

RESEARCH

Open Access



Features of *Ppd-B1* expression regulation and their impact on the flowering time of wheat near-isogenic lines

Antonina A. Kiseleva^{1*}, Elena K. Potokina² and Elena A. Salina¹

From 4th International Scientific Conference "Plant Genetics, Genomics, Bioinformatics and Biotechnology" (PlantGen 2017) Almaty, Kazakhstan. 29 May - 2 June 2017

Abstract

Background: Photoperiod insensitive *Ppd-1a* alleles determine early flowering of wheat. Increased expression of homoeologous *Ppd-D1a* and *Ppd-A1a* result from deletions in the promoter region, and elevated expression of *Ppd-B1a* is determined by an increased copy number.

Results: In this study, using bread wheat cultivars Sonora and PSL2, which contrast in flowering time, and near-isogenic lines resulting from their cross, "Ppd-m" and "Ppd-w" with *Ppd-B1a* introgressed from Sonora, we investigated the putative factors that influence *Ppd-B1a* expression. By analyzing the *Ppd-B1a* three distinct copies, we identified an indel and the two SNPs, which distinguished the investigated allele from other alleles with a copy number variation.

We studied the expression of the *Ppd-A1*, *Ppd-B1a*, and *Ppd-D1* genes along with genes that are involved in light perception (*PhyA*, *PhyB*, *PhyC*) and the flowering initiation (*Vrn-1*, *TaFT1*) and discussed their interactions. Expression of *Ppd-B1a* in the "Ppd-m" line, which flowered four days earlier than "Ppd-w", was significantly higher. We found *PhyC* to be up-regulated in lines with *Ppd-B1a* alleles. Expression of *PhyC* was higher in "Ppd-m". Microsatellite genotyping demonstrated that in the line "Ppd-m", there is an introgression in the pericentromeric region of chromosome 5B from the early flowering parental Sonora, while the "Ppd-w" does not have this introgression. *FHY3/FAR1* is known to be located in this region. Expression of the transcription factor *FHY3/FAR1* was higher in the "Ppd-m" line than in "Ppd-w", suggesting that *FHY3/FAR1* is important for the wheat flowering time and may cause earlier flowering of "Ppd-m" as compared to "Ppd-w".

Conclusions: We propose that there is a positive bidirectional regulation of *Ppd-B1a* and *PhyC* with an *FHY3/FAR1* contribution.

The bidirectional regulation can be proposed for *Ppd-A1a* and *Ppd-D1a*. Using in silico analysis, we demonstrated that the specificity of the *Ppd-B1* regulation compared to that of homoeologous genes involves not only a copy number variation but also distinct regulatory elements.

Keywords: Photoperiod sensitivity, Flowering time, *Ppd-B1*, Phytochrome, Common wheat

* Correspondence: antkiseleva@bionet.nsc.ru

¹The Federal Research Center "Institute of Cytology and Genetics of Siberian Branch of the Russian Academy of Sciences", Prospekt Lavrentyeva 10, Novosibirsk, Russian Federation 630090

Full list of author information is available at the end of the article



Background

Photoperiod sensitivity is an important agronomic trait that influences the wheat heading date, and the *Ppd-1* (*Photoperiod-1*) genes are significant regulators of this process. The *Ppd-1* genes are members of the *Pseudo-Response Regulator* (*PRR*) gene family [1]. There are three *Ppd-1* genes in the hexaploid wheat *Triticum aestivum*, which are located on the short arms of chromosomes 2A, 2B and 2D [2–4] and are designated *Ppd-A1*, *Ppd-B1* and *Ppd-D1*, respectively. Mutant alleles that are responsible for photoperiod insensitivity and, thus, early flowering in short day (SD) conditions are labeled with the suffix “a”.

To date, the photoperiod insensitive (PI) alleles of all the *Ppd-1* genes have been identified. *Ppd-D1a* has a 2089 bp deletion in its promoter region [1]. This allele is widely used in wheat selection, and its influence on the phenotype has been well studied [5, 6]. *Ppd-A1a* dominant alleles were first described using *T. durum* (*Triticum durum*). There are two alleles, both of which are characterized by 1027 bp or 1117 bp deletions in the promoters [4]. The *T. aestivum Ppd-A1a* allele, which was described by Nishida et al. [7], has a 1085 bp deletion in the 5' UTR region similarly to the other PI alleles.

Nishida et al. [7] revealed the PI *Ppd-B1a* allele in the cultivar Winter-Abukumawase with a 308 bp insertion in the promoter region. This insertion was suggested to be a MITE (miniature inverted-repeat transposable element). Another PI *Ppd-B1* mutation is a copy number variation (CNV) [8]. The haploid copy number of the wild-type *Ppd-B1* allele is one, while the PI alleles possess two to four copies. In addition to the copy number, these alleles may have different intercopy regions. Thus, there are three variants of the *Ppd-B1a* alleles based on the differences in the copy number and the intercopy junctions as follows: Recital type, Sonora64/Timstein/C591 type and Chinese Spring type [8]. Kiss et al. [9] ascertained that the phenotypical effect of the *Ppd-B1* loci is associated not only with the copy number but also with the intercopy junction type. Under field conditions, the *Ppd-B1* intercopy junction type was demonstrated to influence the heading date more than the number of copies.

The photoperiod sensitive (PS) *Ppd-1* alleles are expressed during the light period, and the peak of the expression occurs 3 to 6 h after dawn; these alleles are not expressed during the dark period. In contrast, all PI alleles demonstrate a misexpression throughout the 24 period [1, 4, 8]. The *Ppd-1a* alleles are expressed both during the light and dark periods. During the night period, PI *Ppd-1a* alleles were shown to up-regulate *TaFT1* (*Triticum aestivum Flowering Locus T*) expression under SD (Short Day) conditions [10, 11]. *TaFT1* is expressed in the leaves and transfers to the floral meristems to induce flowering [12].

The aim of the current investigation is to study the *Ppd-B1* PI allele with an increased number of copies and characterize the functional specifications of this allele and its interaction with other photoperiod genes. PCR analysis, molecular cloning and sequencing were used for the characterization of the *Ppd-B1* sequence. To analyze the diurnal expression of genes that regulate the heading date, qPCR with SYBR Green I was performed. The PlantPAN 2.0 database [13] was used to determine the putative plant transcription factor binding sites.

Methods

Plant materials

Two pairs of the near-isogenic lines “Ppd-m” and its sister line “Ppd-0^m”, “Ppd-w” and its sister line “Ppd-0^w” and their parental forms Sonora (K-47942, *Vrn-A1/Vrn-B1/Vrn-D1* and *Ppd-A1b/Ppd-B1a/Ppd-D1b*) and PSL2 (Photoperiod Sensitive Line 2, *Vrn-A1/Vrn-B1/vrn-D1* and *Ppd-A1b/Ppd-B1b/Ppd-D1b*), which have different heading dates, were used in this investigation. Lines “Ppd-m” and “Ppd-w” differ from their sister lines by the introgressions on the 2B chromosome from the Sonora variety. This difference was shown previously using SSR analysis [14]. An analysis of specific molecular markers also demonstrated that the near-isogenic lines (NILs) have recessive alleles of *Ppd-D1* and *Ppd-A1* [14]. DNA was extracted from the seedlings using the GeneJET Plant Genomic DNA Purification Mini Kit (Thermo Fisher Scientific, Lithuania) according to the manufacturer’s protocol.

Ppd-B1 intercopy region analysis

Previously reported PCR primers [8] were used to define the *Ppd-B1* intercopy region. The PCR was performed as previously described [8].

SSR genotyping

SSR genotyping of the full genome of NILs was performed in previous study [14]. Unfortunately, there was a mistake in illustration described “Ppd-w” and “Ppd-0^w” lines. “Ppd-w” and “Ppd-0^w” lines do not have an introgression on 5B chromosome. Therefore, to ascertain the 5B chromosome inheritance in the NILs, we used an additional set of markers (*Xgppw358*, *Xbarc74*, *Xgwm213*, *Xgppw2124*). The marker sequences and their annealing temperatures are available [15]. The amplified fragments were analyzed in 5% high-resolution agarose MS-8 (Dia-m, Russia) using ethidium bromide staining.

Primer design, PCR and sequencing of *Ppd-B1*

Previously, we have hypothesized that the *Ppd-B1* allele in the investigated lines might be characterized by sequence mutations in addition to copy number variations [14]. To investigate all possible sequence variations, we

developed primers with a coding region that is specific to all *Ppd-1* genes. The primer sequences are as follows: F2-*Ppd*-exon2-ACCAGGCGTGGGCGTATCT; R2-*Ppd*-exon6-GCTCTAGCTGCCTGTTGGG; F3-*Ppd*-exon6-TGGAGATAGGTGCCCTGG; and R3-*Ppd*-3UTR-GGACCGTCTCTGAATGATCCA. The primers for the promoter region were *Ppd-B1* gene-specific due to the strong differences in the *Ppd-1* promoter region and were designed according to the alignment of 184 sequences from the NCBI database. The primer sequences are as follows: F-*Ppd*-5UTR-CACTCTTATTCCTCTA TGCC and R-*Ppd*-5UTR-CTGTTATTATTGGAATCG TCAG. The reaction mixture was as follows: DNA in a concentration of 5 ng/μL, 1× buffer for Taq-polymerase (pH 8.6, 2.5 mM Mg²⁺), 200 μmoles dNTPs, 0.2 μmoles of each primer, 1 U Taq polymerase (Medigen, Russia), and sterile water up to a volume of 25 μL. The PCR conditions were as follows: 94°C for 3 min, and 35 cycles (94°C for 40 s; 55°C for 30 s; and 72°C for 60 s) and 72°C for 7 min.

The amplicons were recovered using a 1% agarose gel and purified with the kit for DNA elution from agarose gel (Biosilica, Russia). The purified amplicons were ligated into a pAL-TA vector (Evrogen, Russia) with 1 U of T4 DNA Ligase (Thermo Fisher Scientific, UK). The construct was used for the transformation of the *E.coli* Top-10 competent cells, which were prepared according to the CCMB80 protocol [16]. The colonies with the target insert were selected using a blue-white selection with X-Gal/IPTG and PCR with primers to target the sequences. Plasmid DNA was extracted with the Biosilica kit for pDNA extraction (Biosilica, Russia). The sequencing was performed using an ABI PRISM Dye Terminator Cycle Sequencing ready reaction kit (Perkin Elmer Cetus, USA) with M13 primers and extra primers for the following target sequences: F-5UTRad-TTCTTC AACTAGGGCTGGT; R-5UTRad-CGCATAATAGCA CAACCAGC; F-ex4-GTGGCAGTGGTAGTGGAAGT; and F-7ex-ACGCCGCTCAGATGAAGCAA.

Diurnal quantitative expression of the photoperiod genes

Sonora, PSL2, “*Ppd-m*”, and “*Ppd-w*” plants were grown for 21 days after germination under controlled conditions in a climatic chamber Rubarth Apparate (RUMED GmbH) with short days (9 h of light, 20 °C). Three replicate samples from each genotype were harvested into liquid nitrogen at each three-hourly time point over 24 h since the beginning of the light period. RNA was extracted using the Plant RNA MiniPrep (Zymo Research, USA), followed by a DNase treatment with the RNase-Free DNase set (QIAGEN, Hilden, Germany). cDNA was synthesized using RevertAid First Strand cDNA Synthesis (Thermo Fisher Scientific, Lithuania) following the manufacturer’s protocol with 2 μg of total RNA as a

template and Oligo(dT)₁₈ as the primers. In total, 2 μl of the 20-fold dilution of the final cDNA were used for the following analysis.

For the expression analysis, previously published primers for the *TaFT-1*, *Ppd-1* [11], *Vrn-1* [17], *PhyA*, *PhyB* and *PhyC* [18] genes were used. The primers for the *FHY3/FAR1* were designed using the URGI: Traes_5BS_BCC406654.2 sequence. The primer sequences are as follows: F-TaFHY3/FAR1-5B- GCAAACGTCAT CAGGATACA and R-TaFHY3/FAR1-5B- CCTCTTCT CAGCTTTACTTGC. The primers for the 18S rRNA gene [1] were used for normalization. The fluorescence data were collected using ABI 7500fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) with SYBR Green I (Syntol, Russia) as the intercalating dye. The measurements were performed in three technical replicates. The reaction products were checked by a melting curve analysis and 2% agarose gel electrophoresis. The relative expression values were calculated according to the method proposed by Pfaffl [19]. The PCR efficiencies were determined using LinReg software [20]. The expression of target genes was normalized against 18S rRNA.

Statistical analysis

ANOVA with a post hoc Tukey test was used to compare the expression levels. Correlations between the patterns of gene expression were calculated using Pearson coefficient (significance level $P = 0.001$).

Bioinformatic analysis of the gene promoters

The PlantPAN 2.0 [13] database was used to determine the putative plant cis-acting regulatory elements. The 2000 bp upstream TSS (Transcription Start Site), first exon and first intron were analyzed for all sequences except for *TaFT-5D* due to the absence of the sequence of its upstream TSS in the databases.

Results

Sequence analysis of the photoperiod insensitive *Ppd-B1* allele

We have hypothesized that the *Ppd-B1* gene in the PI Sonora, “*Ppd-m*” and “*Ppd-w*” is characterized by not only an increased copy number but also by a nucleotide polymorphism in one of the copies because NILs “*Ppd-m*” and “*Ppd-w*” flowered slightly later than the line with the same background and introgressed *Ppd-D1a* gene [14]. Therefore, we analyzed the sequences of the *Ppd-B1* distinct copies. To investigate the possible polymorphisms, the amplicons that overlapped gene and its promoter were obtained and inserted into a vector to transform *E. coli* and distinct colonies that were regarded to have different copies, which were then sequenced and analyzed.

The results are presented in Fig. 1. Every line in our investigation differed by a single nucleotide indel in the promoter region (-2373 bp) of the *Ppd-B1* allele, which was detected in the cultivars Sonora64, Timstein (DQ885765.2), and Renan (DQ885764.2). Thus, the NILs and the parental cultivar Sonora had the same *Ppd-B1* intercopy region as cultivars Sonora64, Timstein and C591. However, the detected polymorphism provided a distinction among these *Ppd-B1* alleles.

The other SNP (-630 bp) allows for the distinction between the PI and PS NILs. Thus, “Ppd-m” and “Ppd-w” have “C” and the sister lines have “G” upstream the coding region. In exon 3 (+546 bp), the detected SNP distinguished the investigated sequence from another allele by the increased copy number (Chinese Spring).

Diurnal expression analysis

To study the interactions between the *Ppd-B1a* PI allele and other genes that are important for wheat flowering, we used a qRT-PCR assay. We used 21-day-old plants from the late-flowering parental line PSL2, early flowering parental cultivar Sonora with the PI *Ppd-B1a* allele, and NILs “Ppd-m” and “Ppd-w”, representing PSL2 with different alleles at some loci, including *Ppd-B1a*, introgressed from Sonora.

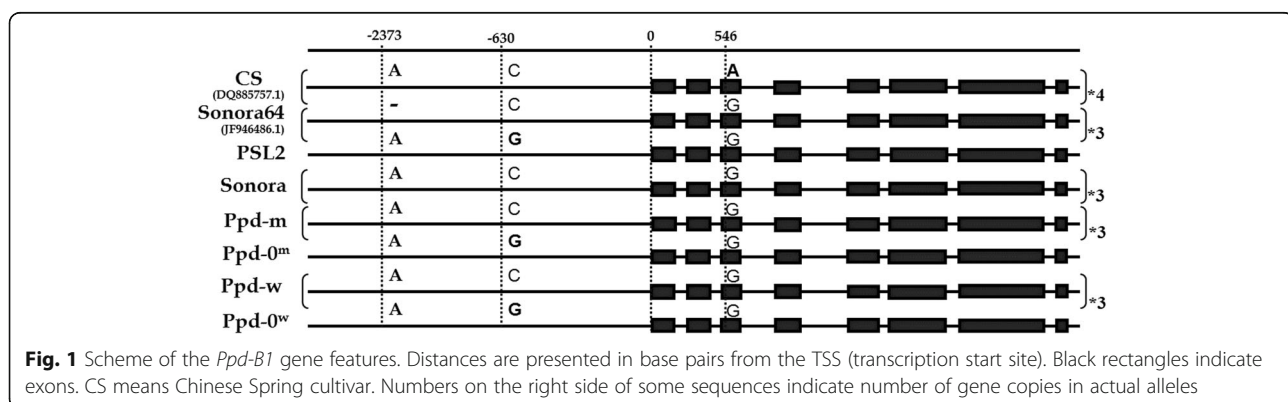
The results of the diurnal expression analysis are presented in Fig. 2. *Ppd-B1* was expressed in the PI lines during the dark period but was not expressed in the photoperiod sensitive PSL2 as expected. The *TaFT1* gene was expressed only in the PI lines, supporting previous investigations [10, 11] in which the influence of the *Ppd-1* dominant alleles on *TaFT1* expression was shown.

The *Ppd-D1* expression was increased in Sonora compare to that in PSL2 and the NILs. This finding may be due to the 5 bp deletion in exon 7. The specificity of *Ppd-A1* is shown in the expression peak shift in “Ppd-m” and “Ppd-w” compared to that in Sonora and PSL2. The shift in the expression peaks in the “Ppd-m” line can be observed in *Ppd-D1*, *TaFT1*, *Vrn-1* and the other genes. The expression patterns of *Vrn-1* did not display any

robust differences between Sonora and “Ppd-w” but was rather higher in Ppd-m. *PhyC* expression decreased since the beginning of the light period and displayed one or two peaks during the dark period. A tendency of an elevated expression during the dark period in the PI lines was observed. The expression patterns of *PhyB* and *PhyA* could be characterized by the two expression peaks as follows: one at the end of the light period and one during the dark period.

We analyzed the correlations (Additional file 1: Table S1) between the expression patterns of the genes involved in light perception (*PhyA*, *PhyB*, *PhyC*) and those involved in the flowering transaction (*Ppd-A1*, *Ppd-B1*, *Ppd-D1*, *Vrn-1*, *TaFT1*) separately during the dark and light periods in the photoperiod sensitive and insensitive lines.

The expression patterns of all the phytochromes significantly correlated with each other, indicating that common factors influence their expression. The *Ppd-1* genes also significantly correlated with each other ($\rho = 0.55-0.85$ in the photoperiod insensitive and $\rho = 0.89-0.97$ in the photoperiod sensitive lines). *TaFT1* expression correlated with the *Ppd-1* genes in the PI lines, and in PSL2, *TaFT1* was not expressed at all. We observed significant correlations between the expression levels of *TaFT1* and *PhyC-5A* ($\rho = 0.75$) and between *TaFT1* and *PhyC-5B* ($\rho = 0.8$) during the light period. The expression patterns of *Vrn-1* significantly correlated with the *Ppd-1* genes ($\rho = 0.89-0.95$) in PSL2. This finding may suggest that there are common factors influencing their expression. In the PI lines, *Vrn-1* correlated with all the phytochromes during the light ($\rho = 0.65-0.79$) and dark ($\rho = 0.56-0.72$) periods. These data are consistent with [21], in which the influence of *PhyC* and *PhyB* on *Vrn-1* expression was shown using RNA-Seq analysis. The correlation between *Ppd-B1* and *PhyC* (*PhyC* in combination and *PhyC-5B* and *PhyC-5A* individually) was significant during the dark period in the PI lines ($\rho = 0.67$).



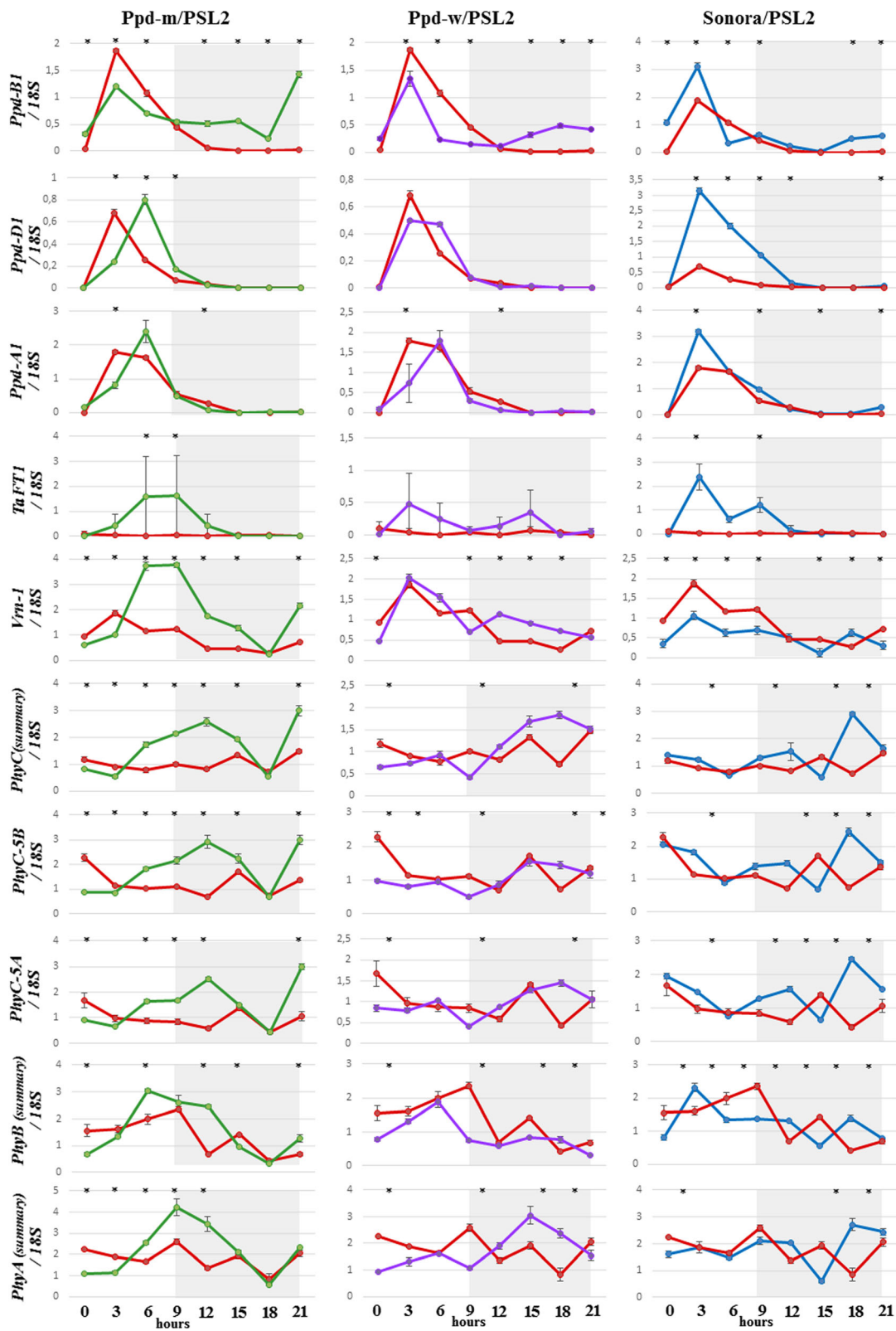


Fig. 2 (See legend on next page.)

(See figure on previous page.)

Fig. 2 Patterns of the diurnal gene expression. Quantitative gene expression data from plants grown in short days (0–9 h light period) in a climatic chamber. Gray shadowing indicates the dark period (9–24 h). The graphs compare the expression between the photoperiod sensitive parental line PSL2 (red) and the photoperiod insensitive NILs (“Ppd-m” (green), “Ppd-w” (purple)) and parent Sonora (blue). Values are expressed as the relative levels normalized against 18S ribosomal RNA. Error bars indicate the SE of the means. Asterisks indicate significant ($P < 0.05$) differences in a one-way ANOVA with a post hoc Tukey test comparing the photoperiod insensitive NILs (“Ppd-m”, “Ppd-w”) and the parent Sonora with the photoperiod sensitive parental line PSL2 in each time point

Features of the expression patterns in the lines with the photoperiod insensitive *Ppd-B1* allele

The expression peaks of all genes except for *Ppd-B1* were shifted in the “Ppd-m” line. The parental forms and “Ppd-w” did not display these expression patterns. Most likely, the genes located in the additional introgressions from Sonora, which are specific to “Ppd-m” but not “Ppd-w”, interact with the PSL2 background genes, which may result in such an effect. Both the “Ppd-m” and “Ppd-w” lines have introgression of *Ppd-B1a* from Sonora, but they differ by four days in their heading date under SD conditions (“Ppd-m” flowered earlier) [14]. “Ppd-m” has the following two supplemental loci from Sonora that were not found in “Ppd-w”: on chromosome 4D nearby the *Xbarc165*, on chromosome 5A nearby the *Xgwm154* and in the pericentromeric region of chromosome 5B (markers *Xbarc74*, *Xgwm67*, *Xgwm371*, and *Xgwm213*). QTLs associated with the heading date were previously identified in the 4D and 5B chromosomes. In chromosome 4D, the QTL was located in the region between markers *wPt8836* and *Xgwm165* [22]. No genes that are located in these areas are known to correspond to the heading time regulation.

In chromosome 5B, the heading date QTL was associated with the *Xgwm371* marker [23]. Later, a QTL associated with candidate genes *FHY3/FAR1*, *AP2/ERF* and *WRKY* was identified in the 5B pericentromeric region [24]. Therefore, chromosome 5B was genotyped using an additional set of SSR markers to ascertain the length of the introgression region. As a result, chromosome 5B in the “Ppd-w” and “Ppd-0^w” lines was completely inherited from PSL2. In the line “Ppd-m”, there is an introgression from the early flowering parental Sonora precisely in the region that carries the *FHY3/FAR1* locus, while in “Ppd-w”, which flowers four days later than Ppd-m, does not have this introgression (Fig. 3). *FHY3/FAR1* is proposed as a good candidate to explain the difference in the heading date.

To examine whether there are any differences in *FHY3/FAR1* expression between lines “Ppd-m” and “Ppd-w”, we applied the diurnal expression analysis and demonstrated significant differences of *FHY3/FAR1* expression. Expression of this gene in “Ppd-m” was higher in 6–9 h after dawn indicating putative reason for the heading time variation (Fig. 4). *FHY3/FAR1* expression pattern correlated with phytochromes and *Vrn-1* at

significant levels. In night *FHY3/FAR1* correlated with *Ppd-B1* in PI lines (“Ppd-m”, “Ppd-w” and Sonora).

Sequence analysis of *Ppd-D1* and *Ppd-A1*

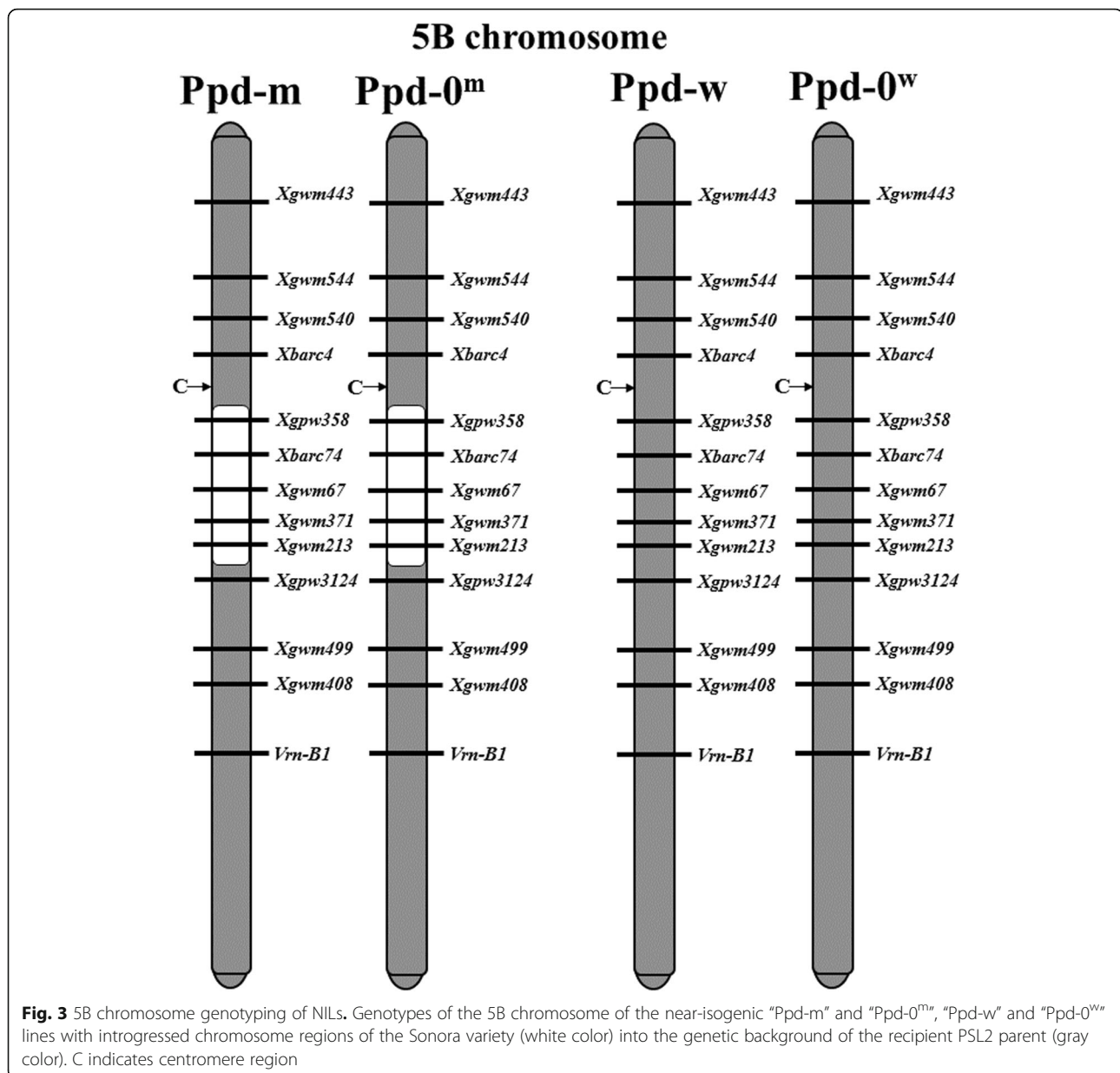
Ppd-D1 of Sonora has a 5 bp deletion that is specific to haplotype IV based on the classification suggested by Guo [25]. Phenotypically, this haplotype is much weaker than haplotype I, which is represented by the dominant PI allele with a 2 kb deletion in the promoter region, but slightly reduces the heading time [25]. However, in contrast to [25], who showed that the expression of the *Ppd-D1* haplotype IV was only 5% higher, Sonora’s *Ppd-D1* expression was statistically significantly higher during the light period than the expression of the PSL’s allele (corresponding to haplotype II). During the dark period, no expression was observed. Most likely, the high expression of Sonora *Ppd-D1* can partially explain the fact that the PI lines flower 7–12 days later than Sonora, because their *Ppd-D1* alleles are inherited from PSL2.

The *Ppd-A1* sequence of PSL2 and the NILs has a SNP (A/C) that has not been previously described in any published *Ppd-A1* sequence. The comparison included 286 sequences of tetra- and hexaploid wheat varieties and was accomplished using the Blastn algorithm.

Discussion

Ppd-B1 is the only *Ppd-1* gene with a dominant PI allele that is associated with the copy number variation. However, little is known about the mechanisms underlying its misexpression. Previously, using near-isogenic lines resulting from a cross of the cultivars Sonora and PSL2, which contrast in flowering time, we have shown that the difference in flowering time was associated with an introgression on chromosome 2B between the markers *Xgwm148* and *Xgwm388* from the PI Sonora variety [14]. *Ppd-B1* is known to be located in this position [26]. Early flowering NILs do not have the PI *Ppd-B1a* allele with a 318 bp insertion in the promoter region that was identified by Nishida et al. [7]. Using Real-Time PCR, we revealed that the NILs and Sonora are characterized by increased copy numbers of *Ppd-B1* [14].

In this study, we have shown that *Ppd-B1a* in the PI lines is characterized by an increased number of copies and possesses an intercopy junction similarly to cultivars Sonora64, Timstein and C591. However, the investigated



Ppd-B1a allele differs from Sonora64, Timstein and C591 allele by an indel in the promoter region.

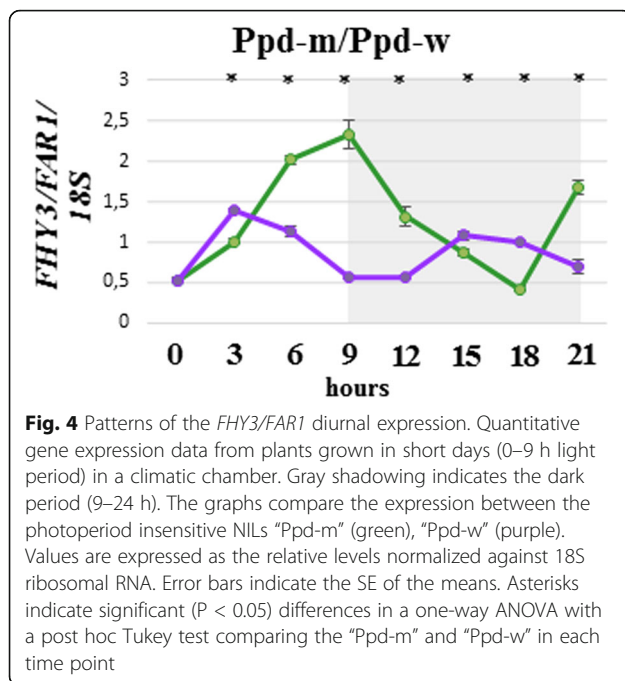
We have also found two SNPs. The SNP in exon 3 (+546 bp) distinguished the investigated sequence from the Chinese Spring allele with an increased copy number.

The SNP (-630 bp) distinguished between the PI and PS NILs. This SNP ("G") was previously detected in cultivars Recital, Paragon and Winter-Abukumawase [1, 8]. It is interesting to note that the *Ppd-B1* gene of the parental cultivar Sonora and all the other tetra- and hexaploid wheats is characterized by "C" in this position. Thus, "G" in this position is rare (4 cultivars, including PSL2).

However, no association between photoperiod sensitivity and this SNP was shown [1, 7, 8].

To study the possible influence of the detected SNPs on the expression of *Ppd-B1a*, the interactions between the investigated *Ppd-B1a* allele and other wheat flowering genes and the activity of these genes in the presence of *Ppd-B1a*, we performed a diurnal expression analysis.

We analyzed the correlations between the expression patterns of genes involved in light perception (*PhyA*, *PhyB*, *PhyC*) and those involved in the flowering transaction (*Ppd-A1*, *Ppd-B1*, *Ppd-D1*, *Vrn-1*, *TaFT1*) separately during the dark and light periods in the photoperiod sensitive and insensitive lines.



TaFT1 expression correlated with the *Ppd-1* genes in the PI lines, but in PSL2, *TaFT1* was not expressed at all. We identified the following cis-elements that corresponded to the RR (Response Regulator) or PRR (Pseudo Response Regulator) regulation: *ARR14*, *RR14*, *PRR4*, *APRR2*, *ARR1*, *RR1* (Myb/SANT, MYB, ARR-B families) and binding site ARR1AT. These findings propose the putative sites for the *TaFT1* regulation through the *Ppd-1* genes.

Significant correlations were found between the expression levels of *TaFT1* and *PhyC-5A* and *TaFT1* and *PhyC-5B* during the light period. During the night, the *TaFT1* expression level was very low, and phytochromes are inactive during the dark. It was suggested that phytochromes influence *TaFT1* expression. Altogether, this finding demonstrated that *Ppd-1* is important but not essential for flowering, and based on the triple *Ppd-1* loss-of-function mutant analysis [27], it was proposed that *TaFT1* is directly regulated by the phytochromes. Most likely, this process is performed with the assistance of *Ppd-1*. In the *TaFT1* promoter, the following binding sites for the transcription factor that is associated with the phytochrome regulation were found: *PIL5*, *POC1*, *PIF3*, *PAP3* (bHLH/bZIP), *FHY3/FAR1* (FAR1), *VOZ2* (VOZ), *SORLIP1AT*, *SORLIP2AT*, *SORLREP5AT*, *SORLIP5AT*, and *RE1ASPHYA3*. The expression correlation and the identification of TFBSs for Phytochrome-regulation in the *TaFT1* promoter suggest that phytochromes are other regulators of *TaFT1*.

We detected a significant correlation between *Ppd-B1* and *PhyC* (*PhyC* in combination and *PhyC-5B* and

PhyC-5A individually) during the dark period in the PI lines.

Phytochromes express in the dark and produce inactive Pr (Red light-absorbing phytochrome) molecules [28, 29] that cannot influence the expression of other genes; thus, the correlation should imply that the expression of *Ppd-B1* during the night period positively influences *PhyC* expression, but *PhyC* does not influence *Ppd-1* during the night. Regarding *PhyA*, the same tendency can be observed, but no significant correlation was found. Because wild type *Ppd-1* reveals no expression during the dark period, it is unable to influence *PhyC* in general. We may hypothesize that *Ppd-1* influences *PhyC* even during the day period, but *PhyC* mRNA degradation in light conceals this putative effect. Previously, it was shown that the NILs with the PI *Ppd-1a* alleles (*Ppd-D1a* or *Ppd-B1a*) have an increased level of phytochrome protein compared to the sister lines with recessive *Ppd-1* alleles [30]. Thus, we suggest that the *Ppd-1a* genes may influence the *PhyC* expression directly or indirectly. To verify this hypothesis, we analyzed the promoter sequences of the *PhyC* genes to detect the possible binding sites for *Ppd-1*.

The binding sites for the response regulators (TFmatrixID_0348 for ARR2, RR2 and TF_motif_seq_0268 for ARR1AT) and the pseudo-response regulators (TF_motif_seq_0252 for APRR4) may be regulated by these pseudo-response regulators, such as *Ppd-1* genes. Some of these TFs correspond to phytochrome-regulation (*EPR1/RVE7*, *POC1/PIF3*, *FHY3/FAR1*, *SORLIP2AT*, and *SORLIP1AT*), suggesting their self-regulation. There were some other TFBSs that were associated with flowering or photoperiod regulation as follows: *GATA12*, *AtHB33*, *AtDOF1*, *SPL3*, *TEM1*, *STM*, *GBOX10NT*, *BSIEGCCCR*, and *IBOXCORE*.

Taken together, the expression correlation data, the *PhyC* promoter analysis and the fact that there are phytochrome protein increments in the lines with the PI *Ppd-1a* alleles, as demonstrated by [30], suggest that *Ppd-B1a* is expressed during night period and positively regulates *PhyC* expression.

“Ppd-m” flowered four days earlier than “Ppd-w”. Using SSR genotyping, we demonstrated that the loci in the 5B pericentromeric region were different in the “Ppd-m” and “Ppd-w” lines. *FHY3/FAR1* is known to be located in this locus [24]. Binding sites for *FHY3/FAR1* were identified in the *Ppd-1*, *PhyC* and *TaFT1* promoters.

Using the diurnal expression analysis, we found that the *FHY3/FAR1* expression was higher in “Ppd-m” than in “Ppd-w”. *FHY3/FAR1* expression pattern correlated with phytochromes during the 24-h period in all the lines and with *Ppd-B1* in night in PI lines.

FHY3/FAR1, known to be involved in phytochrome signaling in *Arabidopsis* and rice [31, 32], control phytochrome accumulation through the *FHY1* regulation in *Arabidopsis* [33]. However, there is no data about *FHY3/FAR1* functions in wheat.

In wheat, the phytochromes (PhyB and PhyC) influence the *Ppd-1* expression [18, 21] and flowering time. *FHY3/FAR1*, which is involved in flowering time control, may contribute to this process. *Ppd-B1a* was suggested to increase *PhyC* expression. Thus, the data propose a bidirectional regulation between the *PhyC* and *Ppd-B1a* genes with a putative *FHY3/FAR1* contribution. This hypothesis requires further investigation and verification. The near-isogenic lines used in this study differ in the 5B pericentromeric region and are a good source for this work.

The lines with *Ppd-D1a* also demonstrated an increase in the phytochrome protein [30]. Therefore, this mechanism is proposed to be common for all *Ppd-1a* alleles. However, the regulation of *Ppd-B1* expression should be different.

Previously, it was suggested that a deletion in the promoter region of *Ppd-A1* and *Ppd-D1* causes the misexpression of the corresponding genes due to the disappearance of the CHE-binding site and impossibility of the repressor CHE (CCA1 HIKING EXPEDITION) to associate with its regulatory element [7]. Then, it could be hypothesized that an increase in the *Ppd-B1* copy number, which was shown to occur in its dominant alleles, results in a misexpression because of the increase in the *Ppd-1* genes with the same quantity of the repressor. However, in this case, an altered expression should be observed in all three *Ppd-1* genes. Previously, published data [11] demonstrated that the *Ppd-B1a* allele shows a misexpression by itself but does not influence *Ppd-A1* and *Ppd-D1* expression. Thus, about the hypothesis regarding the role of the *Ppd-B1* copy number against the repressor's quantity should be rejected. It is interesting to note that there is no *Ppd-B1* allele with a deletion in the promoter region, while all PI alleles of *Ppd-A1* and *Ppd-D1* are characterized by deletions.

To identify the probable factors involved in *Ppd-B1* but not *Ppd-A1* and *Ppd-D1* regulation, we investigated the sequences of the promoter regions of these genes and determined (1) TFBSs (Transcription Factor Binding Sites) that are common to the promoters of all *Ppd-1* homoeologous genes and (2) TFBSs that are specific to *Ppd-B1*.

To detect the TFBSs that are common to the *Ppd-1* genes in the A, B and D genomes, a PlantPan2 multiple promoter analysis was utilized. An in silico promoter sequence analysis revealed that that the *Ppd-1* promoters have many cis-acting elements that are associated with flowering that could be divided into the following three

groups according to their input signal nature: phytochrome-regulated, circadian clock-regulated and other binding sites that are involved in light-regulated development. A detailed description of these transcription factors is summarized in Table 1. The positions of these cis-elements on the *Ppd-1* sequences are presented in Fig. 5.

PhyC and *PhyB* were recently shown to regulate *Ppd-1* expression [18, 21]. These genes were suggested to be important regulators of the photoperiod sensitivity and flowering transaction in wheat [18, 21] and barley [34–36]. Although the chromosome position of *PhyA* in wheat was identified [37], no association between these loci and flowering was described. However, *PhyA* is an important regulator of flowering time in certain other species, such as *Arabidopsis* [38, 39]. Therefore, an investigation of the *PhyA* sequence and functions in cereals may complement the mechanisms of the photoperiod sensitivity and flowering. Here, we may hypothesize that *Ppd-1* expression is regulated by the phytochromes with the assistance of transcription regulators, such as *PIF3*, *PIL5*, *FHY3/FAR1*, *RVE7/EPRI*, and *VOZ2*, and certain TFBSs with unknown transcription factors, such as *SORLIP2AT*, *SORLIP1AT*, and *RE1ASPHYA3*.

The *Ppd-1* genes were suggested to be regulated by the circadian oscillator in wheat [18] and barley [40]. In barley, some genes that are involved in this process are known, while in wheat, no putative circadian affecters are proposed. We identified the TFBSs that might be involved in the circadian clock regulation of the *Ppd-1* genes as follows: *RAV1*, *TEM1*, *TEM2*, *RVE1*, *CCA1*, *LHY*, and *CHE*.

The *Ppd-1* activation by *PhyC* was proposed to be light sensitive throughout the day [41]. Most likely, this process can be mediated by some of the following detected elements: *GATA2*, *GATA12*, *GT-1*, and *AtMYC2* and some binding sites with unknown transcription factors, such as *BOXIIPCCHS*, *TBOXATGAPB*, *IBOXCORENT*, *LREBOXIIPCCHS1*, and *IBOXCORE*.

Binding sites that are specific to *Ppd-B1* and not *Ppd-A1* and *Ppd-D1* are likely associated with the circadian rhythms and flowering time and regulatory elements from the following families: AP2 (*ANT*), C2H2 (*Zat12*), MADS box/*MIKC* (*AGL19*, *FLC*, *MAF2*, *AGL69*, *AGL68*, *FLM*, *AGL6*, *AGL18*, *AGL14*, and *AGL15*), and Lyase Aromatic (*PAL1*) and TFBSs with unknown TF *RBCSGBOXPS*, *REBETALGLHCB21*, *LEAFYATAG*, *SORLIP5AT*, and *MNF1ZMPPC1*. A detailed characterization of these transcription factors is presented in Table 2. The positions of these TFBSs on the *Ppd-B1* sequence are presented in Fig. 6.

The transcription factors that were associated with the flowering transaction included *MADS box* and the *MIKC* family genes. Some of these transcription factors

Table 1 Characterization of the selected transcription factors and the TFBSs with unknown transcription factors that were identified in the promoter regions of the *Ppd-1* genes

Family	Transcription factors/TFBSs with unknown TF	Description
Phytochrome regulated transcription factors		
VOZ	VOZ2	VOZ2 was identified as one of the highly conserved transcriptional factors in land plant genes that are PhyB-interacting factors [58].
bHLH	Phytochrome Interacting Factor3 (PIF3)	The G-box, CACGTG, is a target sequence for PIF3 [59]. PIF3 plays important roles in the Phy-mediated light responses. This factor can regulate the downstream genes either positively or negatively [60]. In <i>Arabidopsis</i> , PIF3 was suggested to play an important role in the control of flowering by the regulation of <i>CO</i> and <i>FT</i> gene expression [61].
bHLH	Phytochrome Interacting Factor3-Like5 (PIL1)	PIL5 interacts with the Pfr forms of Phytochrome A (PhyA) and Phytochrome B (PhyB) [62].
FAR1	Far-Red Elongated Hypokotyls3/Far-Red-Impaired Response1 (FHY3/FAR1)	Transcription factor FHY3/FAR1 modulates PhyA-signaling in higher plants [63]. Other investigations demonstrated that FHY3 plays a principal role in the circadian clock, heading date control and regulation of heading time through <i>ELF4</i> (<i>EARLY FLOWERING4</i>) [64].
Myb/SANT; MYB-related	RVE7/EPR1	<i>RVE7/EPR1</i> is regulated by both PhyA and PhyB and negatively regulates flowering [65].
–	SORLIP2AT and SORLIP1AT	Sequences over-represented in the light-induced promoters SORLIP2AT and SORLIP1AT were identified in the PhyA-induced promoters [66].
–	RE1ASPHYA3	RE1ASPHYA3 (RE1, putative repressor element) is a highly conserved motif in the most monocot <i>PhyA</i> promoters and is responsible for the Pfr-directed repression [67]; this motif was detected in certain other genes [68].
Circadian-clock regulated transcription factors		
AP2/RAV/B3	RAV1	<i>RAV1</i> is a negative component in the regulation of plant development [69].
AP2/RAV/B3	TEMPRANILLO1 (TEM1) TEMPRANILLO2 (TEM2)	<i>TEM1</i> and <i>TEM2</i> genes act as direct repressors of <i>FT</i> [70].
MYB-related	REVEILLE1 (RVE1)	RVE1 is a morning-phased transcription factor that integrates the circadian clock and auxin pathways [71].
Myb/SANT; MYB-related	REVEILLE 8 (RVE8)	<i>RVE8</i> promotes the expression of some evening element that contains clock genes and forms a negative feedback loop with <i>PRR5</i> [72, 73].
MYB	Circadian Clock Associated 1 (CCA1); Late Elongated Hypocotyl (LHY)	The MYB transcription factors <i>CCA1</i> and <i>LHY</i> are some of the key genes in the central oscillator of the plant circadian clock [74]. <i>LHY</i> and <i>CCA1</i> negatively regulate <i>TOC1</i> expression.
TCP	CCA1 HIKING EXPEDITION (CHE)	The TCP transcription factor <i>CHE</i> is a clock component that is partially redundant with <i>LHY</i> in the repression of <i>CCA1</i> [75].
Other light regulated transcription factors		
GATA/tify	GATA2	GATA2 directly regulates genes that respond to light [76].
GATA/tify	GATA12	GATA12 is involved in the regulation of many light-responsive genes [77, 78].
MADF; Trihelix	GT-1	GT-1 may act as a light-responsive transcription factor [79].
bHLH	AtMYC2	AtMYC2 acts as a negative regulator of blue light-mediated photomorphogenic growth and blue and far-red-light-regulated gene expression [80].
–	BOXIIPCCHS	BOXIIPCCHS was suggested to be essential for light regulation [29, 81].
–	TBOXATGAPB	Mutations in TBOXATGAPB cause a reduction in light-activated transcription [82, 83].
–	IBOXCORENT	I-box core motif IBOXCORENT in the conserved DNA modular arrays is associated with the light-responsive promoter regions [84].

Table 1 Characterization of the selected transcription factors and the TFBSs with unknown transcription factors that were identified in the promoter regions of the *Ppd-1* genes (Continued)

Family	Transcription factors/TFBSs with unknown TF	Description
-	LREBOXIIPCCHS1	Light responsive element LREBOXIIPCCHS1, which was detected in parsley, is required for light responsiveness [85].
-	IBOXCORE	Conserved sequence in the 5' UTR of light-regulated genes IBOXCORE may be involved in the regulation of transcription by light and the circadian clock. This sequence was detected in both monocots and dicots [86].

contribute to flowering induction, and others contribute to flowering repression. For example, *AGL19*, *AGL6* and *AGL14* are positive regulators of the flowering transition [42–44]. In contrast, the majority of the identified MADS transcription factors, i.e., *FLC*, *MAF2*, *AGL69*, *AGL68*, *FLM*, *AGL15* and *AGL18*, negatively regulate the transition from vegetative to reproductive development [45–52]. Gu et al. [50] demonstrated that *FLC*, *MAF3* (*AGL70*), *FLM* (*MAF1*, *AGL27*), *MAF2* (*AGL31*) and *MAF4* (*AGL69*) interact with each other and form nuclear complexes that are responsible for flowering repression.

Most of these TFs contribute to the same binding site, TFmatrixID_0503, except for *AGL15*, which is associated with the TF_motif_seq_0105 sequence. Motif TFmatrixID_0503 corresponded to the *MADS* transcription factors not only in *Arabidopsis* but also in *Brachpodium distachyon*, *Oryza sativa* and *Sorghum bicolor*. These transcription factors were shown to be involved in the flowering transaction and flowering time formation in

rice [53, 54]. For example, *OsMADS50* is a positive regulator of flowering, and *OsMADS56* negatively influences the flower transition [54]. *OsMADS7* and *OsMADS8* are involved in flowering time modulation [55]. *MADS* genes were identified in wheat [56]. Some of them are homologs of rice *OsMADS8* (*OsMADS24*) and *OsMADS7* (*OsMADS45*), which can influence the flowering time [57]. However, no data are available regarding the binding sites for the wheat *MADS* genes; thus, we can only propose that their association is at the same sites as those for the *Arabidopsis* and rice *MADS* genes.

Based on the data regarding the regulatory elements in the *Ppd-B1* promoter and the common TFs in the homoeologous *Ppd-1* genes, we may propose that the *MADS* genes play a major role in the misexpression of *Ppd-B1a* with an increased number of copies. Many of these genes are known to be flowering repressors. Most likely, *Ppd-B1* with an increased copy number continues to express during the night period; thus, the quantity of the repressors remains the same.

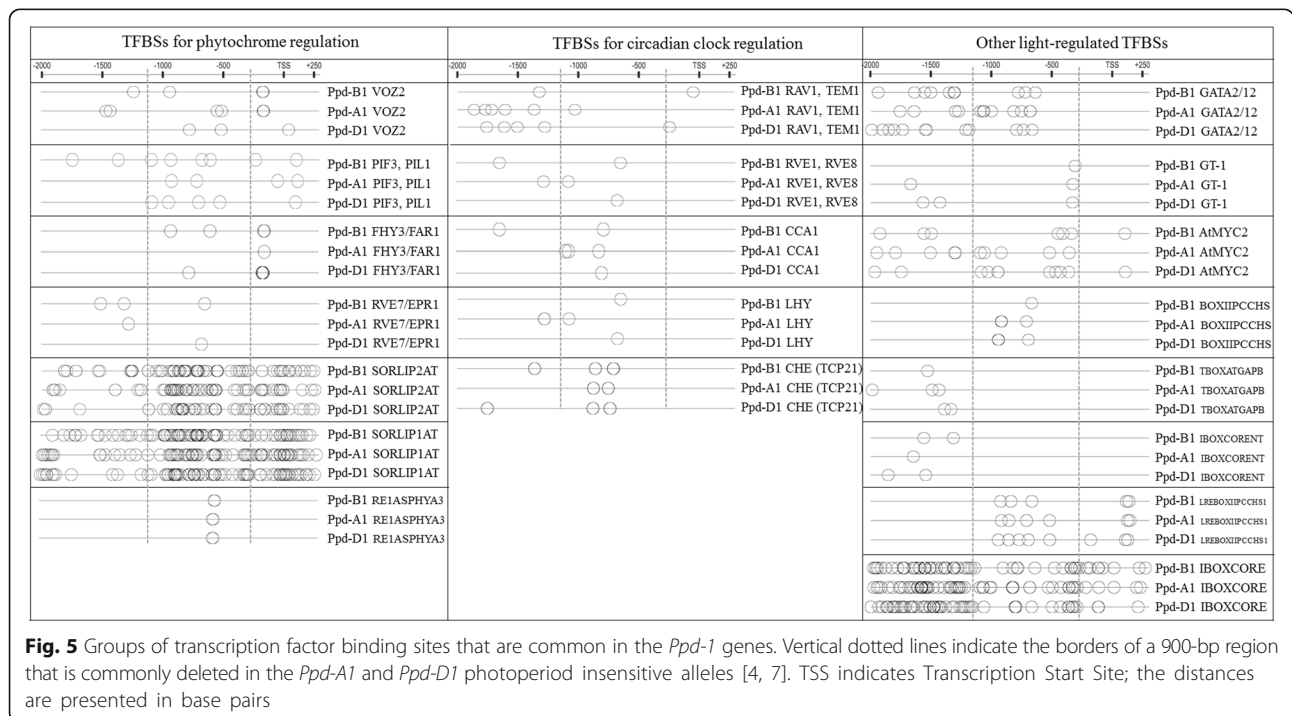


Table 2 Characterization of the selected transcription factors and the TFBSs with unknown transcription factors that were identified in the promoter regions of *Ppd-B1*

Family	Transcription factors/TFBSs with unknown TF	Description
AP2	AINTEGUMENTA (ANT)	ANT initiates floral organ development [87]. It was shown to play a critical role in regulating the ovule and female gametophyte development [88].
C2H2	RESPONSIVE TO HIGH LIGHT 41 (Zat2)	<i>Zat12</i> was originally isolated as a light stress-response cDNA [89]; then, it was suggested to be able to regulate transcripts involved in the response to high-light, cold and oxidative stress [90].
MADS box/MIKC	AGL19	AGL19 controls (promotes) flowering downstream of a cold-perception pathway and acts independently of FT and SOC1 [42].
MADS box/MIKC	FLOWERING LOCUS C (FLC)	FLC acts as an inhibitor of flowering [45].
MADS box/MIKC	MAF2 (AGL31)	<i>MAF2</i> (<i>AGL31</i>), a paralog of <i>FLC</i> , is another flowering repressor that acts in non-inductive photoperiods [46, 47].
MADS box/MIKC	AGL69 (MAF5)	<i>MAF5</i> is normally repressed. Overexpression of <i>MAF5</i> under a non-inductive day length causes late-flowering [48].
MADS box/MIKC	AGL68 (MAF4)	<i>MAF4</i> represses the transition to flowering [49, 50].
MADS box/MIKC	FLM (AGL27, MAF1)	FLM acts as a flowering inhibitor [51].
MADS box/MIKC	AGL6	AGL6 was suggested to be able to act as a flowering repressor or activator, depending on the context [43].
MADS box/MIKC	AGL14 (XAANTAL2, XAL2)	XAL2 is essential for flowering induction. XAL2 promotes flowering in response to different signals and is important for the maintenance and differentiating of flowering meristems [44].
MADS box/MIKC	AGAMOUS-like 15 (AGL15)	AGL15 and AGL18 are floral transition repressors. The <i>agl15 agl18</i> mutants were characterized by a partial suppression of the photoperiod pathway [52].
MADS box/MIKC	AGAMOUS-like 18 (AGL18)	
Lyase Aromatic	Phenylalanine Ammonia-Lyase (PAL1)	PAL1 is a light response element. These motifs are conserved at similar positions in several elicitor or light-responsive genes from different species [91].
–	RBCSGBOXPS	RBCSGBOXPS binding site, identified in <i>Parsley</i> , is involved in light responsiveness [92].
–	REBETALGLHCB21	REBETALGLHCB21, first found in the <i>Lemna dibba Lhcb</i> genes, is necessary for phytochrome regulation. These elements are likely to function by repressing the promoter activity in the dark [93].
–	SORLIP5AT	SORLIP5AT are PhyA-induced motifs that are overrepresented in light-induced genes. These elements, which predominate in the early responsive promoters, are more likely to have the fewest steps in the signal transduction cascade to gene expression [66].
–	MNF1ZMPPC1	MNF1ZMPPC1 is involved in the light-dependent transcriptional control of gene expression [94].

Thus, we have identified a set of putative transcription factors that regulate all homoeologous *Ppd-1* genes. We divided these transcription factors into three groups according to the input signal as follows: phytochrome-regulated, circadian clock-regulated and other light-regulated. However, there are several *Ppd-B1* specific

factors and *MADS* genes that are known to be flowering repressors and are most likely *Ppd-B1* regulators. Our future prospects include the verification of the involvement of the detected transcription factors in *Ppd-B1* regulation, and the discussed NILs are a relevant model for such studies.



Fig. 6 Groups of transcription factor binding sites that are specific to *Ppd-B1*. TSS indicates Transcription Start Site; the distances are presented in base pairs. Different colors designate different TFBSs or TFBSs with unknown TF

Conclusions

The results of this study suggest that there is a positive bidirectional regulation of *Ppd-B1a* and *PhyC* with an *FHY3/FAR1* contribution. The bidirectional regulation can be proposed for *Ppd-A1a* and *Ppd-D1a*. Using in silico analysis, we demonstrated that the specificity of the *Ppd-B1* regulation compared to that of homoeologous genes involves not only a copy number variation but also distinct regulatory elements.

Additional files

Additional file 1: Table S1. Correlation coefficients of the expression patterns of the flowering genes. Bold font indicates significant values ($P = 0.001$). PI means Photoperiod Insensitive and PSL means Photoperiod Sensitive samples. (XLSX 26 kb)

Abbreviations

AGL: Agamous-Like; ANT: Aintegumenta; *ARR*: *Arabidopsis Response Regulator*; CCA1: Circadian Clock Associated 1; cDNA: DNA complementary to RNA; CHE: CCA1 Hiking Expedition; CNV: Copy number variation; *FHY3/FAR1*: *Far-Red Elongated Hypokotyls3/Far-Red-Impaired Response1*; FLC: Flowering Locus C; FLM: Flowering Locus M; LHY: Late Elongated Hypocotyl; MAF: Mads Affecting Flowering; MITE: Miniature inverted-repeat transposable element; NILs: Near-Isogenic Lines; PAL1: Phenylalanine Ammonia-Lyase; *Phy*: *PhytochromeA*; PI: Photoperiod insensitive; PIF3: Phytochrome interacting Factor3; PIL1: Phytochrome Interacting Factor3-Like5; *Ppd-1*: *Photoperiod-1*; *PRR*: *Pseudo-Response Regulator*; PS: Photoperiod sensitive; PSL2: Photoperiod Sensitive Line 2; qPCR: Quantitative PCR; QTLs: Quantitative Trait Loci; RAV1: Related to ABI3/VP1; RE1ASPHYA3: RE1 putative repressor element; RR: Response Regulator; rRNA: DNA coding for rRNA; RVE1: Reveille1; RVE7/EPR1: Reveille 7/Early Phytochrome Responsive 1; RVE8: Reveille8; SD: Short day; SORLIP2AT: SORLIP1AT: SORLIP5AT: Sequences over-represented in the light-induced promoters; SSR: Simple Sequence Repeat; *TaFT1*: *Triticum aestivum Flowering Locus T*; TEM1: Tempranillo1; TEM2: Tempranillo2; TFBS: Transcription Factor Binding Sites; TSS: Transcription Start Site; VOZ2: Vascular Plant One-Zinc Finger1; *Vrn-1*: *Vernalization-1*; Zat2: Responsive To High Light 41

Acknowledgments

We are grateful to Prof. V. A. Koshkin for providing seeds of Sonora, PSL2 and NILs, used in this study. We thank SB RAS Genomics Core Facility and Joint Access Center for Artificial Plant Cultivation ICG SB RAS.

Funding

This study is supported by Russian Scientific Foundation (<http://rscf.ru/>) grant № 14–14–00161. The work at the Joint Access Center for Artificial Plant Cultivation ICG SB RAS was supported by the IC&G Budgetary Project No. 0324–2016–0001. Publication of this article has been funded by the Russian Scientific Foundation (Project No. 14–14–00161).

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

About this supplement

This article has been published as part of *BMC Plant Biology* Volume 17 Supplement 1, 2017: Selected articles from PlantGen 2017. The full contents of the supplement are available online at <https://bmcpantbiol.biomedcentral.com/articles/supplements/volume-17-supplement-1>.

Authors' contributions

Conceptualization: AK, EP, ES. Formal analysis and investigation: AK. Project administration, supervision: ES. Writing – original draft preparation: AK. Writing – review and editing: AK, EP, ES. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details

¹The Federal Research Center "Institute of Cytology and Genetics of Siberian Branch of the Russian Academy of Sciences", Prospekt Lavrentyeva 10, Novosibirsk, Russian Federation 630090. ²N.I. Vavilov Research Institute of Plant Genetic Resources, B.Morskaya Street 42-44, St. Petersburg, Russian Federation 190000.

Published: 14 November 2017

References

- Beales J, Turner A, Griffiths S, Snape JW, Laurie DAA. Pseudo-response regulator is misexpressed in the photoperiod insensitive *Ppd-D1a* mutant of wheat (*Triticum aestivum* L.). *Theor Appl Genet.* 2007;115:721–33.
- Law CN, Sutka J, Worland AJA. Genetic study of day-length response in wheat. *Heredity* (Edinb). 1978;41:185–91.
- Scarth R, Law CN. The location of the photoperiod gene, *Ppd2* and an additional genetic factor for ear-emergence time on chromosome 2B of wheat. *Heredity* (Edinb). 1983;51:607–19.
- Wilhelm EP, Turner AS, Laurie DA. Photoperiod insensitive *Ppd-A1a* mutations in tetraploid wheat (*Triticum durum* Desf.). *Theor Appl Genet.* 2009;118:285–94.
- Worland T, Snape JW. Genetic basis of worldwide wheat varietal improvement. In: William A, Alain B, Maarten G, editors. *World wheat book, a history of wheat breeding*. Paris: Lavoisier Publishing; 2001. p. 61–7.
- Seki M, Chono M, Matsunaka H, Fujita M, Oda S, Kubo K, et al. Distribution of photoperiod-insensitive alleles *Ppd-B1a* and *Ppd-D1a* and their effect on heading time in Japanese wheat cultivars. *Breed Sci.* 2011;61:405–12.
- Nishida H, Yoshida T, Kawakami K, Fujita M, Long B, Akashi Y, et al. Structural variation in the 5' upstream region of photoperiod-insensitive alleles *Ppd-A1a* and *Ppd-B1a* identified in hexaploid wheat (*Triticum aestivum* L.), and their effect on heading time. *Mol Breed.* 2013;31:27–37.
- Díaz A, Zikhali M, Turner A, Isaac P, Laurie D. Copy number variation affecting the *Photoperiod-B1* and *Vernalization-A1* genes is associated with altered flowering time in wheat (*Triticum aestivum*). *PLoS One.* 2012;7:e33234.
- Kiss T, Balla K, Bányai J, Veisz O, Karsai I. Effect of different sowing times on the plant developmental parameters of wheat (*Triticum aestivum* L.). *Cereal Res Commun.* 2014;42:239–51.
- Kitagawa S, Shimada S, Murai K. Effect of *Ppd-1* on the expression of flowering-time genes in vegetative and reproductive growth stages of wheat. *Genes Genet Syst.* 2012;87:161–8.
- Shaw LM, Turner AS, Laurie DA. The impact of photoperiod insensitive *Ppd-1a* mutations on the photoperiod pathway across the three genomes of hexaploid wheat (*Triticum aestivum*). *Plant J.* 2012;71:71–84.
- Yan L, Fu D, Li C, Blechl A, Tranquilli G, Bonafede M, et al. The wheat and barley vernalization gene *VRN3* is an orthologue of FT. *Proc Natl Acad Sci U S A.* 2006;103:19581–6.
- The Plant Promoter Analysis Navigator. <http://plantpan2.itps.ncku.edu.tw/index.html>. Accessed 5 May 2017.
- Kiseleva AA, Eggi EE, Koshkin VA, Sitnikov MN, Roder M, Salina EA, et al. Detection of genetic determinants that define the difference in photoperiod sensitivity of *Triticum aestivum* L. near-isogenic lines. *Russ J Genet.* 2014;50:701–11.
- GrainGenes 2.0. <http://wheat.pw.usda.gov/GG3/>. Accessed 5 May 2017.
- Hanahan D, Jessee J, Bloom FR. Plasmid transformation of *Escherichia coli* and other bacteria. *Methods Enzymol.* 1991;204:63–113.
- Shcherban AB, Khlestkina EK, Efreanova TT, Salina EA. The effect of two differentially expressed wheat VRN-B1 alleles on the heading time is

- associated with structural variation in the first intron. *Genetica* 2013;141:133–41.
18. Chen A, Li C, Hu W, Lau MY, Lin H, Rockwell NC, et al. Phytochrome C plays a major role in the acceleration of wheat flowering under long-day photoperiod. *Proc Natl Acad Sci U S A*. 2014;111:10037–44.
 19. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res*. 2001;29:e45.
 20. Ramakers C, Ruijter JM, Lekanne Deprez RH, Moorman AFM. Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. *Neurosci Lett*. 2003;339:62–6.
 21. Pearce S, Kippes N, Chen A, Debernardi JM, Dubcovsky J. RNA-seq studies using wheat *PHYTOCHROME B* and *PHYTOCHROME C* mutants reveal shared and specific functions in the regulation of flowering and shade-avoidance pathways. *BMC Plant Biol*. 2016;16:141.
 22. Griffiths S, Simmonds J, Leverington M, Wang Y, Fish L, Sayers L, et al. Meta-QTL analysis of the genetic control of ear emergence in elite European winter wheat germplasm. *Theor Appl Genet*. 2009;119:383–95.
 23. Hanocq E, Niarquin M, Heumez E, Rousset M, Le Gouis J. Detection and mapping of QTL for earliness components in a bread wheat recombinant inbred lines population. *Theor Appl Genet*. 2004;110:106–15.
 24. Kiseleva AA, Shcherban AB, Leonova IN, Frenkel Z, Salina EA. Identification of new heading date determinants in wheat 5B chromosome. *BMC Plant Biol*. 2016;16(s1):35–46.
 25. Guo Z, Song Y, Zhou R, Ren Z, Jia J. Discovery, evaluation and distribution of haplotypes of the wheat *Ppd-D1* gene. *New Phytol*. 2010;185:841–51.
 26. Mohler V, Lukman R, Ortiz-Islas S, William M, Worland AJ, van Beem J, et al. Genetic and physical mapping of photoperiod insensitive gene *Ppd-B1* in common wheat. *Euphytica*. 2004;138:33–40.
 27. Shaw LM, Turner AS, Herry L, Griffiths S, Laurie DA. Mutant alleles of *Photoperiod-1* in wheat (*Triticum aestivum* L.) that confer a late flowering phenotype in long days. *PLoS One*. 2013;8:e79459.
 28. Kendrick RE, Kronenberg GHM. *Photomorphogenesis in plants*. Dordrecht: Springer Netherlands; 1994.
 29. Terzaghi WB, Cashmore AR. Light-regulated transcription. *Annu Rev Plant Physiol Plant Mol Biol*. 1995;46:445–74.
 30. Koshkin VA, Lisker IS, Merezhko AF, Kosareva IA, Dragavtsev VA. Influence of genes *Ppd* on phytochrome, photoperiodic sensitivity, growth and development of isogenic lines in wheat. *Dokl. Russ. Acad. Agric Sci*. 2004;1:3–4.
 31. Li J, Li G, Wang H, Wang Deng X. Phytochrome signaling mechanisms. *The Arabidopsis Book*. 2011;29:e0148.
 32. Mongkolsirawatana C, Pongtongkam P, Peyachoknagul S. *silico* promoter analysis of photoperiod-responsive genes identified by DNA microarray in rice (*Oryza sativa* L.). *J Nat Sci*. 2009;43:164–77.
 33. Genoud T, Schweizer F, Tscheuschler A, Debrieux D, Casal JJ, Schäfer E, et al. *FHY1* mediates nuclear import of the light-activated phytochrome A photoreceptor. *PLoS Genet*. 2008;4:e1000143.
 34. Hanumappa M, Pratt L, Cordonnier-Pratt M, Deitzer GA. Photoperiod-insensitive barley line contains a light-labile phytochrome B. *Plant Physiol*. 1999;119:1033–40.
 35. Szucs P, Karsai I, von Zitzewitz J, Mészáros K, Cooper LLD, YQ G, et al. Positional relationships between photoperiod response QTL and photoreceptor and vernalization genes in barley. *Theor Appl Genet*. 2006;112:1277–85.
 36. Nishida H, Ishihara D, Ishii M, Kaneko T, Kawahigashi H, Akashi Y, et al. *Phytochrome C* is a key factor controlling long-day flowering in barley. *Plant Physiol*. 2013;163:804–14.
 37. Ogihara Y, Shimizu H, Hasegawa K, Tsujimoto H, Sasakuma T. Chromosome assignment of four photosynthesis-related genes and their variability in wheat species. *Theor Appl Genet*. 1994;88:383–94.
 38. Johnson E, Bradley M, Harberd NP, Whitelam CC. Photoresponses of light-crown *phyA* mutants of *Arabidopsis*. *Plant Physiol*. 1994;105:141–9.
 39. Neff MM, Chory J. Genetic interactions between phytochrome a, phytochrome B, and cryptochrome 1 during *Arabidopsis* development. *Plant Physiol*. 1998;118:27–35.
 40. Faure S, Turner AS, Gruszka D, Christodoulou V, Davis SJ, von Korff M, et al. Mutation in the circadian clock gene *EARLY MATURITY 8* adapts domesticated barley (*Hordeum vulgare*) to short growing seasons. *Proc Natl Acad Sci U S A*. 2012;109:4–9.
 41. Song YH, Shim JS, Kinmonth-Schultz HA, Imaizumi T. Photoperiodic flowering: time measurement mechanisms in leaves. *Annu Rev Plant Biol*. 2015;66:441–64.
 42. Schönrock N, Bouveret R, Leroy O, Borghi L, Köhler C, Grissem W, et al. Polycomb-group proteins repress the floral activator *AGL19* in the *FLC*-independent vernalization pathway. *Genes Dev*. 2006;20:1667–78.
 43. Koo SC, Bracko O, Park MS, Schwab R, Chun HJ, Park KM, et al. Control of lateral organ development and flowering time by the *Arabidopsis thaliana* *MADS-box* gene *AGAMOUS-LIKE6*. *Plant J*. 2010;62:807–16.
 44. Pérez-Ruiz RV, García-Ponce B, Marsch-Martínez N, Ugartechea-Chirino Y, Villajuana-Bonequi M, de Folter S, et al. *XAANTAL2* (*AGL14*) is an important component of the complex gene regulatory network that underlies *Arabidopsis* shoot apical meristem transitions. *Mol Plant*. 2015;8:796–813.
 45. Michaels SD, Amasino RM. Loss of *FLOWERING LOCUS C* activity eliminates the late-flowering phenotype of *FRIGIDA* and autonomous pathway mutations but not responsiveness to vernalization. *Plant Cell*. 2001;13:935–41.
 46. Scortecci KC, Michaels SD, Amasino RM. Identification of a *MADS-box* gene, *FLOWERING LOCUS M*, that represses flowering. *Plant J*. 2001;26:229–36.
 47. Ratcliffe OJ, Kumimoto RW, Wong BJ, Riechmann JL. Analysis of the *Arabidopsis MADS AFFECTING FLOWERING* gene family: *MAF2* prevents vernalization by short periods of cold. *Plant Cell*. 2003;15:1159–69.
 48. Kim DH, Sung S. The plant homeo domain finger protein, *VIN3-LIKE 2*, is necessary for photoperiod-mediated epigenetic regulation of the floral repressor, *MAF5*. *Proc Natl Acad Sci U S A*. 2010;107:17029–34.
 49. Gu X, Jiang D, Wang Y, Bachmair A, He Y. Repression of the floral transition via histone H2B monoubiquitination. *Plant J*. 2009;57:522–33.
 50. Gu X, Le C, Wang Y, Li Z, Jiang D, Wang Y, et al. *Arabidopsis FLC* clade members form flowering-repressor complexes coordinating responses to endogenous and environmental cues. *Nat Commun*. 2013, 1947:4
 51. Scortecci K, Michaels SD, Amasino RM. Genetic interactions between *FLM* and other flowering-time genes in *Arabidopsis thaliana*. *Plant Mol Biol*. 2003;52:915–22.
 52. Adamczyk BJ, Lehti-Shiu MD, Fernandez DE. The *MADS* domain factors *AGL15* and *AGL18* act redundantly as repressors of the floral transition in *Arabidopsis*. *Plant J*. 2007;50:1007–19.
 53. Lee S, Kim J, Han J-J, Han M, An G. Functional analyses of the flowering time gene *OsMADS50*, the putative *SUPPRESSOR OF OVEREXPRESSION OF CO 1/AGAMOUS-LIKE 20 (SOC1/AGL20)* ortholog in rice. *Plant J*. 2004;38:754–64.
 54. Ryu C-H, Lee S, Cho L-H, Kim SL, Lee Y-S, Choi SC, et al. *OsMADS50* and *OsMADS56* function antagonistically in regulating long day (LD)-dependent flowering in rice. *Plant Cell Environ*. 2009;32:1412–27.
 55. Kang HG, Jang S, Chung JE, Cho YG, An G. Characterization of two rice *MADS box* genes that control flowering time. *Mol Cells*. 1997;7:559–66.
 56. Zhao T, Ni Z, Dai Y, Yao Y, Nie X, Sun Q. Characterization and expression of 42 *MADS-box* genes in wheat (*Triticum aestivum* L.). *Mol Gen Genomics*. 2006;276:334–50.
 57. Shitsukawa N, Tahira C, Kassai KI, Hirabayashi C, Shimizu T, Takumi S, et al. Genetic and epigenetic alteration among three homoeologous genes of a class. E *MADS Box* gene in hexaploid wheat *Plant Cell*. 2007;19:1723–37.
 58. Yasui Y, Mukougawa K, Uemoto M, Yokofuji A, Suzuri R, Nishitani A, et al. The Phytochrome-interacting *VASCULAR PLANT ONE-ZINC FINGER1* and *VOZ2* redundantly regulate flowering in *Arabidopsis*. *Plant Cell*. 2012;24:3248–63.
 59. Shin J, Park E, Choi G. *PIF3* regulates anthocyanin biosynthesis in an *HY5*-dependent manner with both factors directly binding anthocyanin biosynthetic gene promoters in *Arabidopsis*. *Plant J*. 2007;49:981–94.
 60. Kim J, Yi H, Choi G, Shin B, Song P, Choi G. Functional characterization of *PIF3* in phytochrome-mediated light signal transduction. *Plant Cell*. 2003;15:2399–407.
 61. Oda A, Fujiwara S, Kamada H, Coupland G, Mizoguchi T. Antisense suppression of the *Arabidopsis PIF3* gene does not affect circadian rhythms but causes early flowering and increases *FT* expression. *FEBS Lett*. 2004;557:259–64.
 62. Oh E, Kim J, Park E, Kim J-H, Kang C, Choi G. *PIL5*, a phytochrome-interacting basic helix-loop-helix protein, is a key negative regulator of seed germination in *Arabidopsis thaliana*. *Plant Cell*. 2004;16:3045–58.
 63. Hudson ME, Lisch DR, Quail PH. The *FHY3* and *FAR1* genes encode transposase-related proteins involved in regulation of gene expression by the phytochrome A-signaling pathway. *Plant J*. 2003;34:453–71.
 64. Li G, Siddiqui H, Teng Y, Lin R, Wan X, Li J, et al. Coordinated transcriptional regulation underlying the circadian clock in *Arabidopsis*. *Nat Cell Biol*. 2011;13:616–22.
 65. Kuno N, Möller SG, Shinomura T, Xu X, Chua N-H, Furuya M. The novel MYB protein *EARLY-PHYTOCHROME-RESPONSIVE1* is a component of a slave circadian oscillator in *Arabidopsis*. *Plant Cell*. 2003;15:2476–88.

66. Hudson ME, Quail PH. Identification of promoter motifs involved in the network of Phytochrome A-regulated gene expression by combined analysis of genomic sequence and microarray data. *Plant Physiol.* 2003;133:1605–16.
67. Bruce WB, Deng XW, Quail PHA. Negatively acting DNA sequence element mediates phytochrome-directed repression of *phyA* gene transcription. *EMBO J.* 1991;10:3015–24.
68. Ngai N, Tsai FY, Coruzzi G. Light-induced transcriptional repression of the pea *AS1* gene: identification of cis-elements and transactors. *Plant J.* 1997;12:1021–34.
69. YX H, Wang YH, Liu XF, Li JY. Arabidopsis *RAV1* is down-regulated by brassinosteroid and may act as a negative regulator during plant development. *Cell Res.* 2004;14:8–15.
70. Castillejo C, Pelaz S. The balance between *CONSTANS* and *TEMPRANILLO* activities determines *FT* expression to trigger flowering. *Curr Biol.* 2008;18:1338–43.
71. Rawat R, Schwartz J, Jones MA, Sairanen I, Cheng Y, Andersson CR, et al. REVEILLE1, a Myb-like transcription factor, integrates the circadian clock and auxin pathways. *Proc Natl Acad Sci U S A.* 2009;106:16883–8.
72. Rawat R, Takahashi N, Hsu PY, Jones MA, Schwartz J, Salemi MR, et al. REVEILLE8 and PSEUDO-REPONSE REGULATOR5 form a negative feedback loop within the Arabidopsis circadian clock. *PLoS Genet.* 2011;7:e1001350.
73. Farinas B, Mas P. Functional implication of the MYB transcription factor RVE8/LCL5 in the circadian control of histone acetylation. *Plant J.* 2011;66:318–29.
74. Mizoguchi T, Wheatley K, Hanzawa Y, Wright L, Mizoguchi M, Song HR, et al. *LHY* and *CCA1* are partially redundant genes required to maintain circadian rhythms in Arabidopsis. *Dev. Cell.* 2002;2:629–41.
75. Pruneda-Paz JL, Breton G, Para A, Kay SAA. Functional genomics approach reveals CHE as a component of the Arabidopsis circadian clock. *Science.* 2009;323:1481–5.
76. Luo XM, Lin WH, Zhu S, Zhu JY, Sun Y, Fan XY, et al. Integration of light- and brassinosteroid-signaling pathways by a GATA transcription factor in Arabidopsis. *Dev. Cell.* 2010;19:872–83.
77. Franco-Zorrilla JM, López-Vidriero I, Carrasco JL, Godoy M, Vera P, Solano R. DNA-binding specificities of plant transcription factors and their potential to define target genes. *Proc Natl Acad Sci U S A.* 2014;111:2367–72.
78. Manfield IW, Devlin PF, Jen C, Westhead DR, Gilmartin PM. Conservation, convergence, and divergence of light-responsive, circadian-regulated, and tissue-specific expression patterns during evolution of the Arabidopsis GATA gene family. *Plant Physiol.* 2007;143:941–58.
79. Green PJ, Yong MH, Cuzzo M, Kano-Murakami Y, Silverstein P, Chua NH. Binding site requirements for pea nuclear protein factor GT-1 correlate with sequences required for light-dependent transcriptional activation of the *rbcS-3A* gene. *EMBO J.* 1988;7:4035–44.
80. Yadav V, Mallappa C, Gangappa SN, Bhatia S, Chattopadhyay SA. Basic helix-loop-helix transcription factor in Arabidopsis, MYC2, acts as a repressor of blue light-mediated photomorphogenic growth. *Plant Cell.* 2005;17:1953–66.
81. Block A, Dangel JL, Hahlbrock K, Schulze-Lefert P. Functional borders, genetic fine structure, and distance requirements of cis elements mediating light responsiveness of the parsley chalcone synthase promoter. *Proc Natl Acad Sci U S A.* 1990;87:5387–91.
82. Chan CS, Guo L, Shih MC. Promoter analysis of the nuclear gene encoding the chloroplast glyceraldehyde-3-phosphate dehydrogenase B subunit of Arabidopsis thaliana. *Plant Mol Biol.* 2001;46:131–41.
83. Yamamoto YY, Ichida H, Matsui M, Obokata J, Sakurai T, Satou M, et al. Identification of plant promoter constituents by analysis of local distribution of short sequences. *BMC Genomics.* 2007;8:67.
84. Martinez-Hernandez A, Lopez-Ochoa L, Arguello-Astorga G, Herrera-Estrella L. Functional properties and regulatory complexity of a minimal *rbcS* light-responsive unit activated by phytochrome, cryptochrome, and plastid signals. *Plant Physiol.* 2002;128:1223–33.
85. Schulze-Lefert P, Dangel JL, Becker-André M, Hahlbrock K, Schulz W. Inducible in vivo DNA footprints define sequences necessary for UV light activation of the parsley chalcone synthase gene. *EMBO J.* 1989;8:651–6.
86. Borello U, Ceccarelli E, Giuliano G. Constitutive, light-responsive and circadian clock-responsive factors compete for the different I box elements in plant light-regulated promoters. *Plant J.* 1993;4:611–9.
87. Elliott RC, Betzner AS, Huttner E, Oakes MP, Tucker WQ, Gerentes D, et al. *AINTEGUMENTA*, an *APETALA2*-like gene of Arabidopsis with pleiotropic roles in ovule development and floral organ growth. *Plant Cell.* 1996;8:155–68.
88. Klucher KM, Chow H, Reiser L, Fischer RL. The *AINTEGUMENTA* gene of Arabidopsis required for ovule and female gametophyte development is related to the floral homeotic gene *APETALA2*. *Plant Cell.* 1996;8:137–53.
89. Iida A, Kazuoka T, Torikai S, Kikuchi H, Oeda KA. Zinc finger protein RHL41 mediates the light acclimatization response in Arabidopsis. *Plant J.* 2000;24:191–203.
90. Davletova S, Davletova S, Schlauch K, Schlauch K, Coutu J, Coutu J, et al. The zinc-finger protein *zat12* plays a central role in reactive oxygen and abiotic stress signaling in Arabidopsis. *Plant Physiol.* 2005;139:847–56.
91. Lois R, Dietrich A, Hahlbrock K, Schulz WA. Phenylalanine ammonia-lyase gene from parsley: structure, regulation and identification of elicitor and light responsive cis-acting elements. *EMBO J.* 1989;8:1641–8.
92. Gilmartin PM, Sarokin L, Memelink J, Chua NH. Molecular light switches for plant genes. *Plant Cell.* 1990;2:369–78.
93. Degenhardt J, Tobin EMADNA. Binding activity for one of two closely defined phytochrome regulatory elements in an *Lhcb* promoter is more abundant in etiolated than in green plants. *Plant Cell.* 1996;8:31–41.
94. Morishima A. Identification of preferred binding sites of a light-inducible DNA-binding factor (MNF1) within 5'-upstream sequence of C4-type phosphoenolpyruvate carboxylase gene in maize. *Plant Mol Biol.* 1998;38:633–46.

Submit your next manuscript to BioMed Central and we will help you at every step:

- We accept pre-submission inquiries
- Our selector tool helps you to find the most relevant journal
- We provide round the clock customer support
- Convenient online submission
- Thorough peer review
- Inclusion in PubMed and all major indexing services
- Maximum visibility for your research

Submit your manuscript at
www.biomedcentral.com/submit

