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ALLELIC VARIATION AT THE *VRN-1* LOCUS OF POLISH CULTIVARS OF COMMON WHEAT (*TRITICUM AESTIVUM* L.)

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At a molecular level, the length of the vernalization period of common wheat (*Triticum aestivum* L.) is determined mainly by three loci: *VRN-1*, *VRN-2* and *VRN-3*. In hexaploid wheat, the *Vrn-A1*, *Vrn-B1* and *Vrn-D1* genes are dominant for spring growth habit and epistatic to the alleles for winter growth habit. We used DNA markers to determine the *VRN-1* genotypes of 43 common wheat cultivars from the Polish register. All of the 30 examined winter wheat cultivars carried the recessive *vrn-A1* allele, and all of the 13 analyzed spring cultivars carried the dominant *Vrn-A1a* allele. Moreover, 13 winter and 11 spring cultivars carried the dominant *Vrn-B1* allele. These results confirmed that the hexaploid wheat growth habit is determined mainly by the *VRN-A1* locus.

Key words: *VRN-1* locus, vernalization, allelic variation, *Triticum aestivum* L., DNA markers.

INTRODUCTION

Low-temperature activity at certain stages of cereal development is necessary for flowering and kernel formation. The vernalization process consists in the acquisition or acceleration of the plant's flowering ability by cold treatment (Chouard, 1960). Vernalization occurs when air temperature oscillates between 0°C to 10°C and lasts for a few weeks (Flood and Halloran, 1984). Physiological studies showed that the vernalization response center is in the tip of a shoot (Amasino, 2004).

Winters in Poland have become milder in the last few years, and the winter period is often split into two or three cold subperiods separated by thaws (Kożuchowski and Degirmendžić, 2005). These changes influence plants' vernalization process, which affects crop production. This problem is especially important for winter cereal production. Winter bread wheat (*Triticum aestivum* L.) is the most important crop for Polish agriculture. In 2009, the area of its production reached over 2 million hectares, while the spring wheat production area amounted to only about 340,000 hectares (Central Statistical Office, 2010). At a molecular level, the length of the vernalization period for common wheat is determined mainly by three loci: *VRN-1*, *VRN-2* and *VRN-3*. The most important mechanism regulating the vernalization requirement is based on epistatic interactions between *VRN-1* and *VRN-2* loci. The product of *VRN-2* expression is a repressor for *VRN-1*. As the vernalization process reduces the abundance of the *VRN-2* product, *VRN-1* transcription gradually increases, leading to the competence to flower. According to this model, even a single functional copy of the *VRN-2* product can stop flowering (Yan et al., 2003, 2004a).

In hexaploid wheat, the *Vrn-A1*, *Vrn-B1* and *Vrn-D1* genes are dominant for spring growth habit and epistatic to the alleles for winter growth habit (Stelmakh, 1987). The *Vrn-A1* gene was mapped on the long arm of chromosome 5A near the *Fr1* gene (Snape et al., 2001), and it is strongly linked to three RFLP markers: *Xwg644*, *Xpsr426* and *Xpsr2021* (Korzun et al., 1997; Sarma et al., 1998; Sutka et al., 1999; Snape et al., 2001). Snape et al. (2001) also established the position of the *Vrn-D1* gene in the distal part of the long arm of chromosome 5D, and showed that this gene is closely linked to *Xgwm212* and *Xgwm292* microsatellite markers. The *Vrn-B1* gene was mapped in the distal part of the long arm of chromosome 5B and is closely linked to two microsatellite markers: *Xgwm408* and *Xgwm604* (Leonova et al., 2003; Tóth et al., 2003).

Allelic variation at the *VRN-A1* locus is related to mutations within the promoter sequence (Yan et al.,

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TABLE 1. Primers used in PCR reactions for identification of *Vrn* alleles in common wheat cultivars

Primer	Sequence 5´→ 3´	Identified allele	Reference
VRN1AF	GAAAGGAAAAATTCTGCTCG	*Vrn-A1a, Vrn-A1b, vrn-a1*	Yan et al., 2004b
VRN1R	TGCACCTTCCCCCGCCCCAT		
Intr1/A/F2	AGCCTCCACGGTTTGAAAGTAA	*Vrn-A1c*	Fu et al., 2005
Intr/A/R3	AAGTAAGACAACACGAATGTGAGA		
Intr1/B/F	CAAGTGGAACGGTTAGGACA	*Vrn-B1*	
Intr1/B/R3	CTCATGCCAAAAATTGAAGATGA		
Intr1/D/F	GTTGTCTGCCTCATCAAATCC	*Vrn-D1*	
Intr1/D/R3	GGTCACTGGTGGTCTGTGC		

TABLE 2. Thermal profiles of PCR reactions for identification of *Vrn* alleles

Step	Allele			
	Vrn-A1a, Vrn-A1b, vrn-a1	*Vrn-A1c*	*Vrn-B1*	*Vrn-D1*
Preliminary denaturation	94°C, 4´	94°C, 5´	94°C, 5´	94°C, 5´
Denaturation	94°C, 1´	94°C, 30´´	94°C, 30´´	94°C, 30´´
Primer annealing	56°C, 1´	57.2°C, 30´´	57°C, 30´´	61.2°C, 30´´
Extension	72°C, 1´20´´	72°C, 1´10´´	72°C, 1´	72°C, 1´40´´
Final extension	72°C, 7´	72°C, 10´	72°C, 10´	72°C, 10´
Number of cycles	40	38	38	38
Expected product size	500 bp or 650/750 bp	1170 bp	709 bp	1671 bp

2004b) or deletions within the first intron of this gene (Fu et al., 2005). For the *VRN-B1* and *VRN-D1* loci, changes in promoter sequence were not observed; their allelic variation is determined only by deletion within the first intron sequence (Fu et al., 2005).

The *VRN-2* locus has so far been described only for *Triticum monococcum* L. (Yan et al., 2004a). Its characteristics in hexaploid wheat have not been reported. Yan et al. (2006) also identified the *Vrn-B3* gene, which is a flowering promoter, and established its position on the short arm of chromosome 7B. This gene is closely linked to *ABC158* and *GWM569* microsatellite markers. Different genes which control the vernalization process and influence the transition to the generative phase have been identified on chromosomes 3B (Miura and Worland, 1994), 6A, 6B and 6D (Islam-Faridi et al., 1996), but the mechanisms of their activity are not precisely determined.

Allelic variation at the *VRN-1* locus has been examined in common wheat cultivars from countries including the United States (Fu et al., 2005; Stelmakh, 1998), Canada (Stelmakh, 1998; Iqbal et al., 2007) and China (Zhang et al., 2008). In view of the lack of information on the occurrence of *Vrn* alleles in Polish wheat cultivars, here we examined the *VRN-1* genotypes of 43 common wheat cultivars from the Polish register.

MATERIALS AND METHODS

We used 43 common wheat cultivars from the Polish register for this study. The winter cultivars are Alcazar, Anthus, Batuta, Bogatka, Boomer, Dorota, Finezja, Flair, Fregata, Izyda, Kobiera, Legenda, Ludwig, Muza, Nadobna, Naridana, Nutka, Olivin, Ostka Strzelecka, Rapsodia, Rubens, Rywalka, Satyna, Slade, Sława, Smuga, Sukces, Tonacja, Trend and Turnia. Spring cultivars include Bombona, Bryza, Griwa, Hewilla, Histra, Kosma, Monsun, Napola, Parabola, Radunia, Triso, Zebra and Żura.

Total DNA from 5-day-old seedlings was extracted according to the CTAB method (Doyle and Doyle, 1987), with modifications.

The *Vrn* alleles were identified by means of DNA markers. Two STS-PCR (sequence tagged site-polymerase chain reaction) methods were utilized. The first, based on analysis of the *Vrn-A1* gene promoter region, allows the *Vrn-A1a*, *Vrn-A1b* and *vrn-A1* alleles to be identified (Yan et al., 2004b). The second, based on analysis of the presence of a deletion in the first intron of *Vrn-1*, allows the *Vrn-A1c*, *Vrn-B1* and *Vrn-D1* alleles to be determined (Fu et al., 2005). The sequences of the applied primer sets are given in Table 1.

PCR reactions were performed in a 20 µl volume containing 1× PCR buffer (Fermentas), 1.8 mM

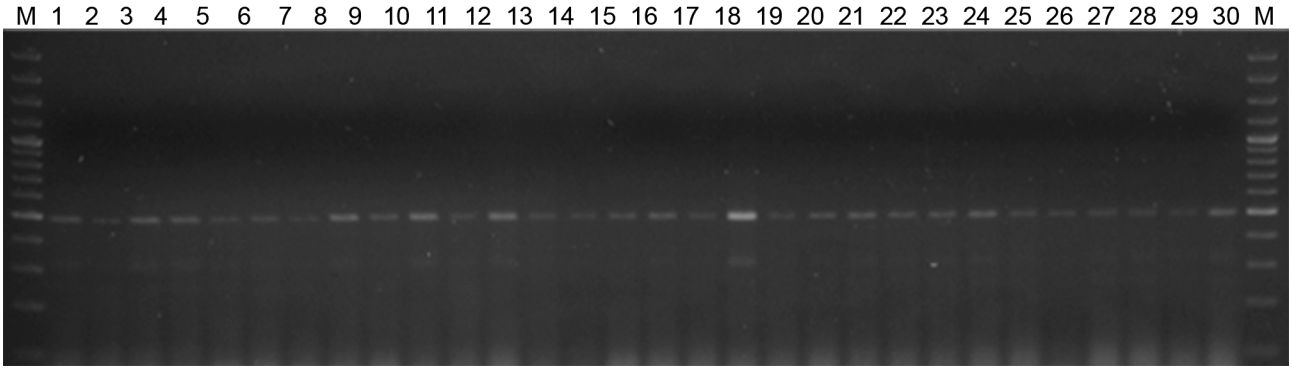

Fig. 1. Results of amplification obtained for winter wheat cultivars in PCR with VRN1AF/VRN1R primer pair: M – GeneRuler^{TM} 100 bp Plus DNA Ladder marker, 1 – Alcazar, 2 – Anthus, 3 – Batuta, 4 – Bogatka, 5 – Boomer, 6 – Dorota, 7 – Finezja, 8 – Flair, 9 – Fregata, 10 – Izyda, 11 – Kobiera, 12 – Legenda, 13 – Ludwig, 14 – Muza, 15 – Nadobna, 16 – Naridana, 17 – Nutka, 18 – Olivin, 19 – Ostka Strzelecka, 20 – Rapsodia, 21 – Rubens, 22 – Rywalka, 23 – Satyna, 24 – Slade, 25 – Sława, 26 – Smuga, 27 – Sukces, 28 – Tonacja, 29 – Trend, 30 – Turnia.

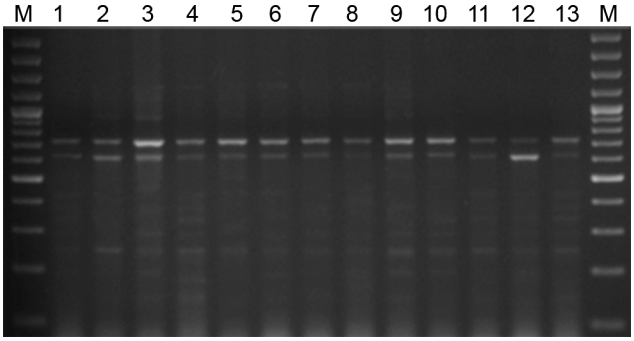

Fig. 2. Results of amplification obtained for spring wheat cultivars in PCR with VRN1AF/VRN1R primer pair: M – GeneRuler^{TM} 100 bp Plus DNA Ladder marker, 1 – Bombona, 2 – Bryza, 3 – Griwa, 4 – Hewilla, 5 – Histra, 6 – Kosma, 7 – Monsun, 8 – Napola, 9 – Parabola, 10 – Radunia, 11 – Triso, 12 – Zebra, 13 – Żura.

$MgCl_2$, 0.2 mM of each dNTP, 5 pmol of each primer, 0.4 U *Taq* DNA Polymerase (Fermentas) and 50 ng template DNA. Table 2 gives the thermal profiles of the reactions. For PCR a Tprofessional Basic (Biometra) thermocycler was used.

The amplification products were separated by electrophoresis on 1.5% agarose gels and visualized under UV light with ethidium bromide. We used GeneRuler^{TM} 100 bp Plus DNA Ladder marker (Fermentas).

RESULTS

After separation of the PCR products on 1.5% agarose gel, a 500 bp DNA band in all of the 30 examined winter wheat cultivars was observed. The presence of this product confirmed the occurrence of the recessive *vrn-A1* allele (Fig. 1). In the same reaction, two bands – 650 bp and 750 bp – were amplified for all of the 13 analyzed spring cultivars. This confirmed the occurrence of the dominant *Vrn-A1a* allele in these cultivars (Fig. 2). None of the 43 examined common wheat cultivars carried the *Vrn-A1b* or *Vrn-A1c* alleles.

The amplification reaction with the primer pair Intr1/B/F and Intr1/B/R3 allowed the *Vrn-B1* allele to be identified. After electrophoresis, a 709 bp DNA band was observed for 13 winter cultivars: Batuta, Bogatka, Finezja, Flair, Fregata, Izyda, Rapsodia, Rubens, Satyna, Smuga, Sukces, Tonacja and Turnia (Fig. 3). Among the 13 analyzed spring cultivars, 11 carried the dominant *Vrn-B1* allele: Griwa, Hewilla, Histra, Kosma, Monsun, Napola, Parabola, Radunia, Triso, Zebra and Żura (Fig. 4). The rest of the examined cultivars contained the recessive *vrn-B1* allele. In winter wheat cv. Izyda, amplification showed another product 10 bp smaller (Fig. 3). This may indicate rearrangement within the sequence of the analyzed DNA fragment, or the occurrence of a new, different allelic form of the *Vrn-B1* gene.

After the PCR reaction with Intr1/D/F and Intr1/D/R3 primers, no amplification products were observed in any cultivars, neither with winter nor with spring growth habit. This result confirmed the presence of the recessive *vrn-D1* allele in all 43 analyzed common wheat cultivars.

DISCUSSION

Information about the *Vrn* genotype is important in considering the frost tolerance and low temperature response of cereals. The occurrence of *Vrn* alleles has been described for many wheat cultivars from different regions of the world. In this study we characterized

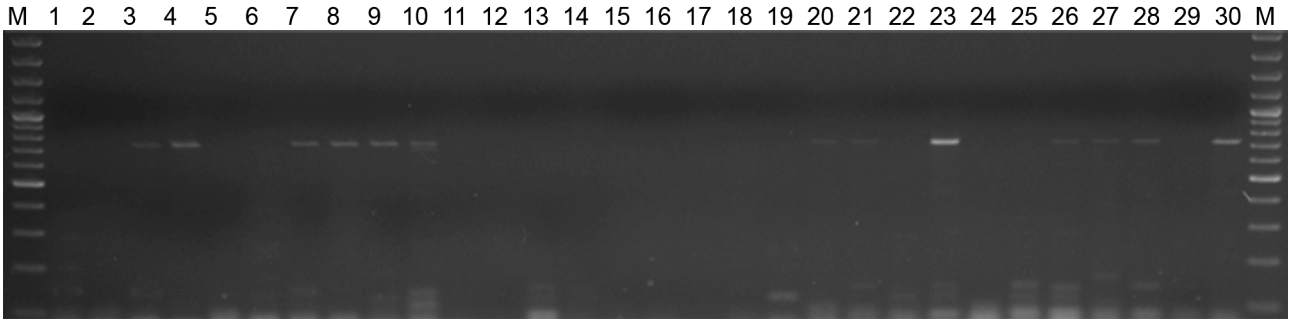

Fig. 3. Amplification products obtained for winter wheat cultivars in PCR with Intr1/B/F and Intr1/B/R3 primer pair: M – GeneRuler^{TM} 100 bp Plus DNA Ladder marker, 1 – Alcazar, 2 – Anthus, 3 – Batuta, 4 – Bogatka, 5 – Boomer, 6 – Dorota, 7 – Finezja, 8 – Flair, 9 – Fregata, 10 – Izyda, 11 – Kobiera, 12 – Legenda, 13 – Ludwig, 14 – Muza, 15 – Nadobna, 16 – Naridana, 17 – Nutka, 18 – Olivin, 19 – Ostka Strzelecka, 20 – Rapsodia, 21 – Rubens, 22 – Rywalka, 23 – Satyna, 24 – Slade, 25 – Sława, 26 – Smuga, 27 – Sukces, 28 – Tonacja, 29 – Trend, 30 – Turnia.

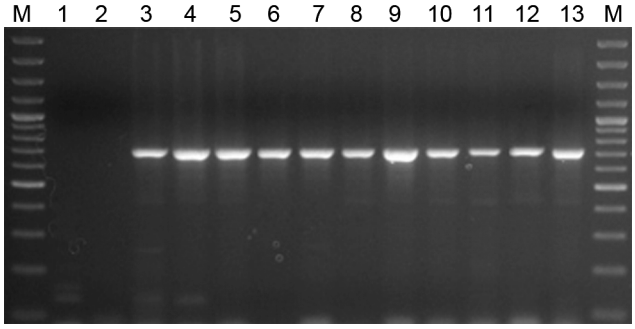

Fig. 4. Amplification products obtained for spring wheat cultivars in PCR with Intr1/B/F and Intr1/B/R3 primer pair: M – GeneRuler^{TM} 100 bp Plus DNA Ladder marker, 1 – Bombona, 2 – Bryza, 3 – Griwa, 4 – Hewilla, 5 – Histra, 6 – Kosma, 7 – Monsun, 8 – Napola, 9 – Parabola, 10 – Radunia, 11 – Triso, 12 – Zebra, 13 – Żura.

the *VRN-1* locus of common wheat cultivars from the Polish register.

According to Stelmakh (1987), hexaploid wheat cultivars with winter growth habit are homozygous for the recessive alleles at the three *VRN-1* loci. However, subsequent work showed that in some cases the dominant *Vrn-B1* or *Vrn-D1* allele is not sufficient to determine spring growth habit, and the plants require low temperature activity; this is described as a facultative type (Sun et al., 2009). The main factor determining flowering initiation is the level of the *VRN-1* transcript (Distelfeld et al., 2009; Dhillon et al., 2010). Sometimes the transcript level probably is too low despite the occurrence of the dominant *Vrn* allele, and this inhibits flowering.

Our results suggest that some Polish bread wheat cultivars described as winter ones display a facultative growth habit due to the occurrence of the dominant *Vrn-B1*, but physiological studies are required to confirm this. It has been shown that within the three analyzed loci, the dominant *Vrn-B1* has the smallest effect on plant traits (Stelmakh, 1993; Eagles et al., 2010).

Yan et al. (2004b) described the dominant *Vrn-A1* allele in 26 lines of hexaploid wheat. A meticulous analysis showed that 18 of them carried the *Vrn-A1a* allele and 6 carried the *Vrn-A1b* allele. In two lines, IL369 from Afghanistan and IL162 from Egypt, a new allele named *Vrn-A1c* was identified. The same authors analyzed the *VRN-A1* locus in 200 lines of hexaploid wheat with different growth habits (68 winter and 132 spring), and confirmed the presence of the recessive *vrn-A1* allele for all tested winter cultivars. Within the lines with spring growth habit, 55% carried the *Vrn-A1a* allele and only 6% the *Vrn-A1b* allele. For the remaining lines they confirmed the occurrence of the recessive *vrn-A1* allele. These results are similar in kind to ours. All the examined winter wheat cultivars from the Polish register carried the recessive *vrn-A1* allele, and spring cultivars carried the dominant *Vrn-A1a* allele.

Fu et al. (2005) characterized the *VRN-1* locus of 117 spring wheat cultivars from Argentina and California. The dominant *Vrn-A1* allele was identified in ~56.5% and *Vrn-D1* in ~42% of them, regardless of region of origin. The frequency of the *Vrn-B1* allele for cultivars from Argentina amounted to 66.1%, and 49.1% for cultivars from California. The most common allelic combination was *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, observed for 48.4% of the examined cultivars. Stelmakh (1987) did similar studies of 45 wheat cultivars from the U.S.A. and Canada. In these cultivars, the presence of the dominant *Vrn-A1* allele was described for 91.1% of the analyzed cultivars, *Vrn-B1* for 60%, and *Vrn-D1* for only 6.7% of them. Further analysis of 40 spring wheat cultivars and lines from western Canada (Iqbal et al., 2007) showed that 34 of them carried the dominant *Vrn-A1a* allele. The *Vrn-A1b* allele was identified in cv. Rescue and its substitu-

TABLE 3. *Vrn* genotypes of analyzed common wheat cultivars from Polish register

Cultivar	*VRN locus*		
	VRN-A1	*VRN-B1*	*VRN-D1*
Alcazar	*vrn-A1*	*vrn-B1*	*vrn-D1*
Anthus	*vrn-A1*	*vrn-B1*	*vrn-D1*
Batuta	*vrn-A1*	*Vrn-B1*	*vrn-D1*
Bogatka	*vrn-A1*	*Vrn-B1*	*vrn-D1*
Bombona	*Vrn-A1a*	*vrn-B1*	*vrn-D1*
Boomer	*vrn-A1*	*vrn-B1*	*vrn-D1*
Bryza	*Vrn-A1a*	*vrn-B1*	*vrn-D1*
Dorota	*vrn-A1*	*vrn-B1*	*vrn-D1*
Finezja	*vrn-A1*	*Vrn-B1*	*vrn-D1*
Flair	*vrn-A1*	*Vrn-B1*	*vrn-D1*
Fregata	*vrn-A1*	*Vrn-B1*	*vrn-D1*
Griwa	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*
Hewilla	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*
Histra	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*
Izyda	*vrn-A1*	*Vrn-B1**	*vrn-D1*
Kobiera	*vrn-A1*	*vrn-B1*	*vrn-D1*
Kosma	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*
Legenda	*vrn-A1*	*vrn-B1*	*vrn-D1*
Ludwig	*vrn-A1*	*vrn-B1*	*vrn-D1*
Monsun	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*
Muza	*vrn-A1*	*vrn-B1*	*vrn-D1*
Nadobna	*vrn-A1*	*vrn-B1*	*vrn-D1*
Napola	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*
Naridana	*vrn-A1*	*vrn-B1*	*vrn-D1*
Nutka	*vrn-A1*	*vrn-B1*	*vrn-D1*
Olivin	*vrn-A1*	*vrn-B1*	*vrn-D1*
Ostka Strzelecka	*vrn-A1*	*vrn-B1*	*vrn-D1*
Parabola	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*
Radunia	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*
Rapsodia	*vrn-A1*	*Vrn-B1*	*vrn-D1*
Rubens	*vrn-A1*	*Vrn-B1*	*vrn-D1*
Rywalka	*vrn-A1*	*vrn-B1*	*vrn-D1*
Satyna	*vrn-A1*	*Vrn-B1*	*vrn-D1*
Slade	*vrn-A1*	*vrn-B1*	*vrn-D1*
Sława	*vrn-A1*	*vrn-B1*	*vrn-D1*
Smuga	*vrn-A1*	*Vrn-B1*	*vrn-D1*
Sukces	*vrn-A1*	*Vrn-B1*	*vrn-D1*
Tonacja	*vrn-A1*	*Vrn-B1*	*vrn-D1*
Trend	*vrn-A1*	*vrn-B1*	*vrn-D1*
Triso	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*
Turnia	*vrn-A1*	*Vrn-B1*	*vrn-D1*
Zebra	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*
Żura	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*

*additional DNA fragment amplification

tion line RC5D only. Four of the analyzed cultivars possessed the recessive *vrn-A1* allele. The dominant *Vrn-B1* allele was observed in half of the analyzed forms, and dominant *Vrn-A1c* and *Vrn-D1* alleles were not found.

In a study of *Vrn* genes in 278 Chinese common wheat cultivars, Zhang et al. (2008) confirmed the presence of the dominant *Vrn-A1a* allele in 68 examined cultivars and of *Vrn-A1b* in 8 of them. In 202 cultivars they found the recessive *vrn-A1* allele. The dominant *Vrn-A1c* allele was not present in any of the analyzed cultivars. The dominant *Vrn-B1* allele was present in 73 of them, and the dominant *Vrn-D1* allele in 105. Iwaki et al. (2000) gave similar frequencies of *Vrn* alleles in wheat cultivars from East Asia.

An examination of 272 wheat cultivars from different geographical regions demonstrated that differences in *Vrn* genotypes are connected with their origin. In European common wheat cultivars the most frequent allele is *Vrn-A1*, the dominant *Vrn-B1* allele is of moderate frequency, and the dominant *Vrn-D1* allele is very rare (Iwaki et al., 2001).

Here we showed that the *Vrn* genotypes of common wheat cultivars from the Polish register are similar to those obtained for cultivars from the U.S.A. and Canada. The frequencies of *Vrn* alleles differed from those given for wheat cultivars from Asia and South America.

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