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ASSESSMENT OF RELATIONSHIP IN CHICKPEA (CICER ARIENTINUM L.) BASED ON PRODUCTIVITY CHARACTERISTICS AND SSR MARKERS IN AZERBAIJAN

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Abstract

Genetic diversity and marker–trait associations related to yield components were assessed in 43 chickpea (*Cicer arietinum* L.) accessions from a germplasm collection using winter-sown seeds in Azerbaijan. The accessions originated from Azerbaijan, Iran, and the International Center for Agricultural Research in the Dry Areas (ICARDA). Molecular genotyping was conducted using ten simple sequence repeat (SSR) markers. Yield and its components were evaluated across eight traits in field experiments. Correlation analysis revealed that yield was primarily influenced by plant height, the number of branches, and the formation of a high number of pods. These traits showed their highest values in chickpea genotypes. SSR analysis detected between four and seven alleles per marker. The highest levels of polymorphism were observed in markers TA46 (PIC = 0.88), H1A12 (PIC = 0.84), and H3E04 (PIC = 0.82), while the lowest were recorded in NCPGR6 (PIC = = 0.42) and H4E04 (PIC = 0.44). Cluster analysis grouped the accessions into five distinct clusters. Several unique alleles identified through this analysis may serve as molecular identifiers (passport markers) for specific genotypes. These informative markers can be useful for chickpea breeding programs focused on yield improvement. Breeding lines with sufficient genetic distance from this study can be used as appropriate parents to get more heterotic recombinants. Correlation analysis was performed between 10 SSR primers and yield components, informative SSR primers related to important trait of plant height were found.

Keywords: *SSR, molecular markers, yield-related components, cluster, correlation*

Introduction

Chickpea (*Cicer arietinum* L.) is an annual, self-pollinated diploid legume plant species from the Fabaceae family, providing high-quality plant protein to more than 30% of the world's population. In 2018, 17.2 million tons of chickpeas were produced from 17.8 million hectares of land worldwide (JHA, *et al*., 2021). As in other parts of the world, chickpea production in Azerbaijan is increasing year by year, and in 2022, it covered more than 6,189 thousand hectares. Particularly in the southern regions of Azerbaijan, chickpeas are successfully cultivated under non-irrigated conditions on an area of 4,642 thousand hectares (https://www.stat.gov.az/source/agriculture). The ability of chickpea plants to withstand drought and moisture deficiency is key to their successful cultivation under non-irrigated conditions in Azerbaijan (Mazkirat, *et al*. ., 2023, R_core_team, 2023). Additionally, chickpea plants can tolerant cold stress from low temperatures and even short periods of freezing. Therefore, winter chickpeas are also used in central regions, where winters are mildly cold and the plants can grow and overwinter under these conditions.

The genetic diversity in germplasm collections of crops, including chickpea, can be an essential resource for identifying and utilizing genotypes adapted to specific regional growing conditions, leading to their successful application in breeding programs (Serekpayev, *et al*., 2016; SARI, *et al*., 2023). The use of molecular markers in genetic diversity studies can further enhance the effective utilization of germplasm collections (Fayaz, et al. 2021).

The discovery of molecular markers closely linked to key breeding traits has enabled the implementation of marker-assisted selection (MAS) (Bradbury, *et al*., 2007). In the breeding of chickpea and other legume crops, microsatellite markers (SSRs) have been successfully used for assessing genetic diversity and facilitating selection processes, including MAS (Bradbury, *et al*., 2007; Liu, *et al*. 2005; Serrote, *et al*. 2020; Varshney, *et al*., 2014). SSR markers exhibit codominant inheritance, which is particularly important for analyzing hybrid populations (Liu et al. 2005). The high polymorphism of SSR loci and their widespread distribution across the genome provide valuable information on genetic variation among studied germplasm collections (Bhattarai, *et al*., 2021; Serekpayev, *et al*., 2016).

The objectives of this study were: (1) to assess the genetic diversity in a chickpea germplasm collection from Azerbaijan using SSR markers, and (2) to analyze associations between SSR allelic variants and yield components in chickpea genotypes to improve productivity under winter and spring sowing conditions.

Materials and methods

In this study, 43 chickpea (*Cicer arietinum* L.) accessions were used from the legume germplasm collection maintained by the National Gene Bank of the Genetic Resources Institute of ARSEM. The chickpea accessions originated from Azerbaijan, Iran, and the International Center for Agricultural Research in the Dry Areas (ICARDA) (Table 1). Local improved varieties – Narmin and Jamila were obtained by selecting from ICARDA introductions in different years. Genotypes were planted in 2023–2025 and evaluated according to international descriptors. The planting scheme of the samples was as follows: in chickpea the length of the spots was 5 meters, width 2.0 meters, row spasing 30 cm, and inter seed spasing 5cm. Each chickpea genotype was planted in two replicates using a completely randomized block design. A total of 100 seeds were sown per genotype, and the local standard variety Jamila was used as a control for comparison. The following traits were measured: plant height, number of branchs, number of productive branches, the height to first pod, number of pods per plant, seed yield per plant, 100 seed weight, seed yield per $1m^2$.

Molecular Analysis

For each genotype, genomic DNA was extracted from fresh leaves using CTAB protocol by DOYLE and DOYLE (1987). The quality and quantity of extracted DNA was determined by measuring absorbance at 260 and 280 nm using spectrophotometer. The DNA concentration was diluted to 100 ng/μL using ddH2O. PCR was carried out in a total volume of 15 μL containing 1.5 μL of 10 × PCR buffer, 2 mM $MgCl_2$, 0.15 μM of each dNTP, 0.7 μM of each primer, 0.5 U of Taq

DNA polymerase. The reactions were carried out in a Bio-Rad iCycler system (Bio-Rad Laboratories, Hercules, CA, USA) with the following program: an initial step of 94 °C for 3 min; 30 cycles of 94 °C for 1 min; 55 °C for 1 min and 72 °C for 1 min; and a final step of 72 °C for 5 min (Varshney, 2005).

PCR products were visualized on 8% polyacrylamide gel at a constant rate of 200 V for 70 min and detected via staining with ethidium bromide. Electrophoresis images were obtained using BIO-RAD gel documentation system.

Table 1. *Origin and morphological characteristics of studied chickpea germplasm collection*

No.	Genotypes	Origin	No.	Genotypes	Origin
1	Narmin	Azerb.	23	Jalilabad 11	Azerb.
2	Jamila St.	Azerb.	24	Flip 97–32	ICARDA
3	Ağdenli	Russian	25	Shamakha 25	Azerb.
4	F.13–154 C	ICARDA	26	Yardımlı 29	Azerb.
5	F.13–227 C	ICARDA	27	Agdash18	Azerb.
6	F.13–234 C	ICARDA	28	Sultan	Azerb.
7	F.13–320 C	ICARDA	29	Yardımlı 27	Azerb.
8	F.13–358 C	ICARDA	30	Masallı 30	Azerb.
9	F.13–364 C	ICARDA	31	Masallı 51	Azerb.
10	F. 88–85 C	ICARDA	32	Bilasuvar 58	Azerb.
11	F. 93–93 C	ICARDA	33	Lerik 33	Azerb.
12	F.13–53	ICARDA	34	Absheron 35	Azerb.
13	F.13–55	ICARDA	35	Lankaran 1	Azerb.
14	F.11–08 C	ICARDA	36	Lankaran 2	Azerb.
15	F.11–01 C	ICARDA	37	Flip03–48	ICARDA.
16	Ordubad 39	Azerb.	38	Jalilabad50	Azerb.
17	Ordubad 41	Azerb.	39	İran 48	İran
18	Qusar 43	Azerb.	40	Jalilabag 55	Azerb.
19	Qusar 44	Azerb.	41	Sabirabad59	Azerb.
20	Agstafa 42	Azerb.	42	Ordubad 47	Azerb.
21	F. 03–22	ICARDA	43	Yardımlı 28	Azerb.
22	Bakı 30	Azerb.			

Ten SSR markers, previously used and recommended for the study of chickpea genetic diversity, were employed to assess the genetic variation among the studied chickpea genotypes (Table 2). To score each polymorphic band, the SSR loci patterns were evaluated using binary codes: '1' for the presence of a band and '0' for its absence. A data matrix based on band presence or absence was generated for further analysis. The number of alleles, major allele frequency, genetic diversity, and polymorphism information content (PIC) were calculated using to WEIR (1990). Cluster analysis was performed using the unweighted pair group method with arithmetic mean (UPGMA), and dendrograms were conducted on the genetic similarity index using NTSYS-pc 2.1 (Rohlf, 2004).

Results and discussion

Chickpea samples were studied and evaluated in a comparative manner alongside the regionalized new standard variety, Jamila. The vegetation period of the plants ranged from 230 to 240 days. The flowering phase occurred between April 15 and April 30, and

the pod formation phase was recorded from May 5 to May 18. Plant height ranged from 55 to 89 cm, with the number of branches varying between 2 and 5. The height of the first bean ranged from 19 to 45 cm, and the number of pods per plant varied from 24 to 65. The weight of 100 seeds ranged from 20.6 to 48.5 g, and the weight of a single seed ranged from 2.0 to 6.0 g. The yield per square meter ranged from 205.5 to 521.7 g. Among the studied chickpea accessions, the following samples outperformed the standard variety in 2–3 key productivity parameters: F.13–320 C, F.13–227 C, F.13–358 C, F.13–364 C, F.88–85 C, F.93–93 C, F.13–53, and Jalilabad 11.

Table 2. *Correlation coefficients among the 8 studied traits in 39 chickpea accessions in the germplasm collection*

Traits	Plant height	Height to first pod (cm)	Number of branches	Number of productive nodes	Number of pods per plant	Seed yield per plant (g)	100 seed weight (g)
Height to first pod (cm)	0.635**						
Number of branches	0.362*	0.075					
Number of productive branches	0.09	0.146	0.115				
Number of pods per plant	0.334*	0.281	0.474**	0.714***			
Seed yield per plant	0.488**	0.691***	0.185	0.690***	0.712***		
100 seed weight	0.404**	0.319*	0.116	0.224	0.423**	0.224	
Yield (g/1m^2)	0.586**	0.218	0.271	0.236	0.257	0.361*	0.252

** 0.01 < p < 0.05; ** 0.001 < p < 0.01; and *** p < 0.001*

Correlation Analysis

The correlation analysis of the yield-related traits in the studied chickpea accessions revealed significant relationships (Table 1). Plant height was positively correlated with first bean height (r = 0.635**), number of productive branches (r = 0.362*), seed mass per plant (r = 0.488**), yield per square meter (r = 0.586**), and the number of pods per plant (r = 0.334*). Seed mass per plant also showed strong correlations with the number of productive branches (r = 0.712***), the number of pods per plant (r = 0.69***), and yield per square meter (r = 0.361*). Additionally, a significant correlation was found between plant height and first bean height (r = 0.635***). The correlation analysis indicated that seed mass, number of pods, and number of fertile branches per plant were positively correlated with yield. These traits were most pronounced in taller, more branched plants. Such chickpea plants are likely to be well-suited for winter cultivation, as they can take advantage of early spring rains while avoiding drought stress during the reproductive stages, which are particularly sensitive to heat and moisture deficits (Lassner, *et al.*, 1989; Serekpayev, *et al.*, 2016).

SSR Markers Analysis

The genetic polymorphism of the studied chickpea germplasm was assessed using 10 SSR primers. Rare alleles were observed at certain loci, with an average polymorphic information content (PIC) value of 0.68 and a Nei genetic diversity index of 0.62. The highest PIC values were recorded for prim-

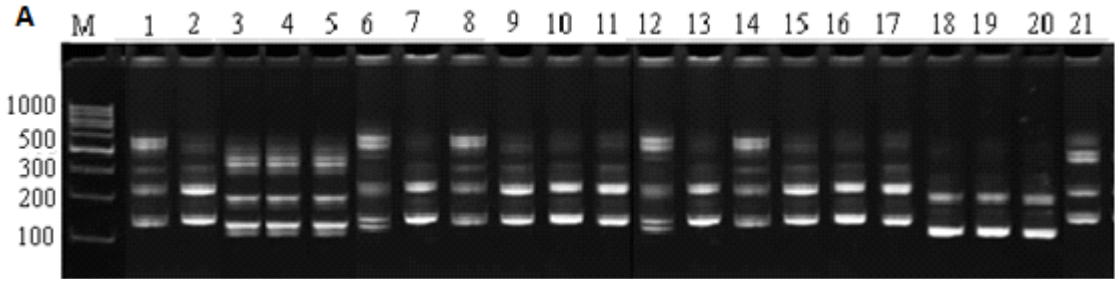
ers TA46 (0.88), H1A12 (0.84), and H3E04 (0.82), while the lowest values were observed for primers NCPGR6 (0.42) and H4E04 (0.44). The results were in accordance to those reported in previous studies (Singh et al., 2023). Seven alleles with high gene diversity and PIC values were identified using the TA46 and H3E04 primers, while only four alleles were recorded with the H4E04 and NCPGR6 primers (Table 2).

Table 3. *Genetic diversity parametrs of SSR primers in the studied chickpea germplasm*

Primer	Motif	Expected allele size, bp	Alleles	PIC	Nei genetic diversity index
TA46	(TAA)22	145–176	7	0.88	0.73
TA142	(TTA)15	128–142	5	0.79	0.70
TA71	(AAT)32	184–232	5	0.72	0.66
TA22	(ATT)40	200–280	6	0.52	0.65
H3E 04	$(TTA)_{36}$ $(CTA)_{5}$	174–216	7	0.82	0.65
H4E 04	$(TTA)_{56}$ $(TTTA)_{3}$	107–114	4	0.44	0.36
H1B09	(TAA)14 (AT)3	181–213	6	0.80	0.73
H1A 12	(TAA)29	129–330	6	0.84	0.77
NCPGR12	(CT)35	213–259	5	0.53	0.44
NCPGR6	(CA)12	230–268	4	0.42	0.47
Mean			5.5	0.68	0.62

A total of 55 amplicons were identified across all SSR primers, with an average of 5.5 amplicons per primer. The size of these alleles ranged from 107 to 330 bp. Among the SSR markers, the Nei genetic diversity index varied from 0.36 (H4E04) to 0.77 (H1A12), with an average genetic diversity of 0.62 cross all primers. This indicates high allelic diversity among the SSR primers selected for the study (Table 2). Based on the overall results, primers TA142, TA46, TA71, H1A12, H1B09, and H3E04 can be considered the most polymorphic microsatellite primers for chickpea genotypes.

Figure 1. *SSR amplification products*



*from (**A**)-TA46; 1-Narmin, 2-Camila, 3-Sultan, Ağdenli, 4-F.13–154 C, 5-F.13–227 C, 6-F.13–234 C, 7-F.13–320 C, 8-F.13–358 C, 9-F.13–364 C, 10-F. 88–85 C, 11-F. 93–93 C, 12-F.13–53, 13-F.13–55, 14-F.11–08 C, 15-F.11–01 C, 16-Ordubad 39, 17-Ordubad 41,18-Qusar 43, 19-Qusar 44, 20-Ağstafa 42, 20-Flip 03–22, 21-Bakı 30*

Cluster Analysis

In the present study, UPGMA was applied to differentiate chickpea accessions based on their genetic distance. The analysis revealed a clear distribution of the studied genotypes into three distinct clusters based on the allelic composition of the analyzed markers (Figure 3).

The first cluster comprised 6 accessions, including genotypes characterized by specific

alleles of 230 and 214 bp for the TA71 and H3E04 primers, respectively. Within this cluster, five accessions were of Azerbaijani origin, one was from Iran, and the remaining accessions were from ICARDA. Notably, ICARDA-introduced Flip 13–55 (№ 13) clustered closely with the local genotype Sultan 98–178 (№ 28), while IRAN 48 (№ 39) was closely related to the local Agdash18 (№ 27). Additionally, the ICARDA-introduced Flip 97–32 (№ 24) and Jamila variety (№ 2) from Azerbaijan were also grouped within the first cluster, indicating a close genetic relationship.

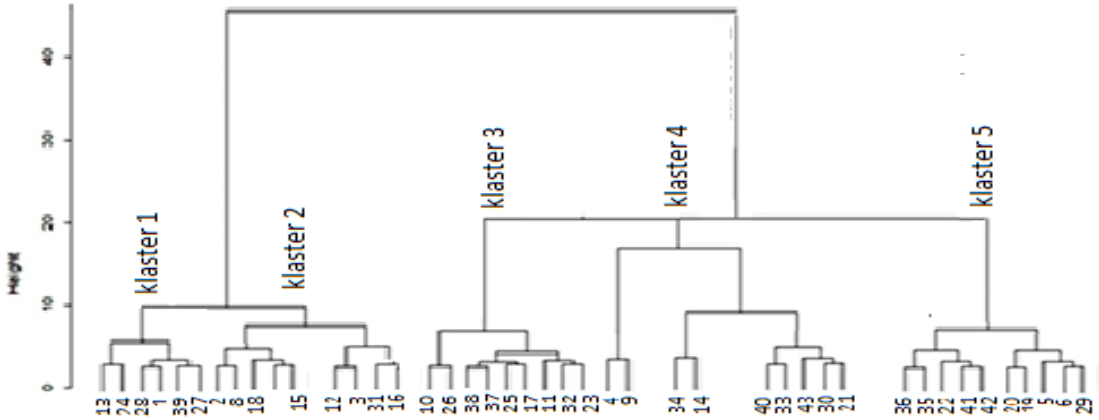
The second cluster consisted of nine chickpea accessions, which carried specific alleles of the TA46 and TA142 markers, with amplicon sizes of 146 and 142 bp, respectively. This cluster included five accessions of Azerbaijani origin (Ordubad 39, Yardimli 27, Masalli 30, Masalli 51, and Jalilabad 11) and two accessions of ICARDA origin (Flip 13–227 and Flip 03–22). Among these, the genetic similarity index between Flip 03–22 and Ordubad 39 was particularly high.

The third cluster included nine chickpea accessions, all of which shared a common allele of 181 bp, detected with the H1B09 primer. Three of the accessions were from ICARDA, while the remaining five were from Azerbaijan.

The fourth cluster also contained nine accessions, which exhibited specific alleles of 193 bp and 213 bp, detected with the H1B09 primer.

The fifth cluster was comprised of ten local accessions (Ordubad 47, Sabirabad 59, Baku 30, Lankaran 1, and Lankaran 2), all of which carried the 114 bp allele of the H4E04 primer. Notably, the Lankaran 1 (№ 35) and Lankaran 2 (№ 36) accessions had a high genetic similarity to each other, yet were located at a relatively greater genetic distance from the other accessions in this cluster.

Figure 2. *Dendrogram derived from UPGMA cluster analysis of 10 SSR marker alleles of 43 chickpea accessions distributed in five clusters*



Marker–Trait Association (MTA) Analysis between SSR Markers and Morphological Traits

As a result of marker–trait association analysis, several significant correlations were found between yield-related traits of first pod height, number of fertile tillers, number of pods per plant, 100-seed mass, seed mass per plant, plant height and NCPGR 12, TA71, TA142, and TA46 SSR primers. (Table 3). Primer TA71 showed positive correlation with three traits and other primers with two traits each were identified as promising markers. Our results are consistent with the results obtained by S. Mazkirat and colleagues (Mazkirat et al., 2023).

Table 4. *Marker–trait associations with MLM models using TASSEL*

Traits	SSR marker	Allels (bp)	pValu
Plant height	TA46	160	0,036*
Height to first pod	TA142	144	0,006
Number of branches	TA142	255	0,017

Traits	SSR marker	Allels (bp)	pValu
Number of productive nodes	TA46	166	0.008
Number of pods per plant	TA142	155	0.014
Seed weight per plant	TA71	246	0.022
	NCPGR12	215	0.010
100 seed weight (g)	TA71	249	0.250
1 m^2 yeald	TA71	233	0.003

*The significance level of the associations is indicated as follows: * 0.01< p <0.05; and ** 0.001 < p < 0.01*

Primer TA142 showed a positive correlation with the number of fertile branches and number of pods per plant, primer TA71 with mass of 100 seeds and mass of seeds per plant and yield, and primer NCPGR 12 with traits of mass of seeds per plant and mass of 100 seeds.

TA46 marker was associated with number of pods per plant and number of branches per plant. Thus, the study of genetic diversity and relationship between SSR primers and yield traits in chickpea plant collection is important for development of breeding strategy under specific agro-ecological conditions.The importance of SSR markers in the study of genetic diversity was also mentioned in previous studies (Singh, *et al.* 2022, Varshney, *et al.*, 2014). Using eleven SSR markers, 9 main productivity traits of 39 chikpea samples of different origins were studied, genetic diversity in the chikpea plant was evaluated, and a significant relationship between SSR markers and productivity indicators was emphasized (Liu, et al., 2005). Our study included markers used by previous researchers, and the polymorphism was lower than the results obtained in other studies, which is mainly due to the small number of samples. Specific alleles of TA46, TA71, H1A12, H3E04, H1B09 and TA142 primers were recorded in the chikpea samples we studied. A polymorphic information index (PIC) was calculated to demonstrate the utility of the primers. The UPGMA method, which is the most reliable type of cluster analysis, was used, and the samples were grouped into 5 clusters. The reliability of this method depends on the number of samples and primers. No clear differentiation was observed between the samples according to their origin. This result was also recorded in other studies, which is due to gene flow, exchange of genetic material and mutations occurring during hybridizations (Liu et al., 2005, Serrote et al., 2020).

Conclusion

High allelic diversity was recorded during the SSR marker study of 43 chikpea samples cultivated in Azerbaijan. Moderate genetic diversity (0.62) was observed with all SSR primers. Primers TA142, TA46, TA71, H1B09, H1A12 and H3E04 were recorded as the most polymorphic microsatellite primers for chikpea genotypes. Correlation analysis revealed several significant correlations between yield indicators and NCPGR 12, TA71, TA142, and TA46 SSR primers.

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