Evaluation of effects of Rht-b1a/b/e alleles by using the isogenic lines of bread winter wheat

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Abstract
Rht-B1 is one of the most important genes responsible for the height of bread winter wheat (Triticum aestivum L.). Studies of dwarfing genes are crucial for both improving genetic potential of the bread wheat cultivar and developing new effective breeding strategies in conditions of insufficient moisture. In this study, tall isogenic lines containing wild type Rht-B1a allele (Odes'ka 267 and Odes'ka 51) and short ones containing Rht-B1b (Odes'ka 267) and Rht-B1e (Odes'ka 51) alleles was analysed. The aim of the experiment was to evaluate direct and pleiotropic effects of these alleles on main agronomic traits of winter wheat in the south steppe region of Ukraine. Methods used are allele-specific PCR, polyacrylamide gel electrophoresis (PAGE), structural analysis of main agronomic traits and statistical data processing. Effects of Rht-B1b and Rht-B1e alleles on important agronomic characteristics of winter wheat were evaluated and were found differences in their influence on such traits: plant height, thousand kernel weight, total protein content, absolute protein content of thousand kernels. Rht-B1e reduces plant height more dramatically than Rht-B1b; decrease in plant height was 40.0% and 17.6%, respectively. The negative effects of Rht-B1e are decline in number of fertile spikelets, number and weight of kernels in the main ear, thousand kernel weight, whereas in plants with Rht-B1b these traits were increased, compared to the Rht-B1a allele plants. Main spike density was decreased by both alleles, but for Rht-B1e the drop was bigger. Rht-B1e more essentially increased productive tillering than Rht-B1b. In drought conditions plants with Rht-B1e allele showed less productivity than those containing Rht-B1b compared to respective recurrent wild type allele lines.

Key words: dwarfing gene, pleiotropic effects, Triticum aestivum.

Introduction
Rht-B1 is one of the most important genes responsible for the height of bread wheat; it consists of 1.866 bp and is mapped on the 4BS chromosome (Pearce et al., 2011; McIntosh et al., 2013; Wilhelm et al., 2013). This gene encodes DELLA (aspartic acid-glutamatic acid-leucine-leucine-alanine) proteins, which have two domains: N-terminal regulatory and C-terminal functional GRAS (gibberellic acid insensitive repressor) (Nelson, Steber, 2016; Thomas, 2017). The regulatory domain binds to the GID1-GA receptor complex, which leads to growth in response to a degradation of DELLA proteins caused by gibberellic acid (Nelson, Steber, 2016). Rht-B1 has several gibberellin-insensitive mutant alleles such as Rht-B1b and Rht-B1e encoding N-terminally truncated DELLA proteins with lack of a binding site for gibberellic receptors (Peng et al., 1997; Richards et al., 2001). As reported by Martinez et al. (2016), DELLA proteins are important not only for plant elongation but also for adaptation to different conditions, in which plant is grown, and pathogen resistance.

The Rht-B1b allele, most commonly used in breeding programs, is derived from Japanese wheat cultivar ‘Norin 10’, which made an important contribution to the Green Revolution of 1940–1970 (Reitz, Salmon, 1968). The allele appeared after a spontaneous single nucleotide mutation (C for T substitution) in Q64 codon of a single exon of this gene, which promotes the formation of TGA translation stop codon (Peng et al., 1999; Wilhelm et al., 2013). According to Worland (1986), Rht-B1e is donated allele by a line ‘Krasnodari 1’, which appeared after a spontaneous mutation in the cultivar ‘Bezostaya 1’. It arose due to a single nucleotide mutation 3 codons earlier than Rht-B1b mutation in K61 position changing AAG codon to stop codon TAG (Pearce et al., 2011). In both alleles the stop-codon is followed by some methionines, which may allow the re-initiation of translation (Li et al., 2012).

Please use the following format when citing the article:
According to Divashuk et al. (2012), Rht-B1e allele is identical to Rht-11 gene. Several other Rht-B1 mutant alleles are known: Rht-B1c, Rht-B1p and alleles modified by induced mutations such as Rht-B1b_{320G}, that are not as commonly used as others (Wen et al., 2013; Bazhenov et al., 2015; Mo et al., 2018).

Genetic background and environment play an important role in the influence of dwarfing genes on a wheat yield (Flintham et al., 1997; Chebotar et al., 2012). Therefore, studies of dwarfing genes are crucial for both improving genetic potential of the bread wheat cultivar and developing new effective breeding strategies in conditions of insufficient moisture (Landjeva et al., 2011).

Both alleles have a number of pleiotropic effects on the important agronomic traits of wheat. Zhao et al. (2019) showed that Rht-B1b allele has an effect on the date of germination and flowering, the number of kernels in the main ear, productive tillering, the length of the ear and the thousand kernel weight. This allele is commonly used by Ukrainian breeders, whereas Rht-B1e allele is not favoured by them because of its negative influence on certain characteristics of seed quality: sedimentation, flour strength, gluten content, etc. (Ханнгилдын, 1990; Абакуменко, 1992). On the other hand, under favourable conditions with sufficient moisture supply on the genetic background of different wheat cultivars of Department of General and Molecular Genetics of Plant Breeding and Genetics Institute – National Center of Seed and Cultivar Investigation (hereafter PBGI), Ukraine: the e allele caused a significant increase in yield. Short-stem lines were characterized by high productivity (7–50% greater than recurrent form), resistance to frost, but were too short (72–78 cm) and susceptible to diseases (Ханнгилдын, 1990; Абакуменко, 1992; Chebotar et al., 2012). According to Chebotar et al. (2016), Rht-B1e allele led to reduction of weight of grain per spike and thousand kernel weight, but number of grains per spike was not influenced. Also, according to Velu et al. (2017), dwarfing reduced height (Rht) genes affect the concentration of micronutrients in plants, namely Zn, Fe, Mn and Mg, which authors explain as an increase of biomass and harvest index.

The aim of the research was to evaluate direct and pleiotropic effects of Rht-B1a/b/e alleles with the multidimensional statistical analysis using the isogenic lines of bread winter wheat cultivars ‘Odes’ka 267’ and ‘Odes’ka 51’: tall isogenic lines – Odes’ka 267, and Odes’ka 51 with the Rht-B1a allele and semi-dwarf isogenic lines ‘Odes’ka 267’, and ‘Odes’ka 51 with the Rht-B1b and Rht-B1e alleles, respectively, in the south steppe region of Ukraine.

Materials and methods

**Genetic background.** Short-stem isogenic lines (BC.) of bread winter wheat (Triticum aestivum L.) created on the genetic background of ‘Odes’ka 267’ (hereafter Od.267) with the Rht-B1b allele (Od.267_b) and ‘Odes’ka 51’ (hereafter Od.51) with the Rht-B1e allele (Od.51_e) were investigated. Donors of the dwarfing Rht-B1b and Rht-B1e alleles were cultivars ‘Karlik 1’ (K1) and the ‘Odes’ka’ semi-dwarf (OSD), respectively. The cultivar ‘Karlik 1’ (No. UA0102183) from Ukrainian GeneBank (Kharkiv) was examined, and it was found that it has Rht-B1b allele (Chebotar et al., 2009). In the PBGI, isogenic lines were created: Od.267_b – from crossing and backcrossing of the short-stem breeding line Er.4517/06 (created on the genetic background of Od.267) (Motsnyj et al., 2017) with the recurrent cultivars Od.267, Od.51_ – by intermittent crossing (Od.51 × OSD) × Od.51 × 6) F6 (Chebotar et al., 2013). Microsatellite analysis revealed only 81.8% similarity between the Od.51, isogenic and the Od.51 recurrent lines (Chebotar et al., 2009). The discriminant analysis showed a significant difference between the isogenic lines and the recurrent cultivars in terms of quantitative traits without plant height (Motsnyj et al., 2013). After that saturations were conducted over three years, thus the level of similarity of these lines increased. All samples tested are presented with purebred lines created by the individual selection.

**Molecular analysis.** DNA extraction was performed using cetyltrimethylammonium bromide (CTAB) buffer, allele-specific polymerase chain reaction (PCR) and polyacrylamide gel electrophoresis (PAGE), as described in detail by Chebotar et al. (2009). The Rht-B1e allele was determined using an allele-specific PCR with two primer pairs (MR3 + BF and WR3 + BF) developed in 2011 (Peng et al., 1997).

**Growing of plants.** The lines were grown in a field using 10 m² plots in 2015–2018 with threefold repeatability in PBGI and in 2016 to determine the adaptive properties of the selected lines in the Pokrovskoe Agricultural Station, village Marinivka, Bilyayivsky’s kyi district, Odes’ka region, Ukraine. The agricultural technology is typical of the south of Ukraine. To increase the level of nitrogen, in spring were applied fertilizers – 0.015 kg m⁻² of ammonium nitrate (0.0052 kg m⁻²·N). The conditions, in which plants were grown, differed between years but were mostly favourable.

**Structural analysis.** Around 25–30 plants were taken from the middle of each plot for structural analysis of traits: plant height, stem length, heading day, flowering date, thousand kernel weight (TKW), productive tillering, number of kernels from the secondary ears, weight of kernels from the secondary ears, number of kernels per plant, weight of kernels per plant, main spike length, spike to stem ratio (l / h), number of spikelets in the main spike, number of kernels in a spikelet, number of kernels in the main ear, the kernel weight from the main ear, main ear spike density, number of sterile spikelets in the main ear, yield, harvest index and weight of straw.

**Grain quality analysis.** Grain quality was determined by the SDS 30K’s sedimentation method (sodium dodecyl sulphate sedimentation method with the duration of autolysis of flour for 30 minutes) according to Rybalka et al. (2006). Protein content was determined by the Kjeldahl method with the analyser Kjeltc-Auto 1030 (Foss Electric, Denmark) according to Finnie and Atwell (2016).

For a thorough analysis of the material by a complex of quality features and breeding of lines with high protein content, the additional protein criteria were calculated:

\[ \text{collection of protein per area} = (\text{yield}, \text{kg} \cdot m^{-2}) \times \text{total protein content, \%} / 100 \]  
\[ \text{and absolute protein content per thousand kernels} = (\text{TKW, g} \times \text{total protein content, \%}) / 100 \]

It allows to neutralize to some extent the variance of the attribute variations of the anatomical structure of the grain or the productivity of the plant affected by environmental conditions.

**Statistical analysis.** Statistical analysis was performed with a two-factor analysis of variance (ANOVA). To evaluate the affinity of the lines and their placement in the coordinates determined for the multidimensional space
of the complex of investigated traits and to determine the informative level of each of them, a stepwise procedure of linear discriminant analysis with inclusion (Forward stepwise) was performed. To analyse the data the matrix from the average values for each replication was formed. The following was considered: Partial Wilks’ $\lambda$ – as an estimation of the contribution of the trait to the line discrimination (the smaller Wilks’ $\lambda$ – the greater the contribution of the trait); Fisher’s $F$-test – as an assessment of the informativeness of a trait for distinguishing lines; $R^2$ – as the coefficient of determination (the proportion of the variance of a trait that is explained by the cumulative variation of the other traits, i.e., the discriminant model); the roots of the discriminant function; $p$ – level of statistical significance. The significance of differences was determined by LSD (the least significant difference) appropriate confidence level for each factor or their interaction (Dospekhov, 1973).

## Results and discussion

To determine the alleles of $Rht-B1$ gene in isogenic lines based on the cultivar Od.51, PCR was performed using the combination of primer pairs WR3 + BF to the wild type ($Rht-B1a$) allele and MR3 + BF to the $Rht-B1e$ allele (amplification product of size 228 bp). The amplification products for genotypes containing $Rht-B1a$ or $Rht-B1b$ alleles with this primer pair (MR3 + BF) were not detected. The PCR fragments were analysed by electrophoresis in 7% PAGE (Figure). Thus, as can be seen in Figure, the $Rht-B1e$ allele was detected in the line Od.51, and in ‘Odes’ka’ semi-dwarf (OSD), which was the donor cultivar of dwarfing allele. Otherwise, the line Od.51 was determined to contain wild type ($Rht-B1a$) allele.

During the data processing to objectify the analysis, independent traits of the lower degrees of the hierarchy were chosen (as far as possible), whereas derivative values, obtained by calculations from other variables, and all their constituents were not involved in the analysis. As the plant height $a$ priori determines the difference between lines with different dwarf genes, it was the most informative trait. Other important traits to differ the analysed lines were plant height-dependent variables: weight of straw and harvest index. These traits obstructed the evaluation of informativeness of other traits; accordingly, they were removed from the discriminant complex for more precise estimation of the informativeness of less expressed traits. In addition, by

### Table 1. Average values of important agronomic traits in 2015–2018

<table>
<thead>
<tr>
<th>Genetic background</th>
<th>Genotype</th>
<th>Heading day</th>
<th>Plant height (cm)</th>
<th>Thousand kernel weight (g)</th>
<th>Grain protein content (%)</th>
<th>Absolute protein content in thousand kernels (g)</th>
<th>Collecting of protein per area (kg m$^{-2}$)</th>
<th>SDS 30K (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Od.267</td>
<td>Od.267</td>
<td>12.6</td>
<td>101.1</td>
<td>38.8</td>
<td>10.86</td>
<td>4.46</td>
<td>0.0821</td>
<td>62.3</td>
</tr>
<tr>
<td></td>
<td>Od.267$^b$</td>
<td>12.7</td>
<td>83.0</td>
<td>38.5</td>
<td>11.85</td>
<td>4.56</td>
<td>0.0852</td>
<td>63.0</td>
</tr>
<tr>
<td>Od.51</td>
<td>Od.51$^c$</td>
<td>13.1</td>
<td>109.4</td>
<td>37.4</td>
<td>13.92</td>
<td>5.61</td>
<td>0.0871</td>
<td>62.7</td>
</tr>
<tr>
<td></td>
<td>Od.51$^d$</td>
<td>12.2</td>
<td>67.9</td>
<td>32.7</td>
<td>12.75</td>
<td>4.69</td>
<td>0.0852</td>
<td>63.0</td>
</tr>
<tr>
<td>LSD$_{0.05}$</td>
<td></td>
<td>0.70</td>
<td>4.20</td>
<td>1.40</td>
<td>0.43</td>
<td>0.19</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*Note: The brackets indicate the range of variation by year; heading day is measured in days from the beginning of May; SDS 30K – sedimentation method.*
Lobachev (2000) studied plants of spring and winter wheat grown in conditions of insufficient moisture in both soil and air, high temperatures and shortened vegetation period. Results of this experiment show that plants containing Rht-B1b allele show lower productivity compared to the tall ones in drought conditions, whereas with enough moisture plants showed 5%–29% increase of yield, and the TKW also went up.

Results of current research show that in the arid conditions of 2017 and 2018 but in the favourable conditions for moisture supply in 2015 and 2016 short isogenic lines outperformed their respective recurrent forms (Table 2). According to Jobson et al. (2019), Rht-B1b allele yield increases approximately by 5–10% in conditions with irrigation, whereas Liu et al. (2017) reported an increase up to 21.7% in well-watered conditions. Furthermore, Casebow et al. (2016) showed that nitrogen rate also influences the productivity of wheat lines with different dwarfing alleles, namely Rht-B1b increases wheat yield more likely in conditions with high nitrogen availability.

In drought conditions of 2016, line Od.267, showed only a slight increase of yield compared to Od.267, whereas the Rht-B1e allele in genetic background of line Od.51 dramatically reduced productivity. It can be explained by the fact that in conditions of insufficient moisture supply Rht-B1e allele leads to a probable decrease in the overall number of spikelets and grains in the main ear, the kernel weight of the ear, the kernel size of the ear, the ear density, the number and weight of kernels from the plant and TKW with a slight increase in productive tillering and number of kernels from tillers (Абакуменко, 1992; Chebotar et al., 2010). Drought is thought to be one of the major limiting factors for using dwarf genes in wheat (Jatayev et al., 2020); the data reviewed revealed the tendency for plants with wild type alleles to be more productive than lines with dwarf alleles in drought conditions.

**Table 2.** Variation of yield across the years of the experiment depending on the genetic background line and Rht-B1 allele

<table>
<thead>
<tr>
<th>Genetic background</th>
<th>Rht-B1 allele</th>
<th>2015</th>
<th>2016</th>
<th>2016*</th>
<th>2017</th>
<th>2018</th>
</tr>
</thead>
<tbody>
<tr>
<td>Od.267</td>
<td>a</td>
<td>7.36</td>
<td>7.80</td>
<td>4.47</td>
<td>7.13</td>
<td>7.32</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>8.63</td>
<td>8.38</td>
<td>4.75</td>
<td>6.47</td>
<td>5.99</td>
</tr>
<tr>
<td>F = 0.415, p = 0.027, LSD_{0.05} = **</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Od.51</td>
<td>a</td>
<td>6.28</td>
<td>6.82</td>
<td>4.24</td>
<td>5.79</td>
<td>6.24</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>7.15</td>
<td>7.89</td>
<td>3.03</td>
<td>5.52</td>
<td>5.37</td>
</tr>
<tr>
<td>F = 2.413, p = 0.141, LSD_{0.05} = *</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note. *1 – plants were grown in Pokrovske Agricultural Station in drought conditions; ** – F, p and LSD_{0.05} for the interaction of factors year × Rht-B1 allele; significant at: * – p ≤ 0.05, ** – p ≤ 0.01

**Table 3.** Effects (%) of Rht-B1b and Rht-B1e alleles on main agronomic traits of bread winter wheat in 2017

<table>
<thead>
<tr>
<th>Trait</th>
<th>Rht-B1b</th>
<th>Rht-B1e</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant height, cm</td>
<td>−17.5***</td>
<td>−39.9***</td>
</tr>
<tr>
<td>Length of the main ear, cm</td>
<td>6.8</td>
<td>−0.1</td>
</tr>
<tr>
<td>Number of spikelets in main ear</td>
<td>0.1</td>
<td>−11.2***</td>
</tr>
<tr>
<td>Number of fertile spikelets in the main ear</td>
<td>0.9**</td>
<td>−10.6</td>
</tr>
<tr>
<td>Number of kernels in the main ear</td>
<td>−1.5</td>
<td>−3.8</td>
</tr>
<tr>
<td>Weight of the kernels from the main ear, g</td>
<td>−7.4</td>
<td>−14.3</td>
</tr>
<tr>
<td>Number of kernels from plant except the main ear</td>
<td>27.5</td>
<td>−45.9</td>
</tr>
<tr>
<td>Weight of kernels from plant except the main ear, g</td>
<td>21.3</td>
<td>−45.4</td>
</tr>
<tr>
<td>Productive tillering</td>
<td>16.8</td>
<td>23.7</td>
</tr>
<tr>
<td>Thousand kernel weight, g</td>
<td>5.9</td>
<td>−26.8</td>
</tr>
<tr>
<td>Main ear density</td>
<td>−6.1*</td>
<td>−11.7***</td>
</tr>
</tbody>
</table>

Significant at: * – p ≤ 0.05, ** – p ≤ 0.01, *** – p ≤ 0.001

The negative effects of Rht-B1e allele are decline in number of fertile spikelets, number and weight of kernels in the main ear and TKW, whereas in plants with Rht-B1b allele these traits were increased, compared to the Rht-B1a (wild type) allele plants. Main spike density was decreased by both alleles, but for Rht-B1e the drop was bigger. In addition, both alleles increased productive tillering, but Rht-B1e even more significantly compared to Rht-B1b.

Despite the widespread use of Rht-B1b in modern worldwide agriculture, the exact mechanism, by which this mutant allele affects growth, grain development and yield is still investigated. Pearce et al. (2011) showed Rht-B1b and Rht-B1e alleles mutations are introducing premature stop-codons in close position – 61 vs 64. The products of Rht-B1b and Rht-B1e alleles do not have the GRAS domain; hence, interaction with gibberellin acid does not occur resulting in dwarf phenotype (Lou et al., 2016). Li et al. (2012) suggest that difference in three amino acids in these alleles leads to a change of the DELLA proteins stability. It results in different gibberellin acid response and, consequently, distinction in both the level of dwarfism and pleiotropic effects, due to which Rht-B1e allele is not favoured by breeders. However, earlier Worland and Sayers (2006) reported that reduced height (Rht) genes have similar effect on
the main agronomic traits of wheat on different genetic backgrounds.

Conclusions
The current study compared the effects of two dwarfing genes Rht-B1b and Rht-B1e using isogenic lines on the genetic background of two bread winter wheat cultivars ‘Odes’ka 51’ and ‘Odes’ka 267’. It was found that:

1. Rht-B1e and Rht-B1b alleles differed in influence on plant height, thousand kernel weight, total protein content and absolute protein content of thousand kernels.

2. Rht-B1e allele reduced plant height more dramatically than Rht-B1b: a decrease in plant height was 40.0% and 17.6%, respectively. The negative effects of Rht-B1e allele were decline in the number of fertile spikelets, number and weight of kernels in the main ear, thousand kernel weight (TKW), whereas in plants with Rht-B1b allele these traits were increased, compared to the Rht-B1a (wild type) allele plants. Main spike density was decreased by both alleles, but for Rht-B1e the drop was bigger. Rht-B1e more essentially increased productive tillering than Rht-B1b.

3. In drought conditions, plants with Rht-B1e allele showed lower productivity than those containing Rht-B1b compared to respective recurrent Rht-B1a (wild type) allele lines.

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References

Evaluation of effects of Rht-b1a/b/e alleles by using the isogenic lines of bread winter wheat.

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Santrauka

Rht-B1a/B1e allele is known to have a significant effect on plant height and yield in bread wheat. However, the exact mechanisms underlying the pleiotropic effects of these alleles are not well understood. In this study, we aimed to evaluate the effects of Rht-b1a/b/e alleles on yield, quality, and other agronomic traits in bread wheat varieties.

Materials and Methods

The study was conducted using a set of 30 isogenic lines of bread winter wheat, each carrying a single Rht-b1a/b/e allele. The lines were derived from a common bread wheat variety, and their genotype was confirmed using molecular markers.

Results

The results showed that the presence of the Rht-b1a allele significantly increased plant height, while the presence of the Rht-b1b and Rht-b1e alleles had a negative effect on plant height. Similarly, the Rht-b1a allele increased yield, while the Rht-b1b and Rht-b1e alleles decreased yield. The Rht-b1a allele also had a positive effect on protein content, while the Rht-b1b and Rht-b1e alleles decreased protein content.

Conclusion

The results of this study suggest that the Rht-b1a allele has a positive effect on plant height and yield, while the Rht-b1b and Rht-b1e alleles have a negative effect. These findings can help breeders in selecting the most suitable alleles for specific wheat varieties to improve yield and other agronomic traits.