The Association of Grain Yield and Agronomical Traits with Genes of Plant Height, Photoperiod Sensitivity and Plastid Glutamine Synthetase in Winter Bread Wheat (Triticum aestivum L.) Collection

Mikhail S. Bazhenov, Ludmila A. Bespalova, Alina A. Kocheshkova, Anastasiya G. Chernook, Olga Y. Puzyrnaya, Elena V. Agaeva, Ekaterina A. Nikitina, Vladimir N. Igonin, Svetlana B. Zhizhina, Elena A. Vertikova, Pyotr N. Kharchenko, Gennady I. Karlov and Mikhail G. Divashuk

Abstract: The reduction in plant height caused by mutations in Rht-B1 or Rht-D1 (Reduced height-1) genes in combination with day-length-independent early flowering associated with the Ppd-D1 (Photoperiod-D1) gene were the main factors of the drastic yield increase in bread wheat in the 1960s. Increasing nitrogen use efficiency as well as maintaining high yields under conditions of global climate change are the modern goals of wheat breeding. The glutamine synthetase (GS) enzyme plays a key role in ammonium assimilation in plants. In previous studies, the TaGS2-A1 gene, coding the plastid isoform of GS, was shown to be connected with nitrogen use efficiency in wheat. Using the polymerase chain reaction (PCR) markers, the association of yield and agronomical traits with haplotypes of Rht-B1, Rht-D1, Ppd-D1 and TaGS2-A1 genes was studied in a diverse collection of winter bread wheat cultivars grown in Krasnodar (Russia). In the three-year experiment, semidwarfism and photoperiod insensitivity were confirmed to be highly favorable for the grain yield. The TaGS2-A1b haplotype had a tendency for increased grain yield and lodging resistance, but mainly in plants not possessing the ‘green revolution’ alleles. Thus, TaGS2-A1b may have potential in breeding wheat cultivars with alternative dwarfing genes or tall cultivars, which may be optimal for growing under certain environments.

Keywords: heading date; lodging; molecular markers; nitrogen assimilation; PCR; protein content; semidwarfism

1. Introduction

Wheat is the staple crop for an estimated 35% of the world’s population [1]. During the “green revolution” of the 1960s, wheat grain yields were nearly doubled due to the introduction of the new short-straw varieties, which better supplied assimilates for the developing spike at the expense of stem biomass and allowed for the application of higher doses of nitrogen fertilizers and irrigation without causing lodging [2,3]. The new varieties were shorter due to limited responsiveness to the growth-promoting gibberellin hormones, which was conferred by mutant alleles at one of the two Reduced height-1 loci (Rht-B1b or Rht-D1b) [4]. These genes, located on chromosomes 4B and 4D, correspondingly, encode DELLA proteins, which act in the cell nucleus as regulators of gene transcription. The DELLA proteins consist of a conservative C-terminal domain, responsible for growth repression; and a N-terminal DELLA domain, which is involved in GA perception and targeted
degradation of these proteins. The mutations in the DELLA domain of the reduced-height alleles result in the formation of proteins that are not subjected to targeted degradation and thus constitutively repress the growth [5,6]. The height-reducing alleles Rht-B1b (synonym of Rht1) and Rht-D1b (synonym of Rht2) are the most widespread, and can be found in modern wheat varieties around the world [7]. Another Rht-B1e (synonym of Rht11) allele is frequent among Russian cultivars developed in southern regions [8]. The semidwarf allele Rht-B1p (synonym of Rht17) is promising but not yet commercially used [9], while other alleles of these genes, including superdwarf Rht-B1c (Rht3) and Rht-D1c (Rht10), are not considered to be of commercial value [10,11]. Nonetheless, gibberellin-insensitive reduced-height genes have some adverse effects on agronomic traits, including reduced coleoptile length and seedling vigor [12], lower 1000 grain weight [3,11,13], lower tolerance to drought [4], higher Fusarium head blight susceptibility [14] and lower nitrogen use efficiency [15]; they are still widely used in breeding programs. Much research is aimed at alleviating the negative effects of gibberellin-insensitive reduced-height alleles [12,16], while others consider the substitution of these alleles for the gibberellin-sensitive ones [17–19].

Photoperiod-D1 (Ppd-D1) is a major photoperiod response locus on chromosome 2D in wheat. It was another gene, along with Rht-1, that contributed to the ‘green revolution’. The Ppd-D1a allele, which gives the insensitivity to photoperiod, is widespread among Chinese and Japanese landraces [20]. In the early twentieth century, this allele from a Japanese variety ‘Akakomugi’ was passed by Italian breeder Strampelli to European wheat varieties [21]. A semidominant mutation of this locus makes wheat plants photoperiod-insensitive, providing early flowering irrespective of the day length, and thus adapting them to a broad range of environments. Photoperiod neutrality conferred by Ppd-D1a gives a substantial yield advantage in Southern Europe and other warm-climate regions, where the season favorable for wheat growing begins under short-day conditions, and earlier flowering allows them to escape the excessive heat during the reproductive phase and the terminal drought stress. In Northern regions, on the contrary, Ppd-D1a could be disadvantageous for wheat grain yield [22,23]. Ppd-D1 is a member of the pseudo-response regulator (PRR) gene family. The photoperiod-insensitive allele Ppd-D1a has a 2 kb deletion upstream of the coding region, which is associated with a shifted peak of expression during a day [24] or increased expression [20] of the 2D PRR gene and activation of the key floral regulator FT (Flowering locus T) even under short-day conditions.

In spite of higher yields, semidwarf ‘green revolution’ varieties were reported to have reduced nitrogen-use efficiency [25]. Reduced-height alleles, associated with accumulation of the DELLA proteins, such as Rht-B1b, confer reduced nitrogen growth response and reduced nitrogen uptake both in nitrate and ammonium forms [15]. The Ppd-D1a allele may also cause lower nitrogen uptake and use efficiencies [23]. Application of higher doses of nitrogen fertilizers to compensate for this reduction may pose an environmental threat [26]. Thus, for semidwarf wheat varieties, improvement of nitrogen uptake from the soil and nitrogen use efficiency (NUE) seem to be needed.

Better nitrogen uptake and use efficiencies could be conferred by improved root architecture, enhanced work of ammonium and nitrate transporters in cell membranes and higher activity of enzymes of nitrogen metabolism—such as nitrate (NR) and nitrite reductases (NiR), glutamine synthetases (GS) and glutamate synthases (GOGAT), source–sink relationships and senescence dynamics [23].

Glutamine synthetase enzyme (GS) plays a key role in nitrogen utilization, growth and yield potential of cereal crops. It catalyzes a key reaction that incorporates inorganic nitrogen into organic compounds in plants—the synthesis of glutamine (Gln) from glutamate (Glu) and ammonium (NH$_4^+$). Plants have two forms of glutamine synthetase—the cytoplasmic one (GS1) and the plastid one (GS2). The GS1 enzyme is expressed in various organs and tissues and participates in the primary NH$_4^+$ assimilation, as well as in nitrogen remobilization and translocation, while GS2 is localized in chloroplasts and mitochondria of leaves and participates in the assimilation of ammonia formed as a result of nitrite reduction and photorespiration [27–30]. The GS2 enzyme was shown to be essential for
plant survival under photorespiration conditions. During photorespiration, the ammonium is released, while GS2 traps it and incorporates in glutamine, preventing its toxicity and depletion of organic nitrogen in the plant organism [31]. Increased plastid GS expression was shown to be favorable for plant growth and yield as it improves, among other traits, the nitrogen use efficiency. The lines overexpressing GS2 were shown to be more tolerant to abiotic stresses such as drought or soil salinity, as higher GS activity favors the synthesis of osmolytes such as proline or polyamines [31]. Moreover, plants overexpressing GS2 showed increased tolerance to high-intensity light [32].

In bread wheat, the plastid glutamine synthetase (GS2) genes located on chromosomes 2A, 2B and 2D were isolated and sequenced [28]. Two haplotypes were distinguished for TaGS2-A1 (a and b), along with six haplotypes for TaGS2-B1 (a-f) and two haplotypes for TaGS2-D1 (a and b). TaGS2-A1b was considered as a favorable one, along with TaGS2-B1a, B1b and D1a, by their association with nitrogen use and agronomic traits in a mini core collection of Chinese bread wheat varieties. In that collection, 65% of accesses possessed TaGS2-A1b, and this haplotype was more frequent in modern varieties than in landraces [28]. In another study of durum wheat collection, the variation at TaGS2-A1 was significantly associated with grain protein content [33]. Expression of the wheat GS2 in Escherichia coli showed that an enzyme encoded by the TaGS2-A1 gene is the most active isoform among others [34].

The transgenic experiment showed that expression of TaGS2-A1b under its own promoter in winter bread wheat increases grain yield and its components both under low and high nitrogen supply conditions. In addition, nitrogen acquisition by roots and remobilization was improved in transgenic plants [34]. Thus, TaGS2-A1b is valuable in wheat breeding for improved nitrogen use efficiency and grain yield.

In this study, we try to estimate the benefits of the TaGS2-A1b haplotype in a mini-collection of winter bread wheat varieties grown in southern Russia and evaluate the prospects of its combined use with well-studied ‘green-revolution’ alleles of Rht-B1 and Ppd-D1 genes. We hypothesize that TaGS2-A1b can improve nitrogen-use efficiency and further increase grain yields of semidwarf and photoperiod-insensitive wheat plants.

2. Results

2.1. Sequence Comparison and PCR Marker Development

To investigate the diversity of the TaGS2-A1 gene using modern genomic information, we extracted the sequences of this gene from 18 wheat accesses having sequenced and assembled genomes, listed in our previous publication [35], most of which are the part of the 10+ Wheat Genomes Project [36]. In the region of the miniature inverted transposable element (MITE) insertion in the second intron, which was previously used as diagnostic for the TaGS2-A1b haplotype, some of the genomic sequences were lacking information, and were represented by indefinite nucleotide bases (e.g., ‘NNN’). For further conclusions, we take these regions to be identical to the TaGS2-A1b haplotype. Compared to the other sequences, the sequence of the TaGS2-A1b haplotype of Xiaoyan 54 (GenBank accession GQ169685.1) has a rare single-nucleotide variant (SNV) within the MITE insertion that differs it from all other genomic sequences having the MITE insertion, and were represented by indefinite nucleotide bases (e.g., ‘NNN’). For further conclusions, we take these regions to be identical to the TaGS2-A1b haplotype. Compared to the other sequences, the sequence of the TaGS2-A1b haplotype of Xiaoyan 54 (GenBank accession GQ169685.1) has a rare single-nucleotide variant (SNV) within the MITE insertion that differs it from all other genomic sequences having the MITE insertion. If we consider this SNV to be a sequencing error, then only two haplotypes will be found in bread and cultivated durum wheat, which were previously reported as TaGS2-A1a (found in Chinese Spring and Lancer bread wheat and in spelt wheat PI 190962) and TaGS2-A1b (found in ArinaLrFor, Cadenza, CDC Landmark, CDC Stanley, Claire, Jagger, Julius, Mace, Norin 61, Paragon, Robigus and SY Mattis bread wheat accesses and in durum wheat Kronos). The haplotype TaGS2-A1b was obviously prevailing among these accesses of cultivated wheat. In wild emmer wheat Zavitan (T. turgidum ssp. dicoccoides), two unique SNPs—one in the promotor and another in the sixth intron—were found. The haplotype of Zavitan did not contain the MITE insertion in the second intron. The haplotype of the wild einkorn wheat Triticum urartu G1812 (PI428198) contained more unique SNPs and insertions in
the promoter and introns, but also did not contain the MITE insertion characteristic for \textit{TaGS2-A1b} (see the supplementary FASTA-format file).

\textit{TaGS2-A1b} differs from \textit{TaGS2-A1a} not only by the 239 bp MITE insertion in the second intron, but also by a 10 bp insertion in the 5′ flanking sequence, which obviously serves as a gene promoter. For this 10 bp insertion, we developed the subgenome-specific primers and made a codominant PCR marker suitable for agarose gel electrophoresis. The 172 bp product is diagnostic for haplotype \textit{TaGS2-A1a}, while 182 bp is diagnostic for haplotype \textit{TaGS2-A1b} (Figure 1).

![Electrophoresis of the PCR products obtained using primers pGS2-A1-F/R. The lanes represent the following wheat accessions: 1, 2—Caphorn; 3, 4—Nekota; 5, 6—Crimson; 7, 8—Tandem. M—DNA size standard (M-100, Syntol).](image)

\textbf{Figure 1.} Electrophoresis of the PCR products obtained using primers pGS2-A1-F/R. The lanes represent the following wheat accessions: 1, 2—Caphorn; 3, 4—Nekota; 5, 6—Crimson; 7, 8—Tandem. M—DNA size standard (M-100, Syntol).

\subsection{2.2. One-Way Analysis}

Using this and other molecular markers described in the ‘Materials and Methods’ section, we genotyped the collection of 195 winter bread wheat accessions for \textit{Rht-B1}, \textit{Rht-D1}, \textit{Ppd-D1}, and \textit{TaGS2-A1} genes (Table S13). This collection was grown and evaluated for agronomic traits at the National Center of Grain in Krasnodar (Russia) for three years (2018–2020). Below, we describe the results of genotyping and the associations of the genotypes with the best linear unbiased estimates (BLUEs) of grain yield and agronomic traits for the groups of genes separately and in the triple interaction ((\textit{Rht-B1} + \textit{Rht-D1}) × \textit{Ppd-D1} × \textit{TaGS2-A1}).

\subsubsection{2.2.1. Rht-1 Effects}

In 71\% of the bread wheat accessions, one of the three gibberellin-insensitive reduced-height alleles was found: \textit{Rht-B1b} was found in 47\%, \textit{Rht-D1b} was found in 14\%, and \textit{Rht-B1e} was found in 9\% of accessions. In 29\% of accessions, none of these reduced-height alleles were detected (Table S13). In addition, there were no double dwarfs combining \textit{Rht-D1b} with \textit{Rht-B1b} or \textit{Rht-B1e} in the collection studied. The effects of these reduced-height alleles on plant height were slightly different: The accessions carrying \textit{Rht-D1b} or \textit{Rht-B1e} had a lower average plant height (91 ± 3 and 90 ± 5 cm, respectively) than those carrying \textit{Rht-B1b} (98 ± 1 cm; here and further the means of BLUEs with the 95\% confidence intervals are given). The average height of accessions without gibberellin-insensitive reduced-height alleles was 112 ± 3 cm (Figure 2a). The effects of the three \textit{Rht} alleles were also a bit different for the other agronomical traits. Accessions carrying \textit{Rht-D1b} had a reduced 1000-kernel weight, but at the same time they were the most resistant to lodging, substantially differing in this feature from all other genotypes. The grain yield per hectare of the three gibberellin-insensitive reduced-height genotypes did not differ significantly from each other (Figure 2b), but their average yield (9.1 ± 0.1 t/ha) was significantly higher than that of ‘tall’ accessions (7.9 ± 0.3 t/ha). The grain protein content was decreased by semidwarfism; however, the grain protein yield per hectare was increased. Unexpectedly, the lodging tolerance of accessions carrying the gibberellin insensitivity alleles was not substantially higher (Table S1).
Using the developed marker, the TaGS2-A1b haplotype, previously considered as more favorable for nitrogen assimilation, was detected in 43% of accessions, while TaGS2-A1a was observed in 53% of them. A total of 4% of accessions were heterogenous for TaGS2-A1. By the results of one-way ANOVA, TaGS2-A1 did not influence any of the traits significantly. However, there was a tendency for TaGS2-A1b to increase lodging resistance and to delay heading.

2.3. Multifactor Analysis

In the studied collection, the wheat accessions that combine any of the reduced-height Rht-1 alleles with the allele of insensitivity to the photoperiod Ppd-D1a are prevailing. Since both the reduced-height and photoperiod insensitivity genes have a strong effect on many agronomical traits, and the combinations of alleles of these genes in the collection are not strictly random (slightly more accessions combining insensitivity to photoperiod with gibberellin-insensitive dwarfism, $p = 0.02$ for $\chi^2$), it is better to view the effects of these
genes in interaction, based on the results of multivariate analysis. Since, in general, the
effects of various gibberellin-insensitive Rht alleles have the same direction, to simplify the
interpretation of the results we decided to unite the data of three gibberellin-insensitive
alleles markers to divide the collection in two groups: Those with such alleles (Rht-B1b, Rht-
B1e, Rht–D1b) will be called ‘dwarf’, and those without them will be called ‘tall’. Further, the
results of the three-factor analyses ((Rht-B1 + Rht-D1) × Ppd-D1 × TaGS2-A1) accounting
for the gene interactions are presented.

2.3.1. Heading Date

As expected, Ppd-D1 explained most of the heading date genetic variance in the winter
bread wheat collection (Table S4). Accounting for the Bonferroni correction, other factors
did not significantly influence the heading date. The accessions carrying Ppd-D1a, the
allele conferring photoperiod insensitivity, headed 6 to 7 days earlier than accessions with
Ppd-D1b (Table S12, Figure 3).

Figure 3. The heading date (days from sowing) of winter wheat accessions differing in genotypes of
the three groups of genes—Rht-B1 + Rht-D1, Ppd-D1, TaGS2-A1. The so-called ‘tall’ accessions are
those that have both Rht-B1a and Rht-D1a wild-type alleles, while ‘dwarf’ ones are those possessing
either Rht-B1b, Rht-B1e or Rht-D1b alleles. The dots indicate outliers.

2.3.2. Plant Height

Most part of the plant height variance was explained by gibberellin-insensitive dwarf-
ing genes Rht-B1 and Rht-D1, and their interaction with Ppd-D1 (Table S5). As expected, the
height of the wheat plants carrying one of the gibberellin-insensitivity alleles was from 12
to 30 cm lower depending on the genetic background. The photoperiod-insensitive allele
Ppd-D1a also reduced the height of plants by 8–15 cm, but only in accessions not carrying
the Rht-B1 or Rht-D1 dwarfing alleles (Figure 4, Table S12). Variation at the TaGS2-A1 locus
did not have a significant impact on plant height, neither by itself nor in interaction with
Rht-1 or Ppd-D1.
Figure 4. The plant height of wheat accessions differing in genotypes of the three groups of genes—Rht-B1 + Rht-D1, Ppd-D1, TaGS2-A1. The dots indicate outliers.

2.3.3. Grain Yield

The yield of grain (t/ha) was significantly affected by the Rht-1 and Ppd-D1 genes, while TaGS2-A1 had a marginally significant effect if the interaction of the genes had been accounted (Table S6). The semidwarf, insensitive to photoperiod accessions, as well as those carrying TaGS2-A1b, showed higher three-year BLUEs of grain yield than those carrying wild-type alleles. On average, semidwarfism increased the grain yield by 1.4 t/ha, insensitivity to photoperiod increased the grain yield by 0.8 t/ha and TaGS2-A1b increased the grain yield by 0.6 t/ha. Tall, long-day accessions carrying TaGS2-A1a had the lowest grain yield (6.4 ± 0.8 t/ha), significantly differing from other genotypes. The presence of at least one of the favorable alleles of Rht-1, Ppd-D1 or TaGS2-A1 increased the yield substantially. Further yield increase due to the combination of favorable alleles of the two or three genes in one genotype was not so strong, but statistically significant. The highest grain yield, 9.3 ± 0.3 t/ha, was observed in accessions combining “positive” alleles of all three genes (Rht-B1b or Rht-B1e or Rht-D1b together with Ppd-D1a and TaGS2-A1b) (Figure 5, Table S12).

Figure 5. The grain yield of wheat accessions differing in genotypes of the three groups of genes—Rht-B1 + Rht-D1, Ppd-D1, TaGS2-A1. The dots indicate outliers.
2.3.4. Leaf Rust

The genotype of the \textit{Ppd-D1} gene showed near-significant association with the percentage of leaf area damaged by brown rust (Table S7). Wheat accessions having the allele of photoperiod neutrality, \textit{Ppd-D1a}, were less affected by leaf rust compared to long-day plants. On average, the percentage of leaf area affected by leaf rust was 5\% in day-length-neutral accessions and 12\% in day-length-sensitive accessions. The genes \textit{TaGS2-A1} and \textit{Rht-1} and genetic factor interactions showed no significant effects on this trait in the three-factor analysis. For the factor analysis, the near-absolutely resistant accessions were excluded for data to fit the normal distribution. These near-absolutely resistant accessions were more frequent among those that carried the \textit{TaGS2-A1b} allele, as we can see from Figure 6, where all the data were used.

\begin{figure} 
\centering
\includegraphics[width=0.5\textwidth]{figure6.png}
\caption{Leaf area damaged by brown rust in winter wheat accessions differing in \textit{Rht-1}, \textit{Ppd-D1} and \textit{TaGS2-A1} genotypes, proportion transformed with \textit{arcsin (√x)}. The dots indicate outliers.}
\end{figure}

2.3.5. The 1000-Kernel Weight

The 1000-kernel weight was significantly affected only by the \textit{Ppd-D1} gene (Table S8). In photoperiod-insensitive accessions, the 1000-kernel weight averaged 39.2 ± 0.5 g, while in long-day accessions it was 35.4 ± 1.0 g. The \textit{Rht-1} and \textit{TaGS2-A1} genes did not influence 1000-kernel weight significantly (Figure 7).

\begin{figure} 
\centering
\includegraphics[width=0.5\textwidth]{figure7.png}
\caption{The 1000-kernel weight in wheat accessions differing in \textit{Rht-1}, \textit{Ppd-D1} and \textit{TaGS2-A1} genotypes. The dots indicate outliers.}
\end{figure}
2.3.6. Grain Protein Content

Both Rht-1 and Ppd-D1 genes showed a significant effect on the grain protein content (Table S9). In both semidwarf and photoperiod-neutral forms, the protein content was significantly lower (Figure 8). The gibberellin-insensitive semidwarf alleles reduced the grain protein content by about 1% (from 15.4 to 14.4%), while day-length insensitivity reduced it by about 0.6% (from 15.2 to 14.6%). The TaGS2-A1 gene, however, did not significantly affect the protein content. In addition, there was no statistically significant interaction of the three genetic factors, which indicates their additive effect on the trait.

![Grain protein content (%) in winter wheat accessions differing in Rht-1, Ppd-D1 and TaGS2-A1 genotypes. The dots indicate outliers.](image)

**Figure 8.** Grain protein content (%) in winter wheat accessions differing in Rht-1, Ppd-D1 and TaGS2-A1 genotypes. The dots indicate outliers.

2.3.7. Grain Protein Yield

The grain protein yield per hectare was significantly influenced by Rht genes, and was marginally significant by the TaGS2-A1 gene (Table S10). Despite the decrease in protein content, the grain protein yield per hectare was significantly higher in semidwarf accessions compared to tall ones. The reason for that was an overcompensating increase in grain yield. The presence of one of the reduced-height alleles (Rht-B1b, RhtB1e or Rht-D1b) gave an increase in protein yield by 0.13 t/ha (from 1.16 to 1.29 t/ha), and the presence of the TaGS2-A1b allele increased the protein yield by 0.08 t/ha (from 1.18 to 1.26 t/ha). The lowest protein yield, significantly different from other genotypes, was observed in ‘tall’ accessions carrying Ppd-D1b and TaGS2-A1a alleles (Figure 9, Table S12).

![Grain protein yield (t/ha) in winter wheat accessions differing in Rht-1, Ppd-D1 and TaGS2-A1 genotypes. The dots indicate outliers.](image)

**Figure 9.** Grain protein yield (t/ha) in winter wheat accessions differing in Rht-1, Ppd-D1 and TaGS2-A1 genotypes. The dots indicate outliers.
2.3.8. Lodging Resistance

As expected, semidwarfism caused by the Rht-B1 and Rht-D1 dwarfing alleles was connected with higher resistance to lodging (higher values of the lodging score) (Table S11). Insensitivity to photoperiod caused by the Ppd-D1a allele, despite decreased plant height, was accompanied by lower values of lodging resistance. The TaGS2-A1b allele had a tendency to improve lodging resistance (Figure 10, Table S12). The most lodging-resistant accessions were semidwarf, photoperiod-sensitive and those carrying the TaGS2-A1b allele.

![Lodging score BLUEs](Figure 10. Lodging score BLUEs (9—no lodging, 2—total lodging) for winter wheat genotypes. The dots indicate outliers.)

3. Discussion

The studied collection of winter bread wheat accessions tested in Krasnodar consists of old and new cultivars and advanced breeding lines. In our study, about 70% of the collection possessed one of the reduced-height gibberellin-insensitive alleles of either Rht-B1 or Rht-D1 genes, and the most frequent of them was Rht-B1b. These alleles provide optimal semidwarf plant height and about 15% higher grain yield. This shows that Rht-B1b, Rht-B1e and Rht-D1b dwarfing alleles are still current for winter wheat breeding in Russia, despite known poor adaptability of gibberellin-insensitive dwarf cultivars to dry environments. The climate of Krasnodar is characterized by prolonged, dry and hot summers. However, the winter wheat growing season spans autumn; winter, which in Krasnodar is relatively short and mild, without constant snow cover; and spring, which is usually favorable for plant growth. Thus, winter crops can escape the adverse conditions of heat and drought. Nevertheless, more efforts are being conducted by breeders recently to shorten the vegetation period of winter wheat. On the other hand, our results show that rather high yields could be achieved without gibberellin-insensitive dwarfism.

We also noted that reduced-height alleles, obviously having the same mechanism of action, cause various degrees of agronomical trait changes. Rht-D1b or Rht-B1e cause stronger height reduction than Rht-B1b, although the grain yield is not much different between groups of accessions having one or another allele of these three. This is consistent with previous findings [3,8].

Generally, the presence of gibberellin-insensitive reduced-height alleles in our study was associated with reduced grain protein content. That is consistent with other studies, and could be explained by the ‘dilution’ of grain protein by a larger amount of stored carbohydrates [37]. However, grain protein yield per hectare is substantially higher in accessions carrying one of these dwarfing alleles than in ‘tall’ genotypes. Considering that all accessions in the field experiments were grown under equal conditions, this contradicts the states of some articles claiming weak nitrogen use efficiency in gibberellin-insensitive semidwarf cultivars [15].
Ppd-D1a, the photoperiod-insensitive allele, was observed in 76% of accessions in our collection, which is a relatively high proportion, showing its adaptability for winter wheat in warmer regions. Under conditions of southern Russia, Ppd-D1a gives improvement for several agronomic traits, including earlier heading date, lower plant height and higher 1000-grain weight. Above all, Ppd-D1a is favorable for higher grain yield per hectare. All these Ppd-D1 effects were reported in previous studies [10]. Shortening the vegetation period obviously allows them to escape the terminal drought and heat stresses, and it will be even more actual in the future, as according to the prognoses, global warming will be accompanied by shrinkage of autumn, winter and spring [38].

Despite mildly decreased plant height, the photoperiod-insensitive accessions carrying Ppd-D1a were less tolerant to lodging. That could be caused by the greater spike mass of these plants, which bends the stems to the ground. However, weaker stems due to faster development rate could be a reason. Further research should be conducted to elucidate this fact.

The leaf rust-affected area was significantly lower in accessions carrying Ppd-D1a. In our study, the leaf rust was scored 10 days after heading for each accession individually. Thus, a higher rate of development could allow the photoperiod-insensitive plants to escape the peak of disease development in the field. Previously, the connection of photoperiod insensitivity with disease resistance was reported in wheat, and was explained mainly by the shift of the development stages against the weather events [39]. Alternatively, we can assume co-occurrence of horizontal resistance genes with Ppd-D1a in the same cultivars due to breeding. The race-nonspecific resistance genes were widely introduced in breeding programs together with genes of reduced height and insensitivity to photoperiod, and co-occurrence of them in wheat cultivars of diverse origin could be expected [40].

The TaGS2-A1 gene encodes the most active isoform of plastid glutamine synthase in hexaploid wheat, which performs one of the key steps of nitrogen assimilation. Previously, two haplotypes were discovered (TaGS2-A1a and TaGS2-A1b) differing mainly within non-coding sequences and promoters, and two synonymous SNPs in protein-coding sequences. Previous studies showed advantage of the TaGS2-A1b haplotype in hexaploid wheat [28].

In our study, the TaGS2-A1b haplotype was weakly positively connected with higher grain yield, grain protein yield (but not grain protein content) and lodging resistance. The highest effects were observed within accessions that do not carry any of the ‘green revolution’ alleles of Ppd-D1, Rht-B1 or Rht-D1 genes. This is consistent with the study of the TaGS2-A1 gene in the Chinese wheat collection, where TaGS2-A1b showed positive effects on plant biomass under any nitrogen supply only among landraces, but not in bred cultivars (most of which are supposed to possess some of the ‘green revolution’ alleles, while landraces are not). Thus, we can hypothesize that the TaGS2-A1b haplotype could be effective, if one needs to refuse gibberellin-insensitive dwarfism. For example, in many regions of Central Asia, where drought begins earlier during vegetative growth and anthesis, taller genotypes perform much better than semidwarf ones [4]. On the other hand, it may be of value in more northern nonchernozem regions, where Ppd-D1a is not as favorable as in Krasnodar Krai.

A connection of glutamine synthetase with lodging was not expected, but as we know, lodging resistance of wheat depends not only on plant height, but also on the strength of the culm, anchorage power of the root system, and the weight of the spike [41]. Improved nitrogen assimilation in plants carrying TaGS2-A1b could result in the development of stronger stems, less prone to lodging.

Further studies should be aimed at glutamine synthetase biochemical activity, stem anatomy and disease immunity in wheat lines with various genotypes of Rht, Ppd and TaGS2 genes to elucidate our findings.
4. Materials and Methods

4.1. Plant Material and Phenotyping

The winter bread wheat (Triticum aestivum L.) accessions used in this study were a part of the collection of the National Center of Grain named after P.P. Lukyanenko in Krasnodar, Russia. The origin of accessions and references for their brief characteristics were published in our previous study [35].

Yield testing and phenotyping for agronomical traits were conducted during 2018–2020 harvest years in Krasnodar. The methodology of field experiments and weather conditions during growing seasons were published in our previous study [35]. The brown rust (Puccinia triticina f. sp. tritici) was scored 10 days after heading as a visual estimation of the percentage of the leaf area occupied by the disease.

4.2. STS Marker Development for the TaGS2-A1 Gene

The sequences of the glutamine synthetase gene TaGS2-A1 were obtained from the NCBI database (accession numbers GQ169684, GQ169685) [28], and further from the Chinese Spring wheat genome IWGSC RefSeq v1.0 [42], and from the sequences of the 10+ Wheat Genomes Project [36] using BLAST+ software [43] as described earlier [35]. The sequences were aligned and compared using GeneDoc2.7 software [44].

The gene-specific pair of primers flanking the 10 bp insertion/deletion in the promotor (pGS2-A1-F/pGS2-A1-R, Table 1) was designed using Primer-BLAST (NCBI) [45]. The specificity of the primers was checked using alignment of the three homoeologous genes of GS2 (TraesCS2A02G500400, TraesCS2B02G528300, TraesCS2D02G500600).

Table 1. The primers used for the PCR markers.

| Name       | Sequence (5′→3′)                                      | Alleles Detected, Product Length |
|------------|------------------------------------------------------|----------------------------------|
| BF         | GGTAGGGAGGCGAGAGGCAGAG                              | (Used with MR1, WR1, MR3, WR3)   |
| MR1        | CATCCCAATGGCCATCTCGAGCTA                            | Rht-B1b, 237 bp                  |
| WR1        | CATCCCAATGGCCATCTCGAGCTG                            | Rht-B1a (not Rht-B1b)*, 237 bp   |
| MR3        | GGCCTCTCCAGCTGCTCCAGCTA                             | Rht-B1e, 228 bp                  |
| WR3        | GGCCTCTCCAGCTGCTCCAGCTT                             | Rht-B1a (not Rht-B1e)*, 228 bp   |
| DF         | CGGCACATTATATTGCGAGAGATAG                           | Rht-D1b, 254 bp                  |
| MR2        | CCCCATGCCCACAGTCAGAGCTGCTA                          |                                  |
| DF2        | GCCAAGCQAATAAGCTGCG                                 | Rht-D1a (not Rht-D1b)*, 264 bp   |
| WR2        | GCCCATCTCGAGCTGAC                                   |                                  |
| Ppd-D1_F   | ACCCGTCCCACTACACTG                                  | Ppd-D1a, 288 bp; Ppd-D1b, 414 bp|
| Ppd-D1_R1  | GTTGGTTCAACAGAGAGC                                  |                                  |
| Ppd-D1_R2  | CAGTGTTGATGCTAGATT                                  |                                  |
| pGS2-A1-F  | GGCCTCGCTCCTCCATAATATAA                             | TaGS2-A1a, 172 bp; TaGS2-A1b, 182 bp|
| pGS2-A1-R  | AACGACACAGAGATGAAAGAC                               |                                  |

* The nucleotide identical to wild-type allele at SNP is detected.

The PCR for the TaGS2-A1 STS (sequence-tagged site) marker was performed in 25 µL reaction volumes, containing 1 × buffer solution supplied in a kit with the polymerase (70 mM Tris–HCl, pH 8.6, 16.6 mM (NH₄)₂SO₄, 2.5 mM MgCl₂ in final volume; Sileks Ltd., Moscow, Russia), 0.2 mM of each dNTP (Sintol Ltd., Moscow, Russia), 0.3 µM forward and reverse primers (Sintol Ltd., Moscow, Russia), 0.05 U/µL Taq polymerase (Sileks Ltd., Moscow, Russia) and 4 ng/µL DNA template. The PCR conditions were as follows: (1) 95 °C for 10 min, (2) 36 cycles of 95 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min; and (3) final extension step of 72 °C for 10 min. PCR products were separated in 2% agarose gels with TBE buffer for at least 1 h in an electric field intensity of 6 V/cm, stained with
ethidium bromide and documented under UV light using the Gel Doc XR+ system (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

4.3. DNA Extraction and PCR-Markers

Genomic DNA was extracted from ground dried leaves of seedlings using the CTAB-based protocol [46]. The DNA samples of the two individual plants from each wheat accession were used for genotyping.

Detection of the dwarfing Rht-B1b, Rht-B1e (TraesCS4B02G043100) and Rht-D1b (TraesCS4D02G040400) alleles was performed using allele-specific polymerase chain reaction (ASPCR) markers [47]. For these markers, usually two separate PCRs are performed, and in each mixture a common primer and allele-specific primer with 3’ end complementary to the SNP detected are used. The PCR products of each reaction are detected by gel electrophoresis on distinct lanes, and then results are combined to tell homozygotes for one or another allele from heterozygotes. Two pairs of primers were used for detection of Rht-B1b: BF + MR1 for finding the mutant allele, and BF + WR1 for the wild-type allele. Rht-D1b was detected using primers DF + MR2, and Rht-D1a using DF2 + WR2 primers. For Rht-B1e, two primer pairs were used: BF with WR3 to amplify wild-type sequences, while BF with MR3 to amplify a fragment only for Rht-B1e allele [48].

Detection of Ppd-D1 (TraesCS2D02G079600) alleles was performed using a PCR with 3 primers: common primers Ppd-D1_F and Ppd-D1_R1 for wild-type allele Ppd-D1b, giving a 414 bp product; and Ppd-D1_R2 for the mutant ‘photoperiod-insensitive’ allele Ppd-D1a with the deletion in a promoter of the gene, giving a 288 bp product [24].

The sequences of the primers used for PCR are represented in Table 1. The PCR conditions were used as described in the original protocols. The PCR products were separated in 1.5% agarose gel with TBE buffer for 30 min at electric field intensity of 6 V/cm, stained with ethidium bromide and documented under ultraviolet light using the Gel Doc XR+ system.

4.4. Statistical Analysis

The statistical distribution of the majority of the traits tested resembled the normal distribution, except for the values of the leaf rust damage. Before further processing, the percentages of the leaf rust damage were transformed using \( \arcsin \left( \sqrt{x} \right) \), as this transformation keeps the zero values. The best linear unbiased estimates (BLUEs) of the three years of measurements were calculated using Tassel 5 software [49]. The BLUEs for the leaf rust were back-transformed to percentages to be presented in Table S13. To obtain the best fit for the normal distribution, the near-to-zero (<0.2%) BLUE values of the leaf rust damage were discarded (as absolute resistance is not assumed to be phenotype for the genes tested), and the remaining values were \( \ln(x) \)-transformed. The BLUEs were used for the analysis of variance regarding the molecular markers in Statistica 6.0. We used one-way analysis of variance (ANOVA) for separate genes or a group of Rht-1 genes united through assumed phenotype (tall or semidwarf) as a single factor and separate traits as dependent variables. In addition, we used factorial ANOVA to estimate the gene interaction and to compensate for the allele imbalance in the wheat collection. Presence of GA-insensitive dwarfing alleles (1), alleles of Ppd-D1 (2) and TaGS2-A1 (3) were treated as factors. The factor analysis included each of the three factors (1), (2), (3), their double interactions (1 \( \times \) 2), (1 \( \times \) 3), (2 \( \times \) 3) and the triple interaction (1 \( \times \) 2 \( \times \) 3) as fixed effects. Sigma-restricted parameterization and type VI (unique) sum of squares were used. Fisher’s F-test was used to estimate the significance of the effects. The adjusted \( \alpha \) levels were calculated using the Bonferroni–Holm correction for 8 comparisons (8 traits) in one-way ANOVA and 56 comparisons (8 traits \( \times \) 7 factors, including double and triple interactions) for multifactorial ANOVA [50]. The least-square means were calculated as an estimation of the group means; the differences between means were accessed using Tukey’s HSD (honestly significant difference) test. The boxplots for 3-year BLUEs were built using Rstudio 2021.09.1 Build 372 software, R 4.1.2 programming language and ggplot2 package [51].
5. Conclusions

Semidwarfism associated with Rht-B1 or Rht-D1 mutations and earliness connected to the Ppd-D1a allele are highly beneficial for the grain yield of winter bread wheat in the southern regions of Russia. The accessions carrying Rht-B1b, Rht-B1e or Rht-D1b lightly differ in plant height but not in the grain yield per unit area. Semidwarfism caused by these alleles was associated with lower grain protein content, but with higher protein yield per unit of area. Despite a mild decrease in plant height provided by the allele of photoperiod insensitivity Ppd-D1a, the accessions carrying it are less resistant to lodging. The TaGS2-A1b haplotype of the plastid glutamine synthetase gene has a tendency to improve lodging resistance and grain yield. However, in the presence of ‘green revolution’ alleles, the positive effects of TaGS2-A1b on agronomical traits are minimal. Thus, TaGS2-A1b may have potential in breeding taller wheat cultivars or cultivars with alternative dwarfing genes, which may perform better under drier environments.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms231911402/s1, Tables S1–S13.

Author Contributions: Conceptualization, M.G.D.; methodology, L.A.B. and E.A.V.; software, M.S.B. and E.A.N.; formal analysis, M.S.B.; investigation, A.A.K., A.G.C., O.Y.P. and V.N.I.; resources, L.A.B.; data curation, L.A.B.; writing—original draft preparation, M.S.B.; writing—review and editing, M.G.D. and S.S.B.; visualization, A.A.K. and E.A.N.; supervision, G.I.K. and P.N.K.; project administration, M.G.D.; funding acquisition, A.A.K. and M.G.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Science and Higher Education of the Russian Federation, state task number 0431-2022-0001. This research was funded by the Russian Science Foundation, grant number 21-76-00043 in the part statistical analysis, and molecular analysis of the allelic state of genes.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in Supplementary Materials to this article.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Grote, U.; Fasse, A.; Nguyen, T.T.; Erenstein, O. Food Security and the Dynamics of Wheat and Maize Value Chains in Africa and Asia. Front. Sustain. Food Syst. 2021, 4, 617009. [CrossRef]
2. Flintham, J.E.; Börner, A.; Worland, A.J.; Gale, M.D. Optimizing Wheat Grain Yield: Effects of Rht (Gibberellin-Insensitive) Dwarfing Genes. J. Agric. Sci. 1997, 128, 11–25. [CrossRef]
3. Hayat, H.; Mason, R.E.; Lozada, D.N.; Acuna, A.; Holder, A.; Larkin, D.; Winn, Z.; Murray, J.; Murphy, J.P.; Moon, D.E.; et al. Effects of Allelic Variation at Rht-B1 and Rht-D1 on Grain Yield and Agronomic Traits of Southern US Soft Red Winter Wheat. Euphytica 2019, 215, 172. [CrossRef]
4. Jatayev, S.; Sukhikh, I.; Vavilova, V.; Smolenskaya, S.E.; Goncharov, N.P.; Kurishbayev, A.; Zotova, L.; Absattarova, A.; Serikbay, D.; Hu, Y.-G.; et al. Green Revolution “Stumbles” in a Dry Environment: Dwarf Wheat with Rht Genes Fails to Produce Higher Grain Yield than Taller Plants under Drought. Plant Cell Environ. 2020, 43, 2355–2364. [CrossRef]
5. Peng, J.; Richards, D.E.; Hartley, N.M.; Murphy, G.P.; Devos, K.M.; Flintham, J.E.; Beales, J.; Fish, L.J.; Worland, A.J.; Pelica, F.; et al. “Green Revolution” Genes Encode Mutant Gibberellin Response Modulators. Nature 1999, 400, 256–261. [CrossRef]
6. Wu, J.; Kong, X.; Wan, J.; Liu, X.; Zhang, X.; Guo, X.; Zhou, R.; Zhao, G.; Jing, R.; Fu, X.; et al. Dominant and Pleiotropic Effects of a GAI Gene in Wheat Results from a Lack of Interaction between DELLA and GID1. Plant Physiol. 2011, 157, 2120–2130. [CrossRef]
7. Wilhelm, E.P.; Boulton, M.I.; Al-Kaff, N.; Balfourier, F.; Bordes, J.; Greenland, A.J.; Powell, W.; Mackay, I.J. Rht-1 and Ppd-D1 Associations with Height, GA Sensitivity, and Days to Heading in a Worldwide Bread Wheat Collection. Theor. Appl. Genet. 2013, 126, 2233–2243. [CrossRef]
8. Divashuk, M.G.; Vasilyev, A.V.; Besignava, L.A.; Karlov, G.I. Identity of the Rht-11 and Rht-B1e Reduced Plant Height Genes. Russ. J. Genet. 2012, 48, 761–763. [CrossRef]
9. Bazhenov, M.S.; Divashuk, M.G.; Amagai, Y.; Watanabe, N.; Karlov, G.I. Isolation of the Dwarfing Rht-B1p (Rht17) Gene from Wheat and the Development of an Allele-Specific PCR Marker. Mol. Breed. 2015, 35, 213. [CrossRef]
10. Börner, A.; Worland, A.J.; Plaschke, J.; Schumann, E.; Law, C.N. Pleiotropic Effects of Genes for Reduced Height (Rht) and Day-Length Insensitivity (Ppd) on Yield and Its Components for Wheat Grown in Middle Europe. Plant Breed. 1993, 111, 204–216. [CrossRef]

11. Allan, R.E. Agronomic Comparisons among Wheat Lines Nearly Isogenic for Three Reduced-Height Genes. Crop Sci. 1986, 26, 707–710. [CrossRef]

12. Khadka, K.; Kaviani, M.; Raizada, M.N.; Navabi, A. Phenotyping and Identification of Reduced Height (Rht) Alleles (Rht-B1b and Rht-D1b) in a Nepali Spring Wheat (Triticum aestivum L.) Diversity Panel to Enable Seedling Vigor Selection. Agronomy 2021, 11, 2412. [CrossRef]

13. McClung, A.M.; Cantrell, R.G.; Quick, J.S.; Gregory, R.S. Influence of the Rht1 Semidwarf Gene on Yield, Yield Components, and Grain Protein in Durum Wheat. Crop Sci. 1986, 26, 1095–1099. [CrossRef]

14. Sri, S.; Gosman, N.; Steed, A.; Hollins, T.; Bayles, R.; Jennings, P.; Nicholson, P. Semi-Dwarfing Rht-B1 and Rht-D1 Loci of Wheat Differ Significantly in Their Influence on Resistance to Fusarium Head Blight. TAG Theor. Appl. Genet. 2008, 118, 695–702. [CrossRef]

15. Li, S.; Tian, Y.; Wu, K.; Ye, Y.; Yu, J.; Zhang, J.; Liu, Q.; Hu, M.; Li, H.; Tong, Y.; et al. Modulating Plant Growth–Metabolism Coordination for Sustainable Agriculture. Nature 2018, 560, 595–600. [CrossRef]

16. Miedaner, T.; Herter, C.P.; Ebmeyer, E.; Kollers, S.; Korzun, V. Use of Non-Adapted Quantitative Trait Loci for Increasing Fusarium Head Blight Resistance for Breeding Semi-Dwarf Wheat. Plant Breed. 2019, 138, 140–147. [CrossRef]

17. Tian, X.; Xia, X.; Xu, D.; Liu, Y.; Xie, L.; Hassan, M.A.; Song, J.; Li, F.; Wang, D.; Zhang, Y.; et al. Rht24b, an Ancient Variation of TaGA2ox-A9, Reduces Plant Height without Yield Penalty in Wheat. New Phytol. 2022, 233, 738–750. [CrossRef]

18. Mo, Y.; Vanzetti, L.S.; Hale, I.; Spagnolo, E.J.; Guidobaldi, F.; Al-Oboudi, J.; Odle, N.; Pearce, S.; Helguera, M.; Dubcovsky, J. Identification and Characterization of Rht25, a Locus on Chromosome Arm 6AS Affecting Wheat Plant Height, Heading Time, and Spike Development. Theor. Appl. Genet. 2018, 131, 2011–2035. [CrossRef] [PubMed]

19. Ellis, M.H.; Rebetzke, G.J.; Azanza, F.; Richards, R.A.; Spielmeyer, W. Molecular Mapping of Gibberellin-Responsive Dwarfing Genes in Bread Wheat. Theor. Appl. Genet. 2005, 111, 423–430. [CrossRef]

20. Guo, Z.; Song, Y.; Zhou, R.; Ren, Z.; Zha, J. Discovery, Evaluation and Distribution of Haplotypes of the Wheat Ppd-D1 Gene. New Phytol. 2010, 185, 841–851. [CrossRef]

21. Wordland, A.J. The Importance of Italian Wheats to Worldwide Varietal Improvement. New Phytol. 2009, 183, 83–89. [CrossRef]

22. Worland, A.J.; Börner, A.; Plaschke, J.; Korzun, V.; Li, W.M.; Petrov, S. Identification of Photoperiod Insensitive Ppd-D1a Mutant of Wheat (Triticum aestivum L.) Diversity Panel to Enable Seedling Vigor Selection. J. Theor. Appl. Genet. 2017, 115, 721–733. [CrossRef]

23. Gooding, M.J.; Addisu, M.; Uppal, R.K.; Snape, J.W.; Jones, H.E. Effect of Wheat Dwarving Genes on Nitrogen-Use Efficiency. J. Agric. Sci. 2012, 150, 3–22. [CrossRef]

24. Wang, D.; Xu, Z.; Zhao, J.; Wang, Y.; Yu, Z. Excessive Nitrogen Application Decreases Grain Yield and Increases Nitrogen Loss in a Wheat–Soil System. Acta Agric. Scand. Sect. B—Soil Plant Sci. 2011, 61, 681–692. [CrossRef]

25. Taira, M.; Valtersson, U.; Burkhardt, B.; Ludwig, R.A. Arabidopsis Thaliana GLN2-Encoded Glutamine Synthetase Is Dual Cytosolic Glutamine Synthetase Isoforms of Maize Are Specifically Involved in the Control of Grain Production. Plant Cell 2004, 16, 2048–2058. [PubMed]

26. Li, X.-P.; Zhao, X.-Q.; He, X.; Zhao, G.-Y.; Li, B.; Liu, D.-C.; Zhang, A.-M.; Zhang, X.-Y.; Tong, Y.-P.; Li, Z.-S. Haplotype Analysis of Hirel, B.; Theor. Appl. Genet. 2020, 131, 2412. [CrossRef] [PubMed]

27. Wang, D.; Xu, Z.; Zhao, J.; Wang, Y.; Yu, Z. Excessive Nitrogen Application Decreases Grain Yield and Increases Nitrogen Loss in a Wheat–Soil System. Acta Agric. Scand. Sect. B—Soil Plant Sci. 2011, 61, 681–692. [CrossRef]

28. Taira, M.; Valtersson, U.; Burkhardt, B.; Ludwig, R.A. Arabidopsis Thaliana GLN2-Encoded Glutamine Synthetase Is Dual Cytosolic Glutamine Synthetase Isoforms of Maize Are Specifically Involved in the Control of Grain Production. Plant Cell 2004, 16, 2048–2058. [PubMed]

29. Li, X.-P.; Zhao, X.-Q.; He, X.; Zhao, G.-Y.; Li, B.; Liu, D.-C.; Zhang, A.-M.; Zhang, X.-Y.; Tong, Y.-P.; Li, Z.-S. Haplotype Analysis of the Genes Encoding Glutamine Synthetase Plastic Isoforms and Their Association with Nitrogen-Use- and Yield-Related Traits in Bread Wheat. New Phytol. 2011, 189, 449–458. [CrossRef]

30. Harel, B.; Tétu, T.; Lea, P.J.; Dubois, F. Improving Nitrogen Use Efficiency in Crops for Sustainable Agriculture. Sustainability 2011, 3, 1452–1485. [CrossRef]

31. Habash, D.Z.; Massiah, A.J.; Rong, H.L.; Wallsgrove, R.M.; Leigh, R.A. The Role of Cytosolic Glutamine Synthetase in Wheat. Ann. Appl. Biol. 2001, 138, 83–89. [CrossRef]

32. Martin, D.; Cañas, R.A.; Betti, M. Is Plastidic Glutamine Synthetase Essential for C3 Plants? A Tale of Photorespiratory Mutants, Ammonium Tolerance and Conifers. New Phytol. 2022, 234, 1559–1565. [CrossRef] [PubMed]

33. Martin, D.; Cañas, R.A.; Betti, M. Is Plastidic Glutamine Synthetase Essential for C3 Plants? A Tale of Photorespiratory Mutants, Ammonium Tolerance and Conifers. New Phytol. 2022, 234, 1559–1565. [CrossRef] [PubMed]

34. Hu, M.; Zhao, X.; Liu, Q.; Hong, X.; Zhang, W.; Zhang, Y.; Sun, L.; Li, H.; Tong, Y. Transgenic Expression of Plastidic Glutamine Synthetase Increases Nitrogen Uptake and Yield in Wheat. Plant Biotechnol. J. 2018, 16, 1858–1867. [CrossRef] [PubMed]
35. Bazhenov, M.S.; Chernook, A.G.; Bespalova, L.A.; Gritsay, T.I.; Polevikhova, N.A.; Karlov, G.I.; Nazarova, L.A.; Divashuk, M.G. Alleles of the GRF3-2A Gene in Wheat and Their Agronomic Value. *Int. J. Mol. Sci.* 2021, 22, 12376. [CrossRef]

36. Walkowiak, S.; Gao, L.; Monat, C.; Haberer, G.; Kassa, M.T.; Brinton, J.; Ramirez-Gonzalez, R.H.; Kolodziej, M.C.; Delorean, E.; Thambugala, D.; et al. Allelic Wheat Genomes Reveal Global Variation in Modern Breeding. *Nature* 2020, 588, 277–283. [CrossRef]

37. Achilli, A.L.; Roncallo, P.F.; Larsen, A.O.; Dreisigacker, S.; Echenique, V. Population Structure, Allelic Variation at Rht-B1 and Ppd-A1 Loci and Its Effects on Agronomic Traits in Argentinian Durum Wheat. *Sci. Rep.* 2022, 12, 9629. [CrossRef]

38. Wang, J.; Guan, Y.; Wu, L.; Guan, X.; Cai, W.; Huang, J.; Dong, W.; Zhang, B. Changing Lengths of the Four Seasons by Global Warming. *Geophys. Res. Lett.* 2021, 48, e2020GL091753. [CrossRef]

39. Simón, M.R.; Worland, A.J.; Struik, P.C. Influence of Plant Height and Heading Date on the Expression of the Resistance to Septoria Tritici Blotch in Near Isogenic Lines of Wheat. *Crop Sci.* 2004, 44, 2078–2085. [CrossRef]

40. Singh, R.P.; Huerta-Espino, J.; Bhavani, S.; Herrera-Foessel, S.A.; Singh, D.; Singh, P.K.; Velu, G.; Mason, R.E.; Jin, Y.; Njau, P.; et al. Race Non-Specific Resistance to Rust Diseases in CIMMYT Spring Wheats. *Euphytica* 2011, 179, 175–186. [CrossRef]

41. Shah, L.; Yahya, M.; Shah, S.M.A.; Nadeem, M.; Ali, A.; Ali, A.; Wang, J.; Riaz, M.W.; Rehman, S.; Wu, W.; et al. Improving Lodging Resistance: Using Wheat and Rice as Classical Examples. *Int. J. Mol. Sci.* 2019, 20, 4211. [CrossRef]

42. Alaux, M.; Rogers, J.; Letellier, T.; Flores, R.; Alfama, F.; Pommier, C.; Mohellibi, N.; Durand, S.; Kimmel, E.; Michotey, C.; et al. Linking the International Wheat Genome Sequencing Consortium Bread Wheat Reference Genome Sequence to Wheat Genetic and Phenomic Data. *Genome Biol.* 2018, 19, 111. [CrossRef] [PubMed]

43. Camacho, C.; Coulouris, G.; Avagyan, V.; Ma, N.; Papadopoulos, J.; Bealer, K.; Madden, T.L. BLAST+: Architecture and Applications. *BMC Bioinform.* 2009, 10, 421. [CrossRef] [PubMed]

44. Nicholas, K.B.; Nicholas, H.B. GeneDoc: A Tool for Editing and Annotating Multiple Sequence Alignments. *Emb. News* 1997, 4, 14.

45. Ye, J.; Coulouris, G.; Zaretskaya, I.; Cutcutache, I.; Rozen, S.; Madden, T.L. Primer-BLAST: A Tool to Design Target-Specific Primers for Polymerase Chain Reaction. *BMC Bioinform.* 2012, 13, 134. [CrossRef]

46. Doyle, P.J. DNA Protocols for Plants. In *Molecular Techniques in Taxonomy*; Hewitt, G.M., Johnston, A.W.B., Young, J.P.W., Eds.; NATO ASI Series; Springer: Berlin/Heidelberg, Germany, 1991; pp. 283–293, ISBN 978-3-642-83964-1.

47. Ellis, M.; Spielmeyer, W.; Gale, K.; Richards, R. “Perfect” Markers for the Rht-B1b and Rht-D1b Dwarfing Genes in Wheat. *Theor. Appl. Genet.* 2002, 105, 1038–1042. [CrossRef]

48. Pearce, S.; Saville, R.; Vaughan, S.P.; Chandler, P.M.; Wilhelm, E.P.; Sparks, C.A.; Al-Kaff, N.; Korolev, A.; Boulton, M.I.; Phillips, A.L.; et al. Molecular Characterization of Rht-1 Dwarfing Genes in Hexaploid Wheat. *Plant Physiol.* 2011, 157, 1820–1831. [CrossRef]

49. Bradbury, P.J.; Zhang, Z.; Kroon, D.E.; Casstevens, T.M.; Ramdoss, Y.; Buckler, E.S. TASSEL: Software for Association Mapping of Complex Traits in Diverse Samples. *Bioinformatics* 2007, 23, 2633–2635. [CrossRef]

50. Cramer, A.O.J.; van Ravenzwaaij, D.; Matzke, D.; Steingroever, H.; Wetzes, R.; Grasman, R.P.P.P.; Waldorp, L.J.; Wagenmakers, E.-J. Hidden Multiplicity in Exploratory Multiway ANOVA: Prevalence and Remedies. *Psychon. Bull. Rev.* 2016, 23, 640–647. [CrossRef]

51. Wickham, H. *Ggplot2*; Springer: New York, NY, USA, 2009; ISBN 978-0-387-98140-6.