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Allelic Variation of *VRN-1* Locus in Iranian Wheat Landraces

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Abstract
Wheat is a crop with spring and winter types and wide adaptability to different climate conditions. The wide adaptability of wheat is mainly controlled by three groups of genetic factors and among them vernalization (*VRN*) genes play pivotal role in determining spring and winter types. In this study, 395 Iranian wheat landraces were characterized with specific primer pairs designed based on *VRN-1* promoter and intron regions. Using the specific primers for *Vrn-A1c* allele, two fragments were amplified in 35 genotypes. Based on MADS-Box and promoter regions of *VRN-1* gene specific primers, two new fragments were amplified in Iranian wheat landraces which has not been reported previously. *Vrn-A1b* allele determining spring habit was the most frequent allele, whereas *Vrn-A1c* showed less frequency. Frequency of dominant allele *Vrn-A1b*, in winter genotypes was higher than that of spring type. It supports the presence of other regulatory sites outside of the *VRN* promoter region.

Keywords: Earliness *per se* genes; Landraces; Photoperiod; Spring and winter growth habit

Introduction

Wheat landraces represent an important source of genetic variation that can be used to improve commercial varieties by means of introducing new alleles or combination of genes (Ciaffi *et al.* 1992). Primary habitats of wheat ancestors are situated in the northern and eastern parts of the Fertile Crescent and modern wheat cultivars were evolved from their ancestors which mostly were distributed in these areas (Harlan and Zohari 1996).

The adaptability of common wheat to wide range of environments and climate conditions is due to variation in vernalization requirement genes and day length for the control of ear emergence (Yan *et al*. 2004a). Based on vernalization requirement, wheat genotypes are classified into winter and spring types. In hexaploid wheat, vernalization requirement is primarily controlled by three orthologous of *VRN-1* genes, *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, which are located on the long arms of chromosomes 5A, 5B, and 5D, respectively (Law *et al*. 1976; Worland 1996; Dubcovsky *et al*. 1998; Barrett *et al*. 2002; Iwaki *et al*. 2002; Yan *et al*. 2003). In the spring wheat different dominant *Vrn* alleles have differential effects on flowering time. Goncharov (2004) reported that wheat genotypes with dominant *Vrn-A1* allele flower earlier, whereas presence of dominant *Vrn-D1*, *Vrn-D5* and/or *Vrn-B1* results in late flowering under non-vernalization condition. It was found that altering the flowering time and different combinations of dominant *Vrn* alleles in wheat may cause variation

in plant height and yield components (Stelmakh 1992; Stelmakh 1998).

Different mutations in the *VRN-1* locus caused expression of the dominant spring growth habit. For example, dominant *Vrn-A1* allele conferring spring growth habit originated from mutations either in the promoter or intron region of recessive *vrn-A1* allele which control winter growth habit in diploid, tetraploid and hexaploid wheat (Yan *et al*. 2004b; Fu *et al*. 2005; Dubcovsky *et al*. 2006; Pidal *et al*. 2009). In *Triticum monococcum*, the promoter region of *Vrn-A^{m}1*, (*Vrn-A^{m}1a*, *Vrn-A^{m}1b*, *Vrn-A^{m}1g*) have different length of deletions, and also one bp deletion at the CArG-Box region of *Vrn-A^{m}1f* allele was identified (Yan *et al*. 2003; Dubcovsky *et al*. 2006; Pidal *et al*. 2009). In addition to similar deletions in CarG-box region of *Vrn-A1d*, and *Vrn-A1e* alleles, a deletion in VRN-box *Vrn-A1b* was reported in tetraploid wheat (Yan *et al*. 2004b; Pidal *et al*. 2009). Yan *et al*. (2004a) found an insertion of a fold back repetitive element and a duplicated region in the promoter of dominant *Vrn-A1a*. They demonstrated that *Vrn-A1a* allele differed from the recessive *vrn-A1* allele in isoline Triple Dirk-C by the insertion of a 222-bp fold back element in the larger fragment and a 131-bp fold back element in the smaller fragment. Their findings suggest that the duplication of the promoter region occurred after the insertion of the fold back element. The *Vrn-A1b* allele has several single nucleotide polymorphisms and deletions in the promoter region. The *Vrn-A1c* allele was reported from IL369 wheat genotype from Afghanistan, IL162 from Egypt (Yan *et al*. 2004a) and Pavon-76 and NR-287 from Pakistan (Iqbal *et al*. 2011). This rare allele shows a large deletion in the first intron (Fu *et al*. 2005). Iqbal *et al*. (2011) in the study of wheat genotypes from Pakistan could identify *Vrn-A1c* allele, but they did not find any deletion in the first intron of *Vrn-A1* in the two genotypes which *Vrn-A1c* allele was detected. Fu *et al*. (2005) used primer pair Intr1/A/F2 and Intr1/A/R3 to detect deletion in the first intron of *VRN-A1* and primer pair Intr1/C/F and Intr1/AB/R as a positive control to identify genotypes lacking this deletion. Using these primer pairs, they could identify both presence and absence of first intron deletion in Afghanian landrace IL369. They also confirmed the presence of eight unique SNPs, five unique one-bp indels in promoter, introns 1, 2, 4 and, 6 as well as exon 7 regions, and one large 5504-bp deletion in the first intron of dominant *Vrn-A1* allele from IL369.

Yan *et al*. (2003) reported that deletions in the *VRN-A^{m}1* promoter of diploid wheat were associated with the spring growth habit. Yan *et al*. (2004a) and Fu *et al*. (2005) in analysis of the dominant *Vrn-A1* alleles from the hexaploid landrace IL369 and tetraploid cultivar Langdon did not identify any variation in the promoter region of the gene compared with its respective recessive alleles.

Tranquilli and Dubcovsky (2000) reported that vernalization requirement in wheat and barley is controlled by the epistatic interaction between *VRN-1* and *VRN-2* loci. In the winter genotypes, vernalization up-regulates *VRN-1* gene which is dominant for spring growth habit (Danyluk *et al*. 2003; Trevaskis *et al*. 2003; Yan *et al*. 2003), whereas vernalization process decreases the abundance of the *VRN-2* product (Yan *et al*.

2004a). Based on this molecular model the *VRN-2* transcription product is a repressor for the *VRN-1*. A single functional copy of *VRN-2* product is sufficient to stop flowering (Yan *et al*. 2003, 2004b). However mutation in the VRN-2 protein causes an inactive repressor, and also mutations that alter the *VRN-1* recognition site for VRN-2 repressor are associated with the dominant spring growth habit in *VRN-1* locus. Consequently, transcription of *VRN-1* gradually increases, leading to competence to flower.

In our best knowledge, no study has been performed to analyze the allelic variation at the vernalization requirement genes on Iranian wheat landraces. In view of the lack of information on the occurrence of *Vrn* alleles in Iranian wheat landraces, here we examined the *VRN-1* genotypes of 395 wheat landraces collected from various regions of Iran.

Materials and Methods

Plant material

The plant materials consisted of 395 Iranian wheat landraces, including 154 spring, 193 winter, 46 with unknown growth habit and two facultative genotypes as well as two standard cultivars, Chinese Spring and Thatcher. Seeds of the plant materials were obtained from gene bank of International Maize and Wheat Improvement Center (CIMMYT).

DNA marker analysis

Leaf tissues from 10 greenhouse grown seedlings per genotype were pooled and genomic DNA was isolated using the CTAB method (Saghai-Maroof *et al*. 1984). We used *Vrn-A1* allele-specific markers based on promoter or intron 1 mutations (Table 1) described by Yan *et al*. (2004a), Fu *et al*. (2005) and Golovnina *et al*. (2010). PCR was performed in a 10 µl volume in a BioRad thermocycler containing 0.6 µl of each of the 5 µmol/l forward and reverse primers, 4 µl PCR ready MasterMix (Amplicon), 3 µl sterile water, 2.8 µl template DNA. PCR programs for each primer pair is given in Table 1. PCR products were separated on 2% agarose gel at 100V, stained with ethidium bromide and subsequently visualized using UV light. For detecting the exact size of DNA bands, we used 50/100 bp plus ladder (Fermentas). In addition, 4% polyacrylamide gel was used to determine exact size of *Vrn-A1b* allele. Amplification experiments were repeated to confirm allelic composition result.

Results and Discussion

VRN-1 promoter region marker

Allelic variation at the promoter region of *VRN-1* gene in 395 Iranian wheat landraces were tested with primers VRN1AF and VRN1R. Amplification of genomic DNA from the promoter region of the landraces using these primers showed the presence of PCR products with the length of 480, 650 and 750-bp (Figure 1) which were also reported by Yan *et al*. (2004a). Amplification of two 650 and 750-bp fragments in 16 genotypes including 10 winter, five spring and one facultative genotypes confirmed the occurrence of the dominant *Vrn-A1a* allele in these landraces. Thatcher and nine spring, five

Table1. Primer sequences, annealing temperatures and expected PCR product sizes for detecting alleles at the *VRN1* loci in wheat

Marker	Primer	Sequence5-3	Expected size (bp)	Annealing temperature	PCR profile*
VRN-A1 Promoter region	VRN1AF	GAAAGGAAAAATTCTGCTCG	500	55	Touch down
	VRN1-R	TGCACCTTCCC(C/G)CGCCCCAT			
IL 369 *VRN-A1* Deletion	Intr1/A/F2	AGCCTCCACGGTTTGAAAGTAA	1170	57.2	57.2 Ramp
	Intr1/A/R3	AAGTAAGACAACACGAATGTGAGA			
VRN-A1 Non-deletion	Intr1/C/F	GCACTCCTAACCCACTAACC	1068	62	62 Ramp
	Intr1/AB/R	TCATCCATCATCAAGGCAAA			
	AP1_ProDel_F1	ACAGCGGCTATGCTCCAG	152		Touch down
	AP1_ProDel_R1	TATCAGGTGGTTGGGTGAGG			
	AP1_2F	CTGTGGTGTGTGTTTGTGGCGAGAG	200		Touch down
	AP1_2R	ACCCTACGCCCCTACCCTCCAACAC			

*Touch down: 1, 95℃, 5 min; 2, 96℃, 1 min; 3, 68℃, 5 min, -2.0℃/cycle; 4, 72℃, 1 min; 5, go to step 2, 4 more times; 6, 96℃, 1 min; 7, 58℃, 2 min, -2.0℃/cycle; 8, 72℃, 1 min; 9, go to step 6, 4 more times; 10, 96℃, 1 min; 11, 50℃, 1 min; 12, 72℃, 1 min; 13, go to step 10, 24 more times; 14, 72℃, 5 min; 15, 4℃, 5 min.

Ramp: 1, 94℃, 5 min; 2, 94℃, 30 s; 3, 0.5℃/s to annealing TM; 4, annealing TM 30 s; 5, 0.2℃/s to 72℃; 6, 72℃, 30s; 7, go to step 2, 39 more times; 8, 72℃, 5 min; 9, 4℃, 5 mim.

winter, and three unknown genotypes showed only 750-bp fragment and in 28 landraces including 25 spring and three winter genotypes a 650-bp fragment was only amplified. Amplification of 480-bp fragment in 334 genotypes consisted of 176 winter, 117 spring, 40 unknown and one facultative genotypes demonstrated that they carried dominant spring habit *Vrn-A1b* allele. *Vrn-A1b* indicates promoter deletions (no intron deletion) (Fu *et al*., 2005). In 13 genotypes consisted of 11 spring and two winter landraces both 480 and 650-bp bands were observed which was not reported in the previous studies. In addition, three winter and one spring genotypes were heterozygote for 480 and 750-bp fragments. The recessive *vrn-A1* allele was not amplified in any of the 395 examined Iranian wheat landraces.

Yan *et al*. (2003) classified the presence of insertions or deletions in the *VRN-A1* promoter as dominant *Vrn-A1* and their absence as recessive *vrn-A1*. Yan *et al*. (2004a) characterized the allelic variation at prompter region in the polyploid wheat and reported amplification of 650 and 750-bp fragments in wheat genotypes carrying dominant *Vrn-A1a* allele. They found that dominant *Vrn-A1a* allele differ from the recessive *vrn-A1* allele by insertion of a 222-bp foldback element in the large fragment and a 131-bp foldback in the smaller fragment.

IL 369 *VRN-A1* Deletion

To identify *VRN-A1* intron 1 deletion, we used the primer pair Intr/A/F2 and Intr/A/R3. This primer pair amplified PCR products of 1170-bp in 21 genotypes consisted of 18 spring, two winter, and one unknown growth habit. In addition, a new allele of 710-bp was detected in 11 spring, two winter, and one genotype with unknown growth habit (Figure 2).

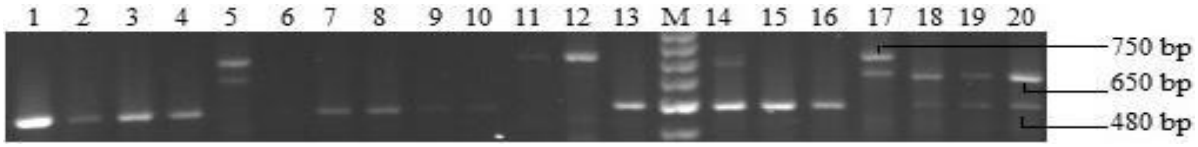

Figure 1. Banding pattern of *Vrn-A1* locus in some Iranian wheat landraces based on primer pair VRN1AF and VRN1R. *Vrn-A1a*: 650 bp +750 bp, *Vrn-A1b*: 480 bp, *Vrn-A1j*: 650 bp, *Vrn-A1k*: 750 bp. M: GeneRuler 50 bp plus DNA ladder marker (Fermentas)

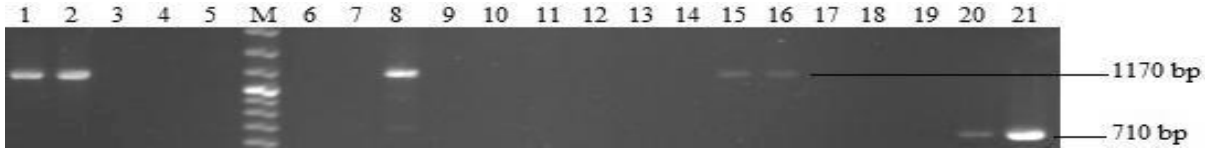

Figure 2. Banding pattern of *Vrn-A1c* locus in some Iranian wheat landraces based on primer pair Intr1/A/F2 and Intr1/A/R3. A new allele *Vrn-A1cb* was detected. *Vrn-A1c*: 1170 bp, *Vrn-A1cb*: 710 bp. M: GeneRuler 100 bp plus DNA ladder marker (Fermentas)

Yan *et al*. (2004a) in the analysis of allelic variation at the *VRN-1* promoter region in the polyploid wheat, in addition to the *Vrn-A1a* and *Vrn-A1b* alleles, identified a new allele named *Vrn-A1c* with size 1170-bp in IL369 and IL162, landraces from Afghanistan and Egypt, respectively. They reported that IL369 has a dominant *Vrn-A1* allele with an identical promoter region to the recessive *vrn-A1* allele. Iqbal *et al*. (2011) by analyzing allelic variation at the *Vrn-A1* locus of 59 Pakistani spring wheat cultivars amplified 1170-bp allele in the advanced breeding lines of NR-287 and Pavon-76 only. Zhang *et al*. (2008) reported that *Vrn-A1c* allele is common among Chinese tetraploid spring genotypes. Santra *et al*. (2009) by genetic and molecular characterization of vernalization genes *Vrn-A1* in spring wheat germplasm from the Pacific Northwest region of the USA did not observe *Vrn-A1c* allele in any of the 117 genotypes.

Vrn-A1 non-deletion marker

The primer pair Intr1/C/F and Intr1/AB/R was used to amplifiy non-deletion *Vrn-A1* marker in Iranian wheat landraces. Using this primer pair, a 1068-bp fragment was amplified in 389 genotypes including 153 spring, Chinese Spring cv., 189 winter, 45 unknown and two landraces with facultative growth habit. The result indicates that all the Iranian landraces carry recessive *vrn-A1* allele (Figure 3).

Zhang *et al*. (2008) in the analysis of allelic variation at the vernalization gene *Vrn-A1* in Chinese wheat cultivars used two primer pairs Intr1/A/F2 and Intr1/A/R3, and Intr1/C/F and Intr1/AB/R, for the *Vrn-A1* first intron to distinguish between two alleles of *Vrn-A1* gene. They reported amplification of a 1068-bp fragment in all cultivars tested using the primer pair Intr1/C/F and Intr1/AB/R, whereas no PCR product was produced using primer pair Intr1/A/F2 and Intr1/A/R3. These results indicate that the large intron 1 deletion (*Vrn-A1c* allele) was not present in the Chinese cultivars. Iqbal *et al*. (2007) reported that in Canadian spring wheat cultivars, *Vrn-A1b* and *vrn-A1* (500-bp) alleles differ in 20 bp. Nowak and Kowalczyk (2010) also confirmed the presence of recessive *vrn-A1* allele in all of the examined winter wheat cultivars from the Polish register. Golovnina *et al*. (2010)

with molecular characterization of vernalization loci *VRN1* in the wild and cultivated wheats found that the majority of the wild wheats have a winter growth habit, suggesting that the recessive *vrn-A1* allele with an intact *VRN1* promoter is the ancestral character.

Allelic variation at the *VRN*1 promoter region

PCR screening of *VRN1* promoter region of Iranian wheat landraces was provided with primer pairs AP1_ProDel_F1/AP1_ProDel_R1 and AP1_2F/AP1_2R. The first primer pair amplified the region flanking the 48-bp deletion. The expected PCR product size for the *vrn-Am1b* allele carrying the 48-bp deletion is 104 bp, whereas for *Vrn-Am1f* and the wild-type *vrn-Am1* alleles are 151 bp and 152 bp, respectively (Yan *et al*. 2003; Pidal *et al*. 2009). Using primer pair AP1_ProDel_F1 and AP1_ProDel_R1, PCR product of 152 bp was observed in 134 spring, Chinese Spring cv., 189 winter, 41 unknown and one facultative accession. In addition, we could amplify a novel 400 bp in 18 spring, eight winter and four unknown genotypes which may be due to large insertion in this region (Figure 4). Seven winter accession (Ardabil2, Saghez1, Saghez2, Ghazvin7, Kermanshah3, Sabzvar8, Torbat-Heidarieh3), and one spring genotype (Mashhad6) were heterozygote for these fragments.

Golovnina *et al*. (2010) by molecular characterization of *VRN1* locus in 27 accessions belonging to four diploid wheat species (*T. urartu*, *T. boeoticum*, *T. monococcum* and *T. sinskajae*), seven goatgrass accessions belonging to *Aegilops speltoides* and *Ae. squarrosa* (syn. *Ae. tauschii*) together with 17 accessions of seven polyploid species belonging to three known sections (*Dicoccoides*, *Triticum*, *Timopheevii*) using primer pair AP1_ProDel_F1/ AP1_ProDel_R1 amplified the expected size of 152 bp in the majority of the studied wheat accessions and in one goatgrass species, *Ae. Speltoides*. No PCR products was found in *Ae. squarrosa* accessions. Out of 27 wheat accessions, 10 showed PCR products of the lower size, which can be explained by deletions in the promoter region. Pidal *et al*. (2009) reported that primer pair AP1_ProDel_F1 and AP1_ProDel_R1 in diploid wheat (*T. monococcum*) amplified the region flanked by 48-bp deletion in *VRN1* promoter. They identified a 104-bp fragment for *vrn-Am1b* with 48-bp deletion as well as 151 and 152-bp fragments for *Vrn-Am1f* and wild type *vrn-Am1* alleles, respectively.

Golovnina *et al*. (2010) extracted all available *VRN1* promoter sequences belonging to different wheat genomes (A, B, D) from GenBank and aligned together with primer sequences. They found a 17-bp deletion in D genome near the region complementary to the reverse primer (AP1_ProDel_R1), and a duplicated fragment (CCTCAC) near this region in A genome. Therefore, they developed a new primer (AP1_2F/AP1_2R) for amplification of D genome. In our study, a PCR product of 400 bp was amplified in 375 Iranian wheat landraces including 141 spring, 187 winter, 45 unknown, and two facultative growth habits using primer pair AP1_2F and AP1_2R (Figure 5).

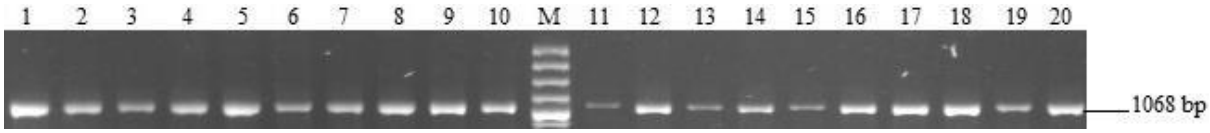

Figure 3. Banding pattern of non-deletion *Vrn-A1* locus in some Iranian wheat landraces based on primer pair Intr1/C/F and Intr1/AB/R. M: GeneRuler 100 bp plus DNA ladder marker (Fermentas)

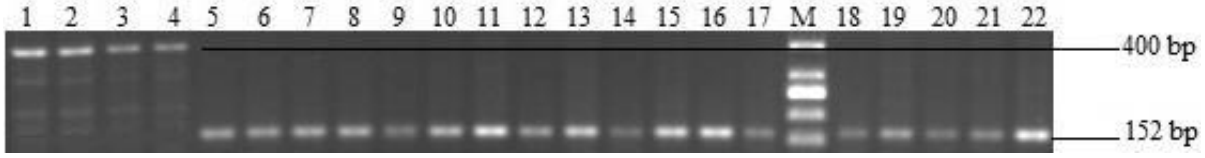

Figure 4. Banding pattern of *Vrn-1* promoter region in some Iranian wheat landraces based on primer pair AP1_ProDel_F1 and AP1_ProDel_R1. M: GeneRuler 50 bp plus DNA ladder marker (Fermentas)

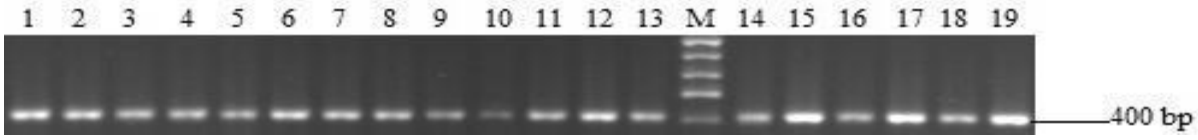

Figure 5. Banding pattern of *Vrn-1* promoter region in some Iranian wheat landraces based on primer pair AP1_2F and AP1_2R. M: GeneRuler 50 bp plus DNA ladder marker (Fermentas)

Distribution of *VRN-1* locus alleles in Iranian wheat landraces

Among the detected *VRN-1* alleles, *Vrn-A1b* allele was the most frequent allele (84.56%) and combination of *Vrn-A1j/Vrn-A1cb* was the least frequent (0.25%) in the Iranian wheat landraces (Tables 2 and 3). The frequency of dominant allele *Vrn-A1b* in the spring and winter genotypes were 35.03% and 52.70%, respectively. Fifteen spring and two winter accessions carried both *Vrn-A1b* and *Vrn-A1c* alleles. Most of these accessions (15) are from east and southeast of Iran. *Vrn-A1b* along with the novel *Vrn-A1cb* allele were amplified in 11 spring and two winter genotypes. These findings show their strength in fulfillment of spring growth habit in Iranian wheat landraces. In our study, the presence of some allelic combination in the winter and spring wheat landraces was not in agreement with those of reported in previous studies. This indicates accurate field and greenhouse evaluations is necessary for determination of growth habit.

Iwaki *et al*. (2001) by studying 272 wheat cultivars from different geographical regions demonstrated that the dominant *Vrn-A1* allele in the European common wheat cultivars is the most frequent. Iqbal *et al*. (2007) in the analysis of 40 spring wheat cultivars from Canada confirmed the presence of *Vrn-A1a* allele in 34 spring wheats. The *Vrn-A1b* allele was found in the Rescue cv. and two of its substitution lines RC5D and CR5A. Four of their examined cultivars carried winter habit *vrn-A1* allele.

Table 2. Distribution of *VRN-1* alleles in wheat landraces with different growth habit

	Growth habit				
		Spring	Winter	Facultative	Unknown
Allelic combination	Total	No.	No.	No.	No.
Vrn-A1a	16	5	10	1	0
Vrn-A1b	334	117	176	1	40
Vrn-A1c	21	18	2	0	1
Vrn-A1cb	14	11	2	0	1
Vrn-A1j	28	25	3	0	0
Vrn-A1k	18	9	5	0	3
Vrn-A1b Vrn-A1c	18	15	2	0	1
Vrn-A1b Vrn-A1cb	14	11	2	0	1
Vrn-A1b Vrn-A1j	13	11	2	0	0
Vrn-A1b Vrn-A1k	4	1	3	0	0
Vrn-A1c Vrn-A1j	3	3	0	0	0
Vrn-A1j Vrn-A1cb	1	1	0	0	0

Table 3. Allelic variation at *VRN-A1* locus in Iranian wheat landraces

Genotype	*Vrn-A1*	Genotype	*Vrn-A1*	Genotype	*Vrn-A1*
Iran1	*Vrn-A1b*	Birjand1-w	*Vrn-A1b*	Kerman2-w	*Vrn-A1b*
Urmia1-w	*Vrn-A1b*	Bojnourd2-w	*Vrn-A1b*	Sirjan1-w	*Vrn-A1b*
Iran2	*Vrn-A1b*	Torbat-Heidar1-w	*Vrn-A1b*	Kerman3-w	*Vrn-A1b*
Iran3	*Vrn-A1b*	Bojnourd3-s	*Vrn-A1b*	Kerman4-w	*Vrn-A1b Vrn-A1c*
Iran4	*Vrn-A1b*	Feridan1-s	-	Shahreza7-w	*Vrn-A1b*
Malayer1-w	*Vrn-A1b*	Borujen1-w	*Vrn-A1b*	Shiraz6-w	*Vrn-A1b*
Arak1-w	*Vrn-A1b*	Yazd1-w	*Vrn-A1b*	Moghan (Garmi)1-w	*Vrn-A1b*
Iran5	*Vrn-A1b*	Yazd2-w	*Vrn-A1b*	Urmia5-w	*Vrn-A1b*
Iran6	*Vrn-A1k*	Shahre-Kord1-w	*Vrn-A1b*	Ardabil2-w	*Vrn-A1j*
Sanandaj1-s	*Vrn-A1k*	Shahreza1-w	*Vrn-A1b*	Tabriz1-w	*Vrn-A1a*
Dareh-Gaz1-w	*Vrn-A1b*	Shahreza2-w	*Vrn-A1b*	Mianeh1-w	*Vrn-A1b*
Kermanshah1-s	*Vrn-A1b*	Shirvan1-w	*Vrn-A1k*	Bandar-Abbas1-w	*Vrn-A1b*
Gazvin1-s	*Vrn-A1a*	Iran8	*Vrn-A1b*	Shiraz7-s	-
Shah-Abad1-s	*Vrn-A1b, Vrn-A1cb*	Shahreza3-w	-	Lenjan1-w	*Vrn-A1b*
Kerend1-s	*Vrn-A1b, Vrn-A1cb*	Borujen3-w	*Vrn-A1b*	Esfahan3-w	*Vrn-A1b*
Saveh1-s	*Vrn-A1b*	Borujen4-w	*Vrn-A1b*	Urmia6-w	*Vrn-A1b*
Gazvin2-s	*Vrn-A1a*	Semirom1-s	*Vrn-A1b*	Urmia7-w	*Vrn-A1b*
Gazvin3-w	*Vrn-A1b*	Ghoochan2-s	*Vrn-A1b*	Ghoochan3-f	*Vrn-A1b*
Gilane-Gharb1-w	*Vrn-A1b*	Birjand3-s	*Vrn-A1b*	Iran10	*Vrn-A1b*
Gilane-Gharb2-w	*Vrn-A1b*	Yazd3-w	*Vrn-A1b*	Lenjan2-w	*Vrn-A1b*
Ilam1-w	*Vrn-A1b*	Yazd4-w	*Vrn-A1b*	Esfahan4-w	*Vrn-A1b*
Ilam2-w	*Vrn-A1b*	Shahreza4-w	*Vrn-A1b*	Esfahan5-w	*Vrn-A1b*
Malayer2-w	*Vrn-A1b*	Birjand4-w	*Vrn-A1b*	Esfahan6-w	*Vrn-A1b*
Hamedan1-s	*Vrn-A1b*	Varamin1-w	*Vrn-A1b*	Mashhad1-w	*Vrn-A1b*
Gorgan1-s	*Vrn-A1b, Vrn-A1cb*	Semirom2-w	*Vrn-A1b*	Ghoochan4-w	*Vrn-A1b*
Kashmar1-w	*Vrn-A1b*	Shahreza5-w	*Vrn-A1b*	Mashhad2-s	*Vrn-A1b*
Kashmar2-w	*Vrn-A1b*	Shahreza6-w	*Vrn-A1b*	Najaf-Abad1-w	*Vrn-A1b*
Sabzvar1-w	*Vrn-A1b*	Shiraz1-w	*Vrn-A1b*	Torbat-Jam2-s	*Vrn-A1b*
Sabzvar2-w	*Vrn-A1b*	Shiraz2-s	*Vrn-A1b, Vrn-A1c*	Torbat-Jam3-w	*Vrn-A1b*

Table3. Continued

Genotype	*Vrn-A1*	Genotype	*Vrn-A1*	Genotype	*Vrn-A1*
Ardakan1-w	*Vrn-A1b*	Shiraz3-s	*Vrn-A1b, Vrn-A1c*	Torbat-Jam4-w	*Vrn-A1b*
Iran7	-	Iran9	*Vrn-A1b*	Damghan1-w	*Vrn-A1b*
Sabzvar3-w	*Vrn-A1b*	Fasa1-s	*Vrn-A1b*	Shah-Abad2-w	*Vrn-A1b*
Torbat-Jam1-w	*Vrn-A1b*	Niriz1-w	*Vrn-A1b*	Sanandaj2-w	*Vrn-A1b*
Ghoochan1-w	*Vrn-A1b*	Shiraz4-w	*Vrn-A1b*	Zanjan1-w	*Vrn-A1b*
Esfahan1-w	*Vrn-A1b*	Shiraz5-s	*Vrn-A1b, Vrn-A1c*	Zanjan2-s	*Vrn-A1b*
Ardakan2-w	*Vrn-A1b*	Hasht-Rood1-w	*Vrn-A1a*	Mashhad3-s	*Vrn-A1b*
Neishabour1-w	*Vrn-A1b*	Kerman1-w	*Vrn-A1b*	Esfahan7-w	*Vrn-A1b*
Neishabour2-s	*Vrn-A1b*	Ardabil1-s	*Vrn-A1a*	Sanandaj3-s	*Vrn-A1k*
Dastjerd1-s	*Vrn-A1b*	Urmia2-f	*Vrn-A1a*	Iran11	*Vrn-A1b*
Esfahan2-w	*Vrn-A1b, Vrn-A1k*	Urmia3-w	*Vrn-A1b*	Khonsar1-w	*Vrn-A1b*
Bojnourd1-w	*Vrn-A1b, Vrn-A1j*	Urmia4-w	*Vrn-A1b, Vrn-A1c*	Damghan2-w	*Vrn-A1b*
Torbat-Jam5-v	*Vrn-A1b*	Shah-Abad4-s	*Vrn-A1b*	Toyserkan1-w	*Vrn-A1a*
Naghadeh1-s	*Vrn-A1k*	Gazvin5-w	*Vrn-A1b*	Toyserkan2-s	*Vrn-A1k*
Iran12	-	Gazvin6-s	*Vrn-A1b*	Torbat-Heidari2-s	*Vrn-A1b*
Esfahan8-w	*Vrn-A1b*	Gazvin7-w	*Vrn-A1a*	Hamedan3-w	*Vrn-A1b*
Esfahan9-w	*Vrn-A1a*	Saghez2-w	*Vrn-A1a*	Iran14	*Vrn-A1b*
Borujerd1-w	*Vrn-A1b*	Shah-Abad5-w	-	Sabzvar5-w	*Vrn-A1b*
Borujerd2-s	*Vrn-A1b*	Sabzvar4-s	*Vrn-A1b*	Iran15	*Vrn-A1b*
Urmia8-w	*Vrn-A1b*	Ghoochan9-s	*Vrn-A1b, Vrn-A1c*	Sabzvar6-s	*Vrn-A1b*
Mahabad1-s	*Vrn-A1b, Vrn-A1k*	Torbat-Jam6-s	*Vrn-A1b*	Sabzvar7-s	*Vrn-A1b*
Mahabad2-s	*Vrn-A1b*	Birjand8-w	*Vrn-A1b*	Iran16	*Vrn-A1k*
Ghoochan5-s	*Vrn-A1b*	Birjand9-s	*Vrn-A1b*	Sabzvar8-w	*Vrn-A1b, Vrn-A1k*
Ghoochan6-s	*Vrn-A1a*	Semirom3-w	*Vrn-A1b*	Iran17	*Vrn-A1k*
Mashhad4-s	*Vrn-A1b, Vrn-A1j*	Ardestan1-w	*Vrn-A1b*	Sabzvar9-s	*Vrn-A1b*
Mashhad5-w	*Vrn-A1b*	Rafsanjan1-w	-	Bojnourd6-s	*Vrn-A1b*
Fooman1-s	*Vrn-A1b, Vrn-A1j*	Torbat-Jam7-w	*Vrn-A1b*	Iran18	*Vrn-A1b*
Birjand5-w	*Vrn-A1b*	Neishabour3-w	*Vrn-A1b*	Iran19	*Vrn-A1b*
Birjand6-w	*Vrn-A1b*	Shirvan2-w	*Vrn-A1b*	Sabzvar10-w	*Vrn-A1b, Vrn-A1j*
Birjand7-w	*Vrn-A1b*	Iran13	-	Kashmar3-s	*Vrn-A1b, Vrn-A1j*
Feridan2-w	*Vrn-A1b*	Arak2-s	*Vrn-A1b*	Yazd5-s	*Vrn-A1b, Vrn-A1j*
Bojnourd4-s	*Vrn-A1b, Vrn-A1j*	Ghasre-Shirin1-w	*Vrn-A1b*	Iran20	*Vrn-A1b*
Bojnourd5-s	-	Ghasre-Shirin2-w	*Vrn-A1b*	Yazd6-w	*Vrn-A1b*
Dareh-Gaz2-s	-	Gilane-Gharb3-w	*Vrn-A1b*	Sabzvar11-w	*Vrn-A1b*
Ghoochan7-s	*Vrn-A1b*	Gilane-Gharb4-s	*Vrn-A1b*	Iran21	*Vrn-A1b, Vrn-A1cb*
Sarakhs1-s	*Vrn-A1k*	Gazvin8-s	*Vrn-A1b*	Iran22	*Vrn-A1b*
Shahrud1-s	*Vrn-A1b*	Mahidasht1-w	*Vrn-A1b*	Sabzvar12-w	*Vrn-A1b*
Tabas1-w	*Vrn-A1b*	Gorgan2-s	*Vrn-A1b*	Sabzvar13-s	*Vrn-A1b, Vrn-A1j, Vrn-A1cb*
Meimeh1-w	*Vrn-A1b*	Kermanshah2-w	*Vrn-A1b*	Feridan3-w	*Vrn-A1b*
Meimeh2-w	*Vrn-A1b*	Sanandaj4-s	*Vrn-A1b*	Sabzvar14-s	*Vrn-A1b, Vrn-A1j*
Ghoochan8-s	*Vrn-A1b*	Shah-Abad-Gharb1-v	-	Iran23	*Vrn-A1b*
Esfahan10-w	*Vrn-A1b*	Saveh2-w	*Vrn-A1b*	Ardakan3-s	*Vrn-A1b*
Shahrud2-s	*Vrn-A1b, Vrn-A1cb*	Hamedan2-w	*Vrn-A1b*	Iran24	*Vrn-A1b*
Meimeh3-w	*Vrn-A1b*	Sanandaj5-s	*Vrn-A1b, Vrn-A1c*	Mashhad7-s	*Vrn-A1b*
Esfahan11-w	*Vrn-A1b*	Mahidasht2-s	*Vrn-A1b*	Najaf-Abad4-w	*Vrn-A1b*
Shahrud3-s	*Vrn-A1b*	Kermanshah3-w	*Vrn-A1a*	Iran25	*Vrn-A1b*
Semnan1-w	*Vrn-A1b*	Sanandaj6-s	-	Iran26	*Vrn-A1b*
Najaf-Abad2-s	*Vrn-A1b*	Maragheh1-w	*Vrn-A1b*	Iran27	*Vrn-A1b*
Najaf-Abad3-v	*Vrn-A1b*	Kermanshah4-w	*Vrn-A1b*	Ghoochan10-w	*Vrn-A1b*

Table 3. Continued

Genotype	*Vrn-A1*	Genotype	*Vrn-A1*	Genotype	*Vrn-A1*
Shah-Abad3-s	*Vrn-A1b*	Sanjabi1-w	*Vrn-A1b*	Esfahan12-w	*Vrn-A1b, Vrn-A1c*
Mashhad6-s	*Vrn-A1a*	Divan-Dareh1-w	*Vrn-A1b*	Iran28	*Vrn-A1b*
Saghez1-w	*Vrn-A1a*	Malayer3-s	*Vrn-A1b*	Iran29	*Vrn-A1b*
Gazvin4-w	*Vrn-A1b*	Nahavand1-w	*Vrn-A1b*	Ardakan4-w	*Vrn-A1b*
Mashhad8-w	*Vrn-A1b*	Astara1-w	*Vrn-A1b*	Yazd7-s	*Vrn-A1b*
Mashhad9-w	*Vrn-A1b, Vrn-A1c*	Shahi1-w	*Vrn-A1b*	Ghoochan13-s	*Vrn-A1b*
Mashhad10-s	-	Esfahan14-w	*Vrn-A1a*	Tabas4-s	*Vrn-A1b, Vrn-A1*
Sabzvar15-s	*Vrn-A1b*	Torbat-Jam8-s	-	Iran41	*Vrn-A1b*
Sabzvar16-w	*Vrn-A1b*	Fariman1-w	*Vrn-A1b*	Hamedan7-w	*Vrn-A1b*
Mashhad11-w	*Vrn-A1b*	Gonabad1-w	*Vrn-A1b*	Tabas5-s	*Vrn-A1j, Vrn-A1*
Iran30	*Vrn-A1b*	Gorgan3-s	-	Esfahan16-s	*Vrn-A1j*
Mashhad12-w	*Vrn-A1b*	Semnan2-s	-	Saghez3-s	*Vrn-A1j*
Ghoochan11-w	*Vrn-A1b*	Shah-Abad6-w	*Vrn-A1a*	Fariman2-w	*Vrn-A1b*
Iran31	*Vrn-A1b*	Mashhad13-s	*Vrn-A1b*	Iran42	*Vrn-A1b*
Iran32	*Vrn-A1b*	Gazvin9-w	*Vrn-A1b*	Bojnourd13-w	*Vrn-A1b*
Neishabour4-w	*Vrn-A1b*	Sabzvar17-w	*Vrn-A1b*	Sabzvar19-s	*Vrn-A1b*
Bojnourd7-w	*Vrn-A1b*	Ardakan5-w	*Vrn-A1b*	Iran43	*Vrn-A1b*
Iran33	*Vrn-A1b*	Bojnourd11-w	*Vrn-A1b, Vrn-A1*	Niriz4-w	*Vrn-A1b*
Shahre-Kord3-w	*Vrn-A1b*	Shahre-Kord5-w	*Vrn-A1b*	Shiraz8-s	*Vrn-A1b, Vrn-A1c*
Neishabour5-w	*Vrn-A1b*	Torbat-Heidar4-w	*Vrn-A1b*	Shiraz9-s	*Vrn-A1b, Vrn-A1c*
Neishabour6-w	*Vrn-A1b*	Naein1-w	*Vrn-A1b*	Maragheh2-s	*Vrn-A1b, Vrn-A1*
Bojnourd8-s	*Vrn-A1b*	Shahre-Kord6-w	*Vrn-A1b*	Iran44	*Vrn-A1b*
Bojnourd9-w	*Vrn-A1b*	Semirom4-w	*Vrn-A1b*	Urmia9-w	*Vrn-A1b*
Bojnourd10-s	*Vrn-A1b*	Shirvan3-s	*Vrn-A1b, Vrn-A1*	Babol1-w	*Vrn-A1b*
Neishabour7-w	*Vrn-A1b*	Dareh-Gaz3-s	*Vrn-A1b, Vrn-A1*	Esfahan17-w	*Vrn-A1b*
Iran34	*Vrn-A1b*	Ghoochan12-s	*Vrn-A1j*	Damghan3-w	*Vrn-A1b*
Hamedan4-s	*Vrn-A1j*	Ghasre-Shirin3-s	*Vrn-A1j*	Iran45	*Vrn-A1b*
Iran35	*Vrn-A1b*	Malayer4-s	*Vrn-A1j, Vrn-A1*	Gazvin12-w	*Vrn-A1b*
Iran36	*Vrn-A1b*	Mahi-Dasht3-s	*Vrn-A1j, Vrn-A1*	Iran46-s	*Vrn-A1b*
Iran37	*Vrn-A1b, Vrn-A1c*	Kermanshah5-w	*Vrn-A1b*	Iran47-s	*Vrn-A1k*
Iran38	*Vrn-A1b*	Gazvin10-s	*Vrn-A1j*	Hamedan8-w	*Vrn-A1b*
Tabas2	*Vrn-A1b, Vrn-A1c*	Varamin2-s	*Vrn-A1k*	Iran48	*Vrn-A1b*
Iran39	*Vrn-A1b*	Iran40	*Vrn-A1b*	Gazvin13-w	*Vrn-A1b*
Shahre-Kord4-s	*Vrn-A1b*	Gilane-Gharb5-s	*Vrn-A1b*	Iran49-s	*Vrn-A1b*
Niriz2-w	*Vrn-A1b*	Hamedan6-s	*Vrn-A1k*	Iran50-s	*Vrn-A1b*
Shah-Roud4-w	*Vrn-A1b*	Esfahan15-s	*Vrn-A1b*	Hamadan9-w	*Vrn-A1b*
Hasht-Rood2-s	*Vrn-A1j*	Sanjabi2-w	*Vrn-A1b*	Tehran1-s	*Vrn-A1b*
Arak3-s	*Vrn-A1j*	Neishabour8-s	*Vrn-A1b, Vrn-A1*	Birjand11-s	*Vrn-A1b, Vrn-A1*
Sanandaj7-w	*Vrn-A1b*	Birjand10-w	*Vrn-A1b*	Sarakhs2-s	*Vrn-A1b*
Hamedan5-s	*Vrn-A1j*	Ghasre-Shirin4-s	*Vrn-A1b*	Iran51-s	*Vrn-A1b, Vrn-A1*
Tabas3-s	-	Shah-Abad7-s	*Vrn-A1b*	Iran52-s	*Vrn-A1j*
Esfahan13-w	*Vrn-A1b*	Bojnourd12-w	*Vrn-A1b*	Zanjan3-s	*Vrn-A1j*
Borujen5-w	*Vrn-A1b*	Kashmar4-s	*Vrn-A1b*	Shahrood5-s	*Vrn-A1b*
Torbat-Heidar3-w	*Vrn-A1k*	Kashmar5-w	*Vrn-A1b*	Semnan3-s	*Vrn-A1b*
Borujen6-w	*Vrn-A1b*	Sabzvar18-s	*Vrn-A1b*	Kerman5-s	*Vrn-A1b, Vrn-A1*
Zahedan1-s	*Vrn-A1b, Vrn-A1c*	Mashhad14-s	*Vrn-A1b*	Kerman10-s	*Vrn-A1b*
Zahedan2-s	*Vrn-A1b, Vrn-A1c*	Shahre-Kord8-s	*Vrn-A1b*	Kerman11-s	*Vrn-A1b*
Zahedan3-s	*Vrn-A1b, Vrn-A1c*	Mashhad15-s	*Vrn-A1b, Vrn-A1*	Esfahan23-s	*Vrn-A1b*
Zahedan4-s	*Vrn-A1b, Vrn-A1c*	Mashhad16-s	*Vrn-A1b, Vrn-A1*	Esfahan24-s	*Vrn-A1b*
Esfahan18-s	*Vrn-A1b*	Mashhad17-s	*Vrn-A1b, Vrn-A1*	Yazd8-s	*Vrn-A1b*

Table 3. Continued

Genotype	*Vrn-A1*	Genotype	*Vrn-A1*	Genotype	*Vrn-A1*
Esfahan19-s	*Vrn-A1b*	Mashhad18-s	*Vrn-A1b, Vrn-A1c*	Tehran2-s	*Vrn-A1b*
Esfahan20-s	*Vrn-A1b*	Mashhad19-s	*Vrn-A1b, Vrn-A1c*	Chinese spring	-
Esfahan21-s	*Vrn-A1b*	Kerman7-s	*Vrn-A1b*	Thatcher	*Vrn-A1k*
Esfahan22-s	*Vrn-A1b*	Kerman8-s	*Vrn-A1b*		
Shahre-Kord7-	*Vrn-A1b*	Kerman9-s	*Vrn-A1b*		

In this study the frequencies of *Vrn-A1* alleles differed from those obtained for wheat cultivars from Europe, America and even Asia. Complementary studies are necessary to investigate the role of other genetic systems, especially earliness *per se*, and *VRN2* in determination of flowering time and adaptation in Iranian wheat landraces.

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