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Pre-anthesis development and number of fertile florets in wheat as affected by photoperiod sensitivity genes *Ppd-D1* and *Ppd-B1*

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Summary

Lengthening the late reproductive phase (LRP) of stem elongation in wheat (*Triticum aestivum* L.), by changing its photoperiod sensitivity independently of the preceding phases, would improve the yield potential through increasing spike weight and the number of fertile florets at anthesis. This paper presents results of a two-year field experiment designed to determine the impact of *Ppd-D1* and *Ppd-B1* on (i) the duration of three pre-anthesis developmental phases, and (ii) spike weight and the number of fertile florets at anthesis under two photoperiods during the LRP (natural and an extension of six hours over that). Near isogenic lines of Mercia and single chromosome recombinant lines of Cappelle Desprez were used. Under natural photoperiod, *Ppd-D1* hastened time to anthesis ca. 500 °C d in both backgrounds by reducing each of the three pre-anthesis phases. *Ppd-B1* hastened the time to anthesis under natural photoperiod by 178 °C d, mainly by reducing the early reproductive phase. The response to photoperiod of the LRP under extended daylength depended on the *Ppd* locus present: *Ppd-D1* was insensitive while *Ppd-B1* and the recessive controls were sensitive. For all lines, photoperiod treatments and years, the number of fertile florets was associated with spike dry weight at anthesis ($R^2 \cong 80\%$, $p < 0.01$) which, in turn, was positively related to the intercepted radiation accumulated during the LRP ($R^2 \geq 45\%$, $p < 0.05$). Changing the duration of the LRP through extended photoperiod or through *Ppd-D1* produced similar results in both backgrounds and years. Thus, altering the duration of the LRP by manipulating photoperiod sensitivity may be an alternative to changing the fertile floret number in wheat. Nevertheless, as no particular allele was responsible for the photoperiod sensitivity only during the LRP, new alleles should be studied to identify the control of photoperiod sensitivity of individual phases to fine-tune the pre-anthesis wheat development.

Introduction

The life cycle of wheat from emergence to anthesis can be divided into phases depending on the main organs that are developing (see review by Slafer & Rawson, 1994). During the vegetative phase, the crop initiates leaves until the floral initiation, which is generally estimated as the formation of the first double ridge in the apex. In the subsequent early reproductive phase, the spikelets are differentiated until the initiation of the terminal spikelet. Finally, during the late reproductive phase, when the stem internodes elongate, the floret primordia are initiated reaching their maximum number, and then, some florets degenerate while others reach the fertile stage at anthesis. Timing and duration of these phases are determined by the cultivar's

sensitivity to photoperiod and temperature. All the developmental phases prior to anthesis may be sensitive to photoperiod, and sensitivity of each individual phase may be partially independent of the others (e.g. Slafer & Rawson, 1994, 1996; Miralles & Richards, 2000; González et al., 2003a). Because of that independence, the duration of individual phases could be fine-tuned to optimize them without affecting the anthesis date (Halloran & Pennell, 1982).

The pre-anthesis late reproductive phase of stem elongation is particularly important for yield (Fischer, 1975, 1985) because during this phase the number of fertile florets at anthesis is determined. This, in turn, sets the final number of grains which is the most important component of wheat yield. The relationship between the spike dry weight and the number of fertile florets at anthesis (Fischer & Stockman, 1980; Brooking & Kirby, 1981; Stockman et al., 1983; Slafer & Andrade, 1993), and the coincidence of spike and stem growth with floret degeneration (Kirby, 1988), led to the suggestion that wheat yield is restricted by the availability of assimilates for spike growth. This idea is supported by the success in breeding for yield, which improved dry matter partitioning to the spikes during the late reproductive phase (Siddique et al., 1989; Slafer et al., 1990; Youssefian et al., 1992; Slafer & Andrade, 1993; Calderini et al., 1995; Miralles & Slafer, 1995). As further increments in dry matter partitioning are unlikely (Slafer et al., 1999), increasing the yield potential might depend on the improvements in the dry matter accumulation during the late reproductive phase. The key trait to enhance assimilate availability for spike growth and to improve wheat yield would be to increase the duration of the late reproductive phase without changing the anthesis date, through manipulation of the photoperiod sensitivity of individual developmental phases (Slafer et al., 1996, 2001). In recent years empirical evidence supporting this idea has been obtained: changing the length of the late reproductive phase, by artificial photoperiod manipulations in controlled and field experiments, resulted in parallel changes in the number of fertile florets and grains per unit land area, without affecting any other phases (Miralles & Richards, 2000; Whitechurch & Slafer, 2002; González et al., 2003a). Genetic manipulation of the photoperiod sensitivity during the late reproductive phase would be strongly accelerated (or even only made possible) by learning the genetic controls underlying the photoperiod sensitivity of individual phases.

Photoperiod response in wheat is mainly determined by a series of homoeologous loci *Ppd-D1* (formerly *Ppd1*), *Ppd-B1* (*Ppd2*) and *Ppd-A1* (*Ppd3*), located on the group 2 chromosomes: 2D, 2B and 2A, respectively (Welsh et al., 1973; Law et al., 1978; Scarth & Law, 1983). The dominant alleles confer photoperiod-insensitivity and, in most cases, the insensitive ranking (measured as days to flowering) is *Ppd-D1* > *Ppd-B1* (Scarth & Law, 1984; Law, 1987; Worland, 1996; Stelmakh, 1998; Worland et al., 1998). Despite many studies that have been conducted to understand the differences among these genes, the physiological effect of *Ppd-D1* and *Ppd-B1* on wheat development and yield is still poorly understood (see review by Snape et al., 2001). Many studies concentrated on the impact of *Ppd-D1* and *Ppd-B1* on the crop cycle (see examples in Table 1), on some numerical components of yield, and on the likelihood of ending the life cycle under favourable environmental conditions. Only a few studies that reported on the impact of *Ppd-D1* and *Ppd-B1* on the time to anthesis or heading actually manipulated photoperiod, and even fewer did so under field conditions (Table 2).

The impact of the *Ppd* genes on duration of individual pre-anthesis phases was assessed only in exceptional cases (Table 2). It is not clear whether the same relationship between the duration of the late reproductive phase and the number of fertile florets at anthesis is observed when changing the photoperiod environment (Miralles et al., 2000; González et al., 2003a) or, alternatively, the photoperiod sensitivity during this specific phase. The goals of the present work were to study, under well-characterized photo-thermal conditions in field plots, the impact of *Ppd-D1*, *Ppd-B1* and their respective recessive controls on (i) development during different pre-anthesis phases, and (ii) the number of fertile floret at anthesis and its eco-physiological determinants under two contrasting photoperiod environments during the late reproductive phase.

Materials and methods

General description

The experiments were carried out during the 2001 and 2002 growing seasons in the experimental field of the Department of Plant Production, Faculty of Agronomy, University of Buenos Aires (35°35′S, 59°29′W, 25 m a.s.l.), Argentina. The soil was a silt clay loam (Argiudol) and during both years 120 Kg of N (urea) were applied, split into two doses: the first at the beginning of tillering and the second at the start of

Table 1. Reported impact of the alleles *Ppd-D1* and *Ppd-B1* on time to anthesis (AN) or heading (HD), spikelet number per spike (SN) and spikelet fertility (SF, grain number per spikelet).

Agronomic characteristic			Description	
AN/HD	SN	SF	Experiment	Reference
(a) Allele *Ppd-D1*				
−8.0	−2.0	+	Mercia (Nil), sowing (UK)	Butterworth et al. (unpublished)
−6.9	−2.2	+0.42	CD (Mara 2D) England } (MCRL), sowing	Worland et al. (1998)
−4.5	−1.0	+0.35	CD (Mara 2D) Germany } (MCRL), sowing	
−7.3	−2.0	+0.46	CD (Ciano 67 2D) England } (MCRL), sowing	
−4.8	−2.6	−0.03	CD (Ciano 67 2D) Germany } (MCRL), sowing	
−3.5			CD (Besostaya I 2D), (SL), sowing	Law and Worland (1997)
−5.4	−1.0		CD (Mara 2D) } (SL), sowing (Yugoslavia)	Worland (1996)
−4.3	−0.2		CD (Timstein 2D) } (SL), sowing (Yugoslavia)	
−7.0	−0.9		CD (Ciano 67 2D) } (SL), sowing (Yugoslavia)	
−3.5	−1.2	+	CD (Mara 2D) (MCRL), sowing (middle Europe)	Börner et al. (1993)
−8.5	−2.0	+	CD (Mara 2D) (MCRL), sowing (Yugoslavia)	Worland et al. (1988)
−3.2			CD (Besostaya I 2D) (SL), sowing	Law (1987)
			CD (Mara 2D) (MCRL), sowing	Law (1987)
−5.6			England	
−8.1			Hungary	
−4.3			Rumania	
−5.0			Yugoslavia	
(b) Allele *Ppd-B1*				
−3.0		+	Mercia (Nil), sowing (UK)	Butterworth et al. (unpublished)
−5.8	−0.3	+0.47	CD (Chinese Spring 2B), England } (MCRL), sowing	Worland et al. (1998)
−4.3	−0.9	+0.16	CD (Chinese Spring 2B) Germany } (MCRL), sowing	
+1.8			CD (Besostaya I 2B) (SL), sowing	Law and Worland (1997)
−6.2			CD (Chinese Spring 2B) (MCRL), sowing (UK)	Worland (1996)
−0.6			CD (Besostaya I 2B) (SL), sowing	Law (1987)
			CS[a], sowing	Law (1987)
−1.0			East Germany } Long days (?h)	
−3.0			Switzerland } Long days (?h)	
−2.0			Netherlands } Long days (?h)	
−1.0			Hungary } Long days (?h)	
−1.5			England } Long days (?h)	
−2.0			Rumania } Long days (?h)	
−5.0			Poland } Short days (?h)	
−12.0			India } Short days (?h)	
−13.0			Argentina } Short days (?h)	
−14.0			Australia } Short days (?h)	

Note. CD: Capelle Desprez, SL: substitution line, MCRL: monochromosomic recombinant line, Nil: near isogenic line; CS: Chinese Spring. The effects of *Ppd-D1* and *Ppd-B1* were expressed as difference from the recessive control in days for AN/HD, or in number of organs for the other characters.

[a]vs. CS (Lutescens 2B)

stem elongation after the terminal spikelet initiation. To avoid water stress, natural rainfall of the season was supplemented with irrigation, as needed. Weeds were manually removed when necessary, and fungicides and insecticides were applied to prevent plant damage.

Plant material

Three isogenic lines of cv. Mercia (Experiment 1) and two single chromosome recombinant lines of cv. Cappelle Desprez (Experiment 2) were used. The *Ppd* constitution of the lines is listed in Table 3. In the Mercia isogenic lines, M_{Ppd-D1} carries the *Ppd-D1* allele on chromosome 2D from cv. Ciano 67, while M_{Ppd-B1} carries the *Ppd-B1* allele on chromosome 2B from cv. Chinese Spring. These isogenic lines were developed by back-crossing to the recipient cultivar for eight generations (Snape John, pers. com.). The Cappelle Desprez recombinant lines were derived from the cross between cv. Cappelle Desprez [Mara 2D] and cv. Cappelle Desprez (see Law, 1966).

Table 2. Reported impact of the alleles *Ppd-D1* and *Ppd-B1* on wheat development from studies where photo-thermal conditions and some developmental phases were analysed

Agronomic characteristic						Description	
AN/HD	VP		ERP	LRP	FLN	SN	Experiment	Reference
(a) Allele *Ppd-D1*								
							Mercia (Nil), sowing (UK)	Foulkes et al. (2004)[c]
−15.0 (−188)							10.4 h (average photoperiod emergence-AN)	
		−29.0 (−208)					8.3 h (" " " GS3.1) 1998	
				+14.0 (+20)			13.4 h (" " GS3.1-AN)	
−8.0 (−102)							10.6 h (emergence-AN)	
		−9.0 (−105)					9.0 h (" " " GS3.1) 1999	
				+1.0 (+3)			15.1 h (" " GS3.1-AN)	
							CD (MCRL), sowing (UK)	
−12.0 (−160)							10.6 h (average photoperiod emergence-AN)	
		−22.0 (−146)					8.4 h (" " " GS3.1) 1998	
				+10.0 (−14)			13.6 h (" " GS3.1-AN)	
−6.0 (−74)							10.7 h (" " emergence-AN)	
		−5.0 (−59)					9.0 h (" " " GS3.1) 1999	
				−1.0 (−15)			15.3 h (" " GS3.1-AN)	
							Mironovskaya 808 (congenic line, donor Besostaya I)	Stelmakh (1998)
							Glasshouse, 60 days vernalisation	
−11.1							8 h	
−26.6							12 h	
−20.0							16 h	
−8.6							20 h	
−5.1							24 h	
							CD (Besostaya I 2D) (SL), Glasshouse	Law (1987)
−16.5					−1.2	−1.9	8 h	
−4.5					−0.3	−0.2	24 h	
	0.0	−7.0		> −3.0	0.0	−2.7	CS (Ciano 67 2D) (SL) Glasshouse, 4 week vernalisation	Scarth et al. (1985)[d]
							8 h photoperiod, 18 °C	

(*Continued on next page*)

Table 2. (*Continued*)

Agronomic characteristic						Description	
AN/HD	VP	ERP	LRP	FLN	SN	Experiment	Reference
(b) Allele *Ppd-B1*							
(−250)						CS[a] 12.3 h (average photoperiod till AN)	Whitechurch & Slafer (2002)
(−25)						≥14.5 h till 19.4 h (“ “ “)	
	(−55.0)			−0.53		11.3 h (average photoperiod VP)	
	(+63.0)			−0.25		18.0 h (“ “ “)	
		(−25)			−3.0	12.1 h (average photoperiod ERP)	
		(+12.5)			−1.8	18.3 h (“ “ “)	
			(−170)			13.5 h (average photoperiod LRP)	
			(−25)			19.5 h (“ “ “)	
						Field, artificial manipulation of photoperiod	
−0.2	+0.2		−0.3			CS[b] 10.8 h (at emergence)	Whitechurch & Slafer (2001)
+3.8	−2.2		+6			18.0 h (constant through all cycle)	
−20.2	−13.8		−6.3	−1.6		CS[a] 10.8 h (at emergence)	
−0.5	−5.2		+4.7	−0.3		18.0 h (constant through all cycle)	
						Field, artificial manipulation of photoperiod	
						Mironovskaya 808 (congenic line, donor Besostaya I)	Stelmakh (1998)
						Glasshouse, 60 days vernalisation	
−2.7						8 h	
+2.2						12 h	
−1.8						16 h	
+5.6						20 h	
+0.1						24 h	
						CD (Besostaya I 2B) (SL), Glasshouse	Law (1987)
−1.7						8 h	
−1.3						24 h	
−20.0	−4.0	−30.0	+14.0	−0.9	+0.6	Homozygous recombinant lines from Scarth and Law (1983)	Scarth et al. (1985)
						8 h photoperiod, 18 °C	

Note. For duration of vegetative (VP), early reproductive (ERP) and late reproductive (LRP) phases, the effects of *Ppd-D1* and *Ppd-B1* were presented as the difference in days or (°C d) from the recessive control. When data is between columns it means that the whole phase was measured. FLN means final leaf number, for other details please Table 1.

[a]vs. CS (Synthetic 2B), SL.

[b]vs. CS (Cappelle Desprez 2B), SL.

[c]The thermal duration was reanalysed using 0 °C as base temperature and average photoperiod was calculated from original data gently provided by John Foulkes.

[d]vs. CS (*ppd-D1 Ppd-B1*).

Table 3. Ppd constitution of near isogenic lines (Mercia) and monochromosomic recombinant lines (Cappelle Desprez)

	Genome		
Background	D	B	A
Mercia			
$M_{recessive}$	*ppd-D1*	*ppd-B1*	*ppd-A1*
M_{Ppd-B1}	*ppd-D1*	*Ppd-B1*	*ppd-A1*
M_{Ppd-D1}	*Ppd-D1*	*ppd-B1*	*ppd-A1*
Cappelle Desprez			
$C_{recessive}$	*ppd-D1*	*ppd-B1*	*ppd-A1*
C_{Ppd-D1}	*Ppd-D1*	*ppd-B1*	*ppd-A1*

Treatments and experimental design

To avoid any interaction between the photoperiod and vernalisation responses, plants were vernalised for 47 days (2001) or 58 days (2002) in a cold chamber ($3.5 \pm 0.4\,^{\circ}$C, 8 h daylength) and transplanted to the field at 360 (in 2001) and 320 (2002) plants per m^2. As 6 to 8 weeks below 5 °C are considered sufficient to complete vernalisation of most wheat cultivars (Davidson et al., 1985; Griffiths et al., 1985), it was assumed that the vernalisation response was saturated with the pre-treatment applied. The dates of transplanting (TR, 6 August 2001 and 2 September 2002) were considered to be the beginning of the experiments.

The experiments were conducted in two years, and in both years they were similarly managed. Plants grew under natural photoperiod from the beginning of the experiment until the terminal spikelet initiation (TS). From TS to anthesis (AN) two photoperiod treatments were applied: (i) NP + 0, where plants remained under the natural daylength and (ii) NP + 6, a photoperiod extended by 6 h over the natural daylength. Low intensity lights were used for the photoperiod extension; they were turn on before sunset and turned off 6 h after twilight using automatic timers. The average daylength of NP + 0 (during the late reproductive phase) ranged from 14.1 h to 14.8 h (across all lines).

Both experiments during both years were arranged in a factorial combination of lines *x* photoperiod treatments, using a split-plot design with three replicates. The main plots were the photoperiod treatments and the lines were the sub-plots. Each experiment was analyzed as a split-plot combined over years. The sub-plots were independent, i.e. each sub-plot was an individual plot separated by 2 m wide streets from the others, and consisted of 9 rows, 0.15 m apart and 1.2 m long.

Response variables and analyses

Two or three times a week, one plant per plot was randomly harvested and its main shoot was dissected to determine the timing of the double ridge formation (DR) and the terminal spikelet initiation (TS) in the apical meristem, as described by Gardner et al. (1985). The timing of anthesis (AN, when the first anthers were visible) was registered when 50% of plants in a sub-plot reached that stage. Immediately after transplanting, two plants per plot were randomly selected and tagged, and the number of leaves emerging on the main shoot was registered two or three times a week until anthesis (Haun, 1973). One or two times a week, the radiation (PAR, photosynthetic active radiation) above and below the canopy was measured (LI-191 S, LICOR) in each sub-plot to estimate the interception of the incident radiation. The intercepted radiation accumulated during the late reproductive phase was calculated summing up the product of the radiation intercepted at different times during this phase and the daily incident radiation. At anthesis, 0.5 m of the central row of each sub-plot was harvested to estimate the total dry matter per m^2 (TDM) and some of its sub-components (main shoot stems –MST-, main shoot spikes -MSP, and tiller spikes – TSP). The partitioning of the total dry matter to reproductive organs (main shoot + tiller spikes – PDM-) and the spike to stem ratio in main shoots (MSP:MST) were also calculated. Also at anthesis, five spikes per sub-plot were collected and the numbers of spikelets and fertile florets per spikelet were counted to calculate the total number of fertile florets per spike. Florets were considered fertile when the style was curved outwards and well-developed stigmatic branches were spread wide, with either pollen grains present on them (stage 10 in the scale of Waddington et al., 1983) or anthers green/yellow.

ANOVA analyses ($\alpha = 0.05$) were performed for each experiment using a split-plot design combined over years and means were compared by LSD ($\alpha = 0.05$). Phyllochron, i.e. the thermal time interval between emergence of two successive leaves, was calculated for each sub-plot from the regression between thermal time from transplant (Y) and number of emerged leaves (X) (TableCurve 2D). Linear and bilinear models were tested but, considering the R^2 and the random distribution of residuals, all resulting fittings were bilinear ($R^2 \geq 99\%$, $p \leq 0.0001$). The model used was:

$$Y = a + bx(x \leq c) + bc(x \geq c) + d(x - c)(x > c)$$

The parameters shown here were: (i) the slopes "b" and "d", representing the phyllochron of the first and the last emerging leaves, respectively, and (ii) the inflection point "c", i.e. the leaf number where the phyllochron changed. Logarithmic functions were used to estimate the evolution of intercepted radiation ($R^2 > 60\%$, $p < 0.05$ for all regressions). Linear regressions were used to asses the relationship between different eco-physiological variables involved in the control of the fertile floret number. A base temperature of 0 °C was used for the calculation of the thermal time.

Results

Phenology

Duration of the pre-anthesis phases, leaf numbers and phyllochron under natural field conditions (NP + 0)

As expected, the time to anthesis depended on the *Ppd* constitution of the lines. For both years, the duration of the crop cycle to anthesis was, in decreasing order: $M_{recessive} > M_{Ppd\text{-}B1} > M_{Ppd\text{-}D1}$ (Table 4). Similarly, in the other genetic background, $C_{recessive}$ reached anthesis later than $C_{Ppd\text{-}D1}$ (Table 4). For both backgrounds and years, duration of TR-DR was longer in $M_{recessive}$ and $C_{recessive}$ than in $M_{Ppd\text{-}D1}$ and $C_{Ppd\text{-}D1}$, respectively (Table 4). Similar effects of *Ppd-D1* vs. *ppd-D1* were evident in the subsequent developmental phases DR-TS and TS-AN (Table 4). $M_{Ppd\text{-}B1}$ showed duration of TR-DR and TS-AN similar to or slightly shorter than $M_{recessive}$, depending on the year (Table 4). Duration of DR-TS, however, was consistently reduced in $M_{Ppd\text{-}B1}$ relative to $M_{recessive}$ (Table 4).

Table 4. Time to anthesis and duration of the different pre-anthesis developmental phases, under natural field conditions (NP + 0)

		Thermal time (°C d)			
Year	Line	TR-AN	TR-DR	DR-TS	TS-AN
(a) Mercia					
2001	$M_{recessive}$	1470 b	504 a	264 b	702 ab
	M_{Ppd-B1}	1352 c	504 a	174 d	67 4b
	M_{Ppd-D1}	1028 d	284 c	220 c	523 c
2002	$M_{recessive}$	1565 a	490 a	344 a	731 a
	M_{Ppd-B1}	1326 c	454 b	217 c	655 b
	M_{Ppd-D1}	1055 d	305 c	232 c	518 c
(b) Cappelle Desprez					
2001	$C_{recessive}$	1609 a	504 a	406 a	699 a
	C_{Ppd-D1}	1125 b	389 b	170 c	566 b
2002	$C_{recessive}$	1632 a	484 a	441 a	707 a
	C_{Ppd-D1}	1106 b	356 b	250 b	501 c

Note. TR: transplant, DR: double ridge formation, TS: terminal spikelet initiation, AN: anthesis. For meaning of lines nomenclature please see Table 3.
Unlike letters within column and background indicate statistical differences (LSD $\alpha = 0.05$).

Table 5. Total number of leaves and spikelets per spike

Year	Line	Leaves	Spikelets
(a) Mercia			
2001	$M_{recessive}$	11.9 a	20.5 a
	M_{Ppd-B1}	11.1 b	19.3 a
	M_{Ppd-D1}	9.4 d	16.3 c
2002	$M_{recessive}$	11.8 a	18.8 b
	M_{Ppd-B1}	10.5 c	18.9 b
	M_{Ppd-D1}	8.9 d	16.5 c
(b) Cappelle Desprez			
2001	$C_{recessive}$	13.0 a	20.4 a
	C_{Ppd-D1}	10.2 c	17.7 c
2002	$C_{recessive}$	12.1 b	19.1 b
	C_{Ppd-D1}	9.7 d	17.4 c

Unlike letters within column and background indicate statistical differences (LSD $\alpha = 0.05$). For meaning of lines nomenclature please see Table 3.

The total number of leaves was correlated with the duration of TR-DR, i.e. the longer the phase the greater the number of leaves. Thus, $M_{recessive}$ and $C_{recessive}$ showed higher number of leaves than $M_{Ppd\text{-}D1}$ and $C_{Ppd\text{-}D1}$ (Table 5). Although $M_{Ppd\text{-}B1}$ showed the final leaf number to be statistically smaller than $M_{recessive}$, the difference was negligible (Table 5). The number of spikelets per spike decreased as the duration of DR-TS was reduced from $M_{recessive}$ and $C_{recessive}$ to $M_{Ppd\text{-}D1}$ and $C_{Ppd\text{-}D1}$, respectively (Table 5). Even though $M_{Ppd\text{-}B1}$ had the shortest DR-TS phase (Table 4), its total number of spikelets per spike was similar to $M_{recessive}$ (Table 5), with the longest DR-TS phase (Table 4).

Leaf emergence fitted a bilinear model in all lines and in both years, i.e. phyllochron of the early emerging leaves was smaller than that of the late emerging leaves. Phyllochron of the early emerging leaves ranged between lines and years from 73 to 93 °C d, without a clear trend associated with the *Ppd* constitution (Figure 1a). Considering both years, phyllochron of the late emerging leaves ranged from 115 to 170 °C d in the Cappelle Desprez background, and between 125 to 148 °C d in the Mercia background (Figure 1a). Contrary to the early emerging leaves, phyllochron of

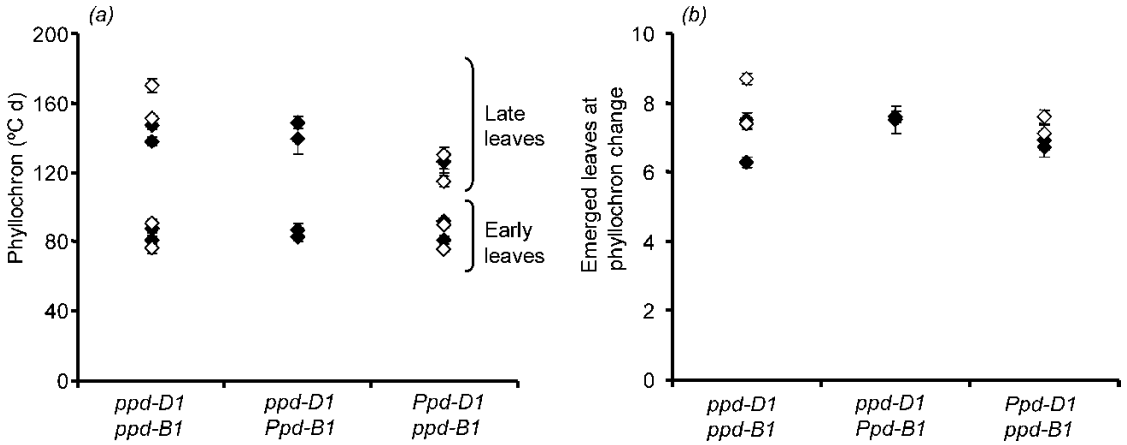

Figure 1. (a) Phyllochron of early and late emerging leaves, and (b) number of leaves already emerged when phyllochron changed, as affected by *Ppd* alleles in Mercia (♦) and Cappelle Desprez (◇). Both years included. Means ± S.E.

the late emerging leaves tended to be greater as duration of TS-AN increased from *Ppd-D1* through *Ppd-B1* to the recessive controls *ppd-D1-ppd-B1* (Figure 1a). The number of leaves already emerged when phyllochron changed between the early and late emerging leaves varied between some lines and years, but it was not associated with the *Ppd* constitution. The change of phyllochron took place between the emergence of the 7th and 8th leaf in most lines and years (Figure 1b).

Response to photoperiod during the late reproductive phase (TS-AN)

Ppd-D1 was almost insensitive to photoperiod, that is, no major variation was observed between the NP + 0 and NP + 6 treatments, disregarding the experimental year and genetic background (Figure 2a and b). In contrast, all lines carrying *ppd-D1* ($M_{recessive}$, $C_{recessive}$ and $M_{Ppd\text{-}B1}$) showed some degree of photoperiod sensitivity during the late reproductive phase, regardless of whether the gene on chromosome 2B was dominant (*Ppd-B1*) or recessive (*ppd-B1*). When all these sensitive lines were exposed to extended photoperiod, duration of TS-AN was similarly reduced to ca. 550 °C d (averaging across years, Figure 2a and b). This duration of TS-AN was similar to that of the photoperiod insensitive lines carrying *Ppd-D1*.

The duration of TS-AN depends on (i) the number of leaves emerging during this particular phase and (ii) their rate of emergence (whose reciprocal is

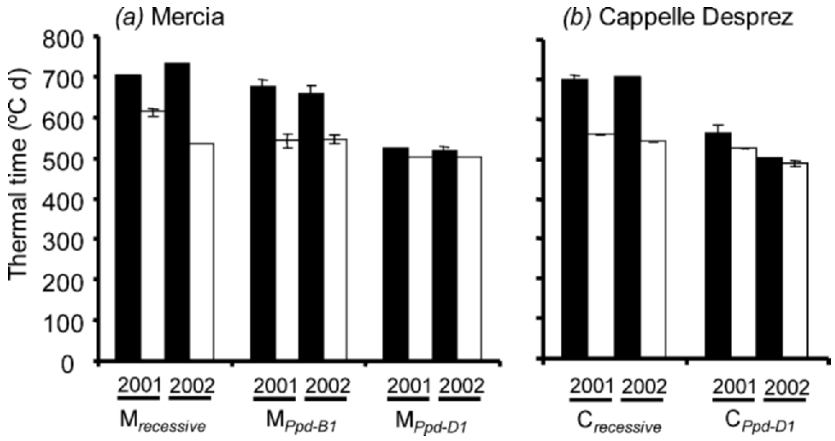

Figure 2. Duration of the late reproductive phase under natural (NP + 0, ■) and extended (NP+6, □) photoperiod in (a) Mercia and (b) Cappelle Desprez. Both years included. Means ± S.E. For meaning of lines nomenclature please see Table 3.

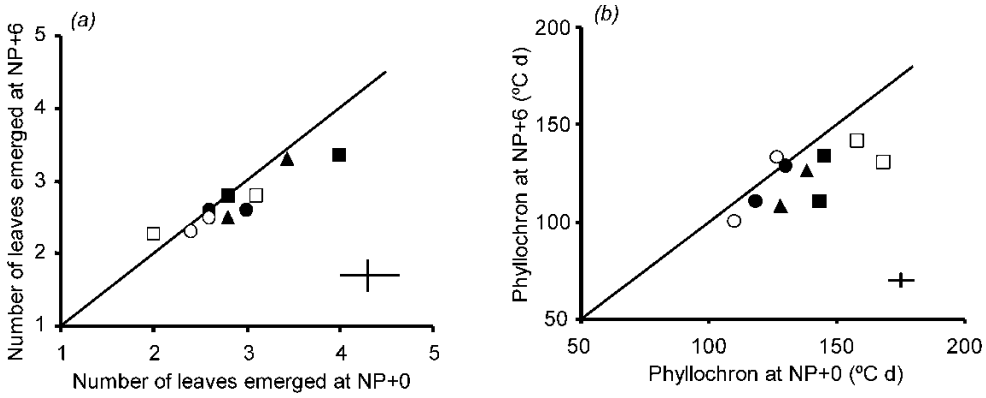

Figure 3. (a) Number and (b) phyllochron of leaves emerged during the late reproductive phase under extended (NP + 6) vs. natural (NP + 0) photoperiod. Data for both years in $M_{recessive}$ (■), $C_{recessive}$ (□), $M_{Ppd\text{-}B1}$ (▲), $M_{Ppd\text{-}D1}$ (●) and $C_{Ppd\text{-}D1}$ (○). Lines on the bottom right indicate S.E.

the phyllochron). The number of leaves emerged during the TS-AN phase varied between years but no impact of lines or photoperiod treatments was observed (Figure 3a). Phyllochron of these leaves was similar within the Mercia lines exposed to NP + 6, averaging 129 ± 6.3 and 109 ± 7.7 °C d during 2001 and 2002, respectively (Figure 3b). In Cappelle Desprez, also under NP + 6, phyllochron was similar between lines during 2001, averaging 138 ± 9.2 °C d but it differed between lines in 2002, being 100 ± 3.6 and 131 ± 15.2 °C d for $C_{Ppd\text{-}D1}$ and $C_{recessive}$, respectively (Figure 3b). The lines that were sensitive to photoperiod during the TS-AN phase ($M_{recessive}$, $C_{recessive}$, and $M_{Ppd\text{-}B1}$) increased the phyllochron of leaves emerging under NP + 0, while those that were insensitive ($M_{Ppd\text{-}D1}$ and $C_{Ppd\text{-}D1}$) showed similar phyllochron between the photoperiod treatments (Figure 3b).

Dry matter production and number of fertile florets at anthesis

Dry matter production at anthesis

The pre-anthesis late reproductive phase occurred in October and November of both years. Average temperature, incident radiation, and the average photothermal coefficient (Q) for these months are shown in Table 6. There were considerable differences between years, particularly for November: in 2001 the monthly incident radiation was higher (25%) and the average temperature was lower (13%) than in 2002, producing large differences in Q (53% higher in 2001 than 2002).

Total dry matter per m^2 at anthesis (TDM) depended on lines and years in both backgrounds

Table 6. Mean daily temperature (T), monthly accumulated incident radiation (R), and average daily photo-thermal coefficients (Q, the ratio between R and T above a threshold of 4.5 °C, see Fischer, 1985) during October and November for 2001 and 2002 experimental years

Year	Month	T (°C)	R (MJ m^{-2})	Q (MJ (m^2 °C d)$^{-1}$)
2001	October	18.4	356	0.85
	November	19.8	622	1.40
2002	October	19.6	400	0.86
	November	21.8	470	0.91

(Table 7). In general, all lines produced less TDM during 2002 than 2001. Within each year and independently of the photoperiod treatments, $M_{recessive}$ and $C_{recessive}$ produced more TDM than $M_{Ppd\text{-}D1}$ and $C_{Ppd\text{-}D1}$, respectively, while $M_{Ppd\text{-}B1}$ had an intermediate production. Photoperiod treatments had only a slight impact on TDM, which tended to decrease under NP + 6 vs. NP + 0 in the most sensitive lines ($M_{recessive}$ and $C_{recessive}$). The partitioning of the total dry matter to the spikes (PDM) varied with years, photoperiod treatments and lines (Table 7). The PDM was greater during 2002 than 2001, and differences between lines and photoperiod treatments were more evident. Under NP + 0, $M_{recessive}$ and $C_{recessive}$ had higher PDM than $M_{Ppd\text{-}D1}$ and $C_{Ppd\text{-}D1}$, respectively, but these differences were not evident under NP + 6. $M_{Ppd\text{-}B1}$ had an intermediate PDM, which did not change when altering photoperiod.

In general, $M_{recessive}$ and $C_{recessive}$ produced higher spike dry weights per m^2 (main shoot spike –MSP- and tiller spike -TSP-) than $M_{Ppd\text{-}D1}$ and $C_{Ppd\text{-}D1}$, while

Table 7. Dry matter production at anthesis.

Year	Photoperiod	Line	TDM ($g\,m^{-2}$)	PDM (%)	MSP ($g\,m^{-2}$)	TSP ($g\,m^{-2}$)	MST ($g\,m^{-2}$)	MSP:MST (%)
(a) Mercia								
2001	NP + 0	$M_{recessive}$	972 a	14 de	116 a	22 bcde	394 a	29 de
		$M_{Ppd\text{-}B1}$	733 bcd	16 bcd	105 ab	14 cde	312 c	33 cd
		$M_{Ppd\text{-}D1}$	508 def	15 cde	66 def	9 e	235 d	28 de
	NP+6	$M_{recessive}$	683 bcde	14 de	84 bcd	11 de	335 bc	25 e
		$M_{Ppd\text{-}B1}$	846 ab	14 cde	109 ab	14 cde	372 ab	29 de
		$M_{Ppd\text{-}D1}$	468 ef	13 e	53 f	7 e	206 e	26 de
2002	NP + 0	$M_{recessive}$	755 abc	23 a	105 ab	65 a	186 efg	57 a
		$M_{Ppd\text{-}B1}$	621 bcdef	19 b	89 abc	31 bc	200 ef	45 b
		$M_{Ppd\text{-}D1}$	388 f	16 bcd	58 ef	6 e	138 g	42 b
	NP+6	$M_{recessive}$	650 bcde	17 bc	74 def	38 b	188 efg	40 bc
		$M_{Ppd\text{-}B1}$	566 cdef	19 b	75 def	29 bcd	170 fg	44 b
		$M_{Ppd\text{-}D1}$	414 f	16 bcd	61 ef	8 e	158 fg	39 bc
(b) Cappelle Desprez								
2001	NP + 0	$C_{recessive}$	1043 a	14 c	130 a	17 ab	466 a	28 de
		$C_{Ppd\text{-}D1}$	695 bc	13 cd	88 bc	6 c	353 b	25 ef
	NP+6	$C_{recessive}$	799 b	12 d	83 bc	10 bc	379 b	22 f
		$C_{Ppd\text{-}D1}$	533 cde	12 d	57 d	4 c	267 c	21 f
2002	NP + 0	$C_{recessive}$	608 bcde	21 a	104 b	25 a	200 cde	52 a
		$C_{Ppd\text{-}D1}$	426 e	17 b	68 cd	6 c	164 f	42 b
	NP+6	$C_{recessive}$	650 bcd	15 bc	64 cd	17 ab	250 cd	33 cd
		$C_{Ppd\text{-}D1}$	481 de	15 bc	82 bc	10 bc	178 de	36 c

TDM: total dry matter, PDM: total partitioning to the spikes (main shoot + tillers), MSP: main shoot spike, TSP : tiller spike, MST: main shoot stem, MSP:MST: spike to stem ratio in main shoot. NP + 0 and NP+6 stand for natural and extended photoperiod treatments, respectively. Unlike letters within column and background mean statistical differences (LSD $\alpha = 0.05$).

$M_{Ppd\text{-}B1}$ showed a similar spike dry weight to $M_{recessive}$ (Table 7). Nevertheless, spike dry weights differed not only between lines but also between years and photoperiod treatments. $M_{recessive}$ and $C_{recessive}$ increased MSP under NP + 0 respect to NP+6 by 26–29% and 36–38 %, respectively, for 2001–2002 (Table 7). In contrast, $M_{Ppd\text{-}B1}$, $M_{Ppd\text{-}D1}$ and $C_{Ppd\text{-}D1}$ showed no significant change in MSP associated with the photoperiod treatments (except for $C_{Ppd\text{-}D1}$ and $M_{Ppd\text{-}B1}$ during 2001, where a small change took place, see Table 7). The general response to photoperiod of TSP was similar to MSP (Table 7). The number of tiller spikes per m^2 was highly variable and only the differences between lines were clear (average tiller spikes per m^2: 187a, 129ab and 75b for $M_{recessive}$, $M_{Ppd\text{-}B1}$ and $M_{Ppd\text{-}D1}$, respectively; and 87a and 57b for $C_{recessive}$ and $C_{Ppd\text{-}D1}$ respectively; different letters within background indicate statistical difference, LSD 0.05).

Stem dry weight of the main shoot per m^2 (MST) was affected by lines and years but it was not modified by the photoperiod treatments (Table 7). In general, $M_{Ppd\text{-}D1}$ and $C_{Ppd\text{-}D1}$ had lighter stems than the remaining lines and all lines partitioned less dry matter to stems during 2002 than 2001, resulting in great differences in the ratio between main shoot spikes and stems (MSP:MST, see Table 7). Within each year, and mainly as a consequence of variation in MSP, $M_{recessive}$ and $C_{recessive}$ increased the MSP:MST ratio under NP + 0 vs. NP+6 and some change was observed in $C_{Ppd\text{-}D1}$ (Table 7).

Relationship between spike dry weight and accumulated intercepted radiation

Variation in the total spike dry matter production per m^2 (MSP+TSP) for all lines, years and photoperiod treatments, was associated with the intercepted radiation accumulated during the late reproductive phase (Figure 4a). Considering both years, all lines, and photoperiod treatments, the intercepted radiation accumulated during the late reproductive phase ranged from 200 to 550 $MJ\,m^{-2}$. Neither photoperiod treatments

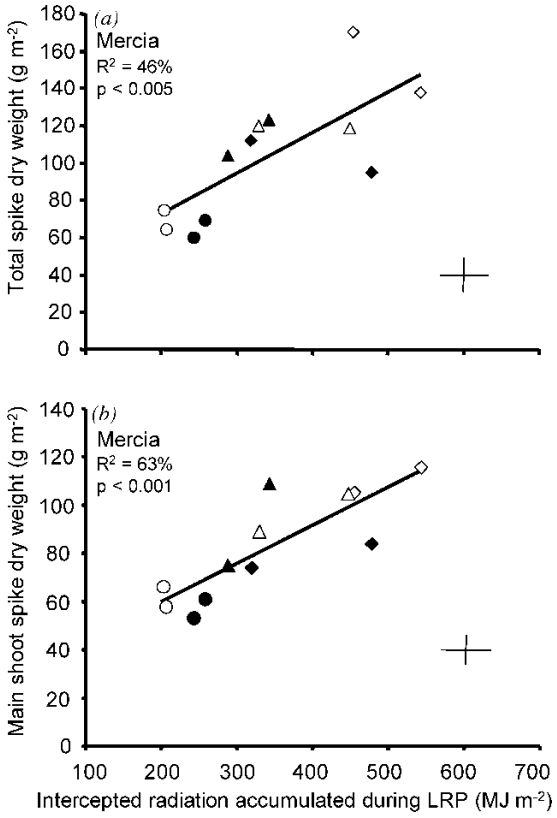

Figure 4. Relationship between (a) total (MSP + TSP) and (b) main shoot (MSP) spike dry weight at anthesis, with intercepted radiation accumulated during the late reproductive phase (LRP), for both years, in $M_{recessive}$ (◇◆), $M_{Ppd\text{-}B1}$ (△▲) and $M_{Ppd\text{-}D1}$ (○●). Open and closed symbols stand for natural (NP + 0) and extended (NP+6) photoperiod treatments. Lines in the bottom right of figure indicate S.E. Cappelle Desprez showed similar response showing for (a) $R^2 = 46\%$ $p < 0.05$ and for (b) $R^2 = 45\%$ $p < 0.05$ (data not shown).

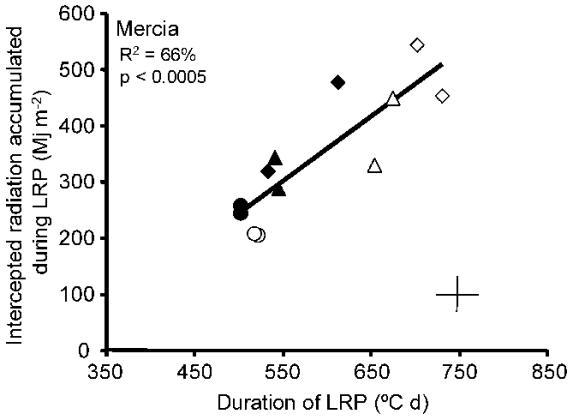

Figure 5. Relationship between intercepted radiation accumulated during the late reproductive phase (LRP) and duration of the late reproductive phase, for both years in Mercia. The meaning of symbols is similar to Figure 4. Lines in the bottom right of figure indicate S.E. Cappelle Desprez showed similar response ($R^2 = 49\%$ $p < 0.05$, data not shown).

nor years modified the interception of the incoming radiation. Nevertheless, it was different between lines. Those with recessive alleles intercepted between 93% ($M_{recessive}$) and 96% ($C_{recessive}$) of the incoming radiation at anthesis, while $M_{Ppd\text{-}B1}$ had 90% interception at that time. The lines with *Ppd-D1* intercepted less radiation than the other lines, reaching 81% ($M_{Ppd\text{-}D1}$) and 85% ($C_{Ppd\text{-}D1}$) at anthesis. Almost 50% of variation observed in the intercepted radiation accumulated during the late reproductive phase was determined by the duration of this phase (Figure 5), which was altered by the interaction between the *Ppd* alleles and the photoperiod treatments. The final spike weight per m^2 at anthesis, which is determined during the late reproductive phase, is affected by (i) the survival of tillers that set the number of tiller spikes per m^2 and (ii) the growth of individual spikes which determines the final weight at anthesis. As described earlier, the number of tiller spikes per m^2 was greater in the most photoperiod-sensitive lines (i.e. those with *ppd-D1*). When these tiller spikes were disregarded, the dry weight of main shoot spikes (MSP) was also highly associated with the intercepted radiation accumulated during the late reproductive phase (Figure 4b). Thus, $M_{recessive}$ and $C_{recessive}$, which under NP + 0 had a longer late reproductive phase than the lines with dominant *Ppd* alleles (Figure 2), accumulated more intercepted radiation during this phase (Figure 5) resulting in an increased MSP (Figure 4b). Although $M_{Ppd\text{-}B1}$ accumulated a different amount of radiation during the late reproductive phase (Figure 5), no variation in MSP was observed (Figure 4b). The lines with no photoperiod response during the late reproductive phase ($M_{Ppd\text{-}D1}$ and $C_{Ppd\text{-}D1}$) did not show a clear trend in the accumulated intercepted radiation and MSP (Figures 5 and 4b).

Number of fertile florets in main shoot spikes and its relationship with spike dry weight at anthesis

The number of fertile florets per spike differed among lines, photoperiod treatments and years (Table 8). Under NP+6 almost no difference was observed between *ppd-D1* and *Ppd-D1*: $M_{recessive}$ and $C_{recessive}$ had similar number of fertile florets per spike as $M_{Ppd\text{-}D1}$ and $C_{Ppd\text{-}D1}$, respectively (except for 2002 where the difference between $M_{recessive}$ and $M_{Ppd\text{-}D1}$ was 9%,Table 8). Meanwhile, $M_{Ppd\text{-}B1}$ had a higher number of fertile florets per spike than other lines under NP + 6, with

Table 8. Fertile florets at anthesis in main shoot spikes

		Mercia		Cappelle Desprez	
Year	Photoperiod	Line	Fertile florets spike -1	Line	Fertile florets spike -1
2001	NP + 0	$M_{recessive}$	46.7 abc	$C_{recessive}$	47.4 a
		$M_{Ppd\text{-}B1}$	49.9 a	–	–
		$M_{Ppd\text{-}D1}$	40.1 bcd	$C_{Ppd\text{-}D1}$	37.5 bc
	NP+6	$M_{recessive}$	36.4 d	$C_{recessive}$	33.6 c
		$M_{Ppd\text{-}B1}$	52.5 a	–	–
		$M_{Ppd\text{-}D1}$	36.7 d	$C_{Ppd\text{-}D1}$	33.0 c
2002	NP + 0	$M_{recessive}$	52.2 a	$C_{recessive}$	45.2 ab
		$M_{Ppd\text{-}B1}$	52.1 a	—	–
		$M_{Ppd\text{-}D1}$	38.9 cd	$C_{Ppd\text{-}D1}$	39.0 bc
	NP+6	$M_{recessive}$	46.8 ab	$C_{recessive}$	39.5 abc
		$M_{Ppd\text{-}B1}$	51.1 a	–	–
		$M_{Ppd\text{-}D1}$	36.9 d	$C_{Ppd\text{-}D1}$	35.9 c

NP + 0 and NP+6 stand for natural and extended photoperiod treatments, respectively. Unlike letters within column and background mean statistical differences (LSD $\alpha = 0.05$).

this difference being greater in 2001 than 2002. Under NP + 0, $M_{recessive}$ and $C_{recessive}$ produced more fertile florets per spike than $M_{Ppd\text{-}D1}$ and $C_{Ppd\text{-}D1}$ (14 to 25%), while $M_{Ppd\text{-}B1}$ had a number similar to $M_{recessive}$ (Table 8). Thus, when the recessive alleles were present ($M_{recessive}$ and $C_{recessive}$) the number of fertile florets per spike increased under NP + 0 relative to NP+6 (Table 8). In contrast, when the dominant allele was present ($M_{Ppd\text{-}B1}$, $M_{Ppd\text{-}D1}$ and $C_{Ppd\text{-}D1}$) the number of fertile florets per spike did not respond to photoperiod treatments (Table 8).The response of the number of fertile florets per spike to photoperiod was mainly explained by changes in the number of fertile florets per spikelet, as the proportion of spikelets bearing florets was not modified by photoperiod treatments (except for $C_{recessive}$ during 2001, data not shown).

As expected, the dry weight of main shoot spikes per m^2 at anthesis (MSP) explained almost 80% of variation in the number of fertile florets per m^2 within each background, considering all lines, years and photoperiod treatments (Figure 6). The lines that were sensitive to the photoperiod treatments during the late reproductive phase and, consequently, increased the spike dry weight at anthesis under NP + 0 ($M_{recessive}$ and $C_{recessive}$), produced higher number of fertile florets per m^2 associated with the increased spike dry weight at anthesis (Figure 6). The number of fertile florets per m^2 increased under NP + 0 *vs*. NP+6 by 17–24% in

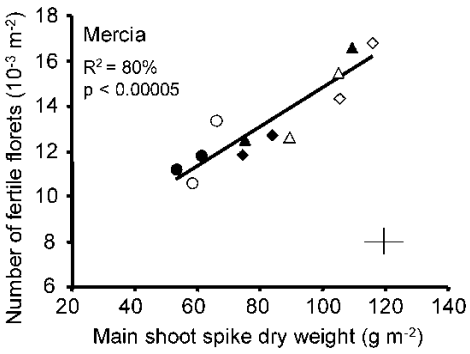

Figure 6. Relationship between number of fertile florets and spike dry weight in main shoot spikes (MSP), for both years in Mercia. The meaning of symbols is similar to Figure 4. Lines in the bottom right of figure indicate S.E. Cappelle Desprez showed similar response ($R^2 = 83\%$ $p < 0.001$, data not shown).

$M_{recessive}$ and by 15–34% in $C_{recessive}$ (depending on the year). In contrast, lines with a dominant allele showed changes in the spike dry weight and fertile florets per m^2 between years but no effect of the photoperiod treatments was observed (Figure 6).

Discussion

Phenology

Time to anthesis and leaf emergence

Time to anthesis was more affected by *Ppd-D1* than *Ppd-B1*, agreeing with previous studies (Tables 1 and 2). *Ppd-D1* reduced the time to anthesis similarly in both backgrounds (476 °C d in Mercia and 505 °C d in Capelle Desprez, compared to the recessive controls and averaged across years). This effect was greater. i.e. 23 to 24 days under the natural photothermal conditions of the present study, than that usually reported for *Ppd-D1* but coincides with the one estimated by Stelmakh (1998) under similar day-lengths (Tables 1 and 2; see 12–16 h photoperiod in Stelmakh 1998). *Ppd-B1* reduced time to anthesis by 178 °C d (averaged across years), equivalent to 6–10 days, depending on the year. This effect agrees with several previous reports (Law, 1987; Worland, 1996; Worland et al., 1998; see Table 1) but disagrees with others (Scarth et al., 1985; Law & Worland, 1997; Stelmakh, 1998; Whitechurch & Slafer, 2001, 2002; see Table 1 and 2).

Leaf emergence fitted a bi-linear model agreeing with many studies reported for commercial cultivars (Baker et al., 1986; Hay & Delécolle, 1989; Cao &

Moss, 1991a,b; Jamieson et al., 1995; Calderini et al., 1996; Slafer & Rawson, 1997; Miralles & Richards, 2000; González et al., 2002). Phyllochron of early emerging leaves was not affected by the *Ppd* constitution. However, phyllochron of the late emerging leaves tended to increase in the lines that were sensitive to photoperiod during the late reproductive phase. This result agrees with previous reports for commercial photoperiod sensitive cultivars where phyllochron of late emerging leaves was responsive to the photoperiod environment (Slafer & Rawson, 1997; Miralles & Richards, 2000; González et al., 2003a). Even though lines showed strong differences in development, they had similar leaf numbers when phyllochron changed. That is, phyllochron change took place at a relatively constant number of emerged leaves (at 7th–8th leaf), disregarding any particular stage of apex development or final leaf number (Stapper & Fischer, 1990; Jamieson et al., 1995; Kirby, 1995; Slafer, 1995; Slafer & Rawson, 1997; Miralles & Richards, 2000; González et al., 2002).

Pre-anthesis developmental phases

Ppd-D1 shortened the duration of all pre-anthesis developmental phases. Under natural field conditions (NP + 0), *Ppd-D1* reduced (i) 202 and 121 °C d the duration of TR-DR (representing between 12.5 and 7.5 calendar days), (ii) 213 and 78 °C d the duration of DR-TS (from 10 to 3 calendar days), and (iii) 196 and 169 °C d the duration of TS-AN (i.e. from 8 to 6.6 calendar days) for Mercia and Cappelle Desprez, respectively (averaged across years). These results partially contrast with those of Scarth et al. (1985) and Foulkes et al. (2004). In Scarth et al. (1985), *Ppd-D1* highly affected the duration of the early and late reproductive phases, but it did not alter the duration of the vegetative phase (see Table 2). In Foulkes et al. (2004) *Ppd-D1* modified duration of development prior to stem elongation (from seedling emergence to GS31), but it did not affect the late reproductive phase (see Table 2). These inconsistencies make us cautious in extrapolating the results as it appears that the environmental background in which the *Ppd* effects are tested may affect the results. The number of differentiated leaves and spikelets per spike decreased as *Ppd-D1* reduced TR-DR and DR-TS phases, respectively, meaning that neither rate of leaf nor rate of spikelet differentiation were greatly affected by this allele (Scarth et al., 1985; Snape et al., 2001).

The *Ppd-B1* effects on time to anthesis under natural photo-thermal conditions were the consequence of the reduction of the early reproductive phase by ca. 5 days. *Ppd-B1* had almost not effect on the vegetative and late reproductive phases. Scarth et al. (1985) also reported an effect of *Ppd-B1* on the early reproductive phase and negligible effects on the vegetative phase, however, duration of the late reproductive phase increased as a consequence of the early formation of the terminal spikelet (see Table 2). The results reported in this paper conflict with previous results of our lab (Whitechurch & Slafer, 2002) where lines with *Ppd-B1* were insensitive to photoperiod during the vegetative and the late reproductive phases, but they were as sensitive as the recessive controls during the early reproductive phase (see Table 2). Due to the relatively small effect of *Ppd-B1* it is likely that results may be highly influenced by the experimental conditions (genetic background, type of line, environmental background, etc.). *Ppd-B1* slightly affected the final number of leaves, which was consistent with its small impact on either duration of the vegetative phase (Scarth et al., 1985) or rate of leaf differentiation (Scarth et al., 1985, Whitechurch & Slafer, 2002). Despite that the early reproductive phase was reduced by *Ppd-B1*, the number of spikelets was similar to the recessive control, agreeing with Scarth et al. (1985), who observed higher rate of spikelet differentiation associated with *Ppd-B1*. Nevertheless, Whitechurch & Slafer (2002) reported that *Ppd-B1* decreased rate of spikelet differentiation by 24% (under 12.1 h).

Response to photoperiod during the late reproductive phase

The response of the late reproductive phase to the extended photoperiod (NP+6) agreed with the results observed under natural field conditions (NP + 0). *Ppd-D1* was insensitive in both backgrounds, while *Ppd-B1* was as sensitive as the recessive control. Photoperiod sensitivity was associated with phyllochron without an evident relationship with the number of leaves emerging during this phase. This contrasts with the results of Whitechurch and Slafer (2002). However, Miralles and Richards (2000) and González et al. (2003a), showed changes in phyllochron when the late reproductive phase was exposed to contrasting photoperiods.

The lack of agreement between the different studies about the impact of *Ppd* alleles may be a consequence of several factors. From the genetic point of view, factors such as (i) the line used, i.e. near isogenic, single chromosome recombinant or substitution line; (ii) the donor of the dominant alleles (see Scarth & Law, 1984), (iii) the recipient or background, and (iv) the presence

of yet unknown photoperiod response genes interacting with the already known genes (Snape et al., 2001), would form a complex net of interactions that may affect the *Ppd* response. From the physiological point of view, the precise description of the environment and the expression of time in degree-days would help to extrapolate results (Slafer & Rawson, 1994).

Number of fertile florets at anthesis and their main ecophysiological determinants

Most previous papers on the effects of the *Ppd* genes on wheat yield potential focused on the adaptation to the target environment and the numerical components of yield. For instance, it was reported that *Ppd-D1* and *Ppd-B1* reduced the number of spikelets per spike, but increased spikelet fertility, resulting in no impact on the final number of grains per spike (e.g. Worland et al., 1988; Börner et al., 1993; Worland, 1996; Worland et al., 1998). This numerical approach may be unsound for predicting the effect of manipulating a trait to alter wheat yield because numerical components are usually negatively correlated (Fischer, 1984; Slafer, 2003, and several papers quoted therein). Therefore, we also analysed the spike dry weight at anthesis, the most significant eco-physiological determinant of fertile florets (and grains) per m^2 when yield is altered by different factors such as (i) shading at different phenophases (Fischer, 1985; Thorne & Wood, 1987; Savin & Slafer, 1991; Abbate et al., 1997; Demotes-Mainard et al., 1999; Demotes-Mainard & Jeuffroy, 2004), (ii) genetic differences including semi-dwarfing genes and cultivars released at different eras (Fischer & Stockman, 1980; Brooking & Kirby, 1981; Stockman et al., 1983; Siddique et al., 1989; Slafer & Andrade, 1993; Miralles et al., 1998), (iii) nutrient availability (Fischer, 1993; Abbate et al., 1995; Demotes-Mainard et al., 1999; Demotes-Mainard & Jeuffroy, 2001; Prystupa et al., 2004), and (iv) photoperiod changes (Miralles et al., 2000; González et al., 2003a).

Total dry matter production (TDM) varied according to the length of the cycle to anthesis. Agreeing with Foulkes et al. (2004), the early lines carrying the *Ppd-D1* allele produced less dry matter than lines carrying the *Ppd-B1* or both recessive alleles, which determined a longer period to anthesis. The proportion of the total dry matter that was partitioned to the spikes (PDM) and the spike to stem ratio in main shoots (MSP:MST) were not intrinsic characteristics of the lines but they depended on years and the photoperiod treatments. These three factors, i.e. lines, years and photoperiod treatments, altered spike dry weight at anthesis because they modified the intercepted radiation accumulated during the late reproductive phase, when spikes are actively growing. More than 50% variation in the intercepted radiation accumulated during the late reproductive phase was explained by duration of this phase, despite different incident radiation during both years. Thus, as *Ppd-D1* lines were almost insensitive to photoperiod, they had shorter duration of the late reproductive phase than the recessive controls, and hence, less radiation was available for spike growth reducing its final weight and the number of fertile florets at anthesis. Nevertheless, the differences between the *Ppd-D1* and the *ppd-D1* lines depended on the photoperiod environment. Under extended daylength, the *ppd-D1* lines behaved similarly to the *Ppd-D1* lines because duration of the late reproductive phase and spike dry weight were similar, yielding no differences in the number of fertile florets at anthesis. That is, these alleles may alter wheat yield by modifying spike growth during the late reproductive phase, when the fate of the floret primordia is being determined. The response of these variables, i.e. dry matter partitioning, duration of the late reproductive phase and accumulated intercepted radiation, agrees with previous studies where sensitive and insensitive commercial cultivars were exposed to contrasting photoperiod environments only during the late reproductive phase (Miralles et al., 2000; González et al., 2003a). The manipulation of the photoperiod sensitivity during the late reproductive phase would be an alternative approach in the improvement of wheat yield (Slafer et al., 1996, 2001) because changing the photoperiod environment produced the same results as altering the photoperiod sensitivity by the *Ppd* alleles. Nevertheless, a longer duration of the late reproductive phase associated with a higher accumulated intercepted radiation did not always result in a higher spike dry weight (see results of the *Ppd-B1* line). In a previous report where commercial cultivars were exposed to short photoperiod (González et al., 2003b), the longer duration the of late reproductive phase resulted in a higher spike dry weight at anthesis due to the longer spike growth period without variation in the spike growth rate. In the present study, it remains unclear whether there was a trade-off between duration and the rate of spike growth yielding no difference in spike weight at anthesis in *Ppd-B1*. The impact of higher spike dry weight at anthesis on fertile floret number has been usually associated with an increased floret primordia survival (Siddique et al., 1989; Youssefian et al., 1992; Miralles et al., 1998, 2000; González et al., 2003b). Considering that the

effects of *Ppd* genes on fertile florets where highly associated with the dry weight of spikes at anthesis, it is possible that they may have increased floret survival.

Conclusion

Ppd-D1 was insensitive to photoperiod during the three pre-anthesis developmental phases, while *Ppd-B1* was insensitive only during the early reproductive phase. However, more genetic backgrounds should be tested. Whenever *ppd-D1* was present, a direct response to photoperiod during the late reproductive phase was observed independently of the presence of *ppd-B1* or *Ppd-B1*. This response was associated with phyllochron of the leaves emerging after the terminal spikelet initiation. Although no allele was particularly linked with the photoperiod sensitivity during the late reproductive phase, the likelihood of *Ppd-B1* of being associated with the early reproductive phase and the direct response to photoperiod during the late reproductive phase reinforce the idea that photoperiod sensitivity of individual phases may be partially independent of each other. Altering the photoperiod environment during the late reproductive phase had a similar impact on the fertile floret number at anthesis as changing the photoperiod sensitivity by the *Ppd-D1*/*ppd-D1* alleles. Although the incident radiation and the photo-thermal coefficient during the late reproductive phase were contrasting between years, longer duration of the late reproductive phase consistently yielded a higher spike dry weight and fertile floret number at anthesis. Thus, increasing photoperiod sensitivity during the late reproductive phase (e.g. through *ppd-D1* allele in the present study) seems to be an alternative way to increase the fertile floret number and wheat yield potential. However, we may need to learn more on the interactions of these alleles with both the genetic background and the environment before this knowledge may be translated to practice. New alleles, photoperiod environments and experimental approaches should be tested to identify the control of photoperiod sensitivity of particular phases to fine-tune pre-anthesis wheat development in actual breeding.

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