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RESEARCH ARTICLE

Chickpea (*Cicer arietinum* L.) genetic diversity detected by Inter Simple Sequence Repeat markers

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ABSTRACT: Genetic relationships among twenty-three Chickpea genotypes include seven Chickpea cultivars, and 16 wild genotypes were analysed by 10 ISSR markers. On average, 11 bands per primer were observed, and 83.4 % polymorphic bands were shared in ISSR analysis. The results of clustering demonstrated the efficiency of ISSR markers (particularly ISSR1 (UBC-834) and ISSR8 (UBC-864)) for reporting appropriate parents for breeding programs to biotic and abiotic stresses. Principle Coordinate Analysis (PCoA) clustered 23 Chickpea genotypes in two groups by which six cultivars separated in one group. Genetic data extracted from ISSR analysis are practical in Chickpea to choose parental sources for inbreeding programs.

KEYWORDS: Chickpea, Inter Simple Sequence Repeat (ISSR), Genetic diversity, PCoA

INTRODUCTION

Chickpea (*Cicer arietinum* L. 2n=16) is the most important legume crops after common bean (*Phaseolus vulgaris* L.), in semi-arid regions such as Asia and Africa (FOASTASTIC, 2018). Chickpea crop contributes to soil fertility in soil as a result of nitrogen fixation [2, 12]. Chickpea along with other important pulse crops are considered as the significant components in developing countries diets due to rich nutritional value and healthy benefits [4, 11]. Chickpea is a suitable source of protein, carbohydrate, dietary fibre and along with micronutrients calcium, magnesium, potassium, phosphorus, iron, zinc and manganese [14, 15] as well as vitamin A [20]. The study of genetic diversity of chickpea germplasm is one of the fundamental experiments for the release of cultivars and the implementation of breeding projects. However, genetic diversity is still a challengeable point for plant breeding programs. Structure of genetic diversity in various crops has been clarified by molecular markers [7, 18, 22]. In addition, molecular markers are pivotal to identify genetic relationships among genotypes [8] and to evaluate diversity in particular genetic regions such as resistant genes to disease [25]. The result of Diapari *et al.* proved potential ability of molecular markers in chickpea which are associated to iron and zinc [6].

ISSR (inter simple sequence repeat) technique is reliable, cost-efficient, highly polymorphic and most importantly does not need DNA sequence knowledge [3]. ISSR markers have already been used in chickpea [5, 10, 17, 19]. Also, relationships in a chickpea collection have been studied using ISSR markers by Iruela *et al.*, 2002 [9]. Their results illustrated that the pattern of variable distribution amongst species was linked to both growth habits and geographic origin. Also the Kabuli and Desi varieties of cultivated chickpea were distinguished by

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cluster analysis, although there was no apparent association between the groupings and the geographical origin of the cultivated accessions. ISSR approach carried out to determine intera and inter species genetic diversity among *Cicer* species. The ISSR markers were used by Sudapak to evaluate among *Cicer* species and the results indicated that annuals of the second crossability group (*C. pinnatifidum*, *C. bijugum*, and *C. judaicum*) are genetically closed to the perennial *C. incisum*, whereas *C. reticulatum* is the wild species that is most closely related to the cultivated chickpea. Interestingly, the ISSR marker information was substantially greater than earlier estimations based on RAPD and AFLP data. In that study, the ISSR markers information was greater than the information obtained from the RAPD and AFLP markers [23]. Recently, Janghel *et al.,* (2021) were used 25 ISSR markers and some Agro-morphological traits for analysis 45 elite chickpea lines. They found that the dendrogram created by the molecular markers was different from the morphological traits. Molecular markers divided the chickpea genotypes into two main groups, while based on the agronomic traits of this genotype, they were divided into five groups. They admitted that the ISSR marker is a remarkably effective tool in recognizing genetic diversity and it can be useful to determining suitable parent material in chickpea breeding progress for further improvement [10].

The object of this study is at first to evaluate genetic relationships of 23 chickpea accessions collection in which some of them are resistant to abiotic and biotic stress, and secondly to identify efficient parents for breeding programs in chickpea.

MATERIALS AND METHODS

plant material

A total of 23 genotypes includes ten cultured genotypes in Iran, ten biotic tolerant genotypes, and three abiotic tolerant genotypes, were studied. Germplasm was obtained from Plant Sciences Research Centre, Ferdowsi University of Mashhad, Iran (Table1).

DNA Extraction and ISSR Analysis

The youngest leaves of seedlings were applied to extract DNA using the genomic DNA Purification Kit. The quality and quantity of DNA were checked by spectrophotometer (Eppendorf, Biophotometer) and 1% agarose gel electrophoresis. 43 ISSR primers (UBC primers) were screened for consistency and their ability to produce polymorphism in Chickpea genotypes. Ten primers were chosen for fingerprinting based on the high number of polymorphic fragments generated (Table 2). Polymerase chain reaction (PCR) contained 30 ng of template DNA, mM Tris-HCl (pH 8.8 at 25 °C), 50 mM of KCl, 0.8% (v/v) Nonidet P40, 2 mM MgCl2, 0.25 µM of primer, 200 mM dNTP mix and 0.6 U Taq-DNA polymerase. PCR products were mixed with four µl of 6X loading dye (0.25% Bromophenol Blue, 0.25% Xylene Cyanol, and 40% Sucrose w/v) and loaded on 1.75% agarose gel, then stained with ethidium bromide and visualized under UV light. Amplification was carried out for 37 cycles, each containing denaturation at 94°C for 45s, annealing at 55°C for 45s and the extension at 72°C for 2 min. An initial denaturation step at 94°C for 5 min and a final synthesis step for 5 min at 72°C were also included.

Table 1. List of Chickpea accessions used for diversity analysis.

No	Genotype	General name	Plant Characteristics
1	MCC 361	Sefide jam	CCI, MM
2	MCC 397	Korosh	CCI, MLM
3	MCC 362	Siahe kaka	CCI
4	MCC 395	Pirouz	CCI, MM
5	MCC 950	Hashem	CCI, MM
6	MCC 252	ILC482	CCI, MLM
7	MCC 358	KARAJ 12-60-31	CCI, MM
8	MCC 49	-	CCI
9	MCC 736	-	CCI
10	MCC 737	-	CCI
11	MCC 776	-	DT, MLM
12	MCC 875	-	DT, MLM
13	MCC 878	-	DT, MM
14	MCC 879	-	DT, MLM
15	MCC 13	-	DT, MM
16	MCC 385	-	DT, MM
17	MCC 5	-	DT
18	MCC 133	-	RA
19	MCC 106	-	RA
20	MCC 270	-	RA
21	MCC 53	-	CT
22	MCC 258	-	CT
23	MCC 428	-	CT

CCI= commonly cultured in Iran, DT= Drought tolerant, RA= Resistant to Ascochyta blight, CT=Cold tolerant, MM= Medium in Maturity, MLM= Medium-late in maturity.

Data Analysis

ISSR PCR fragments were scored for the presence (1) or absence (0) by TotalLab software ver TL120. The number

Table 2. Details of selected of ISSR primers used in the present study

No	name	sequence	b.p	T.b	n.p	P.p	PIC	MI
ISSR1	UBC-834	(AG)8 YT	230-1220	15	13	86	0.148	12.728
ISSR 2	UBC-855	(AC)8 YT	300-2000	12	10	83	0.124	10.292
ISSR 3	UBC-841	(GA)8Y C	350-2000	7	4	57	0.107	6.099
ISSR 4	UBC-811	(GA)8 C	340-1300	5	5	100	0.193	19.3
ISSR 5	UBC-830	(TG)8 G	300-1400	4	4	100	0.162	16.2
ISSR 6	UBC-828	(TG)8 A	600-2000	7	5	71	0.131	9.301
ISSR 7	UBC-807	(AG)8 T	250-1200	10	10	100	0.127	12.7
ISSR 8	UBC-864	(ATG)6	480-1900	12	12	100	0.133	13.3
ISSR 9	UBC-840	(GA)8 YT	500-1900	8	6	75	0.119	8.925
ISSR 10	UBC-836	(AG)8 YA	300-1500	8	5	62	0.084	5.208
Average				8.8	7.4	83.4	0.1328	11.4053

P.p- Percent polymorphic bands; b.p- Approximate size of bands; PIC- polymorphic information content; n.p-number of polymorphic bands; MI- marker index; T.b- Total number of bands.

of total bands (T.b) and Percentage of polymorphism band (P.b) were estimated by PopGen software ver 32. Polymorphic information content (PIC) and marker index (MI) were calculated as flow: PIC= $\sum$ [2pi (1-pi)], where pi is the band frequency for the ith band and MI= P.b × PIC for each primer. Cluster analysis was performed with the help of NTSYS software ver2.02. Based on the comparison between each dendrogram and similarity matrix by the Mantel matrix correspondence test [17], the highest co-phonetic correlation coefficient (0.86) was determined between Jaccard's similarity coefficients with the UPGMA clustering algorithm. For studying diversity between populations, Principle Coordinate Analysis (PCoA) was calculated using GenAlEx software 6.5 [18]. Cluster analysis for ISSR Data was performed by SplitsTree4 software Ver. 4.11.3 based on Neighbor-net distance.

RESULTS

Polymorphic Analysis

Out of 10 polymorphic ISSR primers, ISSR1 (UBC-824) and ISSR8 (UBC-864) have amplified the maximum number of bands (13, 12) and produced the highest marker index (12.728, 13.3 respectively). However, ISSR4 (UBC-811) showed the most top polymorphic information content (0.193). The number of the repeated motifs for primers were nine di-nucleotides and one tri-nucleotide (ATG)6 (ISR8). All bands (12 bands) produced by ISR8 were polymorphic.

Genetic similarity

Genetic similarities and ISSR-based UPGMA dendrogram were calculated based on Jaccard's similarity coefficient. The average similarity among Chickpea varied from 66.7% to 100%. The lowest genetic similarity was observed between genotypes 6 and 2, and the highest one belongs to genotypes 11 and 12.

Cluster Analysis

ISSR-based UPGMA dendrogram was performed for the molecular data in which genotypes 5 (Hashem) and 13 (Drought tolerant genotype) are indicated in one subgroup (Fig. 1). It provides opportunities for breeding programs for drought-resistant, especially in semi-arid regions like Iran. Also, genotypes 7 (KARAJ 12-60-31) and 19 (Resistant to Ascochyta blight) are depicted in another distinct subgroup, which illustrates an appropriate selection for resistance to the Ascochyta blight program. Although cluster analysis by Spilt tree4 based on Neighbor-net distance (Fig. 3) was presented genotypes in clusters closely similar to UPGMA, there was few differences between UPGMA and Neighbor-net cluster output, as an illustration, genotype 6 (ILC482) was closed to genotype 22 (cold tolerant). PCoA analysis based on ISSR result expresses diversity among chickpea genotypes. The result of the cluster analysis showed that the genotypes were separated into two major groups (Fig. 2). Group1 includes; Sefide jam, Korosh, Siahe Kaka, Pirouz, Hashem, and ILC482. The rest of the genotypes located in group 2. This analysis divided genotypes into

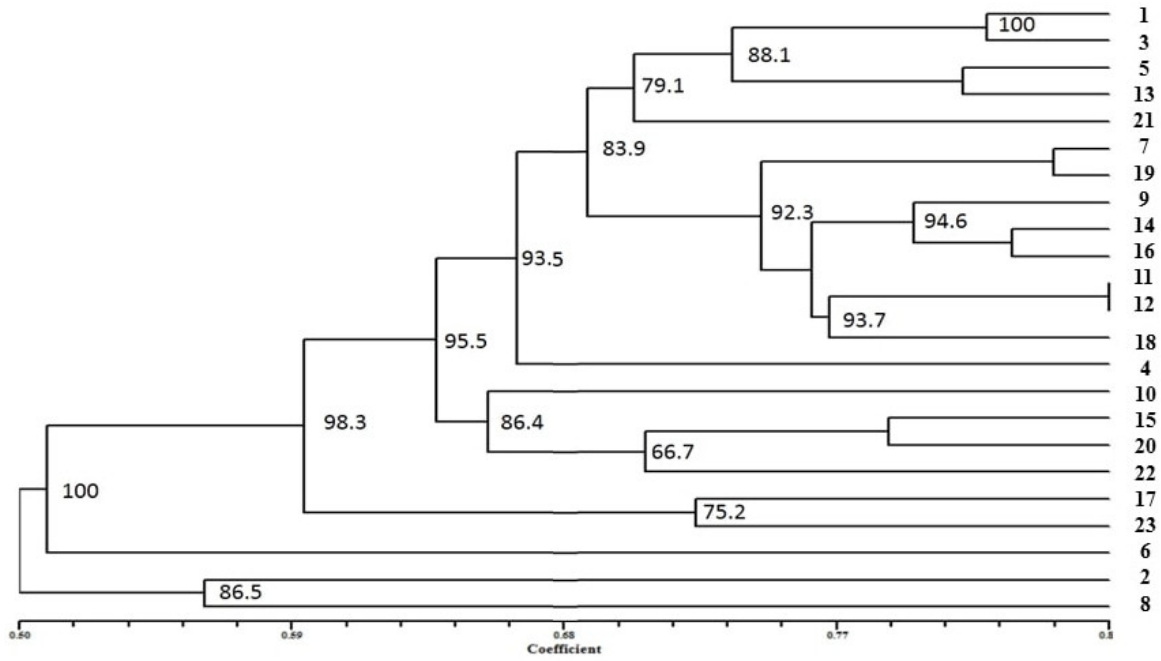

Figure 1. UPGMA clustering of 23 Chickpea genotypes based on Jaccard's similarity using ISSR markers.

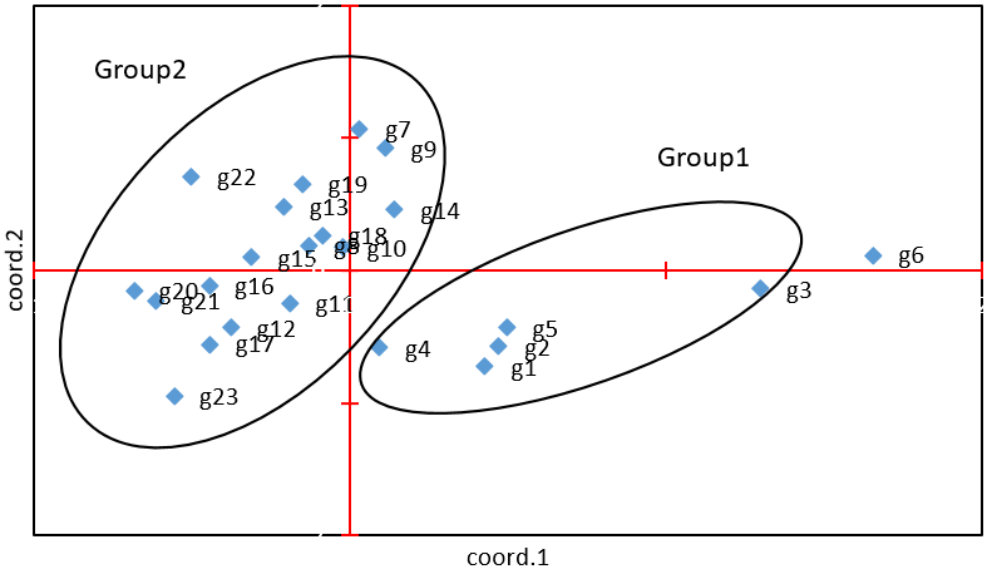

Figure 2. Relationships among 23 Chickpea genotypes virtualized by PCoA using ISSR markers; ◆: genotypes. The details of genotypes are presented in Table 1.

cultivars and wild accessions that declare better Principle Coordinate Analysis ability in showing genetic relationships rather than UPGMA and Neighbor-net.

DISCUSSION

The genetic variation among parents causes genetic differentiation to be seen in subsequent generations during the plant breeding progress and increases the chances of success in genetic improvement [24]. Molecular markers have proven to be useful in the genetic identification, assessment, and analysis of feature mutations [1]. In present study, ISSR analysis results show that there is remarkable diversity among the chickpea germplasm, especially, there is a suitable genetic distance between LIC-482 and others genotypes. Also, Previous studies illustrated that ILC 482 has some desirable traits such as grain yield (1752 kg/h), tolerance to dehydration stress [16], and tolerance to cold stress in the seedling stage [13, 21]. Selecting this genotype as a parent can increase the success rate of the breeding program. This success prediction is based on the

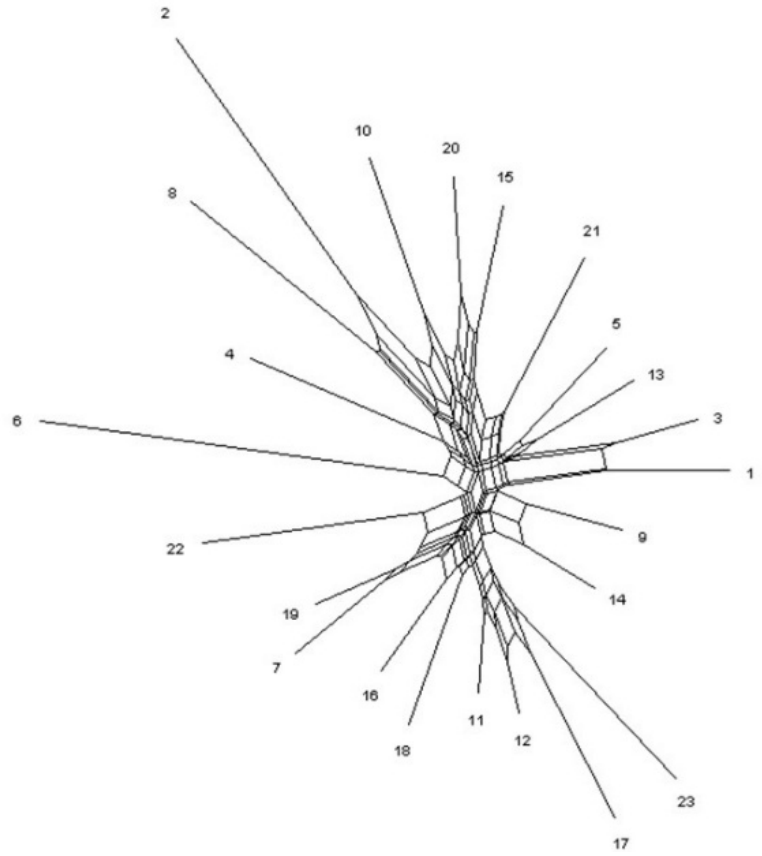

Figure 3. Genetic relationship among 23 genotypes of Chickpea using ISSR with split tree based on Neighbor-net distance.

possibility of significant segregation in the F_2 generation which is caused by genetic distance. As a whole, this study investigated the most polymorphic ISSR primers in Chickpea genotypes which prepare the necessary information for future genetic and breeding programs in this species. However, in future research, more primers may be used to understand germplasm thoroughly.

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شناسایی تنوع ژنتیکی ژرم‌پلاسم نخود (*Cicer arietinum* L.) با استفاده از نشانگر ISSR

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چکیده

نخود دومین محصول مهم حبوبات بعد از لوبیا می‌باشد که کشت آن در مناطق آسیا و آفریقا متداول است. روابط ژنتیکی بین ۲۳ ژنوتیپ نخود شامل ده رقم (متداول) و ۱۳ ژنوتیپ وحشی با استفاده از ۱۰ نشانگر ISSR مورد تجزیه و تحلیل قرار گرفت. به طور متوسط یازده باند در هر آغازگر مشاهده شد که ۸۳/۴ درصد از باندهای حاصله چندشکل بود. نتایج خوشه‌بندی کارایی مطلوب نشانگرهای ISSR بخصوص نشانگرهای ISSR1 و ISSR8 جهت شناسایی والدین به منظور استفاده در برنامه‌های به‌نژادی افزایش تحمل به تنش‌های زنده و غیرزنده را نشان داد. همچنین تجزیه به مختصات اصلی (PCoA) ژنوتیپ‌های نخود را در دو گروه اصلی تفکیک کرد و نشان داد که ژنوتیپ ۶ (ILL482) در هیچ یک از این گروه ها قرار نمی‌گیرد. نتایج حاصل از این مطالعه می‌تواند اطلاعات اولیه برای برنامه‌های ژنتیکی و اصلاحی آینده را فراهم کند.

کلمات کلیدی: Neighbor-net distance، UPGMA، تنوع ژنتیکی، تجزیه به مختصات اصلی