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ORIGINAL PAPER

Earliness per se QTLs and their interaction with the photoperiod insensitive allele *Ppd-D1a* in the Cutler × AC Barrie spring wheat population

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Abstract Earliness per se regulates flowering time independent of environmental signals and helps to fine tune the time of flowering and maturity. In this study, we aimed to map earliness per se quantitative trait loci (QTLs) affecting days to flowering and maturity in a population developed by crossing two spring wheat cultivars, Cutler and AC Barrie. The population of 177 recombinant inbred lines (RILs) was genotyped for a total of 488 SSR and DArT polymorphic markers on all 21 chromosomes. Three QTLs of earliness per se affecting days to flowering and maturity were mapped on chromosomes 1B (*QEps.dms-1B1* and *QEps.dms-1B2*) and 5B (*QEps.dms-5B1*), in individual environments and when all the environments were combined. A QTL affecting flowering time (*QFlt.dms-4A1*) was identified on chromosome 4A. Two grain yield QTLs were mapped on chromosome 5B, while one QTL was mapped on chromosome 1D. The population segregated for the photoperiod insensitive gene, *Ppd-D1a*, and it induced earlier flowering by 0.69 days and maturity by 1.28 days. The photoperiod insensitive allele *Ppd-D1a* interacted in an additive fashion with QTLs for flowering and maturity times. The earliness per se QTL *QFlt.dms-5B.1* inducing earlier flowering could help to elongate grain filling duration for higher grain yield. Hence, chromosome 5B possesses promising genomic regions that may be introgressed for higher grain yield with earlier maturity through marker-assisted selection in bread wheat.

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Introduction

Early maturity in wheat (*Triticum aestivum* L.) is an important breeding objective in regions where the growing season is short and days are long (>14 h), such as the Northern Great Plains of Canada and the USA. The development of early maturing cultivars is also important to avoid frost damage, which can affect both yield and grain quality (Iqbal et al. 2007). Flowering time of wheat is the outcome of a complex interaction of genes that regulate growth habit and earliness. Major gene classes that determine flowering time include vernalization (*Vrn*), photoperiod (*Ppd*) and earliness per se (*Eps*). Different alleles at the *Ppd* loci divide cereals into photoperiod sensitive and insensitive, while *Vrn* genes divide them into winter and

spring types (Distelfeld et al. 2009). Insensitivity to photoperiod and vernalization is due to deletion mutations in the genomic regions involved in plant responses to environmental signals (Yan et al. 2004; Beales et al. 2007; Santra et al. 2009). A two Kbp deletion mutation upstream of the *Ppd-D1* coding region is supposed to have altered the transcriptional start site or has caused removal of the regulatory element (Beales et al. 2007); changing photoperiod sensitive cultivars to insensitive ones.

Earliness per se (*Eps*) genes are considered to be of smaller effect and are not involved in the *Vrn* and *Ppd* complexes (Miura and Worland 1994). Ford et al. (1981) reported some 'other genes' controlling flowering time in wheat apart from vernalization and photoperiod genes and called them "earliness genes". Earliness per se genes have been reported to be strong enough to induce earlier flowering, even in the presence of *Vrn* and *Ppd* genes (van Beem et al. 2005).

The intricate flowering gene network and allo-hexaploid nature of bread wheat has challenged scientists attempting to quantify individual gene effect. To avoid confounding effects of different genomes, studies on *monococum* wheat have been conducted. Bullrich et al. (2002) mapped a major QTL in a *Triticum monococum* accession located close to the SSR marker *Xwg 241* on the long arm of chromosome 1A. Minor flowering difference due to *Eps* genes have been reported by Laurie et al. (1995), Worland (1996a, b) and Kato et al. (1999).

Earliness per se genes can induce early flowering by initiating floral primordia with minimum vegetative growth (Kato and Wada 1999). The effect of *Eps* genes on different phases of early reproductive and vegetative growth was investigated by Lewis et al. (2008). Their study revealed that *Eps-A^{m}1-e* can activate transition of vegetative apices to reproductive apices 35 days earlier than *Eps-A^{m}1-l*. Time from double ridge formation to terminal spikelet was longer in lines carrying *Eps-A^{m}1-l* allele. This prolonged duration resulted in increased grain yield as the lines with late allele *Eps-A^{m}1-l* produced more spikelets per spike than those with the early flowering allele *Eps-A^{m}1-e* (Lewis et al. 2008). Hence, earliness per se genes can alter different growth phases and some other pleiotropic effects are attributed to these genes. In another study, a major *Eps* gene was reported on chromosome 3A that was responsible for significant variation for plant height, 1,000 grain weight and number of grains per plant (Shah et al. 1999). Similar findings of pleiotropic effect of vernalization, photoperiod and earliness per se genes have been reported (Worland and Snape 2001). Earliness per se genes are also considered to play their role in adaptability (Snape et al. 2001). The *Eps* genes are important from a practical breeding viewpoint due to their high broad sense heritability (0.90–0.99) (Kato and Wada 1999) and additive type of gene action (Bullrich et al. 2002).

This study was designed to map QTLs for earliness per se by crossing two spring wheat cultivars maturing significantly apart from each other (Iqbal et al. 2006). The earlier maturing parent Cutler possessed the photoperiod insensitive allele (*Ppd-D1a*), while the late maturing parent AC Barrie possessed the photoperiod sensitive allele (*Ppd-D1b*) (Iqbal et al. 2006). However, both the parents are vernalization insensitive and possess similar vernalization alleles at the three *Vrn-1* loci (Iqbal et al. 2006; Iqbal et al. 2007). This yielded an opportunity to study the interaction between the photoperiod and earliness per se genes. In this study, we aimed to: (i) identify QTLs affecting flowering, maturity, plant height and grain yield in a Canadian spring wheat population derived from a cross between early and relatively late maturing cultivars; (ii) studying the effect of *Ppd-D1a* in a population where RILs share similar genetic background; (iii) investigate the interaction between the *Ppd-D1a* and earliness per se QTLs to improve the understanding of the flowering gene complex. This information will help to develop elite breeding material aimed for early maturing and high yielding cultivars in the region.

Materials and methods

Development of recombinant inbred lines

A population was developed by crossing two Canadian spring wheat cultivars, AC Barrie and Cutler. AC Barrie was one of the most widely grown and high yielding cultivars of Canadian Western Red Spring wheat. It has been characterized as having high grain yield and protein content, late maturing (compared to Cutler) and resistance to some diseases (McCaig et al. 1995). Cutler is an early maturing, semi-dwarf cultivar from the Canadian Prairie Spring class. Cutler was bred for regions where early maturity was of prime concern (Briggs et al. 1991) and possesses the vernalization genes *Vrn-A1a-vrn-B1-vrn-D1* and the photoperiod insensitive allele *Ppd-D1a*. AC Barrie, the comparatively late parent possesses *Vrn-A1a-vrn-B1-vrn-D1* genes and the photoperiod sensitive allele *Ppd-D1b*. Cutler and AC Barrie were crossed to produce F_1 seeds, and to subsequently develop a population of 177 $F_{6:7}$ recombinant inbred lines (RILs). The population ($F_{6:7}$) was originally used in the first field and green house experiment in 2007. Thereafter, heads were taken and re-grown for experimental use annually.

Greenhouse evaluation

Phenotypic analysis of parents and RILs was completed after satisfying vernalization and photoperiod

requirements. The sprouted seeds were vernalized for 42 days at 1 °C in the dark. At the end of the vernalization treatment, seedlings of similar size were transplanted into 12.5-cm diameter pots (two plants per pot). The experiment was arranged as a randomized complete design with four replications, each consisting of one pot per treatment. The population was grown in a greenhouse maintained at 25 °C and 18-h photoperiod (plants received both natural light and artificial illumination). Plants were watered every second day and fertilized biweekly with a 200 ppm solution of a water-soluble commercial fertilizer (15–30–15:N–P_2O_5–K_2O). To remove any confounding effects of differential growth during vernalization treatment, the date of second leaf unfolding was recorded for all plants. The date of heading was subsequently recorded for all plants as the date when the spike completely emerged from the flag leaf. Days to heading was recorded as the number of days from second leaf unfolding to the date of heading.

Field evaluation

Field experiments were conducted at or near the University of Alberta South Campus Crops Research facility in Edmonton, Canada (53°19′N, 113°35′W and 723.3 m elevation) in 2007, 2008, and 2011. In 2007 and 2011, the experiment was seeded on May 27 and May 14, respectively, while in 2008, the complete experiment was grown twice, one planted on May 07 (early) and one on June 04 (late) to assess any confounding effects of vernalization requirement. Each experiment was grown in a randomized complete block design with two replications. Plots consisted of 2-m long double rows with a row spacing of 22.5 cm in 2007 and 2008, while in 2011 the plot size was 1.35 × 1.8 m with six rows each spacing 22.5 cm. The parents, RILs and ten check cultivars Superb, AC Intrepid, CDC Go, AC Foremost, Lovitt, AC Mckenzie, AC Crystal, AC Splendor, and Peace were planted. Data were recorded on days to flowering, maturity, and grain yield. Flowering was recorded when 50 % of the spikes had emerged out of the flag leaf. Physiological maturity was determined when 50 % of the peduncles in a plot had completely lost green color. Days to flowering and maturity were converted into growing degree days by summing the average daily temperatures (over a base temperature of 0 °C) from the date of seeding to the date when flowering or maturity was recorded. Plant height was measured as height (cm) from ground level to the tip of spike (excluding awns) at maturity. Grain yield per plot was converted to t/ha. Fertilizers (N–P_2O_5–K_2O:11–52–0) were applied at the rate of 36 kg/ha at the time of sowing and other standard agronomic practices were undertaken throughout the growing season to obtain even crop stands.

DNA extraction

Seed was sown in trays containing commercial soil (Sunshine-LA4 Sun Grow Horticulture, Canada) and placed in a growth chamber for 7–10 days. Growing conditions were maintained at 21 °C day and 19 °C night and a 16-h photoperiod. Young plant tissue (100–150 mg.) was harvested and flash frozen in liquid nitrogen. Tissue was stored at −80 °C until DNA was extracted. Leaf tissue from a single plant was ground in liquid nitrogen. DNA of 177 RILs along with parents was extracted according to the protocol suggested by DArT (http://www.diversityarrays.com). DNA was quantified by NanoDrop® (ND-1000). DNA was diluted to 50–100 ng/µl. 100-µl DNA solution was aliquoted to 96-well microtitreplates and sent to Diversity Arrays Technology (DArT), Pty Ltd. Australia for marker genotyping with high-density arrays around 7,000 cloned sequences. The Diversity Arrays Technology genotyping followed protocols previously described by Akbari et al. (2006).

SSR genotyping

Three to four plants from selected RILs, parents and F_1 were grown in a controlled environment chamber. Genomic DNA was extracted from leaves of 7–10 day old plants using the Extract-N-Amp™ Plant PCR Kit (Sigma-Aldrich, Oakville, Canada; Cat# XNAP), following the protocol provided by the manufacturer. One hundred and two microsatellite loci from all 21 chromosomes of wheat were selected for genotyping. These were selected based on polymorphic differences when tested against the parents AC Barrie and Cutler. The forward primer of each primer pair was fluorescently labeled using either 6-FAM, NED or VIC (Applied Biosystems). Polymerase chain reaction was performed in a 20-µL volume in a GeneAmp® 9700 thermal cycler (Applied Biosystems; Foster City, CA, USA). The reaction mixture contained 0.5 µL each of the 5 µM forward and reverse primers (2 primer pairs were used in the same reaction to amplify 2 loci), 10-µL Extract-N-Amp™ PCR ReadyMix (Sigma-Aldrich, Cat# E3004), 4-µL sterile water, and 4-µL DNA extract. After initial denaturation at 94 °C for 2 min, 35 cycles at 94 °C for 1 min, 47–66 °C (depending on the primer pair used) for 1 min and 72 °C for 2 min were performed, followed by a final extension at 72 °C for 10 min. The PCR products (0.5 µL) were mixed with 0.10-µL Gene Scan™ 500-LIZ (Applied Biosystems) size standard and 9.4-µL Hi-Di Formamide, denatured at 94 °C for 2 min and chilled on ice for 2 min. Capillary electrophoresis was performed using ABI 3730 DNA Analyzer and products sizes were determined using Gene Mapper v. 3.7 software (Applied Biosystems).

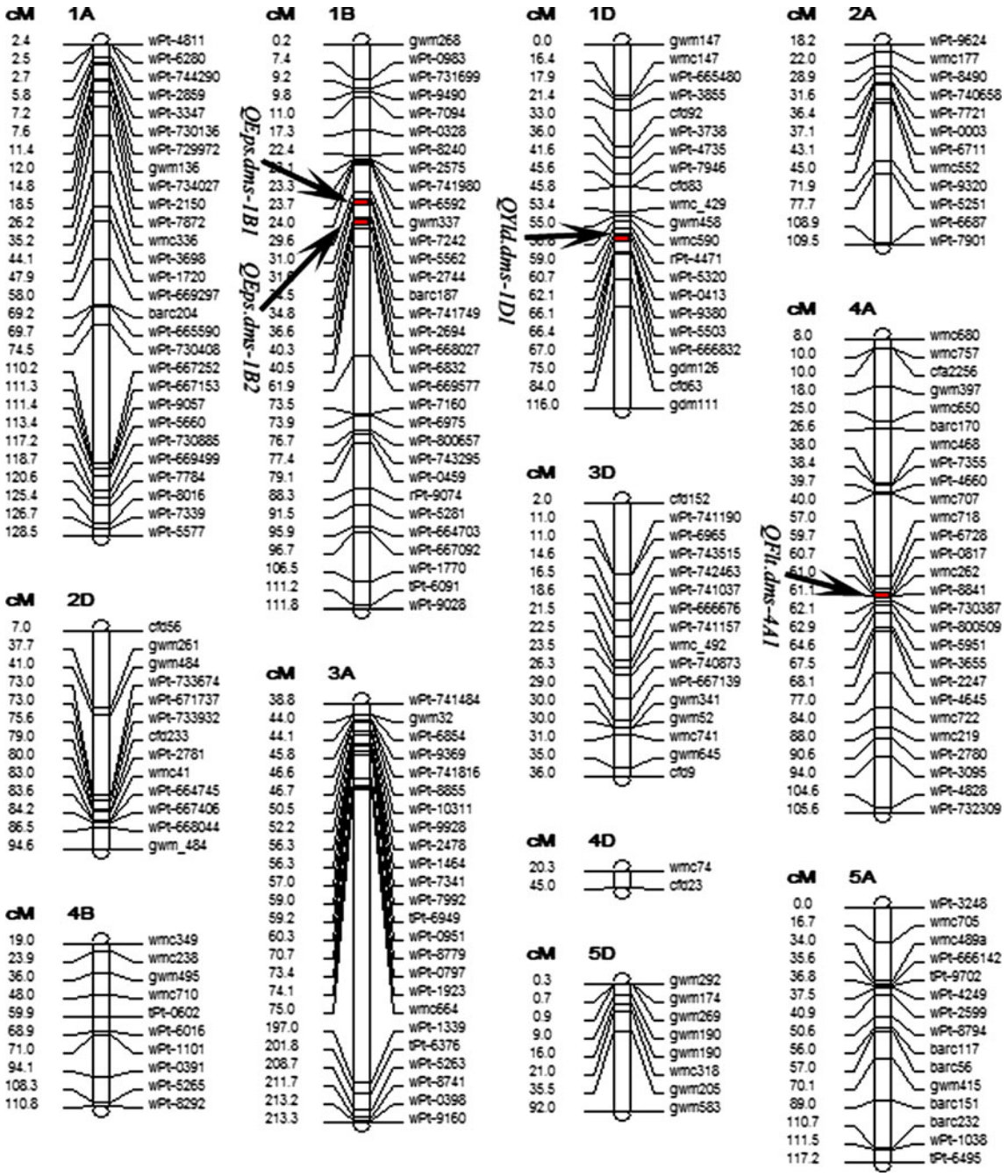

Fig. 1 DArT and SSR linkage map for Cutler × AC Barrie population with chromosomal regions showing the genomic regions involved in controlling days to flowering, maturity and grain yield

Statistical analysis

The data were analyzed using PROC MIXED in SAS statistical software package version 9.2 (SAS Institute Inc., Cary, NC, USA). The lines were considered as a fixed effect, whereas the effect of the block, incomplete block nested in block and seeding environments were considered as random effects.

Multivariate Restricted Estimation of Maximum Likelihood (REML) method was used for estimation of genetic and phenotypic correlation coefficients in PROC MIXED of SAS (Holland 2006). In the correlation analysis, the lines were considered as a random effect along with year, block, and incomplete block. The genetic ($\hat{r}_{g(xy)}$) and phenotypic ($\hat{r}_{p(xy)}$) correlations between the trait x and y were estimated as follows:

$$\hat{r}_{g(xy)} = \frac{\hat{\sigma}_{G(xy)}}{\sqrt{\hat{\sigma}^2_{G(x)} \cdot \hat{\sigma}^2_{G(y)}}},$$

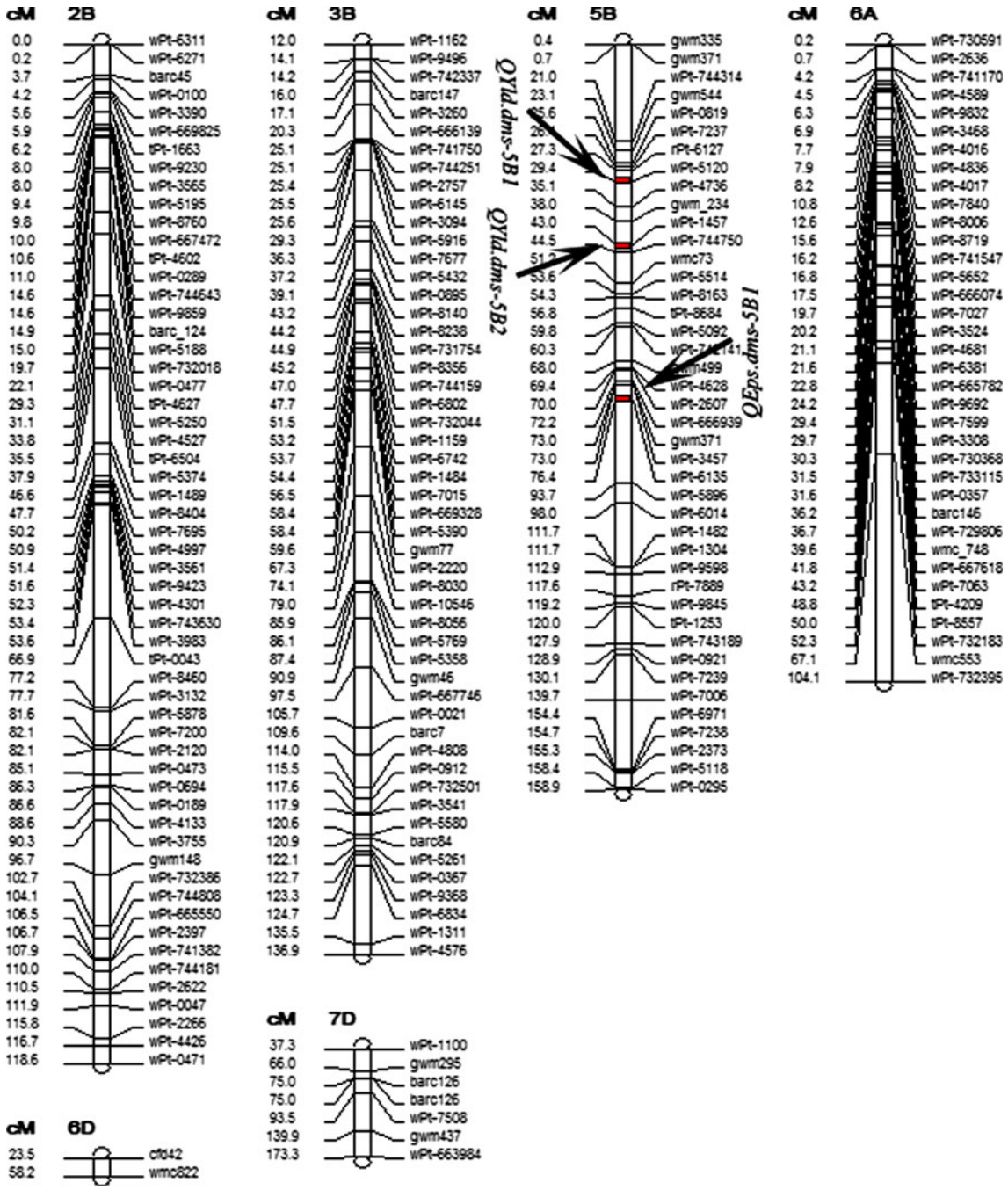

Fig. 2 DArT and SSR linkage map for Cutler × AC Barrie population with chromosomal regions showing the genomic regions involved in controlling days to flowering, maturity and grain yield

and

$$\hat{r}_{\mathrm{P}(xy)} = \frac{\hat{\sigma}_{\mathrm{G}(xy)}}{\sqrt{\hat{\sigma}^2_{\mathrm{P}(x)} \cdot \hat{\sigma}^2_{\mathrm{P}(y)}}} = \frac{\hat{\sigma}_{\mathrm{G}(xy)} + \hat{\sigma}_{\mathrm{GE}(xy)} + \hat{\sigma}_{\mathrm{e}(xy)}}{\sqrt{\hat{\sigma}^2_{\mathrm{G}(x)} + \hat{\sigma}^2_{\mathrm{GE}(x)} + \hat{\sigma}^2_{\mathrm{e}(x)}} \cdot \sqrt{\hat{\sigma}^2_{\mathrm{G}(y)} + \hat{\sigma}^2_{\mathrm{GE}(y)} + \hat{\sigma}^2_{\mathrm{e}(y)}}},$$

where $\hat{\sigma}_{\mathrm{G}(xy)}$, $\hat{\sigma}_{\mathrm{P}(xy)}$, $\hat{\sigma}_{\mathrm{GE}(xy)}$ and $\hat{\sigma}_{\mathrm{e}(xy)}$ are the estimated genetic, phenotypic, genotype × environment and error covariances, respectively, between the two traits (x and y); while $\hat{\sigma}^2_{\mathrm{G}}$ is genetic variance, $\hat{\sigma}^2_{\mathrm{P}}$ is phenotypic variance, and $\hat{\sigma}^2_{\mathrm{GE}}$ and $\hat{\sigma}^2_{\mathrm{e}}$ are the estimated genotype × environment and error variances calculated for both traits (Holland 2006). To test whether genetic and phenotypic correlation coefficients, differed from zero significantly, the coefficients were Z transformed as suggested by (Fisher 1925): $Z_{xy} = \left[\ln\left(1 + r_{xy}\right) - \ln\left(1 - r_{xy}\right)\right]/2$. The Z_{xy} variable was

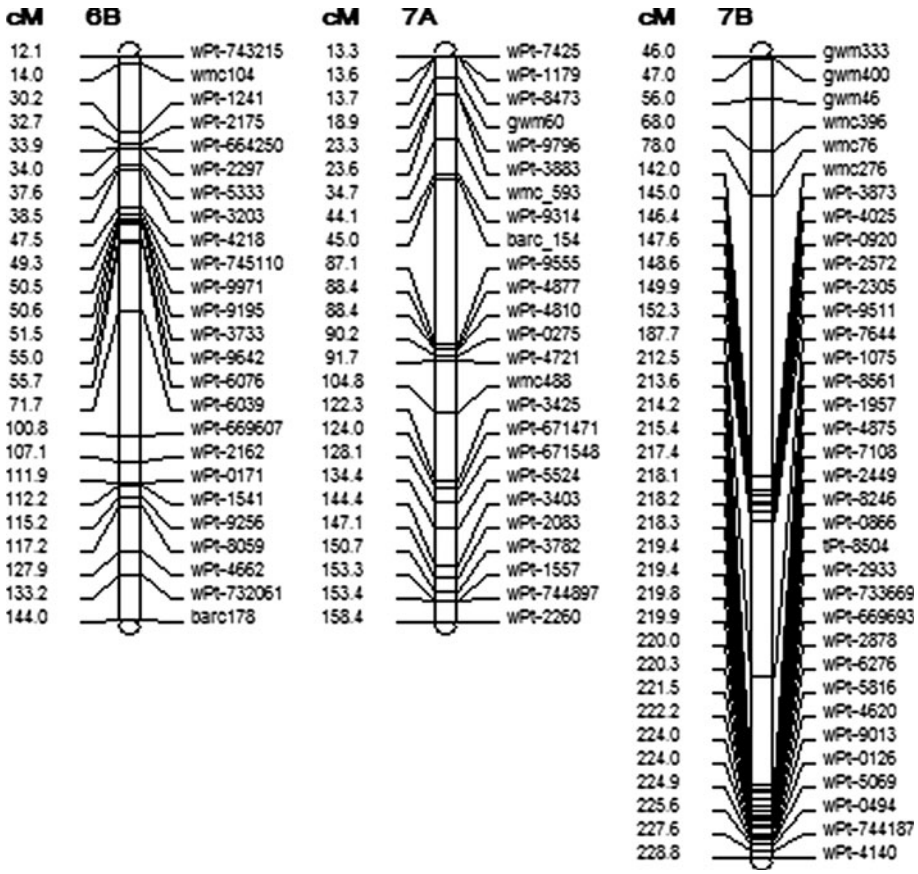

Fig. 3 DArT and SSR linkage map for Cutler × AC Barrie population

examined with $Z' = \frac{Z_{xy}}{1/\sqrt{n-3}}$ where n is the total number of recombinant inbred lines in the study.

PROC MIXED of SAS (SAS Institute Inc. 2003) was used for better estimation of lsmeans (Yang 2010) for QTL analysis using four traits of interest; days to flowering, days to maturity, plant height, and grain yield. Incomplete block nested within block was considered as random in estimating the individual seeding environment, while incomplete block nested within block and seeding environment were considered as random effects to estimate lsmeans for pooling the seeding environments.

The QTL and *Ppd-D1a* gene interaction was studied by identifying the lines having the QTL-linked marker and *Ppd-D1a* scores present versus absent. The CONTRAST and ESTIMATE commands were used in PROC MIXED analysis of SAS by comparing the lines with alternate molecular variants at each QTL and *Ppd-D1a* allele with the lines: (a) having both QTL-linked marker and *Ppd-D1a*, (b) having *Ppd-D1a* allele only, and (c) having QTL-linked marker only. The coefficients for CONTRAST and ESTIMATE were orthogonal, hence the alpha values were not adjusted.

QTL analysis

One hundred and seventy-seven recombinant inbred lines (RILs) were genotyped using SSR and DArT markers. The RILs were grown in four different environments, 2007, 2008 early, 2008 late, and 2011. QTL analysis was performed on least square means for individual years and on combined least square means over all sites. The lines were treated as fixed effects. For QTL analysis, WinQTL Cart 2.5 software (Wang et al. 2010) was used to perform composite interval mapping.

To determine QTL threshold levels, 1000 permutations were carried out using WinQTL prior to QTL analysis at the 0.05 significance level to avoid any obvious Type II error, and the walking distance was one centimorgan (cM). The QTLs were named according to a catalog of gene symbols given by McIntosh et al. (2003).

Results

Overall, the environmental conditions varied significantly in terms of growth temperature and rainfall quantity and

Table 1 Mean temperature, precipitation and degree days data for the year 2007, 2008, and 2011 growing season; Edmonton AB, Canada

	Years	May	June	July	August	September
Temperature (°C)	2007[a]	11	16	21	15	11
	2008	13	16	18	18	12
	2011	13	15	17	17	15
	30 years average	12	16	18	17	11
Precipitation (mm)	2007	58	55	45	23	29
	2008	47	34	80	16	21
	2011	11	139	113	21	15
	30 years average	49	87	92	69	44
Degree days	2007	354	489	660	469	322
	2008	394	481	557	562	372
	2011	400	452	529	531	435
	30 years average	363	465	542	515	340

Data from Environment Canada

[a] Degree days: sum of average daily temperatures over a base temperature as 0 °C

distribution in all four seeding environments (Table 1). Therefore, a higher proportional contribution of environmental variation was noted in analysis of variance, followed by the genotypes and genotype × environment interaction (data not shown).

As expected, the earlier flowering parent Cutler flowered and matured earlier, and was shorter than AC Barrie. It also produced less grain yield (1.06 t/ha) than the higher yielding parent AC Barrie. Transgressive segregation was observed in the population as some of the lines flowered and matured earlier than the early maturing parent Cutler, and others later than AC Barrie. This was also found true for plant height, as some of the lines were shorter than Cutler and others were taller than AC Barrie (Table 2). The overall contribution of environmental variation was higher, indicating significant environmental effects on the traits under study (data not shown). Broad sense heritability estimates were high for days to flowering, maturity and plant height and were medium for grain yield (Table 2). Days to flowering was strongly correlated with days to maturity and moderately correlated with grain yield (Table 3). The photoperiod insensitive allele (*Ppd-D1a*) induced earlier flowering and maturity by 0.7 and 1.3 days, respectively, compared to the photoperiod sensitive allele (*Ppd-D1b*) (Table 4).

In total, 488 markers (102 Simple sequence repeat (SSR) markers and 386 DArT markers (of 7,000 clones) were used for mapping the population. The linkage map was constructed by DArT (http://www.diversityarrays.com). Initially, 566 DArT markers were polymorphic for the population; however, 180 DArT markers were either distorted or redundant and were discarded. The map spanned a distance of 2,279.13 cM and covered all 21 wheat chromosomes with an average distance of 4.67 cM between the markers. A total of seven QTLs were identified in this study (Table 5, Figs. 1, 2, 3). Three QTLs of earliness per se affecting both days to flowering and maturity were found on chromosomes 1B and 5B, in individual and combined environments. The QTL found on chromosome 1B (*QEps.dms-1B1*) was mapped at 31.8 cM and its late allele delayed flowering by 0.6 day in the field and 2.57 days in the greenhouse. This corresponded to a maturity delay of 0.8–2 days. The second QTL on 1B *QEps.dms-1B2* was detected only when the data was converted to degree days. This QTL was mapped at 35.8 cM and the effect was delayed flowering by approximately 0.8 days (12.9 heat units) and maturity by approximately 0.8–3 days (21.2–43.1 heat units) (Table 5). The earliness per se QTL found on chromosome 5B was positioned on 72.1–76.1 cM in different environments, and was mapped on 76.1 cM in combined data. The early allele of this QTL *QEps.dms-5B1* induced earlier flowering by 0.93 day in the field and 1.99 days in greenhouse, and earlier maturity by 0.74–1.63 days. A flowering time QTL was detected on chromosomes 4A (*QFlt.dms-4A1*) and induced earlier flowering by 0.5 day (Table 5). The first grain yield QTL was found on chromosome 1D at 62.2 cM and increased grain yield by 0.38 t/ha. The two grain yield QTLs (*QYld.dms-5B1*and *QYld.dms-5B2*) were found on chromosome 5B at 29.5, 43.1 cM positions, respectively. These QTLs increased grain yield by 0.29, 0.31 t/ha, respectively (Table 5).

The combined effect of the earliness per se QTL *QEps.dms-5B1* and *Ppd-D1a* on days to flowering and maturity was almost equal to their sum (Table 6), suggesting that *Ppd-D1a* and earliness per se QTLs interacted in an additive fashion. The interaction followed a similar trend when the QTLs with delaying affect (*QEps.dms-1B1* and *QEps.dms-1B2*) interacted with *Ppd-D1a* additively. The interaction effects on flowering and maturity were equal to their sum and were non-significant due to opposite direction of effect. The specific alleles at QTLs (*QEps.dms-1B1* and *QEps.dms-1B2*) delayed the time to flowering and maturity, while *Ppd-D1a* induced earlier flowering and maturity. Similar results were found for interaction between the flowering time QTL *QFlt.dms-4A1* and *Ppd-D1a* (Table 6).

Table 2 Mean and ranges of days to flowering, days to maturity, plant height and grain yield among parents, checks and recombinant inbred lines (RILs) from the Cutler × Barrie population during six seeding environments

Trait	Environment	Parents		Checks ($n = 10$)	RILs population ($n = 177$)			Heritability
		Cutler Mean	Barrie Mean		Mean	Minimum	Maximum	
Flowering (days)	2007	48	48	49	50 ± 0.11	45	55	–
	2008 early	53	55	55	55 ± 0.08	51	58	–
	2008 late	43	47	46	46 ± 0.13	41	56	–
	2011	56	61	59	58 ± 0.14	51	71	–
	Greenhouse 2007	36	43	39	41 ± 0.09	30	47	–
	Greenhouse 2008	36	47	38	41 ± 0.10	31	63	–
	Overall	51	53	53	52 ± 0.13	41	71	0.67
Flowering (degree days[a])	2007	810	810	829	850 ± 2.2	742	958	–
	2008 early	780	814	817	815 ± 1.8	735	889	–
	2008 late	708	739	754	758 ± 2.3	678	945	–
	2011	735	814	785	773 ± 2.53	662	974	–
	Overall	756	806	795	796 ± 1.4	662	974	0.68
Maturity (days)	2007	77	83	83	85 ± 0.22	77	99	–
	2008 early	94	95	96	95 ± 0.13	79	103	–
	2008 late	87	90	91	91 ± 0.24	78	107	–
	2011	114	118	115	119 ± 2.6	106	125	–
	Overall	95	98	99	100 ± 0.92	77	125	0.61
Maturity (degree days)	2007	1,311	1,402	1,400	1,418 ± 2.8	1,311	1,616	–
	2008 early	1,509	1,534	1,544	1,535 ± 2.52	1,424	1,698	–
	2008 late	1,560	1,647	1,617	1,622 ± 5.4	1,043	1,852	–
	2011	1,598	1,652	1,616	1,617 ± 4.97	1,717	1,783	–
	Overall	1,506	1,569	1,551	1,556 ± 2.9	1,043	1,852	0.51
Height (cm)	2007	73	80	78	75 ± 0.43	56	96	–
	2008 early	88	96	88	89 ± 0.51	65	112	–
	2008 late	78	96	88	90 ± 0.48	68	111	–
	2011	84	101	92	94 ± 0.81	62	126	–
	Overall	81	94	87	87 ± 0.29	56	126	0.83
Yield (t/ha)	2007	3.74	5.38	4.31	3.42 ± 0.04	1.41	5.48	–
	2008 early	8.37	8.83	9.47	9.27 ± 0.06	2.94	12.47	–
	2008 late	4.57	5.93	5.83	5.42 ± 0.05	1.98	8.43	–
	2011	6.84	6.38	7.22	7.48 ± 0.05	2.92	9.84	–
	Overall	5.69	6.75	6.72	6.08 ± 0.05	1.41	12.47	0.24

[a] Degree days: sum of average daily temperatures over a base temperature as 0 °C

Discussion and conclusion

The Cutler × AC Barrie population was developed for a better understanding of the flowering gene complex. Here, we report four main findings: (1) three earliness per se and one flowering time QTLs were mapped on chromosomes 1B, 4A, and 5B; (2) earliness per se and flowering time QTLs interact in an additive fashion with photoperiod insensitive gene *Ppd-D1a*; (3) photoperiod insensitive gene *Ppd-D1a* reduced days to flowering and maturity, but did not alter plant height and grain yield; (4) there is a positive genetic correlation between days to flowering, days to maturity and grain yield, while a negative correlation exists between days to maturity and plant height.

The QTL *QEps.dms-5B.1* found in this study is positioned between 72.6 and 76.1 cM and most probably is the same QTL that has been reported in previous studies. Hanocq et al. (2007) mapped a meta QTL (MQTL) on chromosome 5B having a position of 76.5 cM on the bread wheat consensus map (Somers et al. 2004) with a

Table 3 Phenotypic and genotypic correlation coefficients among days to flowering, days to maturity, plant height, and grain yield (seeding environments combined)

	Flowering (days)	Flowering Heat Units	Height (cm)	Maturity (days)	Maturity Heat Units	Yield (t/ha)
Flowering (days)		0.99** ±0.06	0.05 ±0.09	0.87** ±0.03	0.87** ±0.04	0.45** ±0.03
Flowering (Degree days)	0.98** ±0.03		0.05 ±0.03	0.86** ±0.03	0.88** ±0.03	0.11 ±0.03
Height (cm)	0.07 ±0.05	0.07 ±0.06		-0.28** ±0.09	-0.22** ±0.05	0.34** ±0.10
Maturity(days)	0.67** ±0.03	0.67** ±0.03	-0.17** ±0.05		0.99** ±0.01	0.47 0.12
Maturity (Degree days)	0.63** ±0.03	0.84** ±0.03	-0.15* ±0.03	0.93** ±0.01		0.07 ±0.08
Yield (t/ha)	0.13** ±0.01	0.16** ±0.03	0.25** ±0.04	0.08 ±0.04	0.09 ±0.04	

Values above the line are genotypic correlations and below the line are phenotypic correlations

*** Significance at $P < 0.001$, *ns* non-significant

Table 4 Effect of the photoperiod (*Ppd-D1*) dominant and recessive alleles on various agronomic traits

Trait	Insensitive allele (*Ppd-D1a*)	Sensitive allele (*Ppd-D1*)	Difference
Days to flowering (days)	49.8 ± 0.28	50.3 ± 0.27	−0.7*
Days to flowering (degree days)	799.7 ± 2.1	813.5 ± 2.3	−13.8**
Days to maturity (days)	89.5 ± 0.38	90.8 ± 0,39	−1.3**
Days to maturity (degree days)	1,517.3 ± 3.2	1,537.9 ± 3.2	−20.6**
Height (cm)	87.2 ± 0.96	85.0 ± 0.94	2.2^{ns}
Yield (t/ha)	5.92 ± 0.18	6.10 ± 0.17	−0.18^{ns}

ns not significant

* Significance at $P < 0.05$

** Significance at $P < 0.01$

confidence interval from 71.6 to 81.4 cM. This meta QTL was declared as one of the most accurate MQTL by pooling eight QTLs. The QTL (*QFlt.dms-5B.1*) identified in this study is located at 72.6 cM having a distance of 0.2 cM from the closest DArT marker (*wPt-666939*) and about 0.4 cM from the SSR marker *Xgwm-371* and is, therefore, mapped within the confidence interval described by Hanocq et al. (2007). They also claimed the MQTL location without ambiguity in this region on chromosome 5B. Some other earliness per se QTLs have also been reported on 5B chromosome e.g., a QTL reported by Tooth et al. (2003) is located in close proximity of SSR marker *Xgwm49* between 16 and 21 cM on long arm of the 5B chromosome, while another QTL on the same chromosome detected by Hanocq et al. (2004) is positioned between 38 and 48 cM region explaining 6.8 % of the total variation explained in the population under study. Shindo et al. (2003) reported three QTL inducing early flowering of 1.7, 1.6, and 1.4 days which are linked with SSR markers *Xwec78, Xrz630b* and *Xgwm234* on chromosome 5B. Two of the earliness per se QTLs found in this study on chromosome 1B are located in close proximity to each other on 31.8 and 35.8 cM, close to SSR marker *Xbarc187*, and are involved in delaying days to flowering and maturity. Wang et al. (2009) reported QTLs delaying days to flowering and maturity on chromosome 1B in a Chinese winter wheat population. Lin et al. (2008) found flowering time QTLs at about 106 cM on long arm of chromosome 1B close to SSR marker *Xbarc-80*. They reported earlier flowering by 0.9–2.4 days induced by the QTL in the population developed by crossing 'Nanda 2419' and 'Wangshuibai'. They also reported a flowering time QTL on chromosome 1D close to SSR marker *Xbarc-62* or *Xgwm-232*. However, QTL reported on chromosome 1B in this study are novel and, to the best of our knowledge, have not been reported previously.

The parents of the QTL mapping population we used differ in their time of flowering and maturity, in part, due to different photoperiod alleles on the *Ppd-D1* locus, as AC Barrie carries photoperiod sensitive (*Ppd-D1b*) allele, while Cutler is photoperiod insensitive. Different plant growth stages differ in their response to photoperiod

Table 5 Summary of quantitative trait loci (QTL) identified for days to flowering, days to maturity, plant height, and grain yield of 177 recombinant inbred lines evaluated in combined and individual seeding environments during 2007, 2008 (early and late), 2011, and in a greenhouse

	QTL	Trait	Years	Chromosome	Map position	Closest DArT Marker	$d1$[a]	Closest SSR marker	$d2$[a]	LOD	R^2 (%)[b]	Additive effect
1	*QEps.dms-1B1*	Flowering	Combined	1B	31.8	Wpt-2744	0.16	*barc187*	2.7	4.79	9.8	0.64
		Flowering	Greenhouse	1B	32.5	Wpt-2744	0.54	*barc187*	2.0	3.52	9.8	2.57
		FLWHT	2011	1B	31.8	Wpt-2744	0.16	*barc187*	2.7	4.07	11.3	11.17
		FLWHT	Combined	1B	31.8	Wpt-2744	0.16	*barc187*	2.7	3.66	7.3	10.31
		Maturity	Combined	1B	31.8	Wpt-2744	0.16	*barc187*	2.7	4.40	8.5	0.85
		Maturity	2007–2008	1B	32.9	Wpt-2744	1.17	*barc187*	2.6	4.58	15.8	1.96
		MATHT	2008L	1B	31.8	Wpt-2744	0.16	*barc187*	2.7	5.20	10.7	34.49
		MATHT	Combined	1B	31.8	Wpt-2744	0.16	*barc187*	2.7	5.38	11	17.44
		MATHT	2008L	1B	31.8	Wpt-2744	0.16	*barc187*	2.7	4.98	10.2	32.09
2	*QEps.dms-1B2*	FLWHT	2011	1B	35.8	Wpt-2694	0.79	*barc187*	1.3	3.69	12.2	12.88
		MATHT	2008L	1B	35.8	Wpt-2694	0.79	*barc187*	1.3	5.23	18.1	43.11
		MATHT	Combined	1B	35.8	Wpt-2694	0.79	*barc187*	1.3	5.84	9.8	21.23
3	*QEps.dms-5B1*	Flowering	2011	5B	76.1	Wpt-6135	0.34	*gwm371*	3.1	3.81	9.87	−0.93
		Flowering	Greenhouse	5B	72.1	Wpt-666939	0.10	*gwm371*	0.9	3.88	15.3	−1.99
		FLWHT	Combined	5B	76.1	Wpt-6135	0.34	*gwm371*	3.1	4.71	11.2	−17.75
		FLWHT	2007–2008	5B	72.1	Wpt-666939	0.10	*gwm371*	0.9	4.83	8.3	−12.68
		Maturity	2007	5B	72.4	Wpt-666939	0.20	*gwm371*	0.6	7.03	12.7	−1.63
		Maturity	2007–08	5B	72.4	Wpt-666939	0.20	*gwm371*	0.6	3.8	4.4	−0.74
		MATHT	2007	5B	72.2	Wpt-666939	0.01	*gwm371*	0.8	7.22	13.4	−17.87
4	*QFlt.dms-4A.1*	Flowering	2007–2008	4A	61.2	Wpt-8841	0.10	*wmc262*	0.2	3.44	5.6	−0.51
5	*QYld.dms-1D1*	Yield	2008 early	1D	62.2	Wpt-0413	0.11	*wmc590*	5.4	4.16	9.1	0.38
6	*QYld.dms-5B1*	Yield	2011	5B	29.5	Wpt-5120	0.08	*gwm234*	8.5	3.74	8.1	0.29
7	*QYld.dms-5B2*	Yield	2011	5B	43.1	Wpt-1457	0.05	*wmc73*	8.1	4.92	9.5	0.31

LOD logarithm of odds, *FLWHT* flowering degree days, *MATHT* maturity degree days

[a] $d1$, 2: distance from the closest DArT/SSR marker

[b] R^2: total variation explained (%)

insensitivity (Slafer and Rawson 1994). Therefore, developmental acceleration induced by insensitive photoperiod genes could be either from emergence to floral initiation (Davidson et al. 1985), and/or reduced spikelet primordial initiation period (Rawson and Richards 1993) or accelerated terminal spikelet to flowering stage (Slafer and Rawson 1994; Snape et al. 2001). In this study, the dominant photoperiod allele (*Ppd-D1a*) induced 0.7 day earlier flowering and 1.3 days earlier maturity (Table 4). Foulkes et al. (2004) reported earlier flowering in lines with *Ppd-D1a* by 9–12 days in winter wheat. Similarly, Worland (1996a, b) and Worland and Sayers (1996) reported 8 and 6–14 days earlier flowering, respectively, in British germplasm. In spring wheat, the photoperiod insensitive near isogenic lines headed 1.3–3.1 days earlier in the Northern Great Plains of America and Canada (Lanning et al. 2012). In another study on Canadian germplasm, earlier flowering and maturity of 1.5–5.8 days was reported due to photoperiod insensitive gene *Ppd-D1a* (Kamran et al. 2013). Earliness per se QTLs and *Ppd-D1a* interacted in an additive type of gene action. This suggests that accumulation of the earliness per se QTLs together with *Ppd-D1a* can help to further reduce days to flowering and, thus, elongate the grain filling duration for higher yields. This information will add to our understanding about the interaction in the flowering gene complex.

In general, earliness per se QTLs affected days to flowering and maturity in both directions by reducing or delaying the flowering and maturity times without environmental signals. These QTLs interacted with *Ppd-D1a* in an additive fashion, and *Ppd-D1a* reduced the days to flowering and maturity. The mapping results also indicate that the B genome contributed most of the genetic variation in this population, as most of the QTLs identified in this study were found on this genome. Hence, this population can be further explored for B genome mapping studies, and

Table 6 Comparison of the lines with *Ppd-D1a* and flowering QTLs to explain interaction between the photoperiod insensitive allele *Ppd-D1a* and the identified QTLs in the study for days to flowering, days to maturity, plant height, and grain yield

QTL	Comparison	Flowering (days)	FLWHT	Height (cm)	Maturity (days)	MATHT	Yield (t/ha)
QEps.dms-1B1	Effect of the QTL only[a]	0.6	10.1**	0.7	1.7	40.0**	0.08
	Effect of *Ppd-D1a* only[b]	−0.7	−13.4**	−1.8	−0.9	−17.2*	−0.12
	Effect of both *Ppd-D1a* and QTL	0.4	6.0	−0.3	0.4	13.1	−0.14
QEps.dms-1B2	Effect of the QTL	0.7	13.7**	0.5	1.6	29.6**	0.1
	Effect of *Ppd-D1a*	−0.5	−10.7**	−1	−0.3	−17.5*	−0.09
	Effect of both *Ppd-D1a* and QTL	0.3	3.5	−1.4	0.9	12.3	−0.23
QEps.dms-5B1	Effect of the QTL	−0.8**	−12.9**	−4.5	−0.6	−9.4	−0.34
	Effect of *Ppd-D1a*	−0.3	−5.1	−3.8	−0.2	−0.9	−0.13
	Effect of both *Ppd-D1a* and QTL	−1.3**	−23.7**	−4.7	−0.7	−29.1**	−0.49
QFlt.dms-4A1	Effect of the QTL	−0.8*	−12.6**	−3.4	−0.3	−6.7	−0.17
	Effect of the *Ppd-D1a*	−0.3	−11.6*	−4.9	−0.9	−15.5*	−0.24
	Effect of both *Ppd-D1a* and QTL	−1.1**	−21.4**	−1.9	−1.3	−24.0**	−0.31

Contrast coefficients: (QTL only, *Ppd-D1a* only, QTL and *Ppd-D1a* both, no QTL and *Ppd-D1a*)

FLWHT flowering degree days, *MATHT* maturity degree days

* indicates significance at $P < 0.05$

** indicates significance at $P < 0.01$

[a] The effect of QTL was estimated by comparing the lines with QTL present vs. absent (1 0 0 −1)

[b] The effect of Ppd-D1a was estimated by comparing the lines with Ppd-D1a present vs. absent (0 1 0 −1); the interaction effect was estimated by comparing the lines with both Ppd-D1a and the QTL present vs. absent (0 0 1 −1)

the parents Cutler and AC Barrie possess polymorphic regions especially for flowering and maturity.

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