Effects of Rht17 in combination with Vrn-B1 and Ppd-D1 alleles on agronomic traits in wheat in black earth and non-black earth regions

Pavel Yu. Kroupin1,2, Gennady I. Karlov1,2, Ludmila A. Bespalova3, Elena A. Salina4, Anastasiya G. Chernook1,2, Nobuyoshi Watanabe5, Mikhail S. Bazhenov1,2, Vladimir V. Panchenko3, Lubov A. Nazarova6, Victor Ya. Kevtunenko3 and Mikhail G. Divashuk1,2,6*

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Abstract

Background: Plant height is an important wheat trait that is regulated by multiple genes, among which Rht is of the utmost value. In wheat, Rht-B1 (Rht17) is a mutant allele of the Rht gene that encodes for a DELLA-protein and results in the development of gibberellin-insensitive plants with a dwarfing phenotype. The pleiotropic effects of dwarfing genes on yield are highly dependent on both the genetic background and the environmental conditions. In Russia, the Central Non-Black Earth Region and Krasnodar Krai are two economically important regions that require differing management for sustainable wheat production for food, feed and industry. The purpose of our study was to compare the pleiotropic effects of Rht-B1 on the main valuable agronomic traits in the F3:4 families of the spring bread wheat Chris Mutant/Novosibirskaya 67 in the genetic background of Vrn-B1a/vrn-B1 (spring/winter phenotype) and Ppd-D1a/Ppd-D1b (insensitivity/sensitivity to photoperiod) alleles in a field experiment in Moscow and Krasnodar Krai.

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Background

Wheat is an important food crop and one of the main sources of calories for humanity. The main factor behind the significant increase in crop yield in the 40th–70th years of the twentieth century, the so-called “Green revolution”, was the development of semi-dwarf wheat varieties (with shorter stature) through the introgression of allelic variants of Rht genes, including Rht-B1b, which determine plant height and result in a semi-dwarf phenotype. Thanks to the expression of these alleles, it became possible to increase grain yield through better lodging resistance under irrigation and high doses of nitrogen fertilizers, leading to an increase in the number of grains in each spikelet and grain per unit area.

Wild-type alleles Rht-A1a, Rht-B1a, and Rht-D1a encode DELLA proteins that inhibit the growth of plant cells through negative regulation of the gibberellin signaling pathway, while the degradation of the DELLA protein is promoted by active forms of gibberellins [1]. The phenotypic manifestation of the wild-type allele Rht-B1a is a tall plant; presently, at least 15 alleles of this gene are known, of which Rht-B1c results in a dwarf phenotype, Rht-B1b, Rht-B1e, Rht-B1f, Rht-B1h, Rht-B1p a semi-dwarf phenotype, while plants with Rht-B1g, Rht-B1i-1 are tall plants (overgrowth mutants) compared to the wild-type allele Rht-B1a [2–6]. Alleles Rht-B1b and Rht-B1e contain a premature stop codon, which is proposed to result in the translation of a form of DELLA protein resistant to proteolysis, and thus permanently inhibiting cell growth [2, 7]. Thus, the dwarfism of plants caused by such mutations of Rht-1 genes cannot be compensated by the use of exogenous gibberellic acid (GA). Previously we have shown that the dwarfing allele formerly named Rht17, now designated Rht-B1p, also contains a single-nucleotide substitution that leads to a premature stop codon, so this probably has a similar mechanism of action to other GA-insensitive alleles of Rht-B1.

Despite the similarities in the mechanism of action of GA-insensitive alleles of the Rht-B1 locus, their effect on plant height and distribution in the genetic regions vary considerably [2, 5, 8–10]. An excessively strong decrease in height is caused by Rht-B1c (about 50%), which inhibits its widespread use in breeding practice [11, 12]. The most common Rht-B1b dwarfing allele leads to a moderate decrease in plant height in the range of 10–25% [13–15]. Locally distributed in Southern Europe is the allele Rht-B1d, which reduces height by 10–17% [12, 16]; in East Europe, Rht-B1e reduces height by 30% [8, 17, 18]; and in China, Rht-B1h produces a 10% height reduction (2). In plants bearing the Rht-B1p allele, the effect of plant height reduction is 30–33% in the vegetation experiment in bread wheat [7, 19]. It is known that the inheritance of Rht-B1p, like that of the dwarfing alleles with a moderate effect, Rht-B1b and Rht-B1e, is partially recessive relative to the dominant wild-type allele Rht-B1a [7, 20–22].

Dwarfing alleles have an impact not only on plant height, but also have a pleiotropic effect on the timing of plant development and productivity elements. In dwarf plants, assimilate partitioning is improved, making possible the survival of a higher number of florets, thereby increasing the number of grains per spike and improving harvest index [11, 23]. However, GA-insensitivity caused by a number of dwarfing alleles of Rht-B1 leads to a decrease in the length of the coleoptile from 19 to 27%, that prevents deep seeding in regions with insufficient soil moisture [19, 24–26]; and also to the development of grains with a lower mass [15, 27]. The effect of dwarf plant genes depends on weather and climatic conditions as well as the genetic background [13, 15, 17, 27]. Therefore, it can be expected that the influence of different
dwarfing alleles at the Rht-B1 locus on the elements of productivity will also be different in different growing conditions and in different genetic environments. The effect of Rht-B1p on elements of crop structure in spring bread wheat has not been studied and needs to be addressed.

The timing of the onset of such developmental phases as stem elongation, heading and flowering are important adaptive traits of wheat and are controlled by three different signaling pathways: the vernalization (VRN), photoperiod (PPD), and earliness per se (EPS) pathways [28–30]. Thus, the optimal combination of alleles of these genes plays a main role in the formation of phenological types of plants most suitable for and adapted to agroclimatic regions.

VRN-1 genes encoding MADS-box transcription factors are involved in the regulation of apical transition from the vegetative to reproductive phase [31, 32]. Of the three homologous genes VRN-A1, VRN-B1 and VRN-D1, located on chromosomes 5A, 5B and 5D respectively, VRN-A1 has the strongest influence on the phenotype and has an epistatic effect relative to VRN-B1 and VRN-D1. The wild type of the alleles of these genes is associated with a winter growth habit (recessive vrn-A1, vrn-B1 and vrn-D1). Dominant alleles at these loci lead to a spring type growth habit (the transition to the generative phase in such plants occurs without exposure to low positive temperatures) and contain mutations in the promoter and/or the 1st intron relative to alleles of the wild type [33–36]. In regions with cold climates, including Western and Eastern Siberia, for spring wheat with dominant alleles associated with a spring habit (Vrn-A1a in combination with Vrn-B1a or Vrn-B1c), this combination allows the plant to complete its lifecycle after a relatively short vegetative period and escape autumn frosts [37–39]. For southern latitudes, varieties with one allele of Vrn-B1a or Vrn-D1a can have a definite advantage, as they ripen later, and a longer vegetative period can provide higher yields [37].

In general, spring wheat is a long-day plant. In addition to the VRN genes, the timing of heading in wheat is also determined by the allelic condition of the Ppd genes regulating the sensitivity of the plant to photoperiod. Furthermore, Ppd genes may be regulatory elements for other genes that are involved in light perception (PhyA, PhyB, PhyC) and flowering initiation (Vrn-1, TaFT1) [40–42]. According to their influence on the rate of plant development, these genes correlate with each other as follows: Ppd-D1 (strong influence) > Ppd-B1 > Ppd-A1 (weak influence) [43–46]. The dominant allele Ppd-D1a serves as the main source of insensitivity to photoperiod, which allows plants to flower at the optimal time in a short day. As a result, this allele is common in wheat in southern Europe, where it is exposed to short-day conditions [37, 47]. In regions with long daylight hours, this allele has no significant adaptive advantage and is extremely rare; the Ppd-D1b allele is most widespread in such regions [37, 38].

Variation in Rht, VRN, and Ppd genes can become a tool for the breeder to fine-tune the phenotype for specific agro-ecological conditions, including soil type, temperature, rainfall, and daylight hours. It is known that various allelic variants of Vrn and Ppd influence not only the duration of wheat phenophases and sensitivity to temperature and light conditions, but also the height of the plant and productivity elements [48–51]. Thus, combining different allelic variants of these genes is a way to develop wheat varieties that are best adapted to the local conditions in terms of straw height, the requirements of temperatures and daylight hours, and the ability to avoid adverse conditions during flowering, heading, ripening and harvesting of grain.

To ensure the economic stability of the Russian regions, it is necessary to develop their own agricultural production, especially wheat, which is supplied both to the domestic and foreign markets for baking, livestock feed, and for starch and alcohol production. The territory of Russia is characterized by a wide variety of agro-climatic conditions. Some regions are more suitable for cultivating winter wheat, others for spring wheat. Spring wheat occupies about half of the crop area under wheat in Russia, and one-third of gross wheat grain harvest. For specific agro-climatic conditions, it is important to choose the combination of straw height and the time of heading/flowering so that the plant can escape stressful conditions in the form of drought or frost, while giving the maximum possible crop of the best quality.

The aim of our work was to study the pleiotropic effect of the Rht-B1p allele (in comparison with Rht-B1a) on plant height and the main economically significant traits in interaction with the alleles of the vernalization genes Vrn-B1 and sensitivity to photoperiod Ppd-D1 in spring bread wheat. For field experiments, we selected two regions with contrasting conditions: Moscow and Krasnodar. These areas are characterized by contrasting agro-climatic conditions. The duration of the period with average daily temperatures above 10°C in Moscow does not exceed 138–140 days, while in the Krasnodar region it lasts for 180–187 days. The soils are sod-podzolic in Moscow and chernozems in Krasnodar. Despite the fact that both regions often receive sufficient and even excessive rainfall per year, the climate of these regions is characterized by sharp fluctuations and unpredictable droughts.

**Results**
The allelic state of Rht-B1, Rht-D1, Rht-8, Ppd-D1, VRN-A1, VRN-B1, and VRN-D1 was revealed via molecular
analysis. The following genotypes were revealed for the parental plants: Chris Mutant: Rht-B1p Rht-B1p/crRht-D1a Rht-D1a/crRht-8a Rht-8a/crPpd-D1a Ppd-D1a/crVrn-A1a Vrn-A1a/crvrn-B1 vrn-B1/crvrn-D1 vrn-D1; Novosibirskaya 67: Rht-B1a Rht-B1a/crRht-D1a Rht-D1a/crRht-8a Rht-8a/crPpd-D1b Ppd-D1b/crVrn-A1a Vrn-A1a/crvrn-B1 vrn-B1/crvrn-D1 vrn-D1. Therefore, in the F₃:₄ families derived from the cross Chris Mutant/Novosibirskaya 67, segregation in the following three loci occurred: Rht-B1 (Rht-B1a/Rht-B1p), Ppd-D1 (PpdD1a/PpdD1b) and VRN-B1 (Vrn-B1a/vrn-B1). For convenience in describing results and comparing values of agronomic traits, we adopted the following abbreviations for the studied alleles:

- Rht-B1a – R (tall plants, dominant allele).
- Rht-B1p – r (semi-dwarf plants, recessive allele).
- Vrn-B1a – V (spring habit, dominant allele).
- vrn-B1 – v (winter habit, recessive allele).
- Ppd-D1a – P (photoperiod insensitive, dominant allele).
- Ppd-D1b – p (photoperiod sensitive, recessive allele).

The abbreviations for the genotypes used in the comparison and description of the revealed phenotypic effects are listed in Table 1.

### Plant height, internode length, and culm weight

Both in Moscow and in Krasnodar, a significant difference in height was revealed between plants homozygous for the Rht-B1p dwarfing allele (semi-dwarf plants) and plants homozygous for the wild-type allele Rht-B1a (tall plants). Rht-B1p, compared to the wild-type allele, reduced the height by an average of 21 cm (28%) in Moscow, and by 25 cm (30%) in Krasnodar (Table 2, Fig. 1). At the same time, the difference in height between plants with the RRvv and rrvv genotypes was 25.5 cm (33%), and among families with the spring habit allele Vrn-B1a (RRVV and rrVV), was 19.9 cm (27%) in Moscow; and 28.5 cm (34%) and 25.2 cm (30%) in Krasnodar, respectively. The two-way analysis of variance of the height between plants with differing sensitivity to photoperiod demonstrated that the difference in height was 18.9 cm between RRpp and rrp plants (25%) and 19.9 cm between RRPP and rPP plants (27%) in Moscow; and 32.6 cm (38%) and 25.2 cm (30%) in Krasnodar, respectively (Table 2).

The decrease in plant height as a whole was due to a significant reduction in the length of each individual internode; however, the length of the peduncle (first upper internode) was most strongly changed. In plants homozygous for the Rht-B1p allele, the peduncle was shorter by 10.6 cm (32%) in Moscow and 12.1 cm (34%) in Krasnodar when compared to plants homozygous for Rht-B1a grown under the same conditions (Table 2).

In our study, wheat plants had from four to five visible (elongated) internodes on the main stem; while in Moscow, five internodes were found in 37% of tall plants (RR), and 20% of semi-dwarf plants (rr). The 7% variation in plant height in the experiment in Moscow could therefore be explained by the number of internodes. In Krasnodar, five internodes of the main stem accounted for 91% of tall plants (RR), and 63% of semi-dwarf plants (rr). The number of internodes explained 15% of the variability of the plant height in the experiment in Krasnodar.

The presence of the dominant allele of the Vrn-A1a gene in all the studied wheat plants means that they all have a spring habit. However, among them, there is segregation of the VRN-B1 gene, which theoretically can affect the rate of plant development and other agronomically important traits. In our study, families with and without the dominant allele Vrn-B1a (contributing to the spring habit), did not differ significantly from each other in plant height in either Moscow or Krasnodar (Additional file 1 Table S1).

Variation in alleles of the Ppd-D1 gene regulating plant response to day length had a small effect on plant height. In Moscow, Ppd-D1a demonstrated the tendency in plant height reduction, whilst in Krasnodar (short daylight hours) it slightly increased height (Additional file 1 Table S1). However, this difference in height did not play a major role in the field experiment. Two-way analysis of variance on the interaction between Rht-B1 and Ppd-D1 in Moscow found no significant differences in height between plants. In Krasnodar, in the background of the dwarfing allele Rht-B1p, an increase in plant height under the action of an allele insensitive to daylength, Ppd-D1a, was 6.3 cm (12%) (Table 2).

Semi-dwarf plant forms had a naturally smaller culm biomass. In plants carrying Rht-B1p, compared to plants with Rht-B1a, the biomass of the culm on average was 0.5 g less in both regions, which accounted for a 22 and 14% decrease in this trait in Moscow and Krasnodar, respectively. Statistically significant phenotypic differences in the culm biomass between plants with alternate VRN-B1 alleles were not revealed. In Moscow, Ppd-D1a

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**Table 1** The abbreviations for the genotypes in families F₃:₄

| Genotype          | Abbreviations |
|-------------------|---------------|
| Rht-B1a Rht-B1a Vrn-A1a Vrn-A1a Vrn-B1a Vrn-B1a | RRVV          |
| Rht-B1a Rht-B1a Vrn-A1a Vrn-A1a vrn-B1 vrn-B1  | RRvv          |
| Rht-B1p Rht-B1p Vrn-A1a Vrn-A1a Vrn-B1a Vrn-B1a| nrVv          |
| Rht-B1p Rht-B1p Vrn-A1a Vrn-A1a vrn-B1 vrn-B1  | rrvv          |
| Rht-B1a Rht-B1a Ppd-D1a Ppd-D1a                 | RRPP          |
| Rht-B1a Rht-B1a Ppd-D1b Ppd-D1b                 | RRpp          |
| Rht-B1p Rht-B1p Ppd-D1a Ppd-D1a                 | rPP           |
| Rht-B1p Rht-B1p Ppd-D1b Ppd-D1b                 | rpp           |
reduced the culm biomass by 0.2 g; however, this effect was not observed after considering particular differences in the two-way analysis of Rht-B1/Ppd-D1 (Table 2, Additional file 1 Tables S1).

Structure and productivity of the spike
In a one-way analysis, plants with the Rht-B1p dwarfing allele had a shorter and more compact spike compared to tall plants. While plants with the allele of photoperiodic insensitivity, Ppd-D1a, had less spike compactness than plants with a genotype sensitive to photoperiod. The relationship between the length and spike compactness and the alleles of the VRN-B1 gene as a whole does not show a single trend, but rather depends on the growing region and genotype for the Rht-B1 gene. The strongest influence on spike compactness among the studied genes in Moscow came from Rht-B1, while in Krasnodar it was the Ppd-D1 gene (Additional file 1 Table S5).

Semi-dwarf plants had, on average, lower main spike productivity (grain weight per main spike) both in Moscow (by 0.24 g, 20%) and in Krasnodar (by 0.09 g, 6%) compared to tall plants. Only in the conditions of a short day in the Krasnodar Krai, the allele of photoperiodic insensitivity Ppd-D1a contributed to mitigation of the negative effect of Rht-B1p on spike productivity (Additional file 1 Table S2). In Krasnodar, in plant families with the photoperiod insensitive allele Ppd-D1a, the decrease in spike productivity associated with Rht-B1p was 6% (0.08 g), and in the absence of Ppd-D1a, it was 15% (0.21 g). In Moscow conditions, Ppd-D1a had no effect on spike productivity. Plants differing in alleles of the VRN-B1 gene did not differ significantly in spike productivity (Table 3).

### Table 2 Mean values of biometric traits for main shoot in families F₃:₄ Chris Mutant/Novosibirskaya 67 grouped by Rht-B1 x VRN-B1 and Rht-B1 x Ppd-D1 alleles

| Genotype | Plant height, cm | Peduncle length, cm | Main shoot biomass, g |
|----------|------------------|---------------------|-----------------------|
|          | Moscow           | Krasnodar           | Moscow               | Krasnodar           | Moscow          | Krasnodar       |
| RRVV     | 73.4 ± 1.5a      | 84.1 ± 1.0a         | 32.9 ± 1.1a          | 36 ± 0.7a           | 2.3 ± 0.1a      | 3.6 ± 0.1a      |
| RRvv     | 77.2 ± 3.3a      | 83.7 ± 1.5a         | 34.7 ± 2a            | 36.6 ± 1.6a         | 2.5 ± 0.2a      | 3.7 ± 0.2a      |
| rVV      | 53.5 ± 1.4b      | 58.9 ± 1.2b         | 22.7 ± 0.9b          | 24 ± 0.5b           | 1.8 ± 0.1b      | 3.1 ± 0.3b      |
| rvv      | 51.7 ± 3.9b      | 55.1 ± 6.4b         | 20.9 ± 3.1b          | 23.2 ± 4.3b         | 1.7 ± 0.3b      | 2.4 ± 0.9ab     |
| RRPP     | 73.4 ± 1.5a      | 84.1 ± 1.0a         | 32.9 ± 1.1a          | 36 ± 0.7a           | 2.3 ± 0.1a      | 3.6 ± 0.1a      |
| RRpp     | 74.9 ± 1.8a      | 85.2 ± 1.2a         | 33.5 ± 1.3a          | 35.3 ± 0.8a         | 2.4 ± 0.1a      | 3.8 ± 0.2a      |
| rrPP     | 53.5 ± 1.4b      | 58.9 ± 1.2b         | 22.7 ± 0.9b          | 24 ± 0.5b           | 1.8 ± 0.1b      | 3.1 ± 0.3b      |
| rpp      | 56 ± 2.6b        | 52.6 ± 1.2c         | 24.6 ± 1.5b          | 21.3 ± 0.8c         | 1.9 ± 0.2b      | 2.9 ± 0.3b      |

Mean values± confidence intervals at 0.01 significance level are shown

*Mean values designated with the same letters have no significant differences within each group of four genotypes (RRVV, RRvv, rVV, rvv and RRPP, RRpp, rrPP, rpp) as calculated using two-way ANOVA

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![Fig. 1](image_url) Mean length of spike and internodes in the families F₂:₄ derived from the cross Chris Mutant/Novosibirskaya 67: R – Rht-B1a, r – Rht-B1p; V – Vm-B1a, v – vm-B1; P – Ppd-D1a, p – Ppd-D1b. The 4th upper internode is not depicted. Total plant height differs from the mean plant height in Table 4 due to polymorphism in the number of internodes between plants.
The productivity of the ear depends on the number of grains and the average weight of 1000 grains. In Krasnodar, plants with different allelic states of the studied genes Rht-B1, Ppd-D1, and VRN-B1 did not differ significantly in the grain number per spike. In Moscow, allele Ppd-D1a reduced the grain number per spike by 3–4 grains (9%) (Additional file 1 Table S2). On average, plants carrying Vrn-B1a and vrn-B1 did not significantly differ in the grain number per spikelet, however, the presence of the dominant allele Vrn-B1a mitigated the contrast between short and tall plants, generating a 5% reduction (1–2 kernels) in RRVV vs rrVV (plants with Vrn-B1a) compared with a 17% reduction (6 grains) in RRvv vs rrvv (plants with vrn-B1). The influence of the studied alleles on the 1000 grain weight is similar to the above-described influence on the productivity of the spike as a whole. In Moscow, Rht-B1p significantly and independently of the allelic condition of the VRN-B1 and Ppd-D1 genes, reduces this productivity by 16–18%. But in Krasnodar, the presence of the Ppd-D1a allele tended towards mitigation of the negative effect of Rht-B1p. Therefore, the dwarfing allele Rht-B1p in the absence of the allele Ppd-D1a led to a decrease in 1000 grain weight by 13% (RRPP and rrpp), but in the presence of the allele Ppd-D1a, 8% only (RRPP and rrpp) (Table 3).

Thus, the alleles insensitive to photoperiod Ppd-D1a and Rht-B1p, showed in our experiments the opposite effect on 1000 grain weight and grain weight per spike, which allows us to consider Ppd-D1a as a compensator for the negative effect of Rht-B1p on plant productivity under the conditions of Krasnodar. Moreover, both alleles, Rht-B1p and Ppd-D1a, showed a tendency to increase the harvest index compared to wild-type alleles Rht-B1a and Ppd-D1b (Additional file 1 Table S3).

### Table 3

Mean values of biometric traits of spike productivity traits in families F3:h Chris Mutant/Novosibirskaya 67 grouped by Rht-B1 × VRN-B1 and Rht-B1 × Ppd-D1 alleles

| Genotype  | Grain weight in main spike, g | Grain number per main spike, pcs. | Thousand grain weight, g |
|-----------|-------------------------------|----------------------------------|--------------------------|
|           | Moscow                        | Krasnodar                        | Moscow                   | Krasnodar                   | Moscow                        | Krasnodar                   |
| RRVV      | 1.17 ± 0.1a                   | 1.43 ± 0.1a                      | 35.2 ± 1.3ab             | 48.5 ± 1.5a                 | 32.9 ± 0.7a                   | 29.5 ± 0.8a                 |
| RRvv      | 1.25 ± 0.1a                   | 1.51 ± 0.1a                      | 37.7 ± 3a                 | 48.6 ± 3.9a                 | 32.8 ± 1.9a                   | 31.4 ± 1.8a                 |
| rrVV      | 0.95 ± 0.1b                   | 1.35 ± 0.1b                      | 33.6 ± 1.5b               | 50.2 ± 1.4a                 | 27.6 ± 0.8b                   | 27 ± 0.7b                   |
| rrVV      | 0.9 ± 0.2b                    | 1.07 ± 0.5b                      | 31.4 ± 4.8b               | 42.6 ± 14a                  | 28.3 ± 1.7b                   | 24.8 ± 5.2b                 |
| RRpp      | 1.17 ± 0.1a                   | 1.43 ± 0.1a                      | 35.2 ± 1.3bc              | 48.5 ± 1.5a                 | 32.9 ± 0.7a                   | 29.5 ± 0.8a                 |
| RRPP      | 1.24 ± 0.1a                   | 1.41 ± 0.1ab                     | 38.1 ± 1.7a               | 49.5 ± 1.7a                 | 32.1 ± 1.1a                   | 28.5 ± 0.8a                 |
| RRPP      | 0.95 ± 0.1b                   | 1.35 ± 0.1b                      | 33.6 ± 1.5b               | 50.2 ± 1.4a                 | 27.6 ± 0.8b                   | 27 ± 0.7b                   |
| rpp       | 1.01 ± 0.1b                   | 1.2 ± 0.2c                       | 37.5 ± 3.2ab              | 47.6 ± 3.7a                 | 26.2 ± 1.3b                   | 24.8 ± 2c                   |

Mean values designated with the same letters have no significant differences within each group of four genotypes (RRVV, RRvv, rrVV, rrvv and RRPP, RRp p, rrPP, rrpp) as calculated using two-way ANOVA.

Mean values ± confidence intervals at 0.01 significance level are shown.

*Mean values designated with the same letters have no significant differences within each group of four genotypes (RRVV, RRvv, rrVV, rrvv and RRPP, RRp p, rrPP, rrpp) as calculated using two-way ANOVA.

**Heading, the sum of effective temperatures and daylight hours**

In Moscow, heading time in semi-dwarf plant families (rr) came slightly later than in tall families (RR) among plants with Vrn-B1a. Also, semi-dwarf plants (rr) in families with the Vrn-B1a allele required a higher sum of active temperatures (>10 °C) and active light days (>12 h) for heading of 5 and 3%, respectively, compared to the tall plants (RR). Under the climatic conditions of Krasnodar, no differences were found between families homozygous for the alleles of the Rht-B1 and VRN-B1 genes (Table 4).

In Moscow, the two-way analysis of the interaction of Rht-B1 and Ppd-D1 showed that heading in semi-dwarf plants (rr) came a little later compared to tall plants (RR) in families with photoperiod sensitivity allele Ppd-D1b and photoperiod insensitivity allele Ppd-D1a. The sum of active temperatures and daylight hours are higher for semi-dwarf plants (rr) than tall plants (RR) by an average of 4 and 3%. Tall plants carrying the allele of insensitivity Ppd-D1a (RRPP) headed a bit earlier than those with the photoperiod sensitive allele (RRpp), and their demand for the sum of active temperatures and daylight days decreased by 3 and 2%, respectively (Table 4).

Under the conditions of Krasnodar, we identified a significant difference in heading time between plants homozygous for different alleles of Ppd-D1. Families with the Ppd-D1a insensitivity allele were earlier than those with the Ppd-D1b sensitivity allele, the difference was 7 days among tall plants (RRPP and RRpp), and 5 days among semi-dwarf plants (rrPP and rrpp). Additionally, in the presence of the photoperiod-insensitive allele, Ppd-D1a, the demand for active temperatures and daylight days decreased by an average of 13 and 10%, respectively (Table 4, Additional file 1 Table S4).
Gene interaction analysis

To identify the most significant relationships between the allelic condition of the studied genes, we conducted a pairwise regression analysis for each pair of genes: Rht-B1 and VRN-B1, Rht-B1 and Ppd-D1, for each region of the field experiment. Correlation coefficients for Rht-B1 in both pairs of comparison coincided, or were very close, within each region of the field experiment, so we demonstrate here the average value of correlation coefficients (Table 5).

In Moscow, a strong negative correlation was shown between the presence of Rht-B1p in the genotype and plant height (explaining 50% of the variability). There was a moderate negative correlation between the presence of Rht-B1p and main culm weight, length of first internode, 1000 grain weight (20% of the variability), vegetative part of the main shoot, and grain weight of the main spike (8% of the variability). A moderate positive correlation was found between the presence of Rht-B1p and the requirements for the sum of active

### Table 4
Mean values of heading date, sum of active (> 10 °C) temperatures and active (> 12 h) light days from sowing to heading date in families F₃:₄ Chris Mutant/Novosibirskaya 67 grouped by Rht-B1 × VRN-B1 and Rht-B1 × Ppd-D1 alleles

| Genotype | Heading date, days from sowing to heading | Sum of active (> 10 °C) temperatures to heading date, °C | Sum of active (> 12 h) light days to heading date, h |
|----------|-------------------------------------------|--------------------------------------------------------|-----------------------------------------------------|
|          | Moscow | Krasnodar | Moscow | Krasnodar | Moscow | Krasnodar |
| RRRV     | 52.7 ± 0.7a | 65 ± 2.3a | 810 ± 15b | 861 ± 48a | 875 ± 12b | 883 ± 35a |
| RRvv     | 53.5 ± 1.4ab | 62.3 ± 6a | 828 ± 30ab | 854 ± 132a | 890 ± 24ab | 841 ± 90a |
| rrVV     | 54.4 ± 1a | 64.6 ± 1.3a | 847 ± 21a | 854 ± 28a | 906 ± 18a | 877 ± 20a |
| rrvv     | 54.2 ± 2ab | 66 ± 4.1a | 842 ± 44ab | 884 ± 104a | 901 ± 35ab | 898 ± 80a |
| RRPP     | 52.7 ± 0.7c | 65 ± 2.3b | 810 ± 15c | 861 ± 48b | 875 ± 12c | 883 ± 35b |
| RRpp     | 53.7 ± 0.8bc | 71.9 ± 3.7a | 831 ± 17bc | 1003 ± 76a | 893 ± 14bc | 988 ± 56a |
| rrPP     | 54.4 ± 1ab | 64.6 ± 1.3b | 847 ± 21ab | 854 ± 28b | 906 ± 18ab | 877 ± 20b |
| rripp    | 55.1 ± 1.2a | 70 ± 5.2a | 861 ± 25a | 967 ± 104a | 917 ± 20a | 959 ± 80a |

Mean values ± confidence intervals at 0.01 significance level are shown

*Mean values designated with the same letters have no significant differences within each group of four genotypes (RRVV, RRvv, rrVV, rrvv and RRPP, RRp p, rrPP, rripp) as calculated using two-way ANOVA

### Table 5
Correlation coefficients between the presence of the Rht-B1p, Vrn-B1a, and Ppd-D1a alleles in homozygotes and valuable agronomic traits in families F₃:₄ Chris Mutant/Novosibirskaya 67

| Agronomic trait | Rht-B1p | Vrn-B1a | Ppd-D1a |
|-----------------|---------|---------|---------|
|                 | Moscow | Krasnodar | Moscow | Krasnodar | Moscow | Krasnodar |
| Plant height (PH) | −0.71a | −0.90a | −0.03 | 0.02 | −0.06a | 0.04a |
| Internode number (IN) | −0.13a | −0.27a | −0.05 | 0.02 | 0.03 | −0.15a |
| Peduncle length (PL) | −0.59a | −0.86a | −0.01 | −0.01 | −0.06a | 0.09a |
| Main spike length (MSL) | −0.15a | −0.11a | −0.01 | 0.14a | −0.02 | −0.01 |
| Spikelet number per main spike (SN) | 0.003 | −0.05 | −0.04 | −0.02 | −0.15a | −0.26a |
| Spike compactness (SC) | 0.16a | 0.06a | −0.03 | −0.15a | −0.13a | −0.23a |
| Main spike weight (MSW) | −0.29a | −0.11a | −0.04 | −0.01 | −0.09a | 0.04 |
| Main culm weight (MCW) | −0.54a | −0.19a | −0.08a | 0.004 | −0.10a | −0.04 |
| Main shoot biomass (MSB) | −0.40a | −0.21a | −0.05 | 0.00 | −0.10a | −0.02 |
| Grain weight per main spike (GW) | −0.29a | −0.15a | −0.02 | −0.01 | −0.08a | 0.08a |
| Grain number per main spike (GN) | −0.09a | 0.06 | −0.02 | 0.04 | −0.16a | 0.01 |
| Grain number per spikelet (GNS) | −0.11a | 0.09 | 0.01 | 0.05 | −0.09a | 0.16a |
| Thousand grain weight, g (W) | −0.45a | −0.29a | −0.01 | −0.07 | 0.08a | 0.14a |
| Harvest index (HI) | 0.12a | 0.07 | 0.05 | 0.02 | 0.02 | 0.10a |
| Heading date (H) | 0.47a | −0.06a | −0.06 | 0.19a | −0.35a | −0.63a |
| Sum of active (> 10 °C) temperatures to heading date, °C (SAT) | 0.47a | −0.05a | −0.06 | 0.19a | −0.36a | −0.63a |
| Sum of active (> 12 h) light days to heading date, h (SAD) | 0.47a | −0.06a | −0.06 | 0.19a | −0.35a | −0.64a |

*a correlations significant at the 0.05 significance level
temperatures and active daylight hours, as well as heading date (20% of the variability). For Ppd-D1a, a moderate negative correlation with the requirements for the sum of active temperatures, active daylight hours, and heading date (12% of the variability) were revealed (Table 5, Fig. 2).

In Krasnodar, a strong negative correlation was shown between the presence of the Rht-B1p in the plant genotype and plant height (explaining 81% of the variability) and the peduncle length; a moderate negative correlation was recorded between the presence of Rht-B1p and 1000 grain weight (9% of the variability), and the number of internodes. Moderate negative correlations between the presence of Ppd-D1a and the requirements for the sum of active temperatures, active daylight hours, and heading date (40% of the variability) and the number of spikelets (40% of the variability) were also revealed (Table 5, Fig. 2).

Thus, both in Moscow and Krasnodar, aside from the reduction in plant height, Rht-B1p showed a negative impact on the 1000 grain weight. The association between the presence of Rht-B1p and the grain weight per main spike was moderate in Moscow and weak in Krasnodar. The association between the presence of Rht-B1p and the grain number per main spike was weak in both regions of the field experiment. Thus, in Krasnodar the selection for plants of spring wheat that carry Rht-B1p, with a slight decrease of 1000 grain weight and grain number per main spike is more likely, as the correlation between the presence of Rht-B1p and these characteristics is weak (Table 5, Fig. 2).

The Principal Component Analysis (PCA) performed for Rht-B1p and Vrn-B1a demonstrated the following tendencies (Fig. 3a and c). The Rht-B1p and plant height (PH) vectors are directed opposite to each other; “RR” families (empty and filled circles) are located near the PH vector (plant height), which generally describes them as tall plants. Conversely, “rr” families (half-circles and a dot) are in the opposite direction, close to the Rht-B1p vector. As can be seen in the biplot in Fig. 3a (Moscow), the circles that are most productive (i.e. close to vectors GW, GN, W) are dark circles (RRvv) and with a dot (rrvv), that is, plants with the recessive vrn-B1 allele. At the same time, in the biplot for Krasnodar, no tendencies can be observed for the distribution of the circles in relation to the vectors of the productivity elements.

**Fig. 2** Correlation coefficients between the presence of the Rht-B1p, Vrn-B1a, and Ppd-D1a alleles in homozygotes and valuable agronomic traits in families F2s Chris Mutant/Novosibirskaya 67 in field tests in Moscow (violet line) and Krasnodar (green line). Blue and red represent negative and positive correlations respectively. PH, plant height; IN, internode number; PL, peduncle length; MSL, main spike length; SN, spikelet number; SC, spike compactness; MSW, main spike weight; MCW, main culm weight; MSB, main shoot biomass; GW, grain weight per main spike; GN, grain number per main spike; GNS, grain number per spikelet; W, thousand grain weight; HI, harvest index; H, heading date; SAT, sum of active (> 10 °C) temperatures to heading date; SAD, sum of active (> 12h) light days to heading date
In addition, no patterns for scattering with respect to the heading date vector (H) were detected for the groups of genotypes. Thus, selection for productive semi-dwarf plants in Moscow is more achievable among plants with \textit{Vrn-A1/vrn-B1} alleles, and in Krasnodar among those with both variants of the \textit{Vrn-A1/vrn-B1} and \textit{Vrn-A1/Vrn-B1a} alleles.

The PCA analysis of the interaction between \textit{Rht-B1p} and the photoperiod-insensitive allele \textit{Ppd-D1a} demonstrated the following trends (Fig. 3b and d): \textit{Rht-B1p} and plant height (PH) vectors are directed opposite to each other; “RR” families (empty and filled circles) are located near the PH vector (plant height), which generally describes them as tall plants. Conversely, “rr” families (half circles and with a dot) are located in the opposite direction, close to the \textit{Rht-B1p} vector. As can be seen in the biplot in Fig. 3b, the most productive (i.e. close to vectors GW, GN, W) are dark circles (RRpp) and with rrpp; that is, plants with the sensitivity allele \textit{Ppd-D1b}. However, in the biplot in Fig. 3d (Krasnodar), the most productive families are represented by empty (RRPP) and a half (rRPP) circles; that is, plants with insensitivity \textit{Ppd-D1a}. Thus, it is more likely to select for more productive semi-dwarf plants among the families with the photoperiod-sensitive allele \textit{Ppd-D1b} in Moscow, and with the photoperiod-insensitive allele \textit{Ppd-D1a} in Krasnodar.

**Discussion**

The application of new dwarfing alleles in wheat breeding in combination with the alleles of vernalization and photoperiod sensitivity allows for an expansion in the adaptability of this crop to various cultivation conditions. The yield of wheat is to higher extent is determined by the combination of the allelic variants of \textit{Rht}, \textit{Vrn} and \textit{Ppd} referred to as “core genes” or “adaptation genes” by some authors [52, 53]. In our research, we evaluated the phenotype of the new dwarfing allele \textit{Rht-}}
B1p in spring bread wheat in combination with the alleles of vernalization gene VRN-B1 and the photoperiod-sensitive gene Ppd-D1 in two regions, Moscow and Krasnodar, which differ in the length of daylight and soil and climatic conditions. In our study, the variation in valuable agronomic traits in semi-dwarf plants bearing the Rht-B1p allele associated with the phenotypic manifestation of the Ppd-D1a and Ppd-D1b alleles was more diverse than that associated with the Vrn-B1a and vrn-B1 alleles. The stronger phenotypic effects of Ppd-D1 in Krasnodar can be explained by the short daylight conditions in which the effect of the photoperiod-insensitive gene becomes evident. Under the conditions of a long day in Moscow, the influence of the allelic condition of this gene is not noticeable.

Among the GA-insensitive dwarfing alleles of Rht-B1, the most common allele is Rht-B1b. The effect of Rht-B1b on plant height is well studied and varies in the range of 10–25% [14, 15, 27, 54, 55]. In our studies, the difference in height between plants with the allele Rht-B1p and Rht-B1a ranged between 27 and 34%. This difference in height between Rht-B1p and Rht-B1a homozygous plants was similar to that observed in segregating population in the same cross in a F2 vegetative experiment [7], as well as when comparing mutant forms of the Mutant Chris, donor of Rht-B1p (previously designated as Rht17), with the original wheat variety Chris [19]. Noteworthy, the phenotypic effect of Rht-B1p on plant height is comparable to Rht-B1e. Analysis of the nucleotide sequences of the alleles showed that stop codons caused by point mutations are found nearby in Rht-B1e and Rht-B1p [7], which may be the probable reason for the close phenotypic manifestation of these alleles. The Rht-B1e allele was detected in 16.5% of accessions from the collection from southern Russia [8]. Rht-B1p can be used in breeding in conjunction with Rht-B1e, after replacing the negative genetic background of the Chris Mutant.

In our experiments, the Ppd-D1a allele in Moscow did not have a statistically significant effect on plant height, although we did note a tendency toward reduced plant height in the experiment in Moscow by 2–5%. In a field experiment in Krasnodar, semi-dwarf plants carrying Rht-B1p with the allele Ppd-D1a were significantly taller than those with allele Ppd-D1b by 12%; that is, the photoperiod-insensitive allele had an effect opposite to the height-reducing effect of Rht-B1p. Li et al. (2013) showed that the Rht-B1h allele reduced plant height only in plants with the Ppd-D1a allele [2]. According to the published data, Ppd-D1a reduces height in the range of 4–15% [9, 17, 49–51, 56]. There is an assumption that the effect of Ppd-D1a is due to its linkage to the Rht-8c dwarfing allele, since this allele also leads to photoperiod insensitivity and to accelerated flowering and heading [50]. However, we have shown that both parent forms carry the wild-type allele Rht-8a, which when present in the genome leads to a tall plant phenotype. Therefore, in our opinion, the difference in height under the influence of this allele Ppd-D1a, is first of all due to the change in the duration of the vegetative phase, which in turn leads to a change in the habit of the plant.

In our studies in both regions of the field experiment, weak negative correlation between spikelet number per spike and the presence of photoperiod-insensitive allele Ppd-D1a in plants and was observed. This tendency agrees with many previous studies [43, 50, 51].

In our studies in Moscow, Rht-B1p on average negatively affected grain number per main spike due to a decrease in grain number per spikelet. Moreover, this trait was also influenced by the interaction with the VRN-B1 gene. In Krasnodar, there was a tendency to an increase in grain number per spike due to an increase in grain number per spikelet and spikelet number per main spike in plants with Rht-B1p, but only in the genetic background of Ppd-D1a and Vrn-B1a. This suggests a multidirectional interaction between the alleles of Ppd-D1, Rht-B1 and VRN-B1 genes, depending on the region of the experiment.

In our field experiment in Moscow, a negative influence of the photoperiod-insensitive allele Ppd-D1a on the grain number per spike was shown (a decrease of 8–11%, a weak but significant negative correlation). While in Krasnodar, in contrast, grain number per spike was greater in semi-dwarf plants with Ppd-D1a. Many studies show that Ppd-D1 affects 1000-grain weight, vegetative weight, harvest index and grain yield [57, 58]. Alleles of photoperiod sensitivity have both negatively and positively influenced grain number per spike, as well as the percentage of fertile flowers in different experiments [50, 51, 59].

Under the influence of Rht-B1p, 1000 grain weight decreased, which is especially noticeable in the PCA biplots where the Rht-B1p and 1000 grain weight vectors have opposite directions (Fig. 3). In our experiments, 1000 grain weight decreased under the influence of Rht-B1p by 14–18% in Moscow and by 9–21% in Krasnodar when different groups of genotypes were compared. A number of studies have shown that Rht-B1b reduces 1000 grain weight (or the weight of individual grains), although in a number of experiments the effect was not statistically significant [14, 15, 27, 55, 60]. With a decrease in plant height, the availability of assimilates to the upper flowers in the spikelet increases, whereas in tall plants, as a rule, they do not produce grain. As a result, the number of grains in the ear increases. There is also another side: this leads to a decrease in 1000 grain weight, the grain becomes smaller due to increasing competition for assimilates. In addition, this may be
because the straw itself as a source of the organic matter becomes shorter, generating a deficit for grain filling.

We found that Vrn-B1a tends to mitigate the negative effect of Rht-B1p on grain number per spike in both regions and 1000 grain weight in Krasnodar. We also demonstrated in the experiments in both regions that Ppd-D1a can partially compensate for the negative effect of Rht-B1p on 1000 grain weight, for 4–9% in Krasnodar and for 2–5% in Moscow, which is in agreement with literature [50, 51, 61].

In Moscow and Krasnodar, grain weight per main spike from plants homozygous for Rht-B1p was lower compared to that of plants homozygous for Rht-B1a. The influence of dwarfing on the productivity of plants or spike depends on a large number of multidirectional factors. In the studies by Chen et al. [50, 51], the maximum grain yield per plant was observed in genotypes of tall plants, although semi-dwarf plants with a combination of a GA-sensitive gene Rht-5 and photoperiod-insensitive alleles had the same yield as that of tall plants [50]. It should be considered that despite the higher potential yield from tall plants, this is not always achievable, especially with intensive cultivation technology where the risk of lodging is high.

Flowering time is the key factor in the adaptation of plants to various environmental conditions and is critical for avoiding the risks of drought, frost or heat during the reproductive period of development. GA-insensitive short-stem alleles generally do not affect flowering time themselves [12, 15]. Nevertheless, some authors observe some effect of Rht-B1 region on earliness that might be associated with putative genes located in the vicinity of Rht-B1 [52, 62]. Later flowering / heading is observed in plants with GA-sensitive dwarfing genes [50, 51, 63]. Ppd-D1a had a much greater effect in Krasnodar, where Ppd-D1a accelerated heading by 5–7 days. It is known from the literature that Ppd-D1a accelerates flowering on short days [9, 50, 64, 65], which was also observed in our experiments.

Dwarfing alleles reduce the stem height and thereby allow the spike to carry a heavier load. This in turn improves resistance to lodging and, as a result, allows for an increase in the density of standing through a higher seeding rate, ultimately generating increased grain yield per unit area. In our experiments in both Moscow and Krasnodar, the decrease in plant height in spring wheat due to Rht-B1p was very strong (about 30%) and the additional effects of the VRN-B1 and Ppd-D1 alleles on plant height (even when statistically significant) do not change the overall effect of the gene. In this regard, the mutual influence of Rht-B1p, VRN-B1 and Ppd-D1 on productivity elements is of particular interest.

The negative effects of dwarfing alleles can be overcome by combining them with alleles of vernalization and day length sensitivity. The length of the straw is a function of the rate of development of the plant, which depends on the response of tissues to hormones and environmental signals (day length, temperature). Different combinations of alleles from genes regulating these reactions can be made to fine-tune developmental phases and achieve maximum plant productivity. The strategy of choosing the right combination of genes should be developed for each region individually.

For Moscow, the most promising option for selection of semi-dwarf productive plants will be a combination of Rht-B1p + Ppd-D1b. For Krasnodar, a promising option for selection of semi-dwarf productive plants will be a combination of Rht-B1p + Ppd-D1a. It follows from the following results: i) with different confidence probability Ppd-D1a has a rather negative effect on productivity in Moscow and a positive impact in Krasnodar (the results of the analysis of variance); ii) a weak significant negative correlation in Moscow and positive correlation in Krasnodar between the elements of productivity and Ppd-D1a (the results of the regression analysis); and iii) most productive families were in the direction opposite to the Ppd-D1a vector in Moscow and in the direction of Ppd-D1a in Krasnodar (the results of the PCA analysis).

Differences in the influence of Rht-B1p on the grain number per spike in Moscow and Krasnodar can be explained as follows: Moscow is characterized by podzol, humus-poor soils, whereas the soils of Krasnodar are typical chernozems. Probably, the Rht-B1p allele studied by us has a positive effect on the number of grains in plants only at high soil fertility. It should be remembered that during the “Green revolution” dwarfing alleles Rht-B1b and Rht-D1b were introduced in order to increase yield and lodging resistance at high doses of fertilizers, irrigation, and intensive cultivation technology. It is also noteworthy that the positive effects of Rht-B1p were manifested in the experiment in Krasnodar only in plants with Ppd-D1a.

In its effect on plant height and productivity elements, Rht-B1p is close to Rht-B1e, which is distributed among 16.5% of varieties in the southern regions of Russia, as well as among some varieties in Ukraine [8, 10, 18]. Thus, Rht-B1p can also be used on an equal footing with other dwarfing alleles in the breeding of wheat in the Krasnodar Krai.

Thus, Rht-B1p significantly reduces the height of plants, but it can also reduce their productivity. Alleles of genes that regulate developmental type and sensitivity to day length can mitigate unwanted manifestations of Rht-B1p in favorable regions. At the same time, in Krasnodar, the interaction of Rht-B1p and Ppd-D1a genes influencing valuable agronomic traits is most pronounced at short day length.
Conclusions
In summary, we estimated the effects of Rht-B1p on plant height and traits of agronomic value in spring bread wheat in Moscow and Krasnodar. Plant height was reduced on average by 21 cm (28%) and 25 cm (30%), respectively, Ppd-D1 tended to strengthen the dwarfing effect in Moscow and mitigate it in Krasnodar. Grain weight per main spike was reduced by Rht-B1p in Moscow and to lesser extent in Krasnodar. Thousand grain weight was reduced on average by 5.3 g (16%) and 2.9 g (10%) in Moscow and Krasnodar, respectively. The harvest index was increased due to Rht-B1p on average by 6 and 10% in Moscow and Krasnodar, respectively. In Krasnodar, Ppd-D1a triggered heading 6 days earlier and partially compensated for the loss of grain weight per main spike and thousand grain weight due to Rht-B1p. Among semi-dwarf plants with Rht-B1p, the most productive were observed among families with Ppd-D1b in Moscow and Ppd-D1a in Krasnodar. In its effect on plant height and productivity elements, Rht-B1p is close to Rht-B1e, which is distributed among varieties in the southern regions of Russia and Ukraine. Thus, Rht-B1p can be used on an equal footing with other dwarfing alleles in wheat breeding in the Krasnodar Krai.

Methods
Plant material
For the initial plant material, we used seeds of the F2 population of Chris Mutant/Novosibirskaya 67, kindly provided by Prof. Nobuyoshi Watanabe (College of Agriculture, Ibaraki University) [7]. The seeds of parental plants Chris Mutant (accession number Cltr 17,241) and Novosibirskaya 67 (accession number 48601) are available at Germplasm Research International Network and N.I. Vavilov Research Institute of Plant Industry (VIR) (Saint Petersburg, Russia), respectively. The parental plants were analyzed for the following alleles of Rht, Vrn, and Ppd in their genotypes: Rht-B1a (wild type), Rht-B1b, Rht-B1e, and Rht-B1p for Rht-B1; Rht-D1a (wild type) and Rht-D1b for Rht-D1; Rht-8a (wild type)/Rht-8c for Rht-8; vrn-A1, Vrn-A1a, and Vrn-A1b for VRNA-I1; vrn-B1, Vrn-B1a, and Vrn-B1c for VRN-B1; vrn-D1, and Vrn-D1a for VRN-D1; Ppd-D1a and Ppd-D1b for Ppd-D1 (see Molecular analysis and Table 6). The following allelic states were revealed: Chris Mutant: Rht-B1p Rht-B1p\cancel{cRht-D1a} Rht-D1a\cancel{cRht-8a} Rht-8a\cancel{cPpd-D1a} Ppd-D1a\cancel{cVrn-A1a} Vrn-A1a\cancel{cvrn-B1} vrn-B1 \cancel{cvrn-D1} vrn-D1; Novosibirskaya 67: Rht-B1a Rht-B1a\cancel{cRht-D1a} Rht-D1a\cancel{cRht-8a} Rht-8a\cancel{cPpd-D1b} Ppd-D1b\cancel{cVrn-A1a} Vrn-A1a\cancel{cvrn-B1a} Vrn-B1a\cancel{cvrn-D1} vrn-D1. Therefore, the F2 population Chris Mutant/Novosibirskaya 67 segregated in the following three loci: Rht-B1 (Rht-B1a/Rht-B1p), Ppd-D1 (Ppd-D1a/Ppd-D1b), and Vrn-B1 (Vrn-B1a/vrn-B1).

The plants of the F2 population of Chris Mutant/Novosibirskaya 67 were planted in pots at 10 seeds per pot and grown in a greenhouse under identical lighting with dosed watering and fertilization. The allelic state of Rht-B1 was identified for each individual F2 plant (see Molecular analysis and Table 6). Plants homozygous for Rht-B1p and Rht-B1a were selected and threshed manually when they achieved complete ripeness. Seeds of F3, harvested from individual F2 plants, were labelled as a single family. Each family was divided into two equal parts and sown in two climatic regions in field plot tests, in Moscow and Krasnodar. During growth, for each individual F3 plant the allelic state of Rht-B1, VRN-B1 и Ppd-D1 was identified using molecular markers (see Molecular analysis and Table 6).

Molecular analysis
Genomic DNA was extracted from leaves using a CTAB method [73]. PCR was performed in a 25 μL reaction volume, containing 70 mM Tris–HCl buffer (pH 8.6), 16.6 mM (NH4)2SO4, 2.5 mM MgCl2, 0.2 mM of each dNTP, 10%v/v dimethyl sulfoxide, 0.3 μM forward and reverse primers (Sintol Ltd., Moscow, Russia), 1.25 U of Coloured Taq-polymerase (Sileks Ltd., Moscow, Russia) and 100 ng of template DNA. The PCR conditions were as recommended by the authors of the molecular markers (Table 6). The PCR reaction was performed in a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, California, USA). The PCR products were separated in a 1.5% agarose gel in TBE buffer using GeneRuler 100 bp DNA Ladder (Thermo Fisher Scientific, Waltham, Massachusetts, USA) as a molecular weight marker, and stained with ethidium bromide for subsequent visualization in Gel Doc XR+ (Bio-Rad Laboratories, Inc., Hercules, California, USA). The size of the PCR products amplified from molecular marker Xgwm261, linked to Rht-8, were measured using fragment analysis in a Genetic Analyzer ABI-3130XL (Applied Biosystems, Foster City, California, USA).

Field experiment
The field experiment was performed in the Field Experimental Station, Russian State Agrarian University—Moscow Timiryazev Agricultural Academy, Moscow (55°50’ N, 37°33’ E, hereinafter – Moscow) and in a plot of land at the National center of grain named after P.P. Lukyanenko in Krasnodar (45°41’ N, 38°55’ E, hereafter referred to as Krasnodar) in 2018. The duration of daylight in Moscow was 15:35 at sowing day (May 5), increasing to 17:33 (June 24) and decreasing to 14:49 at the final date of harvesting (August 8); the average daylight duration was 16:40 (long photoperiod). The duration of daylight in Krasnodar was 12:10 at sowing day (March 21) which increased to 15:34 at the final date of
| Gene | Primer sequence | Allele | Expected size of the product, bp | Reference |
|------|----------------|--------|----------------------------------|-----------|
| Rht-B1 | Rht-B1p-WF: 5′ ACATGGCGGACGTGGTGC 3′ Rht-B1-R1: 5′ GCCGAGAGAGGACGAT 3′ | Rht-B1a | 425 | [7, 66] |
| | Rht-B1p-F: 5′ ACATGGCGGACGTGGTGTG 3′ Rht-B1-R1: 5′ GCCGAGAGAGGACGAT 3′ | Rht-B1p (Rht17) | 425 | |
| | BF: 5′ GGTAGGAGGGCGAGGGCGGAG 3′ WR1: CATCCCCATGGCCATCTCGAGCTG | Rht-B1a | 237 | [67] |
| | BF: 5′ GGTAGGAGGGCGAGGGCGGAG 3′ MR1: 5′ CATCCCCATGGCCATCTCGAGCTA 3′ | Rht-B1b (Rht1) | 228 | [66] |
| | BF: 5′ GGTAGGAGGGCGAGGGCGGAG 3′ W3: 5′ GGCATCTCCAGCTGCAGCTA 3′ | Rht-B1e (Rht11) | 228 | |
| | BF: 5′ GGTAGGAGGGCGAGGGCGGAG 3′ M3: 5′ GGCATCTCCAGCTGCAGCTA 3′ | Rht-B1a | 237 | |
| | DF2: 5′ GCAGCGAAAAAGCCTCGG – 3′ WR2: 5′ GCCATCTCCAGCTGCAGCTA 3′ | Rht-D1a | 264 | [67] |
| | DF: 5′ GCAGCGAAAAAGCCTCGG – 3′ MR2: 5′ CCCATGGCCATCTCGAGCAGCTA – 3′ | Rht-D1b (Rht2) | 254 | |
| | Ppd-D1_F: ACGCTCCCACATCCTG | Ppd-D1a | 288 | [68] |
| | Ppd-D1_R2: CACTCGTGGATGCTGAGATT | Ppd-D1b (Rht2) | 414 | |
| | Ppd-D1_R1: GTGGGTCAACAGAGCAC | | | |
| VRN-A1 | VRN1AF: GAAAGGAAAAATTCTGCTCG VRN1-IN1R: GCAGGAATCGGAAATCGGA | Vrn-A1a | 965 + 876 | [69, 70] |
| | | Vrn-A1b | 714 | |
| | VRN1AF: GAAAGGAAAAATTCTGCTCG | Vrn-A1a | 734 | [69, 70] |
| VRN-B1 | Intr1/B/F: CAAGTGGAAACCGTTAGGACA Intr1/B/R3: CTCATGCAAAAAATTGAGATGA | Vrn-B1a | 709 | [70] |
| | Intr1: ATCATCTTTCACCAAGGG | Vrn-B1a | 1124 | [71] |
| | Intr1/B/R3: CTCATGCAAAAAATTGAGATGA | Vrn-B1c | 737 | |
| | Intr1/B/F: CAAGTGGAAACCGTTAGGACA | Vrn-B1a | 1149 | [70] |
| VRN-D1 | Intr1/D/F: CAAAATGAAAAAATGAGGAGCA | Vrn-D1 | 1671 | [70] |
harvesting (June 30); the average duration of a day was 14:23 (short photoperiod). Sowing was performed using a breeding cassette drill SKS-6-10 with the following parameters: length of plot 1 m; width of plot, 90 cm; width between the rows, 30 cm (Moscow) or 40 cm (Krasnodar); and distance between the plots, 50 cm. Individual plants were labelled and one leaf from each plant was used for DNA extraction to determine the allelic state of VRN-B1 and Ppd-D1, as well as for verification of the allelic state of Rht-B1 (see Molecular analysis and Table 6).

The plots were treated with pesticides to control pests, the weeds were removed manually. Plants of each family were harvested manually at complete ripeness (August 8 in Moscow and June 30 in Krasnodar). The spikes were threshed using a spike thresher MKS-1 M (MZOK Company, Moscow, Russia). The weather conditions in 2018 in Moscow and Krasnodar are displayed in Table 7.

Phenotyping
In total, 82 families (1035 plants) and 73 families (731 plants) were analyzed in Moscow and Krasnodar, respectively. The following traits were measured in each individual plant: plant height (PH, cm), length of each internode (cm), internode number (IN), main spike length (MSL, cm), spikelet number per main spike (SN), main spike weight (MSW, g), main culm weight (MCW, g), grain weight per main spike (GW, g), and grain number per main spike (GN). The following parameters were calculated for each individual plant: spike compactness (SC, number of spikelets per 10 cm of main spike length), main shoot biomass (MSB, sum of MSW and MCW, g), grain number per spikelet (GNS, GN divided by SN), thousand grain weight (W, thousandfold GWS divided by GNS, g), and harvest index (HI, GW divided by MSB). The heading date was recorded for each family when not less than 80% of the plants came to heading, and based on this date the sum of active (> 10 °C) temperatures to heading date (SAT, °C) and sum of active (> 12 h) light days to heading date (SAD, h) were calculated. The seeds were counted with the use of a Seed Counter [74].

Statistical analysis
For each phenotypic trait the mean value and confidence interval at a level of significance of 0.01 were calculated. The statistical evaluation of the data was carried out by three one-way ANOVA analyses for each locus (Rht-B1, VRN-B1, and Ppd-D1) and by two two-way ANOVA analyses for the pairwise interaction between loci (Rht-B1 and VRN-B1, Rht-B1 and Ppd-D1). The comparisons

| Month | Moscow May 5 – August 19 | Krasnodar March 21– June 30 |
|-------|-------------------------|-----------------------------|
|       | Sum of active (> 10 °C) temperatures, °C | Mean monthly temperature, °C | Sum of precipitation, mm | Sum of active (> 10 °C) temperatures, °C | Mean monthly temperature, °C | Sum of precipitation, mm |
| March | – – – | 142 | 6.4 | 38 |
| April | – – – | 2845 | 13.8 | 26 |
| May | 3288 | 16.1 | 105 | 4731 | 19.4 | 43 |
| June | 3914 | 17.3 | 107 | 5407 | 24.1 | 11 |
| July | 5097 | 20.5 | 190 | – | – |
| August | 2926 | 20.3 | 39 | – | – |
| Total | 15,225 | – | 441 | 13,125 | – | 118 |
between means were detected using a least significant differences (LSD) test at the level of significance of 0.01 and 0.05 (for heading time). Pairwise comparisons of the interaction between Rht-B1 and VRN-B1 were performed among families homozygous for the photoperiod-insensitive allele Ppd-D1a. Pairwise comparisons of the interaction between Rht-B1 and Ppd-D1 were performed among families homozygous for the Vrn-B1a allele. ANOVA, Correlation and Principal Components Analysis were performed using Statistica 12.0 software (StatSoft, Inc., Tulsa, Oklahoma, USA).

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s12870-020-02514-0.

Additional file 1: Table S1. Mean values of biometric traits for the main shoot in families F44 Chris Mutant/Novosibirskaya 67 grouped by Rht-B1, VRN-B1, and Ppd-D1 alleles. Table S2. Mean values of biometric traits of spike productivity traits in families F44 Chris Mutant/Novosibirskaya 67 grouped by Rht-B1, VRN-B1, and Ppd-D1 alleles. Table S3. Mean values of harvest index and main spike traits in families F34 Chris Mutant/Novosibirskaya 67 grouped by Rht-B1, VRN-B1, and Ppd-D1 alleles. Table S4. Mean values of heading date, sum of active (>10 °C) temperatures and active (>12 h) light days from sowing to heading date in families F34 Chris Mutant/Novosibirskaya 67 grouped by Rht-B1, VRN-B1, and Ppd-D1 alleles. Table S5. Mean values of spike parameters in families F44 Chris Mutant/Novosibirskaya 67 grouped by Rht-B1, VRN-B1, and Ppd-D1 alleles (upper part, one-way ANOVA) and by Rht-B1 × VRN-B1 and Rht-B1 × Ppd-D1 alleles (lower part, two-way ANOVA).

Abbreviations
GA: Gibberellin acid; R: Rht-B1a; r: Rht-B1p; V: Vrn-B1a; v: Vrn-B1; P: Ppd-D1a; p: Ppd-D1b; PH: Plant height; IN: Internode number; MSL: Main spike length; SN: Spikelet number per main spike; MSW: Main spike weight; MOW: Main culm weight; GW: Grain weight per main spike; GN: Grain number per main spike; SC: Spike compactness; MSB: Main shoot biomass; GNS: Grain number; GW: Grain weight per main spike; GN: Grain number per main spike; SAD: Sum of active (>10 °C) temperatures to heading date; SAD: Sum of active (>12 h) light days to heading date.

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Authors’ contributions
MGD made the conception of the work and designed the experiments. AGC, VWP, LAN, VYK, and EAS performed the experiments. NW helped with the plant material acquisition. PYK, MSB, AGC, and MGD conducted data analysis and contributed towards writing the manuscript. LAB and EAS helped in interpreting results. MGD and GIK supervised the findings of the work. PYK, GIK, LAB, EAS, AGC, NW, MSB, VWP, LAN, VYK, MGD have read and approved the manuscript.

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Availability of data and materials
All data generated or analysed during this study are included in this published article and Additional file 1. The seeds of the parental plant Chris Mutant are available at Germplasm Research International Network (https://npgrweb.ars-grin.gov/fringlobal/search.aspx), accession number 172491; the seeds of the parental plant Novosibirskaya 67 are available at N.I. Vavilov Research Institute of Plant Industry (VRI), Saint Petersburg, Russia (http://db.vir.nw.ru/verdb/mainldb, accession number 48601). The studied recombinant isogenic families with different allelic combinations of the allelic state of Rht-B1 (Rht-B1a/Rht-B1p), Ppd-D1 (Ppd-D1a/Ppd-D1b), and VRN-B1 (Vrn-B1a/vrn-B1) loci designated M17/1-M17/81 are being maintained and available upon request from All-Russia Research Institute Of Agricultural Biotechnology (iabi@iabiac.ru, divashuk@gmail.com) and at the National center of grain named after P.P. Lukyanenko (knish@knish.ru).

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1. Laboratory of Applied Genomics and Crop Breeding, All-Russia Research Institute of Agricultural Biotechnology, Timiryazevskaya str. 42, Moscow 127550, Russia. 2. Centre for Molecular Biology, Russian State Agrarian University–Moscow Timiryazev Agricultural Academy, Timiryazevskaya street, 49, Moscow 127550, Russia. 3. Department of Breeding and Seed Production of Wheat and Triticale, National center of grain named after P.P. Lukyanenko, Central Estate of KNILSh, Krasnodar 350012, Russia. 4. Kurchatov Genomics Center, Institute of Cytology and Genetics SB RAS, Prospekt Lavrentyeva 10, Novosibirsk 630000, Russia. 5. College of Agriculture, Ibaraki University, 3-21-1 Chuo, Ami, Inashiki, Ibaraki 300-0393, Japan. 6. Kurchatov Genomics Center-ARRIAB, All-Russia Research Institute of Agricultural Biotechnology, Timiryazevskaya str. 42, Moscow 127550, Russia.

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