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Altered Expression of an *FT* Cluster Underlies a Major Locus Controlling Domestication-Related Changes to Chickpea Phenology and Growth Habit

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Flowering time is a key trait in breeding and crop evolution, due to its importance for adaptation to different environments and for yield. In the particular case of chickpea, selection for early phenology was essential for the successful transition of this species from a winter to a summer crop. Here, we used genetic and expression analyses in two different inbred populations to examine the genetic control of domestication-related differences in flowering time and growth habit between domesticated chickpea and its wild progenitor *Cicer reticulatum*. A single major quantitative trait locus for flowering time under short-day conditions [*Days To Flower* (*DTF)3A*] was mapped to a 59-gene interval on chromosome three containing a cluster of three *FT* genes, which collectively showed upregulated expression in domesticated relative to wild parent lines. An equally strong association with growth habit suggests a pleiotropic effect of the region on both traits. These results indicate the likely molecular explanation for the characteristic early flowering of domesticated chickpea, and the previously described growth habit locus *Hg*. More generally, they point to de-repression of this specific gene cluster as a conserved mechanism for achieving adaptive early phenology in temperate legumes.

Keywords: chickpea, domestication, florigen, flowering, growth habit, legume, photoperiod, QTL

INTRODUCTION

The timing of flowering is a critical trait for crop adaptation, and as such has significant implications for yield and economic output (Jung and Muller, 2009; Nelson et al., 2010). The wild forms of many crops have a strong environmental requirements for flowering, ensuring that seed development occurs under favorable conditions. However, such requirements often constitute a physiological barrier for adaptation to wider agro-ecological ranges, and in general, domestication and subsequent diversification has involved selection of variants in which these requirements have been modified. A well-known example is wheat (*Triticum aestivum* L.), where relaxation of

photoperiod and vernalization responses has allowed the development of spring cultivars (Trevaskis et al., 2003; Yan et al., 2003; Fu et al., 2005; Beales et al., 2007; Díaz et al., 2012; Kippes et al., 2015, 2016). Similar adaptations have been reported in many other species (Nakamichi, 2015), including legumes, where a loss-of-function mutation in the circadian clock gene *ELF3* overcame the obligate LD requirement of pea (*Pisum sativum* L.), permitting its conversion from a winter to a spring crop at higher latitudes (Weller et al., 2012). Similarly, a mutation at the *Ppd* locus in the short-day species common bean (*Phaseolus vulgaris* L.) enabled summer cropping and broad global adaptation of this crop (Wallace et al., 1993; Weller et al., 2019).

Chickpea (*Cicer arietinum* L.) is a major grain legume, ranking third in global production after bean and pea (FAO, 2016). It is more drought-tolerant than other cool season legumes, and its relative importance is projected to increase in future due to global population growth and climate change (Bar-El Dadon et al., 2017; Muehlbauer and Sarker, 2017). Despite being domesticated in parallel with other long day vernalization-responsive legumes (pea, lentil) and cereals (wheat, barley) (Zohary and Hopf, 2000), the domestication history of chickpea is distinct from these other species (Abbo et al., 2003a). One key difference is the decline of chickpea in the archeological record between the Neolithic period, approximately 9000 years before present (ybp) and the early Bronze Age (approximately 5000 ybp) (Abbo et al., 2003b). A second key difference is that across its center of origin, chickpea has traditionally been grown as a summer crop (Abbo et al., 2003b), and varieties with the winter annual habit typical of wild chickpea are notably absent. This contrasts with other species domesticated in the Fertile Crescent region over the same period, such as barley and pea, in which a significant proportion of the domesticated germplasm retains the ancestral, wild phenology (Saisho et al., 2011; Weller et al., 2012).

The reasons for these two differences are not known, but it is thought that chickpea was neglected as a winter crop in favor of other pulses, as a result of its inherently greater susceptibility to Ascochyta blight, a fungal disease caused by *Ascochyta rabiei*. This disease can cause total crop failure, particularly during humid Mediterranean winter conditions (Siddique et al., 2000; Millan et al., 2003; Sharma and Ghosh, 2016) and its impact would likely have intensified as planting densities increased with cultivation. This pressure may have motivated attempts by early farmers to shift cultivation from autumn sown, over-winter crop (when most precipitation occurs in this region) to a spring-sown summer crop that matures in the predominantly dryer summer season. In such a scenario the selection of earlier-flowering genotypes able to complete their life cycle prior to the onset of summer drought would likely have been essential (Kumar and Abbo, 2001), and the increase in the frequency of archaeobotanical remains of chickpea in the Bronze Age is suggested to reflect the success of this transition (Kumar and Abbo, 2001; Abbo et al., 2003a).

Early phenology continues to be important in present-day chickpea cultivation, as a large proportion of the global chickpea crop is grown in short season environments exposed to end-of season stresses that reduce their productivity (Kumar and Abbo, 2001; Muehlbauer and Sarker, 2017). In Mediterranean and semi-arid environments, where chickpea is grown under rain-fed conditions and matures into summer, terminal drought is the most common cause of yield loss (Zhang et al., 2000; Turner et al., 2001; Siddique et al., 2003; Berger and Turner, 2007). In higher-latitude continental temperate environments like western Canada, the short growing season is instead limited by declining temperatures, delayed maturity and increased potential for frost damage at the sensitive phase of pod development (Croser et al., 2003; Berger J.D. et al., 2004; Clarke and Siddique, 2004; Anbessa et al., 2007). In both situations, early flowering and maturity is thus an important primary escape strategy (Siddique et al., 2003; Berger J.D. et al., 2004; Berger et al., 2006) Hence, genetic control of this trait has been a topic of increasing interest (e.g., Gaur et al., 2008; Ridge et al., 2017).

Several flowering time loci have been reported in chickpea from both classical and quantitative trait locus (QTL) analyses. These include four major loci; *Photoperiod* (Or et al., 1999), *Early flowering 1* (*Efl1*), *Efl3*, and *Efl4* (Kumar and Van Rheenen, 2000; Hegde, 2010; Gaur et al., 2014), and several QTL that appear recurrent in different populations. One prominent example is a "hot-spot" on linkage group (LG) four (Cobos et al., 2007; Varshney et al., 2014; Daba et al., 2016; Mallikarjuna et al., 2017). Another important genomic region is the central portion of chromosome 3 between markers TA6 and TA64, in which flowering time QTL have been reported from all wide crosses investigated for this trait (Cobos et al., 2009; Aryamanesh et al., 2010; Das et al., 2015; Samineni et al., 2015), as well as in several other intraspecific populations (Hossain et al., 2010; Hamwieh et al., 2013; Daba et al., 2016; Mallikarjuna et al., 2017).

In this study we aimed to elucidate the genetic basis of changes in flowering time that occurred early in chickpea crop evolution, through QTL analysis and candidate gene evaluation in two recombinant inbred populations between *Cicer arietinum* and its wild progenitor *C. reticulatum*. Our results point to a strong genetic association between the early flowering and erect growth habit typical of domesticated chickpea, and the elevated expression of a cluster of *FT* genes on chromosome 3. We conclude that a *cis*-acting genetic change leading to deregulated expression of this gene cluster may have played a key role in the prehistoric shift in phenology and farming practice integral to chickpea evolution under domestication.

MATERIALS AND METHODS

Plant Material

CRIL2 is a recombinant inbred line (RIL) population developed from an interspecific cross between *C. arietinum* (accession ICC4958) and *C. reticulatum* (PI489777) by Tekeoglu et al. (2000), Winter et al. (2000), Muehlbauer and Sarker (2017) at the United States Department of Agriculture (USDA), Agricultural Research Service and Washington State University, United States. ICC4958 is an early-flowering desi chickpea type with an erect growth habit, while the wild parent PI489777 is a Turkish accession with prostrate growth habit and late flowering typical of wild chickpea.

Three other recombinant inbred populations were used in this study, developed by the chickpea breeding group in IFAPA (Institute of Agricultural and Fisheries Research and Training, Centro Alameda del Obispo, Cordoba, Spain) and University of Córdoba, Spain. RIP12 is an interspecific population consisting of 88 $F_{6:7}$ RILs derived from a cross between the kabuli cultivar ICCL81001 and a *C. reticulatum* accession, as described in Cobos et al. (2009). RIP5 (102 RILs) and RIP8 (113 RILs) are two $F_{6:8}$ RIL populations derived from reciprocal crosses between the early flowering desi landrace WR315 and the late kabuli accession ILC3279 (Iruela et al., 2007; Ali et al., 2015).

Growing Conditions and Phenotypic Evaluation

Four plants of each of the CRIL2 parents and 124 RILs were grown under long day (LD) or short day (SD) conditions in an automated phytotron at the University of Tasmania between December 2015 and April 2016. Plants under SD received 8 h (8 AM–4 PM) of natural daylight and were then moved to complete darkness inside the phytotron. Plants under LD received natural daylight, extended throughout the growing season with artificial light from high-pressure sodium lamps (50 $\mu molm^{-2}$ s^{-1}) to provide a total photoperiod of 18 h. Night temperature inside the phytotron was maintained at 16°C. Flowering time was recorded as the number of days from seedling emergence to opening of the first flower (DTF) on each individual plant. Lines remaining vegetative at 130 days were assigned a nominal DTF value of 130 in subsequent analyses. Branching tendency was quantified at 3 weeks after emergence and expressed as the ratio of total branch length to main shoot length (branching index, BI) to normalize for differences in general vigor and stem elongation. Growth habit (GH) was scored using a four-category scale (values from 1 to 4), according to the angle of the branches from the vertical axis at harvest stage, as follows: (1) prostrate (branches 0–10° above horizontal), (2) semi-prostrate (10–45°), (3) semi-erect (45–70°), and (4) erect (>70°). For all three traits, the mean value from the four replicate plants was used for analysis.

RIP12 was sown in March in the field at the IFAPA site in Cordoba (latitude/longitude/altitude: 37°53′N/4°47′W/117 m) over four different seasons (2001, 2004, 2008, and 2014). Plots consisted of 2 m-long rows set 0.5 m apart, each sown with 20 plants of each RIL. Every fifth row was sown with one of the parent lines as a check. In 2001, a greenhouse trial was also conducted to assess flowering time under natural short day conditions (Cobos et al., 2009). RIP5 was sown in the field in March 2003 at two different sites: the IFAPA site in Cordoba and the IFAPA Venta del Llano site (Mengibar, Jaen, Spain; latitude/longitude/altitude: 37°57′N/3°48′W/280 m). In this trial, RILs were randomly distributed in four blocks and parents were included as reference in each trial. The unit plot was two rows of 2 m, with 10 seeds/m and 0.7 m between rows (Ali et al., 2015). RIP8 was sown in the field in February 2003 at the IFAPA site in Cordoba with two replications, in which RILs were distributed randomly into four blocks with 20 lines per block. Four check lines were included in each block following a Latin square design to verify environmental homogeneity. The plot unit was three rows, 4 m long, with 0.5 m between rows and a density of 20 plants m^{-2}. For these three populations, days from sowing to 50% flower was recorded (DTF). The data obtained from each of the two trials of RIP8 were analyzed separately. Information about the photoperiod experienced by RIP12, RIP5, and RIP8 during the different growing seasons can be found in **Supplementary Table 5**.

Molecular Markers

Both markers from previous linkage maps and new markers developed specifically for this study were used for map construction and QTL analysis. Polymorphisms in target genes across chickpea LG3 and LG4 were identified by sequencing of the parental accessions or from information available in previous reports (Saxena et al., 2014), and used to design 27 high-resolution melt (HRM) markers (**Supplementary Table 1**) that were added to the markers previously genotyped in the RIP12, RIP5, and RIP8 populations previously described in Iruela et al. (2007), Cobos et al. (2009), Ali et al. (2015), respectively. In the case of CRIL2, the HRM markers were combined with a subset of 210 molecular markers selected from a dense map incorporating 2956 markers (**Supplementary Figure 1**; von Wettberg et al., 2018), to provide an even distribution [approximately 1 marker/5 centiMorgan (cM)] of high-quality (minimal missing data) markers (**Supplementary Table 1**).

Genetic Mapping and QTL Analysis

Linkage analysis in each population was performed using JoinMap v4.0 (Van Ooijen, 2006). Markers were grouped with a minimum logarithm of odds (LOD) value of 3.0, and the regression algorithm was used for mapping, using default options and the Kosambi function for the estimation of genetic distances (Kosambi, 1943). The initial maps were reviewed and problematic markers were removed where necessary based on the following criteria: Chi-square goodness-of-fit threshold (>1); nearest neighbor fit; genotype probability function; and the level of segregation distortion compared to surrounding markers. Following the removal of problematic markers, the maps were re-calculated and the process repeated where necessary, until maps with robust order were produced.

The numbering of the LGs followed the chickpea consensus genetic map (Millan et al., 2010), based on the presence of markers in common with the consensus map itself or others marker of known position, using the Cool Season Food Legume Database[1].

Quantitative trait locus analysis was performed using MapQTL6.0 software (Van Ooijen, 2009). First, interval mapping was carried out to detect putative QTL associated with the variation in each trait. For each putative QTL, the marker closest to the LOD peak and two markers either side of this were used in Automatic Cofactor Selection (ACS) to select the best cofactor for subsequent Multiple QTL Mapping (MQM) analysis. The MQM function was employed iteratively with each new cofactor selection until all QTLs for a specific trait were determined. In both interval and MQM mapping, putative QTL were declared at

[1]https://www.coolseasonfoodlegume.org

a chromosome-wide threshold ($p < 0.05$) based on permutation testing with 1000 permutations.

RNA Extraction and qPCR

For the expression study, the six parental lines of the four populations (RIP5 and RIP8 share the same parental accessions, and therefore were represented only once) were grown in an automated phytotron at the University of Tasmania under SD (8 h) and LD (16 h) conditions. For quantitative reverse-transcriptase PCR (qRT-PCR), dissected apical buds and the uppermost fully expanded leaflets were harvested. Each sample consisted of pooled material from two plants, harvested at midday at 2–4 weeks after seedling emergence. RNA extraction, cDNA synthesis and gene expression determination were performed as described in Sussmilch et al. (2015) using the primers indicated in **Supplementary Table 2**. The expression level of tested genes was normalized against *ACTIN* using the ΔΔCt method.

Statistical Analysis

Statistical analysis was conducted using IBM SPSS Statistics (version 22), including box-plot and frequency distribution graphs. Correlation between traits was measured using Spearman's rank correlation coefficient, and statistical significance was tested by paired or independent *t*-test, according to the nature of the data.

RESULTS

A Major Locus Controls Flowering in the CRIL2 Interspecific Reference Population

We initially characterized flowering time in the CRIL2 reference population under controlled 8-h SD and 18-h LD conditions in an automated phytotron. Phenotypic values obtained are summarized in **Supplementary Figure 2**. Under LD, the difference in flowering time between the parental lines was not significant, with both flowering between 30 and 33 days after emergence. In contrast, under SD, ICC4958 flowered at around 60 days while PI489777 remained vegetative until the experiment was terminated 130 days after sowing. Thus, under these conditions, ICC4958 shows a moderate, quantitative response to photoperiod, whereas the wild line shows an obligate requirement for LD.

Among the RILs, the mean DTF under LD conditions was intermediate between the two parents while the range was substantially wider, with 12 days difference between the minimum and maximum values. Under SD, flowering time in the CRIL2 population showed a clear bimodal distribution, with a significant proportion of lines (68 out of 124) failing to initiate flowering by 130 days after sowing, like the wild parent. All RILs flowered considerably later under SD than under LD ($p < 0.001$) but, interestingly, phenotypic values for DTF in the two conditions were significantly correlated (with only 56 RILs able to flower in both LD and SD considered; $rs[56] = 0.500$, $p < 0.001$), indicating that part of the variation is independent of photoperiod. Transgressive segregation, particularly toward earliness, was observed under both photoperiods (**Supplementary Figure 2**), suggesting that alleles associated with early flowering have been contributed from both parents.

Consistent with the phenotypic homogeneity observed for flowering time in CRIL2 under LD, QTL analysis under these conditions revealed only one minor QTL, *DTF3C* (**Table 1**), located at the top of LG3 (**Figure 1**). In contrast, under SD conditions, a major effect QTL, *DTF3A*, was found in the middle of LG3 (LOD 50.2, PVE 85). As the peak markers for these loci are separated by only around 10 cM, and the effective population size for the LD analysis is relatively small, the possibility that the loci may be the same cannot be excluded. However, as it is also not trivial to prove, we have adopted a conservative interpretation and assigned them distinct names.

Quantitative trait locus analysis was also performed using a subset of the population formed by those 56 RILs that were able to flower under both SD and LD. Interestingly, no significant QTL were found in this case, supporting the idea that only QTL *DTF3A* is acting in CRIL2 grown under SD. However, these results should be interpreted with caution, considering the small population size.

Mapping Identifies the *FT* Cluster as Strong Positional Candidates for *DTF*3A

Several previous studies have reported major flowering QTLs in the central region of chromosome 3 between markers TA6 and TA64 (summarized in **Supplementary Figure 3**), indicating this as a particularly important genomic region (Weller and Ortega, 2015). We scanned this region for genes similar to known flowering time genes in other species and added 18 additional markers to the CRIL2 linkage map, including 13 within the TA6-TA64 interval (**Supplementary Figure 3** and **Supplementary Table 1**). This confirmed the presence of *DTF3A* within this interval and narrowed its location to a smaller interval flanked by markers SUVH4 and CDF2d (**Figure 1**), that corresponds to a physical distance of 1.4 Mbp and contains 124 annotated genes, according to the reference genome. Many of the flowering-related genes annotated in this region lie outside of this interval and were thus considered to be unlikely candidates, including *SOC1a* (*SUPPRESSOR OF CONSTANS OVEREXPRESSION 1*), *COLh* (*CONSTANS-LIKE h*), *AG* (*AGAMOUS*)*-like*, *LUX* (*LUX ARRHYTHMO*)*-like*, *CDF* (*CYCLING DOF FACTOR*), and *WRKY* (**Supplementary Figure 3**). However, the analysis confirmed the presence of a cluster of *FT* genes directly under the QTL peak, and a marker for one of these, *FTa1,* showed the strongest association with SD flowering time among all the markers tested (**Table 1**).

The dramatic delay in flowering of the PI489777 parent line and the bimodal distribution of the flowering phenotypes in CRIL2 under SD suggested that the QTL could also be analyzed as a single Mendelian locus, to refine its position. **Figure 2** illustrates all recombinants identified in the CRIL2 population across the LG3A region, and shows that *DTF3A* can be further delimited to a region of 0.8 Mb between markers *SUVH4* and *GATA9*/*ING2*

TABLE 1 | Quantitative trait loci (QTL) identified by multiple QTL mapping for flowering time, growth habit and branching index in four populations grown in different environments.

Population	Place	Year	Trait[a]	Cond[b]	QTL	LOD[c]	PVE[d]	Marker[e]	LG[f]	Early[g]	Late[g]	Thr[h]
CRIL2	Hobart	2016	DTF	LD, P	*DTF*3C	2.9	9.6	S1202p50545	3	29	30.3	2.8
				SD, P	*DTF*3A	50.2	85.2	*FT*a1	3	66.2	128.6	2.6
		2016	GH	SD, P	*GH*3	34	66.6	*FT*a1	3	3.4	1.6	2.6
					*GH*4	5.5	5.9	S360p1277380	4	2.8	2.2	3.1
		2016	BI	SD, P	*BI*3	10.6	33.1	*FT*a1	3	0.4	0.9	2.6
				LD, P	*BI*3	5.4	18.4	*FT*a1	3	0.2	0.5	2.5
RIP12	Cordoba	2001	DTF	GLH	*DTF*3A	10.8	46.9	*FT*a1	3	14.1	39.6	3.1
				Field	*DTF*3A	4.5	22	*FT*a1	3	60.4	68.6	2.9
		2004	DTF	Field	*DTF*3A	14.8	51.1	*FT*a1	3	8.9[i]	21.7[i]	2.9
					*DTF*4B	3.6	9.2	STMS11	4	17.9[i]	12.6[i]	3.3
		2008	DTF	Field	*DTF*3B	6.3	29.6	COLh	3	70.3	76.8	2.9
		2014	DTF	Field	*DTF*3A	8.4	29.8	*FT*a1	3	58.3	64.2	3
					*DTF*4A	5.3	17.3	GAA47	4	63.5	59	2.8
RIP5	Cordoba	2003	DTF	Field	*DTF3D*	9.6	38.7	WRKY	3	60.4	64.8	2.7
	Cordoba				*DTF*3A	3	8.7	*FT*a1/2	3	61.3	63.9	2.7
	Mengibar				*DTF*3A	5.7	26.9	*FT*a1/2	3	64.2	66.7	2.8
RIP8	Rep1	2003	DTF	Field	DTF3D	7.5	29.2	TA125	3	84.3	87.3	2.6
	Rep2				DTF3D	6.8	29.0	TA125	3	84.6	87.3	2.7

[a] *Trait analyzed: DTF, flowering time; GH, growth habit; BI, branching index.* [b] *Condition: LD, long days; SD, short days; P, phytotron; GLH, glasshouse.* [c] *The LOD scores for each QTL.* [d] *PVE, Phenotypic variation explained.* [e] *Marker nearest to the peak LOD score.* [f] *LG, linkage group harboring the QTL.* [g] *Marker genotype class means for early (C. arietinum accessions ICC4958, ICCL81001 and WR315 for CRIL2, RIP12, and RIP5/8, respectively) and late (C. reticulatum accessions PI489777 and Cr5-9 in CRIL2 and RIP12 and C. arietinum ILC3279 in the case of RIP5/8) parents, calculated for the marker with higher LOD.* [h] *Threshold LOD for a 0.995 confidence value, calculated through permutation test for each trait and linkage group.* [i] *Flowering time in 2004 is a relative value, as specified in* ***Supplementary Figure 2****.*

(**Supplementary Table 3**). This region contains only 59 genes, but still includes the *FT* cluster.

Comparison of the DTF3A Region in Other Crosses

The segregation of a major flowering time locus in CRIL2 and several other interspecific populations suggests a potential role for this locus in early crop evolution. However, a lack of common markers has made it difficult to compare the position of QTL between studies and clearly demonstrate their co-location. To investigate the position of *DTF3A* relative to previously described QTLs, and assess the possible relevance of this region at the intraspecific level, we selected three additional populations for parallel analysis through mapping of common markers. RIP12 is another interspecific population, for which a major flowering QTL has been reported in the TA6-TA64 region (Cobos et al., 2009). The intraspecific populations RIP5 and RIP8 were also examined, as preliminary evidence indicated an association of markers in the 3A region with flowering time in this cross (Castro, 2011). Where polymorphisms were available, the genes targeted in CRIL2 were also genotyped and added to the linkage maps in these additional populations (**Supplementary Table 1**) by recalculation of the linkage maps with markers for these genes and previously mapped markers (**Supplementary Figures 4–7**). These maps were then used for QTL analysis of flowering data for the three populations across different locations, years, and environments (**Supplementary Figure 2**), revealing a total of 12 significant flowering QTL (**Table 1**).

In the RIP12 population, analysis over several years, in glasshouse and field environments, yielded seven QTL; five on LG3 and two on LG4 (**Table 1**). The QTL on LG3 were defined by the same interval 3A described above for CRIL2 (**Figure 1**), and the *FTa1* marker again explained the highest proportion of variation (up to 51%). During 2008, a flowering QTL *DTF3B* was detected in a second region of LG3 between markers *FT*a1 and $Q05_{1828}$. Since both the position of the interval (**Figure 1**) and the significance of the QTL (∼30% PVE) are very close to those obtained for *DTF3A* (**Table 1**), it seems highly probable that these two QTL are equivalent.

In the intraspecific populations, two regions on chromosome 3 influenced flowering time. One of these was region *3A*, which was detected in the RIP8 population, with a variable effect on flowering time depending on location, with a strong effect when grown in Mengibar, and a weaker influence in Cordoba (26.9 vs. 8.7% variance explained, respectively). An additional highly significant QTL (*DTF3D*) was detected on LG3, between markers LOB189 and PRT6, in both intraspecific populations (**Figure 1**). Although this QTL was not detected in RIP5 at Mengibar, in situations where it was detected it had a greater effect than *DTF3A* (**Table 1**).

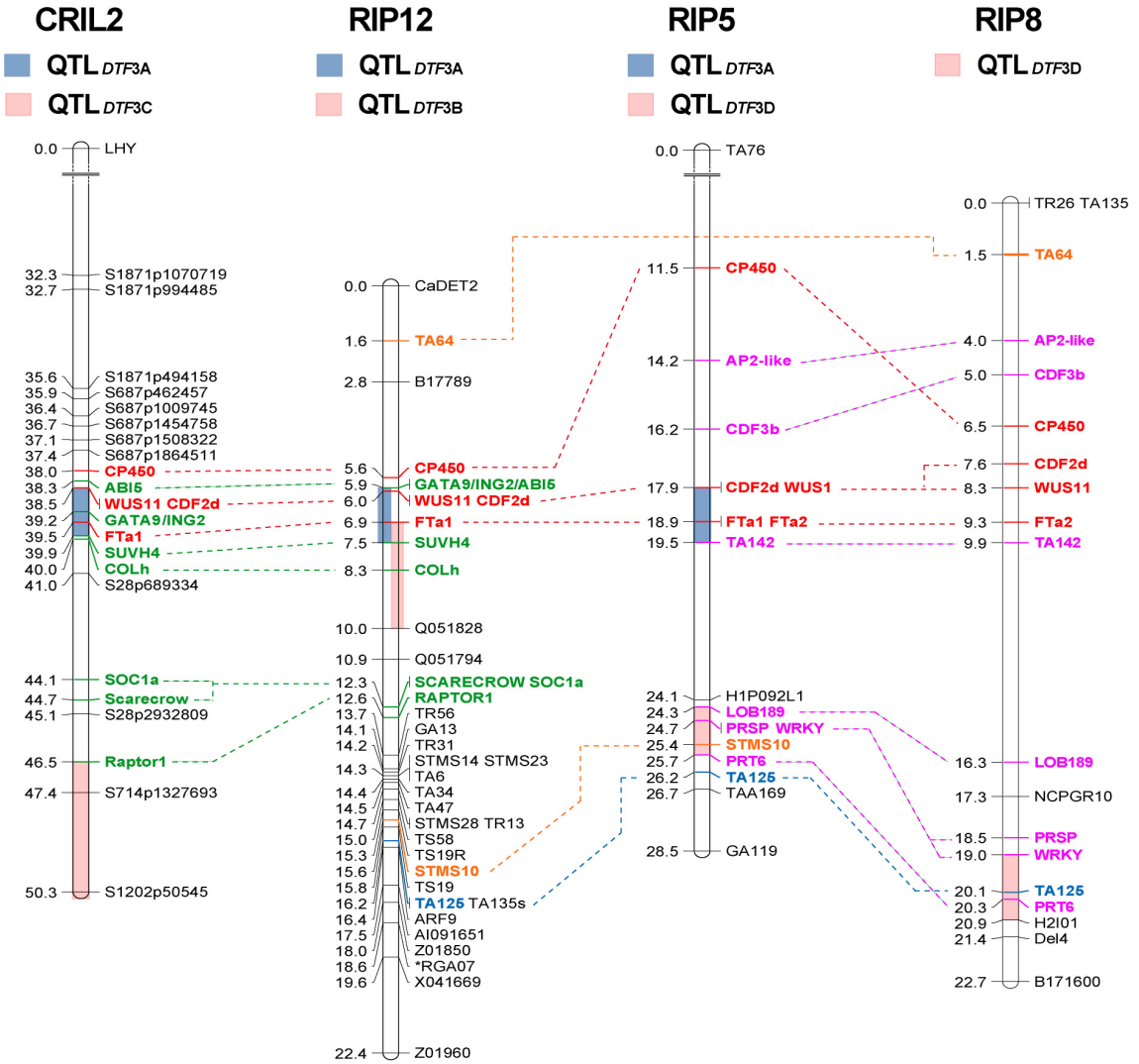

FIGURE 1 | Comparative mapping of flowering time QTLs on chromosome 3. Four regions of chromosome 3 (3A to 3D, colored bars) were found to influence flowering time across three different populations. The length of the bars representing each QTL indicates the two-LOD support interval, which corresponds to a ~95% confidence interval (Van Ooijen, 2006). Only region 3A, in the central portion of the chromosome and containing a cluster of *FT* genes, is consistently detected in both narrow and wide crosses. Numbers at the left of the bars represent genetic distance (in cM). Common markers were used to compare the relative position of the QTLs across populations. Markers common to all populations are shown in red, to three populations in blue and to two populations in orange. Those common to both interspecific populations are shown in green, and to both intraspecific populations, in pink.

FT Genes in Chickpea

In view of the central location of an *FT* gene cluster under the *DTF3A* QTL, we characterized the entire chickpea phosphatidylethanolamine-binding protein (PEBP) family, which includes *FT* genes and the related *TFL1* (*TERMINAL FLOWER 1*) family of flowering repressors (Wickland and Hanzawa, 2015; **Supplementary Figures 8A, 9** and **Supplementary Table 4**). Five chickpea *FT*-like genes were identified in the three previously described legume *FT* subclades; *FTa*, *FTb*, and *FTc* (**Supplementary Figure 10**; Hecht et al., 2011). This analysis confirmed that chickpea, like Medicago, possesses three *FTa* genes, with two of these (*FTa1* and *FTa2*) located together with the single *FTc* gene on chromosome 3 in a tandem arrangement (Hecht et al., 2011; Laurie et al., 2011). Only one other PEBP gene was found on this chromosome (*TFL1a*), while the remaining genes were located on chromosomes 1 (*TFL1b*), 2 (*FTb* and *FTa3*), 6 (*MOTHER OF FT*, *MFT*), and 8 (*TFL1c*) (**Supplementary Figure 8B**). The only difference in the chickpea *FT* family compared to other related legume species is the apparent presence of only a single *FTb* gene, where Medicago and pea each have two highly similar paralogs located in tandem in a conserved genomic location on chromosome 7 and LG5, respectively (Hecht et al., 2011; Laurie et al., 2011). In the broader PEBP family, chickpea possesses single-copy orthologs of the *BFT* (*BROTHER OF FT*) and *MFT* genes, and also of two of the three *TFL1* genes previously described

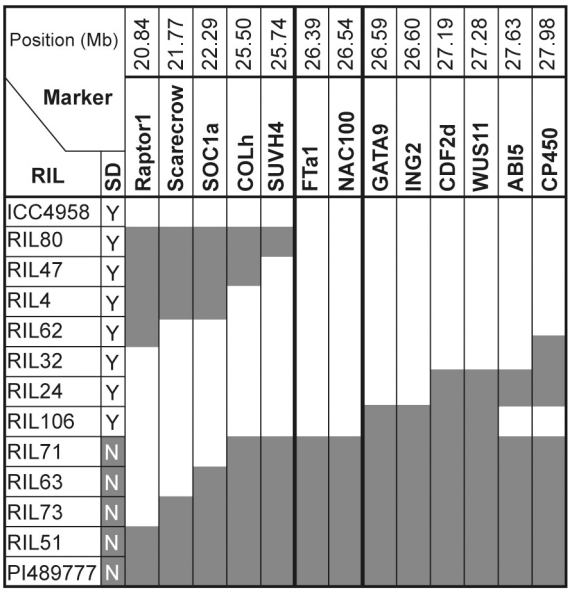

FIGURE 2 | Refinement of the *DTF3A* location. Marker genotypes in recombinant inbred lines from the CRIL2 population showing recombination breakpoints across a 7.14 Mb region of chromosome 3 spanning the *DTF3A* locus. Numbers over the markers correspond to their physical position (in Mb) in the CDC Frontier genome assembly in NCBI (ASM33114v1; Varshney et al., 2013). Alleles from the domesticated parent ICC4958 are shown in white and those from the wild parent PI489777 in gray. Flowering phenotype is shown in the column headed SD and indicates whether the indicated lines flowered (Y) or remained vegetative (N) under an 8h photoperiod. This phenotype showed no recombination between markers *FTa1* and GATA9.

in pea and Medicago, *TFL1a* and *TFL1b*. The third gene, *TFL1c,* was represented by three gene models in the CDC Frontier genome assembly (**Supplementary Table 4**), but was not represented at all in the other available chickpea genome (from ICC4958, assembly ASM34727v3); a discrepancy that will require clarification in future.

Genes in the *FT*a1-*FT*a2-*FT*c Cluster Are Upregulated in Early Accessions

FT genes are well-known as important positive regulators of flowering. This is also true in legumes, where several *FT* genes have been identified and most are capable of promoting flowering when overexpressed in Arabidopsis (Kong et al., 2010; Hecht et al., 2011; Laurie et al., 2011; Sun et al., 2011). Therefore, if one of the *FT* genes in the cluster was the basis for the effect of the *DTF3A* locus, increased activity or expression of one or more of these genes would be expected in the early-flowering parent. To evaluate this possibility, we examined the expression of *FT* genes in the parent lines of the mapping populations. In view of previous reports indicating tissue- and photoperiod-specific expression of *FT* genes in pea and Medicago, we collected samples from leaf and apex tissue under both LD and SD conditions at two timepoints. Expression of the *AP1* homolog *PROLIFERATING INFLORESCENCE MERISTEM* (*PIM*) was used as an indicator of flowering commitment, as previously described for other legumes including chickpea (Hecht et al., 2011; Ridge et al., 2016).

Figure 3 shows that 2 weeks after emergence *PIM* expression in shoot apices was not detectable in any of the accessions. By 4 weeks, *PIM* was expressed significantly above background in all three late parents under LD but not in SD, whereas it was strongly expressed under both LD and SD in the early parents. In parallel, the expression of all three genes in the chromosome 3 *FT* cluster (*FTa1*, *FTa2,* and *FTc*) was elevated in the early parents at 4 weeks under SD and LD. In ICC4958, expression of all three genes was higher than the wild parent even by week 2; i.e., before detectable expression of *PIM*. Similarly, expression of *FTa2* and *FTc* was also elevated in the early parent of RIP12 (ICCL81001) at week 2. However, *FTa2* transcript could not be detected in the early parent of RIP5/8 (WR315), reflecting a complete deletion of the gene (**Supplementary Figure 11**). This result suggests that the elevated expression of *FTa2* seen in the domesticated parents of CRIL2 and RIP12 is unlikely to be solely responsible for the effect of *DTF3A* in these populations. As in pea and Medicago, *FTa1* and *FTc* in chickpea differed in the tissue-specificity of their expression, with *FTa1* expressed strongly in leaves and weakly at the shoot apex, and *FTc* expressed only weakly at the shoot apex. Despite these differences, both genes showed similar expression profiles, with an early upregulation in the domesticated/early flowering parents that preceded *PIM* induction, and they therefore represent good candidates to underlie the QTL.

Significant expression of the single *FTb* gene was seen in 2-week-old plants, but only under LD, and at a similar level in both early and late parents. This is similar to the strongly photoperiod-dependent expression of *FTb* genes previously reported in pea and Medicago (Hecht et al., 2011; Laurie et al., 2011), and indicates that *FTb* misexpression is not a factor in the effect of *DTF3A* under SD. The expression of *FTa3* was restricted to leaf tissue, and only detected at a late developmental phase after commencement of flowering (**Supplementary Figure 12**), suggesting it is unlikely to make a major contribution to the observed differences in flowering time. The expression of *TFL1b* and *TFL1c* was also tested in apical tissue. Whereas expression of *TFL1c* in this tissue did not change significantly, *TFL1b* expression was higher in the wild line under non-inductive conditions and gradually decreased in cultivated and wild accession grown in long photoperiod, consistent with a possible role as a floral repressor. However, the level of expression observed in both genes was very low and the biological significance of these changes is therefore uncertain (**Supplementary Figure 12**).

The DTF3A Locus Coincides With QTL for Plant Architecture

The late-flowering phenotype of wild chickpea is also associated with a prostrate growth habit (GH), reduced apical dominance and an increased number of branches (Singh and Shyam, 1959; Aryamanesh et al., 2010; Ali et al., 2015). Consistent with these reports, we also observed major differences in growth habit between CRIL2 parents and in the CRIL2

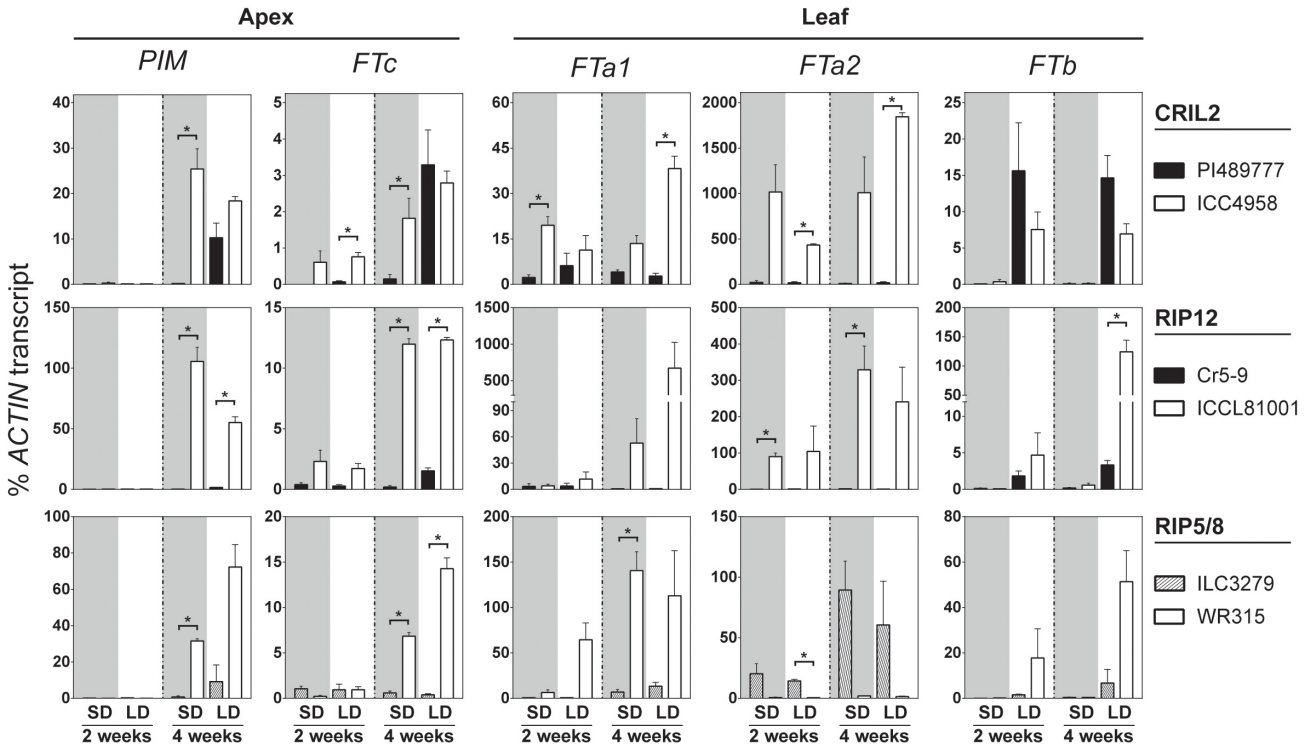

FIGURE 3 | *FT* genes in the LG3 cluster are upregulated in the early parents of the crosses. Relative expression profiles of *FT* genes and the floral indicator *PIM* in the parental lines of the four chickpea populations analyzed. Expression was measured in dissected apical bud or leaves of plants grown from sowing under short (SD, gray background) and long days (LD, white background) for 2–4 weeks. Late flowering lines are shown in black and early parents in white. The average ± SE of two biological replicates (two technical replicates each) is shown, and transcripts were normalized against *ACTIN*. Asterisk indicates significant difference ($p < 0.05$) in the level of expression (*t*-test).

population in SD, which we quantified for genetic analysis using a four step scale (**Supplementary Figures 13A–D**). We also recorded branching propensity in young plants (prior to visible flower initiation) under both SD and LD. Late flowering RILs also showed a shoot architecture that resembled the wild parent, so we investigated the correlation between these three traits (**Supplementary Figure 13**). A highly significant difference ($p < 0.001$) was found between the flowering dates of erect/semierect RILs compared to those with a prostrate/semiprostrate growth habit (**Supplementary Figure 13E**), confirming that in the segregating population, prostrate growth habit is associated with late flowering, as expected. Inspection of individual RILs showed a nearly perfect correlation, with flowering observed in all 53 erect or semi-erect RILs but in only three out of 71 lines categorized as prostrate or semi-prostrate. A strong negative correlation ($r = -0.504$, $p < 0.001$) was found between growth habit and branching index (**Supplementary Figure 13F**), indicating that erect and semi-erect plants in general also had a lower branching index (BI).

BI of the population was generally higher in SD than in LD, as might be expected in view of the longer vegetative growth phase. However, across the population, a strong positive correlation ($r = 0.679$, $p < 0.001$) was found in the BI between photoperiods, suggesting that at this stage (3 weeks old plants) a genetic component of this trait is unrelated to photoperiod. QTL analysis revealed two QTLs for growth habit; a major QTL on LG3 that explained 66% of the variation for this trait, and a minor QTL on LG4. For BI, a single QTL in a similar location was identified under both photoperiods (**Table 1**). Interestingly, the QTL for both GH and BI in chromosome 3 were closely co-located with the *DTF3A* flowering time QTL described above (**Figure 4**). In addition, the physiology of these three QTL is similar with respect to their strong effect under SD and their absence, or minor effect, under LD, as seen in the genotype means for the *FTa1* peak marker shown in **Table 1**.

DISCUSSION

One of the critical events in chickpea evolutionary history is thought to have been its conversion from a winter to a summer crop, likely achieved by Neolithic farmers in an attempt to reduce the incidence of Ascochyta blight, whose onset is favored by the cool, wet conditions that typify Mediterranean winters (Kumar and Abbo, 2001; Abbo et al., 2003a,b). For this shift in the chickpea farming system to succeed, a major modification of phenology toward earliness would have been required in order to match the considerably shorter growing season. This selective pressure is evident today in the typically early flowering

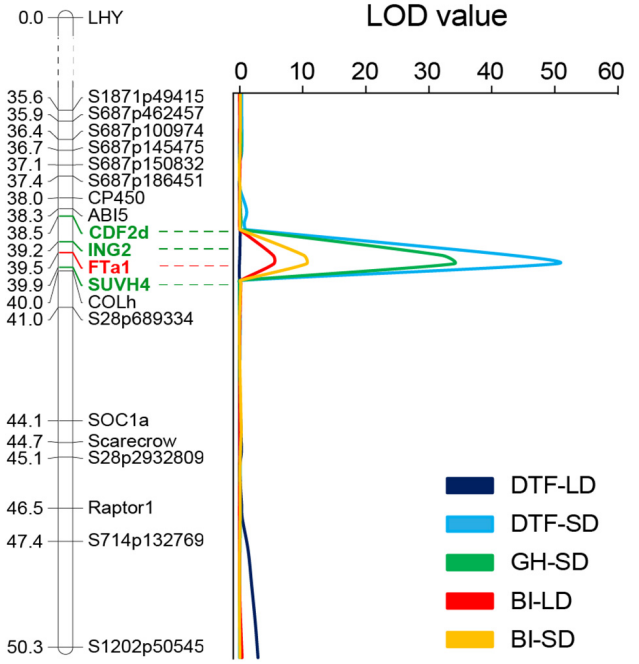

FIGURE 4 | The DTF3A region is also associated with growth habit and shoot branching. Portion of the Linkage Group three from the CRIL2 genetic map showing the perfect co-location of QTLs obtained for flowering time (DTF), growth habit (GH), and branching index (BI) under short (SD) and long days (LD). Numbers at the left of the bar indicate genetic distance (in cM). Markers included in the 3A interval (95% interval confidence) are highlighted in green and the most strongly associated marker (*FT*a1) is shown in red.

phenotype of the domesticated *C. arietinum* relative to wild *Cicer* species (Berger J. et al., 2004).

Our analyses identify a central region of chromosome 3 (referred to as region 3A) that makes a major contribution to this difference in flowering time between domesticated chickpea and its wild progenitor, *C. reticulatum*, in two populations utilizing different *C. arietinum* parents and grown in different conditions. This result is consistent with several previous reports. Das et al. (2015) found a recurrent major QTL on chromosome 3 in an interspecific cross using ICC4958 as the domesticated parent. Aryamanesh et al. (2010) found a major QTL on chromosome 3 defined by the same interval as that reported initially in RIP12 by Cobos et al. (2009) and narrowed in the present study. The fact that these studies use different and unrelated *C. arietinum* accessions suggests that the presence of early alleles at this locus may be a defining feature of domesticated chickpea.

Another interpretation is that the apparent importance of this locus could reflect the fact that the wild parents used in all of these studies are closely related and could conceivably carry a unique variant at this locus that is not representative of the wider *C. reticulatum* germplasm. However, this is discounted by the recent finding of von Wettberg et al. (2018), who examined crosses between a common domesticated parent and 29 newly collected wild accessions representing a much wider diversity, and found that all progenies shared a common major QTL in a 3.55 Mb interval of chromosome 3 encompassing the LG3A region. Interestingly, this region also appears to have a significant effect within domesticated chickpea, as revealed by our analysis of two intraspecific populations, and several other studies (e.g., Hossain et al., 2010; Rehman et al., 2011). However, its effect at this level seems to be more dependent on environment and the influence of other loci, suggesting that additional variation in this region may have also had a role in post-domestication diversification of flowering behavior. Further clarification of this scenario will require a wider analysis in both interspecific and intraspecific contexts, whether in biparental populations or through association approaches.

In addition to late phenology, wild chickpea is also distinguished from domesticated forms by the greater profusion of branches and prostrate growth habit (Ali et al., 2015), and we found that the same chromosomal region 3A also had a significant influence on both traits, particularly under SD conditions, as reflected by the presence in the region of a major QTL for each of these traits (QTL *GH3* and QTL *BI3*). To date, two major loci, *Hg* and *Hg2*, have been reported to determine growth habit differences between *C. arietinum* and *C. reticulatum* (Muehlbauer and Singh, 1987; Kazan et al., 1993; Ali et al., 2015). Interestingly, *Hg* has been mapped to the central region of chromosome 3 by Winter et al. (2000), using a population derived from the same parents as CRIL2, and studies by Cobos et al. (2009), Aryamanesh et al. (2010), Ali et al. (2015) have all reported a locus influencing growth habit in this region. Since the *GH3* QTL we describe here for CRIL2 is located within the intervals reported in these studies, it seems likely that all of these studies are detecting the same locus (*Hg*). Association of flowering with different features of shoot architecture has been previously described in a number of other legume species, including chickpea (Lichtenzveig et al., 2006; Julier et al., 2007; Lagunes Espinoza et al., 2012; González et al., 2016; Yang et al., 2017). In the case of QTL in the chickpea LG3A region, such an association could either represent the action of independent but tightly linked genes, or the pleiotropic effects of a single gene.

The discrete and approximately 1:1 segregation of flowering time in CRIL2 under controlled SD conditions enabled us to map *DTF3A* as a Mendelian trait to a narrower interval, thereby reducing the number of potential candidates. The only remaining clear candidates were a cluster of three *FT* genes orthologous to the *FTa1/a2/c* cluster identified in Medicago and pea by Hecht et al. (2005, 2011). *FT* genes have a widely conserved role as flowering promoters (Wickland and Hanzawa, 2015), and several recent studies show that this is also the case for legume *FTa* and *FTc* genes (Kong et al., 2010; Hecht et al., 2011; Laurie et al., 2011; Sun et al., 2011). We identified elevated expression of genes in the *FT* cluster in the early parents of all three crosses examined (**Figure 3**), implicating the general de-repression of these genes as the likely molecular basis for the *DTF3A* effect. A comparable situation has been recently described in another legume, narrow-leafed lupin (*Lupinus angustifolius*), where a strong ancestral vernalization requirement has restricted

production in warmer regions. This limitation has been overcome by the incorporation of dominant alleles at the major locus *Ku*, which confer de-repressed expression of a tightly linked *FTc* gene and permit flowering in the absence of vernalization (Nelson et al., 2017; Taylor et al., 2018). However, compared to lupin, where only a single *FT* gene is present in this genomic location, the presence of three genes in chickpea is clearly a more complex situation, and raises the question of which of them might be responsible for the QTL effects on photoperiod response, or the QTLs for vernalization response that has been localized to the same genomic region on LG3 (Samineni et al., 2015; Pinhasi van-Oss et al., 2016).

The *FTa1* gene plays a key role in regulation of flowering in both pea and Medicago, as loss-of function mutants show significant impairment of flowering in both species, and overexpression in Medicago confers early flowering and reduced sensitivity to photoperiod and vernalization (Hecht et al., 2011; Laurie et al., 2011). *FTa1* would therefore seem to be the strongest candidate for the causal gene underlying *DTF3A*. Although the role of *FTc* has not been systematically explored in either species, both *MtFTc* and *PsFTc* are strong activators of flowering when overexpressed in Arabidopsis, and their induction in apical tissues correlates closely with flowering (Hecht et al., 2011), suggesting that the higher levels of *CaFTc* expression could also potentially contribute to the earlier flowering of domesticated lines. Intriguingly, the most dramatic expression difference in the two interspecific comparisons was seen for *FTa2*, which was expressed at a low level in *C. reticulatum* parents and over 20 times higher in the domesticated parents. However, despite this striking association with early flowering, *FTa2* was not expressed at all in the early parent of the intraspecific cross, indicating that the early flowering of domesticated relative to wild chickpea cannot result primarily from the high level of *FTa2* expression. Also, in contrast to *FTa1* and *FTc*, *FTa2* from pea or Medicago is much less effective for induction of flowering when expressed in transgenic Arabidopsis, and its endogenous expression patterns are not consistently associated with flowering (Hecht et al., 2011; Laurie et al., 2011). Taken together, these observations suggest that *FTa2* is less likely to be the basis for the interspecific effects of *DTF3A*, but it remains plausible that these effects might reflect general de-repression across the cluster and a functional contribution from all three genes.

The strong photoperiod-dependence of the *DTF3A* effect can also be interpreted in terms of the known role of *FT* genes in mediating of environmental effects on flowering. In both pea and Medicago, photoperiod and vernalization responses appear to be integrated through *FT* genes, but whereas *FTa* genes are regulated by both photoperiod and vernalization, *FTb* genes are strictly regulated by photoperiod (Hecht et al., 2011; Laurie et al., 2011). In chickpea, a similar LD-specific expression of the single *FTb* gene is seen in both wild and domesticated parents (**Figure 3**) and may be sufficient for maximal promotion of flowering, which could provide an explanation for the minimal effect of *DTF3A* under these conditions. In contrast, under non-inductive SD conditions, the absence of *FTb* expression or other inputs would presumably expose any effects of elevated expression of the *FTa/c* cluster.

Whether one or more of the *FT* genes are indeed responsible for the effects of *DTF3A*, it is also of interest to consider what might be the molecular basis of their observed de-repression. The apparently specific effects of the QTL on expression of the underlying *FT* genes suggests a scenario in which the domesticated parents might have undergone modification of either a *cis*-acting or a closely linked *trans*-acting mechanism normally required for repression of the cluster. The absence of other plausible candidates in the defined region favors a *cis*-acting mechanism, and precedent for this is provided by recent studies in two other legumes. In Medicago, insertions in the third intron and 3′ flanking region of *FTa1* confer gain-of-function phenotypes, with elevated *FTa1* expression and dominant early flowering (Jaudal et al., 2013), whereas in narrow-leafed lupin, the derepression of *FTc* expression that underlies the effects of *Ku* alleles is associated with deletions in the *FTc* promoter (Nelson et al., 2017; Taylor et al., 2018). The recently reported role for the polycomb-group protein VRN2 (*VERNALIZATION 2*) in *FTa1* repression in Medicago (Jaudal et al., 2016) points to the likely existence of both epigenetic and transcriptional components to this regulation.

Direct involvement of *FT* genes would also provide an explanation for the association of growth habit and flowering effects with the chromosome 3A region. It is becoming increasingly apparent that *FT* genes, in addition to being major flowering regulators, also affect plant architecture and growth habit across a wide range of plant species including Arabidopsis, tomato, rose and rice (Lifschitz et al., 2006; Tamaki et al., 2007; Hiraoka et al., 2013; Huang et al., 2013; Randoux et al., 2014; Tsuji et al., 2015; Weng et al., 2016). However, the most direct and relevant comparison with chickpea is again provided by Medicago, where *MtFTa1* overexpression converts the prostrate habit of plants grown under SD to a more erect habit typical of LD (Laurie et al., 2011). This effect is clearly similar to that of the corresponding region on chromosome 3A in domesticated chickpea. In contrast, Medicago *fta1* mutants show a highly branched, prostrate phenotype under LD similar to that of wild-type under SD, further emphasizing the multiple roles of *FTa1*. This observation strengthens the case that the major flowering time and growth habit loci in this region of chromosome 3 represent pleiotropic effects of misexpression of genes in the *FT* cluster, and possibly of *FTa1* in particular.

An emerging theme in long day legumes appears to be an important adaptive role for dominant genetic variants in the region of the *FTa/c* cluster that relax the environmental constraints on flowering and permit early flowering (Weller and Ortega, 2015). Whether a common molecular mechanism unites these adaptations and explains their repeated evolution remains to be determined. Among the ancient legume crops, chickpea in particular may represent a unique example in which modification of such a mechanism has been fundamentally important to crop success. Future, more detailed analyses should shed light on its molecular basis and physiological consequences, and its significance for chickpea domestication and adaptation.

AUTHOR CONTRIBUTIONS

JW, RO, VH, and TM conceived the study. JW and RO designed the study. RO, RM, NC-G, RP, JR, and TM carried out the experiments and/or generated the data. RO and JW wrote the manuscript with inputs from the other authors. All authors analyzed the data.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2019.00824/full#supplementary-material

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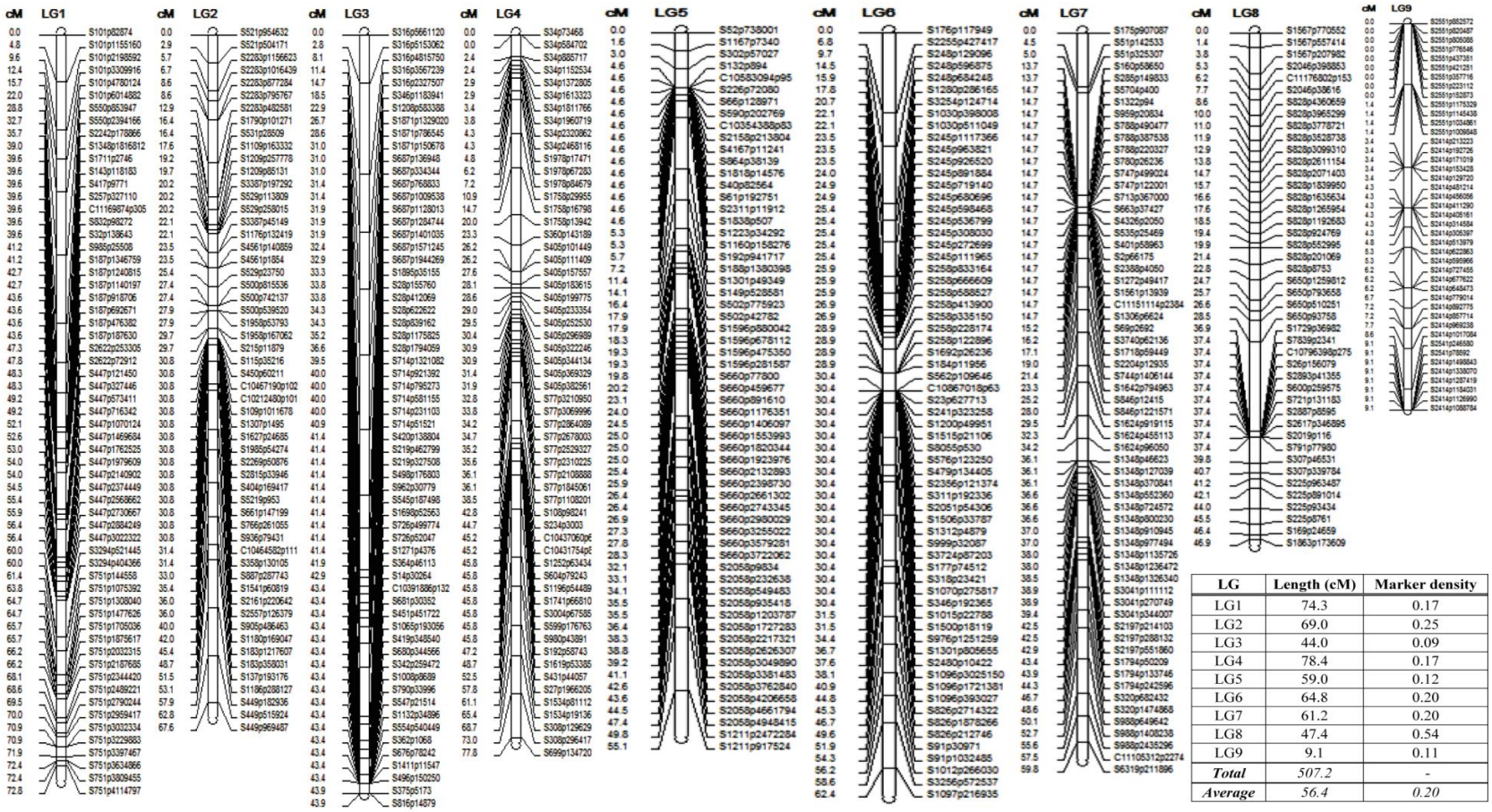
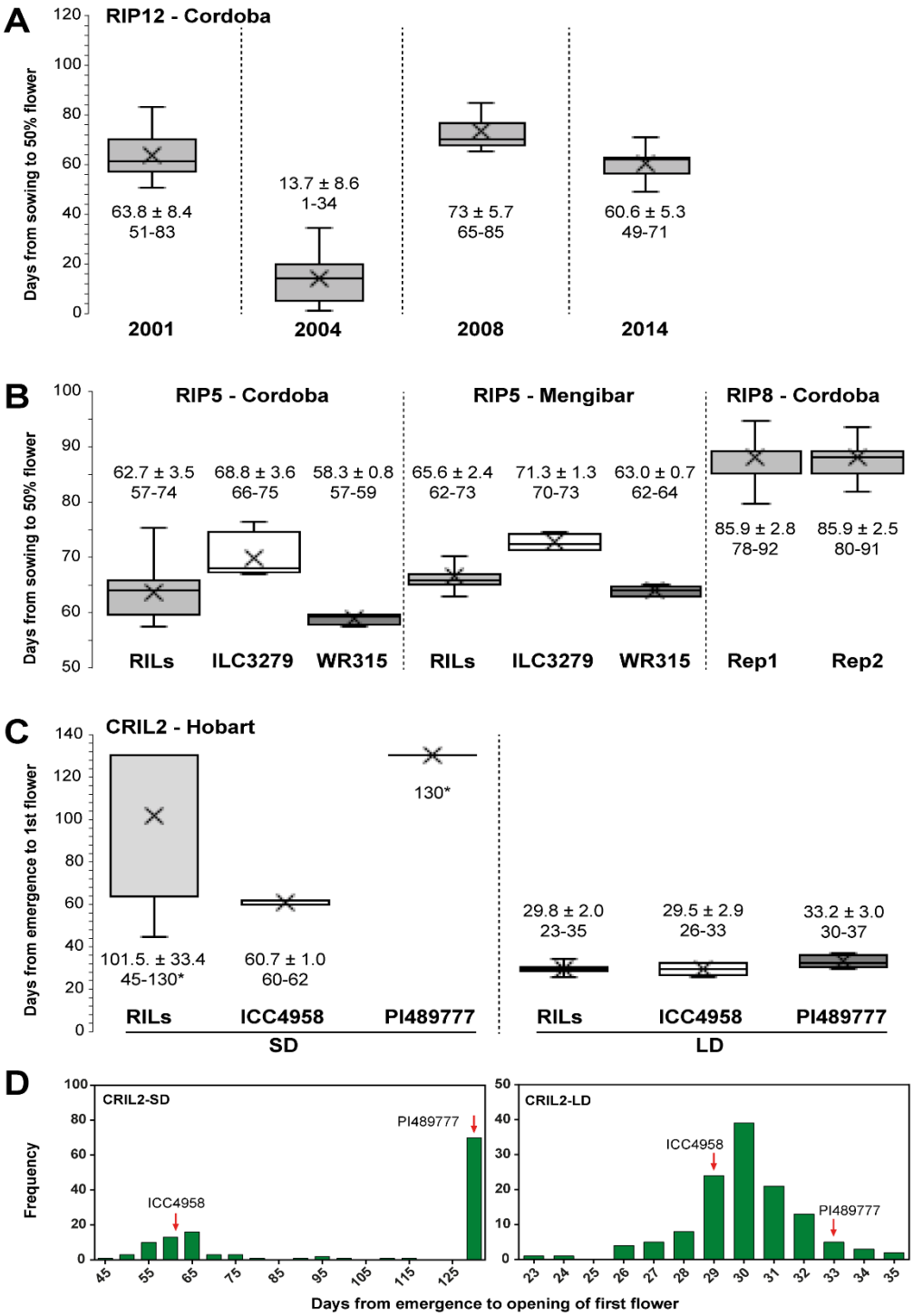
Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

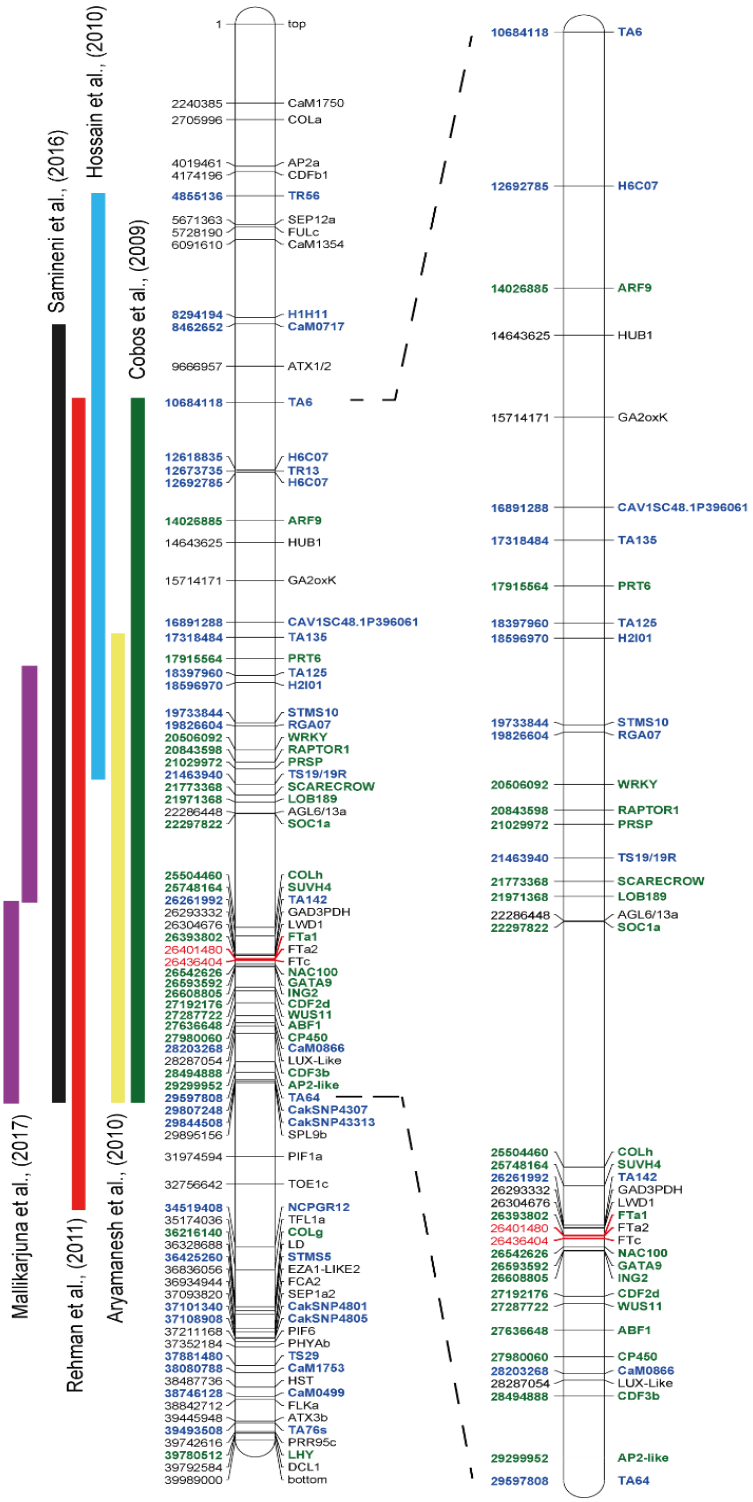


LG	Length (cM)	Marker density
LG1	74.3	0.17
LG2	69.0	0.25
LG3	44.0	0.09
LG4	78.4	0.17
LG5	59.0	0.12
LG6	64.8	0.20
LG7	61.2	0.20
LG8	47.4	0.54
LG9	9.1	0.11
Total	*507.2*	-
Average	*56.4*	*0.20*

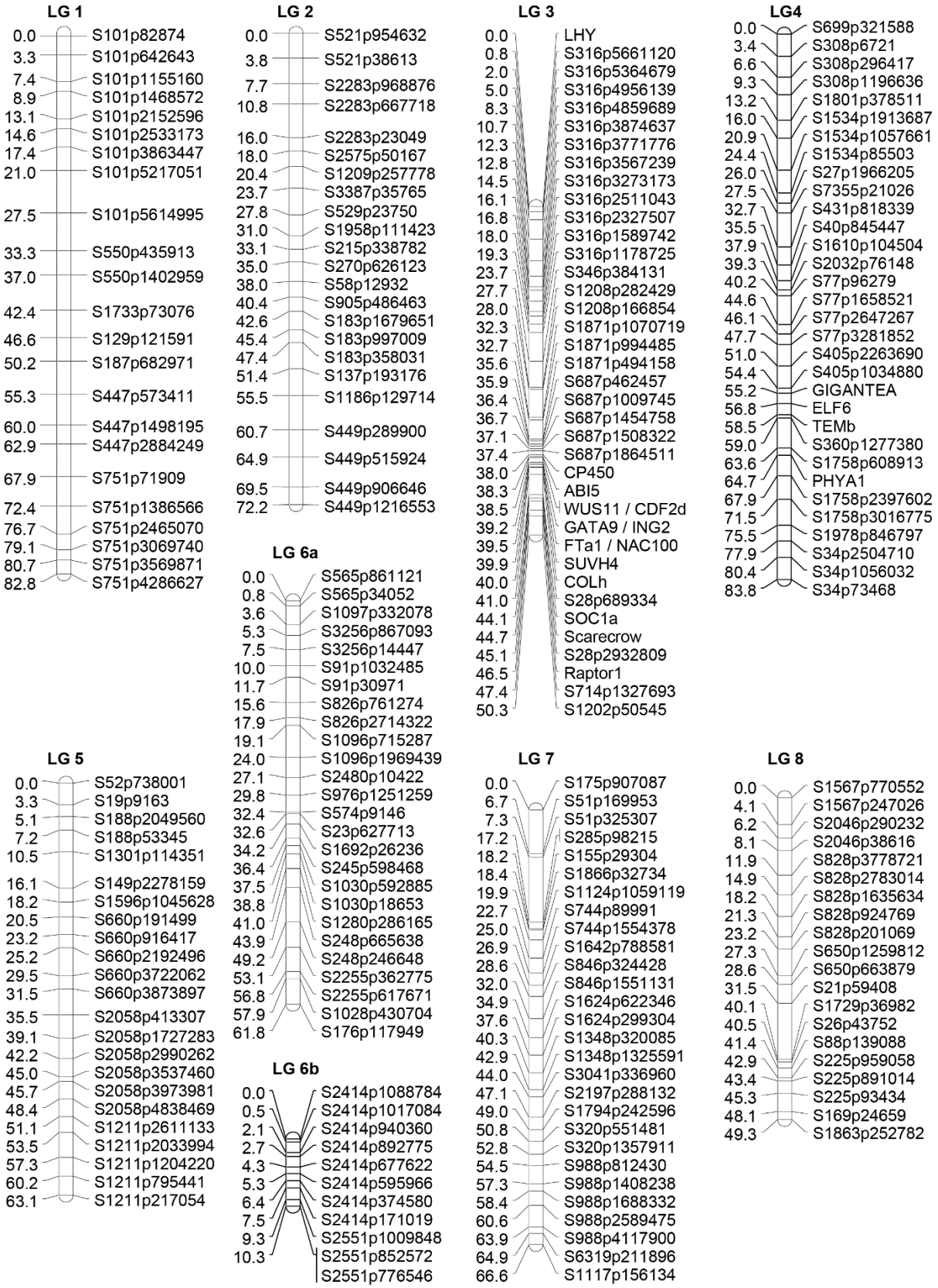
Supplementary Figure 1. High density linkage map of the chickpea interspecific population CRIL2, ICC4958 (*Cicer arietinum*) x PI489777 (*Cicer reticulatum*), which was used to select a subset of markers to contruct genetic maps with the HRM markers designed in this study (Figure S4). The high density map is based on 2956 RAD-GBS SNP markers genotyped in 107 RIL lines (von Wettberg et al. 2018). It comprised nine linkage groups, with a total map length of 507.2cM. The map length of each linkage group varied from 9.1cM (LG9) to 78.4cM (LG4) with an average of 56.36cM (Inset table). A total of 2,956 markers were mapped on all of the 9 linkage groups. The number of markers mapped on an individual linkage group varied from 86 (LG9) to 495 (LG3) with an average of 328.44 markers/LG.



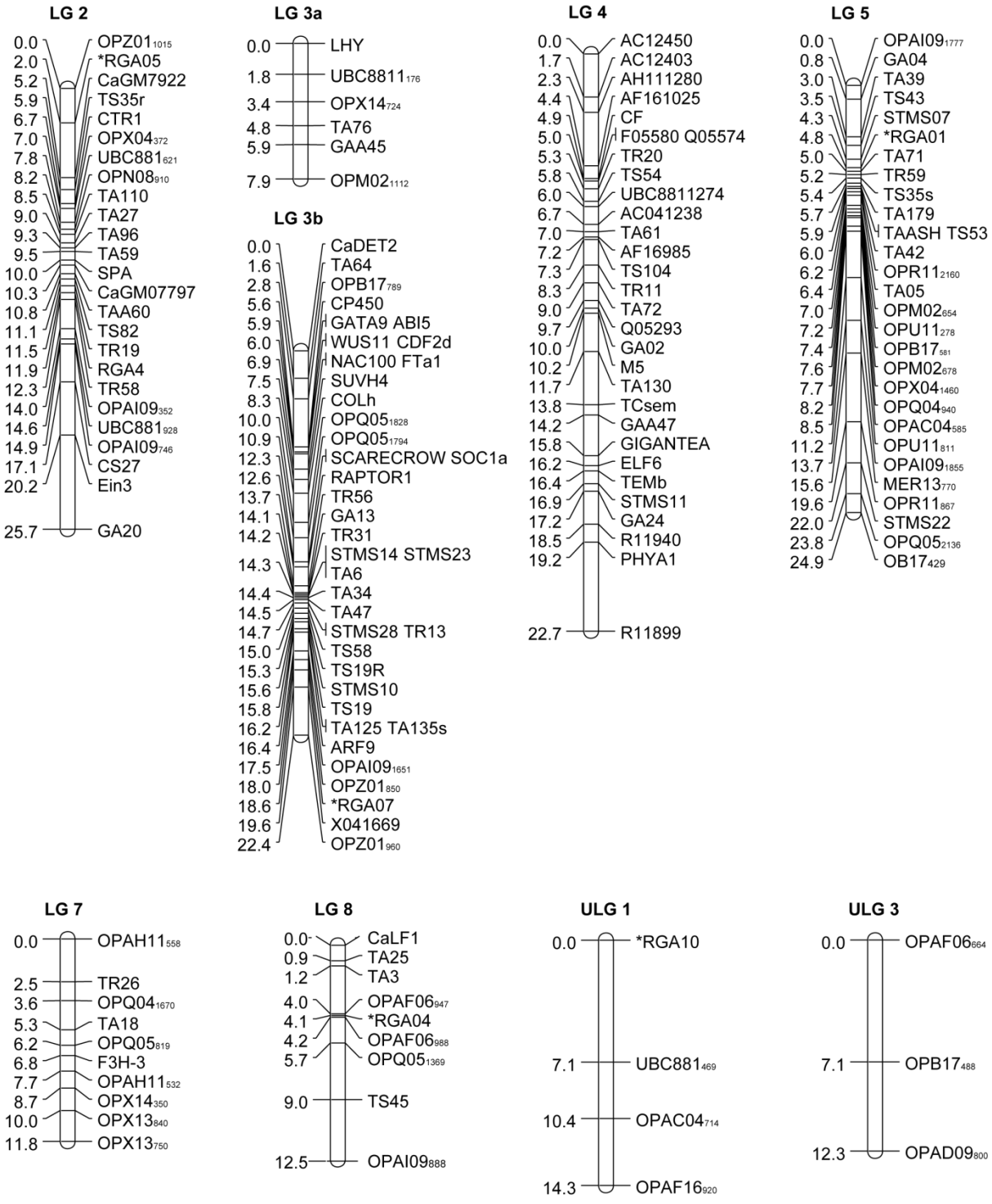
Supplementary Figure 2. Boxplot illustrating the variation in flowering time obtained in different environments for RIP12 **(A)**, RIP5/RIP8 **(B)** and CRIL2 **(C)**. Note that for RIP12, the flowering values during the 2004 season are relative: the date of the first visible flower in any RIL of the population was considered as day 1 and used as reference for the rest of the population. Box edges denote the lower and upper quantiles with mean (cross) and median (line) values in the middle of the box. Numbers under the boxes correspond to mean ± standard deviation and range (minimum-maximum value). **(D)** Frequency distribution of flowering time variation measured in CRIL2 parents and RILs grown under long (LD) and short days (SD). Red arrows indicate the phenotypic values of the parental lines.



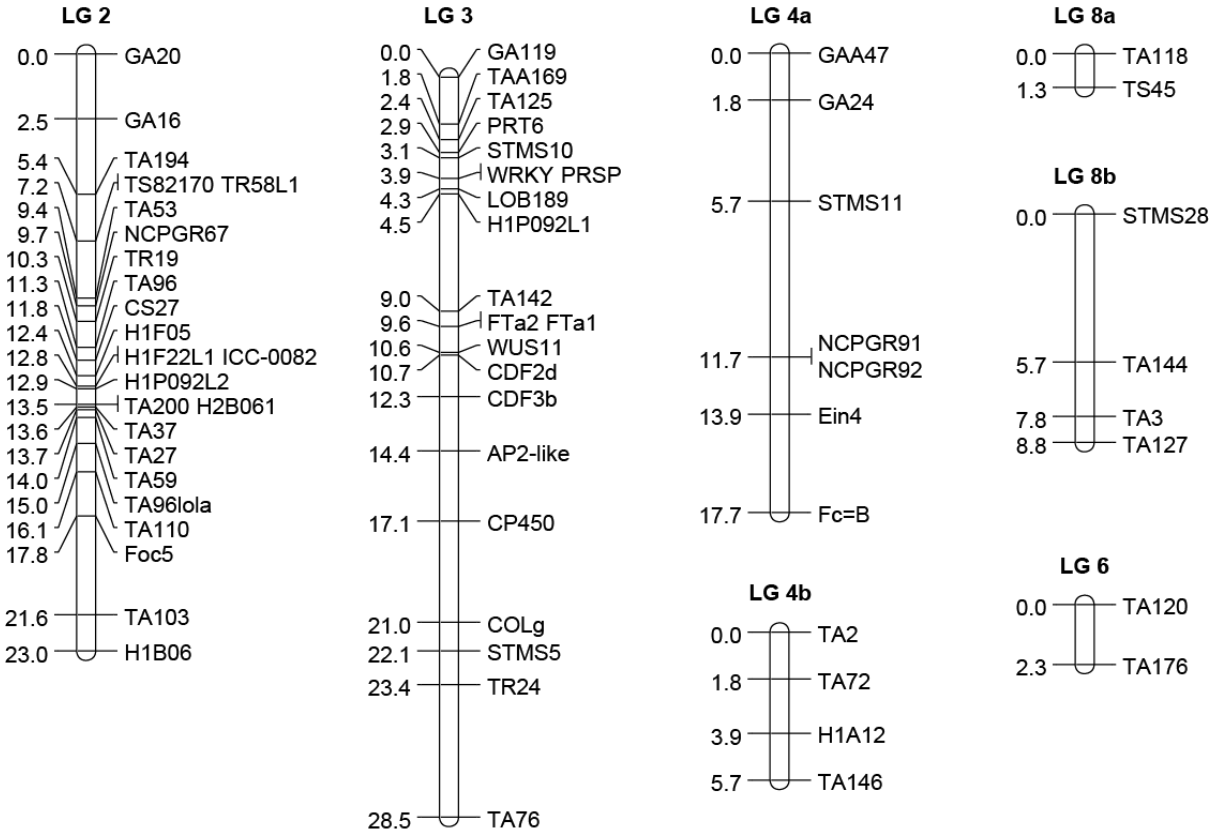
Supplementary Figure 3. Representation of the chickpea chromosome 3 and the region between markers TA6 and TA64, summarizing the different flowering QTL (vertical coloured bars) described to date in the region (Aryamanesh et al. 2010; Cobos et al. 2009; Mallikarjuna et al. 2017; Rehman et al. 2011; Samineni et al. 2016). Numbers on the left of the linkage group indicate physical position in bp, according to the genome assembly of cultivar CDC Frontier (ASM33114v1; Varshney et al., 2013). Interesting markers from previous reports are shown in blue and markers designed for this study are shown in green. Other interesting flowering-related genes are shown in black while the cluster of *FT* genes is highlighted in red.



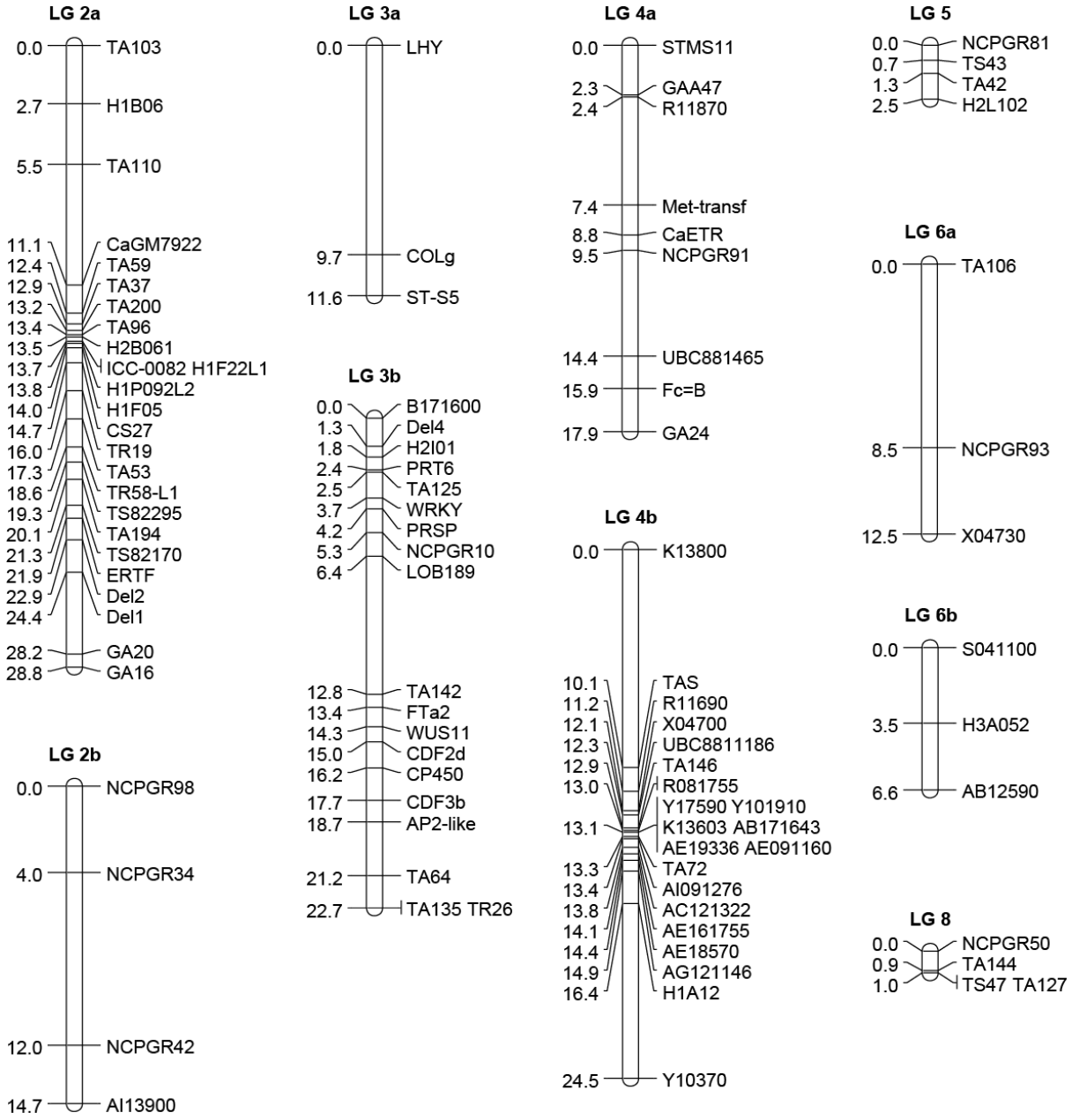
Supplementary Figure 4. Genetic linkage map constructed for CRIL2 (ICC4958 x PI489777). Numbers on the left of each linkage group represent the absolute distances in cM. The map comprises a total of 226 markers, grouped in 9 linkage groups covering a total distance of 540 cM, with an average inter-maker distance of 2.4 cM.



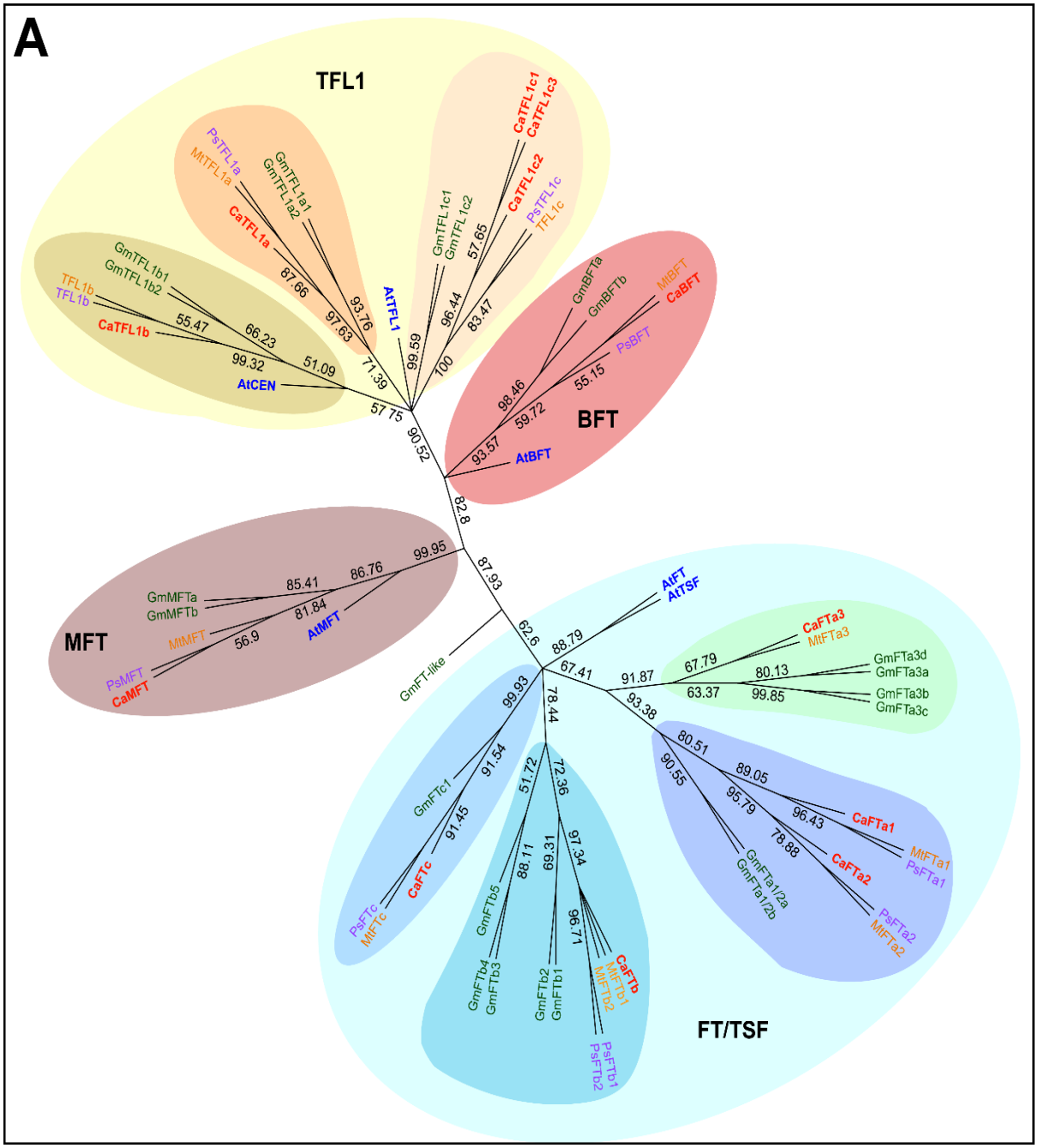
Supplementary Figure 5. Genetic linkage map constructed for RIP12 (ICCL81001 x Cr5-9). Numbers on the left of each linkage group represent the absolute distances in cM. The map comprises a total of 155 markers, grouped in 9 linkage groups covering a total distance of 154.5 cM, with an average inter-maker distance of 1.05 cM.



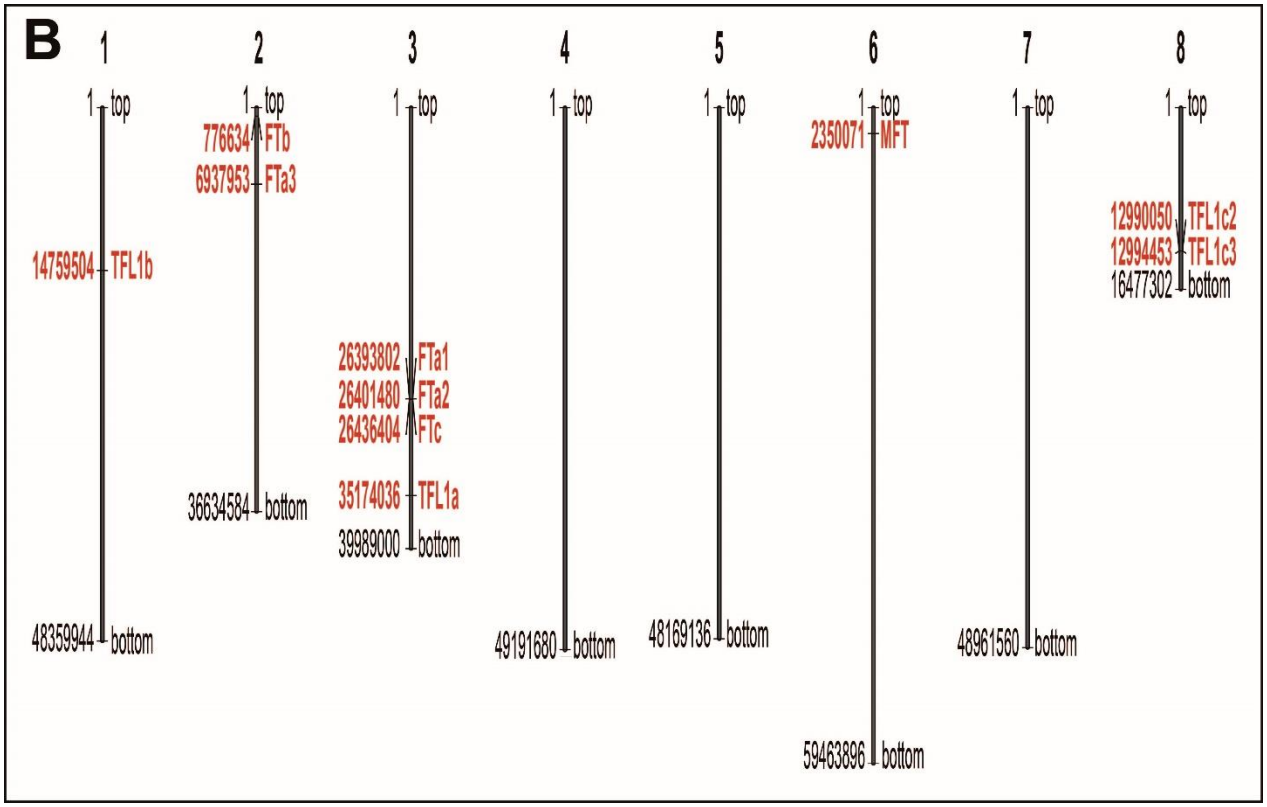
Supplementary Figure 6. Genetic linkage map constructed for RIP5 (WR315 x ILC3279). Numbers on the left of each linkage group represent the absolute distances in cM. The map comprises a total of 64 markers, grouped in 7 linkage groups covering a total distance of 87.3 cM, with an average inter-maker distance of 1.53 cM.



Supplementary Figure 7. Genetic linkage map constructed for RIP8 (ILC3279 x WR315). Numbers on the left of each linkage group represent the absolute distances in cM. The map comprises a total of 95 markers, grouped in 10 linkage groups covering a total distance of 142.7 cM,with an average inter-maker distance of 1.76 cM.

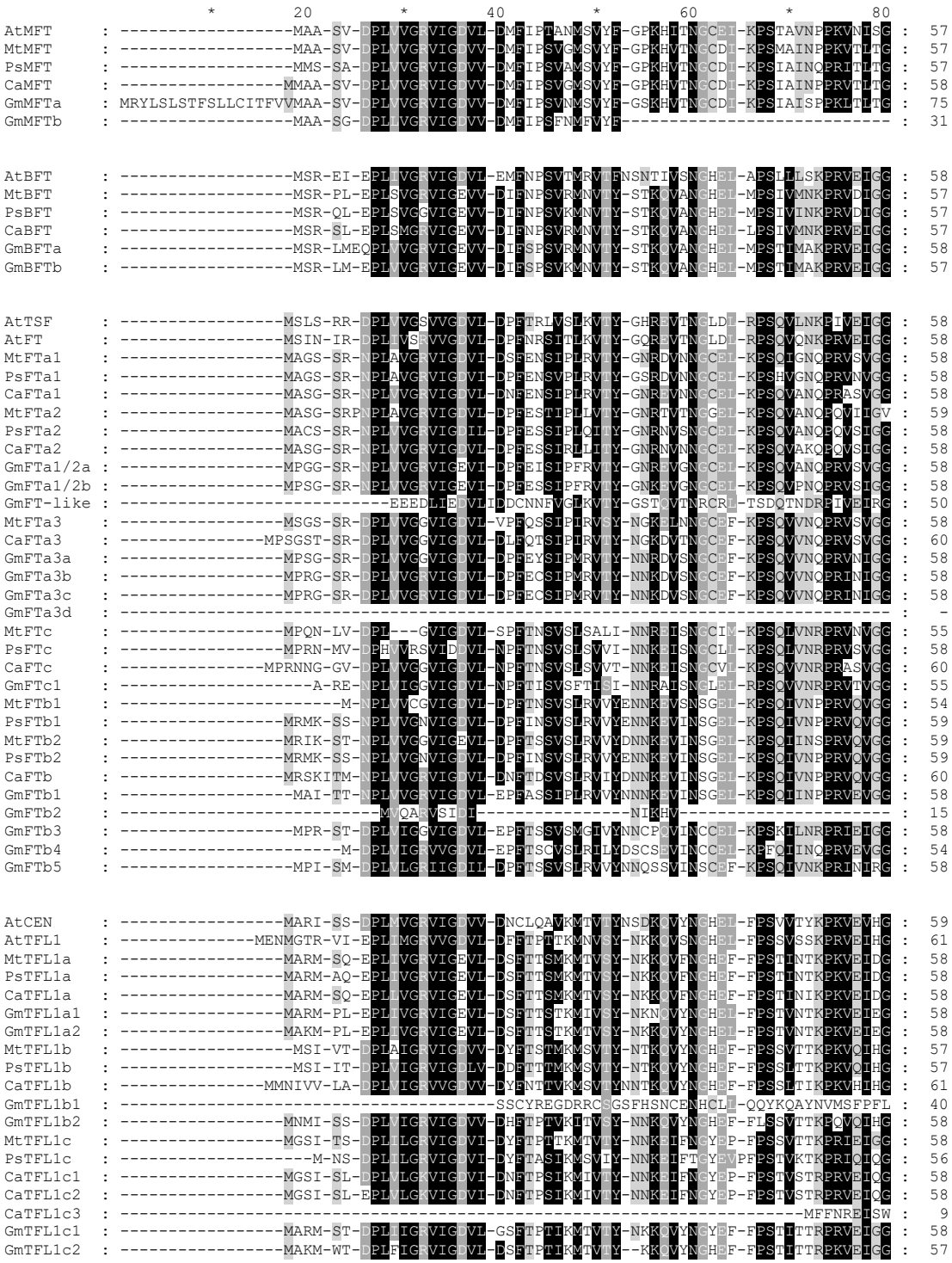


Supplementary Figure 8. (**A**) Maximum parsimony tree derived from the alignment of phosphatidylethanolamine-binding proteins **(**PEBP) in *Arabidopsis thaliana* (At, blue), *Cicer arietinum* (Ca, red), *Glycine max* (Gm, green), *Pisum sativum* (Ps, purple) and *Medicago truncatula* (Mt, orange). Accession number of the PEBP genes can be found in Supplementary Table 4. Sequences were aligned with MAFFT (full alignment can be found in Supplementary Figure 9) and the tree was build using PAUP* in Geneious 8 software. Numbers beside branches represent bootstrap support from 1000 replications. (**B**) In next page; Position of the PEBP genes (in red) in the chickpea reference genome. Bars represent chromosomes, with numbers indicated on top. *BFT* and *TFL1c1* are not included as they were located in an unplaced scaffold (NW_004516558.1 and NW_004516523.1, respectively).

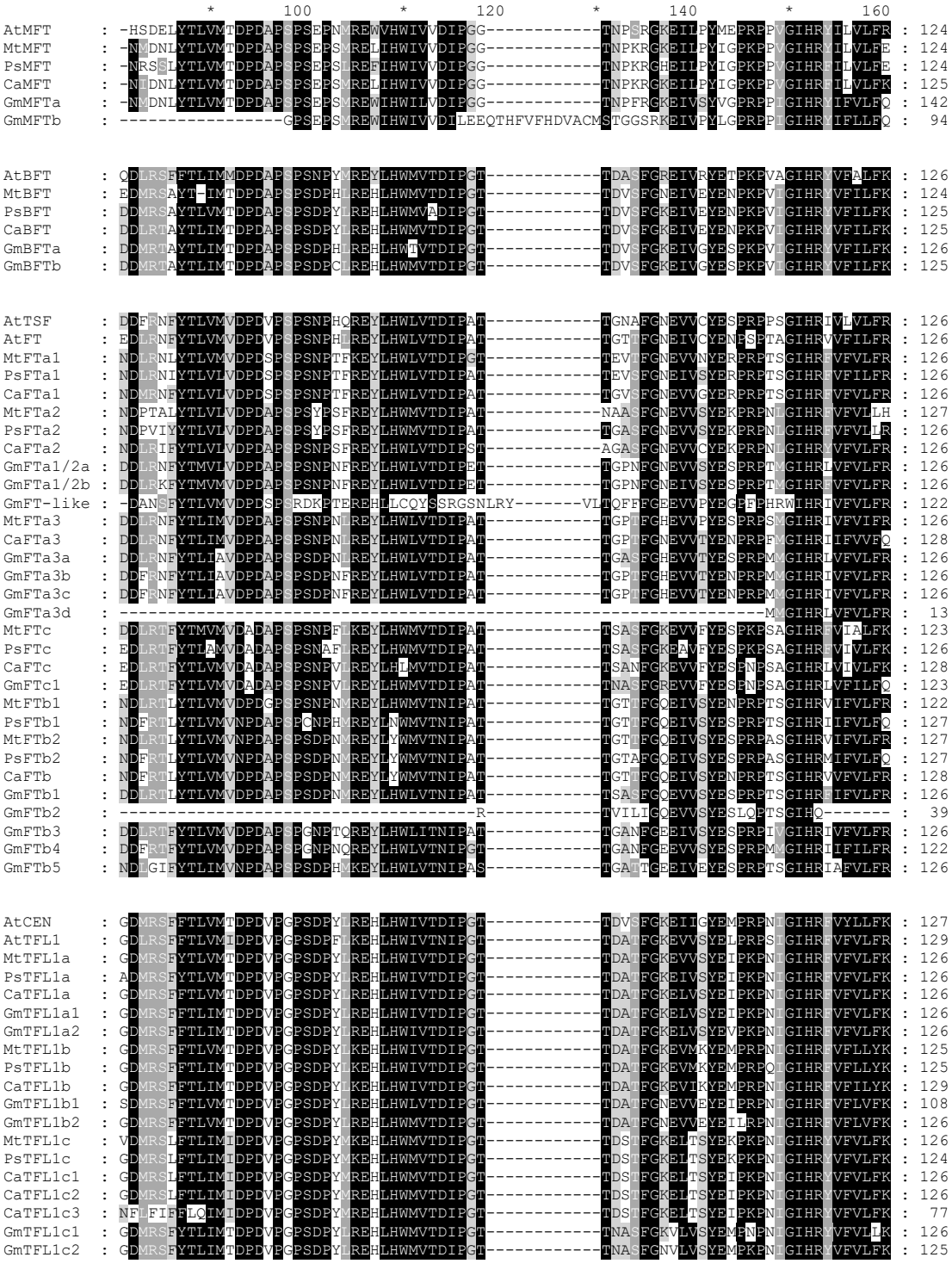


Supplementary Figure 8 B.

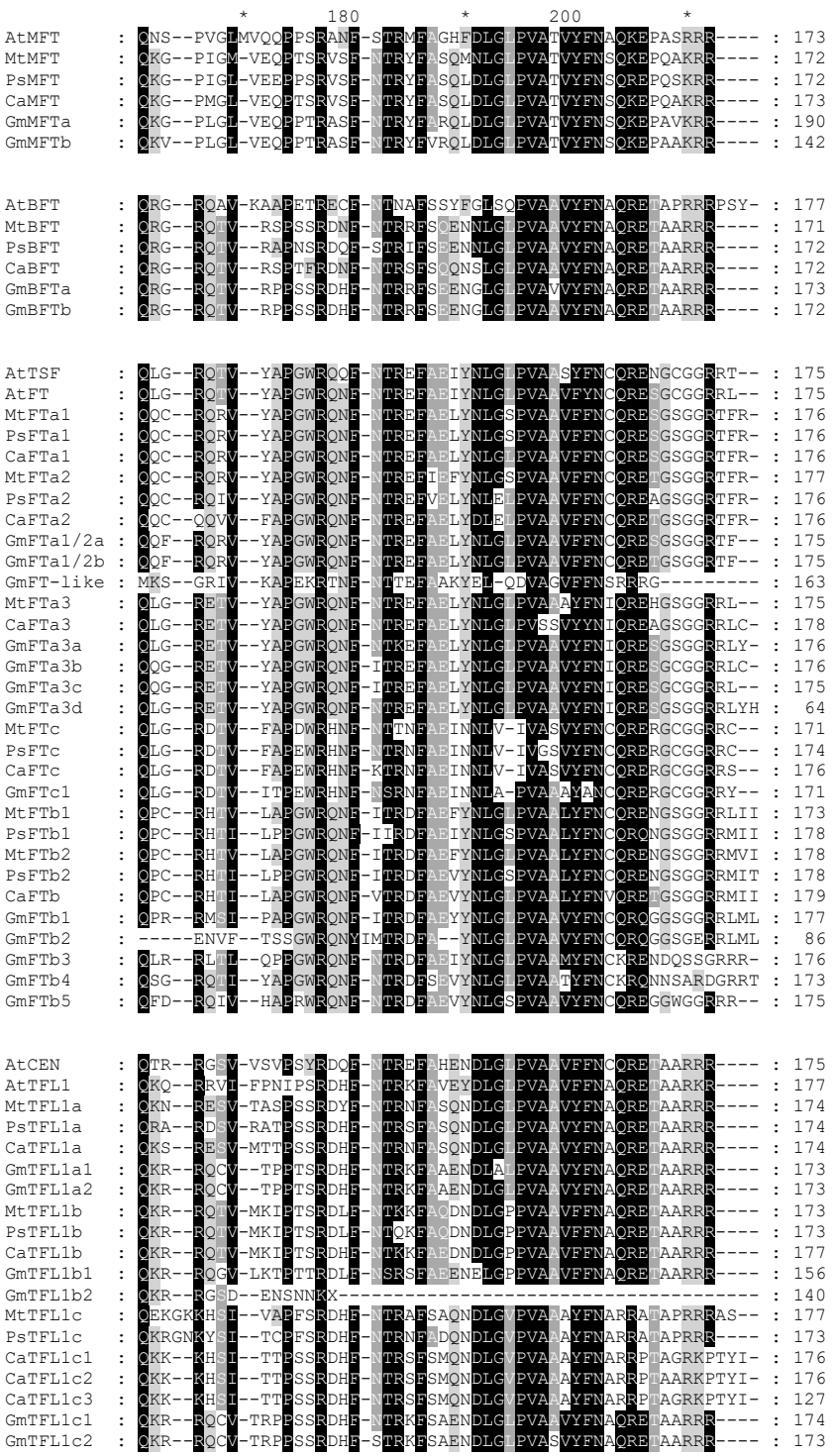
Supplementary Figure 9. Multiple sequence alignment of the PEBP sequences from five species described in Supplementary Table 1. Proteins were aligned in Geneious 8 using MAFFT. Residues conserved in more than 80% of the sites are presented with black background/while letters. Those with a conservation between 60-80% show grey background/white letters and those between 40-60% with grey background/black letters.

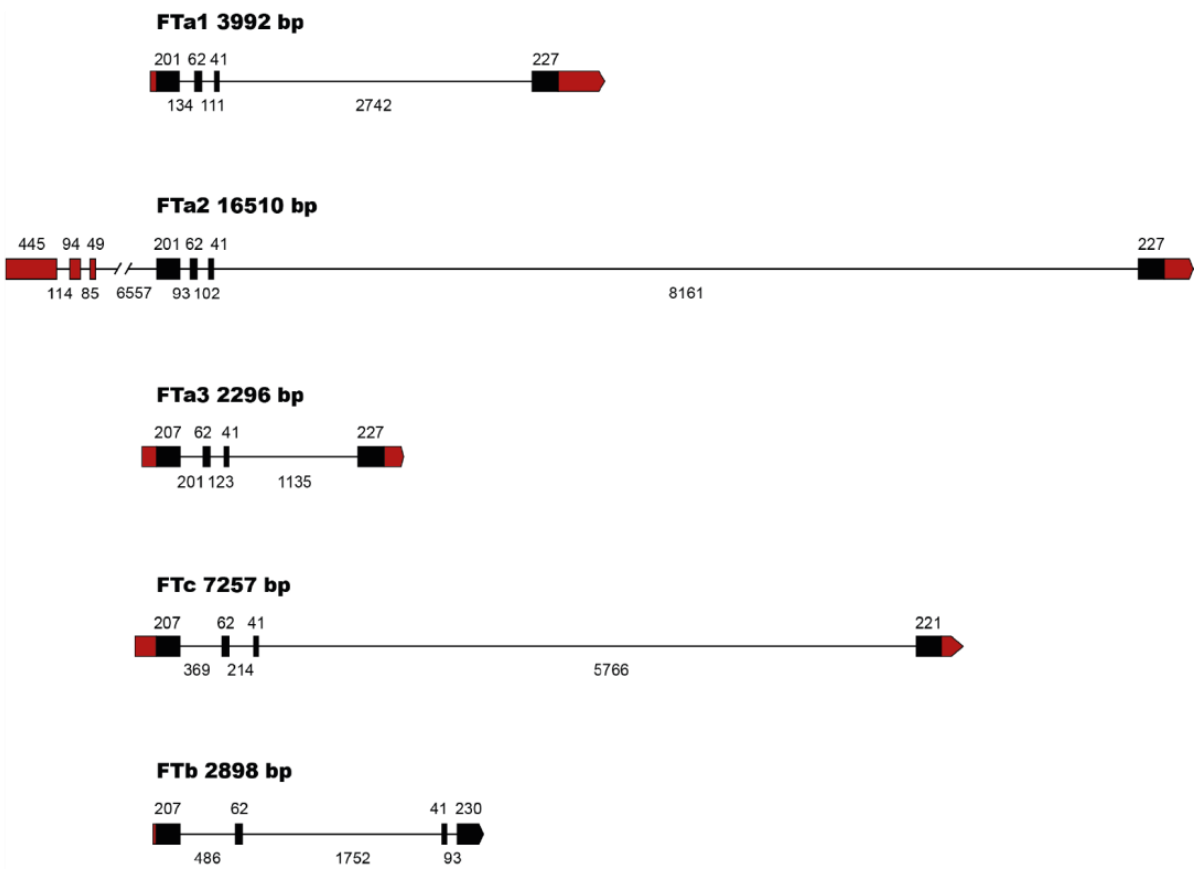


Supplementary Figure 9. Continued

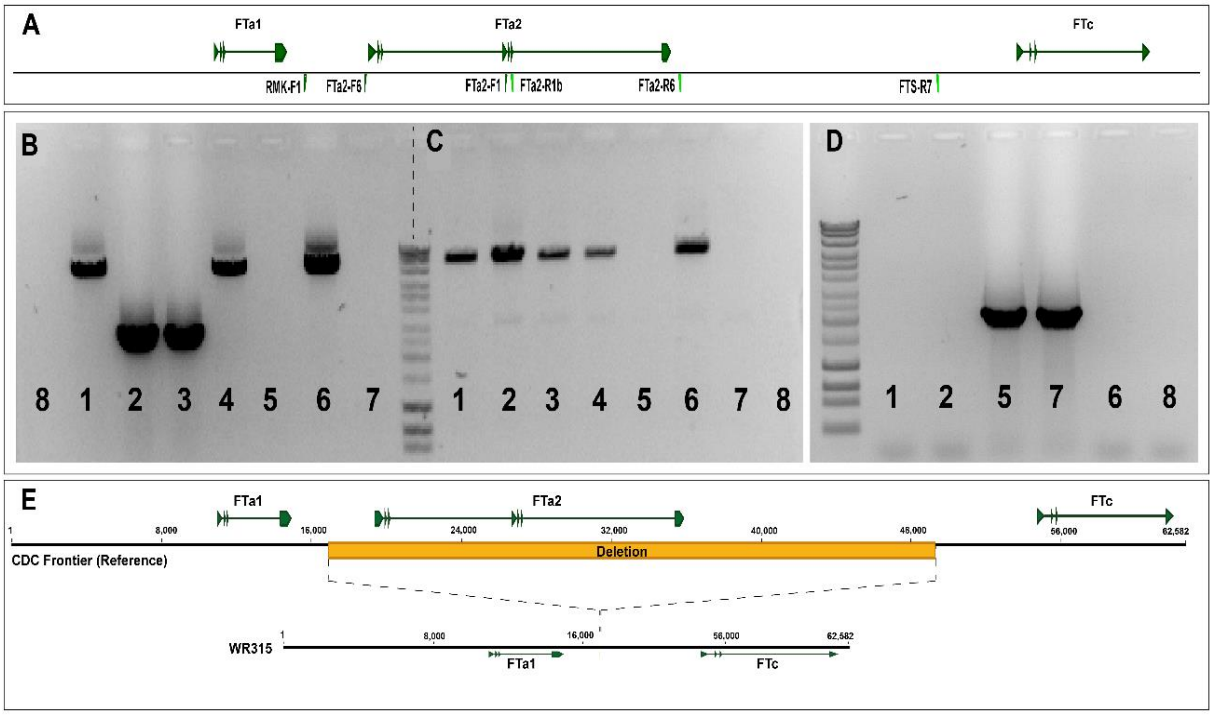


Supplementary Figure 9. Continued

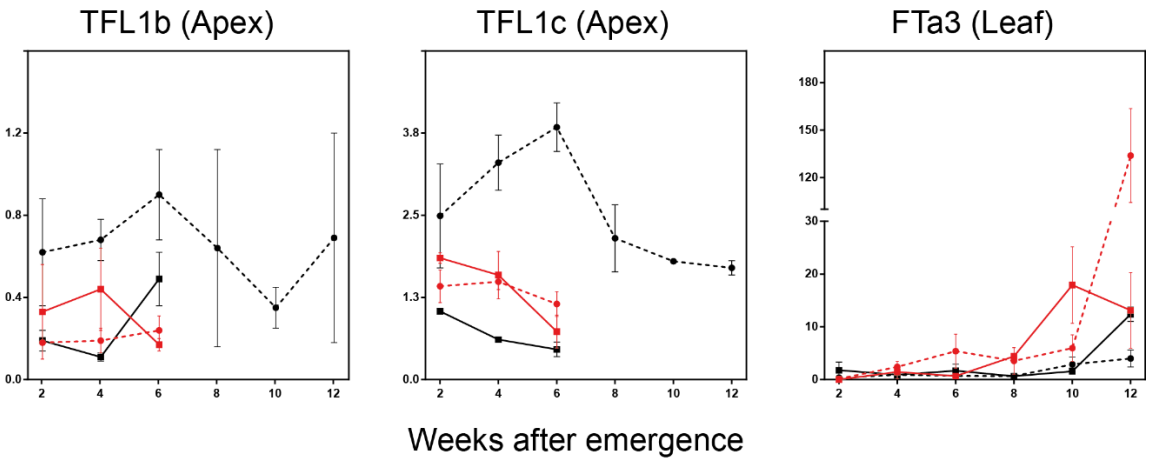




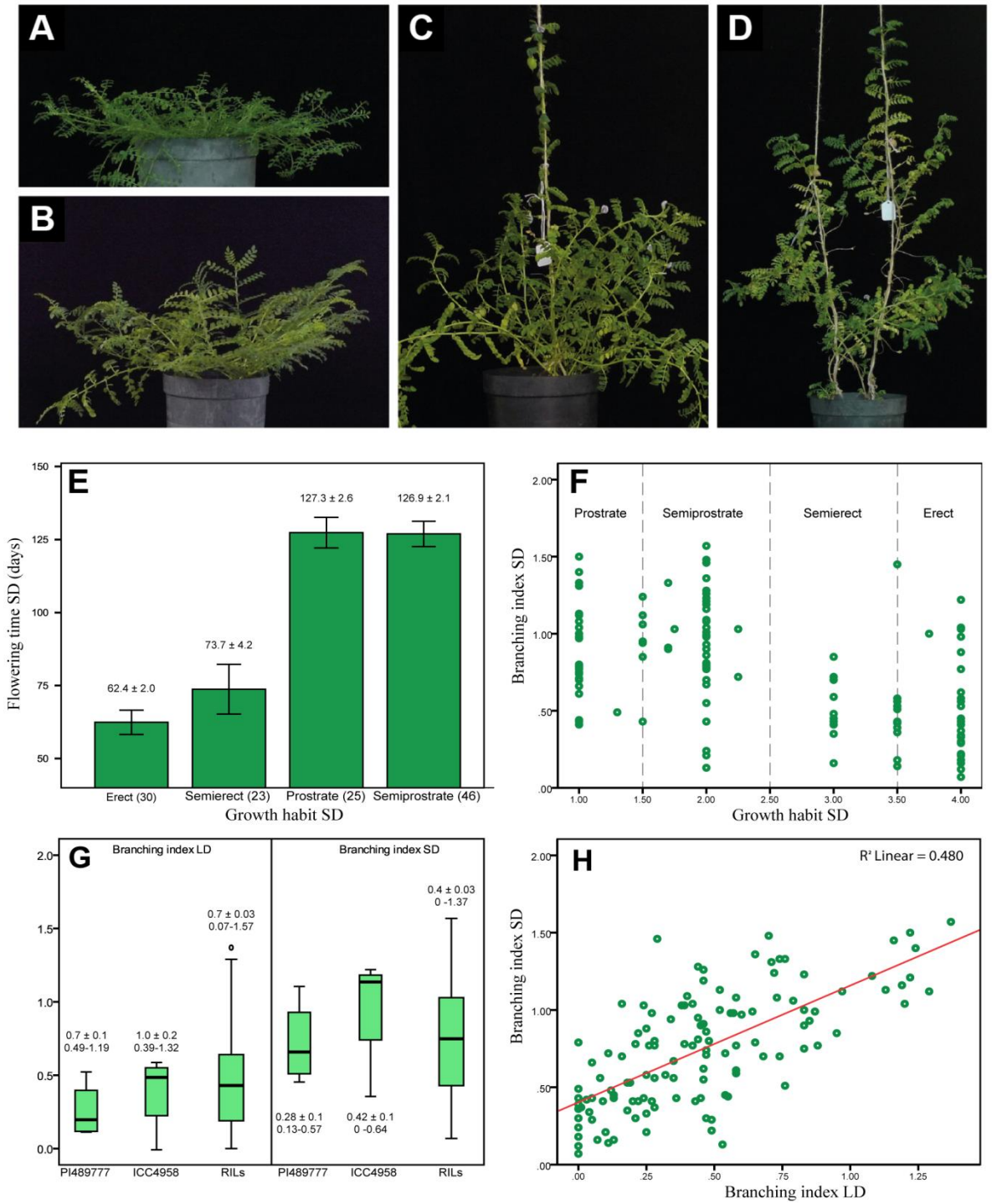
Supplementary Figure 10. Graphic representation of the genomic sequences of the five chickpea *FT* genes. Black boxes indicate exons (sizes over the line, in bp), red boxes the 5' and 3' untranslated regions and lines correspond to introns (sizes under the line).



Supplementary Figure 11. (**A**) Schematic diagram of the region of chromosome 3 containing the *FTa1-FTa2-FTc* cluster. The black line represents genomic DNA sequence with *FTa1*, *FTa2* and *FTc* gene models shown at top in green (triangles representing exons and line corresponding to introns). The position of the primers used are indicated below; forward and reverse primers are presented in dark and light green, respectively. The failure to amplify any region of *FTa2* in some lines, shown by the electrophoresis gel of the products obtained with primers FTa2-F6/FTa2-R1b (**B**) and FTa2-F1/FTa2-R6 (**C**), suggested a deletion of the gene. 7 chickpea accessions were tested, as follows: 1-ICC4958; 2-PI489777; 3-Cr5-9; 4-CA2156; 5-WR315; 6-ILC3279; 7- ICCV2; 8-SDW. To determine the extent of the deletion, several primers were tested in the *FTa1-FTa2* and *FTa2-FTc* intergenic regions. The primer pair RMK-F1/FTS-R7 successfully yielded a band (**D**). Since the expected size of the amplicon in (**D**) is 33.7 kb according to CDC Frontier genome used as reference, the presence of a 2-3 kb band indicated a probable deletion spanning ~ 30kb. Sequencing of the band confirmed the deletion of 32kb that completely eliminates *FTa2* and includes part of the *FTa1-FTa2* and *FTa2-FTc* intergenic regions (**E**). All PCRs were performed in a final volume of 25 µL containing 50 ng template DNA, 5 µL of 5x reaction buffer, 10 mM dNTPs, 0.2 µM of each primer, 50 mM MgCl2, 0.1 µL of MangoTaqTM DNA polymerase (Bioline, Australia) and autoclaved Milli-Q water to final volume. Reactions were performed in a thermal cycler using the following program: an initial denaturation of 5 min at 94°C, followed by 35-40 cycles (94°C for 40 seconds, 58oC annealing temperature and 10 min extension) and a final extension of 10 minutes at 72°C. Primers sequences: FTa2-F6 AAGCCCACAACCCACCTAAGGG; FTa2-R1b ACTAGCCCCAGCAGTTGAAG; FTa2-F1 TAGGCGGAAACGATCTCA GG; FTa2-R6 GCCATAAACCTCTGTCGAACGGC, RMK-F1 ACTGTTCTGCACACAGTGGCTACC; FTS-R7 AGGCCAAAGACAAGATCCCG.



Supplementary Figure 12. Relative expression of flowering-related chickpea genes in CRIL2 parental lines. ICC4958 and PI48977 are represented by red and black lines, respectively. Continuous lines/squares indicate long day condition while dashed lines/circles represent short day. TFL1b and TFL1c expression was measured only in apex and FTa3 expression was measured in both tissues but found only in leaf. The average ± SE of 2 biological and 2 technical replicates is shown, and transcripts were normalized against ACTIN.



Supplementary Figure 13. Variation in growth habit (GH) observed in the CRIL2 population, illustrated by representative examples of the four categories used in the present study: Prostrate (**A**), semi-prostrate (**B**), semi-erect (**C**) and erect (**D**). (**E**) Relationship between GH and flowering phenotype (DTF) of the CRIL2 population grown under SD. Numbers over bars indicate mean DTF ± standard error and those between parentheses correspond to the number of RILs in each category. (**F**) Relationship between GH and branching index (BI) of the CRIL2 population grown under SD. Boxplot illustrating the variation in branching index displayed by CRIL2 parental lines and RILs under LD and SD (**G**). Box edges denote the lower and upper quantiles with median value in the middle of the box. (**H**) Relationship between BI of CRIL2 population grown under SD and LD photoperiod.

Supplementary Table 1. Details of the HRM markers developed in this study for each population, and identity (NCBI GeneID) of the genes targeted by them.

Marker	Population	Fw Sequence	Rv Sequence	GeneID
LHY	CRIL2/RIP12	AAACCAACTAAGCATACCCT	TGAGCATCACTCATTCACCA	101500635
	RIPs8/5	CGTCACACTTGTAATCTTCATTCC	AGTTTTCCCCCTTTAATAATGTGG	
CP450	CRIL2/RIP12	CACAAAATAGAGAACAATGACAGC	ACTTTTCCCTTTGCATGTAGG	101502624
	RIPs8/5	AGAGTTGTATAGTTGTTAAGGATG	GTGTGTGTGTTTATCAATTTAAGC	
CDF2d	CRIL2/RIP12	TGGTTCCAATTAAGTTTCAAGTG	AGTCAAGTGTTTGGTAAGAGTTG	101500722
	RIPs8/5	AGTCGATGCTTAATCTTCAACAGC	AGATCTGCATAAAGATGGTTCC	
WUS11	CRIL2/RIP12	CAGCCTGGTAATTAACTGCATC	ATGATTTTGAGCAATTATTCTGTG	101503157
	RIPs8/5	GCATAACCTAGAGTGATCGAGC	CTACTCTGACTTAATGGGTTCG	
FTa1	CRIL2/RIP12	AATCATCCCCAAGAGATCAA	TGCACAGTCATTGTGTTTCG	101497376
	RIPs8/5	ACTGTTCTGCACACAGTGGCTACC	AAAGAGATCTAACACATTTTGC	
			GAGGAACACGACGCCTCC	
ING2	CRIL2/RIP12	CATCTCATGGCTCTAAGAAAGG	ATTTCTTTAAGCAATTTCTCAATGG	101503708
SUVH4[1]	CRIL2/RIP12	TTCGCCGTCACTACCTCG	GGAGATTAAGCTTCGGAGG	101508428
COLh	CRIL2/RIP12	TGCTACATCATTCACCTAGTAACA	TGCCATGATATAGGAAGTCTTAGTT	101504031
SOC1a	CRIL2/RIP12	AAACAAAGAAAAACGAATGTGTCC	CGACATATAATTCATTGTGGACCG	101510775
SCARECROW[2]	CRIL2/RIP12	GAGACATGTTGTTGAACAGC	CTTGATGGTCCTCTAACAGC	101513767
RAPTOR1[2]	CRIL2/RIP12	CCCAATGCCATCCAAATCGG	CACACAACAACAATACCTAGGG	101514864
ARF9[2]	RIP12	GCAATATGGTGAGAAGAATTTTC	TGAGATAGGCAATTTAGTCCCTG	101491204
ABI5	CRIL2/RIP12	TTAGCACAAGAACGAGCGAGC	GTGAACGAAAAGGTGTTATGAAGG	101490892
GATA9[1]	CRIL2/RIP12	GAGGAATATGTCTTCTTCCATTCC	TGGAAAGAGTAATTTTCCCCCTA	101503040
NAC100[1]	CRIL2/RIP12	CAGGTCTTAGCAATGACACG	GCCCTATTTCTTCCCATGTC	101500623
GIGANTEA	CRIL2/RIP12	TGTTGAGTACTTGATTCAGTTTAC	TCAGTATATAGATGCATACCTCAGC	101511540
ELF6	CRIL2/RIP12	ACCACCCATTTCAGTTTGTTTAC	CCAGGGTCAAAATTATCATAGTCG	101509509
TEMb	CRIL2/RIP12	GAGTTGAGAAAATCTGTTTCAGCG	AGGTTCCGCGGAAAAGACG	101492303
PHYA1	CRIL2/RIP12	ACAATTGCCTTGTAATCGCC	GCCCAAGATATAACTGCTCAG	101506511
CDF3b	RIPs8/5	AACAACCGAAGAAAAATAGG	GAATTTGTATAATGTTTATCTTCG	101499964
COLg	RIPs8/5	AGAGACTCTGAAGGTGTCCC	CAGTGGCTCGGAGAAAGTGG	101499146
AP2-like	RIPs8/5	AACAAACATCGTCACATCACC	CCTTGTGCTATTTAGTGTTCTGC	101502947
LOB189	RIPs8/5	ACAAAATCAATACAAGCAAACC	TGCAACCGTTAGTTTGTTTGG	101508422
PRSP	RIPs8/5	GATACATTTTCGCTCAAACTATG	TGCGTTGAAAAGTGTTTTATTAGC	101491522
PRT6	RIPs8/5	AAATTTTCATTCTCCTAAGACAGT	ACGGTCCAACCCAACGTATA	101506928
WRKY	RIPs8/5	TTCTGAGAGCACCGTGATGG	AGCATCTCCAACTGTAATTAAATG	101511519

(1) Primers designed by Saxena et al (2014)

(2) Primers designed for this study based on SNPs described by Saxena et al (2014)

Supplementary Table 2. Sequence and product size of the primers used to measure the expression of flowering related genes in chickpea

Gene	Fw primer	Rv primer	Product size (bp)		GeneID
			Genomic	cDNA	
ACTIN	ATTGTCTTGAGTGGTGGTTCT	TTCCTCTCTGGTGGTGCTAC	(Verma et al. 2013)		
PIM	GAACTTCAGAGTCTGGAACAGC	CATTGTGCCTGTTGTTGAGC	472	188	101488241
UNI	TGCAACGCGTAACAGTGAACG	ACGAACAATGCCGTGAGTTCTTG	660	169	101503680
FTa1	TTGCCAATCAACCCAGAGCG	AGTGGGGTTACTTGGGCTAGG	236	101	101497376
FTa2	GTTCTGACGGTGGTTCTCTC	CGGAGGTTCACAAAAGAAGG	297	183	101496618
FTc	TGTTGGTGGTGAAGATCTAAGG	ATTCCTGCTGAAGGATTCG	6616	186	101508200
FTb	GGTGAGCTCAAACCCTCCCA	TCCCTCATATTTGGGTCACTAGG	618	131	101505276
FTa3	CCATCCCGGAGCATACACAGTC	TGCACCAAGCCCTAGCAATCC	1460	196	101515383
E1	AACGACAACAACAAGGGATCGG	TGCAGCCAACAAGAGTCTGC	120	120	101497661
TFL1b	TATACCGGGCACAACAGATG	GGAGGTCCAAGGTCATTGTC	584	195	101508699
TFL1c	ACGTTCCTGGCCCAAGTGAT	TGGATCCCTATGTTAGGCTTTGGT	1134	136	101491943

Supplementary Table 3. Description, physical position in the chromosome and GeneID of the 59 genes annotated between markers SUVH4 and GATA9, according to the genome assembly of cultivar CDC Frontier (ASM33114 v1; Varshney et al., 2013), available at the National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov).

Marker	Position	GeneID	Description
SUVH4	25748486	101508428	histone-lysine N-methyltransferase, H3 lysine-9 specific SUVH4
	25765688	101509380	CLAVATA3/ESR (CLE)-related protein 4A-2-like
	25787922	101509702	uncharacterized protein LOC101509702
	25849917	101510346	protein Mpv17-like, partial
	25899924	101510883	N-alpha-acetyltransferase MAK3
	25908342	101511521	cytochrome b-c1 complex subunit Rieske-4, mitochondrial-like
	25911181	101511199	uncharacterized protein LOC101511199
	25920520	101511847	uncharacterized protein LOC101511847
	25929005	101512385	uncharacterized protein LOC101512385
	25945170	101512699	LRR receptor-like serine/threonine-protein kinase RPK2
	25987693	101513246	UPF0496 protein At3g19330-like
	26006715	101513575	putative serine/threonine-protein kinase isoform X2
	26029555	101514543	chalcone synthase
	26038727	101505971	uncharacterized protein LOC101505971
	26049105	101514869	jasmonate O-methyltransferase-like isoform X2
	26056836	101506278	jasmonate O-methyltransferase-like
	26073603	101506600	3,7-dimethylxanthine N-methyltransferase-like
	26105741	101515408	7-methylxanthosine synthase 1-like
	26119620	101506937	ethylene-responsive transcription factor LEP
	26135351	101515744	nudix hydrolase 12, mitochondrial
	26162323	101488713	calcium-binding mitochondrial carrier protein SCaMC-1-like
	26169052	101489051	uncharacterized protein LOC101489051
	26187333	101489383	histidine biosynthesis bifunctional protein hisIE, chloroplastic
	26191225	101489915	uncharacterized protein LOC101489915
	26198363	105851804	glycine-rich cell wall structural protein 1-like
	26211557	101490249	syntaxin-22-like
	26221867	101490568	syntaxin-22-like
	26223872	101490780	uncharacterized protein LOC101490780
	26228217	101491110	DNA cross-link repair protein SNM1 isoform X1
	26233285	101491643	uncharacterized protein LOC101491643
	26237155	101491949	uncharacterized protein LOC101491949
	26259086	101492622	uncharacterized protein LOC101492622
	26268535	101492953	probable xyloglucan endotransglucosylase/hydrolase protein B
	26275124	101493288	probable xyloglucan endotransglucosylase/hydrolase-like precursor
	26293522	101493610	glyceraldehyde-3-phosphate dehydrogenase A, chloroplastic

Supplementary Table 3. Continued

	26300947	101493929	pentatricopeptide repeat-containing protein At3g26630, chloroplastic
	26304938	101494250	WD repeat-containing protein LWD1
	26309206	105851806	pentatricopeptide repeat-containing protein At3g26630, chloroplastic-like
	26315891	101494555	acetyl-coenzyme A synthetase, chloroplastic/glyoxysomal
	26325185	101494873	eukaryotic peptide chain release factor subunit 1-3
	26331419	101495197	importin-13 isoform X2
	26353855	101495954	INO80 complex subunit D-like
	26365580	101496291	omega-hydroxypalmitate O-feruloyl transferase
FTa1	26393854	101497376	protein FLOWERING LOCUS T-like
	26409508	101496618	protein HEADING DATE 3A-like isoform X1
	26437711	101508200	protein FLOWERING LOCUS T-like
	26446273	101497706	transmembrane and coiled-coil domain-containing protein 1-like
	26454060	101498244	aquaporin SIP1-2-like
	26459034	101498578	apyrase 2-like
	26465157	101499141	pentatricopeptide repeat-containing protein At5g15010, mitochondrial
	26476855	101499461	nucleoside-triphosphatase-like
	26505866	101499961	NAC transcription factor 29
	26513981	101500308	uncharacterized protein LOC101500308
NAC100	26542626	101500623	NAC domain-containing protein 100-like
	26551883	101500931	U11/U12 small nuclear ribonucleoprotein 65 kDa protein
	26560819	101501672	thioredoxin-like 1-1, chloroplastic
	26568175	101501985	CCR4-NOT transcription complex subunit 3 isoform X1
	26586013	101503370	glucan endo-1,3-beta-glucosidase 8-like
GATA9	26593826	101503040	GATA transcription factor 9-like

Supplementary Table 4. Accession number of the PEBP genes from five plant species; *Arabidopsis thaliana*, *Medicago truncatula*, *Pisum sativum* (Pea), *Glycine max* (soybean) and *Cicer arietinum* (chickpea). Soybean accessions were obtained from Wang et al. (2015) and the protein sequences retrieved from the Soybean Knowledge Database [http://soykb.org; Joshi et al. (2012)]. Proteins sequences retrieved from the accessions listed were used in the alignment displayed in Supplementary Figure 10.

Arabidopsis		Medicago		Pea	Chickpea		Soybean	
MFT	AT1G18100	Medtr8g106840		PsCam040701	LOC101504081		**GmMFTa**	Glyma05g34030
							GmMFTb	Glyma08g05650
BFT	AT5G62040	Medtr0020s0120		PsCam044479	LOC101507903		**GmBFTa**	Glyma09g26550
							GmBFTb	Glyma16g32080
ATC	AT2G27550	-		-	-		-	
TFL1	AT5G03840	**TFL1a**	Medtr7g104460	AY340579	**TFL1a**	LOC101506075	**GmTFL1b1**	Glyma12g30940
		TFL1b	Medtr2g086270	AY340580	**TFL1b**	LOC101508699	**GmTFL1b2**	Glyma13g39360
		TFL1c	Medtr1g060190	AY343326	**TFL1c1**	LOC101495644	**GmTFL1a1**	Glyma03g35250
					TFL1c2	LOC101491943	**GmTFL1a2**	Glyma19g37890
					TFL1c3	LOC101492277	**GmTFL1c1**	Glyma10g08340
							GmTFL1c2	Glyma13g22030
FT	AT1G65480	**FTa1**	Medtr7g084970	HQ538822	**FTa1**	LOC101497376	**GmFTc1**	Glyma19g28400
TSF	AT4G20370	**FTa2**	Medtr7g085020	HQ538821	**FTa2**	LOC101496618	**GmFTa3a**	Glyma16g26660
		FTa3	Medtr6g033040	-	**FTa3**	LOC101515383	**GmFTa3b**	Glyma16g04830
		FTb1	Medtr7g006630	HQ538824	**FTb**	LOC101505276	**GmFTa3c**	Glyma16g26690
		FTb2	Medtr7g006690	HQ538825			**GmFTa3d**	Glyma02g07650
		FTc	Medtr7g085040	HQ538826	**FTc**	LOC101508200	**GmFTa1/2a**	Glyma19g28390
							GmFTa1/2b	Glyma16g04840
							GmFTb1	Glyma08g47820
							GmFTb2	Glyma18g53670
							GmFTb3	Glyma18g53680
							GmFTb4	Glyma18g53690
							GmFTb5	Glyma08g47810
							GmFT-like	Glyma08g28470

Supplementary Table 5. Information about sunrise and sunset times (h = hours, m = minutes) in Cordoba, Spain (Latitude, Longitude: 37 52 51, - 4 46 44) and Jaen, Spain (Latitude, Longitude: 37 45 59, - 3 47 21) during the growing seasons of RIP12, RIP5 and RIP8. Source: Observatorio Astronómico Nacional, Instituto Geográfico Nacional, Ministerio de Fomento, Spain.

Cordoba, 2001

Day	February		March		April		May		June	
	Sunrise	Sunset	Sunrise	Sunset	Sunrise	Sunset	Sunrise	Sunset	Sunrise	Sunset
	h m	h m	h m	h m	h m	h m	h m	h m	h m	h m
1	823	1843	751	1913	804	2042	723	2110	659	2136
2	823	1844	749	1914	803	2043	722	2111	658	2136
3	822	1845	748	1915	801	2044	721	2112	658	2137
4	821	1846	747	1916	800	2045	720	2112	658	2138
5	820	1847	745	1917	758	2046	719	2113	657	2138
6	819	1848	744	1918	757	2047	718	2114	657	2139
7	818	1849	742	1919	756	2048	717	2115	657	2139
8	817	1850	741	1920	754	2048	716	2116	657	2140
9	816	1851	739	1921	753	2049	715	2117	657	2140
10	815	1853	738	1921	751	2050	714	2118	656	2141
11	814	1854	736	1922	750	2051	713	2119	656	2141
12	812	1855	735	1923	748	2052	712	2120	656	2142
13	811	1856	733	1924	747	2053	711	2121	656	2142
14	810	1857	732	1925	745	2054	710	2121	656	2143
15	809	1858	730	1926	744	2055	709	2122	656	2143
16	808	1859	729	1927	743	2056	708	2123	656	2143
17	807	1900	727	1928	741	2057	707	2124	656	2144
18	805	1901	726	1929	740	2058	707	2125	657	2144
19	804	1902	724	1930	738	2059	706	2126	657	2144
20	803	1903	723	1931	737	2059	705	2127	657	2144
21	802	1904	721	1932	736	2100	704	2127	657	2145
22	800	1905	720	1933	734	2101	704	2128	657	2145
23	759	1906	718	1934	733	2102	703	2129	658	2145
24	758	1908	717	1935	732	2103	702	2130	658	2145
25	756	1909	815	2036	731	2104	702	2131	658	2145
26	755	1910	814	2037	729	2105	701	2131	658	2145
27	754	1911	812	2037	728	2106	701	2132	659	2145
28	752	1912	811	2038	727	2107	700	2133	659	2145
29			809	2039	726	2108	700	2134	700	2145
30			808	2040	724	2109	659	2134	700	2145
31			806	2041			659	2135		

Cordoba, 2004

Day	February		March		April		May		June	
	Sunrise	Sunset	Sunrise	Sunset	Sunrise	Sunset	Sunrise	Sunset	Sunrise	Sunset
	h m	h m	h m	h m	h m	h m	h m	h m	h m	h m
1	824	1842	750	1913	804	2042	723	2110	658	2136
2	823	1843	749	1914	803	2043	722	2111	658	2136
3	822	1844	748	1915	801	2044	721	2112	658	2137
4	821	1845	746	1916	800	2045	720	2113	657	2138
5	820	1846	745	1917	758	2046	718	2114	657	2138
6	819	1847	743	1918	757	2047	717	2115	657	2139
7	819	1848	742	1919	755	2048	716	2115	657	2139
8	818	1849	740	1920	754	2049	715	2116	657	2140
9	816	1851	739	1921	752	2050	714	2117	656	2140
10	815	1852	737	1922	751	2051	713	2118	656	2141
11	814	1853	736	1923	749	2051	712	2119	656	2141
12	813	1854	734	1924	748	2052	711	2120	656	2142
13	812	1855	733	1925	746	2053	711	2121	656	2142
14	811	1856	731	1926	745	2054	710	2122	656	2143
15	810	1857	730	1927	744	2055	709	2123	656	2143
16	809	1858	728	1927	742	2056	708	2123	656	2143
17	807	1859	727	1928	741	2057	707	2124	656	2144
18	806	1900	725	1929	739	2058	706	2125	657	2144
19	805	1901	724	1930	738	2059	706	2126	657	2144
20	804	1903	722	1931	737	2100	705	2127	657	2145
21	803	1904	721	1932	735	2101	704	2128	657	2145
22	801	1905	719	1933	734	2102	704	2128	657	2145
23	800	1906	718	1934	733	2103	703	2129	658	2145
24	759	1907	716	1935	731	2103	702	2130	658	2145
25	757	1908	715	1936	730	2104	702	2131	658	2145
26	756	1909	713	1937	729	2105	701	2132	659	2145
27	755	1910	712	1938	728	2106	701	2132	659	2145
28	753	1911	810	2039	727	2107	700	2133	659	2145
29	752	1912	809	2040	725	2108	700	2134	700	2145
30			807	2040	724	2109	659	2134	700	2145
31			806	2041			659	2135		

Cordoba, 2014

Day	February		March		April		May		June	
	Sunrise	Sunset	Sunrise	Sunset	Sunrise	Sunset	Sunrise	Sunset	Sunrise	Sunset
	h m	h m	h m	h m	h m	h m	h m	h m	h m	h m
1	823	1842	751	1912	805	2042	723	2110	659	2136
2	823	1843	750	1913	803	2043	722	2110	658	2136
3	822	1845	748	1914	802	2044	721	2111	658	2137
4	821	1846	747	1915	800	2045	720	2112	658	2137
5	820	1847	745	1916	759	2046	719	2113	657	2138
6	819	1848	744	1917	757	2046	718	2114	657	2139
7	818	1849	742	1918	756	2047	717	2115	657	2139
8	817	1850	741	1919	754	2048	716	2116	657	2140
9	816	1851	740	1920	753	2049	715	2117	657	2140
10	815	1852	738	1921	751	2050	714	2118	656	2141
11	814	1853	737	1922	750	2051	713	2119	656	2141
12	813	1855	735	1923	748	2052	712	2120	656	2142
13	811	1856	734	1924	747	2053	711	2120	656	2142
14	810	1857	732	1925	746	2054	710	2121	656	2143
15	809	1858	731	1926	744	2055	709	2122	656	2143
16	808	1859	729	1927	743	2056	708	2123	656	2143
17	807	1900	728	1928	741	2057	708	2124	656	2144
18	806	1901	726	1929	740	2058	707	2125	657	2144
19	804	1902	724	1930	739	2058	706	2126	657	2144
20	803	1903	723	1931	737	2059	705	2126	657	2144
21	802	1904	721	1932	736	2100	705	2127	657	2145
22	800	1905	720	1933	735	2101	704	2128	657	2145
23	759	1906	718	1934	733	2102	703	2129	658	2145
24	758	1907	717	1935	732	2103	703	2130	658	2145
25	756	1908	715	1935	731	2104	702	2130	658	2145
26	755	1909	714	1936	729	2105	701	2131	658	2145
27	754	1910	712	1937	728	2106	701	2132	659	2145
28	752	1911	711	1938	727	2107	700	2133	659	2145
29			709	1939	726	2108	700	2133	700	2145
30			808	2040	725	2109	659	2134	700	2145
31			806	2041			659	2135		

Cordoba, 2003

Day	February		March		April		May		June	
	Sunrise	Sunset	Sunrise	Sunset	Sunrise	Sunset	Sunrise	Sunset	Sunrise	Sunset
	h m	h m	h m	h m	h m	h m	h m	h m	h m	h m
1	824	1842	752	1912	805	2042	724	2109	659	2135
2	823	1843	750	1913	804	2043	723	2110	658	2136
3	822	1844	749	1914	802	2043	722	2111	658	2137
4	821	1845	747	1915	801	2044	720	2112	658	2137
5	820	1846	746	1916	759	2045	719	2113	657	2138
6	819	1848	744	1917	758	2046	718	2114	657	2138
7	818	1849	743	1918	756	2047	717	2115	657	2139
8	817	1850	742	1919	755	2048	716	2116	657	2140
9	816	1851	740	1920	753	2049	715	2117	657	2140
10	815	1852	739	1921	752	2050	714	2117	656	2141
11	814	1853	737	1922	750	2051	713	2118	656	2141
12	813	1854	736	1923	749	2052	712	2119	656	2142
13	812	1855	734	1924	748	2053	711	2120	656	2142
14	811	1856	733	1925	746	2054	710	2121	656	2142
15	810	1857	731	1926	745	2054	709	2122	656	2143
16	808	1859	730	1927	743	2055	709	2123	656	2143
17	807	1900	728	1928	742	2056	708	2124	656	2144
18	806	1901	727	1929	740	2057	707	2124	656	2144
19	805	1902	725	1930	739	2058	706	2125	657	2144
20	803	1903	724	1931	738	2059	705	2126	657	2144
21	802	1904	722	1931	736	2100	705	2127	657	2145
22	801	1905	720	1932	735	2101	704	2128	657	2145
23	800	1906	719	1933	734	2101	704	2128	657	2145
24	758	1907	717	1934	732	2103	703	2129	658	2145
25	757	1908	716	1935	731	2104	702	2130	658	2145
26	756	1909	714	1936	730	2105	702	2131	658	2145
27	754	1910	713	1937	729	2106	701	2132	659	2145
28	753	1911	711	1938	727	2106	701	2132	659	2145
29			710	1939	726	2107	700	2133	659	2145
30			808	2040	725	2108	700	2134	700	2145
31			807	2041			659	2135		

Cordoba, 2008

Day	February		March		April		May		June	
	Sunrise	Sunset	Sunrise	Sunset	Sunrise	Sunset	Sunrise	Sunset	Sunrise	Sunset
	h m	h m	h m	h m	h m	h m	h m	h m	h m	h m
1	824	1842	750	1913	804	2042	723	2110	658	2136
2	823	1843	749	1914	803	2043	722	2111	658	2137
3	822	1844	748	1915	801	2044	721	2112	658	2137
4	821	1845	746	1916	800	2045	720	2113	658	2138
5	820	1846	745	1917	758	2046	718	2114	657	2138
6	819	1847	743	1918	757	2047	717	2115	657	2139
7	818	1848	742	1919	755	2048	716	2116	657	2139
8	817	1850	740	1920	754	2049	715	2116	657	2140
9	816	1851	739	1921	752	2050	714	2117	657	2141
10	815	1852	737	1922	751	2051	713	2118	656	2141
11	814	1853	736	1923	749	2052	712	2119	656	2142
12	813	1854	734	1924	748	2052	711	2120	656	2142
13	812	1855	733	1925	746	2053	711	2121	656	2142
14	811	1856	731	1926	745	2054	710	2122	656	2143
15	810	1857	730	1927	744	2055	709	2123	656	2143
16	809	1858	728	1928	742	2056	708	2123	656	2143
17	807	1859	727	1928	741	2057	707	2124	656	2144
18	806	1900	725	1929	739	2058	706	2125	657	2144
19	805	1902	724	1930	738	2059	706	2126	657	2144
20	804	1903	722	1931	737	2100	705	2127	657	2145
21	802	1904	721	1932	735	2101	704	2128	657	2145
22	801	1905	719	1933	734	2102	704	2128	657	2145
23	800	1906	718	1934	733	2103	703	2129	658	2145
24	759	1907	716	1935	731	2104	702	2130	658	2145
25	757	1908	715	1936	730	2104	702	2131	658	2145
26	756	1909	713	1937	729	2105	701	2132	659	2145
27	755	1910	712	1938	728	2106	701	2132	659	2145
28	753	1911	710	1939	726	2107	700	2133	659	2145
29	752	1912	709	1940	725	2108	700	2134	700	2145
30			807	2041	724	2109	659	2134	700	2145
31			806	2041			659	2135		

Jaen, 2003

Day	February		March		April		May		June	
	Sunrise	Sunset	Sunrise	Sunset	Sunrise	Sunset	Sunrise	Sunset	Sunrise	Sunset
	h m	h m	h m	h m	h m	h m	h m	h m	h m	h m
1	820	1838	747	1908	801	2038	720	2105	655	2131
2	819	1839	746	1909	800	2039	719	2106	655	2132
3	818	1840	745	1910	758	2039	718	2107	654	2132
4	817	1842	743	1911	757	2040	717	2108	654	2133
5	816	1843	742	1912	755	2041	716	2109	654	2134
6	815	1844	740	1913	754	2042	715	2110	654	2134
7	814	1845	739	1914	752	2043	713	2111	653	2135
8	813	1846	737	1915	751	2044	712	2111	653	2135
9	812	1847	736	1916	749	2045	711	2112	653	2136
10	811	1848	735	1917	748	2046	710	2113	653	2136
11	810	1849	733	1918	747	2047	709	2114	653	2137
12	809	1850	732	1919	745	2048	708	2115	653	2137
13	808	1852	730	1920	744	2049	708	2116	653	2138
14	807	1853	729	1921	742	2049	707	2117	653	2138
15	805	1854	727	1922	741	2050	706	2118	653	2139
16	804	1855	726	1923	739	2051	705	2119	653	2139
17	803	1856	724	1924	738	2052	704	2119	653	2139
18	802	1857	723	1925	737	2053	703	2120	653	2140
19	801	1858	721	1926	735	2054	703	2121	653	2140
20	759	1859	720	1927	734	2055	702	2122	653	2140
21	758	1900	718	1927	733	2056	701	2123	653	2140
22	757	1901	717	1928	731	2057	700	2124	654	2141
23	756	1902	715	1929	730	2058	700	2124	654	2141
24	754	1903	713	1930	729	2059	659	2125	654	2141
25	753	1904	712	1931	727	2100	659	2126	654	2141
26	752	1905	710	1932	726	2100	658	2127	655	2141
27	750	1906	709	1933	725	2101	657	2127	655	2141
28	749	1907	707	1934	724	2102	657	2128	655	2141
29			706	1935	722	2103	656	2129	656	2141
30			804	2036	721	2104	656	2130	656	2141
31			803	2037			656	2130		