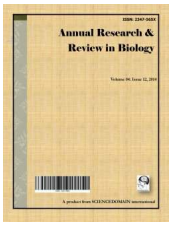

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Genetic Diversity Assessment in Chickpea Using Morphological Characters and Start Codon Targeted (SCoT) Molecular Markers

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Authors' contributions

This work carried out in collaboration between both authors. Author KMSA performed the experiment. Author RT wrote the manuscript draft and managed the field and laboratory experiments. Both authors read and approved the final manuscript.

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ABSTRACT

Genetic diversity in 35 chickpea genotypes were studied using morphological characters and 14 start codon targeted (SCoT) markers. Results of variance analysis and descriptive statistics for morphological traits indicated that the genotypes differed significantly for all studied characteristics. A dendrogram was constructed from morphological traits and the genotypes were grouped into six clusters. Fourteen SCoT primers yielded 135 bands, of which 100 bands were polymorphic. Number of polymorphic bands varied from 6 to 9, with an average of 7.14 bands per primer. PIC values ranged from 0.27 (SCoT22) to 0.46 (SCoT15), with an average value of 0.36 per primer. Cluster analysis Based on SCoT-PCR markers grouped 35 chickpea genotypes into three major clusters. Results showed a weak relationship between morphological divergence and molecular diversity pattern. Overall, we found relatively high genetic diversity in examined chickpea genotypes using morphological and SCoT molecular markers. Findings of this study can be useful for breeder for selective genotypes and specific traits in breeding programs in chickpea.

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1. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one most important cool season food legume in the world with high nutritive value [1]. Chickpea is a one of the most important sources of proteins for human and livestock feeding [2]. Assessment of germplasm diversity in crops is the basic and fundamental for crop breeding and conservation of genetic resources and is very important part of breeding programs for select the best parents in breeding hybrids [2-3]. The importance of diverse germplasm collections to improve genetic potential of the crops and in improving biotic and abiotic stresses has been well recognized [4]. Genetic diversity measurement can be investigated by different methods including the conventional methods like as morphological traits and biochemical and molecular methods [5]. Morphological traits assessment within germplasm are the most common and important tools for taxonomic and germplasm classification in crops. Compared with other methods, morphological diversity characterization is simple and inexpensive. Biochemical markers are very dependent and effectible from environment and most of genes in plants did not translated to proteins [6]. However, most dependent and affectability of morphological and biochemical traits from environments are the most weakness for using only these markers in plants classification [6]. Genetic diversity using DNA-based molecular markers provide very powerful, robustness and reliable tools for genetic diversity analysis in crop germplasm. Different molecular markers technology for chickpea genetic diversity has been used like as RAPD [7], AFLP [8], SSR [9-10]. Recently, a novel molecular marker technique called start codon targeted (SCoT) polymorphism was developed by Collard and Mackill [11]. These markers were developed based on the short conserved region flanking the ATG start codon in plant genes. SCoT markers are very reproducible [12]. The aims of his study were to investigate the genetic diversity of different chickpea accessions using morphological and SCoT-PCR markers, and, thereby, establish if there is any definite relationship between morphological and DNA-based molecular diversity.

2. MATERIALS AND METHODS

Thirty five chickpea (*Cicer arietinum* L.) advanced breeding lines were chosen for the study based on morphological and SCoT-PCR based molecular markers (Table 1). A field experiment was conducted at the experimental farm of Agriculture Faculty, Garmian University, Kifri, Iraq (34°41′0″N44°58′0″E) in spring 2015. Field experiments were laid out in randomized complete block design (RCBD) with three replications. Seeds were hand drilled and each line was sown in four rows of 3 m, with row to row distance of 0.30 m. Five plants were randomly chosen from each plot to measure the morphological traits like as: number of seeds per plant, number of pods per plant, plant height (cm), seed yield (gr/plant) and 100-seed weight (gram). For molecular diversity analysis, total genomic DNA was extracted from 2 g fresh leaves of each genotype following a CTAB extraction protocol. For SCOT-PCR analysis, 14 primer sequences employed that have been reported previously [11] (Table 2). PCR amplification was performed in 20 µl reaction containing 30 ng sample DNA, 4 µM primer, 250 µM of each dNTP, 2 mM $MgCl_2$ and 1.5 unit of Taq DNA polymerase (Cinnagene, Iran). PCR cycles were carried out in a Eppendorf thermo cyclers as follows: 94℃ for 5 min, followed by 32 cycles of denaturation at 94℃ for 1 min, annealing at 49℃ for 1 min, and extension at 72℃ for 2 min. A final extension cycle at 72℃ for 10 min was followed. PCR products were separated on 1.4% agarose gels, stained with ethidium bromide. For morphological data analysis, variance analysis for morphological characteristics was calculated at the 5% probability level using SAS software [13]. Cluster analysis was done using NTSYS version 2.0 and Un-weighted Neighbor Joining method as the clustering algorithm [14]. In SCoT-PCR analysis, amplified bands were scored visually for the presence (1) and absence (0) of bands. Nei,s genetic distance [15] was determined among the genotypes and used for grouping of the genotypes by UNJ (Un-weighted Neighbor Joining) cluster method [14]. For both morphological and molecular clusters the fit of dendrograms obtained were checked by bootstrapping using 100 replications. Bootstrap values were calculated by free tree + tree view (version 1.6.6 for Windows).

Table 1. List of chickpea genotypes for genetic diversity analysis by morphological and SCoT markers

Number	Genotype	Number	Genotype
1	FLIP97-706C	19	FLIP05-40C
2	FLIP03-17C	20	FLIP05-44C
3	FLIP03-31C	21	FLIP05-46C
4	FLIP03-63C	22	FLIP05-58C
5	FLIP03-74C	23	FLIP05-59C
6	FLIP03-87C	24	FLIP05-74C
7	FLIP03-128C	25	FLIP05-87C
8	FLIP03-134C	26	FLIP05-110C
9	FLIP03-135C	27	FLIP05-142C
10	FLIP03-141C	28	FLIP05-143C
11	FLIP04-2C	29	FLIP05-150C
12	FLIP04-19C	30	FLIP05-153C
13	FLIP05-16C	31	FLIP05-160C
14	FLIP05-18C	32	FLIP82-150C
15	FLIP05-21C	33	FLIP88-85C
16	FLIP05-22C	34	FLIP93-93C
17	FLIP05-26C	35	ILC482
18	FLIP05-33C		

Table 2. SCoT primer sequences and details for genetic diversity analysis in chickpea

Primer	Sequence ◌3 ◌5	%CG	Annealing temperature
SCoT1	CAACAATGGCTACCACCA	50	49℃
SCoT2	CAACAATGGCTACCACCC	55	49℃
SCoT6	CAACAATGGCTACCACGC	55	49℃
SCoT11	AAGCAATGGCTACCACCA	50	49℃
SCoT12	ACGACATGGCGACCAACG	61	49℃
SCoT13	ACGACATGGCGACCATCG	61	49℃
SCoT 14	ACGACATGGCGACCACGC	66	49℃
SCoT15	ACGACATGGCGACCGCGA	66	49℃
SCoT19	ACCATGGCTACCACCGGC	66	49℃
SCoT20	ACCATGGCTACCACCGCG	66	49℃
SCoT22	AACCATGGCTACCACCAC	55	49℃
SCoT28	CCATGGCTACCACCGCCA	66	49℃
SCoT35	CATGGCTACCACCGGCCC	72	49℃
SCoT36	GCAACAATGGCTACCACC	55	49℃

3. RESULTS AND DISCUSSION

3.1 Morphological Diversity

Analysis of variance and descriptive statistics showed highly significant differences among genotypes for measured morphological traits (Tables 3 and 4). Descriptive statistics for showed that grain yield (g/plant) ranged from 3.67 to 22.77 with a mean value of 12.78 g/plant. High differences between the maximum and minimum mean values were found for all other traits. Among traits, number of seeds per plant and number of pods per plant ranged from 14 to 109 and 8 to 57, respectively (Table 3). High differences between the maximum and minimum mean values were found for other traits. Characterization of morphological and agronomical traits is a fundamental step for effective utilization of germplasm collections in breeding programs [16-17]. A dendrogram was constructed from the standardized value of morphological traits. The genotypes were grouped into six clusters (Fig. 1). The first cluster including eight genotypes, which these genotypes showed higher seed yield and number of pods per plant in compare to other genotypes. The other clusters contain five to six genotypes with moderate yield and 100-seed weight. In our analysis, we were able to define groups

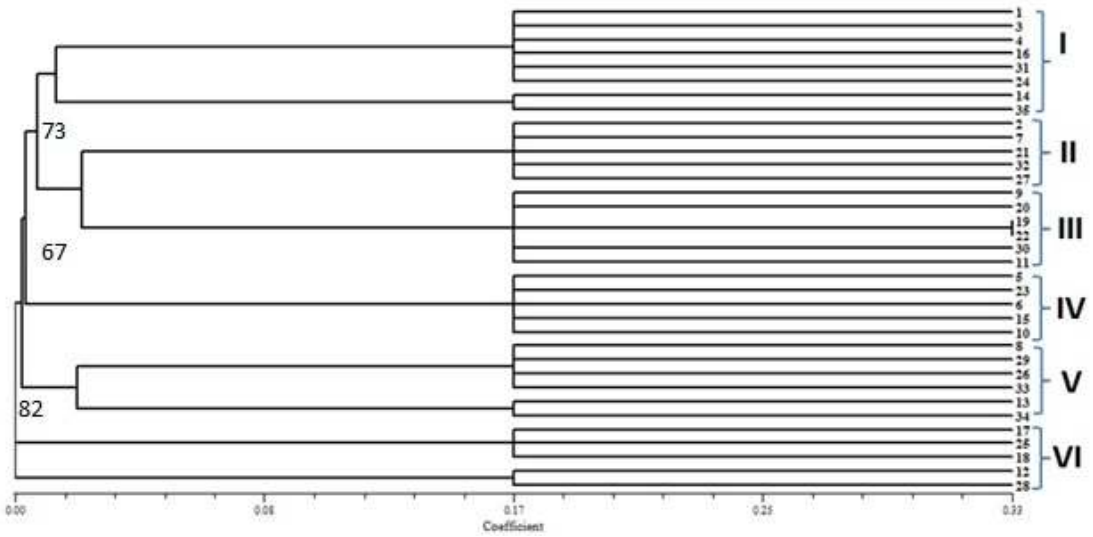

Fig. 1. Dendrogram of the 35 chickpea genotypes based on the dissimilarity matrix developed using morphological characters. Only bootstrap values higher than 50% are presented

Table 3. Analysis of variance for seed yield and morphological traits in 35 chickpea genotypes

Source of variation	Mean of square					
	df	Number of pods/plant	Number of seeds/plant	Seed yield (g/plant)	100-seed weight (gr)	Plant height
Replication	2	217.5	392.75	41.18*	616.2*	325.18
Genotype	34	567.6**	601.91**	50.85*	937.17**	899.2**
Error	68	232.5	306.7	21.57	318.02	416.08

Table 4. Descriptive statistics for morphological traits in 35 chickpea genotypes

Variable	Min	Max	Mean	Variance	CV%
Number of pods/plant	8	57	19.32	109.27	12.77
Number of seeds/plant	14	109	39.97	559.1	18.36
Seed Yield (g/plant)	3.67	22.77	12.78	139.87	10.48
100-seed weight	26.57	39.2	26.531	35.11	11.32
Plant height	24	51	36.77	177.14	19.31

of genotypes that were significantly different from each other for characters of interest. These finding is in agreement with previous studies on Iranian chickpea accessions using morphological traits [18].

3.2 SCoT-PCR Analysis

A set of 14SCoT primers were used to genetic diversity analysis in 36 chickpea genotypes. All markers showed sharp and distinguished polymorphic bands pattern between genotypes. By optimizing PCR profile and the chemical concentrations, each of the primers used produced distinct banding patterns. An amplification and diversity pattern obtained by primers SCoT6 (a) and SCoT28 is presented in Fig. 2. Among the 35 chickpea accessions, 14 SCoT primers yielded 135 clear and distinct bands. Out of 135 bands, 100 bands were polymorphic and the polymorphism percentage averaged to 72.4% across all the genotypes. The number of bands varied from 7 (SCoT14) to 12 (SCoT6 and SCoT19), with an average of 9.85 bands per primer (Table 5). The level of polymorphism observed with SCoT markers was higher than previous studies in chickpea by other molecular markers such as RAPD [3] and ISSR [2], indicating that SCoT markers has more discernible potential than RAPD and ISSR for genetic diversity and germplasm discrimination in chickpea. PIC values ranged from 0.27(SCoT22)

to 0.46 (SCoT15), with an average value of 0.36 per primer (Table 5). We recorded relatively acceptable high PIC value for SCoT markers for discrimination between chickpea lines which is contrary to the previous studies in chickpea [1-2]. Based on un-weighted neighbour-joining method, a dendrogram for genetic relationships among the chickpea genotypes were constructed. Only bootstrap values higher than 50% are presented. If the bootstrap value was less than 50%, it cannot sufficiently provide the meaning and polymorphic phylogeny of the accessions. The 35 chickpea genotypes fell under four groups (Fig. 3). Cluster I contained 11 genotypes and showed relatively similar grouping pattern with cluster I and II that obtained by morphological characters Clusters II, III and V contained 9, 12 and 2 genotypes, respectively (Fig. 3). Similarity between genotypes clustering in SCoT analysis and morphological based clustering was relatively low. The values of mantel test correlation showed a non-significant positive correlation (r=0.18) between the SCoT-PCR and morphological dendrogram. Genetic diversity analysis using molecular markers provide useful information on the history and biology of genotypes, but it does not necessarily reflect what may be observed with respect to agronomic traits [19]. In this study, we evaluated 14 SCoT markers in 36 chickpea genotypes. The SCoT-PCR analysis showed high genetic diversity, detecting a total of 100 polymorphic bands with an average of 7.14 polymorphic bands per primer. We found that there was no strong relationship between morphological and molecular diversity pattern. The rate of diversity for morphological characters and SCoT-PCR based markers was different; we anticipate that the source of detected diversity is different. There could be many reasons for the lack of correlation between SCoT and morphological distances, such as the low number of genotypes assessed in this study or low number of SCoT markers [20-21]. The relationship between molecular markers and phenotypic traits could be significant if the markers were linked to selected loci [22-23]. Also, many previous researches on different plants indicated that morphologically similarities are not necessarily genetically so, as different gene pools could result in similar phenotypes.

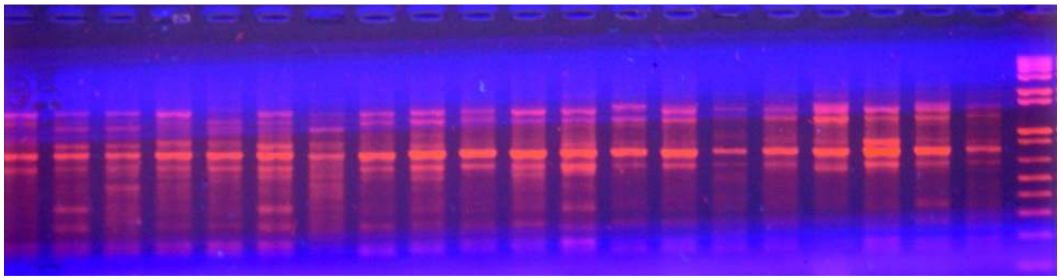

Fig. 2. SCoT amplification profile for primer SCoT28 on chickpea genotypes

Table 5. Total number of amplified bands, polymorphism bands, percentage of polymorphism bands and PIC values in chickpea genotyes as revealed by SCoT markers

Primer	No. of amplified bands	No. of polymorphic bands	Polymorphism%	PIC value
SCoT1	10	7	70	0.35
SCoT2	11	8	72	0.39
SCoT6	12	8	66	0.41
SCoT11	8	6	75	0.31
SCoT12	10	7	70	0.33
SCoT13	10	8	80	0.36
SCoT 14	7	6	85	0.44
SCoT15	11	9	81	0.46
SCoT19	12	8	66	0.41
SCoT20	10	7	70	0.39
SCoT22	9	6	66	0.27
SCoT28	11	8	72	0.39
SCoT35	8	6	75	0.32
SCoT36	9	6	66	0.29
Mean	9.85	7.14	72.4	0.36

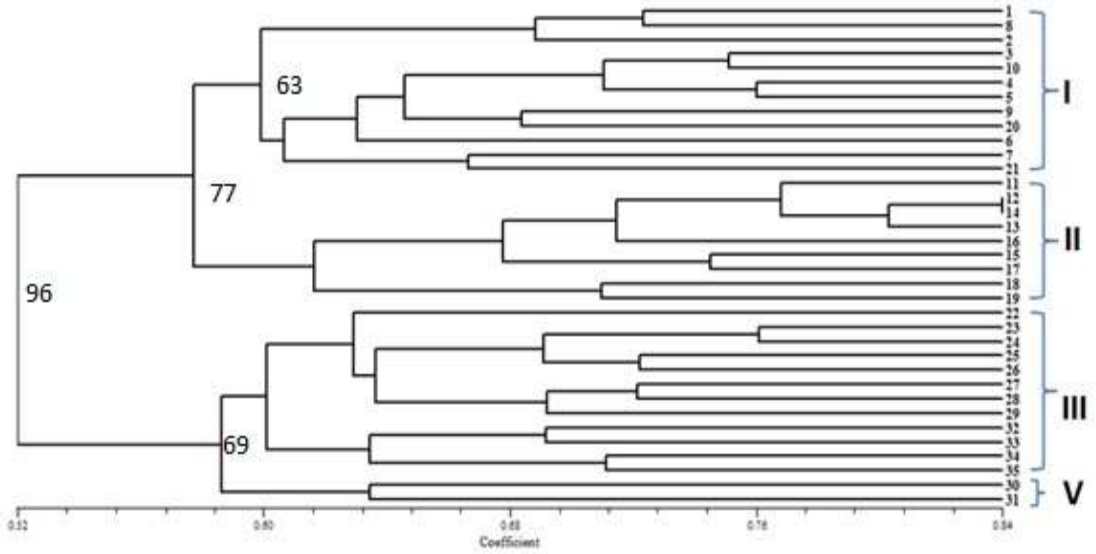

Fig. 3. Dendrogram of the 35 chickpea genotypes based on the dissimilarity matrix developed using SCoT markers. Only bootstrap values higher than 50% are presented

4. CONCLUSION

In conclusion, our results demonstrate that high genetic diversity in both morphological and molecular levels exists between the chickpea genotypes. The genetic variation detected in this study can be useful for future breeding programs in chickpea. Therefore, the diversity pattern and clustering obtained for these chickpea genotypes, based on morphological traits and SCoT-PCR molecular markers will be useful tool for breeders to plan crosses for positive agronomic characters by choosing genotypes with appropriate diversity. In this study, the amount of polymorphisms and efficiency of generating polymorphism observed by SCoT was high and demonstrated the high efficiency of SCoT markers for genetic diversity analysis in chickpea. On the basis of these data, dendrograms were created and both the markers showed different clustering patterns revealing genetic variation pattern among chickpea genotypes. The differences in the pattern of dendrograms when two markers were used had also been reported [24-25]. The current study confirmed the importance of novel gene-based molecular studies (cheap, fast and informative markers) that can be used beside the morphological data in detecting genetic variation among genotypes in selecting diverse parents to carry out a new crossing programme successfully.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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