

Genetic interactions among flowering loci influence phenology, vigour, and yield in chickpea

Molecular Breeding

R. Ortega Martinez^{ab}, L. Lake^{cde}, J.E. Hayes^d, J. L. Weller^{ab}, R. French^f, V. O. Sadras^{cde}

a School of Natural Sciences, University of Tasmania, Sandy Bay Campus, Hobart, Tasmania, Australia

b ARC Centre of Excellence for Plant Success in Nature and Agriculture, Australia

c South Australian Research and Development Institute, Australia

d Waite Research Institute, School of Agriculture, Food and Wine, The University of Adelaide, Australia

e College of Science and Engineering, Flinders University, Australia

f Department of Primary Industries and Regional Development, Merredin, Western Australia, Australia

Corresponding author: Raul Ortega Martinez. E-mail address: rortega@utas.edu.au.

Supplemental Table 1.

Primer sequences (5' – 3') for Kompetitive Allele Specific Polymorphism (KASP™) markers to characterise alleles at phenology loci *CaELF3a*, *CaLG3a* and *CaLG3b*. Primer sequences for *CaELF3a* were sourced directly from Ridge et al. (2017) and target the deletion verified as conferring loss of function. Sequences for *CaLG3a* were sourced from Nguyen et al. (2022). *CaLG3b* was characterised using four markers spanning a broad genetic interval.

Marker name	Locus	Allele-specific primer 1	Allele-specific primer 2	Common primer
CaELF3a	<i>CaELF3a</i>	GAAGGTGACCAAGTTC ATGCTGATGAAGAGAG GGAAAGATGATGAG	GAAGGTCGGAGTCAAC GGATTGGATGAAGAGAG GGAAAGATGATGAT	CCTCCTTTCTCC GTATCACCAACAT
Ca3_34805805	<i>CaLG3a</i>	GAAGGTGACCAAGTTC ATGCTTATTTTACTTATT TGTTTGGTATCATCTAC	GAAGGTCGGAGTCAAC GGATTATTTTACTTATTT GTTTGGTATCATCTAG	TCTAAAAGTAGTT TTAAGTACAATCT TCTA
Ca3_LG3b1	<i>CaLG3b</i>	GAAGGTGACCAAGTTC ATGCTTTTAGAAGGACT TAAGCATTACCTCAT	GAAGGTCGGAGTCAAC GGATTAGAAGGACTTAA GCATTACCTCAC	CCCTCTATATCTT TTCATGAGATCCT TCTA
Ca3_LG3b2	<i>CaLG3b</i>	GAAGGTGACCAAGTTC ATGCTCAAATAAGTAAA CAACAAGCATCTGCC	GAAGGTCGGAGTCAAC GGATTGCAAATAAGTAAA CAACAAGCATCTGCT	CACGAGCAAAA CTCATTGTTTAAA CCGTT
Ca3_LG3b3	<i>CaLG3b</i>	GAAGGTGACCAAGTTC ATGCTATAATAGTTCCCA TTAATACTGAACTGTAA TA	GAAGGTCGGAGTCAAC GGATTATAGTTCCCATTA ATACTGAACTGTAATG	CTTGATTACAGAT AAAGATAAAGGG CACAA
Ca3_LG3b4	<i>CaLG3b</i>	GAAGGTGACCAAGTTC ATGCTGAAGAAAAGCA GTTTTCAAAGCTGGT	GAAGGTCGGAGTCAAC GGATTGAAGAAAAGCAG TTTTCAAAGCTGGA	GAAGTTTGAAGTGA AGGCAATAGACC ATAT

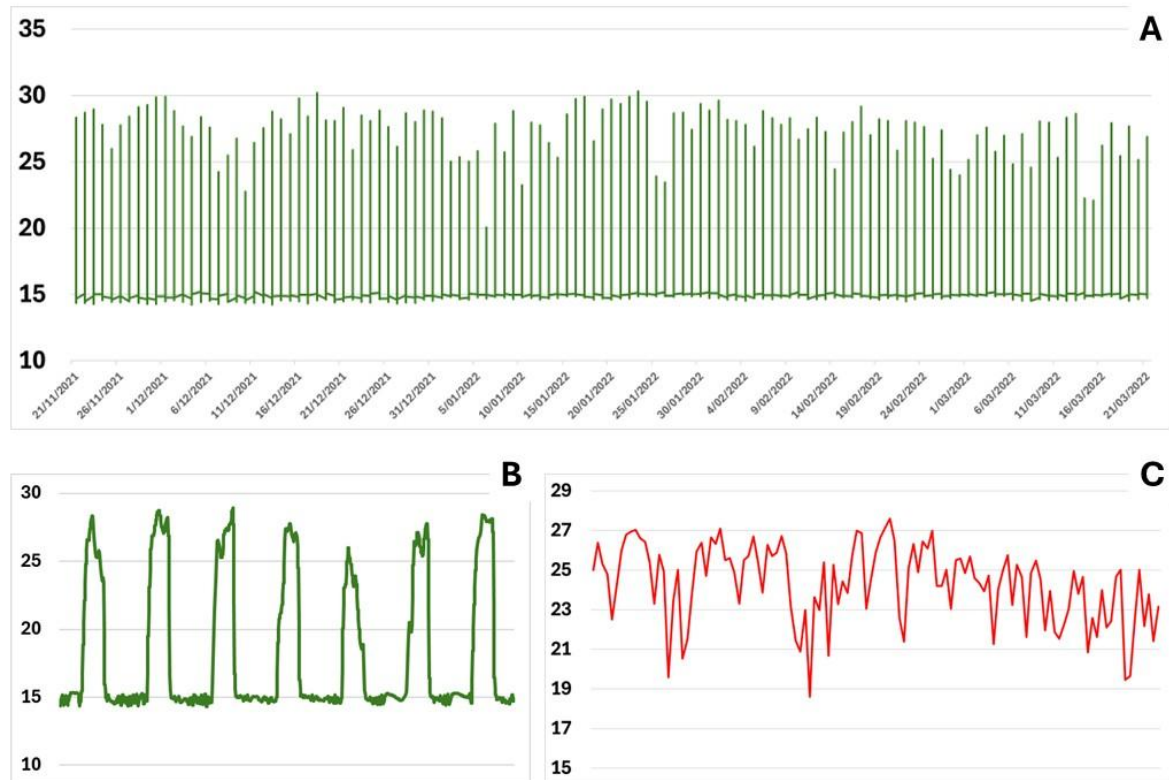
Mean, Standard deviation (SD), Minimum (Min), Maximum (Max), Range (Ran) and Median (Med) values for 10 phenological traits in parental lines and Near-Isogenic Lines grown under long-day (LD) and short-day (SD) conditions. Traits include days from emergence to floral initiation (DFI), floral development (DFD), and pod development (DPD); corresponding node-based traits (NFI, NFD, NPD); and developmental intervals between stages (DFDELAY, DPDELAY, NFGAP, NPGAP).

[illegible]

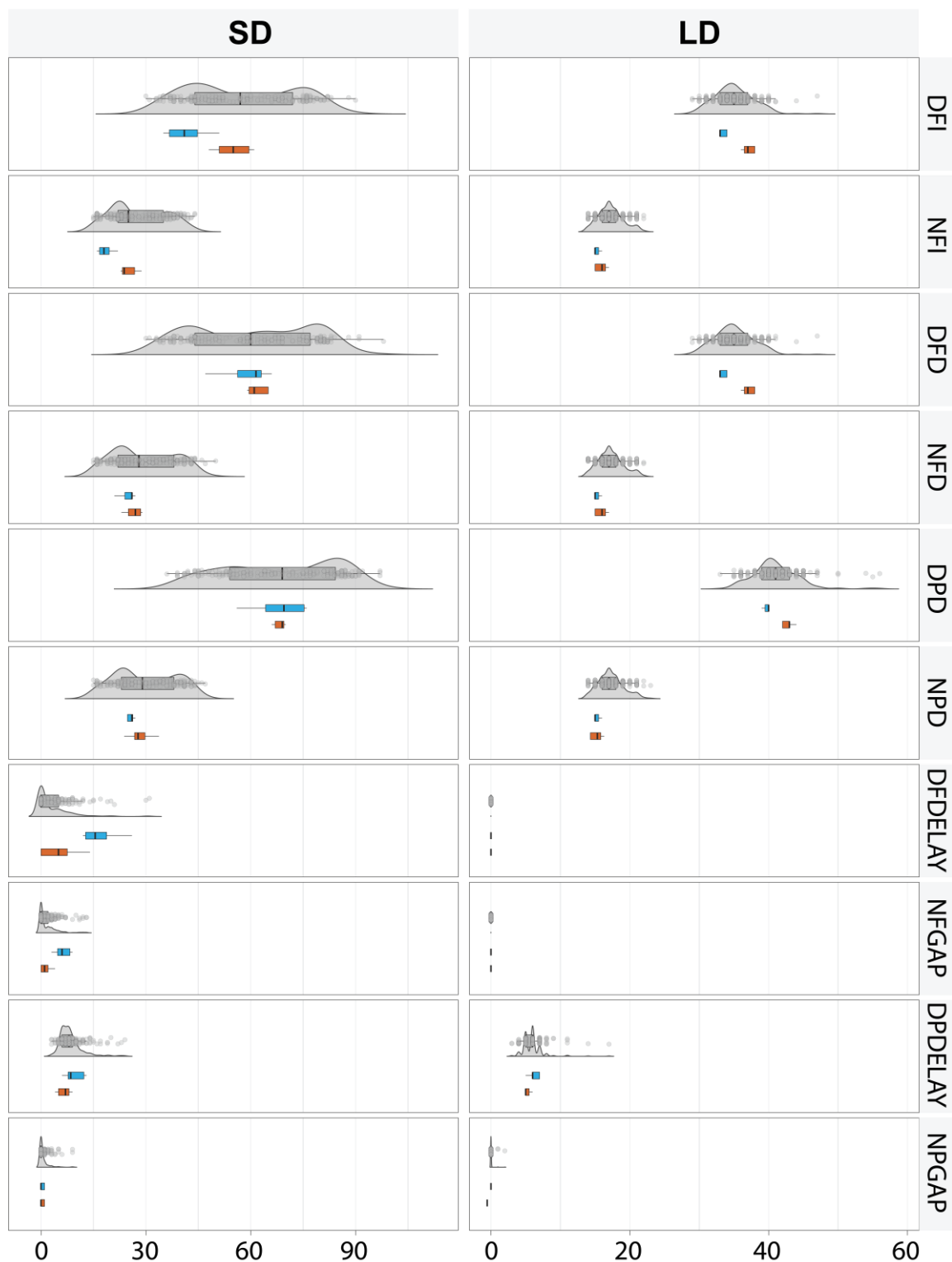
Supplemental Table 4.

Summary of *CaLG3a*, *CaLG3b*, and *ELF3a* effect on ten phenological traits in chickpea lines grown under long-day (LD) and short-day (SD) photoperiods. For each trait and condition, all possible contrasts were calculated as (*Genesis836* allele value - *Rupali* allele value) across NIL pairs differing for each locus. The table reports the mean difference between contrasts, standard deviation (SD), standard error (SE), and the number of contrasts (Count) performed for each locus.

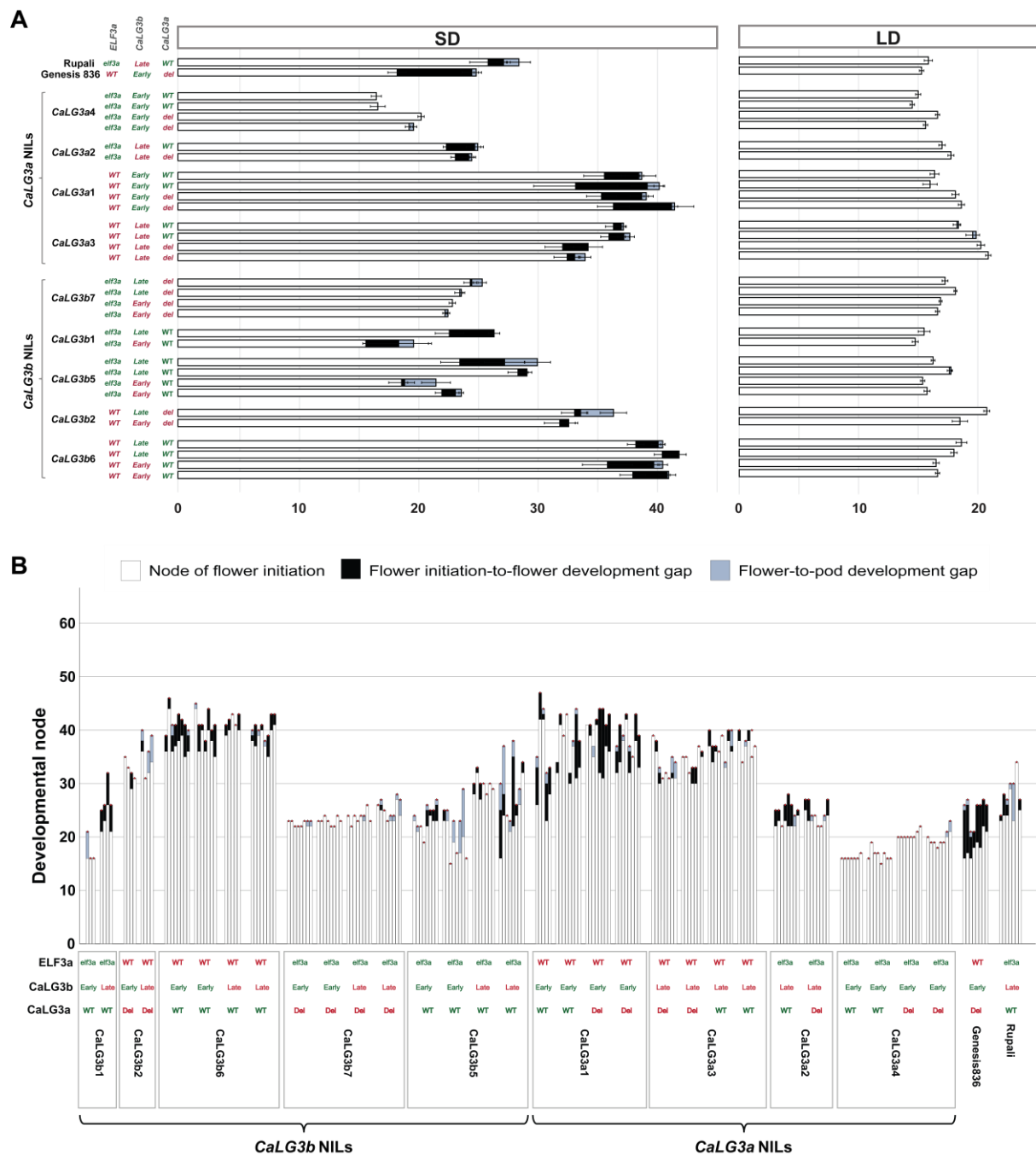
Trait	Locus	<i>CaLG3a</i>				<i>CaLG3b</i>				<i>ELF3a</i>			
	Condition	Mean Diff	SD	SE	Count	Mean Diff	SD	SE	Count	Mean Diff	SD	SE	Count
DFI	LD	2.18	0.93	0.26	13	-1.50	1.57	0.42	14	0.45	1.96	0.26	57
	SD	0.71	4.87	1.35	13	-6.15	7.47	2.00	14	25.69	8.70	1.15	57
DFD	LD	2.18	0.93	0.26	13	-1.50	1.57	0.42	14	0.45	1.96	0.26	57
	SD	0.01	4.51	1.25	13	-6.89	9.36	2.50	14	27.09	11.64	1.54	57
DPD	LD	1.64	1.30	0.36	13	-1.80	1.36	0.36	14	1.17	2.29	0.30	57
	SD	0.55	4.77	1.32	13	-6.50	8.85	2.37	14	25.69	11.95	1.58	57
DFDELAY	LD	0.00	0.00	0.00	13	0.00	0.00	0.00	14	0.00	0.00	0.00	57
	SD	-0.71	2.96	0.82	13	-0.75	3.75	1.00	14	1.40	5.09	0.67	57
DPDELAY	LD	-0.54	0.95	0.26	13	-0.30	0.93	0.25	14	0.72	0.81	0.11	57
	SD	0.02	1.29	0.36	13	0.39	2.33	0.62	14	-1.11	2.48	0.33	57
NFI	LD	1.66	0.71	0.20	13	-1.38	0.67	0.18	14	1.90	0.87	0.12	57
	SD	0.29	3.12	0.87	13	-3.28	2.82	0.75	14	14.28	4.02	0.53	57
NFD	LD	1.66	0.71	0.20	13	-1.38	0.67	0.18	14	1.90	0.87	0.12	57
	SD	0.23	2.92	0.81	13	-3.30	3.47	0.93	14	15.71	5.17	0.69	57
NPD	LD	1.60	0.74	0.21	13	-1.39	0.69	0.18	14	1.92	0.91	0.12	57
	SD	0.55	3.00	0.83	13	-3.39	3.03	0.81	14	15.40	4.99	0.66	57
NFGAP	LD	0.00	0.00	0.00	13	0.00	0.00	0.00	14	0.00	0.00	0.00	57
	SD	-0.06	1.20	0.33	13	-0.02	1.62	0.43	14	1.44	2.46	0.33	57
NPGAP	LD	-0.06	0.11	0.03	13	-0.02	0.05	0.01	14	0.02	0.08	0.01	57
	SD	-0.01	0.43	0.12	13	-0.09	1.34	0.36	14	-0.12	1.11	0.15	57
Contrast:		<i>LG3a^{Del}</i> - <i>LG3a^{WT}</i>				<i>LG3b^{Early}</i> - <i>LG3b^{Late}</i>				<i>ELF3a^{WT}</i> - <i>elf3a</i>			



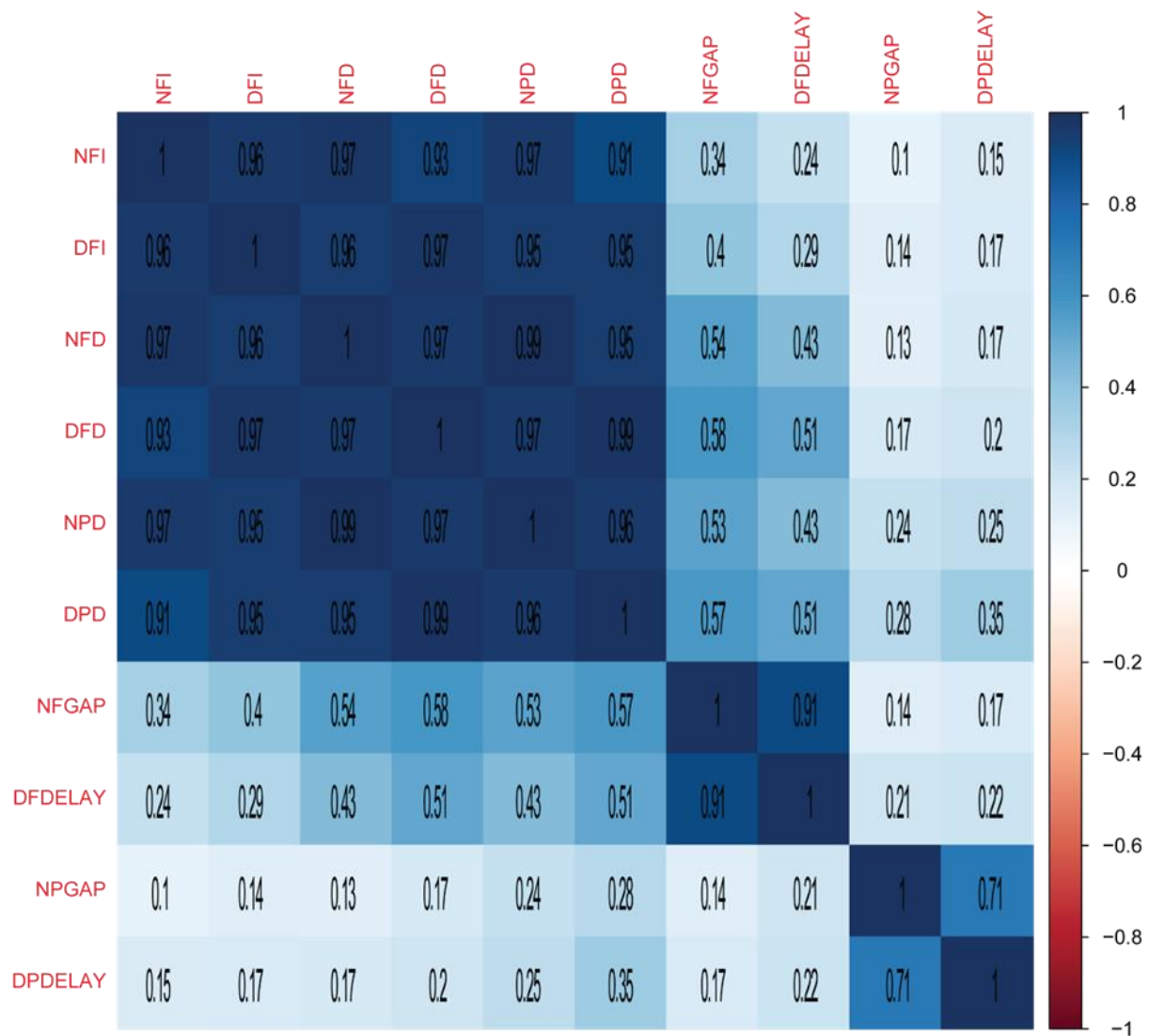
Supplemental Figure 1. Temperature variation (in °C, measured every 10 minutes) experienced by plants grown in controlled environment facilities (Experiment 1) during the scoring period from 21st November 2021 to 21st March 2022 (**A**). Plants were kept inside a phytotron at constant 15 °C for 16 hours/day (4 PM to 8 AM), while subjected to daily variation for 8 hours/day when outside the phytotron (8AM to 4 PM), as shown in (**B**) for a one-week interval, from 21/Nov/2021-12:00 AM to 28/Nov/2021-12:00 AM. (**C**) Average daily temperature during the whole scoring period (November 2021-March 2022). Only temperatures recorded between 8AM to 4 PM (plants exposed to variation) were plotted.



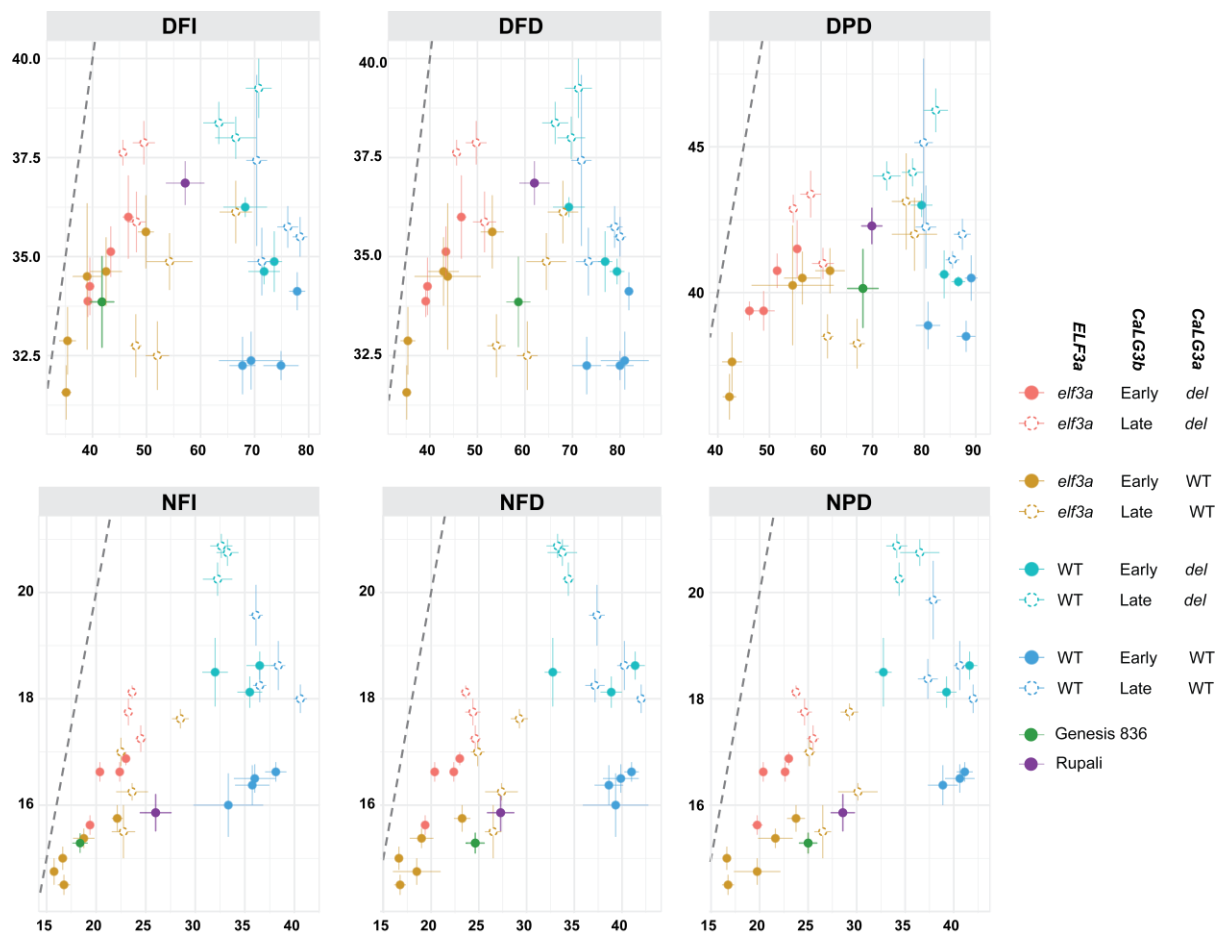
Supplemental Figure 2. Phenotypic variation under long-day (LD, right panel) and short-day (SD, left panel) conditions for chickpea Near-isogenic lines (NILs) and their parental lines. Within each panel, NILs distribution is represented by a grey half violin plot, with overlaid boxplot displaying spread and jittered points representing individual plants. Parents are shown as boxplots only, with Genesis836 in blue and Rupali in orange. The x-axis is the measured value for the trait in each facet, which include node or days to key phenological events, as follows: NFI/NFD/NPD = node of flower initiation/flower development/pod development, respectively; DFI/DFD/DPD = their counterparts measured in days. NFGAP/DFDELAY = interval between flower initiation and flower opening (nodes/days); NPGAP/DPDELAY = interval between flower and pod development (nodes/days).



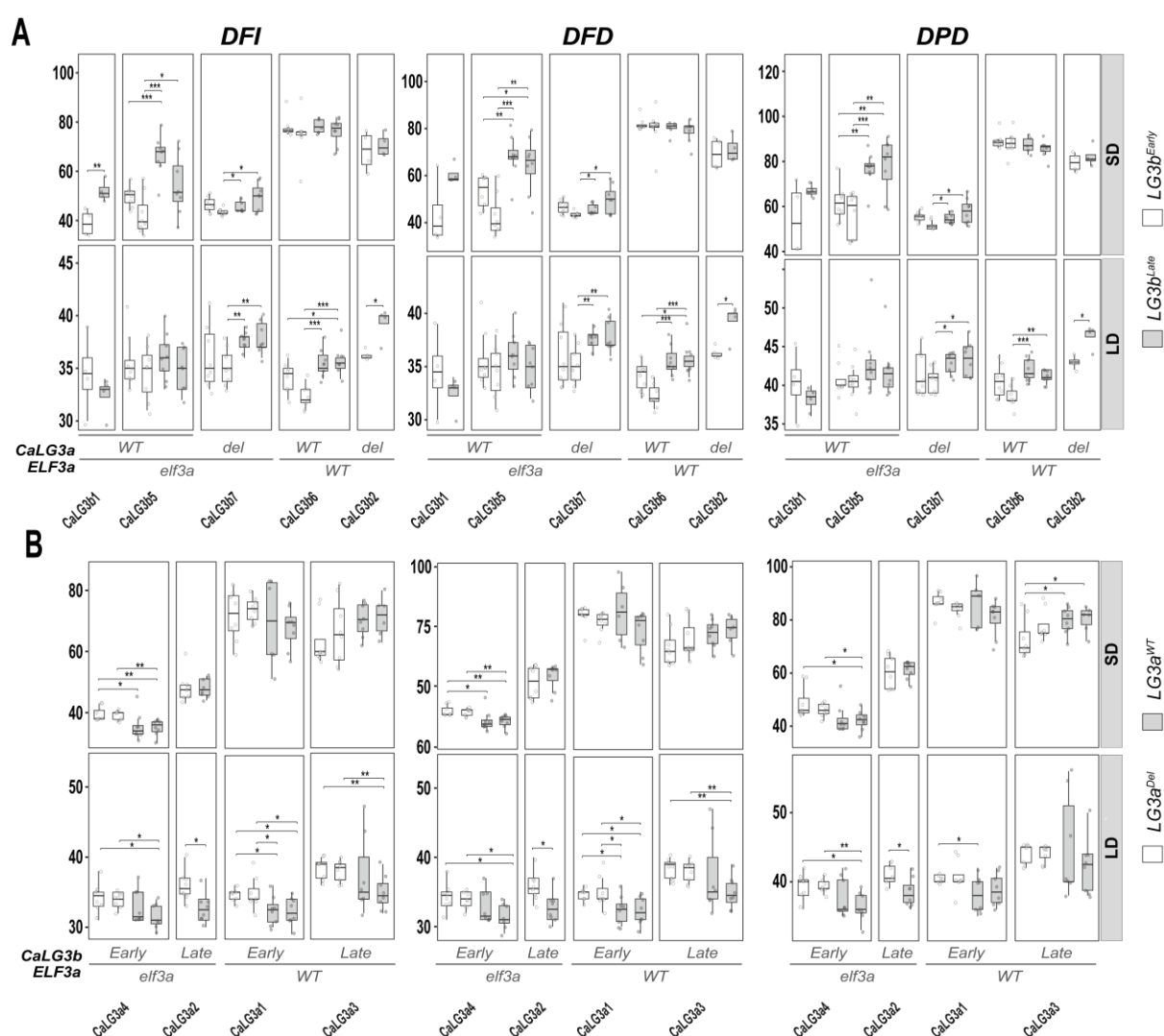
Supplemental Figure 3. (A) Variation in the node of flower initiation, flower development, and pod development for the 30 Near-Isogenic Lines and the parental accessions Rupali and Genesis 836 grown under long-day (LD) and short-day (SD) conditions. Bars represent mean values $\pm$ standard error. **(B)** Displays the same data but each bar represents an individual plant instead of averages values, to highlight the incidence of aborted flowers (black columns). Allelic composition at *ELF3a*, *CaLG3a*, and *CaLG3b* is shown for all lines, with the colour of the allele indicating whether it promotes (green) or delays (red) phenology in this study.



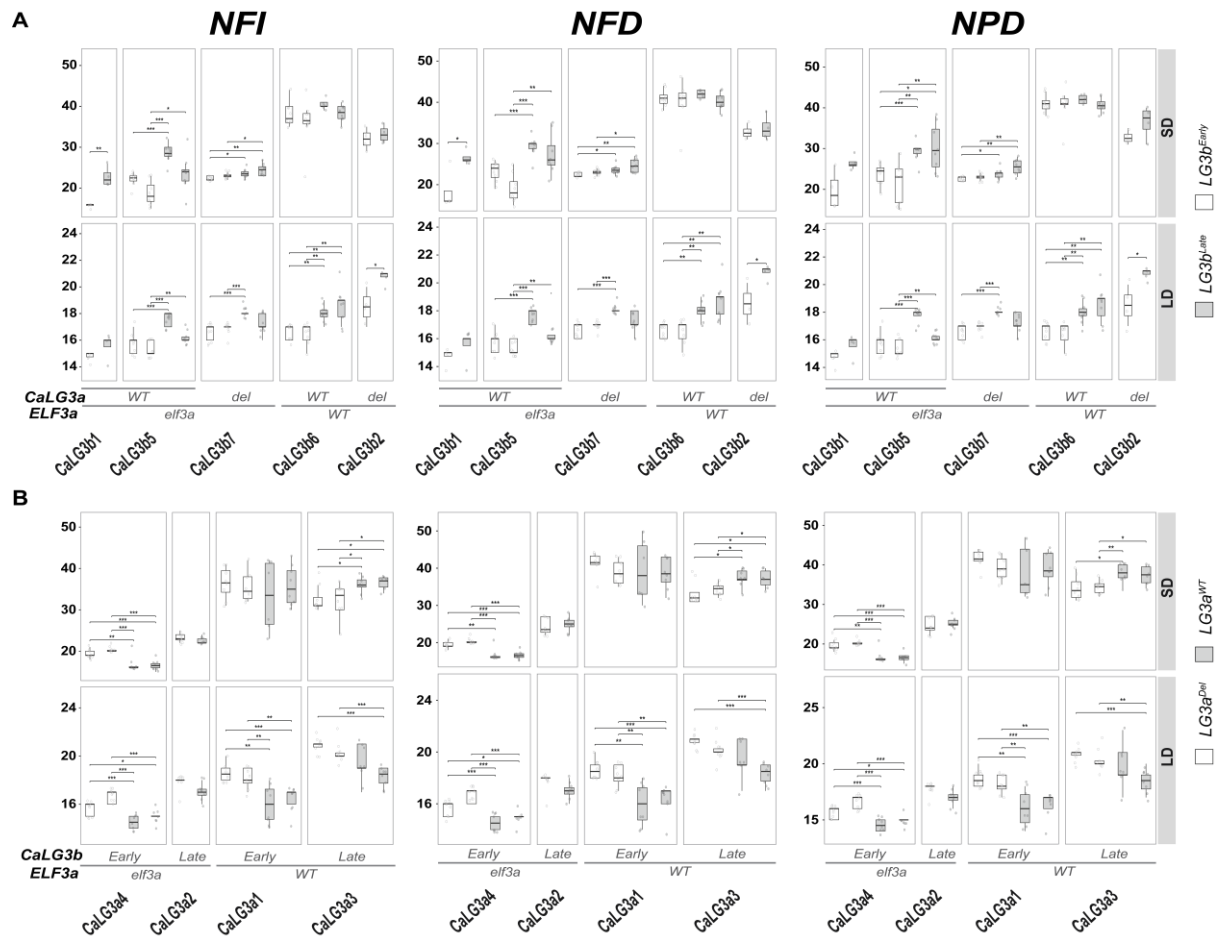
Supplemental Figure 4. Correlation matrix of phenological trait values recorded for NILs and parent genotypes *Genesis836* and *Rupali* in a controlled environment facility under two daylength regimes. Traits include days to flower initiation (DFI), flower development (DFD), pod development (DPD), and node counts (NFI, NFD, NPD), as well as developmental gaps and delays (NFGAP, DFDELAY, NPGAP, DPDELAY). Colour intensity represents Pearson correlation coefficients, ranging from -1 (strong negative correlation, red) to +1 (strong positive correlation, blue).



Supplemental Figure 5. Comparison of six phenological traits measured under long-day (LD; Y-axis) and short-day (SD; X-axis) photoperiods in 30 chickpea near-isogenic lines (NILs) and parental controls. Traits include days from emergence to flower initiation (DFI), flower development (DFD) and pod development (DPD), and node positions for flower initiation (NFI), flower development (NFD), and pod development (NPD). Each point represents the mean value for a particular NIL, with vertical and horizontal error bars indicating standard deviation under LD and SD conditions, respectively. Colours and bold/dashed lines denote genotype at *ELF3a*, *CaLG3b*, and *CaLG3a*, as indicated in the legend. The dashed diagonal line represents the 1:1 relationship between LD and SD measurements (photoperiod insensitivity).



Supplemental Figure 6. Variation in days from emergence to flower initiation (DFI), flower development (DFD) and pod development (DPD) across Near-Isogenic Lines (NILs) segregating for *CaLG3a* (**A**) or *CaLG3b* (**B**) and grown under long day (LD) or short-day (SD) conditions. The genotype of each NIL for other loci is indicated at the bottom. Grey boxes represent alleles from *Rupali* and white boxes alleles from *Genesis836*. Points represent individual observations and asterisks denote significant differences between allelic classes.



Supplemental Figure 7. Variation in node of flower initiation (NFI), node of flower development (DFD) and node of pod development (DPD) across Near-Isogenic Lines (NILs) segregating for *CaLG3b* (**A**) or *CaLG3a* (**B**) and grown under long day (LD) or short-day (SD) conditions. The genotype of each NIL for other loci is indicated at the bottom. Grey boxes represent alleles from Rupali and white boxes alleles from Genesis 836. Points represent individual observations and asterisks denote significant differences between allelic classes.

