生物工程学报
journals.im.ac.cn
cjb@im.ac.cn

Chin J Biotech 2010, November 25; 26(11): 1539−1545
Chinese Journal of Biotechnology ISSN 1000-3061

三个小麦春化基因的时空表达特性分析

袁秀云[1,2]，李永春[1]，孟凡荣[3]，王潇[1]，尹钧[1]

1 河南农业大学 国家小麦工程技术研究中心，郑州 450002
2 郑州师范高等专科学校，郑州 450044
3 河南农业大学生命科学学院，郑州 450002

摘　要：为明确小麦春化基因的时空表达特性，以中国春和洛旱 2 号小麦品种为试验材料，利用半定量 RT-PCR 技术，分析了 3 个春化基因 *VERNALIZATION1* (*VRN1*)、*VRN2* 和 *VRN3* 的时空表达特性。结果表明，*VRN1* 在中国春的三叶期叶片和根、灌浆期的茎秆和旗叶、花药、胚珠和发育的种子中均有不同程度的表达。在开花前，表达水平呈上升趋势，而花后呈降低的趋势，在干种子和萌发种子的胚芽中没有检测到表达；在洛旱 2 号中，除了在三叶期的叶片和根中没有检测到表达外，*VRN1* 的表达特性与中国春有相同的趋势。*VRN2* 只在三叶期的叶片和萌发种子的胚芽中表达，在其他检测的组织中没有表达；*VRN3* 的表达与 *VRN1* 的时空表达特性相似，但在根中未检测到表达。这一结果为进一步分析普通小麦品种春化发育的分子调控机理提供了重要信息。

关键词：小麦，春化基因，时空表达，半定量 RT-PCR

Spatiotemporal expression patterns of three vernalization genes in wheat

Xiuyun Yuan[1,2], Yongchun Li[1], Fanrong Meng[3], Xiao Wang[1], and Jun Yin[1]

1 *National Engineering Research Center for Wheat*, *Henan Agricultural University*, *Zhengzhou* 450002, *China*
2 *Zhengzhou Normal College*, *Zhengzhou* 450044, *China*
3 *College of Life Science*, *Henan Agricultural University*, *Zhengzhou* 450002, *China*

Abstract: To identify spatiotemporal expression patterns of vernalization genes in common wheat, we analyzed expression characteristics of several vernalization genes (*VRN1*, *VRN2* and *VRN3*) in the wheat cultivars ‘Chinese spring’ and ‘Luohan 2’ by RT-PCR. The *VRN1* gene was expressed at different levels in the leaves and roots at the 3-leaf stage, stems, flag leaves at the grain-filling stage, anthers, ovules, and developing seeds in ‘Chinese spring’. Expression of *VRN1* increased before flowering date, then decreased after flowering time. Expression of *VRN1* was not detected in dry seeds or seeds germination. Expression patterns of *VRN1* in ‘Luohan 2’ were similar to those in ‘Chinese spring’, except that it was not expressed in roots or in the leaves at the 3-leaf stage in ‘Luohan 2’. Expression of *VRN2* was only detected in the leaves at the 3-leaf stage and in the embryo buds during seeds germination. The spatiotemporal expression of *VRN3* was similar to that of *VRN1*, except that *VRN3* was not expressed in roots. These results improved our understanding of the molecular regulation of vernalization genes in common wheat.

Keywords: common wheat, vernalization gene, spatiotemporal expression, semi-quantitative RT-PCR

Received: December 30, 2009; **Accepted:** July 12, 2010
Supported by: National Natural Science Foundation of China (No. 30671261).
Corresponding author: Jun Yin. Tel: +86-371-63558203; E-mail: xmzxyj@126.com
国家自然科学基金（No. 30671261）资助。

Vernalization is defined as the requirement for prolonged exposure to low temperatures to accelerate flowering. It is critical for floral induction in vernalization-requiring plants. Several genes and the interactions among them are responsible for flowering in the vernalization response[1-2]. *VERNALIZATION1* (*VRN1*), *VRN2* and *VRN3* are the main genes involved in the vernalization response in common wheat (*Triticum aestivum* L.)[3]. The *VRN1* gene promotes flowering. It shows high similarity to the meristem identity gene *AP1* in *Arabidopsis*[4], which encodes a MADS-box transcription factor that is essential for the initiation of the transition from vegetative to reproductive apices[4-7]. The expression of *VRN1* can be induced by low temperatures in the leaves and apex tissues. The *VRN-1* gene has three orthologous genes located in the middle of the long arms of chromosomes 5A, 5B, and 5D in common wheat[8-12]. *VRN2* represses flowering, and encodes a protein with a zinc finger motif and a CCT (CONSTANS, CONSTANS-LIKE, and TIMING OF CAB1-1) domain[13]. The expression of *VRN2* can be inhibited by low temperatures and short-days (SD). *VRN3*, a homolog of the *FLOWERING LOCUS T* gene (*FT*) of *Arabidopsis*, is located on the short arm of chromosome 7B in diploid wheat *Triticum monococcum* L.[14-15]. *VRN3* is a mobile promoter of flowering[16-18]. Its protein is produced in leaves when wheat is exposed to the long days and is transported to the shoot apex where it up-regulates expression of *VRN1* and promotes floral development through interacting with TaFDL2, a bZIP transcription factor[19]. Although much progress has been made on uncovering the genes involved in vernalization, the mechanisms by which these genes are regulated remain largely unknown[20].

Previous studies have mainly focused on the expression of vernalization genes in leaves and apex tissues in wheat. Systematical analyses of spatiotemporal expression patterns have not been reported. We systematically analyzed the expression patterns of three *VRN* genes in roots, stems, leaves, flowers, and seed tissues during seeds development and germination. The results will increase our understanding of gene expression during vernalization and its molecular regulatory mechanisms in common wheat.

1 Materials and methods

1.1 Plant materials

Seeds of 'Chinese Spring' (spring wheat) and 'Luohan 2' (winter wheat) were germinated in Petri dishes with water at room temperature. The embryos and endosperm tissues of germinating seeds were collected at various time points (6, 12, 24, 48, 72, and 120 h). Leaves and roots were harvested when plants reached the 3-leaf stage, and were immediately frozen in liquid nitrogen and stored at −80°C until analysis. In addition, mature seeds, stems, flag leaves, anthers, ovules and developing seeds were collected at 5, 10, 15, 20, and 30 days after pollination (DAP) from field-grown wheat varieties 'Chinese Spring' and 'Luohan 2' for analysis of spatiotemporal expression patterns.

1.2 RNA Extraction

Total RNA was extracted from leaves, roots and stems using the TRIzol method (Invitrogen) according to the manufacturer's instructions. Total RNA was extracted from anthers, ovules, embryos and endosperm tissues using the method described by Zhu Yun *et al*[21]. The integrity of RNA samples was assessed by agarose gel (0.8%) electrophoresis. Concentration and purity of RNA were determined from the A_{260}/A_{280} ratio using a UC800 nucleic acid-protein analyzer (Beckman Co., USA).

Equal amounts (2 μg) of RNA were reverse transcribed into cDNA in a 20 μL reaction mixture containing 50 mmol/L Tris-HCl (pH 8.3), 75 mmol/L $MgCl_2$, 10 mmol/L DTT, 50 μmol/L dNTP, 200 U M-MLV reverse transcriptase (TaKaRa) and 50 pmol Oligo-dT(15) nucleotides. The mixture was incubated at 42°C for 60 min and finally denatured at 95°C for 5 min.

1.3 RT-PCR analysis of *VRN1*, *VRN2* and *VRN3*

Specific primers were designed based on the sequences of wheat vernalization genes in GenBank. In addition, primers specific to the wheat actin gene were also designed as an endogenous control (Table 1). RT-PCR was performed using *Taq* DNA polymerase (TaKaRa). The PCR program was as follows: 5 min denaturation at 94°C, followed by 28 cycles of a denaturation step at 94°C, an annealing step at 53°C−60°C, and an extension step at 72°C. Each step was 50 s long, and the final extension step was at 72°C for 7 min. The PCR products of amplification were separated using 1.5% agarose gels and stained with ethidium bromide.

2 Results

2.1 Expression patterns of three VRN genes in wheat tissues

The spatial expression patterns of *VRN1*, *VRN2* and

VRN3 were characterized using semi-quantitative RT-PCR. The vernalization genes were expressed differently in various tissues of the wheat cultivars 'Chinese Spring' and 'Luohan 2'. In 'Chinese Spring', low levels of *VRN-A1* and *VRN-D1* transcripts were detected in leaves and roots at the 3-leaf stage, while transcripts of *VRN-B1* were not detected in these tissues. High levels of *VRN-A1*, *VRN-B1* and *VRN-D1* transcripts were detected in the stems and flag leaves at the grain-filling stage, anthers, ovule tissues, and transcript abundance tended to decrease from stems to ovules. Transcripts of *VRN2* were detected only in the leaves at the 3-leaf stage, but not in other tissues (including roots, stems, flag leaves at the grain-filling stage, anthers and ovules). *VRN3* transcripts were not detected in the roots at the 3-leaf stage, but were detected with increasing levels in leaves at the 3-leaf stage, stems,and flag leaves at the grain-filling stage (Fig. 1). The level of expression tended to decrease from anthers to ovules.

In 'Luohan 2', expressions of *VRN-A1*, *VRN-B1* and *VRN-D1* were not detected in the leaves or roots at the 3-leaf stage. However, these genes were expressed at high levels in the stems, flag leaves at the grain-filling stage, anthers and ovule tissues.

The pattern of *VRN2* transcription in 'Luohan 2' was similar to that in 'Chinese Spring'. We did not detect *VRN3* transcripts in leaves or roots at the 3-leaf stage. However, high levels of *VRN3* transcripts were observed in the stems and flag leaves at the grain-filling stage, and lower levels in anthers and ovule tissues (Fig. 1).

Table 1 RT-PCR primers used to detect expression of VRN1, VRN2, and VRN3 in common wheat

Genes	Primer sequence (5′−3′)	Product size (bp)	Annealing temperature (°C)	Accession No.
VRN-A1	CCACCGAGTCATGTATGG ACA GAGCTGGTTTGAGGCTGAGTT	403	53.3	AY747600 AY747601
VRN-B1	ACCGAGTCATGTATGGACAAAAT TCCTCTGCCCTCTCTCCTGA	488	53.6	AY747604 AY747603
VRN-D1	CTGAAGGCGAAGGTTGAGACA CGCTGGATGAATGCTGGTAGC	348	54.8	AY747606 AY747597
VRN2	CCATGTCATGCGGTTTGTG CGCCTCTTCCTCTTCTCCC	475	56.5	AY485969
VRN3	ATGGCCGGTAGGGATAGGG GCCGTGGGTAGATCAATTGTACAT	546	59.7	DQ890162 CJ509289
Actin	GTTCCAATCTATGAGGGATACACG C GAACCTCCACTGAGAACAACATTACC	422	58.5	AB181991

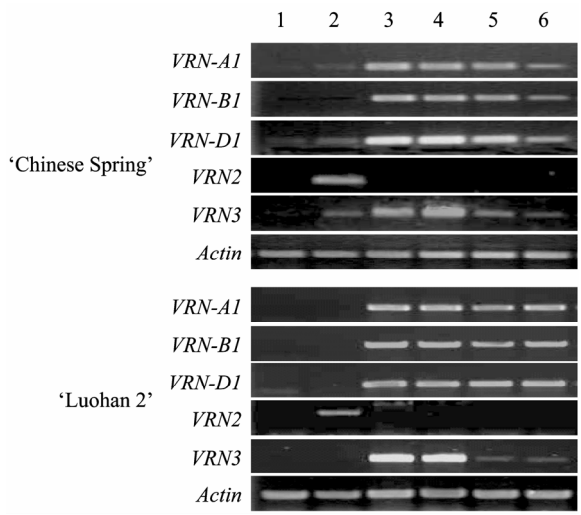

Fig. 1 Expression of vernalization genes in various tissues. 1: roots at the 3-leaf stage; 2: leaves at the 3-leaf stage; 3: stems at the grain-filling stage; 4: flag leaves at the grain-filling stage; 5: anthers; 6: ovules.

2.2 Expression patterns of VRN genes during seeds development

In 'Chinese spring', the expressions of *VRN-A1*, *VRN-B1* and *VRN-D1* gradually decreased during seeds development (from 5 DAP to 30 DAP). However, *VRN2* expression was not detected in seeds of 'Chinese Spring'. The expression pattern of *VRN3* was the same as that of *VRN1*. Expression patterns of *VRN1*, *VRN2,* and *VRN3* during seeds development in 'Luohan 2' were similar to those in 'Chinese Spring' (Fig. 2)

2.3 Expression patterns of vernalization genes during seeds germination

During seeds germination of 'Chinese Spring', *VRN-A1*, *VRN-B1*, *VRN-D1,* and *VRN3* were not expressed in dry seeds, germinating embryos or embryo buds, or endosperm at any of the tested times. No *VRN2* transcripts were detected in the seeds, germinating

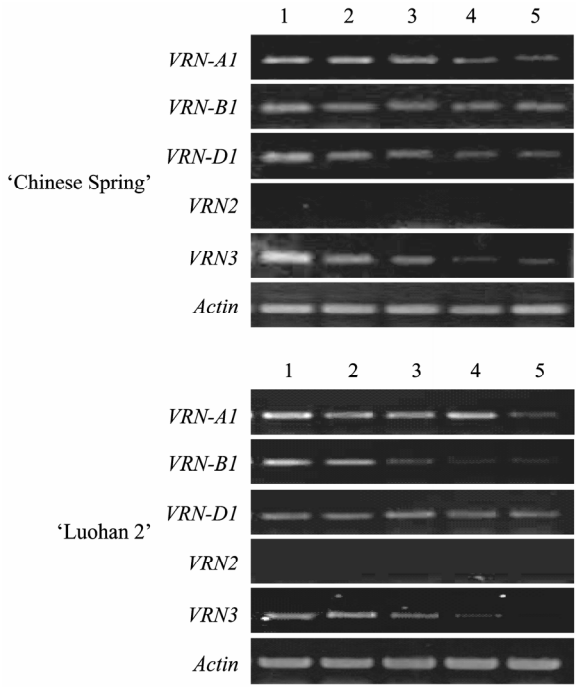

Fig. 2 Expression of vernalization genes during seeds development. 1: seed at five days after pollination (DAP); 2: seed at 10 DAP; 3: seed at 25 DAP; 4: seed at 20 DAP; 5: seed at 30 DAP.

embryos, or endosperms at 6, 12, 24, or 48 h. However, *VRN2* transcripts were detected in the embryo buds of germinating seeds at 72 and 120 h, with higher levels of transcripts detected at 120 h than at 72 h (Fig. 3). *VRN1* and *VRN3* were not transcribed during seeds germination. Transcription of VRN2 was initiated in the embryo buds, and transcript levels increased with the embryo buds developing.

During seeds germination of 'Luohan 2', the expression profiles of *VRN-A1*, *VRN-B1*, *VRN-D1* and *VRN3* were similar to those in 'Chinese Spring'. *VRN2* transcripts were detected only in the embryo buds of germinating seeds at 120 h, but not in seeds or any of the germinating embryo tissues at 12, 24, 48, or 72 h (Fig. 4).

3 Discussion

3.1 Relationships between expression of VRN genes and *VRN1* alleles in wheat

VRN1 and *VRN2* are normally expressed in leaves and apex tissues[4,13,22]. Our results showed that *VRN1*

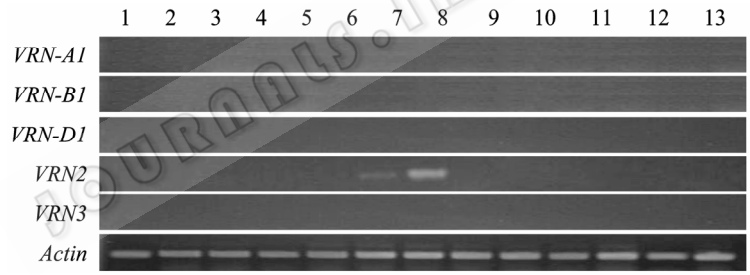

Fig. 3 Expression characteristics of vernalization genes during seed germination of Chinese spring.1: seeds; 2: embryos at 6 h germination; 3: embryos at 12 h germination; 4: embryos at 24 h germination; 5: embryos at 48 h germination; 6: embryo buds at 72 h germination; 7: embryo buds at 120 h of germination; 8: endosperms at 6 h germination; 9: endosperms at 12 h germination; 10: endosperms at 24 h germination; 11: endosperms at 48 h germination; 12: endosperms at 72 h germination; 13: endosperms at 120 h germination.

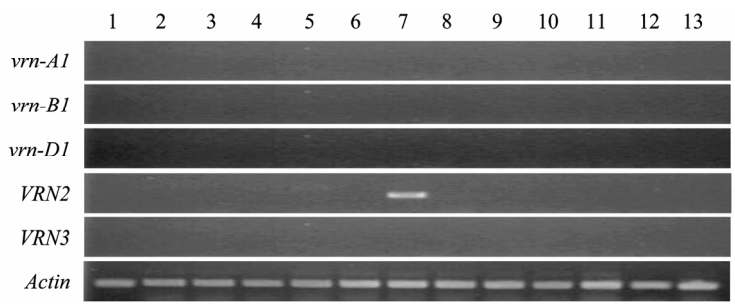

Fig. 4 Expression characteristics of vernalization genes during seed germination of 'Luohan No2'. 1: seeds; 2: embryos at 6 h germination; 3: embryos at 12 h germination; 4: embryos at 24 h germination; 5: embryos at 48 h germination; 6: embryo buds at 72 h germination; 7: embryo buds at 120 h of germination; 8: endosperms at 6 h germination; 9: endosperms at 12 h germination; 10: endosperms at 24 h germination; 11: endosperms at 48 h germination; 12: endosperms at 72 h germination; 13: endosperms at 120 h germination.

was expressed not only in leaves at the 3-leaf stage, but also in roots at the 3-leaf stage and stems at the grain-filling stage, anthers, ovules, and developing seeds in 'Chinese Spring'. In addition, *VRN1* was expressed in stems, anthers, ovules, and developing seeds in 'Luohan 2'. *VRN2* transcripts were detected in embryo buds and leaves at an early developmental stage. The expression pattern of *VRN3* was similar to that of *VRN1*, except that *VRN3* was not expressed in roots. This result was consistent with the findings of Hemming *et al*[23]. 'Chinese Spring' has two recessive *vrn-1* alleles (*vrn-A1 vrn-B1*) and one dominant *Vrn-1* allele (*Vrn-D1*)[15,24-25]. The winter wheat cultivar 'Luohan 2' has all three recessive *vrn-1* alleles (*vrn-A1 vrn-B1* and *vrn-D1*)[26-27]. *VRN1* was expressed in roots and leaves at the 3-leaf stage in 'Chinese Spring', but not in 'Luohan 2'. This result suggested that the expression characteristics of *VRN1* are related to its alleles in winter and spring wheat. Loukoianov *et al*[22] indicated that in varieties of wheat that require vernalization to flower, *VRN1* is induced by exposure to low temperatures and is expressed at low basal levels without vernalization. In some varieties of wheat that have dominant *Vrn1* alleles, the dominant alleles reduce or remove the requirement for vernalization, and are expressed at high basal levels without vernalization treatment.

Expression patterns of *VRN2* were similar in spring wheat ('Chinese Spring') and winter wheat ('Luohan 2'). This result indicated that its expression was not related to the *Vrn-1* alleles. The *VRN3* gene was expressed in leaves of plants at the 3-leaf stage in 'Chinese Spring', but not in 'Luohan 2'. It was not expressed in roots of spring or winter wheat. This result indicated that expression of *VRN3* may be determined by its own genotype, not by *Vrn-1* alleles.

3.2 Relationship between expression of VRN genes and developmental stage

The *VRN1* promotes the initiation of inflorescences, and the transition from vegetative to reproductive development at the shoot apex[23]. Dominant *Vrn-1* alleles are expressed at early stage of wheat development, whereas recessive *vrn-1* alleles are expressed at a later stage[4,7]. *VRN1* was not expressed in roots or leaves of plants at the 3-leaf stage in 'Luohan 2', which requires vernalization to flower. This is because at this stage, 'Luohan 2' has not passed through the vernalization process. However, *VRN1* was expressed in 'Chinese Spring' (which carries the *Vrn-D1* allele) in the same tissues and at the same stage. This result suggested that expression of *VRN1* in winter wheat was influenced by the developmental stage. The *VRN1* was expressed in the stems, leaves, and flower tissues after transition of vegetative development to reproductive development. After pollinating, the expression of *VRN1* tends to decrease with the seeds developing. When the seeds is fully mature, transcription of *VRN1* ceases.

The expression pattern of *VRN2* was completely different from that of *VRN1*. The *VRN2* gene was not expressed in root tissues, and was expressed in leaves at the 3-leaf stage and embryo buds in both winter and spring wheat varieties. This indicates that *VRN2* maintains the vegetative state until the requirements for vernalization are met before initiation of flowering. According to the hypothetical model proposed by Hemming *et al*, *VRN1* acts in the vernalization response pathway, and is induced by low temperature independently of *VRN2* and *FT* (*VRN3*), which act in the day-length response pathway. The *VRN2* gene delays flowering by down-regulating expression of *FT*, which integrates vernalization and day-length responses[23]. Our results showed that *VRN2* was expressed in the embryo buds and leaf tissues at the 3-leaf stage, in spite of expression of *VRN1* in leaves of spring wheat at the 3-leaf stage. We propose that the expression of *VRN2* is related to the developmental stage, and is not associated with *VRN1*. The expressions of *VRN1* and *VRN3* appeared to be related to their own genotypes.

Previous studies have not explained the mechanism of action of *VRN1* in promoting flowering. Moreover, there is diversity in the composition of *VRN1* alleles in the A, B and D genomes of common wheat, therefore it would be worthwhile to conduct further research on their expression in varieties with different alleles. Previous studies on expression of *VRN2* and *VRN3* have mainly focused on diploid wheat (*T. monococcum* L.), while their molecular roles in common wheat remain unclear. Recently, Distelfeld et al. described the genetic and molecular characterization of the *VRN2* loci in tetraploid wheat[28]. Their results provide information that will be useful for exploring the characteristics of *VRN2* alleles in common wheat. Because of the value of cultivation and breeding of common wheat, it would be useful to study the alleles of *VRN2* and *VRN3* in common wheat to understand how genes that regulate vernalization responses contribute to the control of flowering.

4 Conclusion

We analyzed expression characteristics of several vernalization genes (*VRN1*, *VRN2* and *VRN3*) in the wheat cultivars 'Chinese spring' and 'Luohan 2' by RT-PCR. The *VRN1* gene was expressed in vegetative organs (roots, leaves and stems) and regenerative organs (anthers, ovules and developing seeds). The exact patterns of its expression depended on the genotype and the stage of development. The *VRN2* gene was expressed only in the leaf tissues, and its expression depended on the developmental stage. Expression of the *VRN3* gene was similar to that of the *VRN1* gene, except that *VRN3* was not expressed in the roots at the 3-leaf stage.

REFERENCES

[1] Cockram J, Jones H, Leigh FJ, *et al*. Control of flowering time in temperate cereals: genes, domestication, and sustainable productivity. *J Exp Bot*, 2007, **58**(6): 1231−1244.

[2] Colasanti J, Coneva V. Mechanisms of floral induction in grasses: something borrowed, something new. *Plant Physiol*, 2009, **149**(1): 56−62.

[3] Trevaskis B, Hemming MN, Dennis ES, *et al*. The molecular basis of vernalization-induced flowering in cereals. *Trends Plant Sci*, 2007, **12**(8): 352−357.

[4] Yan L, Loukoianov A, Tranquilli G, *et al*. Positional cloning of the wheat vernalization gene *VRN1*. *Proc Natl Acad Sci USA*, 2003, **100**(10): 6263−6268.

[5] Trevaskis B, Bagnall DJ, Ellis MH, *et al*. MADS box genes control vernalization-induced flowering in cereals. *Proc Natl Acad Sci USA*, 2003, **100**(22): 13099−13104.

[6] Danyluk J, Kane NA, Breton G, *et al*. TaVRT-1, a putative transcription factor associated with vegetative to reproductive transition in cereals. *Plant Physiol*, 2003, **132**(4): 1849−1860.

[7] Shitsukawa N, Ikari C, Shimada S, *et al*. The einkorn wheat (*Triticum monococcum*) mutant, maintained vegetative phase, is caused by a deletion in the *VRN1* gene. *Genes Genet Syst*, 2007, **82**(2): 167−170.

[8] Law CN, Worland AJ, Giorgi B. The genetic control of ear emergence time by chromosomes 5A and 5D of wheat. *Heredity*, 1976, **36**(1): 49−58.

[9] Nelson JC, Sorrells ME, Van Deynze AE, *et al*. Molecular mapping of wheat: major genes and rearrangements in homologous groups 4, 5, and 7. *Genetics*, 1995, **141**(2): 721−731.

[10] Barrett B, Bayram M, Kidwell K. Identifying AFLP and microsatellite markers for vernalization response gene *Vrn-B1* in hexaploid wheat (*Triticum aestivum* L.) using reciprocal mapping populations. *Plant Breeding*, 2002, **121**(5): 400−406.

[11] Iwaki K, Nishida J, Yanagisawa T, *et al*. Genetic analysis of *Vrn-B1* for vernalization requirement by using linked dCAPS markers in bread wheat (*Triticum aestivum* L.). *Theor Appl Genet*, 2002, **104**(4): 571−576.

[12] McIntosh RA, Yamazaki Y, Devos KM, *et al*. Catalogue of gene symbols for wheat//Pogna NE, Romano M, Pogna E, *et al*, eds. Proceedings of the 10th International Wheat Genetics Symposium. Rome: Instituto Sperimentale per la Cerealicoltura, 2003, **4**: 1−34.

[13] Yan L, Loukoianov A, Blechl A, *et al*. The wheat *VRN2* gene is a flowering repressor down-regulated by vernalization. *Science*, 2004, **303**(5664): 1640−1644.

[14] Law CN, Wolfe MS. Location of genetic factors for mildew resistance and ear emergence time on chromosome 7B of wheat. *Can J Genet Cytol*, 1966, **8**: 462−470.

[15] Yan L, Fu D, Li C, *et al*. The wheat and barley vernalization gene *VRN3* is an orthologue of *FT*. *Proc Natl Acad Sci USA*, 2006, **103**(51): 9581−19586.

[16] Jaeger KE., and Wigge PA. FT protein acts as a long-range signal in *Arabidopsis*. *Curr Biol*, 2007, **17**(12): 1050−1054.

[17] Corbesier L, Vincent C, Jang S, *et al*. FT protein movement contributes to long-distance signaling in floral induction of *Arabidopsis*. *Science*, 2007, **316**(5827): 1030−1033.

[18] Mathieu J, Warthmann N, Kuttner F, *et al*. Export of FT protein from phloem companion cells is sufficient for floral induction in *Arabidopsist*. *Curr Biol*, 2007, **17**(12): 1055−1060.

[19] Li C, and Dubcovsky J. Wheat FT protein regulates *VRN1* transcription through interactions with FDL2. *Plant J*, 2008, **55**(4): 543−554.

[20] Colasanti J, Coneva V. Mechanisms of floral induction in grasses: something borrowed, something new. *Plant Physiol*, 2009, **149**(1): 56−62.

[21] Zhu Y, Wang M, Jia ZW, *et al*. An effective method for extracting total RNA from young ears of maize. *Chin Bull Bot*, 2007, **24**(5): 624−628.
朱昀, 王猛, 贾志伟, 等. 一种从富含多糖的玉米幼穗中提取 RNA 的方法. 植物学通报, 2007, **24**(5): 24−628

[22] Loukoianov A, Yan L, Blechl A, *et al*. Regulation of *VRN1* vernalization genes in normal and transgenic polyploid wheat. *Plant Physiol*, 2005, **138**(4): 2364−2373.

[23] Hemming MN, Peacock WJ, Dennis ES, *et al*.

Low-temperature and daylength cues are integrated to regulate *FLOWERING LOCUS T* in barley. *Plant Physiol*, 2008, **147**(1): 355−366.

[24] Fu D, Szücs P, Yan L, *et al*. Large deletions within the first intron in *VRN1* are associated with spring growth habit in barley and wheat. *Mol Genet Genomics*, 2005, **273**(1): 54−65.

[25] Sherman JD, Yan L, Talbert L, *et al*. A PCR marker for growth habit in common wheat based on allelic variation at the *VRN-A1* gene. *Crop Sci*, 2004, **44**(5): 1832−1838.

[26] Yang ZQ, Yin J, Zhou R, *et al*. Study on vernalization character of different genotypes of wheat from Huanghuai wheat production area. *J Triticeae Crops*, 2006, **26**(2): 82−85.
杨宗渠, 尹钧, 周冉, 等. 黄淮麦区不同小麦基因型的春化发育特性研究. 麦类作物学报, 2006, **26**(2): 82−85.

[27] Yuan XY, Li YC, Meng FR, *et al*. Allelic composition of the vernalization gene *VRN1* in 21 wheat (*Triticum aestivum* L.) cultivars from Huanghuai wheat production area. *J Triticeae Crops*, 2009, **29**(5): 760−765.
袁秀云, 李永春, 孟凡荣, 等. 黄淮麦区 21 个小麦品种中春化基因 VRN1 的组成分析. 麦类作物学报, 2009, **29**(5): 760−765.

[28] Distelfeld A, Tranquilli G, Li C, *et al*. Genetic and molecular characterization of the *VRN2* loci in tetraploid wheat. *Plant Physiol*, 2009, **149**(1): 245−257.