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Molecular Characterization of Ten *Cicer arietinum* L. Genotypes Using SCOT Marker

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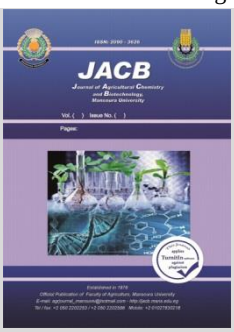

ABSTRACT

The up-to-date technique of start codon targeted (SCoT) polymorphism (sites around start codon) is efficient in genetic diversity analysis and fingerprinting. In the current study, 94 amplified fragments were obtained using 10 SCoT Primers with 10 genotypes of *Cicer arietinum* L with 35% polymorphism, where the polymorphic bands were 33 and 61 bands were monomorphic. The SCoT data analysis showed genetic similarity ranging from 84 to 98%. A dendrogram separated the studied ten chickpea genotypes into two major clusters, divided into two main sub-clusters. The difference in Principal Coordinate Analysis (PCA) established genetic variance between the investigated samples. In four quadrants, the PCA variable demonstrated clustering for the 10 genotypes of chickpea that were examined.

Keywords: Genetic diversity, SCoT molecular marker, *Cicer arietinum* L.

INTRODUCTION

A self-pollinated diploid, Chickpea (*Cicer arietinum* L.) is grown in semi-arid regions. grown in semi-arid regions. It is a crucial legume food crop grown in a wide variety of climatic regions around the world (Considine *et al.*, 2017). It is rich in vitamins, minerals, carbohydrates, vegetarian proteins, and other constituents. In most rural communities with low incomes, play a critical role in the human feed (Varshney *et al.*, 2013). It improves soil fertility through nitrogen fixation (Roy *et al.*, 2010). Traditional breeding protocols based on morphological characteristics that are capable of characterizing genotypes on the basis of their phenotypical features, which have been helpful in improving legumes (Gatti *et al.*, 2016). The production of Chickpea has been restricted by numerous Biotic stresses and abiotic stresses (Croser *et al.*, 2003). There is a desire to grow genotypes of chickpeas with superior quality and quantity of seed, and tolerance to stress for feeding the rising global population. At the DNA level, molecular markers reflect genetic diversity and can visualize the accurate genetic diversity between genotypes (Cui *et al.*, 2017). Some advancement has been made in the genetic enhancement of chickpea through the use of gene transfer techniques. (Das, and Parida 2014 and Leonetti *et al.*, 2018).

Collard and Mackill (2009) identified SCoT polymorphism depending on the short-conserved plant gene regions surrounded by the start codon translation of ATG (Sawant *et al.*, 1999).

SCoT markers are reproducible and dominant in general. Primer length and annealing temperature are not the only variables assessing the reproducibility, Collard and Mackill (2009). They are dominant markers; moreover, during the amplification, a number of co-dominant markers were produced. (Gorji *et al.*, 2011). SCoT technique used for assessing structure, genetic diversity, and for the mapping of quantitative trait loci (QTL) and fingerprinting of DNA of many crop cultivars (Cabo *et al.*, 2014; Heikrujam *et al.*, 2015 and Satya *et al.*, 2015).

The aim of the current study was to characterize and assess genetic diversity among and within 10 genotypes of chickpea via the application of 10 SCoT Primers.

MATERIALS AND METHODS

Sampling and extraction of DNA

The 10 various chickpea genotypes were obtained by the Egyptian Agriculture Research Center (ARC) and the International Center for Agricultural Research in the Dry Areas (ICARDA), all of which originated from different zones (Table 1). Extraction of genomic DNA from fresh leaves was done using QIAGEN DNeasy Plant DNA extraction Mini Kit (cat. No. 69104). Isolation of DNA on the basis of the protocol of Doyle and Doyle 1990, was used to get high-quality chickpea genotypes DNA.

Table 1. List of the ten chickpea genotypes with their origin.

No.	Genotype	Origin
1	Giza 88	Egypt
2	Giza 531	Egypt
3	Giza 2	Egypt
4	FLIP 06-64 C	Iran
5	FLIP 87-59 C	Iran
6	FLIP 05-67 C	Iran
7	FLIP 06-102 C	Iran
8	70755	ICARDA
9	71775	ICARDA
10	71783	ICARDA

SCoT-PCR Reactions

Detection of polymorphism among and within the genotypes was according to Collard and Mackill (2009); names and sequences are listed in Table 2. Volume of 25 µL

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with 1X PCR buffer, 1.5 mM MgCl2, 0.2 mM dNTPs, 1 µM primer, 1 U Taq DNA polymerase, and 30 ng template DNA were amplified. Based on the sequences of consensus of codon region of the translation initiation, primer sequences were designed.

Table 2. List of SCoT primers used.

Code	Primer Sequence 5'-3'
SCoT-1	ACGACATGGCGACCACGC
SCoT-2	ACCATGGCTACCACCGGC
SCoT-3	ACCATGGCTACCACCGCA
SCoT-4	ACAATGGCTACCACTGAC
SCoT-5	ACAATGGCTACCACTGCC
SCoT-6	ACAATGGCTACCACTACC
SCoT-7	CAACAATGGCTACCACCG
SCoT-8	CCATGGCTACCACCGGCA
SCoT-9	CCATGGCTACCACCGGCG
SCoT-10	CCATGGCTACCACCGGCC

A: Adenine, T: Thymine, G: Guanine and C: Cytosine

PCR Products detection and Thermocycling profile

Amplification of PCR was done in Perkin-Elmer/Gene Amp® PCR System 9700 (PE Applied Biosystems) to complete 35 cycles at 94ºC for 5 min after initial denaturation. Every step was 1 min of denaturation at 94ºC, 1 min of annealing at 50ºC, and 1.5 min of elongation at 72ºC. In the last cycle, the extension segment was expanded to 7 min at 72ºC. Electrophoresis was done to resolve amplification products in 1.5% agarose gel having 0.5 ug ethidium bromide mL^{-1} in 1X TBE buffer at 95 v. As a molecular size norm, a DNA ladder of 100bp was used. Products from PCR were visualized in UV light and photographed by a Gel Documentation System (BIO-RAD 2000).

Data Analysis

Banding given by SCoT were evaluated in order to assess genetic relationship. The presence of clear and distinct amplifications was rated as '1' and '0' for the absence of bands. The same versatility bands were considered similar. The genetic similarity coefficient between genotypes has been calculated using the Dice coefficient. (Sneath, and Sokal 1973).

Dice formula: $GS_{ij} = 2a/(2a+b+c)$

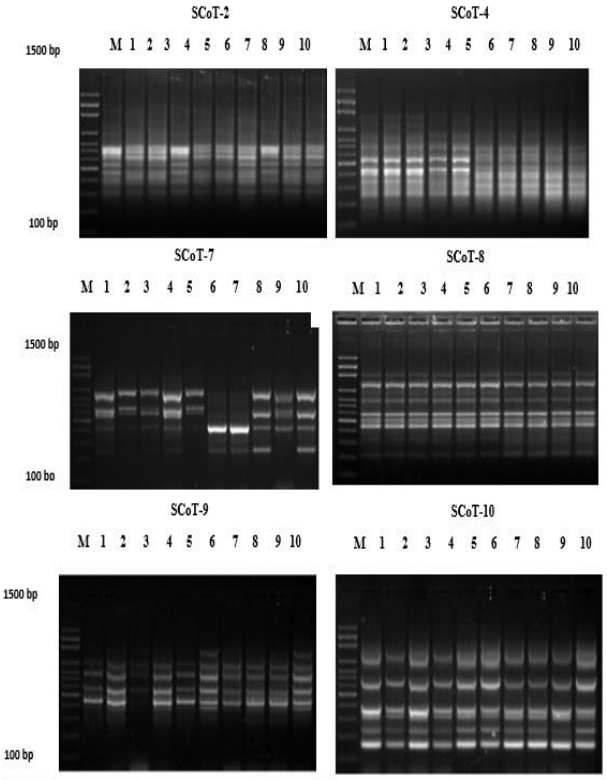
Where

the genetic resemblance between individuals I and j is GSij; a is the number of bands shared by I and j; b is the number of bands present in I and absent in j and c is the number of bands present in j and absent in i.

A matrix of similarity was employed to organize data into and develop taxonomies. Firstly, when each accession is a cluster of its own, the distances between accessions were specified by the distance selected (Dice coefficient). Upon conducting several accessions, as an average between accession pairs, the distance between two clusters was computed. Such a technique is called by Sneath and Sokal (1973), the Unweighted Pair Group Method using Arithmetic Average (UPGMA).

RESULTS AND DISCUSSION

A total of 10 primers were tested for selective amplification of DNA fragments of 10 genotypes of *Cicer arietinum* L. Levels of polymorphism are listed in Table 3. The 10 SCoT primers produced reliable PCR products (Figure 1). Data analysis recorded a total of 94 amplified fragments using 10 SCoT Primers with 35% of polymorphism. A maximum of 13 fragments was amplified with primers SCoT-2, while a minimum of four fragments was amplified with the primers SCoT-1. The polymorphic bands were 33 and 61 bands were monomorphic.



M: marker, (1): Giza 88, (2): Giza 531 (3): Giza 2, (4): FLIP 06-64C, (5): FLIP 87-59C, (6): FLIP 05-67C, (7): FLIP 06-102 C, (8): 70755, (9): 71775 and (10): 71783.

Figure 1. Amplification of the ten Chickpea genotypes with SCoT-PCR primers No. 2, 4, 7, 8, 9 and 10.

Table 3. Chickpea polymorphic SCoT primers statistics used.

Code	Total No. of amplified bands	No. of Monomorphic Bands	No. of Polymorphic bands	Polymorphism %
SCoT – 1	4	3	1	25
SCoT – 2	13	6	7	54
SCoT – 3	9	6	3	33
SCoT -4	11	9	2	18
SCoT -5	7	6	1	14
SCoT -6	9	4	5	56
SCoT – 7	9	1	8	89
SCoT – 8	11	10	1	9
SCoT – 9	9	5	4	44
SCoT – 10	12	11	1	8
Total	94	61	33	35

Sadhu, *et al.,* (2020) assessed the genetic fidelity of *in vitro* chickpea via (SCoT) and (ISSR) molecular marker. Amom, *et al.,* (2020) used SCoT to analyze genetic polymorphism among 50 economic genotype bamboos. Shekhawat, *et al.,* (2018) reported that 12 SCoT markers produced a total of 156-amp icons over 8 to 17 per primer,

of which 114 (73%) were polymorphic. Hajibarat, *et al.*, (2015) assessed diversity among 48 chickpea cultivars using SCoT with simple sequence repeat (SSR) and conserved DNA-derived polymorphism (CDDP). For the SSR, SCoT, and CDDP markers, the average polymorphism information content (PIC) values were 0.47, 0.45 and 0.45, respectively, indicating that 3 different types of markers were equivalent for the evaluation of genotype diversity.

Genetic similarity and dendrogram analysis

For the presence (1) and absence (0) of bands for all genotypes, amplified bands obtained with molecular markers were visually graded. The genetic similarity estimates for the primers used in Chickpea ranged between 84 and 98 % based on Jaccard's coefficient. Giza 88 and FLIP 06-64C revealed the closest relationship with 98% similarity (Table 4). The lowest percentage (84%) was between Giza 2 and 70755.

The dendrogram separated the genotypes into two major clusters. The first contained two main sub-clusters; sub-cluster one having two genotypes (FLIP 05-67C) and (71783), and sub-cluster two of (70755) and (71775), in addition to (Flip 06-102C). The second cluster could be divided into two sub-clusters; sub-cluster one containing (Giza 88) and (Flip 06-64C). Sub-cluster two contained (Giza 531), (Giza 2), and (FLIP 87-59C) as shown in Figure 2.

In this study, the current SCoT analysis was able to establish the phylogenetics between the genotypes successfully. Shekhawat, *et al.*, (2018) used SCoT for genetic diversity and De Giovanni, *et al.*, (2017) studied the genetic distance of a global chickpea germplasm collection. Amom, *et al.*, (2020) used UPGMA dendrograms of different bamboo genotypes.

Table 4. Genetic similarity between the ten chickpea genotypes, as determined using SCoT data using ten primers.

	Giza 88	Giza 531	Giza 2	FLIP 06-64 C	FLIP 87-59 C	FLIP 05-67 C	FLIP 06-102 C	70755	71775	71783
Giza 88	100									
Giza 531	96	100								
Giza 2	95	97	100							
FLIP 06-64 C	98	95	95	100						
FLIP 87-59 C	95	97	96	95	100					
FLIP 05-67 C	86	86	86	87	88	100				
FLIP 06-102 C	85	86	86	85	88	94	100			
70755	89	86	84	90	86	92	91	100		
71775	85	86	85	86	87	91	91	95	100	
71783	86	85	85	86	85	96	91	95	95	100

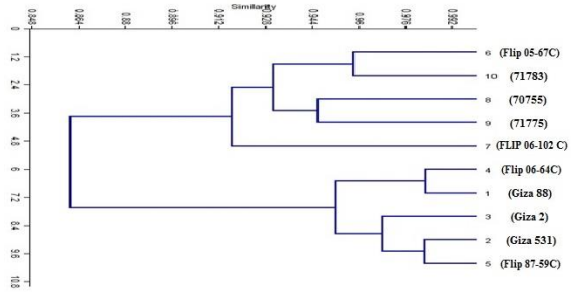
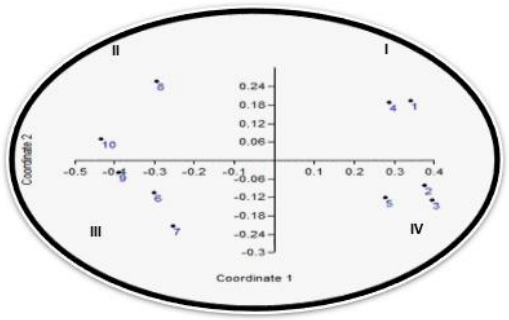


Figure 2. Dendrogram generated among the ten Chickpea genotypes using SCoT analysis based on UPGMA clustering method and Jacquared`s coefficient.

The principal coordinate analysis (PCA):

PCA computes the change of basis on the data, using variance/covariance analysis. It is used to decrease the dimensionality of large data (Abdi and Williams 2010). It is a variable showing clustering in four-quadrant variables. Results showed that the genotypes 1 (Giza 88) and 4 (FLIP 06-64C) set in quadrant I. 8 (70755) and 10 (71783) set in quadrant II. 6 (FLIP 05-67C), 7 (FLIP 06-102C) and 9 (71775) set in quadrant III, while the three genotypes 2 (Giza 531), 3 (Giza 2) and 5 (FLIP 87-59C) are located in quadrant IV. Figure 3 indicates that the 10 genotypes are apart from each other.

these results Consistent with those of Farahani, *et al.*, (2019) who reported that PCA and discriminant analysis of principal component (DAPC) were reliable with those of the analysis of the cluster and population structure in chickpea. Seyedimoradi, *et al.*, (2020) mentioned that Chickpea breeding lines were divided into four different groups by cluster analysis, indicating agreement with model-based structure. Tyagi, *et al.*, (2020) showed that the principal components analysis of 20 morphological characteristics was 72.70% variability, with important positive associations between fruit characteristics in sponge gourd.



(1): Giza 88, (2): Giza 531 (3): Giza 2, (4): FLIP 06-64C, (5): FLIP 87-59C, (6): FLIP 05-67C, (7): FLIP 06-102 C, (8): 70755, (9): 71775 and (10): 71783.

Figure 3. PCA plot for two axes clarified (Coordinates), four quadrants were defined.

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التوصيف الجزيئي لعشره طرز وراثيه لنبات الحمص (*Cicer arietinum* L.) باستخدام تقنيه SCoT Marker

احمد محمد سراج الدين

قسم الوراثة والهندسة الوراثية ـ كلية الزراعة ـ جامعة بنها ـ مصر

تم استخدام تقنيه تعدد الأشكال (SCoT - marker) ، والتي تعد أسلوب جديد للمعلمات الجزيئية والتي تستهدف المنطقة المحيطة بكودون البدء والتي أثبتت كفاءتها في تحليل التنوع الجيني والبصمه الوراثيه. في هذه الدراسة ، كان العدد الإجمالي للحزم المتحصل عليها من العشر طرز الوراثيه والتي تمثل نبات الحمص (*Cicer arietinum* L.) 94 حزمه، والنسبة المئوية للمواقع متعددة الأشكال 35٪. بلغ عدد الحزم متعددة الأشكال 33، بينما بلغ عدد الحزم احاديه الشكل 61.أظهر تحليل بيانات SCoT أن التشابه الوراثي يتراوح ما بين 84 إلى 98٪. تم دراسه درجه القرابه الوراثيه (dendrogram) للطرز الجينية العشرة المدروسة للحمص حيث تم تقسيمها إلى مجموعتين رئيسيتين، وكل مجموعه مقسمة إلى مجموعتين فرعيتين. كما أظهر تحليل مكونات التباين الأساسية على مستوى الأنواع بين العينات المدروسة توزيعا مختلفا للعينات حسب درجة التشابه والإختلاف.