# Analysis of genetic diversity of chickpea cultivars using STMS markers

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***Abstract:*** *A set of 38 chickpea cultivars were assessed in this study at the molecular level using 8 STMS (Sequence-Tagged Microsatellite Site) markers. This set includes advanced lines from the Tunisian breeding program, Tunisian varieties and reference lines from ICARDA (International Centre for Agricultural Research in Dry Areas, ICRISAT (International Crops Research Institute for the Semi-Arid-Tropics) and other countries. A total of 58 alleles were generated from the amplification of the 8 microsatellite sites studied with an average value of 7.25 alleles per locus, which explain a high level of polymorphism within the cultivars studied. All the cultivars studied showed a unique genetic profile, each one using the genotypic combination of all loci studied. Only three STMS markers (TA59, TA64, and TA71) from the eight studied showed the highest number of alleles, the effective number of alleles and the highest values of power of discrimination allow us the unambiguous discrimination of all the cultivars studied.*

*The Dice coefficient of similarity was calculated between all pairs of accessions and UPGMA analysis was performed from the genetic similarity matrix, and allowed the arrangement of all genotypes according to their genetic relationships. The genetic similarity among cultivars observed in this work, led to their separation according to their pedigree, their morphological characteristics and their environment adaptation for biotic and abiotic stresses.*

*The results of this work suggest that microsatellite markers are valuable tools for molecular characterization and analysis of diversity in chickpea.*

***Keywords:*** *Chickpea, STMS markers, genetic diversity, genetic relationships*

## I.  Introduction

The genus Cicer contains 44 species including 35 perennial and eight wild species and one domesticated chickpea [1].  The cultivated chickpea (Cicer arietinum L.) is a member of the Fabaceae family. It is a dicotyledonous plant, diploid, with 8 pairs of chromosomes (2n=16) and genome size of 732 Mb. *Cicer arietinum* was one of the first grain legumes domesticated in the old world [2]. It is most probably originates from Turkey and Syria [3]. Chickpea is the third most important pulse crop in the world after bean and pea, with a cultivated area of 13.54 million ha, production of 13.1 million tons and productivity of about 967.6 kg/ha [4]. However, there is a large gap between the potential yield of this crop and current yields obtained. This is due particularly to biotic and abiotic stresses and poor crop management. The knowledge of genetic diversity of chickpea germplasm is fundamental for chickpea breeding and conservation of genetic resources and can provide practical information for the selection of parental material and thus, could be a useful tool and a valuable aid in planning crop improvement program [5].

During the last decades, researchers have started to investigate chickpea variability at the molecular level using molecular markers. DNA-based markers systems such as restriction fragment length polymorphisms (RFLPs), RAPD, SSRs or microsatellites, AFLPs, SNPs have become available. Among these marker systems, SSRs are commonly employed for diversity analysis in pulses [6]. In fact, DNA-based molecular markers are tools that enable plant breeders to directly evaluate genetic variation between genotypes without any concern of environmental factors and effects on gene expression levels.

For chickpea, 174 microsatellite markers have been developed and characterized by [7], [8]and another 10 by [9] so far. More recently, a set of 233 and 13 new microsatellite markers were generated for chickpea by [10] and [11] respectively. Microsatellites markers, or STMS, which are codominant and highly polymorphic markers easily detected with PCR procedure, appear as the best available choice of markers for chickpea assessment.

The principal aims of this study are to assess the genetic diversity of some Tunisian varieties, to choose among improved lines candidates for registration in Tunisia allowing diversity and to choose among genetic resources carrying important traits appropriate sources to include in the crossing bloc in the coming seasons.

# II.    Materials And Methods

## 2.1, Plant material

Thirty eight chickpea (C. arietinum L.) genotypes were used in this study (Table 1). They include twelve advanced lines obtained from the Tunisian chickpea breeding program (V1, V2, V3, V4, V5, V6, V7, V8, V9, V10, V12 and Béja2), seven Tunisian commercial varieties (Amdoun1, Chétoui, Béja1, Nayer, Kasseb, Bouchra and Nour), a Spanish variety (Blanco Lechoso), three reference lines (ILC482, JG62 and WR315) and 15 ILC (ICARDA Landrace Chickpea) accessions (ILC72, ILC191, ILC195, ILC200, ILC215, ILC267, ILC523, ILC1254, ILC1278, ILC3363, ILC3856, ILC4296, ILC4637, ILC3279 and F.10-65).

**Table 1:** Varieties,  pedigree lines and their origins

| Varieties | Pedigree | Origin |
|---|---|---|
| Kasseb* | FLIP83-46C | Improved line from ICARDA |
| Blanco Lechoso |  | Spain |
| Chétoui* | ILC3279 | Russia |
| Amdoun 1* | Amdoun1 | Selection from Tunisian local population |
| Béja 1* | (Amdoun1xILC3279)xILC200 | ICARDA – INRAT |
| Bouchra* | FLIP84-79C | Improved line from ICARDA |
| Nayer* | FLIP84-92C | Improved line from ICARDA |
| ILC482 | ACC. N°267780-68 | Turkey |
| WR315 | WR315 | ICRISAT |
| JG62 | JG62 | ICRISAT |
| Béja 2 | (Amdoun1xILC482)xILC191 | ICARDA – INRAT |
| V1 | X96TH86-W9-W1-A1-A1-A1-W1 | ICARDA – INRAT |
| V2 | X96TH62-A4-A1-W1-A1-A1-A1-A1 | ICARDA – INRAT |
| V3 | X97TH85-W1-A1-W2-W1-W2-A1 | ICARDA – INRAT |
| V4 | X96TH61-A4-W2-A2-A1-W1-W1-A1 | ICARDA – INRAT |
| V5 | X96TH24-A2-A1-A1-W2-W1-A1-A1 | ICARDA – INRAT |
| V6 | X96TH61-A5-W1-A2-A1-W2-W3-A1 | ICARDA – INRAT |
| V7 | X98TH86-A4-A1-W1-A2-A1 | ICARDA – INRAT |
| V8 | X96TH86-A4-A1-A1-A1-A1-A1 | ICARDA – INRAT |
| V9 | X96TH62-A3-W1-A2-W1-A1-A1-A1-A1 | ICARDA – INRAT |
| V10 | X96TH61-A4-W2-A1-W1-A1-A1-A1-A1 | ICARDA – INRAT |
| V11 (Nour*) | X96TH61-A3-W1-A2-W1-A1-W1-W1 | ICARDA – INRAT |
| V12 | X96TH63-A2-A1-A1-A1-W1-A1-A1 | ICARDA – INRAT |
| ILC72 | INIA | Spain |
| ILC191 | Vysokoroshyj 30 – Krasvadar 1286 | Russia |
| ILC195 | Vysokoroshyj 30 – Krasvadar 1286 | Russia |
| ILC200 | Steponj 1 | Russia |
| ILC215 | PI222770 | Iran |
| ILC267 | PI358930 | Iran |
| ILC523 | F33 : Improved line | Egypt |
| ILC1254 | G156 | Morocco |
| ILC1278 | PI 268376 | Afghanistan |
| ILC3363 | CA41 | Spain |
| ILC3856 | Pch 128 | Morocco |
| ILC4296 | Surutato | Mexico (INIAM) |
| ILC4637 | FLIP81-79C | Improved line from ICARDA |
| ILC3279 | Steponj 1- Krasvadar 1335 | Russia |
| F.10-65 |  | ICARDA |

\* Registered varieties in the Tunisian catalogue of plant varieties

**2.2, DNA extraction and amplification**

About 100 mg of young leaves was harvested and frozen in liquid nitrogen. DNA was isolated using [12] protocol. PCR reactions were carried out in 20µl (one reaction containing: 25-50 ng of plant genomic DNA, 1 x Buffer (50 mM KCl, 10mM Tris-HCl, 0.1% Triton X-100), 1.5 mM MgCl₂, 250 mM of each dNTP, 2mM of forward and reverse primers, and 0.5 units of Taq DNA polymerase (GoTaq® Flexi, Promega). After denaturing the DNA for 1 min at 95°C, the reaction mixture was subjected to 35 cycles of the following temperature profile: 94°C for 2 min, 56°C for 50 s and 60°C for 50 s, followed by a final extension at 60°C for 5 min. Amplification products were loaded on polyacrylamid gels in 1 x TBE buffer and visualized by ethidium bromide staining. Fragment sizes were estimated with 25 bp DNA step Ladder of Promega DNA sizing markers.

**Table 2**: Characteristics of the 8 STMS primer pairs (Forward (F) and Reverse (R)) used in this study.

| Primers | Motif | Ta (°C) | Size | Source |
|---------|-------|---------|------|--------|
| TA27 | (TAA)21 | 56 | 241 | [8] |
| TA59 | (TAA)29 | 56 | 258 | [8] |
| TA64 | (TAA)39 | 56 | 239 | [8] |
| TA71 | (AAT)32 | 56 | 225 | [8] |
| TA72 | (ATT)36 | 56 | 256 | [8] |
| TA96 | (AT)3(TTA)30(AT)3 | 56 | 275 | [8] |
| TA194 | (TTA)21 | 56 | 132 | [8] |
| GAA47 | (GAA)11 | 56 | 169 | [8] |

**2.3, Data analysis**

The number of alleles per locus was counted basing on gel profile analysis, and designated as presence (1) or absence (0) of allele in a binary matrix.

The ability of a marker to discriminate between two random cultivars was estimated for each locus with the power of discrimination calculated as $PD=1-\sum g_i^2$, were $g_i$ is the frequency of i$^{th}$ genotype [13]. Those parameters served to evaluate the information given by the microsatellites markers.

Data were analyzed using NTSYS-pc, version 2.1 program [14]. The presence (1) or absence (0) of amplified fragments was recorded for each cultivar. A similarity matrix was generated with the SIMQUAL module using the Dice coefficient [15]. Similarity data were processed through the unweighted pair group method (UPGMA) cluster analysis and finally depicted in a dendrogram representing the genetic relationships between the 38 chickpea genotypes studied.

# III.    Results And Discussion

**3.1, Genetic diversity of microsatellites markers**

Thirty-eight accessions of chickpea were analyzed with 8 polymorphic STMS markers. All loci analyzed in this work are multiallelic. The number of alleles detected per locus ranged from 2 (GAA47) to 11 (TA71), with a total of 58 alleles for all loci and an average of 7.25 alleles per locus (Table 3) resulting in a high level of polymorphism. The differences in size between the alleles of the same locus varied between 2 and 54 base pairs (bp) and the difference in size between 2 consecutive alleles varied between 2 and 12 bp.

**Table 3**: Parameters of variability calculated for the 8 STMS markers in 38 chickpea cultivars

| Locus | Number of loci | N | Ne | ≠ Genotypes | PD |
|-------|---------------|---|-----|-------------|-----|
| TA27 | 1 | 8 | 3.9 | 8 | 0.74 |
| TA59 | 1 | 8 | 7.8 | 8 | 0.87 |
| TA64 | 1 | 10 | 7.3 | 10 | 0.86 |
| TA71 | 1 | 11 | 8.4 | 11 | 0.88 |
| TA72 | 1 | 3 | 2.8 | 3 | 0.74 |
| TA96 | 1 | 6 | 5.3 | 6 | 0.81 |
| TA194 | 1 | 10 | 5.0 | 10 | 0.80 |
| GAA47 | 1 | 2 | 1.0 | 2 | 0.02 |
| Total | 8 | 58 | 41.5 | 58 | - |
| Average | 1 | 7.25 | 5.18 | 7.25 | 0.71 |

N: number of alleles; Ne: Effective number of alleles; ≠ Genotypes: number of different genotypes; PD: Power of discrimination

In this present work, STMS markers have been distinguished from others markers that have shown in other works on chickpea lower levels of polymorphism such as the case of RAPDs [16] which confirms the effectiveness of these STMS markers in the analysis of genetic diversity. All STMS markers used in this study except GAA47 showed discrimination power values (PD) very similar and roughly 0.8. These values and the values of the effective number of alleles (Ne) for each locus allow us to identify the best markers. Indeed, the most polymorphic STMS markers are those with the highest number of effective alleles (Ne) and the values of the highest PD. Based on the values of table 3, the markers TA59, TA64, and TA71 are the most polymorphic

ones. These three markers allowed to separate completely all studied accessions and can be considered as very reliable markers to be used in future work on genetic diversity for chickpea

**3.2, Genetic relationships among chickpea cultivars based on STMS variation**

The assessment of genetic diversity among 38 accessions analyzed in the present work was made possible by analyzing the variability between 8 microsatellite markers studied. The dendrogram (Fig. 1) was obtained from the binary matrix (0/1) of the absence or presence of an allele for each locus using the UPGMA clustering method and using the Dice coefficient [15].

The 38 accessions studied were divided into groups or clusters according to their genetic similarity. The dendrogram of Fig.1 shows two large groups with a genetic similarity (GS) of 0.17 which is equivalent to a genetic distance (D= 1 - SG) of 0.83. The first group is divided into two subgroups: the first one is composed of the genotypes V1, Chétoui, ILC3856, ILC3279 and F10-65. The classification of Chétoui and ILC3279 in the same sub-group is logical since Chétoui (variety registered in Tunisia in 1987) is itself ILC3279. The second sub-group is further subdivided into 2 clusters; the first one is represented by the genotypes V6, V8, ILC3363, ILC482, Blanco Lechoso, ILC200, ILC1278, ILC4637, ILC195, and ILC1254. The combination of these different accessions at this first cluster can be explained firstly, by the fact that both advanced V6 and V8 lines have in their pedigree some of these ILC such as, ILC1278 and ILC1254 which explains their positioning in the same cluster. On the other hand, some of the ILC share the same geographical origin and probably have agronomic characters or adaptation to similar environmental conditions. Further studies to compare agro-morphological traits should be undertaking. The second cluster contains the genotypes ILC215, Bouchra, ILC72, ILC267, Kasseb, Nayer, WR315, ILC191, JG62 and ILC4296. The clustering of Bouchra, Kasseb, Nayer, ILC215 and ILC72 is predictable since the first three varieties are from the same cross ILC72 X ILC215 performed in Tel Hadia in Syria in 1980. They were introduced at the F8 generation and have been selected against various biotic and abiotic stresses in different soil and climatic regions to be finally registered in the Tunisian official catalogue as varieties for winter sowing.

The second group of the dendrogram consists of the remaining genotypes studied (V2, V3, ILC523, V4, V5, V7, V9, V10, V12, Beja1, Beja2, Amdoun1). Within this group, we can observe that the genotypes Beja1, Amdoun1, and Beja2 show a high genetic similarity, this is not surprising since Beja1 and Beja2 present in their pedigree the variety Amdoun1. On the other hand, these two genotypes also have similar morpho-agronomic characters [17] which explain their genetic similarity. The grouping of 8 improved lines (V2, V3, V4, V5, V7, V9, V10 and V12) in this second group can be attributed to a similar adaptation of these lines with environment conditions. Indeed, these lines were selected for their tolerance/resistance to ascochyta blight and fusarium wilt. On the other hand, many of these lines have common ancestors in their pedigree.
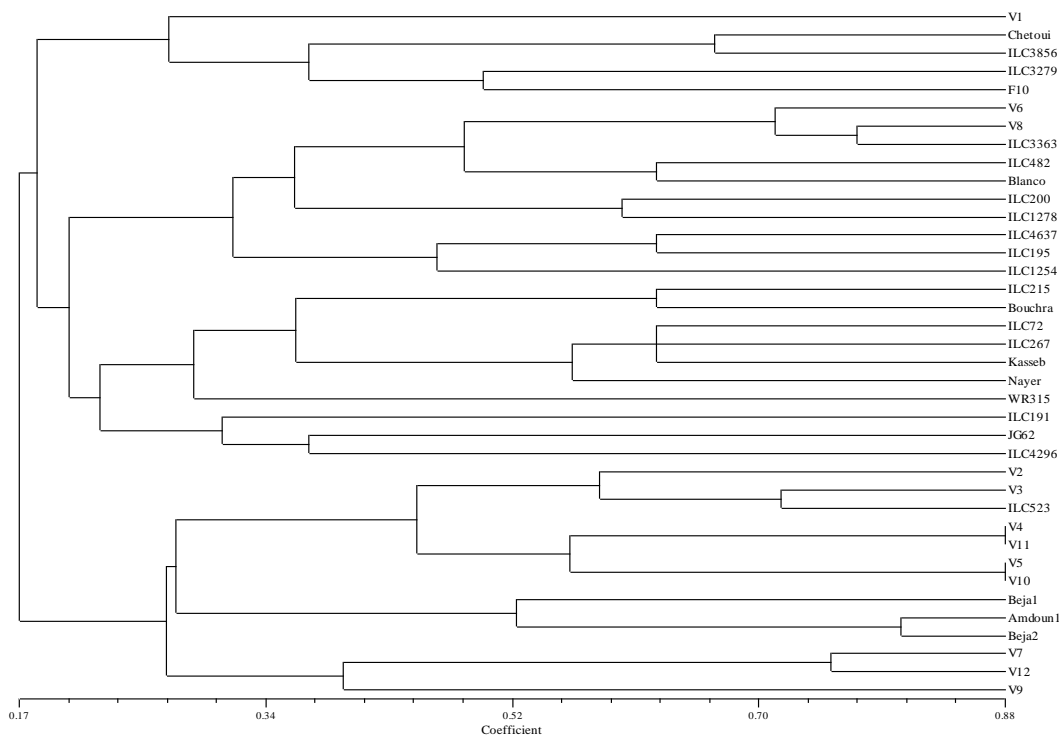


**Fig. 1:** Dendrogram UPGMA of 38 accessions of chickpea using 8 STMS markers and coefficient of Dice (Nei et Li, 1979). The coefficient mentioned in the below of the figure correspond to genetic similarity.

# IV. Conclusion

To achieve certain breeding goals, molecular characterisation of chickpea cultivars seems to be of great interest for the Tunisian breeding program. In fact, this will guaranty a correct identification of the cultivars studied without influence of environmental factors that may affect phenotypic characterization. Moreover, the study of genetic diversity is very useful to choose parental genotypes for crosses, to optimize germplam management and to maximize diversity.

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