Linkages between wheat development and growth: it's in the genes

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Abstract
There has been some anecdotal evidence that suggests rate of development and the accumulation of biomass may be associated, with reports early-maturing cultivars grow faster than later-maturing cultivars. Rate of development is largely determined by responses to photoperiod and vernalisation controlled by the *PPD1* and *VRN1* genes. *PPD1* and *VRN1*, are now known to be regulatory genes, influencing traits in addition to phenology, though effects on crop growth have not previously been reported in field crops. The effects of *Ppd-B1, Ppd-D1, Vrn-A1, Vrn-B1* and *Vrn-D1* genes on anthesis date and crop growth rate were measured on forty-seven lines from a doubled-haploid population derived from the cross between cv. Janz and cv. Diamondbird. The lines were grown in replicated field experiments at Wagga Wagga and Yanco in 2010. These genes accounted for 75% of the genetic variance in anthesis date. Presence of the winter allele at either *Vrn-A1* or *Vrn-B1* delayed anthesis, whilst genotypes with winter alleles at all three *VRN1* loci (*Vrn-A1v + Vrn-B1v +Vrn-D1v*) caused the largest delay in anthesis date and were classified as winter types. Presence of the winter allele *Vrn-B1v* consistently reduced biomass and slowed crop growth rate compared to the spring allele *Vrn-B1a.* However, the suppression of growth reported for *Vrn-B1v* was independent of alleles at other *VRN1* loci, suggesting the effect of *VRN1* genes on plant growth is a pleiotropic effect of these genes, rather than a direct association with development *per se*. The faster growth associated with *Vrn-B1a* may explain the yield advantage of cultivars with this allele in some environments reported in a previous study.

Keywords
Phenology, vernalisation, photoperiod, epistasis.

Introduction
An observed link between early growth and rate of development of wheat has been reported in previous studies. One possible explanation for this association is the influence of the *VRN1* and *PPD1* genes which regulate phasic development through responses to vernalisation and photoperiod. Genotypes responsive to vernalisation or photoperiod require a period of cold temperatures or long days respectively to progress from vegetative to reproductive development. The *VRN1* genes have been described as MADS box transcription genes that act as regulatory genes, and influence plant traits other than development (Trevaskis 2010). They have been associated with freezing tolerance and higher grain yield (Eagles et al. 2014). The *PPD1* genes are pseudo-response regulator genes (Diaz et al. 2012) and less is known about the effect of *PPD1* genes on traits other than phasic development. The objective of this research was to determine the effect of *VRN1* and *PPD1* genes on anthesis date and crop growth rate and how these genes may influence the association between early growth and development in wheat.

Methods
Genotypes
Progeny of a doubled haploid population from a cross between cv. Janz and cv. Diamondbird (NSW DPI, Wagga Wagga) were screened for maturity and plant height. Forty-seven doubled haploid (DH) lines with a range in anthesis date were selected for this study. Double-dwarf and tall lines were excluded. The selected doubled-haploid lines were classified for *PPD1* and *VRN1* genes based on methods described in Cane et al. (2013). The lines showed allelic variation for *Ppd-B1, Vrn-A1* and *Vrn-B1*. The alleles present were labelled and described according to Cane et al. (2013), and summarised in Table 1.

Table 1. Designated nomenclature and description of alleles present in the experimental genotypes.

Designated nomenclature	Allele description
Ppd-B1b	One-copy allele, responsive to photoperiod
Ppd-B1c	Four-copy allele, elevated expression regardless of photoperiod
Ppd-D1a	Elevated expression regardless of photoperiod
Vrn-A1a	Common spring allele, generally unresponsive to vernalisation
Vrn-A1v	Winter allele, vernalisation responsive
Vrn-B1a	Spring allele, does not require vernalisation though may be responsive
Vrn-B1v	Winter allele, vernalisation responsive
Vrn-D1v	Winter allele, vernalisation responsive

Field experiments

Two field experiments were conducted at Yanco (34.62°S, 146.43°E, 352.9 mm AAR) and Wagga Wagga (35.05°S, 147.35°E, 550 mm AAR) in southern NSW. The Yanco experiment was sown on May 12 2010, following 100 mm pre-irrigation and was an incomplete-randomised-block design (treatments: genotypes) with 3 replications. The Wagga Wagga experiment was sown on May 18 2010 as a randomised-complete-block design (treatments: genotypes) with 3 replications. Both experiments were sown using a cone seeder with 18 cm row spacing, in plots measuring 20 m x 1.44 m. Plant densities of 100 plants m^{-2} at the Yanco site and 150 plants m^{-2} at the Wagga Wagga site were targeted by adjusting sowing rates according to the seed weight of the different genotypes. 90 kg ha^{-1} of monoammonium phosphate (10% N, 21.8% P) fertiliser was sown with the seed. Two destructive dry matter harvests were taken in each plot when approximately 50% of the genotypes had reached DC14 and DC31, which corresponded to the stages just before floral initiation and at early stem elongation. At each harvest time, four samples of 0.5 m x 2 rows were taken in each plot and plant number recorded. Samples were dried at 70°C for 48 h and weighed. Crop growth rate (CGR) was calculated by dividing the difference between two harvests by time (days). Anthesis date (DC65) was recorded for each plot.

Statistical analyses

Statistical methods were based on mixed-model methods used by Cane et al. (2013). A basic statistical analysis was conducted first using lines, from which variance components were estimated using the REML directive in Genstat. For this analysis, lines, experiments, lines x experiments and replications within experiments were included in the random part of the mixed model. When included, *PPD1* and *VRN1* genes were fixed in the mixed model and lines included as random effects in the model. There were large epistatic effects of the photoperiod and vernalisation genes on anthesis date, thus the effect of combinations of alleles rather than the effect of individual genes are presented. Paired comparisons were conducted to enable a more accurate assessment of the effects of *Ppd-B1*, *Vrn-A1* and *Vrn-B1* alleles present in the doubled-haploid population.

Results

The variation in *PPD1* and *VRN1* genes in the DH population accounted for 75% of the genotypic variance in anthesis date. There were significant epistatic effects of *PPD1* and *VRN1* genes on anthesis date (Table 2). Pairwise comparisons of *Ppd-B1b* and *Ppd-B1c* showed the *Ppd-B1c* allele, derived from Janz; reduced time to anthesis in all comparisons, with the greatest difference being 3.2 days in the winter genotypes (*bavvv* versus *cavvv*). The effect of the winter alleles of *Vrn-A1* and *Vrn-B1* (*Vrn-A1v* and *Vrn-B1v*) was to delay anthesis compared with the spring *Vrn-A1a* and *Vrn-B1a* alleles. The greatest effect was when either *Vrn-A1a* or *Vrn-B1a* was substituted into a winter genotype (*Vrn-A1v* + *Vrn-B1v* + *Vrn-D1v*), resulting in a delay of up to 6.3 days *(baavv* versus *bavvv*).

The variation in *PPD1* and *VRN1* genes accounted for 42% of the genotypic variance in crop growth rate in the DH population. When alleles of *Vrn-A1* and *Vrn-B1* were compared across alleles of *Ppd-B1* (indicated by the # in the genotype code, Table 3), the effects of *Vrn-A1* on crop growth rate were not significant. However, genotypes with *Vrn-B1a* had faster crop growth rates than genotypes with the winter *Vrn-B1v* allele, with many of the differences either statistically significant or approaching significance (Table 3). Pairwise comparisons of *Vrn-B1a* and *Vrn-B1v* showed that *Vrn-B1v* reduced crop growth rate compared to *Vrn-B1a*, however only the comparison between *caaav* and *caavv* was statistically significant (Table 4). In

that comparison, *Vrn-B1v* slowed crop growth rate by 6.3 kg ha^{-1}day^{-1} compared with *Vrn-B1a*, which equates to a 19% reduction.

Table 2. Pairwise comparisons of mean days to anthesis for *Ppd-B1, Vrn-A1* and *Vrn-B1* alleles in the DH population across two sites (Yanco and Wagga Wagga) in 2010. *Differences significantly greater than zero (P≤0.05).

Genotype comparison	Sowing to anthesis (days)	Difference	s.e.d
Ppd-B1			
baaav	146.7		
caaav	144.1	**-2.6***	1.0
baavv	148.5		
caavv	146.9	-1.6	1.3
bavav	148.6		
cavav	147.0	-1.6	0.9
bavvv	154.8		
cavvv	151.6	**-3.2***	1.1
Vrn-A1			
ba***a***av	146.7		
ba***v***av	148.6	**1.9***	0.8
ca***a***av	144.1		
ca***v***av	147.0	**2.9***	1.0
ba***a***vv	148.5		
ba***v***vv	154.8	**6.3***	1.2
ca***a***vv	146.9		
ca***v***vv	151.6	**4.7***	1.3
Vrn-B1			
baa***a***v	146.7		
baa***v***v	148.5	1.8	1.0
caa***a***v	144.1		
caa***v***v	146.9	**2.8***	1.3
bav***a***v	148.5		
bav***v***v	154.8	**6.2***	1.0
cav***a***v	147.0		
cav***v***v	151.6	**4.6***	1.0

Note: description of the *PPD1* and *VRN1* alleles in Table 1.

Table 3. Pairwise comparisons of mean crop growth rate (CGR) comparing alleles of *Vrn-A1* and *Vrn-B1* in DH population across two field sites (Yanco and Wagga Wagga) in 2010. *Ppd-B1* is represented as # in genotype code to indicate adjustment for this gene. *Differences significantly greater than zero (P≤0.05).

Genotype comparison	CGR (kg ha^{-1}day^{-1})	Difference	s.e.d
Vrn-A1			
#a***a***av	35.8		
#a***v***av	35.0	-0.8	1.4
#a***a***vv	31.9		
#a***v***vv	32.0	0.1	1.8
Vrn-B1			
#aa***a***v	35.8		
#aa***v***v	31.9	**-3.9***	1.7
#av***a***v	35.0		
#av***v***v	32.0	**-3.0***	1.5

Note: description of the *PPD1* and *VRN1* alleles in Table 1.

Table 4. Pairwise comparisons of mean crop growth rate (CGR) comparing *Vrn-B1a* and *Vrn-B1v* in the presence of *PPD1*, *Vrn-A1* and *Vrn-D1* alleles in the DH lines across two field sites (Yanco and Wagga Wagga) in 2010. *Differences significantly greater than zero (P≤0.05).

Genotype comparison	CGR (kg ha^{-1}day^{-1})	Difference	s.e.d
*baa**a**v*	36.3		
*baa**v**a*	34.9	-1.4	2.2
*caa**a**v*	38.9		
*caa**v**v*	32.6	**-6.3***	2.8
*bav**a**v*	37.6		
*bav**v**v*	35.0	-2.6	2.3
*cav**a**v*	36.5		
*cav**v**v*	32.4	-3.2	2.3

Note: description of the *PPD1* and *VRN1* alleles in Table 1.

Discussion

There were epistatic effects of *Ppd-B1*, *Vrn-A1* and *Vrn-B1* alleles on time to anthesis as reported in Cane et al. (2013) for heading date. *Vrn-A1* and *Vrn-B1* similarly affected anthesis date, however only *Vrn-B1* had an effect on crop growth rate, with the *Vrn-B1a* allele resulting in faster crop growth rates than the winter *Vrn-B1v* allele. These results suggest that growth suppression may be a pleiotropic effect of the regulatory nature of *VRN1* genes rather than a direct association with phasic development. It has been observed that expression of the *VRN1* genes down-regulate *CBF* genes (e.g. Deng et al. 2015), which when over-expressed, resulted in plants with increased freezing tolerance and slowed growth in *Arabidopsis* (Achard et al. 2008) and in barley (Soltész et al. 2013). This slowed growth was reported as being a consequence of interactions between the *CBF* genes and the gibberellin biosynthetic pathway, which provides a possible functional genetics explanation for the slowed growth of *Vrn-B1v* reported in this paper. Furthermore, faster crop growth rates and reduced time to anthesis associated with *Vrn-B1a* may provide a physiological explanation for the yield-enhancing effect of *Vrn-B1a* reported by Eagles et al. (2014).

Conclusion

This paper reports the effect of *PPD1* and *VRN1* genes on anthesis date and crop growth rate of wheat. The observed slowed growth of later-maturing lines is likely due to pleiotropic effects of the *VRN1* genes, rather than a direct link between wheat development and growth. Increased understanding of these genes will provide information for targeted breeding programs.

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