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# Effects of *Rht-B1b* and *Ddw1* Dwarfing Genes in Two Connecting Populations of Spring Triticale under Greenhouse Experiment Conditions

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**Abstract:** Dwarfing genes not only reduce the height of triticale plants, but also have pleiotropic effects on important agronomic traits. An important task for breeding is to evaluate the effects of gibberellin responsive (GAR) and gibberellin-insensitive (GAI) dwarfing genes in one genotype. In the greenhouse experiment, we evaluated the effects of the GAI gene *Rht-B1b* of wheat and the GAR gene *Ddw1* of rye on height and the main agronomic traits in two connecting populations derived from crossing *Ddw1* donors (cv. ‘Mudrets’ and cv. ‘Valentin 90’) with a *Rht-B1b* donor (cv. ‘Dublet’). The results show a strong decrease in height under the influence of *Ddw1* in both populations by more than 30%. In this case, *Rht-B1b* in the presence of *Ddw1* does not lead to a significant decrease in the height of the spring triticale; thus, this is not likely to be included in breeding programs in order to further reduce the height in the presence of *Ddw1* in the spring triticale germplasm. However, *Ddw1* reduces the 1000 grain weight, while *Rht-B1b* increases the grain number per spike and grain number per spikelet. Thus, our studies have demonstrated the negative effect of *Ddw1* on spring triticale productivity of the main spike in the greenhouse experiment, which can be partially compensated by *Rht-B1b*.

**Keywords:** triticale; breeding; germplasm; dwarfing genes; agronomic traits; *Ddw1*; *Rht-B1*; wheat; rye; wide hybridization; PCR; fragment analysis

## 1. Introduction

Triticale plays an increasingly important role in agriculture. Products from this crop are in demand in the livestock sector as feed, in the alcohol-production industry, and have good prospects in the baking industry. Due to its high ecological plasticity, triticale is capable of producing stable yields under unstable agriculture. Triticale is grown in Europe, and among the leaders of its production are Germany, Poland, Belarus, France, and Russia [1].

One of the strategies to increase the productivity of triticale may be optimization of the plant architecture defined by tillering, stature, and spike morphology [2]. Plant architecture is one of the

most important agronomic traits that determines the adaptability of a plant for cultivation, the yield index, and the potential grain yield [3]. One of the important components of plant architecture is height [2]. Since triticale combines the genetic material of two species, wheat and rye, it is possible to influence plant height by introducing dwarfing genes from both the wheat and rye genomes. In general, dwarfing genes decreasing the height of the stem lead to the redistribution of assimilants from stem to spike, which allows more flowers to form and survive in the spike [4–7]. Short-stem (dwarf and semi-dwarf) forms have a higher harvest index both due to a decrease in aboveground biomass and due to an increase in the number of grains. Finally, crop losses in lodging-resistant forms of wheat are lower. Dwarf plants are considered to be more responsive to favorable cultivation conditions, while tall forms have an advantage in dry and hot weather conditions [8]. Dwarfing genes of wheat and rye origin are divided into two classes according to their response to exogenous gibberellic acid (GA): GAI gibberellin insensitive and GAR gibberellin responsive. To date, 24 genes that reduce plant height have been identified in bread wheat [9,10]. The gibberellin-insensitive gene *Rht-B1b* is one of the genes successfully introduced as a result of the Green Revolution and is widely distributed throughout the world [11]. The effect of the gibberellin-insensitive gene *Rht-B1b* on height can vary from about 10 to 25% in bread wheat and from about 25 to 35% in durum wheat compared to the wild-type allele *Rht-B1a* [6,12–17]. Wheat varieties and lines with *Rht-B1b* compared to *Rht-B1a* show a higher grain yield compared to wild type [6,15–18]. *Rht-B1b* increases the number of grains in the spike [17,18] and, as a result, the number of grains per square meter [15–17]. A number of studies have shown that *Rht-B1b* reduces the average mass of a single grain, although in a number of experiments the effect was not significant [12,13,15–17]. Also, an increase in harvest index due to an increase in grain yield and a decrease in the total aboveground biomass is shown [15,17]. Reductions of flag leaf area under the influence of *Rht-B1b* have not been shown [16,17]. Thus, *Rht-B1b* in wheat increases the yield by increasing the number of grains in the spike and per square meter, although the grain becomes smaller.

In rye, plant height is also determined by a variety of genetic factors [19,20]. To date, 14 different rye dwarfing genes are known, of which the GAR gibberellin-responsive dominant *Ddw1* gene, first identified in the rye EM-1 mutant form, is of the greatest breeding value [21,22]. Unlike GAI gibberellin-insensitive genes, GAR gibberellin-responsive genes do not reduce the length of the coleoptile, allowing deep seeding under the conditions of insufficient soil moistening. The introgression of *Ddw1* in winter triticale led to a decrease in height, but the resulting forms were later in development, reducing the linear dimensions of the leaves, reducing grain yield and resistance to *Fusarium* head blight [23–25].

Assessment of the interaction effects of GAR gibberellin-responsive and GAI gibberellin-insensitive dwarfing genes is important for selecting the most optimal combination of them in the genome of spring triticale and obtaining the optimum plant height for a particular growing area. The combination of genes with different mechanisms of action can enhance the positive effects of various dwarfing genes [16,17]. It should be taken into consideration that their effects on rye and wheat may differ from those in triticale, where they interact with each other in one plant organism combining the genetic environment of two different species. The goal of our study is to evaluate the effects of *Rht-B1b* and *Ddw1* on height and a number of important agronomic traits in two connected populations of spring triticale in F2 under a greenhouse conditions experiment.

## 2. Materials and Methods

### 2.1. Plant Material

As parental forms, triticale cultivars bearing contrasting combinations of rye and wheat dwarfing alleles were chosen. Spring triticale cv. ‘Dublet’ with genotype *ddw1 ddw1 Rht-B1b Rht-B1b* (Danko Hodowla Roślin Sp. z o.o., Poland) was used as the paternal form and winter triticale cv. ‘Mudrets’ (M) and cv. ‘Valentin 90’ (V90) with genotype *Ddw1 Ddw1 Rht-B1a Rht-B1a* (Lukyanenko Agricultural Research Institute, Russia) were used as the maternal forms.

## 2.2. Hybridization

The seeds of the parental cultivars were sown into the vegetation pots at 10 seeds per pot in the greenhouse of the Center for Molecular Biotechnology (Russian State Agrarian University—Moscow Timiryazev Agricultural Academy). For vernalization, the maternal plants at tillering were placed into a spring chamber for 60 days and then returned to the greenhouse. When maternal plants were heading the anthers in their spike were removed manually with fine forceps, the spikes were isolated with butter paper bags. The maternal plants were pollinated by placing a spike of a cut flowering paternal plant under a paper bag. The cut stem of the paternal plant was placed in water to keep it alive as long as possible.

## 2.3. Growth Conditions

Growing F<sub>1</sub> and F<sub>2</sub> plants was carried out in the same greenhouse as the parental plants under the same lighting conditions with five plants per pot, provided with watering to maintain 60–70% soil moisture and the single application of 2 g of fertilizers per pot at the tillering stage (N 16%, P<sub>2</sub>O<sub>5</sub> 16%, K<sub>2</sub>O 16%). Since the maternal form in both combinations was winter, in the segregating F<sub>2</sub> population there was a certain amount of winter forms, which we selected out according to the phenotype. A total of 318 plants of the ‘Mudrets’ × ‘Dublet’ (M×D) and 407 plants of the ‘Valentin 90’ × ‘Dublet’ (V90×D) F<sub>2</sub> segregating populations were analyzed.

## 2.4. Phenotyping

After full ripening and harvesting, a structural analysis of each individual plant was carried out according to the following phenotypic characters: plant height (cm), length of peduncle (first upper internode), 2nd and 3rd upper internodes, 2nd and 1st lower internodes (cm); main spike length (cm), spikelet number per main spike, main spike density (calculated as tenfold spikelet number per main spike divided by main spike length), grain weight per main spike (g), grain number per main spike, grain number per spikelet (grain number per main spike divided by spikelet number per main spike), 1000-grain weight (calculated as thousandfold grain weight per main spike divided by grain number per main spike, g), number of fertile tillers; and number of internodes. Additionally, heading date (days after sowing) and flowering date (days after sowing) were registered in growing plants.

## 2.5. Molecular analysis

DNA was extracted from each individual plant in F<sub>1</sub> and F<sub>2</sub> generations at tillering, and the allele status of the *Rht-B1* and *Ddw1* genes was determined using molecular markers. Genomic DNA was isolated from individual vegetative plants tissue using the CTAB method [26]. Primers BF, MR1 and WR1 were used to identify the *Rht-B1a* (wild type) and *Rht-B1b* (dwarfing type) alleles; PCR was performed in accordance with the conditions recommended by the authors of the molecular marker [27] (Table 1). The PCR products were separated in 2% agarose gel in TBE buffer and stained with ethidium bromide for subsequent visualization in a UV transilluminator. The presence of *Ddw1* was determined using primers developed on the sequence of the microsatellite locus REMS1218, closely linked to the gene [28] (Table 1). The determination of REMS1218 microsatellite alleles was carried out using PCR according to the protocol proposed by the authors of the molecular marker and subsequent fragment analysis using 3130xl Genetic Analyzer (Applied Biosystems, USA).

**Table 1.** Primers used for the identification of allelic state of the *Rht-B1* and *Ddw1* genes.

Gene	Primer	Source	Expected Amplicon Size
<i>Rht-B1</i>	BF 5' GGTAGGGAGGCGAGAGGCGAG 3'	Ellis et al., 2002 [27]	BF+MR1: 237 bp for <i>Rht-B1b</i>
	MR1 5' CATCCCCATGGCCATCTCGAGCTA 3'		BF+WR1: 237 bp for <i>Rht-B1a</i>
	WR1 5' CATCCCCATGGCCATCTCGAGCTG 3'		
<i>Ddw1</i>	F 5' GTGCTATGATAAGTACCTGC 3'	Tenhola-Roininen et al., 2010 [29]	317 bp for <i>ddw1</i>
	R 5' GGC TAA GGG AAC TCG CATTG 3'		317+321 bp for <i>Ddw1</i>

## 2.6. Statistical Analysis

For each phenotypic trait, the mean, standard deviation and range were determined. All data were processed in the Statistica 8.0 (StatSoft) program by regression and analysis of variance (ANOVA); to determine the significance of the differences between the mean values we used the least significance difference (LSD) criterion at a 95% confidence level. When analyzing the interaction of *Rht-B1b* and *Ddw1*, the effect of each of the alleles and their total effect were taken into account as follows:

$$\text{Effect of } RhtB1b (\%) = \frac{\text{Mean}(02) - \text{Mean}(00)}{\text{Mean}(00)} 100,$$

$$\text{Effect of } Ddw1 (\%) = \frac{\text{Mean}(20) - \text{Mean}(00)}{\text{Mean}(00)} 100,$$

$$\text{Effect of } RhtB1b + Ddw1 (\%) = \frac{\text{Mean}(22) - \text{Mean}(00)}{\text{Mean}(00)} 100.$$

where.

- 00 – *ddw1 ddw1 Rht-B1a Rht-B1a* (absence of both dwarfing genes, wild type, tall plant),
- 02 – *ddw1 ddw1 Rht-B1b Rht-B1b* (the presence of only one dwarfing gene *Rht-B1b* from wheat),
- 20 – *Ddw1 Ddw1 Rht-B1a Rht-B1a* (the presence of only one dwarfing gene *Ddw1* from rye),
- 22 – *Ddw1 Ddw1 Rht-B1b Rht-B1b* (the presence of both dwarfing genes)

Fractional ratios of internodes (%) were calculated as the length of a given internode divided by the total plant height expressed in percent.

## 3. Results

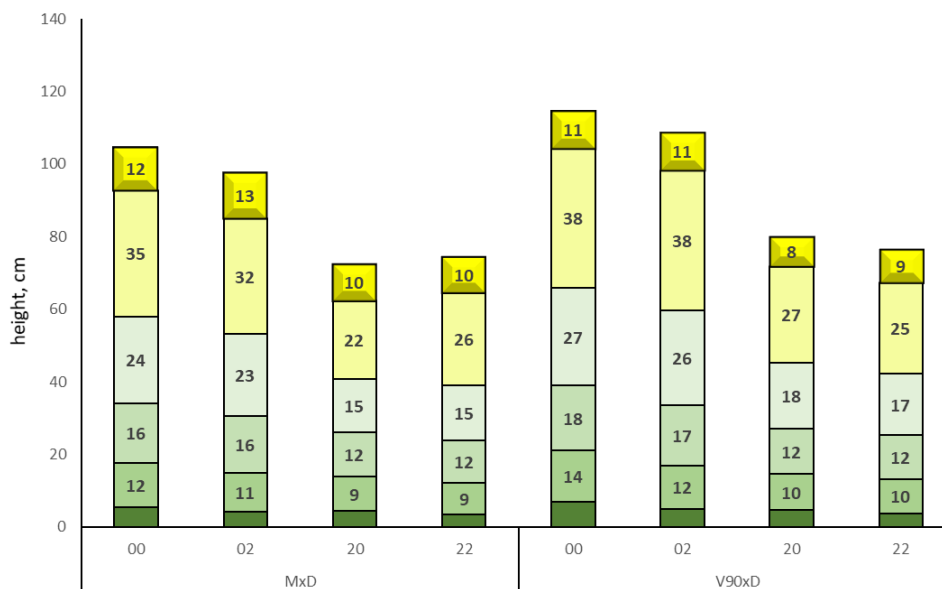
### 3.1. Molecular Analysis

Each plant of F<sub>1</sub> and F<sub>2</sub> generations in Mudrets' × 'Dublet' (M×D) and 'Valentin 90' × 'Dublet' (V90×D) populations were genotyped at tillering. The allelic status of *RhtB1* (Supplementary Figure S1) and *Ddw1* (Supplementary Figure S2) was detected using PCR molecular analysis. Based on the results of molecular analysis, the F<sub>2</sub> plants were categorized into four groups according to the presence or absence of dwarfing alleles, namely *ddw1 ddw1 Rht-B1a Rht-B1a* (00), *ddw1 ddw1 Rht-B1b Rht-B1b* (02), *Ddw1 Ddw1 Rht-B1a Rht-B1a* (20), and *Ddw1 Ddw1 Rht-B1b Rht-B1b* (22).

### 3.2. Plant Height.

The average plant height without dwarfing genes (genotype 00) differed in two populations by almost 15 cm, 104.7 cm in 'Mudrets' × 'Dublet' (M×D) and 118.7 cm in 'Valentin 90' × 'Dublet' (V90×D) (Table 2, Supplementary Table S1). Apparently, this is the reason for the absence of a statistically significant effect of *Rht-B1b* on height in the M×D population, although, in general, plants with genotype 02 (that is, only with *Rht-B1b*) were slightly lower than plants with genotype 00. In the V90×D population, the effect of *Rht-B1b* had a significant effect and reduced plant height by 11.2 cm

(9.4%). At the same time, *Ddw1* significantly reduced height by 38.9 cm (37.2%) in M×D, and by 35.8 cm (30.2%) in V90×D. When both dwarfing genes were present, no additional effect on the decrease in height was found. Thus, *Rht-B1b* does not reduce the height of plants additionally to the effect of *Ddw1*. The effect of *Ddw1* is manifested at each internode in both populations; the effect of *Rht-B1b* is statistically significant only at the two lower internodes (Figure 1, Supplementary Table S1). Although there is some tendency to reduce the length of each individual internode, the additional presence of *Rht-B1b* in the presence of *Ddw1* does not give a statistically significant effect, as in the case of height. It should be noted that there is a decrease in the number of internodes in the case of the presence of only *Ddw1* in the M×D population. In both populations, under the influence of both *Ddw1* and *Rht-B1b* genes, there is a proportional decrease in internodes (Table 3). Thus, the ratio of the internode length to the height of the plant is maintained on average within the following limits: 33% (peduncle): 22% (2nd upper internode); 15–16% (3rd upper internode); 12% (2nd lower internode); and 5% (1st lower internode). Thus, we can say that the ratio between the internodes is a rather conservative factor, and the *Ddw1* and *Rht-B1b* alleles do not have a significant effect on this indicator.



**Figure 1.** The effects of *Ddw1* and *Rht-B1b* on length distribution across main internodes in ‘Mudrets’ × ‘Dublet’ (M×D) and ‘Valentin 90’ × ‘Dublet’ (V90×D) spring triticale. From top to bottom: main spike, peduncle (1st upper) internode, 2nd upper internode, 3rd upper internode; 2nd lower internode; 1st lower internode; vertical axis: 00, *ddw1 ddw1 Rht-B1a Rht-B1a*; 02, *ddw1 ddw1 Rht-B1b Rht-B1b*; 20, *Ddw1 Ddw1 Rht-B1a Rht-B1a*; 22, *Ddw1 Ddw1 Rht-B1b Rht-B1b*.

**Table 2.** Effects of *Ddw1* and *Rht-B1b* on several agronomic traits in the spring triticale population F<sub>2</sub> ‘Hongor’ × ‘Dublet’ and × ‘Valentin 90’ × ‘Dublet’ in the greenhouse experiment.

Genotype	Plant Height, cm		Grain Number per Spike		1000-Grain Weight, g		Heading Time (Days after Sowing)	
	Mudrets × Dublet	Valentin 90 × Dublet	Mudrets × Dublet	Valentin 90 × Dublet	Mudrets × Dublet	Valentin 90 × Dublet	Mudrets × Dublet	Valentin 90 × Dublet
00 **	104.7 ± 26.0 a	118.7 ± 19.1 a	35.1 ± 14.7 ab	49.3 ± 14.7 a	46.0 ± 11.1 a	43.6 ± 6.9 a	49.1 ± 5.9 a	50.9 ± 7.4 a
02	100.1 ± 24.5 a	107.5 ± 11.7 b	40.7 ± 12.7 a	53.1 ± 16.4 a	42.1 ± 8.8 ab	41.1 ± 7.3 a	47.0 ± 5.1 a	50.8 ± 8.2 a
20	65.8 ± 19.6 b	82.9 ± 14.7 c	31.6 ± 11.7 b	44.5 ± 13.1 a	36.1 ± 8.4 c	39.9 ± 9.6 a	54.4 ± 9.8 b	52.6 ± 6.8 a
22	73.7 ± 13.1 b	77.6 ± 10.3 c	40.7 ± 10.6 a	50.6 ± 17.9 a	37.6 ± 8.9 bc	35.1 ± 5.7 b	51.3 ± 3.7 ab	48.9 ± 3.3 a
Contrasts								
02vs00	-4.6 (-4.4%)	<b>-11.2 * (-9.4%)</b>	5.6 (15.8%)	3.8 (7.8%)	-3.9 (-8.5%)	-2.5 (-5.8%)	-2.1(-4.3%)	-0.1 (-0.3%)
LSD0.05	12.4	9.6	8.5	10.5	6.2	6.1	4.4	5.1
20vs00	<b>-38.9 * (-37.2%)</b>	<b>-35.8 * (-30.2%)</b>	-3.5 (-10%)	-4.8 (-9.7%)	<b>-9.9 * (-21.5%)</b>	-3.7 (-8.5%)	<b>5.3 * (10.8%)</b>	1.7 (3.4%)
LSD0.05	13.0	9.4	9.0	10.9	6.5	6.2	4.4	5.1
22vs00	<b>-31.0 * (-29.6%)</b>	<b>-41.1 * (-34.6%)</b>	5.5 (15.7%)	1.3 (2.7%)	<b>-8.4 * (-18.2%)</b>	<b>-8.5 * (-19.4%)</b>	2.2(4.4%)	-2.0 (-3.9%)
LSD0.05	13.6	9.7	9.1	10.5	6.6	6.0	4.9	5.4

\* Significant differences ( $p = 0.05$ ) are marked with asterisks (\*) and typed in bold; the letters demonstrate if the means are different; \*\* 00—*ddw1 ddw1 Rht-B1a Rht-B1a* (absence of both dwarfing genes, wild type, tall plant), 02—*ddw1 ddw1 Rht-B1b Rht-B1b* (the presence of only one dwarfing gene *Rht-B1b*), 20—*Ddw1 Ddw1 Rht-B1a Rht-B1a* (the presence of only one dwarfing gene *Ddw1*), 22—*Ddw1 Ddw1 Rht-B1b Rht-B1b* (the presence of both dwarfing genes); LSD0.05, least significant difference at 95% confidence level; SD, standard deviation.

**Table 3.** Fractional ratios of the internode length in the ‘Mudrets’ × ‘Dublet’ and ‘Valentin 90’ × ‘Dublet’ F<sub>2</sub> populations in the greenhouse experiment (%).

Internode *	Mudrets × Dublet					Valentin 90 × Dublet				
	Genotype **				F0.05	Genotype				F0.05
	00	02	20	22		00	02	20	22	
Peduncle length	31.7	32.7	34.6	33.3	0.68	32.3	35.7	32.3	32.2	1.47
2nd UIN	22.7	22.6	20.6	22.9	0.30	22.7	24.4	21.8	22.0	1.25
3rd UIN	15.7	18.5	15.8	15.6	0.70	15.1	15.4	15.1	15.5	0.96
2nd LIN	10.7	14.4	11.7	11.7	0.50	11.5	11.0	11.6	12.4	0.81
1st LIN	4.1	6.6	4.8	5.2	0.68	5.9	4.6	5.5	4.6	1.95
	F0.05 (df1 = 4, df2 = 269) = 2.41					F0.05 (df1 = 4, df2 = 273) = 2.40				

\* UIN, upper internode; LIN, low internode; \*\* genotype designation the same as in Table 2; F0.05, Fisher criterion at 95% confidence level.

### 3.3. The Structure and Productivity of the Main Spike

In general, the presence of *Rht-B1b* did not affect the main spike length in both populations. The presence of *Ddw1* somewhat reduced the spike length. This was statistically significant only in genotype 02 (*Ddw1 Ddw1 Rht-B1a Rht-B1a*) in the V90×D population (Supplementary Table S1). None of the studied dwarfing genes had a statistically significant effect on the spikelet number per main spike in any of the populations (Supplementary Table S1). In the M×D population, the main spike density in plants with the genotype 22 (both dwarfing genes) was found to be 6.8 units (33.6%) more than that of 00 (absence of both dwarfing genes) and 7.9 units (40.9%) more than 02 (*ddw1 ddw1 Rht-B1b Rht-B1b*). In the V90×D population, in plants with the 20 genotype, the spike density is 11.3 units (45.8%) more than 00 genotype, and 10.2 units (39.5%) more than 02 genotype (Supplementary Table S1). Thus, in both populations, an increase in the spike density occurs because of a decrease in the spike length in the M×D population due to the interaction of the *Ddw1* and *Rht-B1b* alleles, and in the V90×D population only due to *Ddw1*.

When analyzing the influence of two dwarfing alleles on the grain weight per main spike, it was found that in the M×D population a significant difference from the 00 genotype (*ddw1 ddw1 Rht-B1a Rht-B1a*) has only plants with 20 genotype (*Ddw1 Ddw1 Rht-B1a Rht-B1a*) with its grain weight per spike less by 0.52 g (−31.5%) (Supplementary Table S1). No significant differences in the grain weight per spike between homozygotes in the V90×D population were found. We conducted a statistical analysis of the effects of *Rht-B1b* without taking into account the presence/absence of *Ddw1* and the effects of *Ddw1* without taking into account the presence/absence of *Rht-B1b* (independent analysis, Supplementary Table S2). An independent analysis demonstrated that *Ddw1* reduces the grain weight per spike in both populations; *Rht-B1b* increases the grain weight per spike in the M×D population.

The effect of dwarfing genes on the grain number per main spike in both populations showed similar trends: In the group of plants with genotype 02 (with *Rht-B1b*), the grain number per spike was slightly higher, and in the group of plants with genotype 20 (with *Ddw1*), it was slightly lower than in the wild type (genotype 00) (Table 2, Supplementary Table S1). At the same time, the presence of both *Rht-B1b* and *Ddw1* in different populations gave a different effect: In the M×D population, the grain number per spike of was at the level of genotype 02 (with *Rht-B1b*) and in the population of V90×D at the level of genotype 00 (absence of both dwarfing genes). These differences were statistically insignificant, so we conducted an independent statistical analysis of the effects of *Rht-B1b* and *Ddw1* (Supplementary Table S2). An independent analysis showed that *Ddw1* in both populations does not affect the grain number per spike. It was also demonstrated that *Rht-B1b* significantly increases the grain number per spike in the M×D population by 0.29 pcs (18.96%). In the population of V90×D, the significant effect of *Rht-B1b* is not observed; however, the difference between homozygotes for *Rht-B1* is close to the critical value of 4.7 g (10.1%) with LSD0.05 = 5.2. This effect is confirmed by the



significant weak correlation found using the regression analysis (0.20) and the significant Spearman's coefficient (0.15).

In the M×D population, plants with the 22 genotype (*Ddw1 Ddw1 Rht-B1b Rht-B1b*) have a grain number per spikelet of 0.37 units (27.0%) more than plants with the 20 genotype (*Ddw1 Ddw1 Rht-B1a Rht-B1a*), and plants with the 02 genotype (*ddw1 ddw1 Rht-B1b Rht-B1b*) have a grain number per spikelet of 0.49 units (26.3%) more than the 20 genotype (*Ddw1 Ddw1 Rht-B1a Rht-B1a*) (Supplementary Table S1). In the V90×D population, none of the dwarfing alleles showed a statistically significant effect on the grain number per spikelet, but we have noted the tendency that plants carrying the dwarfing gene *Rht-B1b* have a somewhat greater grain number per spikelet. Thus, the presence of the *Rht-B1b* allele in both populations increases the grain number per spikelet, while we have not revealed the statistical effect of *Ddw1*, although there is a tendency of decreasing this trait by *Ddw1* (Supplementary Table S1).

The 1000 grain weight decreased in both populations under the influence of *Ddw1* (Supplementary Table S1; Table 2). In the M×D population, the presence of *Ddw1* reduces the 1000 grain weight by 9.9 g (21.5%) and together with *Ddw1* with *Rht-B1b* by 8.4 g (18.2%). At the same time, the 1000 grain weight in plants with genotypes 20 (*Ddw1 Ddw1 Rht-B1a Rht-B1a*) and 22 (*Ddw1 Ddw1 Rht-B1b Rht-B1b*) is not significantly different. In the V90×D population, a statistically significant decrease in the 1000 grain weight is observed only with the simultaneous presence of *Ddw1* and *Rht-B1b*; the decrease relative to the 00 genotype (*ddw1 ddw1 Rht-B1a Rht-B1a*) is 8.5 g (19.4%). Thus, in both populations, *Ddw1* reduces the 1000 grain weight (Table 2, Supplementary Table S1).

The influence of dwarfing genes on tillering capacity was not found in both populations (Supplementary Table S1).

### 3.4. Flowering and Heading.

The 20 homozygotes (*Ddw1 Ddw1 Rht-B1a Rht-B1a*) came into heading 5.3 days later than the 00 homozygotes (*ddw1 ddw1 Rht-B1a Rht-B1a*) in the M×D population. In the V90×D population, none of the dwarfing alleles had a statistically significant effect on heading or flowering (Table 2, Supplementary Table S1).

## 4. Discussion and Conclusions

The task of the breeder is primarily to increase the adaptability of the plant to the environment, that is, in the model of the variety (ideotype), the key factor is the height and flowering period, which most closely matches the environment [16]. Dwarfing genes affect not only the height, but also the significant agronomic traits associated with it. A tall stem accumulates more assimilants than a short one; therefore, during the remobilization, tall plants form a larger and more filled grain. In addition, dwarf plants are formed due to the slower elongation of the stem, which leads to a later heading and flowering, and consequently, to lower yields [12]. Thus, the selection of dwarfing genes that would provide optimal flowering and heading periods and stem length for specific growing conditions is an important and critical task.

We analyzed the effect of *Ddw1* and *Rht-B1b* on the height of spring triticale plants in two connected populations. This provided an assessment of the manifestation of these genes in a different genetic background. This shows that *Ddw1* reduces the height of spring triticale plants in both by approximately 30%. In winter triticale, this value was on average about 20 cm (20–25%) [25,30], and in diploid rye, the reduction can reach 40% [21,29]. In our experiment, *Rht-B1b* in the M×D population did not have a statistically significant effect on height, which may be due to the strong influence of the genetic environment on plant height. In the V90×D population, *Rht-B1b* reduced height only in the absence of *Ddw1* (about 9%). In this case, *Rht-B1b* did not give an additional effect of reducing the height with *Ddw1*. The absence of the effect of *Rht-B1b* with *Ddw1* can be explained by the strong masking effect of *Ddw1*, in which a slight decrease in height due to *Rht-B1b* is not noticeable. The reduction in the length of internodes due to *Ddw1* occurs proportionally, that means the ratio of internodes



between themselves is maintained. This is not typical for all GAR gibberellin responsive alleles: *Rht-12* (probable ortholog of *Ddw1*) disproportionately changes the peduncle length.

*Ddw1* in the population 'Mudrets' × 'Dublet' significantly reduced the spike length only in the presence of *Rht-B1b*, and in the population of 'Valentin 90' × 'Dublet' in its absence, while the decrease in both cases was about 20%. According to Kobylansky and Solodukhina (2015), in rye, under the influence of *Ddw1*, on the contrary, an increase in the spike length occurs [21]. *Rht-B1b* reduces the sensitivity of tissues to the GA, which leads to less cell stretching; *Ddw1* has a different mechanism of action. Probably, under the influence of the surrounding genetic background, there is a different interaction of these mechanisms in different populations. In both populations, the grain weight per spike was reduced by 12–18% due to *Ddw1*; the number of grains was not affected by *Ddw1*, and the weight of 1000 grains decreased by 12–13%. Rebetzke et al. (2012) showed that, under the influence of *Rht12* (a possible *Ddw1* ortholog of wheat), there is a decrease in the mass of one grain by about 12%, which is comparable with our data [16]. Kobylansky and Solodukhina (2015) showed that the number of grains in the spike increases in rye under the influence of *Ddw1*, which is not observed in our experiments [21]. The effect of *Rht-B1b* on the grain weight and the amount of grains in the spike of common wheat greatly depends on the test conditions and the genetic environment and can be both positive and negative [18]. The 1000 grain weight (or the average grain weight) in bread wheat under the influence of *Rht-B1b* may decrease significantly or may not statistically significantly differ from *Rht-B1a* depending on the genetic environment and experimental conditions. [12,13,15,16]. Our experiment also shows the dependence of the *Rht-B1b* effect on the surrounding genotype. In the M×D population, *Rht-B1b* eliminates the negative effect of *Ddw1* in relation to the grain weight per spike due to an increase in the grain number per spike and spikelet and does not significantly reduce the 1000 grain weight. In the V90×D population, *Rht-B1b*, on the other hand, enhances the effect of *Ddw1* on reducing the 1000 grain weight. The presence of *Ddw1* led to a later heading and flowering in the M×D population (Supplementary Table S2); no such effect was observed in the V90×D population. Kalih et al. (2015) [31] and Wolski et al. (1996) [23] also wrote that *Ddw1* leads to later periods of flowering and heading in triticale. Later flowering and heading may be the result of a smaller grain weight, as is the case, for example, for the GAR gibberellin-responsive *Rht-5* wheat gene [32–34].

Thus, our studies show the negative effect of *Ddw1* in spring triticale on the productivity of the main spike in the greenhouse experiment, which can be partially compensated by *Rht-B1b*. There is no positive effect of *Ddw1* on the number of grains, as it happens in rye, but we have observed that the gene at least does not reduce their number. A decrease in the 1000 grain weight under the influence of *Ddw1* was expected. *Rht-B1b* increased the grain number per main spike and the grain number per spikelet, which may be due to faster delivery of assimilants to the spike, greater survival of distant spikelets, and increased fertility. In general, we found that the effect of *Ddw1* and *Rht-B1b* on height and agronomic characters depends on the population.

In triticale, combining dwarfing GAR and GAI genes from wheat and rye genomes increases the possible variations in their effects and enables the search for more appropriate variants. The combination of GAR and GAI dwarfing genes is an important direction in breeding of short-stem cereals as it provides an opportunity to achieve the compensating or/and additive effects. For example, Kurkiev (2008) [35] demonstrated additive effects on reducing plant height in triticale plants with both 2R/2D substitutions and rye and wheat dwarfing genes; Rebetzke et al. (2012) [15] and Wang et al. (2014) [36] showed that the plants of wheat having both GAI and GAR dwarfing genes was shorter than that with a single of the studied genes, whereas Liu et al. (2017) [17] in wheat, and we in our study of triticale did not find additive effects between GAR and GAI genes on plant height. In wheat, the combination of GAR and GAI genes does not additively reduce 1000 grain weight (or average single kernel weight) even if GAR alone severely reduces it [17,36]. In our study, the interaction of *Rht-B1b* and *Ddw1* resulted in a significant drop in the 1000 grain weight in the V90×D population while there was no additional reduction of this trait in GAR+GAI plants in the M×D population. In wheat, the grain number per spike in lines with the combination of the GAR and GAI gene was less than in lines

with the single dwarfing genes [35]; in our study, in the V90×D population, the grain number per spike was significantly higher in plants with the combination of the GAR and GAI genes than in plants with the GAR *Ddw1* gene; this is an example of the compensating effect between the genes.

In summary, in the studied spring triticale populations, *Ddw1* was found to decrease plant height with the greatest contribution of peduncle, to decrease the grain weight per main spike and the 1000 grain weight, while *Rht-B1b* is associated with an increase in the grain number per main spike. Individual effects may be due to a specific set of minor genes in each population that affect the studied traits.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2077-0472/9/6/119/s1>, Figure S1: PCR analysis of F<sub>2</sub> plants for the allelic state of *Rht-B1* using the marker by Ellis et al., 2002 [27], Figure S2: Fragment analysis of F<sub>2</sub> plants for the allelic state of *Ddw1* using the marker by Tenhola-Roininen et al., 2010 [29], Table S1: Effects of *Ddw1* and *Rht-B1b* in the spring triticale population F<sub>2</sub> ‘Mudrets’ × ‘Dublet’ and × ‘Valentin 90’ × ‘Dublet’ in the greenhouse experiment, Table S2: Independent effects of *Ddw1* and *Rht-B1b* in the spring triticale population F<sub>2</sub> ‘Mudrets’ × ‘Dublet’ and × ‘Valentin 90’ × ‘Dublet’ in the greenhouse experiment.

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