ALLELE VARIATION IN LOCI FOR ADAPTIVE RESPONSE AND PLANT HEIGHT AND ITS EFFECT ON GRAIN YIELD IN WHEAT


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ABSTRACT

In the present study the microsatellite marker Xgwm261, linked to Rht 8 gene as well as allele specific markers for Ppd-D1 (Ppd1), Vrn-1A and Vrn-1D loci, and the gibberellic acid (GA) test were employed to examine the distribution of semi-dwarf and photoperiod response and vernalization genes in 21 old and modern Bulgarian, 4 introduced cultivars and 11 advanced breeding lines from the wheat germplasm collections of DAI, General Toshevo, Institute of Genetics, Sofia and IPGR, Sadovo. Several allele variants were identified at locus Xgwm261, among which the 192 bp allele can serve as a diagnostic marker for the presence of Rht 8 gene. The Ppd-D1a, vrn-A1 and vrn-D1 alleles were found in almost all wheat genotypes illustrating the relationship between the photoperiod and vernalization response and the adaptability to the regional environments. The photoperiod insensitivity allele (Ppd-D1a) was found in 93.3% of analyzed modern cultivars released during 1960-2003 and has been most probably introduced from the Ukrainian cultivar Bezostaya1 (192 bp allele at locus Xgwm261), which is the main donor of Rht 8 gene in Bulgarian wheat. All studied modern Bulgarian wheat cultivars excluding cv. Laska (vrn-A1, Vrn-D1) and cv. Gladiator 113 (Vrn-A1a, vrn-D1) were of winter type (vrn-A1 and vrn-D1). Allele Vrn-A1c determining also spring type was not found in the studied set of the old and modern Bulgarian wheat cultivars. The association between specific alleles at the Rht-B1, Xgwm261, Ppd-D1, Vrn1 loci with plant height, heading time and some yield related traits was also examined. The results suggested that allele combination Ppd-D1a/vrn-A1, vrn-D1 led to earlier heading than the allele combination Ppd-D1b/vrn-A1, vrn-D1.

The recombinant genotypes obtained from the crosses between photoperiod sensitive spring and photoperiod insensitive winter type wheat behave differently under autumn and spring sowing. Under early spring sowing the earliest heading dates and yield per m² were observed in lines with Ppd-D1a/vrn-A1, vrn-D1 and Ppd-D1b/vrn-A1, vrn-D1 allele combinations.


Keywords: adaptation, allele variation, vernalization genes (Vrn), photoperiod response (Ppd) and plant stature (Rht), polymerase chain reaction (PCR), wheat (T. aestivum L.)

Abrasreviations: DAI - Dobroudja Agricultural Institute, General Toshevo, Bulgaria; IPGR - Institute of Plant Genetic Resources, Sadovo, Bulgaria;

Introduction

The adaptation of wheat (T. aestivum L.) to diverse environmental conditions is greatly influenced by variation in flowering time (30) that is mainly controlled by few major genes. These include genes involved in vernalization response (Vrn genes), photoperiod response (Ppd genes) and developmental rate genes (earliness per se, Eps genes). The expression of the first two groups of genes is environmentally dependant while the third one is not influenced by the environmental conditions.

Cultivated wheat (T. aestivum L.) is usually classified as spring or winter growth habit according to the effect of low temperature on flowering time. Winter wheat requires a cold period to induce flowering and is normally planted in the autumn for harvesting in the next year while spring wheat flowers without cold treatment and is typically sown and harvested in the same year. Winter and spring habit are controlled by three major loci, referred as Vrn-A1, Vrn-B1 and Vrn-D1 located on chromosomes 5A, 5B and 5D, respectively (24, 36). The molecular genetics mechanism of vernalization requirement in cereal crops including wheat is well understood and reviewed by Cockram et al. and Trevaskis et al. (3, 27). The spring habit alleles at these loci are dominant while recessive alleles at all three loci determine winter growth habit. The spring Vrn-A1 allele provides complete insensitivity to vernalization whereas spring Vrn-B1 and Vrn-D1 alleles provide a reduced vernalization requirement relative to winter alleles.

Photoperiod response in wheat is described as sensitive in the case when wheat genotypes require long days for induction of flowering, while the insensitive genotypes flower independently of the day length. Photoperiod response is closely associated with adaptability and grain yield in wheat varieties (11, 31, 32, 33). Photoperiod insensitive wheat varieties are widespread worldwide. However such varieties
are more abundant in regions where spring wheat is grown as a crop over the winter period and autumn sown winter wheat needs to mature in the following year before onset of high temperatures as in the case of southern Europe. Several studies showed that in all environments photoperiod insensitive wheat tends to mature earlier than sensitive ones. Photoperiod response in wheat is controlled by three loci (\textit{Ppd-D1}, \textit{Ppd-B1} and \textit{Ppd-A1}) on chromosomes 2D, 2B and 2A respectively (20, 28). The dominant alleles (\textit{Ppd-D1a}, \textit{Ppd-B1a} and \textit{Ppd-A1a}) of these genes confer photoperiod insensitivity, whereas the recessive alleles (\textit{Ppd-D1b}, \textit{Ppd-B1b} and \textit{Ppd-A1b}) confer photoperiod sensitivity. The \textit{Ppd-D1a} allele is generally considered the most potent, followed by \textit{Ppd-B1a} and \textit{Ppd-A1a} (2). The dominant \textit{Ppd-B1a} allele confers an intermediate level of insensitivity and was found to explain 23 to 45% of the variation for heading date in a double haploid population tested in France (23). However recent works (2, 26) showed that B1a could be as strong as \textit{Ppd-D1a}.

Several of the genes controlling growth habit have recently been cloned and sequenced (1, 6, 22, 35). Development of molecular markers for determination of allele variation in major loci controlling growth habit allows efficient characterization of the germplasm. These marker systems also contribute to a great extent to knowledge that facilitates both studying of the allele variation effect on the agronomically important traits and for managing of selection activities during breeding process.

The increase of yield in Bulgarian wheat in the middle of the last century was mainly attributed to the introduction of the dwarfing genes (\textit{Rht} 8 and \textit{Rht} 1) and photoperiod insensitivity (8) in the breeding programs. Several studies on identification, distribution and effect on agronomic traits of the semi-dwarfing \textit{Rht} alleles in Bulgarian bread wheat showed that the allele 192 bp at the microsatellite locus \textit{Xgwm261}, which is linked to \textit{Rht} 8 and \textit{Ppd-D1} genes (2DS chromosome) prevailed in Bulgarian cultivars developed during the last 50 years (8, 37). Among the studied yield components, the spike density (number of spikes per unit area) is the most closely correlated to wheat grain yield in Bulgaria. The expression of this trait shows strong environmental effects and greatly depends on the damages due to freezing, drought and vegetative diseases at the different ontogenetic stages of plants.

One of the ways to solve this problem is to reduce the duration of the vegetative period of plants, which strongly depends on the genes, responsible for climate adaptation as well as the genes controlling earliness (\textit{Eps}). The accelerated development could be obtained by reduced vernalization requirements in combination with insensitivity to day length. However this can decrease the frost-resistance and the vegetative and productive potential of the plants.

The lack of more complete information of the allele variation at the major loci for growth habit and their effect on maturity and other productive agronomic traits in Bulgarian bread wheat varieties and newly developed breeding lines motivated this study. It is also necessary in order to predict the optimal gene and allele combinations for a stable high yield under different climatic and agro-ecological conditions of the country. The latest will permit that vegetative-stage frost damage is avoided and will allow long grain-filling period before the onset of dry and hot summer conditions.

The objective of the present study was to identify alleles of the \textit{Ppd} and \textit{Vrn} genes in Bulgarian varieties and breeding lines of wheat and to associate them with some yield components.

### Materials and Methods

#### Plant material

A total of 20 Bulgarian and 4 foreign (Bezostaya1, Skorosopelka 35, Mercia, Cappelle Desprez) wheat cultivars from the gene bank collections of the Institute of Plant Genetic Resources (IPGR) and Dobroudja Agricultural Institute (DAI), General Toshevo, Bulgaria were included in this study. Five of the Bulgarian cultivars (Sadowska ranozrekja, Karnobatska ranozrekja, Yubileina 2, Belia, No301) were old. The rest were developed in the period 1960-2003. Eleven breeding lines obtained in the Institute of Genetics, BAS, Sofia and DAI, G. Toshevo were also included in the study. The latest were selected from the cross between spring and winter type of wheat as follows: PS1, PS7 and C218-MO88: Gladiator113 x Roussalka; PS2 and PS9 (DH): (Gladiator113 x F3Roussalka); MO26/07, MO27/07, MO28/07, MO30 and MO37: MO88 x K106.

#### Microsatellite analysis

Microsatellite analysis of \textit{Xgwm261} locus was performed as described in Roder et al. (1998). Amplification was carried out using Cy5' labelled forward and unlabelled reverse primers developed for microsatellite locus \textit{Xgwm261}. Fragment analysis was carried out on automated laser fluorescence (AFL Express II) sequencer (Ameracham Biosciences). Size of the fragments was estimated by the program Allele locator, version 1.03 (Ameracham Biosciences).

\textbf{Ppd-D1}

Alleles of a pseudo-response regulator \textit{Ppd-D1} on chromosome 2D, which confer sensitivity or insensitivity to day length, were detected using primers described in (1). Following that the photoperiod insensitive allele was labelled as \textit{Ppd-D1a}. The alternative allele, which infer photoperiod sensitivity, was designated \textit{Ppd-D1b}.

\textbf{Vrn-A1}

Dominant spring alleles (\textit{Vrn-A1a} and \textit{Vrn-A1b}) due to variation in the promoter region of the \textit{Vrn-A1} locus were identified utilizing genome-specific primers (VRN1AF and VRN1-INT1R) described by (35). The primer combination Intr1/A/F2/Intr1/A/R3 was used to differentiate dominant spring allele \textit{Vrn-A1c} from \textit{Vrn-A1} (recessive winter allele).

\textbf{Vrn-D1}

\textit{Vrn-D1} intron-1 alleles were detected using primers Intr1/D/F, Intr1/D/R3, and Intr1/D/R4 (7). Primer pair Intr1/D/F, Intr1/D/R3 was used for amplification of the dominant spring
allele Vrn-D1a while primer pair Intr1/D/F and Intr1/D/R4 was used for amplification of the recessive winter allele vrn-D1.

**Gibberellin test**

The semi-dwarf breeding lines isolated from the hybrid Gladiator 113 (spring cultivar with Rht-B1b gene) x Roussalka (winter) were subjected to seedling GA-response test following the method described by (9).

**Results and Discussion**

Allele combinations of the major growth habit (Ppd and Vrn) and semidwarf (Rht8, Rht-1) genes in Bulgarian and foreign bread wheat varieties (Triticum aestivum L.) and their effect on the heading date

Allele variation in loci for plant stature, photoperiod sensitivity and vernalization response

The determination of alleles at the major loci controlling growth habit in wheat becomes feasible with the development of PCR-based markers for the major genes controlling wheat development and maturity. Many of these markers are diagnostic across a range of germplasm such as Rht genes that confer the semi-dwarf stature, the Vrn genes and the Ppd-D1 gene determining vernalization requirements and photoperiod sensitivity.

In this study the allele variation in the major loci responsible for adaptive response and plant stature and their effect on the heading date was determined in 21 old and modern Bulgarian cultivars as well as several introduced wheat cultivars using recently developed allele specific DNA markers. Screening of Bulgarian wheat germplasm collections of the DAI, G. Toshevo and IPGR, Sadovo showed variation in most of the major growth habit loci.

In particular, the old Bulgarian cultivars obtained through selection from local forms are characterized with tall stem and possess the allele 174 bp at the closely located to Rht8 gene locus Xgwm 261-2DS (Table 1). The other tall alleles such as 212 bp and 164 bp at this locus were observed only in cvs. Belia and Sadovska ranozreika. In contrast the modern cultivars are of semi-dwarf stature resulting from the presence of either Rht8 gene alone (allele 192 bp, at locus Xgwm 261

**TABLE 1**

<table>
<thead>
<tr>
<th>Cultivars and breeding lines</th>
<th>Rht allele: Xgwm 261 locus</th>
<th>Ppd-D1 locus</th>
<th>VrnAI locus (Vrn-A1a, Vrn-A1b, Vrn-A1c)</th>
<th>VrnD1 locus</th>
<th>Heading date (days after 1st of May)</th>
</tr>
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<tbody>
<tr>
<td>Sadoska ranozrejka</td>
<td>Rht-B1a; 164</td>
<td>Ppd-D1a (In)</td>
<td>vrn-A1</td>
<td>Vrn-D1</td>
<td>8</td>
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<tr>
<td>Karnobatska ranozrejka</td>
<td>Rht-B1a; 174</td>
<td>Ppd-D1b (S)</td>
<td>Vrn-A1b</td>
<td>vrn-D1</td>
<td>19</td>
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<tr>
<td>Yubileina 2</td>
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<td>Ppd-D1a (In)</td>
<td>vrn-A1</td>
<td>vrn-D1</td>
<td>22</td>
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<td>Rht-B1a; 212</td>
<td>Ppd-D1b (S)</td>
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<tr>
<td>No 301</td>
<td>Rht-B1a; 174</td>
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<td>vrn-A1</td>
<td>vrn-D1</td>
<td>25</td>
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<td>Roussalka</td>
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<td>Aglika</td>
<td>Rht-B1d; 202</td>
<td>Ppd-D1a (In)</td>
<td>vrn-A1</td>
<td>vrn-D1</td>
<td>12</td>
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<td>vrn-A1</td>
<td>vrn-D1</td>
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<tr>
<td>Sadovo 1</td>
<td>Rht8; 192</td>
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<td>vrn-D1</td>
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<td>Pliska</td>
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<td>Ppd-D1a (In)</td>
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<td>vrn-D1</td>
<td>15</td>
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<tr>
<td>Galateja</td>
<td>Rht-B1b; 202</td>
<td>Ppd-D1a (In)</td>
<td>vrn-A1</td>
<td>vrn-D1</td>
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<td>vrn-D1</td>
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<td>Milena</td>
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<td>vrn-A1</td>
<td>vrn-D1</td>
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<tr>
<td>Laska</td>
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<tr>
<td>Svilena</td>
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<td>vrn-D1</td>
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<td>Pobeda</td>
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<td>Ppd-D1a (In)</td>
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<td>vrn-D1</td>
<td>19</td>
</tr>
<tr>
<td>Slaveja</td>
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<td>vrn-A1</td>
<td>vrn-D1</td>
<td>19</td>
</tr>
<tr>
<td>Karat</td>
<td>Rht?; 174</td>
<td>Ppd-D1a (In)</td>
<td>vrn-A1</td>
<td>vrn-D1</td>
<td>19</td>
</tr>
<tr>
<td>Lozen</td>
<td>Rht-B1b; 174</td>
<td>Ppd-D1a (In)</td>
<td>vrn-A1</td>
<td>vrn-D1</td>
<td>20</td>
</tr>
<tr>
<td>Gladiator 113</td>
<td>Rht-B1b; 174</td>
<td>Ppd-D1b (S)</td>
<td>Vrn-A1a</td>
<td>vrn-D1</td>
<td>25</td>
</tr>
<tr>
<td>Skorospelka 35</td>
<td>Rht B1a; 192</td>
<td>Ppd-D1a (In)</td>
<td>vrn-A1</td>
<td>vrn-D1</td>
<td>15</td>
</tr>
<tr>
<td>Bezostaya 1</td>
<td>Rht8; 192</td>
<td>Ppd-D1a (In)</td>
<td>vrn-A1</td>
<td>vrn-D1</td>
<td>20</td>
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<tr>
<td>Mercia</td>
<td>Rht B1a; 174</td>
<td>Ppd-D1b (S)</td>
<td>vrn-A1</td>
<td>vrn-D1</td>
<td>28</td>
</tr>
<tr>
<td>Cappelle Desprez</td>
<td>Rht B1a; 174</td>
<td>Ppd-D1b (S)</td>
<td>vrn-A1</td>
<td>vrn-D1</td>
<td>31</td>
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</tbody>
</table>
also known as Rht 8c which is diagnostic for the presence of Rht 8 gene) or from the combination of Rht 8c with Rht-B1a, Rht-B1b or Rht-B1d alleles at the Rht-1 locus on the 4B chromosome. The combination of Rht 8c with Rht-B1b or Rht-B1d alleles at the Rht-1 locus was found in 31% of the modern Bulgarian cultivars. In this study Rht 8 gene alone was found in Bulgarian cultivar Sadovo and the Ukrainian one- Bezostaya1, which is the main donor of this gene in the modern Bulgarian wheat germplasm. Similar results have been obtained in another study (8) reporting the distribution and the effect of the semi-dwarfing Rht alleles on agronomic traits in Bulgarian bread wheat cultivars released in the period 1925-2003 using gibberellic acid (GA) test and microsatellite traits in Bulgarian bread wheat cultivars released in the period 1987-2003. 

Among them, 37 carry Rht 8 alone, and 27 possess various combinations of Rht 8 and a GA-insensitive allele as follows: 17 genotypes with Rht 8 + Rht-B1d; 6 genotypes with Rht8 + Rht-D1b and 4 genotypes having Rht 8 + Rht-B1b combination (8). The authors reported the presence of the 174 bp allele at locus Xgwm 261 (2D) in seven Bulgarian cultivars but only one of them was sensitive to photoperiod.

The prevalence of wheat cultivars carrying combination of two different Rht genes in Bulgarian breeding and agriculture, previously reported in (8), was confirmed in the present study. For example, cv. Enola (Rht-B1d+Rht 8) and cv. Aglika (Rht-B1d+202bp allele at locus Xgwm 261) were widely grown (19% and 10% respectively in 2009), followed by cvs. Sadovo 1 (8.5%), Pobeda (6.1%) and Milena (3.7%).

Among the introduced cultivars only cv. Bezostaya 1 is characterized with the presence of Rht 8 gene alone. The remaining three cultivars- the Ukrainian cv. Skorospelka 35 and the French cvs. Cappelle Desprez and Mercia possess alleles Rht-B1a + Rht 8c or Rht-B1a+174 bp at loci Rht-B1 (4B) and Xgwm 261 (2D).

The photoperiod sensitive allele Ppd-D1b was found in three of the tested here old Bulgarian cultivars. The remaining two were photoperiod-insensitive as carrying allele Ppd-D1a at the locus Ppd-D1 (2DS). In contrast most of the modern Bulgarian wheat cultivars (developed during period 1960-2003) were photoperiod insensitive. This allele was found in 93.3% of analyzed cultivars and has been probably introduced from the main donor of Rht 8 gene in Bulgarian wheat- cv. Bezostaya1 (Table 1). In the group of the introduced wheat genotypes the Ukrainian ones were photoperiod insensitive while the western Europeans were photoperiod sensitive.

Most of the old cultivars were of winter type, carrying the recessive alleles (designated here as vrn-A1 and vrn-D1) at the vernalization loci on chromosome 5. Only cv. Karnobatska ranozrejka (Vrn-A1b) and cv. Sadovska ranozrejka (Vrn-D1) were classified as spring type (absence of vernalization requirement) and facultative type (intermediate vernalization requirement) of wheat. All modern Bulgarian cultivars, with the exception of two- cv. Laska (vnr-A1, Vrn-D1) and cv. Gladiator 113 (Vrn-A1a, vrn-D1) were of winter type (Table 1). The studied introduced cultivars were of winter type. Allele Vrn-A1c which has been shown to be responsible for the spring growth habit (7), was not detected in the tested Bulgarian bread wheat germplasm. Yan et al. (34) using the developed DNA markers specific for Vrn-A1a and Vrn-A1b identified Vrn-A1a in 55% and Vrn-A1b in 6% of 132 spring wheat genotypes from Argentina, Canada, and the USA. The Vrn-A1a allele was reported to be present in most of spring cultivars from China (36). The Vrn-A1c allele has been discovered only in the spring hexaploid landrace IL369 from Afghanistan but was common among tetraploid spring genotypes (7).

**Effect of allele combinations at loci for plant stature, photoperiod sensitivity and vernalization response on heading dates**

Four major combinations of alleles of the photoperiod sensitivity and vernalization genes were identified in the old Bulgarian cultivars: Ppd-D1b/vrn-A1, vrn-D1; Ppd-D1b/vrn-A1b, vrn-D1; Ppd-D1a/vrn-A1, Vrn-D1 and Ppd-D1a/vrn-A1, vrn-D1. Among them cv. Sadovska ranozrejka carrying the allele combination Ppd-D1a/vrn-A1, Vrn-D1 showed earliest heading date.

The major combination of alleles of the photoperiod sensitivity Ppd-D1 and Vrn-A1 and Vrn-D1 loci in the modern Bulgarian wheat gene pool is Ppd-D1a/vrn-A1, vrn-D1 (86.6% in this study). The study clearly demonstrated that both the modern Bulgarian and the introduced wheat cultivars having the above mentioned allele combination are characterized with earlier heading in comparison to these with Ppd-D1b/ vrn-A1, vrn-D1. The prevalence of genotypes with the allele combination Ppd-D1a/vrn-A1, vrn-D1 in Bulgarian breeding programs could be explained by their high adaptability to the local climatic conditions, avoiding vegetative-stage frost damage and allowing long grain-filling period before the onset of dry and hot Summer.

Variation in the heading dates was also observed among the modern photoperiod insensitive winter wheat cultivars (Ppd-D1a/vrn-A1, vrn-D1). Some of these cultivars such as Roussalka, Kristal and Aglika have early heading dates. The observed variation in the heading dates in the cultivars with Ppd-D1a/vrn-A1, vrn-D1 allele combination could be related to a combined influence of other growth habit and developmental genes. Blake et al. (2) reported association of the insensitive alleles at Ppd-D1 and Ppd-B1 with earlier heading under field conditions and shorter stature for two of three studied spring populations showing segregation at these loci. The authors observed that Ppd-B1 has the strongest effect on the heading date, followed by Ppd-D1. Earlier heading of the tested insensitive genotypes under field conditions reflected the ability of the examined by them genotypes to initiate flowering before the onset of long days in Summer.

In our study earliest heading date was shown only by the old Bulgarian cultivar Sadovska ranozrejka, which could be probably due to the allele combination 164 bp at locus Xgwm 261 (Rht8)/Ppd-D1a/vrn-A1, vrn-D1. We admit that
in this case the presence of the spring allele at locus \(Vrn-D1\) accelerates the flowering time by shortening the vernalization requirement.

Allele combinations of the major growth habit (\(Ppd\) and \(Vrn\)) genes in Bulgarian wheat breeding lines of the cross Spring x Winter cultivars and their effect on the yield components

Several recently initiated breeding programs in the Institute of Genetics involved production of spring x winter wheat crosses. These programs aimed at increasing both the genetic diversity and improving of some traits such as yield, earliness as well as development winter-spring and spring wheat varieties.

The effect of different allele combinations at loci responsible for plant stature and growth habit on maturity and yield was studied in several breeding lines. These genotypes were obtained from the cross between spring and winter Bulgarian cultivars cvs. Gladiator 113 and Roussalka (Table 1) as well as between the spring and winter breeding lines C218-Mo88 and K106. Both cvs. Gladiator and C218-Mo88 were shown to carry the following allele combinations: 174 bp allele at locus \(Xgwm\) 261/\(Ppd\)-\(D1b\)/\(Vrn\)-\(A1a\), \(vrn\)-\(D1\) while cv. Roussalka and K106 carried the allele combination: 192 bp allele at locus \(Xgwm\) 261/\(Ppd\)-\(D1a\)/\(vrn\)-\(A1\), \(vrn\)-\(D1\).

**TABLE 2**

Effect of allele combinations in lines obtained from the cross between spring and winter type of wheat on their heading date under autumn and spring sowing

<table>
<thead>
<tr>
<th>Breeding lines</th>
<th>261 locus (bp)</th>
<th>Ppd-D1 locus</th>
<th>Vrn-A1 locus</th>
<th>Vrn-D1 locus</th>
<th>Heading date</th>
<th>Stem length</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Autumn sowing</td>
<td>Spring sowing</td>
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<tr>
<td>Gladiator 113</td>
<td>174</td>
<td>Ppd-D1b (S)</td>
<td>Vrn-A1a</td>
<td>vrn-D1</td>
<td>May 26</td>
<td>June 4</td>
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<tr>
<td>Russalka</td>
<td>192</td>
<td>Ppd-D1a (In)</td>
<td>vrn-A1</td>
<td>vrn-D1</td>
<td>May 11</td>
<td>-</td>
</tr>
<tr>
<td>PS1</td>
<td>192</td>
<td>Ppd-D1a (In)</td>
<td>Vrn-A1a</td>
<td>vrn-D1</td>
<td>May 13</td>
<td>May 26</td>
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<tr>
<td>PS2</td>
<td>174</td>
<td>Ppd-D1b (S)</td>
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<td>vrn-D1</td>
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<td>May 27</td>
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<td>164</td>
<td>Ppd-D1b (S)</td>
<td>Vrn-A1a</td>
<td>vrn-D1</td>
<td>May 25</td>
<td>June 1</td>
</tr>
<tr>
<td>PS9</td>
<td>164</td>
<td>Ppd-D1b (S)</td>
<td>Vrn-A1a</td>
<td>vrn-D1</td>
<td>May 23</td>
<td>May 28</td>
</tr>
<tr>
<td>C 218-MO88</td>
<td>174</td>
<td>Ppd-D1b (S)</td>
<td>Vrn-A1a</td>
<td>vrn-D1</td>
<td>May 23</td>
<td>May 28</td>
</tr>
<tr>
<td>K 106</td>
<td>192</td>
<td>Ppd-D1a (In)</td>
<td>vrn-A1</td>
<td>vrn-D1</td>
<td>May 17</td>
<td>-</td>
</tr>
<tr>
<td>MO26/07</td>
<td>192</td>
<td>Ppd-D1b (S)</td>
<td>Vrn-A1a</td>
<td>vrn-D1</td>
<td>May 23</td>
<td>May 28</td>
</tr>
<tr>
<td>MO27/07</td>
<td>174</td>
<td>Ppd-D1a (In)</td>
<td>Vrn-A1a</td>
<td>vrn-D1</td>
<td>May 14</td>
<td>May 24</td>
</tr>
<tr>
<td>MO28/07</td>
<td>192</td>
<td>Ppd-D1a (In)</td>
<td>vrn-A1a</td>
<td>vrn-D1</td>
<td>May 14</td>
<td>May 27</td>
</tr>
<tr>
<td>MO30</td>
<td>174</td>
<td>Ppd-D1a (In)</td>
<td>vrn-A1</td>
<td>vrn-D1</td>
<td>May 16</td>
<td>June 3</td>
</tr>
<tr>
<td>MO37</td>
<td>174/192</td>
<td>Ppd-D1b (S)</td>
<td>vrn-A1</td>
<td>vrn-D1</td>
<td>May 28</td>
<td>June 4</td>
</tr>
</tbody>
</table>

**TABLE 3**

Effect of allele variation at major growth habit and photoperiod sensitivity loci on some agronomic traits in wheat lines with different \(Ppd-D1\) and \(Vrn\) combinations in spring sowing

<table>
<thead>
<tr>
<th>Allele combinations</th>
<th>Spike length</th>
<th>Spikelets / spike</th>
<th>Grains /spike</th>
<th>Yield / spike (g)</th>
<th>Grains no. / spike</th>
<th>100 grain weight (g)</th>
<th>Heading date (days after 1st of May)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ppd-D1a + Vrn-A1a</td>
<td>9.10± 0.69</td>
<td>18.5±2.21</td>
<td>54.03±6.23</td>
<td>2.40±0.50</td>
<td>2.92±0.17</td>
<td>44.6±5.98</td>
<td>25.67</td>
</tr>
<tr>
<td>Ppd-D1a + vrn-A1a</td>
<td>9.56±1.63</td>
<td>17.2±3.03</td>
<td>41.8±12.9</td>
<td>1.96±0.35</td>
<td>2.43±0.20</td>
<td>46±3.27</td>
<td>34</td>
</tr>
<tr>
<td>Ppd-D1b +Vrn-A1a</td>
<td>10.83±3.18</td>
<td>19.73±2.03</td>
<td>51.1±5.7</td>
<td>2.3±0.57</td>
<td>2.48±0.26</td>
<td>45.3±9.07</td>
<td>28.8</td>
</tr>
<tr>
<td>Ppd-D1b + vrn-A1a</td>
<td>10.7±0.76</td>
<td>19.6±1.34</td>
<td>55±13.56</td>
<td>2.81±0.44</td>
<td>34±3.24</td>
<td>35</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Distribution of the \(Ppd\)-\(D1\) and \(Xgwm\) 261 allele combinations in lines selected from the crosses: Gladiator 113 x Roussalka and MO88 x K106.
Among the selected genotypes from both crosses these with allele combinations Ppd-D1a/192 bp allele and Ppd-D1b/174 bp at loci Ppd-D1 and Xgwm 261 predominated (Fig. 1, Table 2). In total 40% of the selected genotypes were recombinants carrying allele combinations Ppd-D1b/192 bp and Ppd-D1a/174 bp at the above mentioned loci. About a half of the obtained lines from both crosses were of spring type carrying allele Vrn-A1a (Fig. 2, Table 2) on the chromosome 5A. Among them those with allele combinations Ppd-D1b/Vrn-A1a and Ppd-D1a/Vrn-A1a prevailed.

In spring and autumn sowing under field conditions the lines with allele combinations Ppd-D1a/Vrn-A1a, vrn-D1 showed earliest heading and reduced plant height in comparison to all others (Fig. 3, Fig. 4, Table 2).

Among lines carrying different allele combinations at loci Rht 8, Rht-B1 (Rht1) and Ppd-D1 those with combination rht 8, Rht-B1b (Rht 1), Ppd-D1a were with shortest plant height while those with rht8, Rht-B1a (rht1) and Ppd-D1b were the tallest (Fig. 4). Our study showed that the allele combinations Ppd-D1a/Vrn-A1a and Ppd-D1b/Vrn-A1a have favorable impact on some characteristics of the yield such as early ear emergence and high yield/m² under early spring sowing (Fig. 5, Table 3).

**Fig. 2.** Distribution of the Ppd-D1 and Vrn1 allele combinations in lines selected from crosses: Gladiator 113 x Roussalka and MO88 x K106

**Fig. 3.** Effect of allele combinations at major habit genes on ear emergence in wheat lines

**Fig. 4.** Effect of different Rht and Ppd-D1 allele combinations on plant height of wheat lines

**Fig. 5.** Effect of different Ppd-D1 and Vrn allele combinations on yield (g/m²) in wheat lines

**Conclusions**

Selection for earlier flowering and photoperiod insensitivity is an important prerequisite for improvement of the adaptation abilities of the newly developed wheat cultivars to many environments.

Allele combinations at the growth habit (Ppd-D1, Vrn-A1, Vrn-D1) and plant height (Rht 8, Rht-1) loci in Bulgarian bread wheat were determined using recently developed diagnostic molecular markers for genes controlling growth habit and plant stature.

Bulgarian wheat cultivars vary for photoperiod, vernalization, and height-reducing genes, with several combinations of alleles defined in most widespread cultivars. The higher frequency of Ppd-D1a, vrn-A1, vrn-D1, Rht 8 and Rht 1 allele combination in the modern Bulgarian cultivars was found.

The recombinant genotypes obtained from the crosses between photoperiod sensitive spring and photoperiod insensitive winter type of wheat behaved differentially under autumn and spring sowing. Under spring sowing the earliest
heading dates and yield per m² were observed only in lines with Ppd-D1a/Vrn-A1, vrn-D1 and Ppd-D1b/Vrn-A1, vrn-D1 allele combinations.

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

REFERENCES