

SN Nayak^{1,2}, N Varghese¹, H Zhu³, R Horres⁴, T Nepolean¹, G Srivani¹, R Jungmann⁴, HK Choi¹, S Datta⁵, PM Gaur¹, PB Kavi Kishor², G Kahl⁴, DA Hoisington¹, A Kilian⁶, P Winter⁷, DR Cook³ and RK Varshney^{1,*}

¹International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India; ²Osmania University, Hyderabad, India; ³University of California, Davis, CA, USA; ⁴University of Frankfurt, Germany; ⁵Indian Institute of Pulse Research (IIPR), Kanpur, India; ⁶Diversity Arrays Technology Pvt Ltd, Australia; ⁷GenXPro GmbH, Frankfurt, Germany
*Author for correspondence: r.k.varshney@cgiar.org

Abstract

In order to increase the genomic resources in chickpea, a novel set of microsatellite or simple sequence repeat (SSR) and Diversity Array Technology (DART) markers were developed. A total of 1,655 SSR markers from SSR-enriched library (311) and mining the BAC-end sequences (1,344); and an extended DART array with 15,360 features were developed. Newly developed SSR and DART markers were tested for polymorphism on parental genotypes of an inter-specific mapping population (*Cicer arietinum* ICC 4958 × *C. reticulatum* PI 489777) comprising of 131 RILs. A total of 305 SSR markers (52 from enriched library and 253 markers from BAC-end sequences) and 675 DART markers were found polymorphic and segregation data were obtained. In addition, genotyping data for several hundred published markers from University of Frankfurt as well as novel gene based SNP markers from UC-Davis were assembled. In total, genotyping information has become available for 1,693 markers on this inter-specific mapping population. MAPMAKER/EXP was used to calculate map and here we present the most comprehensive genetic map of chickpea comprising 1,393 marker loci. This map should be useful for trait mapping as well as anchoring the (future) physical map in chickpea.

Development of novel SSR markers

Novel SSR markers (1,655) were developed using two strategies (Table 1)
 ➤ SSR-enriched genomic library (311): markers designated as ICRISAT Chickpea Microsatellite (ICCM)
 ➤ BAC-end sequences (1,344): markers designated as Cicer arietinum Microsatellite (CaM)

Table 1. Development of novel microsatellite markers in chickpea

Details	SSR enriched library (ICCM)	BAC-end sequences (CaM)
Number of clones sequenced	288	25,000
Sequences surveyed	457	46,270
Amount sequence data (bp)	286,718	33,217,120
SSRs identified	643	6,845
SSR frequency	1/ 450 bp	1/ 4.9 kb
Primers pairs designed	311	4,964
Primers pairs synthesized	311	1,344
Polymorphic markers in ICC 4958 and PI 489777	52	253

SSR polymorphism survey

➤ Novel SSR markers (311-ICCM and 1,344-CaM) and H series SSR (233) markers (Lichtenzweig et al. 2005, Theor. Appl. Genet.,110:492-510) were screened for polymorphism detection between ICC 4958 and PI 489777. In total 357 markers were found polymorphic (Table 2) and were genotyping data were obtained on 131 RILs

Table 2. Polymorphic status of microsatellite markers in ICC 4958 and PI 489777

Marker names	Total markers	Scorable markers	Polymorphic markers	Percent polymorphism	No. of markers mapped
ICCM	311	225	52	23.1	47
CaM	1,344	1,214	253	20.8	196
H series	233	153	52	34	46
Total	1,888	1,592	357	22.4	289

Diversity Array Technology (DART) genotyping

➤ An extended DART array with 15,360 features was developed
 ➤ 675 DART markers were found polymorphic between ICC 4958 and PI 489777 and genotyping data were obtained on 131 RILs and 520 of them (77 %) of DART markers were mapped
In addition, genotyping data for 581 markers from University of Frankfurt (Germany) and for 76 markers from University of California, Davis (USA) were assembled

Comprehensive inter-specific genetic map of chickpea

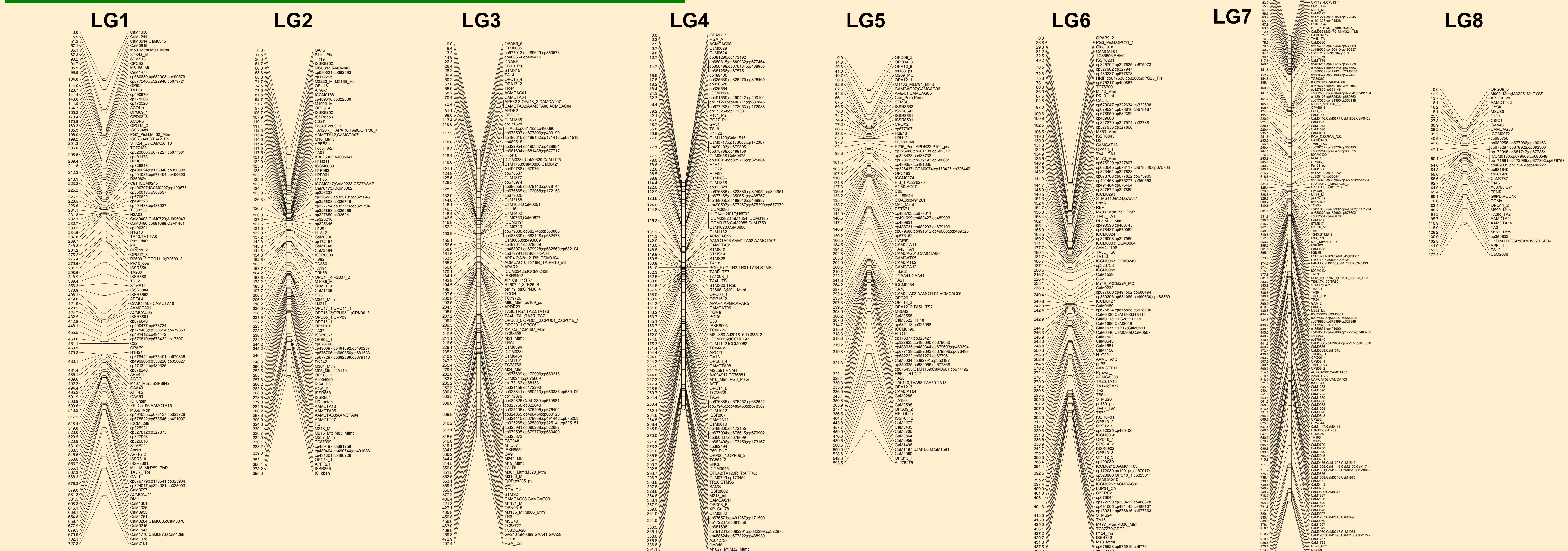


Figure 1. High density genetic map of chickpea with 1,393 marker loci

Segregation data assembled for 1,693 marker loci and were used to construct linkage map using MAPMAKER/EXP V. 3 programme. Marker loci were assigned to linkage groups at max LOD 16 and the ungrouped marker loci were tried at lower LOD 6. Marker order was determined using 'Compare' and 'Try' commands. The map distances were calculated applying the Kosambi mapping function.

Salient features of genetic map

- Total number of marker loci mapped: 1,393 include 423 SSRs; 520 DARTs; 183 gene-based marker loci; 84 RAPDs; 69 AFLPs and 114 other marker loci
- Number of marker loci per linkage group: 58 (LG8) to 284 (LG7)
- Average number of marker loci per linkage group: 174
- Total map distance: 4243.8 cM
- Average map distance per linkage group: 530.48 cM
- Inter-loci distance: 1.99 cM (LG4) to 4.22 cM (LG1) with an average of 3.05 cM

Acknowledgements

Technical help from Abdul Gafoor and Prathima Juvvadi in SSR genotyping is acknowledged. Financial support from Tropical Legume I of Generation Challenge Programme (GCP) of CGIAR, National Fund of Indian Council of Agricultural Research (ICAR) and Department of Biotechnology (DBT), Government of India, are greatly acknowledged. Council of Scientific and Industrial Research (CSIR), Government of India is acknowledged for providing fellowship to SNN.