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The study of genetic relationships between in landrace chickpea collected from north-west of Iran using SCoT molecule marker

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Abstract

This study was carried out using 10 SCoT primers, to genetic relationships evaluation for 38 accessions of 4 population's chickpea of the north-west of Iran. In addition, 95 bands were scored using these primers, that polymorphism were showed for 65 bands. Primer $SCoT_{12}$ with 14 bands had the highest and primer $SCoT_{35}$ with 5 bands had the lowest number of bands. The lowest percent of polymorphism belonged to $SCoT_{11}$ (33%) and the primer of $SCoT_{13}$ had the highest percent of polymorphism (100%). AMOVA revealed that 12% of the total variance was due to differences between populations and 88% was due to differences within populations. Sanandaj population with the Shannon's information index (I) and Nei's gene diversity (He) had the highest variety between reviewed population, and Kurdistan population had the lowest variety. In addition the population of Kurdistan had the highest distance with Qorve and the population of Kurdistan had the highest similarity with Sanandaj. These results were confirmed by cluster analysis and principal coordinate analysis of populations. Cluster analysis and Scatter plot based on first and second axis from principal coordinate analysis for accessions, showed that genetic variation did not agreement with the geographical distribution.

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Introduction

Chickpea (*Cicer arietinum* L.) is a cool season grain legume with high nutritive value and serves as an important cheap source of protein in developing countries diet in addition to improving land fertility. Chickpea is a diploid plant with an estimated haploid genome size of about 740 Mb and 2n=2x=16 chromosomes (Arumuganathan and Earle, 1991; Coram *et al.,* 2007). In addition to the importance of this crop for the poor, it is also an important food for the affluent populations to alleviate major food related health problems (Charles *et al.,* 2002; McIntosh and Topping, 2000). However, more research is necessary to elucidate and extend the food and nutraceutical benefit of this important food legume through breeding (Millan *et al.,* 2006). A rich and diverse germplasm collection is the backbone of every successful crop improvement programs. The genetic variability is the raw material of crop breeding industry on which selection acts to evolve superior genotypes. Morphological characteristics are the strongest determinants of the agronomic value and taxonomic classification of plants. Compared with other methods, morphological evaluations are direct, inexpensive and easy. However, errors can arise; furthermore, morphological estimations are more dependent on environment. Neutral, DNA-based molecular markers allow a more precise and environment independent way to evaluate the genetic diversity of a particular species. DNA-based molecular markers such as random amplified polymorphic DNA RAPD; (Chowdhury *et al.,* 2002; Iruela *et al.,* 2002; Sant *et al.,* 1999; Sudupak *et al.,* 2002) and RFLP (Powell *et al*., 1996) were unable to reliable address the genetic variation within chickpea (Hernández *et al.,* 2001; Ratnaparkhe *et al.,* 1995). Although RAPD markers were recently shown to be useful for genetic diversity in chickpea, they require minimum of 30 or more primers and may cause genotyping errors (Rao *et al,* 2007). Genetic fingerprinting in chickpea, for a long time hampered by the little variability in chickpea's genome, is today facilitated by highly polymorphic, co-dominant microsatellite-based markers (Nguyen *et al.,* 2004; Serret *et al.,* 1997; Sethy *et al.,* 2006). In recent years, many new alternative and promising marker techniques have been developed in line with the rapid growth of genomic research (Gupta and Rustgi, 2004). With initiating a trend away from random DNA markers towards gene-targeted markers, a novel marker system called Start codon targeted (SCoT) (Collard and Mackill, 2009) was developed based on the short conserved region flanking the ATG start codon in plant genes. SCoT markers are generally reproducible, and it is suggested that primer length and annealing temperature are not the sole factors determining reproducibility. Here, the use of SCoT polymorphism markers technique for studying genetic diversity was reported for the first time in chickpea genotypes. Genetic variations based on Molecular markers for between and within different species of Chickpea were reported by many researchers (Saeed *et al.,* 2011; Talebi *et al.,* 2009; Ahmad *et al.,* 2010). Objectives of the present study are as follows: (1) to determine the potential of this methodology to generate polymorphic markers in chickpea; (2) to identify the relationships between different chickpea populations using SCoT molecular markers.

Materials and methods

Plant Materials

In this research 38 accessions from 4 populations of landrace chickpea collected from north-west of Iran were prepared from gen bank Tehran, Iran (Table 1).

DNA Extraction and SCoT Method

Total genomic DNA was extracted for young leaves of greenhouse-grown plants using a modified CTAB (Murry and Tompson, 1980) with modification described by De la Rosa *et al.,* (2002). Quality and quantity of extracted DNA were examined using 0.8% agarose gel. PCR amplification was performed in 20 µl reaction containing 1× PCR buffer, 30 ng sample DNA, 2.5 µM primers, 200 µM of each dNTP, 1.5–2.5 mM MgCl2 and 1.5 unit of Taq DNA polymerase

(Cinnagene, Iran). Template DNA was initially denatured at 92°C for 5 min, followed by 35 cycles of PCR amplification under the following parameters: denaturation for 30 seconds at 95°C, primer annealing for 30 seconds at the temperature based on primer temperature (temperatures of annealing in this study was 50, 55 and 60 °C) and primer extension for 1 min at 72°C. A final incubation for 5 min at 72°C was performed to ensure that the primer extension reaction proceeded to completion. The PCR amplified products were separated by electrophoresis on a 1.5% agarose gels using TBE buffer. The gels were put in the ethidium bromide for 30-45 min and visualized by gel document.

Statistical Analysis

SCoT bands were treated as binary characters and coded accordingly (presence =1, absence = 0). Number of bands scored, Number of polymorphic bands, Percentage of polymorphic bands was calculated for each primers and each accessions. Polymorphism information content (PIC) was measured for each primer (Anderson *et al.,* 1993). Data were analyzed using POPGEN 32 and Nei's gene diversity (He) (Nei, 1973), Shannon's information index (I) (Shannon, 1948) were calculated for each of populations. Cluster analysis, similarity matrix and principal coordinate analysis axis were carried out for 38 accession using DARwin5 and Gen ALEX 6.2.

Results

SCoT Polymorphism

Primers sequences, code, number of scored bands (NSB), number of polymorphic bands (NPB), percent of polymorphic bands (PPB) and polymorphism information content (PIC) were showed for SCoT primers in table 2. Polymorphism was observed for all primers used in this study. In addition, the number of 95 bands was scored that polymorphism was observed for 65 of them. $SCoT_{12}$ primer with 14 bands had the highest and primer $SCoT_{35}$ with 5 bands had the lowest number of bands. Band pattern of accessions for $Scot_{11}$ was showed in Figure 1.

Table1. List of 38 selected accessions of landrace chickpea of the north-west of Iran.

Number	Gen bank cod	Origin	Number	Gen bank cod	Origin
1	40-4937	Marivan	21	40-4937	Qorve
2	40-4938	Marivan	22	40-4938	Qorve
3	40-5023	Marivan	23	40-5023	Sanandaj
4	40-5024	Marivan	24	40-5024	Sanandaj
5	40-5025	Marivan	25	40-5025	Sanandaj
6	40-5026	Marivan	26	40-5026	Sanandaj
7	40-5132	Marivan	27	40-5132	Sanandaj
8	40-5370	Marivan	28	40-5370	Sanandaj
9	40-5371	Marivan	29	40-5371	Sanandaj
10	40-5373	Marivan	30	40-5373	Sanandaj
11	40-5378	Qorve	31	40-5378	Sanandaj
12	40-5379	Qorve	32	40-5379	Sanandaj
13	40-5249	Qorve	33	40-5249	Kurdistan
14	40-5250	Qorve	34	40-5250	Kurdistan
15	40-5252	Qorve	35	40-5252	Kurdistan
16	40-5372	Qorve	36	40-5372	Kurdistan
17	40-5372	Qorve	37	40-5375	Kurdistan
18	40-5377	Qorve	38	40-5377	Kurdistan
19	40-4937	Qorve			
20	40-4938	Qorve			

Average of polymorphism percent was 64% which, the lowest percent of polymorphism belonged to $SCoT_{11}$ (33%) and the highest percent of polymorphism was 100% for primer $SCOT_{13}$. The average of PIC for all primers was 0.34 that the highest value of PIC was related to $SCoT_{11}$, $SCoT_1$ and $SCoT_2$ primers and the lowest belonged to $SCoT_{28}$ (Table 2).

Table 2. SCoT primers used in this study and some summary results.

PIC	Percentageof polymorphic bands(PPB)	No. of polymorphic bands	No. of bands scored	Primer sequence	SCoT code
0.35	90%	9	10	5'-AACCATGGCTACCACCAC-3'	SCoT $_{22}$
0.32	100%	11	11	5'-ACGACATGGCGACCATCG-3'	SCoT $_{13}$
0.27	75%	9	12	5'-CCATGGCTACCACCGCCA-3'	SCoT $_{28}$
0.33	40%	2	5	5'-CATGGCTACCACCGGCCC-3'	SCoT $_{35}$
0.31	44%	4	9	5'-ACCATGGCTACCACCGCG-3'	SCoT $_{20}$
0.30	62%	5	8	5'-GCAACAATGGCTACCACC-3'	SCoT $_{36}$
0.42	33%	2	6	5'-AAGCAATGGCTACCACCA-3'	SCoT $_{11}$
0.42	62%	8	13	5'-CAACAATGGCTACCACCC-3'	SCoT $_{2}$
0.36	79%	11	14	5'-ACGACATGGCGACCAACG-3'	SCoT $_{12}$
0.41	57%	4	7	5'-CAACAATGGCTACCACCA-3'	SCoT $_{1}$
0.34	64%	6.5	9.5		Average

Table 3. Molecular variance analysis.

S.O.V	Df	SS	MS	Est. Var.	Var%	Stat	Value	P
Between populations	3	73.325	24.442	1.486	12%	PhiPT	0.123*	0.010
Within populations	34	359.333	10.569	10.569	88%			
Total	37	432.658		12.055	100%			

Est.Var; Calculated variance for Within and between group.

Var%; Percent of variance of each source to total variance.

Analysis of molecular variance (AMOVA) was performed for SCoT bands to determine of significant difference between populations of accessions based on origin (Table 3). AMOVA revealed that 12% of the total variance was due to differences between populations and 88% was due to differences within populations.

Table 4. Statistical analysis of genetic diversity of 4 populations.

Population	Number of simple	Percentage of polymorphic bands (PPB)	No. of polymorphic bands	Nei's gene diversity (He)	Shannon's information index(I)
Marivan	10	90.77	59	0.28 (0.016)	0.43 (0.021)
Qorve	12	73.85	48	0.24 (0.019)	0.36 (0.026)
Sanandaj	10	93.85	61	0.29 (0.016)	0.44 (0.021)
Kurdistan	6	64.62	42	0.22 (0.020)	0.34 (0.028)

Genetic variation

Shannon's information index (I), Nei's gene diversity (He), number and percentage of polymorphic bands were calculated for each population (Table 4).

The genetic diversity of Sanandaj was relatively high, and the PPB, He and I of Sanandaj were 93.58%, 0.29 and 0.44, respectively and followed by Marivan and Qorve. Population of Kurdistan had the lowest genetic diversity among the populations, that is, PPB = 64.26%, He = 0.22 and I = 0.34.

Genetic relationships

Genetic distance for populations based on Dice's

coefficient (Table 5) showed that the highest of distance are between Kurdistan and Qorve population (0.134) and the lowest of distance relates to Kurdistan and Sanandaj population (0.014). These results were confirmed by cluster analysis by UPGMA methods and principal coordinate analysis of populations (Fig. 2, 3).

Table 5. Dice's coefficient distance for population.

population	Marivan	Qorve	Sanandaj	Kurdistan
Marivan	****	0.866	0.971	0.961
Qorve	0.143	****	0.874	0.832
Sanandaj	0.029	0.134	****	0.986
Kurdistan	0.039	0.183	0.014	****

Cluster analysis

UPGMA hierarchical clustering for grouping accessions based on Dice's coefficient (Fig. 4) were identified the three distinctive groups. Grouping of accessions indicated that genetic variations do not agreement with the geographical distribution of accessions. Therefore considering of genetic distance between these groups, using of the first and third group accessions can be useful in breeding programs, to utilization of heterosis.

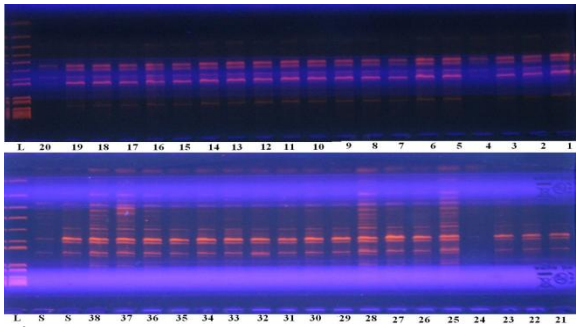

Fig. 1. The band pattern of landrace chickpea of the west of Iran accessions using $SCoT_{11}$ primer.

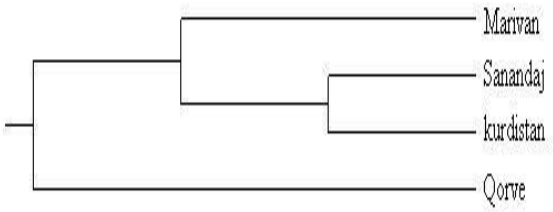

Fig. 2. Dendrogram of cluster analysis for populations by UPGMA method.

Principal coordinate analysis

Scatter plot for accessions based on first and second axis from principal coordinate analysis (Fig. 5) showed that genetic variation did not matching with the geographical distribution of accessions. These results confirmed by cluster analysis and similarity matrix.

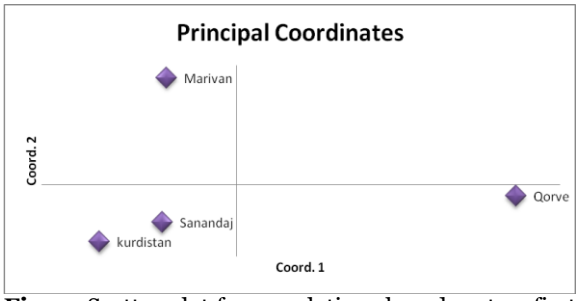

Fig. 3. Scatter plot for populations based on two first axes from principal coordinate analysis.

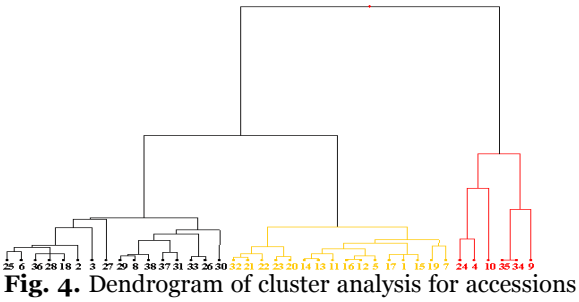

Fig. 4. Dendrogram of cluster analysis for accessions based Dice's coefficient by UPGMA.

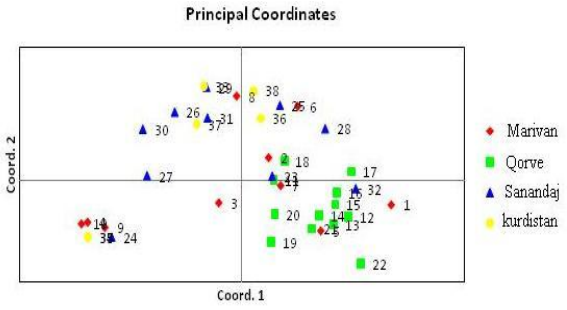

Fig. 5. Scatter plot for accessions based on two first axes from principal coordinate analysis.

Discussion

Although chickpea has a narrow genetic base, with the discovery of diverse molecular markers from different targets regions of the genome, it is now possible to conduct extensive molecular diversity study in this important crop to identify genetically diverse germplasm with beneficial traits for use in crop improvement programs. The assessment of genetic diversity is important not only for crop improvement but also for efficient management and conservation of germplasm resources. The current study confirmed the importance of molecular studies data in detecting genetic variation among genotypes in selecting diverse parents to carry out a new crossing program successfully. We believe that there needs a molecular markers studies as a complementary studies for the morphological traits in the field. It was revealed that genetic pattern of chickpea populations can be determined using SCoT in the short–term. Genetic fingerprinting in chickpea, for a long time hampered by the little variability in chickpea's genome, can be facilitated by highly polymorphic functional markers such as SCoT. Results indicated that there was a well variation between studying population considering to Shannon's information index (I) and Nei's gene diversity (He) which, it was revealed that Sanandaj population had highest variation between its accessions and the lowest belongs to Kurdistan population. We found relatively acceptable genetic diversity within available Iranian landrace chickpea accessions which is contrary to the findings of Iruela *et al.*, (2002), who reported low level of genetic diversity within *C. arietinum* compared to the wild species. Primer $SCoT_{13}$ showed a high polymorphism (100%) which indicated a high ability in the reviewing of genetic variation and increased variety of studying population. Considering polymorphism information content (PIC), the primers $SCoT_{11}$, $SCoT_1$ and $SCoT_2$ can be used to analysis of genome of other accession of landrace chickpea of Iran in the future researches. These results suggested the presence of a considerable polymorphism at studied molecular markers and revealed a high level of genetic diversity in the existing chickpea germplasm. Genetic diversity in 7 species *cicer* was studied by Talebi *et al.,* (2009) based on RAPD marker and they reported a different PIC for used primers. Similarity and distance were observed based on Dice's coefficient between populations. Kurdistan population had the highest distance with Qorve and the Sanandaj population had the highest similarity with Kurdistan. We may use more distant genotypes for future hybridization to improve the degree of genetic polymorphism available in Iran. Studied accessions were clustered in tree groups based on UPGMA clustering method. Grouping of accessions based on cluster analysis and principal coordinate analysis indicated that genetic variations do not in agreement with the geographical distribution of accessions. Therefore considering of genetic distance between these groups, using of the first and third group accessions can be useful in breeding programs, to utilization of heterosis. There are several possible explanations for such results: some of them connected with nature and structure of different molecular markers that designed from various regions of genome. Another problem was the possibility of overestimating genetic similarity because fragments with the same size could have different origins (Talebi *et al.*, 2008). The efficiency of SCoT markers for fingerprinting of genotypes is relatively the same. In general, these techniques could be used in conjunction with each other for diagnostic fingerprinting of chickpea. The present study showed existence of high genetic diversity in Iranian landrace chickpea germplasm accessions. The magnitude and pattern of genetic variation detected in this study can be useful for more systematic germplasm management and utilization in breeding programs (Tanya *et al.*, 2011).

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