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Physiological determinants of fertile floret survival in wheat as affected by earliness *per se* genes under field conditions

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ABSTRACT

Variations in wheat yield are largely explained by changes in grain number per m^2 which is linked to the number of fertile florets at anthesis. This, in turn is the outcome of developmental processes which control floret initiation and mortality. Earliness '*per se*' (*Eps*) genes are involved in fine-tuning time to anthesis in wheat (and other cereals) but their effect on development prior to anthesis is less well studied. We aimed to determine effects of *Eps* genes on spike fertility, quantifying aspects of floret developmental which influence this trait. Field experiments were carried out to record floret primordia generation/degeneration dynamics in near isogenic lines (NILs) with contrasting *Eps* alleles (late flowering *vs* early flowering alleles; *Eps*-late and *Eps*-early, respectively) derived from the Avalon x Cadenza (AxC) cross with the *Eps* gene on either chromosome 1D or 3 A and from the Spark x Rialto (SxR) cross with the *Eps* gene on chromosome 1D. *Eps* NILs varied in spike fertility: *Eps*-late alleles increased fertility. Although the effect was in general slight, the magnitude was affected by the particular alleles and the cross used to produce the NILs. Differences in the number of fertile florets were explained by differences in the dynamics of floret development. NILs with *Eps*-late alleles improved the development of a small number of labile florets allowing them to complete their development to become fertile florets instead of dying, as in lines carrying early alleles. Thus, these alleles improved floret fertility mainly through reducing the rate of floret mortality with no influence on the dynamics of floret primordia initiation or in maximum number of floret primordia. Therefore, *Eps* genes could be exploited in wheat breeding not only to fine-tune time to anthesis but also to improve spike fertility.

1. Introduction

Increased wheat yields are an urgent priority for global food security (Reynolds et al., 2012). Yield is determined by the number of grains per unit land area and their average weight. Grain size is a minor source of variation for yield improvements (Slafer et al., 2014). It has been widely reported that wheat yield depends far more on the number than on the weight of the grains (Peltonen-Sainio et al., 2007; Sadras, 2007), and that grain number is the main factor limiting wheat yield potential (e.g. Fischer, 2011). Consequently, the improvement of agronomic performance of wheat (and other crops) the number of grains, of which spike fertility is a critical player, must be increased (Slafer et al., 2014). The number of grains is defined from sowing to anthesis with a critical period from the emergence of the penultimate leaf (20–30 days before anthesis) until *c.* 7–10 days post anthesis (Fischer, 1985; Slafer and Savin, 1991), while the average seed weight is defined from a few days before anthesis until maturity (Calderini et al., 2001; Ugarte et al., 2007). Understanding the mechanisms involved in defining the grain number would allow the identification of traits which might be critical for efforts to increase grain yield. Although some fertile florets may not set grains, grain set under field conditions usually exceeds 70% (Ferrante et al., 2013; Savin and Slafer, 1991), which is expected in a cleistogamous species. Therefore, grain number is largely defined by the number of fertile florets, which in turn depends on the dynamics of floret generation/degeneration. This process begins with floret primordia development but, as development proceeds, involves varying levels of floret mortality/survival during the late reproductive phase. Thus, studying genotypic differences in the dynamics of floret generation/degeneration could be critical for the further improvement of yield.

Earliness '*per se*' (*Eps*) genes are important for fine-tuning the adaptation of wheat (Herndl et al., 2008) and therefore are common in

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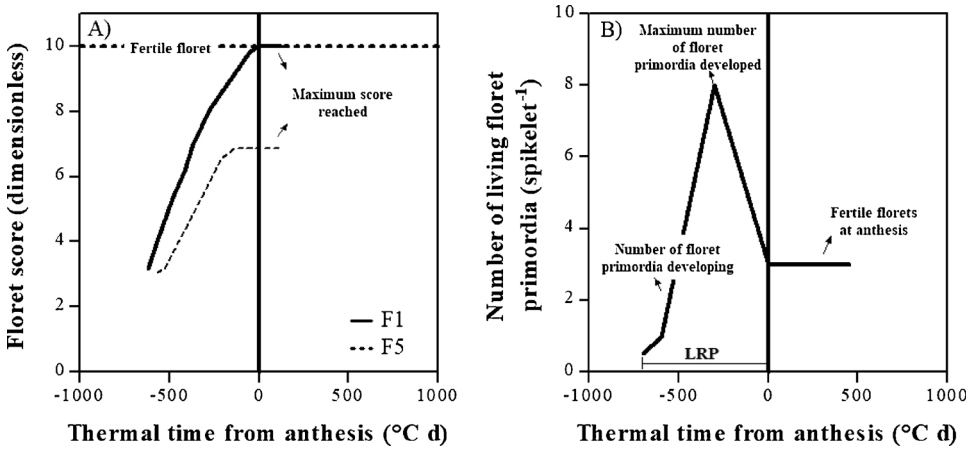

Fig. 1. Schematic diagrams of the dynamics of (A) floret development and (B) number of living florets through thermal time from anthesis (negative values represent the period before anthesis). In panel A we exemplified the expected dynamics of developmental progress for two contrasting floret positions, e.g. the floret most proximal to the rachis (i.e. F1) and the fifth floret (i.e. F5). In all cases these dynamics are strongly bi-linear with a growing phase in which the floret primordium develops and a plateau indicating the maximum score of development reached by that primordium (that can be a fertile floret if it reached 10, or the maximum stage it reached before starting its degeneration). Panel B shows the typical dynamics in which during the late reproductive phase (LRP) the number of living floret primordia increases until a maximum number is reached followed by a floret mortality phase and finishing with the final number of floret primordia that reached the fertile floret stage at anthesis.

germplasm adapted to different regions (e.g. Appendino et al., 2003; Worland et al., 1994). As the magnitude of their effects is frequently minor (Griffiths et al., 2009) it has been unusual to find studies reporting on their likely impact on number of organs developed. An exception is the earliness *per se* locus *Eps-A^{m}1*of diploid wheat *Triticum monococcum* L. (Valárik et al., 2006), with a rather large effect on phenology, that also affected spike fertility (Lewis et al., 2008). Although, to the best of our knowledge, this was never studied before, the effect on spike fertility might indicate an impact of these genes on the dynamics of floret development. If *Eps* genes in hexaploid wheat could affect these dynamics they could directly affect spike fertility, and breeding programs might exploit *Eps*-late alleles to increasing floret fertility, in addition to any use they may have for fine-tuning time to anthesis.

To the best of our knowledge there have been no studies reporting the effects of *Eps* genes in hexaploid wheat on the dynamics of floret development and in setting a particular level of the spike fertility. In the companion paper (Ochagavía et al., 2018) we reported the effects of *Eps* genes on the duration of different phases as well as on the dynamics of leaf and spikelet initiation. These *Eps* genes were those identified in Griffiths et al. (2009) and characterised for the effects on phenology by Zikhali et al. (2014) and Farré et al. (2016). In this paper, we studied their effect under field conditions on the dynamics of floret development. We also showed the consequences of these effects on spike fertility by quantifying changes in the number of fertile florets at anthesis.

2. Materials and methods

2.1. General description, treatments and design

Details of these experiments are available in the companion paper (Ochagavía et al., 2018). To recap briefly, fields experiments were carried out from 2012-13 to 2014-15 in Lleida, NE Spain. Sowing dates and rates were always within the optimum and the experiments were irrigated to avoid water stress.

Treatments consisted of wheat NILs, developed at John Innes Centre (Farré et al., 2016; Zikhali et al., 2014), differing in earliness *per se* alleles that resulted from the crosses of Avalon x Cadenza (AxC) or Spark x Rialto (SxR), each pair of NILs carrying either the early or the late allele in Chromosome 1D (in both AxC and SxR) or in 3 A (AxC). NILs, within each of the three groups (*Eps-D1* of AxC, *Eps-D1* of SxR, and *Eps3 A* of AxC) were arranged in a completely randomised design with eighteen (for NILs from AxC) and twelve (NILs coming from SxR) replications. Even though the lines are derived from UK material, they are well adapted to our environment. Due to the relatively strong winter of Lleida, wheat crops sown in fall are exposed to a sufficiently long period of vernalising temperatures (in these experiments more than 10 weeks of vernalising temperatures; Ochagavía et al., 2018) and consequently they flowered in spring, like cultivars released by local breeding programs do.

2.2. Measurements and analyses

As explained in the companion paper (Ochagavía et al., 2018), the stages of terminal spikelet and anthesis were determined allowing estimation of the duration of the late reproductive phase, which is the stage when florets develop. Thermal time was calculated using the mean air temperature and a base temperature of 0 °C.

Floret development dynamics were determined as in Prieto et al. (2018). Briefly, from terminal spikelet onwards, one representative plant per plot (i.e. 18 plants of each NIL of the AxC cross and 12 plants of each NIL of the SxR cross) was randomly sampled and taken to the lab two or three times a week, the main spikes dissected under a microscope (Leica MZ 7.5, Leica Microscopy System Ltd., Heerbrugg, Switzerland), and each individual floret primordium from the central spikelets was characterised for stage of development following the scale described by Waddington et al. (1983). Pistil development progress was observed and recorded in each floret primordium from the early stages until W10, when florets were considered fertile or, in the case of floret primordium that did not reached W10, until the maximum 'Waddington stage' was reached. Florets were numbered from 1 to n, from the closest to the most distal positions respect to the rachis, respectively. The score used for describing the pistil development was plotted against the thermal time from anthesis (Fig. 1A).

The number of living floret primordia within spikelets was counted at each sampling time in the central spikelets allowing representation of the dynamics of floret primordia generation/degeneration. Floret generation phase lasts until the maximum number of floret primordia is reached. From then on, floret mortality or a degeneration phase takes place until the surviving primordia constitute the number of fertile florets, which is set near to anthesis. Plotting these dynamics facilitates the comparison, not only of differences in the maximum and final number of florets, but also the length of each phase among genotypes (Fig. 1B). To start counting a floret primordium as a living floret it should have reached the stage of development of W 3.5; i.e. the first stage when an individual floret primordium can be identified (Waddington et al., 1983).

Samples of aboveground biomass were taken at anthesis from each replicate of each genotype from a sample area of 0.5 m long of a central row, which had been labelled shortly after seedling emergence. Thus ensuring that the plant density and uniformity was that expected and that the interplant variability within the sample was minimised. From