Identification of stable heat tolerance QTLs using inter-specific recombinant inbred line population derived from GPF 2 and ILWC 292

Ashutosh Kushwah¹, Dharminder Bhatia¹, Inderjit Singh¹, Mahendar Thudi², Gurpreet Singh³, Shayla Bindra¹, Suruchi Vij¹, B. S. Gill¹, Chellapilla Bharadwaj⁴, Sarveet Singh¹*, Rajeev K. Varshney²,5*

1 Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India, 2 Center of Excellence in Genomics and Systems Biology (CEGSB), International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, 3 Regional Research Station, Faridkot, Punjab Agricultural University, Ludhiana, Punjab, India, 4 Division of Genetics, ICAR-Indian Institute of Agricultural Research (IARI), New Delhi, India, 5 State Agricultural Biotechnology Centre, Centre for Crop and Food Innovation, Food Futures Institute, Murdoch University, Murdoch, Western Australia, Australia

* sarveet62@pau.edu (SS); r.k.varshney@cgiar.org, rajeev.varshney@murdoch.edu.au (RKV)

Abstract

Heat stress during reproductive stages has been leading to significant yield losses in chickpea (Cicer arietinum L.). With an aim of identifying the genomic regions or QTLs responsible for heat tolerance, 187 F₈ recombinant inbred lines (RILs) derived from the cross GPF 2 (heat tolerant) × ILWC 292 (heat sensitive) were evaluated under late-sown irrigated (January-May) and timely-sown irrigated environments (November-April) at Ludhiana and Faridkot in Punjab, India for 13 heat tolerance related traits. The pooled ANOVA for both locations for the traits namely days to germination (DG), days to flowering initiation (DFI), days to 50% flowering (DFF), days to 100% flowering (DHF), plant height (PH), pods per plant (NPP), biomass (BIO), grain yield (YLD), 100-seed weight (HSW), harvest index (HI), membrane permeability index (MPI), relative leaf water content (RLWC) and pollen viability (PV) showed a highly significant difference in RILs. The phenotyping data coupled with the genetic map comprising of 1365 ddRAD-Seq based SNP markers were used for identifying the QTLs for heat tolerance. Composite interval mapping provided a total of 28 and 23 QTLs, respectively at Ludhiana and Faridkot locations. Of these, 13 consensus QTLs for DG, DFI, DFF, PH, YLD, and MPI have been identified at both locations. Four QTL clusters containing QTLs for multiple traits were identified on the same genomic region at both locations. Stable QTLs for days to flowering can be one of the major factors for providing heat tolerance as early flowering has an advantage of more seed setting due to a comparatively longer reproductive period. Identified QTLs can be used in genomics-assisted breeding to develop heat stress-tolerant high yielding chickpea cultivars.
Introduction

Chickpea (*Cicer arietinum* L.) or Garbanzo beans is a cool season food legume, originated from South-Eastern Turkey [1]. It is the second most consumed grain legume after dry bean grown worldwide. It is a self-pollinated diploid (*2n = 2x = 16*) crop with genome size of 738 Mb [2]. Chickpea is a nutrient-rich legume crop that contains 17–31% protein and significant amount of essential amino acids, vitamins and minerals [3]. It is free from anti-nutritional factors thereby the consumer preference for this legume is increasing. Despite of its economic importance, neither the area under cultivation nor productivity has increased to a desired level to meet the current demands. This sluggish pace of production trend is due to several abiotic and biotic constraints that have been challenging the crop [4,5]. Among abiotic stresses, heat stress is considered as one of the major constraints that affects the chickpea production. Chickpea is grown in winter season (November to April) in Northern India and experiences a high temperature (>35˚C) stress during the reproductive phase (mid-February to April). Studies on the impact of climate change on chickpea production underlined the effect of warmer temperatures on crop development and the yield. For example, rise in temperature of 1˚C reduces the chickpea yield up to 301 kg/ha in India [6]. The comparatively narrow genetic base of chickpea makes it vulnerable to high temperature which has a detrimental effect on its production [7]. Thus, there is a vital call for developing chickpea varieties that are heat resilient.

The effects of heat stress during the vegetative and reproductive growth stages using agronomic, morphological, phenological and physiological parameters have been studied in major crops such as wheat [8]; rice [9] and cotton [10], while limited studies have been conducted in chickpea [11]. In several studies, reproductive stage of the crop plant has been observed as the most sensitive stage of plant to heat stress [12]. Pod formation and seed set are adversely affected in chickpea if temperature rises above the threshold level, leading to reduction in grain yield [11,13]. Tissue hydration, crucial for physiological processes, measured by relative leaf water contents (RLWCs), is reduced during seedling, early flowering and pod development stages in chickpea subjected to stress [14]. Severe heat stress raises the temperature at cellular level, causes damage to the cell walls and increases the electrolyte leakage [15,16] thus, serve as an important adaptation to carry signals for induction of programmed cell death and assists to assimilate remobilization for development of seeds [16].

Heat stress tolerance is a complex trait and thus, an effective and simple screening method having well-defined traits for selecting heat-tolerant genotypes under field conditions is indispensable [17]. Genotype by environment (*G × E*) interaction also hampers the direct selection of heat-tolerant genotypes. Visual inspection, selection for physiological attributes related to plant response to high temperature, empirical selection for yield and marker assisted selection (MAS) are the four important selection methods which are used to improve heat tolerance through breeding [18]. Due to instability and poor heritability, lower genotypic variance for seed yield under stress [19], quantitative nature of traits, prevalence of linkage between desired and undesired genes [20] and complex genetic background of traits [21], breeding for yield under heat stress condition by means of conventional approaches has not been fairly successful over the years. Under such circumstances, molecular breeding seems to be a better strategy that can be deployed by targeting heat tolerance related traits.

In chickpea until 2005, about 150 SSR markers and sparse genetic maps were available which have limited efficacy for trait dissection. During last decade, chickpea research community has decoded the chickpea genome [2,22] and developed several genomic [23–25] and transcriptomic resources [26,27] that have transformed chickpea from “orphan legume crop” to “genomics resource rich legume crop” [28]. Now, several high-density genetic maps, physical maps and consensus maps are available for trait dissection [29–34] which provide new
opportunities for accelerating research for faster genetic gains in chickpea. With the rapid development in next generation sequencing technologies, accelerated genotyping platforms such as genotyping by sequencing (GBS) has become a widely used approach in genetic studies. Backed by power of NGS, GBS is a high throughput and low cost genotyping method that can mine thousands of SNPs across the genome in number of individuals in a mapping population in very less time [35]. Restriction-site-associated DNA sequencing (RAD-seq) has been effectively applied for development of SNP markers, high-density genetic map construction, QTL mapping, phylogenetic research and population genetics [36]. Double Digestion Restriction-site-Associated DNA sequencing (ddRAD-seq) technique, developed [37], can adjust number of fragments by utilizing two different restriction enzymes [38] and exclusively uses size selection for recovering the appropriate number of regions, which arbitrarily distributed throughout the genome and maximizes the ability of multiplexing of numerous samples [39].

Chickpea is known to have narrow genetic base as compared to most other legumes [40,41]. Due to relatively low levels of polymorphism, inter-specific crosses between *C. arietinum* and *C. reticulatum* have been the primary focus for genetic studies [42]. The amount of polymorphism in an inter-specific mapping population varied from 16% to 36%, whereas 9.5% only in an intra-specific mapping population [23]. High-resolution genetic linkage maps can also be constructed by exploiting the inter-specific polymorphisms between *C. arietinum* and *C. reticulatum* [43]. Variation detection based on SNPs has also shown the similar trends. Thus, an inter-specific mapping population from a cross between GPF 2 (*C. arietinum*) and ILWC 292 (*C. reticulatum*) has been used in the present study to identify the key genomic regions of heat tolerance related traits using ddRAD-seq based genotyping and phenotyping in contrasting environmental conditions. Chickpea cultivar GPF2 is a semi erect, medium tall cultivar released by Punjab Agricultural University, Ludhiana, Punjab and recommended for cultivation in Punjab state and in North Western Plains Zone of India. Another parent of RILs, ILWC 292 (*C. reticulatum*) is the wild species of chickpea having semi prostrate growth habit. After evaluating the RIL population under late-sown irrigated and timely-sown irrigated environments at two locations and generating ddRAD-Seq data, this study reports a genetic map for the above mentioned population and identification of QTLs associated with heat tolerance. Some of these QTLs, after validation, should be useful in genomics-assisted breeding for heat stress tolerance in chickpea.

**Materials and methods**

**Plant materials and phenotyping**

A total of 187 recombinant inbred lines (RILs) segregating for heat tolerance related traits from an inter-specific cross of cultivar GPF 2 (heat tolerant) × *C. reticulatum* acc ILWC 292 (heat sensitive) developed using single seed descent method. The RIL population along with parents was planted during winter’s season of 2017–18 in alpha lattice design (17 × 12) under timely-sown (November-April) and late-sown (January-May) conditions with three replications at two locations, i.e., Ludhiana and Faridkot. The Ludhiana (30.9010˚ N, 75.8573˚ E) and Faridkot (30.6769˚ N, 74.7583˚ E) sites are categorized as a semi-arid sub-tropical region and semi-arid dry region, respectively. Both sites comprise loamy sand with 59.8% sand and 16.5% clay (Typic Ustorthents). The average annual rainfall is 700 mm at Ludhiana and 450 mm at Faridkot, of which more than 70% occurs from July to September. Each RIL was sown in paired rows of 2 m length at 30 cm × 10 cm spacing. The late-sown chickpea was exposed to terminal heat stress because the conserved soil moisture recedes as the season progresses and the temperature rises [44,45]. Thus, heat tolerance related traits have been studied in late-sown irrigated condition, using the timely-sown irrigated condition as a control. During the
screening of heat tolerance, irrigation was provided to avoid the confounding effect of drought stress. The daily maximum temperatures for late-sown as well as timely-sown conditions during the reproductive phase at both locations (Ludhiana and Faridkot) were recorded (Fig 1).

Phenotypic data were collected for a total of 13 heat tolerance related traits viz., days to germination (DG), days to flowering initiation (DFI), days to 50% flowering (DFF), days to 100% flowering (DHF), plant height (PH), number of pods per plant (NPP), biomass (BIO), yield (YLD), 100-seed weight (HSW), harvest index (HI), membrane permeability index (MPI), relative leaf water content (RLWC) and pollen viability (PV). Randomly five plants were selected to record the observations on PH, NPP, BIO and YLD in each plot. The data on DG, DFI, DFF, DHF and HSW were recorded on plot basis. HI was calculated as:

Harvest Index (HI) = (seed yield/total shoot biomass) × 100
Pollen viability test was studied by collecting the pollen samples at the time of 50% flowering. The pollen viability was observed by using 2% acetocarmine stain described by [46]. The MPI was determined according to the method described earlier [47] and modified [48] using following formula:

\[ \text{Membrane permeability index (MPI)} = [1 - (C1/C2)] \times 100 \]

Where, \( C1 \) = Initial electrical conductivity at (40˚C); \( C2 \) = Final electrical conductivity at (100˚C).

The RLWC was calculated by the formula [49] using following formula:

\[ \text{RLWC (%)} = (\text{FW-DW} / \text{TW-DW}) \times 100 \]

Where, FW = Fresh weight, DW = Dry weight, TW = Turgid weight.

**Analysis of variance (ANOVA), best linear unbiased predictor(s) (BLUPs) and correlation coefficient analysis**

The ANOVA was calculated for individual environments using mixed model analysis to estimate the contribution made by each factor to the total variation using SAS-software version 9.3 [50]. The data from timely-sown and late-sown conditions were used to estimate BLUPs using the residual maximum likelihood algorithm (ReML) in R package lmer [51]. BLUPs were estimated for 13 traits and scatter plots were drawn for all the traits using BLUPs to find the correlation between two locations i.e., Ludhiana and Faridkot.

**QTL analysis**

The RIL population was genotyped with ddRAD-seq [37] that used restriction enzymes \textit{Pst1} and \textit{Msp1} (Thermo Fisher Scientific, MA, United States). The ddRAD-seq data analysis of RILs for SNP discovery and development of linkage map has already been described earlier [52]. The QTL analysis has been performed with the composite interval mapping (CIM) method executed in the Windows QTL Cartographer V2.5 software package [53] using genotypic and phenotypic data. The CIM analysis was run using forward and backward stepwise regression. For each trait, experiment-wise significance thresholds \((p = 0.05)\) were determined with 1000 permutations for QTL detection. The position of the QTLs was identified on the basis of its logarithm of odds (LOD) peak location with 95% confidence interval. The LOD score of 3 has been adapted as threshold LOD value. The percentage of phenotypic variance and additive effect described by QTLs was also estimated. The phenotypic contribution \((R^2)\) was estimated as the percentage of variance explained by each QTL in proportion to the total phenotypic variance, while additive effect was estimated to find the positive or negative effect for the respective trait.

**Results**

**Phenotypic performance of the mapping population**

The RILs along with parents were evaluated in timely-sown (non-stress) and late-sown (heat-stress) conditions at Ludhiana and Faridkot. The late-sown condition in chickpea exposed the RIL population to heat stress during reproductive stage as the maximum temperature crossed the threshold limit during that period at both the locations (Fig 1). Significant variation was observed for heat stress related traits among the RILs as well as the parents under timely-sown and late-sown conditions (Table 1, Fig 2, S1 and S2 Figs). The contrast analysis of parents for all the traits depicts that there were highly significant differences between parents under timely-sown and late-sown conditions (Table 1). All the traits were significantly affected by heat stress environment, except HSW and HI which were moderately affected. The pooled
Table 1. Mean performance of RILs evaluated under timely-sown and late-sown conditions based on pooled data from Ludhiana and Faridkot.

| Trait                              | Environment | ILWC 292 | GPF 2 | Contrast between parents | Mean Coefficient of variation (%) | Range          | Genotypic variance | Genotype × location interaction variance |
|------------------------------------|-------------|----------|-------|--------------------------|-----------------------------------|----------------|-------------------|----------------------------------------|
| Days to germination                | Timely sown | 12.34    | 8.12  | 44.33 **                 | 9.33                              | 5.05           | 8.12–12.34        | 1.50**                                 |
|                                    | Late sown   | 15.53    | 10.67 | 75.00 **                 | 13.12                             | 9.23           | 10.63–16.16       | 5.09**                                 |
| Days to flowering initiation       | Timely sown | 90.33    | 82.8  | 78.87 **                 | 85.99                             | 2.01           | 78.91–90.33       | 1.96**                                 |
|                                    | Late sown   | 82.82    | 75.86 | 11.53                    | 75.42                             | 4.15           | 69.17–83.49       | 6.01**                                 |
| Days to 50% flowering              | Timely sown | 94.26    | 86.46 | 171.50 **                | 89.46                             | 2.08           | 82.35–94.26       | 2.24**                                 |
|                                    | Late sown   | 84.74    | 78.76 | 19.17 **                 | 78.15                             | 3.97           | 72.26–86.11       | 9.92**                                 |
| Days to 100% flowering             | Timely sown | 98.08    | 89.78 | 171.50 **                | 93.1                              | 1.96           | 86.67–98.08       | 2.28**                                 |
|                                    | Late sown   | 88.27    | 82.66 | 10.77                    | 81.99                             | 3.86           | 75.75–90.05       | 8.77**                                 |
| Plant height (cm)                  | Timely sown | 42.42    | 58.87 | 65.43 **                 | 45.68                             | 10.47          | 33.82–58.87       | 4.05**                                 |
|                                    | Late sown   | 21.82    | 43.9  | 125.85 **                | 29.33                             | 32.08          | 15.14–48.88       | 45.96**                                |
| Number of pod per plant            | Timely sown | 43.53    | 68.54 | 133.10 **                | 47.39                             | 21.75          | 25.13–75.07       | 18.64**                                |
|                                    | Late sown   | 20.3     | 43.96 | 117.41 **                | 29.26                             | 31.29          | 14.65–48.28       | 35.46**                                |
| Biomass per plant (g)              | Timely sown | 76.78    | 113.32| 55.17 **                 | 81.33                             | 15.47          | 51.55–113.70      | 10.35**                                |
|                                    | Late sown   | 38       | 76.26 | 131.55 **                | 57.03                             | 30.29          | 30.16–90.32       | 64.94**                                |
| Yield per plant (g)                | Timely sown | 27.91    | 49.74 | 232.07 **                | 32.14                             | 27.07          | 14.13–54.69       | 18.16**                                |
|                                    | Late sown   | 12.35    | 33.39 | 125.87 **                | 19.52                             | 43.33          | 8.35–38.07        | 53.39**                                |
| 100 seed weight (g)                | Timely sown | 11.27    | 16.18 | 1629.73 **               | 14.22                             | 16.11          | 9.79–18.42        | 20.41**                                |
|                                    | Late sown   | 9.32     | 15.85 | 174.08 **                | 13.06                             | 22.24          | 7.92–18.20        | 46.74**                                |
| Harvest index (%)                  | Timely sown | 36.63    | 43.55 | 15.81 **                 | 38.98                             | 16.48          | 22.49–52.86       | 12.52**                                |
|                                    | Late sown   | 32.61    | 43.97 | 22.90 **                 | 32.85                             | 16.1           | 21.75–44.87       | 19.04**                                |
| Membrane permeability index        | Timely sown | 42.23    | 28.81 | 150.71 **                | 39.42                             | 12.12          | 28.70–50.76       | 15.49**                                |
|                                    | Late sown   | 52.71    | 38.03 | 12.71 **                 | 46.28                             | 15.82          | 32.23–59.71       | 27.38**                                |
| Relative leaf water content (%)    | Timely sown | 65.28    | 88.31 | 178.91 **                | 74.85                             | 9.37           | 59.06–89.94       | 6.07**                                 |
|                                    | Late sown   | 47.72    | 78.61 | 290.43 **                | 62.41                             | 17.05          | 45.96–79.89       | 29.92**                                |
| Pollen viability (%)               | Timely sown | 81.29    | 93.09 | 27.36 **                 | 84.74                             | 7.4            | 70.99–94.77       | 10.83**                                |
|                                    | Late sown   | 69.43    | 85.34 | 36.61 **                 | 73.55                             | 15.59          | 58.98–93.13       | 22.49**                                |

* = Significant at 5% probability level  
** = Significant at 1% probability level.

https://doi.org/10.1371/journal.pone.0254957.t001
Fig 2. Graphical representations of RILs for the various traits in chickpea using pooled phenotypic data between timely-sown and late-sown condition at Ludhiana and Faridkot.

Ludhiana

Faridkot

https://doi.org/10.1371/journal.pone.0254957.g002
ANOVA for both locations in timely-sown as well as late-sown conditions for all the traits showed highly significant differences in RILs for genotypic variance (Table 1). Significant differences were also observed for genotype \( \times \) location (\( G \times L \)) interaction variance for all the traits, except DG, DFI, DFF and DHF.

Correlation between locations

To identify the QTLs that could be consistent at two different locations, BLUPs (Best Linear Unbiased Predictors) for genotypes were identified for both the locations (S1 Table). Even though there was significant \( G \times L \) interaction, the scatter plots using BLUP values showed highly significant relationship between locations for most of the traits except PH, HI and RLWC which showed moderately high correlation coefficient (Fig 3). Correlation coefficient (\( r^2 \)) ranged from 0.67 for RLWC to 0.94 for DFF between the two locations.

QTLs identified for heat-stress tolerance

Generation of genotyping data for 1365 filtered and parental polymorphic SNPs and construction of linkage map has been described earlier [52]. Here, genotypic data of 1365 informative SNPs, linkage map distances and BLUP values of two different locations were used to identify QTLs for heat stress tolerance related traits. A total of 28 QTLs for Ludhiana and 23 QTLs at...
Faridkot were identified for 13 different traits (Tables 2 and 3; Fig 4, S3 Fig). Out of these, 13 stable QTLs for DG, DFI, DFF, DHF, PH, YLD and MPI have been identified at both the locations (highlighted with bold in Tables 2 and 3). Four QTL clusters containing QTLs for DG, DFI, DFF, DHF, PH and MPI were identified on chromosome 1, 2, 4 and 6 on the same genomic position at both the locations (Fig 4, S2 Fig). All of these QTLs were distributed on seven linkage groups, while linkage group on chromosome 8 harbours no QTL. Maximum QTLs were present on chromosomes 1 and 4 at both the locations. The highest phenotypic variation was observed for biomass (13.71%) at Ludhiana and days to 50% flowering (18.30%) at Faridkot. The highest LOD value was observed for days to germination (5.27) at Ludhiana and days to 50% flowering (7.26) at Faridkot. QTLs having positive or negative additive effect for a particular trait imply that the increase in the proportion of the phenotypic variation of that particular trait is contributed by the allele from GPF 2 or \textit{C. reticulatum} acc ILWC 292, respectively.

**Discussion**

Heat stress is increasingly becoming a severe constraint to chickpea production due to the changing scenario of chickpea cultivation and expected overall increase in global temperatures.

| Trait | Chr. QTL name | LOD | Additive effect | Phenotypic variation explained(%) | Left flanking marker | Right flanking marker |
|-------|---------------|-----|----------------|----------------------------------|---------------------|----------------------|
| Days to germination | 5 qdg-01 | 3.53 | 0.0828 | 8.98 | CNC_021164.1.40739029 | CNC_021164.1.39765508 |
| | 6 qdg-02 | 5.27 | 0.0985 | 11.3 | CNC_021165.1.18056125 | CNC_021165.1.513774 |
| | 7 qdg-03 | 2.3 | 0.0529 | 5.59 | CNC_021166.1.28474588 | CNC_021166.1.8118822 |
| Days to flowering initiation | 1 qdfi-01 | 3.59 | 0.5876 | 9.21 | CNC_021160.1.35858685 | CNC_021160.1.8023246 |
| | 4 qdfi-02 | 3.88 | -0.4694 | 9.94 | CNC_021163.1.11351378 | CNC_021163.1.11351447 |
| Days to 50% flowering | 1 qdff-01 | 3.93 | 0.6375 | 10.13 | CNC_021160.1.35858685 | CNC_021160.1.8023246 |
| | 3 qdff-02 | 3.29 | -0.4136 | 7.9 | CNC_021162.1.21073044 | CNC_021162.1.39357194 |
| | 4 qdff-03 | 3.71 | -0.4681 | 9.25 | CNC_021163.1.11351378 | CNC_021163.1.11351447 |
| | 6 qdff-04 | 3.07 | 0.469 | 6.8 | CNC_021165.1.8056125 | CNC_021165.1.513774 |
| Days to 100% flowering | 1 qdhf-01 | 3.58 | 0.5381 | 9 | CNC_021160.1.35858685 | CNC_021160.1.8023246 |
| | 3 qdhf-02 | 3.11 | -0.4143 | 7.6 | CNC_021162.1.21073044 | CNC_021162.1.39357194 |
| | 4 qdhf-03 | 3.52 | -0.4674 | 8.72 | CNC_021163.1.13462111 | CNC_021163.1.11351378 |
| | 4 qdhf-04 | 2.5 | -0.412 | 5.7 | CNC_021163.1.48163277 | CNC_021163.1.48245021 |
| Plant height (cm) | 2 qph-01 | 3.66 | -0.431 | 7.93 | CNC_021161.1.31430073 | CNC_021161.1.9956999 |
| | 4 qph-02 | 3.56 | 0.5285 | 10.2 | CNC_021163.1.38540774 | CNC_021163.1.38370939 |
| Number of pod per plant | 1 qnpp-01 | 4.53 | -1.4312 | 12.76 | CNC_021160.1.41330352 | CNC_021160.1.41044856 |
| | 3 qnpp-02 | 4.02 | 1.3582 | 11.77 | CNC_021162.1.38774358 | CNC_021162.1.29358942 |
| Biomass per plant (g) | 1 qbio-01 | 4.41 | -1.635 | 12.22 | CNC_021160.1.41330352 | CNC_021160.1.41044856 |
| | 4 qbio-02 | 3.33 | 1.642 | 13.71 | CNC_021163.1.11351378 | CNC_021163.1.11351447 |
| Yield per plant (g) | 2 qyld-01 | 3.84 | -1.1401 | 10.28 | CNC_021161.1.31430073 | CNC_021161.1.9956999 |
| | 3 qyld-02 | 3.1 | 1.0001 | 8.6 | CNC_021163.1.38774358 | CNC_021163.1.29358942 |
| 100 seed weight (g) | 6 qhsw-02 | 3.38 | -0.2103 | 8.56 | CNC_021165.1.41420023 | CNC_021165.1.41646826 |
| Membrane permeability index | 2 qmpi-01 | 2.52 | 0.5386 | 6.69 | CNC_021161.1.3663690 | CNC_021161.1.31430073 |
| | 4 qmpi-02 | 3.21 | -0.8137 | 10.07 | CNC_021163.1.38540774 | CNC_021163.1.38370939 |
| Relative leaf water content (%) | 7 qrlwc-01 | 3.34 | 0.158 | 9.15 | CNC_021166.1.25250619 | CNC_021166.1.42984094 |
| Pollen viability (%) | 2 qpv-01 | 4.54 | -1.151 | 11.28 | CNC_021161.1.3663690 | CNC_021161.1.31430073 |
| | 4 qpv-02 | 3.33 | 1.2303 | 13.59 | CNC_021163.1.11351447 | CNC_021163.1.12812015 |
| | 4 qpv-03 | 3.61 | 0.9206 | 8.92 | CNC_021163.1.12812016 | CNC_021163.1.12811935 |

https://doi.org/10.1371/journal.pone.0254957.t002
due to climate change. A threshold temperature of 35˚C was found to be critical in differentiating heat tolerant and heat sensitive genotypes in chickpea under field conditions [44]. Chickpea suffers heavy yield losses when exposed to heat stress at the reproductive stage. In this study, late-sown condition was proved to be an ideal condition for heat tolerance screening as the temperature at the time of pod setting crossed the threshold limit (Fig 1). Late sowing exposes the chickpea to terminal heat stress condition as the season progresses; temperature increases and the conserved moisture depleted from the soil [45]. Heat stress during pod development reduced the seed yield at higher rate as compared to heat stress during early flowering [11]. However, earliness is the most significant trait offering tolerance to heat and drought stress. Thus, late sowing is effective for heat tolerance screening in chickpea [54].

Interspecific RIL population and its parents showed significant differences for yield and yield contributing traits and physiological traits in late-sown as compared to timely-sown condition. Most of the morphological and physiological traits were significantly affected by heat stress environment in some previous studies [44,55–58]. Overall, there was reduction in seed yield in RILs under heat stress conditions. Low pollen viability in the RILs could be one of the major causes of reduced seed yield during heat stress environment [59]. Pollen sterility was reported to be one of the major reasons for poor pod setting during pre-anthesis high temperature stress [60]. Low pollen viability, indehiscent anthers and other anther abnormalities are associated with poor pod set during pre-anthesis high temperature stress [61]. Whereas, high temperature stress during post-anthesis is related with poor pollen germination, pollen tube growth and fertilization [62,63]. Development of male and female reproductive parts like

Table 3. Summary of the QTLs associated with the heat stress related traits at Faridkot.

| Trait                     | Chr. | QTL name | LOD | Additive effect | Phenotypic variation explained (%) | Left flanking marker | Right flanking marker |
|---------------------------|------|----------|-----|----------------|-----------------------------------|----------------------|-----------------------|
| Days to germination       | 7    | qdg-01   | 4.20| 0.0067         | 8.9                               | CNC_02116.1.20474588 | CNC_02116.1.81188822 |
|                           | 6    | qdg-02   | 2.93| 0.0069         | 6.7                               | CNC_02116.1.18056125 | CNC_02116.1.513774   |
| Days to flowering initiation | 1    | qdfi-01  | 5.39| 0.3836         | 13.68                             | CNC_02116.1.35885685 | CNC_02116.1.8023246  |
|                           | 4    | qdfi-02  | 3.19| -0.2153        | 7.64                              | CNC_02116.1.11351378 | CNC_02116.1.11351447 |
| Days to 50% flowering     | 1    | qdff-01  | 4.46| 0.4609         | 14.72                             | CNC_02116.1.33682907 | CNC_02116.1.35885685 |
|                           | 1    | qdff-02  | 7.26| 0.5151         | 18.3                              | CNC_02116.1.35885685 | CNC_02116.1.8023246  |
|                           | 4    | qdff-03  | 2.50| -0.2456        | 6.49                              | CNC_02116.1.11351378 | CNC_02116.1.11351447 |
|                           | 6    | qdff-04  | 3.09| 0.2894         | 7.07                              | CNC_02116.1.18056125 | CNC_02116.1.513774   |
| Days to 100% flowering    | 1    | qdhf-01  | 3.75| 0.3482         | 9.06                              | CNC_02116.1.35885685 | CNC_02116.1.8023246  |
|                           | 4    | qdhf-02  | 4.58| -0.2849        | 9.94                              | CNC_02116.1.13462111 | CNC_02116.1.11351378 |
|                           | 4    | qdhf-03  | 3.76| -0.3236        | 8.07                              | CNC_02116.1.48163277 | CNC_02116.1.48245021 |
| Plant height (cm)         | 2    | qph-01   | 2.28| -0.7371        | 6.19                              | CNC_02116.1.31430073 | CNC_02116.1.99560999 |
| Yield per plant (g)       | 1    | qyld-01  | 3.22|-1.193          | 7.86                              | CNC_02116.1.18096653 | CNC_02116.1.3078636  |
| 100 seed weight (g)       | 4    | qhsw-01  | 3.35|-0.1654         | 8.9                               | CNC_02116.1.44507694 |                      |
|                           | 5    | qhsw-02  | 3.73| 0.178          | 11.11                             |                      |                       |
| HI                        | 1    | qhi-01   | 3.72| 0.4889         | 7.82                              |                      |                       |
|                           | 1    | qhi-02   | 3.07| 0.4633         | 7.58                              |                      |                       |
|                           | 1    | qhi-03   | 3.09| -0.3231        | 8.36                              |                      |                       |
| Membrane permeability index | 1    | qmpi-01  | 3.29| 0.8733         | 8.04                              |                      |                       |
|                           | 2    | qmpi-02  | 2.52| 0.7048         | 7.81                              |                      |                       |
|                           | 4    | qmpi-03  | 3.55| 0.8254         | 9.23                              |                      |                       |
| Pollen viability (%)      | 5    | qpv-01   | 3.46|-1.1695         | 12.16                             |                      |                       |

https://doi.org/10.1371/journal.pone.0254957.t003
pollen and stigma are the most sensitive organs to heat stress in reproductive biology [64]. Pod set percentage was reduced at high temperatures in chickpea and concluded that pollen viability is the major reason of sterility under high temperature stress at anthesis in chickpea [57]. Thus, study of pollen grains may help to expect the genetic variations present among the genotypes for heat tolerance at reproductive phase. Late-sown condition adversely affected the physiological traits such as RLWC, MPI and morphological traits like plant height, total dry matter, grain yield and test weight as compared to controlled conditions [65]. Generally, reduced water availability is frequently associated with heat stress under field conditions [66]. The RLWC has been reduced due to increase in transpiration under heat stress condition [67,68]. Heat stress can reduce the grain yield by disturbing both source and sink relationship for photosynthate assimilates [17].

Variances due to G × L interaction were highly significant for all the traits except DG, DFI, DFF, DHF, which were non-significant at both locations. Both the locations had almost similar rise in temperature under late-sown condition with very little differences. Significant G × L interaction could be due to other factors than the temperature. To encounter these differences, BLUP values for genotypes for both the locations were also estimated taking location as random effect. BLUP values of RIL population for both the locations showed high correlations with each, thus showing that these can be used for further QTL analysis to find the consistent QTLs at both the locations. A highly significant genetic and genotype × environment interaction variance for pooled analysis of two heat stress environments for days to 50% flowering, pod setting percentage, biomass, number of filled pods, yield, harvest index, 100-seed weight and total number of seeds was reported [58]. Further, a highly significant genetic and genotype × environment interaction variance across the heat stress environments was also reported [69].
A little progress could be made to breed cultivars harbouring complex quantitative traits through conventional selection due to polygenic control and higher genotype x environment interaction [70]. Thus, mapping QTLs for complex quantitative traits is an important pre-requisite for understanding their genetic architecture and precise transfer in the background of commercial cultivars. A total of 28 QTLs at Ludhiana and 23 QTLs at Faridkot were identified for 13 traits in the RIL population evaluated under timely-sown and late-sown conditions. Out of these, 13 stable QTLs for DG, DFI, DFF, DHF, PH, YLD and MPI were identified at both the locations. The stable QTLs for DG have been reported first time in our study on chromosome 6 and 7. Though DG is not affected by heat stress under late-sown condition, but QTL represents the genotypic differences in RIL population for this trait, which can be used in marker assisted breeding programmes. A stable QTL for seed yield under heat stress conditions was identified on chromosome 2.

Early flowering has an advantage of more pod setting before the occurrence of heat stress due to comparatively longer reproductive phase. Thus, early flowering can be one of the major factors for providing tolerance against heat stress. The stable QTLs for flowering harbour on chromosome 1 and 4, suggesting that these loci confer flowering time in chickpea. The QTLs for seed yield were earlier reported [71] who identified three QTLs, while Rehman et al., [72] identified two QTLs located on chromosome 1. For seed yield, one QTL on chromosome 4 [73] and for seed weight, two QTLs on chromosome 4 and 8 [74] were mapped. Using GBS approach four QTLs for yield per plant located on chromosome 4, 6, 7 and 8 were reported [75]. Two QTLs for seed weight on chromosome 6 (LOD = 2.6) and 7 (LOD = 2.7) and two QTLs for plant height on chromosome 1 (LOD = 3.25) and 3 (LOD = 2.7) were identified [76]. QTL for 100-seed weight was identified [77] on chromosome 4 which was in accordance to our results. Several QTLs for plant height, number of pods per plant, 100-seed weight, biomass, harvest index and yield which were also at the same locus as identified in our study [31]. Likewise, several QTLs have been found [78] for plant height, number of pods per plant, 100-seed weight and yield which were at the same locus as identified in our study. More recently, 77 QTLs (37 major and 40 minor) were reported for 12 of 13 heat tolerance related traits, including a genomic region on CaLG07 harbours QTLs explaining >30% phenotypic variation for days to pod initiation, 100 seed weight [79]. Four QTL clusters containing QTLs for DG, DFI, DFF, DHF, PH and MPI identified on chromosome 1, 2, 4 and 6 on the same genomic position at both the locations. A total of nine QTL clusters for drought tolerance related traits identified [31], out of which one major cluster, present on chromosome 4, was referred as “QTL-hotspot”. Jaganathan et al. [33] refined this “QTL-hotspot” region by genotyping-by-sequencing (GBS) approach and identified 49 SNP markers in this region. Further, Kale et al. [34] partitioned this “QTL-hotspot” into two regions “QTL-hotspot_a” and “QTL-hotspot_b” and identified four promising candidate genes responsible for drought stress tolerance in chickpea. QTL clusters identified in our study can be targeted for marker-assisted breeding for introgression into elite cultivars to enhance heat stress tolerance.

QTLs for multiple traits identified at both the locations co-localised at same genomic position. These QTL regions could be prime target in breeding programme for improving chickpea cultivars under heat stress conditions. QTLs for multiple traits for single location were also identified, however no strong signals could be observed for other location. This could be due to significant variation observed for genotype x location. However, the present study has identified the potential genomic regions for important agronomic and physiological traits that could be used in further breeding programme. These identified QTLs will serve as a potential tool for identification of candidate genes with the recent advances in genomics and transcriptomics resources in chickpea.
Conclusions
This study illustrated the presence of significant differences in interspecific RIL population and its parents for yield and yield contributing traits and physiological traits in late-sown as compared to timely-sown condition. Reduction in seed yield during heat stress could be associated with low pollen viability in the RILs. A total of 28 QTLs at Ludhiana and 23 QTLs at Faridkot location were identified for 13 traits using SNP genotyping by ddRAD-Seq and BLUPs in the RIL population evaluated under timely-sown and late-sown conditions. Out of these, 13 stable QTLs for 7 traits were identified at both the locations. The stable QTLs for days to germination have been reported first time in the present study. The stable QTLs for flowering suggesting that these loci confer flowering time in chickpea and early flowering has an advantage of more pod setting before the occurrence of heat stress due to comparatively longer reproductive phase. Four QTL clusters containing QTLs for multiple traits identified on the same genomic region at both locations which would be the prime target in breeding programme for improving heat stress tolerance in chickpea.

Supporting information
S1 Fig. Graphical representations of RILs for the various traits in chickpea using pooled phenotypic data between timely-sown and late-sown condition at Ludhiana.
(TIF)
S2 Fig. Graphical representations of RILs for the various traits in chickpea using pooled phenotypic data between timely-sown and late-sown condition at Faridkot.
(TIF)
S3 Fig. Logarithm of odds ratio (LOD) curves obtained by composite interval mapping for quantitative trait loci (QTLs) mapped for different traits in RIL population (GPF 2 × C. reticulatum acc ILWC 292) in heat stress environment.
(JPG)
S1 Table. Best linear unbiased prediction value (BLUPs) for RIL population of pooled phenotypic data between timely-sown and late-sown conditions at Ludhiana.
(DOCX)
S2 Table. Best linear unbiased prediction value (BLUPs) for RIL population of pooled phenotypic data between timely-sown and late-sown conditions at Faridkot.
(DOCX)

Acknowledgments
We thank all cooperation partners and employees which contributed to this study.

Author Contributions
Conceptualization: Inderjit Singh, B. S. Gill, Sarvjeet Singh.
Data curation: Ashutosh Kushwah, Gurpreet Singh, Shayla Bindra, Suruchi Vij, Sarvjeet Singh.
Formal analysis: Ashutosh Kushwah, Dharmander Bhatia, Mahendar Thudi.
Funding acquisition: Sarvjeet Singh, Rajeev K. Varshney.
Investigation: Ashutosh Kushwah, Gurpreet Singh, Shayla Bindra, Suruchi Vij.
Methodology: Inderjit Singh, B. S. Gill, Sarvjeet Singh.

Project administration: Rajeev K. Varshney.

Resources: Inderjit Singh, B. S. Gill.

Software: Dharminder Bhatia, Mahendar Thudi, Sarvjeet Singh.

Supervision: Chellapilla Bharadwaj, Sarvjeet Singh, Rajeev K. Varshney.

Validation: Dharminder Bhatia, Sarvjeet Singh.

Visualization: Chellapilla Bharadwaj, Rajeev K. Varshney.

Writing – original draft: Ashutosh Kushwah, Sarvjeet Singh.

Writing – review & editing: Mahendar Thudi, Rajeev K. Varshney.

References

1. Ladizinsky G. A new Cicer from Turkey. Notes Royal Botanical Garden Edinburgh 1975; 34: 201–02.
2. Varshney RK, Mohan SM, Gaur PM, Gangarao NVPR, Pandey MK, Bohra A, et al. Achievements and prospects of genomics-assisted breeding in three legume crops of the semi-arid tropics. Biotechnology Advances. 2013; 10: 1016–22. https://doi.org/10.1016/j.biotechadv.2013.01.001 PMID: 23313999
3. Fayaz H, Rather I, Wani A, Tyagi S, Pandey R, Mir, R. Characterization of chickpea gene pools for nutrient concentrations under agro-climatic conditions of North-Western Himalayas. Plant Genetic Resources: Characterization and Utilization. 2019; 17(5): 464–467.
4. Thudi M, Upadhyaya HD, Rathore A, Gaur PM, Krishnamurthy L, Poonkkiwai M, et al. Genetic dissection of drought and heat tolerance in chickpea through genome-wide and candidate gene-based association mapping approaches. PLoS ONE. 2014; 9(5): e96758. https://doi.org/10.1371/journal.pone.0096758 PMID: 24801366
5. Mir AH, Bhat MA, Dar SA, Sofi PA, Bhat PA, Mir RR. Assessment of cold tolerance in chickpea (Cicer spp.) grown under cold/freezing weather conditions of North-Western Himalayas of Jammu and Kashmir, India. Physiology and Molecular Biology of Plants. 2021; 27: 1105–1118. https://doi.org/10.1007/s12298-021-00997-1 PMID: 34092953
6. Kalra N, Chakraborty D, Sharma A, Rai HK, Jolly M, Chander S, et al. Effect of increasing temperature on yield of some winter crops in northwest India. Current Science. 2008; 94(1): 82–88.
7. Abbo S, Berger JD, Turner NC. Evolution of cultivated chickpea: four genetic bottlenecks limit diversity and constrain crop adaptation. Functional Plant Biology. 2003; 30: 1081–87. https://doi.org/10.1071/FP03084 PMID: 32689090
8. Sharma KD, Panu RK, Behl RK. Effect of early and terminal heat stress on biomass partitioning, chlorophyll stability and yield of different wheat genotypes. Proceedings of the International Conference on sustainable crop production in stress environments: Management and genetic options. 2005; 187–94.
9. Weerako on WMW, Maruyama A, Ohba K. Impact of humidity on temperature induced grain sterility in rice (Oryza sativa L.). Journal of Agronomy and Crop Science. 2008; 194: 134–40.
10. Cottee NS, Tan DKY, Bange MP, Cothren JT, Campbell LC. Multi-level determination of heat tolerance in cotton (Gossypium hirsutum L.) under field conditions. Crop Science. 2010; 50: 2553–64.
11. Wang J, Gan YT, Clarke F, McDonald CL. Response of chickpea yield to high temperature stress during reproductive development. Crop Science. 2006; 46: 2171–78.
12. Toker C, Canci H. Selection for drought and heat resistance in chickpea under terminal drought conditions. 4th International Food Legumes Research Conference: Food Legumes for Nutritional Security and Sustainable Agriculture, 2006; 18–22.
13. Gaur PM, Jukanti AK, Srinivasan S, Chaturvedi SK, Basu PS, Babbar A, et al. Climate change and heat stress tolerance in chickpea. In Climate change and plant abiotic stress tolerance 2014 (pp. 839–56). Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.
14. Rahbarian R, Khavari-nejad R, Ganjeali A. Drought stress effects on photosynthesis, chlorophyll fluorescence and water relations in tolerant and susceptible chickpea (Cicer arietinum L.) genotypes. Acta Biologica Cracoviensia Series Botanica. 2011; 53: 47–56.
15. Bhattacharjee S. An inductive pulse of hydrogen peroxide pretreatment restores redox-homeostasis and mitigates oxidative membrane damage under extremes of temperature in two rice cultivars (Oryza sativa L., Cultivars Ratna and SR 26B). Plant Growth Regulation. 2012; 68: 395–410.
16. Pouresmael M, Khavari-Nejad RA, Mozafari J, Najafi F, Moradi F. Efficiency of screening criteria for drought tolerance in chickpea. Archives of Agronomy and Soil Science. 2013; 59(12): 1675–93.

17. Devasirvatham V, Gaur PM, Mallikarjunna M, Tokachichu RN, Trethowan RM, Tan DKY. Effect of high temperature on the reproductive development of chickpea genotypes under controlled environments. Functional Plant Biology. 2012; 139:1009–18. https://doi.org/10.1071/FP12033 PMID: 32480850

18. Howarth CJ. Genetic improvements of tolerance to high temperature. In Abiotic stresses-Plant resistance: tolerance through breeding and molecular approaches 2005 (pp. 277–300). The Haworth Press, USA.

19. Ludlow MM, Muchow RC. A critical evaluation of traits for improving crop yields in water limited environments. Advances in Agronomy. 1990; 43: 107–53.

20. Flowers TJ, Koyama ML, Flowers SA, Sudhakar C, Singh KP, Yeo AR. QTL: their place in engineering tolerance of rice to salinity. Journal of Experimental Botany. 2000; 51(342): 99–106. PMID: 10938800

21. Turner NC, Wright GC, Siddique KHM. Adaptation of grain legumes (pulses) to water-limited environments. Advances in Agronomy. 2001; 71: 193–231.

22. Jain M, Misra G, Patel RK, Priya P, Jhanwar S, Khan AWet al. A draft genome sequence of the pulse crop chickpea (Cicer arietinum L.). The Plant Journal. 2013; 74: 715–29. https://doi.org/10.1111/tpj.12173 PMID: 23489434

23. Nayak SN, Zhu H, Varghese N, Datta S, Choi HK, Horres R, et al. Integration of novel SSR and gene-based SNP marker loci in the chickpea genetic map and establishment of new anchor points with Medicago truncatula genome. Theoretical and Applied Genetics. 2010; 120: 1415–41. https://doi.org/10.1007/s00122-010-1265-1 PMID: 20998978

24. Agarwal G, Sabbavarapu MM, Singh VK, Thudi M, Sheelamary S, Gaur PM, et al. Identification of a non-redundant set of 202 in silico SSR markers and applicability of a select set in chickpea (Cicer arietinum L.). Euphytica. 2015; 205(2): 381–394.

25. Thudi M, Khan AW, Kumar V, Gaur PM, Katta AVSK, Garg V, et al. Whole genome re-sequencing reveals genome wide variations among parental lines of mapping populations in chickpea (Cicer arietinum L.). BMC Plant Biology. 2016; 6(1): 10. https://doi.org/10.1186/s12870-015-0690-3 PMID: 26822060

26. Hiremath PJ, Farmer A, Cannon SB, Woodward J, Kudapa H, Tuteja R, et al. Large-scale transcriptome analysis in chickpea (Cicer arietinum L.), an orphan legume crop of the semi-arid tropics of Asia and Africa. Plant Biotechnology Journal. 2011; 9(8): 922–31. https://doi.org/10.1111/j.1467-7652.2011.00625.x PMID: 21615673

27. Kudapa K, Azam S, Sharpe AG, Tar’an B, Li R, Deonovic B, et al. Comprehensive transcriptome assembly of chickpea (Cicer arietinum L.) using Sanger and next generation sequencing platforms: development and applications. PLoS ONE 2014; 9(1): e86039. https://doi.org/10.1371/journal.pone.0086039 PMID: 24465857

28. Varshney RK. Exciting journey of 10 years from genomes to fields and markets: Some success stories of genomics-assisted breeding in chickpea, pigeonpea and groundnut. Plant Science. 2016; 242: 98–107. https://doi.org/10.1016/j.plantsci.2015.09.009 PMID: 26566828

29. Millan T, Winter P, Jüngling R, Gil J, Rubio J, Cho S, et al. A consensus genetic map of chickpea (Cicer arietinum L.) based on 10 mapping populations. Euphytica. 2010; 175(2): 175–89.

30. Gujaria N, Kumar A, Dauthal P, Dubey A, Hiremath P, Prakash A, et al. Development and use of genic molecular markers (GMMs) for construction of a transcript map of chickpea (Cicer arietinum L.). Theoretical and Applied Genetics. 2011; 122(8):1577–89. https://doi.org/10.1007/s00122-011-1556-1 PMID: 21384113

31. Varshney RK, Thudi M, Nayak SN, Gaur PM, Kashiwagi J, Krishnamurthy L, et al. Genetic dissection of drought tolerance in chickpea (Cicer arietinum L.) Theoretical and Applied Genetics. 2014; 127(2): 445–62. https://doi.org/10.1007/s00122-013-2230-6 PMID: 24326458

32. Gaur R, Jeena G, Shah N, Gupta S, Pradhan S, Tyagi AK, et al. High density linkage mapping of genomic and transcriptomic SNPs for synteny analysis and anchoring the genome sequence of chickpea. Scientific Reports. 2015; 5: 13387. https://doi.org/10.1038/srep13387 PMID: 26303721

33. Jaganathan D, Thudi M, Kale S, Azam S, Roorkiwal M, Gaur PM, et al. Genotyping-by-sequencing based intra-specific genetic map refines a ‘QTL-hotspot’ region for drought tolerance in chickpea. Molecular Genetics and Genomics. 2015; 290: 559–71. https://doi.org/10.1007/s00438-014-0932-3 PMID: 25344290

34. Kale SM, Jaganathan D, Ruperao P, Chen C, Punna R, Kudapa H, et al. Prioritization of candidate genes in ‘QTL-hotspot’ region for drought tolerance in chickpea (Cicer arietinum L.). Scientific Reports. 2015; 5: 15296. https://doi.org/10.1038/srep15296 PMID: 26478518

35. Bhatia D, Wing RA, Singh K. Genotyping by sequencing, its implications and benefits. Crop Improvement. 2013; 40(2): 101–11.
36. Mir RR, Hiremath PJ, Riera-Lizarazu O, Varshney RK. Evolving molecular marker technologies in plants: From RFLPs to GBS. In: Diagnostics in Plant Breeding 2013 (pp. 229–247). Springer Science, New York.

37. Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE. Double Digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS ONE. 2012; 7(5): e37135. https://doi.org/10.1371/journal.pone.0037135 PMID: 22675423

38. Puritz JB, Matz MV, Toonen RJ, Weber JN, Bolnick DI, Bird CE. Demystifying the RAD fad. Molecular Ecology. 2014; 23: 5937–42. https://doi.org/10.1111/mec.12965 PMID: 25319241

39. Mir RR, Varshney RK. Future prospects of molecular markers in plants. In: Molecular Markers in Plants 2013 (pp. 169–190). Blackwell Publishing Ltd, Oxford, UK.

40. Stephens A, Lombardi M, Cogan NOI, Forster JW, Hobson K, Materne M, et al. Genetic marker discovery, intraspecific linkage map construction and quantitative trait locus analysis of ascochyta blight resistance in chickpea (Cicer arietinum L.). Molecular Breeding. 2014; 33(2): 297–313.

41. Kushwah A, Gupta S, Bindra S, Johal N, Singh I, Bharadwaj C., et al. Gene pyramiding and multiple character breeding. In Chickpea: Crop wild relatives for enhancing genetic gains 2020a (pp. 131–165). Elsevier Academic Press.

42. Singh R, Sharma P, Varshney RK, Sharma SK, Singh NK. Chickpea improvement: role of wild species and genetic markers. Biotechnology and Genetic Engineering Reviews. 2008; 25: 267–314. https://doi.org/10.5661/bger-25-267 PMID: 21412359

43. Thudi M, Bohra A, Nayak SN, Varghese N, Shah TM, Pennetts RV, et al. Novel SSR markers from BAC-end sequences, DAR arrays and a comprehensive genetic map with 1,291 marker loci for chickpea (Cicer arietinum L.). PLoS ONE. 2011; 6: e27275. https://doi.org/10.1371/journal.pone.0027275 PMID: 22102885

44. Gaur PM, Srinivasan S, Gowda CLL, Rao BV. Rapid generation advancement in chickpea. eJournal of SAT Agricultural Research. 2007; 3(1):1–3.

45. Hamwieh A, Imtiaz M. Identifying water-responsive and drought-tolerant chickpea genotypes. Crop and Pasture Science. 2015; 66: 1003–11.

46. Alexander MP. Differential staining of aborted and non-abortion pollen. Biotechnic and Histochemistry 1969; 44: 117–122.

47. Premachandra GS, Sangroka T, Ogatta S. Cell membrane stability as indicators of drought tolerance as affected by applied nitrogen in soybean. The Journal of Agricultural Science. 1990; 115: 63–66.

48. Sairam RK. Effect of moisture stress on physiological activities of two contrasting wheat genotypes. Indian Journal of Experimental Biology. 1994; 32: 584–93.

49. Slavik B. Methods of studying plant water relations. Springer-Verlag, Berlin and New York, 1974; 449.

50. SAS Institute Inc. SAS Campus Drive Care, NC 27513, USA 2002.

51. Bates D, Maechler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software. 2015; 67(1): 1–48.

52. Kushwah A, Bhatia D, Rani U, Yadav IS, Singh I, Bharadwaj C., et al. Molecular mapping of quantitative trait loci for ascochyta blight and botrytis grey mould resistance in an inter-specific cross in chickpea (Cicer arietinum L.). Molecular Breeding. 2021; 71(2).

53. Wang S, Basten CJ, Zeng ZB. Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC 2007.

54. Toker C, Luch C, Tejera NA, Serraj R, Siddique KHM. Abiotic Stresses. In Chickpea Breeding and Management 2007 (pp. 474–496) CAB International, UK.

55. Canci H, Toker C. Evaluation of yield criteria for drought and heat resistance in chickpea (Cicer arietinum L.). Journal of Agronomy and Crop Science. 2009; 195(1): 47–54.

56. Krishnamurthy L, Gaur PM, Basu PS, Chaturvedi SK, Tripathi S, Vadez V, et al. Large genetic variation for heat tolerance in the reference collection of chickpea (Cicer arietinum L.) germplasm. Plant Genetic Resources. 2011; 9: 59–61.

57. Devasirvatham V, Gaur PM, Mallikarjuna N, Tokachichu R, Trehowan R, Tan DKY. Reproductive biology of chickpea response to heat stress in the field is associated with the performance in controlled environments. Field Crops Research. 2013; 142: 9–19.

58. Paul PJ, Samineni S, Sajja SB, Rathore A, Das RR, Chaturvedi SK, et al. Capturing genetic variability and selection of traits for heat tolerance in a chickpea recombinant inbred line (RIL) population under field conditions. Euphytica. 2018; 214: 27.

59. Kushwah A, Bhatia D, Singh G, Singh I, Bindra S, Vij S, et al. Phenotypic evaluation of genetic variability and selection of yield contributing traits in chickpea recombinant inbred line population under high temperature stress. Physiology and Molecular Biology of Plants. 2021b; (in press).
60. Devasirvatham V, Tan DKY, Trethowan RM, Gaur PM, Mallikarjuna N. Impact of high temperature on the reproductive stage of chickpea. Proceeding of the 15th Australian Society of Agronomy Conference, Lincoln, New Zealand 2010.

61. Sakata T, Higashitani A. Male sterility accompanied with abnormal anther development in plants–genes and environmental stresses with special reference to high temperature injury. The International Journal of Plant Developmental Biology. 2008; 2: 42–51.

62. Prasad PVV, Craufurd P, Kakani VG, Wheeler TR, Boote KJ. Influence of high temperature during pre-and post-anthesis stages of floral development on fruit-set and pollen germination in peanut. Functional Plant Biology. 2001; 28: 233–40.

63. Kakani VG, Prasad PVV, Craufurd PQ, Wheeler TR. Response of in vitro pollen germination and pollen tube growth of groundnut (Arachis hypogaea L.) genotypes to temperature. Plant, Cell and Environment. 2002; 25: 1651–61.

64. Nayyar H, Bains T, Kumar S. Low temperature induced floral abortion in chickpea: Relationship with abscisic acid and cryoprotectants in reproductive organs. Environmental and Experimental Botany. 2005; 53: 39–47.

65. Kumar S, Kumar A, Kumar RR, Roy RK, Agrawal T. Genetic variability of chickpea genotypes under heat stress condition: correlation and path analysis: based analysis. Indian Journal of Ecology. 2017; 44(4): 59–64.

66. Simões-Araújo JL, Rumjanek NG, Margis-Pinheiro M. Small heat shock proteins genes are differentially expressed in distinct varieties of common bean. Brazilian Journal of Plant Physiology. 2003; 15(1): 33–41.

67. Tsukaguchi T, Kawamitsu Y, Takeda H, Suzuki K, Egawa Y. Water status of flower buds and leaves as affected by high temperature in heat tolerant and heat-sensitive cultivars of snap bean (Phaseolus vulgaris L.). Plant Production Science. 2003: 6: 4–27.

68. Wahid A, Close TJ. Expression of dehydrins under heat stress and their relationship with water relations of sugarcane leaves. Biologia Plantarum. 2007: 51: 104–09.

69. Upadhyaya HD, Dronavalli N, Gowda CLL, Singh S. Identification and evaluation of chickpea germplasm for tolerance to heat stress. Crop Science. 2011; 51: 2079–94.

70. Kushwah A, Bindra S, Singh I, Dixit GP, Sharma P, Srinivasan S, et al. Advances in chickpea breeding and genomics for varietal development and trait improvement in India. In Accelerated Plant Breeding 2020b (pp. 31–66) Springer.

71. Jingade P, Ravikumar RL. Development of molecular map and identification of QTLs linked to Fusarium wilt resistance in chickpea. Journal of Genetics. 2015; 94(4): 723–29. https://doi.org/10.1007/s10528-015-0589-7 PMID: 26690528

72. Rehman AU, Malhotra RS, Bett K, Tar’an B, Bueckert R, Warkentin TD. Mapping QTL associated with traits affecting grain yield in Chickpea (Cicer arietinum L.) under terminal drought stress. Crop Science. 2011; 51(2): 450–63.

73. Cobos MJ, Rubio J, Fernández-Romero MD, Garza R, Moreno MT, Millán T, et al. Genetic analysis of seed size, yield and days to flowering in a chickpea recombinant inbred line population derived from a Kabuli × Desi cross. Annals of Applied Biology. 2007; 151(1): 33–42.

74. Cobos MJ, Winter P, Kharrat M, Cubero JI, Gil J, Millán T, et al. Genetic analysis of agronomic traits in a wide cross of chickpea. Field Crops Research. 2009; 111(1–2): 130–36.

75. Verma S, Gupta S, Bandhiwal N, Kumar T, Bharadwaj C, Bhatia S. High-density linkage map construction and mapping of seed trait QTLs in chickpea (Cicer arietinum L.) using Genotyping-by-Sequencing (GBS). Scientific Reports. 2015; 5: 17512. https://doi.org/10.1038/srep17512 PMID: 26631981

76. Gupta S, Kumar T, Verma S, Bharadwaj C, Bhatia S. Development of gene-based markers for use in construction of the chickpea (Cicer arietinum L.) genetic linkage map and identification of QTLs associated with seed weight and plant height. Molecular Biology Reports. 2015; 42: 1571–80. https://doi.org/10.1007/s11033-015-3925-3 PMID: 26446030

77. Jamalabadi JG, Saidi A, Karami E, Kharkesh M, Talebi R. Molecular mapping and characterization of genes governing time to flowering, seed weight, and plant height in an intraspecific genetic linkage map of chickpea (Cicer arietinum L.). Biochemical Genetics. 2013; 51(5–6): 387–97. https://doi.org/10.1007/s10528-013-9571-3 PMID: 23371372

78. Gowda SJM, Radhika P, Mhase LB, Jamadagni BM, Gupta VS, Kadoo NY. Mapping of QTLs governing agronomic and yield traits in chickpea. Journal of Applied Genetics. 2011; 52: 9–21. https://doi.org/10.1007/s13353-010-0016-y PMID: 21181334

79. Jha UC, Nayyar H, Palakurthi R, Jha R, Valluri V, Bajaj P, et al. Major QTLs and Potential Candidate Genes for Heat Stress Tolerance Identified in Chickpea (Cicer arietinum L.). Frontiers in Plant Science 2021; https://doi.org/10.3389/fpls.2021.655103