Wheat pre-anthesis development as affected by photoperiod sensitivity genes (*Ppd-1*) under contrasting photoperiods

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TITLE

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RUNNING TITLE

Wheat development and photoperiod sensitivity genes (*Ppd-1*)

HIGHLIGHTS

Photoperiod insensitive alleles shortened all pre-anthesis phases (*Ppd-D1a* > *Ppd-A1a* > *Ppd-B1a*) and no one was associated with a particular phase. No additive effect when stacking them was observed.

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Wheat regulates duration of phases leading to anthesis date, and hence yield potential, through day-length (photoperiod). Genes controlling the response to photoperiod (*Ppd-1*): (i) provided varying magnitudes of insensitivity, (ii) were not particularly associated to duration –photoperiod sensitivity- of any specific phase and (iii) show no additive effect when stacked. Further investigation with different allelic variants should be carried on for tailoring time to anthesis and duration of each particular phase to improve wheat yield potential facing climate change.

ABSTRACT

Fine-tuning wheat phenology is of paramount importance for adaptation. Better understanding on how genetic constitution modulates the developmental responses during pre-anthesis phases would help to maintain or even increase yield potential as temperature increases due to climate change. The photoperiod-sensitive cultivar Paragon, and four near isogenic lines with different combinations of insensitivity alleles *(Ppd-A1a, Ppd-B1a, Ppd-D1a* or their triple stack*)* were evaluated under short (12h) and long (16h) photoperiods. Insensitivity alleles hastened time to anthesis and duration of the three pre-anthesis phases (vegetative, early reproductive and late reproductive), following the *Ppd-D1a* > *Ppd-A1a* > *Ppd-B1a* ranking of strength. Stacking them intensified the

insensitivity, but had no additive effect over that of *Ppd-D1a*. The late reproductive phase was the most responsive, even exhibiting a qualitative response. Leaf plastochron was not affected but spikelet plastochron increased according to *Ppd-1a* ranking of strength. Earlier anthesis resulted from less leaves differentiated and a fine-tuning effect of accelerated rate of leaf appearance. None of the alleles affected development exclusively during any particular pre-anthesis phase, which would be ideal for tailoring time to anthesis with specific partitioning of developmental time into particular phases. Other allelic variants should be further tested to this purpose.

KEYWORDS: final leaf number, insensitivity alleles, ontogenesis, phenology, primordia dynamics, spikelet number.

ABBREVIATIONS: AN, anthesis, EM, emergence; ERP, early reproductive phase; Exp, experiment; FL, flag leaf; FLN, final leaf number; Gen, genotype; LAR, leaf appearance rate; LP, long photoperiod; LPDR, leaf primordia differentiation rate; LRP, late reproductive phase; NIL, near isogenic line; Phot, photoperiod; *Ppd-1*, *Photoperiod-1* genes; SP, short photoperiod; SPDR, spikelet differentiation rate; SPKLTS SPK^{-1}, spikelets per spike; TPDA, total primordia differentiated in the apex; ToCR, timing of change of rate; TS, terminal spikelet; VP, vegetative phase.

77 INTRODUCTION
78
79 The phenological pattern determining anthesis date plays a major role in wheat
80 (*Triticum aestivum* L.) adaptation (Worland 1996; Snape *et al.* 2001) maximising grain
81 yield for a given environment. This is because phenology of the crop defines resource
82 capture and stress avoidance opportunities during the pre- and post-anthesis periods of
83 yield generation (Fischer 1975; Evans 1978). For instance, rising global temperatures
84 because of climate change (IPCC 2014) are predicted to reduce yield by 6% per ºC of
85 temperature increment, solely based on accelerated developmental rates (Asseng *et al.*
86 2015; García *et al.* 2015), particularly during pre-anthesis.
87 Wheat undergoes three phenophases before reaching anthesis: the vegetative
88 phase (VP) from seed imbibition to floral initiation, when leaf primordia are
89 differentiated; the early reproductive phase (ERP) from then to the differentiation of the
90 terminal spikelet (TS), when spikelet primordia initiation takes place; and the late
91 reproductive phase (LRP) from TS to anthesis, when florets develop within the spikelets
92 whilst the stems and spikes grow (Slafer 2012). Manipulating the duration of these
93 phases, i.e. increasing duration of LRP, which includes the pre-anthesis critical period for
94 yield generation, might be an avenue to raising wheat yield potential (Slafer *et al.* 1996;
95 2001; Miralles *et al.* 2000; González *et al.* 2003; 2005a; 2011), helping to overcome part
96 of the predicted yield losses due to global warming.
97 From physiological studies exposing wheat to contrasting photoperiod
98 environments, it has been suggested that photoperiod sensitivity of each phenophase
99 might be under –at least partially– independent genetic control (Halloran and Pennell
100 1982; Slafer and Rawson 1994a; Slafer *et al*. 1996; 2001; González *et al.* 2002); which
101 would allow for manipulating sensitivity to photoperiod of a particular phenophase –and
102 with it, its duration– without significantly affecting duration of other phases. Studies on
103 the differences in rates of development comparing isogenic lines for *Photoperiod-1 (Ppd-*
104 *1*, photoperiod sensitivity genes) have been far less common (Foulkes *et al.* 2004;
105 González et al. 2005b, Matsuyama *et al.* 2015; Ochagavía *et al*. 2017), and cases in
106 which the effects of these alleles included the study of developmental processes in each
107 of these phases –instead of simply time to heading or anthesis– are almost inexistent
108 (González et al. 2005b). Such studies are critical to understand the impact that particular

109 *Ppd-1* genes have on developmental processes occurring in each phenophase, which is
110 relevant to design the best genetic combination to optimise adaptation and yield potential.
111 The *Ppd-1* genes are a homeoallelic series of loci located in short arms of
112 chromosome 2 of the A, B and D genomes (Scarth and Law 1983; 1984): *Ppd-A1*, *Ppd-*
113 *B1* and *Ppd-D1*, respectively (McIntosh *et al.* 2003). Early investigations pointed at them
114 as the main source variation in response to photoperiod amongst wheat genotypes *(Law et*
115 *al.* 1978; Scarth and Law 1984). The wild type allele, *Ppd-1b,* is associated with
116 photoperiod sensitivity (i.e. late flowering under short days) whilst semi-dominant
117 mutations, *Ppd-1a,* provide levels of insensitivity to photoperiod (Bentley *et al.* 2011;
118 Shaw *et al.* 2012). More recent investigations deploying near-isogenic-lines (NILs)
119 confirmed the multi-allelism proposed by Scarth and Law (1984) and suggested a ranking
120 of insensitivity for time to anthesis as being *Ppd-D1a* > *Ppd-A1a* > *Ppd-B1a (e.g.* Díaz *et*
121 *al.* 2012; Shaw *et al.* 2012; Bentley *et al.* 2013). Response to photoperiod has been
122 reported to be controlled as well by other genes in the long arm of chromosome 6B
123 (Islam-Faridi et al. 1996), chromosome 3D, possibly 3A and 3B too (Miura and Worland
124 1994) and 1A and 1B (Law et al. 1998). More recently, a report locates a Ppd-B2 in the
125 short arm of chromosome 2B (Khlestkina et al. 2009). Their effect, however, is relatively
126 small when compared to that of Ppd-1 genes.
127 As mentioned above, there were few studies registering *Ppd-1* effects on
128 physiological process during pre-anthesis phases. Not only there were few but also they
129 provided inconsistent results (see revision on Tables 1 and 2 in González *et al.* 2005b).
130 For instance, *Ppd-D1* has been associated with duration of the three pre-anthesis phases
131 (González *et al.* 2005b); or mainly with the early phases, VP+ERP, but without impact on
132 LRP (Foulkes *et al.* 2004), or only during ERP (Scarth *et al.* 1985), or even in the VP and
133 LRP but not in the ERP (Ochagavía *et al*. 2017). *Ppd-B1* has been associated with
134 duration of ERP (Scarth *et al.* 1985; González *et al.* 2005b), or VP+ERP with less or no
135 impact on LRP (Whitechurch and Slafer 2002; Matsuyama *et al.* 2015), or with all the
136 phases (Ochagavía *et al*. 2017). Finally, as far as we are aware there has been ony one
137 paper reporting on effects of *Ppd-A1* on these different phases, being the VP and ERP
138 affected; Ochagavía *et al*. 2017).
139 We found no reports on primordia differentiation dynamics in response to
140 differences in duration of pre-anthesis phases using isogenic lines for *Ppd-1* genes either.

141 However, Scarth *et al*. (1985) using chromosomes substitution lines observed that *Ppd-*
142 *D1a* did not modify the final number of leaves nor the rate of spikelet initiation resulting
143 in less spikelets per spike due to reduced ERP duration. For *Ppd-B1a*, rate of leaf
144 primordia differentiation was not altered resulting in less leaves produced, but spikelet
145 primordia differentiation rate was increased. Together with shorter duration of ERP it
146 resulted in no change in spikelet count per spike (Scarth et al., 1985). All in all,
147 considering the few reports available on the effects of *Ppd-1a* alleles on spikelets per
148 spike, there seem to be a generalised agreement in that the most extreme phenotypes
149 differ by *ca.* 2 to 3.5 spikelets per spike, regardless of whether the studies were in
150 controlled (Scarth *et al.* 1985) or field conditions (González *et al.* 2005b, Matsuyama *et*
151 *al.* 2015). Likewise, the impact of *Ppd-1* genes on the leaf appearance rate (LAR) seems
152 to have been only exceptionally considered so far (González *et al.* 2005b). This is
153 relevant as LAR, together with final leaf number, determines the time to flag leaf
154 appearance, which largely determines time to anthesis.
155 In this study, we assessed the individual effects of *Ppd-A1a*, *Ppd-B1a* and *Ppd-*
156 *D1a* and their triple stacking not only on time to anthesis and final number of leaves, but
157 also on duration of different pre-anthesis phenophases (VP, ERP and LRP), dynamics of
158 leaf and spikelet primordia differentiation, and leaf appearance rate.

MATERIALS AND METHODS

Experiments, treatments and design

We conducted two independent experiments at the University of Lleida (Catalonia, Spain) in 2015. In each of these experiments, treatments consisted of the factorial combination of five wheat (*Triticum aestivum* L.) genotypes and two contrasting photoperiod conditions. The genotypes were the wild type with only *Ppd-1b* alleles, Paragon (a spring cultivar, Winfield *et al.* 2010), and four NILs for *Ppd-1* genes (Table 1), kindly provided by the John Innes Centre (UK). *Ppd-1a* alleles from GS-100, Chinese Spring and Sonora 64 (*Ppd-A1a, Ppd-B1a* and *Ppd-D1a*, respectively) were introgressed into the photoperiod-sensitive cultivar Paragon by crossing with each of these genotypes, and then backcrossing with Paragon as the recurrent parent to develop BC4 families. The detailed procedure is described by Bentley *et al.* (2011). Photoperiod treatments consisted of two contrasting regimes: one with days relatively short (12 h light/12 h dark, or neutral day, henceforth referenced to as short photoperiod or SP), and another with long days (16 h light/8 h dark, hereafter long photoperiod or LP), which were applied in individual growth chambers in consecutive runs. Care was taken to ensure same daily incident radiation (*c.* 2.36 MJ m^{-2} day^{-1}) for both treatments by turning off some of the lamps in the chamber set with long days. Temperature in both photoperiodic conditions and in both experiments was constant at 16ºC. Each experiment was arranged as a completely randomised design: all genotypes were equally distributed between chambers and randomly set within them. Number of replicates depended on the response variable (see below).

–TABLE1–

Plants were grown in pots (235 ml) filled with a 7:3 mixture of peat and manure-based soil amendment. One seed per pot was sown after coating with the recommended dose of insecticide and fungicide, irrigated and left at room temperature until seedling emergence. Fifty per cent more pots than those required to fully fill the chambers were sown for each genotype to select for the experiments those with evenly emerged plants,

starting the experiment at seedling emergence. Number of pots per genotype ranged from 38 to 54 depending on *Ppd-1* genetic constitution and photoperiod treatment, i.e. the short photoperiod and the genotypes expected to be more sensitive had more pots than the others, to allow for more dissections. The pot was considered the experimental unit. Twice a week pots were rearranged inside the chambers to avoid border effects. Macro- (P, K) and micro-nutrients (B, Cu, Fe, Mn, Zn) were applied once at the beginning of the experiments, adding 0.01 ml of Manvert's 0-17-19 per pot with irrigation water as supplement. Each pot was periodically irrigated using an automatic drenching gun at a fixed dose. Insects and diseases were prevented spraying insecticides and fungicides.

Measurements, response variables and analyses

In each experiment, eight plants per genotype within each photoperiod treatment were identified and labelled immediately after seedling emergence (EM). In these plants, we determined the stages of flag leaf emergence (FL) and anthesis (AN) (Zadoks *et al.* 1974). Also, we recorded periodically (two-three times a week) the number of leaves appeared on the main shoot (Haun 1973) from EM to FL, when the final leaf number (FLN) was counted. At AN, when the experiment finished, the number of spikelets per spike was also recorded. Therefore, for all these traits there were eight replicates –per genotype x photoperiod combination- in each of the experiments.

Thermal time from EM to each particular stage was computed using 0ºC as base temperature. To estimate leaf appearance rate (LAR) a linear model relating the cumulative number of appeared leaves with thermal time from EM was fitted considering all the observations in each genotype x photoperiod combination (Eq. 1, *b*: LAR). The segmental linear model was fitted instead (Eq. 2) when evidence of lack of linear fit was detected through the analysis of the distribution of residuals. In this case, early leaves appeared at a faster rate (b) than the late-appearing leaves (d), being (c) the timing when the change of rate occurred.

Equation 1: $Y = a + b\,x$

Equation 2: $Y = a + b\,x\,(x \leq c) + b\,c\,(x>c) + d\,(x-c)\,(x>c)$

For each experiment, two plants per genotype x photoperiod treatment were randomly sampled twice or thrice a week –depending on developmental rate for each genotype x photoperiod combination–and dissected under binocular microscope. In each case, from EM to TS, we counted the number of primordia and determined the stage of development of the apex (Kirby and Appleyard 1981). Following each sampling, the remaining plants were rearranged to keep a canopy-like structure within the growth chamber. The cumulative number of primordia was related to thermal time by fitting a segmental linear model for estimation of primordia differentiation rates also by Eq.2 but with different interpretation of parameters: *b* and *d* mostly represent leaf and spikelet primordia differentiation rates, respectively, whilst c indicates the timing of change in primordia differentiation rates. Model adequacy was tested by using replicates test (GraphPad Prism version 6.00). Time to beginning of reproductive development (or floral initiation –FI) was estimated *a posteriori,* as the moment when the first reproductive primordium (collar, i.e. the first one in excess of FLN) was initiated for each plant. Duration of pre-anthesis phases was calculated as the difference in thermal time between the following stages: VP = FI -EM, ERP = TS-FI and LRP = AN-TS.

Analyses of variance were performed to assess the effects of experiments, main factors genotypes and photoperiod treatments, and the interactions genotype x photoperiod and experiment x genotype x photoperiod. Means of response variables were compared by Tukey's test ($\alpha = 0.05$) when found to significantly differ from one another using Infostat (Di Rienzo *et al.* 2015). Regression analyses were performed with GraphPad Prism version 6.00. The 95% Confidence Interval was used to determine statistical significance of differences amongst means for LAR, primordia differentiation rates and timing for change in these rates.

RESULTS

Analyses of variance consistently showed that the main effects of both photoperiod and genotype were highly significant for all traits (Table 2). Their interaction, although of a lower impact than the main factors, was also highly significant (Table 2), as expected when growing in contrasting photoperiods genotypes produced to differ in photoperiod sensitivity. On the other hand, the differences between the two independent experiments was not significant for most traits, and for the few cases in which it was, differences between experiments were negligible compared to those among genotypes or between photoperiod regimes (mean squares of the effects of the experiments represented at most 7.6 and 2.3% of the genotype and photoperiod mean squares respectively; Table 2). Also, the triple interaction (genotype x photoperiod x experiment) was always extremely small, beyond of non-significant (Table 2). This implies that the minor differences between experiments did not alter the effects of the main factors nor that of the genotype x photoperiod interaction. Therefore, means across experiments were used to describe each genotype x photoperiod performance in terms of duration of phases and number of leaves and spikelets, and data from both experiments were fitted together when describing leaf appearance or primordia differentiation dynamics.

–TABLE 2–

In the genotypes with at least one *Ppd-1a* allele, all plants developed normally until anthesis regardless of the photoperiodic condition. Contrastingly, in the genotype with the three *Ppd-1b* alleles, Paragon, some plants reached anthesis normally whilst others failed to develop towards that stage, particularly under short photoperiod when only 31% of the plants reached anthesis (25% in experiment 1 and 38% in experiment 2). The plants that did not reach anthesis by the end of the experiment (*c.* 2300ºC d after seedling emergence), would have not reached it either should the experiments had lasted longer, as they showed stalled post-TS development (see supplemental material, SM1). Thus, most of the plants of Paragon exhibited a qualitative response to photoperiod during LRP, whilst the response was quantitative for the earlier phases of development.

In the rest of the Results of this paper, we concentrated on the quantitative differences. For that purpose, the LRP and the whole period EM-AN for Paragon were analysed considering only the plants that developed normally until AN.

Time to anthesis, final leaf number and leaf appearance rate

Depending on photoperiod treatments, genotypes differed for the complete pre-anthesis cycle duration: Paragon reached AN significantly later than the NILs possessing insensitivity alleles in both short and long photoperiod, though the difference was rather large (>1000ºC d later than the triple insensitive NIL) under short photoperiod but relatively marginal (*c.* 200ºC d) under long photoperiod (Figure 1). Differences amongst NILs with insensitivity alleles were statistically significant only in short days. Under such condition, the strength of the alleles for producing insensitivity to photoperiod was *Ppd-D1a* > *Ppd-A1a* > *Ppd-B1a*. When the three *Ppd-1a* alleles were introgressed together the insensitivity was the strongest –though the difference with *Ppd-D1a* was statistically significant only with $\alpha = 0.10$.

–FIGURE 1–

Duration of the cycle from EM to AN was related to both duration from EM to flag leaf appearance –FL– (R^2=0.99, P<0.001) and duration of peduncle elongation –FL to AN–, albeit much less strongly (R^2=0.43, P=0.04). Genotypes differed little amongst them for duration of peduncle elongation, as values ranged from *c.* 270 to 335ºC d under short photoperiod and from *c.* 225 to 280ºC d when photoperiod was long. In contrast, very large differences were observed amongst genotypes for duration from EM to FL, ranging from *c*. 832 to 1921ºC d under short photoperiod and from *c.* 771 to 913ºC d under long photoperiod. FLN was highly and positively associated with duration EM-FL (R^2=0.93, P<0.001), mainly setting time to FL appearance. The ranking of strength amongst *Ppd-1a* alleles for the durations of FL-AN and EM-FL was similar to that observed on the duration of the EM-AN phase.

309 Duration from EM to FL comes as result of final leaf number (FLN) and the rate
310 at which those leaves appear (leaf appearance rate, LAR, which determines the
311 phyllochron). In nine out of the ten combinations of genotypes and photoperiod
312 conditions, the dynamics of leaf appearance was adequately fitted by a linear regression
313 (Figure 2). On the other hand, when data from Paragon under 12 h of photoperiod were
314 fitted with a linear regression the distribution of residuals was not at random (Figure 2,
315 inset on the left panel), indicating the appropriateness of a segmental linear regression in
316 this case. When this bi-linear regression was fitted, it was clear that late leaves (from *c.*
317 the seventh onwards) appeared at a significantly lower rate than the early leaves (Figure
318 2). Each of the *Ppd-1a* alleles, and their triple combination, accelerated the LAR
319 significantly, in either long or short photoperiod. The magnitude of such effect was minor
320 in long photoperiod (phyllochron ranged from 122ºC d in the triple insensitive to 132ºC d
321 in Paragon; Figure 2, right panel) and much more noticeable in short photoperiod (from
322 128ºC d in the triple insensitive to 163ºC d in the early-leaves and 200ºC d in the late
323 leaves of Paragon, Figure 2).

324

325 *–FIGURE 2–*

326

327 Considering the ten combinations of genotype x photoperiod, FLN was negatively
328 associated with LAR (R^2=0.69 P=0.003; Figure 3). However, the overall relationship was
329 strongly driven by the response to photoperiod of each genotype (Figure 3). Within each
330 photoperiod regime differences between genotypes in LAR were independent of those in
331 FLN, except for Paragon under short photoperiod. This cultivar under short photoperiod
332 exhibited both higher FLN and lower LAR than the NILs (Figure 3). Thus, when
333 analysing the effects of *Ppd-1a* alleles, as compared with the triple insensitive, under
334 short photoperiod they varied little in FLN (*c.* 0.7 leaves) and their differences in LAR
335 explained most differences in EM-FL (Figure 4, R^2=0.99, P=0.022). When including
336 Paragon, the difference in EM-FL duration was simultaneously due to an increased FLN
337 and decreased LAR (Figure 4, R^2= 0.97, P=0.017 and R^2=0.99 P=0.075, respectively).
338 The curvilinear trend indicates that the increase in EM-FL was much larger than the
339 decrease in LAR, whilst this was not the case for the relationship with FLN, which

followed a linear trend (Figure 4). This implies that the large delay in FL in Paragon under short photoperiod was chiefly due to the effect of the sensitivity alleles on FLN, complemented by a relatively minor change in LAR. Under long photoperiod, differences in EM-FL were significant only between Paragon and the genotypes with insensitivity alleles, yielding non-significant relationships with LAR and FLN (Figure 4, R^2=0.36, P=0.400; R^2=0.19, P=0.561, respectively).

–FIGURE 3–

–FIGURE 4–

Duration of vegetative, early and late reproductive phases

The insensitivity alleles sped up developmental rates of all phases under short photoperiod, thus shortening VP, ERP and LRP for genotypes carrying any of them alone or their triple combination (Table 2, Figure 5 bottom panels). Under long photoperiod, the effects of *Ppd-1a* alleles were milder than under short photoperiods, but still significant at least when comparing the extreme cases of Paragon and the NIL carrying the triple insensitivity (Figure 5 top panels).

Under short photoperiod, Paragon showed the longest duration of VP, followed by P(CS-2B) and P(GS-100-2A). The latter had similar VP duration to P(S64-2D) and the triple insensitive NIL (Figure 5 bottom-left panel). This means that for the duration of VP *Ppd-A1a* and *Ppd-D1a* had similar strength, and *Ppd-B1a* was the weakest allele. Under long photoperiod, although noticeably reduced, differences in duration of VP were still detected when comparing the triple insensitive and P(GS-100-2A) to Paragon (Figure 5 top-left panel).

For ERP, it was again observed that under short photoperiod Paragon was the longest (*c.* 540ºC d) and NILs carrying *Ppd-D1a*, *Ppd-A1a* and the three insensitivity alleles were the shortest. Although the NIL carrying the *Ppd-B1a* tended to show an intermediate duration (similar to that observed in VP), it was not statically different from the other NILs (Figure 5 bottom-middle panel). Long photoperiod treatment lessened the

differences amongst genotypes. Even though significant differences in duration of the ERP were detected between P(S64-2D) and Paragon, the actual difference was rather minor (*c.* 70ºC d) and there was no difference at all between Paragon and the NIL with the triple insensitivity (Figure 5 top-middle panel).

Single and triple *Ppd-1a*-bearing combinations also shortened LRP, and the magnitude of their effect was greater than for previous VP and ERP phases (Figure 5, right panel). Whilst this shortening was significant for every genotype with at least one *Ppd-1a*, there was a differential effect depending on the particular alleles involved. The ranking in LRP duration under short photoperiod was identical to that of the whole period to anthesis: Paragon > *P(CS-2B)* > *P(GS-100-2A)* > *P(S64-2D)* ≥ *Triple Insensitive* (Figure 5 bottom-right panel). Under long photoperiod, only the triple stacking of insensitivity alleles shortened the LRP significantly (reducing it by *c.* 200ºC d) when compared with Paragon (Figure 5 top-right panel).

Every genotype responded to photoperiod shortening all of the three pre-anthesis phases, although only for P(CS-2B) –the NIL with the weakest allele– and Paragon –with all three *Ppd-1b* alleles– was such response always statistically significant. Also, the magnitude of such response was greater for the LRP than for earlier phases.

–FIGURE 5–

Leaf and spikelet number

Genotypes carrying any combination of insensitivity alleles produced significantly less primordia than Paragon under short photoperiod (Table 3); whilst the differences were less clear and inconsistent under long photoperiod (Tables 2, 3). The effect of insensitivity alleles on the number of primordia was due to reductions in both vegetative (leaves) and reproductive (spikelets) primordia.

–TABLE 3–

Differences in FLN were detected amongst genotypes grown at 12 h photoperiod, as even P(CS-2B), the NIL that evidenced the weakest insensitivity in time to anthesis, produced fewer leaves than Paragon but more than the triple insensitive genotype; whereas P(S64-2D) and P(GS-1002A) were intermediate amongst the genotypes but did not differ significantly from either of them (Table 3). Under long photoperiod, most of such differences disappeared, as the FLN ranged only from six to seven across all genotypes. Furthermore, the slight differences were not clearly related to the *Ppd-1* allele composition: there were no differences between Paragon and either the triple insensitive or P(S64-2D), whilst the genotype that had the weakest *Ppd-1a* allele in terms of phenology –P(CS-2B)– produced the lowest FLN (Table 3).

Similarly, under short photoperiod the number of spikelets initiated was reduced by the introgression of insensitive alleles, with Paragon and the triple insensitive genotype showing the highest and lowest number of spikelets, respectively (Table 3). Differences were much smaller –and not significant– when grown in 16 h photoperiod, although with a consistent trend for genotypes carrying at least one *Ppd-1a* allele having fewer spikelets than Paragon (Table 3). It is noteworthy that, considering photoperiod response as the difference between primordia production under short vs. long photoperiod, every NIL responded when FLN was the response variable, but only Paragon significantly did so for the number of spikelets.

To assess the importance of phase duration on determining number of structures achieved, a linear regression was fitted to the relationship between number of structures differentiated during a particular phase and its duration (Figure 6). FLN was significantly related to the duration of the VP (Figure 6a). Although the relationship was strongly influenced by a single data-point –Paragon, short photoperiod–, it was still significant if that data-point were excluded from the analysis (R^2=0.71, P=0.004), mainly due to the photoperiod treatments and the interaction with genotypes. Differences in FLN were completely unrelated to duration of VP amongst genotypes under long photoperiod, even when including Paragon (R^2=0.17, P=0.495). In contrast, under short photoperiod differences amongst all genotypes in FLN were related to their differences in duration of VP (R^2=0.98, P=0.002) (Figure 6a). When photoperiod treatments are compared within

each genotype, differences in FLN were mostly related to differences in duration of VP; i.e. the response to photoperiod of each of the lines in terms of duration of VP translated in a parallel response of FLN (Figure 6a).

The relationship between the number of spikelets per spike and duration of ERP was much weaker than that between FLN and VP. Not only did the overall relationship have a lower coefficient of determination (R^2=0.82, Figure 6b) but it also strongly depended upon the single response of Paragon to photoperiod, as removal of this particular data-point rendered the relationship non-significant (R^2=0.25, P=0.175). The change in duration of ERP between short and long photoperiod within each genotype did not translate in differences in spikelets per spike (Figure 6b), except for Paragon in which the shortening of the ERP when grown under long photoperiod was followed by a reduction, albeit small, in number of spikelets.

–FIGURE 6–

Primordia differentiation dynamics

The relationships between the cumulative number of primordia differentiated in the apex from emergence to TS and thermal time were always bi-linear (Figure 7). For the presentation and discussion of these results we assumed the first slope represented the leaf primordia differentiation rate and the second slope, the spikelet initiation rate. Comparing timing of FI in Figure 2 with the arrowheads in Figure 7 reveals that all leaves were initiated at the rate represented by the first slope and that the vast majority of the spikelets were initiated at the rate of the second slope (although a few initial spikelets were differentiated at the same rate of the leaf primordia).

The leaf primordia differentiation rate was similar amongst all genotypes and photoperiods (Figure 7, Table 4). Averaging across all genotypes in both photoperiods, these rates represented a leaf plastochron (interval between differentiations of two consecutive primordia) of 51.4±6.5°C d. In contrast, genotypes differed in the timing when the change in rate of primordia differentiation occurred. This timing was also

affected by photoperiod treatments. The lines with insensitivity alleles advanced the timing of this change in short photoperiod compared to Paragon, whilst long photoperiod advanced this timing in Paragon and P(CS-2B) (Table 4). For the rest of genotypes with *Ppd-1a* alleles long photoperiod effect was not significant, but a consistent trend to advance this timing was observed (Figure 7, Table 4).

The spikelet initiation rate was modified by the interaction between genotypes and photoperiod treatments. It was increased under long compared to short photoperiod only for Paragon and P(CS-2B), whilst for the other genotypes the differences were not significant. When grown under 16 h photoperiod, this rate was similar amongst all genotypes, averaging across them a spikelet plastochron of 16.3±1.0°C d. Adjustment in the rate at which reproductive primordia were differentiated when the ERP was shortened, due to either photoperiod or *Ppd-1a* constitution under short days, allowed for the –at least, partial– compensation on final number of spikelets.

–FIGURE 7–

–TABLE 4–

476 DISCUSSION

477

478 *Time to anthesis and duration of pre-anthesis phases*

479

480 The response to short photoperiod was not only quantitatively large but also
481 qualitative. For the most sensitive genotype, Paragon, only a small proportion (*c.* a third)
482 of plants reached AN when grown under short days. The plants that did not reach AN had
483 their development stalled after TS. It has been previously reported that not a single plant
484 of Paragon reached anthesis when grown under 10 h photoperiod (after 120 days of
485 experiment) (Bentley *et al.* 2011). Other authors reported similar responses when strongly
486 sensitive cultivars were exposed to short photoperiod (Pugsley 1966; Halse and Weir
487 1970; Slafer and Rawson 1996) or non-inductive vernalizing conditions (González *et al.*
488 2002). The noteworthy fact that the qualitative response occurred during post TS
489 formation was in agreement with previous results (e.g. Slafer and Rawson 1996 for
490 sensitivity to photoperiod; González *et al.* 2002 for sensitivity to vernalisation). This
491 indicates that sensitivity to environmental cues might increase as development progress,
492 which was also clear with the quantitative response observed in those plants that did
493 develop until anthesis (see below).

494 Considering the quantitative response –i.e. restricting the analysis to the plants of
495 Paragon that did reach AN–, different strength in terms of AN hastening was observed for
496 each insensitivity allele, being the effect of *Ppd-D1a* > *Ppd-A1a* > *Ppd-B1a*. This is in
497 agreement with (i) the ranking proposed by latest investigations evaluating the same
498 alleles, also using NILs with Paragon background (Díaz *et al.* 2012; Shaw *et al.* 2012;
499 Bentley *et al.* 2013), and (ii) with the conclusion by Langer *et al.* (2014) who determined
500 that *Ppd-D1a* is the allele with strongest effect in time to anthesis in European wheat.

501 Whilst major differences in EM-AN duration were associated with changes in
502 both FLN and LAR, fine-tuning of AN date was still accomplished by changes in LAR,
503 even when no significant differences in FLN could be detected. Insensitivity alleles
504 accelerated LAR of leaves emerging prior to the seventh –following the ranking stated
505 above– in both short and long photoperiods, which shortened EM-FL duration beyond the
506 effect of these alleles on FLN, something that, as far as we are aware, has never been
507 reported before –for the particular effects of *Ppd-1a* alleles. This simultaneous effect on

FLN and LAR reveals that time to AN could be coarsely adjusted by changes in FLN and fine-tuned by changes in developmental rates given by further changes in LAR. The result that *Ppd-1a* alleles reduced FLN agrees with González *et al.* (2005c), whilst our description of their effect on LAR contrasts with their report: they found no impact of *Ppd-D1a* and *Ppd-B1a* on phyllochron of early emerging leaves.

Every *Ppd-1a* allele shortened each of the pre-anthesis phenophases, VP, ERP and LRP under short photoperiod, following a very similar ranking of magnitude to that observed for the whole cycle to AN. Whilst no previous records exist –to the best of our knowledge– on *Ppd-A1a* effects on duration of pre-anthesis phenophases, we found both agreeing and conflicting results for *Ppd-D1a* and *Ppd-B1a* effects in preceding literature. For *Ppd-D1a*, González *et al.* (2005c) also found effects on all three phases, whilst others reported effects only on duration of ERP or during EM-TS phase (Scarth *et al.* 1985 and Foulkes *et al.* 2004 respectively). Similarly, whilst *Ppd-B1a* has been found to shorten EM-TS (Whitechurch and Slafer 2002; Matsuyama *et al.* 2015) or even only ERP (Scarth *et al.* 1985; González *et al.* 2005b), no previous reports on it shortening LRP –as it here did– have been found.

In addition, our results not only showed that *Ppd-1a* alleles reduce the duration of all pre-anthesis phases but also that the most responsive was LRP. Under short photoperiod, this increase in responsiveness was so critical that the late reproductive phase exhibited a qualitative response to photoperiod in most of the plants of the most sensitive genotype. On the other hand, all of the plants showed a quantitative response for the VP and ERP. The few plants that developed normally to anthesis showed a large increment of the duration of LRP. Even under long photoperiod, in which durations of VP and ERP were somewhat affected, LRP was much longer in Paragon than in the NILs with *Ppd-1a* alleles. This stronger responsiveness of LRP than earlier phases agrees with physiological experiments in which sensitive cultivars were subjected to different photoperiods (e.g. Slafer and Rawson 1995). Not only did we prove that all of the pre-anthesis phases (VP, ERP or LRP) were responsive to the action of *Ppd-1a* alleles, but also that responsiveness to them differ throughout the cycle, as it does to photoperiod (Slafer and Rawson 1994a).

Contrasting results from previous reports on *Ppd-1a* effects may be due to (i) the use of whole chromosomes substitution lines (Scarth and Law 1984; Scarth *et al.* 1985;

Whitechurch and Slafer 2002) in which too many other genes might have affected the results; (ii) the possible interaction with different degrees of vernalisation satisfaction when using winter-habit cultivars (Foulkes *et al.* 2004; González *et al.* 2005b); and/or (iii) interactions between *Ppd-1* and the genetic background of the material on which *Ppd-1* have been tested on (Kiss *et al.* 2014). Sources of variation in the ranking mentioned –beyond the stated above– could be different alleles for a given locus, i.e. the functional polymorphism in *Ppd-B1* (Tanio and Kato 2007; Nishida *et al.* 2013) or copy number variation in *Ppd-B1* as well (Beales *et al.* 2007; Bentley *et al.* 2011; Díaz *et al.* 2012; Shaw *et al.* 2012; Nishida *et al.* 2013; Muterko *et al.* 2015; Matsuyama *et al.* 2015). Ochagavía *et al*. (2017) grew a more comprehensive collection of NILs in the field and in general found similar, though not identical, results on the effects on developmental phases, revealing again that any ranking on effects would be susceptible to the combination of genetic (e.g. using different NILs) and environmental (field condition in which photoperiod lengthens throughout the growing season) backgrounds.

Finally, in the conditions of our study we found no evidence of any particular *Ppd-1* allele affecting developmental rates of any particular pre-anthesis phases. Previous physiological studies proposed that duration of different pre-anthesis phases could be manipulated, given their photoperiodic sensitivity seemed to be under independent genetic control (Slafer *et al.* 1996; 2001; González *et al.* 2002). In this experiment, none of the tested alleles, affected developmental rates exclusively during any particular pre-anthesis phase, which would be ideal for tailoring time to anthesis with specific partitioning of developmental time into particular phases. Three main systems controls wheat development to anthesis *Vrn*, Eps and *Ppd* genes, of which *Ppd-1* is only a part (Stelmakh, 1997; Kamran, *et al.* 2014). In the present work, only some combinations of *Ppd-1* were studied. Other loci of minor impact have been found to affect photoperiod sensitivity (Law *et al.* 1998, Cockram 2007, Khlestkina *et al.* 2009); the impact of them on particular pre-anthesis phases remains unknown. Also the impact of any Ppd-1 genes have been discussed to interact with the genetic and environmental backgrounds, and therefore with our results we can definitively conclude that such an independent control of the analysed alleles was not found but could not discard that under different background conditions or if introgressed in another background these alleles might not affect any of the three phases considered differentially. As research continues on the

572 genetic controls of anthesis in general and on photoperiod sensitivity in particular, new
573 combinations of *Vrn*, *Eps* and *Ppd* genes will be made available to further test this
574 hypothesis.
575
576 *Number of primordia differentiated in the apex and their dynamics*
577
578 Insensitivity alleles differently affected leaf and spikelet primordia differentiation
579 rates. They had no effect on leaf differentiation rate and, consequently, their effect on
580 FLN largely reflected those on VP duration. This coincides with the only other known
581 report on effects of *Ppd-1* genes on primordia differentiation (Scarth *et al.* 1985)
582 comparing *Ppd-B1a* and *Ppd-B1b* chromosome substitution lines. The leaf plastochron
583 values we determined (*c.* 50ºC d leaf^{-1}) was within the range of those previously reported
584 in the literature (Evans and Blundell 1994; Miralles and Richards 2000; González *et al.*
585 2002). In addition, this is in line with physiological models of response of FLN to
586 environmental factors. These models assume that leaf plastochron would be insensitive to
587 photoperiod whilst the period of leaf differentiation (VP) would be sensitive, so that the
588 relative change in duration of VP would be paralleled by the same relative change in FLN
589 (e.g. Miglietta 1989; Slafer and Rawson 1994b; Slafer 2012).
590 Insensitivity alleles did, however, accelerate spikelet differentiation when ERP
591 was shortened, as well as they hastened the timing to change of rate in primordia
592 differentiation. Thus, even with ERP being shorter, a partial compensation in number of
593 spikelets was observed. These findings coincide with the only other known report of *Ppd-*
594 *1* genes on primordia dynamics (Scarth *et al.* 1985) in the case of *Ppd-D1a*, but not for
595 *Ppd-B1a,* for which they found full compensation for number of spikelets. This is of
596 particular interest as breeding to optimise pre-anthesis phases duration could then enlarge
597 LRP at the expense of ERP (Slafer *et al.* 2001) without negatively affecting the number
598 of spikelets per spike, a numerical component of yield.
599 The spikelet plastochron was also in accordance with previous reports (Rahmann
600 1980; Scarth *et al.* 1985; Evans and Blundell 1994; Miralles and Richards 2000,
601 González *et al.* 2002), averaging 18.5ºC d spikelet^{-1}. Also, as therein described, spikelet
602 plastochron was reduced when exposed to longer photoperiods for all genotypes but the
603 triple insensitive, albeit significant responses (*c*. -2.5ºC d spikelet^{-1} h) were only detected

for the most sensitive ones, Paragon and P(CS-2B). As a result, there was much less variation in the number of structures produced, owing mainly to remaining variations in duration of ERP.

Generally, fewer spikelets were produced when development of the spike initiation phase was hastened. This holds regardless of whether the hastening of development of this phase is the result of the introgression of *Ppd-1a* alleles or exposure to longer photoperiod. González *et al.* (2005b) observed a similar relationship for *Ppd-1a* NILs on two different winter backgrounds in field conditions, and spikelet number per spike ranged from *c.* 20 (*Ppd-1b* genotype) to 16,4 or 17,6 for insensitive genotypes. Matsuyama *et al.* (2015) found their most sensitive genotype to produce 17,7 to 18,7 spikelets per spike depending on year and site combination, while insensitive genotypes produced 15,5 spikelets per spike. Scarth *et al.* (1985), working with chromosome substitution lines under contrasting photoperiods in a growth chamber experiment at constant 18°C, showed a decline of nearly 3 spikelets when introgressing a chromosome with *Ppd-D1a* in Chinese Spring background. Average spikelet count in short days was however considerably higher than in the present study, possibly due to much shorter photoperiod (8 h) used by Scarth *et al.* (1985) than in experiment (12 h).

Linking the insensitive phenotype to the role of Ppd-1a in the flowering pathway

The effects of insensitivity alleles (*Ppd-1a*) on phasic development and rates of leaf and spikelet initiation were comparable to that of longer, more inductive photoperiods, the magnitude of such effect being dependent on the strength of the allele (González *et al.* 2005b). Considering recent molecular studies of the flowering pathway, and particularly how *Ppd-1* genes interact with it, this seems unsurprising. Mutant alleles (*Ppd-1a*) show altered patterns of expression of the mutated gene, promoting high transcript levels –as long days would– throughout the dark period, which is associated with elevated, flower-inducing *TaFT1* (wheat's orthologue of *FLOWERING LOCUS T*) transcript levels even under non-inductive photoperiod (Turner *et al.* 2005; Beales *et al.* 2007; Díaz *et al.* 2012; Shaw *et al.* 2012). As a result, in the present study we found that

636 the NIL with the triple combination of all *Ppd-1a* alleles showed little variation between
637 photoperiods in either phase duration, number of primordia differentiated at the apex or
638 the rate at which they are initiated and expanded (leaves). On the other hand, the
639 performance of the rest of the genotypes was very similar to that of the triple insensitive
640 NIL when in long photoperiods. Also, it was under short photoperiod that *Ppd-1a* effects
641 -and differences among genotypes- were the most notorious.
642 By no means was anthesis –or any of the phenophases or other developmental
643 processes described– in the NIL carrying *Ppd-1a* in all three genomes hastened to the
644 same extent as the sum of the three single allele's individual effect. Whilst the triple
645 insensitive genotype consistently showed to have the strongest insensitivity, it was
646 difficult to find significant differences between it and P(S64-2D), carrying the strongest
647 single allele. This has been previously recognised by Shaw *et al.* (2012) –for heading date
648 only– when working with single, double and triple NILs of the same origin. Whilst they
649 detected a direct relationship between increasing number of insensitivity alleles and
650 *TaFT1* expression levels, flowering was not always accordingly hastened –in strong
651 double and triple insensitive genotypes, grown under 10 h photoperiod. They suggested a
652 rate-limiting process downstream of *TaFT1*. The same mechanism may be explaining not
653 only anthesis date, but also all the other traits we measured in the present study. Shaw *et*
654 *al*. (2012) showed that *Ppd-1* transcription products are not genome-specific as of their
655 downstream targets –i.e. *Ppd-1a* mutations on any given genome regulate the expression
656 of downstream targets at all three genomes. Thus, high transcript levels from a single
657 "strong" *Ppd-1a* allele might already upregulate *TaFT* to levels that saturate the response
658 observed. The lack of strong additive effects among *Ppd-1a* observed in the present
659 paper, i.e. no further response was observed by stacking alleles on duration of sub-phases
660 or number of structures generated (leaves and spikelets) is in line with this molecular
661 model.
662
663 *Concluding remarks*
664
665 In conclusion, *Ppd-1a* alleles hastened anthesis both under short and long
666 photoperiod, each providing different levels of insensitivity. The ranking on strength of
667 the insensitivity alleles for anthesis was *Ppd-D1a (Sonora 64) > Ppd-A1a (GS-100) >*

668 *Ppd-B1a (Chinese Spring)*. All of the pre-anthesis phases (VP, ERP or LRP) were
669 sensitive to the action of *Ppd-1a* alleles, but not equally so: unlike what is commonly
670 assumed –that early phases might be more sensitive– the magnitude of the effects of these
671 alleles increased with advances in development. The increment in sensitivity was so
672 critical that the late reproductive phase exhibited a qualitative response in the most
673 sensitive genotype under short photoperiod, whilst previous phases only exhibited
674 quantitative responses. Furthermore, photoperiod insensitivity alleles may affect time to
675 anthesis not only through their effects on FLN but also through an additional, fine-tuning
676 adjustment, through effects on phyllochron. These effects are not trivial as they may be
677 responsible for the quantitative response to photoperiod of the late reproductive phase.
678 Stacking of *Ppd-1a* alleles intensified the insensitivity, but the cumulative effect was far
679 from being additive. We also showed that every combination of either one or three *Ppd-*
680 *1a* alleles, on Paragon background, responded to photoperiod; the magnitude of the
681 response varying according to the strength of the alleles. None of the tested alleles
682 affected developmental rates exclusively during any particular pre-anthesis phase, which
683 would be ideal for tailoring time to anthesis with specific partitioning of developmental
684 time into particular phases. The effect of other allelic variants should be further tested to
685 this purpose.

686 SUPPLEMENTARY DATA

687

688 Fig. S1. Detail on the qualitative response of Paragon to short photoperiod.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Table 1: Allelic composition for Ppd-1 of each genotype.

Genotype	Allelic composition		
Paragon	*Ppd-A1b*	*Ppd-B1b*	*Ppd-D1b*
P(GS-100-2A)	*Ppd-A1a*	*Ppd-B1b*	*Ppd-D1b*
P(CS-2B)	*Ppd-A1b*	*Ppd-B1a*	*Ppd-D1b*
P(S64-2D)	*Ppd-A1b*	*Ppd-B1b*	*Ppd-D1a*
Triple insensitive	*Ppd-A1a*	*Ppd-B1a*	*Ppd-D1a*

a: photoperiod-insensitive allele; b: photoperiod-sensitive allele

The A, B and D genomes of the five genotypes compared in the study: Paragon (a spring cultivar with the three sensitive alleles), and its four NILs: Paragon (P) with Ppd-A1a, Ppd-B1a, or Ppd-D1a, and with the three of them together (triple insensitive). The donors of the sensitive alleles were GS-100, Chinese Spring and Sonora 64, respectively.

*Table 2: Mean squares for the main effects of experiment (Exp), photoperiod (Phot) and genotype (Gen) and interactions Gen*Phot and Gen*Phot*Exp for ANOVA tests performed on durations of phases and on number of structures produced in the apex of the main shoot.*

Source of variation	Duration (10^{-3} ºC d)					Structures produced (leaves and/or spikelets)		
	EM-AN	EM-TS	VP	ERP	LRP	TPDA	FLN	SPKLTS SPK^{-1}
Experiment	83.5 ***	3.2 *	0.2 ns	2.0 ns	10.0 *	0.5 ns	0.2 ns	0.1 ns
Photoperiod	4514.2 ***	302.8 ***	49.5 ***	107.5 ***	429.7 ***	334.2 ***	136.9 ***	43.2 ***
Genotype	1091.4 ***	69.7 ***	13.7 ***	24.6 ***	199.9 ***	118.7 ***	22.6 ***	41.2 ***
Gen*Phot	511.2 ***	42.7 ***	8.1 ***	14.6 ***	94.4 ***	62.4 ***	16.9 ***	15.6 ***
Gen*Phot*Exp	3.4 ns	0.4 ns	0.1 ns	0.5 ns	2.0 ns	1.0 ns	0.2 ns	1.1 ns

*Durations considered were those of the cycle from seedling emergence (EM) to anthesis (AN) or to terminal spikelet (TS) and of each pre-anthesis phases (vegetative phase –VP–, early reproductive phase –ERP– and late reproductive phase –LRP–). Numbers of structures considered were total number of primordia differentiated at the apex –TPDA– and its components: final leaf number –FLN– and spikelets per spike –SPKLTS SPK^{-1}. On the right of each mean square it is indicated whether the effect was statistically significant (*P<0.05, **P<0.01, and ***P<0.001) or not (ns).*

Table 3: Final leaf number (FLN), number of spikelets per spike (SPKLTS SPK^{-1}) and total number of primordia differentiated in the apex (TPDA) for each genotype, Paragon and its 4 NILs, grown under either long (16 hours) or short (12 hours) photoperiod.

Photoperiod	Genotype	FLN (leaves)	SPKLTS SPK^{-1} (spikelets)	TPDA (primordia)
16 hours	Triple Insensitive	6.8 b	13.6 a b c	20.4 a b
	P(S64-2D)	7.0 b c	13.5 a b	20.5 a b
	P(GS-100-2A)	6.6 a b	13.5 a b	20.1 a
	P(CS-2B)	6.3 a	13.8 a b c	20.0 a
	Paragon	7.0 b c	14.7 b c	21.7 b c d
12 hours	Triple Insensitive	7.5 c d	13.3 a	20.8 a b
	P(S64-2D)	8.1 e	14.1 a b c	22.2 c d
	P(GS-100-2A)	7.9 d e	13.9 a b c	21.9 b c d
	P(CS-2B)	8.2 e	14.9 c	23.1 d
	Paragon	11.6 f	18.3 d	29.9 e

Different letters within columns indicate statistically significant differences amongst genotypes and photoperiods (Tukey α=0.05).

Table 4: Leaf primordia differentiation rate (LPDR), spikelet primordia differentiation rate (SPDR) and timing of change of rate (ToCR).

Photoperiod	NIL	LPDR *(leaves [ºC d]$^{-1}$)*	SPDR *(spikelets [ºC d]$^{-1}$)*	ToCR *(ºC d from EM)*
16 hours	Triple Insensitive	0.021 a	0.058 a	210 a b c
	P(S64-2D)	0.018 a	0.058 a	150 a
	P(GS-100-2A)	0.021 a	0.060 a	198 a b
	P(CS-2B)	0.015 a	0.065 a	195 a b
	Paragon	0.018 a	0.066 a	210 b c
12 hours	Triple Insensitive	0.020 a	0.076 a	272 b c d
	P(S64-2D)	0.022 a	0.049 a b	256 a b c d
	P(GS-100-2A)	0.021 a	0.048 a b	267 b c d
	P(CS-2B)	0.019 a	0.041 b	294 c d
	Paragon	0.022 a	0.041 b	390 d

The three variables –LPDR, SPDR and ToCR– were parameters estimated from the segmental linear regression between number of primordia differentiated in the apex and thermal time from emergence (EM) for each genotype when grown under long (16 h) or short (12 h) photoperiod. Shared letters within columns indicate that the CI95 of the parameter estimation overlapped.

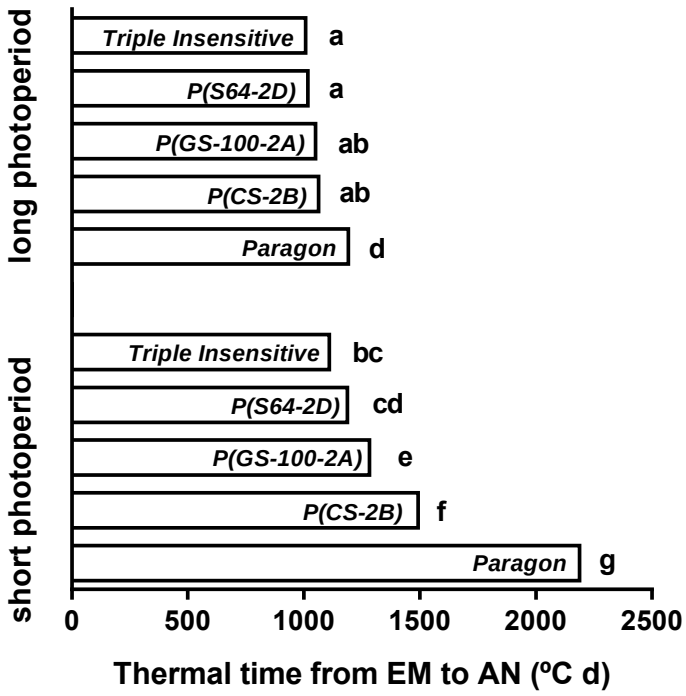

Figure 1: Duration of the whole pre-anthesis cycle. Thermal time from seedling emergence (EM) to anthesis (AN) for Paragon and each of the NILs in both photoperiods. Shared letters at the end of the bar indicates that the difference was not statistically significant between genotypes and across photoperiod treatments (Tukey, α=0.05). Data are means of two independent experiments.

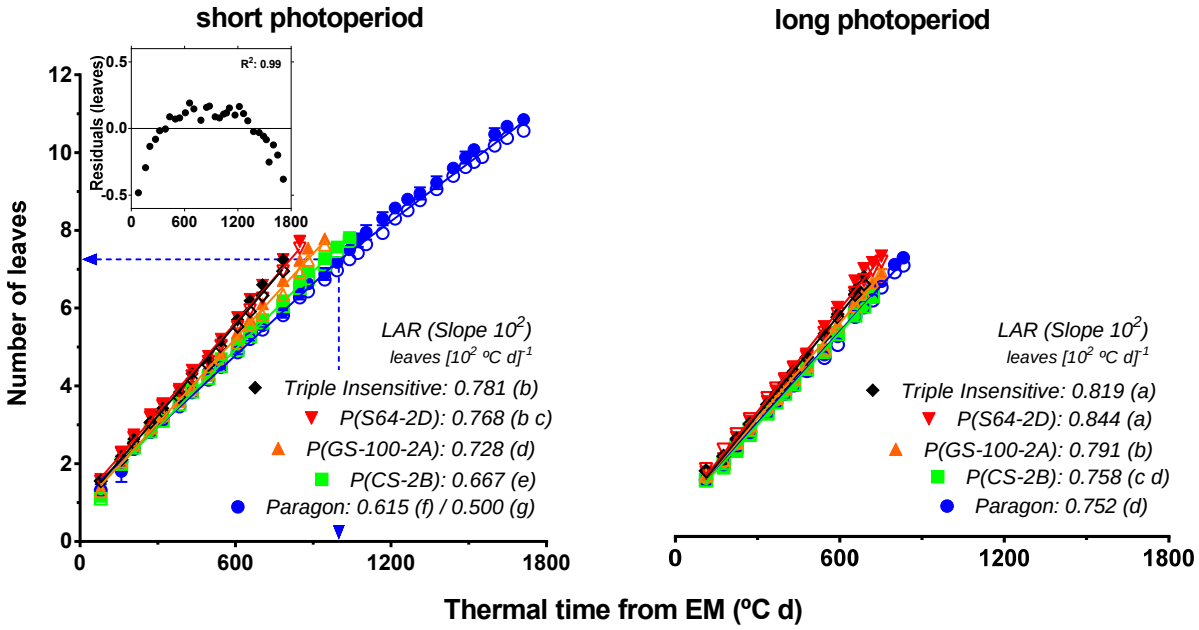

Figure 2: Leaf appearance dynamics. Relationship between leaves appeared on the main shoot in experiments 1 and 2 (closed and open symbols, respectively) and thermal time from emergence (EM). The error bars in data-points stand for the corresponding SEM. Slopes (inset, bottom right) indicate leaf appearance rates (LAR, leaves [10^2 ºC d]$^{-1}$). Lines were fitted by either linear or segmental-linear regression, being R^2 >0.97 (P<0.001) for all of them. Shared letters between any two LARs indicate that the CI95 of parameter estimation (slopes) overlapped. The arrowed dotted line indicates the number of leaves (ordinate) appeared and time (abscissa) when the change in slope for Paragon in short days occurred. The relationship between the residuals of the linear regression between the number of leaves and thermal time (inset, top left) justified the need for a segmental-linear regression in this case

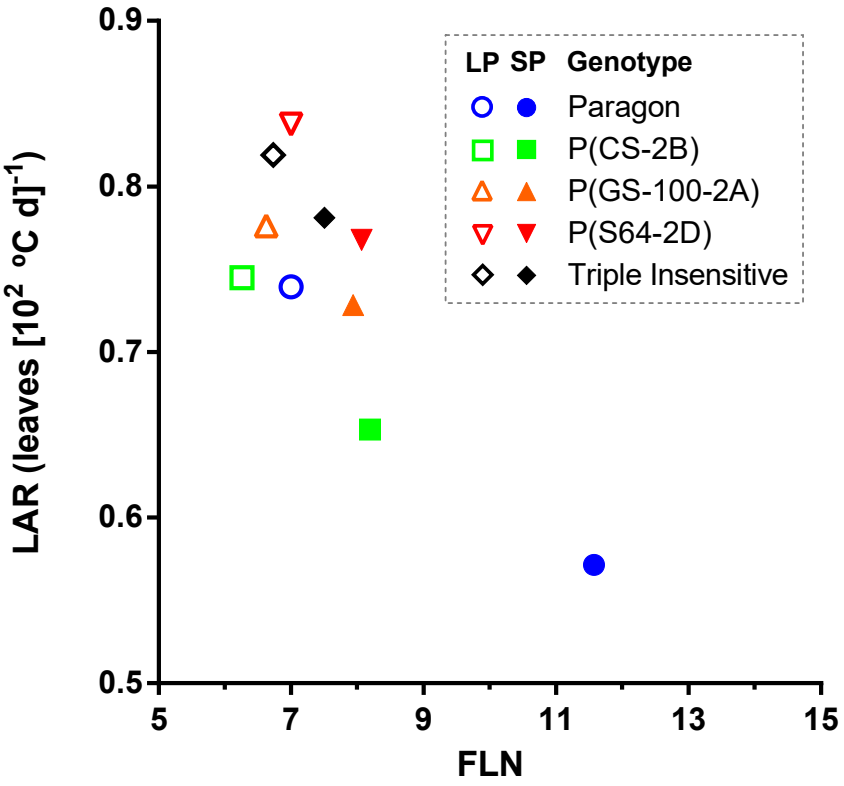

Figure 3: Relationship between leaf appearance rate (LAR) and final leaf number (FLN) under long (LP) and short photoperiod (SP). LAR for Paragon in short photoperiod was calculated as the weighted average of LAR values for early and late appearing leaves.

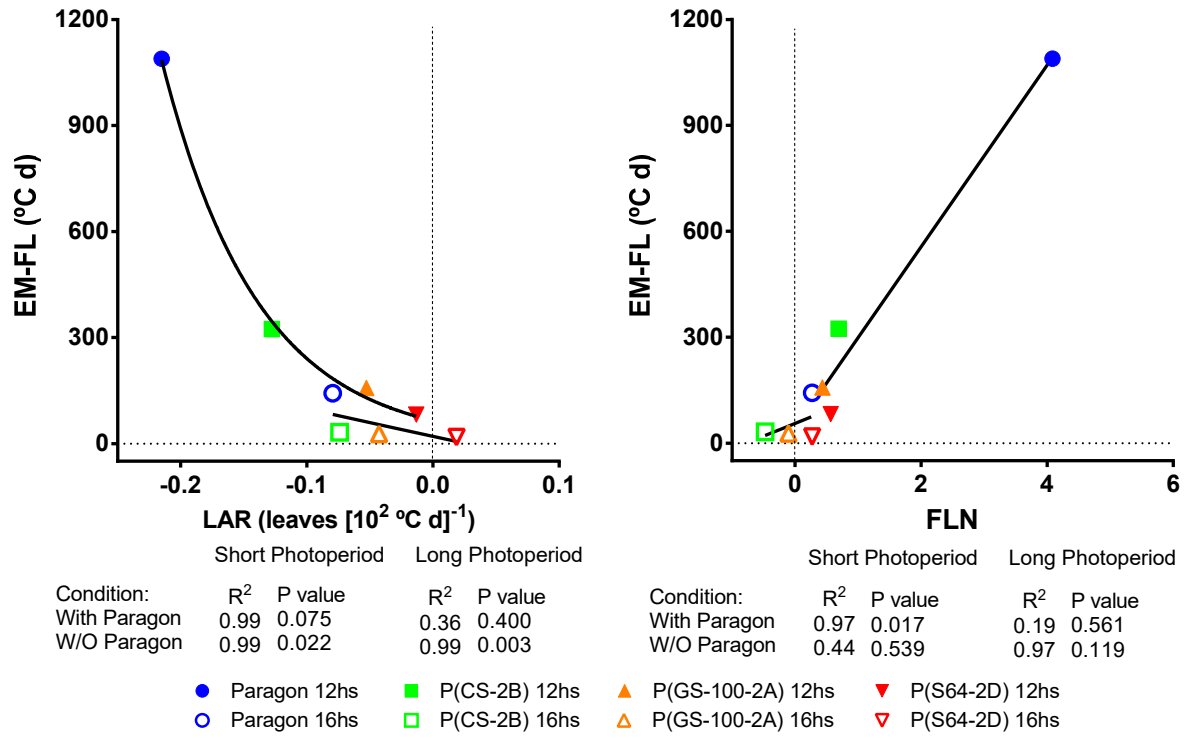

Figure 4: Differences in thermal time from seedling emergence to flag leaf appearance (EM-FL) between each genotype and the triple insensitive NIL plotted against either leaf appearance rate (LAR, left panel) or final leaf number (FLN, right panel). Regressions (exponential in the case of the relationship with LAR under short photoperiod, linear in the three other cases) were fitted for each photoperiod treatment including and excluding Paragon, but only the lines from the ones including Paragon are shown.

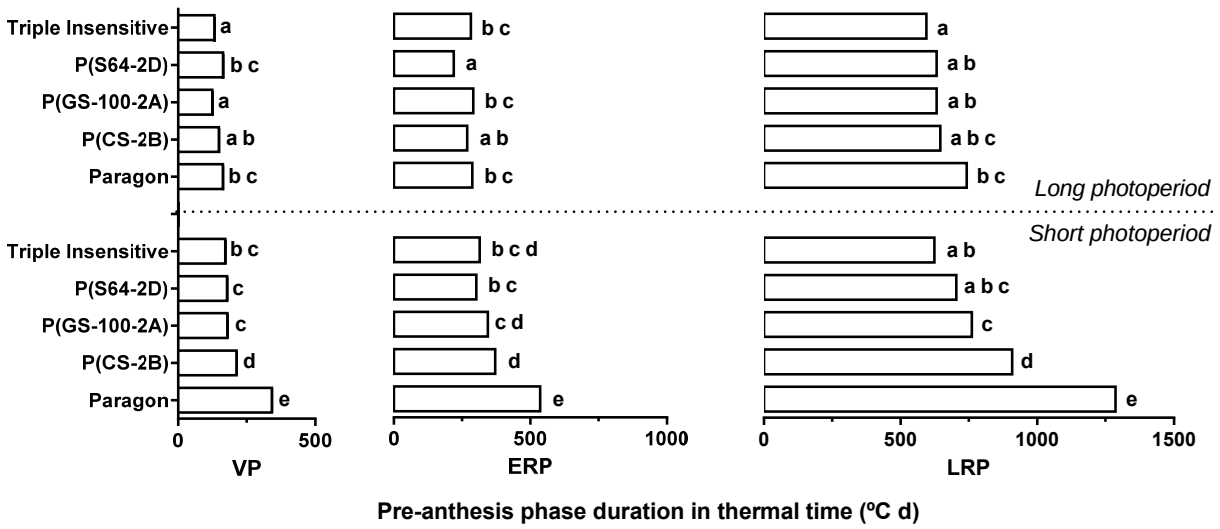

Figure 5: Pre-anthesis phases duration. Defined as vegetative phase (VP, left panel), early reproductive phase (ERP, central panel) and late reproductive phase (LRP, right panel). Bars with no shared letters for a specific phase are significantly different (Tukey α = 0.05). Each bar shows the average of two independent experiments.

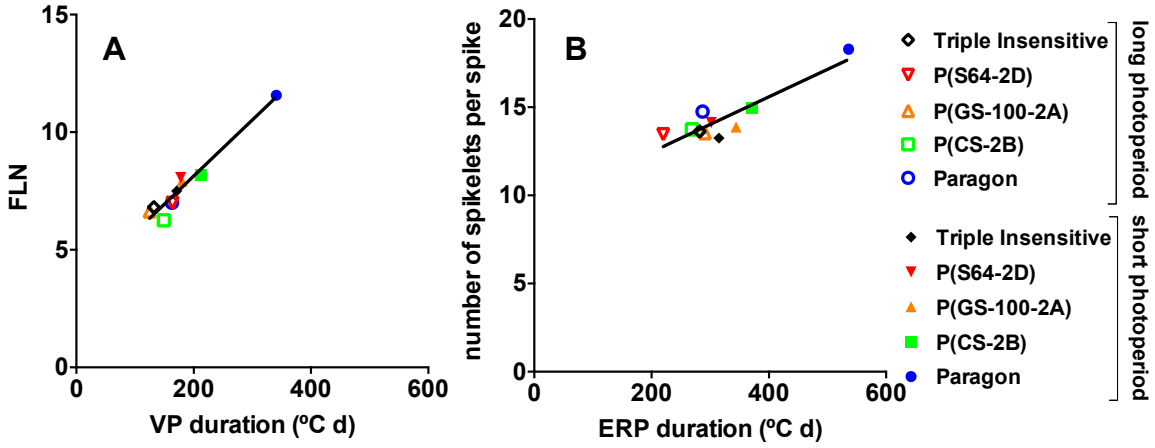

Figure 6: Relationship between number of structures differentiated and the duration of the phase during which they were differentiated. Panel a) final leaf number (FLN) and duration of the vegetative phase (VP), (R^2=0.95, P<0.001); and Panel b) number of spikelets per spike and duration of the early reproductive phase (ERP); (with Paragon 12h, R^2=0.825, P<0.001; without Paragon 12h, R^2=0.25, P<0.175).

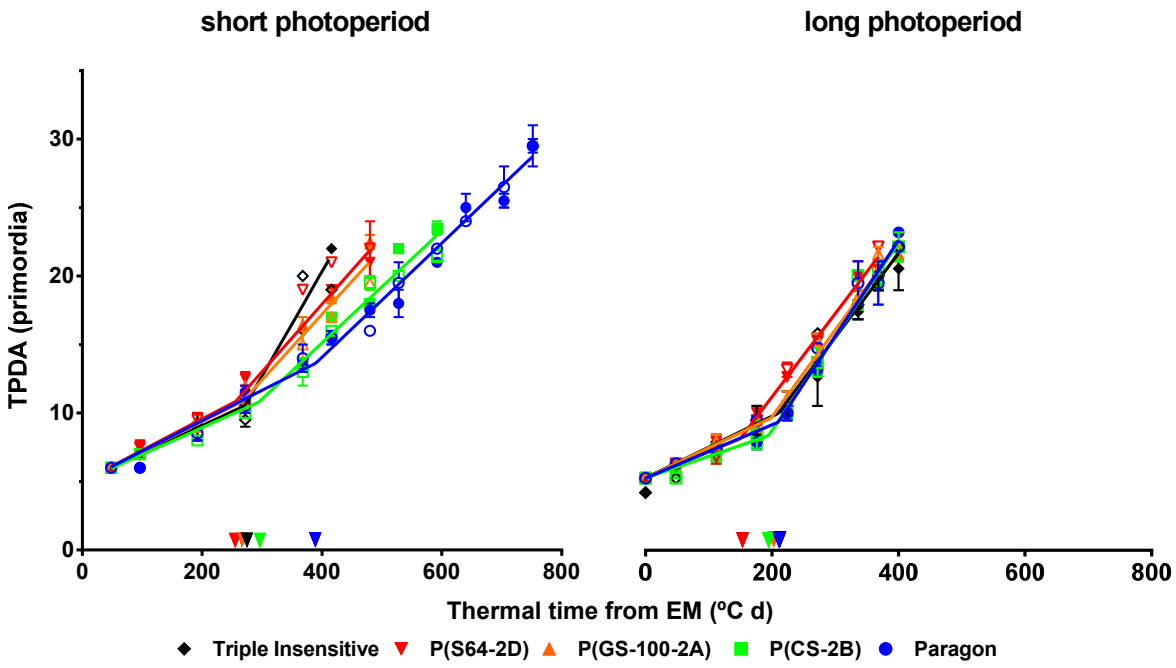

Figure 7: Primordia differentiation dynamics. Relationship between total primordia number differentiated in the apex (TPDA) and thermal time from EM in both short and long photoperiod (left and right panels). Each data-point is the average of the replicated plants determined in each sample in both Exp. 1 (closed symbols) and Exp. 2 (open symbols), and the error bars in each of them stand for the corresponding SEM. Data-points for each genotype and photoperiodic condition were fitted with a segmental linear regression (R^2 >0.98, P<0.001 in all cases). Error bars for each data-point stand for SEM (when not visible it was smaller than the diameter of the symbol). The model fitted yielded two primordia differentiation rates: a slower first slope –mostly for leaves–, and second faster one –mostly for spikelets. Arrowheads indicate timing of change of rate for each case.