Mining allelic variation associated with beneficial traits in composite collection of chickpea (*Cicer arietinum* L.)

HD Upadhyaya[1], M Baum[2], SL Dwivedi[1], SM Udupa[2], HK Buhariwalla[3], BJ Furman[2], S Chandra[1], D Hoisington[1], JH Crouch[3], CLL Gowda[1] and S Singh[1]

1. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, 502 324, AP, India. 2. International Center for Agricultural Research in the Dry Areas, PO Box 5466, Aleppo, Syria. 3. International Center for Maize and Wheat Improvement, Apdo, Postal 6-641, 06600 Mexico, DF Mexico.

About chickpea

Chickpea is the 4th largest grain-legume crop in the world covering an area of 10.38 million ha, with a production of 8.57 million t, and productivity of 0.83 t ha^{-1}. There are two types of chickpeas – *desi,* widely grown in South Asia and Africa, and *kabuli*, widely grown in the Mediterranean region. Countries with the largest chickpea production are India, Turkey, Pakistan, and Iran in Asia, Ethiopia in Africa, and Mexico in North and Central America. Major constraints to chickpea productivity are *Ascochyta rabei, Botrytis cinerea, Fusarium oxysporum, Fusarium solani, Rhizoctonia bataticola, Helicoverpa armigera, Liriomyza cicerina,* drought and salinity, and fluctuation in temperature. There is a large variation in most of the morphological/agronomic traits and resistance to biotic and abiotic stresses of chickpea germplasm.

Trait variation in chickpea germplasm

Chickpea germplasm and marker

Global composite collection: A composite collection of 3000 accessions was formed that consists of core collection representing germplasm held at the ICRISAT genebank (Upadhyaya et al. 2001), accessions representing ICARDA genebank, and cultivars/breeding lines, trait-based unique germplasm and wild *Cicer* species compatible with *Cicer arietinum*.

Global composite collection of chickpea

Germplasm description	Number of accessions
Core collection developed at ICRISAT	1956
Cultivars/breeding lines	39
Trait-based unique germplasm	276
ICARDA accessions	709
Wild *Cicer* (C. *echinospermum* and C. *reticulatum*)	20

Marker: From the preliminary screening of 200 SSRs on 288 diverse chickpea germplasm (chickpea mini core – 211 accessions + 57 kabuli types + 20 wild *Cicer* accessions), 50 polymorphic SSRs were selected for genotyping the global composite collection.

SSRs to study genetic structure of global composite collection of chickpea

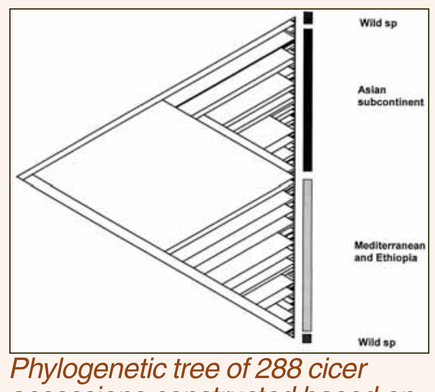
Source	Number of markers	Allele size (bp)	Annealing temperature (°C)	Repeat motifs
Huttel et al. 1999	3	174-241	65–60	di- and tri-nucleotide
Winter et al. 1999	42	132-436	55, 60–55, 65–60	di- and tri-nucleotide
Niroj et al. 2003	5	195-306	65–60	di-nucleotide

Summary of progress achieved in 2004

Preliminary analysis reveals a large allelic diversity and a total of 873 alleles, with an average of 25 alleles per locus. The dinucleotide motifs detected a lower number of alleles (average 11 alleles) than the trinucleotide motifs (average 27 alleles). Similarly, gene diversity with dinucleotide motif was lower (average 0.723) than with trinucleotide motif (0.898). The mean gene diversity of all the SSR markers was 0.873. The UPGMA dendogram revealed two main groups, one consisting mainly of accessions from the Asian subcontinent and the other from the Mediterranean and Ethiopia. The wild species were split into two groups flanking two ends of the chickpea accessions.

Gene diversity in global composite collection of chickpea

Institution	Number of markers and genotypes	# Alleles per locus (range)	Gene diversity
ICRISAT	35 SSRs and 288 genotypes	25 (6-44)	0.723 (di-nucleotide repeat motifs) and 0.898 (tri-nucleotide repeat motifs)
ICARDA	15 SSRs and 288 genotypes	28 (14-55)	0.912 (tri-nucleotide repeat motifs)



Phylogenetic tree of 288 cicer accessions constructed based on UPGMA clustering of shared allele distance

Discriminant function analysis (DFA) detected similarities in clustering patterns between phenotype- [22 traits data on 210 mini core accessions (Upadhyaya and Ortiz 2001)] and genotype-based (data from 40 SSRs markers on 210 mini core accessions) clusters. Most of the individuals were assigned with a high degree of confidence to the original (phenotypic) clusters. Only 27% of the individuals were re-assigned into new clusters according to marker data, which were mainly identified within clusters 4, 6 and 7 of the mini-core.

Summary of progress achieved in 2005

At ICRISAT, DNA was extracted from 2716 accessions and part of the DNAs were sent to ICARDA. The 2716 accessions have been genotyped using 35 SSRs. The marker data is being tabulated to detect missing data. Markers with large number of missing data will be repeated to generate full data set on 2716 accessions.

Binning and marker quality index

Quality index (Sw)	Binning quality (allele size)
0.00–0.30	Accurate binning
0.31–0.40	Binning likely
>0.45	Binning and allele sizing unacceptable

A (C language) software program has been developed for allele binning based on the Idury and Cardon (1997) algorithm to provide a statistical measure of the fit of the determined "raw" alleles to an expected size, based on the SSR repeat unit. Markers that show significant deviations from the expected fit will either be eliminated or re-genotyped before including them into the final analysis. A good marker is the one that has an Sw value less than 0.30 and an allelic drift value of zero.

Future outlook

Using genotyping data a representative reference collection of 300 accessions (10% of the composite collection) will be selected that will be evaluated for drought tolerance and agronomic traits. The breeders will have opportunity to use trait-specific genetically diverse accessions in their programs to enhance the genetic potential of chickpea. The genetically diverse accessions will also be available for structural and functional genomics studies.

Reference

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