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Vernalisation and photoperiod sensitivity in wheat: The response of floret fertility and grain number is affected by vernalisation status

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ABSTRACT

The impact of different allelic combinations of *VERNALIZATION1 (VRN1)* and *PHOTOPERIOD1 (PPD1)* on the development of reproductive structures, spike dry weight (SDW) and its links to the duration of stem elongation (SE) and yield components was assessed using Near Isogenic Lines (NILs) of the cultivar Sunstate for both genes. Emphasis was on observations on the main shoot and tillers, linking to plant and canopy level in Steinfort at al. (2016). Experiments under controlled and irrigated field conditions relevant to the northern wheat growing region of Australia were designed.

In vernalised plants, the number of fertile florets in the main shoot spike was positively related to the number of fertile spikelets, the duration of SE, the SDW at anthesis and the sum of the radiation intercepted during SE, while no associations were found under non vernalised and slow vernalising field conditions. Vernalised plants also produced more grains in the average tiller spike, compared to non vernalised plants, particularly under short days. Both main shoot and tiller grain number per spike contributed to the higher grain number per plant observed in vernalised plants in Steinfort et al. (2016), as spike number was similar. In the field, higher yield of lines with spring alleles of VRN1 was underpinned by longer spikes, with fewer spikelets but more fertile florets per spikelet in the main shoot spike, and more grains per tiller. When non vernalised, winter lines had an apex that grew slower, shorter spikes and higher spikelet density compared to the other genotypes. The utilisation of sensitivity to VRN1 or PPD1 to increase the duration of the SE and the number of fertile florets and grains is a more complex process than originally proposed.

1. Introduction

Wheat is an annual crop that requires the exposure to cold temperatures and long days in order to transition from the vegetative to the reproductive phase. The successful adaptation of wheat worldwide has been achieved in part due to genotypic differences at the *VERNALISATION1* (VRN1) (Flood and Halloran, 1986) and *PHOTOPERIOD1* (PPD1) (Miralles and Slafer, 1999) genes, responsible for the control of the vernalisation and photoperiod response. Allelic variations of VRN1 and PPD1 in interaction with the environment, determine the plant lifeís cycle and the duration of the developmental phases. Vernalisation response affects primarily the length of the vegetative phase until the plant reaches double ridge or the initiation of floral primordia (Allard et al., 2012; Flood and Halloran, 1986; Steinfort et al., 2016), but has also some effect on

Abbreviations: DC31, decimal code detection of the first node on the Zadoks scale; DC65, anthesis defined by the Zadoks scale; LD, long day; LSD, least significant difference; NILs, near isogenic lines; PAR, photosynthetically active radiation; PPD1, *PHOTOPERIOD1*; REML, restricted maximum likelihood; SD, short day; SDW, spike dry weight; SD1, first sowing date; SD2, second sowing date; SE, stem elongation; VRN1, *VERNALIZATION1*.

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the early reproductive phase from double ridge to terminal spikelet and the duration of stem elongation (SE) (Gonzalez et al., 2002, 2003b; Steinfort et al., 2016). Photoperiod response also affects the length of the vegetative and early reproductive phases (Rawson and Richards, 1993) but the main effect is on the length of the SE phase (Miralles and Richards, 2000). In a previous study utilising near isogenic lines with different combinations of VRN1 and PPD1, a longer SE was observed under short days in plants with photoperiod sensitive genotypes, more effectively when genotypes with one VRN1 spring alleles were vernalised (Steinfort et al., 2016).

Exposure to long photoperiods in sensitive lines resulted in shorter durations of SE, lighter spikes at anthesis and a linear decrease in total number of spikelets per spike and the number of fertile florets per spikelet (Miralles and Richards, 2000). Photoperiod sensitive lines also produced heavier main shoot spikes at anthesis leading to a higher number of fertile florets independently of vernalisation in field experiments under natural versus extended photoperiod during SE (Gonzalez et al., 2003b). A longer duration of SE is expected to increase the amount of assimilates available for spike growth and positively impact the development of fertile florets and grains (Gonzalez et al., 2011a, 2005; Slafer, 1996; Slafer et al., 2001) in accordance with the model proposed by Fischer (1984).

The links between floret ontogeny, fertility, carbohydrate availability and ovary properties have been discussed extensively. In terms of development, wheat has the potential to initiate an indeterminate number of floret primordia per spikelet, but only up to 12 florets have been reported depending on the position of the spikelet within the spike (Sibony and Pinthus, 1988). From those, just one to four and occasionally five florets will become fertile (Fischer, 1984). Floret initiation starts in the basal florets of the central spikelets and continues acropetally within each spikelet and acropetally and basipetally within the spike (Sibony and Pinthus, 1988). The number of fertile florets at anthesis is determined during SE, in parallel to spike growth. Florets between the third and fifth position within the spikelet are likely to arrest their development by autophagy, presumably due to competition for assimilates (Gonzalez et al., 2003a, 2005; Kirby, 1988; Sibony and Pinthus, 1988), while distal florets are usually aborted before the start of the maximum rate of spike growth (Ghiglione et al., 2008; Gonzalez et al., 2011a, 2005). A different theory by Bancal (2009) proposed that a floret will become a grain only if its ovary is capable of diverting nutrients, based on its width, which was related to the relative growth rate of different floret primordia, suggesting that floret autophagy is more likely to be a sink not a source problem. From a molecular experiment, Ghiglione et al. (2008) found that decreases on the length of the spike growth period triggered by long photoperiod in sensitive genotypes, caused an increase in the autophagy of floret primordia, also coinciding with the findings of Gonzalez et al. (2003a, 2005). Indirect evidence that floret fertility was associated to an increase in the level of water soluble carbohydrates (WSC) in the spike of the main shoot, such as glucose and fructose, was also found, when the flag leaf was immersed directly in a sucrose solution (Ghiglione et al., 2008). Dreccer et al. (2014a), working under contrasting photoperiods and temperatures during SE, observed that recombinant inbred lines with a high content of WSC in the stem, produced up to 10 more fertile florets per spike of the main shoot and 9% more fertile florets per spikelet than their counterparts with low WSC content.

In a previous paper using NILs with allelic combinations of VRN1 and PPD1 in contrasting environments, Steinfort et al. (2016) observed that the duration of SE was weakly associated with the increase in spike weight m^{-2} at flowering in field experiments but this did not translate into higher grain number m^{-2}; which in turn was associated with more grains per spike. Additionally, high yields were obtained by vernalised plants in the glasshouse and genotypes with spring vernalisation alleles in the field. Following that evidence, we investigated more closely floret fertility and grain number at the single culm level to study the link with other observations at the tiller, plant and canopy level presented in Steinfort et al. (2016).

2. Materials and methods

2.1. Germplasm

NILs were obtained by crossing donors of different VRN1 or PPD1 alleles to the spring cultivar Sunstate followed by four rounds of recurrent backcrossing and selection of targeted donor (see Table 1 in Steinfort et al., 2016 and Table 1, this paper). Primers and conditions for allelic detection were presented, and also a list consisting of 3000 wheats lines screened for the detection of novel deletion alleles with the primer set BT472/BT474 (Ben Trevaskis, pers. comm.) (Steinfort et al., 2016). The genotypes included different combinations of the VRN-A1, VRN-B1 and VRN-D1 spring (A, B and D) and winter (a, b and d) alleles, the spring form being the common intron deletions of VRN1, described by Yan et al. (2004) and Fu et al. (2005). In the field experiment further allelic variation in VRN1 was included, the A1a allele (ABDi) with a promoter insertion, and Ai1, Ai2, and B2 novel alleles with intron deletions. Photoperiod sensitivity was conferred by the full length promoter PPD-D1 (s1) allele and photoperiod insensitivity by the deletion PPD-D1 (i) allele described by Beales et al. (2007). Most of the lines were insensitive at PPD-B1 (3 copies) as described by Diaz et al. (2012), only two being sensitive (1 copy) and one unknown composition.

2.2. Experiments

Two glasshouse and two field experiments were conducted at Gatton, (Queensland, Australia, 27.55° S lat, 152.33° E long). A full description is given in Steinfort et al. (2016). At the glasshouse each experiment consisted of a randomised complete block design with three replicates. Eight NILs were grown at two vernalisation levels (0 and 7 weeks) at short (SD = 11 h) and long (LD = 17 h) photoperiod. Plants were grown in 22 L rectangular (43 cm L x 32 cm W) containers filled with University of California mix (Chandler, 1979), with a plant density of 300 plants m^{-2} in a mini canopy with four rows. Imbibed seeds were vernalised at 5.5 °C and 8/16 h (day/night) while non vernalised seeds were sown six weeks later. Vernalised seedlings were transplanted when the second leaf emerged at the end of the seventh week to coincide with the same phenological stage of the non vernalised seedlings. The amount of daily PAR was standardised with the use of radiation blockage curtains that closed after 11 h daylength; temperature was set at 22/12 °C (day/night). The experiment was drip irrigated and fully fertilised weekly and kept insect and disease free.

Field trials were arranged in a randomised complete block design with a single factor (genotype) and two replicates and were sown at optimal (24 May 2012) and late sowing (27 July 2012) dates, referred to as SD1 and SD2 respectively, to extend the temperature and photoperiod range. A plant density of 150 plants m^{-2} was used in the plots that were 7 rows wide, spaced at 0.25 m and 7 m long. The soil was a black Vertosol, with 225 kgN ha^{-1} at sowing. Plots were irrigated, and kept pest and disease free. On both experiments, thermal time was calculated using the sum of the average daily temperature every 15 min minus a base temperature of 0 °C. Global radiation data from the local meteorological station (Australian Bureau of Meteorology, 2014 (http://www.bom.gov.au/silo)) was used to calculate the photosynthetically active radiation (PAR) (Pinker and Laszlo, 1992). The radiation sum was calculated during the SE phase and was used to compare the total amount