The floral integrator *WFT* in wheat: expression profiles of three homoeologous genes

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ABSTRACT

Flowering is a very important event that is crucial for reproductive success in higher plants. There are four flowering pathways in *Arabidopsis* such as photoperiodic pathway, vernalization pathway, the autonomous pathway and gibberellin pathway. *FLOWERING LOCUS T (FT)* known as florigen plays a central role in flowering of *Arabidopsis*, and the *FT* homologues of many species have been isolated and analysed until now. In wheat (*Triticum aestivum* L.), the following three components of flowering exist: photoperiod sensitivity, vernalization requirement and earliness *per se*. It has reported that flowering induction under long day condition is occurred by high levels of *WFT (Wheat FT)* expression. Wheat is a hexaploid species with AABBDD genome. Wheat genome, therefore, contains three homoeologous genes derived from A, B and D genomes. And then, the expression of *WFT* may include that of three, two or one homoeologous genes. To investigate the expression levels of each *WFT-A, WFT-B,* and *WFT-D,* we carried out expression analysis using cv. Chinese Spring (CS) grown under long day or short day conditions. The expression of *WFT* was started at 6 weeks (6-leaf stage) or 8 weeks (7-leaf stage) after sowing under long day or short day conditions, respectively. Interestingly, both under long day and short day conditions, the expression level of *WFT-B* was highest of three homoeologous genes. On the other hand, chromosome substitution line CS (Hope7B) showed the early flowering (*Vrn-B3*) in compared with CS. In addition, the flowering time in Hope is later than CS. It is likely that *WFT-B* derived from B genome plays a major role in flowering of wheat.

INTRODUCTION

Flowering time is affected by several environmental factors such as day-length (photoperiod) and temperature. In *Arabidopsis*, there are the photoperiodic pathway, the vernalization pathway, the autonomous pathway and the gibberellin pathway (Bernier and Périlleux 2005). Information on the molecular and genetic mechanisms of flowering has been mainly obtained by using *Arabidopsis*. The GI-CO-FT proteins act as the core of the photoperiodic pathway, and then their regulation results in *FT* expression under long day condition (Turck et al. 2008). *FLOWERING LOCUS T (FT)* is known as an integrator of the flowering pathway in *Arabidopsis*, and it was recently reported that the FT protein moves from leaf to apical meristems as a 'florigen' (Jaeger et al. 2007).

On the other hand, OsGI-Hd1-Hd3a proteins, which are orthologs of *Arabidopsis* GI-CO-FT, work on the photoperiodic pathway in rice. Moreover, Hd3a protein is a mobile signal similar to the *Arabidopsis* FT, and is a key factor of flowering in rice (Tamaki et al. 2007).

In wheat (*Triticum aestivum* L.), *WFT (TaFT)* is also a key factor of flowering, like *Arabidopsis* and rice (Yan et al. 2006). Wheat is a hexaploid species with AABBDD genome. Therefore, the wheat genome contains three homoeologous genes derived from the A, B and D genomes. Thus, the expression of *WFT* may include that of three, two or one homoeologous genes. Actually, Shitsukawa et al. (2007) reported that only *WLHS1-D* has the functional role in wheat although there are three homoeologous genes of *WLHS1 (LEAFY HULL STERILE1)*, which is homolog of *OsMADS1* in rice. It has reported that chromosome substitution line CS (Hope7B) showed the early flowering (*Vrn-B3*) compared with CS (Law 1966). These reports indicate that there are the functional differences among three homoeologous *FT* genes, *WFT-A, B*, and *D*.

In this study, we carried out an expression analysis of the three homoeologous genes of *WFT* using *Triticum aestivum* L. cv. Chinese Spring (CS), Hope, chromosome substitution line CS (Hope7B), and CS nullisomic/tetrasomic (NT) lines to reveal the function of *WFT-A, B,* and *D*.

MATERIALS AND METHODS

Plant materials and growth condition

Triticum aestivum L. cv. Chinese Spring (CS), Hope, chromosome substitution line CS (Hope7B), and CS nullisomic/tetrasomic (NT) lines were used in this study to investigate the differences in the expression pattern of three homoeologous genes. These lines were grown in a growth chamber for long day (16 h Light/ 8 h Dark), or for short day conditions (12 h Light/ 12 h Dark) at 20±3ºC (175μmol·m^{-2}·s^{-1}). For expression analysis through the developmental stages leaf samples were collected at each-leaf stages. For analysis of circadian oscillation of *FT* expression, leaves of CS at 6-leaf stages were sampled each 4 h after the start of the dark phase. Numbers of days from seed sowing to the appearance of the first panicle were observed, to check flowering time.

Genomic PCR

Genomic DNA was extracted from the leaves of CS and NT lines, and used as template for genomic PCR with each *WFT-A, B*, and *D* specific primer pairs. PCR was performed for 35 cycles under the following conditions: denaturation, 94ºC, 30 sec; annealing 65ºC, 30 sec; extension 72ºC, 50 sec.

Expression analysis

Total RNAs were extracted from leaves by Get pure RNA Kit (Dojindo, Kumamoto, Japan). First-strand cDNA was synthesized from 1 μg of each RNA samples in a 20 μl reaction solution using the TaKaRa RNA RCR kit (AMV) Ver. 3.0 (Takara Bio Inc., Shiga, Japan).

Quantitative PCR (Q-PCR) was carried out by using Mx3000P® (Stratagene Japan Inc., Tokyo, Japan) with Brilliant® II SYBR® Green QPCR Master Mix (Stratagene Japan Inc.). First, *WFT-A, B*, and *D*, and *TaActin* as an internal standard were amplified with genome-specific primers, and the amplified fragments were cloned into pCR2.1-TOPO vector (Invitrogen, Carlsbad, CA, USA). Standard curve was calculated with each diluted plasmid series using the 'SYBR Green with Dissociation Curve' as experiment type on Mx3000P®. Q-PCR products of *WFT-A, B, D* and *TaActin* from leaf samples were amplified with each specific primer pair by the 'Comparative Quantitation with Calibrator' as experiment type on Mx3000P®. Q-PCR was performed for 40 cycles under following conditions after 94ºC, 10 min: denaturation, 94ºC, 30 sec; annealing, 65ºC, 1 min; extension, 72ºC, 50 sec. Finally, the copy numbers of each gene were obtained from standard curve. The values of *WFT-A, B,* and *D* were normalized using that of *TaActin*.

RESULTS AND DISCUSSION

Identification of the specific primers for WFT-A, B, and D

We performed the genomic PCR using the genomic DNAs of CS nullisomic/tetrasomic (NT) lines as templates, to confirm whether each *WFT-A, B*, and *D* primers could amplify specific fragments of *WFT-A, B*, and *D*. Amplified products of *WFT-A, B*, and *D* did not observe in the lines without A, B, and D genome, respectively. Therefore, these primers were used in expression analysis of three homoeologous genes.

CS (Hope7B) line showed early flowering phenotype.

To reveal the functional differences among three homoeologous genes, chromosome substitution line CS (Hope7B), CS, and Hope were grown under long day conditions. CS (Hope7B) showed early flowering compared with CS, while the flowering time in Hope is later than that in CS (Table 1). CS nullisomic/tetrasomic (NT) lines grown under long day condition were also used to check the flowering time. N7DT7B (Nulli-7D Tetra-7B) line showed the earliest flowering among NT lines, whereas no significant changes in flowering time of other NT lines was observed in comparison to CS (Table 2). These results suggested that *WFT-B* derived from B genome plays a major role in flowering of wheat.

Expression pattern and diurnal oscillation of WFT-A, B, and D through developmental stage

To investigate the expression levels of each *WFT-A, B*, and *D*, we carried out expression analysis using cv. Chinese Spring (CS) grown under long day or short day conditions. The expression of *WFT* was started at 6-leaf stage (6 weeks) or 7-leaf stage (8 weeks) after sowing under long day or short day conditions, respectively. Interestingly, both under long day and short day conditions, the expression level of *WFT-B* was highest among three homoeologous genes (Table 3). Moreover, CS (Hope7B), CS, and Hope were grown under long day conditions for expression analysis of three homoeologous genes. In CS and Hope, three homoeologous *FTs* were expressed at the same time, #-leaf stage and #-leaf stage, respectively. However, only *WFT-B* mRNA in CS (Hope7B) was observed in earlier stage (1-leaf stage) prior to the expression of other two homoeologous *FTs*, *WFT-A* and *D* (Table 1). For the analysis of diurnal oscillation of *WFT-A, B*, and *D*, the samples of CS at 6- or 7-leaf stages were collected every 4 h for 2 days during long day and short day conditions, respectively. The transcript level of *WFT-B* was always higher than those of two homoeologous genes in CS.

Table1 The flowering time and initiation stage of *WFT* expression in CS, CS (Hope7B), and Hope

		Initiation stage of *WFT* expression (-leaf stage)		
	Flowering time (day)	*WFT-A*	*WFT-B*	*WFT-D*
CS	71.13±0.295	6	6	6
CS (Hope7B)	48.25±0.977	3	1	3
Hope	>100	7	7	7

Numbers of day from seed sowing to the appearance of the first panicle were observed.
The mean of copy number is shown with SE.

Table2 The flowering time in nullisomic/tetrasomic lines of CS (day)

	LD
CS	70.0±1.40
N7AT7B	73.8±0.60
N7AT7D	69.4±1.76
N7BT7A	76.5±0.90
N7BT7D	74.0±1.45
N7DT7A	66.1±2.15
N7DT7B	50.5±2.26

The mean of copy number is shown with SE.

These results indicate that the most functional *FT* gene copy in wheat is *WFT-B* and the early flowering phenotype in CS (Hope7B) is caused by the exceptional early expression of *WFT-B* derived from Hope in CS background. Yan et al. (2006) reported that Hope *WFT-B* had a retrotoronsposon insertion in the promoter region, which might affect the promoter activity. The difference between the promoter activity of Hope *WFT-B* and CS *WFT-B* in CS background could be a reason for the early flowering of CS (Hope7B).

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Table3 Expression levels of *WFT-A, B,* and *D* in CS at 10-leaf stage (/Actin)

	LD	SD
WFT-A	12.4±1.60	0.156±0.0406
WFT-B	68.4±14.3	3.32±2.41
WFT-D	7.40±0.356	0.117±0.0140

The mean of copy number is shown with SE.