Panhandle Research and Extension Center | Agricultural Research Division of IANR

2009

Genetic and Molecular Characterization of Vernalization Genes *Vrn-A1, Vrn-B1,* and *Vrn-D1* in Spring Wheat Germplasm from the Pacific Northwest Region of the U.S.A.

Dipak K. Santra
University of Nebraska-Lincoln, dsantra2@unl.edu

M. Santra
Washington State University

R. E. Allen
USDA-ARS, Pullman, WA

K. G. Campbell
USDA-ARS, Pullman, WA

K. K. Kidwell
Washington State University,

Follow this and additional works at: https://digitalcommons.unl.edu/panhandleresext

Part of the Agriculture Commons

Santra, Dipak K.; Santra, M.; Allen, R. E.; Campbell, K. G.; and Kidwell, K. K., "Genetic and Molecular Characterization of Vernalization Genes *Vrn-A1, Vrn-B1,* and *Vrn-D1* in Spring Wheat Germplasm from the Pacific Northwest Region of the U.S.A." (2009). *Panhandle Research and Extension Center.* 30.
https://digitalcommons.unl.edu/panhandleresext/30

Genetic and Molecular Characterization of Vernalization Genes *Vrn-A1*, *Vrn-B1*, and *Vrn-D1* in Spring Wheat Germplasm from the Pacific Northwest Region of the U.S.A.

D. K. SANTRA[1,3,4], M. SANTRA[1], R. E. ALLAN[2], K. G. CAMPBELL[2] and K. K. KIDWELL[1]

[1]Department of Crop and Soil Sciences, Washington State University, Pullman, WA 99164-6420, USA; [2]US Department of Agriculture, Agricultural Research Service, Wheat Genetics, Quality, Physiology and Disease Research Unit, Pullman, WA 99164-6420, USA; [3]Present address: Department of Agronomy and Horticulture, University of Nebraska-Lincoln Panhandle Research and Extension Center, 4502 Avenue I, Scottsbluff, NE 69361, USA; [4]Corresponding author, E-mail:dsantra2@unl.edu

Abstract

The objective of this study was to determine the *Vrn-1* allelic composition of spring wheat germplasm from the Pacific Northwest region of the USA. Individual plants from 56 spring wheat lines were crossed to near-isogenic tester lines carrying the dominant allele *Vrn-A1*, *Vrn-B1* or *Vrn-D1*. F_2 progeny were evaluated for growth habit in the field and *Vrn-1* allelic composition was determined through chi-square analysis. Lines also were analysed with DNA sequence-based *Vrn-1* allele-specific markers. A majority of the germplasm carried the dominant allele *Vrn-A1a* alone or in combination with *Vrn-B1*, *Vrn-D1* or *Vrn-B3* alleles. *Vrn-B1* and *Vrn-D1* were almost always associated with other dominant *Vrn-1* allele(s). Based on DNA sequence analysis, a novel *Vrn-B1* allele referred to as *Vrn-B1b*, which carried a single nucleotide polymorphism (SNP) and a 36 bp deletion, was identified in cultivar 'Alpowa'. These results will be useful to wheat breeders for choosing parents with different *Vrn-1* alleles for crossing to maximize diversity at the *Vrn-1* loci with an expectation of identifying superior *Vrn-1* allelic combinations for cultivar improvement.

Keywords: *Triticum aestivum* — near isogenic line — vernalization — spring growth habit — wheat breeding

Vernalization involves induction of flowering as a result of exposing wheat (*Triticum aestivum* L.) plants to temperatures between 4 and 6°C for 4–6 weeks (Porter and Gawith 1999). Major genes for vernalization (*Vrn*) and photoperiod (*Ppd*) response determine flowering habit in wheat. Winter-habit wheat genotypes require vernalization to flower, whereas spring types flower without vernalization either in the presence of *Ppd-1*, the gene for photoperiod insensitivity, or once the long day photoperiod requirement is met. In wheat, vernalization requirement is primarily controlled by alleles at three orthologous loci, *Vrn-A1*, *Vrn-B1* and *Vrn-D1,* which are located on the long arms of chromosomes 5A, 5B and 5D, respectively (Law et al. 1975, Galiba et al. 1995, Dubcovsky et al. 1998). Collectively, these three genes are referred to as *Vrn-1* loci. Dominant alleles of each locus are designated as *Vrn-A1*, *Vrn-B1*, and *Vrn-D1*, whereas the recessive alleles are designated as *vrn-A1*, *vrn-B1*, and *vrn-D1*. The presence of a dominant *Vrn-1* allele in any genome confers spring growth habit, whereas the presence of recessive alleles in the homozygous state across *Vrn-1* loci confers winter growth habit. *Vrn-A1* eliminates vernalization requirement, and is epistatic to *Vrn-B1* and *Vrn-D1*, both of which have small residual

vernalization requirements (Pugsley 1971). Additional vernalization-responsive genes, the *Vrn-2* series located on chromosomes 4B, 4D and 5A, *Vrn-B3* (located on chromosome 7BS) and *Vrn-D5* (located on chromosome 5D) have been identified in wheat (Goncharov 2003, Kato et al. 2003, Yan et al. 2006). These genes interact to bridge the link between responses to cold and day length to regulate plant development (Trevaskis et al. 2007). Allelic differences at *Vrn-1* loci are the most frequent source of variation in growth habit among all the *Vrn* genes identified in cultivated wheat.

In recent years, the *Vrn-1* alleles were cloned and characterized at the DNA sequence level (Yan et al. 2003, 2004, Fu et al. 2005). Various mutations in the *Vrn-1* gene resulted in expression of the dominant spring growth habit. At *Vrn-A1*, three distinct mutations were reported to confer spring growth habit: (i) insertions within the promoter region; (ii) deletions within the promoter region; and (iii) large deletions in intron 1. The dominant alleles at this locus, which are associated with promoter insertions, deletions and the deletion in intron1, are designated as *Vrn-A1a*, *Vrn-A1b*, and *Vrn-A1c*, respectively (McIntosh et al. 2007). Large deletions within intron 1 of the *Vrn-B1* and *Vrn-D1* genes also resulted in spring growth habit (Fu et al. 2005). Dominant alleles at these loci, which are associated with such mutations, are designated as *Vrn-B1* and *Vrn-D1* (McIntosh et al. 2007). Based on DNA sequence data, Polymerase chain reaction (PCR)-based markers were developed for each *Vrn-1* allele. Yan et al. (2004) developed DNA markers specific for *Vrn-A1a* and *Vrn-A1b* based on the presence of insertions or deletions in the *Vrn-A1* promoter. They identified *Vrn-A1a* in 55% and *Vrn-A1b* in 6% of 132 spring wheat genotypes from Argentina, Canada, and the U.S.A. The *Vrn-A1*a allele also was present in a high proportion of spring cultivars from China (Zhang et al. 2008). Fu et al. (2005) developed DNA markers, specific for the *Vrn-A1c*, *vrn-A1*, *Vrn-B1*, *vrn-B1*, *Vrn-D1*, and *vrn-D1* alleles, based on the presence or absence of deletions in intron 1 in the dominant and recessive alleles, respectively. These markers were used to determine *Vrn-1* allelic compositions of 117 spring wheat genotypes from Argentina and California. *Vrn-A1a/b* was the predominant allele, either alone or in combination with other *Vrn-1* alleles, followed by *Vrn-B1* and *Vrn-D1*. *Vrn-A1c* was not identified in any of the 117 genotypes. Iqbal et al. (2007a) identified *Vrn-A1a* in 34 of 40

Canadian spring wheat genotypes, followed by *Vrn-B1* in 20 genotypes and *Vrn-A1b* in one genotype. *Vrn-A1c* and *Vrn-D1* were not identified in any of the 40 genotypes.

Vernalization and photoperiod response genes play significant roles in geographical adaptation and yield potential of wheat cultivars (Gororo et al. 2001). Several reports have been published concerning *Vrn-1* allelic composition of wheat germplasm and its effect on grain yield. Stelmakh (1990, 1998) analysed a global collection of 647 spring wheat genotypes, and reported that *Vrn-A1* was present alone or in combination with *Vrn-B1* and/or *Vrn-D1* in 81% of the genotypes tested. *Vrn-D1* was the predominant allele in spring wheat genotypes adapted to regions closest to the equator. In another survey of European genotypes, Stelmakh (1993) reported that the average grain yield per plant across environments was highest for spring wheat genotypes with *Vrn-A1* and/or *Vrn-B1*, whereas cultivars with all three dominant alleles (*Vrn-A1 Vrn-B1 Vrn-D1*) gave the lowest grain yields. If temperature and/or moisture stress occurred during grain filling, the highest grain yields were reported for photoperiod insensitive wheat genotypes with *Vrn-D1* in combination with either *Vrn-A1* or *Vrn-B1*. van Beem et al. (2005) concluded that *Vrn-D1* was the most frequent allele in globally important CIMMYT wheat genotypes, adapted to tropical and sub-tropical regions. Iqbal et al. (2007b) reported that the predominance of *Vrn-A1a* in Canadian spring wheat genotypes resulted in early maturity, permitting avoidance of late-season frost damage during the short growing season in northern, high latitudes. However, a grain yield penalty was detected for early maturing spring wheat cultivars (Iqbal et al. 2007b). Clearly, *Vrn*-allelic composition is involved in regional adaptation and agronomic performance of wheat genotypes. Therefore, optimizing *Vrn-1* allelic composition for specific production environments may offer possibilities for developing spring wheat cultivars with higher yield potential.

Most of the annual precipitation in the Pacific Northwest (PNW) region of the USA. occurs during the winter. This region also is characterized by high latitude and a short growing season. Winter wheat is the primary non-irrigated field crop grown in the PNW, and has higher grain yield potential than spring wheat grown in the same region. However, spring wheat is a valuable component of conservation tillage systems for the region (Schillinger and Young 2004). The economic viability of spring wheat production would be enhanced if grain yield potential was increased; this might be achieved by manipulating allelic composition at the *Vrn-1* loci. Breeders often use diverse germplasm in crosses as donors of genes for disease resistance and other useful traits. Knowledge of the most appropriate *Vrn-1* allelic combinations in spring wheat genotypes for maximizing yield potential adapted to the PNW will allow breeders to more rapidly select the best *Vrn-1* allelic combination using either marker-assisted selection or phenotypic selection. The objective of this study was to determine the *Vrn-1* allelic composition of elite, adapted spring wheat cultivars and advanced breeding lines from the PNW that are used as parents for cultivar development through genetic segregation and DNA marker analyses.

Materials and Methods

Plant materials: Fifty-six elite spring wheat cultivars and advanced breeding lines from Washington State University (24 entries), University of Idaho (18), Oregon State University (eight) and two private

breeding companies [WestBred, LLC (five) and Northrup King (one)] that were submitted for field testing in the 1994 Tri-State Nursery were evaluated (Table 1). This germplasm will be referred to as 'lines' in the remainder of this report. Triple Dirk D (*Vrn-A1 vrn-B1 vrn-D1*), Triple Dirk B (*vrn-A1 Vrn-B1 vrn-D1*), and Triple Dirk E (*vrn-A1 vrn-B1 Vrn-D1*), near isogenic lines (NILs) for *Vrn-A1*, *Vrn-B1* and *Vrn-D1*, respectively, developed from the cultivar 'Triple Dirk' (Pugsley 1972), were used as homozygous testers to cross with each line to develop F$_2$ populations for genetic segregation analysis. Individual plants from

Table 1: Fifty-six spring wheat lines evaluated for *Vrn-1* allelic composition by genetic segregation and/or DNA marker analyses

Line (accession no.[1])	Market class[2]	Origin/source
Alpowa (PI566596)	SWS	Washington State University
Calorwa (PI566594)	Club	
Edwall (PI477919)	SWS	
Penawawa (PI495916)	SWS	
Spillman (PI506350)	HRS	
Urquie (CItr17413)	SWS	
Wadual 94 (PI566595)	SWS	
Wakanz (PI506352)	SWS	
Wampum (CItr17691)	HRS	
Wawawai (PI574598)	SWS	
WA7764	HRS	
WA7766 (PI574537)	SWS	
WA7778	HWS	
WA7780	SWS	
WA7798	HRS	
WA7799	HRS	
WA7800	HRS	
WA7803	SWS	
WA7804	SWS	
WA7805	SWS	
WA7806	SWS	
WA7807	SWS	
WA7808	SWS	
WA7809	SWS	
Centennial (PI537303)	SWS	University of Idaho
Copper (PI502644)	HRS	
Fielder (CItr17268)	SWS	
Idaho 377s (PI591045)	HWS	
Owens (CItr17904)	SWS	
Pomerelle (PI592983)	SWS	
Treasure (PI468962)	SWS	
Whitebird (PI592982)	SWS	
ID461	HRS	
ID464	HRS	
ID470	HWS	
ID471	SWS	
ID489	HRS	
ID490	HWS	
ID491	HRS	
ID493	HWS	
ID495	SWS	
ID496	SWS	
ORS85010	HRS	Oregon State University
OR488348	HWS	
OR488528	HWS	
O4895019	HRS	
O4895103	HRS	
OR490041	HRS	
OR491028	HRS	
OR492002	HRS	
Express (PI573003)	HRS	WestBred, LLC
Sprite (PI337605)	SWS	
Vanna (PI587199)	SWS	
Westbred 926	HRS	
Westbred 936 (PI587200)	HRS	
Klasic (PI486139)	HWS	Northrup King

[1]Accession numbers are listed when available.
[2]Club, soft white spring club; HRS, hard red spring; HWS, hard white spring; SWS, soft white spring.

each of the three Triple Dirk NILs were crossed to three plants from each of the 56 spring lines. F_1 plants were grown in the greenhouse and F_2 seed was harvested separately from 2 to 5 F_1 plants per line × tester combination.

Field experiment: When available, 200 seeds of each F_2 family in each line × tester combination were planted in a plot consisting of 4 rows that were 6 m in length with 20 cm between rows. Up to four different F_2 families were evaluated per cross. Because of an unusually cold, wet spring, the planting date was 7 June, 1996, at Spillman Agronomy Farm, Pullman. Stand counts were recorded on June 18 and 27, 1996, and phenotypic data for growth habit type was collected 97 days after planting.

Genetic segregation analysis: Expected segregation ratios for growth habit among individuals within F_2 families differed depending on the allelic compositions at *Vrn-1* loci of the parents. Each line was assigned to one of 11 genotypes. If the parents shared the same *Vrn-1* allele, all F_2 progenies were expected to be spring type. To detect a single plant with winter growth habit with an expected segregation ratio of 63 : 1 ($P \geq 0.05$), 191 F_2 plants needed to be evaluated; 47 plants were required to recover one winter type when the expected ratio was 15 : 1 ($P \geq 0.05$) (Sedcole 1977). Chi-square analysis was conducted to determine the likelihood of agreement between observed and expected phenotypic ratios. Homogeneous data within cross combinations were pooled.

DNA marker analysis: Leaf tissues from 10 seedlings per line were pooled and genomic DNA was isolated using the CTAB method (Anderson et al. 1992). We used *Vrn-A1* allele-specific markers based on promoter or intron 1 mutations described by Yan et al. (2004) and Fu et al. (2005). PCR primers were synthesized by MWG-Biotech (High Point, NC, USA) and PCR was conducted according to Yan et al. (2004) and Fu et al. (2005). Triple Dirk *Vrn*-NILs were included as controls. *Vrn-B3* allele-specific marker analysis was conducted following Zhang et al. (2008). PCR amplified products were separated in 2% agarose gels using 0.5× TBE buffer and visualized under UV transillumination. *Vrn-1* and *Vrn-B3* allelic identities were determined by comparing the size of the amplified DNA bands with the size of the bands expected for each *Vrn-1* allele. Amplification experiments were repeated to confirm allelic composition results.

Determination of *Vrn-1* allelic frequencies: The frequency of a dominant allele at a particular *Vrn-1* locus was determined using only those lines where results for both DNA marker and genetic segregation analyses were identical. The frequencies of *Vrn-1* allelic haplotypes were determined using only those lines where results for both DNA marker and genetic segregation analyses were identical across the three *Vrn-1* loci.

Cloning and sequencing of *Vrn-B1* allele specific DNA marker: The DNA band amplified by the *Vrn-B1* allele-specific primer pair was gel-purified using the Wizard® SV Gel (Cat. No. A9281; Promega Corp., Madison, WI, USA) and PCR Clean-Up System, and the ligation reaction was set up using pGEM®-T Easy vector (Cat. no. A1120; Promega Corp.). Transformation was performed by electroporation using ElectroMAXDH10B™ cells (Cat. no. 18290-015; Invitrogen Corp., Carlsbad, CA, USA). Recombinant plasmids were extracted from cultured white colonies selected on LB/ampicillin/IPTG/X-Gal plates and plasmids were purified using Wizard® *Plus* SV Minipreps DNA Purification System (Cat. no. A1330; Promega Corp.) followed by quantification with DNA spectrophotometry. Insertion of the PCR product into the plasmid was verified by restriction enzyme digestion with *Eco*RI or *Not*I (New England BioLabs, Inc., Ipswich, MA 01938, USA) and subsequent analysis by agarose gel electrophoresis (data not shown). Four independent recombinant plasmids were sequenced at the Molecular Biology Core Facility at Washington State University on an ABI Prism 377 DNA sequencer using the dideoxy sequencing

method (Applied Biosystems, Foster City, CA, USA). Sequence data for *Vrn-B1* and *vrn-B1* from Triple Dirk B and Triple Dirk C, respectively, from the NCBI database (http://www.ncbi.nlm.nih.gov/Genbank/index.html), were used for sequence comparisons with BLAST (http://www.ncbi.nlm.nih.gov/blast).

Nomenclature of the dominant *Vrn-A1* allele: Genetic segregation analysis using *Vrn*-NILs was used to determine whether the *Vrn-1* alleles were dominant or recessive. Possible dominant alleles at the *Vrn-A1* locus included *Vrn-A1a*, *Vrn-A1b* or *Vrn-A1c* depending on the presence of the specific mutations, which could be determined only through allele-specific DNA marker analysis. Therefore, Triple Dirk D (*Vrn-A1*) was used as the tester to identify and designate any of the three possible dominant *Vrn-A1* alleles in genetic segregation results. For DNA marker analysis, primers specific for *Vrn-A1a*, *Vrn-A1b* or *Vrn-A1c* were used to determine which specific dominant *Vrn-A1* allele was detected.

Results
Vrn-1 allelic compositions based on genetic segregation

Vrn-1 allele composition was determined ($P \geq 0.05$) for 35 of the 56 lines from segregation analysis of growth habit (Table 2). Because of flooding of part of the field we were unable to obtain segregation analyses on the other 21 F_2 populations. By pooling data across families within line x tester combinations, progeny sizes of the 35 F_2 populations were adequate for acceptable statistical tests. A *Vrn-A1* allele was present in 29 of the 35 lines, 10 of which carried the *Vrn-A1* alone, and 19 carried the allele in combination with other dominant *Vrn* alleles. The remaining six lines carried the recessive *vrn-A1* allele. The dominant *Vrn-B1* allele was not present alone in any of the 35 lines; however, it was identified in combination with other dominant *Vrn-1* alleles in 11 lines. The recessive *vrn-B1* allele was present in 24 lines. The dominant *Vrn-D1* allele was found alone in two lines, and was identified in combination with other dominant *Vrn* alleles in nine lines. The recessive *vrn-D1* allele was present in 24 lines. Only two lines, Calorwa and WA7807, carried all three dominant *Vrn-1* alleles.

Segregation ratios revealed the presence of an unknown dominant *Vrn* allele in nine lines. We temporarily designated this dominant allele as *Vrn-X*. Presumably it was one of the other known vernalization genes (e.g. *Vrn-B3* or *Vrn-D4*). Six lines carried *Vrn-X* in combination with *Vrn-A1* and three lines carried *Vrn-X* with *Vrn-D1*. *Vrn-B3* allele-specific marker analysis was conducted on these nine lines to determine if *Vrn-X* could be *Vrn-B3*. The *Vrn-X* allele in five lines (Fielder, Idaho 377s, Pomerelle, Whitebird, and Vanna) was *Vrn-B3*.

Vrn-1 allelic compositions based on DNA marker analysis

All 56 spring wheat lines were evaluated using PCR-based *Vrn-1* allele-specific markers. Prior to evaluating the lines, we verified the specificity of these PCR primers for each *Vrn-1* allele using the Triple Dirk *Vrn*-NILs. All PCR primers amplified bands in each of the Triple Dirk *Vrn*-NILs as expected, from the original reports (data not shown). Allelic identity results based on DNA marker analysis of the 35 lines for which we obtained genetic segregation data (presented in Table 2) were compared, and lines for which genetic segregation and marker analysis results at *Vrn-1* loci did not align are presented in Table 3. *Vrn-1* allelic compositions of the 21 lines determined solely on DNA marker analysis are given in Table 4.

Table 2: Segregation for growth habit in F_2 populations from crosses of spring wheat lines and *Vrn-1* near isogenic line (NIL) testers

Line	NIL tester	No. F_1 families	No. spring types	No. winter types	P (15 : 1)	P (63 : 1)	Allelic composition[1]
Calorwa	*Vrn-A1*	4	376	0	0.00	0.01	Vrn-A*1*
	Vrn-B1	4	578	0	0.00	0.00	Vrn-B*1*
	Vrn-D1	4	352	0	0.00	0.02	*Vrn-D1*
Urquie	*Vrn-A1*	4	333	0	0.00	0.02	*Vrn-A1*
	Vrn-B1	3	120	7	0.73	0.00	*vrn-B1*
			105	12	0.07	0.00	
			82	5	0.85	0.00	
	Vrn-D1	4	322	25	0.46	0.00	*vrn-D1*
Wawawai[2]	*Vrn-A1*	3	315	0	0.00	0.03	*Vrn-A1*
	Vrn-B1	1	98	4	0.33	0.05	*vrn-B1*
	Vrn-D1	4	410	24	0.54	0.00	*vrn-D1*
WA7764	*Vrn-A1*	4	519	0	0.00	0.00	*Vrn-A1*
	Vrn-B1	2	202	16	0.51	0.00	*vrn-B1*
	Vrn-D1	4	460	25	0.32	0.00	*vrn-D1*
WA7778	*Vrn-A1*	4	555	0	0.00	0.00	*Vrn-A1*
	Vrn-B1	4	521	0	0.00	0.00	*Vrn-B1*
	Vrn-D1	3	344	8	0.00	0.28	*vrn-D1*
WA7780[2]	*Vrn-A1*	4	485	5	0.00	0.33	*vrn-A1*
	Vrn-B1	4	454	10	0.00	0.30	*vrn-B1*
	Vrn-D1	4	465	13	0.00	0.04	*Vrn-D1*
							Vrn-X
WA7799	*Vrn-A1*	4	390	0	0.00	0.01	*Vrn-A1*
	Vrn-B1	4	391	36	0.06	0.00	*vrn-B1*
	Vrn-D1	3	272	20	0.67	0.00	*vrn-D1*
WA7800	*Vrn-A1*	4	493	0	0.00	0.01	*Vrn-A1*
	Vrn-B1	3	397	0	0.00	0.01	*Vrn-B1*
	Vrn-D1	4	381	10	0.00	0.11	*vrn-D1*
WA7804[2]	*Vrn-A1*	4	498	0	0.00	0.00	*Vrn-A1*
	Vrn-B1	3	392	11	0.00	0.06	*vrn-B1*
	Vrn-D1	4	566	0	0.00	0.00	*Vrn-D1*
WA7805[2]	*Vrn-A1*	4	508	0	0.00	0.00	*Vrn-A1*
	Vrn-B1	4	520	10	0.00	0.55	*vrn-B1*
	Vrn-D1	4	387	11	0.00	0.05	*vrn-D1*
							Vrn-X
WA7806[2]	*Vrn-A1*	3	282	0	0.00	0.03	*Vrn-A1*
	Vrn-B1	3	295	11	0.06	0.00	*vrn-B1*
	Vrn-D1	2	222	8	0.08	0.02	*vrn-D1*
WA7807[2]	*Vrn-A1*	4	450	0	0.00	0.01	*Vrn-A1*
	Vrn-B1	3	342	0	0.00	0.02	*Vrn-B1*
	Vrn-D1	4	358	0	0.00	0.02	*Vrn-D1*
Centennial	*Vrn-A1*	4	527	0	0.00	0.00	*Vrn-A1*
	Vrn-B1	4	524	0	0.00	0.00	*Vrn-B1*
	Vrn-D1	4	428	12	0.00	0.05	*vrn-D1*
Copper[2]	*Vrn-A1*	4	470	0	0.00	0.01	*Vrn-A1*
	Vrn-B1	3	388	16	0.06	0.00	*vrn-B1*
	Vrn-D1	2	219	15	0.92	0.00	*vrn-D1*
Fielder[2]	*Vrn-A1*	3	462	0	0.00	0.01	*Vrn-A1*
	Vrn-B1	4	476	3	0.00	0.10	*vrn-B1*
	Vrn-D1	3	374	7	0.00	0.67	*vrn-D1*
							Vrn-X[3]
Idaho 377s[2]	*Vrn-A1*	4	660	0	0.00	0.00	*Vrn-A1*
	Vrn-B1	4	556	3	0.00	0.05	*vrn-B1*
	Vrn-D1	4	529	8	0.00	0.86	*vrn-D1*
							Vrn-X[3]
Owens	*Vrn-A1*	4	569	0	0.00	0.00	*Vrn-A1*
	Vrn-B1	4	569	0	0.00	0.00	*Vrn-B1*
	Vrn-D1	2	279	3	0.00	0.50	*vrn-D1*
Pomerelle[2]	*Vrn-A1*	4	450	0	0.00	0.01	*Vrn-A1*
	Vrn-B1	4	382	8	0.00	0.44	*vrn-B1*
	Vrn-D1	3	321	4	0.00	0.63	*vrn-D1*
							Vrn-X[3]
Treasure[2]	*Vrn-A1*	3	370	0	0.00	0.02	*Vrn-A1*
	Vrn-B1	3	223	14	0.83	0.00	*vrn-B1*
	Vrn-D1	4	448	24	0.30	0.00	*vrn-D1*
Whitebird[2]	*Vrn-A1*	4	525	0	0.00	0.00	*Vrn-A1*
	Vrn-B1	2	142	4	0.08	0.25	*vrn-B1*
	Vrn-D1	3	242	5	0.01	0.56	*vrn-D1*
							Vrn-X[3]
ID461	*Vrn-A1*	4	541	0	0.00	0.00	*Vrn-A1*
	Vrn-B1	3	368	16	0.09	0.00	*vrn-B1*
	Vrn-D1	4	127	5	0.24	0.04	*vrn-D1*
			140	10	0.83	0.00	
			33	5	0.08	0.00	

Table 2: Continued

Line	NIL tester	No. F_1 families	No. spring types	No. winter types	P (15 : 1)	P (63 : 1)	Allelic composition[1]
			61	4	0.97	0.00	
ID489[2]	Vrn-A1	4	415	0	0.00	0.01	Vrn-A1
	Vrn-B1	3	310	22	0.78	0.00	vrn-B1
	Vrn-D1	2	226	11	0.31	0.00	vrn-D1
ID493	Vrn-A1	4	543	0	0.00	0.00	Vrn-A1
	Vrn-B1	3	306	0	0.00	0.03	Vrn-B1
	Vrn-D1	3	419	6	0.00	0.80	vrn-D1
ID496[2]	Vrn-A1	4	496	0	0.00	0.01	Vrn-A1
	Vrn-B1	4	501	12	0.00	0.16	vrn-B1
	Vrn-D1	2	222	3	0.00	0.78	vrn-D1
							Vrn-X
ORS85010[2]	Vrn-A1	3	410	0	0.00	0.01	Vrn-A1
	Vrn-B1	4	510	11	0.00	0.31	vrn-B1
	Vrn-D1	2	248	0	0.00	0.05	Vrn-D1
OR488528	Vrn-A1	2	289	5	0.00	0.85	vrn-A1
	Vrn-B1	2	297	0	0.00	0.03	Vrn-B1
	Vrn-D1	4	543	0	0.00	0.00	Vrn-D1
O4895019	Vrn-A1	3	322	0	0.00	0.02	Vrn-A1
	Vrn-B1	4	591	0	0.00	0.00	Vrn-B1
	Vrn-D1	3	330	6	0.00	0.74	vrn-D1
O4895103	Vrn-A1	4	586	0	0.00	0.00	Vrn-A1
	Vrn-B1	4	531	25	0.09	0.00	vrn-B1
	Vrn-D1	2	259	16	0.77	0.00	vrn-D1
OR491028	Vrn-A1	4	632	0	0.00	0.00	Vrn-A1
	Vrn-B1	3	423	0	0.00	0.01	Vrn-B1
	Vrn-D1	4	589	7	0.00	0.44	vrn-D1
OR492002	Vrn-A1	4	486	29	0.56	0.00	vrn-A1
	Vrn-B1	4	125	7	0.65	0.00	vrn-B1
			123	9	0.79	0.00	
			110	4	0.23	0.09	
			84	15	0.00	0.00	
	Vrn-D1	4	472	0	0.00	0.01	Vrn-D1
Express	Vrn-A1	4	114	3	0.10	0.38	vrn-A1
			106	5	0.45	0.01	
			89	10	0.11	0.00	
			84	5	0.81	0.00	
	Vrn-B1	3	368	30	0.29	0.00	vrn-B1
	Vrn-D1	4	327	0	0.00	0.02	Vrn-D1
Sprite[2]	Vrn-A1	3	291	9	0.02	0.04	vrn-A1
	Vrn-B1	3	223	6	0.02	0.20	vrn-B1
	Vrn-D1	3	260	0	0.00	0.04	Vrn-D1
							Vrn-X
Vanna[2]	Vrn-A1	2	250	4	0.00	0.99	vrn-A1
	Vrn-B1	3	365	7	0.00	0.62	vrn-B1
	Vrn-D1	4	482	0	0.00	0.01	Vrn-D1
							Vrn-X[3]
Westbred 936	Vrn-A1	4	525	0	0.00	0.00	Vrn-A1
	Vrn-B1	4	480	0	0.00	0.01	Vrn-B1
	Vrn-D1	4	521	3	0.00	0.07	vrn-D1
Klasic[2]	Vrn-A1	4	547	0	0.00	0.00	Vrn-A1
	Vrn-B1	2	325	4	0.00	0.61	vrn-B1
	Vrn-D1	4	531	0	0.00	0.00	Vrn-D1

Homogeneous results for F_1 families were pooled.
[1] *Vrn-A1* could be *Vrn-A1a*, *Vrn-A1b* or *Vrn-A1c*, which can be distinguished only by marker analysis.
[2] Lines for which genetic segregation data did not agree with the prediction by allele-specific polymerase chain reaction analysis.
[3] *Vrn-X* was identified as *Vrn-B3* based on allele-specific marker analyses.

DNA marker vs. genetic segregation results in 35 genotypes

Vrn-1 allelic compositions based on genetic segregation and DNA marker analyses across the three *Vrn-1* loci (*Vrn-A1*, *Vrn-B1* and *Vrn-D1*) (data not shown) were identical for 17 lines. The results were not consistent for *Vrn-B1* or *Vrn-D1* loci in the remaining 18 lines (Table 3). Among the 17 lines with consistent results from genetic segregation and DNA marker analyses, *Vrn-A1a* was present in 13 lines, *Vrn-A1b* in one line, and *vrn-A1* was identified in three lines; *Vrn-B1* and *vrn-B1* were identified in 10 and 7 lines, respectively, whereas *Vrn-D1* was detected in four lines and *vrn-D1* was identified in the remaining 13 lines.

For the 18 lines with inconsistent results, *Vrn-1* allelic identities based on genetic segregation and DNA marker analyses were considered separately for each *Vrn-1* locus. At *Vrn-A1*, allelic identity results based on both analyses matched in all 18 lines (Table 3). Alleles *Vrn-A1a* and *vrn-A1* were identified in 15 and 3 lines, respectively.

At *Vrn-B1*, allelic identity results based on both analyses matched only for WA7807 and Klasic. Among 16 lines, *Vrn-B1* was identified in 13 lines, and three lines (WA7804, Copper and ID489) appeared to carry both *Vrn-B1* and *vrn-B1* based on DNA marker analysis; however, based on genetic segregation data all 16 lines carried *vrn-B1* (Table 3). The *Vrn-B1*

Table 3: Genotypes of 18 wheat accessions for which phenotypic segregation data and predictions based on marker analysis did not match

Genotype	*Vrn-A1* allele composition based on		*Vrn-B1* allele composition based on		*Vrn-D1* allele composition based on	
	Marker analysis	Genetic analysis	Marker analysis	Genetic analysis	Marker analysis	Genetic analysis
Wawawai	*Vrn-A1a*	*Vrn-A1a*	***Vrn-B1***	***vrn-B1***	*vrn-D1*	*vrn-D1*
WA7780	*vrn-A1*	*vrn-A1*	***Vrn-B1***	***vrn-B1***	*Vrn-D1*	*Vrn-D1*
WA7804	*Vrn-A1a*	*Vrn-A1a*	***Vrn-B1, vrn-B1***	***vrn-B1***	*Vrn-D1*	*Vrn-D1*
WA7805	*Vrn-A1a*	*Vrn-A1a*	***Vrn-B1***	***vrn-B1***	*vrn-D1*	*vrn-D1*
WA7806	*Vrn-A1a*	*Vrn-A1a*	***Vrn-B1***	***vrn-B1***	*vrn-D1*	*vrn-D1*
WA7807	*Vrn-A1a*	*Vrn-A1a*	*Vrn-B1*	*Vrn-B1*	***Vrn-D1, vrn-D1***	***Vrn-D1***
Copper	*Vrn-A1a*	*Vrn-A1a*	***Vrn-B1, vrn-B1***	***vrn-B1***	*vrn-D1*	*vrn-D1*
Fielder	*Vrn-A1a*	*Vrn-A1a*	***Vrn-B1***	***vrn-B1***	*vrn-D1*	*vrn-D1*
Idaho 377s	*Vrn-A1a*	*Vrn-A1a*	***Vrn-B1***	***vrn-B1***	*vrn-D1*	*vrn-D1*
Pomerelle	*Vrn-A1a*	*Vrn-A1a*	***Vrn-B1***	***vrn-B1***	***Vrn-D1, vrn-D1***	***Vrn-D1***
Treasure	*Vrn-A1a*	*Vrn-A1a*	***Vrn-B1***	***vrn-B1***	*vrn-D1*	*vrn-D1*
Whitebird	*Vrn-A1a*	*Vrn-A1a*	***Vrn-B1***	***vrn-B1***	*vrn-D1*	*vrn-D1*
ID489	*Vrn-A1a*	*Vrn-A1a*	***Vrn-B1, vrn-B1***	***vrn-B1***	*vrn-D1*	*vrn-D1*
ID496	*Vrn-A1a*	*Vrn-A1a*	***Vrn-B1***	***vrn-B1***	***Vrn-D1***	***vrn-D1***
ORS85010	*Vrn-A1a*	*Vrn-A1a*	***Vrn-B1***	***vrn-B1***	*Vrn-D1*	*Vrn-D1*
Sprite	*vrn-A1*	*vrn-A1*	***Vrn-B1***	***vrn-B1***	*Vrn-D1*	*Vrn-D1*
Vanna	*vrn-A1*	*vrn-A1*	***Vrn-B1***	***vrn-B1***	*Vrn-D1*	*Vrn-D1*
Klasic	*Vrn-A1a*	*Vrn-A1a*	*vrn-B1*	*vrn-B1*	***Vrn-D1, vrn-D1***	***Vrn-D1***

Differences are shown in bold script.

Table 4: Allelic identities at the *Vrn-1* loci based on DNA marker prediction for 21 spring wheat lines for which phenotypic segregation data were not available

Line	*Vrn-A1* allele	*Vrn-B1* allele	*Vrn-D1* allele
Alpowa	*vrn-A1*	*Vrn-B1b*[1]	*vrn-D1*
Edwall	*Vrn-A1b*	*Vrn-B1*	*Vrn-D1, vrn-D1*
Penawawa	*Vrn-A1b*	*Vrn-B1*	*vrn-D1*
Spillman	*Vrn-A1a*	*vrn-B1*	*vrn-D1*
Wadual 94	*Vrn-A1a*	*vrn-B1*	*Vrn-D1*
Wakanz	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*
Wampum	*Vrn-A1a*	*vrn-B1*	*vrn-D1*
WA7766	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*
WA7798	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*
WA7803	*Vrn-A1a*	*Vrn-B1*	*Vrn-D1*
WA7808	*vrn-A1*	*Vrn-B1*	*Vrn-D1*
WA7809	*Vrn-A1a*	*Vrn-B1*	*Vrn-D1*
ID464	*Vrn-A1a*	*vrn-B1*	*vrn-D1*
ID470	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*
ID471	*Vrn-A1b*	*Vrn-B1*	*vrn-D1*
ID490	*Vrn-A1b*	*Vrn-B1*	*vrn-D1*
ID491	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*
ID495	*Vrn-A1b*	*Vrn-B1*	*vrn-D1*
OR488348	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*
OR490041	*vrn-A1*	*vrn-B1*	*Vrn-D1*
Westbred 926	*Vrn-A1a*	*vrn-B1*	*vrn-D1*

[1]New allele.

allele-specific marker (PCR-amplified 709 bp fragment) was sequenced in 12 of the 13 lines and the resulting sequences matched the published sequence. This indicated that the DNA marker results were correct, and suggested that genotypes deduced from the genetic segregation results were incorrect for these lines. As leaf tissues from 10 individual seedlings of each of the three lines (WA7804, Copper and ID489) were bulked for DNA isolation for the original PCR analyses, individual seedlings from each of the three lines were analysed. All three lines were genetically heterogeneous for the *Vrn-B1* locus.

In the case of the *Vrn-D1* locus, allelic identity results based on both DNA marker and genetic segregation analyses matched in 14 lines; among them, five lines carried *Vrn-D1* and nine lines carried *vrn-D1*. Marker analysis revealed that ID496 carried *Vrn-D1*, whereas genetic analysis indicated that

the *vrn-D1* allele was present. The PCR fragment amplified by the *Vrn-D1* allele-specific marker in ID496 was sequenced, and it matched the published sequence. The remaining three lines carried both *Vrn-D1* and *vrn-D1* based on marker analysis (Table 3). Analyses of 10 seedlings in each of these three lines confirmed genetic heterogeneity. *Vrn-D1* allelic composition obtained from DNA marker and genetic segregation analyses agreed for 31 of the 34 lines.

DNA marker analysis results for 21 lines

Vrn-1 allelic compositions of 21 lines were determined solely from DNA marker analysis (Table 4). *Vrn-A1a*, *Vrn-A1b*, and *vrn-A1* were identified in 12, six, and three lines, respectively. *Vrn-B1* was identified in 14 lines and *vrn-B1* was present in six lines. A unique *Vrn-B1* allele identified in cultivar 'Alpowa' will be discussed below. Based on marker fragment size, the *Vrn-D1* and *vrn-D1* alleles were detected in five and 15 lines, respectively, whereas Edwall carried both *Vrn-D1* and *vrn-D1*. Marker analyses of individual seedlings showed that Edwall was heterogeneous at this locus.

Vrn-1 allele frequencies in 35 spring wheat lines

Vrn-A1a was the most frequent allele present in spring wheat lines developed by the three universities, whereas *Vrn-D1* was the most frequent allele in lines developed by private breeding companies. *Vrn-D1* was absent in the lines developed by the University of Idaho. When lines from all four programmes were considered together, *Vrn-A1a* was the most frequent allele, followed by *Vrn-B1* and *Vrn-D1*.

We analysed *Vrn-1* allelic haplotypes in 17 of 35 lines for which the *Vrn-1* allelic composition results from DNA marker and genetic segregation analyses were identical. Six of seven possible haplotypes of *Vrn-1* alleles were identified. The most frequent haplotype 'Vrn-A1a/b Vrn-B1 vrn-D1' was identified in eight lines followed by 'Vrn-A1a/b vrn-B1 vrn-D1' in five lines. Each of the four remaining haplotypes was identified in one line from each of the four breeding programmes. The haplotype 'vrn-A1 Vrn-B1 vrn-D1' was not detected in any of the 17 lines.
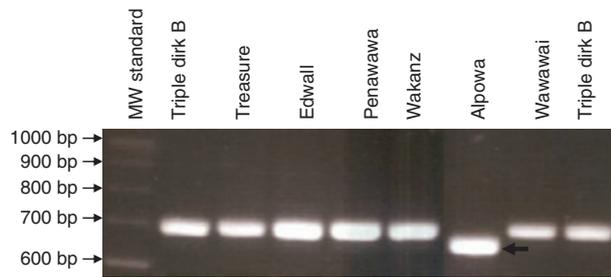
Fig. 1: Polymerase chain reaction amplification with *Vrn-B1* allele-specific primers Intr1/B/F and Intr1/B/R3 in Triple Dirk B and six spring wheat lines. The amplified DNA product in 'Alpowa' was smaller than the amplified products in other lines

Novel *Vrn-B1* allele identified in 'Alpowa'

A fragment, smaller than the predicted 709 bp for *Vrn-B1* from Triple Dirk B was detected in 'Alpowa' (Fig. 1). Ten individual seedlings of 'Alpowa' were evaluated to confirm the presence of this smaller DNA band. We cloned and sequenced the DNA band amplified by the *Vrn-B1* allele-specific primers in 'Alpowa' to determine its exact size and sequence differences relative to the band amplified by Triple Dirk B. The band from 'Alpowa' was 673 bp in length. A SNP (G–C) at position 1656 and a 36 bp deletion from 1661 to 1696 bp was identified within the 673 bp fragment from 'Alpowa' when aligned with that from Triple Dirk B. We designated this new allele as *Vrn-B1b* and the sequence of this allele was deposited in GeneBank (http://www.ncbi.nlm.nih.gov/Genbank/index.html; accession number FJ766015). The 36 bp deletion was located 314 bp downstream of the large deletion in intron 1 (1341–8190 bp), in the *Vrn-B1a* (previously, *Vrn-B1*) sequence for Triple Dirk C (Fig. 2).

Discussion
Discrepancies in *Vrn-1* allelic composition

Vrn-1 allelic compositions of wheat lines in previous reports were based on genetic segregation or DNA marker analyses. Here, we determined *Vrn-1* allelic combinations from both genetic segregation and DNA marker analyses for 35 lines. An additional 21 lines were genotyped by DNA markers. Nine lines had a *Vrn* gene that was not a *Vrn-1* allele. The gene in five of the lines was identified as *Vrn-B3* by marker analyses; the gene in the remaining four lines was not identified.

Vrn-1 allele-specific markers used in our study were developed from known DNA sequence information. Therefore, we expected the DNA markers to be 100% diagnostic for allele composition at the *Vrn-1* loci. We conclusively determined the allelic compositions at *Vrn-A1*, *Vrn-B1* and *Vrn-D1* loci for 35, 19 and 31 lines, respectively. However, there were inconsistencies in results between genetic segregation and marker analyses for 13 lines at *Vrn-B1* and for one line at *Vrn-D1*. The possibility that these inconsistencies resulted from alternative mutations in *Vrn-B1* and *Vrn-D1* alleles that disrupted function was excluded by comparison of sequence data of the allele-specific PCR-amplified DNA fragments from the lines with that of the cloned genes. It is more likely that growth habit was inaccurately phenotyped because of the late June planting date, which reduced the likelihood of satisfying residual vernalization requirements of the *Vrn-B1* and *Vrn-D1* alleles or epistatic interactions among *Vrn-1* genes (Pugsley 1972, Stelmakh 1993). Some progeny possessing *Vrn-B1* or *Vrn-D1* as heterozygotes might have been inaccurately scored as winter growth habit because of the delayed flowering in the very short growing season of that particular year.

Of the 56 lines evaluated in this study, 16 were previously characterized for *Vrn-1* allele identity exclusively based on marker analyses (Yan et al. 2004, Fu et al. 2005), and our results aligned with previously reported results in 15 of 16 cases. For the cultivar 'Calorwa', we identified *Vrn-A1a*, *Vrn-B1* and *Vrn-D1*, whereas Fu et al. (2005) reported *Vrn-A1a*, *vrn-B1* and *vrn-D1*. As different seed sources were used the possibility of genetic heterogeneity between sources cannot be excluded.

Heterogeneity at *Vrn-1* loci

We detected heterogeneity within several lines at *Vrn-1* loci based on marker analyses of 10 individual seedlings from each population, whereas genetic segregation analysis on three plants failed to detect such heterogeneity. Although wheat cultivars are often assumed to be homozygous and homogeneous because of the self-pollinating nature of the species, residual heterogeneity within cultivars is highly likely because of the selection strategies used in early population advancement (Knott 1987). Detection of such heterogeneity based on genetic segregation analysis is difficult when phenotypic variation is not obvious. Functional markers are useful for detecting such heterogeneities as many individuals can easily be analysed in an efficient manner. Therefore, to accurately determine *Vrn-1* alleles at the cultivar population level it is important to analyse individual seedlings in instances where bulk DNA samples are heterogeneous for the marker.

Vrn-1 allelic composition and spring wheat cultivar improvement

Frequencies of dominant *Vrn-1* alleles were different in spring wheat lines developed by different breeding programmes in the
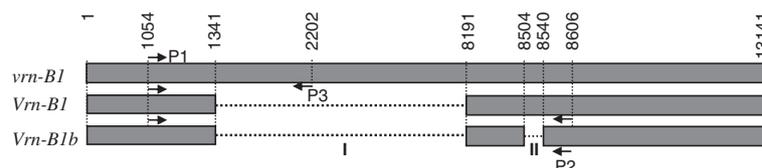

Fig. 2: Schematic representation of *vrn-B1* from Triple Dirk C, *Vrn-B1a* from Triple Dirk B, and *Vrn-B1b* from 'Alpowa'. The *Vrn-B1* allele-specific primer pair Intr1/B/F (P1) and Intr1/B/R3 (P2) amplified 709 bp and 673 bp bands in Triple Dirk B and 'Alpowa', respectively. The *vrn-B1* allele-specific primer pair Intr1/B/F (P1) and Intr1/B/R4 (P3) amplified a 1149 bp band in Triple Dirk C. The large deletion in intron 1 (I) and the novel 36 bp deletion in *Vrn-B1b* (II) are indicated by dashed lines. Sequences of *vrn-B1* (Accession no. AY747604.1) and *Vrn-B1* (AY747603.1) were from the NCBI database (http://www.ncbi.nlm.nih.gov/Genbank/index. html). Nucleotide numbers are based on the sequence of *vrn-B1*

PNW. *Vrn-A1a* was the most frequent allele, either alone, or in combination with other *Vrn* alleles in lines from three of the four programmes, whereas *Vrn-B1* and *Vrn-D1* were rarely found alone, agreeing with other reports (Stelmakh 1998, Yan et al. 2004, Fu et al. 2005, Iqbal et al. 2007a). Higher frequencies of *Vrn-D1* were found in lines developed by private companies and at OSU compared with those from WSU and UI. This may be related to the greater use of parental lines from CIMMYT by private companies and OSU. CIMMYT lines have a high frequency of *Vrn-D1* (van Beem et al. 2005). The presence of the dominant *Vrn-A1a/b* allele results in complete elimination of vernalization, whereas the presence of *Vrn-B1* or *Vrn-D1* allele alone is associated with some residual vernalization response and later flowering (Stelmakh 1993, 1998). Spring wheat lines carrying *Vrn-A1a/b* together with *Vrn-B1* may provide optimum flowering time and yield potential in PNW production environments as this combination of dominant *Vrn-1* alleles was the most frequently identified in this study. However, spring wheat lines carrying *Vrn-D1*, which causes late flowering, may have a yield advantage in production environments like southern Idaho and Oregon where the growing season is longer. Our results will be useful to wheat breeders for identifying parents carrying different *Vrn-1* alleles and for generating genetic diversity at these loci with an expectation of identifying superior *Vrn-1* allele combinations for spring cultivars targeted to specific production environments.

Novel *Vrn-B1* allele

We did not find the *Vrn-B1* allele alone in any of the 56 spring wheat lines evaluated, supporting previous reports (Stelmakh 1990, 1998). However, we identified a novel *Vrn-B1* allele, *Vrn-B1b*, in the cultivar 'Alpowa'. This novel allele was associated with a SNP (G–C), a unique small deletion within intron 1, and a large deletion shared with V*rn-B1a* (Fu et al. 2005). As the nucleotide at this SNP site was the same in both *Vrn-B1* of Triple Dirk B (spring growth habit) and v*rn-B1* of Triple Dirk C (winter growth habit), it is unlikely that the change is associated with vernalization response. The unique 36 bp deletion (8504–8540 bp) detected in *Vrn-B1b* (Fig. 2) is likely to result in spring growth habit type as 'Alpowa' carries winter habit alleles v*rn-A1* and v*rn-D1*. It would be interesting to test whether *Vrn-B1b* influences flowering time in a different manner to other *Vrn-B1* alleles.

Acknowledgements

References

Anderson, J. A., Y. Ogihara, M. E. Sorrells, and S. D. Tanksley, 1992: Development of chromosomal arm map for wheat based on RFLP markers. Theor. Appl. Genet. **83,** 1035—1043.

van Beem, I., V. Mohler, R. Lukman, M. van Ginkel, M. William, J. Crossa, and A. J. Worland, 2005: Analysis of genetic factors influencing the developmental rate of globally important CMMYT wheat cultivars. Crop Sci. **45,** 2113—2119.

Dubcovsky, J., D. Lijavetzky, L. Appendino, and G. Tranquilli, 1998: Comparative RFLP mapping of *Triticum monococcum* genes controlling vernalization requirement. Theor. Appl. Genet. **97,** 968—975.

Fu, D., P. Szucs, L. Yan, M. Helguera, J. S. Skinner, J. von Zitzewitz, P. M. Hayes, and J. Dubcovsky, 2005: Large deletions within the first intron in *VRN-1* are associated with spring growth habit in barley and wheat. Mol. Gen. Genomics. **273,** 54—65.

Galiba, G., S. A. Quarrie, J. Sutka, and A. Morgounov, 1995: RFLP mapping of the vernalization (*Vrn1*) and frost resistance (*Fr1*) genes on chromosome 5A of wheat. Theor. Appl. Genet. **90,** 1174—1179.

Goncharov, N. P., 2003: Genetics of growth habit (spring vs winter) in common wheat: confirmation of the existence of dominant gene *Vrn4*. Theor. Appl. Genet. **107,** 768—772.

Gororo, N. N., R. G. Flood, R. F. Eastwood, and H. A. Eagles, 2001: Photoperiod and vernalization responses in *T. turgidum* x *T. taushii* synthetic hexaploid wheat. Ann. Bot. **88,** 947—952.

Iqbal, M., A. Navabi, R. C. Yang, D. F. Salmon, and D. Spaner, 2007a: Molecular characterization of vernalization response genes in Canadian spring wheat. Genome **50,** 511—516.

Iqbal, M., A. Navabi, D. F. Salmon, R. C. Yang, B. M. Murdoch, S. S. Moore, and D. Spaner, 2007b: Genetic analysis of flowering and maturity time in high latitude spring wheat. Euphytica **154,** 207—218.

Kato, K., M. Yamashita, K. Ishimoto, H. Yoshino, and M. Fujita, 2003: Genetic analysis of two genes for vernalization response, the former *Vrn2* and *Vrn4*, by using PCR based molecular markers. In: N. E. Pogna, M. Romano, E. A. Pogna, and G. Galterio (eds), Proceedings of the 10th International Wheat Genetics Symposium, 971—973. Instituto Sperimentale, Rome.

Knott, D. R., 1987: The application of breeding procedure to wheat. In: Heyne. EG (eds), Wheat and Wheat Improvement, 2nd edn, 419—427. American Society of Agronomy, Madison, WI.

Law, C. N., A. J. Worland, and B. Giorgi, 1975: The genetic control of ear emergence time by chromosomes 5A and 5D of wheat. Heredity **36,** 49—58.

McIntosh, R. A., K. M. Devos, J. Dubcovsky, W. J. Rogers, C. F. Morris, R. Appels, D. J. Sommers, and O. A. Anderson, 2007: Catalogue of gene symbols for wheat (supplement) Available at: http://wheat.pw.usda.gov/GG2/maps.html. (accessed on 5 February 2009).

Porter, J. R., and M. Gawith, 1999: Temperatures and the growth and development of wheat: a review. Eur. J. Agron. **10,** 23—36.

Pugsley, A. T., 1971: A genetic analysis of spring-winter habit of growth in wheat. Aust. J. Agric. Res. **22,** 21—31.

Pugsley, A. T., 1972: Additional genes inhibiting winter habit in wheat. Euphytica **21,** 547—552.

Schillinger, W. F., and D. L. Young, 2004: Cropping systems research in the world's driest rainfed wheat region. Agron. J. **96,** 1182—1187.

Sedcole, J. R., 1977: Number of plants necessary to recover a trait. Crop Sci. **17,** 667—668.

Stelmakh, A. F., 1990: Geographic distribution of *Vrn* genes in landraces and improved varieties of spring bread wheat. Euphytica **45,** 113—118.

Stelmakh, A. F., 1993: Genetic effects of *Vrn* genes on heading date and agronomic traits in bread wheat. Euphytica **65,** 53—60.

Stelmakh, A. F., 1998: Genetic systems regulating flowering response in wheat. Euphytica **100,** 359—369.

Trevaskis, B., M. N. Hemming, E. S. Dennis, and W. J. Peacock, 2007: The molecular basis of vernalization-induced flowering in cereals. Trends Plant Sci. **12,** 351—357.

Yan, L., A. Loukojanov, G. Tranquillo, M. Helguera, T. Fahima, and J. Dubcovsky, 2003: Positional cloning of wheat vernalization gene *VRN1*. Proc. Natl Acad. Sci. USA **100,** 6263—6268.

Yan, L., M. Helguera, K. Kato, S. Fukuyama, J. Sherman, and J. Dubcovsky, 2004: Allelic variation at the VRN-1 promoter in polyploidy wheat. Theor. Appl. Genet. **109,** 1677—1686.

Yan, L., D. Fu, C. Lin, A. Blechl, G. Tranquilli, M. Bonafede, A. Sanchez, M. Valarik, and J. Dubcovsky, 2006: The wheat and barley vernalization gene *VRN3* is an orthologue of *FT*. Proc. Natl Acad. Sci. USA **103,** 19581—19586.

Zhang, X. K., X. C. Xia, Y. G. Xiao, J. Dubcovsky, and Z. H. He, 2008: Allelic variation at the vernalization genes *Vrn-A1*, *Vrn-B1*, *Vrn-D1* and *Vrn-B3* in Chinese common wheat cultivars and their association with growth habit. Crop Sci. **48,** 458—470.