Journal of Cereal Research
Volume 18 (1): 81-92
Homepage: http://epubs.icar.org.in/ejournal/index.php/JWR

Research Article

Effect of Genes Governing Photoperiod and Vernalization Response on Wheat Phenology and Adaptation

Harsimardeep S. Gill, Tavisha Singh, Asish Kumar Padhy, Satinder Singh, Prinka Goyal, Gomti Grover, Puja Srivastava, G S Mavi, Jayesh Singh, V S Sohu andAchla Sharma*

Punjab Agricultural University, Ludhiana, Punjab -141 004

Article history:
Received: 21 Jan., 2026
Revised: 10 Mar., 2026
Accepted: 11 Mar., 2026

Citation:
Gill HS, T Singh, AK Padhy, S Singh, P Goyal, G Grover, P Srivastava, GS Mavi, J Singh, VS Sohu and A Sharma. 2026. Effect of Genes Governing Photoperiod and Vernalization Response on Wheat Phenology and Adaptation. *Journal of Cereal Research* ***18 (1):*** 81-92. http://doi.org/10.25174/2582-2675/2026/175650

Corresponding author:
E-mail: achla@pau.edu

Abstract

Flowering time in wheat is a complex trait that significantly influencestotal yieldandadaption of wheat to different niches, and is primarily under the control of vernalization genes (VRN genes) and photoperiod sensitivity genes (PPD genes). Strategic changes in these alleles have yielded substantial gains in productivity. In the present study, different allelic combinations of vernalization (Vrn) and photoperiod sensitivity (Ppd) genes were identified in two back cross populations derived from winter wheat x spring wheat crosses and were evaluated under various sowing regimes. The BC$_1$F$_6$ progenies for the crosses Chianti/WH1105//PBW698 and Aciento/2*PBW683 showed wide variation for days to flowering, ranging from 95 to 125 days and 90-115 days, respectively. Screening of parental lines for the Vrn1 and Ppd-D1 genes revealed that the parents of the cross Aciento/2*PBW683 were polymorphic for two Vrn1 genes viz. Vrn-B1 and Vrn-D1, while,those of the cross Chianti/WH1105//PBW698 were polymorphic for these loci as well as for the Ppd-D1 gene. The identified allelic haplotypes of Vrn and Ppd genes were evaluated for productivity and phenology across three sowing regimes. Different allelic combinations of Vrn and Ppd genes affected days to flowering, tiller number per plant, and thousand-grain weight. Spikelets per spike were not affected by allelic variation in Vrn1 gene. However, this trait was influenced by allelic variation at the Ppd-D1 gene. These findings provide valuable guidance for wheat breeding programs in India to strategically deploy Vrn and Ppd alleles for developing varieties with improved phenological adaptation and higher productivity under diverse sowing environments.

Key words: Vernalization genes, Photoperiod response, Phenology, Allelic combinations, Wheat

1. Introduction

Wheat (*Triticum aestivum* L.) is sown extensively worldwide,yielding an average of over 760 million tons (FAO, 2024). Climate change is expected to aggravate global food insecurity. By 2070, global population is projected to reach up-to 9.7 billion (Adam, 2021) and around 17 percent reduction in wheat yield is predicted owing to the negative impacts of climate change by 2050 (Knox *et al.,* 2012). Therefore, adaptation is regarded as a key factor that will govern the severity of the projected climate change impacts on global wheat production. In this scenario, the understanding of the genetic basis of traits governing plant adaptability will be crucial for developing wheat varieties adapted to different environments, and thereby ensuring optimum productivity and yield (Foulkes *et al.,* 2011). Depending on climatic conditions, wheat can be classified as autumn, winter, and spring sown, each requiring niche-climate-specific wheat varieties to unlock the yield potential. Vernalization response, photoperiod sensitivity, and earliness per se are the three key determinants of wheat's wider adaptability (Herndl *et al.,* 2008), and these genetic systems (viz., the *Vrn* genes, *Ppd* genes, and *Eps* genes) act together to determine flowering time (Worland *et al.,* 2001).

Flowering time is a major adaptive trait of wheat that determines grain filling under optimal environmental conditions, thus strongly influencing reproductive success and final yield (Arjona *et al.,* 2020). Much of wheat's productivity is determined by allelic diversity within genes regulating vernalization requirement *(Vrn)* and photoperiod sensitivity *(Ppd).* As a photoperiod sensitive crop, wheat flowers only after the fulfilment of critical day length. The *Ppd* genes control photoperiod response,with photoperiod-insensitive alleles inducing flowering regardless of critical day length (Turner *et al.,* 2005). Vernalization governed by *Vrn* genes, regulate the transition from vegetative to reproductive phase in response to temperature (Distelfeld *et al.,* 2009a) and thus determine the spring (no vernalization requirement) and winter (a strong vernalization requirement) growth habit. Wheat has four vernalization genes. *VRN-1* (A1, B1 and D1 located on chromosome 5AL, 5BL and 5DL respectively) and is identified as actual promoter of flowering. *VRN-2* is repressor of flowering, it has three allelic forms i.e. *VRN-A2* (5AL), B2 (4BL) and D2 (4DL). *VRN-3* acts as flowering activator in wheat with three alleles *VRN-A3* (7A), *VRN-B3* (7B) and *VRN-D3* (7D). *VRN-4* (5DS) is known for early flowering in wheat(Kippes *et al.,* 2015; Tan and Yan, 2016). The photoperiod genes (*PPD-1*) include *PPD-A1, PPD-B1*, and *PPD-D1* found on chromosomes 2A, 2B and 2D respectively (Law *et al.,* 1978). The regulation of phasic growth and acclimatization of wheat to specific environments is made possible due to *VRN-1* and *PPD-1* genes (Trevaskis, 2010).In winter wheat, once the vernalization requirement is met, flowering timing is subsequently regulated by photoperiod response, primarily mediated by the *PPD-1* loci (Fjellheim *et al.,* 2014). Therefore, the effects of allelic variants at *VRN-1* and *PPD-1* on plant phenology and agronomic traits will aid in determining the most suitable allele combinations for specific area, enabling better adaptation to changing climate scenario and maximizing productivity. Hence, this research focuses on the determination of the genetic variation at the VRN and PPD loci in winter wheat x spring wheat crosses and to analyse the influence of the allelic variants of *VRN-1* and *PPD-1* genes per se, and in combinations- on phenological development, and yield related traits under different sowing regimes in the Punjab region, using the markers for the main *VRN-1* and *PPD-1* alleles.

2. Materials and methods

2.1. Plant material and experimental setup

A panel of 376 European winter wheat lines,including commercial varieties from Germany, France, England, and other European countries carrying distinct *Vrn* and *Ppd* genes/alleles, was procured from National Institute of Agricultural Botany (NIAB), United Kingdom. The selected European genotypes like Aciento, Chianti, Exotic, and Plethore were crossed as donors and spring wheat cultivars(PBW 343, PBW683, PBW698, PBW 703, and WH 1105) as recipients to develop four backcross-derived populations i.e. ACIENTO/2*PBW683, CHIANTI/WH1105//PBW698, EXOTIC/BWL 0080//HD2967, and PLETHORE/BWL 0831//PBW703. The BC_1F_4 populations (Supplementary table S1), each consisting of 167-203 progenies were used in this study. These populations were sown in the main season (November to April) at the experimental field of the Department of Plant Breeding and Genetics, Punjab Agricultural University(PAU), Ludhiana. These progenies were grown in rows of 1m long rows with a row to row spacing of 30 cm. The lines were monitored for variation in flowering time (time of sowing to the date when more than 50% plants in each line had completed heading).

Based on variation for days to flowering, two populations i.e. ACIENTO/2*PBW683, and CHIANTI/WH1105//PBW698, were chosen for further analysis as they showed a wide variation for flowering time and indication of distinct phenotypic categories. Each selected population was then partitioned into distinct classes with respect to flowering time and were further propagated at PAU off-season Research Station, Keylong (Himachal Pradesh) for rapid generation advancement.

2.2. Molecular characterization of VRN-1 and PPD-1 genes

Progenies of the two crosses i.e. ACIENTO/2*PBW683, and CHIANTI/WH1105//PBW698 along with parental lines and check varieties such as Aardvark, Aciento, Chianti, PBW 343, PBW683, PBW698, PBW550, PBW 703, and WH 1105 etc. were chosen for molecular analysis. A modified CTAB based protocol was used to extract total genomic DNA from young disease-free leaves of selected accessions (Saghai-Maroof *et al.,* 1984). Genotyping was conducted using 13 allele specific PCR markers (Table 1) to detect the presence of dominant and recessive alleles of *Vrn-A1, Vrn-B1, Vrn-D1, Ppd-A1 and Ppd-D1. Vrn-B1, Vrn-D1,* and *Ppd-D1* were also amplified using multiplex PCR with primer pairs and slightly modified protocol (Eagles *et al.,* 2009). For *Vrn-B1* locus,the primers Intr1/B/F, Intr1/B/R3, and Intr1/B/R4 were used in the PCR with annealing at 57°C for 45 seconds with extension time of 70 seconds. Similarly, for *Vrn-D1 locus*, the PCR contained three primers: Intr1/D/F, Intr1/D/R3 and Intr1/D/R4 with annealing at 62°C for 45 seconds with extension time of 90 seconds. In both of the cases, annealing was carried out for 38 cycles with final extension at 72°C for 10 minutes. Different alleles at the *Ppd-D1* locus were identified using primers Ppd-D1_F, Ppd-D1_R1, and Ppd-D1_R2 in PCR with annealing at 55°C for 30 seconds. The reaction was carried out for 40 cycles with a final extension at 72°C for 7 minutes.

Table 1. Allele specific molecular markers and their PCR primers used molecular characterization of *Vrn* and *Ppd* genes

S. No.	Allele	Primer Name	Primer Sequence	Reference
1	*Vrn-A1a*	VRN1AF VRN1-INT1R	5'- GAA AGG AAA AAT TCT GCT CG -3' 5'- GCA GGA AAT CGA AAT CGA AG -3'	Yan *et al.* (2004)
2	*Vrn-A1b*	Ex1/C/F Intr1/A/R3	5'- GTTCTCCACCGAGTCATGGT -3' 5'-AGTAAGACAACACGAATGTGAGA-3'	Fu *et al.* (2005); Yan *et al.* (2004)
3	*Vrn-A1c*	Intr1/A/F2 Intr1/A/R3	5'-AGCCTCCACGGTTTGAAAGTAA-3' 5'-AGTAAGACAACACGAATGTGAGA-3'	Fu *et al.* (2005)
4	*vrn-A1*	Intr1/C/F Intr1/AB/R	5'-GCACTCCTAACCCACTAACC-3' 5'-TCATCCATCATCAAGGCAAA-3'	Fu *et al.* (2005)
5	*Vrn-B1a*	Intr1/B/F Intr1/B/R3	5'-CAAGTGGAACGGTTAGGACA-3' 5'-CTCATGCCAAAAATTGAAGATGA-3'	Santra *et al.* (2009)
6	*Vrn-B1b*	Intr1/B/F Intr1/B/R3	5'-CAAGTGGAACGGTTAGGACA-3' 5'-CTCATGCCAAAAATTGAAGATGA-3'	Santra *et al.* (2009)
7	*vrn-B1*	Intr1/B/F Intr1/B/R4	5'-CAAGTGGAACGGTTAGGACA-3' 5'-CAAATGAAAAGGAATGAGAGCA-3'	Fu *et al.* (2005)
8	*Vrn-D1*	Intr1/D/F Intr1/D/R3	5'-GTTGTCTGCCTCATCAAATCC-3' 5'-GGTCACTGGTGGTCTGTGC-3'	Fu *et al.* (2005)
9	*vrn-D1*	Intr1/D/F Intr1/D/R4	5'-GTTGTCTGCCTCATCAAATCC-3' 5'-AAATGAAAAGGAACGAGAGCG-3'	Fu *et al.* (2005)
10	*Ppd-D1a*	Ppd-D1_F1 Ppd-D1_R2	5'-ACGCCTCCCACTACACTG-3' 5'-CACTGGTGGTAGCTGAGATT-3'	Beales *et al.* (2007)
11	*Ppd-D1b*	Ppd-D1_F1 Ppd-D1_R1	5'-ACGCCTCCCACTACACTG-3' 5'-GTTGGTTCAAACAGAGAGC-3'	Beales *et al.* (2007)
12	*Ppd-A1a*	Ag5del_F1 Ag5del_F2	5'-GTATGCGATTCGCCTGAAGT-3' 5'-TGTCACCCATGCACTCTCTTT-3'	Bentley *et al.* (2011)
13	*Ppd-A1b*	Ag5del_R1 Ag5del_R2	5'-GAGCAAGGGATTGAGACTGC-3' 5'-CTGGCTCCAAGAGGAAACAC-3'	Bentley *et al.* (2011)

2.3. Phenotypic evaluation and derived variables

Evaluation of BC_1F_6 progenies with identified allelic haplotypes of the *Vrn* and *Ppd* loci was conducted for productivity and phenology under three sowing regimes viz., 17 October, 17 November and 17 December during the main season at PAU, Ludhiana. Data for number of morphological traits was recorded. The crop growth stages were determined using the Zadoks' scale (Zadok *et al.,* 1974). Days to flowering was recorded when half of the tillers showed completed head emergence (anthers extruded from the florets and turned color) Plant height (PH), excluding awns, was measured briefly after flowering. Number of tillers, and spikelets per spike (SPS) was determined by the average value of randomly collected sample spikes per plant. The weight of thousand seeds from a random sample of seed harvested per plot was measured in three replications and average was worked out.

2.4. Statistical analysis

Analysis was done using factorial randomized block design using the software SAS version 9.3 to study the interaction between date of sowing and genotypes. The analysis of variance table for factorial RBD was worked out.

3. Results

3.1. Identification of crosses showing different classes for days to flowering

BC_1F_4 progenies derived from four different winter x spring wheat crosses were screened for days to flowering under recommended sowing condition. In the cross Aciento/2*PBW683, there were 199 BC_1F_4 progenies that were screened for variation in days to flowering. Majority of the progenies were observed to be in-between 96 to 115 days and only five progenies were found to be very early in flowering i.e. 90-95 days (Figure 1). A total of 203 BC_1F_4 progenies were observed for differences in flowering time in the cross Chianti/WH 1105//PBW698. The differences in days to flowering ranged from 94 to 127 days (Figure 1). Similarly, 167 BC_1F_4 progenies in the cross Exotic/BWL 0080//HD2967 were observed for variation in days to flowering and most of the progenies were found in the range of 105 days to 110 days showing no significant variation. Similarly, in the cross Plethore/BWL 0831//PBW703, 195 BC_1F_4 progenies were screened and ranged from 90 to 115 days for flowering but majority of progenies was found in the range of 105 to110 days (Figure 1).

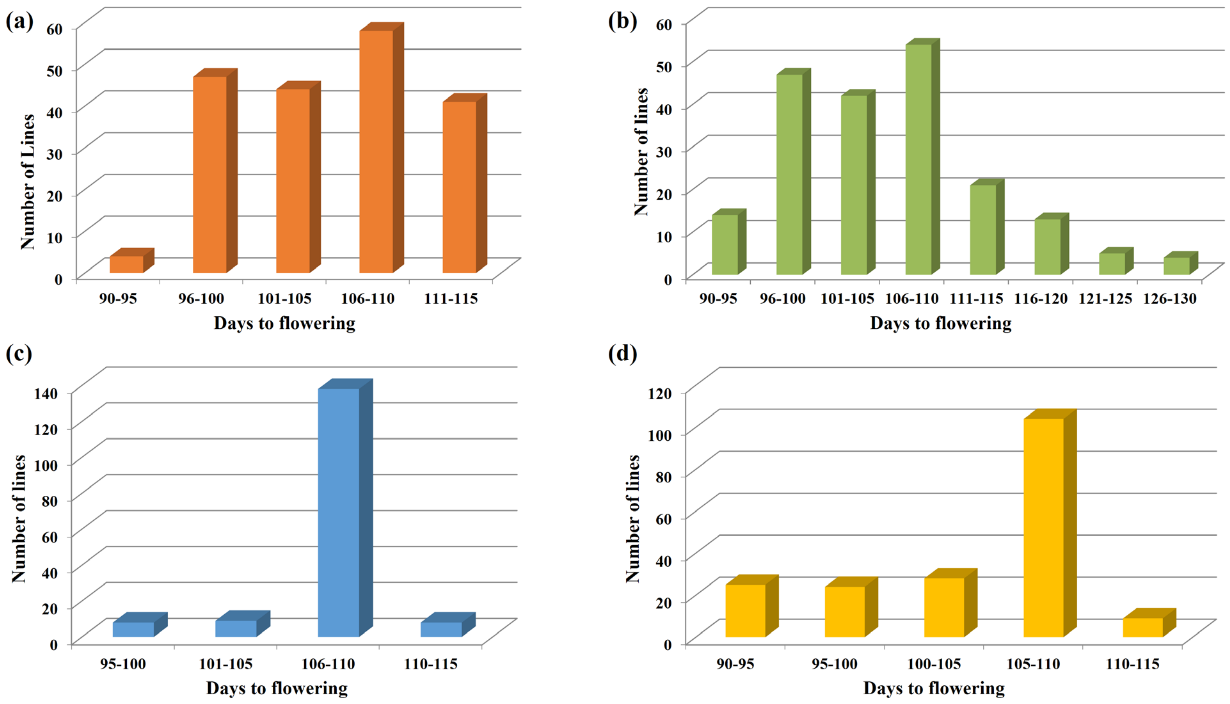

Fig. 1: Distribution of variation in days to flowering in cross (a) Aciento/2*PBW683 (b) Chianti/WH1105//PBW698 (c) Exotic/BWL 0080//HD2967 (d)Plethore/BWL 0831//PBW703

Among these four crosses Chianti/WH 1105//PBW698 showed maximum variation for days to flowering trait followed by the cross Aciento/2*PBW683. In cross Exotic/BWL 0080//HD2967 and Plethore/BWL 0831// PBW703, number of progenies representing a particular class of days to flowering were not widely distributed. Approximately 85% (142 out of 167) of the progenies in cross Exotic/BWL0080//HD2967 were falling in one category only.

Therefore, two crosses viz. Chianti/WH 1105// PBW698 and Aciento/2*PBW683 were selected for the molecular characterization with respect to different allelic combinations for *Vrn and Ppd* genes, followed by their agronomic evaluation.

3.2. Molecular screening of progenies for different allelic combinations of Vrn and Ppd genes

As mentioned in the material and methods section, progenies of the two crosses i.e. ACIENTO/2*PBW683, and CHIANTI/WH1105//PBW698 along with parental lines and check varieties such as Aardvark, Aciento, Chianti, PBW 343, PBW683, PBW698, PBW550, PBW 703, and WH 1105 etc. were selected for molecular analysis owing to their displayed wide range of variation in the flowering time during the agronomic evaluation.

A set of allele specific markers (Table 1) was employed to characterize different vernalization and photoperiod gene combinations. Molecular markers for 3 *Vrn1* loci and 1 *Ppd1* loci were used for documentation of polymorphism between parental lines. The amplicon size of different primers to distinguish between various alleles is given in Table 2. Different alleles at *Vrn-A1* were identified using primers VRN1AF andVRN1-INT1R. The winter allele *vrn-A1* with amplicon size of 734 bp was observed in all the parental lines except in PBW550 (Figure 2). No band was found with spring allele *Vrn-A1a* or *Vrn-A1b*. Primers Intr1/B/F, Intr1/B/R3 and Intr1/B/R4 were used to characterize different alleles at *Vrn-B1*. Among different parental lines, PBW683, PBW343, PBW550, PBW761 and WH1105 amplified around 700 bp fragment indicating the presence of *Vrn-B1a* spring allele. In all other lines, amplicon of 1149 bp was observed which indicates the presence of winter allele *vrn-B1* at this locus (Figure 2). Similarly, *Vrn-D1* was screened using primers Intr1/D/F, Intr1/D/R3 and Intr1/D/R4. The winter lines Aciento and Chianti were only to amplify a 997 bp fragment indicating the presence of winter allele *vrn-D1*. A 1671 bp was observed in all the spring wheat cultivars which refer to the presence of *Vrn-D1* spring allele (Figure 2). *Ppd-D1* alleles were analysed using the markers Ppd-D1_F1, Ppd-D1_R1 and Ppd-D1_R2. Winter wheat line Chianti amplified a fragment of around 414 bp indicating the presence of photoperiod sensitive allele *Ppd-D1b* at *Ppd-D1* locus. All other lines amplified a fragment of 288 bp which showed the presence of photoperiod insensitive allele *Ppd-D1a* (Figure 2).

Table 2: Amplicon size for the various genes with the use of molecular markers used in the current study

S. No.	Gene	Marker	Target Allele	Amplicon size
1	*Vrn-A1*	VRN1AF; VRN1-INT1R	*Vrn-A1a* (Spring type) or *Vrn-A1b* (Spring type) or *vrn-A1* (winter type)	~950bp + 870bp (*Vrn-A1a*) ~714bp (*Vrn-A1b*) ~734bp (*vrn-A1*)
2	*Vrn-B1*	Intr1/B/F; Intr1/B/R3	*Vrn-B1a* (spring type) or *Vrn-B1b* (spring type)	~700bp (*Vrn-B1a*) ~670bp (*Vrn-B1b*)
		Intr1/B/F; Intr1/B/R4	*vrn-B1*(winter type)	~1149bp
3	*Vrn-D1*	Intr1/D/F Intr1/D/R3	*Vrn-D1b* (spring type)	~1671bp
		Intr1/D/F Intr1/D/R4	*vrn-D1*(winter type)	~997bp
4	*Ppd-D1*	Ppd-D1_F1 Ppd-D1_R1 Ppd-D1_R2	*Ppd-D1a* (photoperiod insensitive) or *Ppd-D1b* (photoperiod sensitive)	~288bp (*Ppd-D1a*) ~414bp (*Ppd-D1b*)

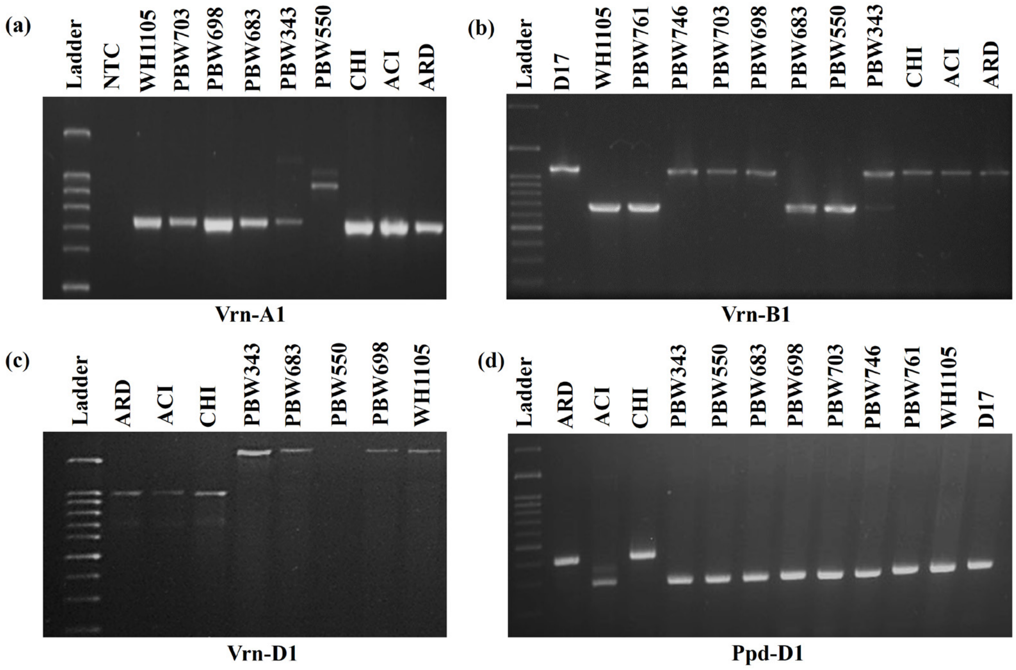

Fig. 2:Amplification of Vrn and Ppd genes in parental lines of winter x spring wheat population.
(a) *Vrn*-A1 (b) *Vrn*-B1 (c) *Vrn*-D1(d) *Ppd*-D1, where, ACI: Aciento, CHI: Chianti, ARD: Aardvark,NTC: No Template Control

3.3. The different allelic combinations for Vrn1 and Ppd-D1 genes identified in parental lines

The screening of parental lines for *Vrn* and *Ppd* genes revealed that the parents of the cross Aciento/2*PBW683 were polymorphic for two *Vrn* genes viz. *Vrn-B1* and *Vrn-D1*. The parental lines of cross Chianti/WH1105//PBW698 were found polymorphic for two *Vrn* genes viz. *Vrn-B1* and *Vrn-D1* as well as for *Ppd-D1* gene. The selected lines of cross Aciento/2*PBW683 were screened with markers for *Vrn-B1* and *Vrn-D1* (Figure 2). Similarly, selected lines of cross Chianti/WH1105//PBW698 were screened with marker for *Vrn-B1*, *Vrn-D1* and *Ppd-D1* (Figure 2). Based on allele specific markers, selected lines including parents as checks were screened in the crosses viz., Aciento/2*PBW683 and Chianti/WH1105//PBW698 with their respective allelic combinations (supplementary table S2 and S3 respectively). Also, different allelic haplotypes of *Vrn* and *Ppd* genes were observed in aforementioned crosses and number of genotypes with each haplotype are tabulated in the form of Table 3.

Table 3: Different allelic haplotypes of *Vrn* and *Ppd* genes observed in the crosses Aciento/2*PBW683 and Chianti/WH1105//PBW698 and number of genotypes with each haplotype

Sr No.	Haplotype for Aciento/2*PBW683	Genotype code	No. of genotypes
1	*vrn-A1, Vrn-B1, Vrn-D1, Ppd-D1a*	*vaaa*	30
2	*vrn-A1, Vrn-B1, vrn-D1, Ppd-D1a*	*vava*	7
3	*vrn-A1, vrn-B1, Vrn-D1, Ppd-D1a*	*vvaa*	**7**
4	*vrn-A1, vrn-B1, vrn-D1, Ppd-D1a*	*vvva*	4
		Total	48
Sr No.	**Haplotype for Chianti/WH1105//PBW698**	**Genotype code**	**No. of genotypes**
1	*vrn-A1, Vrn-B1, Vrn-D1, Ppd-D1a*	*vaaa*	14
2	*vrn-A1, Vrn-B1, vrn-D1, Ppd-D1a*	*vava*	12
3	*vrn-A1, Vrn-B1, vrn-D1, Ppd-D1b*	*vavb*	4
4	*vrn-A1, vrn-B1, Vrn-D1, Ppd-D1a*	*vvaa*	10
5	*vrn-A1, vrn-B1, Vrn-D1, Ppd-D1b*	*vvab*	3
6	*vrn-A1, vrn-B1, vrn-D1, Ppd-D1a*	*vvva*	4
		Total	47

3.4. Evaluation of different allelic combinations of Vrn and Ppd genes for phenology and adaptations under different sowing regimes

The BC_1F_6 lines with identified allelic combinations of *Vrn* and *Ppd* genes were used for evaluation of different morphological traits in the main season. 49 lines for each of the selected cross including the parental lines were sown in randomized complete block design. Different lines with identified allelic combinations of *Vrn* and *Ppd* genes were evaluated in three sowing regimes with first sowing 17 October second sowing on 17 November and third sowing on 17 December. Supplementary Table S4 and S5 summarizes the mean observation for various traits recorded in the experiment for cross Aciento/2*PBW683 and Chianti/WH1105//PBW698 respectively.

Analysis of variance showed significant difference between backcross derived progenies for days to flowering in both the crosses. Differences among different sowing dates were also found to be significant. Analysis also revealed significant results for sowing x genotype interactions. Plant height is another important morphological trait which was studied, and analysis of variance showed significant differences between different sowing regimes as well as backcross derived progenies. Sowing x genotype interaction was also found significant for plant height.

Yield is the primary and most important trait for selection of genotypes for particular sowing conditions. Tillers per plant and thousand grain weight are important traits that attributes to the total grain yield. For cross Aciento/2*PBW683, and Chianti/WH1105//PBW698, analysis of variance for tillers per plant and thousand grain weight (test weight) revealed significant differences between different sowing dates and also between different backcross derived lines. Sowing x genotype interaction was also significant.

*3.5. Effect of different allelic combination of VRN and PPD genes on different traits in Aciento/2*PBW683 and Chianti/WH1105//PBW698 derived BC1F6 lines*

The results from both crosses showed that the different allelic combinations of *Vrn* and *Ppd* genes significantly affected days to flowering, tiller per plant, and thousand grain weight(Figure 3,4). Spikelets per spike were unaffected by *Vrn1* allelic variation (Figure 4) But were influenced by *Ppd-D1* alleles. There was no significant

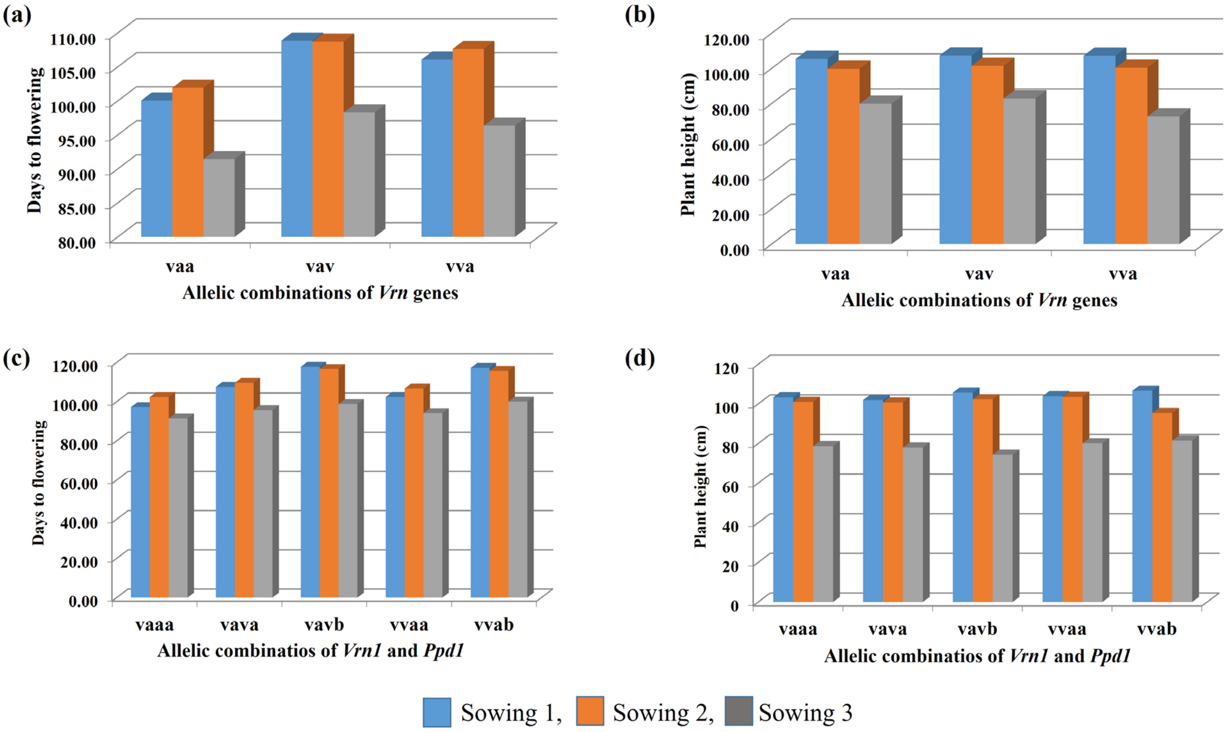

Fig. 3: Bar graph representing the effect of different allelic combinations of *Vrn* and *Ppd* genes in Aciento/2*PBW683 derived lines and Chianti/WH1105//PBW698 derived lines on days to flowering and plant height. (a) Days to flowering in Aciento/2*PBW683 derived lines; (b) Plant Height in Aciento/2*PBW683 derived lines; (c) Days to flowering in Chianti/WH1105//PBW698 derived lines; (d) Plant Height in Chianti/WH1105//PBW698 derived lines

effect of these combinations on plant height. Rather, the plant height was significantly affected by different sowing environments as expected.

The days to flowering were greatly affected by different allelic haplotypes of *Vrn* and *Ppd* genes. Genotypes carrying the winter allele at all three *Vrn* loci viz., *Vrn-A1*, *Vrn-B1* and *Vrn-D1* took longest time to flower. Thus, these genotypes were found to be late in flowering compared to other categories. Substitution of single spring allele at any *Vrn1* locus remarkably reduced the days to flowering. Differential effect of different *Vrn* genes i.e. *Vrn-B1* and *Vrn-D1,* on reduction in days to flowering was observed. The reduction in days to flowering was more when *Vrn-D1* was present instead of *Vrn-B1*. The genotypes which carried two spring alleles at *Vrn-B1* as well as *Vrn-D1* were earliest to flower. The trend was constant across sowing regime 1 and sowing regime 2 but reduced in the third sowing regime (Figure 3).

Except for flowering time, different allelic combinations of *Vrn1* also affected the number of tillers per plant and thousand grain weight. Genotypes with winter alleles at all three *Vrn1* loci exhibited highest tillering. Substitution of one or more spring alleles reduced number of tillers per plant. However, the reduction was comparatively more when single spring allele was present on *Vrn-B1* locus instead of *Vrn-D1* locus. When spring alleles were present at both *Vrn-B1* and *Vrn-D1* loci, genotypes exhibited highest reduction in number of tillers per plant(Figure 4). Highest thousand grain weight was observed in the genotypes with only spring allele at *Vrn-D1* locus and winter alleles at other two loci, followed by the genotypes with sole spring allele at *Vrn-B1*. Combination of two spring alleles at *Vrn-B1* and *Vrn-D1* resulted into decrease in the thousand grain weight (Figure 4).

Allelic variation at the photoperiod gene *Ppd-D1* in the Chianti/WH1105//PBW698 derived lines enabled evaluation of its effects on different agronomic traits. Allelic variation at *Ppd-D1* gene was found to be having notable effect on days to flowering. Presence of photoperiod sensitive allele at this locus resulted in increased numbers of days to flowering. Genotypes with photoperiod sensitive allele significantly delayed

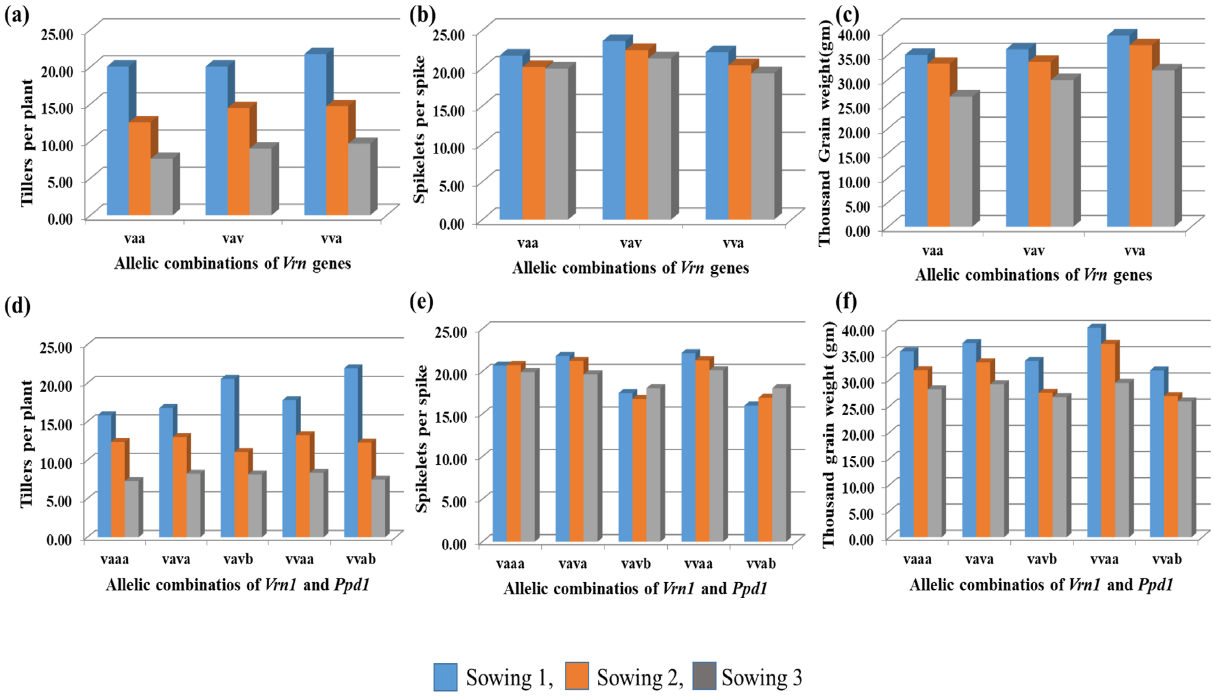

Fig. 4: Bar graph representing the effect of different allelic combinations of *Vrn* and *Ppd* genes in Aciento/2*PBW683 derived lines and Chianti/WH1105//PBW698 derived lines on tiller per plant, spikelets per spike and thousand grain weight. (a) Tiller per plant in Aciento/2*PBW683 derived lines; (b) Spikelets per spike in Aciento/2*PBW683 derived lines; (c) Thousand grain weight in Aciento/2*PBW683 derived lines; (d) Tiller per plant in Chianti/WH1105//PBW698 derived lines; (e) Spikelets per spike in Chianti/WH1105//PBW698 derived lines; (f) Thousand grain weight in Chianti/WH1105//PBW698 derived lines

flowering compared to genotypes with insensitive allele. However, the differences were reduced in sowing regime three which could be owed to the sudden exposure of extended photoperiod in later part of growing season. Additionally, variation in *Ppd-D1* gene also affected other traits of economic importance. Number of tillers per plant was affected by variation in *Vrn1* and *Ppd-D1* genes (Figure 4). Genotypes with photoperiod sensitive allele at *Ppd-D1* showed increased number of tillers per plant. Spikelets per spike were found to be affected by presence of allelic variation at *Ppd-D1* gene. Remarkable decrease was observed when insensitive allele at *Ppd-D1* was substituted by the photoperiod sensitive allele regardless of *Vrn1* alleles. Worland (1996) also reported a reduction in days to heading by 4-8 days due to insensitive alleles at photoperiod gene *Ppd-D1*. It was also suggested that the shortened life cycle results into a reduction in plant height, tiller number and spikelet per ear. Apart from affecting days to flowering, several pleiotropic effects of Photoperiod alleles have been reported such as reduction in plant height (Worland, 1996), reduced number of tillers per plant (Miralles and Richards, 2000; Dyck *et al.,* 2004) and reduction in the number of spikelets per spike (Snape *et al.,* 2001; Dyck *et al.,* 2004). Kamran *et al.* (2013) reported a reduction of about 5 days due to the presence of photoperiod insensitive allele Ppd-D1a.

Discussion

Optimal yield stability across locations requires targeted niche-specific breeding. It can be achieved by understanding the genetic basis of plant (Ortiz-Ferrara et al., 1998). In wheat, flowering time is very complicated which varies continuously is primarily controlled governed by vernalization *(Vrn)*, photoperiod *(Ppd)* and earliness *(Eps)* gene systems (Kamran *et al.,* 2014). Therefore, interaction of these gene families with varying temperature and agroclimatic regions largely determines wheat adaptability and yield potential (Gororo *et al.,* 2001). Vernalization genes, for example, delay ear initiation in winter wheats at high northern latitudes, protecting floral organs from the damage caused by severe cold temperatures. Similarly, in places where summers are too hot for wheat growth, photoperiod insensitive genes increase ear initiation and development with increasing day duration in late winter and early spring to ensure that the reproductive phase is completed before the beginning of high temperatures (Kamran *et al.,* 2014). The genetic variability for anthesis and heading time in bread wheat is predominantly controlled by *Vrn* gene system (70-75%), followed by *Ppd* gene system (20-25%) and earliness per se (approximately 5%) (Stelmakh, 1998).

The *VRN-1* and *PPD-1* allelic variations mainly arise from mutations either in their coding n or e promotor regions, resulting in contrasting phenotypes, such as *PPD-D1b* for photoperiod-sensitive (long day) or *PPD-D1a* for photoperiod-insensitive (day-neutral) .Strategic combinations of these alleles enable fine-tuning of phenological events prior to flowering without considerable modifications in anthesis date (González *et al.,* 2011). Studies confirm that combining *VRN-1* and *PPD-1* allelic variants have resulted in variations in phenological development such as flowering time and agronomic traits (Cane *et al.,* 2013) tillering, plant height, and spikelet number (Dyck *et al.,* 2004). There are several reports documenting the effects of *VRN-1* and *PPD-1* allelic variants on the adaption and agronomic traits of wheat varieties or germplasm (Gomez *et al.,* 2014), and have examined the impact of these phenology genes on phenological traits and yield across diverse longitude and latitude (Arjona *et al.,* 2018, 2020; Chen *et al.,* 2018).

Characterization for different *Vrn1* genes revealed that the spring allele at locus *Vrn-D1* predominated in spring wheat parental lines. This aligns with Kiss *et al.* (2014), who also reported that *Vrn-D1* was present in higher frequencies in Central and South Asian germplasm, largely attributable to CIMMYT introductions (Singh *et al.,* 2013). Van Beem *et al.* (2005) similarly identified *Vrn-D1* as the most frequent *Vrn* gene in the globally important CIMMYT wheat varieties. *Vrn-D1* was found to be the sole spring allele in worldwide grown three spring wheat varieties viz. Attila, Kauz and Pastor. The prevalence of spring allele at *Vrn-D1* in most of parental lines involved in the study corroborates with these findings. Our findings in context to PBW 343 were in corroboration with the findings of Singh *et al.* (2013). The results for PBW 343 for presence of winter allele at *Vrn-A1*,and for the presence of recessive winter allele *vrn-B1* were in agreement with the results reported by Singh *et al.* (2013)and Singh *et al.* (2013). A 1671 bp was observed in all the spring wheat cultivars which refer to the presence of *Vrn-D1* spring allele (Figure 2).

In our findings, substitution of the winter allele for spring allele at *Vrn-D1* locus delays flowering by 5-9 days across three sowing regimes. Eagles *et al.* (2010) also estimated an increase of 4.9 days on substitution of winter for spring at *Vrn-D1*. Stelmakh (1998) reported the differential effect of *Vrn* genes on flowering time, plant height, and several yield components, suggesting that genotypes with a combination of two dominant alleles at different *Vrn* loci were early to flower with higher yield. Genotypes combining three dominant alleles were earliest to flower but with reduced yield. Goncharov (2004) reported that *Vrn-A1* has the largest effect on reducing days to heading followed by *Vrn-D1* and *Vrn-B1*. Similar findings were reported by Zhang *et al.* (2008) while studying allelic variation at *Vrn1* in Chinese wheat cultivars and by Eagles *et al.* (2010) while studying the effect of *Vrn* and *Ppd* genes on Australian wheat cultivars. While studying the effect of different allelic combinations on flowering time across six different agro ecological zones of India, Singh *et al.* (2013) reported a differential effect of spring alleles at *Vrn-A1*, *Vrn-B1*, and *Vrn-D1*. They suggested that the genotypes with spring alleles at all three *Vrn* loci were earliest to flower compared to mono-dominant or di-dominant gene combinations. The *Vrn-A1* was reported more effective than *Vrn-B1* and *Vrn-D1* in reducing the days to flowering. In our results, drastic decrease was observed when insensitive allele at *Ppd-D1* was substituted by the photoperiod sensitive allele regardless of *Vrn1* alleles. Similar to our results, Worland (1996) also reported a reduction in days to heading by 4-8 days due to insensitive alleles at photoperiod gene *Ppd-D1*. It was also suggested that the shortened life cycle results into a reduction in plant height, tiller number and spikelet per ear. Apart from affecting days to flowering, several pleiotropic effects of photoperiod alleles have been reported such as reduction in plant height (Worland, 1996), reduced number of tillers per plant (Miralles and Richards, 2000; Dyck *et al.,* 2004) and reduction in the number of spikelets per spike (Snape *et al.,* 2001; Dyck *et al.,* 2004). Kamran *et al.* (2013) reported a reduction of about 5 days due to the presence of photoperiod insensitive allele *Ppd-D1a*.

Conclusion

The present study demonstrated the significant role of vernalization *(Vrn)* and photoperiod *(Ppd)* gene allelic combinations in regulating wheat phenology and yield-related traits under different sowing regimes. Among the four winter × spring wheat derived populations evaluated, only two crosses i.e. Chianti/WH1105//PBW698 and Aciento/2*PBW683 exhibited sufficient variation for days to flowering and were therefore selected for molecular characterization and agronomic evaluation. Molecular screening confirmed polymorphism for *Vrn-B1, Vrn-D1* and *Ppd-D1* loci, enabling the identification of diverse allelic haplotypes in BC$_1$F$_6$ progenies.

Evaluation of these haplotypes under different sowing environments revealed that allelic variation in *Vrn* and *Ppd* genes significantly influenced days to flowering, tiller number per plant and thousand grain weight. Genotypes carrying winter alleles at all *Vrn* loci showed delayed flowering, whereas the presence of spring alleles, particularly at the *Vrn-D1* locus, accelerated flowering. Similarly, variation at the *Ppd-D1* locus affected flowering time, tiller number and spikelets per spike, highlighting its role in photoperiod response and plant development. However, plant height was largely influenced by sowing environment rather than allelic combinations of these genes. Overall, the results indicate that specific combinations of *Vrn* and *Ppd* alleles can effectively modulate wheat phenology and yield attributes, thereby facilitating better adaptation to diverse sowing conditions. The identification of favourable allele combinations in this study provides valuable genetic resources for breeding wheat cultivars with improved adaptability and productivity under varying agro-climatic conditions, particularly in the wheat-growing regions of north-western India.

Acknowledgements

All authors are grateful for the support received under the ad hoc project, "Transforming India's Green Revolution by Research and Empowerment for Sustainable food supplies (TIGR2ESS)" funded by United Kingdom through UK Research & Innovation (UKRI), CGRF (BBSRC), grant number: BB/P027970/1.

Author contributions

Conceptualization: VSS and AS

Work implementation: HSG, GG and AS

Writing-original draft preparation: AS and TS

Writing-review and editing: AKP, AS, PG, PS, SS, GSM and TS

Supervision & logistics: AS

Conflict of interest

The authors declare no conflict of interest.

Ethical Approval

The article doesn't contain any study involving ethical approval.

Generative AI or AI/Assisted Technologies use in Manuscript Preparation

No

References

1. Adam D. 2021. How far will global population rise? Researchers can't agree. *Nature,* **597**(7877), 462–465. https://doi.org/10.1038/d41586-021-02522-6
2. Arjona JM, C Royo, S Dreisigacker, K Ammar, and D Villegas. 2018. Effect of Ppd-A1 and Ppd-B1 allelic variants on grain number and thousand kernel weight of durum wheat and their impact on final grain yield. *Frontiers in plant science,* **9**, 888.
3. Arjona JM, C Royo, S Dreisigacker, K Ammar, J Subirà and D Villegas. 2020. Effect of allele combinations at PpdL1 loci on durum wheat grain filling at contrasting latitudes. *Journal of Agronomy and Crop Science,* **206**, 64–75.
4. Cane K, HA Eagles, DA Laurie, B Trevaskis, N Vallance, RF Eastwood, N N Gororo, H Kuchel, and PJ Martin. 2013. Ppd-B1 and Ppd-D1 and their effects in southern Australian wheat. *Crop and Pasture Science,* **64**(2), 100-114.
5. Chen S, J Wang, G Deng, L Chen, X Cheng, H Xu and K Zhan. 2018. Interactive effects of multiple vernalization *(Vrn-1)*-and photoperiod *(Ppd-1)*-related genes on the growth habit of bread wheat and their association with heading and flowering time. *BMC Plant Biology,* **18**(1), 1-16.
6. Distelfeld A, C Li and J Dubcovsky. 2009a. Regulation of flowering in temperate cereals. *Curr Opin Plant Biol,* **12**: 178-84.
7. Dubcovsky J, D Lijavetzky, L Appendino, G Tranquilli and JD Dvorak. 1998. Comparative RFLP mapping of Triticum monococcum genes controlling vernalization requirement. *Theor Appl Genet* **97**: 968-75.
8. Dyck JA, MA Matus-Ca ´diz, P Hucl, L Talbert, T Hunt, JP Dubuc, H Nass, G Clayton, J Dobb and J Quick. 2004. Agronomic performance of hard red spring wheat isolines sensitive and insensitive to photoperiod. *Crop Sci,* **44**: 1976-81.
9. Eagles HA, K Cane and V Neil. 2009. The flow of alleles of important photoperiod and vernalisation genes through Australian wheat. *Crop Past Sci,* **60**: 646-57.
10. Eagles HA, K Cane and B Trevaskis. 2011. Veery wheats carry an allele of *VrnA1* that has implications for freezing tolerance in winter wheats. *Plant Breed,* **130**: 413-18.
11. Eagles HA, K Cane, H Kuchel, GJ Hollamby, N Vallance, RF Eastwood, NN Gororo and PJ Martin. 2010. Photoperiod and vernalization gene effects in southern Australian wheat. *Crop Past Sci,* **61**: 721-30.
12. Fjellheim S, S Boden and B Trevaskis. 2014. The role of seasonal flowering responses in adaptation of grasses to temperate climates. *Frontiers in plant science,* **5**, 431.
13. Foulkes MJ, GA Slafer, WJ Davies, PM Berry, R Sylvester-Bradley, P Martre, D F Calderini, S Griffiths and MP Reynolds. 2011. Raising yield potential of wheat. III. Optimizing partitioning to grain while maintaining lodging resistance. *Journal of experimental botany,* 62(2), 469-486.
14. Gomez D, L Vanzetti, M Helguera, L Lombardo, J Fraschina and DJ Miralles. 2014. Effect of Vrn-1, Ppd-1 genes and earliness per se on heading time in Argentinean bread wheat cultivars. *Field Crops Research,* **158**, 73-81.
15. Goncharov NP. 2004. Response to vernalization in wheat: its quantitative or qualitative nature. *Cereal Res Commun,* **32**: 323-30.
16. González FG, II Terrile and MO Falcón. 2011. Spike fertility and duration of stem elongation as promising traits to improve potential grain number (and yield): variation in modern Argentinean wheats. *Crop Science,* **51**(4), 1693-1702.
17. Gororo NN, RG Flood, RF Eastwood and Eagles HA. 2001. Photoperiod and vernalization responses in *T. turgidum* × *T. tauschii synthetic* hexaploidy wheats. *Ann Bot* (Lond), **88**: 947-52.

18. Hernd l M, JW White, S Graef and W Claupein. 2008. Theimpactof vernalization requirement, photoperiod sensitivity and earliness per se on grain protein content of bread wheat (*Triticum aestivum* L.). *Euphytica,* **163**: 309-20.
19. Kamran A, M Iqbal and D Spaner. 2014. Flowering time in wheat (*Triticum aestivum* L.): a key factor for global adaptability. *Euphytica,* **197**: 1-26.
20. Kamran A, HS Randhawa, C Pozniak and D Spaner. 2013. Phenotypic effects of the flowering gene complex in Canadian spring wheat germplasm. *Crop Sci,* **53**: 84-94.
21. Kippes N. 2015. Identification of the VERNALIZATION 4 gene reveals the origin of spring growth habit in ancient wheats from South Asia. *Proceedings of the National Academy of Sciences,* **112**(39): E5401-10.
22. Kiss T, K Balla, O Veisz, L Láng, Z Bedő, S Griffiths, P Isaac and I Karsai. 2014. Allele frequencies in the VRN-A1, VRN-B1 and VRN-D1 vernalization response and PPD-B1 and PPD-D1 photoperiod sensitivity genes, and their effects on heading in a diverse set of wheat cultivars (*Triticum aestivum* L.). *Molecular Breeding,* **34**(2), pp.297-310.
23. Knox J. 2012. Climate change impacts on crop productivity in Africa and South Asia. *Environmental Research Letters,* **7**(034032): 1-8.
24. Law CN, J Sutka and AJ Worland. 1978. A genetic study of day length response in wheat. *Heredity,* **41**: 575-85.
25. Miralles DJ and RA Richards. 2000. Responses of leaf and tiller emergence and primordium initiation in wheat and barley to interchanged photoperiod. *Ann Bot,* **85**: 655-63.
26. Ortiz-Ferrara G, MG Mosaad, V Mahalakshmi and S Rajaram. 1998. Photoperiod and vernalization response of Mediterranean wheats, and implications for adaptation. *Euphytica,* **100**: 377-84.
27. Singh SK, AM Singh, N Jain, GP Singh, AK Ahlawat and I Ravi. 2013. Molecular characterization of vernalization and photoperiod genes in wheat varieties from different agro-climatic zones of India. *Cereal Res Commun,* **41**: 376-38.
28. Snape JW, K Butterworth, E Whitechurch and AJ Worland. 2001. Waiting for fine times: genetics of flowering time in wheat. *Euphytica,* **119**: 185-90.
29. Stelmakh AF. 1998. Genetic systems regulating flowering response in wheat. *Euphytica,* **100**: 359-69.
30. Tan C and L Yan. 2016. Duplicated, deleted and translocated VRN2 genes in hexaploid wheat. *Euphytica,* **208**(2), 277-284.
31. Trevaskis B. 2010. The central role of the VERNALIZATION1 gene in the vernalization response of cereals. *Funct Plant Biol,* **37**: 479-87.
32. Turner A, J Beales, S Faure, RP Dunford and DA Laurie. 2005. The pseudo-response regulator Ppd-H1 provides adaptation to photoperiod in barley. *Science,* **310**:1031-34.
33. Van Beem J, V Mohler, R Lukman, M van Ginkel, M William, J Crossa and AJ Worland. 2005. Analysis of genetic factors influencing the developmental rate of globally important CIMMYT wheat cultivars. *Crop Science,* **45**(5), 2113-2119.
34. Worland AJ. 1996. The influence of the flowering time genes on environmental adaptability in European wheats. *Euphytica,* **89**: 49-57.
35. Worland T and JW Snape. 2001. Genetic basis of worldwide wheat varietal improvement. In: Bonjean AP, Angus WJ (eds) *The world wheat book: A history of wheat breeding.* Pp 61-67 Intercept Ltd., London.
36. Zadoks JC, TT Chang and CF Konzak. 1974. A decimalcode for the growth stages of cereals. *Weed Res.,* **14**(6): 415-421
37. Zhang XK, YG Xiao, Y Zhang, XC Xia, J Dubcovsky and ZH He. 2008. 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. *Crop Sci,* **48**: 458-70.