Duration of developmental phases, and dynamics of leaf appearance and tillering, as affected by source and doses of photoperiod insensitivity alleles in wheat under field conditions

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ABSTRACT

Variation in photoperiod sensitivity in wheat plays a major role in the crop adaptation to wide agronomic environments. Photoperiod insensitivity is provided by *Ppd-Aa, Ppd-B1a* and *Ppd-D1a* alleles. Effects of the genome, doses and source of the particular *Ppd-1a* alleles on time to anthesis has not been analysed simultaneously in the same experiments, even less under field conditions; and the effects on particular phases rather than on considering only the total time to anthesis and on phyllochron have not been considered for this range of allele combinations. We carried out field experiments during two consecutive growing seasons to assess differences in time to anthesis, in its component phases, in final leaf number, phyllochron and tillering across wheat isogenic lines differing in specific *Ppd* alleles and homoeoalleles. In addition to confirming that the introgression of *Ppd-1a* alleles advanced anthesis time (on average by 307, 251 and 191ºC d if there were 3, 2 or 1 insensitivity alleles introgressed, respectively), we found that the variation between photoperiod insensitive genotypes was largely dependent on the varietal source of *Ppd-B1a* which could be stronger or weaker than *Ppd-D1a* depending on the donor considered. All components of time to flowering: the particular sub-phases ($P<0.001$) as well final leaf number ($P<0.001$) and phyllochron of late-appearing leaves ($P<0.05$) were sensitive to *Ppd-1a* alleles, but the strength of particular alleles on particular components was different, so that similar adjustments in time to anthesis could be achieved with different partitioning of developmental time between the considered phases. We also found that although they did not affect phyllochron of the first 7 leaves, that of the leaves appearing later was consistently reduced in lines carrying *Ppd-1a*. Tillering was sensitive too, but not final number of spikes due to compensations between tillering and tiller mortality.

Key words: Leaf number; anthesis; vegetative; reproductive; phyllochron

1. Introduction

Variation on flowering time allows the adaptation of crops to particular environmental conditions. Genetic control of flowering time (anthesis) in wheat is mainly regulated by photoperiod, vernalization and "earliness *per se*" genes (Slafer et al., 2015). Photoperiod response is determined by allelic variation at *Ppd-A1, Ppd-B1* and *Ppd-D1* genes (formerly *Ppd3*, *Ppd2* and *Ppd1*, respectively) located on the short arm of each homoeologous group 2 chromosome (Beales et al., 2007). Photoperiod insensitive wheats flower independently of day length, whilst sensitive wheats delay anthesis when grown under short days (Bentley et al., 2011). Photoperiod insensitive alleles are denoted by adding an *a* suffix, whilst photoperiod sensitive alleles carry a *b* suffix (McIntosh et al., 2003).

Alleles of *Ppd-1* are largely responsible for crop adaptation to mega-environments (Griffiths et al., 2009). In general, *Ppd-D1a* shows the strongest effect in reducing time to anthesis (e.g. Beales et al., 2007; Jones et al., 2017; Kiss et al., 2014; Snape et al., 2001; Yang et al., 2009; Worland, 1996). But the mode of action of the three *Ppd* genes are different. For instance, Tanio and Kato (2007) and Bentley et al. (2011) found that *Ppd-B1a* and *Ppd-A1a* also reduced time to anthesis, and Stelmakh (1998) reported that in their study *Ppd-A1a* showed stronger insensitivity than *Ppd-1* insensitive alleles of the B and D genome. As the ranking in strength of the particular *Ppd* genes is not universal, the source (donors) of the particular insensitivity alleles may play a significant role, beyond the particular genome in which it is introgressed. However, the effect of the source of alleles is largely undocumented. In general, the number of *Ppd-1a* alleles introgressed in the genome (doses) is accepted to be positively related with the strength of the reduction in time to anthesis (i.e. *Ppd* insensitivity alleles have additive effect). But this does not always happen: e.g. under constant short days, lines with two *Ppd* insensitivity mutations flowered earlier than lines with photoperiod insensitivity in only one genome, but not later than lines with insensitive alleles on all three genomes (Shaw et al., 2012). There are almost no studies comparing simultaneously lines with different doses of *Ppd-1a* alleles, and with different sources of some of the alleles which prevents concluding on the relative importance of the *Ppd* alleles, the doses and the source of the alleles in accelerating development towards anthesis under natural photoperiods in field grown plots. Most of what we know on the effects of *Ppd* genes on adaptation relates to the effects on time from sowing or seedling emergence to anthesis, considering it as a single whole period.

However, time to anthesis is composed of different developmental phases in which leaves, spikelets and florets, that will be later sources and sinks determining yield, are being formed (Slafer and Rawson, 1994). These are the vegetative (when leaf primordia are generated), the early reproductive (when spikelet primordia are formed) and the late reproductive phase (when firstly initiation and then degeneration of floret primordia take place). Evidence of genetic variation in duration of these phases as well as of environmental effects on them has been reported (Brown et al., 2013; García et al., 2011; Kirby et al., 1999; Sanna et al., 2014; Slafer and Rawson, 1994). Attempts have been made to identify QTLs related to the duration of specific sub-phases within the variation available in mapping populations (e.g. Borràs-Gelonch et al., 2012; Sanna et al., 2014). It has been far less documented to what degree *Ppd* alleles affect the duration of the phases composing time to anthesis (e.g. González et al., 2005b; Whitechurch and Slafer, 2002) and in these few cases the number of isogenic lines used was rather limited. In particular, the effect of different sources of specific *Ppd* alleles or dosage of *Ppd* genes on the duration of these sub-phases of time to anthesis are, to the best of our knowledge, non-existent. Recognising the effects of particular alleles on vegetative and reproductive phases is relevant not only academically but also potentially useful empirically. This is because there is a compelling range of evidences that whilst crop growth during the late reproductive phase is critical for yield, reducing growth during the vegetative and early reproductive phases does not affect it (e.g. Abbate et al., 1997; Demotes-Mainard and Jeuffroy, 2004; Ferrante et al., 2012; Fischer, 1985; Fischer, 2011; Fischer, 2016; González et al., 2005a; Savin and Slafer, 1991; Slafer, 2003). Therefore, it could be potentially useful for achieving genetic gains in yield to manipulate the durations of vegetative, early reproductive and late reproductive phases (Slafer et al., 2001; Miralles and Slafer, 2007), which could be facilitated if effects of particular alleles on specific phases composing time to anthesis were uncovered.

Photoperiod affects final leaf number (FLN) on main shoots of sensitive genotypes (e.g. Brooking et al., 1995) and in many cases most of the effect on time to heading or anthesis was explained by the effect on FLN (e.g. Brooking et al., 1995; Brooking and Jamieson, 2002; Slafer and Rawson, 1995a). This is in line with the pioneering work of Cooper (1956) who found that photoperiod sensitivity in wheat started from the appearance of first leaf, therefore not possessing a juvenile phase (e.g. Hay and Kirby, 1991; Slafer and Rawson, 1995a). In some models FLN was used to predict time to anthesis, assuming that phyllochron (reciprocal of rate of leaf emergence or the thermal time interval between the emergence of two consecutive leaves) is constant (e.g. Cao and Moss, 1989). However,

some studies demonstrated that phyllochron may vary in response to photoperiod particularly that of the later leaves (Slafer and Rawson, 1997). To what degree the effect of *Ppd* genes on time to anthesis might also be mediated through changes in phyllochron has been much less studied. The effects of *Ppd* genes on phyllochron may be relevant beyond its effects on time to anthesis. Tillering is critical for rapidly intercepting resources by the crop in early stages and determines the final spike number, which is a key component of yield. Tillering is controlled by both genetic and environmental factors (Assuero and Tognetti, 2010; Xie et al., 2016). Furthermore, tillering dynamics are related to the dynamics of leaf appearance (Kirby et al., 1985), but the degree of coordination between the appearance of tillers and leaves may be not constant (Alzueta et al., 2012). Therefore, it is possible that genes affecting phyllochron may also affect tillering dynamics. This likely effect of *Ppd* alleles has not been considered so far.

Therefore, the aim of this work was to quantify the impact of photoperiod insensitivity alleles and homoeoalleles, one of them from different donors, when introgressed in the A, B and/or D genomes of a photoperiod sensitive spring wheat variety on (i) the duration of vegetative and reproductive phases and (ii) the dynamics of appearance of leaves and tillers in field grown wheat near isogenic lines for *Ppd* genes.

2. Materials and methods

2.1. Experimental field conditions

Two field experiments were carried out during the 2012/13 and 2013/14 growing seasons nearby Bell-lloc d´Urgell (41.63°N, 0.78°E) in Catalonia, North-East Spain. In both experiments the soil was classified as a complex of *Calcisol petric* and *Calcisol haplic*, (FAO, 1990). Experiments were sown on 24 November 2012 and 12 November 2013 at a rate of 300 seeds m^{-2}. To minimise the experimental error, a week after emergence we labelled in each plot areas in which samples would have be taken later on in the season. In those areas we aimed to have 240 emerged seedlings per m^2, and when necessary we thinned by hand these areas to have that density with very high uniformity. To double-check this we counted number of adult plants at anthesis (that were 238±5 and 247±4 plants per m^2 in the first and second growing season, respectively). Weeds, diseases and insects were controlled through spraying herbicides, fungicides and insecticides as recommended by their manufacturers.

The mean daily global radiation (MJ m^{-2}), maximum and minimum temperature (°C) and accumulated precipitation (mm) were recorded daily by meteorological stations from the agro-meteorological network of Catalonia. Meteorological stations were located close to the experimental fields. Averaging across the growing season, temperature was *c.* 1ºC warmer in the second than in first growing season (which tended to be cooler than the average of the six previous years; Fig. 1). This difference in temperature between seasons was more marked in April and May (months in which the crop was reaching anthesis stage in all lines) than in earlier parts of the growing season. These particular months were clearly warmer (*c.* 3ºC) in the second than in the first growing season (Fig. 1). Although both growing seasons had a higher accumulated precipitation than the average of the six previous years (264.3 and 303.0 mm for first and second growing season, respectively compared with the average of six previous years 246.5 mm), in order to avoid water stress in critical stages of development, rainfall was supplemented with irrigation. In the first growing season, that had a wetter spring, only one irrigation was given on 16 April 2013. In the second, drier, growing season the field was irrigated both on 26 March and 3 May 2014. Each irrigation consisted of 80 l m^{-2}.

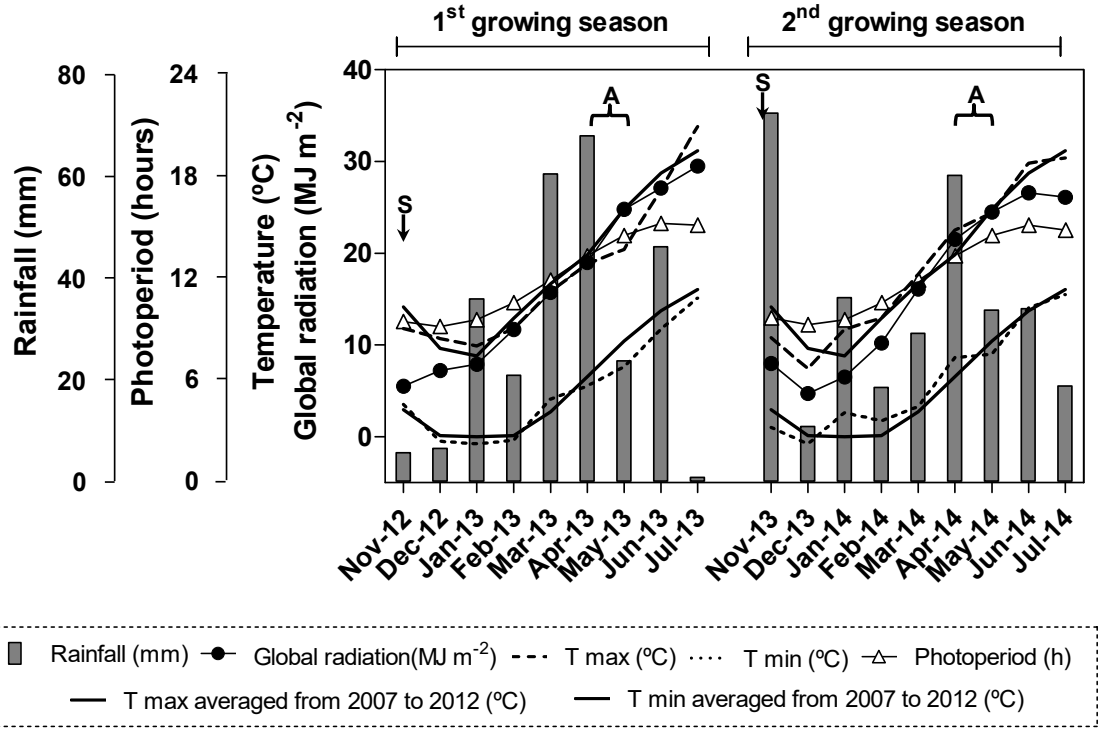


Fig. 1. Accumulated rainfall, maximum and minimum temperature, photoperiod and global incident radiation for monthly periods during the two growing seasons (2012/13, left and

153 2013/14, right). The arrow with the "S" indicates sowing date and the square bracket with
154 the "A" shows the range of dates in which anthesis was reached.

155

156 *2.2. Treatments and design*

157 Treatments consisted of 12 near isogenic lines (NILs; i.e. lines differing in genes of interest
158 but with the same genetic backgrounds) carrying *Ppd-1a* alleles of hexaploid wheat
159 developed at the John Innes Centre (Norwich, UK), according to Shaw et al. (2013). The
160 13 experimental genotypes were the "wild type" Paragon (a wheat cultivar with a strong
161 sensitivity to photoperiod but insensitive to vernalization) and 12 NILs in which *Ppd* alleles
162 were introgressed on chromosome 2 of the A, B and/or D genomes of the wild type (Table
163 1).

164 A sample of the *Ppd-1* homoeoallic was represented by these alleles (i) *Ppd-A1a* from "GS-
165 100" (carrying a 1027 bp promoter deletion) (Wilhelm et al., 2009); (ii) *Ppd-B1a* from
166 either "Chinese Spring" (characterized by one truncated copy and three intact copies in
167 tandem in a 185 kb region), "Sonora 64" (that has three intact copies in tandem), or from
168 "Recital 64" (that presents two intact copies in tandem) (Beales et al., 2007); and (iii) *Ppd-*
169 *D1a* from "Sonora 64" (that has a 2089 bp promoter deletion) (Beales et al., 2007).

Table 1. Near isogenic lines (NILs) with introgressed *Ppd* alleles (indicating the source of insensitive alleles introgressed into the wild type) and the wild type Paragon used in this study. The dose (number of photoperiod insensitivity alleles) introgressed in the wild type, indicating the *Ppd* alleles in each chromosome, and code to name the individual lines throughout this paper are shown (to facilitate the visual impact insensitive alleles are shown in bold type).

Genotypes (Lines; source of "*a*" alleles)	Dose of insensitive alleles	*Ppd* alleles	Code[1]
Paragon (wild type)	0	*Ppd-A1b*, *Ppd-B1b* and *Ppd-D1b*	A_P+B_P+D_P
GS-100 2A	1	***Ppd-A1a***, *Ppd-B1b* and *Ppd-D1b*	$\mathbf{A_{GS}}$+B_P+D_P
Chinese Spring 2B		*Ppd-A1b*, ***Ppd-B1a*** and *Ppd-D1b*	A_P+$\mathbf{B_{CS}}$+D_P
Sonora 64 2B		*Ppd-A1b*, ***Ppd-B1a*** and *Ppd-D1b*	A_P+$\mathbf{B_S}$+D_P
Recital 2B		*Ppd-A1b*, ***Ppd-B1a*** and *Ppd-D1b*	A_P+$\mathbf{B_R}$+D_P
Sonora 64 2D		*Ppd-A1b*, *Ppd-B1b* and ***Ppd-D1a***	A_P+B_P+$\mathbf{D_S}$
GS-100 2A+CS 2B	2	***Ppd-A1a***, ***Ppd-B1a*** and *Ppd-D1b*	$\mathbf{A_{GS}}$+$\mathbf{B_{CS}}$+D_P
GS-100 2A+ Sonora 64 2B		***Ppd-A1a***, ***Ppd-B1a*** and *Ppd-D1b*	$\mathbf{A_{GS}}$+$\mathbf{B_S}$+D_P
GS-100 2A+ Sonora 64 2D		***Ppd-A1a***, *Ppd-B1b* and ***Ppd-D1a***	$\mathbf{A_{GS}}$+B_P+ $\mathbf{D_S}$
Chinese Spring 2B+ Sonora 64 2D		*Ppd-A1b*, ***Ppd-B1a*** and ***Ppd-D1a***	A_P+$\mathbf{B_{CS}}$+$\mathbf{D_S}$
Sonora 64 2B +Sonora 64 2D		*Ppd-A1b*, ***Ppd-B1a*** and ***Ppd-D1a***	A_P+$\mathbf{B_S}$+ $\mathbf{D_S}$
GS-100 2A+ Chinese Spring 2B+Sonora 64 2D	3	***Ppd-A1a***, ***Ppd-B1a*** and ***Ppd-D1a***	$\mathbf{A_{GS}}$+$\mathbf{B_{CS}}$+$\mathbf{D_S}$
GS-100 2A+Sonora 64 2B+Sonora 64 2D		***Ppd-A1a***, ***Ppd-B1a*** and ***Ppd-D1a***	$\mathbf{A_{GS}}$+$\mathbf{B_S}$+$\mathbf{D_S}$

[1]this code denotes the genomes with sensitivity- (in plain text) or insensitivity-alleles (in bold type), indicating the source of such alleles (subscript; P=Paragon, GS=GS 100, CS=Chinese Spring, S=Sonora 64). In the case of the sensitive alleles the source was always the wild type Paragon.

All in all, there were five NILs with only one insensitive allele introgressed, another five NILs with two insensitive alleles and two NILs with the three insensitive alleles, and for some of the cases alternative insensitivity alleles on the B genome from different source cultivars (Table 1). For the purpose of simplicity we refer to particular lines using a code considering whether the allele was sensitive or insensitive in each genome (plain font or bold font when designating the genome, respectively) and the source of the allele as a subscript (Table 1).

Treatments were arranged in a complete randomized design with different number of replications (ranging from one to five replications, depending on availability of seeds) in 2012/13 and in a randomized complete block design with three replications in 2013/14 Experimental plots measured 1.2 x 4 m. Row spacing was 0.2 m.

2.3. Measurements and analyses

Phenological stages of seedling emergence (stage DC10), flag leaf emergence (stage DC39) and anthesis (stage DC65) were determined according the Decimal Code developed by Zadoks et al. (1974). Two other phenological stages were critical for this study: the timing of floral initiation and of terminal spikelet. To determine these stages, plants sampled at random from each plotwere frequently dissected, and the accumulated number of primordia was recorded. The timing of floral initiation measured in thermal time was determined *a posteriori* as the time when the number of primordia initiated in the apex exceeded the final number of leaves emerged on the main shoots (see below). Timing of terminal spikelet was determined as in Waddington et al. (1983). With these data the duration of the whole cycle to anthesis as well as that of its component phases (vegetative, until floral initiation; early reproductive, from then to terminal spikelet; and late reproductive, from terminal spikelet to anthesis) were computed in thermal time (thermal time was calculated as mean air temperature and with base temperature of 0°C, Kirby et al., 1985) for each plot.

At seedling emergence, three plants per plot were selected to represent the particular treatment, minimising the noise from changes in the competitive conditions within and among plots, which could be relevant for tillering dynamics. These plants have to be (i) at the expected plant density in its surrounding area, (ii) uniformly distributed, and (iii) emerged when 50% of emergence of the plot occurred. For that purpose we selected within a week after emergence these three plants in that condition within each plot and

labelled them individually. From then on, we monitored leaf and tiller appearance. From seedling emergence to anthesis, the number of leaves appeared on the main shoot (Haun, 1973) and the number of shoots were recorded once or twice weekly, depending on temperature on these plants. After the appearance of 3-4 leaves, the last expanded leaf in that main shoot was marked to avoid miscounting the accumulated number of leaves due to senescence of the oldest or not recognising the main shoot after the onset of tillering. Throughout the cycle, from seedling emergence to anthesis there were 21 determinations of leaf and tiller number in each of the three plants measured. Means were calculated for each sampling date and plot. No distinction was made between tiller categories, only the total number of tillers. With these values the dynamics of leaf appearance and tillering were analysed for each line.

Dynamics of leaf appearance were obtained by plotting the cumulative number of leaves appeared in the main shoot (Haun, 1973) against thermal time. Although the coefficient of determination for the linear relationship between leaf number and time was statistically significant in all cases, data distribution provided clear evidence of bilinear trends (early leaves appeared at a faster rate than late leaves), and therefore data were fitted using bilinear regression. Phyllochron for the early and late appearing leaves was estimated as the reciprocal of the slopes of the reciprocal bi-linear regressions (thermal time *vs* emerged leaves). So, instead of simply computing the reciprocal of the slope of the dynamics of leaf appearance, we obtained the values of phyllochron together with standard errors which we could then use to establish whether differences in this trait between lines were significant or not. The timing of the break in rate of leaf appearance was fitted to coincide with the emergence of the 7^{th} leaf. This assumption is based on reports the change in phyllochron occurs when the Haun stage is between 6 and 8 (e.g. Calderini et al., 1996; González et al., 2005b; Jamieson et al., 1995; Slafer and Rawson, 1997); and assuming that this threshold is applicable across a very wide range of conditions (Miralles et al., 2001). Thus, the phyllochron of early leaves refers to the pace of leaf appearance corresponding to the first 7 leaves, whilst phyllochron of late leaves refers to that of leaves appearing from then to the flag leaf.

Dynamics of tillering (including tillering and tiller mortality) were analysed using a tetralineal model, as in Alzueta et al. (2012). It has an intercept indicating the onset of tillering, a first upward slope representing the rate of tillering, the maximum number of tillers and the time when this occurs, the onset of tiller mortality, the rate of tiller mortality

241 and lastly the final number of living tillers, which will become the number of spikes. To
242 assess any possible effects on the coordination between tillering and leaf emergence, tiller
243 dynamics was also analysed against the number of emerged leaves, determining the onset
244 of tillering (in terms of number of leaves, or phyllochrons, at the onset of tillering) and
245 tiller appearance rate per emerged leaf.

246 A mix model was fitted for all the analysed traits using statistical software JMP® Pro
247 Version 12.0 (SAS Institute Inc. Cary, NC, USA). Growing seasons, genotypes, and their
248 interaction were considered fixed, whereas blocks nested within growing seasons were
249 identified as random. Differences between *Ppd-1* alleles were tested using analysis of
250 LSMeans Contrast and of Student´s *t* test with significance at $P<0.05$.
251
252
253 **3. Results**

254 There were large environmental (growing seasons) and genotypic differences in most
255 variables measured, indicating the major effect of *Ppd-1a* alleles had on developmental
256 patterns of wheat. These genotypic effects were not restricted to those in total time to
257 anthesis and final leaf number, which are well known, but also highly significant for each
258 of the component phases of time to anthesis (Table 2). The genotype by growing season
259 (GxGS) interaction was also statistically significant in most of the traits analysed; but its
260 magnitude was always smaller than that of the genotypes (Table 2). Considering the two
261 most integrative traits analysed (time to anthesis and final leaf number), the magnitude of
262 the mean squares corresponding to the genotypic effects was between 10 and 25-fold
263 larger than those of the GxGS interaction (Table 2). Although the magnitude of the
264 difference was not that large, the mean squares of the genotypic effects were also
265 consistently greater than those of the GxGS interaction for the duration of the three
266 component phases determining the time to anthesis (Table 2).
267
268 **Table 2.** Mean squares of the effects of the growing season, the genotypes and their interaction
269 on the main traits analysed: (i) thermal time from sowing to anthesis, (ii) duration of its three
270 component phases (vegetative, early reproductive and late reproductive), and (iii) final leaf
271 number.

Source of variation	df	Sowing to anthesis (ºC d)	Vegetative (ºC d)	Early reproductive (ºC d)	Late reproductive (ºC d)	Final leaf number (leaves)

Growing season	1	137,910***	9,679**	201,192***	95 ns	7.02***
Genotypes	12	39,811***	4,093***	11,861***	5,611***	1.25***
GxGS	12	1,517**	3,088***	5,252***	3,230**	0.13 ns

Asterisks indicate the significance level of the F-ratio (**P<0.05, **P<0.01, ***P<0.001*, and ns-non significant).

Therefore, even though the GxGS interaction was statistically significant in many cases, the interaction was not a crossover one: i.e. it was quantitative (the magnitude of the difference between genotypes with different *Ppd-1a* alleles was different between the two growing seasons) but not qualitative (the direction of the effect was the same in both seasons). This can be clearly illustrated with time to anthesis. The cycle was consistently longer in the second growing season but within growing seasons the genotypic effects were rather consistent, and the main reason for the significant GxGS interaction was that in the first season there was not a significant difference between NILs with 2 or 3 *Ppd-1a* alleles, whilst in the second growing season the difference was significant (Supplementary Fig. FS1).

Consequently, in spite of the fact that the GxGS interaction was statistically significant for most traits the effects of the doses, genomes and sources of *Ppd-1a* alleles can be reliably considered averaged across the two growing seasons.

3.1. Duration of time to anthesis

Genotypes carrying insensitive alleles took significantly less time to reach anthesis than wild type-Paragon (Fig. 2, left panels). In general, the larger the dose of insensitivity alleles the stronger the effect on reducing total time to anthesis (Fig. 2, left panels), although the acceleration of developmental rates produced by introgressing *Ppd-1a* alleles was less than proportional with the increase in doses: introgressing 1 allele reduced time to anthesis by *c.* 190ºC d whilst each extra doses advanced anthesis by *c.* 60ºC d more (Fig. 2, left panels).

The strength in reducing time to anthesis of *Ppd-1a* alleles in each particular genome exhibited a clear trend for the insensitivity in the A and D genome to be stronger than that in the B genome (strength of the alleles was *Ppd-D1a*≥*Ppd-A1a*>*Ppd-B1a*, where ≥

indicates that the effect was stronger arithmetically but the difference was not statistically significant at $P<0.05$ (Fig. 2, top middle panels). When comparing the cases in which two doses were introgressed, the stronger effect of insensitivity from the A and D genomes, compared with that in B was confirmed: insensitivity from A and D homoeoalles together was stronger than that conferred by combinations of insensitivity homoeoallele carried on the A and B genomes or the B and D genomes (Fig. 2, bottom middle panels). When comparing allelic effects we fully acknowledge the possibility that alleles linked at the introgressed locus and random background segments maintained in spite of five rounds of backcrossing (~3% donor background expected) might contribute to variation in the observed traits. Although this could be reduced by further backcrossing and the identification of close recombinants it is a question always likely remain using traditional genetic approaches. For future work we propose the use of gene editing technologies to recreate these alleles with no linkage or background effects (Doudna and Charpentier, 2014; Kumar and Jain, 2014; Ran, 2014).

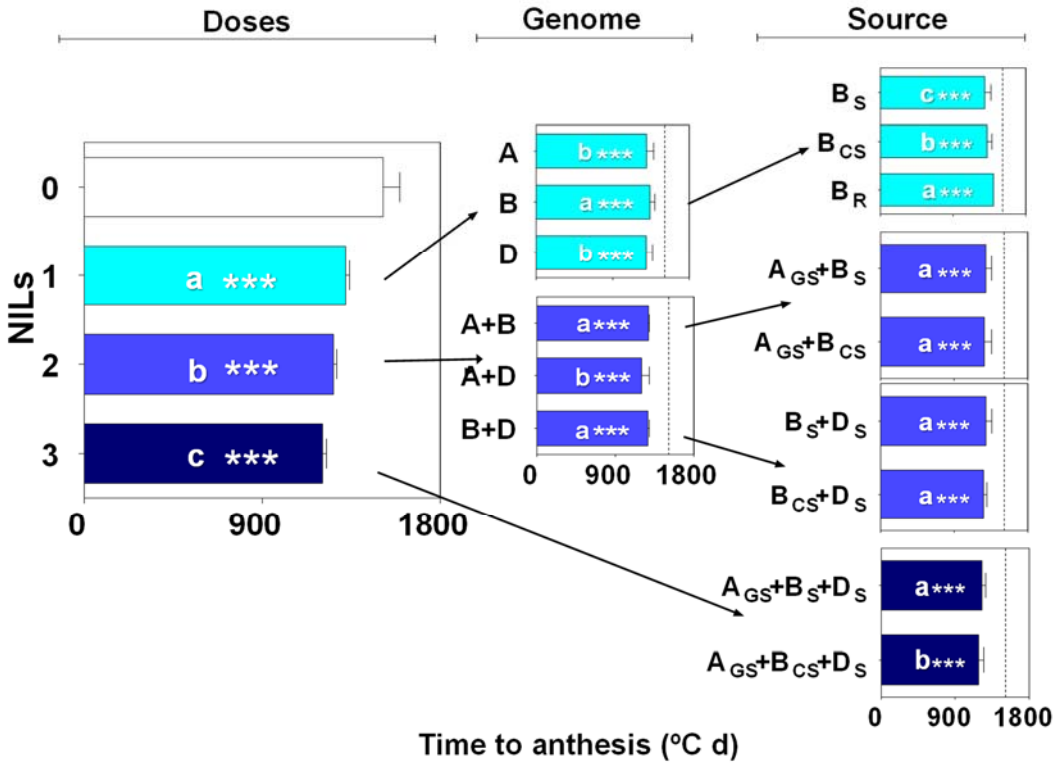


Fig. 2. Duration of the period from sowing to anthesis of the different *Ppd-1a* NILs and the wild type-Paragon (open bar on the left panels, dotted lines in all other panels). On the left the durations are averaged across lines with the same doses of insensitive *Ppd* homoeoalleles including the wild type Paragon with all three alleles sensitive to photoperiod. In the middle, the durations are

identified for the genomes in which the insensitivity was introgressed, in the case of the B genome averaged across the different sources of the allele. On the right the durations are shown for each of the different sources used as donors of *Ppd-B1a* alleles. Asterisks indicate the statistical significance of the differences between lines with insensitivity alleles and Paragon from the LSmeans contrast (*$P<0.05$, **$P<0.01$, ***$P<0.001$ and ns-non significant). Different letters within bars indicate that the differences between lines with different doses of *Ppd-1a* alleles (left), or between lines with different genomes with insensitivity, within doses (middle), or between lines with different sources of *Ppd-B1a* alleles, within doses and genomes (right) are statistically significant ($P<0.05$). Segments in each bar stand for the standard error of the means used to calculate the time to anthesis in each case.

To take into account the effect of the source of the *Ppd-1a* alleles within the same genome, we explored three different sources for the *Ppd-B1a* alleles. *Ppd-B1a* from Chinese Spring was stronger than that from Recital but weaker than that from Sonora 64 (Fig. 2, top right panels). Thus the actual ranking of strength of *Ppd-A1a*, *Ppd-D1a*, and *Ppd-B1a* may well depend more on the source of the specific *Ppd-1a* allele, including flanking genes, than on the genome. Ranking the strength of the studied *Ppd-1a* alleles for the NILs with only one dose was $Ppd\text{-}B1a_S \geq Ppd\text{-}D1a \geq Ppd\text{-}A1a \geq Ppd\text{-}B1a_{CS} > Ppd\text{-}B1a_R$, indicating that *Ppd-B1a* could be the strongest or the weakest of all the *Ppd-1a* analysed in the present study, depending on the source of the allele. For all of the B genome alleles insensitivity is the result of increased copy number. The earliness of $Ppd\text{-}B1a_S$ compared to $Ppd\text{-}B1a_R$ might simply be due to three intact copies versus two. However, $Ppd\text{-}B1a_{CS}$ carries three intact copies and one truncated. Diaz et al 2012 found a similar ranking for the *Ppd-B1a* alleles in the same lines grown under short days. Considering the double and triple insensitive lines, we used two different sources, and in the case of the lines with two *Ppd-1a* alleles introgressed there was no difference depending on whether the *Ppd-B1a* was from Chinese Spring or Sonora 64 (Fig. 2, middle right panels). And, when considering the NILs with *Ppd-1a* alleles in all three genomes, the strength reverted to what was found in the NILs with a single insensitivity allele: *Ppd-B1a* from Chinese Spring was stronger than that from Sonora 64 (Fig. 2, top bottom right panels), revealing that *Ppd-1a* homoeoalleles might display epistatic interaction effects.

3.2. Duration of vegetative and reproductive phases

The introgression of *Ppd-1a* alleles reduced significantly the duration of the vegetative phase respect to that of Paragon, but there was no clear effect of the doses: the reduction was similar with 1, 2 or 3 *Ppd-1a* alleles, *c*. 70ºC d (Fig. 3, top row left panel). The particular genome in which the *Ppd-1a* alleles were introgressed did not affect

356 consistently the magnitude of the effect on the duration of the vegetative phase in the
357 NILs with a single dose, whilst in the NILs with two alleles introgressed it seemed that
358 the joint action of the alleles in genomes A and D were stronger than when the genome B
359 was involved (Fig. 3, top row middle panels). Similarly, in the case of the total time to
360 anthesis, the source of the allele seemed to have been paramount. The duration of the
361 vegetative phase was significantly reduced when the *Ppd-B1a* alleles were obtained from
362 Sonora 64 or Chinese Spring acting as donors, with no significant differences in strength
363 between them, but it was not significantly affected when the *Ppd-B1a* allele came from
364 Recital (Fig. 3, top row right panels). Therefore, a ranking of strength of *Ppd-1a* alleles
365 to reduce the duration of the vegetative phase, considering the NILs with a single dose,
366 was *Ppd-B1a*$_S$≥*Ppd-B1a*$_{CS}$≥*Ppd-D1a*≥*Ppd-A1a*>*Ppd-B1a*$_R$.
367 On the other hand, when *Ppd-1a* alleles were introgressed in only one genome they did
368 not affect significantly (*P=0.15*) the duration of the early reproductive phase (Fig.3,
369 middle row left panel). When the photoperiod insensitivity alleles were introgressed in
370 two or three genomes simultaneously they reduced the length of this phase in 132°C d
371 with respect to the wild type (with no differences between lines with two or three
372 photoperiod insensitivity mutations introgressed). Analysing *Ppd-1a* alleles in each
373 genome separately, it may be suggested that the overall effect of *Ppd-1a* alleles in one
374 genome did not affect the early reproductive phase due to the fact that lines with *Ppd-B1a*
375 or *Ppd-D1a* were similar to the wild type-Paragon, although *Ppd-A1a* shortened this
376 phase (*P=0.03*)(Fig. 3, middle row middle panels). The lack of significant effects of a
377 single dose of *Ppd-B1a* reflected a lack of significant effect of all three sources considered
378 for this allele (Fig. 3, middle row right panels). Although *Ppd-B1a*$_S$ tended to have a
379 stronger effect than A and D genome homoeoalleles when considering a single
380 insensitivity allele, the analysis of the double and triple insensitivities did not confirm
381 that trend (Fig. 3, middle row right panels). Ranking *Ppd-1a* alleles for their strength to
382 reduce the duration of the early reproductive phase was *Ppd-A1a*>*Ppd-B1a*$_S$≥*Ppd-D1a*≥
383 *Ppd-B1a*$_{CS}$>*Ppd-B1a*$_R$, when considering the NILs with only one *Ppd-1a* allele
384 introgressed.
385 When considering the late reproductive phase, again the introgression of *Ppd-1a* alleles
386 reduced significantly the duration of the phase and the effect was similar with 1 or 2 *Ppd-*
387 *1a* alleles introgressed (by *c*. 60ºC d), but when these alleles were in all the genomes the
388 reduction (*c*. 90ºC d) was significantly stronger than when there were 1 or 2 doses (Fig.
389 3, bottom row left panel). The introgressed insensitive homoeoallele affected: the late

reproductive phase, which was slightly reduced by *Ppd-A1a* (*P<0.10*), while it was more clearly reduced by *Ppd-B1a* and particularly *Ppd-D1a* alleles (Fig. 3, bottom row middle panels). The fact that lines carrying *Ppd-D1a* showed the strongest expression of this trait was consistent with results of the NILs with two doses, in which the strongest effect was observed when *Ppd-D1a* acted together with *Ppd-A1a* (followed by *Ppd-A1a* + *Ppd-D1a* and then *Ppd-A1a* + *Ppd-B1a*). When considering the effect of the source of the *Ppd-B1a* allele, when the donor was Recital this phase was not shortened. Reduction was most apparent when the insensitivity came from Chinese Spring and intermediate when the donor was Sonora 64, and this trend was confirmed in most (though not all) the other NILs with 2 and 3 insensitivity alleles (Fig. 3, bottom row right panels). Therefore, a ranking of strength of *Ppd-1a* alleles to reduce the late reproductive phase, considering the NILs with a single dose, was *Ppd-D1a*≥*Ppd-B1a*$_{CS}$ ≥*Ppd-B1a*$_{S}$> *Ppd-A1a* >*Ppd-B1a*$_{R}$.

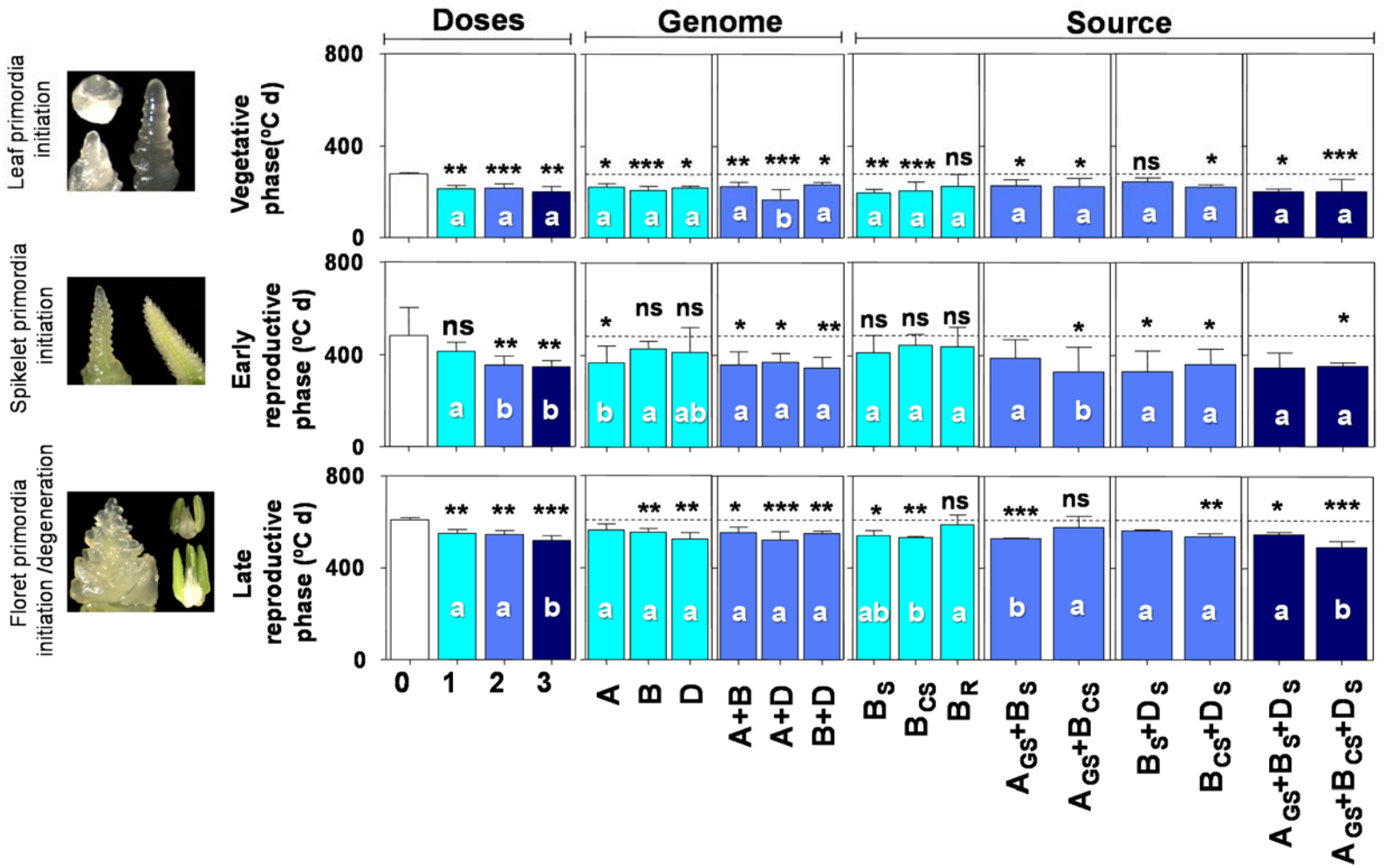


Fig. 3. Duration (in thermal time) of the three component phases of time to anthesis: the vegetative (from seedling emergence to floral initiation; top row of panels), the early reproductive (from floral initiation to terminal spikelet; middle row of panels) and the late reproductive (from terminal spikelet to anthesis; bottom row of panels) of different NILs and wild type- Paragon (open bars on left panels, dotted lines in other panels) averaged across growing seasons. Left panels represent the durations averaged across lines with the same doses of insensitive *Ppd* alleles including the wild type Paragon. In the intermediate panels, the durations are identified for the genomes in which the insensitivity was introgressed, in the case the B genome averaged across the different sources of the allele. In the right panels the durations are shown for each of the different sources used as donors of the *Ppd-B1a* allele. Asterisks indicate the statistical significance of the differences between lines with insensitivity alleles and Paragon from the LSmeans contrast (*$P<0.05$, **$P<0.01$, ***$P<0.001$ and ns-non significant, if nothing is noted on top of a bar it implies that $P<0.10>0.05$). Different letters within bars indicate that the differences between lines within panels are statistically significant ($P<0.05$). Segments in each bar stand for the standard error of the means used to calculate the durations in each case.

As when considering the whole set of NILs the insensitivity alleles reduced all the phases considered, the relationship between the total time to anthesis and the duration of each phase was in all cases positive and significant (Fig. 4, left panel). However, it seemed that overall combinations of doses, genomes and sources of *Ppd-1a* alleles the insensitivity to photoperiod accelerated development towards anthesis more through accelerating the early reproductive phase than the other two phases considered. This is not based on the marginally larger coefficient of correlation but on the clearly smaller slope, which implies that the absolute variation in duration of this phase was larger than that of the vegetative and late reproductive phase (Fig. 4, left panel). If the regressions are analysed reducing the variation from specific *Ppd-1a* alleles, averaging the variables across doses of these alleles introgressed, the proportion of the variation in time to anthesis explained by each of the component phases increased noticeably (Supplementary Fig. FS2), reinforcing the perception that all three component phases are similarly responsible for the response of time to anthesis to the introgression of *Ppd-1a* alleles.

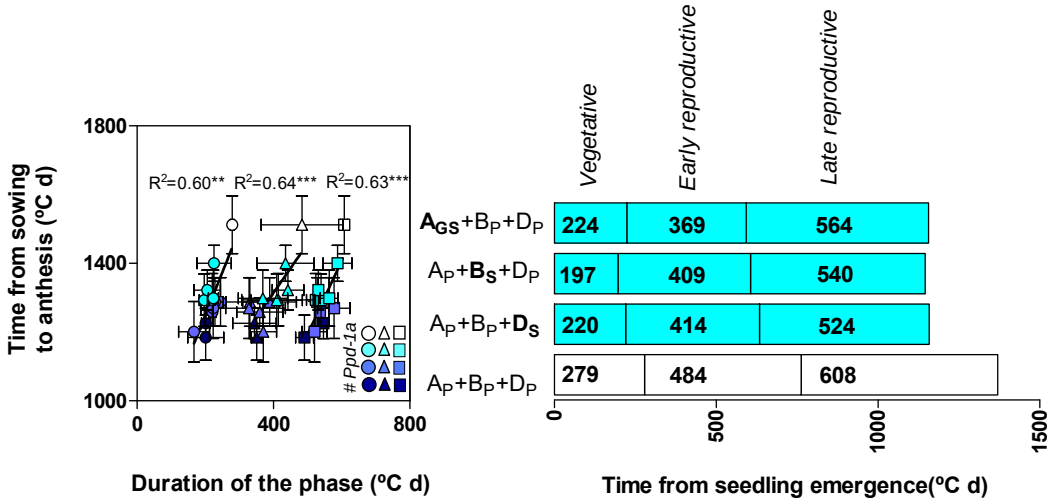


Fig. 4. Left panel: relationship between time from sowing to anthesis and the duration of the three component phases (vegetative, circles; early reproductive, tringles; and late reproductive, squares) for Paragon (open symbols) and the NILs with 1, 2 or 3 doses of *Ppd-1a* alleles (pale, intermediate and dark symbols). The coefficient of determination (R^2) and the level of significance (***P<0.01*, ****P<0.001*) were included. Right panel: duration of the three phases (vegetative, early reproductive and late reproductive, from left to right) in Paragon (open bars) and in three of the NILs with a single introgression of *Ppd-1a* alleles (pale bars). Figures have inside each phase stand for its duration in thermal time.

However, this observation only holds true when considering the effects of overall combinations of doses, genomes and sources of *Ppd-1a* alleles analysed in Fig. 4 (left

panel). More than 35% of variation in time to anthesis was associated with differences in durations of the phases produced by particular *Ppd-1a* alleles (Fig. 4, right panel).

To illustrate this issue with a simple comparison we selected three cases with a single *Ppd-1a* allele introgressed in each of the three genomes in which time from seedling emergence to anthesis was similarly reduced (from *c*. 1370 in Paragon to *c*. 1150ºC d in each of these three NILs) but the partitioning of the time to anthesis into different component phases was quite different (Fig. 4, right panel). When the single dose of *Ppd-1a* introgressed was that from the genome A of GS-100 the reduction in time to anthesis was strongly linked to that of the vegetative (*c*. 20%) and early reproductive phases (*c*. 25%) while the late reproductive phase was only marginally reduced (<10% and only statistically significant at $P<0.10$; see Fig. 3). In contrast, when the allele was donated from the D genome of Sonora 64 the duration of the early reproductive phase was less reduced (*c*. 15% and not statistically significant; see Fig. 3) with the partitioning of accelerated development shifted to the late reproductive phase (*c*. 15% and highly significant; see Fig.3). The effect of *Ppd-B1a* from Sonora 64 had intermediate effects on both reproductive phases and the strongest reduction in the duration of the vegetative phase (Fig. 4, right panel).

3.3.Final leaf number, phyllochrons and its coordination with anthesis

Photoperiod insensitivity alleles caused a decrease in final leaf number (FLN) that was significant in all NILs except one (Table 3). As doses of *Ppd-1a* alleles increased, the magnitude of the reduction in FLN increased as well (Table 3). However, there was not a consistent trend for the strength of *Ppd-1a* of the different genomes for reducing FLN. Analysing the cases with a single dose of these alleles, *Ppd-A1a* and *Ppd-D1a* exhibited the same FLN (*c*. 1 less leaf than Paragon) and *Ppd-B1a* showed more or less effect depending on the specific allele but taking these alleles together it can be said that *Ppd-B1a* had similar strength to that of the other two genomes (Table 3). Regarding the effect of the source of *Ppd-B1a* alleles a clear difference was seen in the single introgression NILs. *Ppd-B1a*$_R$ did not significantly reduce FLN, the reduction was significant at $P<0.05$ for *Ppd-B1a*$_{CS}$ and at $P<0.01$ for *Ppd-B1a*$_S$ (Table 3). However, when combined with *Ppd-A1a* and/or *Ppd-D1a* alleles in NILs with 2 or 3 doses of insensitivity the effect of the source of *Ppd-B1a* (Sonora 64 *vs* Chinese Spring) disappeared (Table 3). Analysing the photoperiod insensitivity alleles introgressed in a single genome, the same ranking of strength was observed as that seen for time to anthesis: *Ppd-B1a*$_S$≥*Ppd-D1a*≥*Ppd-*

A1a>*Ppd-B1a*$_{CS}$>*Ppd-B1a*$_{R}$. Consequently, most (>85%) of the effects of these alleles on time to anthesis were due to their effects on FLN (Fig. 5, left panel).

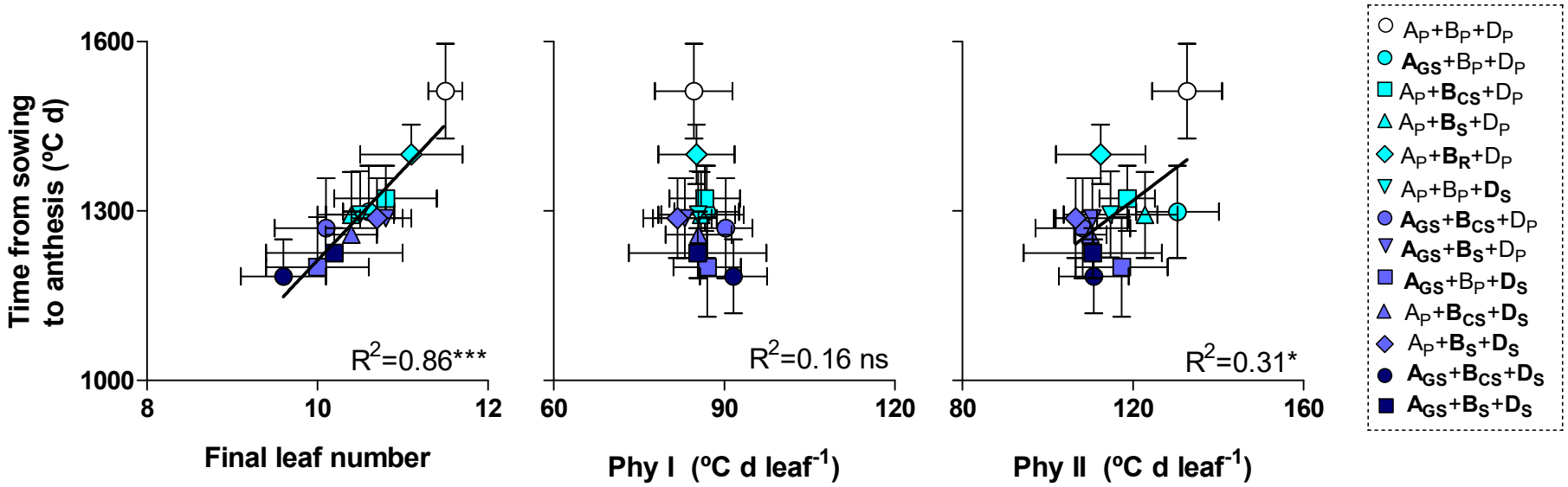
In order to explore whether the *Ppd-1a* alleles affected the dynamics of leaf appearance, in addition to their effects in the number of leaves that appeared before heading and anthesis, we plotted for each case the Haun stage against time, as illustrated for the insensitive wild type and for the NILs with extreme effects on FLN (Supplementary Fig. FS3): A_P+**B_R**+D_P, which reduced FLN marginally and not significantly and **A_{GS}+B_{CS}+D_S**, which produced the strongest reduction in FLN (Table 3). As can be seen in the extreme cases chosen for illustrating the dynamics of leaf appearance, although the coefficient of determination of a linear regression between leaf number and time would have been rather high (ranging from 0.976 to 0.995), using such regression would have not left the residuals distributed at random: in all cases a significant quadratic pattern between residuals and time were evident (Fig. FS3, middle panels). This quadratic component of the regression between residuals of Haun stage revealed clearly that the dynamics of leaf appearance are better represented using a bi-linear model, which does produce a random distribution of residuals (Fig. FS3, right panels). The use of bi-linear regressions increased the coefficient of determination of the relationships between leaf number and time, ranging from 0.978 to 0.998. The bi-linear regression used gave two slopes: quantifying the rate of leaf appearance of the early (first slope) and late leaves (second slope); and the reciprocal of these rates was the phyllochron for early and late leaves. The introgression of *Ppd-1a* alleles did not affect phyllochron of the early leaves (Table 3). The phyllochron of the late leaves was reduced by insensitivity to photoperiod, particularly with 2 and 3 doses of *Ppd-1a* alleles , whilst the effect of a single dose was smaller and only in few cases significant (although the average of all NILs with a single dose showed a phyllochron significantly shorter than Paragon; Table 3). Thus, in general, the higher the dose of insensitivity alleles the stronger the reduction in phyllochron of late leaves up to two doses, there was no significant difference between lines with double or triple insensitivity (Table 3). There was not a clear and consistent effect of any of the particular genomes in which the *Ppd-1a* alleles were introgressed or of the source from where the alleles were introgressed (Table 3).

Table 3. Phyllochron for early (Phyll I) and late leaves (Phyll II) and final leaf number (FLN) of the wild type, Paragon (A_P+B_P+D_P) and all the different *Ppd-1a* NILs, grouped and averaged by doses of insensitivity alleles.

Genotypes (Lines)	FLN	Phyll I (ºC d leaf^{-1})	Phyll II (ºC d leaf^{-1})
A_P+B_P+D_P	11.5±0.2	84.6±06.8	133.5±08.2
A_{GS}+B_P+D_P	10.6±0.3**	86.9±05.7	130.5±09.7
A_P+**B_S**+D_P	10.4±0.4**	85.9±07.5	122.8±07.7
A_P+**B_{CS}**+D_P	10.8±0.6*	86.6±06.2	*118.7±06.5*
A_P+**B_R**+D_P	11.1±0.6 ns	85.1±06.7	*112.5±10.5*
A_P+B_P+**D_S**	10.5±0.4**	85.4±06.5	114.8±11.0
$Average_{SINGLES}$	10.7±0.1**	86.0±01.3	*119.8±01.9*
A_{GS}+B_S+D_P	10.8±0.3*	83.1±05.6	*110.5±08.6*
A_{GS}+B_{CS}+D_P	10.1±0.6***	90.3±04.7	*108.2±11.8*
A_{GS}+B_P+**D_S**	10.0±0.6***	87.0±05.9	117.3±10.8
A_P+**B_S+ D_S**	10.7±0.3*	81.8±06.0	*106.6±08.6*
A_P+**B_{CS}+D_S**	10.4±0.3**	85.5±05.8	*110.0±03.8*
$Average_{DOUBLES}$	10.4±0.2***	85.5±01.8	*110.5 ±01.3*
A_{GS}+B_S+D_S	10.2±0.8***	85.3±12.1	*110.6±16.2*
A_{GS}+B_{CS}+D_S	9.6±0.5***	91.6±05.9	*110.8±08.2*
$Average_{TRIPLES}$	9.9±0.3***	88.5±00.2	*110.7±03.4*

Values indicate means ± standard errors of the means (SEM). Values in italic type show that differences in phyllochron between NILs and wild type-Paragon were larger than their SEMs. Asterisks indicate statistical differences in FLN from LSMeans contrast against the wild type, Paragon (*$P<0.05$, **$P<0.01$, ***$P<0.001$, any symbols $0.10>P>0.05$ and ns-non significant at $P>0.10$).

The advancement of anthesis time produced by insensitivity to photoperiod was therefore independent of any effects on the phyllochron of early leaves (Fig. 5, middle panel), and the main cause of reducing the FLN was complemented by a reduction in phyllochron of the late leaves (Fig. 5, right panel).



518

519 **Fig. 5.** Relationship between the duration of the period from sowing to anthesis and final leaf number (left panel), phyllochron of early (Phy I, middle panel)
520 and late leaves (Phy II, right panel) for the different NILs and Paragon. The coefficient of determination (R^2) and the level of significance (*$P<0.05$, ***$P<0.001$,
521 ns-non significant) were included. Segments stand for the standard error of the means.

522

3.4. Dynamics of tillering and its coordination with leaf appearance

Generally, there were not consistent effects of photoperiod insensitivity alleles on tillering dynamics (Supplementary Table TS1). Overall NILs there was a trend for *Ppd-1a* alleles to advance the onset of tillering, but this was not only minor but also only exceptionally significant statistically, and there was no effect on the rate on the duration of tillering dynamics. Consequently, the maximum number of tillers and the number of spike-bearing tillers at anthesis was not significantly affected (Table TS1).

When tillering dynamics was analysed using phyllochrons (number of emerged leaves), instead of thermal time, we found no evidences of consistent and relevant effects of these alleles on the coordination between tillering and leaf appearance (Table TS1).

4. Discussion

There was a rather large effect of the growing season on the most relevant traits considered. As large effects on duration of developmental phases can be only ascribed to photoperiod and temperature (Hall et al., 2014; Slafer et al., 2015), the large differences between seasons was unexpected. Photoperiod does not change between years and as sowing times were very similar, average photoperiod for each developmental phase was identical between the two growing seasons. Therefore, the factor responsible for the variation between seasons had to be temperature. As we estimated the durations in thermal time, differences in temperatures between seasons should have been accounted for, if it is admitted that the assumptions made in the calculations were correct. We assumed, as most frequently done, universal thresholds (the same for all genotypes and all phases): a base temperature of 0ºC and an optimum temperature that would have been higher than those registered in the field, and then thermal time was simply the summation of daily average between maximum and minimum temperatures. The fact that calculated thermal time differed between seasons reveals that assumptions in calculations would not have been strictly true. As the thermal times in the warmer season were much longer than in the cooler season, it seems likely that the assumption that actual maximum temperatures would have been always lower than the optimum threshold may have been wrong. As developmental rates are reduced by temperature increases beyond the optimum threshold the warmer temperatures would have produced the delay in development. This would support the evidences from studies on the relationships between rates of development and temperature revealing that the optimum temperature might well be

relatively low (Porter and Gawith, 1999; Porter and Semenov, 2005; Slafer and Rawson, 1995b; 1995c). Notwithstanding the growing season effect, the differences between lines were highly significant and even though the interaction of the genotypes (NILs) and growing season was statistically significant; its magnitude was much smaller than the genotypic effect. This implies that the differences between lines were reasonably consistent across seasons, allowing the results be analysed averaged across of both growing seasons (as in Borràs-Gelonch et al., 2009, Gonzalez-Navarro et al., 2016).

4.1. Phenology

We confirmed that the introgression of *Ppd-1a* alleles accelerated the development towards flowering (Bentley et al.,2011,Wilhelm et al., 2009); with an additive effect of combining *Ppd* insensitivity alleles in different genomes (e.g. Jones et al., 2017; Shaw et al., 2012). But, well beyond this confirmation we offered novel results that shed light (i) on the complex nature of the interaction between different *Ppd-1a* alleles, and (ii) on the effects of these alleles on the components of time to anthesis both in terms of sub-phases and in terms of number of leaves initiated and rate of leaf appearance.

Regarding the complex interactions between *Ppd-1a* alleles on responses of anthesis time, they seemed to depend on the cultivar origin, on how many genomes carried photoperiod insensitivity, and on the source of the specific *Ppd-1a* allele. Concerning the particular genome in which a *Ppd-1a* allele was introgressesd, we found that, when Sonora 64 was the donor of *Ppd-B1a*, this allele and *Ppd-D1a* had the greatest effects on advancing anthesis (and *Ppd-B1a* slightly, though not significantly, stronger than *Ppd-D1a*). When considering this particular source of *Ppd-B1a*, our results would somewhat disagree with most of the literature, in which lines with *Ppd-D1a* introgressed flowered significantly earlier than those with *Ppd-A1a* or *Ppd-B1a* alleles (Bentley et al., 2011; Bentley et al., 2013; Díaz et al., 2012; González et al., 2005b; Jones et al., 2017; Worland et al., 1998). In addition, this general finding would be in line with conclusions, from a study comparing 410 European cultivars, that flowering time is mainly controlled by *Ppd-D1* and could then be exploited when relatively large adjustments in flowering time are needed, whilst *Ppd-B1* would be mainly responsible for relatively minor effects and could be exploited only for fine-tuning development to local conditions (Langer et al., 2014). However, the disagreement between our results and those most commonly reported depended is partial and not unique. It is partial because if we considered the strength of

589 *Ppd-B1a* from other sources in our results, its strength would be less than that of *Ppd-*
590 *D1a*, as it is the most frequent case reported (see above references). It is not unique, as
591 examples in which lines with *Ppd-B1a* flowered earlier than or simultaneously with lines
592 carrying *Ppd-D1a* can be also found (e.g. Scarth and Law, 1984; Whitechurch and Slafer,
593 2002). Our results (as well as those from most literature) also conflict those from
594 Stelmakh (1998) who found that *Ppd-A1a* had the strongest insensitivity effect on heading
595 date. The inconsistency in the literature regarding the strength of *Ppd-1a* alleles
596 depending on the particular genome is further reinforced in our own dataset when
597 combining particular *Ppd-1a* alleles with doses: the rankings we could establish
598 considering exclusively lines with a single dose, did not hold when these alleles were
599 combined with other alleles effecting together time to anthesis. We therefore proved what
600 could be inferred from the conflicts in the literature, that even though it is generally
601 accepted that the ranking of strength of photoperiod insensitive alleles is *Ppd-D1a* > *Ppd-*
602 *B1a* > *Ppd-A1a*, the actual strength of each allele would depend not only on the particular
603 genome in which it is introgressed but also (i) on the source of the insensitive allele, (ii)
604 on the interactions of the allele with both other *Ppd-1a* alleles, and (iii) on the genetic and
605 environmental backgrounds in which they are evaluated. These complex interactions
606 could be seen as an inconvenience for generating simple models of actions guiding
607 breeders interested in manipulating time to anthesis in wheat, but it is simultaneously a
608 source of richness in opportunities for fine-tuning adaptation of the crop. Perhaps
609 counting with such richness is behind the extremely wide range of adaptation of wheat,
610 making it a universal crop grown in most regions of the globe (from 60ºN to 40ºS passing
611 through the equator and from sea level to above 3000 m above sea level) (Slafer and
612 Satorre, 1999). We showed explicitly in the present study the relevance of the source of
613 the *Ppd-B1a* alleles, having the weakest and strongest effect on time to anthesis when its
614 donors were Recital and Sonora 64, respectively, and intermediate when it was Chinese
615 Spring. Bentley et al. (2011) and Díaz et al. (2012) in experiments carried out in a
616 photoperiod glasshouse in summer -but controlling photoperiod to be 10 h- in the UK
617 also found a weaker effect of *Ppd-B1a* alleles from Chinese Spring than from Sonora 64,
618 which suggest that even in such contrasting conditions (isolated plants in a glasshouse in
619 summer of the UK in previous studies; canopy plots under field conditions with a normal
620 autumn sowing in NE Spain in the present study) the different hold and therefore it can
621 be postulated the effect of the source in this case would be larger than the source x
622 environment interaction. Then depending how fine the adaptation is to be tuned in a

particular breeding programme, one or other source of *Ppd-B1a* could be more appropriately exploited. This difference in the strength of *Ppd-B1a* alleles to modify time to anthesis was likely reflecting their different polymorphism for these alleles (Díaz et al., 2012).

Regarding the effects of these alleles on the components phases of time to anthesis, an overall view of relationships (all linear, positive and highly significant) would suggest that these alleles affected similarly the vegetative, the early reproductive and the late reproductive phases. With this overall view, the interpretation of the particular genomes, doses and sources made for time to anthesis could be extended to each of the component phases. However, a closer inspection showed that while the duration of the vegetative and the late reproductive phases were significantly reduced by *Ppd-1a* alleles with the exception of *Ppd-B1a* from Recital, the duration of the early reproductive phase was only significantly reduced by a single dosis of insensitivity when the allele was *Ppd-A1a*, which also reduced (albeit slightly) the duration of the vegetative phase, but its effect on the late reproductive phase was only significant at $P<0.1$. Thus, in the background of Paragon at least, *Ppd-A1a* may be used to advance anthesis mainly through reducing the duration of early phases with negligible effects on the duration of the late reproductive phase. This may be relevant as the late reproductive phase is critical for determining yield (e.g. Slafer, 2003), and reducing the growth during the vegetative and early reproductive phases may not affect yield, as proven empirically by Fischer (2016). Oppositely, if the fine-tuning of anthesis date is achieved by introgressing the *Ppd-D1a* considered in the present study, all phases would be reduced but the effect on the early reproductive phase was not significant and that on the late reproductive phase was stronger than that on the vegetative phase. The strong effect of this allele on the late reproductive phase agrees with González et al. (2005b) who found this with different genetic backgrounds and different sources of the insensitivity allele that we used in the present study. Summarizing, the particular pattern of development partitioning through affecting more or less the durations of vegetative, early reproductive and late reproductive phases could be manipulated through the use of particular alleles for insensitivity. This in turn implies that the sensitivity to photoperiod varies through these phases and the specific strength of the sensitivity is affected by particular alleles, genomes and doses, in agreement with what had been theoretically proposed long time ago (Halloran and Pennel, 1982; Slafer and

Rawson, 1994), and with earlier findings from comparing single chromosome substitution lines (Whitechurch and Slafer, 2001).

4.2. Final leaf number, phyllochrons and its coordination with anthesis

Most of the effects of *Ppd-1a* alleles on time to anthesis can be seen as a consequence of the effects on FLN and duration of phyllochron, as suggested by Kirby (1990). *Ppd-1a* alleles consistently reduced FLN, as expected from previous results (Whitechurch and Slafer, 2002; González et al., 2005b). Even though the reduction of FLN by photoperiod insensitivity alleles was the dominant cause of responses of time to anthesis to the introgression of *Ppd-1a* alleles (through genomes, doses and sources), there was also a contribution of the effects of these alleles in reducing phyllochron of late leaves. This is because, we found that there were two rates of leaf appearance; the phyllochron of the first seven leaves was shorter than that of later leaves, as it had been documented before (Slafer and Rawson, 1997). The fact that the phyllochron of the initial leaves was unaffected by the *Ppd-1a* alleles and that of the late leaves was is in line with previous findings by González et al. (2005b) as well as with the effect of modifying photoperiod for a particular sensitive genotype (e.g. Slafer and Rawson, 1997). As the appearance of the late leaves mostly (though not strictly) coincides with the late reproductive phase, it was not surprising that the allele affecting most this phase, *Ppd-D1a*, was also one of the strongest effect on phyllochron of the late leaves. And *Ppd-A1a* which accelerated development mainly of the early phases had negligible effects on phyllochron. In fact, the mechanism by which an allele may modify the duration of the late reproductive phase would be its effect on phyllochron of the late leaves.

4.3. Dynamics of tillering and its coordination with leaf appearance

Ppd-1a alleles did reduce the maximum number of tillers but as there was a complete compensation later with the rates of tiller mortality, it was clear that in the present study these alleles did not cause major differences in the number of spikes. Although, other studies showed that *Ppd-D1a* alleles may have reduced the number of tillers at heading (Li et al., 2002), this is not necessarily affecting the number of spike-bearing tillers and may well not represent a conflicting result (it does agree with our results on the effects on maximum number of tillers). The positive relationship between the maximum number of tillers and tiller mortality rates determining the compensation we found are rather common in the literature (e.g. Sharma, 1995). This is because alleles increasing the

maximum number of tillers differ mainly in the number of late appearing tillers, and it has been since long recognised that late-appearing tillers do not survive to produce spikes (e.g. Davidson and Chevalier, 1990). Thus lines tillering more profusely also exhibit higher rates of tiller mortality, and not necessarily higher number of fertile tillers (e.g. Borràs-Gelonch et al., 2009); which is the basis for the proposition of an ideotype of wheat of limited tillering firstly (Donald, 1968) and more recently the suggestion that introgressing genes inhibiting tillering would be desirable (e.g. Kebrom and Richard, 2013 and references quoted therein).

The main conclusions we reached are:

- the magnitude of the effects of *Ppd-1a* alleles on time to anthesis, depended upon
  i. the specific genome in which they were introgressed,
  ii. the doses of photoperiod insensitivity alleles introgressed, and
  iii. the source of the specific *Ppd-1a* allele.

  For instance, when Sonora 64 was the donor of *Ppd-B1a*, this allele and *Ppd-D1a* had the greatest effects on advancing anthesis. The ranking of strength of photoperiod insensitive alleles that is generally accepted, *Ppd-D1a* > *Ppd-B1a* > *Ppd-A1a*, would depend not only on the particular genome in which it is introgressed but also on the source of the insensitive allele, and on the interactions of the allele with other *Ppd-1a* alleles.
- particular alleles might be used to manipulate the partitioning of developmental time to anthesis into component sub-phases: for instance in the background of Paragon, *Ppd-A1a* may be used to advance anthesis mainly through reducing the duration of early phases with negligible effects on the duration of the late reproductive phase.

- although photoperiod insensitivity alleles advanced anthesis mainly through reducing FLN, there was a complementary contribution by *Ppd-1a* alleles through reducing phyllochron of late leaves (whilst phyllochron of the first seven leaves was unaffected). *Ppd-1a* alleles did reduce the maximum number of tillers but there was a complete compensation with the rates of tiller mortality, therefore these alleles did not cause major differences in the final number of spike-bearing tillers (and might remove potential negative effects of tiller loss on yield)
- these conclusions may have implications for breeding such as (i) depending how fine the adaptation is to be tuned one or other source of insensitivity alleles could be more useful, (ii) if in a program it is preferred advancing anthesis through preferentially accelerating

development in a particular vegetative of reproductive phase, specific insensitivity alleles could be exploited.

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929 **Supplementary material**

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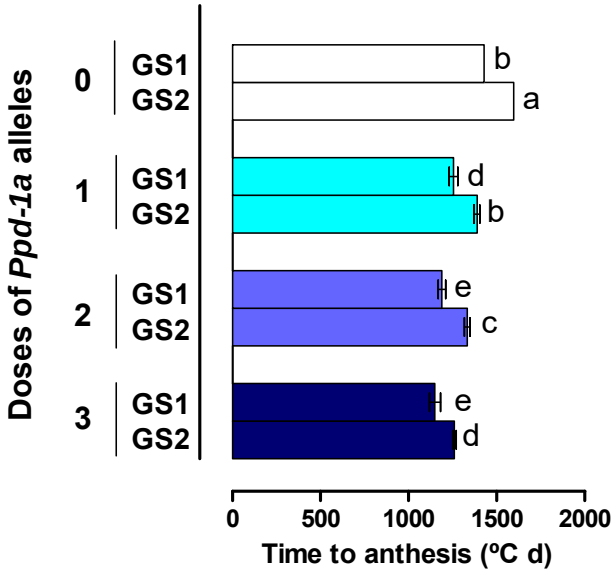

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932 **Fig. FS1.** Duration of the period from sowing to anthesis of the different genotypes averaged across
933 lines with the same doses of insensitive *Ppd* alleles. Different letters indicate that the differences are
934 statistically significant ($P<0.05$). Segments in each bar stand for the standard error of the means
935 (SEM) used to calculate the time to anthesis in the cases where different NILs were averaged.

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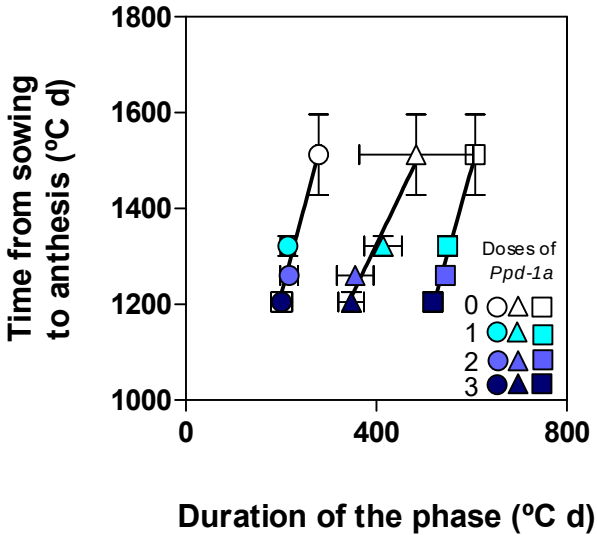
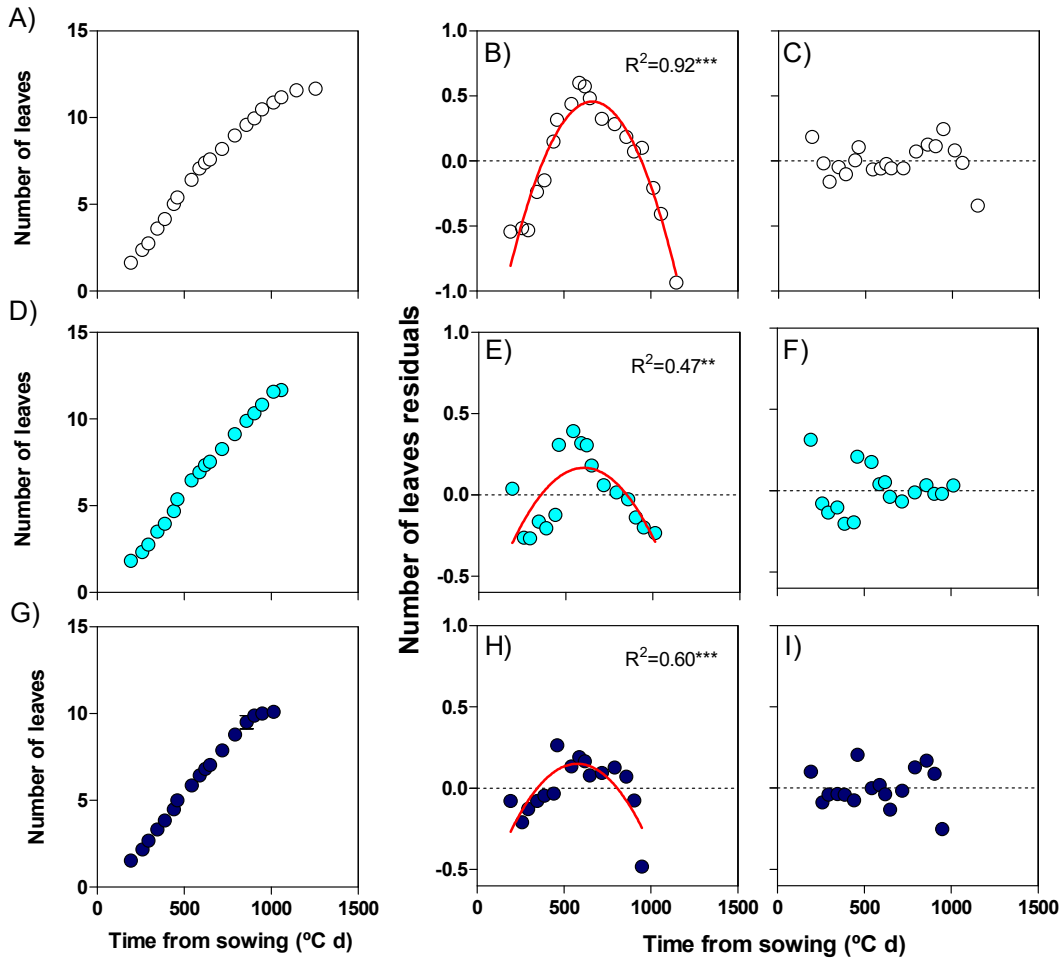


Fig. FS2. Relationship between time from sowing to anthesis and the duration of the three component phases (vegetative, circles; early reproductive, tringles; and late reproductive, squares) for Paragon (open symbols) and its the NILs with 1, 2 or 3 doses of *Ppd-1a* alleles (pale, intermediate and dark symbols). Durations are averaged across lines with the same doses of insensitive *Ppd* alleles.



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944 **Fig. FS3.** Relationship between the number of appeared leaves in the main shoot (Haun stage) and time after
945 sowing (A, D, G) and distribution of the residuals of the linear (B, E, H) or bi-linear (C, F, I) regressions for
946 these dynamics (until the appearance of the flag leaf exclusively) for the wild type Paragon (top panels: A, B,
947 C) and the NILs with the smallest (A_P+**B_R**+D_P, middle panels: D, E, F) and largest (**A_{GS}+B_{CS}+D**s, bottom
948 panels: G, H, I) reductions in FLN.

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Table TS1. Main parameters of the dynamics of tillering based on thermal time or on phyllochrons after sowing for each of the different NILs and wild type-Paragon (A_P+B_P+D_P) as well as for the averages across lines with the same doses of insensitive *Ppd* alleles. These parameters were the timing of the onset of tillering, the tiller appearance rate (TAR), the duration of tillering (TAD), the maximum number of tillers (MNT) reached at the end of tillering (as the average of the values determined from then to the onset of tiller mortaility), and the final number of tillers (FNT) which are the number of fertile (spike-bearing) tillers. Tillers counted do not include the main shoot (to consider the total number of shoots, and spikes, per plant it must be added 1 to the corresponding figures).

Genotypes (Lines)	Onset of tillering (°C d)	TAR (tillers pl^{-1} [100 °C d]$^{-1}$)	TAD (°C d)	MNT (tillers $plant^{-1}$)	FNT (tillers $plant^{-1}$)	Onset of tillering (leaves)	TAR (tillers pl^{-1} $leaf^{-1}$)
A_P+B_P+D_P	347.3±16.1	1.60±0.3	259.2±29.5	4.14±1.1	0.49±0.1	3.41±0.2	1.38±0.1
A_{GS}+B_P+D_P	336.5±11.5	1.55±0.2	217.3±15.8	3.40±0.8 ns	0.92±0.1 ns	3.35±0.2	1.23±0.2
A_P+**B_S**+D_P	322.2±15.9	1.59±0.3	244.9±23.8	3.87±1.1 ns	0.65±0.0 ns	3.18±0.2	1.32±0.4
A_P+**B_{CS}**+D_P	327.5±18.4	1.79±0.3	290.8±19.5	5.03±1.5 ns	0.79±0.1 ns	3.24±0.3	1.42±0.2
A_P+**B_R**+D_P	*297.8±13.4*	1.45±0.2	*338.1±40.2*	4.70±1.0 ns	1.15±0.6 ns	*2.82±0.1*	1.24±0.1
A_P+B_P+**D_S**	338.8±09.4	1.53±0.3	238.4±09.7	3.50±1.6 ns	0.53±0.1 ns	3.45±0.2	1.11±0.2
Average $_{SINGLES}$	324.6±7.3	1.58±0.1	265.9±21.7	4.10±0.3 ns	0.81±0.1 ns	3.21±0.1	1.26±0.1
A_{GS}+B_S+D_P	357.0±13.3	1.31±0.2	243.0±25.5	3.17±0.2 ns	0.81±0.1 ns	3.49±0.3	*0.94±0.1*
A_{GS}+B_{CS}+D_P	335.5±20.1	1.40±0.2	201.1±27.3	2.82±0.1 ns	0.53±0.3 ns	3.28±0.1	1.29±0.2
A_{GS}+B_P+**D_S**	*291.5±09.6*	1.33±0.2	275.7±26.0	3.33±1.2 ns	0.78±0.1 ns	3.03±0.4	*0.99±0.2*
A_P+**B_S+ D_S**	307.1±33.9	1.72±0.2	242.1±27.4	3.83±0.4 ns	1.02±0.1*	3.32±0.5	1.29±0.1
A_P+**B_{CS}+D_S**	367.3±51.7	1.37±0.5	239.2±70.7	3.24±0.2 ns	0.41±0.0 ns	3.73±0.7	1.11±0.3
Average $_{DOUBLES}$	331.7 ±14.4	1.43 ±0.1	240.2±11.8	3.28±0.2 ns	0.71±0.1 ns	3.37±0.1	*1.14±0.1*
A_{GS}+B_S+D_S	288.4±79.3	2.34±1.0	261.7±41.1	4.51±1.9 ns	0.80±0.0 ns	3.15±1.3	1.66±0.6
A_{GS}+B_{CS}+D_S	321.0±34.5	1.42±0.3	213.7±66.4	2.92±0.4 ns	0.64±0.1 ns	3.05±0.2	1.29±0.2
Average $_{TRIPLES}$	*304.7±16.3*	1.88 ±0.5	237.7±24.0	3.71±0.7 ns	0.72±0.1 ns	*3.10±0.1*	1.48±0.2

Values indicate means ± standard errors of the means (SEM), when in italic type differences between NILs and Paragon were larger than their SEMs. Asterisks indicate statistical differences in number of tillers from LSMeans contrast against the wild type, Paragon (*$P<0.05$, ns-non significant at $P>0.10$).