STUDY OF LOCAL AND INTRODUCED CHICKPEA SAMPLES FOR COMPLEX TRAITS AND CHARACTERISTICS BASED ON MOLECULAR MARKERS

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Abstract: Chickpea (*Cicer arietinum* L.) is one of the most important legumes widely cultivated in the world due to its high nutritional value and adaptability to diverse environmental conditions. The evaluation of genetic diversity and agronomically important traits is fundamental for the development of high-yielding and stress-tolerant cultivars. Molecular marker approaches provide reliable tools for assessing genetic variation and elucidating relationships among chickpea genotypes. The present study aimed to evaluate the genetic diversity of local and introduced chickpea genotypes using molecular markers together with the assessment of key morphological and agronomic traits. The combined analyses enabled the characterization of the genetic structure of the studied germplasm and the identification of genetically diverse genotypes with desirable agronomic characteristics. These findings provide valuable information for chickpea breeding programs and the conservation of genetic resources, facilitating the development of improved cultivars with enhanced productivity, adaptability and resistance to biotic and abiotic stresses.

Keywords: *Cicer arietinum*, breeding, genetic diversity, SNP.

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Introduction

Chickpea (*Cicer arietinum* L.) is one of the important legumes cultivated in over 50 countries of the world and is among the major pulse crops used for both human consumption and livestock feed. The crop is widely grown in semi-arid and temperate regions due to its adaptability to diverse environmental conditions and relatively low production costs. Despite its widespread cultivation, productivity is frequently constrained by abiotic and biotic stresses, including drought, salinity, pests and diseases (Yadav et al. 2019). Consequently, the development of high-yielding, stress-tolerant cultivars remains a primary objective of modern chickpea breeding programs.

Chickpea seeds are an excellent source of protein, carbohydrates, vitamins and minerals, making them an important component of human nutrition (Hajibarat et al. 2015). In addition to its nutritional value, chickpea also contributes to sustainable agricultural systems through biological nitrogen fixation, which enhances soil fertility and reduces the need for synthetic nitrogen fertilizers (Choudhary et al. 2012).

Genetic diversity plays a crucial role for the crop improvement, as it provides the genetic variation required for enhancing agronomic performance and adaptation to changing environmental conditions. However, cultivated chickpea possesses a relatively narrow genetic base due to domestication and intensive

selection, limiting the availability of useful alleles for breeding (Mohan et al. 2023). Therefore, the evaluation of local and introduced germplasm is essential for identifying valuable genetic resources that can be incorporated into breeding programs (Nayak et al. 2010).

In Azerbaijan and neighboring countries, chickpea is an economically and nutritionally important legume crop that represents a valuable source of genetic diversity. Characterizing local germplasm alongside introduced genotypes is essential for broadening the genetic base available for breeding and for identifying traits associated with adaptation to regional environmental conditions (Lichtenzveig et al. 2005). Local cultivars may possess unique adaptive characteristics that have evolved under specific agroecological conditions, making them valuable genetic resources for the development of resilient cultivars (Ghaffari et al. 2014).

Traditionally, chickpea germplasm has been characterized using morphological and agronomic traits, including plant height, seed size, pod number, and grain yield. Although these characteristics are useful for describing phenotypic variation, they do not always reflect the underlying genetic diversity because of their sensitivity to environmental factors (Varshney et al. 2010; Abbo et al. 2003). DNA-based markers overcome these limitations by providing stable genetic information that is largely independent of environmental influences. Advances in molecular genetics have enabled the widespread use of DNA markers, which provide a reliable and efficient approach for detecting genetic polymorphism and assessing genetic diversity. Unlike morphological characterization, which is often influenced by environmental conditions, molecular marker technologies provide reliable and reproducible information on genetic diversity, population structure, and relationships among genotypes (Winter et al. 2000; Saeed et al. 2011). Consequently, these techniques have become indispensable tools in plant genetics, breeding, and germplasm conservation.

Among the available molecular markers, Simple Sequence Repeats (SSR) and Single Nucleotide Polymorphism (SNP) markers are widely used due to their high level of polymorphism, reproducibility and reliability (Upadhyaya et al. 2011). These markers facilitate the accurate evaluation of genetic variability, the identification of genetically diverse parental lines, and the analysis of population structure and phylogenetic relationships. In chickpea, molecular marker analysis has been successfully applied to characterize germplasm collections, including SSR, amplified fragment length polymorphisms (AFLP), and SNP markers, and support breeding programs through the identification of genetically valuable genotypes (Winter et al. 2000). Among these, SSR markers have been extensively used because of their high polymorphism, co-dominant inheritance, reproducibility, and broad genome coverage, making them particularly effective for distinguishing closely related genotypes (Yadav et al. 2019; FAO 2021). The application of molecular markers has considerably enhanced the characterization of chickpea germplasm by facilitating the assessment of genetic diversity, population structure, and phylogenetic relationships (Kumar et al. 2023; Ghaffari et al. 2014).

Integrating molecular marker analysis with morphological and agronomic traits provides a comprehensive assessment of genetic diversity and enables the identification of genetically distinct genotypes with desirable breeding attributes. Such integrated approaches support the efficient conservation and utilization of chickpea germplasm while facilitating the selection of promising parental material for cultivar improvement (Saeed et al. 2011). Therefore, the present study aimed to evaluate the genetic diversity of local and introduced chickpea genotypes using molecular markers in combination with morphological and agronomic traits to identify valuable genetic resources for future breeding efforts.

Combining molecular marker analysis with morphological and agronomic characterization provides a more comprehensive

evaluation of chickpea genetic resources than either approach alone. This integrated strategy enables the identification of superior genotypes possessing desirable agronomic traits together with broad genetic diversity, thereby supporting the development of cultivars with improved productivity, environmental adaptability, and resistance to biotic and abiotic stresses (Lichtenzveig et al. 2005; Singh et al. 1997). Additionally, molecular marker approaches contribute to the conservation and effective utilization of chickpea genetic resources, strengthening future breeding efforts aimed at developing resilient and high-performing cultivars (Nayak et al. 2010; Talebi et al. 2008).

Materials and Methods

Plant materials

This study included local and introduced chickpea genotypes. Local genotypes were collected from different regions of Azerbaijan, representing diverse agro-climatic conditions, while introduced genotypes were obtained from international germplasm collections. Morphological traits, including plant height, number of pods per plant, and seed size, were recorded for each genotype prior to molecular analysis.

DNA isolation

Genomic DNA was extracted from young leaf tissues using a modified cetyltrimethylammonium bromide (CTAB) method. Leaf samples were collected and stored at −80°C until further processing. Approximately homogenized leaf tissue was ground in liquid nitrogen and processed using CTAB extraction buffer containing Tris-HCl, EDTA, NaCl, and β-mercaptoethanol. Following cell lysis, proteins and other contaminants were removed by chloroform alcohol extraction. DNA was precipitated with cold isopropanol, washed with 70% ethanol, air-dried, and dissolved in TE buffer (pH 8.0). The quality of extracted DNA was assessed by agarose gel electrophoresis, and DNA concentration and purity were determined using spectrophotometric analysis. DNA samples with an A260/A280 ratio between 1.8 and 2.0 were considered suitable for subsequent molecular analyses.

Determination of DNA purity and concentration

DNA purity and concentration were determined using an ULTROSPEC 3300 PRO spectrophotometer (Amersham, USA). Absorbance values were measured at 260 and 280 nm, and the A260/A280 ratio was calculated to evaluate DNA purity. Samples that did not need the required quality criteria were subjected to repeated extraction to obtain DNA suitable for downstream molecular analysis.

Results and Discussion

The selected SSR markers successfully amplified from all analyzed chickpea genotypes, producing clear and reproducible amplification profiles. Polymorphic loci were identified, demonstrating the effectiveness of the selected markers for detecting genetic variation within the studied germplasm. The observed polymorphism indicates the presence of considerable genetic variability among chickpea genotypes from different origins.

Genetic diversity was evaluated using allele number, observed heterozygosity (Ho), expected heterozygosity (He), and polymorphic information content (PIC). The PIC values demonstrate that several SSR markers were highly informative, and suitable for differentiating closely related genotypes. The variation in diversity parameters among local genotypes reflects difference in their genetic backgrounds and suggests that both local and introduced germplasm contribute valuable variation for chickpea improvement.

The UPGMA dendrogram based on Nei's genetic distance revealed distinct genetic relationships among the studied genotypes. Local and introduced genotypes were distributed into different clusters, reflecting their diverse genetic backgrounds. The clustering pattern suggests that geographic origin and breeding history influenced the genetic structure of the evaluated germplasm. Population structure analysis further supported the observed differentiation among genotypes and demonstrated the presence of distinct

genetic groups. The identification of genetically diverse accessions is important for selecting suitable parental materials and broadening the genetic base available for chickpea breeding. The inclusion of both local and introduced germplasms may provide opportunities to combine locally adapted characteristics with desirable traits from genetically distant materials.

The combined analysis of molecular marker data with morphological and agronomic traits provided a more comprehensive evaluation of chickpea genetic resources. While molecular markers revealed patterns of genetic relatedness, phenotypic evaluation allowed the identification of genotypes with desirable agronomic characteristics. This integrated approach facilitates the selection of promising genotypes, both genetic diversity and agronomic performance.

Local genotypes represent an important source of genetic variation shaped by adaptation to regional environmental conditions, whereas introduced germplasm may provide additional alleles that are not present in local populations. The complementary contribution of these two groups highlights the importance of maintaining diverse germplasm collections for sustainable chickpea improvement.

Conclusion

The present study demonstrates the value of combining SSR marker analysis with morphological and agronomic characterization for evaluating chickpea genetic resources. The detected genetic variation among local and introduced genotypes provides opportunities for identifying diverse parental materials and improving the efficiency of breeding programs. Conservation and effective utilization of both locally adapted and introduced germplasm are essential for maintaining genetic diversity and developing chickpea cultivars with improved productivity, adaptability, and resilience under changing environmental conditions.

Conflict of Interest

Authors declare that there is no conflict of interest.

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