Yield plasticity and molecular diversity analysis in chickpea (Cicer arietinum)

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ABSTRACT

Genetic diversity among 40 chickpea (Cicer arietinum L.) genotypes was investigated using 125 microsatellite (SSR, simple sequence repeat) markers. Twenty five polymorphic markers with average genetic diversity and PIC (Polymorphic Information Content) value of 0.489 and 0.437, respectively, generated a total of 90 alleles. High PIC and gene diversity (H_e) values indicated good variability amongst the chickpea genotypes. Sequential Agglomerative Hierarchical Non-overlapping (SAHN) grouping revealed two main clusters with 29 genotypes in cluster I and 11 genotypes in cluster II. The Cluster analysis did not follow geographical diversity rather it was in agreement for genetic diversity with respect to seed type and parentage/pedigree. Grouping clearly delineated the diverse kabuli and desi genotypes. Molecular variance analysis also indicated 97% variation within the populations and 3% variation among the populations. Principal coordinate analysis (PCoA) divided all the 40 genotypes into three populations based on their seed type and pedigree. The 2D plot largely supported the dendrogram with similar pattern of clustering. It also indicated that the material used was diverse. Thus, the study proved that SSR markers are informative tools for assessing genetic diversity and can be recommended for characterization studies in chickpea.

Key words: Chickpea, Genetic diversity, Microsatellite markers, Molecular variance, Principal coordinate analysis, SAHN grouping.

Chickpea (Cicer arietinum L.) is a highly proteinaceous legume grown all over the world (Patil et al. 2017) and India produces 68% of total world production of around 8.88 mt in an area of 9.21 mha (Kumar et al. 2017). Low genetic diversity in the cultivated chickpea is one of the causes for narrow genetic base leading to lower yield gains in chickpea (Bharadwaj et al. 2011). Drought and heat both limit chickpea production resulting in 40–60% average yield losses globally (Sachdeva et al. 2017). Identification of divergent kabuli and desi pools and crossing between them has been suggested as a way for developing chickpea with broader genetic base by Santosh et al. 2017.

Molecular markers being stable and informative have been used for characterization of crop plants diversity (Bharadwaj et al. 2010, Satyavathi et al. 2005). These are vital for marker assisted breeding programs targeting chickpea yield enhancement (Varshney et al. 2013a, Yadav et al. 2011, Bharadwaj et al. 2010) and to provide information on allelic variation in the breeding material (Jain et al. 2014). Microsatellites being abundant, highly polymorphic, co-dominant, multi-allelic and uniform in distribution across the genome are considered important in plant breeding (Cuevas and Prom 2013). Unique allelic profiles generated using the scored loci differentiates the genotypes based on the genetic data (Konsam et al. 2014).

Thus, SSR markers were used in this study to understand the relationships among chickpea genotypes and assess the extent of genetic variability for use in future breeding programs.

MATERIALS AND METHODS

Forty chickpea genotypes including both desi and kabuli genotypes were selected from the training population set (Table 1). The material was obtained from Pulse Research Laboratory, Indian Agricultural Research Institute, New Delhi and sown under controlled conditions in plastic pots of 13 cm diameter at the National Phytotron Facility, Indian Agricultural Research Institute, New Delhi. Temperature was maintained at 24°C during day and 18°C in the night. Yield traits, viz. seeds harvested from each plant, seed weight and yield of the plant were recorded for all the 40 genotypes.
| Genotype   | Biological status | Pedigree                                                                 | Source          | Seed type |
|------------|-------------------|--------------------------------------------------------------------------|-----------------|-----------|
| ICCV 09313 | Released variety  | ICCV 92311 × ICC 14198                                                  | ICRISAT, India  | Kabuli    |
| ICCV010313 | Released variety  | ICCV 92337 × ICC 14194                                                  | ICRISAT, India  | Kabuli    |
| ICCV08310  | Released variety  | ICCV 95311 × ICC 17109                                                  | ICRISAT, India  | Kabuli    |
| ICCV097309 | Released variety  | (ICC 2588 × ICC 32) × [(ICCC 49 × ICC 15980) × ICCV 3]                 | ICRISAT, India  | Kabuli    |
| ICCV03311  | Released variety  | ICCV 92328 × [(ICCC 32 × ICC 12034) × ICCV 19686]                       | ICRISAT, India  | Kabuli    |
| ICCV01309  | Released variety  | (ICC 4973 × ICC 14196) × ICCV 92329                                     | ICRISAT, India  | Kabuli    |
| ICCV09312  | Released variety  | ICCV 92337 × ICC 7344                                                   | ICRISAT, India  | Kabuli    |
| ICCV9314   | Released variety  | ICCV 92311 × ICC 17109                                                  | ICRISAT, India  | Kabuli    |
| ICCV10304  | Released variety  | ICCV 92311 × ICC 14215                                                  | ICRISAT, India  | Kabuli    |
| ICCV10307  | Released variety  | ICCV 92311 × ICC 17109                                                  | ICRISAT, India  | Kabuli    |
| ICCV10306  | Released variety  | ICCV 92311 × ICC 17109                                                  | ICRISAT, India  | Kabuli    |
| ICCV10316  | Released variety  | ICCV 92337 × ICC 17109                                                  | ICRISAT, India  | Kabuli    |
| ICCV92337  | Released variety  | (ICCV 2 × ICC 12034) × ICC 7344                                        | ICRISAT, India  | Kabuli    |
| ICCV00109  | Released variety  | ICC 18746 × ICCV 10                                                      | ICRISAT, India  | Desi      |
| ICCV03103  | Released variety  | [ICC 92014 × JG 23] × BG 1032                                            | ICRISAT, India  | Kabuli    |
| ICCV09307  | Released variety  | ICCV 92337 × ICC 17109                                                  | ICRISAT, India  | Kabuli    |
| ICCV95423  | Released variety  | (ICC 7676 × ICC 32) × ([ICCC 49 × ICC 15980] × ICCV 3)                  | ICRISAT, India  | Kabuli    |
| ICCV97404  | Released variety  | (ICCV 32 × ICC 4967) × ([ICCC 49 × ICC 15980] × ICCV 3)                 | ICRISAT, India  | Kabuli    |
| ICCV10     | Released variety  | ICC 1376 × ICC 1443                                                      | ICRISAT, India  | Desi      |
| ICC1882    | Genetic stock     | Traditional landrace P1506-4 from ICRISAT                                | ICRISAT, India  | Desi      |
| BGD72      | Released variety  | P1231 × P1265                                                            | IARI, New Delhi | Kabuli    |
| PUSA-1103  | Released variety  | (Pusa 256 × Cicer reticulatum) × Pusa 362                                | IARI, New Delhi | Desi      |
| ICC4958    | Genetic stock     | GW 5/7, a drought tolerant breeding line from ICRISAT                    | ICRISAT, India  | Desi      |
| ICCV00301  | Released variety  | ICCV 92502 × ICCV 2                                                      | ICRISAT, India  | Kabuli    |
| ICCV00302  | Released variety  | FLIP 91-18C × ICCV 2                                                     | ICRISAT, India  | Kabuli    |
| ICCV01301  | Released variety  | GNG 1044 × (ICCC 32 × ICC 12034)                                        | ICRISAT, India  | Kabuli    |
| L-550      | Landrace          | PBG7 × Rabat                                                             | PAU, Ludhiana   | Kabuli    |
| ICCV03403  | Released variety  | (ICCV 4973 × ICC 14196) × ICCV 92329                                   | ICRISAT, India  | Kabuli    |
| C-235      | Released variety  | IP 58 × C1234                                                            |                  | Desi      |
| ICCV03404  | Released variety  | (ICCV 4973 × ICC 14196) × ICCV 92329                                   | ICRISAT, India  | Kabuli    |
| ICCV03310  | Released variety  | BG 70 × ICCV 92329                                                       | ICRISAT, India  | Kabuli    |
| ICCV07301  | Released variety  | ICCC 95334 × (ICCV 2 × ICCV 98506)                                       | ICRISAT, India  | Kabuli    |
| ICCV05312  | Released variety  | ICCV 2 × ICCV 92325                                                      | ICRISAT, India  | Kabuli    |
| ICCV5308   | Released variety  | ICCV 2 × ICCV 92311                                                      | ICRISAT, India  | Kabuli    |
| ICCV5313   | Released variety  | ICCV 2 × ICCV 92325                                                      | ICRISAT, India  | Kabuli    |
| ICCV4310   | Released variety  | (ICCV 4973 × ICC 14196) × ICCV 92329                                   | ICRISAT, India  | Kabuli    |
| PUSA-1003  | Released variety  | Mutant of L532                                                           | IARI, New Delhi | Kabuli    |
| CSG8962    | Released variety  | Selection from GPF 7035                                                  | CSSRI, Karnal   | Desi      |
| ICCV4303   | Released variety  | (ICCV 4973 × ICC 14196) × ICCV 92329                                   | ICRISAT, India  | Kabuli    |
| ICCV2      | Released variety  | [(ICC 5003 × ICC 4953) × ICC 583] × (ICCV 4973 × ICCV 7347)              | ICRISAT, India  | Kabuli    |

**Table 1: Genotypes used in the study**
Fresh young chickpea leaves (2 g) were used for genomic DNA isolation (Tapan et al. 2014). Quality and quantity of DNA was checked using 1% agarose (Sambrook et al. 2001) spectrophotometrically (Thermo Scientific, USA). A total of 125 SSR markers (Sigma-Aldrich) were used for genetic diversity analysis; only 25 markers were found to be polymorphic. Amplification was done in a 10 µl volume reaction master mix using a Veriti Thermal Cycler PCR (Applied Biosystems, Foster City, CA, USA). The PCR mix consisted of 1 µl of 20 ng genomic DNA, 1.6 µl of 10X TBE buffer, 1 µl of 10mM of dNTP mix, 1 µl each of forward and reverse primer and 0.3 µl of 3 U/µl Taq polymerase (Sigma-Aldrich) and the amplification was done as per PCR conditions described by Bhardwaj et al. (2011).

The amplicons were analyzed on 3% agarose gel containing EtBr (10 mg/ml) at a constant voltage of 120V for 3 h using horizontal gel electrophoresis system (Biorad, USA) in 1X TBE buffer. A 100bp DNA ladder (Thermo Scientific, USA) was used as standard to determine the approximate band size of the amplicons. Gel pictures were taken under UV light gel documentation system (UVITECH Imaging System, UK) and phenogram was generated based on Jaccard’s coefficients (Jaccard 1908) by SAHN grouping method (Sokal and Sneath 1963) using the Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc) program Version 2.1 (Rohlf 2000). Power Marker version 3.0 (Liu and Muse 2005) was used to assess diversity indices, viz. alleles generated, PIC values, gene diversity (Hₑ) and major allele frequency (Table 2).

Molecular variance was estimated in GenAlEx 6.5 software. The three broad populations based on seed type, viz. *kabuli* genotypes, *desi* genotypes, and *desi* genotypes of ICRISAT origin were subjected to principal coordinates analysis (PCoA). Distance matrix method based principal coordinates analysis (PCoA) was also done.

**RESULTS AND DISCUSSION**

Narrow genetic variability in chickpea has been a serious concern in advancement for genetic improvement (Konsam et al. 2014). The repeated use of fewer numbers of elite lines with narrow genetic base for developing new breeding lines could have been one of the major reasons for this narrow genetic variability (Bhardwaj et al. 2011). Greater genetic diversity that can be obtained through the use of wider germplasm and use of wild relatives has been reported by many chickpea breeders (Bhardwaj et al. 2011). In this study, 125 SSR markers distributed all over the genome were used to characterize the chickpea genotypes and assess their genetic diversity. Among 125 SSR markers, only 25 were polymorphic and produced 3.6 alleles per locus on an average. PIC values and diversity indices were calculated for each SSR marker (Table 2). Maximum alleles (8) were detected for the locus TA136 while minimum (2) for GAA47. Thirteen highly informative loci with high PIC values not less than 0.4 were generated and the highest PIC (0.7825) was found for the primer TA136. Bhardwaj et al. (2010) also reported high PIC values in SSR analysis due to polymorphism of TAA motifs in chickpea with maximum DI (Diversity Index) and PIC value for TA136 (Udupa et al. 1999). This study could identify SSR markers that can be recommended for diversity analysis in chickpea. High DI and PIC values indicate greater amount of genetic variability at molecular level in chickpea lines analyzed and also the suitability of such SSR markers for characterization studies by many workers (Bharadwaj et al. 2010, Satyavathi et al. 2005). The most diverse genotypes were ICCV9314 and Pusa1103, with a similarity coefficient of 0.163. Such distant lines when crossed are expected to produce higher variability.

AMOVA indicated that 97% of the variations were within the populations while the remaining 3% variations were among the populations. The analysis of variance revealed significant differences in all the genotypes studied (Table 3), indicating existence of sufficient diversity among them which can be utilized to combine the desirable characters through *desi-kabuli* introgression breeding.

There was a wide variation among the genotypes for protein content and yield traits indicating the soundness of the material for diversity studies and using in crossing program (Table 4); these were further grouped on the basis of seed type, viz. *desi* and *kabuli*. These two gene pools represent diverse yet easily crossable lines having higher variation for yield traits, plant type, quality characters and tolerance to various stresses. The *desi* pool in this study showed higher leaf protein, higher yield in comparison to the *kabuli* pool with higher seed size (Table 5). The plant yield ranged from 4.2–46.10 g with an average value of 16.56 g. The mean 100-seed weight was found to be 28.74 g ranging from 11.19–41.76g.

The first and the second component of principal coordinate analysis (PCoA) accounted for 76.14% and 23.86% of the total variations, respectively, indicating the soundness of the biplot generated (Table 6). The PCoA biplot illustrates the differentiation among accessions (Fig 1). The chickpea genotypes from the different sources grouped into three populations may be due to different ancestors or different evolutionary processes or genetic exchanges representing three different gene pools. *Kabuli* and *desi* genotypes distinctly grouped indicating that they were diverse. Furthermore, the ICRISAT *desi* accessions within *desi* group sub-grouped themselves. This was in conformity with dendrogram generated by SAHN grouping wherein all the *desi* genotypes developed by ICRISAT were grouped at closer genetic distance to that of ICRISAT developed *kabuli* lines. The *kabuli* genotypes in cluster I and *desi* genotypes in cluster II represented the populations formed in PCoA. The PCoA done provides an insight into overall diversity unlike tree methods which tend to concentrate more on individual relations. The *kabuli* genotypes grouped in first lower quarter while the *desi* in the second quarter. Further, the *desi* genotypes developed by ICRISAT which were sourced from the training population grouped midway. Wider
| Primer name | Primer sequence | No. of alleles produced | Major allele frequency | Gene diversity | PIC |
|-------------|-----------------|-------------------------|------------------------|---------------|-----|
| TR43        | AGGACGAAACTATTCAAGGTAAGTGA | 3 | 0.7 | 0.465 | 0.4199 |
|             | AATTGAGATTGGATATTAATGGGATAAC  |   |     |   |   |
| TA25        | AGTTTAATGCTGTTGCTCTAAGATAAC  | 3 | 0.7692 | 0.3787 | 0.3434 |
|             | AGGGTACCTTTAATAATCAGAATGAGA  |   |     |   |   |
| NC81        | CGGAATGTCTCAATAAATCA | 3 | 0.8875 | 0.2059 | 0.1958 |
|             | TGTGGTACCTGGGATAACTCC |   |     |   |   |
| GAA47       | CACTCCTCAATGCCAACCTCTCCT | 2 | 0.5897 | 0.4839 | 0.3668 |
|             | AATATGGGATATTGCTGATTGGGG  |   |     |   |   |
| NCPGR69     | GACCGAATGTCCTCATAAATCA | 3 | 0.9125 | 0.1628 | 0.1553 |
|             | GGAGCTGGAAAAACTACAGC |   |     |   |   |
| NCPGR91     | ATGTTACCTTTTCTGGAACCG | 2 | 0.7125 | 0.4097 | 0.3258 |
|             | CTGTTCCTCCTCTCTCCG |   |     |   |   |
| GA6         | ATTTTTCTCCGTTGTGCAC | 3 | 0.6282 | 0.5322 | 0.4724 |
|             | AACGAGCAGAGATGGGAGTGAT |   |     |   |   |
| NCPGR147    | TGTGTGCAACACCTTGACTCATT | 4 | 0.45 | 0.6747 | 0.617 |
|             | CGATGATATTCCTCAGGGAAC |   |     |   |   |
| TS29        | AACATTCATGAACCTACCACTTAAT | 4 | 0.6375 | 0.5122 | 0.4451 |
|             | CCATGATACCTACCCGTCAGTGAGA |   |     |   |   |
| TR31        | CTAAATGCCACATTTACTCTAAATA | 3 | 0.6081 | 0.4869 | 0.3808 |
|             | ATCCATAAAAACAGGGTAACACTTAAAT |   |     |   |   |
| CaM1903     | TGTGTGCAACCTTGACTCATT | 4 | 0.48 | 0.6015 | 0.5224 |
|             | CCATGATACCTACCCGTCAGTGAGA |   |     |   |   |
| CaM1502     | TCGAATGTCATACTCAATGGTG | 3 | 0.6 | 0.515 | 0.4244 |
|             | TTCACTGCCACCCGTTACCA |   |     |   |   |
| TA130       | TCTTTACTTTGGCTTTCAATATG | 3 | 0.7436 | 0.3932 | 0.3335 |
|             | GTATAATCCAGGAAACAA |   |     |   |   |
| NCPGR74     | TCCGTCACACATTTCTACTT | 5 | 0.4459 | 0.7199 | 0.6829 |
|             | CTCTTATGCTGCTGCTGAA |   |     |   |   |
| NCPGR103    | ACAACCTATACATTTTGCG | 3 | 0.6579 | 0.5083 | 0.4557 |
|             | TTGAGATGGAAAAAGGGGAA |   |     |   |   |
| NCPGR77     | TGGACTAAACAAATACGACGA | 3 | 0.7222 | 0.4398 | 0.3988 |
|             | AGGCCACCTCAAATTTATT |   |     |   |   |
| NCPGR107    | AAACTCAATATTGCCCCTTCA | 3 | 0.725 | 0.4363 | 0.3955 |
|             | CCAACCTGGAATGACCTTT |   |     |   |   |
| NCPGR130    | GATCTGGTGAAATAATGGA | 3 | 0.8125 | 0.3184 | 0.2901 |
|             | CAAGCTTTATCAGATGTTCG |   |     |   |   |
| NCPGR138    | ATTCACACATTTGCGTTTG | 3 | 0.75 | 0.4013 | 0.3601 |
|             | TGTGATTATTTGTTGCAATG |   |     |   |   |
| TA8         | AAAATTTGCACCCACAAAAATATG | 3 | 0.7 | 0.4638 | 0.4175 |
|             | CGTAAATATATGCGAGGAAAC |   |     |   |   |
| TR58        | CTCTATATTTGTTTGGTCTGTGTGG | 6 | 0.325 | 0.7616 | 0.7232 |
|             | TAAAATGTGTTAGGGTCGATAATAA |   |     |   |   |
| TA136       | AGATCTGTCAGAGATTTATGTT | 8 | 0.3125 | 0.8075 | 0.7825 |
|             | TGCTGTGAGCTTATACAAACAA |   |     |   |   |
| H3A10       | TTTAAGGCTTCCAGATTTGATTCTCT | 7 | 0.5541 | 0.6377 | 0.6021 |
|             | TCAACATGCTCACCCTAATAAAA |   |     |   |   |
| GAA50       | TTCTGGCCTCCATCAATCCA | 3 | 0.7821 | 0.3646 | 0.3351 |
|             | CCCCCTGGATTTTCATAACCA |   |     |   |   |
| NCPGR99     | ATCATGAAAGCAAAATCTCCAC | 3 | 0.6154 | 0.547 | 0.488 |
|             |
genetic variability between kabuli and desi genotypes for base broadening and greater enhancement of productivity by crossing desi and kabuli lines was reported by Santosh et al. (2017).

SAHN clustering grouped the chickpea genotypes into two major clusters (Table 7, Fig 2). Out of two clusters, the larger cluster I comprised of 29 genotypes whereas cluster II comprised of 11 genotypes. The cluster I consisted of all genotypes which were kabuli type irrespective of their source of breeding. Contrastingly the cluster II was of

Table 3 Analysis of molecular variance (AMOVA) for variation among and within forty genotypes of chickpea

| Source of variation | Df   | SS      | MS      | Estimated variance | %   |
|---------------------|------|---------|---------|--------------------|-----|
| Among pops          | 2    | 36.064  | 18.032  | 0.458              | 3%  |
| Within pops         | 37   | 565.161 | 15.275  | 15.275             | 97% |
| Total               | 39   | 601.225 | 15.732  | 100%               |     |

**Significant at 1%, Df-degree of freedom, SS-sum of square, MS-mean of square.

Table 4 Mean performance of chickpea genotypes for yield traits and soluble protein (leaf)

| Genotypes | Protein content (g/l) | Seeds/pod | 100-seed weight (g) | Plant yield (g) |
|-----------|-----------------------|-----------|---------------------|-----------------|
| ICC1882   | 25.78±0.40            | 1.00±0    | 17.65 ± 0.27        | 22.2± 1.45      |
| ICC4958   | 33.95±0.11            | 1.00±0    | 28.82 ± 0.35        | 18.44 ± 1.07    |
| Pusa1103  | 30.64±0.61            | 1.18±0    | 21.97 ± 0.38        | 24.68 ± 0.99    |
| BGD72     | 31.83±0.59            | 1.00±0    | 16.39 ± 0.19        | 46.10 ± 2.12    |
| Pusa1003  | 24.71±0.36            | 1.02±0    | 16.58 ± 0.34        | 14.60 ± 1.40    |
| CSG8962   | 33.60±0.17            | 1.03±0    | 11.19 ± 0.24        | 24.53 ± 2.29    |
| C235      | 28.65±0.29            | 1.01±0    | 14.22 ± 0.19        | 14.20 ± 1.32    |
| ICCV3310  | 31.46±0.12            | 1.00±0    | 33.17 ± 0.49        | 11.67 ± 1.26    |
| ICCV3311  | 30.49±0.17            | 1.11±0    | 30.59 ± 0.37        | 11.50 ± 0.68    |
| ICCV3403  | 30.53±0.08            | 1.03±0    | 30.93 ± 0.27        | 13.91 ± 1.36    |
| ICCV3404  | 28.43±0.17            | 1.02±0    | 38.71 ± 0.34        | 16.87 ± 0.46    |
| ICCV7301  | 29.47±0.11            | 1.04±0    | 37.29 ± 0.24        | 15.50 ± 1.40    |
| ICCV4303  | 30.67±0.21            | 1.07±0    | 35.95 ± 0.26        | 12.71 ± 0.66    |
| ICCV4310  | 29.76±0.21            | 1.06±0    | 33.61 ± 0.33        | 12.99 ± 1.36    |
| ICCV5312  | 29.54±0.50            | 1.05±0    | 35.71 ± 0.27        | 4.20 ± 1.76     |
| ICCV9312  | 29.86±0.11            | 1.05±0    | 37.29 ± 0.21        | 11.90 ± 2.13    |
| ICCV9313  | 30.35±0.16            | 1.12±0    | 39.24 ± 0.19        | 7.06 ± 0.53     |
| ICCV9314  | 28.95±0.21            | 1.14±0    | 36.45 ± 0.29        | 18.78 ± 2.41    |
| ICCV10313 | 28.49±0.13            | 1.07±0    | 37.55 ± 0.24        | 36.50 ± 1.40    |
| ICCV10    | 32.39±0.28            | 1.15±0    | 19.61 ± 0.15        | 15.47 ± 1.88    |
| ICCV2     | 28.01±0.48            | 1.08±0    | 21.92 ± 0.36        | 17.68 ± 1.95    |
| ICCV92337 | 30.31±0.09            | 1.09±0    | 30.93 ± 0.26        | 8.72 ± 1.39     |
| ICCV8310  | 30.49±0.20            | 1.07±0    | 30.22 ± 0.34        | 8.73 ± 2.06     |
| ICCV97309 | 32.10±0.33            | 1.23±0    | 24.66 ± 0.17        | 14.05± 1.17     |
| ICCV1309  | 30.33±0.17            | 1.18±0    | 30.97 ± 0.23        | 14.28 ± 1.68    |
| ICCV10304 | 29.86±0.09            | 1.21±0    | 22.68 ± 0.32        | 07.80± 0.26     |
| ICCV10307 | 30.59±0.13            | 1.06±0    | 35.24 ± 0.25        | 8.19 ± 1.10     |
| ICCV10306 | 31.20±0.24            | 1.24±0    | 35.53 ± 0.33        | 9.84 ± 2.09     |
| ICCV10316 | 29.78±0.30            | 1.17±0    | 41.76 ± 0.33        | 13.65 ± 1.31    |
| ICCV00109 | 29.71±0.10            | 1.18±0    | 20.87 ± 0.41        | 15.33 ± 1.35    |
| ICCV3103  | 30.34±0.07            | 1.09±0    | 25.42 ± 0.40        | 10.57± 1.57     |
| ICCV9307  | 31.27±0.35            | 1.07±0    | 38.94 ± 0.29        | 11.98 ± 2.08    |
| ICCV95423 | 26.00±0.30            | 1.05±0    | 27.37 ± 0.35        | 41.36 ± 2.33    |
| ICCV97404 | 29.62±0.21            | 1.11±0    | 25.46 ± 0.39        | 25.2 ± 3.67     |

Contd.
### Table 4 (Concluded)

| Genotypes     | Protein content | Seeds/pod | 100-seed weight (g) | Plant yield (g) |
|---------------|-----------------|-----------|---------------------|-----------------|
| ICCV0301      | 30.68±0.27      | 1±0       | 17.95 ± 0.25        | 12.45± 2.45     |
| ICCV0302      | 30.02±0.05      | 1±0       | 31.16 ± 0.48        | 12.52 ± 3.28    |
| ICCV1301      | 29.39±0.20      | 1±0       | 26.54 ± 0.29        | 11.16 ± 2.46    |
| L550          | 29.10±0.22      | 1.06±0    | 17.73 ± 0.28        | 16.70 ± 2.55    |
| ICCV5308      | 30.04±0.60      | 1.04±0    | 37.66 ± 0.49        | 28.80 ± 2.43    |
| ICCV5313      | 30.98±0.11      | 1.19±0    | 33.72 ± 0.29        | 19.45 ± 2.29    |
| Mean          | 29.98           | 1.08      | 28.74               | 16.55           |
| Max.          | 33.95           | 1.24      | 41.76               | 46.10           |
| Min.          | 24.71           | 1.00      | 11.19               | 4.20            |
| CV            | 0.007           | 1.20      | 1.06                | 1.01            |

### Table 5 Differences in desi and kabuli genotypes of chickpea for yield related traits and soluble protein content (leaf)

| Trait      | Desi  | Kabuli |
|------------|-------|--------|
| Leaf protein | 28.14 | 26.12  |
| SPP        | 1.08  | 2.28   |
| 100 SW     | 19.49 | 30.7   |
| Plant yield| 23.82 | 30.7   |
| SPPL       | 144.33| 56.59  |

SPP= seeds/pod

### Table 6 Percentage of variation explained by the first 3 axes

| Axis | 1  | 2  | 3  |
|------|----|----|----|
| %    | 76.14 | 23.86 | 0.00 |
| Cum %| 76.14 | 100.00 | 100.00 |

### Table 7 Clustering analysis of chickpea genotypes based on SAHN grouping

| Major cluster | No. of genotypes | Minor cluster | Minor subgroup genotypes |
|---------------|------------------|---------------|--------------------------|
| I             | 28               | I-A           | ICCV10313, ICCV3311, ICCV5313, L550, ICCV1301, ICCV9307, ICCV3404, ICCV3313 |
|               |                  | I-B           | C235                      |
| II            | 3                | II-A          | (i) ICCV5308, ICCV2, ICCV0302, ICCV97309, ICCV5312, ICCV10316, ICCV4303, ICCV3310, ICCV92337, ICCV9313, ICCV95423, ICCV1309, ICCV97404, ICCV4310, ICCV9312, ICCV10304, ICCV78301, ICCV8310, ICCV9314, ICCV10307, ICCV10306, ICCV3403, P1003 |
|               |                  | II-B          | (i) ICC1882, ICCV10 |
|               |                  | (i) ICC1882, ICCV10 |
|               |                  | (i) ICC1882, ICCV10 |

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Fig 1 Principal coordinate analysis based on a squared Euclidean distance matrix between the individuals.

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desi seed type having brown seed coat with smaller seed and owl head seed shape. Such distinctive separation was also reported by Konsam et al. (2014) who also correlated it to the probable differences in non-reducing raffinose family sugar differences. Bharadwaj et al. (2011) while studying phylogeny in a geographical collection of chickpea genotypes reported a similar distinction between desi and kabuli genotypes using molecular markers. Choumane et al. (2000) inferred that while using microsatellite markers for diversity analysis, close relatives and lines derived from the same cross tend to group together. The microsatellite flanking sequences in these lines are relatively conserved and generally exist in same sequence, hence getting similar genotyping scores. Thus, these lines which may be phenotypically different are actually at molecular level very close and if selected in breeding program, tend to further narrow down the genetic base of chickpea. The grouping based on SSR data, SAHN grouping is in congruence with the PCoA 2D plot delineating kabuli and desi genotypes.
with minor deviations and indicated that the chickpea genotypes grouped in different clusters and there was significant diversity among them.

Breeders generally tend to concentrate more on phenotypic performance and variability which is generally due to genotype and environment interaction (G × E interaction). The various biometric tools help to evaluate only these G × E interactions, genotype effects and environmental effects. Though such genotypes appear phenotypically diverse, being similar at molecular level they do not help to broaden the genetic base and achieve greater yield gains (Glaszmann et al. 2010). The next step perhaps would be to identifying trait specific diverse germplasm and association of markers with trait of interest (Upadhyaya et al. 2011). Advances in the molecular tools available in this orphan semi-arid legume and subsequent sequencing of chickpea genome would greatly aid in the genomics assisted breeding of chickpea (Varshney et al. 2013b). This study would pave way for breeders to select superior parents for breeding program. It further indicates that SSR markers are good indicators of genetic divergence and the diverse chickpea genotypes identified could serve as important sources for enhancing the genetic potential of chickpea.

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