Can allele variation at *PPD1* and *VRN1* gene loci predict flowering time in wheat under controlled conditions?

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Abstract
Ensuring wheat flowers at an optimal time minimises combined damage from frost, drought and heat stresses and maximises yield. Predicting flowering time of diverse cultivars across varying sowing dates and environments is crucial for attaining optimal yields, however there is no reliable way to do this for newly released cultivars. *Photoperiod1* and *Vernalisation1* genes are the major drivers of development in wheat and molecular markers have been developed to identify alleles at these loci. Allelic information has been used to parameterise phenology models, but it remains uncertain how much variation in flowering time can be explained by alleles of these major genes. We grew 13 elite commercial wheat cultivars, selected for diverse phenology and thus allelic variation of the major genes, and 13 near-isogenic lines (NILs) with matching alleles to quantify how much of the variation in time to flowering could be explained by the major genes. The experiment was conducted in four controlled environments (17 or 8-hour photoperiod, ± vernalisation) at a constant temperature of 22°C. NILs explained 97% of variation in time to flowering of elite cultivars under long days without vernalisation, 62% under short days without vernalisation, and less under short and long days with vernalisation (51% and 47%). Long days significantly accelerated time to flowering in all genotypes, while vernalisation hastened flowering in 17 genotypes. Results indicate allelic information of the major genes is not enough to parameterise an accurate model to predict flowering time under field conditions. Further investigation into genetic drivers of development and their interactions with environment are required before a genetically derived parameter estimate model can accurately simulate flowering time.

Key Words
Triticum aestivum, anthesis, thermal time, optimal flowering period.

Introduction

In Australia wheat (*Triticum aestivum*) is traditionally sown following autumn rainfall throughout a diverse range of climates and environments. However, an increase in farm size and cropping programs (Fletcher et al. 2016), combined with a decrease in autumn and in-crop rainfall (Cai et al. 2012), means growers require a suite of cultivars with diverse phenology to suit a wider range of sowing times (Hunt et al. 2019). In turn, crops must flower during an optimal window to ensure yields are maximised (Flohr et al. 2017). The optimal flowering period is defined as the time of year most suitable for wheat growth and when combined damage from drought, heat and frost are minimised (Flohr et al. 2017). Accurately predicting flowering time for the full range of available cultivars from a broad range of sowing times across diverse environments is crucial to ensure best yields are achieved; however, growers currently have no accurate way of achieving this. When new cultivars are released, recommended sowing times are based on data from limited field trials and are specific to the few environments the trials are grown, leaving growers without accurate sowing times for their environments for two or more years.

Time to flowering is determined by the genotype, environment and time of sowing. Genotypic factors are sensitivity to photoperiod, determined by alleles at the homoeologous *Photoperiod1* (*PPD1*) gene loci of *Ppd-D1* and *Ppd-B1* on chromosomes 2D and 2B, respectively (Beales et al. 2007; Díaz et al. 2012); vernalisation requirement, determined by alleles at the homoeologous *Vernalisation1* (*VRN1*) loci of *Vrn-A1*, *Vrn-D1* and *Vrn-B1* on chromosomes 5A, 5D and 5B, respectively (Yan et al. 2004; Fu et al. 2005); and to a lesser extent by various earliness *per se* (*EPS*) genes. Environmental factors that drive these genetic responses are temperature, through accumulated thermal and vernal time, and photoperiod.

Molecular markers for different alleles of *Ppd-B1* (Díaz et al. 2012), *Ppd-D1* (Beales et al. 2007), *Vrn-A1* (Yan et al. 2004), *Vrn-B1* and *Vrn-D1* (Fu et al. 2005) have been identified and allelic information of these major genes has been proposed as a way to accurately predict flowering time (Eagles et al. 2009; Cane et al.

2013). Some studies (Eagles et al. 2010; Cane et al. 2013) and models (Brown et al. 2013; Zheng et al. 2013) have attempted this, but it remains unclear as to how much of the variation in time to flowering can be explained by alleles of these five genes. This study aimed to evaluate the extent that allele variation of the five major *PPD1* and *VRN1* genes present in a common background could predict thermal time to flowering (TTF) in a diverse range of elite commercial wheat cultivars under limiting and/or saturating photoperiod (8 or 17-hour) and vernalisation (±) treatments.

Methods

A full description of methods can be found in Bloomfield et al. (2018), a brief description follows here.

Experiments were conducted in glasshouses and controlled environment rooms at the AgriBio Centre for Agribioscience, Bundoora, Victoria (37.7°S, 145.0°E).

Cultivar selection

Thirteen elite commercial cultivars were selected based on their popularity among growers and diverse phenology observed in the field, and in turn variation in alleles of *Ppd-B1*, *Ppd-D1*, *Vrn-A1*, *Vrn-B1* and *Vrn-D1* (Table 1). Thirteen near-isogenic lines (NILs), developed by introgressing target alleles of *PPD1* and *VRN1* into a common genetic background (cv. Sunstate, BC4 generation; Steinfort et al. 2017), were selected to match the multi-locus genotype (MLG) of each elite cultivar (Table 1).

Table 1. Alleles of the *PPD1* and *VRN1* genes of 13 near-isogenic lines and 13 elite commercial cultivars. Alleles determining response to vernalisation (*VRN1*; *a* = insensitive, *v* = sensitive) and photoperiod (*Ppd-B1*; *a* = insensitive, *b* = sensitive; *Ppd-D1*; *a* and *d* = insensitive, *b* and *c* = sensitive). Genotypes with sensitive alleles at all three *VRN1* loci are classified as winter habit type (W), and genotypes with insensitive allele/s at one or more of the *VRN1* loci are classified as spring habit type (S).

Pair number	Near-isogenic line	Multi-locus genotype					Elite cultivar	Habit type
		PPD1		*VRN1*				
		Ppd-B1	*Ppd-D1*	*Vrn-A1*	*Vrn-B1*	*Vrn-D1*		
1	CSIROW077	*a*	*a*	*a*	*a*	*a*	Young	S
2	CSIROW105	*a*	*a*	*a*	*a*	*v*	Axe	S
3	CSIROW029	*a*	*a*	*a*	*v*	*v*	Gauntlet	S
4	Sunstate A	*a*	*a*	*v*	*a*	*a*	Spitfire	S
5	CSIROW005	*a*	*a*	*v*	*a*	*v*	Mace	S
6	CSIROW007	*a*	*a*	*v*	*v*	*v*	Longsword	W
7	CSIROW018	*a*	*b*	*v*	*a*	*a*	Ellison	S
8	CSIROW087	*a*	*c*	*a*	*v*	*v*	Sunbri	S
9	CSIROW002	*a*	*c*	*v*	*a*	*a*	Trojan	S
10	CSIROW021	*a*	*d*	*v*	*v*	*v*	Rosella	W
11	Sunstate B	*b*	*a*	*v*	*a*	*a*	Scout	S
12	CSIROW003	*b*	*a*	*v*	*v*	*a*	EGA Gregory	S
13	CSIROW073	*b*	*a*	*v*	*v*	*v*	EGA Wedgetail	W

Experimental design

One experiment was conducted in four pseudoreplicated controlled environments consisting of a randomised complete block design with four replicates. Factors were environment (photoperiod, 8 or 17 hours; vernalisation, ±) and genotype. The four environments were long day, no vernalisation (LN); long day, vernalisation (LV); short day, no vernalisation (SN); and short day, vernalisation (SV). Seeds for LV and SV were vernalised in specimen jars in complete darkness at 4.7°C for 8 weeks prior to sowing by method of partial imbibition with 40% water by weight. Three seeds genotype^{-1} replicate^{-1} (two seeds for NIL CSIROW105 due to limited seed) were sown in 200 mm pots containing a standard potting mix with slow release fertiliser. Plants were watered regularly to avoid water stress. All environments were set at constant temperature of 22°C and monitored at 30 min intervals with Tinytag Plus 2 data loggers (Gemini Data Loggers, Chichester, UK) in radiation shields. Plants were monitored daily to record date of emergence and flowering (first visible anthers on one head).

Statistical analyses

Accumulated thermal time (degree days; °Cd) was calculated from logged temperature data. Thermal time from emergence to flowering was analysed using two-way analysis of variance in the GENSTAT 18 software (VSN International Ltd, Hemel Hempstead, UK) assuming a split-plot design with randomised blocks with environment as whole-plot and genotype as subplot.

Results and Discussion

Thermal time to flowering under controlled conditions

NILs explained a large amount of variation in TTF in the LN environment (97%), a moderate amount in SN (62%), and less in SV and LV (51% and 47%) (Figure 1). Long photoperiod (LN) significantly reduced TTF in all genotypes, vernalisation (SV) in 17 genotypes, and the combination of the two (LV) in all genotypes (see Table 5 in Bloomfield et al. 2018). Winter and spring types were clearly separated in both LN and LV, except for the winter types CSIROW007 (NIL in pair 6) and Rosella (elite in pair 10) in LV, which flowered in a similar time as the mid-slow spring types. NILs predicted less variation when only looking at spring types (29-60%). Spring types remained tightly clustered under long days (Figure 1 *a*, *b*) due to having little to no requirement for vernalisation, but they were more spread under short days (Figure 1 *c*, *d*) and photoperiod-sensitive types (presence of sensitive allele at *Ppd-D1* locus) were not clearly distinguished from the winter types. Although only significant in ~65% of comparisons, NILs flowered in less thermal time than their elite pair, except pair 2 under SN and SV, pair 5 under SN and pair 10 under LV (results not shown). Of these 4 exceptions, only elites in pair 5 under SN and pair 10 under LV were significantly faster than the matched NIL. Interestingly, there were significant reductions in TTF under long days in spring types with insensitive alleles at the *PPD1* loci, which has been seen in other controlled environment experiments (Steinfort et al. 2017). Vernalisation only reduced TTF more than photoperiod in the fast winter NIL CSIROW007, and combined effects of vernalisation and photoperiod only accelerated TTF in winter pair 6 and winter elite Rosella, whilst photoperiod-sensitive and partially vernalisation-sensitive spring elite Sunbri had an increased TTF. This raises the question as to whether vernalisation was fully satisfied in all genotypes, and we assume the method of partially imbibing seeds was not fully effective.

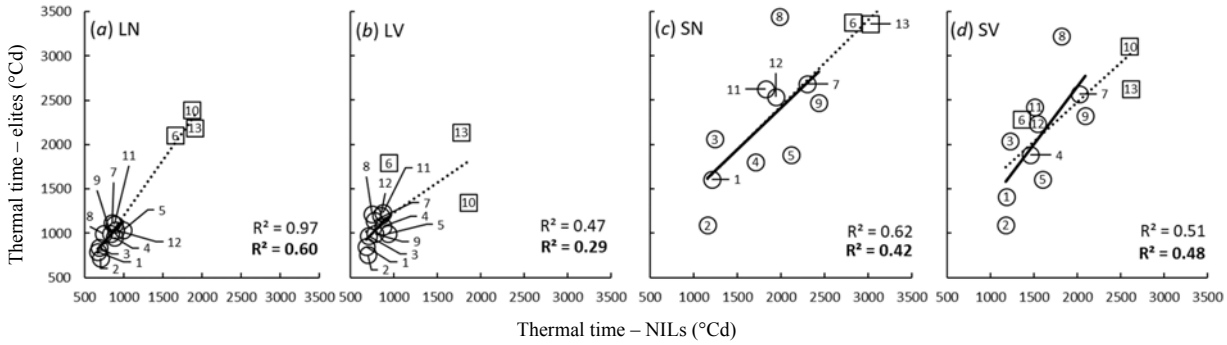

Figure 1. Mean thermal time to flowering for 13 near-isogenic lines (NILs) and 13 elite commercial cultivars with matched multi-locus genotypes (MLGs) for five major *PPD1* and *VRN1* development genes under (*a*) long day, no vernalisation (LN); (*b*) long day, vernalisation (LV); (*c*) short day, no vernalisation (SN); and (*d*) short day, vernalisation (SV). Spring genotypes (○, —, R^2); winter genotypes (□); whole series (●●●, R^2). Numbers indicate MLG-matched NIL and elite pairs (see Table 1). No data point for matched pair 10 in (*c*) SN as no replicates of Rosella reached heading or flowering.

Thermal time to flowering in the field

These results suggest that TTF cannot be accurately predicted by MLG of the five major genes under field conditions. This agrees with findings from Eagles et al. (2010) (with MLG consisting of *Ppd-D1*, *Vrn-A1*, *Vrn-B1* and *Vrn-D1*) and Cane et al. (2013) (all five *PPD1* and *VRN1* genes), who reported 45% and 53% of variation in flowering time, respectively, from multiple sowing dates (late May to mid-June) in 124 field trials across seven sites and 24 years. The stronger relationship between matched pairs without vernalisation indicates that predicting flowering time in the field based on the MLG of the five *PPD1* and *VRN1* loci would be more accurate from early times of sowing (March to early April) when vernal time is not being rapidly accumulated. Zheng et al. (2013) could account for 96% of variation in flowering time in a genetically derived parameter estimate (GDPE) model using *Ppd-D1*, *Vrn-A1*, *Vrn-B1* and *Vrn-D1*, but required cultivar-specific parameters to achieve this. Further parameters using *EPS* genes that act

independently of the *PPD1* and *VRN1* genes could be incorporated in such a GDPE model as future research untangles and quantifies their effects on TTF.

Conclusion

NILs explained a moderate amount of the variation in TTF of elite cultivars with matched *PPD1* and *VRN1* alleles under controlled conditions. Long photoperiods significantly decreased thermal time to flowering while vernalisation had less effect, likely attributed to insufficient/mixed vernalisation by method of partial imbibition. Further quantification of the more minor genetic drivers of flowering time, such as *EPS* genes, are required before a GDPE model can accurately predict flowering time across diverse environments from many times of sowing for new cultivars as they are released.

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References

Beales J, Turner A, Griffiths S, Snape JW and Laurie DA (2007). A Pseudo-Response Regulator is misexpressed in the photoperiod insensitive *Ppd-D1a* mutant of wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics 115, 721-733.

Bloomfield MT, Hunt JR, Trevaskis B, Ramm K and Hyles J (2018). Ability of alleles of *PPD1* and *VRN1* genes to predict flowering time in diverse Australian wheat (*Triticum aestivum*) cultivars in controlled environments. Crop and Pasture Science 69, 1061-1075.

Brown HE, Jamieson PD, Brooking IR, Moot DJ and Huth NI (2013). Integration of molecular and physiological models to explain time of anthesis in wheat. Annals of Botany 112, 1683-1703.

Cai W, Cowan T and Thatcher M (2012). Rainfall reductions over Southern Hemisphere semi-arid regions: the role of subtropical dry zone expansion. Scientific reports 2, 702.

Cane K, Eagles HA, Laurie DA, Trevaskis B, Vallance N, Eastwood RF, Gororo NN, Kuchel H and Martin PJ (2013). *Ppd-B1* and *Ppd-D1* and their effects in southern Australian wheat. Crop and Pasture Science 64, 100-114.

Díaz A, Zikhali M, Turner AS, Isaac P and Laurie DA (2012). Copy number variation affecting the *Photoperiod-B1* and *Vernalization-A1* genes is associated with altered flowering time in wheat (*Triticum aestivum*). PLOS ONE 7, e33234.

Eagles HA, Cane K, Kuchel H, Hollamby GJ, Vallance N, Eastwood RF, Gororo NN and Martin PJ (2010). Photoperiod and vernalization gene effects in southern Australian wheat. Crop and Pasture Science 61, 721-730.

Eagles HA, Cane K and Vallance N (2009). The flow of alleles of important photoperiod and vernalisation genes through Australian wheat. Crop and Pasture Science 60, 646-657.

Fletcher A, Lawes R and Weeks C (2016). Crop area increases drive earlier and dry sowing in Western Australia: implications for farming systems. Crop and Pasture Science 67, 1268-1280.

Flohr BM, Hunt JR, Kirkegaard JA and Evans JR (2017). Water and temperature stress define the optimal flowering period for wheat in south-eastern Australia. Field Crops Research 209, 108-119.

Fu D, Szűcs P, Yan L, Helguera M, Skinner JS, von Zitzewitz J, Hayes PM and Dubcovsky J (2005). Large deletions within the first intron in *VRN-1* are associated with spring growth habit in barley and wheat. Molecular Genetics and Genomics 273, 54-65.

Hunt JR, Lilley JM, Trevaskis B, Flohr BM, Peake A, Fletcher A, Zwart AB, Gobbett D and Kirkegaard JA (2019). Early sowing systems can boost Australian wheat yields despite recent climate change. Nature Climate Change 9, 244-247.

Steinfort U, Trevaskis B, Fukai S, Bell KL and Dreccer MF (2017). Vernalisation and photoperiod sensitivity in wheat: Impact on canopy development and yield components. Field Crops Research 201, 108-121.

Yan L, Helguera M, Kato K, Fukuyama S, Sherman J and Dubcovsky J (2004). Allelic variation at the *VRN-1* promoter region in polyploid wheat. Theoretical and Applied Genetics 109, 1677-1686.

Zheng B, Biddulph B, Li D, Kuchel H and Chapman S (2013). Quantification of the effects of *VRN1* and *Ppd-D1* to predict spring wheat (*Triticum aestivum*) heading time across diverse environments. Journal of Experimental Botany 64, 3747-3761.