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RESEARCH PAPER

Time to flowering and flowering duration in mungbean are unrelated physiological traits with independent genetic controls

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Abstract

Mungbean (*Vigna radiata*), a valuable sub-tropical grain legume, typically has a long, asynchronous flowering window, which increases vulnerability to abiotic stress and complicates harvesting. To facilitate breeding efforts, we conducted an extensive study of days to flowering (DTF) and the novel trait flowering duration (FD) in multi-environment trials. A diverse nested association mapping population was evaluated across four field trials in Queensland, Australia. Extensive phenotypic variation was observed for both DTF (35–70 d after sowing) and FD (20–60 d). Both traits displayed Genotype × Environment interactions, with FD showing stronger environmental interactions than DTF. No relationship was evident between DTF and FD across environments. Genome-wide association studies identified eight quantitative trait loci (QTLs) for DTF and one for FD, with none overlapping. The accumulation of early or late alleles at DTF QTLs was associated with variations in flowering time. These results show for the first time in mungbean that DTF and FD are independent traits with distinct genetic controls and environmental responses, providing a mechanistic understanding of how flowering patterns may be optimised to potentially enhance adaptation and performance in diverse agricultural environments challenged by climate change.

Keywords: Floral initiation, flowering time, flowering duration, genome wide association study (GWAS), genotype × environment interaction, mungbean, phenology, *Vigna radiata*.

Abbreviations: FD, Flowering duration; EF, end of flowering; DT100, days to 100% flowering; DTFF, days to first flower; DTF, days to flowering; MET, multi-environment trial.

Introduction

Mungbean (*Vigna radiata*) is a sub-tropical legume crop grown across diverse production regions (Lambrides and Godwin, 2007). Mungbean has a short duration and also offers significant agronomic benefits by providing a disease break, reducing weed pressure, and enriching soil nitrogen levels through symbiotic nitrogen fixation (Foyer *et al.*, 2019). In Australia, mungbean is the primary summer legume rotation option; however, production is variable across seasons and yields are low with most farmers achieving 0.5–1.5 t ha^{-1} (ABARES, 2022). A key industry objective in Australia is to increase yield stability and achieve 2 t ha^{-1}, which has been attained in research trials and in crop model simulations (Rachaputi *et al.*, 2019; Pasley *et al.*, 2023).

In most production environments, mungbean flowering behaviour typically exhibits a protracted, asynchronous pattern that involves multiple flushes of flowering on the same plant (Lambrides and Godwin, 2007; AMA, 2016). This leads to asynchronous pod maturity, which can complicate harvest in mechanised production systems (Gentry, 2010; Mondal *et al.*, 2011). A long reproductive window also increases susceptibility to abiotic stress such as drought and heat, often leading to yield loss (Rathcke and Lacey, 1985; Van Haeften *et al.*, 2023; Pasley *et al.*, 2024). Flowering initiation and duration are critical factors in mitigating asynchrony in mungbean. Time to flowering is an agronomically important, quantitative trait that has been researched in almost all crop species due to its strong influence on yield (Ducrocq *et al.*, 2009; Du *et al.*, 2021). In contrast, flowering duration remains unexplored in many crops. Unlike time to flowering, which measures only the time to onset, flowering duration quantifies the length of the flowering period. Understanding flowering behaviour in mungbean is important in order to optimise its phenology for a range of sowing windows and environments, and ultimately to avoid environmental stress and maximise yield (Pasley *et al.*, 2024). This in turn requires detailed dissection of flowering traits and their underlying genetic mechanisms.

The onset of flowering and flowering duration are distinct components of plant development, and as such have different controls. Initiation of flowering, which marks the transition from vegetative to reproductive growth, is primarily influenced by genetic factors and environmental cues such as photoperiod and temperature. This transition involves the activation of flowering genes that respond to external signals. Flowering duration encompasses the period from the appearance of the first flower to the end of flowering. It is a complex trait that is influenced by both the initiation and termination of flowering and may be regulated by various factors such as the regulation of the florigen signal, development of floral meristems, flower maturation, temperature, nutrient availability, water availability, and plant health (Jeuffroy and Sebillotte, 1997; Dorji *et al.*, 2020; González-Suárez *et al.*, 2020, 2023; Swentowsky and Robil, 2023). While an extensive body of research on crop species is related to flowering time, little is known about flowering duration. Previous studies conducted in cotton and soybean have identified quantitative trait loci (QTLs) associated with flowering duration that were deemed to be independent of genomic regions associated with time to flowering (Komatsu *et al.*, 2012; Kushanov *et al.*, 2017). While both flowering initiation and duration are interrelated, their genetic and environmental controls could be distinct, requiring separate strategies for breeding and management to optimise flowering and yield.

Flowering is well understood in model and major crop species, and genetic variation in time to flowering and how it is regulated by the environment has been reported in many legume crops (Nelson *et al.*, 2010; Weller and Ortega, 2015; Surkova and Samsonova, 2022). The initiation of flowering in temperate legume crops such as faba bean, pea, chickpea, lupin, and alfalfa is primarily controlled by day length and vernalisation (Ellis *et al.*, 1990; Major *et al.*, 1991; Landers, 1995; Weller *et al.*, 1997; Kim *et al.*, 2009). Tropical grain legumes can also be sensitive to the seasonal and regional effects of photoperiod and temperature, as observed by responses in yield and phenology (Lawn, 1989). Most tropical legumes are facultative short-day plants, such as pigeonpea, cowpea, soybean, and mungbean, although photoperiod-insensitive genotypes have been identified in all these species (Vas Aggarwal and Poehlman, 1977; Summerfield *et al.*, 1985; Carberry *et al.*, 2001; Kantolic and Slafer, 2001; Rebetzke and Lawn, 2006). Soybean is a well-studied, tropical crop that was domesticated from a temperate and strongly photoperiod-sensitive relative (Carter *et al.*, 2004). Previous research in soybean suggests that the environment (photoperiod, water availability) influences not just the initiation of flowering but also the duration of the reproductive phase and therefore yield (Johnson *et al.*, 1960; Han *et al.*, 2006; Nico *et al.*, 2015). Understanding the influence of the environment on the expression of phenological traits is therefore worthy of further investigation.

In mungbean, genotypic and phenotypic variation for time to flowering and duration have been observed in diverse germplasm (Lawn, 1979; Imrie and Lawn, 1990; Lawn and Rebetzke, 2006; Rebetzke and Lawn, 2006). Previous research has also implied that the environment might influence flowering duration as it has been noted that soil moisture content and water availability can result in a lack of flowering synchrony in mungbean (Chauhan and Rachaputi, 2014; Chauhan and Williams, 2018).

The genetic basis of time to flowering in mungbean has been investigated through the identification of genetic regions, candidate genes, and homologous flowering-related genes. Several homologs of known flowering-regulatory genes have been identified in mungbean, including *CONSTANS* (*CO*), *CONSTANS-LIKE1* (*COL1*), and *COL2*. Homologs of the soybean loci *E3* and *J* have been identified as encoding

PHYTOCHROME A (*PHYA*) and *EARLY FLOWERING 3* (*E3*), respectively, which encode proteins involved flowering and growth regulation (Hwang *et al.*, 2017; Liu *et al.*, 2021). In addition, the *CIRCADIAN CLOCK ASSOCIATED 1-like* genes *CCA1L6* and *CCA1L26* have been identified in mungbean (Liu *et al.*, 2022). These flowering-time genes associated with the photoperiod pathway have been mapped to specific QTL regions associated with flowering-time traits, such as days to first flower (first flower observed in the plot) and days to flowering (50% of the plot flowering; Hwang *et al.*, 2017). While combined QTL and phylogenetic analysis and gene identification studies have provided valuable insights into the genetic loci and potential candidate genes, functional validation of QTLs and genes is still lacking. For instance, while the *COL* genes in mungbean have been phylogenetically characterised and shown to exhibit differential expression under varying photoperiods (Liu *et al.*, 2021), and overexpression of *VrCOL2* in Arabidopsis accelerates flowering under short-day conditions, the precise functional roles of the *COLs* in mungbean have not been conclusively confirmed. Similarly, although *VrCCA1L* genes have been identified based on their expression patterns and sequence similarities to Arabidopsis homologs, their direct involvement in regulating flowering time in mungbean has not yet been experimentally validated (Liu *et al.*, 2022). The identification of *COLs* and *CCA1Ls* has revealed promising candidates associated with flowering time in mungbean (Liu *et al.*, 2022); however, further functional studies are needed to validate their specific roles in the regulation of flowering.

Recent studies have identified QTLs for days to first flower and days to flowering in mungbean (Ha *et al.*, 2021; Seo *et al.*, 2023; Amkul *et al.*, 2024; Chiteri *et al.*, 2024). Flowering time genes are located within these QTLs, including *FLOWERING LOCUS C* (*FLC*), *FLOWERING LOCUS M* (*FLM*) (Seo *et al.*, 2023), and *PHYTOCRHOME E*, which is a photoreceptor gene homolog that contributes several functions including flowering-time control (Amkul *et al.*, 2024). These findings indicate a potential functional link between reported candidate genes and observed phenotypic variation for flowering time in mungbean. Studies that link variation in flowering traits to genetic variation provide an important foundation for further work. There is now an opportunity to investigate Genotype × Environment (G×E) interactions of flowering time and flowering duration in mungbean.

This study investigated the physiological and genetic basis for key flowering traits using a diverse Australian mungbean nested association mapping population grown across multiple environments in Southeast Queensland. We examined flowering traits including days to first flower, days to flowering, days to 100% flowering, end of flowering, and flowering duration. Multi-environment trial analyses were conducted to evaluate G×E interactions and subsequent genome-wide association studies were undertaken to identify genomic regions associated with key flowering traits. Our findings provide new phenotypic and genetic information that may be utilised in crop improvement and management efforts to optimise phenology to suit a range of sowing dates and production environments.

Materials and methods

Plant materials

This study was conducted using a nested association mapping (NAM) population of mungbean (*Vigna radiata*) previously developed by the Queensland Department of Primary Industries and the Queensland University of Technology (Noble *et al.*, 2017). This population was specifically chosen for its established genetic and phenotypic diversity across key agronomic traits, such as phenology, canopy architecture, seed size, and yield. The NAM population was developed from 30 diverse donor parents crossed to the reference parent Crystal, resulting in the generation of 2060 lines. For comprehensive genetic analyses, the population and parental lines were genotyped using DArTseq markers (Diversity Arrays Technology Pty Ltd, Canberra, Australia; https://www.diversityarrays.com/), resulting in a dataset of 32 055 high-quality single-nucleotide polymorphisms (SNPs).

Field trials

Four field trials were conducted in Southeast Queensland during 2022 and 2023 (Table 1), located at the Pacific Seeds Foundation Farm, Allora (28°3′44.16″S; 151°57′47.38″E) and The University of Queensland Gatton Campus (27°34′02.6″S/152°19′51.4″E). In the 2022 harvest season, the NAM population with sufficient seed available (1994 genotypes) and the parental lines (30 genotypes) were evaluated in the main trial at Gatton (Gatton22A), and additional trials were conducted at Gatton (Gatton22B) and Allora (Allora22), each of which consisted of the NAM parents and a subset of 369 genotypes of the NAM population (Supplementary Table S1). In the 2023 harvest season, only the NAM parents were evaluated at Gatton (Gatton23). Allora22 and Gatton22B employed an unbalanced replicated design, with the NAM parents replicated four times and the subset of the NAM population replicated twice (Table 1). Gatton22A had a partially replicated design with the NAM parents replicated four times and the entire population having a 1.5 replication. Gatton23 had a completely replicated design with all genotypes having four replications. Two-row plots were sown at all the sites, with plant density standardised at 25 plants m^{-2}. The trials were laid out using a model-based row–column design incorporating genetic relatedness, facilitated by the R package 'od' (Cullis *et al.*, 2020). Seeds were provided with Group 1 inoculum (Easy*Rhiz*™, New Edge Microbials Pty. Ltd, Wodonga, Victoria, Australia) during sowing. A basal application of 25 kg ha^{-1} starter fertiliser was applied at sowing, and weed, insect, and disease management were executed as required throughout the season according to standard practices. Trials were either rainfed (2022) or irrigated (2023; total of 100 mm ha^{-1} supplementary irrigation), with the timing of irrigation determined via continuous monitoring of soil moisture content. Daily meteorological data (minimum and maximum temperature, rainfall) for 2022 at Allora (Station No. 041525) and for 2022 and 2023 at Gatton (Station No. 040082) were sourced from the Bureau of Meteorological station (Supplementary Fig. S1). When most plots within a trial had reached physiological maturity, the crop was desiccated with glyphosate and measurements ceased.

Phenotyping of flowering traits

In all the trials, five flowering traits were systematically recorded as the number of days after sowing, as follows: days to first flower (DTFF), which denotes the number of days until the first flower was observed in the plot; days to flowering (DTF), which was recorded when 50% of the plants in the plot had flowered; days to 100% (DT100) flowering, was recorded when all plants in the plot had flowered; days to end of

Table 1. Summary of four field trials analysed in this study

Trial name	Sowing date	Location*	Germplasm	No. genotypes	No. replicates	No. plots	No. columns.	No. rows
Allora22	21 Jan. 2022	Allora	NAM parents	30	4	672	24	28
			NAM subset	369	2			
Gatton22A	1 Feb. 2022	Gatton	NAM parents	30	4	2840	71	40
			NAM population	1994	1.5			
Gatton22B	2 Feb. 2022	Gatton	NAM parents	30	4	728	28	26
			NAM subset	369	2			
Gatton23	22 Dec. 2022	Gatton	NAM parents	30	4	120	10	12

*Both sites are in Queensland, Australia: Allora, 28°3′44.16″S/151°57′47.38″E; Gatton, 27°34′02.6″S/152°19′51.4″E.

flowering (EF), which was recorded when it was observed that no new flowers were produced on any plants in the plot; and flowering duration (FD), which was determined as the time from DTF to EF (i.e. EF–DTF).

Across the four trials, 29 genotypes that did not have an end of flowering point at physiological maturity (when desiccant was applied) were excluded from the analyses for EF and FD. This approach was chosen rather than assigning minimum nominal values because that would have artificially truncated flowering duration, introducing a systematic bias that would have suggested that flowering ended earlier than biological reality. While slightly reducing the sample size, this exclusion approach preserved data integrity by including only complete flowering cycles with definitive endpoints, allowing for more accurate trait characterisation. Sensitivity analyses comparing results with and without these exclusions showed consistent patterns in the main findings, indicating that this methodological choice did not substantially affect the key conclusions.

Daily thermal time (degree-days, °Cd) for each trait across all trials was determined using previously established equations (Jones and Kiniry, 1986; Hammer *et al.*, 1993), incorporating the temperature data acquired and mungbean-specific base, optimal, and maximum temperatures, set at 7.5 °C, 30 °C, and 40 °C, respectively (Ellis *et al.*, 1994; Robertson *et al.*, 2002; Chauhan and Williams, 2018).

Statistical analysis

Multi-environment trial analysis

Single-site spatial analyses were first conducted to explore within-trial spatial variation followed by a multi-environment trial (MET) analysis to assess the overall genotypic performance across all environments. The data for flowering traits obtained from all the trials were analysed in a MET using a linear mixed model that incorporated the interaction between genotype and environment (G×E). The linear mixed models were fitted in ASReml-R (Butler *et al.*, 2009) as follows:

$$y = X\tau + Zu + e$$

In the model, X denotes the design matrix associated with a vector of fixed effects (τ), which includes environmental and trial-specific factors contributing to field variation. This encompasses linear row and column terms, as well as covariates such as buffer row(s). Conversely, Z represents the design matrix for the vector of random effects (u), which captures the effects of genotype both within and across sites, along with additional random effects, including separate row and column terms. The local spatial trend at each trial was accounted for in the model as a variance structure (u), utilising a separable autoregressive process of order one (AR1×AR1). A base model was fitted to check for outliers using the Additive Outlier Model (AOM) in ASReml-R. Outliers were identified based on studentised residuals exceeding a threshold of 4 and were removed.

To assess the G×E interactions for flowering traits, a range of different genetic variance structure models were examined, including a diagonal model and the corv and corh correlation models, which assumed homogeneous and heterogenous genetic variance and co-variance structures in order to model the G×E co-variance matrix. However, after determining that there was unequal variance between trials, a factor analytic (FA) variance structure was fitted for the interaction (Smith *et al.*, 2001). The number of factors applied within this structure was determined based on the Akaike information criterion (AIC), the Bayesian information criterion (BIC), and the restricted maximum likelihood (REML) log-likelihood ratio as well as the total observed genetic variance accounted for by the model (Supplementary Table S2). To further investigate the G×E interactions influencing the traits, a single overall genotypic best linear unbiased predictor (BLUP) value was estimated for each genotype across all traits to capture the random effects associated with the interactions. Similarly, a single best linear unbiased estimate (BLUE) value was calculated for each genotype from the fixed effects in the model for subsequent analyses. The genotype effect u_g and its associated variance was calculated using the following equation:

$$u_g = (\lambda_1 \otimes I_m)f_1 + (\lambda_2 \otimes I_m)f_2 + (\lambda_3 \otimes I_m)f_3 + \delta$$

where λ_i are the known trial site loadings, I_m is the genetic variance matrix, f_i are the random set of hypothetical factor scores for each genotype, and δ is the residual vector (Smith *et al.*, 2001).

Investigating the relationships among flowering traits

To examine the relationships among all the flowering traits considered in this study (DTFF, DTF, DT100, EF, FD) a principal component analysis (PCA) was performed using the single BLUE for each genotype across all trials. An additional PCA was later undertaken for DTF and FD using a single BLUE for each genotype in each trial to explore trait expression across environments.

Genome-wide association studies

Genome-wide association studies (GWAS) were conducted using SNP marker data and BLUEs for DTF and FD from the MET analyses. Curation of the DArT markers was performed prior to analyses, including removal of markers with a minor allele frequency ≤1%, with greater than >10% heterozygosity, and markers with >20% missing data. 'Soft Impute' was utilised to impute missing values within the marker data matrix via a singular value decomposition approach (Mazumder *et al.*, 2010), resulting in a final curated marker set of 10 864 SNPs. PCA was conducted to analyse the genetic structure of the NAM population based on the genetic dissimilarity between curated markers (Supplementary Fig. S2). The PCA was conducted utilising a genetic distance matrix calculated with Rogers' distance (Rogers, 1972) using the R package 'Selection Tools'. The distribution of the final markers across the 11 chromosomes were as follows: Chr. 1,792; Chr. 2, 919; Chr. 3, 597; Chr. 4, 669; Chr. 5, 1477; Chr. 6, 905; Chr. 7, 1815; Chr. 8, 1433; Chr. 9, 590; Chr. 10, 859; and Chr. 11, 808.

GWAS was performed using the Bayesian-information and linkage-disequilibrium iteratively nested keyway (BLINK) model (Huang *et al.*, 2019)

in the R package 'Genomic Association and Prediction Integrated Tool' (GAPIT; Lipka *et al.*, 2012). The BLINK model effectively incorporates Bayesian information to account for population structure and linkage disequilibrium, making it well-suited for complex populations such as the NAM population used in this study. By iteratively refining the estimates of marker effects while adjusting for genetic relationships, BLINK improves the accuracy of association tests, allowing for robust identification of trait-associated loci. To determine significant marker trait associations representing a QTL, an adjusted false-discovery rate (FDR) of 0.05 was applied, consistent with the default parameter in the GAPIT package and previously published GWAS (Sokolkova *et al.*, 2020; Manjunatha *et al.*, 2023; Chiteri *et al.*, 2024). To determine if any QTLs were associated with both DTF and FD, a linkage disequilibrium (LD) analysis was undertaken for pairs of markers significantly associated with DTF and FD. Pairwise LD between genetic markers was calculated using the 'Population Genetics' package in R (https://CRAN.R-project.org/package=genetics). QTLs were deemed to be linked if the pairwise marker LD was >0.4, with a *P*-value <0.05.

To determine whether the QTLs detected in this study were novel, we compared the positions with previously published QTLs and genes using the *Vigna radiata* reference genome (v. 6; Kang *et al.*, 2014) in the NCBI Genome Data Viewer (https://www.ncbi.nlm.nih.gov/gdv). The QTL regions identified were subjected to a series of bioinformatic analyses to explore potential candidate genes. First, the QTL sequences were compared to the *Vigna radiata* reference genome (v.6) using BLAST with default parameters to identify previously reported regions related to flowering time. Cross-species comparisons were then conducted using BLAST searches against the Phytozome database (v. 13; https://phytozome-next.jgi.doe.gov/), focusing on related legumes (*V. unguiculata*, *Glycine max*, and *Phaseolus vulgaris*) and *Arabidopsis thaliana*. Synteny analysis was performed using the Genome Context Viewer of the Legume Information System (LIS; https://gcv.legumeinfo.org/gcv2/) to identify conserved gene regions between mungbean and the legume Species listed above. For each QTL region, a window of 500 kb upstream and downstream of the peak marker was examined. Candidate genes were identified based on sequence homology (*E*-value threshold $<1\times10^{-5}$) and known associations with flowering time regulation in related species.

To investigate the phenotypic effects associated with the accumulation of early or late alleles for DTF, alleles at each of the QTLs that were identified were assigned to all lines in the population using the most significant marker. The allele associated with a negative effect was considered as contributing to 'early' flowering, whereas the allele associated with a positive effect was considered as contributing to 'late' flowering. The cumulative effect on DTF was determined by summing the number of early and late alleles for each line. The difference between the DTF for groups of lines carrying different numbers of early and late alleles was investigated. In addition, the FD of the same lines in the NAM population were analysed to investigate potential interactions between DTF and FD. For this analysis, the mean flowering duration (°Cd) for each early and late QTL allele count was calculated and compared.

Results

Time to flowering and flowering duration are independent traits

In our initial approach, we investigated a range of flowering traits encompassing DTFF, DTF, DT100, EF, and FD; however, analyses revealed that most of them were closely related (Supplementary Fig. S3). Across all environments, the three time to flowering traits (DTFF, DTF, DT100) were found to have a strong positive correlation, whilst EF and FD were correlated with each other but showed a neutral relationship with DTFF, DTF, and DT100. Given that there were two distinct groups of highly correlated traits, only DTF and FD were examined in further analyses. The NAM population, developed from 30 diverse donor parents crossed to the reference parent Crystal, showed a high degree of phenotypic variation for flowering traits.

Substantial phenotypic variation in DTF and FD was observed in the NAM population (Fig. 1), with both traits displaying moderate-to-high broad sense heritability across all environments (DTF, H^2=0.74; FD, H^2=0.61). All the NAM families segregated for DTF and FD, with transgressive segregation evident in several families. For instance, the DTF of the parents ranged from 675 °Cd (AGG 325955) to 820 °Cd (Putland), whereas in the progeny ranged from 576–1241 °Cd (Supplementary Table S3). Similarly, the FD of the parents ranged from 433 °Cd (AGG 425954) to 782 °Cd (AGG 325977) compared to the progeny range of 163–1101 °Cd. The single-site spatial analyses produced BLUEs (Supplementary Table S3) that reflected both genetic and site-specific environmental effects, resulting in wider value ranges (e.g. 576–1241 °Cd for DTF and 163–1101°Cd for FD). In contrast, the MET analysis integrated data across all four sites, reducing environmental noise and providing more stable genotypic estimates, with narrower ranges observed (e.g. 641–890 °Cd for DTF and 193–917 °Cd for FD; Fig. 1). This reduction in range reflects the removal of site-specific environmental variation, revealing the underlying genetic signal. Unless otherwise stated, all trait values reported from this point forward refer to the MET-derived BLUEs.

Days to flowering and flowering duration display minimal cross-over of G×E interactions

Factor analytic models were fitted with a different number of factors (FA1–FA3) to determine which model accounted for the most genetic variance (Smith *et al.*, 2015). For DTF, the FA2 model was selected according to the fitted parameters (Supplementary Table S2) and accounted for 98% of the genetic variance, whilst the FA3 model was selected as the preferred model for FD and explained 88% of the genetic variance.

Overall, DTF and FD had minimal cross-over of G×E interactions (Fig. 2). Scale-type G×E was observed for both traits; however, FD showed more compared to DTF.

DTF and FD showed a neutral relationship and were confirmed to be independent of each other across all environments (Fig. 3). Principal components one and two accounted for 58.39% and 25.27% of the variation, respectively. DTF was highly correlated in all four environments whereas FD showed moderate correlations across all environments.

Flowering time and flowering duration are associated with independent genomic regions

Eight QTLs were found to be associated with DTF and were located as follows: Chr. 1 (*QDTF.1.1*; position 12 467 500), Chr. 2 (*QDTF.2.1*; position 23 895 882), Chr. 3 (*QDTF.3.1*; position 3

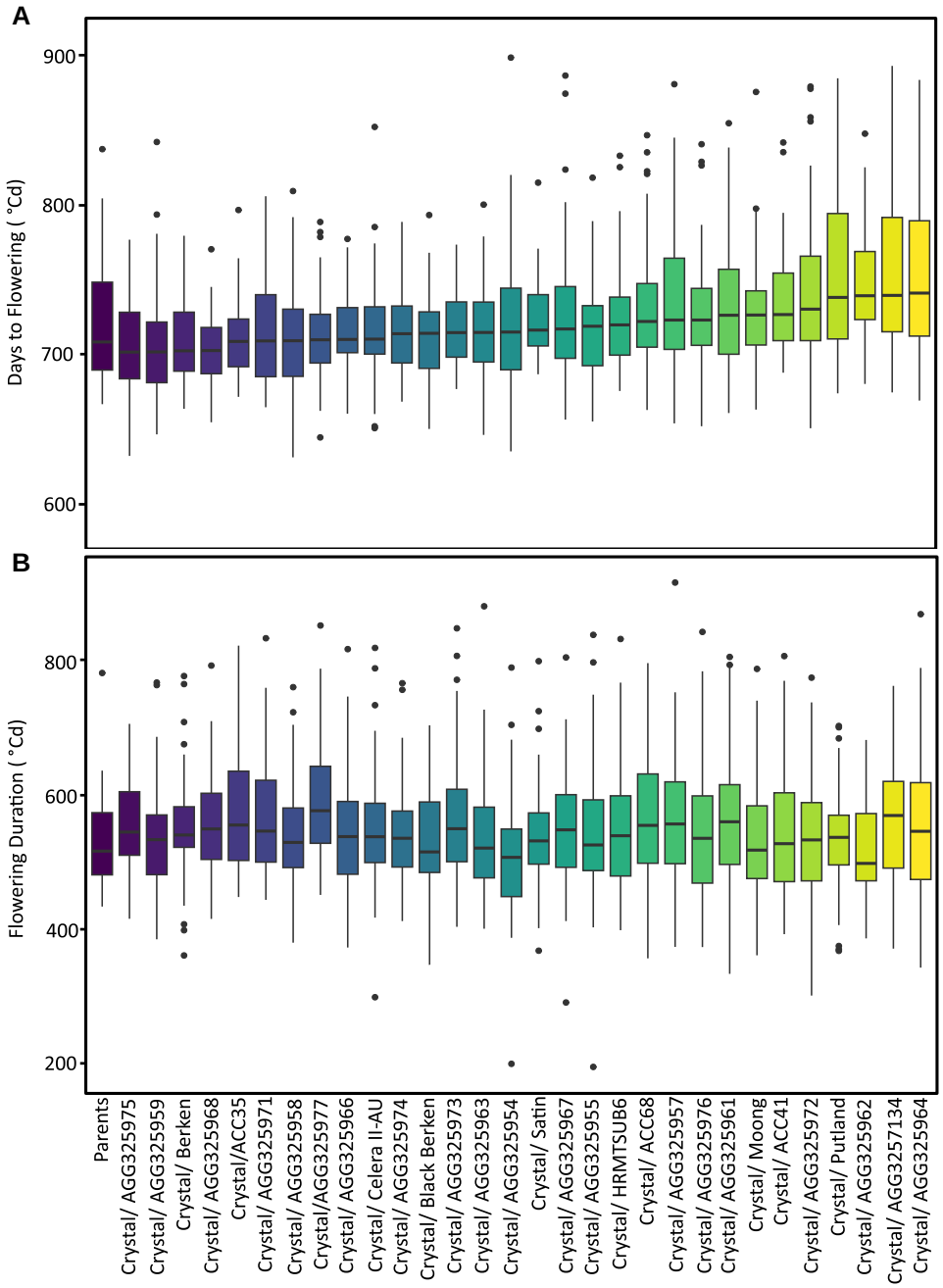

Fig. 1. Phenotypic variation in flowering time and duration for the parents and families of the mungbean nested association mapping population. The population was developed from the 30 diverse donor parents crossed to the reference parent Crystal. (A) Days to flowering and (B) flowering duration, both measured as thermal time. Each boxplot shows the distributional spread across genotypes using the best linear unbiased estimates (BLUEs) for genotypes, calculated across the four environments. Boxplots display the interquartile range (box), median (horizontal line), and values within 1.5× the interquartile range (whiskers); points beyond are shown as outliers. Shading differentiates families of the NAM population and is consistent across panels.

442 005), Chr. 5 (*QDTF.5.1*; position 2 454 971), Chr. 7 (*QDTF.7.1*; position 48 218 974), Chr. 9 (*QDTF.9.1*; position 7 890 386), and Chr. 10 (*QDTF.10.1* and *QDTF.10.2*; positions 15 771 485 and 18 743 804, respectively) (Fig. 4). A single QTL associated with FD was identified on Chr. 4 (*QFD.4.1*; position: 17 944 073). Of the eight DTF QTLs, three were associated with a moderate effect (>10 °Cd) and five were associated with a small effect (Table 2). The FD QTL was associated with a moderate effect (15.9 °Cd). Notably, none of the markers associated with DTF and FD were in linkage disequilibrium, and thus all nine

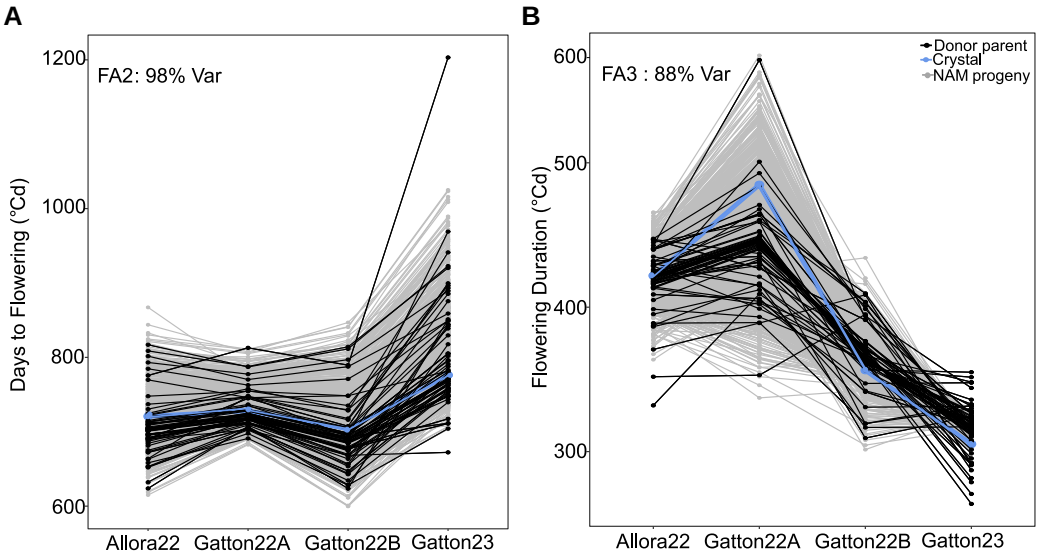

Fig. 2. Genotype by environment interactions in flowering time and duration across the four field trials for the parents and families of the mungbean nested association mapping (NAM) population. The population was developed from 30 diverse donor parents crossed to the reference parent Crystal, and details of the trials are given in Table 1. (A) Days to flowering and (B) flowering duration, both displayed as thermal time. The values are the best linear unbiased predictions (BLUPs) for each genotype calculated across all environments. Factor analytic (FA) models with either two or three factors (FA2 and FA3, respectively) were fitted to model genotype-by-environment interactions by capturing the genetic variance–covariance structure across trials. The percentages of total genetic variance explained by each model are indicated.

QTLs were deemed to be independent. Unexpectedly, early alleles at each of the DTF QTLs were contributed by donor parents that displayed both early and late phenotypes, and this was also the case for late alleles (Table 2). For example, for *QDTF2.1* a total of 15 parent genotypes contributed the allele associated with early flowering, including both the reference parent Crystal and the donor parent Putland, which display divergent phenotypes. The identified QTLs were subjected to preliminary bioinformatic analyses to explore potential gene candidates within the associated regions. First, BLAST searches against the *V. radiata* genome were performed, specifically comparing our QTL sequences with previously identified QTLs for DTF. Second, we conducted cross-species BLAST searches using the Phytozome database, comparing sequences with other legumes (cowpea, soybean, and common bean), and Arabidopsis. These initial analyses did not yield any promising candidates, but synteny analyses between mungbean and the legumes listed above using the LIS Genome Context Viewer did identify promising targets. Specifically, we found *CONSTANS-LIKE 1-like* (a zinc finger protein) and *ZGT* (circadian clock coupling factor) as potential candidates for *QDTF.2.1* and *QFD.4.1*, respectively.

The phenotypic effects associated with accumulation of early or late alleles at the DTF QTLs were determined by examining NAM progeny containing different numbers of the alleles (Fig. 5). These lines were examined to identify the compounding effects of the alleles within the population and their relationship with FD. A clear trend was observed in which the accumulation of early and late alleles resulted in earlier and later DTF, respectively. On average, DTF decreased by 83 °Cd (~5 d) with the accumulation of six early alleles compared to an increase by 123 °Cd (~8 d) with the accumulation of six late alleles. However, variation existed within each class, and the magnitude of change varied substantially with allele number among the genotypes. We also examined the FD of the same lines to investigate potential interactions between it and DTF. The trend line for FD showed an increase from one to two early alleles and then remained more or less steady (Fig. 5). On the other hand, the FD trendline had a less consistent relationship with the change in number of late alleles.

Discussion

Investigation of a diverse mungbean NAM population evaluated across multiple environments revealed distinct genetic architecture governing flowering time and flowering duration. Our analysis demonstrated that while time to flowering traits (DTFF, DTF, DT100) were highly correlated, they were independent of EF and FD (Supplementary Fig. S3). This presents opportunities for breeding programs aiming to optimise flowering for different production environments.

While DTFF and DTF are strongly correlated in mungbean (see also Hwang *et al.*, 2017), their relative utility for breeding applications requires careful consideration. Although DTFF might provide valuable insights into the initial floral response (Bhakta *et al.*, 2017; Aguilar-Benitez *et al.*, 2021; Seo *et al.*, 2023), field-based measurements are subject to environmental

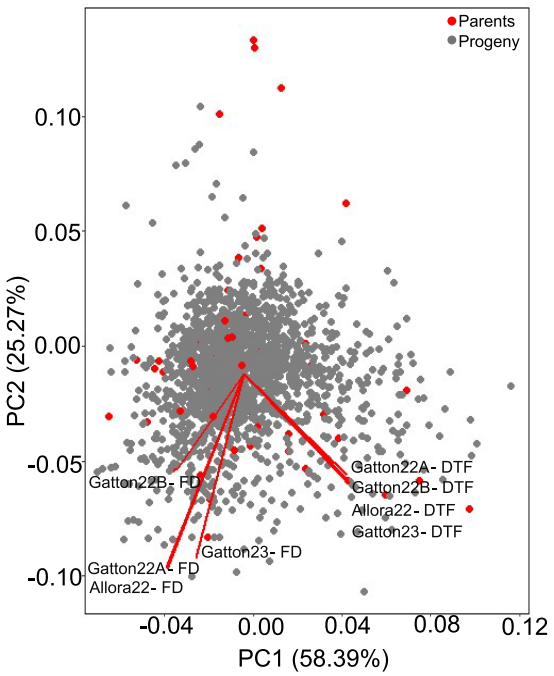

Fig. 3. Relationships between days to flowering (DTF) and flowering duration (FD) across the four field trials for the parents and families of the mungbean nested association mapping population. The population was developed from 30 diverse donor parents crossed to the reference parent Crystal, and details of the trials are given in Table 1. Principal component (PC) analysis was performed using best linear unbiased estimates (BLUEs) for each trait in each environment.

variability, plant competition effects, and the potential for genetic contamination. In contrast, DTF provides a more reliable flowering time criterion, as evidenced by generally higher heritability (Supplementary Table S3). The robust data we obtained for DTF provides sufficient resolution for developing mungbean varieties with targeted flowering times.

Research examining the relationship between DTF and FD remains limited in crop species, with studies in indeterminate crops primarily focused on associations between flowering time and maturity (Bernard, 1971; Cober *et al.*, 1996; Anbessa *et al.*, 2007; Watanabe *et al.*, 2009; Kong *et al.*, 2014; Miranda *et al.*, 2020). While physiological maturity, EF, and FD share phenological dependencies and might be associated, they encompass distinct biological processes as maturity reflects the culmination of pod development and seed filling, EF marks the termination of flower production, and FD specifically quantifies the temporal window of flower production. In this study, EF and FD in mungbean were associated (Supplementary Fig. S3); however their relationships with maturity were not investigated. It is likely that the relationships among maturity, EF, and FD will vary with both genotype and environment due to the influence of temperature and rainfall on pod development and the occurrence of multiple flowering flushes. FD represents a novel trait worthy of investigation regardless of its correlation with other traits as it captures the length of the flowering period and therefore influences stress adaptation, resource allocation patterns, and the flexibility of desiccation practices in farming systems.

The NAM population provides a rich source of diversity for flowering time

The NAM population, developed from genetically diverse donor parents (Noble *et al.*, 2017), displayed extensive variation in DTF across multiple environments (Fig. 1). The transgressive segregation observed in the NAM progeny suggests that the parents probably carried diverse allelic variation for DTF. While some families exhibited consistent early (AGG325975 and AGG 325954) or late DTF (AGG 325966, ACC68), most exhibited substantial within-family variation. This genetic diversity provides valuable breeding material for selecting lines with targeted early and late DTF phenotypes.

Our multi-environment trial (MET) analyses revealed low-to-moderate G×E interactions for DTF (Fig. 2A), with a high correlation of DTF at the population level across the different environments (Fig. 3). This stability probably reflects the similar geographic locations and sowing dates of the two field sites. Environmental variation in temperature and rainfall (Supplementary Fig. S1) contributed to the observed G×E for DTF. Temperature effects on mungbean flowering exhibit genotype-specific responses (Vas Aggarwal and Poehlman 1977), with flowering typically delayed by temperatures below 24 °C and accelerated above 30 °C (Summerfield and Lawn, 1987; Imrie and Lawn, 1990; Ellis *et al.*, 1994). Although the photoperiod was constant across the environments in this study, previous research has demonstrated widespread photoperiod sensitivity in mungbean germplasm, ranging from absolute day neutrality to short-day flowering responses (MacKenzie *et al.*, 1975; Lawn and Russell, 1978; Summerfield and Lawn, 1987; Imrie and Lawn, 1990).

Given the genotype-specific temperature sensitivities, the diverse germplasm evaluated in this study presents challenges in the application of thermal-time calculations. While cardinal temperatures were derived from previously published studies (Robertson *et al.*, 2002; Chauhan and Williams, 2018) and align with the recently updated APSIM mungbean model parameterised for Australian cultivars (Pasley *et al.*, 2023), further research is needed. Specifically, determination of cardinal temperatures and photoperiod responses across diverse mungbean germplasm (e.g. the NAM parents) would enhance the accuracy of flowering time predictions and modelling approaches.

Flowering duration: an underexplored trait with high environmental plasticity

Flowering duration in the NAM population exhibited substantial phenotypic plasticity (Fig. 1B), which has important implications for mungbean adaptation and yield development. While mean FD for each family ranged from 501 °Cd to

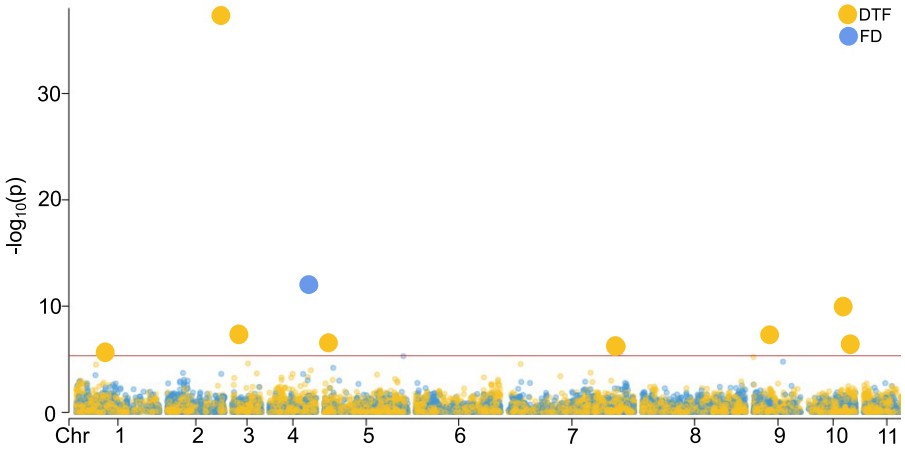

Fig. 4. Marker–trait associations for days to flowering (DTF) and flowering duration (FD) within the mungbean nested association mapping population. The red line represents the significance threshold, corresponding to an adjusted *P*-value of the false-discovery rate of 0.05. Quantitative trait loci above the threshold are represented by the larger dots.

Table 2. Summary of the significant quantitative trait loci associated with days to flowering and flowering duration

QTL	Trait	Chr no.	Position	SNP position	$-Log_{10}P$	Phenotypic variance explained (%)	Effect (°Cd)	Effect (days)	Alternate allele	Source line
QDTF.1.1	DTF	1	12 467 500	6 164 337	5.6	2.26	−5.1	−0.29	C	AGG 325971, AGG 325957, AGG 325972, AGG 325961, Berken, Black Berken, AGG 325966, AGG 327134, Crystal, AGG 325962, AGG 325973, AGG 325968, AGG 325976, Moong, Putland, Satin
QDTF.2.1	DTF	2	23 895 882	6 178 887	37.5	11.26	−18.7	−1.06	T	AGG 325975, Berken, Black Berken, Celera II AU, AGG 325966, Crystal, AGG 325962, AGG 325973, AGG 325968, AGG 325963, AGG 325976, Moong, AGG 325974, Putland, Satin
QDTF.3.1	DTF	3	3 442 005	6 180 070	7.3	6.11	−8.6	−0.49	G	AGG 325958, AGG 325959, AGG 325954, ACC35
QFD.4.1	FD	4	17 944 073	6 161 199	11.9	5.86	15.9	0.91	G	AGG 325961, AGG 325955, AGG 325959, AGG 325954, Crystal, AGG 325968, Satin
QDTF.5.1	DTF	5	2 454 971	6 176 790	6.5	3.61	8.3	0.47	G	AGG 325972, AGG 325961, AGG 327134, AGG 325964, ACC35
QDTF.7.1	DTF	7	48 218 974	6 161 696	6.1	1.41	−5.8	−0.33	A	AGG 325975, AGG 325958, AGG 325955, AGG 325959, AGG 325954, AGG 325964, AGG 325968, Putland, ACC35, ACC41, ACC68
QDTF.9.1	DTF	9	7 890 386	6 171 844	7.3	16.36	14.8	0.84	C	Putland
QDTF.10.1	DTF	10	15 771 485	6 161 248	9.9	13.91	−8.7	−0.49	A	AGG 325971, AGG 325957, AGG 325972, AGG 325955, AGG 325959
QDTF.10.2	DTF	10	18 743 804	19 053 807	6.3	2.71	−14.2	−0.81	A	AGG 325971, AGG 325975, Berken, Black Berken, Celera II AU, AGG 325966, AGG 325955, Crystal, AGG 325973, AGG 325968, AGG 325963, AGG 325976, Moong, AGG 325974, Putland, ACC35

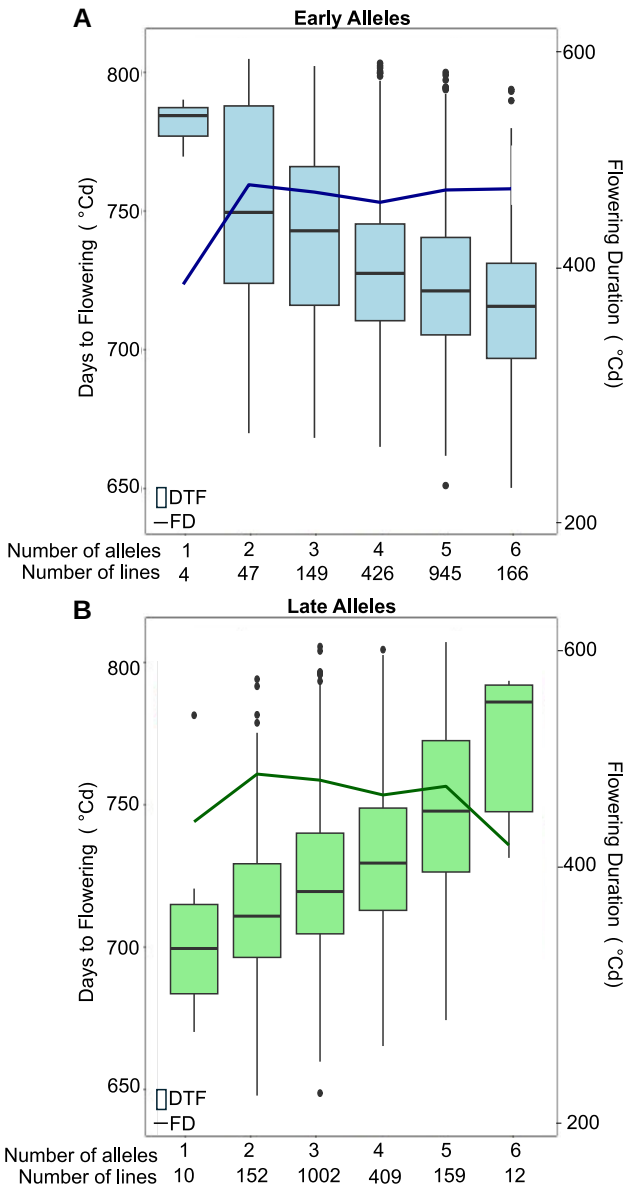

Fig. 5. The phenotypic effects associated with accumulation of alleles at quantitative trait loci for early and late days to flowering for progeny lines within the mungbean nested association mapping population. Effects of increasing numbers of (A) early alleles and (B) late alleles on days to flowering and flowering duration, both measured as thermal time. The numbers of lines in each case are indicated. Each boxplot shows the best linear unbiased estimates (BLUEs) for each genotype, calculated across the four environments. Boxplots display the interquartile range (box), median (horizontal line), and values within 1.5× the interquartile range (whiskers) to show the distributional spread of the data; points beyond are shown as outliers.

572° Cd, there was considerable within-family variation. For instance, within the Crystal/AGG 325954 family, FD ranged from 199–790 °Cd, representing a considerable difference in reproductive strategy. Short FD genotypes concentrate flower production within a narrow window, which is potentially beneficial in environments with terminal drought stress. In contrast, extended FD genotypes allow for multiple flowering flushes, providing resilience against intermittent stress but potentially complicating harvest.

The MET analyses revealed a higher degree of scale-type G×E for FD compared to DTF, suggesting greater environmental plasticity (Fig. 2). This was also reflected in a moderate heritability for FD (Supplementary Table S3) that might partly have been due to the derivation of FD, which would have compounded the errors associated with phenotyping the DTF and EF traits. Environmental effects on FD were evident through coordinated responses in reproductive development. For example, in the Gatton23 trial, higher temperatures and lower rainfall (Supplementary Fig S1) resulted in shorter FD, demonstrating how water availability and temperature modify vegetative growth and branching, and therefore floral production. These environmental responses might also align with other physiological mechanisms, such as onset of senescence in vegetative tissues, as water limitation and/or high temperatures can accelerate senescence, redirecting resource allocation from flower production to seed development in existing pods (Descamps *et al.*, 2021; Sharma *et al.*, 2016).

The regulation of FD involves complex whole-plant signalling networks that integrate environmental cues with developmental processes. This includes hormone signalling, sugar metabolism, and genetic networks that coordinate the cessation of flowering (González-Suárez *et al.*, 2020; Izawa, 2021). Further understanding of G×E interactions and the complex physiological mechanisms modulating FD is particularly important for mungbean breeding programs, as FD directly influences both stress adaptation and harvest timing.

Days to flowering and flowering duration are under independent genetic control

Our evaluation of the NAM population across four environments represents the largest study of flowering time traits in mungbean to date. The GWAS analyses revealed eight QTLs for DTF on Chrs 1–3, 5, 7, 9, and 10, and one for FD on Chr. 4 (Fig. 4). The absence of shared genetic controls between DTF and FD is consistent with the lack of phenotypic relationships observed across the different environments (Fig. 3). Despite the extensive size and diversity of the mapping population, only one QTL was detected for FD, probably reflecting the environmental plasticity of this trait and complex genetic control where many genes with small effects contribute to trait expression.

The QTL regions identified in this study do not overlap with reported loci in previous mungbean GWAS and mapping studies, and therefore represent novel genomic regions (Somta *et al.*, 2015; Ha *et al.*, 2021; Manjunatha *et al.*, 2023; Amkul *et al.*, 2024). These studies have reported flowering time QTLs across different chromosomal regions: Manjunatha *et al.* (2023) identified associations on Chr 2, Ha *et al.* (2021) detected significant QTLs on Chr. 2; and Amkul *et al.* (2024) reported on a QTL on

Chr. 4. In contrast, our study identified QTLs in distinct positions on CHRs 1–4 (FD), 7, 9, and 10 (Table 2). Of particular interest, the QTLs *DAYS-TO-FLOWERING3-1* (*DTF3-1*) and *DAYS-TO-FIRST-FLOWERING3-1* (*DFF3-1*), originally mapped to Chr. 3 in the draft mungbean genome assembly (VC1973A v1) but later reassigned to Chr. 4 in the updated and improved genome reference assembly (VC1973A v2) are located in a different region from our FD QTL on Chr. 4. Importantly, the candidate genes identified within our QTL regions have well-established connections to flowering regulation in other plant species. The *CONSTANS-LIKE 1-like* gene identified in *QDTF.2.1* belongs to a family of transcription factors known to regulate photoperiodic flowering in diverse species including other legumes, Arabidopsis, and lotus (Putterill *et al.*, 1995; Liang *et al.*, 2009; Wu *et al.*, 2014; Yang *et al.*, 2014) Similarly, *circadian clock coupling factor ZGT*, identified in *QFD.4.1*, was originally characterised as a light- and circadian clock-regulated gene in tobacco and exhibits rhythmic expression in maize, and it has been implicated in integrating circadian rhythms with flowering time regulation in model plants (Xu and Johnson, 2001; Jończyk *et al.*, 2011). These findings are consistent with conserved flowering regulation mechanisms observed in other legume crops and provide molecular support for the phenotypic associations observed in our study.

Flowering in plants involves complex genetic networks (Amasino and Michaels, 2010; Srikanth and Schmid, 2011; Bolouri Moghaddam and Van den Ende, 2013; Weller and Ortega, 2015). The identification of novel QTLs in this study probably reflects differences in genotypic diversity, the number of field trials conducted, environmental conditions, the genotyping platform, the quality of the reference sequence, and analytical approaches compared with other studies. For instance, most previous studies have evaluated diverse germplasm accessions originating from Asia (Manjunatha *et al.*, 2023; Seo *et al.*, 2023), while a recent study by Chiteri *et al.* (2024) included one Asian cultivar in common with this study (Moong) and eight (unnamed) Australian genotypes. The novel QTLs identified here, enabled by the genetic diversity of the NAM population, hold significant potential for breeding applications, particularly in Australia. While this study provides robust insights into mungbean flowering genetics through four field trials conducted over two years in Southeast Queensland, it should be noted that the expression of these flowering-related QTLs might vary in other mungbean-producing regions with different temperature regimes, and further validation across diverse environments would strengthen the broader applicability of our findings.

Opportunities for improving days to flowering and flowering duration

To explore the potential of manipulating DTF in mungbean through targeted breeding, we investigated the cumulative effect of early and late alleles at the QTLs mapped in this study (Table 2; Fig. 5). Both the QTL stacking scenarios demonstrated linear trends for DTF, with accumulation of additional alleles shifting flowering time in the desired direction (either earlier or later). This highlights the potential for crop improvement programs to take advantage of diverse germplasm and manipulate DTF to match a wide range of sowing dates and production environments to increase yield. While there are opportunities to alter DTF in both directions, our analysis indicated greater potential for delaying flowering (by 123 °Cd) compared to advancing it (by 83 °Cd), probably reflecting biological constraints related to DTF where a minimum accumulation of thermal units is necessary to develop an optimum canopy size and initiate flowering. In addition, as early flowering is a key target for production systems, the germplasm that we studied has most likely been subjected to historic selection pressure for this trait. This was reflected in the higher frequency of early alleles compared to late in the population, with 166 lines carrying six early alleles whilst only 12 lines carried six late alleles (Fig. 5). This was further skewed by the inclusion of Crystal as the reference parent of the NAM population, which had a relatively early flowering time of 734 °Cd.

Analysis of QTL effects on FD further emphasised the genetic independence of these traits (Fig. 5). There was an initial increase in FD with increasing numbers of early alleles before it became more-or-less stable, while late-flowering alleles displayed a less consistent pattern, suggesting potential compensatory effects. This distinction between DTF and FD suggests strategic manipulation of flowering time is possible without inadvertently affecting flowering duration.

Interestingly, the parental lines exhibited complex allelic combinations contributing to flowering time. Late-flowering parents such as Late-flowering parents such as Putland (820 °Cd) and AGG 3257134 (805 °Cd; Fig. 1) contributed alleles for both late and early flowering. Putland contributed alleles at both a late-flowering QTL (*QDTF.9.1*) and early-flowering QTLs (*QDTF.1.1*, *QDTF.2.1*, *QDTF.7.1*, *QDTF.10.2*), while AGG 327134 contributed early-flowering alleles at *QDTF.1.1* and *QDTF.5.1* (Table 2). Similarly, early-flowering parents Crystal (734 °Cd) and AGG 325968 (681 °Cd) contributed alleles for both early and late flowering. These mixed allelic contributions suggest that the specific combination of alleles might be important for trait expression, and therefore epistasis might influence the flowering time phenotype. Given the polygenic nature of DTF, a whole-genome approach such as genomic selection might prove more effective than marker-assisted selection for breeding applications (Voss-Fels *et al.*, 2019).

Selection for flowering traits could also consider the QTL for FD on Chr. 4 (*QFD.4.1*). Seven parental lines, including Crystal, carry this long FD allele. Extended FD might enable multiple flowering flushes, potentially increasing yield under favourable conditions; however, in mechanised harvest systems, extended FD could complicate harvest timing and lead to yield and quality penalties (Gentry, 2010). Further research is required to quantify the value of specific DTF and FD configurations, which will probably depend on production-system features such as water

availability (irrigated, rainfed), sowing time including heat-stress risk (increased temperatures during critical periods for yield development), harvest method (mechanised, hand harvest), and agronomic rotation requirements (e.g. double-cropping).

In conclusion, this study has established that DTF and FD in mungbean represent genetically independent traits with distinct environmental responses. The identification of their separate QTLs, coupled with the observed genotype × environment interactions, provides a foundation for understanding mungbean flowering behaviour. These findings open new avenues for breeding programs to develop mungbean varieties with optimised flowering patterns, potentially enhancing crop resilience and productivity across diverse agricultural environments.

Supplementary data

The following supplementary data are available at *JXB* online.

Fig. S1. Daily maximum and minimum temperatures and rainfall for the Gatton22, Allora22, and Gatton23 trials.

Fig. S2. Principal component analysis of the NAM population based on genetic markers.

Fig. S3. Principal component analysis showing the relationships among the five flowering traits for the NAM population evaluated across the four field trials.

Table S1. Composition of NAM families and subset details across the four environments.

Table S2. Goodness-of-fit for the multi-environment trial analysis models for days to flowering and flowering duration.

Table S3. The mean, minimum, and maximum values, and broad-sense heritability for days to flowering and flowering duration for all four field trials.

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Author contributions

CD: data capture, data curation;, formal analysis, investigation, writing—original draft; CD, SVH, LTH, and MRS: conceptualisation and design; SVH, HR, ED, and SA: advice on data analyses; LTH, MRS, KM, EAD, CB, MU, SVH, HR, ED, and SA writing—review and editing; LH, MRS, KM, EAD, CB, MU: supervision; CD, SVH, HR, LH, MRS, and TN: methodology; MRS: funding acquisition.

Conflict of interest

The authors declare that they have no conflicts of interest in relation to this work.

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Data availability

All primary data supporting the findings of this study are available via The University of Queensland eSpace repository at https://doi.org/10.48610/9cf4a4d.

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