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Virus-induced gene silencing-based functional verification of six genes associated with vernalization in wheat

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ABSTRACT

Vernalization requirement is an important characteristic in crop breeding. Wheat is a widely grown crop in the world that possesses enormous economic significance. To better understand the gene networks in vernalization process, we performed a high-throughput RNA sequencing analysis comparing the transcriptomes of spring and winter wheat cultivars, with and without vernalization (unpublished data). In this study, we selected six unigenes (CL14010, CL12788, CL176, Unigene 16777, CL8746 and Unigene10196) from our transcriptome analysis based on their expression differences to further characterize their function. Transient silencing of the six unigenes individually were achieved through virus-induced gene silencing (VIGS) using BSMV vector. The period from germination to spike differentiation were recorded and compared between plants underwent VIGS silencing and the control. Our result showed that VIGS of the six unigenes significantly shortened the period from seedling to double ridge (DR) stage. Resulting in SD period ranging from 59.8 ± 0.60 to 65.8 ± 0.48 days, compared to 85.0 ± 0.73 days in the control. The results indicated that these six unigenes function as suppressors in vernalization process and silence or down-regulation of these genes promoted flower development in wheat. Further characterization of these six unigenes and their function in vernalization and flowering control is needed.

1. Introduction

Flowering in many plants requires a prolonged period of cold exposure in the winter, a process called vernalization. Vernalization requirement is an important characteristic in crop breeding, enabling greater geographic distribution of crops [1]. Studying the molecular basis of plant response to vernalization will help further understand plants' transformation from vegetative to reproductive growth, and eventually, help in the breeding of high yield crop varieties.

The molecular study of vernalization has been focused on three plant species: Arabidopsis, barley and wheat [2]. In Arabidopsis, vernalization triggers the down-regulation of the MADS-box transcription factor, flower locus C (FLC) [3]. However, there is no FLC homolog in wheat. Instead, genetic studies have uncovered three genes in wheat that are required for the vernalization process, *VRN1*, *VRN2* and *VRN3*. While VRN1 and VRN2 have no homologs in Arabidopsis, *VRN3* is a homolog of the *Flowering locus T* (*FT*) gene in Arabidopsis [4]. Despite the uncovering of these key players, the detailed molecular mechanism and gene network of the vernalization process in wheat remain unknown.

In recent years, virus-induced gene silencing (VIGS) has developed into an efficient genetic tool in identifying gene function in a number of dicots, including tobacco [5], pea [6], Arabidopsis [7], tomato [8]; as well as some monocots, such as barley [9], wheat [10] and corn [11]. VIGS is a natural defense response in plant triggered by virus infection [12]. Upon virus infection, the post-transcriptional gene silencing (PTGS) is activated in the host which degrades the double-stranded RNA produced by the virus

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into short interfering RNAs (siRNAs). If the host defense response is triggered by the recombinant virus carrying sequence corresponding to the gene of interest, its homologous endogenous mRNAs becomes the target for degradation [13,14]. Two vectors have been developed and are widely used in gene silencing in monocots, one is barley stripe mosaic virus (BSMV) used for barley and wheat [9,10]; the other is brome mosaic virus (BMV), suitable for barley, rice and corn [11].

To better understand genes involved in vernalization process, we conducted a high-throughput RNA sequencing analysis of *Triticum aestivum*, comparing the transcriptome of spring and winter cultivars with and without vernalization (unpublished data). Here, we selected six genes (Table 1) that are mostly differentially down-regulated after vernalization to further verify their function through VIGS. Silencing of the Phytoene Desaturase (*PDS*) gene, which causes a photobleached leaf phenotypes was used as a positive control.

2. Materials and methods

2.1. Plant materials and growth conditions

Full seeds of wheat (*T. aestivum* L. cv. Jing 841) were fully soaked and seeded in petridish, and were transferred into pots containing sterilized vermiculite after germination. They were cultivated in growth chambers under controlled condition (20 °C, 14 h/10 h day/night photoperiod). The seedlings with consistent growth were selected at the two leaves stage, and were then inoculated with the recombinant plasmids BSMV-VIGS.

2.2. RNA extraction and reverse transcription

Three new leaves from each wheat plant and a total of nine leaves from three individual plants from each treatment were harvested for RNA extraction. RNA was harvested on day 0 and every 7 days after BSMV-VIGS inoculation or in the control plants until 35 days post inoculation (dpi).

Total RNA was isolated using RNAiso Plus kit (TaKaRa, Dalian, China) according to the manufacture's instruction. Total RNA were dissolved in DEPC-treated ddH2O and stored at −80 °C. The integrity of RNA was assessed by 1.0% agarose gel electrophoresis. Total RNA was quantified with Agilent Bioanalyser 2000 CE (Agilent, USA). Total RNA was reverse-transcribed into cDNA in a 20 μl reaction using PrimeScript RT kits (TaKaRa, Dalian, China).

2.3. Quantitative real-time PCR (qRT-PCR)

qRT-PCR was performed using first-strand cDNA as template using 2 × SYBR® *Premix Ex Taq*™ II(TaKaRa, Dalian, China). The reaction contained 10 μl 2x SYBR, 0.4 μM each of both forward and reverse primers (Table 2), and 100 ng cDNA template. PCR was performed using Bio-Rad iQ5 (Biorad, USA) under the following conditions: initial denaturation for 30 s at 95 °C, followed by 40 cycles of 5 s at 95 °C, 20 s at 60 °C. *β-actin* was used as internal control. The $2^{-\triangle\triangle Ct}$ method used to calculate relative changes in gene expression determined from qRT-PCR experiments. Where $\triangle\triangle Ct=(C_{T,Target}-C_{T,Actin})$Time $x-(C_{T,Target}-C_{T,Actin})$ Time 0. Time x was any time point and Time 0 represented the 1 × expression of the target gene normalized to β-actin [15].

Table 1
The six unigenes that were identified from high-throughput RNA sequencing analysis (unpublished data) due to their differential expression upon vernalization.

Gene ID	Best match	Fold change (with vs. without vernalization)	P-Value
CL14010.contig2	*VER2*	−12.983	7.63E-28
CL12788.contig3	ZPR1	−12.745	3.93E-109
CL176.contig1	CDT1	−12.698	1.19E-53
Unigene16777	ATXR6-like	−11.871	3.13E-16
CL8746.contig1	*WCO1*(B-box)	−11.751	7.62E-18
Unigene10196	*WRKY*	−11,.337	8.22E-29

2.4. BSMV plasmid and and construction of recombinant vector

BSMV containes a tripartite genome of α, β and γ which has been sequenced. The three genomes were cloned into a transcription vector. The BSMV constructs were than transcribed into RNA in vitro using T7 RNA polymerase. Every RNA has a cap structure at the 5 'end [16] and a tRNA-like structure at the 3′ end [17]. A large number of polypeptide which may be the component of the virus replicase (RNA-dependent RNA polymerase, RdRp) might be encoded by the 5' end of the α and β ORF. Equal amount of the three RNA were inoculated into wheat leaf. Target genes were cloned into BSMV-γ through the *Nhe*I restriction site by molecular cloning.

2.5. BSMV in-vitro transcription and inoculation

The recombinant plasmids of BSMV:00, BSMV: PDS, and BSMV plasmids containing the six unigenes (with BSMV-α or BSMV-γ backbone) were linearized via *Mlu*I digestion. The recombinant plasmids with BSMV-β backbone were linearized by *Spe*I, at 37 °C for 6 h. The linearizing products were purified and were transcribed in vitro using RiboMAXTM Large Scale RNA Production System –T7 and Ribo m7G Cap Analog (Promega, USA).

Plants with consistent growth were chosen at the two leaves stage. BSMV RNA synthesized in vitro was inoculated onto the second leave (10 μl on each leaf). The leaves were kept moisturized by spraying with RNase-free water. The seedlings were then covered by preservative film for 3 days and cultured in illumination incubator (14 h photoperiod at 25 °C). The control plants CK received no treatment.

2.6. Detection of target gene expression after VIGS

Total RNA was isolated every 7 days after inoculation until the photobleaching phenotype of BSMV:PDS disappeared (around 35 dpi). Expression of target genes was analyzed by qRT-PCR.

Table 2
Primers used for qPCR analysis.

Gene ID	Primers
Unigene10196	GTCTCTCTTTTGCTACGGCAGTTT
	GCAGCATAGGGACTGCAACTATG
CL12788.contig3	ACGAAGCCGCTAACGCATC
	ATTGTGAAAATATGTGAGGCTCTGG
Unigene16777	ATGATGGTCAGGTCCTTGATGTG
	TCTTCAGCAACGACCTCACCTA
CL176.contig1	CTGAACCTGGTCCGCATAGC
	CCACCTCTAAAAACGGTCAAGC
CL8746.contig1	GATGCTGGACTGGACCGTATTG
	GTACCTTCACAGTCTGCAATTGTGA
CL14010.contig2	TAATGGCATCCGCACAAAGGTT
	CCCAAAGAACCCGACGATGCT
PDS	CTGACCAGAGTAAAGCAAAGATT
	TCGCCGGCCAGATAGAA
β-actin	TTTGAAGAGTCGGTGAAGGG
	TTTCATACAGCAGGCAAGCA

2.7. Measurement of chlorophyll content

Chlorophyll content was measured by Chlorophyll Meter SPAD (Soil and Plant Analyzer Development)-502 (Konica Minolta sensing Inc., Japan). SPAD measurements were performed on leaves along the direction of the vein at the base, middle and tip of each leaf and the three values was averaged. The SPAD values obtained from the measurement were then converted to chlorophyll content. Three new leaves from each wheat plant and a total of nine leaves from three individual plants were measured for each treatment.

3. Results

3.1. Phenotype of BSMV: PDS recombinant vector after inoculation

BSMV vectors carrying a 263-bp coding sequence of *PDS* gene (BSMV:PDS) was used as a positive control to examine the silencing effect of VIGS (Fig. 1). At 5 dpi, the second leaves began to develop the chlorotic phenotype; the photobleaching phenotype was obvious at 7 dpi (Fig. 1A, a), indicating that the virus has successfully infected the plant leaves. The base of the stem of new leaves showed albino symptoms, and other leaves appeared green with yellow bleach spots at 14 dpi (Fig. 1A, b). The photobleaching phenotype of new leaves remained at 21 dpi (Fig. 1A, c). By 28 dpi, the phenotype gradually subsided (Fig. 1A, d and e), but there were still macula on leaves. Fig. 1B shows the enlarged image of the infected and the control leaf at 14 dpi. Blank control leaf was green as normal, negative control BSMV:00 had linear yellow spots, while leaf inoculated with BSMV:PDS showed significant bleaching leaf spot.

Consistent with the chlorosis phenotype in BSMV:PDS infected plants, chlorophyll content exhibited a similar trend (Fig. 2A). Chlorophyll content first decreased significantly at 3 leaves stage (3 L, which also correlates to 7 dpi) reached its minimum at 14 dpi, then gradually increased and stabilized from 5 L to 8 L (Fig. 2A). Chlorophyll content in the negative control BSMV:00 showed a similar trend, although to a much lesser degree.

3.2. The expression of target gene after BSMV recombinant vector inoculation

qRT-PCR were employed to verify whether the chlorosis phenotype was due to endogenous *PDS* silencing. RNA was extracted every 7 days after BSMV recombinant vector inoculation, and target gene expression was detected by qRT-PCR. BSMV:PDS was used as positive control, BSMV:00 as negative control.

The expression of *PDS* in BSMV:00 were stable from 0 dpi until 35 dpi (Fig. 2B). However, PDS rapidly decreased in BSMV:PDS at 7 dpi, was almost undetectable at 14 and 21 dpi, and then slowly rise at 28 dpi. PDS expression recovered to about half of the original level at 35 dpi. The trend of PDS expression change was consistent with the change of chlorophyll content (Fig. 2A). Therefore, our result suggested that the photobleaching phenotype of these plants were the result of the silencing of endogenous *PDS*.

The expression of unigenes CL14010, CL12788, CL176, Unigene 16777 and CL8746 showed similar expression pattern after BSMV VIGS inoculation as BSMV:PDS (Fig. 3A–E). Their expression rapidly decreased at 7 dpi, and remained at very low level at 14 and 21 dpi, and started to increase at 28 and 35 dpi. For Unigene 10196, its expression remained nearly undetectable from 7 dpi until 35 dpi (Fig. 3F).

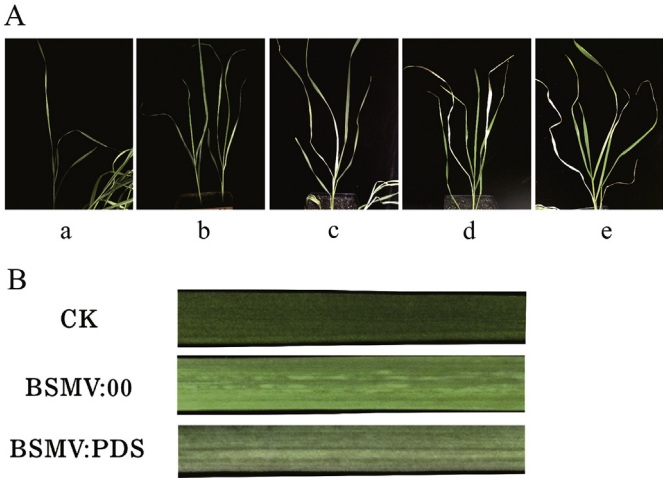

Fig. 1. Wheat plants inoculated with BSMV:PDS. A: leaf phenotype from 7 to 35 dpi. (a) 7 dpi. (b)14 dpi. (c)21 dpi. (d)28 dpi. (e)35 dpi. B: magnified image of leaf at 14 dpi after BSMV:PDS inoculation showing chlorotic phenotype.

3.3. Effect of BSMV VIGS of the six unigenes on proceeding of spike differentiation

The duration from germination to spike differentiation in wheat is determined by each variety's growth characteristics and vernalization sensitivity. If cold condition is not sufficient, single ridge (SR) and double ridge (DR) stage will prolong, which leads to heading delay. Thus, the time from germination to DR stage is an important indicator and measurement for the vernalization process in wheat.

The germination to DR stage duration was 85.0 ± 0.73 days in the control (CK), and was 83.8 ± 0.60 and 84.3 ± 0.80 respectively in BSMV:00 and BSMV:PDS. There was no statistical difference between the three lines ($p > 0.05$). However, BSMV mediated VIGS of the six unigenes resulted in shorter period from germination to DR stage, ranging from 59.8 ± 0.60 to 65.8 ± 0.48 days (Table 3). Silencing of the Unigene CL14010 resulted in the shortest duration, 59.8 ± 0.60 days. The differences between the spike differentiation of the six unigenes and that of the control was significantly different ($p < 0.05$). There were no significant difference among BSMV:CL12788, BSMV:CL18746, BSMV:Unigene10196, BSMV:CL8746 and BSMV:CL176.

4. Discussion

A proper control for each VIGS experiment is critical to monitor the silencing effect. PDS gene is often used as a positive control as its silencing results in a rapidly visible photobleaching phenotype [18]. In our experiment, severe albino phenotype was observed after BSMV:PDS inoculation at the fourth leaves stage where as the chlorophyll content was also minimum. The albino area of new leaf were reduced after fifth leaves, correlated with an increase in chlorophyll content. qPCR result confirmed that the expression of *PDS* rapidly decreased after BSMV:PDS inoculation, reached its minimum at 14 dpi, then slowly increased. The phenotypic and expression results indicated that the endogenous *PDS* was silenced successfully by BSMV recombinant vector carrying the *PDS* fragment. However, infection by the negative control, BSMV:00 also produced linear yellow spots initially after viral infection, indicating that BSMV itself also caused yellow spot symptom. Consistently, the chlorophyll content also showed a slight decrease after BSMV:00 inoculation. The symptoms triggered by BSMV:00 virus infection gradually disappeared with the activation of plant defense response mechanism, corresponding to increased and stable chlorophyll content.

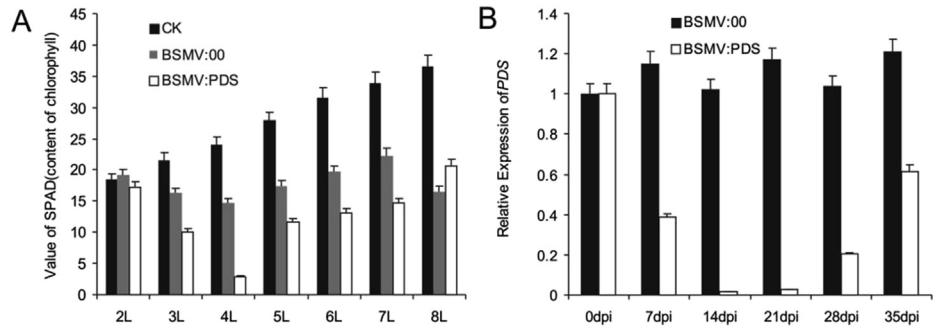

Fig. 2. Chlorophyll content and relative expression in wheat plant from 0 to 35dpi with BSMV:PDS. A: Chlorophyll content from two leaves stage (2L) to 8L after inoculation. B: The relative expression of *PDS* after inoculation by qRT-PCR analysis, β-actin was used as an internal control.

qPCR analysis showed that expression of all of the six target unigenes decreased to various degree after inoculation of BSMV recombinant vector carrying the target fragment. This result suggested that silencing of the endogenous target genes were indeed induced successfully. Silencing of the six unigenes influenced the proceeding of spike differentiation, and the growth period from germination to DR stage was greatly shortened. As a key stage of development, DR stage marks the initiation of spike differentiation and the end of vegetative growth [19].

We identified a number of vernalization-responsive unigenes from our vernalization transcriptome analysis (unpublished data). In this study, we selected six genes that were most significantly down-regulated upon vernalization to do further functional characterization. Our result showed that silencing or down-regulation of these six genes promoted the proceeding of spike differentiation, indicated that these genes might inhibit floral development either directly or indirectly. Silencing these genes earlier in development can partially bypass the requirement for vernalization.

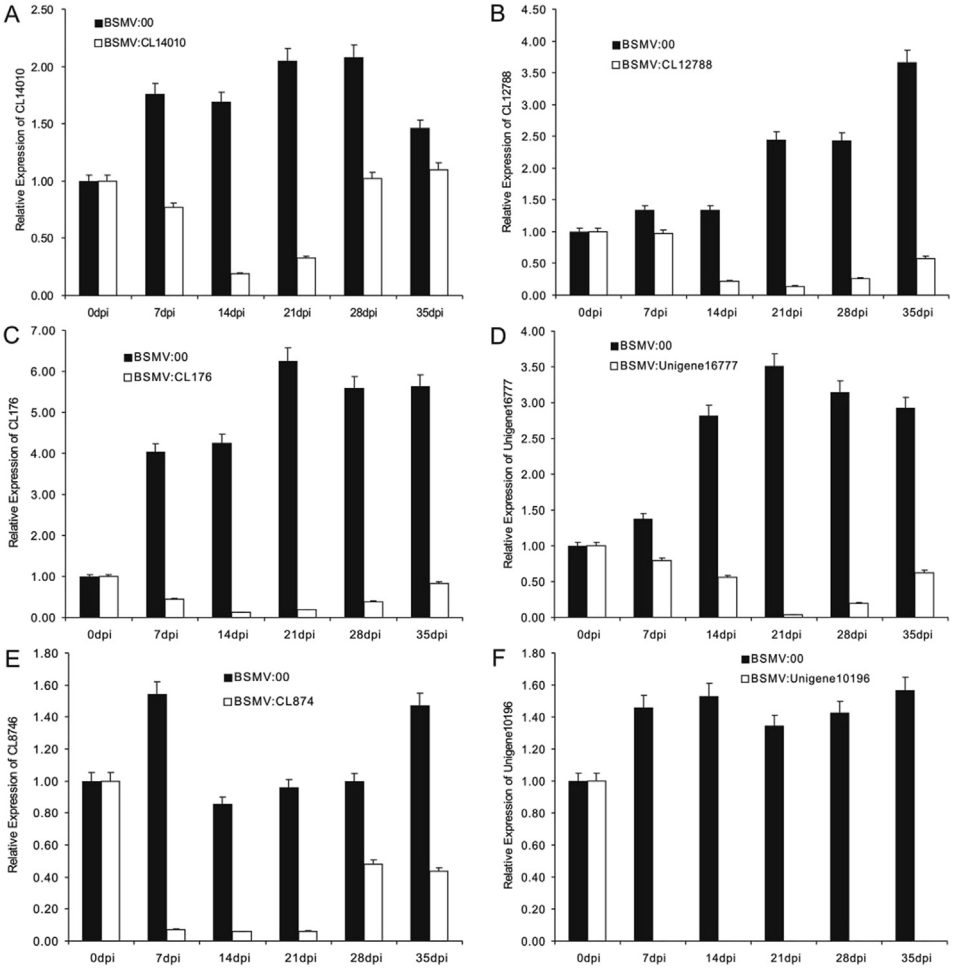

Fig. 3. Expression analysis of the six target genes after BSMV recombinant vector inoculation by qRT-PCR analysis. β-actin was used as an internal control (A) CL14010. (B) CL12788. (C) CL176. (D) Unigene 16777. (E) CL8746. (F)Unigene10196.

Table 3
The duration from seedling to double ridge (DR) after BSMV VIGS of the six unigenes.

BSMV recombinant vector	Intervals from germination to DR stage (days)
CK	85.0 ± 0.73^{a}
BSMV:00	83.8 ± 0.60^{a}
BSMV:PDS	84.3 ± 0.80^{a}
BSMV:CL14010	59.8 ± 0.60^{d}
BSMV:CL12788	65.0 ± 0.58^{bc}
BSMV:CL176	64.8 ± 0.70^{bc}
BSMV:Unigene16777	64.3 ± 0.42^{c}
BSMV:CL8746	65.8 ± 0.48^{bc}
BSMV:Unigene10196	65.2 ± 0.31^{bc}

Note: Data expressed as mean ± standard deviation (N = 5), different lower case letters indicated significant difference, $P \leq 0.05$.

CL14010.contig2 was best matched with the vernalization-related gene *VER2*, an important lectin with a jacalin-like domain in the C-terminal. VER2 can be induced by both jasmonic acid (JA) and vernalization in wheat [20]. Xing et al. [21] reported that the phosphorylation of VER2 is associated with O-linked β-N-acetyl-glucosamine (O-GlcNAc) signaling during vernalization. Our result that CL14010.contig2 was down-regulated during vernalization, indicating that it may be a repressor for flowering (Table 1). VIGS silencing of CL14010.contig2 shortened the development time from germination to flowering, further confirmed its essential function in the vernalization process.

CL12788.contig3 was predicted to encode a zinc finger protein ZPR1-like protein. Zinc finger protein ZPR1 is an evolutionarily conserved protein expressed in both the cytoplasm and nucleoplasm. ZPR1 subcellular distribution changes during cell cycle, indicating its important role in cell cycle progression and cell proliferation [22,23]. ZPR1 deficient leads to blockage of S phase progression and results in cell cycle arrest at G1/G2 phase [22–24]. Transcription deficient of ZPR1 also results in a decrease of histone gene expression [22–24].

CL176.contig1 is highly similar with the CDT1-like protein (Cdc10-dependent transcript 1) in *Brachypodium distachyon*. CDT1 was a key regulator for DNA replication and is required for completion of mitosis and the maintenance of genome stability in eukaryotic cells [25]. It is possible that the down-regulation of CL176.contig1 during vernalization (Table 1) is because the vernalization condition is unfavorable for DNA replication and cell proliferation. However, it is also possible that CL176.contig1 is directly involved in development regulation.

Unigene16777 was similar with the histone-lysine N-methyltransferase ATXR6-like. Histone lysine methylation is mainly involved in heterochromatin formation and transcriptional regulation of gene imprinting. The role of histone-lysine methylation in gene expression regulation has become a hot topic in epigenetics studies. In Arabidopsis, the decreased expression *FLC* by vernalization correlates with the changes of histone modifications in *FLC* chromosome [1]. A long-term silencing effect might be created through both histone methylation and DNA methylation systems, and transmitted by DNA replication. It is possible that Unigene16777 function as a lysine methyltransferase and is involved in transcription regulations of genes that are involved in vernalization process.

CL8746.contig1 was best match with *WCO1* (Wheat CONSTANS 1). Flowering time in plant is controlled by circadian clock signals integrates with optical signals. This process is jointly determined by the abundance of *CO* transcripts as well as the stability of CO protein. *CO* directly induce the expression of FLOWERING LOCUS T (*FT*), whose transcript is specifically induced under long days [26], and promote flowering in Arabidopsis [27]. The down-regulated expression of CL8746.contig1 during vernalization might be due to the vernalization treatment, which was conducted under darkness (Table 1). The down-regulation of CL8746.contig1 by dark treatment might in turn activate genes that promote flowering development.

Unigene10196 was similar with the transcription factor WRKY 63 (*WRKY63*) in *Oryza sativa*. *WRKY* transcription factors are a large gene family which includes 74 members in Arabidopsis and about 100 members in rice [28]. *WRKY* is an integral part of the signal network in many plants, and plays an important role in the regulation of seed development and senility [29]. It has been reported that *WRKY* may be involved in cold hardening in wheat [30]. There are close relations between *AtWRKY6* and senility and defense response in Arabidopsis [31]. *WRKY* gene encoded a class of transcriptional repressor of GA signaling pathway in rice [32], a pathway involved in flowering. It is possible that down regulation of Unigene10196 activated the GA pathway thus promoted flowering.

In conclusion, we successfully suppressed expression of six vernalization-related unigenes by VIGS. As candidate genes for vernalization regulation, silence or down-regulation of the six unigenes promoted flower development. Whether these six unigenes were directly involved in the vernalization regulation or through interacting with other key regulators still need further validation.

Conflict of interest

The authors declare no conflict of interest.

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References

[1] R. Bastow, J.S. Mylne, C. Lister, Z. Lippman, R.A. Martienssen, C. Dean, Vernalization requires epigenetic silencing of FLC by histone methylation, Nature 427 (2004) 164–167.
[2] S. Sung, R.M. Amasino, Remembering winter: toward a molecular understanding of vernalization, Annu. Rev. Plant Biol. 56 (2005) 491–508.
[3] S.D. Michaels, R.M. Amasino, FLOWERING LOCUS C encodes a novel MADS domain protein that acts as a repressor of flowering, Plant Cell. 11 (1999) 949–956.
[4] B. Trevaskis, M.N. Hemming, E.S. Dennis, W.J. Peacock, The molecular basis of vernalization-induced flowering in cereals, Trends Genet. 12 (2007) 352–357.
[5] M.T. Ruiz, O. Voinnet, D.C. Baulcombe, Initiation and maintenance of virus-induced gene silencing, Plant Cell. 10 (1998) 937–946.
[6] G.D. Constantin, B.N. Krath, S.A. MacFarlane, M. Nicolaisen, I. Elisabeth Johansen, O.S. Lund, Virus-induced gene silencing as a tool for functional genomics in a legume species, Plant J. 40 (2004) 622–631.
[7] T. Dalmay, A. Hamilton, E. Mueller, D.C. Baulcombe, Potato virus X amplicons in arabidopsis mediate genetic and epigenetic gene silencing, Plant Cell. 12 (2000) 369–379.
[8] Y. Liu, M. Schiff, S. Dinesh-Kumar, Virus-induced gene silencing in tomato, Plant J. 31 (2002) 777–786.
[9] S. Holzberg, P. Brosio, C. Gross, G.P. Pogue, Barley stripe mosaic virus-induced gene silencing in a monocot plant, Plant J. 30 (2002) 315–327.
[10] S.R. Scofield, L. Huang, A.S. Brandt, B.S. Gill, Development of a virus-induced gene-silencing system for hexaploid wheat and its use in functional analysis of the Lr21-mediated leaf rust resistance pathway, Plant Physiol. 138 (2005) 2165–2173.
[11] X.S. Ding, W.L. Schneider, S.R. Chaluvadi, M.R. Mian, R.S. Nelson, Characterization of a brome mosaic virus strain and its use as a vector for gene silencing in monocotyledonous hosts, Mol. Plant Microbe Interact. 19 (2006) 1229–1239.
[12] F. Ratcliff, B.D. Harrison, D.C. Baulcombe, A similarity between viral defense and gene silencing in plants, Science 276 (1997) 1558–1560.

[13] P. Brodersen, O. Voinnet, The diversity of RNA silencing pathways in plants, Trends Genet. 22 (2006) 268–280.
[14] T.M. Burch-Smith, J.C. Anderson, G.B. Martin, S.P. Dinesh-Kumar, Applications and advantages of virus-induced gene silencing for gene function studies in plants, Plant J. 39 (2004) 734–746.
[15] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods 25 (2001) 402–408.
[16] A. Agranovsky, V. Dolja, V. Kagramanova, J. Atabekov, The presence of a cap structure at the 5′-end of barley stripe mosaic virus RNA, Virology 95 (1979) 208–210.
[17] Y.V. Kozlov, V. Rupasov, D. Adyshev, S. Belgelarskaya, A. Agranovsky, A. Mankin, S.Y. Morozov, V. Dolja, J. Atabekov, Nucleotide sequence of the 3′-terminal tRNA-like structure in barley stripe mosaic virus genome, Nucleic Acids Res. 12 (1984) 4001–4009.
[18] M. Kumagai, J. Donson, G. Della-Cioppa, D. Harvey, K. Hanley, L. Grill, Cytoplasmic inhibition of carotenoid biosynthesis with virus-derived RNA, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 1679–1683.
[19] E. Kirby, Ear development in spring wheat, J. Agric. Sci. 82 (1974) 437–447.
[20] W.-d Yong, Y.-y Xu, W.-z Xu, X. Wang, N. Li, J.-s Wu, T.-b Liang, K. Chong, Z.-h Xu, K.-h Tan, Vernalization-induced flowering in wheat is mediated by a lectin-like gene VER2, Planta 217 (2003) 261–270.
[21] L. Xing, J. Li, Y. Xu, Z. Xu, K. Chong, Phosphorylation modification of wheat lectin VER2 is associated with vernalization-induced O-GlcNAc signaling and intracellular motility, PLoS One 4 (2009) e4854.
[22] L. Gangwani, Deficiency of the zinc Finger protein ZPR1 cause defects in transcription and cell cycle progression, J. Biol. Chem. 281 (2006) 40330–40340.
[23] X. Ye, Y. Wei, G. Nalepa, J.W. Harper, The cyclin E/Cdk2 substrate p220NPAT is required for S-phase entry, histone gene expression, and Cajal body maintenance in human somatic cells, Mol. Cell. Biol. 23 (2003) 8586–8600.
[24] G. Gao, A.P. Bracken, K. Burkard, D. Pasini, M. Classon, C. Attwooll, M. Sagara, T. Imai, K. Helin, J. Zhao, NPAT expression is regulated by E2F and is essential for cell cycle progression, Mol. Cell. Biol. 23 (2003) 2821–2833.
[25] D. Varma, S. Chandrasekaran, L.J. Sundin, K.T. Reidy, X. Wan, D.A. Chasse, K.R. Nevis, J.G. DeLuca, E. Salmon, J.G. Cook, Recruitment of the human Cdt1 replication licensing protein by the loop domain of Hec1 is required for stable kinetochore-microtubule attachment, Nat. Cell. Biol. 14 (2012) 593–603.
[26] A. Samach, H. Onouchi, S.E. Gold, G.S. Ditta, Z. Schwarz-Sommer, M.F. Yanofsky, G. Coupland, Distinct roles of CONSTANS target genes in reproductive development of Arabidopsis, Science 288 (2000) 1613–1616.
[27] R. Hayama, G. Coupland, The molecular basis of diversity in the photoperiodic flowering responses of arabidopsis and rice, Plant Physiol. 135 (2004) 677–684.
[28] S. Berri, P. Abbruscato, O. Faivre-Rampant, A.C. Brasileiro, I. Fumasoni, K. Satoh, S. Kikuchi, L. Mizzi, P. Morandini, M.E. Pè, Characterization of WRKY co-regulatory networks in rice and Arabidopsis, BMC, Plant Biol. 9 (2009) 120.
[29] P.J. Rushton, I.E. Somssich, P. Ringler, Q.J. Shen, WRKY transcription factors, Trends Plant Sci. 15 (2010) 247–258.
[30] V. Talanova, A. Titov, L. Topchieva, I. Malysheva, Y.V. Venzhik, S. Frolova, Expression of WRKY transcription factor and stress protein genes in wheat plants during cold hardening and ABA treatment, Russ. J. Plant Physiol. 56 (2009) 702–708.
[31] S. Robatzek, I.E. Somssich, A new member of the Arabidopsis WRKY transcription factor family, AtWRKY6, is associated with both senescence- and defence-related processes, Plant J. 28 (2001) 123–133.
[32] Z.L. Zhang, Z. Xie, X. Zou, J. Casaretto, T.H. Ho, Q.J. Shen, A rice WRKY gene encodes a transcriptional repressor of the gibberellin signaling pathway in aleurone cells, Plant Physiol. 134 (2004) 1500–1513.