

Research Article**Vernalization and Photoperiod Genes in Iranian Wheat Cultivars****Seyyed Hamid Reza Ramazani**Department of Agronomy and Plant Breeding Sciences,
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ABSTRACT

Wheat (*Triticum aestivum* L.) can avoid the deleterious effects of low temperatures by using environmental adaptation strategies such as vernalization requirement and photoperiod reaction. Awareness of the genetic factors influencing growth and flowering patterns is necessary for introducing new varieties to specific environments. We performed morphological and genetic studies of 104 lines and cultivars of Iranian wheat genotypes, including four durum genotypes, obtained from national wheat breeding programmes. We used sequence-tagged site (STS)-PCR with specific primers to identify alleles affecting the sensitivity to vernalization and photoperiod response at the *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, *Ppd-A1*, *Ppd-B1*, and *Ppd-D1* loci. Some morphological traits such as percentage germination, growth habit, final leaf number (FLN), ear length, and days to ear emergence were also measured. Results showed that FLN and days to ear emergence are the best morphological traits to study wheat flowering time. Allelic variation showed that *Vrn-D1* is more frequent than other genes in Iranian wheat genotypes, and so most Iranian genotypes are vernalization-insensitive. In addition, most genotypes were photoperiod-insensitive because of the semi-dominant mutation allele, *Ppd-D1a*. Based on allelic variation and morphological traits, we identified five classes of Iranian genotypes. The allelic variation study and morphology evaluation of this germplasm showed that the majority of Iranian cultivars and breeding lines are spring varieties and insensitive to day length.

Keywords: Anthesis, Compatibility, Cluster analysis, Genetic diversity, GIS.**INTRODUCTION**

Cold temperatures at the end of winter or beginning of spring are one of the major reasons for wheat yield reduction in temperate regions of Iran (Anonymous, 2005). Appointment of harmony between growth phases of wheat with climate changes is a strategic way to increase wheat production per unit area (Cockram *et al.*, 2007). Iran is the biggest wheat producer of the Middle Eastern countries (FAO, 2012), and has introduced most of its wheat germplasm through national experiments or worldwide wheat breeding programmes such as CYMMYT and ICARDA.

Vernalization is controlled by the vernalization (*Vrn*) genes. The vernalization requirement of wheat is mostly controlled by three *Vrn* genes, *Vrn-A1*, *Vrn-B1*, and *Vrn-D1* (which are located on the long arms of chromosomes 5A, 5B and

5D, respectively), and their allelic combination is very important for determination of the vernalization demand of wheat genotypes. It has been shown that the allelic distribution of these three genes is different in different environments (Langer *et al.*, 2014; Kiss *et al.*, 2014); these three *Vrn-1* genes perform additively, with the dominant allele of *Vrn-A1* exerting the strongest effect (Galiba *et al.*, 1995; Worland 1996; Barrett *et al.*, 2002; Yan *et al.*, 2003). Allelic variation for these loci at the time of segregation of *Vrn-1* This gene, which produces a zinc finger protein, has been isolated from diploid wheat and has not been found in the *Arabidopsis thaliana* genome. The dominant allele of *Vrn-1* is not inhibited by *Vrn-2*, and this produces a spring wheat (which has no need for vernalization) (Yan *et al.*, 2004). *Vrn-2*, as an

inhibitor gene, is activated by deletion of *Vrn-1*. Spring wheat can also be produced by lack of effective mutations in the *Vrn* genes. These mutations allow winter alleles (*Vrn-1*) to provide sufficient regulation without vernalization (Fu *et al.*, 2005). Some mutations of *Vrn* genes and their functions are given in Table 1.

Table 1 presented.

Photoperiod sensitivity is controlled by the *Ppd-A1*, *Ppd-B1*, and *Ppd-D1* genes, which are located on the homologous group 2 chromosomes (Kato and Yokoyama, 1992; Worland *et al.*, 1998; Dubcovsky *et al.*, 2006; Yang *et al.*, 2009; Guo *et al.*, 2010; Bentley *et al.*, 2011). The *Ppd* genes encode a type of pseudo response regulator (PRR) protein, which is involved in the activation of the photoperiod pathway. The Iranian climate is divided to four zones for wheat cultivation according to temperature and growth type: Zone I, the warm and humid climate of Caspian Sea beaches; Zone II, the dry and warm climate of the southern areas; Zone III, a temperate climate; and Zone IV, a cold climate (Jalal Kamali *et al.*, 2012). Thus, we aimed to genotype Iranian wheat germplasms using specific allelic markers for loci affecting cold requirement (*Vrn-A1*, *Vrn-B1*, and *Vrn-D1*) and day length sensitivity (*Ppd-A1*, *Ppd-B1*, and *Ppd-D1*) to determine the genetic and allelic diversity of Iranian genotypes. Marker-assisted selection should help breeders in segregate generations to breed genotypes that are better adapted to specific sites.

MATERIAL AND METHODS

Plant material

In total, 104 wheat cultivars and lines were obtained from the national wheat breeding programmes of Iran for this study. Four of these genotypes (Yavarous, Karkhe, Arya, and Dena) were durum genotypes. Bread wheat is hexaploid (AABBDD) but durum is tetraploid (AABB), thus the *Vrn-D1* and *Ppd-D1* loci do not exist in durum genotypes.

Phenotyping

Vernalization was performed using the water-deficiency method (Purvis and Gregory, 1952).

Seeds were disinfected with sodium hypochlorite 1%, then washed in sterile water, and placed on filter paper in 10 cm petri dishes. Seeds were kept at 50% humidity and 2–4°C in a dark refrigerator for 60 days of vernalization (Streck *et al.*, 2003). After that, plantlets with the same length of growth were translocated to the farm (Van Beem *et al.*, 2005).

Plants were cultivated using two treatments of photoperiod and temperature by sowing on two different dates in the Center of Agricultural and Natural Resources Research of South Khorasan (latitude: 35.52 and longitude: 59.13, with 1280 m elevation) in 2013–2014. Dates of planting were determined by the method of Herndl *et al.*, (2008) as April 3 and May 6, with the former used for applying the day length treatment and the latter one having a lesser effect on day length. This experiment was performed with three replications. Treatments were contained date of planting, vernalization at two levels (with and without vernalization) and genotypes, planted on a row 1.5 m in length with 50 plants. Germination, ear length at the time of maturity, and plant growth type (prostrate, semi-prostrate, moderate, semi-erect and erect) were recorded to measure reaction to vernalization and day length.

Genotyping

Allelic variation for *Vrn* and *Ppd* loci on Iranian wheat genotypes was performed in the biotechnology laboratory of CIMMYT using STS-PCR specific primers (Table 1). DNA of each genotype was extracted using the CTAB method (Doyle and Doyle, 1987). Agarose gel electrophoresis of PCR products was performed, and bands were visualized using ethidium bromide. Based on analysis of the location of the gene *Vrn-A1*, the alleles *Vrn-A1a*, *Vrn-A1b*, and *vrn-A1* were identified (Yan *et al.*, 2004b). Then, based on defects in the first intron of the gene *Vrn-1*, the alleles *Vrn-A1c*, *Vrn-B1*, and *Vrn-D1* were determined (Beales *et al.*, 2007; Fu *et al.*, 2005; Yan *et al.*, 2006).

Data analysis

Cluster analysis using measured traits and allelic variation was performed by the square of Euclidean distance criteria and the Ward

method, using PAST software (Hammer, Harper and Ryan, 2001).

RESULTS and Discussion

Morphological traits

Analysis of variance showed that planting date, vernalization, and genotype were significant for all measured traits except germination, which was not affected by vernalization (data not shown). Mean comparison showed that vernalization led to fewer days to ear emergence, consistent with the results of previous studies (Flood and Halloran, 1984; Goncharov, 2004; Trevaskis *et al.*, 2007).

The greatest ear length and FLN were observed with non-vernalized treatment as continues growing of some winter genotypes. As noted previously, vernalization demand is a way to allow acclimatization of the genotype to different conditions (Cockram *et al.*, 2007; Mahfoozi *et al.*, 2006), so that a genotype with vernalization demand will be encouraged to stimulate anthesis after exposure to cold temperature with a specific photoperiod and enough water, thus it will be more successful than plants without this demand. FLN and days to ear emergence after vernalization, which are the main traits used to quantify the vernalization demand of genotypes (Wall & Cartwright, 1974; Davidson *et al.*, 1985; Hoogendoorn, 1985; Ortiz-Ferrara, 1998), were also significantly different between genotypes in this study (Table 2).

Table 2 presented.

Means comparison between genotypes under vernalized conditions (Table 2) showed that the longest ear (5.833 cm) was in a Roshan cultivar and the shortest (1.167 cm) was in a spring backcross of Roshan. Ear length of the winter backcross of Roshan was 4 cm, which was significantly lower than that of Roshan itself. This comparison indicated the inaccuracy of this trait to quantify vernalization requirement, owing to its inconsistent variation in genotypes with the same origin (Roshan). In addition, germination that is not affected by vernalization cannot be a reliable trait to quantify vernalization requirement. The growth type of wheat as a qualify trait can be easy to cause by

human error or can be severely affected by environment, making this trait unsuitable for quantifying vernalization demand. Thus, germination, growth type, and ear length, as indicated by previous studies (Miura & Worland, 1994; Ortiz-Ferrara, 1998) cannot be relied upon for quantifying vernalization requirement.

Means comparison between genotypes under vernalized conditions (Table 2) showed that the highest FLN occurred in Omid and Arya cultivars (8.333), which are located near each other in the cluster analysis (Fig. 1), while the Arge cultivar had the lowest FLN.

Fig. 1 presented

In addition, the highest number of days to ear emergence was found in Rasad (72.667 days), Karaj 3, and Ohadi (both 66.000 days), which are also located near each other in the cluster analysis (Fig. 1), while the lowest number of days to ear emergence (47.667 days) was found in a Parsi cultivar. These results showed that FLN and days to ear emergence, as well as response to temperature, day length, and *EPS* characteristics are suitable for quantifying vernalization requirement, as Herndl *et al.*, (2008) reported, because there was harmony between their morphological means and allelic variation in vernalization and photoperiod loci (Table 2, Fig. 1).

Allelic variation

Our results showed that allelic variation for vernalization genes is high, and about half the genotypes had the dominant *Vrn-A1* allele, while half of them had the *Vrn-B1* allele but about two-thirds of them had the dominant allele of *Vrn-D1*. Thus, most of these genotypes do not require cold treatment for initiation of flowering (Barrett *et al.*, 2002; Yan *et al.*, 2003; Yan *et al.*, 2004; Fu *et al.*, 2005). Nine of the genotypes had the non-dominant allele for *Vrn-A1*, *Vrn-B1*, and *Vrn-D1*, therefore they were winter types (Barrett *et al.*, 2002; Yan *et al.*, 2003) (Table 3).

Table 3 presented.

There were only a few genotypes sensitive to day length (Table 2). Most of the genotypes had the dominant allele of *Ppd-A1*, which confers sensitivity to day length; the exceptions were the three durum genotypes Yavarus, Arya, and Dena, which had *Ppd-A1a*. In addition, the

insensitive *Ppd-D1a* allele (the semi-dominant mutation has been identified as the major source of earliness in wheat varieties worldwide; Kumar *et al.*, 2012) was the most frequent allele in the *Ppd-D1* loci. All genotypes had the insensitive allele at the *Ppd-B1* loci (Table 2). Thus, according to the genotyping results of the *Ppd* alleles, most of the genotypes should be insensitive to day length. It should be noted that most of the studied genotypes are spring types, and in agreement with the report of Mohammadi *et al.*, (2012), most Iranian wheat genotypes have only a minimum requirement for cold treatment. In addition, there were significant differences between the four durum cultivars for all measured traits, despite having the same alleles for *Vrn-A1*, *Vrn-B1*, *Ppd-A1*, and *Ppd-B1*. This result suggests the contribution of some unknown genetic elements such as *EPS* genes to flowering time.

Clustering

Based on the morphological traits under vernalized conditions and the allelic variation for *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, *Ppd-A1*, *Ppd-B1*, and *Ppd-D1* genes, a cluster with cophenetic correlation of 0.72, comprising five major classes, was drawn (Fig. 1). Based on the fact that many of the factors affecting the flowering pathway in wheat are unknown, and even under the best conditions only half of the variation in flowering time is accounted for by genetic variation (Li and Dubcovsky 2008; Kiss *et al.*, 2014; longer *et al.*, 2014), precise distinguishing of winter types and other types on the basis of allelic variation of the known loci is difficult. Therefore, using only these loci to predict the growth habit of a genotype can be misleading, and phenotypic evaluation is essential. The earliest genotypes in Iran were placed in class IV of the dendrogram (Fig. 1) because of the high frequency of earliness alleles at vernalization loci in this group and the high frequencies of alleles for day length insensitivity (Table S1), especially for the locus of *Ppd-D1* as it is the main photoperiod gene (Kumar *et al.*, 2012).

Table S1 presented.

By contrast, genotypes with the longest period to ear emergence are located in classes II and V,

owing to their photoperiod sensitivity and alleles of vernalization loci (Table S1). The other classes (I and III) are intermediate for earliness and their distinction back to their different alleles with the same function.

Geographical distribution

The cold region of Iran (zone IV) (Jalal Kamali *et al.*, 2012) is much more suitable for winter cultivars than the other zones, and spring wheat varieties are mainly cultivated in the other regions of Iran (Table S2), especially the warm southern and warm northern regions (zones I and II).

Table S2 presented.

The long-term average temperatures in January have an important effect on determination of the four climates in Iran (Jalal Kamali *et al.*, 2012). Based on the dominant cultivar cultivated in different regions of Iran, cultivars with *vrn-A1* in clusters II and V are mainly cultivated in the north-western and north-eastern regions of Iran, while cultivars with *Vrn-A1* are the most frequently grown types in the central and southern regions. Although this difference seems to be due to the January temperature, some cultivars grown in regions with the same January temperature had different vernalization alleles. Hence, distribution of wheat growth habit and *Vrn* loci cannot be determined on the basis of winter temperature because maintenance of genetic variation for acclimatization traits in microclimate areas is necessary. Although most of the Iranian wheat genotypes are spring types (Table S2), wheat cultivation is mainly performed in autumn (Mahfoozi *et al.*, 2006; Ramazani *et al.*, 2015). This further complicates flowering time by other factors such as *EPS* genes. Thus, genotypes with different combinations of these genes can produce adaptations to a specific site or cold winter independently of vernalization or photoperiod sensitivity genes (Iwaki *et al.*, 2000). Generally, cultivars with the *vrn-A1* allele, which are found in the north-western and north-eastern regions of Iran, enter flowering early under warm conditions without vernalization, and they are also cultivated in warm regions with fair or firm winters (Berry *et al.*, 1986). The *Vrn-B1* gene was observed

mainly in cultivars in the western regions, whereas the *Vrn-D1* gene was found mainly in cultivars in the southern and northern regions. In addition, heterogeneity of *Vrn-B1* with the recessive allele was observed in most parts of Iran (Table S1, Fig. 2).

Fig. 2 presented

As Fig. 3 shows, the insensitive vernalization alleles of *Vrn-A1* locus (b and w) are very common among Iranian wheat genotypes, and according to the results for this locus, most Iranian genotypes are spring type and grown mainly in the central, south, and western regions of Iran, while its recessive alleles are more frequent in the east, north and north-west of Iran. *Vrn-B1* and *Vrn-D1* loci had insensitive vernalization alleles in most regions of Iran except the eastern regions for *vrn-B1* and the western regions for *vrn-D1* (Fig. 3).

Fig. 3 presented.

Taken together, these results show that the most frequent vernalization alleles adapted to the agro-climates of Iran are *Vrn-A1w*, *Vrn-B1a*, and *Vrn-D1a*. Distribution of *Ppd* genes in different regions of Iran showed that in all regions of Iran, *Ppd-A1* had photoperiod-sensitive alleles but *Ppd-B1* had photoperiod-insensitive alleles. The *Ppd-D1* gene was dominant and photoperiod-insensitive in the south and north of Iran, and it was heterozygous in the other regions, except in the eastern parts of Iran, where the sensitive allele was found. In addition, allelic diversity for *Ppd* loci at regional level (Fig. 3) showed that *Ppd-A1* had sensitive alleles in most parts of Iran except some southern outskirts, but *Ppd-B1* had insensitive alleles in most regions of Iran except some northern and north-western regions. The *Ppd-D1* locus had insensitive alleles in northern, western, and southern regions, but had sensitive alleles in eastern regions of Iran. The *Ppd-D1* locus was heterozygous in central regions of Iran. On the whole, the most frequent photoperiod sensitivity alleles found in verified genotypes in Iranian climates were *Ppd-D1a*, *Ppd-B1b*, and *Ppd-A1w*.

The puzzle of wheat flowering time has attracted the attention of many researchers around the world. Although flowering time has a role, it is

by no means the only cause of cultivar adaptation to different environments. In addition, it is still not possible to determine with confidence when flowering will happen, and much more information is needed to understand the flowering pathway, the vernalization and photoperiod sensitivity genes and their functions, the *EPS* genes and regulatory genes and their interactions, and finally the interaction of these genes with the environment. Overall, it seems that phenotypic evaluation of genotypes is essential in order to understand their growing habit. The best morphological traits to quantify the function of flowering time genes are FLN and days to ear emergence under either vernalized or non-vernalized conditions. Our allelic variation study showed that *Vrn-D1* is more frequent than other genes in Iranian wheat genotypes, and the majority of genotypes studied were photoperiod-insensitive. In addition, given the importance of the *Ppd-D1* allele (the semi-dominant mutation *Ppd-D1a* allele that is insensitive to day length) on determination of genotypes for day length insensitivity (Kumar *et al.*, 2012), most genotypes were insensitive to day length, except for Arvand, Omid, Tabasi, Sorkhtokhm, Mahooti, Homa, Ohadi, and Karim. Despite many unknown factors affecting flowering time of wheat, we were able to distinguish five classes of Iranian genotypes based on their allelic variation and morphological traits. However, this classification is unable to distinguish winter types from others. Other genes affecting earliness need to be determined and assessed in order to allow precise distinction between genotypes for site-specific adaptation on the basis of flowering time. The most frequent alleles of vernalization in the four zones of Iran for wheat cultivation are *Vrn A1/vrn A1*, *Vrn B1/vrn B1*, and *Vrn D1/vrn D1* for Zone I; *Vrn A1*, *Vrn B1/vrn B1*, and *Vrn D1* for Zone II; *Vrn A1/vrn A1*, *Vrn B1/vrn B1*, and *Vrn D1/vrn D1* for Zone III; and *vrn A1*, *Vrn B1*, and *Vrn D1* for Zone IV. Photoperiod insensitivity alleles were also dominant in all zones of the Iranian climate. Finally, the allelic variation study and morphological evaluation in this study showed that most of the Iranian

cultivars and breeding lines were spring type and insensitive to day length.

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Table 1: Genes and their alleles affecting on vernalization and photoperiod in wheat.

Gene	Chr.	Allele	Multiple	Reference variety	Effect on flowering	References
<i>Vrn-A1</i>	5A	a	Promoter deletion		Earliness	Yan <i>et al.</i> , 2004
		b	Deletion of 20 additional bp	Marquis	Delaying	Yan <i>et al.</i> , 2004
		v	SNP on exon 4 in winter allele	Jagger	Earliness	Eagles <i>et al.</i> , 2009, Chen <i>et al.</i> , 2010
		w	Winter allele/ wild type		Delaying, resistant to winter cold	Yan <i>et al.</i> , 2004
<i>Vrn-B1</i>	5B	a	Deletion of intron 1		Earliness	Yan <i>et al.</i> , 2004
		b	36 bp deletion in intron 1	Alpowa	Unknown	Milec <i>et al.</i> , 2011, Sandra <i>et al.</i> , 2009
		w	Winter allele/ wild type		Delaying	Yan <i>et al.</i> , 2004
<i>Vrn-D1</i>	5D	a	Deletion of intron 1		Earliness	Yan <i>et al.</i> , 2004
		w	Winter allele/ wild type		Delaying	Yan <i>et al.</i> , 2004
<i>Ppd-D1</i>	2D	a	Deletion of 2 kbp of upstream 5 sequence	Ciano67	Insensitivity/earliness	Beales <i>et al.</i> , 2007
		b (M)	mariner translocation in intron 1	Mercia	Sensitivity/ Delaying	Beales <i>et al.</i> , 2007
		b (N)	Deletion of 5 bp in exon	Nor star	Sensitivity/ Delaying	Beales <i>et al.</i> , 2007
		w	wild type	Chinese Spring	Sensitivity/ Delaying	Beales <i>et al.</i> , 2007
<i>Ppd-B1</i>	2B	a	Deletion of 2 kbp of upstream 5 sequence, 3 fold addition of	Sonora64/Ti mstein	Insensitivity/earliness	Diaz <i>et al.</i> , 2012

		genes copy number				
<i>Ppd-A1</i>	2A	b	Same haplotype with different copy number of the genes	Paragon/Chayenne/Renan	Insensitivity and sensitivity/ Earliness	Diaz <i>et al.</i> , 2012
		a	Upstream deletion of 1027 (GS100) /1117(GS105)	Durum wheat	Insensitivity/Earliness	Wilhelm <i>et al.</i> , 2009
		b	Deletion of exon 5	Cappelle-Desprez	Without effect allele	Beales <i>et al.</i> , 2007
		c	insertion of 1.2 kbp	Chinese Spring	Sensitivity allele/ Non definite	Beales <i>et al.</i> , 2007
		w	wild type		Sensitivity allele/ non definite	Beales <i>et al.</i> , 2007

Table 2: Alleles of genotypes for studied genes and means for morphological traits in vernalized condition.

Cultivar/ line	Genes						Morphological traits Cultivar/ line at vernalized condition				
	<i>Vrn.A</i>	<i>Vrn.B</i>	<i>Vrn.D</i>	<i>Ppd.A</i>	<i>Ppd.B</i>	<i>Ppd.D</i>	Germination (%)	Final leaf No.	Growth type	Ear length	Days to ear emergenc
	w	a/b/v/	a/b/w	a/w	a/b/w	a/b					
Karaj 1	v	a	a	w	b	.	90.00	6.67	4.00	3.33	59.33
Karaj 2	w	.	a	w	b	a	90.00	6.67	4.33	3.67	53.33
Karaj 3	av	a	a	w	b	b	80.00	6.67	4.67	4.00	66.00
Azadi	av	a	a	w	b	a	86.67	6.00	4.67	3.00	55.00
Ghods	av	a	a	w	b	a	83.33	6.67	4.67	4.33	52.33
Mahdavi	.	a	w	w	b	a	83.33	6.67	4.33	2.17	56.33
Nicknejad	av	a	w	w	b	a	83.33	7.00	4.33	2.50	50.00
Pishtaz	.	b	a	w	a	a	83.33	6.33	4.00	4.00	50.00
Shiraz	av	w	w	b	a	a	83.33	6.00	4.33	4.00	56.00
Marvdasht	av	a	w	w	b	a	80.00	6.67	4.00	3.00	55.67
Sepahan	av	b	w	w	a	a	100.00	6.33	3.33	3.00	51.67
Bahar	av	a	a	w	a	a	76.67	7.00	4.00	2.00	56.67
Parsi	w	.	a	w	a	a	100.00	6.33	3.67	3.00	47.67
Sivand	.	b	a	w	b	a	90.00	6.33	3.33	2.33	55.33
WS-82-9	.	b	a	w	b	a	93.33	6.67	1.67	1.83	52.67
WS-85-10	v	a	a	b	a	a	83.33	7.00	3.00	1.67	57.67
WS-86-14		a	a	w	a	a	90.00	7.33	2.67	2.33	50.67
Bezostaya	w	.	w	w	b	a	86.67	7.00	3.33	3.00	57.00
Navid	av	.	w	w	b	b	90.00	7.00	4.00	3.33	60.00
Alamoot	w	.	w	w	b	a	90.00	7.67	4.33	4.33	57.67
Alvand	.	a	w	w	b	a	96.67	7.67	5.00	4.33	51.33
Zarin	.	a	w	w	b	a	86.67	7.00	2.33	2.33	57.67
Uroom	v	w	a	w	b	a	90.00	6.67	3.67	3.00	52.33
Zare	w	w	.	w	b	a	83.33	7.33	3.33	3.67	57.67
Mihan	v	w	a	.	b	a	93.33	6.67	1.67	2.67	55.33
MV-17	w	w	w	w	b	a	90.00	6.67	3.67	1.33	56.67
Gaspard	w	w	a	w	b	b	96.67	6.67	4.33	4.67	59.67
Gaskogen	w	w	w	w	b	.	93.33	7.67	2.67	3.00	55.00
Soissons	w	w	w	.	a	a	96.67	7.00	5.00	3.67	56.33
Shahriar	av	.	w	w	a	a	96.67	7.33	4.33	4.33	56.33
Tous	w	a	w	w	b	a	86.67	6.67	4.67	3.33	55.33
C-85-3	w	w	a	w	b	b	86.67	6.33	4.33	2.33	57.00
C-85-6	w	.	a	w	a	a	86.67	7.33	4.33	4.00	59.67
C-86-3	w	.	w	w	b	a	90.00	6.00	1.33	1.67	56.33
C-86-5	w	w	w	w	b	b	86.67	6.33	4.33	4.00	56.00
C-86-6	w	a	w	w	a	.	86.67	7.67	3.33	2.67	56.67
Pishgam	v	w	a	.	b	a	93.33	8.00	3.67	3.00	54.33

Continued table 2: Alleles of genotypes for studied genes and means for morphological traits in vernalized condition.

Cultivar/ line	Genotypes						Morphological traits at vernalized condition				
	<i>Vrn.A1</i> a/b/v/w	<i>Vrn.B1</i> a/b/w	<i>Vrn.D1</i> a/w	<i>Ppd.A1</i> a/b/w	<i>Ppd.B1</i> a/b	<i>Ppd.D1</i> a/b/w	Germinati on (%)	Final leaf No. (FLN)	Growt h type	Ear lengt h	Days to ear emergenc e
Tajan	v	.	a	w	a	a	90.00	6.67	3.67	3.67	48.67
Shiroodi	w	.	.	b	a	a	90.00	7.67	3.00	3.33	49.67
Darya	av	.	a	w	b	a	93.33	7.00	2.33	2.67	48.33
Arta	w	.	a	w	a	a	93.33	7.00	3.33	2.67	49.67
Morvarid	.	w	a	w	b	a	86.67	6.67	2.67	1.50	51.67
Gonbad	w	w	a	w	b	a	90.00	7.67	1.67	1.67	56.67
Arvand	w	b	a	w	.	w	73.33	7.00	4.67	1.33	54.33
Chenab	av	a	a	w	a	a	86.67	7.33	3.67	2.00	54.33
Bayat	v	a	w	w	ab	a	76.67	6.67	4.00	3.33	60.00
Falat	w	a	a	b	b	a	96.67	7.00	1.33	1.83	50.00
Hirmand	b	b	w	w	b	a	80.00	7.67	3.33	1.67	53.33
Darab 2	av	a	w	w	a	a	96.67	6.00	2.67	2.67	49.67
Atrak	w	w	a	w	b	a	90.00	6.33	1.00	2.00	48.00
Chamran	w	w	a	b	a	a	66.67	6.00	3.00	1.83	49.00
Star	a	a	a	w	b	a	86.67	6.67	3.33	2.33	51.67
Dez	.	.	a	w	a	a	96.67	7.33	1.00	3.00	50.67
Vee/Nac	a	w	a	w	a	a	73.33	6.00	1.00	2.00	54.67
LineA	v	a	a	w	a	a	80.00	6.67	1.00	1.00	57.00
S-78-11	a	w	w	w	b	a	70.00	6.33	1.33	2.00	56.33
Aflak	a	a	w	w	a	a	76.67	7.00	2.00	1.67	57.67
Chamran 2	v	b	.	b	b	a	76.67	7.67	3.33	2.67	56.67
S-84-14	v	a	a	w	b	a	70.00	7.67	2.00	1.50	58.00
S-85-19	.	a	a	b	a	.	66.67	6.67	1.00	1.33	52.33
S-87-18	a	a	.	w	b	a	90.00	7.33	4.00	2.33	48.67
S-87-19	.	w	a	b	a	a	76.67	7.00	2.00	1.33	53.00
S-87-20	.	a	a	.	b	a	66.67	7.67	3.33	1.50	53.67
Roshan	w	.	a	w	a	w	96.67	7.67	5.00	5.83	52.67
B.C .	w	w	a	w	a	a	86.67	6.00	1.00	1.17	54.67
B.C .	w	w	.	.	a	w	73.33	7.33	4.67	4.00	58.67
Maroon	a	b	a	w	a	a	90.00	6.00	3.00	3.00	48.67
Kavir	a	a	w	w	a	w	93.33	6.67	4.00	4.00	48.33
Hamoon	w	a	w	b	a	w	83.33	8.00	3.00	2.00	56.33
Bam	w	a	.	w	a	w	86.67	6.00	3.67	2.33	55.33
Neishabor	w	a	w	w	a	b	86.67	6.33	3.67	2.50	54.67
Sistan	w	a	.	w	a	w	96.67	7.33	4.33	3.00	55.67
Arg	w	a	w	w	a	b	93.33	5.67	3.33	3.17	55.00
MS-84-13	a	a	.	w	a	w	93.33	8.00	1.33	1.33	58.67
Yavarous*	v	w	*	a	b		90.00	7.00	3.67	3.00	52.00
Karkheh	v	w	.	.	b		93.33	7.33	3.67	1.00	57.00
Arya	v	w	.	a	b		80.00	8.33	4.67	2.17	48.00
Dena	v	w	.	a	b		86.67	6.00	4.00	3.83	53.33
Omid	w	w	a	w	b	w	60.00	8.33	2.00	1.33	58.00
Azar2	w	.	a	w	b	a	86.67	7.00	3.67	2.67	51.00
Zagross	w	a	a	w	b	a	90.00	6.33	2.33	4.00	48.67

Continued table 2: Alleles of genotypes for studied genes and means for morphological traits in vernalized condition.

Cultivar/ line	<i>Vrn-A1</i>	<i>Vrn-B1</i>	<i>Vrn-D1</i>	<i>Ppd-A1</i>	<i>Ppd-B1</i>	<i>Ppd-D1</i>	Morphological traits at vernalized condition				
	<i>a/b/v/w</i>	<i>a/b/w</i>	<i>a/w</i>	<i>a/b/v/w</i>	<i>a/b</i>	<i>a/b/w</i>	Germination (%)	Final leaf No. (FLN)	Growth type	Ear length	Days to ear emergence
Homa	w	w	w	w	b	w	90.00	7.33	3.00	1.33	51.00
Rasad	w	w	w	w	b	.	76.67	7.00	3.00	1.67	72.67
Ohadi	w	w	w	w	b	w	76.67	7.33	4.00	1.33	66.67
Koohdasht	a	a	a	w	b	a	90.00	6.33	2.67	1.50	56.33
Gahar	w	a	a	b	a	b	86.67	6.33	2.67	1.33	56.33
Seimare	v	a	.	w	b	.	76.67	7.67	4.00	2.67	48.00
Karim	v	.	w	w	b	w	80.00	7.67	4.00	2.33	49.33
C-85-D8	av	a	a	w	b	.	80.00	7.00	2.00	2.17	57.33
C-85-D9	av	a	w	w	a	b	83.33	6.67	1.67	1.67	56.67
C-85-D13	av	w	w	w	a	.	96.67	6.67	1.33	1.67	57.00
Moghan 3	w	a	a	b	b	a	86.67	7.00	1.00	2.00	51.67
Golestan	w	b	a	.	a	.	86.67	6.67	3.00	2.33	53.00
Rasool	av	a	a	b	b	a	93.33	7.00	1.33	3.00	49.00
Pastor	.	a	a	b	b	a	83.33	6.67	3.33	2.33	55.67
Rijaw	v	.	.	w	b	a	83.33	7.00	2.67	2.00	49.33
Sabalan	w	a	w	w	b	a	90.00	7.00	5.00	3.33	54.33
Sween 220	w	.	a	w	b	a	76.67	7.67	3.00	1.67	58.67
Tabasi	w	w	a	w	b	w	86.67	7.67	3.00	1.33	59.67
Sorkhtokhm	w	.	a	w	b	w	83.33	6.33	2.33	3.00	51.00
Mahooti	w	w	a	w	b	w	76.67	7.00	2.67	3.67	56.33
Sholeh	w	.	a	w	a	w	60.00	6.67	5.00	3.00	57.67
Sardari	w	w	w	w	b	.	76.67	8.00	4.67	2.67	58.00
Baran	w	w	a	w	b	a	83.33	7.33	4.33	2.33	51.67
Mean							85.51	6.93	3.22	2.60	54.51
LSD (P<0.05)							16.47	1.05	1.27	1.49	4.82

* Durum genotypes which they don't have DD genome and so loci of *Vrn-D1* and *Ppd-D1*.**Table 3.** Frequency of dominant allele at vernalization loci.

Abbreviation letters	Allelic composition			Genotypes (%)
ABD	<i>Vrn-A1</i>	<i>Vrn-B1</i>	<i>Vrn-D1</i>	23
ABd	<i>Vrn-A1</i>	<i>Vrn-B1</i>	<i>vrn-D1</i>	11
AbD	<i>Vrn-A1</i>	<i>vrn-B1</i>	<i>Vrn-D1</i>	11
Abd	<i>Vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	4
aBD	<i>vrn-A1</i>	<i>Vrn-B1</i>	<i>Vrn-D1</i>	12
aBd	<i>vrn-A1</i>	<i>Vrn-B1</i>	<i>vrn-D1</i>	9
abD	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>Vrn-D1</i>	21
Abd	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	9
Total				100

Vrn and *vrn* show insensitive vernalization alleles and sensitive vernalization alleles, respectively.

Table S1. Characteristics of classes defined in dendrogram

Classes	<i>Vrn.A1</i>	<i>Vrn.B1</i>	<i>Vrn.D1</i>	<i>Ppd.A1</i>	<i>Ppd.B1</i>	<i>Ppd.D1</i>	Morphological traits				
	<i>a/b/v/w</i>	<i>a/b/w</i>	<i>a/w</i>	<i>a/b/w</i>	<i>a/b</i>	<i>a/b/w</i>	Germination (%)	FLN	Growth type	Ear length	Days to ear emergence
Class1	w/v*	w/a	a	w/b	a/b	a	69.25	7.33	3.00	1.85	53.10
Class2	w/av	w/a/b	w/a	w/b	a/b	a/w	79.80	7.01	3.43	2.52	57.47
Class3	w/v/a	a/w	a/w	w/a/b	b/a	a/b	89.50	6.74	3.04	2.44	53.33
Class4	w/av/b	a/b/w	a/w	b/w	b/a	a/w/b	87.50	6.79	3.73	2.69	52.72
Class5	w/av	w/a/b	w/a	w	b/a	a/w/b	92.34	6.95	3.35	2.91	55.16

*; alleles are written based on their high frequency from left to right at the noted class.

Table S2. Geographical distribution percentage of spring wheat cultivars in four different climates of Iran

Climate	No. Cultivars	Spring (%)	Long term means of January temperature (°C)	Long-term average January temperature of some cities (°C)*
North- warm (I)	16	87.5	7.02	Rasht (6.67), Sari (7.39), Gorgan (7.04)
South- warm (II)	25	100	12.5	Ahwaz (12.37), Bushehr (15.22), Bandar Abbas (17.37), Zahedan (7.12), Jiroft and Kahnooj (10.5)
Temperate (III)	19	79	3.07	Shiraz (6.08), Kerman (4.54) Esfahan (3.22), Sanandaj (0.22), Shahrekord (-2.16), Ilam (4.0), Yasooj (2.52), Tehran (4.3), Yazd (5.88), Semnan (3.71), Kermanshah (2.03), Birjand (3.5), Mashhad (2.07)
Cold (IV)	22	55	-1.75	Tabriz (-1.57), Urmia (-2.28), Hamadan (-2.62), Zanjan (-2.67), Ardebil (-3.28), Qazvin (-0.4), Bojnourd (-0.54)

*Temperatures are extracted from Meteorological Organization of Iran leading to 2014.

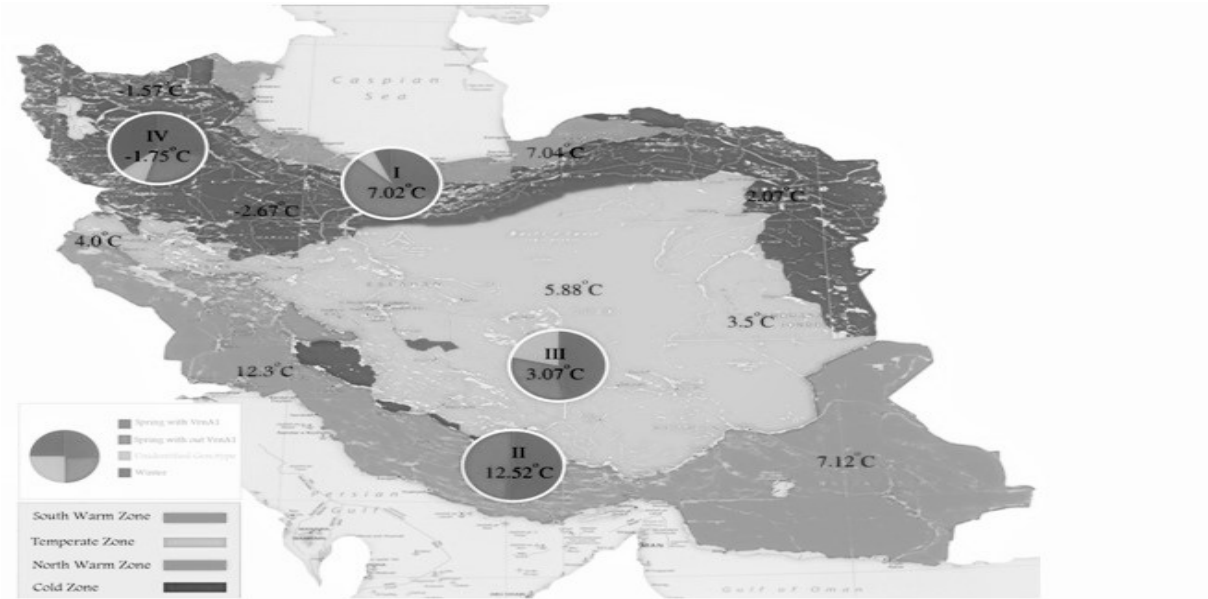


Figure 2. Eco-geographical diversity plan of growth habit of wheat cultivars in four different climates of Iran. The radial parts of the graph, blue, red, green, and purple indicating spring cultivars with genes *Vrn-A1*, percentage of spring cultivars without genes *Vrn-A1*, percentage of anonymous cultivars and winter cultivars percentage, respectively. In this figure, long-term average temperature of January is displayed. (Data of temperatures are extracted from the Meteorological Organization of Iran leading to 2014. Cereal Department Research of Seed and Plant Improvement Institute is the reference of climate classification).

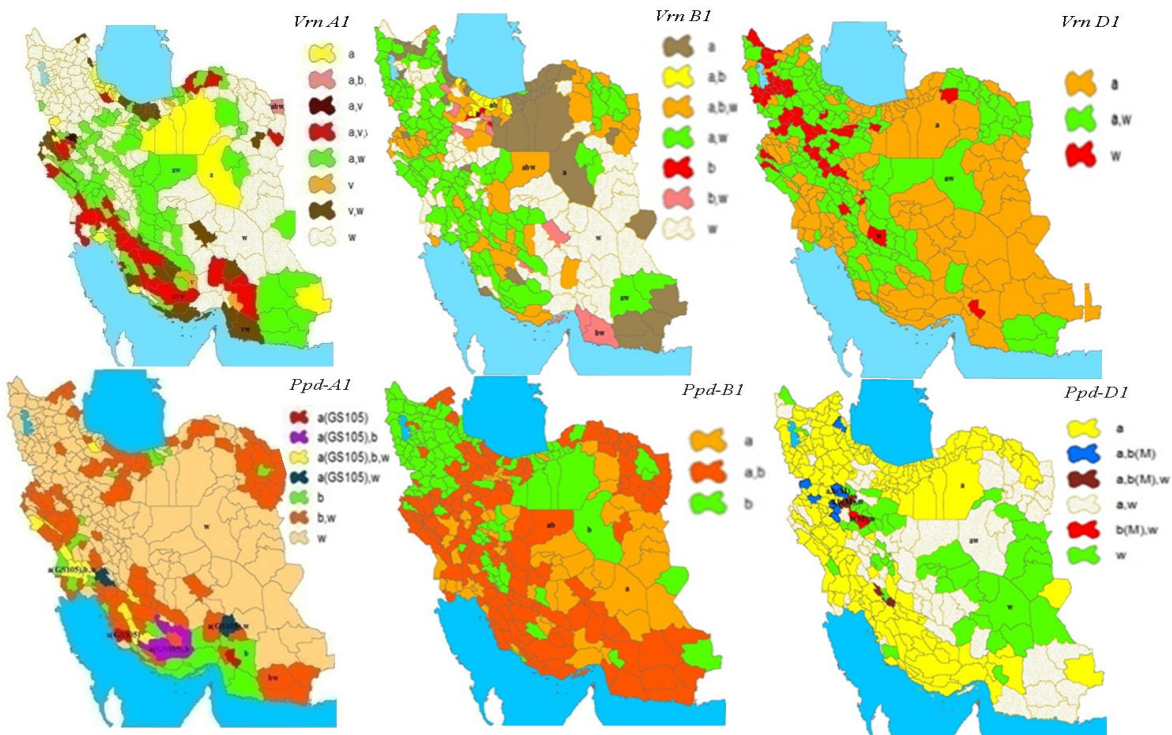


Figure 3. Allelic diversity of *Vrn 1* and *Ppd* genes at different parts of Iran. Phenotype of these abbreviations was determined in table 1.