

## Supplementary information

### Information included in the Supplementary Material

1. Cloning and sequencing of wheat *CO* and *FT* homologues.
2. Primer sequences and reaction conditions for quantitative RT-PCR assays.

#### 1. *TaCO1* and *TaFT1* assay development

Barley contains two genes (*HvCO1* and *HvCO2*) closely related to the *A. thaliana CONSTANS* gene (Griffiths *et al.*, 2003). Due to the extensive colinearity between the wheat and barley genomes, it is reasonable to assume that the two barley genes might also be present in wheat as homeologous series. One series, *TaHd1* (homologous to *HvCO2*) has been described by Nemoto *et al.* (2004) but the second (homologous to *HvCO1*) has not. Using an RT-PCR assay developed for barley *HvCO1*, an amplification product was obtained from wheat cDNA. Direct sequencing of this amplicon revealed a fragment of 457bp, 97.1% homologous to the equivalent region in *HvCO1*. The sequencing trace files contained a number of double base calls (7 of 457, underlined below) indicative of amplification from more than one homoeologous genome simultaneously.

#### Wheat RT-PCR *CO1* sequence

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CMRCAGCAG CAGCAGCAGC AGYAGCAGAT AYTTTGGTGA AGTGGATCAG  
TACTTTGACC TTGTCGGGTA CAATTCTTAC TATGATAGCC ACATGAACAA  
CCAAGAGCAG TATGTGATGC AAGAACAACA ACATCTGCAG CAGATGCAAA  
AGGAGTACGC GGAGCAGCAG ATGCAAAAAGG AGTACGTGGA GAAGGAAGGG  
AGYGAGTGCA TAGTACCTTC GCAGTCTGCA ATTGTGAGCA GGCCACATCA  
GAGTGGTTAT GCACCACTTG TARGGCAGA GCAGGCTGCC TCCGTGACTG  
CTGGGGTTAG TGCTTACACA GATTCCGTCA ACAACAGCAT ATCTTTCTCA  
ATGGAGGCGG GTATAGTMCC GGACAATACG GTCCAGTCCA GCATCCTGAG  
ACCAGCTGGA GCCATCGGTC WCTTCTCAAG TCCTTCACTT CAGACACCAC  
TTCACTTCA
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The RT-PCR amplicon was homologous to seven wheat EST sequences (DR733387, CJ659269, CJ550649, CJ550682, CJ551655, CJ658279, CJ658312) of which the last five included an identical 3' UTR. A primer pair for the 3' UTR (forward 5' aaggcaccttagcacctacg 3'; reverse 5' tgctagctgcttttcgtaa 3') assigned the gene to 7B using 'Chinese Spring' nullisomic-tetrasomic lines (Fig. S1) consistent with the 7H location of the equivalent gene in barley.

Wheat *TaFTI* sequences were amplified using the *HvFTI* primers described in Turner et al. (2005). Sequencing confirmed that the correct gene was amplified and was equivalent to wheat *FTI* (GenBank AY705794).

## 2. PCR primers for quantitative RT-PCR assays

Table S1. Primer sequences, amplicon size and optical read temperatures for Quantitative RT-PCR assays.

Assay	Primers 5' to 3' sequences	Product size [Reaction efficiency]	Optical read temp.	Primer derivation	Associated wheat sequences
18s rRNA	18S_F: atacgtgcaacaaaccc 18S_R: ctacctccccgtgtca	288bp [1.47]	81°C	<i>Arabidopsis thaliana</i> (1)	AY049040
<i>TaCOI</i>	HvCO1Bf : ccataatcgcaaggcaac HvCO1MidR1: tggettctctctccttgagc	497bp [1.44]	81°C	from barley AF490467 (2)	
<i>TaGI</i>	Gi_F: caattgccacaccaagtgcta Gi_R: tgatgaattcagaggtaacaaacca	497bp [1.36]	81°C	from barley: AF411229 (3)	AF543844 (4) AY679114 (4) AY679115 (4)
Group 2 <i>PRR</i> (non- specific)	HvPRR72_F5_1: ttgagctgagcctgaagag HvPRR72_R8: tatagctaggtgcgtggcg	A 992 bp B 892 bp D 873 bp [1.22]	81°C	This paper	
2A <i>PRR</i> gene	TaPRR72_AgF5: cggtctccaatcaaggcc HvPRR72_R6_1: tctccccgaagtctctc	655 bp [1.42]	81°C	This paper	
2B <i>PRR</i> gene	TaPRR72_BgF1: agacgattcattccgctcc HvPRR72_R6_2:	659 bp & 552 bp* [1.24]	81°C	This paper	

	agcagcaccatttgagagg				
2D PRR gene	HvPRR72_F2: gatgaacatgaaacggg TaPRR72_DgR2: gtctaaatagtaggtactagg	209 bp [1.37]	81°C	This paper	
<i>TaFT1</i>	HvHd3a_F2: ccaaccttagagagtatctcact HvHd3a-R2: ccctggtgtgaagtctgg	155 bp [1.28]	81°C	from barley DQ100327 (5)	AJ577367 (6)

\* A small quantity of a splice variant (confirmed by sequencing and comparison to genomic DNA) smaller than the full-length transcript was occasionally amplified by this assay. The real-time PCR analysis was configured to detect the sum of amounts of both transcripts.

(1) Tyler et al. (2005). (2) Griffiths et al. (2003). (3) Dunford et al. (2005). (4) Zhao et al. (2005). (5) Turner et al. (2005). (6) Ciaffi et al. (2005).

Specificity of the 2A, 2B and 2D PRR assays was confirmed by direct sequencing of the PCR products and by amplification from 'Chinese Spring' nullisomic-tetrasomic lines (Fig S2). Reaction Efficiency and Optical Read Temperature are defined in the materials and methods section of the main manuscript.

### References cited in Supplementary Information

Ciaffi M, Paolacci AR, D'Aloisio E, Tanzarella OA, Porceddu E (2005) Identification and characterization of gene sequences expressed in wheat spikelets at the heading stage. *Gene* 346: 221-230

Dunford RP, Griffiths S, Christodoulou V, Laurie DA (2005) Characterisation of a barley (*Hordeum vulgare* L.) homologue of the *Arabidopsis* flowering time regulator *GIGANTEA*. *Theor Appl Genet* 110: 925-931

Griffiths S, Dunford RP, Coupland G, Laurie DA (2003) The evolution of *CONSTANS*-like gene families in barley (*Hordeum vulgare*), rice (*Oryza sativa*) and *Arabidopsis thaliana*. *Plant Physiol* 131: 1855-1867

Nemoto Y, Kisaka M, Fuse T, Yano M, Ogihara Y (2003) Characterization and functional analysis of three wheat genes with homology to the CONSTANS flowering time gene in transgenic rice. *Plant Journal* 36: 82-93.

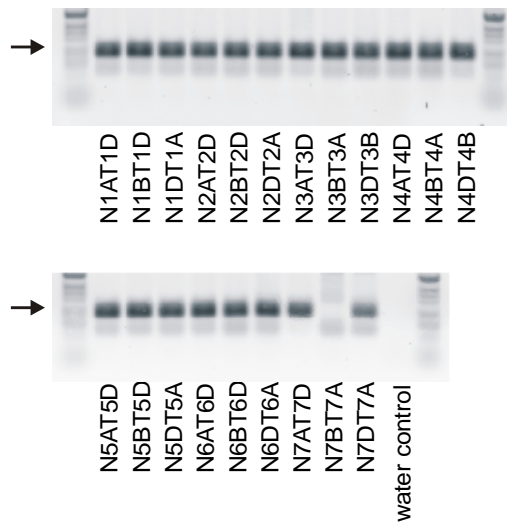
Turner A, Beales J, Faure S, Dunford RP, Laurie DA (2005) The pseudo-response regulator *Ppd-H1* provides adaptation to photoperiod in barley. *Science* 310: 1031-1034

Tyler L, Thomas SG, Hu JH, Dill A, Alonso JM, Ecker JR, Sun TP (2005) DELLA proteins and gibberellin-regulated seed germination and floral development in *Arabidopsis*. *Plant Physiol* 135: 1008-1019

Zhao XY, Liu MS, Li JR, Guan CM, Zhang XS (2005) The wheat *TaG11*, involved in photoperiodic flowering, encodes an *Arabidopsis GI* ortholog. *Plant Mol Biol* 58: 53-64

## Supplementary figures

**Fig. S1.** PCR amplification of a genome specific amplicon from the 3' UTR of *TaCO1* in 'Chinese Spring' nullisomic-tetrasomic lines. The respective nullisomic chromosome is indicated below each track and the arrow shows the expected band size. Absence of the band in the nullisomic 7B line shows that the product derives from this chromosome.



**Fig. S2.** PCR amplification of *PRR* gene amplicons from 'Chinese Spring' nullisomic-tetrasomic lines of the group 2 chromosomes. This shows that the amplicons used to measure the expression of the 2A, 2B and 2D *PRR* genes are specific to their respective chromosomes.

