RESEARCH

Allelic Variation at the Vernalization Genes *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, and *Vrn-B3* in Chinese Wheat Cultivars and Their Association with Growth Habit

X. K. Zhang, Y. G. Xiao, Y. Zhang, X. C. Xia, J. Dubcovsky, and Z. H. He*

ABSTRACT

Information on the distribution of vernalization genes and their association with growth habit is crucial to understanding the adaptability of wheat (*Triticum aestivum* L.) cultivars to different environments. In this study, 278 Chinese wheat cultivars were characterized with molecular markers for the vernalization genes *Vrn-A1*, *-B1*, *-D1*, and *-B3*. Heading time was evaluated in a greenhouse under long days without vernalizaton. The dominant *Vrn-D1* allele showed the highest frequency in the Chinese wheat cultivars (37.8%), followed by the dominant *Vrn-A1*, *-B1*, and *-B3* alleles. Ninety-two winter cultivars carried recessive alleles of all four vernalization loci, whereas 172 spring genotypes contained at least one dominant *Vrn* allele. All cultivars released in the North China Plain Winter Wheat Zone were winter type. Winter (53.0%), spring (36.1%), and early-heading (10.9%) cultivars were grown in the Yellow and Huai River Valley Winter Zone. Most of the spring genotypes from this zone carried only the dominant *Vrn-D1* allele, which was also predominant (64.1%) in the Middle and Lower Yangtze Valley Winter Zone and Southwestern Winter Wheat Zone. In three spring-sown wheat zones, all cultivars were early-heading spring types that frequently possessed the strongest dominant *Vrn-A1a* allele and combinations with other dominant *Vrn* gene(s). The *Vrn-D1* allele is associated with the latest heading time, *Vrn-A1* the earliest, and *Vrn-B1* intermediate values. The information is important for breeding programs in countries interested in using Chinese wheats.

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Abbreviations: PCR, polymerase chain reaction; UV, ultraviolet.

Common wheat (*Triticum aestivum* L.) is one of the most widely cultivated food crops in the world and is grown over a wide range of elevations, climatic conditions, and soil fertility (Bushuk, 1998). The wide adaptability of wheat is largely governed by three groups of genetic factors— vernalization (*Vrn*) genes (vernalization requirement), photoperiod (*Ppd*) genes (photoperiod sensitivity), and genes controlling earliness per se (*Eps*) (Kato and Yamagata, 1988)—that act together to determine flowering time and hence the basic adaptation of a genotype for a particular environmental condition (Worland, 1996; Worland et al., 1998). Vernalization genes determine growth habits, which divide wheat into winter and spring classes. Winter cultivars are mainly adapted to areas with average January temperature between –7 and 4°C, whereas spring cultivars are adapted to areas with temperatures below or above this range (Iwaki et al., 2000, 2001). The different frequencies of *Vrn* alleles observed in different parts of the world suggest that these allele combinations have an adaptive value (Gotoh, 1979; Stelmakh, 1990; Iwaki et al., 2000, 2001; Goncharov, 1998;

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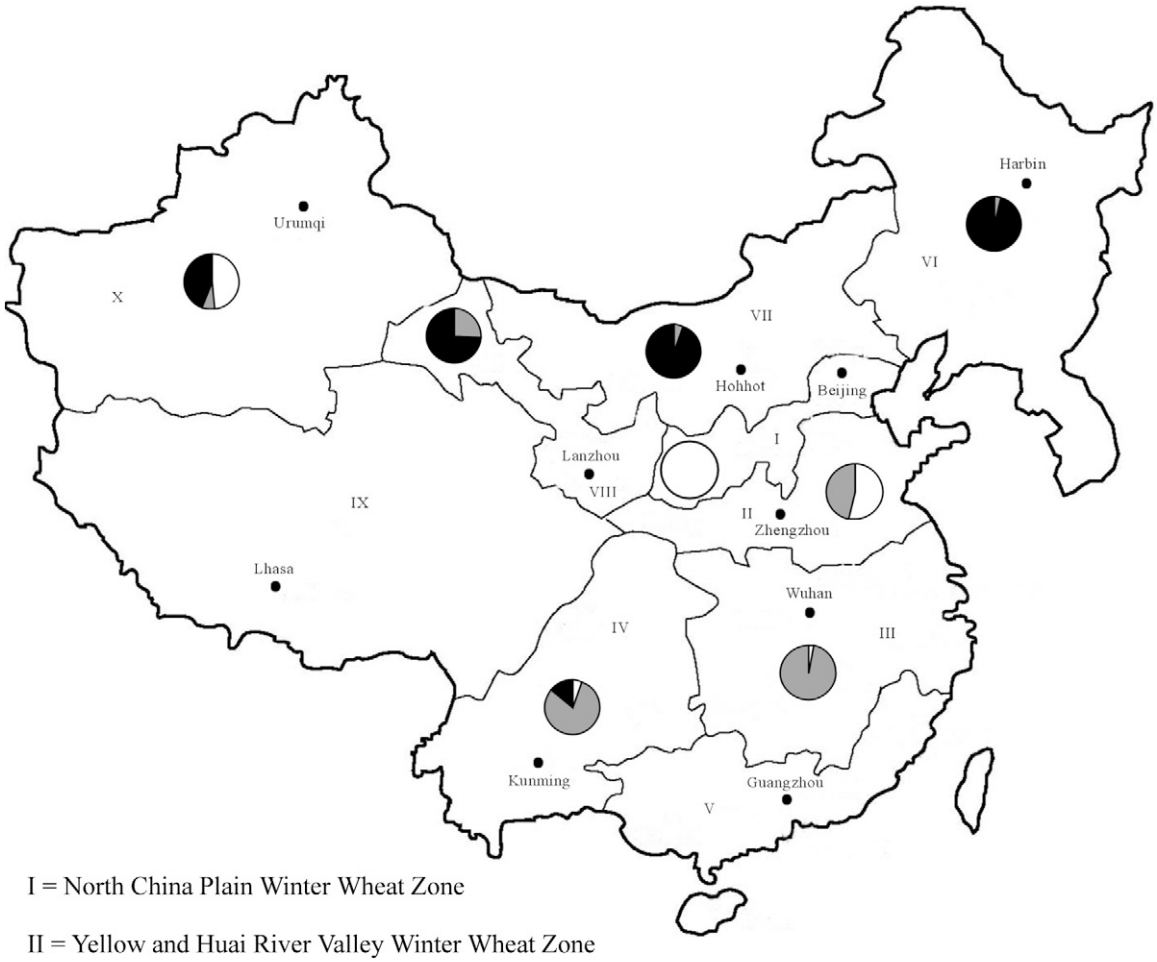
Stelmakh, 1998). For example, the dominant *Vrn-A1* allele is frequently observed in improved cultivars from Europe and Siberia, whereas higher frequencies of dominant *Vrn-D1* allele were found in commercial cultivars from countries situated nearer the equator (Stelmakh, 1990), such as Japan (Gotoh, 1979), the Central Asian Republic of the Former Soviet Union (Stelmakh, 1990), and China (Iwaki et al., 2000, 2001), or in Mediterranean climates (Fu et al., 2005). Important germplasm from the International Maize and Wheat Improvement Center (CIMMYT) have also been classified for their phasic gene constitution, allowing conclusions about the frequencies of occurrence of certain gene combinations (Van Beem et al., 2005). Therefore, an understanding of the vernalization genes present in wheat breeding programs is useful when developing cultivars broadly adapted to different regions.

Various studies showed that the vernalization requirement is genetically controlled by at least five loci, *Vrn-A1* (formerly *Vrn1*), *Vrn-B1* (*Vrn2*), *Vrn-D1* (*Vrn3*), *Vrn4*, and *Vrn-B3* (*Vrn5*), in global sets of commercial cultivars (Pugsley, 1971, 1972; McIntosh et al., 1998; Goncharov, 2003; Yan et al., 2006). The three major vernalization genes *Vrn-A1*, *Vrn-B1*, and *Vrn-D1* are located on the homeologous chromosomes 5A, 5B, and 5D in common wheat, respectively (Pugsley, 1971; Law et al., 1976; Worland, 1996; Barrett et al., 2002; Yan et al., 2003), and *Vrn-B3* is located on chromosome arm 7BS (Law and Wolfe, 1966; Yan et al., 2006). The spring alleles from these genes are epistatic to the winter alleles, and, therefore, the winter habit is observed only when all the genes have recessive alleles (Pugsley, 1971). The *Vrn-A1a* allele is the most potent allele for spring growth habit, providing complete insensitivity to vernalization, whereas *Vrn-B1*, *Vrn-D1*, and *Vrn4* result in a partial elimination of the vernalization requirement (Pugsley, 1971, 1972).

Detection of vernalization genes by traditional genetic methods is time consuming. Fortunately, the recent cloning of wheat vernalization genes (Yan et al., 2003, 2006) has facilitated the development of gene-specific markers or functional markers (also known as perfect or diagnostic markers). These markers provide a unique opportunity to screen large collections of wheat germplasm for allelic diversity at the *Vrn* genes. Yan et al. (2003) used diploid wheat *T. monococcum* ($2n = 14$, A^mA^m) to clone the *Vrn-A^{m}1* gene, using a positional cloning approach to show that this gene is similar to the *Arabidopsis* meristem identity gene *APETALA1*. The different dominant *Vrn-A1* alleles were identified in polyploid wheats. The most abundant one in common wheat, *Vrn-A1a*, has an insertion of a foldback repetitive element and a duplicated region in the promoter (Yan et al., 2004). The less frequent *Vrn-A1b* allele shows several single nucleotide polymorphisms and deletions in the promoter region (Yan et al., 2004), whereas the rare *Vrn-A1c* allele has a large deletion in the first intron (Fu et al., 2005). The *Vrn-A1c* allele was found only in the spring hexaploid landrace IL369 from Afghanistan but is common among tetraploid spring genotypes. The dominant *Vrn-B1* and *Vrn-D1* alleles for spring growth habit are also characterized by large deletions in the first intron of the same gene (Fu et al., 2005). A dominant allele for spring growth habit was recently identified in the cultivar Hope and was designated *Vrn-B3* on the basis of its orthology with the barley *Vrn-H3* gene (Yan et al., 2006). The dominant *Vrn-B3* allele has a retrotransposon insertion in the promoter region of a gene similar to *Arabidopsis FT* (Yan et al., 2006).

China is the largest wheat producer in the world. Ten major wheat agroecological zones have been recognized in China (Fig. 1) on the basis of differences in wheat types, growing season, presence of major biotic and abiotic stresses, and cultivar responses to temperature and photoperiod (Zhuang, 2003). At present, autumn-sown wheats account for about 90% of production and acreage and include zones I (4%), II (60%), III (13%), IV (10%), and V (minor area of production). Spring-sown wheats represent 7% of the wheat acreage in China and are grown in zones VI, VII, and VIII. Zones IX and X cover less than 3% of the total wheat area and include both spring- and fall-sown wheats. Average January temperatures and wheat-growing periods vary greatly among different Chinese wheat regions (Jin, 1986, 1997; Zhuang, 2003). The spring-sown wheat regions are well defined, and only spring cultivars are found in these regions. However, the autumn-sown regions include both winter and spring cultivars, resulting in some confusion in cultivar classification. As an example, spring-type cultivars from Zones II and III are sometimes planted in the northern part of Zone II, resulting in severe losses due to winter damage (Jin, 1986, 1997; Dong and Zheng, 2000; Zhuang, 2003). Therefore, information on the distribution of vernalization genes in Chinese wheats is crucially important for developing widely adapted cultivars and for providing information to extension workers and farmers.

Previously, vernalization genotypes and growth habits of 42 Chinese wheat landraces were studied by crossing them with near-isogenic lines of 'Triple Dirk' carrying different vernalization genes (Gotoh, 1979; Iwaki et al., 2000, 2001). However, the vernalization genotypes and growth habits of wheat cultivars released in China from the 1960s to the present are mostly unknown. The aims of this study are (i) to characterize allelic variations at the four vernalization loci *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, and *Vrn-B3* among the major Chinese wheat cultivars released since the 1960s using molecular markers; (ii) to test heading times of these cultivars under controlled conditions; and (iii) to analyze the relationships between vernalization genotypes, geographic distribution, and planting times for these cultivars. This information is expected to be useful for improving the adaptation of wheat cultivars in Chinese breeding programs targeting different environments and



I = North China Plain Winter Wheat Zone

II = Yellow and Huai River Valley Winter Wheat Zone

III = Middle and Lower Yangtze Valley Winter Wheat Zone

IV = Southwestern Winter Wheat Zone

V = Southern Winter Wheat Zone

VI = Northeastern Spring Wheat Zone

VII = Northern Spring Wheat Zone

VIII = Northwestern Spring Wheat Zone

IX = Qinghai-Tibetan Plateau Spring and Winter Wheat Zone

X = Xinjiang Winter and Spring Wheat Zone

White, gray, and black cake graphs show the proportions of winter cultivars with recessive alleles, spring cultivars without and with dominant *Vrn-A1* allele, respectively.

Figure 1. Distribution of growth habit and vernalization allele combinations among China's different wheat (*Triticum aestivum* L.) zones.

for foreign breeding programs interested in using Chinese wheat germplasm.

MATERIALS AND METHODS

Plant Material

A total of 278 Chinese wheat cultivars collected from eight major zones were used to identify their vernalization genotypes using polymerase chain reactions (PCR) methods, and the growth habits of 266 of them were assessed in the greenhouse (see Table A1 in Appendix). They include landmark landraces, leading cultivars, and 12 well-known introductions that had significant impact on Chinese wheat production and breeding after 1960. The number of entries in various zones was based on wheat acreage and number of cultivars developed by the local breeding programs (Table 1). Cultivars Thatcher (*Vrn-A1a*), Chinese Spring (*Vrn-D1*), and Hope (*Vrn-B3*) were used as controls.

DNA Extraction and Molecular Marker Analysis

Genomic DNA was extracted from seeds following the procedure of Gale et al. (2001). Sequences of nine specific primer

sets for the amplification of allelic variations at *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, and *Vrn-B3* loci have been published before (Yan et al., 2004, 2006; Fu et al., 2005) and are summarized in Table A2 in the Appendix. These primers were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. (Shanghai, China; http://www.sangon.com).

Polymerase chain reaction was performed in an MJ Research PTC-200 thermal cycler (Waltham, MA). The PCR conditions for the primer pair VRN1AF and VRN1-INT1R were as follows: 1X PCR buffer with 1.5 mM of $MgCl_2$, 150 μM of each dNTPs, 2 pmol of each primer, 1 unit of *Taq* DNA polymerase (Tiangen Biotech Co., Beijing, China) and 50 to 100 ng of template DNA in 20 μL of final volume. Thermocycling conditions were an initial denaturation at 94°C for 10 min, followed by 38 cycles of 45 s at 94°C, 45 s at 50°C, 1 min at 72°C, and with a final extension step at 72°C for 5 min. Amplified PCR fragments were separated on a 2.5% agarose gel at 80 V for 4 to 5 h, stained with ethidium bromide, and visualized using ultraviolet (UV) light. Thatcher (*Vrn-A1a*) and Chinese Spring (*vrn-A1*) were used as controls in the test. For the other eight primer sets we used 10 pmol of each primer and the PCR conditions were similar to those for the VRN1AF/VRN1-INT1R primer pair except for the annealing temperatures and extension times (see Table A2). The amplified PCR fragments were separated on a 1% agarose gel at 150 V, stained with ethidium bromide, and visualized using UV light.

Greenhouse Experiment

Heading times of 266 wheat cultivars were evaluated following the methods of Stelmakh (1987), Iwaki et al. (2001), and Beales et al. (2005) with minor modifications. Cultivars were grown in a greenhouse under a 16-h-daylength regime and a temperature of 18 ± 3°C to avoid natural vernalization. For each cultivar, five germinated seeds were sown in soil-filled containers at a space of 2.5 cm between plants in a row and 6 cm between rows. Days to heading were recorded during 6 mo in the greenhouse.

RESULTS

Allelic Frequencies at the *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, and *Vrn-B3* Loci

The specific allele combinations identified in the 278 wheat cultivars are shown in Table A1. First, all cultivars were tested with primers VRN1AF and VRN1-INT1R for the *Vrn-A1* promoter region. A total of 68 cultivars from Zones IV, VI, VII, VIII, and X showed PCR fragments identical to those in Thatcher (965 bp and 876 bp), indicating the presence of the dominant *Vrn-A1a* allele (Fig. 2A, Table A1). Only eight cultivars (Fan 7, Chuanyu 12, and Jingmai 11 from Zone IV, Dabaipi from Zone VII, Longchun 8, Longchun 21, and Ganmai 8 from Zone VIII, and Xinchun 9 from Zone X) showed the 714-bp fragment characteristic of the *Vrn-A1b* allele (Fig. 2A, Table A1). The remaining 202 cultivars exhibited the 734-bp fragment characteristic of the dominant allele *Vrn-A1c* or the recessive *vrn-A1* allele (Fig. 2A). To distinguish between these two alleles, all cultivars were tested using the two primer pairs Intr1/A/F2 and Intr1/A/R3, and Intr1/C/F and Intr1/AB/R, for the *Vrn-A1* first intron. A 1068-bp fragment was amplified in all cultivars tested using the primer pair Intr1/C/F and Intr1/AB/R (Fig. 2C), whereas no PCR product was produced using primer pair Intr1/A/F2 and Intr1/A/R3 (Fig. 2B). These results indicate that the large intron 1 deletion (*Vrn-A1c* allele) was not present in the Chinese cultivars and that the 202 cultivars with the 734-bp amplification product carried the recessive *vrn-A1* allele (Table A1).

A total of 73 cultivars have the dominant *Vrn-B1* allele as indicated by the amplification of a 709-bp fragment using primers Intr1/B/F and Intr1/B/R3 (Fig. 3A). The other 205 cultivars showed a 1149-bp amplification product with primers Intr1/B/F and Intr1/B/R4, which

Table 1. Distribution of growth habits and combination of dominant alleles at *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, and *Vrn-B3* loci in various Chinese wheat (*Triticum aestivum* L.) zones.

Classification	Zone†								Total
	I	II	III	IV	VI	VII	VIII	X	
No. of all cultivars	32	86	29	35	24	17	27	28	278
No. of cultivars tested in the greenhouse	32	75	29	35	24	17	27	27	266
% late-heading cultivars	100	51.2	3.4	5.7	–	–	–	46.4	33.1
% early-heading cultivars	–	36.0	96.6	94.3	100	100	100	50.0	62.6
Overall % within spring cultivars									
Vrn-A1	–	–	–	15.2	95.8	94.1	74.1	85.7	44.2
Vrn-B1	–	6.7	11.1	42.4	75.0	58.8	70.4	50.0	42.4
Vrn-D1	–	93.3	96.3	78.8	50.0	29.4	14.8	28.6	61.0
Vrn-B3	–	–	–	–	8.3	–	–	–	1.2
Regional % within spring cultivars and average heading time									
Vrn-D1 alone‡ = 54 d§ (36–109 d)	–	93.3	88.9	51.5	–	–	3.7	14.3	41.9
Vrn-B1 alone = 47 d (36–73 d)	–	6.7	3.7	15.2	–	–	11.1	–	6.4
Vrn-B1 + *Vrn-D1* = 42 d (36–49 d)	–	–	7.4	18.1	4.2	5.9	11.1	–	7.6
Subtotal spring without *Vrn-A1*	–	100	100	84.8	4.2	5.9	25.9	14.3	55.9
Vrn-A1 alone = 39 d (33–46 d)	–	–	–	–	12.5	23.5	26.0	35.7	11.0
Vrn-A1 + *Vrn-B1* = 38 d (32– 49 d)	–	–	–	6.1	33.2	47.1	48.1	35.7	20.9
Vrn-A1 + *Vrn-D1* = 38 d (36–44 d)	–	–	–	6.1	12.5	17.6	–	–	4.6
Vrn-A1 + *Vrn-B1* + *Vrn-D1* = 38 d (33–50 d)	–	–	–	3.0	29.2	5.9	–	14.3	6.4
Vrn-A1 + *Vrn-B1* + *Vrn-B3* = 30 d	–	–	–	–	4.2	–	–	–	0.6
Vrn-A1+*Vrn-B1*+*Vrn-D1*+*Vrn-B3* = 31 d	–	–	–	–	4.2	–	–	–	0.6
Subtotal spring with *Vrn-A1*	–	0	0	15.2	95.8	94.1	74.1	85.7	44.1

†See Fig. 1.

‡Eight cultivars with the *Vrn-D1* allele alone were not tested for heading time.

§Average heading time of tested genotypes with this genotype.

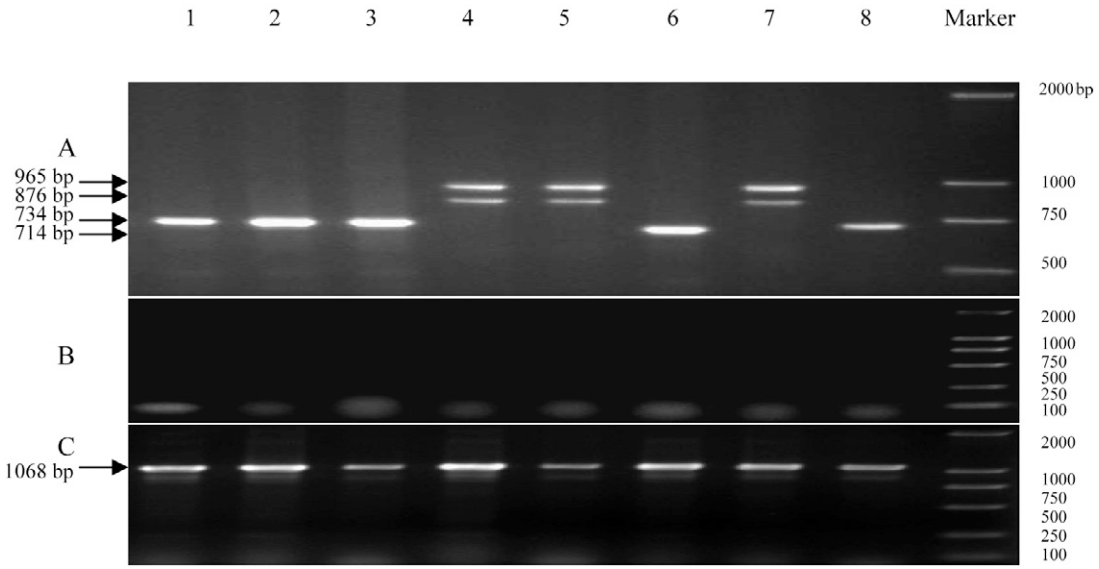


Figure 2. Polymerase chain reaction amplification using primer pairs (A) VRN1AF and VRN1-INT1R, (B) Intr1/A/F2 and Intr1/A/R3, and (C) Intr1/C/F and Intr1/AB/R to detect alleles at the *Vrn-A1* locus. 1, Chinese Spring (*vrn-A1*); 2, Jing 411 (*vrn-A1*); 3, Yumai 2 (*vrn-A1*); 4, Thatcher (*Vrn-A1a*); 5, Xinkehan 9 (*Vrn-A1a*); 6, Longchun 21 (*Vrn-A1b*); 7, Gan 630 (*Vrn-A1a*); 8, Shaan 229 (*vrn-A1*).

is characteristic of the recessive *vrn-B1* allele (Fig. 3B and Table A1).

A 1671-bp fragment was generated from 105 cultivars using primer pair Intr1/D/F and Intr1/D/R3 (Fig. 4A), demonstrating that they carried the dominant *Vrn-D1* allele. Amplification of DNA from the other cultivars using primers Intr1/D/F and Intr1/D/R4 showed a 997-bp band characteristic of the recessive *vrn-D1* allele (Fig. 4B and Table A1).

The dominant *Vrn-B3* allele, defined by the amplification of a 1.14-kb fragment with primers VRN4-B-INS-F and VRN4-B-INS-R, was found only in cultivars Longfumai 1 and Liaochun 10 from Zone VI (Fig. 5A). All other cultivars showed a 1.2-kb amplification fragment using primers VRN4-B-NOINS-F and VRN4-B-NOINS-R, which is characteristic of the recessive *vrn-B3* allele (Fig. 5B, Table A1).

Geographic Distribution of the Different Allele Combinations

The frequencies of the different *Vrn* allele combinations varied greatly across different wheat agroecological zones (Table 1, Fig. 1). Cultivars with recessive alleles at all the analyzed *Vrn* loci represent 38.1% of the cultivars and are mainly concentrated in Zones I, II, and X (Table 1). The other 61.9% includes cultivars with at least one dominant *Vrn* allele, which can be classified as spring. These cultivars are found mainly in Zones III, IV, VI, VII, VIII, and X (Table 1).

Among the cultivars with at least one dominant *Vrn* allele, the frequencies of the different alleles varied across regions (Table 1). The dominant *Vrn-B3* allele is present only in two cultivars from zone VI. Among the *Vrn-1*, alleles *Vrn-D1* showed the highest frequency, followed closely by dominant *Vrn-A1* and *Vrn-B1* alleles (Table 1). The dominant *Vrn-A1* allele is not presented in Zones I,

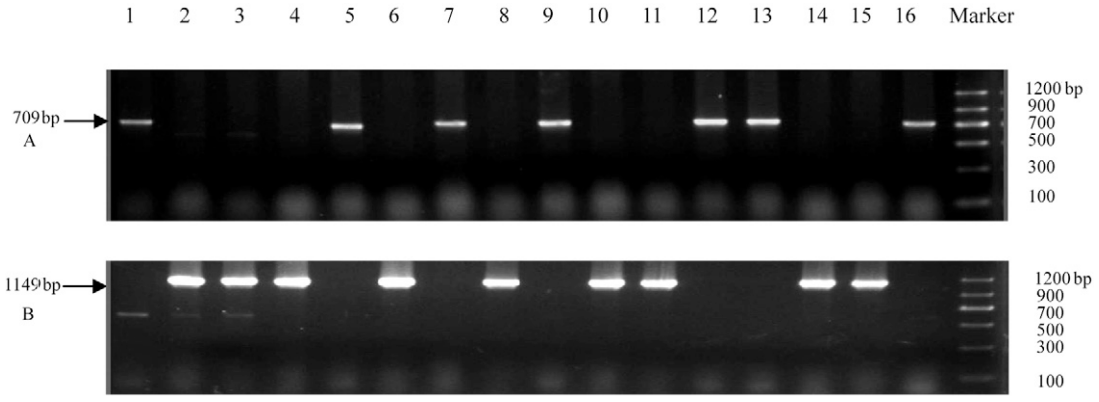


Figure 3. Polymerase chain reaction amplification using primer pairs (A) Intr1/B/F and Intr1/B/R3 and (B) Intr1/B/F and Intr1/B/R4 to detect the dominant (*Vrn-B1*) and recessive (*vrn-B1*) alleles at the *Vrn-B1* locus, respectively. 1, Abbondanza; 2, Dongfanghong 3; 3, Mentana; 4, Mazhamai; 5, Zhengmai 9023; 6, Mianyang 11; 7, Mianyang 15; 8, Miannong 4; 9, Kefeng 3; 10, Xinkehan 9; 11, Longmai 20; 12, CI12203; 13, Jinchun 14; 14, Xuzhou 25; 15, Chinese Spring; 16, Xinchun 12.

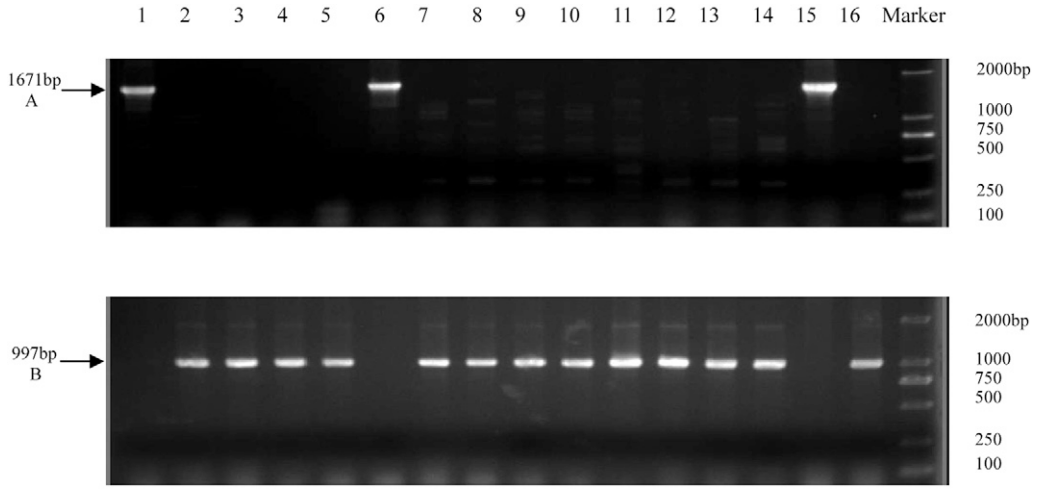


Figure 4. Polymerase chain reaction (PCR) amplification using primer pairs (A) Intr1/D/F and Intr1/D/R3 and (B) Intr1/D/F and Intr1/D/R4 to detect the dominant (*Vrn-D1*) and recessive (*vrn-D1*) alleles at the *Vrn-D1* locus, respectively. 1, Chinese Spring; 2, Beijing 10; 3, Jing 411; 4, Bima 4; 5, Abbondanza; 6, Neixiang 36; 7, Jinan 2; 8, Zhoumai 18; 9, Shijiazhuang 8; 10, Shaannong 7859; 11, Yumai 2; 12, Lumai 1; 13, Lumai 14; 14, Zhengmai 9023; 15, Mentana; 16, Emai 6.

II, and III, and its frequency is low in Zone IV. However, high frequencies are observed in Zones VI, VII, VIII, and X (Table 1, Fig. 1). The dominant allele *Vrn-B1* is not present in Zone I, and low frequencies are observed in Zones II and III. However, high frequencies are observed in Zones IV, VI, VII, VIII, and X. The dominant allele *Vrn-D1* is not present in Zone I, but is present at relatively high frequencies in Zones II, III, IV, VI, VII, VIII, and X.

Among the four autumn-sown wheat zones (I, II, III, and IV), the frequency of dominant *Vrn-D1* allele is the highest, followed by *Vrn-B1* and *Vrn-A1* (*Vrn-B3* is absent) (Fig. 6). In contrast, in three spring-sown wheat zones (VI, VII, and VIII) the frequency of the dominant *Vrn-A1* allele is the highest, followed by *Vrn-B1* and *Vrn-D1*, respectively (Fig. 6). *Vrn-B3* frequency (2.9%) is the lowest.

Frequencies of the different combinations of vernalization genes were also very different among the various wheat agroecological zones (Table A1 and Table 1). In brief, nine combinations of dominant *Vrn* alleles were identified. Among them, the *Vrn-D1* allele alone was the most frequent (72 cultivars), followed by the *Vrn-A1Vrn-B1* (36 cultivars) combination. The distribution of the different allele combinations across the different zones is described in Table 1. In summary, most cultivars released in the autumn-sown wheat regions of south China (Zones III and IV) and north China (Zone II) possessed *Vrn-D1* as a single dominant allele. In contrast, in spring-sown wheat regions, cultivars carried the strongest dominant *Vrn-A1* alleles, and the majority of them included additional dominant *Vrn* alleles at the *Vrn-B1*, *Vrn-D1*, and *Vrn-B3* loci. On the basis of the vernalization alleles found in this study, the vernalization requirement can be ranked from strongest to weaker from Zone I, Zone II, Zone III, Zone IV, with the weakest requirement in the spring-sown spring wheat regions (Zones VI, VII, and VIII).

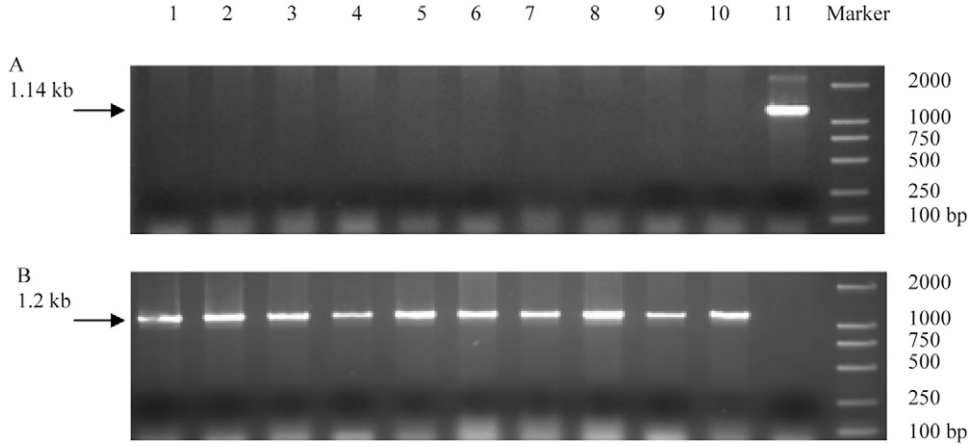


Figure 5. Polymerase chain reaction amplification using primer pairs (A) VRN4-B-INS-F and VRN4-B-INS-R and (B) VRN4-B-NOINS-F and VRN4-B-NOINS-R to detect dominant (*Vrn-B3*) and recessive (*vrn-B3*) alleles at the *Vrn-B3* locus, respectively. 1, Chinese Spring; 2, Shijiazhuang 407; 3, Lumai 21; 4, Xi'an 8; 5, Jinan 2; 6, Shaanong 7859; 7, Sumai 3; 8, Neixiang 36; 9, Neimai 19; 10, Gan 630; 11, Liaochun 10.

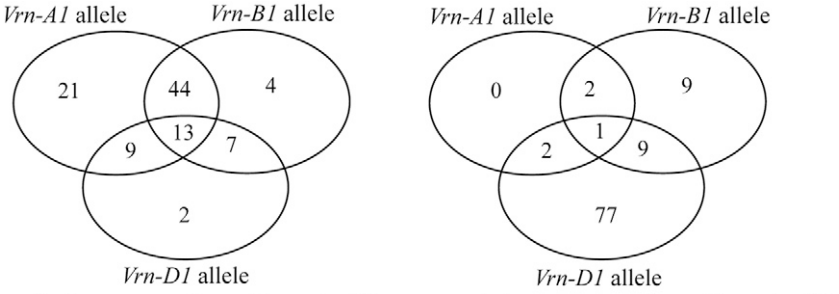


Figure 6. Percentage of different vernalization allele combinations among spring-sown spring and autumn-sown spring cultivars in China.

Growth Habit

Heading dates showed a continuous distribution from 30 d to more than 6 mo after planting in the greenhouse (Table A1). Of 266 cultivars tested in the greenhouse, the 92 cultivars that failed to head within 109 d all possessed recessive vernalization alleles at the four *Vrn* loci as identified by the PCR markers. Most of them were classified as winter cultivars in the literature (Jin, 1986, 1997; Zhuang, 2003). Among the 174 cultivars that headed within 109 d (early heading), 164 carried at least one of the tested dominant vernalization alleles and were classified as spring. The other cultivars, nine from Zone II (Jimai 36, Taishan 1, Lumai 23, Laizhou 953, Weimai 8, Xinmai 9408, Yumai 66, Yumai 70, Xuzhou 14) and one from Zone III (Emai 6), carried recessive alleles at the four vernalization loci. The most likely explanation for this discrepancy is the presence of an unknown allele at the four loci characterized in this study or the presence of a spring allele at the *Vrn4* locus not included in this survey because the gene is still unknown.

All 32 cultivars released in Zone I headed after 109 d, had all three recessive *vrn-1* alleles, and were classified as winter. In Zone II, of 75 cultivars tested in the greenhouse, 44 (58.7%) headed after 109 d. Although both winter and spring types are found in all provinces of Zone II, the late-heading cultivars were mainly cultivated in the provinces of Shandong (79.2%), Shaanxi (63.6%), and Anhui (100.0%), whereas the early-heading cultivars were mostly present in the provinces of Henan and Jiangsu. Most of the cultivars from Zones III and IV (>94%) headed before 109 d. The frequency of early-heading genotypes increased gradually from north to south in the autumn-sown regions. In Zones VI, VII, and VIII, all 68 cultivars tested in the greenhouse headed within 109 d. In Zone X, the frequency of early-heading genotypes was 51.9%. Therefore, it was concluded that spring cultivars in China are more frequent in the high-latitude regions (spring sowing) and in the low-latitude area with warm winters (autumn sowing). Winter cultivars are frequently present in the middle-latitude area with relatively cold winters (autumn sowing).

Relationships between *Vrn* Allele Combinations and Growth Habits

The relationships between vernalization genotypes and heading times in the absence of vernalization are shown in Table 1. The 92 late-heading (winter) cultivars all carried recessive alleles at the four vernalization loci. Different combinations and proportions of vernalization alleles were found in the other 172 early-heading (spring) cultivars. Single dominant alleles were observed for the *Vrn-A1* (11.0%), *Vrn-B1* (6.4%), or *Vrn-D1* (41.9%). We also observed two gene combinations, including *Vrn-A1–Vrn-B1* (20.9%), *Vrn-A1–Vrn-D1* (4.6%), and *Vrn-B1–Vrn-D1* (7.6%), and three dominant allele combinations, including *Vrn-A1Vrn-B1Vrn-D1* (6.4%) and *Vrn-A1Vrn-B1Vrn-B3* (0.6%). In addition, one very early heading cultivar (Liaochun 10) carried all four dominant alleles (*Vrn-A1Vrn-B1Vrn-D1Vrn-B3*).

Days to heading among the different combinations of dominant vernalization alleles are described in Table 1. In summary, the earliest cultivars were those carrying three to four dominant alleles, including the rare *Vrn-B3* allele (average 30 to 31 d to heading), followed by the one-, two- or three-gene combinations, including *Vrn-A1* but not *Vrn-B3* (average 38 d to heading). Lines carrying the *Vrn-B1/Vrn-D1* allelle combination headed approximately 42 d after sowing, whereas those carrying only the *Vrn-B1* (average 47 d) or *Vrn-D1* (average 54 d) were among the latest spring cultivars. On the basis of these data, the strength of the dominant spring *Vrn-1* alleles can be ranked as *Vrn-A1* > *Vrn-B1* > *Vrn-D1*. *Vrn-B3* resulted in the earliest heading times in combination with other dominant *Vrn1* alleles.

DISCUSSION

Effectiveness of Molecular Markers for Identifying Vernalization Alleles

Functional markers (also known as perfect markers) are derived from polymorphic sites within genes that directly affect phenotypic trait variation, and they are ideal tools for marker-assisted selection (Bagge et al., 2007). The vernalization gene markers developed by Yan et al. (2004, 2006) and Fu et al. (2005) are likely functional markers. The observed heading times in the greenhouse experiment and the growth habit determinations from the literature (Jin, 1986, 1997; Dong and Zheng, 2000; Zhuang, 2003) were consistent with the *Vrn* genotypes. However, 10 of the 174 cultivars showed inconsistent results, with early heading in the greenhouse but recessive alleles present at the four *Vrn* alleles characterized in this study. These exceptions are most likely due to the presence of the *Vrn4* locus, which is known to be present in Chinese landraces

(Iwaki et al., 2000, 2001). Unfortunately, this gene has not been cloned, and no markers are currently available to screen these cultivars. An alternative possibility is the presence of new mutations at the *Vrn* loci included in this study outside the region tested with the available primers or the presence of alleles for spring growth habit at unknown vernalization genes. Further investigation of these exceptions may provide new insights on wheat vernalization genes.

Additional exceptions were the early-heading Chinese landraces Jiounong 2 and Ganmai 11 from Zone VIII, which showed three amplification products with primers VRN1A and VRN1-INT1R (data not shown). This result suggests that these landraces may carry a new *Vrn-A1* allele. However, further investigation is needed to confirm this assumption.

In spite of these few exceptions, the distribution of dominant *Vrn-A1*, *Vrn-B1*, and *Vrn-D1* alleles based on molecular markers (Yan et al., 2004; Fu et al., 2005) among modern Chinese cultivars is similar to that reported before for a smaller set of Chinese landraces using crosses with near-isogenic lines of 'Triple Dirk' (Gotoh, 1979; Iwaki et al., 2000, 2001). These results indicate that these molecular markers can be effectively used to detect allelic variations at these four vernalization gene loci.

Reasons for Different Distributions of Growth Habit and Vernalization Genotypes among Wheat Zones of China

The average January temperatures increase from Zone I to II, from II to III, and from III to IV, whereas the length of growth periods decreases in the same direction (Dong and Zheng, 2000; Zhuang, 2003). The frequency of dominant vernalization alleles gradually increases in the same direction (Fig. 1). Only winter cultivars with recessive alleles at the four vernalization loci can survive in the cold winters of Zone I, whereas almost all cultivars released in Zones III and IV with warmer winters are spring types. Most of them carry the single dominant *Vrn-D1* allele, due to the wide utilization of some breeding parents, including Mentana with *Vrn-D1* in Zones III and IV (Stelmakh, 1990; Zhuang, 2003). The *Vrn-D1* allele is the weakest of the dominant *Vrn-1* alleles and has a residual requirement for vernalization that is well suited for fall planted wheats in regions with mild winters.

Zone II is located in the middle of Zones I, III, and IV (Fig. 1). The frequency of winter cultivars planted in Zone II is lower than that in Zone I and higher than those in Zones III and IV. In general, cultivars from the northern part of Zone II have better winter hardiness or freezing resistance than cultivars from the southern part of Zone II (Zhuang, 2003). Winter wheats cannot be cultivated in Zones VI, VII, and VIII, where the average minimum temperature in January and February is too low. In these regions spring wheats are planted in spring to avoid the colder conditions (Wilsie, 1962). The length of growth periods in spring-sown regions (VI, VII, and VIII) is shorter than that in autumn-sown regions (Dong and Zheng, 2000; He et al., 2001; Zhuang, 2003). These characteristics may explain the high frequency of cultivars with the dominant *Vrn-A1* allele in these spring-sown regions, because this allele has the strongest insensitivity to vernalization, conferring a very early heading time that is essential for the adaptation to short growing seasons. Heading time can be further accelerated by the presence of multiple *Vrn* alleles. Lines with multiple *Vrn* alleles are frequent in Zone VI, which has the shortest growing season in China. This region is also the only one where the very early heading *Vrn-B3* allele from Hope was found. Interestingly, Hope is not presented in the pedigrees of Liaochun 10 and Longfumai 1 (Zhuang, 2003). In Zone X, the average January temperatures are very low, so spring cultivars are planted in spring and winter cultivars in autumn. Winter wheats can survive largely because of snowfall in this region.

The large differences in the frequencies of dominant vernalization alleles observed across the different agroecological regions suggest that these distributions are largely determined by environmental factors. Particularly important are the differences in average January temperatures and the length of growth periods among different zones. Cultivars with the most suitable vernalization genes are maintained through long-term natural selection and breeder selection in the wheat breeding programs. In addition to vernalization genes, other genes such as the photoperiod genes and the earliness per se genes play important roles in the determination of heading time in different cultivars. They are the reasons why distributions of growth habit and vernalization genotypes between Chinese and other countries' wheats are different (Stelmakh, 1998; Iwaki et al., 2000, 2001; Fu et al., 2005). The effect of dominant spring *Vrn-1* alleles on heading time in this study differs from that described by Stelmakh (1993). Stelmakh (1993) used three genetic backgrounds to study effects of *Vrn-1* genes on heading date in the field, showing that the *Vrn-B1* allele was associated with the latest heading time, *Vrn-A1* with the earliest, and *Vrn-D1* with intermediate values. The difference could be due to dissimilar genetic backgrounds of cultivars and environments. Therefore, further investigation is needed to understand the distribution of these associated genes and their interactions with the vernalization genes in determining the adaptability of wheat cultivars to the different agroecological regions.

Classification of Autumn-Sown Wheat Zones in China

A genetic definition of growth habit (winter vs. spring) of wheat cultivars on the basis of vernalization genotype is superior to the customary definition based on sowing

time (Crofts, 1989). Autumn-sown wheats in China are traditionally referred to as winter types (Jin, 1986, 1997; Zhuang, 2003), and, therefore, the classification of autumn-sown wheat zones was originally based on sowing time. Our results show that in addition to winter cultivars, Zone II includes spring cultivars with a residual vernalization requirement (presence of *Vrn-D1*). We also showed that the majority of modern cultivars released in Zones III and IV have at least one dominant *Vrn* allele and should be classified as spring type. These results confirmed previous observations from Chinese breeders (Zhuang, 2003). We propose to rename Zone II as the Yellow and Huai River Valley Autumn-Sown Winter and Spring Wheat Zone and Zones III and IV as the Middle and Lower Yangtze Valley Autumn-Sown Spring Wheat Zone, and the Southwestern Autumn-Sown Spring Wheat Zone, respectively. This view is supported by He et al. (2001).

In conclusion, growth habits and distribution of dominant vernalization alleles among various wheat zones in China were significantly different. All cultivars released in Zone I were winter types and carried recessive alleles at the four vernalization loci. In Zone II, both winter and spring cultivars were present, and the latter usually carried a single dominant *Vrn-D1* allele. In Zones III and IV, spring cultivars with the single dominant *Vrn-D1* allele were frequent. In spring-sown Zones VI, VII, and VIII, all cultivars were spring, and most of them carried the strongest dominant vernalization gene *Vrn-A1* plus other dominant gene(s). The distribution of growth habit and vernalization alleles in the different wheat zones of China were largely determined by the severity of the winter temperatures and the length of the growing season.

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Appendix

Table A1. Growth habit and allelic variation at the *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, and *Vrn-B3* loci in Chinese and certain introduced wheat (*Triticum aestivum* L.) cultivars.

Cultivar	Days to heading	*Vrn-A1*	*Vrn-B1*	*Vrn-D1*	*Vrn-B3*	Zone†/ Province
Beijing 8	#‡	R§	R	R	R	I/Beijing
Beijing 10	142	R	R	R	R	I/Beijing
Beijing 837	147	R	R	R	R	I/Beijing
Zhongmai 9	#	R	R	R	R	I/Beijing
Zhongyou 9507	#	R	R	R	R	I/Beijing
Lunxuan 987	#	R	R	R	R	I/Beijing
CA9722	186	R	R	R	R	I/Beijing
CA0045	#	R	R	R	R	I/Beijing
CA8686	147	R	R	R	R	I/Beijing
CA9632	118	R	R	R	R	I/Beijing
Jing 411	#	R	R	R	R	I/Beijing
Jing 9428	146	R	R	R	R	I/Beijing
Fengkang 2	#	R	R	R	R	I/Beijing
Fengkang 8	#	R	R	R	R	I/Beijing
Jingdong 6	#	R	R	R	R	I/Beijing
Jingdong 8	#	R	R	R	R	I/Beijing
Jingdong 10	142	R	R	R	R	I/Beijing
Nongda 139	163	R	R	R	R	I/Beijing
Nongda 183	163	R	R	R	R	I/Beijing
Nongda 3214	#	R	R	R	R	I/Beijing
Nongda 3291	129	R	R	R	R	I/Beijing
Dongfanghong 3	125	R	R	R	R	I/Beijing
Youmangbai 4	#	R	R	R	R	I/Beijing
Jingwang 10	130	R	R	R	R	I/Beijing
Jingshuang 16	#	R	R	R	R	I/Beijing
Early Piemium¶	#	R	R	R	R	I/Beijing
Suyin 10¶	134	R	R	R	R	I/Beijing
Jinmai 60¶	#	R	R	R	R	I/Shanxi
Longjian 196	#	R	R	R	R	I/Gansu
Xifeng 1	180	R	R	R	R	I/Gansu
Xifeng 16	>185	R	R	R	R	I/Gansu
Lantian 9	117	R	R	R	R	I/Gansu
Jimai 26	–#	R	R	D	R	II/Hebei
Jimai 30	59	R	R	D	R	II/Hebei
Jimai 36	69	R	R	R	R	II/Hebei
Jimai 38	–	R	R	R	R	II/Hebei
Shijiazhuang 8	#	R	R	R	R	II/Hebei
Shijiazhuang 407	181	R	R	R	R	II/Hebei
Shi 4185	74	R	R	D	R	II/Hebei
Han 4564	151	R	R	R	R	II/Hebei
Han 6172	#	R	R	R	R	II/Hebei
Heng 7228	55	R	R	D	R	II/Hebei
Youbaomai	132	R	R	R	R	II/Shandong
Taishan 1	106	R	R	R	R	II/Shandong
Taishan 4	>185	R	R	R	R	II/Shandong
Jinan 2	#	R	R	R	R	II/Shandong
Jinan 9	142	R	R	R	R	II/Shandong
Jinan 13	–	R	R	R	R	II/Shandong
Jinan 16	53	R	D	R	R	II/Shandong
Jinan 17	137	R	R	R	R	II/Shandong
Jimai 19	139	R	R	R	R	II/Shandong
Jimai 20	>185	R	R	R	R	II/Shandong
Lumai 1	#	R	R	R	R	II/Shandong
Lumai 7	–	R	R	D	R	II/Shandong
Lumai 14	130	R	R	R	R	II/Shandong
Lumai 15	127	R	R	R	R	II/Shandong
Lumai 21	136	R	R	R	R	II/Shandong
Lumai 22	124	R	R	R	R	II/Shandong
Lumai 23	97	R	R	R	R	II/Shandong
Shannongfu 63	#	R	R	R	R	II/Shandong
Yannong 15	124	R	R	R	R	II/Shandong
Yanyou 361	136	R	R	R	R	II/Shandong
Laizhou 953	100	R	R	R	R	II/Shandong
Zimai 12	137	R	R	R	R	II/Shandong
Weimai 8	90	R	R	R	R	II/Shandong
Huangxiandalibang-mang	>185	R	R	R	R	II/Shandong
Wendengbiansui	#	R	R	R	R	II/Shandong
Qida 195	#	R	R	R	R	II/Shandong
Youzimai	–	R	R	D	R	II/Henan
Pingyuan 50	92	R	R	D	R	II/Henan
Bainong 3217	74	R	R	D	R	II/Henan
Boai 7023	55	R	R	D	R	II/Henan
Neixiang 5	76	R	R	D	R	II/Henan
Neixiang 36	–	R	R	D	R	II/Henan
Zhengmai 9023	46	R	D	R	R	II/Henan
Xinmai 9408	50	R	R	R	R	II/Henan
Yumai 2	143	R	R	R	R	II/Henan
Yumai 7	47	R	R	D	R	II/Henan
Yumai 13	–	R	R	D	R	II/Henan
Yumai 17	74	R	R	D	R	II/Henan
Yumai 18	57	R	R	D	R	II/Henan
Yumai 21	135	R	R	R	R	II/Henan
Yumai 41	129	R	R	R	R	II/Henan
Yumai 49	–	R	R	R	R	II/Henan
Yumai 54	142	R	R	R	R	II/Henan
Yumai 66	74	R	R	R	R	II/Henan
Yumai 70	92	R	R	R	R	II/Henan
Zhoumai 18	142	R	R	R	R	II/Henan
St1472/506¶	–	R	R	D	R	II/Henan
Funo¶	47	R	R	D	R	II/Henan
Mazamai	104	R	R	D	R	II/Shaanxi
Bima 1	–	R	R	D	R	II/Shaanxi
Bima 4	>185	R	R	R	R	II/Shaanxi
Xinong 6028	69	R	R	D	R	II/Shaanxi
Xinong 1376	38	R	R	D	R	II/Shaanxi
Fengchan 3	109	R	R	D	R	II/Shaanxi
Aifeng 3	137	R	R	R	R	II/Shaanxi
Shaannong 7859	118	R	R	R	R	II/Shaanxi
Shaan 229	163	R	R	R	R	II/Shaanxi
Shaanyou225	#	R	R	R	R	II/Shaanxi
Xiaoyan 6	129	R	R	R	R	II/Shaanxi
Xiaoyan 22	#	R	R	R	R	II/Shaanxi
Xian 8	–	R	R	D	R	II/Shaanxi
Jinmai 33	#	R	R	R	R	II/Shanxi
Jinmai 45	#	R	R	R	R	II/Shanxi
Xuzhou 14	86	R	R	R	R	II/Jiangsu
Xuzhou 21	65	R	R	D	R	II/Jiangsu
Xuzhou 25	#	R	R	R	R	II/Jiangsu
Huaimai 16	135	R	R	R	R	II/Jiangsu
Huaimai 17	43	R	R	D	R	II/Jiangsu
Huaimai 18	72	R	R	D	R	II/Jiangsu
Huaimai 20	#	R	R	R	R	II/Jiangsu
Wanmai 18	135	R	R	R	R	II/Anhui
Wanmai 19	180	R	R	R	R	II/Anhui
Wanmai 38	#	R	R	R	R	II/Anhui
Wanmai 40	>185	R	R	R	R	II/Anhui

Table A1. Continued.

Cultivar	Days to heading	*Vrn-A1*	*Vrn-B1*	*Vrn-D1*	*Vrn-B3*	Zone†/ Province
Zhongliang 5	97	R	R	D	R	II/Gansu
Zhongliang 11	86	R	R	D	R	II/Gansu
Yangmai 1	44	R	R	D	R	III/Jiangsu
Yangmai 2	42	R	R	D	R	III/Jiangsu
Yangmai 3	57	R	R	D	R	III/Jiangsu
Yangmai 4	48	R	R	D	R	III/Jiangsu
Yangmai 5	42	R	R	D	R	III/Jiangsu
Yangmai 9	42	R	R	D	R	III/Jiangsu
Yangmai 10	45	R	R	D	R	III/Jiangsu
Yangmai 11	47	R	R	D	R	III/Jiangsu
Yangmai 12	47	R	R	D	R	III/Jiangsu
Yangmai 13	51	R	R	D	R	III/Jiangsu
Yangmai 158	42	R	R	D	R	III/Jiangsu
Wangshuibai	51	R	R	D	R	III/Jiangsu
Sumai 3	45	R	R	D	R	III/Jiangsu
Sumai 6	39	R	D	D	R	III/Jiangsu
Ningmai 9	46	R	R	D	R	III/Jiangsu
Nanjing 9981	49	R	R	D	R	III/Jiangsu
Mentana¶	42	R	R	D	R	III/Jiangsu
Shen 9204	47	R	R	D	R	III/Shanghai
Shen 32109	48	R	R	D	R	III/Shanghai
Jianmai 1	139	R	R	R	R	III/Jiangsu
Een 1	36	R	D	D	R	III/Hubei
Emai 6	91	R	R	R	R	III/Hubei
Emai 11	46	R	R	D	R	III/Hubei
Emai 12	51	R	R	D	R	III/Hubei
Emai 14	36	R	R	D	R	III/Hubei
Huamai 8	40	R	R	D	R	III/Hubei
Wumai 1	49	R	R	D	R	III/Hubei
Wanmai 33	42	R	D	R	R	III/Anhui
Wanmai 48	46	R	R	D	R	III/Anhui
Chengduguangtou	58	R	R	D	R	IV/Sichuan
Yaanzao	46	R	R	D	R	IV/Sichuan
Fan 6	49	R	D	D	R	IV/Sichuan
Fan 7	39	Db[4]	R	D	R	IV/Sichuan
Chuanmai 20	49	R	R	D	R	IV/Sichuan
Chuanmai 22	47	R	R	D	R	IV/Sichuan
Chuanmai 24	49	R	R	D	R	IV/Sichuan
Chuanmai 28	49	R	R	D	R	IV/Sichuan
Chuanmai 107	58	R	R	D	R	IV/Sichuan
Chuanyu 12	34	Db	D	D	R	IV/Sichuan
Chuanyu 14	41	R	D	D	R	IV/Sichuan
Mianyang 11	48	R	R	D	R	IV/Sichuan
Mianyang 15	45	R	D	D	R	IV/Sichuan
Mianyang 19	47	R	R	D	R	IV/Sichuan
Mianyang 20	48	R	R	D	R	IV/Sichuan
Mianyang 26	46	R	R	D	R	IV/Sichuan
Mianyang 28	52	R	R	D	R	IV/Sichuan
Mianyang 29	40	R	R	D	R	IV/Sichuan
Miannong 4	45	R	R	D	R	IV/Sichuan
Abbondanza¶	69	R	D	R	R	IV/Sichuan
Villa Glori¶	#	R	R	R	R	IV/Sichuan
Ardito¶	129	R	R	R	R	IV/Sichuan
Yimai 1	40	R	R	D	R	IV/Yunnan
Yunmai 39	52	R	R	D	R	IV/Yunnan
Yunmai 42	45	R	R	D	R	IV/Yunnan
Yunmai 46	47	R	D	R	R	IV/Yunnan
Fengmai 24	42	R	D	D	R	IV/Yunnan
Fengmai 27	39	R	D	D	R	IV/Yunnan
Fengmai 29	37	Da	R	D	R	IV/Yunnan
Yumai 3	44	R	D	R	R	IV/Yunnan
Demai 3	36	Da	D	R	R	IV/Yunnan
Demai 4	38	R	D	D	R	IV/Yunnan
Jingmai 10	47	R	D	R	R	IV/Yunnan
Jingmai 11	44	Db	D	R	R	IV/Yunnan
Orofen¶	52	R	D	R	R	IV/Yunnan
Kehan 6	36	Da	R	D	R	VI/Heilongjiang
Kefeng 3	37	Da	D	D	R	VI/Heilongjiang
Kefeng 6	36	Da	D	D	R	VI/Heilongjiang
Xinkehan 9	36	Da	R	D	R	VI/Heilongjiang
Longfumai 1	30	Da	D	R	D	VI/Heilongjiang
Longfumai 2	32	Da	D	R	R	VI/Heilongjiang
Longfumai 3	36	Da	R	R	R	VI/Heilongjiang
Longfumai 4	37	R	D	D	R	VI/Heilongjiang
Longfumai 5	37	Da	D	R	R	VI/Heilongjiang
Longfumai 8	38	Da	R	R	R	VI/Heilongjiang
Longfumai 9	41	Da	D	D	R	VI/Heilongjiang
Longfumai 10	43	Da	D	R	R	VI/Heilongjiang
Longfumai 12	32	Da	D	R	R	VI/Heilongjiang
Longfumai 13	38	Da	R	R	R	VI/Heilongjiang
Longfumai 14	38	Da	D	D	R	VI/Heilongjiang
Longmai 12	39	Da	D	R	R	VI/Heilongjiang
Longmai 15	35	Da	D	D	R	VI/Heilongjiang
Longmai 19	39	Da	D	D	R	VI/Heilongjiang
Longmai 20	36	Da	R	D	R	VI/Heilongjiang
Longmai 26	43	Da	D	R	R	VI/Heilongjiang
Longmai 30	37	Da	D	D	R	VI/Heilongjiang
Glenlea¶	37	Da	D	R	R	VI/Heilongjiang
Liaochun 10	31	Da	D	D	D	VI/Liaoning
Xiaobingmai 33	36	Da	D	R	R	VI/Jilin
Menghua 1	35	Da	D	R	R	VII/Inner Mongolia
Mengjian 2	36	Da	D	R	R	VII/Inner Mongolia
Mengmai 22	42	Da	D	R	R	VII/Inner Mongolia
Mengmai 28	36	Da	R	R	R	VII/Inner Mongolia
Neimai 19	35	Da	R	R	R	VII/Inner Mongolia
Bayou 1	37	Da	R	R	R	VII/Inner Mongolia
Zhongzuo 8131-1	36	Da	R	D	R	VII/Beijing
Jinqiang 1	36	Da	D	R	R	VII/Tianjin
Jinqiang 2	36	Da	D	R	R	VII/Tianjin
Dabaipi	42	Db	R	D	R	VII/Hebei
Banong 1	44	Da	R	D	R	VII/Hebei
Kangxuan 9	44	Da	R	R	R	VII/Hebei
Jizhangchun 5	40	R	D	D	R	VII/Hebei
Jinchun 4	33	Da	D	R	R	VII/Shanxi
Jinchun 9	34	Da	D	R	R	VII/Shanxi
Jinchun 12	33	Da	D	D	R	VII/Shanxi
Jinchun 14	37	Da	D	R	R	VII/Shanxi
Ganmai 8	49	Db	D	R	R	VIII/Gansu
Gan 630	36	Da	R	R	R	VIII/Gansu
Longchun 7	46	R	D	D	R	VIII/Gansu
Longchun 8	46	Db	D	R	R	VIII/Gansu
Longchun 9	46	R	D	D	R	VIII/Gansu
Longchun 20	42	Da	R	R	R	VIII/Gansu
Longchun 21	42	Db	D	R	R	VIII/Gansu
Longchun 22	47	R	D	R	R	VIII/Gansu
Longchun 8139	37	Da	D	R	R	VIII/Gansu
Dingxi 1	43	Da	D	R	R	VIII/Gansu

Table A1. Continued.

Cultivar	Days to heading	*Vrn-A1*	*Vrn-B1*	*Vrn-D1*	*Vrn-B3*	Zone†/ Province
Linmai 25	53	R	R	D	R	VIII/Gansu
Minqin 732	40	Da	D	R	R	VIII/Gansu
Weimai 1	39	Da	D	R	R	VIII/Gansu
CI 12203¶	46	Da	D	R	R	VIII/Gansu
Hongtu	34	Da	D	R	R	VIII/Ningxia
Doudi 1	36	R	D	R	R	VIII/Ningxia
Ningchun 4	36	Da	R	R	R	VIII/Ningxia
Ningchun 13	34	Da	D	R	R	VIII/Ningxia
Ningchun 16	33	Da	R	R	R	VIII/Ningxia
Ningchun 18	35	Da	R	R	R	VIII/Ningxia
Ningchun 19	36	Da	R	R	R	VIII/Ningxia
Ningchun 30	35	Da	D	R	R	VIII/Ningxia
Ningchun 37	37	R	D	R	R	VIII/Ningxia
Ningchun 38	36	Da	D	R	R	VIII/Ningxia
Ningchun 39	37	Da	D	R	R	VIII/Ningxia
Gaoyuan 602	39	Da	R	R	R	VIII/Qinghai
Qingchun 533	47	R	D	D	R	VIII/Qinghai
Xinchun 2	45	Da	R	R	R	X/Xinjiang
Xinchun 6	40	Da	R	R	R	X/Xinjiang
Xinchun 7	40	Da	D	R	R	X/Xinjiang
Xinchun 8	46	Da	R	R	R	X/Xinjiang
Xinchun 9	50	Db	D	D	R	X/Xinjiang
Xinchun 11	41	Da	R	R	R	X/Xinjiang
Xinchun 12	40	Da	D	R	R	X/Xinjiang
Xinchun 13	36	Da	D	R	R	X/Xinjiang
Xinchun 14	46	R	R	D	R	X/Xinjiang
Xinchun 15	37	Da	D	R	R	X/Xinjiang
Xinchun 16	35	Da	D	D	R	X/Xinjiang
Xinchun 17	40	Da	R	R	R	X/Xinjiang
Xinchun 18	39	Da	D	R	R	X/Xinjiang
Xindong 14	>185	R	R	R	R	X/Xinjiang
Xindong 15	#	R	R	R	R	X/Xinjiang
Xindong 16	132	R	R	R	R	X/Xinjiang
Xindong 18	>185	R	R	R	R	X/Xinjiang
Xindong 19	#	R	R	R	R	X/Xinjiang
Xindong 20	61	R	R	D	R	X/Xinjiang
Xindong 22	110	R	R	R	R	X/Xinjiang
Xindong 23	#	R	R	R	R	X/Xinjiang
Xindong 24	138	R	R	R	R	X/Xinjiang
Xindong 25	–	R	R	R	R	X/Xinjiang
Xindong 27	112	R	R	R	R	X/Xinjiang
Xindong 28	#	R	R	R	R	X/Xinjiang
Kuihua 1	#	R	R	R	R	X/Xinjiang
Kuidong 4	#	R	R	R	R	X/Xinjiang
Kavkaz¶	>185	R	R	R	R	X/Xinjiang

†Zone I = North China Plain Winter Wheat Zone, Zone II = Yellow and Huai River Valley Winter Wheat Zone, Zone III = Middle and Lower Yangtze Valley Winter Wheat Zone, Zone IV = Southwestern Winter Wheat Zone, Zone VI = Northeastern Spring Wheat Zone, Zone VII = Northern Spring Wheat Zone, Zone VIII = Northwestern Spring Wheat Zone, Zone X = Xinjiang Winter and Spring Wheat Zone.

‡Plants of the cultivar could not head and died from 125 to 185 d after planting in the greenhouse.

§R and D indicate recessive and dominant alleles at the *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, and *Vrn-B4* loci, respectively.

¶Introduced cultivar.

#Growth habit of the cultivar was not investigated in the greenhouse.

Table A2. Primer sequences, expected polymerase chain reaction (PCR) band sizes, and PCR conditions for detecting alleles at the *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, and *Vrn-B3* loci.

Locus	Allele	Primer pairs	Sequence (5'-3')	Expected band size	Annealing temperature	Extending time in each cycle	Reference
				bp	°C		
Vrn-A1	*Vrn-A1a* *Vrn-A1b* *Vrn-A1c* *vrn-A1*	VRN1AF VRN1-INT1R	GAAAGGAAAAATTCTGCTCG GCAGGAAATCGAAATCGAAG	965 + 876 714 734 734	50	1 min	Yan et al., 2004
	Vrn-A1c	Intr1/A/F2 Intr1/A/R3	AGCCTCCACGGTTTGAAAGTAA AAGTAAGACAACACGAATGTGAGA	1170	56	1 min 5 s	Fu et al., 2005
	vrn-A1	Intr1/C/F Intr1/AB/R	GCACTCCTAACCCACTAACC TCATCCATCATCAAGGCAAA	1068	58	1 min 5 s	Fu et al., 2005
Vrn-B1	*Vrn-B1*	Intr1/B/F Intr1/B/R3	CAAGTGGAACGGTTAGGACA CTCATGCCAAAAATTGAAGATGA	709	63	43 s	Fu et al., 2005
	vrn-B1	Intr1/B/F Intr1/B/R4	CAAGTGGAACGGTTAGGACA CAAATGAAAAGGAATGAGAGCA	1149	58	1 min 9 s	Fu et al., 2005
Vrn-D1	*Vrn-D1*	Intr1/D/F Intr1/D/R3	GTTGTCTGCCTCATCAAATCC GGTCACTGGTGGTCTGTGC	1671	65	1 min 30 s	Fu et al., 2005
	vrn-D1	Intr1/D/F Intr1/D/R4	GTTGTCTGCCTCATCAAATCC AAATGAAAAGGAACGAGAGCG	997	63	1 min	Fu et al., 2005
Vrn-B3	*Vrn-B3*	VRN4-B-INS-F VRN4-B-INS-R	CATAATGCCAAGCCGGTGAGTAC ATGTCTGCCAATTAGCTAGC	~1200	63	1 min 10 s	Yan et al., 2006
	vrn-B3	VRN4-B-NOINS-F VRN4-B-NOINS-R	ATGCTTTCGCTTGCCATCC CTATCCCTACCGGCCATTAG	~1140	57	1 min 5 s	Yan et al., 2006