Genetic mapping of flowering time in two interspecific RIL populations of chickpea

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Abstract
Chickpea (*Cicer arietinum*) is grown under diverse environments in Australia and often faces various yield limiting abiotic stresses such as low (chilling) temperatures, drought and heat during the reproductive phase. Understanding the genetic control of flowering time, a key adaptive trait is essential for wider adaptation and productivity of chickpea varieties. Currently, our knowledge on the genetic control of flowering time in Australian chickpea varieties is very limited. We utilised two recombinant inbred line (RIL) populations; RB (derived from an early flowering variety, Rupali and late flowering interspecific breeding line (a backcross derivative from *Cicer echinospermum*), 04067-81-2-1-1) and YB (derived from mid-flowering variety, Yorker and 04067-81-2-1-1). The RB RIL population was evaluated under two sowing times (early and mid-season) at Tamworth and the YB population was evaluated at Wagga Wagga in 2018 to identify genomic regions (quantitative trait loci: QTL) controlling flowering time. One significant QTL was identified on chromosome Ca5 in the RB RIL population and explained up to 16.8% and 11.9% of the phenotypic variation for flowering time in an early and mid-season sowing time, respectively. In the YB RIL population, two significant QTL were identified on chromosomes Ca4 and Ca8 and explained 9.6% and 11.2% of the phenotypic variation. We have also identified that the Australian chickpea varieties Rupali, Sonali and Sona carry the mutated form of the early flowering *ELF3* gene on Ca5 (*caelf3a*). Markers associated with the QTL for early and mid-flowering will accelerate the development of varieties suitable for a wider range of growing environments.

Key Words
Chickpea, flowering time, QTL mapping, *ELF3* gene

Introduction

Chickpea (*Cicer arietinum* L.) is a cool season, diploid grain legume (2n = 2x = 16) with a genome size of 738 Mb (Varshney et al., 2013). Photoperiod and vernalisation requirements are the two most important environmental drivers of the transition from vegetative to reproductive phase (time to flower) in the plant species. The cultivated chickpea is photoperiod sensitive with little or no vernalisation requirement as compared to the wild progenitors; *Cicer. reticulatum* and *C. echinospermum*, which require both vernalisation and photoperiod to initiate the reproductive phase.

In Australia, chickpeas are grown under tropical and temperate environments and exhibit a range of photoperiod responses to initiate flowering and pod setting. Development of early flowering varieties having photoperiod insensitivity or reduced photoperiod response is one of the targets of the chickpea breeding programs. Varieties with a long reproductive and pod-filling period (Or et al., 1999) will be optimal for sowing under rain-fed environments to escape from terminal drought and heat. However, for the temperate environments of Canada, development of early flowering varieties will mitigate the effect of terminal (end of season) frost (Ridge et al., 2017).

At least four major loci (*Efl1* to *Efl4*) and seven quantitative trait loci (QTL) controlling flowering time in chickpea have been identified (Ridge et al., 2017, Mallikarjuna et al., 2017, Kumar and van Rheenen, 2000). Recently, Ridge et al. (2017), identified a chickpea ortholog of *EFL3* gene on chromosome Ca5, which represents the *Efl1* locus. However, there is no information on the flowering time genes in the Australian chickpea varieties. In order to understand the genetic basis of flowering time, we identified QTL for flowering time in two RIL populations derived from an early flowering variety (Rupali) and a mid-flowering variety (Yorker) crossed with an interspecific breeding line. Identification of different QTL and genes associated with flowering time regulation will assist in designing the chickpea ideotypes for a range of

environments.

Methods

Plant material

Two RIL populations; RB (derived from an early flowering variety, Rupali and an interspecific breeding line, 04067-82-2-1-1) and YB (derived from mid-flowering variety, Yorker and 04067-81-2-1-1) were used in this study. Details of the populations have been provided in Amalraj et al. (2018).

Phenotyping and data analysis

The RB RIL mapping population was evaluated at Tamworth (TW) in 2018 for flowering time under field conditions using a split-plot design with two sowing treatments (TOS1-7th May and TOS2-12th June). Plots were arranged in randomised complete blocks within each block of split plots. There were three plots for each line in each treatment. The YB RIL population was grown in 1 m rows with two replications under birdcage at Wagga Wagga (WW) in 2018. Flowering time was recorded as number of days from sowing to 10% (DTF10), 50% (DTF50) and 95% (DTF95) flowering. Statistical analyses were performed using ASREML in R.

QTL analysis in RB RIL and YB RIL populations

The genetic linkage map of RB RIL and YB RIL populations previously developed by Amalraj et al. (2018) was used for QTL analysis using "R/qtl" in R software version 3.3.4. The predicted means for DTF10 and DTF50 in RB and DTF10 and DTF95 in YB populations were used for QTL analysis. We employed the composite interval mapping (CIM) method to identify QTL for flowering time. The statistical significance of the QTL was assessed using permutation test (1,000 replications) and a significance level of $p = 0.05$. The additive effects and per cent phenotypic variance (R^2) of the significant QTL were estimated using the "fitqtl" function in "R/qtl" package. We used 'lodint' to derive the confidence interval for the QTL location based on LOD score.

Association of the caelf3a gene in flowering time

We investigated if the early flowering Australian variety, Rupali carries the mutant form (*caefl3a* allele) of the *CaEFL3a* gene in early flowering Indian variety, ICCV 96029, as reported by Ridge et al. (2017). The sequence data of the *CaEFL3a* gene in 48 Australian chickpea varieties was aligned with the ICCV 96029 (*caefl3a*) and Frontier (*CaEFL3a* allele; late flowering Canadian variety). We developed an allele specific competitive allele specific PCR (KASP) marker to identify breeding lines/varieties with *caefl3a* or *CaELF3a* allele.

Results and Discussion

Flowering time of parental lines and RIL populations

Flowering time in chickpea is a highly variable trait and is affected by soil moisture, photoperiod, temperature, altitude and latitude. At both locations, cool temperatures persisted during the reproductive phase with average daily temperatures at Wagga Wagga significantly lower than Tamworth (Table 1). In the RB population trial at TW, Rupali flowered in 93 days as compared to the interspecific parent (120 days) and the RIL lines took 89 to 130 days to flower in TOS1. In the TOS2 treatment, Rupali took only 66 days to flower as compared to 101 days for the 04067-81-2-1-1.

There was no significant difference in days to flower between parents of the YB RIL population; Yorker (110 days) and 04067-81-2-1-1 (113 days) at WW. But, transgressive segregation for flowering time was observed in the YB RIL population as the RIL lines took 102 days to 118 days to flower.

Table 1. Average temperatures during flowering at Tamworth and Wagga Wagga in 2018

	Average daily temperature (Tamworth, 31.15 S, 150.98 E)	Average daily temperature (Wagga Wagga, 35.05 S, 147.35 E)
July	10.75	8.2
August	11	8.5
September	15.3	11.2
October	20	17.8

Genetic analysis in RB and YB RIL populations

Analysis of the flowering time at Tamworth identified a single QTL (*QRBdtfCa5*) on chromosome Ca5 explaining 16.8% (LOD score 12.3) and 11.9% (LOD score 4.2) of the phenotypic variation in TOS1 and TOS2 treatments respectively. The QTL was mapped in the vicinity of the *CaEFL3* gene reported previously (Ridge et al., 2017). While in the YB RIL population, two significant QTL on chromosomes Ca4 and Ca5 were detected for the flowering time. The significant QTL (*QYBdtfCa4*) on Ca4 (LOD = 3.7) explained 9.6% of the phenotypic variation. Second QTL on Ca8 (*QYBdtfCa8*) explained 11.2% of the phenotypic variation with the LOD score of 4.3.

Identification of caelf3a allele in Rupali

We identified that early flowering varieties; Rupali, Sonali and Sona carry the same 11-bp deletion in the *CaELF3a* gene as in the early flowering Indian variety, ICCV 96029 (Figure 1). This deletion was associated with early flowering (Ridge et al., 2017). Yorker, the mid-flowering parent of the YB RIL population carries the normal version of the gene (*CaEFL3a* allele) as the late flowering Canadian variety, Frontier (Figure 1).

Frontier AAG AGA GGG AAA GAT GAT GA**G AAG ATG ATG A**TG GGT CCA CTT TTT CCT AGA…

Yorker AAG AGA GGG AAA GAT GAT GA**G AAG ATG ATG A**TG GGT CCA CTT TTT CCT AGA…
ICCV 96029 AAG AGA GGG AAA GAT GAT GA……T GGG TCC ACT TTT TCC TAG*
Rupali/Sonali/Sona AAG AGA GGG AAA GAT GAT GA……T GGG TCC ACT TTT TCC TAG*

11-bp deletion

Figure 1. *ELF3* homolog controls the flowering time in chickpea varieties; ICCV 96029-early flowering variety carries the mutated form (11-bp deletion) of the gene (*caelf3a*); late flowering variety, Frontier carries the normal gene (*CaELF3a*) (Ridge et al., 2017). Australian variety Yorker (mid flowering) shares the normal gene as Frontier, while the early flowering varieties Rupali, Sonali and Sona carry the *caelf3a* version of the gene. *11-bp deletion creates premature stop codon altering the function of the gene.

Time to flower is crucial for the adaptation of chickpea varieties to diverse growing environments. Identification of QTL associated with flowering time would be useful for the breeding programs to develop varieties with the phenology suited to the environment. In the present study, we identified a significant QTL on Ca5 in the RB RIL population and, two significant QTL on Ca4 and Ca8 in the YB RIL population.

So far, four genes (efl-1 to efl-4) and at least seven QTL of major effects on flowering time have been reported in chickpea (Mallikarjuna et al., 2017, Kumar and van Rheenen, 2000). Recently, Ridge et al. (2017) identified an early flowering gene (*CaELF3a*) on Ca5 in ICCV 96029. ICCV 96029 carries the mutated form of the gene (*caelf3a*, 11-bp deletion in the gene) and the lines carrying this gene flowered earlier under long days than those carrying the normal gene. But under short days, this gap was large (at least 20 days in flowering time between the lines carrying the *caelf3a* gene and the normal gene). Similar observations were made in RB RIL population at TW in TOS1 (short days during the reproductive phase); the gap between Rupali (*caefl3a*) and 04067-82-2-1-1 was 27 days.

Currently, we are comparing the QTL identified in our study with the previously identified QTL on chromosomes Ca4, Ca5 and Ca8 associated with flowering time.

Conclusion

Our study has revealed at least three genomic regions on chromosomes Ca4, Ca5 and Ca8 associated with flowering time in two Australian chickpea populations. We also identified an association between 11-bp deletion in the early flowering gene (*CaELF3a*) and flowering time in Australian varieties. The KASP markers for the *CaEFL3a* gene can be used to select early flowering breeding lines and trace the lineage of early flowering varieties such as Rupali, Sonali and Sona. The QTL associated with early and mid-flowering can be used to develop varieties with a range of flowering and photoperiod responses.

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