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

Oksana OKHRYMOVYCH[1], Galyna CHEBOTAR[1], Sabina CHEBOTAR[1,2], Ivan MOTSNY[2]

[1]Odessa I. I. Mechnikov National University
Dvoryanskaya 2, 65082 Odessa, Ukraine

[2]Plant Breeding and Genetics Institute – National Center of Seed and Cultivar Investigation (PBGI)
Ovidiopolskaya 3, 65036 Odessa, Ukraine
E-mail: s.v.chebotar@onu.edu.ua

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$_b$) and *Rht-B1e* (Odes'ka 51$_e$) 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-glutamic 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 gibberellin receptors (Peng et al., 1997; Richards et al., 2001). As reported by Martínez 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).

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$_{E529K}$* 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 winter 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. (Хангильдин, 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 (Хангильдин, 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$_a$ and Odes'ka 51$_a$ with the *Rht-B1a* allele and semi-dwarf isogenic lines 'Odes'ka 267$_b$ and 'Odes'ka 51$_e$ 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$_6$) 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$_e$ – by intermittent crossing (Od.51 × OSD) × Od.51 × 6) F$_\infty$ (Chebotar et al., 2013). Microsatellite analysis revealed only 81.8% similarity between the Od.51$_e$ 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 (Motsnyy 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 Pokrovske Agricultural Station, village Marinivka, Bilyayivs'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⁻¹), 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 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 30′K 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 Kjeltec-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:

collection of protein per area = (yield, kg m⁻² × total protein content, %) / 100% and absolute protein content per thousand kernels = (TKW, g × 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 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' λ – as an estimation of the contribution of the trait to the line discrimination (the smaller Wilks' λ – 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$_e$ 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
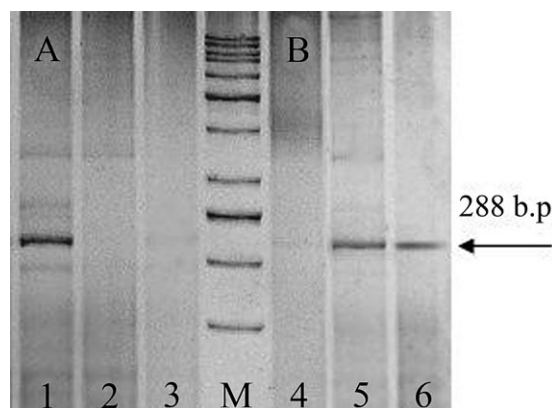
On the tracks of the gel: 1, 4 – Od.51 (*Rht-B1a*), 2, 5 – Od.51$_e$, 3, 6 – OSD ('Odes'ka' semi-dwarf); M – molecular weight marker GeneRuler 50 bp

Figure. Electrophorese in 7% PAGE of amplification fragments obtained by PCR of DNA of isogenic lines of 'Odes'ka 51' (Od.51) with allele-specific primer pairs WR3 + BF to *Rht-B1a* (A), MR3 + BF to *Rht-B1e* (B)

means of ANOVA, it was found that the following traits were important to distinguish the pairs of lines with *Rht-B1a*–*Rht-B1b* and *Rht-B1a*–*Rht-B1e*: heading day, TKW, grain protein content, and absolute protein content in thousand kernels (Table 1). As reported by Guan et al. (2020), *Rht-B1b* is likely to be a candidate gene with pleiotropic effect on the kernel size and TKW in the quantitative trait loci (QTL) cluster on chromosome 4B.

The absence of significant differences between the lines in collecting of protein per area and sedimentation values is explained by the variation of yield depending on the conditions of the year, that is interaction of factors year × line (Table 2).

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

Genetic background	Genotype	Heading day	Plant height cm	Thousand kernel weight g	Grain protein content %	Absolute protein content in thousand kernels g	Collecting of protein per area kg m⁻²	SDS 30′K ml
Od.267	Od.267$_a$	12.6 (9.5–18.0)	101.1 (82.0–108.6)	38.8 (33.9–43.0)	10.86 (9.1–13.2)	4.46 (3.95–4.70)	0.0821 (0.0668–0.0940)	62.3
	Od.267$_b$	12.7 (7.0–16.5)	83.0 (68.0–91.0)	38.5 (32.1–45.0)	11.85 (9.4–13.6)	4.56 (4.25–5.00)	0.0852 (0.0580–0.0965)	63.0
Od.51	Od.51$_a$	13.1 (9.3–20.0)	109.4 (106.4–115.0)	37.4 (30.6–46.0)	13.92 (12.7–15.0)	5.61 (5.40–5.82)	0.0871 (0.0864–0.0877)	62.7
	Od.51$_e$	12.2 (8.3–20.0)	67.9 (63.9–73.0)	32.7 (24.2–40.0)	12.75 (12.1–13.0)	4.69 (4.55–4.83)	847 (742–952)	60.7
LSD$_{0.05}$		0.70	4.20	1.40	0.43	0.19	–	–

Note. The brackets indicate the range of variation by year; heading day is measured in days from the beginning of May; SDS 30′K – 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 shows 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

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

Genetic background	*Rht-B1* allele	2015	2016	2016[1]	2017	2018
Od.267	*a*	7.36	7.80	4.47	7.13	7.32
	b	8.63	8.38	4.75	6.47	5.99
$F = 0.415, p = 0.027, \mathrm{LSD}_{0.05} = **$						
Od.51	*a*	6.28	6.82	4.24	5.79	6.24
	e	7.15	7.89	3.03	5.52	5.37
$F = 2.413, p = 0.141, \mathrm{LSD}_{0.05} = *$						

Note. [1] – plants were grown in Pokrovske Agricultural Station in drought conditions; ** – F, p and $\mathrm{LSD}_{0.05}$ for the interaction of factors year × *Rht-B1* allele; significant at: * – $p \leq 0.05$, ** – $p \leq 0.01$

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$_b$ 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.

Rht-B1e plant height reduced more dramatically than *Rht-B1b* allele in the conditions of 2017 (Table 3), which agrees with the data of Chebotar et al. (2012), where a decrease in plant height was 19% and 31%, respectively, on the genetic background of Ukrainian cultivars in the conditions of the South Ukraine.

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

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

Significant at: * – $p \leq 0.05$, ** – $p \leq 0.01$, *** – $p \leq 0.001$

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 gibberellic 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 gibberellic 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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Rht-b1a/b/e alelių poveikis žieminio kviečio izogeninėms linijoms

O. Okhrymovych[1], G. Chebotar[1], S. Chebotar[1,2], I. Motsny[2]

[1]Odesos I. I. Mechnikovo nacionalinis universitetas, Ukraina
[2]Ukrainos augalų selekcijos ir genetikos instituto Nacionalinis sėklų ir veislių tyrimų centras

Santrauka

Rht-B1 yra vienas svarbiausių genų, atsakingų už paprastojo kviečio (*Triticum aestivum* L.) aukštį. Žemaūgiškumo genų tyrimai yra labai svarbūs, siekiant pagerinti duoninio žieminio kviečio veislių genetinį potencialą ir sukurti naujas veiksmingas selekcines strategijas drėgmės trūkumo sąlygomis. Tirtos aukštaūgės izogeninės linijos, turinčios laukinio tipo *Rht-B1a* alelį (Odes'ka 267 ir Odes'ka 51), ir žemaūgės izogeninės linijos, turinčios *Rht-B1b* (Odes'ka 267b) bei *Rht-B1e* (Odes'ka 51e) alelius. Tyrimo tikslas – įvertinti tiesioginį ir pleiotropinį šių alelių poveikį pagrindinėms kviečių agronominėms savybėms Pietų Ukrainos stepių regione. Taikyti šie metodai: aleliui specifinė PGR, poliakrilamido gelio elektroforezė (PAGE), pagrindinių agronominių požymių struktūrinė analizė ir duomenų statistinis apdorojimas. Tyrimo metu įvertintas *Rht-B1b* ir *Rht-B1e* alelių poveikis kviečių agronominėms savybėms ir nustatyti jų įtakos skirtumai augalų aukščiui, 1000 grūdų masei, suminiam baltymų kiekiui ir absoliučiam 1000 grūdų baltymų kiekiui.

Augalų aukštį labiau sumažino *Rht-B1e* nei *Rht-B1b* alelis – jis sumažėjo atitinkamai 40,0 ir 17,6 %. *Rht-B1e* alelio neigiamas poveikis buvo derlingų varpučių skaičiaus, grūdų skaičiaus bei svorio pagrindinėje varpoje ir 1000 grūdų masės sumažėjimas, o augaluose, turinčiuose *Rht-B1b* alelį, šių požymių vertės padidėjo, palyginti su turinčiais *Rht-B1a* alelį. Pagrindinių varpučių tankį sumažino abu aleliai, tačiau labiau – *Rht-B1e*. Produktyvių krūmijimąsi iš esmės labiau padidino *Rht-B1e* nei *Rht-B1b* alelis. Sausros sąlygomis augalų, turinčių *Rht-B1e* alelį, produktyvumas buvo mažesnis nei turinčių *Rht-B1b*, palyginti su atitinkamomis pasikartojančiomis laukinio tipo alelių linijomis.

Reikšminiai žodžiai: pleiotropinis poveikis, *Triticum aestivum* L., žemaūgiškumo genas.