Molecular Characterization of Vernalization and Photoperiod Genes in Wheat Varieties from Different Agro-climatic Zones of India

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Ninety-nine wheat cultivars from six different agro-climatic zones of India were analyzed for the *Vrn-1, Vrn-2, Vrn-B3, Vrn-4* and *Ppd-D1* composition with DNA sequenced based allele specific or linked markers for the above-mentioned genes. A majority of the germplasm carried the dominant *Vrn-A1a* allele alone or in combination with *Vrn-B1* and *Vrn-D1*. The three dominant genes were cumulatively present in 30 cultivars among all the zones, whereas double dominant combination, *Vrn- A1/Vrn-B1* was identified in 18 cultivars, *Vrn-A1/Vrn-D1* in 6 cvs and *Vrn-B1/Vrn-D1* in 16 cvs. The combination of the dominant alleles of all three genes was most frequent in cvs of Northern Western Plains Zone. Northern Hill Zone had *vrn-B1* and *vrn-D1* alleles in higher proportions compared to the dominant alleles *Vrn-B1* and *Vrn-D1* indicating successful spring/winter wheat cross breeding. All of the cvs had the recessive *Vrn-B3* allele. Most of the cvs had photoperiod insensitive allele in all the zones and only 9% cvs possessed the photoperiod sensitive allele *(b)* of the *Ppd-D1* gene. This information will be useful in selecting parental lines for crossing to maximize diversity at these loci and for future molecular marker assisted breeding for cultivar improvement.

Keywords: diagnostic markers, photoperiod genes, spring wheat, vernalization genes

Introduction

Wheat is the most widely grown crop encompassing different geographic regions ranging from 67º North to 45º South (Kilian et al. 2009). Of the several genes that have played a significant role in adaptation and yield potential of wheat across different regions, vernalization and photoperiod response genes are proposed to be the most important (Stelmakh 1998; Gororo et al. 2001). Vernalization (exposure of wheat plants to temperatures between 4 and 6 ºC for 4–6 weeks) is required for induction of flowering. Wheat plants can be classified as photoperiod sensitive if they require long days for flowering induction or photoperiod insensitive if they can flower irrespective of the day length. Photoperiod influences the expression of genes responsible for developmental changes to flowering stimulus. Agronomically, most wheat cultivars are divided into two distinct groups: win-

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ter and spring types. Both groups may manifest sensitivity or insensitivity to photoperiod. Winter habit wheat genotypes flower after low temperature exposure is given and spring-types genotypes flower without vernalization either in the presence of photoperiod insensitivity genes or after the photoperiod requirement is met. The genes for vernalization (known as *Vrn*) along with others (photoperiod sensitivity genes known as *Ppd* and earliness *per se* known as *Eps*) are the major genes involved in growth and development phases, i.e. tillering, stem elongation, heading, anthesis and maturity (Dubcovsky et al. 1998). Winter habit is considered to be ancestral to spring habit (Flood and Halloran, 1986) and the winter allele of *Vrn-A1* is considered to be ancestral to the spring alleles.

Genes for vernalization and photoperiod requirement are known for a long time. There have been different terminologies and gene designations for the genes involved in vernalization response. Precisely, vernalization is known to be controlled by four major series of genes designated *Vrn-1* (including the *Vrn-A1, Vrn-B1* and *Vrn-D1* genes located on the long arms of chromosomes 5A, 5B and 5D, respectively), *Vrn-2* (consisting of genes located on chromosomes 4B, 4D and 5A), *Vrn-3* consists of three genes *Vrn-A3, Vrn-B3* and *Vrn-D3; Vrn-3* (consisting of genes *Vrn-A3* and *Vrn-B3* and *Vrn-D3*; *Vrn-B3* located on chromosome 7BS (Law and Worland 1997) which has been identified as and FT-like gene (Yan et al. 2006), as have its homologoues *Vrn-A3* and *Vrn-D3* on 7AS and 7DS, respectively (Bonnin et al. 2008), and *Vrn-4* (or *Vrn-D4* located on chromosome 5D). The role of these *VRN* genes in regulating flowering, their structure and homology with those of *Arabidopsis* genes have been reviewed in Distelfeld et al. (2009). Current models of flowering in the temperate cereals suggest that before vernalization, *Vrn-3* is repressed by *Vrn-2*. Long exposures to cold temperature result in the up-regulation of *Vrn-1* and down regulation of *Vrn-2* in the leaves. As the spring approaches, the *Vrn-3* levels are up regulated (a process mediated by photoperiod genes) which travels from leaves to shoot apex and increases the transcription of *Vrn-1* above threshold levels for induction of flowering. Photoperiod response in wheat is similarly found under the control of three orthologous genes (*Ppd1* now *Ppd-D1, Ppd2* now *Ppd-B1* and *Ppd3* now *Ppd-A1*as per the nomenclature proposed by McIntosh et al. 2003) located on the short arm of chromosome 2A, 2B and 2D respectively (Law et al. 1978). The dominant allele of the genes *Ppd-A1a, Ppd-B1a* and *Ppd-D1a* confer insensitivity of wheat to day length while the recessive alleles (*Ppd-A1b, Ppd-B1b* and *Ppd-D1b*) confer sensitivity (Worland et al. 1998). Potency of the insensitivity of these genes has been ranked in the order of *Ppd-D1 > Ppd-B1 > Ppd-A1*.

The first three series of genes have been identified using map-based cloning approaches and validated using mutants and transgenic plants (Yan et al. 2003, 2004, 2006). The allelic differences at *Vrn-1* loci are the most frequent sources of variation in growth habit amongst all these vernalization genes (Santra et al. 2009). Yan et al. (2004) proposed that the three alleles viz., *Vrn-A1a, A1b* and *A1c* have resulted due to insertions within promoter region, deletion within promoter region and large deletions in the intron 1, respectively, of the *Vrn-A1* gene. Based on their DNA sequence data, polymerase chain reaction based markers were also developed for alleles of each *Vrn-1* loci (Yan et al. 2004; Fu et al. 2005). Yan et al. (2006) also developed diagnostic markers for alleles of the gene *Vrn-B3*

while linked markers have been identified for the gene *Vrn-4* (Yoshida et al. 2010). Beales et al. (2007) developed diagnostic markers for *Ppd-D1* that enable to detect variation within *Ppd-D1* locus. Using these markers, a large number of spring and winter habit wheat genotypes grown in different regions around the world have been screened (Fu et al. 2005; Yan et al. 2006; Iqbal et al. 2007a; Zhang et al. 2008; Andeden et al. 2011; Yang et al. 2011; Nowak and Kowalczyk et al. 2011; Milec et al. 2012).

Wheat is the second most important cereal crop in India and is grown under short day conditions. The country is divided into six agroclimatic zones (Nothern Hills Zone NHZ, Nothern Western Plains Zone NWPZ, Nothern Eastern Plains Zone NEPZ, Central Zone CZ, Peninsular Zone PZ and Sothern Hill Zone SHZ) each with somewhat specific climate for wheat growth and four major sowing conditions such as the timely sown irrigated/rainfed/restricted irrigated, late sown irrigated. The food demand is expected to be more than doubled by 2050. Introgression of adaptability and yield contributing genes from different sources is, therefore, very much needed to overcome the yield plateau being observed in varieties developed in current wheat breeding programmes. Winter wheats have a great potential to contribute to thermotolerance and moisture stress as they possess various desirable attributes. Many winter wheats show a stay-green habit even at receding moisture levels and high temperatures, which can be utilized in breeding programmes in India (Nanda and Sohu 1998). The successful release of Indian varieties containing genes of Veery, Atilla, Pastor, Baviacora etc. developed by CIMMYT, are among the best examples of utilization of winter wheat gene pool. However, information on the distribution of *Vrn-1* and *Ppd* alleles in Indian wheat germplasm is scanty (Yang et al. 2009; Eagles et al. 2009, 2011). Due to this, many spring/winter crosses have to be discarded as they lead to progenies that are not adapted and have vernalization requirement. Thus, the knowledge of the allelic distribution of vernalization and photoperiod genes can help accelerate the introgression by predicting the best combinations for enhanced yield potential and adaptation in Indian climate. In this paper, we report the distribution of the alles of five vernalization gene (*Vrn-A1, Vrn-B1, Vrn-D1, Vrn-B3* and *Vrn-D4*) and *Ppd-D1* gene in 99 cvs of India while the information on quality genes in these cultivars is reported elsewhere (Singh et al. 2012).

Materials and Methods

Plant material

Ninety-nine wheat cultivars released by the central varietal release committee and notified by the Indian Gazette were included in this study. They represent the varieties released for the six agro-climatic zones over two and a half decades starting from 1985 to 2011. A brief description of the genotypes is given in Table S1*. All the genotypes were raised in the field during 2009–10 and 2010–11 at Division of Genetics, IARI. Australian cv Janz, among others with published *Vrn* profile (Eagles et al. 2009) and earlier used in a small re-

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* Further details about the Electronic Supplementary Material (ESM) can be found at the end of the article.

search activity in the Indo-Australian program on marker assisted wheat breeding (Prog no. CIM/2010/014) was used as a positive control for different *Vrn* alleles along with molecular weight standard DNA ladder. Gabo, another Australian line was used as a positive control for *Vrn-D4* analysis.

DNA extraction and PCR analysis

Individual plants were raised in glass house from seeds of a head-to-row grown line. Leaf tissue from five such one-month-old plants was pooled and total genomic DNA was extracted by CTAB method as described by Murray and Thompson (1980). The gene specific primers for the characterisation of vernalization and photoperiod alleles were synthesized as described by Fu et al. (2005) and Zhang et al. (2008) and Beales et al. (2007) for *Vrn* genes (Table 1). PCR amplifications were carried out following Yan et al. (2004) and Fu et al. (2005) with minor modifications. For photoperiod alleles, primer combinations and amplification conditions as described by Beales et al. (2007) were followed. For multiplexing, PCR was carried out using primers of the dominant and recessive alleles of a gene following Eagles et al. (2010). Amplification products were resolved on 2% Agarose gel using 1 × TBE buffer, stained with Ethidium bromide and visualised on Gel Documentation System (G-Box, SYNGENE Synoptics, USA) under UV transillumination. Amplification products were scored in relation to molecular weight standard 100 bp DNA ladder Plus (MBI Fermentas) for presence/absence/size of bands.

The cvs were planted in two rows of 2.5 m each with 25 cm spacing between rows on 16th November of each sowing year. Data was recorded on days to heading when heads emerged from 50% of the culms in the field and on height of three plants from each variety on maturity during 2009–10 and 2010–11 crop seasons and averaged over the two years.

Results

Allelic variation at Vrn loci

Ppd-D1 and *Vrn* alleles in the cultivars were deduced following multiplex PCR (Figs 1a and 1b) and primer pairs reported by Eagles et al. (2010). The description of individual genotypes possessing a particular allelic combination is depicted in Table S1 and the zonewise distribution of alleles of the six genes is given in Table 2. Majority of the cultivars carried either single or combination of any of the dominant *Vrn-A1, Vrn-B1* and *Vrn-D1* alleles and having a spring growth habit. Among the 99 cultivars, 57 cultivars carried *Vrn-A1a* allele alone and 18 carried other alleles of *Vrn-A1* gene. *Vrn-A1b* allele was found only in 3 varieties and 39 varieties carried the recessive *vrn-A1*allele.

The dominant *Vrn-B1* allele amplicon size (709 bp) and was observed in 70 cultivars. The remaining 29 cultivars had recessive allele *(vrn-B1)* with 1149 bp product. We did not observe the other dominant allele in any of the cultivars. Sixty-nine cultivars produced a 1670 bp PCR product, demonstrating the dominant *Vrn-D1* allele and 30 cultivars produced 997 bp fragment indicative of recessive allele *(vrn-D1)*. The dominant and

Table 1. Molecular markers for phenological traits used in the present study

Allelic designation	Marker name	Primer pair sequence (5'–3')	PCR conditions (Anneling temp. & extension time)	Amplicon size (bp)	References
Vrn-A1a/A1b /A1c/vrn-A1	VRN1AF VRN-INT1R	GAAAGGAAAAATTCTGCTCG GCAGGAAATCGAAATCGAAG	50ºC , 1 min.	965+876, 714,734,734	Yan et al. 2004 Zhang et al. 2008
Vrn-A1c	Intr1/A/F2 Int1/A/R3	AGCCTCCACGGTTTGAAAGTAA AAGTAAGACAACACGAATGTGAGA	56ºC, 1 min. 5 s	1170	Fu et al. 2005 Zhang et al. 2008
vrn-A1	Intr1/C/F Int1/AB/R	GCACTCCTAACCCACTAACC TCATCCATCATCAAGGCAAA	58ºC, 1 min. 5 s	1068	Fu et al. 2005 Zhang et al. 2008
Vrn-A1a/b/c	BT468-F BT486-R	GGCTATCAGGTGGTTGGGTGAGGAC TGGGGCATCGTGTGGCTG	66ºC, 20 s	*vrn-A1*~ 200, *Vrn-A1a* ~ 400, *Vrn-A1b*~ 180	Eagles et al. 2010
Vrn-B1	Intr1/B/F Int1/B/R3	CAAGTGGAACGGTTAGGACA CTCATGCCAAAAATTGAAGATGA	63ºC, 43 s,	*Vrn-B1a* (709) *Vrn-B1b* (673)	Fu et al. 2005 Zhang et al. 2008
vrn-B1	Intr1/B/F Int1/B/R4	CAAGTGGAACGGTTAGGACA CAAATGAAAAGGAATGAGAGCA	58ºC, 1 min 9 s	1149	Fu et al. 2005 Zhang et al. 2008
Vrn-D1	Intr1/D/F Int1/D/R3	GTTGTCTGCCTCATCAAATCC GGTCACTGGTGGTCTGTGC	65ºC, 1 min 30 s	1671	Fu et al. 2005 Zhang et al. 2008
vrn-D1	Intr1/D/F Int1/D/R4	GTTGTCTGCCTCATCAAATCC AAATGAAAAGGAACGAGAGCG	63ºC, 1 min	997	Fu et al. 2005 Zhang et al. 2008
Vrn-B3	VRN4-B-INS-F VRN4-B-INS-R	CATAATGCCAAGCCGGTGAGTAC ATGTCTGCCAATTAGCTAGC	63ºC, 1 min 10s	~1200	Yan et al. 2006 Zhang et al. 2008
vrn-B3	VRN4-B-NOINS-F VRN4-B-NOINS-R	ATGCTTTCGCTTGCCATCC CTATCCCTACCGGCCATTAG	57ºC, 1 min 5s	~1140	Yan et al. 2006 Zhang et al. 2008
Ppd-D1a	*Ppd-D1-F* *Ppd-D1a-R1* *Ppd-D1a-R2*	ACGCCTCCCACTACACTG GTTGGTTCAAACAGAGAGC CACTGGTGGTAGCTGAGATT	55ºC, 30 s	D1b -414 D1a-288	Beales et al. 2007

recessive alleles of *Vrn-B1* and *Vrn-D1* genes were again confirmed by multiplex PCR, following the protocol of Eagles et al. (2010) and are shown in Figures 1c and 1d.

Figure 1a shows amplification product of *Ppd-D1;* Lane M: 50 bp ladder; 1: HS 207; 2: DL-153-2;
3: PBW343; 4: HD2270; 5: HW 2004; 6: HD 2380; 7: HP 1744; 8: HW1085; 9: HD2643; 10: HD 2781;
11: HS 420; 12: RAJ 1972; 13: HUW 234; 14: WH291; 15: RAJ 2184; 16: PBW175; 17: PBW 154;
18: K9107; 19: WH542; 20: PBW 502; 21: DBW14; 22: RAJ4037; 23: MP4010; 24: Janz (+ve control)

Figure 1b shows amplification product of *Vrn-A1;* Lane M: 50 bp ladder; 1: HS 207; 2: DL-153-2;
3: PBW343; 4: HD2270; 5: KRL-1-4; 6: HD 2380; 7: HP 1744; 8: HW1085; 9: HD2643; 10: HD 2781;
11: HS 420; 12: RAJ 1972; 13: HUW 234; 14: WH291; 15: RAJ 2184; 16: PBW175; 17: PBW 154;
18: K9107; 19: WH542 ; 20: PBW 502; 21: DBW14; 22: RAJ4037; 23: MP4010; 24: Janz (+ve control)
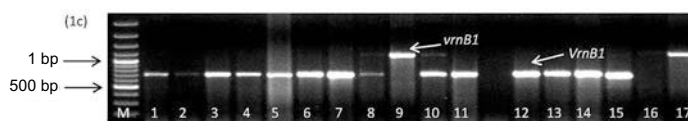
Figure 1c shows amplification of multiplexed PCR of *Vrn-B1*; Lane M: 100 bp plus ladder; 1: HS 207;
2: DL-153-2; 3: CPAN 3004; 4: HD 2270; 5: HP1731; 6: HP 1744; 7: HP 1761; 8: HD2687; 9: PBW343;
10: HD 2781; 11: HW 2045; 12: HUW 213; 13: RAJ 1972; 14: RAJ 2184; 15: PBW 154;
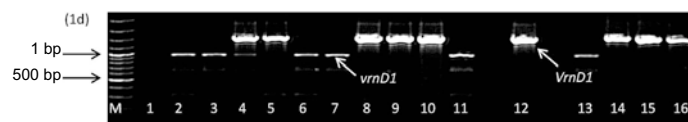16: Without DNA (-ve control); 17: Janz (+ve control)

Figure 1d shows amplification of multiplexed PCR of *Vrn-D1;* Lane M: 100 bp plus ladder; 1: -ve control;
2: Janz (+ve control); 3: HI 1077; 4: HS240; 5: DL-784-3; 6: HP 1744; 7: HP 1761; 8: HS365; 9: PBW343;
10: HD 2781; 11: HW 2045; 12: HUW 234; 13: RAJ 2184; 14: PBW 154; 15: KRL-1-4; 16: UP 2425

The frequency of these *Vrn* alleles varied across six different agro-ecological zones in India. The *Vrn-A1a* allele was the most frequent allele present in spring wheat varieties in North-Western Plain Zone (NWPZ) whereas *Vrn-B1* allele was mostly identified in North-Eastern Plain Zone (NEPZ) and Peninsular Zone (PZ). The Northern Hill Zone (NHZ) had recessive *vrn-B1* and *vrn-D1* alleles in higher proportions compared to the dominant *Vrn-B1* and *Vrn-D1,* frequent in other zones. The three dominant genes were cumulatively present in 29 cvs among all the zones, where as double dominant combination,

Vrn-A1/Vrn-B1 was identified in 18 cvs, *Vrn-A1/Vrn-D1* in 6 cvs and *Vrn-B1/Vrn-D1* in 16 cvs. None of the cvs carried all the recessive alleles (Table 2) in either of the zones. The most frequent combinations of all the dominant alleles were observed in NWPZ followed by NEPZ and PZ. The allelic combination *Vrn-A1/vrn-B1/vrn-D1* was not observed in NWPZ and PZ, while one cultivar JOB666 of the NEPZ and three cvs of NHZ (HS 420, UP1109 and VL804) possessed this allelic combination.

We also screened these varieties for the presence of *Vrn-B3* gene. All of the bread wheat cvs carried 1140 bp fragment indicative of the recessive *Vrn-B3* allele. None of the cvs produced a band of 1200 bp with the primer combination VRN4-B-INS-FVRN4-B-INS-R.

Table 2. Allelic combination of vernalization genes of Indian wheat cultivars in different zones

Allele combination	NWPZ	NEPZ	NHZ	CZ	PZ	SHZ
Vrn-A1Vrn-B1Vrn-D1	11	9	1	3	5	0
Vrn-A1Vrn-B1vrn-D1	6	6	2	3	1	0
Vrn-A1vrn-B1**Vrn-D1**	3	0	1	0	2	0
vrn-A1**Vrn-B1Vrn-D1**	3	6	1	2	3	1
Vrn-A1vrn-B1vrn-D1	0	1	3	1	0	1
vrn-A1**Vrn-B1**vrn-D1	0	1	2	2	0	1
vrn-A1vrn-B1**Vrn-D1**	6	6	4	1	0	0

Allelic variation at Ppd loci

The frequency of photoperiod sensitive allele *Ppd-D1b* was found to be very low in our cvs (Table S1 and Fig. 1a). Photoperiod insensitive allele *Ppd-D1a* was present in 91 cultivars while photoperiod sensitive allele *Ppd-D1b* was present in eight cvs. None of cvs of the NEPZ and Southern Hill Zone (SHZ) possessed *Ppd-D1b* allele.

Discussion

Genetic variation in vernalization and photoperiod genes and their effects on agronomic traits are of great value to wheat breeders since the adaptation of wheat to climatic conditions and its agronomic performance, quality, and yield potential are associated with growth and developmental phases such as days to heading or flowering habits, determined by Vernalization gene *(Vrn),* photoperiod responses, determined by photoperiod sensitivity gene *(Ppd)* and maturity duration determined by earliness *per se* genes *(Eps)* (Dubcovsky et al. 1998). In the present study, we have characterized most of the varieties released in India over past two and a half decades, for vernalization and photoperiod alleles. Dominant *Vrn-A1/Vrn-B1* combination was most prevalent irrespective of the zone. Since the agro-climatic conditions in the six zones vary, the predominance of *Vrn-A1/Vrn-B1* across these zones support the hypothesis of adaptive value of these genes. History suggests the incorporation of dominant *Vrn-D1* gene, in the latter half of the century, in most of the modern cultivars. Zhang et al. (2008) reported *Vrn* alleles in

278 chinese cultivars and showed highest frequency of *Vrn-D1* allele (37.8%), followed by dominant *Vrn-A1, Vrn-B1* and *Vrn-B3* alleles. Dominant *Vrn-D1* allele is introduced through CIMMYT germplasm into the regions near the equator. Study by Lantican et al. (2005) suggests that most of the varieties in developing countries including India released after 1988 contained CIMMYT genes. The preponderance of the dominant *Vrn-D1* allele in our released varieties corroborates these findings. Semi-dwarf wheat varieties Lerma Rojo64 and Sonora64 introduced from CIMMYT germplasm, and used extensively during green revolution, may be responsible for the wide distribution of *Vrn-D1* gene in our present day cultivars. In our study, *Vrn-B1* and *Vrn-D1* alone were present less frequently, i.e. 6% and 16%, respectively, agreeing with other reports (Fu et al. 2005; Iqbal et al. 2007a).

Our results showed that most of the Indian cultivars had spring type growth habit predominantly due to the dominant *Vrn-A1* alleles, *Vrn-A1a* being the highest. Iqbal et al. (2007b) also observed the prevalence of *Vrn-A1a* allele in Canadian varieties leading to spring growth habit. As *Vrn-A1a* confers insensitivity to vernalization, the cultivars with this allele were found to flower and mature earlier compared to the dominant *B1* and *D1* alleles. Dominant *Vrn-A1* allele have been found by others (Fu et al. 2005; Zhang et al. 2008) to be present in high frequency among spring planted spring varieties while *Vrn-D1* is frequent in fall-planted spring wheats. We observed high frequency of *Vrn-D4* also based on the linked SSR marker screening (variety Gabo from Australia served as positive control). Iwaki et al. (2001) also reported that allelic frequency of *Vrn-D4* is relatively high in India compared with other regions. Allelic profile of a few pre and post-green revolution varieties of India has been reported recently (Kumar et al. 2012). However, the allelic constitution for PBW343 reported by us is different from Kumar et al. (2012) and same as Eagles et al. (2011). Barring PBW343, our is the first report on vernalization and photoperiod genes in Indian wheat varieties. Using the primer of Fu et al. (2005), Santra et al. (2009) identified a new allele of *Vrn-B1* in the cultivar 'Alpowa'. This allele expressed a lower amplicon size (673 bp) as compared to the previously identified allele with amplicon size (709 bp). Santra et al. (2009) termed the new allele found in 'Alpowa' as *Vrn-B1b* and the previously existing one was termed as *Vrn-B1a* (previously called *Vrn-B1*). We did not observe *Vrn-B1b* in any of the 99 cultivars studied. Milec et al. (2012) have recently reported the presence of three dominant alleles *Vrn-B1a, Vrn-B1b* and *Vrn-B1c* in Czech cultivars using a set of different primers designed to cover the entire sequence of the gene *Vrn-B1*.

Winter and spring wheats contain different gene pools due to geographical and agro-ecological adaptations. Breeders have augmented the yield potential of spring wheats by harnessing variability from winter wheats. PBW343, a well adapted high yielding cultivar of India, ruling over major areas since the last two decades, contained Attila as a parent which was developed from European and US winter wheats. Northern Hill Zone of India comprises high altitude zone which is characterized by low rainfall during summer and high snowfall during winter. Under these conditions, cultivars which are not affected by relatively high temperature and having genes with mild cold requirements for entry into reproductive phase have been most successful. The semi-winter wheats in this

zone have a moderate degree of sensitivity to low temperature and flower late in the sea-son avoiding extreme temperatures. Successful spring × winter wheat programme is being carried out in this zone (Lakshmi Kant and Gupta 2002), we also observed the prevalence of *Vrn-A1, vrn-B1* and *vrn-D1* alleles in most of the cultivars in this zone.

It is reported that spring alleles at the three loci do not have the same effects on days to heading. Loss of *Vrn-A1* spring allele reduces the days to heading more significantly com-pared to the loss of *Vrn-B1* spring allele. Eagles et al. (2009) also suggested the smaller ef-fects of *Vrn-B1* allele compared to *Vrn-A1* and *Vrn-D1* allele. We observed days to head-ing for the cultivars along with distribution of *Vrn-A1, Vrn-B1* or *D1* alleles in different agro-ecological zones (days to heading classified into five categories very early <80, early (81–90), medium (91–100), late (101–110), very late >110 like Kundu et al. 2006). The genotypes with three dominant genes headed quite early compared to mono or di-domi-nant gene combinations. *Vrn-A1, Vrn-B1* or *Vrn-D1* carrying genotypes were early to me-dium in days to heading in all the zones except the NHZ where the *Vrn-D1* containing ge-notypes headed late to very late in the season. Majority of the genotypes which headed early between 85–90 days had *Vrn-A1/Vrn-B1* combinations irrespective of the zone. These results are in agreement with Zhang et al. (2008) where *Vrn-A1* genotypes were the earliest in heading followed by *Vrn-B1* and *Vrn-D1* genotypes studied by them.

The dominant *Vrn-B3* was not found in Indian cultivars. Zhang et al. (2008) also re-ported low frequency of dominant *Vrn-B3* allele in Chinese cultivars. In addition to this, most of the Indian wheat varieties had photoperiod insensitive allele in all the zones. Our results corroborated the work carried out by Ferrara et al. (1998) who suggested that photoperiod insensitivity led to high-yield potential in improved wheat cvs, thus broaden-ing their adaptation over range of environments especially in Asian, Mediterranean and North African regions. Day length insensitive wheats as indicated by their early to me-dium heading days requirement was frequent in most zones except a few cultivars present in NHZ which headed quite late in the season.

Thus, in this study we identified 39 cultivars across the zones that had a winter allele (designated as *vrn-A1* here) along with spring or winter alleles at one or more *Vrn-1* loci. In a recent study, Chen et al. (2009) found two alleles of *Vrn-A1* in winter cultivars adapted to the Great Plains of the USA. These two alleles were distinguishable by a single nucleotide polymorphism (SNP) in exon 4 of *Vrn-A1*. Eagles et al. (2011) have shown that the allele they termed as *Vrn-A1v* (represented by the cultivar 'Jagger') existed in all the lines with spring allele of *Vrn-A1* (either *Vrn-A1a* or *Vrn-A1b*). The Cultivars from CIMMYT cross 'Veery' and many of its relatives, carried another allele they termed it as *(Vrn-A1w)* which has a transition from C to T and was predicted to change a conserved leucine to phenylalanine. It has been suggested that in addition to their role in flowering regulation, these *Vrn* genes may have other adaptive roles in reference to climate change such as freezing tolerance, yield stabilization etc. (Van Beem et al. 2005; Iqbal et al. 2007b). The *vrn-A1w* allele has been observed in an Indian cv PBW 343 (Eagles et al. 2011). This cv brought a jump in Indian wheat production, has a broad range adaptability and has been grown from Northern eastern to Northern western Plains zones contributing to more than 60% of wheat producing area in India. It is hoped that the knowledge of the

distribution of the vernalization genes in the elite germplasm will provide breeders, the opportunity for more precise identification of parents carrying different *Vrn* genes and maximizing the diversity in their crossing programmes targeting development of spring and winter cultivars.

Acknowledgements

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Electronic Supplementary Material (ESM)

Electronic Supplementary Material (ESM) associated with this article can be found at the website of CRC at http://www.akademiai.com/content/120427/

Electronic Supplementary *Table S1.* Allelic variation of genes in different agro-climatic zones in Indian bread wheat