

# Allele variation in loci for adaptive response in Bulgarian wheat cultivars and landraces and its effect on heading date

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## Abstract

Allele composition at the major growth habit (*Ppd-D1*, *Vrn-1*, *Rht-1* and *Rht8*) loci was determined in 52 Bulgarian bread wheat cultivars and landraces, using recently developed diagnostic molecular markers. The study showed that Bulgarian wheat germplasm varies for photoperiod, vernalization and height-reducing genes. The photoperiod-sensitive allele (*Ppd-D1b*) was the most frequent one in the old cultivars and landraces (90.9%), while the photoperiod-insensitive allele (*Ppd-D1a*) showed the highest frequency in the modern cultivars (96.71%). The alleles conferring winter growth habit (*vrn-A1*, *vrn-B1* and *vrn-D1*) were more common in both the old (72.7%) and the modern (93.3%) wheat genotypes. The spring allele *Vrn-A1c* was not detected in Bulgarian germplasm, while the spring allele *Vrn-B1* was found only in the old genotypes (13.6%). The semi-dwarfing allele *Rht-B1b* was observed in several modern cultivars. Seven allele variants were found in the microsatellite locus *Xgwm261*, closely located to the *Rht8* gene. Among them, alleles of 164, 212 and 216 bp length were specific for the old genotypes studied, while alleles of 192 and 202 bp length were specific for the modern ones. The allele combination *Rht-B1b*//192 or 202 bp allele (*Xgwm261* locus)//*Ppd-D1a*//*vrn-A1*/*vrn-B1*/*vrn-D1* was detected in most of the early-heading modern cultivars. Our study emphasizes on the plasticity of the adaptive response of bread wheat cultivars sown in Bulgaria, as well as on the effect of variation for major growth habit on some yield and reproductive characteristics.

**Keywords:** adaptive response genes (*Ppd-D1*; *Vrn-A1*; *Vrn-B1*; *Vrn-D*; *Rht8* and *Rht-1*); allele composition; molecular markers; wheat (*T. aestivum* L.)

## Introduction

The recently developed allele-specific DNA markers for defining the allele variation in major loci controlling growth habit (vernalization, photoperiod response and plant stature) (Korzun *et al.*, 1998; Ellis *et al.*, 2002; Yan *et al.*, 2004; Fu *et al.*, 2005; Beales *et al.*, 2007) have allowed more efficient characterization of wheat germplasm. These markers have also contributed to the

deeper understanding of the effect of allele variation on the agronomically important traits as well as to more effective management of selection activities during the breeding process. Our previous works on the identification, distribution and effect of the semi-dwarfing *Rht* alleles on some agronomic traits in Bulgarian bread wheat showed that the allele 192 bp at the microsatellite locus *Xgwm 261*, which is linked to *Rht8* gene (2DS), prevailed in the cultivars developed during the last 50 years (Ganeva *et al.*, 2005; Zheleva *et al.*, 2006).

This study was motivated by the lack of more complete information concerning the allele variation at the major loci for growth habit and their effect on maturity and

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other productive agronomic traits in Bulgarian bread wheat varieties. The aim was to define and to assess the allele variation and distribution at loci for growth habit genes (*Vrn*, *Ppd* and *Rht*) in Bulgarian germplasm collections from the Institute of Plant Genetic Resources (IPGR), Sadovo and Dobroudzha Agricultural Institute (DAI), G. Toshevo, Bulgaria. The obtained results could also prove useful for planning and implementation of molecular markers in practical wheat-breeding programmes.

## Material and methods

### Plant material

A total of 52 old and modern Bulgarian wheat genotypes from the genebank collections of the IPGR, Sadovo and DAI, Bulgaria were used in this study. The modern cultivars were developed in the period 1960–2008.

### Microsatellite analysis

Microsatellite analysis of the *Xgwm261* locus was performed as described by Röder *et al.* (1998), using Cy5'-labelled forward and unlabelled reverse primers. Fragment analysis was carried out on AFL Express II sequencer (Amersham Biosciences). The size of the fragments was determined with the program Allele locator, version 1.03.

### *Ppd-D1*

The alleles of the pseudo-response regulator *Ppd-D1* (2D), which determine the sensitivity or insensitivity to

day length, were detected using primers according to Beales *et al.* (2007). Following Beales *et al.* (2007), the photoperiod-insensitive allele was labelled as *Ppd-D1a*. The alternative allele, inferring photoperiod sensitivity, was designated *Ppd-D1b* by analogy.

### *Vrn-A1*

The dominant spring alleles (*Vrn-1Aa* and *Vrn-1Ab*) were identified utilizing genome-specific primers (VRN1AF and VRN1-INT1R) described by Yan *et al.* (2004), by variation in the promoter region of the *Vrn-A1* locus. The primer combination Intr1/A/F2/Intr1/A/R3 was used to differentiate the dominant spring allele *VrnA1c* from *vrn-A1* (the recessive winter allele).

### *Vrn-D1*

*Vrn-D1* intron-1 alleles were detected using Intr1/D/F, Intr1/D//R3 and Intr1/D/R4 primers (Fu *et al.*, 2005). The primer pair Intr1/D/F and Intr1/D//R3 was used for the amplification of the dominant spring allele *Vrn-D1*, while the primer pair Intr1/D/F and Intr1/D/R4 was used for the amplification of the recessive winter allele *vrn-D1*.

### *Rht-1*

The primer pairs BF/WR1 and BF/MR1 (Ellis *et al.*, 2002) were used for the amplification of the alleles *Rht-B1a* (wild type) and *Rht-B1b* (dwarf), respectively.

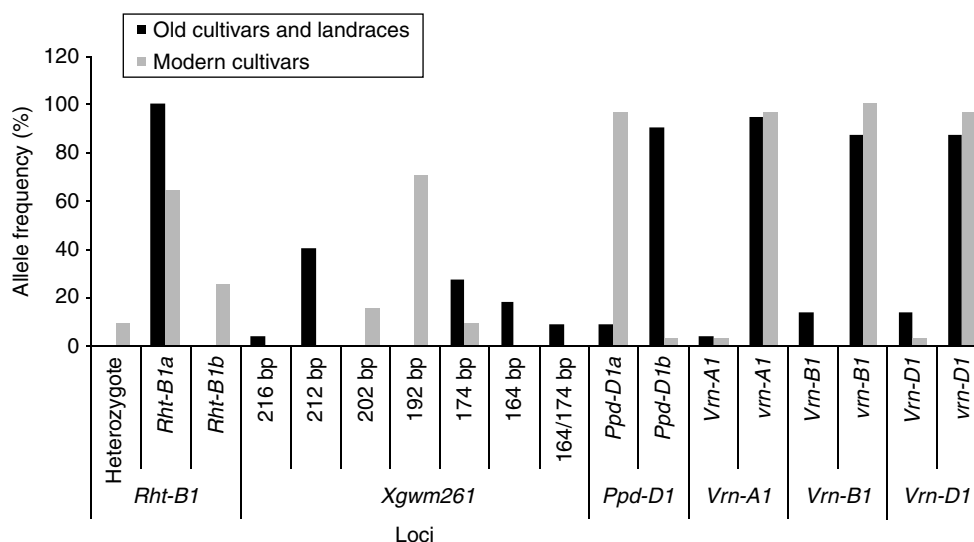


Fig. 1. Distribution of alleles for major growth habit genes among the old and modern Bulgarian wheat genotypes.

**Table 1.** *Rht-1*, *Xgwm261*(*Rht8*), *Ppd-D1* and *Vrn-1* allele composition in the modern Bulgarian bread wheat cultivars (*T. aestivum* L.) and their heading dates<sup>a</sup>

Cultivars and breeding lines	<i>Rht1</i> locus ( <i>Rht-B1a</i> , <i>Rht-B1b</i> )	<i>Xgwm261</i> locus (bp)	<i>Ppd-D1</i> locus ( <i>Ppd-D1a</i> ; <i>Ppd-D1b</i> )	<i>Vrn-A1</i> locus ( <i>Vrn-A1a</i> ; <i>Vrn-A1b</i> ; <i>Vrn-A1c</i> ; <i>vrn-A1</i> )	<i>Vrn-B1</i> locus ( <i>Vrn-B1</i> ; <i>vrn-B1</i> )	<i>Vrn-D1</i> locus ( <i>Vrn-D1</i> ; <i>vrn-D1</i> )	Average heading date (days after 1st of May)
Roussalka	<i>Rht-B1b</i>	192	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>Vrn-D1</i>	8.8
Prostor	<i>Rht-B1a</i>	192	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	10.4
Katya	<i>Rht-B1a/Rht-B1b</i>	192	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	10.4
Galateya	<i>Rht-B1b</i>	202	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	10.4
Kristal	<i>Rht-B1b</i>	192	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	10.6
Pliska	<i>Rht-B1b</i>	202	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	10.8
Enola	<i>Rht-B1b</i>	192	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	11.4
Karat	<i>Rht-B1a</i>	202	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	11.4
Sadovo 772	<i>Rht-B1a</i>	192	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	12
Kaloyan1	<i>Rht-B1a</i>	192	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	14.4
Zlatina2	<i>Rht-B1a</i>	192	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	14.4
Zora	<i>Rht-B1a</i>	192	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	14.4
Momchil	<i>Rht-B1a</i>	192	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	14.4
Ideal	<i>Rht-B1a</i>	192	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	14.6
Progress	<i>Rht-B1a/Rht-B1b</i>	192	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	14.6
Aglika	<i>Rht-B1b</i>	202	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	14.6
Yantar	<i>Rht-B1a</i>	192	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	14.8
Slaveya	<i>Rht-B1b</i>	202	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	14.8
Diamant	<i>Rht-B1a</i>	192	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	15
Kristi	<i>Rht-B1a</i>	192	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	15.2
Laska	<i>Rht-B1a</i>	192	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>Vrn-D1</i>	15.2
Svilena	<i>Rht-B1a</i>	192	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	15.4
Karat	<i>Rht-B1a</i>	174	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	15.6
Dobrud-zhanka	<i>Rht-B1a</i>	192	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	15.6
Sadovo 552	<i>Rht-B1a</i>	174/192	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	15.6
Rekviem	<i>Rht-B1a/Rht-B1b</i>	192	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	15.6
Elitsa	<i>Rht-B1a</i>	192	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	16.2
Bezostaya 1	<i>Rht-B1a</i>	192	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	20
Lozen 6	<i>Rht-B1a</i>	174	<i>Ppd-D1a</i>	<i>vrn-A1</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	20
Gladiator 113	<i>Rht-B1b</i>	174	<i>Ppd-D1b</i>	<i>Vrn-A1a</i>	<i>vrn-B1</i>	<i>vrn-D1</i>	25

<sup>a</sup>The data were recorded during 2005–2009.

## Results and discussion

### **Distribution of the alleles of the major growth habit genes (*Ppd-D1*, *Vrn-1*, *Rht-1* and *Rht8*) within Bulgarian bread wheat genotypes (*Triticum aestivum* L.)**

The assessment of *Vrn-1*(5A/5B/5D), *Ppd-D1*(2DS), *Rht-1*(4B) and *Rht8*(2D) allele variation in 52 old and modern Bulgarian wheat cultivars and landraces showed that the recessive (*vrn*) alleles of *Vrn-1* genes responsible for winter growth habit prevailed in both the old and the modern Bulgarian genotypes (72.7 and 93.3%, respectively).

The dominant alleles *Vrn-A1a* and *Vrn-A1b*, which determine the spring growth habit, were found only in two cultivars, the modern one – ‘Gladiator 113’ and the old one – ‘Karnobatska ranozreika’. A combination of two dominant alleles *Vrn-A1b* and *Vrn-B1* was identified only in cv. ‘Karnobatska ranozreika’. Alleles *Vrn-A1a* and *Vrn-A1b* separately or in combination with other *Vrn-1* alleles have been observed in high proportion in spring wheat genotypes from Argentina, Canada, USA and China (Yan *et al.*, 2004; Fu *et al.*, 2005; Santra *et al.*, 2009). Similar to the results in some other studies (Fu *et al.*, 2005; Iqbal *et al.*, 2007), the allele *Vrn-A1c* was not found in Bulgarian wheat germplasm. On the other hand, the spring allele *Vrn-B1* was found only in the old Bulgarian cultivars and landraces, while *Vrn-D1* allele was detected in a few old and in one modern cultivar. The photoperiod sensitive allele (*Ppd-D1b*) prevailed in the old cultivars (90.9%). A significant increase in the frequency of the photoperiod-insensitive allele (*Ppd-D1a*) was observed in the modern Bulgarian cultivars (96.7%) (Fig. 1). The same tendency has also been observed for the modern Chinese wheat (Yang *et al.*, 2009). In our study, the allele *Rht-1a* (tall type) was found in both old cultivars and landraces as well as in the modern ones. The semi-dwarfing allele (*Rht-1b*) was detected in several modern cultivars. This may be related to the greater use of CIMMYT germplasm in Bulgarian breeding in the previous years (Table 1). At the *Xgwm261* (2D) locus located close to the *Rht8* gene, the alleles of 164, 212 and 216 bp length were found only in the old cultivars and landraces.

### **Allele combinations of the major growth habit genes (*Ppd-D1*, *Vrn-1*, *Rht8* and *Rht-1*) in the modern Bulgarian bread wheat and their influence on some agronomic traits**

The contribution of the allelic composition at the *Vrn*, *Ppd* and *Rht* loci to the variation of the heading date

and other agronomic traits was studied in the modern Bulgarian bread wheat cultivars developed after 1960. In these cultivars, the dominating allele is 192 bp at locus *Xgwm261* (2D), which is considered to be a diagnostic for the presence of *Rht8* gene.

Here, it was confirmed that some of the commercial Bulgarian cultivars carried the *Rht8* gene alone or in combination with the *Rht-1* gene. Similar results have been reported by Ganeva *et al.* (2005), using gibberellin test and isogenic lines of cv. ‘Mercia’. The combination of different *Rht* genes in Bulgarian cultivars could be considered as successful, as these cultivars have found extensive use in the breeding practice. In the last few years, the lands sown with the cultivars ‘Enola’ (*Rht-B1b* + *Rht8*) and ‘Aglika’ (*Rht-B1a* + 202 bp allele at the *Xgwm 261* locus) comprise 19 and 10%, respectively, of the total wheat-sown areas in 2009, followed by ‘Sadovo1’ (8.5%), ‘Pobeda’ (6.1%) and ‘Milena’ (3.7%) (Table 1). The photoperiod insensitivity of the modern Bulgarian wheat cultivars is due to the presence of the *Ppd-D1a* allele, which has been introduced from the main donors of *Rht8* (192 bp allele at the *Xgwm261* locus) in Bulgarian wheat such as the Russian cultivars ‘Bezostaya 1’, ‘Skorospelka’ and ‘Kavkaz’.

All modern Bulgarian wheat cultivars excluding ‘Laska’ (*vrn-A1/vrn-B1/Vrn-D1*) and ‘Gladiator 113’ (*Vrn-A1a/vrn-B1/vrn-D1*) were of the winter type (*vrn-A1/vrn-B1/vrn-D1*). Ten allele combinations for growth habit and plant stature were detected in the old cultivars and landraces. In contrast, the major combination of alleles of the photoperiod sensitivity (*Ppd-D1*) and the *Vrn-1*(5A/5B/5D) genes in the modern Bulgarian wheat gene pool *Ppd-D1a//vrn-A1/vrn-B1/vrn-D1* amounted to 93.1%. The results showed that most of the early-heading cultivars possessed the allelic combination *Rht-B1b//192* or *202 bp* allele at the *Xgwm261* locus//*Ppd-D1a//vrn-A1/vrn-B1/vrn-D1* (Table 1).

Additional studies on the allele variation in other genes affecting the photoperiod sensitivity in wheat (*Ppd-B1* and *Ppd-A1*), the vernalization response (*Vrn-B3* and *Vrn-D4*) and the earliness *per se* gene(s) would generalize to a great extent our knowledge of their effect on maturity and other agronomic traits.

Assessment of the effects of these genes on the heading date and grain yield in diverse Bulgarian production environments is also a task of current priority.

## References

- Beales J, Turner A, Griffiths S, Snape JW and Laurie DA (2007) A pseudo response regulator is misexpressed in the photoperiod insensitive *Ppd-D1a* mutant of wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics* 115: 721–733.

- Ellis MH, Spielmeier W, Gale KR, Rebetzke GJ and Richards RA (2002) Perfect markers for the *Rht-B1b* and *Rht-D1b* dwarfing genes in wheat. *Theoretical and Applied Genetics* 105: 1038–1042.
- Fu D, Szücs P, Yan L, Helguera M, Skinner JS, von Zitzewitz J, Hayes PM and Dubcovsky J (2005) Large deletions within the first intron in *VRN-1* are associated with spring growth habit in barley and wheat. *Molecular Genetics and Genomics* 273: 54–65.
- Ganeva G, Korzun V, Landjeva S, Tsenov N and Atanassova M (2005) Identification, distribution and effect on agronomic traits of the semi-dwarfing *Rht* alleles in Bulgarian common wheat cultivars. *Euphytica* 145: 305–315.
- Iqbal M, Navabi A, Yang RC, Salmon DF and Spaner D (2007) Molecular characterization of vernalization response genes in Canadian spring wheat. *Genome* 50: 511–516.
- Korzun V, Röder MS, Ganal MW, Worland AJ and Law CN (1998) Genetic analysis of the dwarfing gene (*Rht8*) in wheat. Part I. Molecular mapping of *Rht8* on the short arm of chromosome 2D of bread wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics* 96: 1104–1109.
- Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P and Ganal MW (1998) A microsatellite map of wheat. *Genetics* 149: 2007–2023.
- Santra DK, Santra M, Allian RE, Campbell KG and Kidwell KK (2009) Genetic and molecular characterization of vernalization genes *Vrn-A1*, *Vrn-B1*, and *Vrn-D1* in spring wheat germplasm from the Pacific Northwest Region of the USA. *Plant Breeding* 128: 576–584.
- Yan L, Helguera M, Kato K, Fukuyama S, Sherman J and Dubcovsky J (2004) Allelic variation at the *VRN-1* promoter region in polyploid wheat. *Theoretical and Applied Genetics* 109: 1677–1686.
- Yang FP, Zhang XK, Xia XC, Laurie DA, Yang WX and He ZH (2009) Distribution of the photoperiod insensitive *Ppd-D1a* allele in Chinese wheat cultivars. *Euphytica* 165: 445–452.
- Zheleva D, Todorovska E, Jacquemin J-M, Atanassov A, Christov N, Panayotov I and Tsenov N (2006) Allele distribution at microsatellite locus *Xgwm261* marking the dwarfing gene *Rht8* in hexaploid wheat from Bulgarian and Belgian gene bank collections and its application breeding programs. *Biotechnology and Biotechnological Equipment* 20: 45–56.