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Title: QTL detection for flowering time in faba bean and the responses to ambient temperature and photoperiod

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Abstract

Faba bean (*Vicia faba* L.) is a grain legume primarily used for animal feed and human food grown in a range of environments, globally. Time of flowering in faba bean is critical for adaptation to specific environments and is controlled largely by factors such as ambient temperature and photoperiod. The aim of this study was to investigate the genetic control of flowering time and the responses of flowering time to ambient temperature and photoperiod in faba bean. A bi-parental recombinant inbred line (RIL) population (Icarus × Ascot) was evaluated over three years in field trials and three different controlled environments with varying temperatures and photoperiods. QTL analysis identified eight regions of co-localised QTLs associated with days to flower, thermal time to flower and node of first flower; on Chr-I.A/III/V, Chr-I.B.3, Chr-III.1, Chr-III.2, Chr-V.1 and Chr-V.2. Two of the detected regions are common with previously detected QTLs, up to two more are possibly common and the remaining four appear to be novel. For the first time, the associations of these QTLs with ambient temperature and photoperiod response were described. Candidate genes for some of the QTLs were identified using the associations with ambient temperature and photoperiod response together with knowledge extended from other legumes that have a syntenic relationship with faba bean.

Keywords

Days to flower; thermal time to flower; node of first flower; *Vicia faba* L.; genetic control of flowering; pulse; legume

Introduction

Faba bean (*Vicia faba* L.) is a cool-season grain legume used as a protein source in human food (primarily in developing countries of Northern Africa and Asia) and animal feed. Globally, faba bean is grown on over 2 million hectares, making it the fourth most important cool-season legume, behind chickpea (*Cicer arietinum* L.), pea (*Pisum sativum* L.) and lentil (*Lens culinaris* L.) (FAO 2016). In Australian cropping systems, faba bean crops provide a disease break for cereals, fix atmospheric nitrogen, which contributes to sustainable agriculture by reducing the use of fertilizer, and are a profitable grain crop in areas where varieties are well adapted to the environment. One of the most important aspects of plant adaptation is the time of flowering (Patrick and Stoddard 2010). Faba bean plants are particularly susceptible to stresses such as heat, frost and low moisture at the flowering stage (Smith 1982). Therefore, it is important that the time of flowering coincides with a period that minimises exposure to these stresses. The time of flowering is controlled by a plant's genotype and its interactions with the environment, most importantly photoperiod and temperature (both ambient and vernalising).

Flowering studies have used different methods of measurement to evaluate time to flowering, namely, days to flowering (DF), thermal time to flowering (TTF) and node of first flower (NF). Days to flowering is useful for comparing genotypes in one environment or across environments with different photoperiods, at a given temperature. Thermal time is calculated by the equation: $K = \sum_{i=1}^{n}(T_i - T_b)$, where, K is the thermal time (in °C.d); T_i is the mean temperature of the *i*th day; and T_b is the base temperature, below which no plant development occurs. TTF is most useful for comparing genotypes in environments with fluctuating temperatures and across environments with different temperatures. Number of nodes present is a quick visual indicator of the development stage of a plant and NF has regularly been used in flowering studies of *Arabidopsis thaliana* and pea to easily assess the development stage at which flowering occurs (Collins and Wilson 1974; Murfet 1985). Flowering at a higher node indicates onset of flowering at a later developmental stage.

Flowering time in faba bean is responsive to photoperiod and temperature and this response varies with genotype (Evans 1959; Ellis et al. 1988a, c; Ellis et al. 1990; McDonald et al. 1994). In general, the time to flowering decreases with a longer photoperiod as faba bean is a long-day plant (requires long days to flower), but day-neutral genotypes (that eventually

flower regardless of photoperiod) and photoperiod unresponsive genotypes (that flower in the same amount of thermal time regardless of photoperiod) have also been identified (Evans 1959; Ellis et al. 1990; McDonald et al. 1994). Variation in response to ambient temperature has not been as comprehensively studied as response to photoperiod. Ellis et al. (1990) reported that all faba bean genotypes require about the same amount of thermal time to flower (~1000 °C.d). McDonald et al. (1994), however, observed variation in TTF in a wider range of genotypes, with one line flowering in as little as 611 °C.d. Vernalisation has been shown to decrease the time to flowering and variation in response to vernalisation has been observed in faba bean (Ellis et al. 1988a; McDonald et al. 1994), however, the occurrence of a true vernalisation response has been disputed (Ellis et al. 1988b) and it is not covered in the present study - further mention of temperature refers to ambient temperature.

Understanding the genetic control of variation in flowering time of faba bean could be used to improve the efficiency of breeding programs. When introducing new traits (such as disease resistance) to a breeding program, new genetic material is often sourced from exotic regions and is crossed with locally adapted lines. Early stages of the breeding process focus on selection for traits of interest and are often undertaken in environments that do not represent field production conditions, while adaptation traits are often not observed until lines are tested in the field at a later stage. Marker-assisted selection (MAS) could aid earlier, more efficient, selection of lines that have retained adaptation traits and could also be used in targeted breeding for specific environments. In a broad sense, earlier flowering lines are suited to marginal cropping regions with short growing seasons, whereas later flowering lines are better suited to high rainfall environments with longer growing seasons.

While the genetic control of flowering time has been described in depth for model species such as *A. thaliana* and pea (Amasino and Michaels 2010; Weller and Ortega 2015), the same level of knowledge is lacking for faba bean. Recently, high density linkage maps for faba bean have been developed and linked markers have been identified for a number of traits (Torres et al. 2010; Cruz-Izquierdo et al. 2012; Satovic et al. 2013; Kaur et al. 2014; Sallam et al. 2016), but only two studies have detected quantitative trait loci (QTLs) for flowering time in faba bean. Cruz-Izquierdo et al. (2012) detected five QTLs for days to flower in a Vf6 × Vf27 RIL population. Two QTLs were located on chromosome I.B (refined to being chromosome IV by Satovic et al. (2013)) and one on each of chromosomes I.A (refined to chromosome I (Satovic et al. 2013)), III and V. These QTLs were all detected in field studies in 2007, but only the QTLs on chromosomes V and I were detected again in 2008. Sallam et al. (2016) detected nine QTLs for days to flower in the Göttingen Winter Bean Population

(GWBP) grown in the field in one season (2012-13). One QTL was detected on LG02, one on LG03, two on LG04, four on LG05 and one that was unlinked to any groups. It was not reported in either study whether any of the QTLs were associated with response to photoperiod or temperature.

A strong conservation of flowering genes has been observed between *A. thaliana*; and legumes such as pea, *Medicago truncatula*, chickpea, soybean (*Glycine max* L.) and *Lotus japonicus* (Hecht et al. 2005; Weller and Ortega 2015). Faba bean has a high level of macrosynteny with these legumes (Rispail et al. 2010; Cruz-Izquierdo et al. 2012; Kaur et al. 2014; Khazaei et al. 2014), so this synteny could be used to extend knowledge to faba bean, help to compare genomic regions across different populations that use different markers and even help identify candidate genes underlying flowering QTLs detected in faba bean.

The aim of this study was to investigate the genetic control of flowering time and the responses of flowering time to ambient temperature and photoperiod in faba bean. To achieve the aim, this study utilised the Icarus × Ascot recombinant inbred line (RIL) population (Kaur 2014) to detect QTLs for flowering time in the field and controlled environment conditions of differing ambient temperature and photoperiod.

Materials and Methods

Plant materials

Seed for the QTL mapping experiments was obtained from an existing RIL population generated by crossing faba bean cultivars Icarus and Ascot (Kaur et al. 2014). This population was chosen because of the difference in time to flowering of the two parents (where Icarus is late and Ascot early) and subsequent segregation of flowering traits among the population. F_6 derived F_7 seed was used to phenotype the population in the controlled environment experiment and F_6 derived F_8 seed was used to phenotype the population in the field experiment, due to seed availability.

Evaluation of the Icarus × Ascot RIL population in controlled environments

The two parents and 87 RILs were grown in a Dunnair air conditioned plant growth room (Dunnair (Aust.) PTY LTD) with 400W high pressure sodium lamps. Plants were grown under three treatments involving different photoperiods and temperatures (Table 1) to evaluate flowering differences between long days (LD) and short days (SD) and high temperatures (HT)

and low temperatures (LT). Each treatment was a randomised complete block design with three replicates per RIL and two plants per replicate. There were six replicates of each parent with two plants per replicate. Seeds were sown in 0.55 L punnets filled with bark mix potting soil and placed in trays (12 punnets per tray) on benches in the growth room. Plants were watered regularly, monitored every two to three days and scored for date of emergence, date of first open flower and NF. Days to flowering for each plant were recorded as days from emergence to first open flower. Plants that did not flower by the end of the experiment were given the number of days from emergence to the last day of scoring, plus an additional 14 d as a value for analysis. Thermal time to flowering for each plant was calculated using the equation: $K = \sum_{i=1}^{n}(T_i - T_b)$, where, K is the thermal time (in °C.d); T_i is the mean temperature of the *i*th day, and T_b is the base temperature, below which no plant development occurs. For the purposes of this experiment a base temperature of 0 °C was used, in accordance with a similar study by Pierre et al. (2008) to detect QTLs for flowering in *M. truncatula*. The rationale for using 0 °C was that the temperature in the controlled environment was maintained in a range where plant development could occur and because the data were to be used for QTL identification rather than predicting flowering times in other environments. The average DF, TTF and NF of each replicate were adjusted for spatial variation using the Asreml-R package (Gilmour et al. 2009) in the R statistical environment and the best linear unbiased estimators (BLUEs) were used for QTL analysis.

To evaluate photoperiod response (PR), the difference in time to flowering (DF and NF) between the long day, high temperature treatment and the short day, high temperature treatment for each of the lines was used, following the conventions previously described in QTL detection of photoperiod response studies of wheat (*Triticum aestivum* L.) by Sourdille et al. (2000) and barley (*Hordeum vulgare* L.) by Ren et al. (2012). The same principle was used to evaluate ambient temperature response (TR), where the difference in time to flowering (DF, TTF and NF) between the long day, high temperature treatment and the long day, low temperature treatment was used. Thermal time to flowering was only used in QTL detection for TR and not for the individual treatments or for PR because the direct relationship of TTF to the constant temperature of these treatments meant that it did not add any more information than DF.

Table 1 Treatment conditions for the evaluation of the Icarus × Ascot RIL population in controlled environments.

Treatment	Photoperiod	Temperature

1 (LD HT)	18 h	22 °C (±2 °C)
2 (LD LT)	18 h	11 °C (±2 °C)
3 (SD HT)	13 h	22 °C (±2 °C)

LD HT Long day, high temperature; *LD LT* Long day, low temperature; *SD HT* Short day, high temperature

Field evaluation of the Icarus × Ascot RIL population

The two parents and 92 RILs were hand sown in a randomised complete block design in the field over three years (19th May 2011, 30th May 2012 and 20th May 2014) at Turretfield Research Centre, South Australia (-34.5390° S 138.8439° E). Each plot consisted of 18 plants sown over two rows with 25 cm between rows and 25 cm between plants along each row. There were either two (2011 and 2012) or three (2014) replicates per RIL and 10 (2011 and 2012) or 15 (2014) replicates per parent (randomly allocated). Fertiliser was applied at sowing and fungicide was not applied as the plots were also being scored for disease resistance. As a result, ascochyta blight was present, which could have an effect on flowering time. From the first sign of flowering, plots were scored weekly for the number of plants with open flowers and the time (in days) from sowing to 50% flowering (DF) for each plot was calculated by regression analysis. Daily mean temperatures for Turretfield, obtained from the Australian Bureau of Meteorology (Bureau of Meteorology 2014), were used to determine the TTF with a T_b of 0 °C. The DF and TTF of each replicate were adjusted for spatial variation using the Asreml-R package (Gilmour et al. 2009) in the R statistical environment and the BLUEs were used for QTL analysis.

QTL analysis

The genetic linkage map produced by Kaur et al. (2014) from the Icarus × Ascot mapping population was used in this study. This linkage map contained 522 markers, spanning 1216.8 cM over 12 linkage groups (LGs) with an average distance of 2.3 cM between markers. Composite interval mapping (CIM) was used for QTL detection within Windows QTL Cartographer v2.5 (Wang et al. 2012), with the minimum log-of-odds (LOD) for confirming QTL presence determined using 1000 permutations.

The QTL containing regions from this study were compared to the QTLs detected in the Göttingen Winter Bean Population (GWBP) (Sallam et al. 2016) using *M. truncatula* as the

genetic linking bridge, via the process of anchoring the genetic linkage maps from Kaur et al. (2014) and Sallam et al. (2016) to *M. truncatula* using markers for orthologous genes.

Results

Flowering traits in the Icarus × Ascot RIL population

Under field evaluation, the parents of the bi-parental RIL population (Icarus and Ascot) differed by an average of 13 d and 164°C.d in time to flowering (Table 2), with Icarus flowering later. In 2014, flowering of both parents occurred later as compared to 2011 and 2012.

Table 2 Phenotyping of flowering traits of the parents and RILs of the Icarus × Ascot population grown in the field at Turretfield in 2011, 2012 and 2014.

Trait	Year	Icarus	Ascot	RIL Population	
				Mean ± SE	Range
DF (days)	2011	101	88	91 ± 1.0	85 – 98
	2012	102	90	94 ± 1.3	88 – 101
	2014	110	95	104 ± 0.6	100 – 110
	Average	*104*	*91*	*96*	
TTF (°C.d)	2011	1124	967	1014 ± 12.5	931 – 1100
	2012	1069	917	964 ± 14.7	901 – 1057
	2014	1219	1048	1152 ± 7.2	1102 – 1222
	Average	*1137*	*978*	*1043*	

DF days to flowering; *TTF* thermal time to flowering; *SE* standard error (calculated as the average SE of the RILs)

Ascot flowered earlier than Icarus, when expressed in both DF and TTF, in all controlled environment treatments (Table 3). For NF however, Ascot flowered at a lower node than Icarus in the long day, low temperature treatment; the same node in the long day, high temperature treatment; and a higher node in the short day, high temperature treatment. For each treatment and trait in the controlled environments, the RIL population mean was within the parental means, however, the range observed for the progeny extended beyond the parental means (transgressive segregation) in most cases. Icarus was more responsive than Ascot to both ambient temperature and photoperiod when measured in DF or TTF, however, Ascot was more responsive to temperature and photoperiod when measured in NF. Flowering occurred

on a lower node in the long day, low temperature treatment compared to the long day, high temperature treatment, as indicated by the negative values for NF in the TR measure.

Table 3 Phenotyping of flowering traits of the parents and RILs of the Icarus × Ascot population grown in three controlled environments and measures of their ambient temperature response (TR) and photoperiod response (PR).

Trait	Treatment	Icarus	Ascot	RIL Population	
				Mean ± SE	Range
DF (days)	LD HT	30.6	26.4	28.2 ± 0.9	24.6 – 33.1
	LD LT	83.3	58.7	66.7 ± 2.3	55.5 – 88.5
	SD HT	118.7	69.6	81.7 ± 12.3	43.8 – 154.0
TTF (°C.d)	LD HT	673.0	581.0	620.4 ± 20.7	541.2 – 728.2
	LD LT	916.0	646.0	733.7 ± 29.9	610.5 – 973.5
	SD HT	2611.0	1531.0	1797.4 ± 270.2	963.6 – 3388.0
NF	LD HT	12.0	11.8	12.0 ± 0.4	10.3 – 14.1
	LD LT	9.8	6.3	7.9 ± 0.5	6.1 – 10.5
	SD HT	22.8	28.7	26.7 ± 2.9	18.5 – 48.1
DF (days)	TR[a]	52.6	32.3	38.6 ± 2.9	28.7 – 58.2
	PR[b]	88.1	43.1	53.5 ± 12.3	17.0 – 125.2
TTF (°C.d)	TR	242.0	64	114.2 ± 36.4	-14.4 – 307.6
	PR	1938.2	948.2	1177.0 ± 271.0	374.0 – 2754.4
NF	TR	-2.2	-5.4	-4.0 ± 0.6	-7.6 – -1.0
	PR	10.8	17.0	14.7 ± 3.0	6.7 – 36.2

RIL recombinant inbred line; *DF* days to flowering; *TTF* thermal time to flowering; *NF* node of first flower

[a] TR is the difference between long day, high temperature (LD HT) measure and long day, low temperature (LD LT) measure. [TR = (LD LT) – (LD HT)]

[b] PR is the difference between long day, high temperature (LD HT) measure and short day, high temperature (SD HT) measure. [PR = (SD HT) – (LD HT)]

Regions of co-localised QTL for flowering time

In total, 35 QTLs were detected for flowering time in the Icarus × Ascot RIL population (Tables 4, 5 and 6). Due to the interconnected relationship of the traits measured, these QTLs were

clustered in eight regions across six of the linkage groups previously described by Kaur et al. (2014) (Figure 1); one region on each of Chr-I.A/III/V, Chr-I.B.3, Chr-III.1, Chr-III.2 and Chr-V.2; and three regions on Chr-V.1 (referred to as the first, second and third region of Chr-V.1). The QTLs detected within each of these regions overlapped at the 2-LOD, if not the 1-LOD interval (Figure 1).

QTL detection from field evaluation

Four regions of co-localised QTLs were detected for DF in three years of field evaluation, with each individual QTL explaining 7.0% – 38.3% of the phenotypic variation (Table 4 and Figure 1). Two of the QTL regions, one on Chr-I.B.3 (containing *qDF-20011-1*, *qDF-2012-1* and *qDF-2014-1*) and one on the first region of Chr-V.1 (containing *qDF-2011-2*, *qDF-2012-2* and *qDF-2014-3*) were observed in all years for DF. A QTL in the second region of Chr-V.1 (*qDF-2011-3*) was only detected in 2011, and a QTL on Chr-III.2 (*qDF-2014-2*) was only detected in 2014.

QTLs were detected on the same four chromosomal regions for TTF in the field as those for DF (Table 4 and Figure 1). *qTTF-2011-1*, *qTTF-2012-1* and *qTTF-2014-1* were detected on Chr-I.B.3. *qTTF-2011-2*, *qTTF-2012-2* and *qTTF-2014-3* were detected on the first region of Chr-V.1. *qTTF-2012-3* was only observed in 2012 and was detected on the second region of Chr-V.1. *qTTF-2014-2* was only observed in 2014 and was detected on Chr-III.2.

Table 4 Faba bean flowering time QTLs detected over three seasons in the field at Turretfield, SA.

Trait	QTL	Linkage Group	1-LOD interval (cM)	Marker with greatest association	LOD threshold	Maximum LOD	Vp (%)	Add
DF	*qDF-2011-1*	Chr-I.B.3	3.7 - 6.7	SNP_50002190	3.2	5.62	12.2	-1.17
	qDF-2012-1	Chr-I.B.3	3.7 - 12.3	SNP_50002190	3.0	7.34	18.5	-1.08
	qDF-2014-1	Chr-I.B.3	0.4 - 14.3	SNP_50002190	3.1	4.86	11.8	-0.77
	qDF-2014-2	Chr-III.2	23.3 - 36.7	SNP_50000993	3.1	4.69	10.9	-0.74
	qDF-2011-2	Chr-V.1	19.6 - 22.4	SNP_50001709	3.2	11.19	38.3	-2.25
	qDF-2012-2	Chr-V.1	16.4 - 25.7	SNP_50001709	3.0	9.50	29.4	-1.48
	qDF-2014-3	Chr-V.1	5.5 - 18.6	SNP_50000729	3.1	7.25	19.6	-1.00
	qDF-2011-3	Chr-V.1	60.6 - 62.6	SNP_50001804	3.2	3.58	7.0	0.96
TTF	*qTTF-2011-1*	Chr-I.B.3	3.7 - 6.7	SNP_50002190	3.4	5.72	12.0	-15.04
	qTTF-2012-1	Chr-I.B.3	0.4 - 14.3	SNP_50002190	3.0	5.71	14.9	-11.76
	qTTF-2014-1	Chr-I.B.3	0.4 - 12.3	SNP_50002190	3.0	5.23	12.5	-9.03
	qTTF-2014-2	Chr-III.2	24.3 - 37.7	SNP_50000993	3.0	4.37	11.1	-8.47
	qTTF-2011-2	Chr-V.1	19.6 - 22.4	SNP_50001709	3.4	11.13	37.2	-28.70
	qTTF-2012-2	Chr-V.1	16.4 - 19.6	SNP_50000729	3.0	9.31	29.2	-17.54
	qTTF-2014-3	Chr-V.1	5.5 - 18.6	SNP_50000729	3.0	6.30	16.4	-10.29
	qTTF-2012-3	Chr-V.1	58.4 - 62.6	SNP_50001804	3.0	3.26	7.5	8.83

QTL Quantitative trait loci; *LOD* Log of odds; *Vp* Phenotypic variance; *Add* Additive effect (a negative value means the Ascot allele causes earlier flowering); *DF* Days to flower; *TTF* Thermal time to flower

QTL detection from controlled environment evaluation

Five QTL regions were detected for DF in the controlled environment treatment (Table 5 and Figure 1). *qDF-SDHT-1* was detected on Chr-I.B.3; *qDF-LDHT* on Chr-III.1; *qDF-SDHT-2* on Chr-III.2; *qDF-LDLT* and *qDF-SDHT-3* on the first region of Chr-V.1; and *qDF-SDHT-4* on the third region of Chr-V.1.

Five QTL regions were also detected in the controlled environment treatments for NF, although not all the same regions as for DF (Table 5 and Figure 1). *qNF-LDHT-1* was detected on Chr-I.A/III/V; *qNF-LDHT-2* on Chr-III.1; *qNF-SDHT-1* on Chr-III.2; *qNF-LDLT* on the first region of Chr-V.1; and *qNF-SDHT-2* on Chr-V.2.

Table 5 Faba bean flowering time QTLs detected in three controlled environment treatments of varying temperature and photoperiod.

Trait	Treatment	QTL	Linkage group	1-LOD interval (cM)	Marker with greatest association	LOD threshold	Maximum LOD	Vp (%)	Add
DF	LD HT	*qDF-LDHT*	Chr-III.1	153.7-166.6	SNP_50000468	3.0	4.49	14.1	-0.59
	LD LT	*qDF-LDLT*	Chr-V.1	12.1-29.0	SNP_50001709	3.0	9.45	31.9	-3.37
	SD HT	*qDF-SDHT-1*	Chr-I.B.3	17.1-23.4	SNP_50002190	3.1	4.97	16.5	-9.58
		qDF-SDHT-2	Chr-III.2	33.7-43.7	SNP_50000993	3.1	3.25	9.5	7.20
		qDF-SDHT-3	Chr-V.1	9.1-19.6	SNP_50001769	3.1	3.04ns	8.7	-6.74
		qDF-SDHT-4	Chr-V.1	100.6-109.8	SNP_50001325	3.1	3.04ns	8.6	6.84
NF	LD HT	*qNF-LDHT-1*	Chr-I.A/III/V	0-1.0	SNP_50002450	3.1	3.64	11.1	-0.28
		qNF-LDHT-2	Chr-III.1	153.7-161.8	SNP_50000468	3.1	6.98	24.1	-0.40
	LD LT	*qNF-LDLT*	Chr-V.1	15.4-17.4	SNP_50000729	3.3	13.79	46.1	-0.84
	SD HT	*qNF-SDHT-1*	Chr-III.2	35.7-43.7	SNP_50000993	2.9	3.29	11.6	1.90
		qNF-SDHT-2	Chr-V.2	46.1-47.1	SNP_50000225	2.9	2.98	10.0	-1.79

QTL Quantitative trait loci; *LOD* Log of odds; *Vp* Phenotypic variance; *Add* Additive effect (a negative value means the Ascot allele causes earlier flowering); *DF* Days to flower; *NF* Node of first flower; *LD HT* Long day, high temperature; *LD LT* Long day, low temperature; *SD HT* Short day, high temperature; *ns* Not significant

Temperature and photoperiod response QTLs

Two QTL regions were detected for temperature response (Table 6 and Figure 1). *qDF-TR*, *qTTF-TR* and *qNF-TR-2* were detected on the first region of Chr-V.1 for temperature response of DF, TTF and NF, respectively; and *qNF-TR-1* was detected (for temperature response of NF) on Chr-I.A/III/V. Four QTL regions were detected for photoperiod response of days to flower (Table 6 and Figure 1). *qDF-PR-1* was detected on Chr-I.B.3, *qDF-PR-2* on Chr-III.2, *qDF-PR-3* on the first region of Chr-V.1, and *qDF-PR-4* on the third region of Chr-V.1.

Table 6 Faba bean flowering time QTLs detected for ambient temperature response (TR) and photoperiod response (PR) in controlled environment treatments.

Trait	Response	QTL	LG	1-LOD interval (cM)	Marker with greatest association	LOD threshold	Maximum LOD	Vp (%)	Add
DF	TR	*qDF-TR*	Chr-V.1	11.1-28.0	SNP_50001706	3.1	8.88	33.1	-3.50
	PR	*qDF-PR-1*	Chr-I.B.3	0.4-6.7	SNP_50002190	3.1	5.04	16.5	-9.40
		qDF-PR-2	Chr-III.2	33.7-43.7	SNP_50000993	3.1	3.49	10.0	7.31
		qDF-PR-3	Chr-V.1	9.1-19.6	SNP_50001769	3.1	3.24	9.3	-6.89
		qDF-PR-4	Chr-V.1	101.6-108.9	SNP_50001325	3.1	3.39	9.5	7.05
TTF	TR	*qTTF-TR*	Chr-V.1	10.1-28.0	SNP_50001769	3.1	8.39	31.0	-38.90
NF	TR	*qNF-TR-1*	Chr-I.A/III/V	0-11.7	SNP_50002450	3.0	3.50	7.5	0.41
		qNF-TR-2	Chr-V.1	9.1-17.4	SNP_50000729	3.0	11.15	33.2	-0.88
	PR	None detected							

QTL Quantitative trait loci; *LOD* Log of odds; *Vp* Phenotypic variance; *Add* Additive effect (a negative value means the Ascot allele causes earlier flowering); *DF* Days to flower; *TTF* Thermal time to flower; *NF* Node of first flower

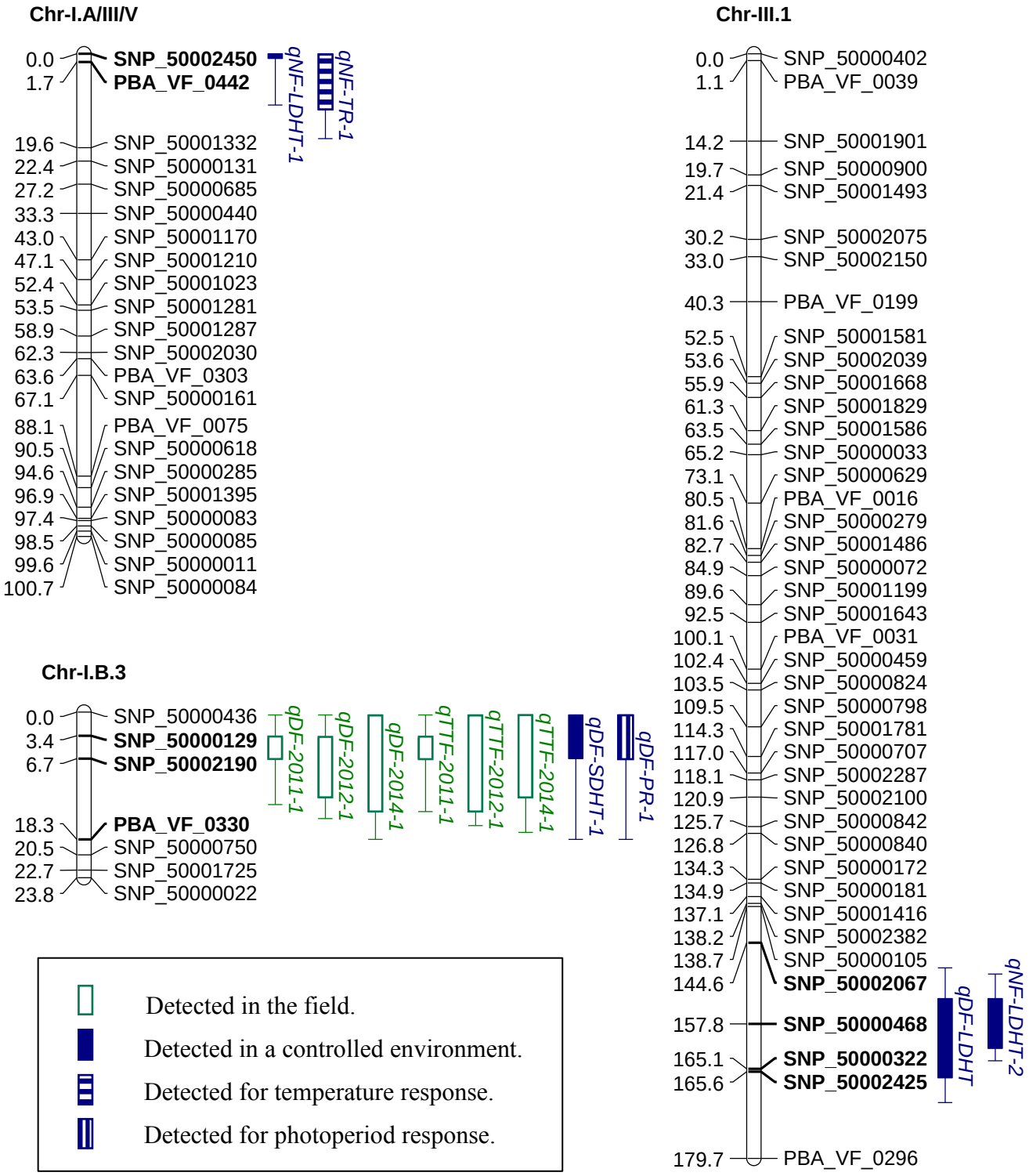

Fig. 1 Genetic linkage map constructed from the Icarus × Ascot faba bean RIL population, showing the QTL positions for flowering time. Marker loci are on the right of the linkage groups (markers in bold are within the QTL interval) and map distances (in cM) are on the left. QTLs are indicated by thick bars for the 1-LOD interval, simple bars for the 2-LOD interval and are positioned relative to the chromosome diagram with their names to the right (linkage groups without QTLs not shown).

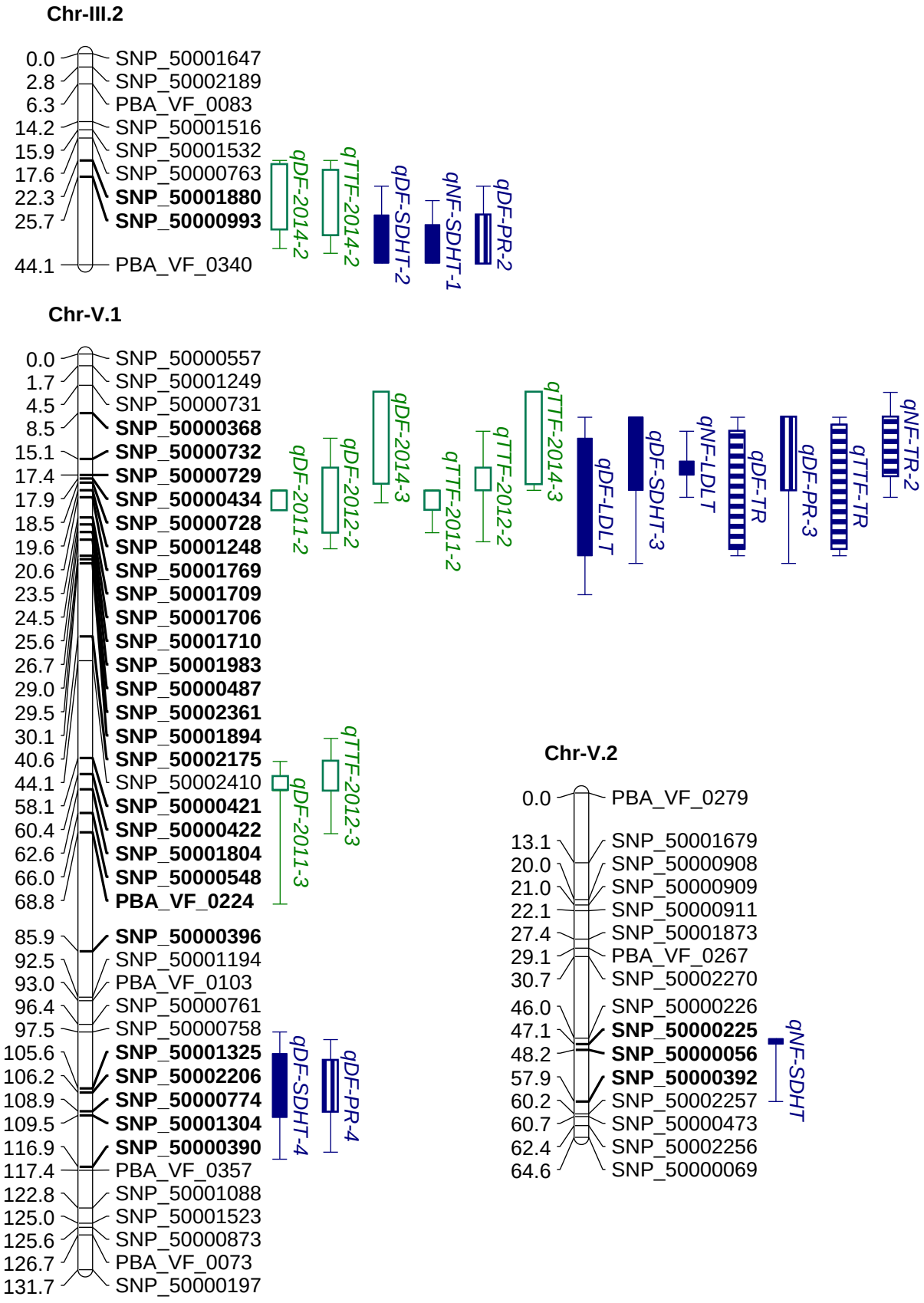
Chr-III.2
0.0 SNP_50001647
2.8 SNP_50002189
6.3 PBA_VF_0083
14.2 SNP_50001516
15.9 SNP_50001532
17.6 SNP_50000763
22.3 SNP_50001880
25.7 SNP_50000993
44.1 PBA_VF_0340
qDF-2014-2
qTTF-2014-2
qDF-SDHT-2
qNF-SDHT-1
qDF-PR-2
Chr-V.1
0.0 SNP_50000557
1.7 SNP_50001249
4.5 SNP_50000731
8.5 SNP_50000368
15.1 SNP_50000732
17.4 SNP_50000729
17.9 SNP_50000434
18.5 SNP_50000728
19.6 SNP_50001248
20.6 SNP_50001769
23.5 SNP_50001709
24.5 SNP_50001706
25.6 SNP_50001710
26.7 SNP_50001983
29.0 SNP_50000487
29.5 SNP_50002361
30.1 SNP_50001894
40.6 SNP_50002175
44.1 SNP_50002410
58.1 SNP_50000421
60.4 SNP_50000422
62.6 SNP_50001804
66.0 SNP_50000548
68.8 PBA_VF_0224
85.9 SNP_50000396
92.5 SNP_50001194
93.0 PBA_VF_0103
96.4 SNP_50000761
97.5 SNP_50000758
105.6 SNP_50001325
106.2 SNP_50002206
108.9 SNP_50000774
109.5 SNP_50001304
116.9 SNP_50000390
117.4 PBA_VF_0357
122.8 SNP_50001088
125.0 SNP_50001523
125.6 SNP_50000873
126.7 PBA_VF_0073
131.7 SNP_50000197
qDF-2011-2
qDF-2012-2
qDF-2014-3
qTTF-2011-2
qTTF-2012-2
qTTF-2014-3
qDF-LDLT
qDF-SDHT-3
qNF-LDLT
qDF-TR
qDF-PR-3
qTTF-TR
qNF-TR-2
qDF-2011-3
qTTF-2012-3
qDF-SDHT-4
qDF-PR-4
Chr-V.2
0.0 PBA_VF_0279
13.1 SNP_50001679
20.0 SNP_50000908
21.0 SNP_50000909
22.1 SNP_50000911
27.4 SNP_50001873
29.1 PBA_VF_0267
30.7 SNP_50002270
46.0 SNP_50000226
47.1 SNP_50000225
48.2 SNP_50000056
57.9 SNP_50000392
60.2 SNP_50002257
60.7 SNP_50000473
62.4 SNP_50002256
64.6 SNP_50000069
qNF-SDHT

Fig. 1 (Cont.)

Two of the QTL containing regions identified in this study were found to be in common locations of the genome as flowering time QTLs detected by Sallam et al. (2016) (Figure 2). The flanking markers of the QTL identified on Chr-III.1 in this study were close to the location of a marker (Vf_Mt1 g056180) associated with days to flower on LG03 in the GWBP. Furthermore, the flanking markers of the first QTL region from this study on Chr-V.1 were found to be co-localised as represented by two of the markers (Vf_Mt g084010 and Vf_Mt g090890) of LG05 associated with days to flower QTL in the GWBP.

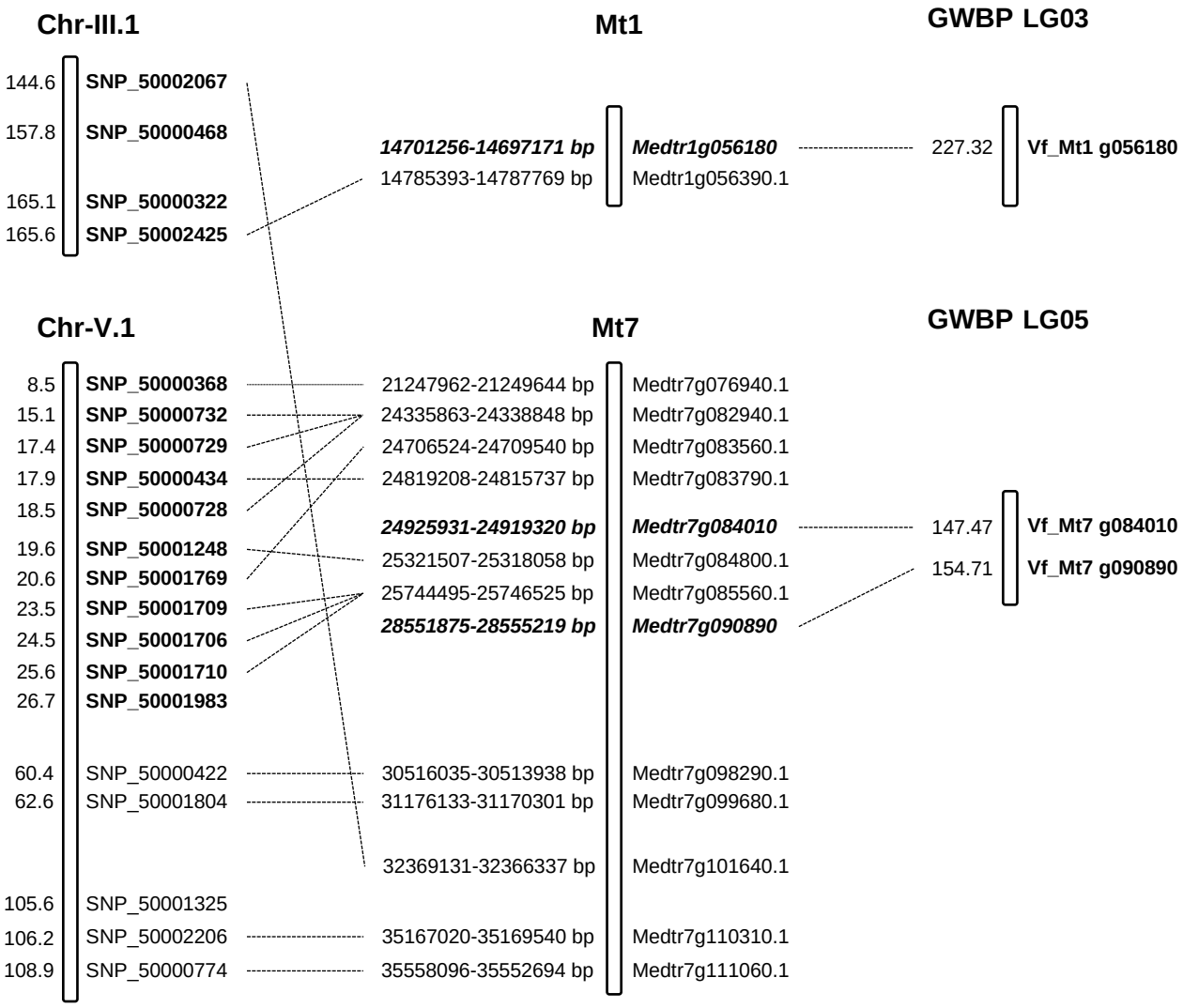

Fig. 2 Synteny comparison between flowering time QTLs from this study with Göttingen Winter Bean Population (GWBP) using *M. truncatula* (v 3.5) as the genetic bridge. The QTL regions identified from this study are represented on the left side of the figure. QTLs that are located in the same chromosome location as found by the GWBP study are shown on the right side of the figure. The marker loci flanking the QTL regions and their distances (in cM)

from both the studies are represented according to their characterised linkage groups. For each faba bean linkage group, their respective position in the *M. truncatula* genome (centre of the image) is as indicated in bp with their respective gene.

Discussion

The main purpose of this study was to investigate the genetic control of flowering time and the responses of flowering time to ambient temperature and photoperiod in faba bean. Evaluation of flowering time in the Icarus × Ascot RIL population grown in different controlled environments and in the field resulted in the detection of eight regions of the faba bean genome on six linkage groups (one on each of Chr-I.A, Chr-I.B, Chr-III.1, Chr-III.2 and Chr-V.2; and three on Chr-V.1) containing QTLs that explain the phenotypic variation in flowering time, and for the first time in faba bean, associations of flowering QTLs with ambient temperature response and photoperiod response were determined.

The most important region of the faba bean genome controlling time to flower in the Icarus × Ascot RIL population is the first region on Chr-V.1. This region explains between 19.6 and 38.3% of the phenotypic variation in DF at Turretfield, with the Icarus allele causing later flowering and was shown to be associated with both photoperiod and temperature response. Cruz-Izquierdo et al. (2012) observed a QTL on chromosome V that explained most of the phenotypic variation in flowering time of faba bean. The QTL region in this study was confirmed to be consistent with a QTL detected by Sallam et al. (2016) and is likely to be consistent with that of Cruz-Izquierdo et al. (2012), however, a lack of common markers prevents confirmation of this. Chr-V.1 is syntenic with *M. truncatula* chromosome 7 (Kaur et al. 2014), which also contains a region that explains most of the phenotypic variation in flowering time (Pierre et al. 2008). The genetic basis of this variation in *M. truncatula* has been suggested as being a cluster of three flowering locus T-like genes (*MtFTa1, MtFTa2* and *MtFTc*) (Laurie et al. 2011; Pierre et al. 2008). FT genes are central to the induction of flowering of legumes and the expression of FT genes has been shown to be influenced by vernalisation and photoperiod in *M. truncatula* (Laurie et al. 2011), and photoperiod in pea and soybean (Hecht et al. 2011; Kong et al. 2010), while ambient temperature influences FT expression in *A. thaliana* (Capovilla et al. 2014). The importance and environmental responsiveness of these FT-like genes make them good candidates, however, other flowering genes that exist within the same QTL interval of *M. truncatula* cannot be ruled out, namely

MtCO, *MtFD* and *MtPKS* (Pierre et al. 2008). This region in faba bean would need to be investigated further, with fine mapping, in order to understand better the genetic basis of flowering time control at this location and to develop diagnostic markers that could be used for MAS.

The QTL region on Chr-I.B.3 was also important in controlling flowering time, explaining between 11.8 and 18.5% of the phenotypic variation in DF at Turretfield. The QTL was associated with photoperiod response and the Icarus allele caused later flowering. This QTL region may be common with one of the two QTLs observed on chromosome I.B by Cruz-Izquierdo et al. (2012). However, a lack of common markers between the linkage maps means this commonality cannot be confirmed. Chr-I.B.3 is syntenic with *M. truncatula* chromosome 8 and chickpea chromosome 8 (Kaur et al. 2014). Three flowering QTLs have been detected on *M. truncatula* chromosome 8 (Pierre et al. 2008) and two on chickpea chromosome 8 (Rehman et al. 2011; Varshney et al. 2014). Sixteen flowering related genes have been identified on *M. truncatula* chromosome 8 by Kim et al. (2013), including the photoperiod pathway genes: Casein kinase alpha 1 (CKA1); Casein kinase II, alpha chain 2 (CKA2); and Chryptochrome-interacting basic-helix-loop-helix 1 (CIB1). These are the most plausible candidate genes, given the association the QTL has with photoperiod response, however, fine mapping would have to be carried out in order to narrow the possibilities. Like the first region on Chr-V.1, this QTL region is a good candidate for further study and MAS.

Two other QTL regions of interest are the ones located on linkage groups Chr-III.1 (which wasn't associated with response to temperature or photoperiod, with the Icarus allele causing later flowering) and Chr-III.2 (which was associated with photoperiod response, with the Ascot allele causing later flowering). The region on Chr-III.1 is in the same location as a QTL identified by Sallam et al. (2016), which corresponds to a position on *M. truncatula* chromosome 1. It is, however, unclear at this stage whether the region on Chr-III.1 is common with the QTL identified on chromosome III by Cruz-Izquierdo et al. (2012). As well as being syntenic with *M. truncatula* chromosome 1, Chr-III.1 is syntenic with chickpea chromosome 4 and pea chromosome 2 (Hecht et al. 2005; Kaur et al. 2014). *M.* truncatula chromosome 1 and chickpea chromosome 4 contain the flowering genes: GI (orthologous to the GIGANTEA gene in *A. thaliana*), Phytochrome A (PHYA), Cryptochrome 2 (CRY2) and Flowering Locus D (FLD) (Deokar et al. 2015; Hecht et al. 2005; Kim et al. 2013; Weller and Ortega 2015). Additionally, pea chromosome 2 contains Photoperiod (PPD) and Late Flowering (LF) (Liew et al. 2014; Weller et al. 1997; Weller and Ortega 2015). From a

phenotypic basis, the most plausible candidate genes for the QTL detected on Chr-III.1 are FLD and LF because they act independently of the environment. Chr-III.2 is largely syntenic with *M. truncatula* chromosome 6 and chickpea chromosome 8 (Kaur et al. 2014). There are no known flowering related QTLs or genes on *M. truncatula* chromosome 6 and, although there are two flowering QTLs on chickpea chromosome 8 (Rehman et al. 2011; Varshney et al. 2014), there are no known flowering genes on this linkage group either. These QTL regions (on Chr-III.1 and Chr-III.2) are mostly of interest from a functional genomics viewpoint, as they had little to no importance in the control of flowering time in the field in this experiment.

This study contributes to the efforts of identifying the genes controlling flowering time in faba bean by confirming the importance of previously detected QTLs, by detecting novel QTLs and by linking QTLs to photoperiod and/or ambient temperature response. Candidate genes have been identified for the major flowering QTLs in faba bean, based on orthologous genes located in syntenic regions (or at least comparative linkage groups) of other legumes and their known associations with photoperiod and temperature. The associations of QTLs in this study with photoperiod and temperature response could be used to narrow the candidate genes in other legumes with syntenic flowering QTLs, now for confirmed syntenic QTLs, and in future for those not yet confirmed. Assuming the mechanism behind the responses are consistent across the legume species.

To further this study, the role of vernalisation in flowering time needs to be evaluated and the genetic basis of any such variation explored. Fine mapping of the important regions for flowering time and validation of the QTLs in multiple environments and genetic backgrounds would need to be carried out to develop reliable markers for the different flowering time traits and to more accurately determine the phenotypic variance attributable to each QTL, along with the interactions with varied, industry significant, environments. From there, marker-assisted selection could be adopted for more efficient and effective selection of flowering traits in a breeding program, leading to the faster development of varieties adapted to environments with specific requirements for time of flowering.

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