألياف والفيتامينات والمعادن بالإضافة إلى المواد الكيميائية المفيدة للصحة. المعلمات الجزيئية هى أدوات أساسية لتحديد التباين الوراثى فى الأنواع المختلفة وهى مفيدة لحفظ الأصول الوراثية وتعريف الأصناف. هذه الدراسة تهدف إلى استخدام علامات جزيئية مختلفة لتوصيف سبعة أنماط جينية من الكينوا بإستخدام ISSR، SCoT ومعلمات الـ DNA-Barcoding وهى متمثلة فى *rbcL* و *rpoC1* للكشف عن تعدد الأنماط الوراثية وتحديد معلمات مميزة لكل نمط جينى. تم استخدام خمسة عشر من بادئات ISSR في هذه الدراسة ،وأعطت ١٧٢ شظيه بمتوسط ١١.٧ شظيه/ بادئ. وكانت عدد الشظايا المتشابهه ٩٠ بمتوسط ٦ شظيه/ بادئ ، وكان متوسط مستوى التباين الوراثى ٤٩.٧ ٪. وتم تقدير كفاءة العلامات ISSR فى الأنماط الجينية المدروسة PIC تتراوح من ٠.٢١ إلى ٠.٨٤ بمتوسط ٠.٦٩. وأيضاً تم استخدام اثنى عشر بادئات من SCoT للتحديد تعدد الأشكال الوراثية بين الأنماط الجينيه السبعة من نبات الكينوا. وأعطت ١٥٧ شظيه بمتوسط ١٣.١ شظيه/ بادئ ، بينما كان إجمالي عدد الشظايا المتشابهه ٨٠ شظيه بمتوسط ٦.٧ شظيه/ بادئ ، وكانت قيم PIC المدروسة تتراوح من ٠.٥٠ إلى ٠.٨٤ بمتوسط ٠.٧٧. لقد نجحنا فى تسجيل جينات الباركود الخاصه بـ *rbcL* و *rpoC1* لأصناف الكينوا السبعة في بنك الجينات NCBI. ووجد أن نسبة التشابه الخاصه بجين *rbcL* من أصناف الكينوا التى تمت دراستها كانت عالية التشابه بجينات *rbcL* لأصناف الكينوا المسجله ببنك الجينات. وكانت نسبة التشابه تتراوح بين ٧٦ ٪ إلى ٨٠ ٪. ومن ناحية أخرى وجد أن نسبة التشابه الخاصه بجين*rpoC1* من أصناف الكينوا التى تمت دراستها كانت عالية التشابه بجينات *rpoC1* لأصناف الكينوا المسجله ببنك الجينات، وكانت نسبه التشابه تتراوح بين ٨٠ ٪ إلى ٨١ ٪.