Development and evaluation of high-density Axiom® CicerSNP Array for high-resolution genetic mapping and breeding applications in chickpea

Manish Roorkiwal1, Ankit Jain1, Sandip M. Kale1, Dadakhalandar Doddamani1, Annapurna Chitikineni1, Mahendar Thudi1 and Rajeev K. Varshney1,2,*

1International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India
2School of Agriculture and Environment & Institute of Agriculture, The University of Western Australia, Crawley, Perth, Australia

Received 20 July 2017; revised 6 September 2017; accepted 10 September 2017.
*Correspondence (Tel +91 40 30713305; fax +91 40 3071 3075; email r.k.varshney@cgiar.org)

Abstract

To accelerate genomics research and molecular breeding applications in chickpea, a high-throughput SNP genotyping platform ‘Axiom® CicerSNP Array’ has been designed, developed and validated. Screening of whole-genome resequencing data from 429 chickpea lines identified 4.9 million SNPs, from which a subset of 70 463 high-quality nonredundant SNPs was selected using different stringent filter criteria. This was further narrowed down to 61 174 SNPs based on p-convert score ≥0.3, of which 50 590 SNPs could be tiled on array. Among these tiled SNPs, a total of 11 245 SNPs (22.23%) were from the coding regions of 3673 different genes. The developed Axiom® CicerSNP Array was used for genotyping two recombinant inbred line populations, namely ICCRIL03 (ICC 4958 × ICC 1882) and ICCRIL04 (ICC 283 × ICC 8261). Genotyping data reflected high success and polymorphic rate, with 15 140 (29.93%; ICCRIL03) and 20 018 (39.57%; ICCRIL04) polymorphic SNPs. High-density genetic maps comprising 13 679 SNPs spanning 1033.67 cM and 7769 SNPs spanning 1076.35 cM were developed for ICCRIL03 and ICCRIL04 populations, respectively. QTL analysis using multilocation, multiseason phenotyping data on these RILs identified 70 (ICCRIL03) and 120 (ICCRIL04) main-effect QTLs on 13 679 SNPs spanning 1033.67 cM and 7769 SNPs spanning 1076.35 cM were developed for ICCRIL03 and ICCRIL04 populations, respectively. QTL analysis using multilocation, multiseason phenotyping data on these RILs identified 70 (ICCRIL03) and 120 (ICCRIL04) main-effect QTLs on genetic map. Higher precision and potential of this array is expected to advance chickpea genetics and breeding applications.

Keywords: chickpea, drought, genotyping, molecular breeding, QTLs, SNP array.

Introduction

Ongoing climatic changes that are affecting crop yields drastically and increasing food demand from ever increasing world population would certainly make the scenario worst by 2050 when the world population would be crossing the count of 9 billion. In order to feed this huge population and meet the future demands, it has become necessary to understand the factors involved in affecting the growth and yield of the crops. Chickpea (Cicer arietinum L., 2n = 16) is the second most important grain legume after common bean and third important pulse grown on low-input marginal lands and known to play a key role in food and nutritional security globally (Jukanti et al., 2012). Besides its ability to fix atmospheric nitrogen that improves the soil nutritional profile, chickpea further plays a pivotal role in yield and nutritional profile of other crops when involved in crop rotation programme (Gan et al., 2010). Currently, global chickpea acreage in 56 countries covers more than 13.98 mha and accounts for a production of 13.23 million tons annually (FAO 2014). With the impact of various abiotic and biotic stresses, current average chickpea productivity is <1 t/ha, which is far less than its actual potential yield of 6 t/ha under optimum growing conditions. Among these abiotic stresses, terminal drought alone is known to reduce the annual production by 40% (Ahmad et al., 2005). During the last five decades, conventional breeding efforts could enhance the chickpea productivity from 0.6 t/ha (1960) to 0.9 t/ha (2014) (FAO 2014). However, this increase is not enough to meet the significantly increasing global demand. Therefore, intervention of modern technology for enhancing the crop productivity has become essential. Success stories of application of genomic approaches in enhancing the production among different crop species confirm the potential of genomics-assisted breeding (GAB) (Kole et al., 2015; Varshney et al., 2005). Inspired by the success of GAB, chickpea community also started to deploy markers for developing superior lines and were able to develop improved lines with enhanced yield under rainfed conditions in JG 11 background (Varshney et al., 2013a) and lines resistant to fusarium wilt and ascochyta blight in the genetic background of C 214 (Varshney et al., 2014a). Very recently, efforts to deploy genomic selection for yield-related traits in chickpea have also been initiated (Roorkiwal et al., 2016). Advances in next-generation sequencing (NGS) and high-throughput genotyping technologies have enabled the use of these technologies at lower cost and offered opportunities to deliver high-throughput data to capture millions of variations in genome level. With the availability of draft genome sequence (Varshney et al., 2013b) and large-scale resequencing efforts (Thudi et al., 2016a,b), large number of single nucleotide polymorphism (SNP) markers have become available. SNPs, due to their abundance at genome-wide level, biallelic and
reproducible nature, are considered to be the most desirable, precise and efficient tools for developing high-density genome scans (Gupta et al., 2008; Wang et al., 1998). SNPs are often efficiently used to determine the functional relevance of genomic regions/candidate genes responsible for complex traits in many crop plants such as rice (Parida et al., 2012), maize (Face et al., 2015; Riedelsheimer et al., 2012; Weber et al., 2008) and barley (Mora et al., 2016).

In order to use huge genomic resources for breeding applications, there is a dire need of low-cost, high-throughput genotyping platforms to construct high-density genetic maps and undertake QTL analysis. Recent development in the array technology has brought down the cost of high-throughput genotyping platforms, thus making it accessible to most of the researchers and breeding communities engaged in genetic studies and crop improvement applications. SNP genotyping platforms can be used for genetic diversity studies, foreground selection, fine mapping, association mapping, genomic selection and evolutionary studies. As a result, SNP arrays with large number of SNPs distributed throughout the genome have been developed and used for various applications including association mapping, genetic diversity and genomic selection in several agronomically important crops (see Rasheed et al., 2017).

With the availability of high-quality chickpea genome (Varshney et al., 2013b) and various large-scale resequencing projects (Thudi et al., 2016a,b; unpublished data), millions of SNPs have become available in chickpea. This information can be further exploited to access the genome-wide sequence variations, which can help to expedite the process of development of improved lines in chickpea. With an objective to utilize SNP resource for chickpea improvement, the current study deals with the development of a high-density SNP array with genome-wide distributed SNPs. This array has been used for genotyping two chickpea intraspecific recombinant inbred line (RIL) populations to assess the utility of the developed SNP array in genetics and breeding applications. In brief, Axiom® CicerSNP Array is expected to provide a solid foundation for establishing the high-throughput genotyping, which is of great importance for research as well as breeding applications.

Results

SNP selection and Axiom® CicerSNP Array design

The resequencing data generated from 429 genotypes were aligned to the chickpea reference genomes for SNP discovery. The alignment of sequencing data resulted in the identification of total 4.9 million SNPs distributed across genome.

As described in the ‘Methods’ section and Figure 1, after applying seven filter criteria on identified SNPs from resequencing data, a total of 70 463 high-quality SNPs were selected. Further on the basis of p-convert value of ≥0.3, generated by in silico validation of SNPs using Axiom GTv1 algorithm, 61 174 SNPs were selected for further processing. Finally, a custom array consisting of a total of 50 590 SNPs was developed that includes final data set of high-quality SNPs along with previously validated 70 and 32 SNPs from QTL-seq (Singh et al., 2016) and skim sequencing (Kale et al., 2015), respectively. In the selected set of SNPs, 22.23% SNPs were from coding region of the genome and spanning across 3673 different genes of eight chickpea linkage groups.

---

Figure 1 Design and development workflow of Axiom® CicerSNP Array. Tiling to array included three major steps (i) SNP detection, (ii) SNP selection that included quality filtration and SNP validation and (iii) final tiling on array.
Genome-wide distribution of selected SNPs of Axiom® CicerSNP Array

Selected 50,590 SNPs cover all the eight pseudomolecules of chickpea and provide a good representation of the whole chickpea genome (Figure 2a,b), with an average of 6323 SNPs/linkage group (LG) (average distance of 6.86 Kb). Maximum number of SNPs have come from CalG04 (16,772; 33.15%), while minimum number of SNPs have come from CalG08 (1,888; 3.73%). As the upstream and downstream regions are not annotated in the chickpea genome assembly, these were not considered while predicting SNP effect using SnpEff software. With respect to genomic region in the Axiom® CicerSNP Array, as expected maximum number of SNPs have come from intergenic regions (31,653; 62.57%) followed by 11,245 SNPs (22.23%) from coding regions and 7,688 SNPs (15.20%) from intronic regions (Figure 2b). The SNPs from coding region cover synonymous coding (8,902; 17.60%), nonsynonymous coding (2,267; 4.48%), stop gained (23), synonymous stop (14), stop lost (10), synonymous start (8), splice site donor (8), splice site acceptor (7) and start lost (6).

Functional annotations of SNPs resulted in detailed classification of involvement of SNP-carrying genes in (i) biological process, (ii) molecular function and (iii) cellular component (Figure 2c). Under ‘molecular function’ category, the majority of SNP-carrying genes were involved in binding and catalytic activities. Under ‘biological process’ category, large number of SNP-carrying genes were found to be involved in ‘cellular and metabolic processes’ and for ‘cellular component’ category, majority of the genes annotated were found to be related to classes ‘cell’ and ‘cell parts’.

Validation and deployment of the Axiom® CicerSNP Array

Two different RIL populations, namely ICCRIL03 and ICCRIL04, were used to demonstrate the application of Axiom® CicerSNP Array. ICCRIL03 consisting of 245 lines was genotyped with the developed Axiom® CicerSNP Array. Three samples were removed from the analysis due to below-threshold standard dish quality control check (DQC < 0.82). Seven additional samples were removed due to a lower call rate (< 97%). An initial performance validation of the Axiom® CicerSNP Array followed the Axiom best practices genotyping workflow. SNPs were classified as described in ‘Methods’ section (Figure 3), and results are summarized in Table 1. Overall, 25.89% SNPs were found to fall in ‘PolyHighResolution’ category, whereas 45.7% SNPs were found to fall in ‘MonoHighResolution’ category. A summary of the distribution of all SNPs in the different classes and on different LGs is shown in Table S1.

In the case of ICCRIL04, consisting of 230 lines, one sample was excluded from the analysis due to lower dish quality control check and 15 samples were removed due to a lower call rates. Overall, 35.7% SNPs were found to fall in ‘PolyHighResolution’ category, large number of SNP-carrying genes were found to be involved in ‘cellular and metabolic processes’ and for ‘cellular component’ category, majority of the genes annotated were found to be related to classes ‘cell’ and ‘cell parts’.

Figure 2  Summary of distribution of SNPs selected for Axiom® CicerSNP Array: (a) SNPs distribution along the eight linkage groups of chickpea; (b) distribution of SNPs in different genomic regions of chickpea genome.
Genotypic data generated using Axiom® CicerSNP Array were used for the construction of genetic maps for ICCRIL03 and ICCRIL04 populations. For ICCRIL03, a total of 15 140 high-quality SNPs were identified, of which 14 034 showed expected 1:1 segregation at \( P \leq 0.01 \). Finally, 13 679 SNPs were successfully mapped to eight linkage groups (CaLG01–CaLG08) covering 1033.67 cM (Table 2). The highest number of markers were mapped on CaLG04 (5179), while the lowest number of markers were mapped on CaLG05 (212). The distribution of marker mapped on eight linkage groups is shown in Figure 4. The total map distance for linkage groups varied from 62.24 cM (CaLG08) to 226.43 cM (CaLG04). The highest average marker density was observed for CaLG01, which had 25 markers/cM followed by 23 markers/cM on CaLG04. The lowest average marker density was observed for CaLG05, which had two markers per cM. In summary, the genetic map has 13 markers/cM on an average (Table 2).

Similarly for ICCRIL04 population, 20 018 high-quality SNPs were identified, of which 8224 SNPs showed 1:1 segregation and 7769 SNPs could be mapped, covering 1076.35 cM across eight linkage groups (Figure 5). Maximum number of markers were mapped to CaLG01 (2001) followed by CaLG07 (1727), whereas minimum number of markers were mapped to CaLG02 (154). Highest average marker density was observed for CaLG04 (19.74 markers/cM), and minimum marker density of 1.85 markers/cM was observed for CaLG02. Overall, the map has an average of 7.22 markers/cM (Table 3).

QTLs for drought tolerance-related traits

QTL analysis using genotyping and phenotyping data resulted in the identification of a large number of QTLs for five groups of drought component traits such as root traits, morphological traits, phenological traits, yield and yield-related traits and transpiration efficiency (TE) using QTL IciMapping software. In the case of ICCRIL03, a total of 70 QTLs were identified that included 26 QTLs for phenological traits, 11 QTLs for morphological traits, 21 QTLs for yield-related traits, 6 QTLs for drought indices, 5 QTLs for root traits and 1 QTL for carbon isotope
Table 2 Features of genetic map developed for ICCRIL03 (ICC 4958 × ICC 1882) population using Axiom® CicerSNP Array and its comparison with earlier studies

| Linkage group | Marker loci mapped | Map Distance (cM) | Marker loci mapped | Map Distance (cM) | Marker loci mapped | Map Distance (cM) |
|---------------|--------------------|-------------------|--------------------|-------------------|--------------------|-------------------|
| Axiom® CicerSNP Array (Current study 2017) | | | | | | |
| CaLG01        | 2610               | 104.02            | 109                | 101.27            | 31                 | 99.27             |
| CaLG02        | 1088               | 97.67             | 90                 | 92.16             | 18                 | 78.50             |
| CaLG03        | 576                | 164.90            | 90                 | 72.78             | 41                 | 28.13             |
| CaLG04        | 5179               | 226.43            | 386                | 112.10            | 45                 | 111.90            |
| CaLG05        | 212                | 94.15             | 39                 | 59.41             | 22                 | 33.24             |
| CaLG06        | 1709               | 193.99            | 160                | 104.36            | 36                 | 123.08            |
| CaLG07        | 1837               | 90.27             | 60                 | 96.59             | 27                 | 96.11             |
| CaLG08        | 468                | 62.24             | 73                 | 88.62             | 21                 | 51.28             |
| Total         | 13 679             | 1033.67           | 1,007              | 727.29            | 241                | 621.51            |
| Average       | 1709.88            | 129.21            | 125.88             | 90.91             | 30.13              | 77.69             |

GBS (Jaganathan et al., 2015)

SSRs (Varshney et al., 2014b)

Figure 4 High-resolution genetic map comprising eight linkage groups (CaLG01–CaLG08), with marked major QTL clusters, using 13 679 SNP markers for ICCRIL03 (ICC 4958 × ICC 1882).

Figure 5 High-resolution genetic map ICCRIL04 (ICC 283 × ICC 8261) with major QTL clusters constructed using 7769 SNPs.
Table 3 Features of genetic map developed for ICCRIL04 (ICC 283 × ICC 8261) population using Axiom® CicerSNP Array and its comparison with earlier studies

| Linkage group | Marker loci mapped | Map distance (cM) | Marker loci mapped | Map distance (cM) |
|---------------|--------------------|-------------------|--------------------|-------------------|
| CalLG01       | 2001               | 183.98            | 16                 | 60.78             |
| CalLG02       | 154                | 83.16             | 16                 | 66.61             |
| CalLG03       | 1063               | 144.19            | 22                 | 69.44             |
| CalLG04       | 1516               | 76.79             | 18                 | 43.95             |
| CalLG05       | 440                | 143.76            | 23                 | 51.51             |
| CalLG06       | 557                | 141.43            | 31                 | 65.29             |
| CalLG07       | 1727               | 200.30            | 24                 | 104.92            |
| CalLG08       | 311                | 102.74            | 18                 | 70.57             |
| Total         | 7769               | 1,076.35          | 168                | 533.07            |
| Average       | 971.13             | 134.54            | 21                 | 66.63             |

*Table 3: Features of genetic map developed for ICCRIL04 (ICC 283 × ICC 8261) population using Axiom® CicerSNP Array and its comparison with earlier studies.

discrimination (δ13C) (Table S3). However, in the case of ICCRIL04, 120 QTLs were identified, namely 48 QTLs for yield-related traits, 41 QTLs for morphological traits, 21 QTLs for phenological traits, 8 QTLs for root traits and 2 QTLs for drought indices (Table S4). Of the 70 QTLs identified in ICCRIL03, 16 QTLs (22.86% of total QTLs identified) were located on CalLG08 followed by CalLG04 and CalLG05 (20%, 14 QTLs each). However for ICCRIL04, 33 QTLs (27.5% of total QTLs) were located on CalLG08 followed by CalLG03 with 25 QTLs (20.83%). In total, 52 major QTLs were identified across all linkage groups for morphological traits, which is quite high in comparison with previous studies (Varshney et al., 2014b). Of these 52 QTLs, 16 QTLs were designated as robust QTLs based on phenotypic variation explained (PVE) > 10%. QTLs for plant height (PHT, cm) were detected in both RIL populations. However, QTLs for primary branches (PBS) and secondary branches (SBS) were specific to ICCRIL03 population, and QTLs for shoot dry weight (SDW, g), plant width (PWD, cm) and plant stand (PS) were specific to ICCRIL04 population.

In the case of ICCRIL03, three QTL clusters were observed. Cluster one located on CalLG04 represented QTLs for 100-seed weight (100-SDW, g), days to 50% flowering (DF), PHT, pods per plant (PDP), while other two clusters were located on CalLG08 responsible for DF, days to maturity (DM), harvest index (HI, %), PHT and DF, DM, PHT traits, respectively. Similarly in the ICCRIL04 population, a total of nine QTL clusters were identified on CalLG01 (one cluster: 100-SDW, SDW), CalLG02 (two clusters: a) HI, PS, yield (YLD, g); (b) biomass (BM, g), drought tolerance index (DTI), PS, YLD), CalLG03 (one cluster for DF and DM and another cluster for DF, DM, HI), CalLG05 (one cluster: DF and seeds per pod (SPD)) and CalLG08 (three clusters: a) DF, DM, HI, PHT, YLD; b) DF, DM, HI, PHT; c) 100-SDW, DF, DM, PHT, POD).

On the basis of location/year, QTLs were further categorized in two categories: (i) QTL for a trait appearing in more than one location was considered as ‘stable’ QTL and (ii) QTL appearing for >1 year or season was considered ‘consistent’ QTL (Tables 4 and 5). PVE for QTLs ranged from 4.8% to 66.49% in case of ICCRIL03 population and 5.49% to 39.32% in case of ICCRIL04 population (Tables S3 and S4).

Root traits

A total of six root traits [root length density (RLD, cm/cm3), root dry weight (RDW, g), rooting depth (RDp, cm), root surface area (RSA, cm2), root volume (RV, cm3) and root dry weight/total plant dry weight ratio (RTR, %)] were analysed and QTLs for five traits one each for RLD, RSA, RV, RTR and RDp in ICCRIL03 with PVE ranging from 6.55% (RSA) to 66.49% (RV) were identified (Table S3). However, in case of ICCRIL04, one QTL was identified each for RLD, RSA, RV and RDp, and 2 QTLs for RDW and 3 QTLs for RTR were identified with PVE ranging from 5.79% (RDL) to 8.08% (RDV) (Table S4). One robust QTL was observed for RV in ICCRIL03 population (Table 4), whereas no robust QTL was observed in case of ICCRIL04 (Table 5). In both the RIL populations, no consistent and no stable robust QTL was observed for root traits (Tables 4 and 5).

Morphological traits

A total of 11 QTLs were identified in ICCRIL03 population for morphological traits that included PHT (7 QTLs), PBS (3 QTLs) and SBS (1 QTL). Overall, PVE ranged from 6.12% (PHT) to 13.51% (PHT) (Table S2). In all, four robust QTLs were observed for PHT (Table 4).

In the ICCRIL04 population, 41 QTLs for morphological traits were identified, of which 17 QTLs were for PHT, 11 QTLs for SDW, 9 QTLs for PS and 4 QTLs for PWD. PVE ranged from 5.49% (SDW) to 17.65% (PHT) among QTLs for morphological traits (Table S4). In total, 12 robust QTLs were observed including two for SDW which were found consistent as well as stable. In the case of PHT, eight robust QTLs were observed, of which two QTLs were consistent as well as stable. However, for PS, two robust QTLs were observed and none of them was stable or consistent (Table 5).

Phenology-related traits

For phenological traits in ICCRIL03 population, a total of 26 QTLs were identified for two traits, viz. DF (13 QTLs) and DM (13 QTLs), with PVE ranging from 4.8% (DF) to 34.82% (DF) (Table S3). Of total 26 QTLs, six robust QTLs were found, three each for DF and DM. Two QTLs each from DF and DM were both consistent and stable (Table 4).

In ICCRIL04 population, a total of 21 QTLs were observed, of which 10 for DF and 11 for DM with PVE ranging from 5.59% (DF) to 39.32% (DF). A total of 12 robust QTLs were identified, six for each DF and DM (Table S4). Three QTLs were found both consistent and stable for DF. However for DM, two stable robust QTLs and one additional stable and consistent QTL were observed (Table 5).

Yield and yield-related traits

In total, 21 QTLs were identified for six traits, viz. 3 QTLs for 100-SDW, 3 QTLs for POD, 1 QTL for SPD, 2 QTLs for BM, 8 QTLs for HI and 4 QTLs for YLD in ICCRIL03 population (Table S4). PVE of identified QTLs was ranged from 5.59% (HI) to 33.6% (SDW). Overall, six QTLs were found to be robust, two for POD, one for HI and three for 100-SDW (of which two were both stable and consistent; Table 4).

In ICCRIL04 population, a total of 48 QTLs were identified for six traits with PVE ranging from 5.49% (YLD) to 35.88% (HI) (Table S4). There were 2 QTLs for SPD, 3 QTLs for POD, 11 QTLs for HI, 6 QTLs for 100-SDW, 13 QTLs each for BM and YLD. A total of 20 QTLs were found to be robust, five for YLD, three each for 100-SDW and BM, one each for SPD and POD and seven for HI.
| Traits                      | Axiom™ CicerSNP Array (Current study 2017) | GBS (Jaganathan et al., 2015) | SSRs (Varshney et al., 2014b) |
|-----------------------------|--------------------------------------------|--------------------------------|-------------------------------|
| Total QTLs                  | Total Consistent Stable PVE (%)            | Total Consistent Stable PVE (%)| Total Consistent Stable PVE (%)|
| Root traits                 |                                            |                                |                               |
| Root length density (RLD, cm/cm³) | 1 – – – | 1 1 1 – 10.65–12.09 | 1 – – – 10.90 |
| Root surface                | 1 – – – | 1 – – 11.04 | 1 – – 10.26 |
| Root volume (RV, cm³)       | 1 – – – | 66.49 | – – – |
| R–T ratio (RTR, %)          | 1 – – – | 1 1 – 10.85–13.56 | 1 – – 16.67 |
| Morphological traits        |                                            |                                |                               |
| Plant height (PHT, cm)      | 3 2 2 2 14.79–34.82 | 3 1 1 1 10.86–67.71 | 2 1 1 10.51–26.87 |
| Shoot dry weight (SDW, g)   | – – – – | 10.11 | 10.78–26.91 | 10.11–34.79 | 13.89–17.59 |
| Phoenological traits        |                                            |                                |                               |
| Days to 50% flowering (DF)  | 3 2 2 10.57–26.10 | 2 1 1 1 10.11–34.79 | 3 1 1 12.13–19.71 |
| Days to maturity (DM)       | 3 2 2 11.45–33.60 | 2 2 2 10.12–60.41 | 2 1 1 10.31–58.20 |
| Yield–related traits        |                                            |                                |                               |
| 100–seed weight (100–SDW, g) | 3 2 2 16.05 | 3 1 – 10.14–25.94 | 3 – – 10.67–14.36 |
| Biomass (BM, g)             | – – – – | 12.56–24.07 | 12.56–24.07 | 10.19–23.18 |
| Harvest index (HI, %)       | 1 – – – | 66.49 | 45.40 | 42.07 |
| Pods/plant (POD)            | 2 – – – | 10.32–13.56 | 10.32–13.56 | 10.32–13.56 |
| Seeds/pod (SPD)             | – – – – | 24.07 | 12.13–19.71 | 12.13–19.71 |
| Yield (YLD, g)              | – – – – | 11.67–18.64 | 11.67–18.64 | 11.67–18.64 |
| Drought indices traits      |                                            |                                |                               |
| Drought tolerance indices (DTI) | – – – – | 10.10–10.76 | 10.10–10.76 | 10.10–10.76 |
| Total                      | 17                          | 39                                | 25                          |

PVE, phenotypic variation explained; –, No robust QTL identified.
Among yield traits, only two QTLs were found consistent for HI (Table 5).

**Drought indices and Transpiration efficiency**

In the case of ICCRL03, a total of six QTLs were identified, namely four QTLs for DTI and two for drought susceptibility index (DSI) with PVE ranging from 5.58% (DSI) to 8.13% (DTI) (Table S3). However, in the case of ICCRL04, only two QTLs for DTI were observed and both were robust (Table 4). Overall, PVE for these QTLs in ICCRL04 population ranged from 18.63% to 28.49% (Table S4). No QTL for drought indices was found stable and/or consistent. In case of carbon isotope discrimination (δ13C) as a surrogacy trait for transpiration efficiency (TE), only single QTL was identified in ICCRL03 with PVE of 5.46% (Table S3), while in the case of ICCRL04, δ13C was not measured.

**Discussion**

Molecular markers have been widely used for genetic diversity assessment, evolutionary and mapping studies (Varshney *et al.*, 2007). Applicability of markers in breeding mainly relies on cost, ease of automation and precision, making SNPs an indispensable choice. Rapid progress in NGS technologies during last decade enabled massive sequence data output at low cost in very less time period (Thudi *et al.*, 2012).

Due to limited availability of genomic resources until past decade, chickpea was considered to be an ‘Orphan Crop’ (Varshney *et al.*, 2012). Chickpea improvement efforts using conventional breeding were able to enhance the chickpea productivity but were not enough to meet increase in the global demand. Availability of large-scale genomic resources and markers has offered an opportunity to utilize GAB for crop improvement to enhance the rate of genetic gain (Varshney *et al.*, 2005). The past decade has witnessed tremendous application of GAB to critically address some of the major issues in legume crops (Varshney *et al.*, 2013c).

For successful and effective deployment of GAB to develop superior chickpea varieties, it becomes important to tap the variations existing in the genome by successful mapping and tagging these variations for agronomically important traits. Success of GAB depends on the level of marker precision and cost for genotyping. In the case of chickpea, different SNP genotyping platforms have been developed for various applications (Gujaria *et al.*, 2011; Hiremath *et al.*, 2012; Roorkiwal *et al.*, 2013, 2014).

In recent past, NGS-based technologies have been effectively used for genome sequencing and resequencing, enabling the identification of millions of SNP markers in chickpea (Kale *et al.*, 2015; Thudi *et al.*, 2016a,b). SNP genotyping arrays are user-friendly and cost-effective for generation and analysis of genotyping data. Considering the utility of these arrays, the present study focused on the development of high-density SNP array and its utility for genetics and breeding applications in chickpea. In brief, whole-genome resequencing of 300 lines of the reference set and >100 lines of elite chickpea varieties at 5–13 x coverage led to the generation of >4.9 million SNPs. With the application of different filter criteria ensuring distribution of SNPs across all the eight linkage groups and with further inclusion of SNPs from skin sequencing, a high-quality ‘Axiom®CicerSNP Array’ was developed. High-density Axiom®CicerSNP Array with 50 590 high-quality nonredundant SNPs produced 95.92% and 93.04% sample success rate, and average QC call rate of 99.81, among two chickpea intraspecific populations ICCRL03 and ICCRL04, respectively. The total missing data in case of ICCRL03 population was 0.5% and 0.4% in ICCRL04 population. On an average, the triplicates showed >99% concordance among the results (includes all calls for triplicates and minimum duplicates).

© 2017 The Authors. *Plant Biotechnology Journal* published by Society for Experimental Biology and The Association of Applied Biologists and John Wiley & Sons Ltd., 16, 890–901
Chickpea crop improvement efforts are severely affected by the narrow genetic diversity among cultivated chickpea accessions. Some of the previously generated intraspecific genetic maps with only a few hundred markers also indicate the narrow genetic base in chickpea (Gaur et al., 2011; Jamalabadi et al., 2013; Millán et al., 2010; Radhika et al., 2007; Varshney et al., 2014b). For instance, Varshney et al. (2014b) after screening ~3000 markers could find only couple of hundred polymorphic markers and ultimately were able to map 241 and 168 markers on ICCRIL03 and ICCRIL04, respectively. With the availability of NGS technology, Jaganathan et al. (2015) using GBS approach could identify 828 novel SNPs and were able to integrate additional 743 SNPs to develop a genetic map with 1007 mapped markers spanning around 727.29 cM. However, the current study by using SNP array successfully mapped 13 679 markers spanning around 1033.67 cM (Table 2). Similarly, another advanced map was developed in the current study using 46-fold more markers (7769) spanning 1076.35 cM map distance in comparison with genetic map constructed by Varshney et al. (2014b) for ICCRIL04 (Table 3). Varying levels of marker densities were recorded for different LGs in both the maps and the average intermarker distances were 0.08 cM and 0.14 cM in the case of ICCRIL03 and ICCRIL04, respectively. Genetic maps generated in current study will not be only useful in ordering future genetic maps but also a higher marker density obtained here will aid in the selection of appropriate markers for various molecular breeding applications.

QTL analysis includes identification of markers/genomic regions, associated with the genetic variation influencing economically important traits (Mackay et al., 2009). Markers associated with QTLs aid in improving the accuracy of genetic selection by identifying favourable genotypes. Use of Axiom® CicerSNP Array on two RIL populations resulted in identifying a large number of QTLs for five groups of drought tolerance-related traits such as root traits, morphological traits, phenological traits, yield and yield-related traits and TE. In total, 70 QTLs for ICCRIL03 and 120 QTLs for ICCRIL04 were identified. In the case of ICCRIL04, more than twofold robust QTLs (46) were identified in comparison with the previous study (Varshney et al., 2014b). In the present study, 13 main-effect QTLs for root traits were identified. Unlike our previous study (Varshney et al., 2014b) where 18 main-effect QTLs for root traits were observed distributed all over the linkage group except for CalG02, we observed the absence of main-effect QTLs for root traits on CalG01, CalG02 and CalG06.

While analysing for both RIL populations (ICCRIL03 and ICCRIL04), common QTLs were identified for four root traits (RLD, RSA, RTR and RdP). However, it was interesting to note that except for RLD, QTLs for these traits were identified on different linkage groups in the RIL populations. Additionally, QTLs for RV and RDW were found only in ICCRIL03 and ICCRIL04, respectively. The possible reason behind this contrasting identification of QTLs in two RIL populations could be either the presence of several small-effect QTLs or break of robust QTLs into many small-effect QTLs due to higher number of markers in the current study.

SNP arrays have been used for the validation of genome sequence assembly by comparing genetic position with the physical position (Sim et al., 2012). As an application of the Axiom® CicerSNP Array, while working on different mapping populations comparison of genetic with physical positions of the SNP can be used for further validation and improvement of assembled chickpea genome. Some studies in maize and apple have clearly shown the possibility of improvement of genome assembly using large number of markers by applying array-based genotyping for high-resolution genetic mapping (Bianco et al., 2016; Ganal et al., 2011). In addition, high-density SNP arrays have also been used for various breeding applications and high-resolution genetic mapping using genome-wide association studies (GWAS). Several SNP arrays including medium- to high-throughput have been developed and used for GWAS and breeding applications in groundnut (Pandey et al., 2017), rice (Chen et al., 2014; McCouch et al., 2010; Singh et al., 2015; Yu et al., 2014) and wheat (Maccacari et al., 2015).

From the cost perspective, SNP array platforms are found to be efficient and cost-effective in comparison with other genotyping platforms. In the case of chickpea, 828 unique SNPs were identified across 208 RILs using GBS with a cost US $40–45 per sample (Jaganathan et al., 2015). Similarly, application of skm sequencing costing about US $100, resulted in the identification of >80 K SNPs across 232 lines (Kale et al., 2015). However, use of the Axiom® CicerSNP Array can provide genotyping data for 50 590 SNPs for a cost of US $52 per sample as per the negotiated agreement between Affymetrix and ICRISAT for the chickpea community. Therefore, SNP array developed in the present study has a potential to generate low-cost data points. Furthermore, SNP arrays produce robust and reliable data with less missing values, in comparison with GBS where the presence of large proportion of missing values and the presence of false homozygotes can be observed (Bianco et al., 2016). It is also important to mention that SNP array data are generated in simple allele calls and can directly be used for further analysis, suggesting minimal computational skills requirement. However, advanced computational skills are required to handle GBS and skm sequencing data, to analyse the generated data that further aid to the postprocessing cost to the data analysis (Bajgain et al., 2016). Hence based on these parameters, it can be concluded that the Axiom® CicerSNP Array developed under this study will be a valuable tool to dissect agronomically important traits and for various molecular breeding applications in chickpea. Implementation of developed array in ongoing genomic selection attempts in chickpea (Roorkiwal et al., 2016) will further enhance the precision and accuracies of the genomic predictions.

In summary, the SNP array developed in this study is a most awaited genomic resource for global chickpea research community and will eventually accelerate the process and precision of understanding trait genetics and molecular breeding for chickpea improvement.

Methods

Plant material and phenotypic evaluation

Two different RIL populations, namely ICCRIL03, developed by crossing ICC 4958 (a drought-tolerant genotype) and ICC 1882 (a drought-sensitive genotype), and ICCRIL04 generated by crossing ICC 4958 (a drought-tolerant genotype) and ICC 1882 (a drought-sensitive genotype), and ICCRIL04 developed by crossing ICC 8261 (a drought-tolerant genotype) and ICC 283 (a drought-sensitive genotype), were used in this study.

Genomic DNA from parental genotypes and RILs were isolated using high-throughput mini-DNA extraction (Cuc et al., 2008). Estimation of quality and quantity of DNA was made using spectrophotometer (Shimadzu UV160A, Japan).

Phenotyping data were collected for 20 different drought-tolerant traits that included six root traits, six yield and yield-related traits, five morphological traits, two physiological traits and one physiological trait for both the RIL populations (ICCRIL03...
SNP identification and array design

In order to select the SNPs for array, the resequencing data available from a diverse set of 429 chickpea accessions from other studies were used (unpublished). SNPs from earlier studies were filtered using ‘in-house’ multiple sequence alignments scripts and SNPs with no other SNP in the flanking 35 bp on both sides were selected. These selected SNPs were then subjected to seven different kinds of filters, namely (i) no SSR in flanking region, (ii) no indel in flanking region, (iii) only biallelic nature SNPs, (iv) minor allele frequency of 0.05, (v) GC% to be 40%–70%, (vi) no denaturing code permitted in the region and (vii) SNP quality score ≥30 to select best SNPs for the array. High-quality SNPs resulted from these assorted criteria were further subjected to in silico validation that involved preliminary screening of each selected SNP on the basis of p-convert values generated using Affymetrix power tool (APT) AxiomGTv1 algorithm to ensure a high-quality final array. Probability of SNP conversion represented by p-convert value was predicted using random forest model for both forward and reverse probes of each SNP. The model takes into account factors such as sequence of the probe, binding energy and expected reverse probes of each SNP. The model was trained using factors that identified significant SNPs from the selected SNPs using R/qtl program (Broman et al., 2003). The results obtained were compared with earlier studies to check the accuracy and further refinement.

Genotyping with Axiom®CicerSNP Array

In order to utilize the developed Axiom®CicerSNP Array, high-quality DNA extracted from fresh leaves of both the populations, ICCRIL03 with 245 lines and ICCRIL04 with 230 lines along with parental genotypes, were used for genotyping. In brief, high-quality total genomic DNA (100 ng) was randomly fragmented (25–125 bp) and fragmented DNA was purified before hybridizing with Axiom®CicerSNP Array. Under stringent conditions, nonspecific random ligations bound to targets were washed off. In order to identify multicolour ligation event at the array surface which points towards polymorphic nucleotide, array was stained, imaged and processed using GeneTitan® Multi-Channel (MC) instrument to generate the data. Allele calling was performed using generated ‘CEL files’ with Axiom Analysis Suite (1.1.0.616). Axiom best practice workflow was followed in order to get high-quality genotyping results (Figure 1). Samples with DQC < 0.82 and QC call rate <97% were not considered for further analysis. SNP QC was performed under default parameter for diploid species type, that is cr-cutoff ≥97; fld-cutoff ≥3.6; het-so-cutoff ≥−0.1; het-so-otv-cutoff ≥−0.3; hom-1cutoff ≥0.6; hom-1-of-2-cutoff ≥0.3. The whole data were analysed at an ‘Inbred penalty score’ of 12. The results generated were further processed using the SNPPolisher package that classifies SNPs into different classes as per Bassil et al. (2015). These different classes include MonoHighResolution (SNPs with genotyping data with QC pass but monomorphic across the genotypes studied), PolyHighResolution (genotyping data passed all QC with polymorphic SNPs), NoMinorHom (data passed all QC but only two clusters could be observed), Off-Target Variant (data with low-intensity cluster resulted from mismatches between the probe and the sequences for that group of genotypes) and Other (SNP with genotyping data with no clear cluster pattern and could not be assigned any of the other classes). PolyHighResolution SNPs were further filtered out for reproducibility and variance. In addition, markers with complex genotypes causing cluster splits are further filtered into additional classes using ‘Variance’ filters.

QTL analysis

The QTL analysis was carried out using QTL ICiMapping V3.3 software (Meng et al., 2015). For this, the genotyping data obtained as a result of application of Axiom®CicerSNP Array in the current study and the phenotyping data for 20 drought tolerance-related traits (as mentioned in Varshney et al., 2014b) were used. Composite interval mapping was carried out using ICIM-ADD mapping method keeping other parameters as default. The LOD threshold was set using 1000 permutations and P value ≤0.05. The results obtained were compared with earlier studies to check the accuracy and further refinement.

Acknowledgements

The authors are thankful to Bill & Melinda Gates Foundation (Tropical Legumes III) and Department of Agriculture Cooperation & Farmer’s Welfare, Ministry of Agriculture & Farmer’s Welfare, Government of India, for financial assistance. The work reported in this article was undertaken as a part of the CGIAR Research Program on Grain Legumes. ICRI SAT is a member of the CGIAR.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

M.R. performed most of the experiments; D.D. and M.R. selected and filtered SNPs for designing array; S.M.K., A.J. and M.R. analysed genotyping data and conducted genetic analysis; A.C.
and M.T. contributed genetic material and sequencing data; M.R., A.J. and R.K.V. interpreted the results and wrote the manuscript; R.K.V. conceived, designed and supervised the study.

References
Ahmad, F., Gaur, P.M. and Croser, J. (2005) Chickpea (Cicer arietinum L.). In Genetic Resources, Crop Improvement and Crop Improvement – Grain Legumes, Vol 1 (Singh, R.J. and Jauhar, P.P., eds), pp. 187–217. Boca Ratno: CRC Press.

Bagijn, P., Rouse, M.N. and Anderson, J.-A. (2016) Comparing genotyping-by-sequencing and single nucleotide polymorphism chip genotyping for quantitative trait loci mapping in wheat. Crop Sci. 56, 232–248.

Bassil, N.V., Davis, T.M., Zhang, H., Ficklin, S., Mittmann, M., Webster, T., Mahoney, L. et al. (2016) Development and preliminary evaluation of a 90 K Axiom SNP array for the allo-octolpid cultivated strawberry Fragaria ananassa. BMC Genomics, 16, 155. http://doi.org/10.1186/s12864-015-1310-1

Bianco, L., Cestaro, A., Linsmith, G., Muranty, H., Denance, C., Théron, A., Poncet, C. et al. (2016) Development and validation of the Axiom®Apple480K SNP genotyping array. Plant J. 86, 62–74. https://doi.org/10.1111/tpj.13145

Broman, K.W., Wu, H., Sen, S. and Churchill, G.A. (2003) R/qtl: QTL mapping in experimental crosses. Bioinformatics, 19, 889–890.

Chen, H., Xie, W., He, H., Yu, H., Chen, W., Li, J., Yu, R. et al. (2014) A high-density SNP genotyping array for rice biology and molecular breeding. Mol Plant, 7, 541–553.

Conesa, A., Gótz, S., García-Gómez, J.M., Terol, J., Talón, M. and Robles, M. (2005) Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics, 21, 3674–3676.

Cuc, L.M., Mace, E.S., Crouch, J.H., Quang, V.D., Long, T.D. and Varsney, R.K. (2008) Isolation and characterization of novel microsatellite markers and their application for diversity assessment in cultivated groundnut (Arachis hypogaea). BMC Plant Biol. 8, 55.

FAO 2014. http://www.fao.org/faostat/en/#data/QC

Gan, Y., Johnston, A.M., Knight, J.D., McDonald, C. and Stevenson, C. (2010) Nitrogen dynamics of chickpea: effects of cultivar choice, N fertilization, Rhizobium inoculation, and cropping systems. Can. J. Plant Sci. 90, 055666.

Ganal, M.W., Durstewitz, G., Polley, A., Bajgain, P., Rouse, M.N. and Anderson, J.A. (2016) Comparing genotyping-by-sequencing to the B73 reference genome. Can. J. Plant Sci. 96, 563–567.

McCouch, S.R., Zhao, K., Wright, M., Tung, C.W., Ebana, K., Thomson, M., Reynolds, A. et al. (2010) Development of genome-wide SNP assays for rice. Breed. Sci. 60, 524–535.

Meng, L., Li, H., Zhang, L. and Wang, J. (2015) QTL IciMapping: integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. Crop J. 3, 269–283.

Murali, F., Winter, P., Jüngling, R., Gil, J., Rubio, L., Cho, S., Cobos, M.I. et al. (2017) A consensus genetic map of chickpea (Cicer arietinum L.) based on 10 mapping populations. Euphytica, 175, 175–189.

Mora, F., Quiral, Y.A., Matus, I., Russell, J., Waugh, R. and del Pozo, A. (2016) SNP-based QTL mapping of 15 complex traits in barley under rain-fed and well-watered conditions by a mixed modeling approach. Front. Plant Sci. 7, 909.

PACE, J., Gardiner, C., Romay, C., Ganapathysubramanian, B. and Lübberstedt, T. (2015) Genome-wide association analysis of seedling root development in maize (Zea mays L.). BMC Genom. 16, 1.

Paridey, M.K., Agarwal, G., Kale, S.M., Cleengen, J., Nayak, S.N., Srisowathi, M., Chittikoni, A. et al. (2017) Development and evaluation of a high density genotyping ‘Axiom®Arachis’ array with 58 K SNPs for accelerating genetics and breeding in groundnut. Sci. Rep. 7, 40577.

Parida, S.K., Mukerji, M., Singh, A.K., Singh, N.K. and Mohapatra, T. (2012) SNPs in stress-responsive rice genes: validation, genotyping, functional relevance and population structure. BMC Genom. 13, 1.

Radhika, P., Gowda, S.J.M., Kadod, N.Y., Mhaise, L.B., Jamadagni, B.M., Sainani, M.N., Chandra, S. et al. (2007) Development of an integrated intraspecific map of chickpea (Cicer arietinum L.) using two recombinant inbred line populations. Theor. Appl. Genet. 115, 209–216.

Rashed, A., Hao, Y., Xia, X., Khan, A., Xu, Y., Varsney, R.K. and He, Z. (2017) Crop breeding chips and genotyping platforms: progress, challenges and perspectives. Mol. Plant. 10, 1047–1064. pii:S1674-2052(17)30174-0.

Riedelsheimer, C., Lisek, J., Czedik-Eysenberg, A., Altmann, T. et al. (2015) Genome-wide association mapping of leaf metabolic profiles for dissecting complex traits in maize. Proc. Natl Acad. Sci. USA, 109, 8872–8877.

Roorkiwal, M., Sawargoonkar, S.L., Chitikineni, A., Thudi, M., Saxena, R.K., Upadhyaya, H.D., Vales, M.I. et al. (2013) Single nucleotide polymorphism genotyping for breeding and genetics applications in chickpea and pigeonpea using the BeadXpress platform. Plant Genome, 6, 2.

Roorkiwal, M., Nayak, S.N., Thudi, M., Upadhyaya, H.D., Brunel, D., Mournet, P., This, D. et al. (2014) Allele diversity for abiotic stress responsive candidate genes in chickpea reference set using gene based SNP markers. Front. Plant Sci. 5, 248.

Roorkiwal, M., Rathore, A., Das, R.R., Singh, M.K., Jain, A., Srinivasan, S., Gaur, P.M. et al. (2016) Genome-enabled prediction models for yield related traits in chickpea. Front. Plant Sci. 7, 1666.

Sim, S.C., Durstewitz, G., Pieske, J., Wieseke, R., Ganul, M.W., Van Deynze, A., Hamilton, J.P. et al. (2012) Development of a large SNP genotyping array and genome selection of high-density genetic maps in tomato. PLOS ONE, 7, e40563.

Singh, J., Jayaswal, P.K., Pandä, K., Mandal, P., Kumar, V., Singh, B., Mishra, S. et al. (2015) Single-copy-based 50 K SNP chip for genetic studies and molecular breeding in rice. Sci. Rep. 5, 11600.

Singh, V.K., Khan, A.W., Jaganathan, D., Thudi, M., Roorkiwal, M., Takagi, H., Garg, V. et al. (2016) QTL-seq for rapid identification of candidate genes for 100-seed weight and root total plant dry weight ratio under rainfed conditions in chickpea. Plant Biotechnol. J. 14, 2110–2119.
Thudi, M., Li, Y., Jackson, S.A., May, G.D. and Varshney, R.K. (2012) Current state-of-art of sequencing technologies for plant genomics research. Brief. Funct. Genomics, 11, 3–11.

Thudi, M., Khan, A.W., Kumar, V., Gaur, P.M., Katta, K., Garg, V., Roorkiwal, M. et al. (2016a) Whole genome re-sequencing reveals genome-wide variations among parental lines of 16 mapping populations in chickpea (Cicer arietinum L.). BMC Plant Biol. 16, 10.

Thudi, M., Chitikineni, A., Liu, X., He, W., Roorkiwal, M., Yang, W., Jian, J. et al. (2016b) Recent breeding programs increased genetic diversity in both desi and kabuli varieties of chickpea (Cicer arietinum L.). Sci. Rep. 6, 38636.

Varshney, R.K., Mahendar, T., Aggarwal, R.K. and Börner, A. (2007) Genic molecular markers in plants: development and applications. In Genomics-Assisted Crop Improvement (Varshney, R.K., Tuberosa, R. eds.), pp. 13–29. Netherlands: Springer.

Varshney, R.K., Ribaut, J.M., Buckler, E.S., Tuberosa, R., Rafalski, J.A. and Langridge, P. (2012) Can genomics boost productivity of orphan crops? Nat. Biotechnol. 30, 1172–1176.

Voorrips, R.E. (2006) MapChart 2.2: Software for the Graphical Presentation of Linkage Maps and QTLs. Wageningen, the Netherlands: Plant Research International.

Wang, D.G., Fan, J.B., Siao, C.J., Berno, A., Young, P., Sapolsky, R., Ghandour, G. et al. (1998) Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. Science, 280, 1077–1082.

Yu, H., Xie, W., Li, J., Zhou, F. and Zhang, Q. (2014) A whole-genome SNP array (RICE6K) for genomic breeding in rice. Plant Biotechnol. J. 12, 28–37.

**Supporting information**

Additional Supporting Information may be found online in the supporting information tab for this article:

| Table S1 | Linkage group wise SNP data summary in ICCRILO3 (ICC 4958 × ICC 1882) population. |
| Table S2 | Linkage group wise SNP data summary in ICCRILO4 (ICC 283 × ICC 8261) population. |
| Table S3 | Details on QTLs identified for the 17 traits and two drought indices in ICC 4958 × ICC 1882 population. |
| Table S4 | Details on QTLs identified for the 17 traits and one drought indices in the ICC 283 × ICC 8261 population. |