Article

Compensatory Effect of the ScGrf3-2R Gene in Semi-Dwarf Spring Triticale (x Triticosecale Wittmack)

Anastasiya G. Chernook 1,*, Mikhail S. Bazhenov 1, Pavel Yu. Kroupin 1, Aleksey S. Ermolaev 1, Aleksandra Yu. Kroupina 1, Milena Vukovic 1, Sergey M. Avdeev 2, Gennady I. Karlov 1 and Mikhail G. Divashuk 1

1 All-Russia Research Institute of Agricultural Biotechnology, 127550 Moscow, Russia
2 Moscow Timiryazev Agricultural Academy, Russian State Agrarian University, 127434 Moscow, Russia

* Correspondence: irbis-sibri@yandex.ru

Abstract: The dwarfness in many triticale cultivars is provided by the dominant Ddw1 (Dominant dwarf 1) allele found in rye. However, along with conferring semi-dwarf phenotype to improve resistance to lodging, this gene also reduces grain size and weight and delays heading and flowering. Grf (Growth-regulating factors) genes are plant-specific transcription factors that regulate plant growth, including stem growth, in terms of length and thickness, and leaf and fruit size. In this work, we partially sequenced the rye gene ScGrf3 on chromosome 2R homologous to the wheat Grf3 gene, and found multiple polymorphisms in intron 3 and exon 4 complying with two alternative alleles (haplotypes ScGrf3-2Ra and ScGrf3-2Rb). For the identification of these, we developed a codominant PCR marker. Using a new marker, we studied the effect of ScGrf3-2R alleles in combination with the Ddw1 dwarf gene on economically valuable traits in F4 and F5 recombinant lines of spring triticale from the hybrid combination Valentin 90 x Dublet, grown in the Non-Chernozem zone for 2 years. Allele ScGrf3-2Ra was associated with greater thousand-grain weight, higher spike productivity, and earlier heading and flowering, which makes ScGrf3-2R a perspective compensator for negative effects of Ddw1 on these traits and increases prospects for its involvement in breeding semi-dwarf cultivars of triticale.

Keywords: triticale; dwarfing genes; Ddw1; ScGrf3-2R; molecular marker

1. Introduction

Triticale (x Triticosecale Wittmack) is an artificially created genus of cultivated plants, and is an intergeneric amphidiploid wheat (Triticum ssp.) and rye (Secale ssp.). Hexaploid triticale (2n = 6x = 42; BBAARR) remains the most commercially demanded type of this species, for which the first cultivars were registered in 1968 [1]. Triticale was developed to combine the high-yield potential and end-product quality of wheat with the adaptivity of rye [2]. Most triticale consumption is as forage and feed grain. At the same time, this natural resource crop has great potential for the use of its processed products in the production of biofuels (ethanol and biogas), the supply and production of the chemicals, paper, construction, and plastics industries, food production (beer and kvass), and bakery and rusk products [3–5].

The breeding process has improved the feed qualities of triticale and eliminated undesirable traits such as pre-harvest sprouting. However, lodging has long been a problem of growing this crop. Lodging reduces the efficiency of photosynthesis, promotes pre-harvest sprouting and disease development, slows maturation, and hampers harvesting [6,7]. The solution of the lodging problem was found in the introduction of semi-dwarfing genes into commercial wheat cultivars. Dwarfing genes reduce plant height and increase yield by redistributing plant resources in favor of the developing head [8,9]. However, these genes also tend to reduce the grain weight, impair the absorption of nitrate nitrogen from soil, and reduce the drought resistance [10,11].
Plants height in triticale can be reduced using either wheat or rye dwarfing genes, or both. Dwarfing genes are classified depending on their response to exogenous gibberellic acid (GA): gibberellin-insensitive and gibberellin-sensitive. To date, 27 genes reducing the height of bread wheat stem have been identified [10,12,13]. The gibberellin insensitivity Rht-B1b (synonym with Rht-1, Reduced height-1) gene was the first to be successfully introduced during the “Green Revolution”. It is widely distributed in wheat and triticale varieties worldwide [14]. The decrease in plant height due to Rht-B1b varies from 10% to 25% in common wheat, and from 25% to 35% in durum wheat, in comparison to the carriers of the wild-type allele Rht-B1a [15–21]. Semi-dwarf cultivars and breeding lines of wheat carrying Rht-B1b show higher yield than wild-type tall plants [15,19–21]. Among the 14 rye dwarf genes known today [22], Ddw1 (Dominant dwarf 1) has the highest agronomic value. The dominant allele of this gene reduces the height of diploid and tetraploid rye plants by 40% and 55%, respectively, but at the same time reduces the grain weight and spike productivity, and delays heading and flowering [23–28]. Ddw1 is successfully used in European triticale breeding programs [8,29,30]. In rye, Ddw1 manifests pleiotropically by shortening the stem internode, and increasing tillering, size of leaves and spikes, number of spikes, and grains per spike [31].

Growth-regulating factors (GRFs) are plant transcription proteins that participate in the regulation of growth and development. The Grf gene was first found in rice as OsGRF1; it encodes a protein that regulates the response to gibberellin, which promotes stem elongation [32]. Since the discovery of the first Grf, they have been reported in various species. In particular, nine members of the Grf family have been identified in Arabidopsis thaliana [33,34], 14 in maize [35], and 12 in rice [36]. In Arabidopsis thaliana, Grf knockout mutants are characterized with smaller and narrower leaves compared to wild-type genotypes [33,37,38]. In rice, the suppression of Grf3, Grf4, and Grf5 leads to dwarfism and delay in inflorescence development [39], while increased Grf expression results in a significant increase in the length of the panicle, and an increase in the length, width, and weight of the caryopsis [40–43]. Grf1 overexpression in maize increases the number of dividing cells, resulting in larger leaves, although plant height is reduced [44].

In bread wheat, 30 TaGRFs were identified in 12 linkage groups and classified into four phylogenetic groups [45,46]. Previously, we assessed the TaGrf3-2D sequence diversity in common wheat (Triticum aestivum, BBAADD) and Ae. tauschii (DD), and revealed its influence on grain weight and size in wheat [26]. The effect of the allelic state of TaGrf3-2A on thousand-grain weight and spike weight in Ddw1-carrying triticale was shown, and the possibility of using TaGrf3-2A to compensate for the negative effects of Ddw1 was demonstrated [47]. In addition, the TaGrf3-2Ab allele that highly likely originated from bread wheat, Bezostaya 1, was associated with earlier heading and better grain performance in Krasnodar Krai [48].

Thus, recent studies have proved the significance of TaGrf3 as a potential means of compensating for the negative effects of dwarfism in wheat and triticale. However, the Grf genes in rye have not yet received sufficient attention. In this work, we studied the effect of the rye ScGrf3-2R gene on important valuable agronomic traits in spring triticale.

2. Results
2.1. Partial Sequencing of ScGrf3-2R

The search for a gene homologous to wheat’s TaGrf3-2A in the rye Lo7 genome sequence led to the discovery of the most similar fragment to it on chromosome 2R, designated ScGrf3-2R. Based on the found sequence and sequences of TaGrf3 wheat gene homologs, we designed primers specific for the rye genome for one of the gene fragments. Using these, we amplified the ScGrf3-2R gene fragment in two rye varieties, Novaya Era and Saratovskaya 7, and in two triticale varieties, Khongor and Dublet. Amplicon sequence analysis showed that both rye and triticale have two different alleles of this gene. The allele found in the Lo7 reference rye genome, in addition to the allele present in Saratovskaya 7 and Dublet, was designated ScGrf3-2Ra, and the allele found in Khongor was designated...
ScGrf3-2Rb. Novaya Era was found to be heterozygous, and both ScGrf3-2Ra and ScGrf3-2Rb were found in it.

The sequenced gene fragment covers most of the third intron and the last fourth exon of the gene, including a small part of the 3’UTR. It should be noted that ScGrf3-2Ra and ScGrf3-2Rb alleles are very different. In the third intron, there are single nucleotide polymorphisms (SNPs) and 10 insertions/deletions (indels) ranging in size from 1 to 28 nucleotides. The coding sequence of the fourth exon contains one double-nucleotide and four single nucleotide polymorphisms, and an insertion of a triplet of nucleotides in ScGrf3-2Rb. At the same time, three of the four SNPs in the coding sequence are synonymous, and the remaining polymorphisms lead to changes in the amino acid sequence. The last exon encodes the C-terminal fragment of the protein, which does not contain conserved domains. According to the PROVEAN prediction, the found changes should not significantly affect the functioning of the protein (Table 1).

Table 1. Prediction of the significance of the detected amino acid substitutions for the functionality of the rye GRF3 protein using the PROVEAN online service.

| Variant             | PROVEAN Score | Prediction (Cutoff = −2.5) |
|---------------------|---------------|---------------------------|
| T263S               | 0.440         | Neutral                   |
| G319_F320insG       | 0.079         | Neutral                   |
| N359S               | 0.759         | Neutral                   |

Therefore, we identified an allelic polymorphism in the 4th exon between the ScGrf3-2Ra and ScGrf3-2Rb alleles. Even synonymous nucleotide substitutions can affect translation efficiency, since different tRNAs will be required for different alleles [49]. In the case of ScGrf3-2Ra and ScGrf3-2Rb, this concerns both nonsynonymous (T263S, N359S) and synonymous substitutions, resulting in non-isoaccepting codons (T290, F313). In addition, the found substitutions and insertion G319_F320insG are located at the carboxylic end of the GRF protein, in which the function of transcription activation via protein–protein interaction was revealed [32,33,40]. In the third intron, we found indels ranging in size from 1 to 28 nucleotides, which can affect the efficiency of gene expression and also lead to phenotypic differences [50]. Thus, we can assume that the revealed differences in the nucleotide and deduced amino acid sequence may be functional for the studied GRF protein.

2.2. Development of Allelic-Specific PCR Marker for ScGrf3-2R

To determine the allelic state of ScGrf3-2R in triticale lines, we developed a codominant Sequence-Tagged Site (STS) marker based on the presence of insertions/deletions in the third intron of the gene (Figure 1).

Figure 1. STS marker design based on polymorphisms in the 3rd intron of the ScGrf3-2R gene. The sequences of the flowing accessions are shown: triticale, Dublet (ScGrf3-2Ra), and Khongor (ScGrf3-2Rb); rye, Novaya Era (both alleles), and Saratovskaya 7 (ScGrf3-2Ra); reference genome sequence of rye Lo7 (ScGrf3-2Ra). The sequences of the designed primers are highlighted in color.

Primers were designed using Primer-BLAST (NCBI) and their specificity was preliminary tested based on the alignment between rye and wheat homologous genes. The
expected amplicon size is 220 bp for ScGrf3-2Ra (Saratovskaya 7, Dublet) and 180 bp for ScGrf3-2Rb (Novaya Era, Khongor). Conditions for PCR amplification are shown in the Materials and Methods section. The observed results of testing STS marker on plant material were consistent with those expected (Figure 2).

**Figure 2.** Application of the allelic-specific STS marker for the ScGrf3-2R gene. An example of electrophoresis of PCR products amplified from DNA of spring triticale F₃ plants of the hybrid combination Dublet x Valentin 90. M-100-5 (ZAO Sintol, Moscow, Russia) was used as a molecular weight marker. Expected amplicon sizes: 220 bp for ScGrf3-2Ra, 180 bp for ScGrf3-2Rb.

Parental triticale varieties of the hybrid combination Valentin 90 x Dublet differ in the allelic state of ScGrf3-2R and the Ddw1 dwarf gene. The spring variety of triticale Dublet carries Ddw1 (wild type, tall plant) and ScGrf3-2Ra, whereas the winter variety Valentin 90 has Ddw1 (dwarfing allele, short plant) and ScGrf3-2Rb. All recombinant lines derived from F₃ plants used in this study were selected for a spring habit. For genotyping recombinant lines for ScGrf3-2R, we used the STS marker developed in this study, and for genotyping for Ddw1, the REMS1218 microsatellite marker was applied.

### 2.3. Association between Genotypes and Valuable Agronomic Traits

To assess association between the allelic state of Ddw1 and ScGrf3-2R, on one hand, and the studied plant traits, on the other hand, we studied individual effects of Ddw1 and ScGrf3-2R (Supplementary Table S1) and their complex interaction (Supplementary Table S2).

#### 2.3.1. Individual Effects of Ddw1 and ScGrf3-2R

The dominant dwarfing gene Ddw1 significantly reduced the height of spring triticale plants by 28.3 cm (31%) and 27.6 cm (29.7%) in 2018 and 2019, correspondingly, confirming the data of our previous studies. Additionally, a significantly lower productivity of the main spike was observed in the Ddw1 carriers in comparison to the lines with the wild-type allele. The grain weight per spike was lower in short plants than in tall wild-type plants in both years by 0.15 g (6% and 8%, respectively). The decrease in spike productivity was mainly due to a decrease in the thousand-grain weight (TGW): in Ddw1-carrying lines, TGW was 4 g (10%) and 3.8 g (7%) lower in 2018 and 2019, respectively, compared to tall lines. Due to Ddw1 the number of grains in the main spike decreased in both years insignificantly, by 1–2 pieces (2–3%) (Supplementary Table S1).

In both years of the field experiment, ScGrf3-2Ra demonstrated positive effect on the productivity of the main spike. The grain weight in the main spike in the ScGrf3-2Ra-carrying lines was 0.17 g (9%) and 0.3 g (12%) higher in 2018 and in 2019, respectively, than in the ScGrf3-2Rb-carriers. The increase in productivity occurred both due to an increase in the grain number per spike by 3 pcs (7%) and 5 pcs (9%) in 2018 and 2019, respectively, and due to an increase in the TGW by 1 g (2%) in both years. The allelic state of ScGrf3-2R showed significantly affected plant height: triticale lines carrying ScGrf3-2Ra were lower by 8 cm (10%) in 2018 and 3.7 cm (5%) in 2019 compared to lines carrying ScGrf3-2Rb.
Spring triticale plants homozygous for ScGrf3-2Ra had more compact spikes compared to ScGrf3-2Rb in both years of field trials, which was due to a decrease in the spike length and an increase in the spikelet number per spike (Supplementary Table S2).

2.3.2. Interaction of Ddw1 and ScGrf3-2R

Tall plants (ddw1 ddw1) carrying ScGrf3-2Ra had a significantly higher grain number per spike, by 9 pcs (22%) in 2019 and by 6 pcs (13%) in 2018, compared to tall plants carrying ScGrf3-2Rb; among semi-dwarf lines (Ddw1 Ddw1), the trend was the same, but the effect was not statistically significant (p < 0.05) in both years (Figure 3, Supplementary Table S2).

![Figure 3](image_url)

**Figure 3.** Grain number per main spike (pieces) in recombinant triticale lines with different combinations of Ddw1 and ScGrf3-2R alleles in 2018 (a) and 2019 (b). Letters denote significantly different groups at a significance level of 0.05 according to Tukey’s test. The rectangles show the interval between the 1st and 3rd quartiles, the vertical lines show the maximum and minimum values, the horizontal line inside the rectangle indicates the median, dots show the trait values of individual plants.

In tall plants of spring triticale (ddw1 ddw1) grown in 2018, ScGrf3-2Ra significantly increased the grain weight in spike by 0.5 g (25%); among short plants (Ddw1 Ddw1) this effect was not statistically significant (p < 0.05), but the trend persisted. In 2019, both tall (ddw1 ddw1) and short (Ddw1 Ddw1) plants homozygous for ScGrf3-2Ra had higher grain weight in spikes, by 0.3 g (11–13%), compared to the ScGrf3-2Rb carriers (Supplementary Table S2).

In 2018, ScGrf3-2Ra increased TGW both in tall (ddw1 ddw1) and short (Ddw1 Ddw1) plants by 2.4 g (6%) and 2.3 g (6%), respectively. Short plants (Ddw1 Ddw1) homozygous for ScGrf3-2Ra in 2019 had a significantly higher TGW, by 2.1 g (4%), compared to short plants with the ScGrf3-2Rb genotype (Figure 4, Supplementary Table S2).

In 2018, in short plants (Ddw1 Ddw1), ScGrf3-2Ra reduced plant height by 6.1 cm (9%) relative to ScGrf3-2Rb; in tall plants (ddw1 ddw1), the same tendency was not statistically significant (p < 0.05). In 2019, in tall lines (ddw1 ddw1), plant height was reduced due to ScGrf3-2Ra by 6.8 cm (7%), whereas in short lines the same tendency was observed, although it was not statistically significant (p < 0.05). Plants homozygous for both Ddw1 and ScGrf3-2Ra were the shortest, with a mean height of 59.8 cm (2018) and 64.4 cm (2019). The number of internodes in spring triticale plants remained equal to five during two years of the field experiment in all studied genotypes, the decrease in plant height was achieved by uniformly reducing the length of each individual internode, and the interaction between ScGrf3-2Ra and Ddw1 almost always resulted in shorter internodes (Supplementary Table S2). In both years of the experiment, plants homozygous for both ScGrf3-2Ra and Ddw1 had the highest harvesting index (Supplementary Table S2). Therefore, the evaluation of the ScGrf3-2R effects on agronomic traits in spring triticale demonstrated...
that the ScGrf3-2Ra allele exhibits a positive effect on plant productivity, i.e., increases grain number and grain weight per spike, and TGW. Additionally, ScGrf3-2Ra showed its ability to mitigate the negative effect of Ddw1 on productivity traits.

Figure 4. Thousand-grain weight (grams) in recombinant triticale lines with different combinations of Ddw1 and ScGrf3-2R alleles in 2018 (a) and 2019 (b). Letters denote significantly different groups at a significance level of 0.05 according to Tukey’s test. The rectangles show the interval between the 1st and 3rd quartiles, the vertical lines show the maximum and minimum values, the horizontal line inside the rectangle indicates the median, dots show the individual values of individual plants.

In both the 2018 and 2019 experiments, short plants (Ddw1-genotype) headed and flowered 3–4 days and 2–4 days later, correspondingly, than tall plants (ddw1-genotype). In short plants (Ddw1 Ddw1), ScGrf3-2Ra accelerated heading by 7 days in 2018 and by 3 days in 2019, and hastened flowering by 8 days in 2018 and by 3 days in 2019. Among tall plants (ddw1 ddw1), ScGrf3-2Ra accelerated flowering and heading in both years, albeit not statistically significant (p < 0.05, Supplementary Table S2). Therefore, the ScGrf3-2Ra allele leads to a reduction in the transition from the vegetative to the generative phase, despite the negative effect of the dominant dwarfism gene Ddw1, which delays it.

3. Discussion

Dwarfing genes, and Ddw1 in particular, are known to have pleiotropic effects on plant traits. In our previous works in spring triticale [22,23,27], Ddw1, in addition to lowering the height, was shown to affect productivity traits, i.e., reduce grain weight and number per spike, and thousand-grain weight (TGW), and also lead to later heading.

In this study, we evaluated the manifestation of ScGrf3-2R and Ddw1 in spring triticale recombinant F4 and F5 lines grown in a two-year field experiment in the Non-Chernozem zone, so the effects of gene alleles under different weather conditions were analyzed. In both years, the effect of the ScGrf3-2R and Ddw1 allelic state was stable and unidirectional.

We showed that the allelic state of ScGrf3-2R in semi-dwarf plants carrying Ddw1 affects plant productivity, TGW, and grain weight per spike, demonstrating a partial compensatory effect of ScGrf3-2R against Ddw1. In our previous study [26], we described the effect of TaGrf3-2D allelic state on grain weight and size in the common wheat collection, and this confirms our assumption about similar effects of the ScGrf3-2R gene on plant traits. The dwarfing effect of Ddw1 on plant height was 27–28%, which is generally consistent with previous studies [23–27]. The ability of the ScGrf3-2Ra allele to mitigate the negative effect of the Ddw1 allele on productivity traits (TGW, grain number per spike) is promising for the development of new semi-dwarf varieties of spring triticale with increased productivity.

As a result of our experiment, ScGrf3-2Ra demonstrated the ability to reduce plant height in spring triticale. Thus, in the breeding of semi-dwarf Ddw1-carrying triticale
varieties, which are resistant to lodging and are demanded for intensive cultivation technologies, ScGrf3-2Ra not only maintains the dwarf stature, but can also decrease it; however, this supplementary dwarfing effect may be disguised by Ddw1.

One of Ddw1’s drawbacks, both in rye and triticale, is reported to be the delay in heading and flowering [51]. Accelerated transition from the vegetative to the generative phase may be possible in water-deficit and hot regions to escape summer drought and produce large-filled grain. In addition, the earliness per se helps avoid the rain and fog period at harvest, leading to pre-harvest sprouting. In our study, semi-dwarf plants (Ddw1 Ddw1) carrying ScGrf3-2Ra headed and flowered earlier than those with ScGrf3-2Rb, which suggests the possibility of its use in breeding new varieties of spring triticale.

In the present study, a molecular STS allelic-specific marker was developed that can be applied for selecting desirable ScGrf3-2R alleles in breeding programs of triticale and rye to improve their agronomic valuable traits.

The ScGrf3-2R gene is homologous to wheat and rice Grf3 genes, which are characterized by increased expression in the stem and flowers. The TaGRF3 gene and its homologs TaGRF15 and TaGRF23 in bread wheat have a higher level of expression in the stem and spike than in other organs [52,53]. In rice, OsGRF3 represses the promoter of the KNOX gene Oskn2, and the latter is expressed during shoot, inflorescence, and floral development [39,54]. OsGRF3 also serves as a transcription factor for the OsbHLH35 gene, which regulates the development of anthers [55]. In our study, polymorphism in ScGrf3-2R was found to be associated with phenotypic differences in the parameters of stem (plant height) and spike (time of heading and flowering, number and weight of grains per spike) in triticale. This suggests that ScGrf3 may also participate in the regulation of plant height, reproductive organ development, and time of heading and flowering in triticale.

The Ddw1 and Rht12 genes are probable orthologues [56,57]; the GA2oxidase genes are colocalized with these genes [56,58], and this suggests that their dwarfism probably works in the same way and is associated with the work of this enzyme. Therefore, it would be interesting to compare their effects on triticale traits, although keeping in mind that the experiments in different studies were performed under different conditions and genetic background. In this and our previous studies, plant height was decreased by 30–37% due to Ddw1 and in the study of Hao et al. [59] by 37.7% due to Rht12, which demonstrates their similar effects on plant height. Productive traits in triticale are affected negatively by both genes but less dramatically by Ddw1 compared to Rht12: spikelet number per spike was reduced by 0–2% due to Ddw1 vs. 12.8% due to Rht12, grain number per spike decreased by 0–3% vs. 25.1%, and thousand-grain weight was reduced by 9–14% vs. 14.5%. Moreover, harvest index increased by 5–6% due to Ddw1 and decreased by 16% due to Rht12 [24,25,27]. It can be preliminarily concluded that the Ddw1 gene is more beneficial for breeding of semi-dwarf triticale than Rht12, although additional direct comparison studies are required.

The latest data reveal a trend in which the height of triticale plants decreased between 1982 and 2010, and since 2011 there has been an increase in the share of higher genotypes in the cultivar market. This can be explained by an increase in demand for forage triticale varieties with high biomass yield, as plant height is one of the main factors affecting biomass yield [8]. The results of our research demonstrate that incorporation of selection for ScGrf3-2Ra in breeding schemes potentially can help to increase the supply of semi-dwarf grain triticale varieties in the current market. The same analysis of breeding trends showed that developmental stages did not change over time in triticale cultivars, but there was a slight trend towards earlier heading in later varieties [8]. The selection for ScGrf3-2Ra may help to strengthen this trend and contribute to the development of cultivars with earlier heading.

Therefore, our findings may be useful for the development of more productive earlier semi-dwarf grain triticale varieties. These varieties could help promote this crop to be among the top-four grains, along with wheat, rice, and corn, which will allow us to appreciate the benefits of triticale in terms of sustainable agriculture and functional food.
4. Materials and Methods

4.1. Plant Material

The varieties of winter rye Novaya Era (N.I. Vavilov All-Russian Institute of Plant Industry, St. Petersburg) and Saratovskaya 7 (Federal Agrarian Scientific Center of the South-East, Saratov), and varieties of hexaploid spring triticale Dublet (Danko Hodowla Roslin, Poland) and Khongor winter triticale (P.P. Lukyanenko National Grain Center, Krasnodar) were used for partial sequencing of the ScGrf3-2R gene.

The progenies of the F3 plants of the Valentin 90 (Ddw1 Ddw1 ScGrf3-2Rb ScGrf3-2Rb) x Dublet (ddw1 ddw1 ScGrf3-2Ra ScGrf3-2Ra) intercross were used as material for studying the effect of the ScGrf3-2R gene on agronomical traits, in interaction with the Ddw1 dwarfing gene.

During 2016–2017, generations F2 and F3 were grown in the field under spring sowing with mass selection of spring forms. In 2018–2019, field experiments of homogeneous spring-type families were carried out. Thus, 121 lines were created and used in this study.

4.2. Field Conditions

The field experiment was carried out in 2018 and 2019 at the field experimental station of Russian State Agrarian University—Moscow Timiryazev Agricultural Academy (55°50′30.8″ N 37°33′24.1″ E, Moscow, Russia). The seeds of the F4 and F5 generations were sown in the first week of May in 2018 and 2019, respectively, on single-row plots 1 m long with a row spacing of 30 cm. In general, 2019 was more favorable for the spring triticale yield; in July of 2018 there was an increased amount of precipitation compared to 2019, but it was within the climatic norm (according to ten-year data) (Table 2) [60]. An increased level of precipitation during the anthesis stage, which took place in the first week of July for some lines, could reduce the grain set, while during grain filling and ripening stages, it could favor disease development such as root rot, Septoria, and Fusarium head blight [61].

Table 2. The weather conditions in Moscow during field experiments.

| Month | Amount of Precipitation, mm | Norm, mm | Average Temperature, °C | Norm, °C |
|-------|-----------------------------|----------|--------------------------|---------|
|       | 2018 | 2019 | 10 Years | 2018 | 2019 | 10 Years |
| May   | 44   | 58   | 61       | 16.1 | 16.2 | 13.6 |
| June  | 54   | 55   | 78       | 17.2 | 19.6 | 17.3 |
| July  | 85   | 64   | 84       | 20.3 | 16.7 | 19.7 |
| August| 20   | 48   | 78       | 19.8 | 16.4 | 17.6 |

4.3. Analysis of Valuable Agronomic Traits

The study of agronomical traits was carried out in 10 individual plants from each hybrid line, F4 and F5. The date of the heading and flowering was determined by the onset of the corresponding stage in at least 80% of the plants in the row. Plant height was measured at the main shoot from the tillering node to the top of the spike except for the awns. The length of the main spike and each internode, the number of internodes, the spikelet number, grain number and grain weight in the main spike, and the stem and spike weight were measured in the main shoot. In the plant as a whole, the number of spikes per plant (productive tillering) was determined, in addition to the weight of grain in the secondary shoots (tillers). The thousand-grain weight (TGW) was determined as the thousand-fold ratio of the grain weight from the main spike to the number of grains from the main spike. Spike compactness was defined as the ten-fold ratio of the total number of spikelets in the main spike to the length of the main spike. The harvest index (HI) was calculated as the ratio of the grain mass per plant to the total mass of the plant at harvest.

A total of 1716 and 1794 individual plants were analyzed in 2018 and 2019, respectively.
4.4. DNA Extraction, PCR and Sequencing

Plant genomic DNA was extracted from dried leaves according to the protocol using cetyltrimethylammonium bromide (CTAB) [62]. The expected rye ScGrf3-2R gene sequence was found in the Lo7 reference rye genome assembly using BLAST (Nucleotide-Nucleotide BLAST 2.8.1+) [63] and the TaGrf3-2A (TraesCS2A02G435100) gene sequence from wheat [64]. The boundaries of exons and introns of the gene were predicted using the AUGUSTUS online service (https://bioinf.uni-greifswald.de/augustus/submission.php, accessed on 3 April 2022) [65]. Primers for amplification of large gene fragments were selected using the Primer-BLAST (NCBI) online service. We amplified the region of the 3rd intron and the 4th exon of the ScGrf3-2R gene in the studied accessions of triticale and rye using the primers designed (GRF-2R_107L: TTCTGGGTCCATATTTTAGCCCG, GRF-2R_107R: CAGCTCACAGACACGTTTGTAC). The PCR was performed in 25 µL reaction volumes containing 70 mM Tris–HCl buffer (pH 9.3), 16.6 mM (NH₄)₂SO₄, 2.5 mM MgCl₂, 0.2 mM each dNTP, 30 pM forward and reverse primers (Syntol, Moscow, Russia), 0.04 U/µL LR (long reading) Plus polymerase (Sileks, Moscow, Russia), 0.02 U/µL Taq polymerase (Sileks), and 4 ng/µL DNA template; amplification was performed on a Bio-Rad T100 (Hercules, California, USA). The PCR conditions were as follows: (1) 95 °C—10 min, (2) 45 cycles 95 °C—30 s, 60 °C—30 s, 72 °C—4 min; (3) final elongation 72 °C—10 min.

PCR products were separated in 1.5% agarose gel with TBE buffer (90 mM Tris-HCl, pH 8.3, 90 mM boric acid, 0.1 mM EDTA), using a GeneRuler 100 bp Plus DNA size standard (Thermo Fisher Scientific, Waltham, Massachusetts, USA) in Bio-Rad Sub-Cell horizontal electrophoresis chamber in conjunction with a Power Pac Basic, Bio-Rad power supply. The gels were stained with ethidium bromide and visualized using the Gel Doc XR+ system (Bio-Rad Laboratories, Hercules, California, USA) under ultraviolet light.

PCR products were sequenced by fragments using the Illumina MiSeq new generation platform at LLC «Genomed» (Moscow, Russia). DNA libraries were prepared using the Swift 2S Turbo DNA Library Kit (Swift Biosciences, USA), with the amplicons labeled with DNA barcodes. Sequence assembly from the studied sequences was carried out as described earlier, combining de novo assembly with alignment of contigs to the reference genome region [66]. A preview of the alignment was performed using the Integrative Genomics Viewer 2.12.2 [67]. The resulting sequences were compared with each other using GeneDoc 2.7 software [68]. The translation of the protein-coding nucleotide sequences into amino acids was carried out using the GeneDoc 2.7 program. The prediction of the functional significance of the detected amino acid substitutions in the protein was carried out using the PROVEAN online service [69]. Conserved domains of the proteins were annotated by searching the Conserved Domain Database (NCBI) [70].

4.5. Molecular Markers

The allelic state of the Ddw1 gene was determined using primers for the REMS1218 microsatellite sequence (F: 5'CGCACAAACAAAAACACGAC-3', R: 5'CACAACAAACACGACGTGAC-3') [71] and subsequent fragment analysis on an Applied Biosystems™ 3130 genotyping analyzer (USA). Amplification conditions were as follows: (1) 94 °C—5 min, (2) 35 cycles 94 °C—30 s, 60 °C—30 s, 72 °C—1 min; (3) final elongation 72 °C—5 min.

To determine the haplotypes of the ScGrf3-2R gene, we used the STS marker designed by us. PCR with primers SCGrf3-2R-HF: CCTGCTTTAAATGTGCAGCAAC, SCGrf3-2R-HR: AGACTTGCAGCATAGTGACCAA was carried out in a volume of a 25 µL mixture containing 70 mM Tris–HCl buffer (pH 9.3), 16.6 mM (NH₄)₂SO₄, 2.5 mM MgCl₂, 0.2 mM each dNTP, 30pM forward and reverse primers (Syntol, Moscow, Russia), 0.05 U/µL Taq stained polymerase (Sileks, Moscow, Russia), 4 ng/µL DNA template. PCR conditions were as follows: (1) 95 °C for 10 min, (2) 36 cycles for 95 °C—30 s, 60 °C—30 s, 72 °C—1 min; and (3) final elongation at 72 °C for 10 min; amplification was performed on a Bio-Rad T100 (USA). PCR products were separated in a Bio-Rad Sub-Cell horizontal electrophoresis chamber in conjunction with a Power Pac Basic, Bio-Rad power supply (USA) in a 2%
agarose gel with TBE buffer (Tris, boric acid, EDTA) in the presence of the molecular weight marker M-100 (Syntol, Moscow, Russia), stained with ethidium bromide and visualized using the Gel Doc XR+ system (Bio-Rad Laboratories, Hercules, California, USA). The expected sizes of amplicons are 220 and 180 bp.

4.6. Statistical Analysis

Statistical analysis was performed using R 4.1.2 [72]. A two-way ANOVA was performed using the car 3.1-0 package [73]. False Discovery Rate (FDR) p-value correction of 0.05 was used to identify false positive significant patterns in ANOVA. Pairwise comparisons were performed using the Tukey criteria from the agricolae 1.3-5 package [74]. Only plants homozygous for both studied genes, Ddw1 and ScGrf3-2R, were analyzed. Pairwise comparison of mean values carried out in one-way analysis between homozygous genotypes Ddw1, Ddw1 (short plants) and ddw1, ddw1 (tall plants), ScGrf3-2Ra, ScGrf3-2Ra and ScGrf3-2Rb, ScGrf3-2Rb, in two-way analysis—between dihomozygous genotypes, Ddw1 Ddw1 ScGrf3-2Ra ScGrf3-2Ra, Ddw1 Ddw1 ScGrf3-2Rb ScGrf3-2Rb, ddw1 ddw1 ScGrf3-2Ra ScGrf3-2Ra, and ddw1 ddw1 ScGrf3-2Rb ScGrf3-2Rb. Graphs of agronomical traits depending on the genotype were constructed using the ggplot2 3.3.6 package [75]. The significance of the difference between the means was estimated at the 95% confidence level.

5. Conclusions

In our work, we developed and tested a molecular STS allelic-specific marker that enables distinguishing the allelic state of the ScGrf3-2R gene. In our two-year field experiments in the Non-Chernozem zone, we showed a statistically significant effect of the ScGrf3-2R allelic state on important agronomic valuable traits, such as grain number and weight in main spike, thousand-grain weight, and heading and flowering time in semi-dwarf (Ddw1 Ddw1) spring triticale plants. While maintaining the semi-dwarf phenotype, ScGrf3-2R can partially compensate for the negative effects of Ddw1 on yield-related traits.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/plants11223032/s1, Table S1: Mean values of plant traits measured in spring triticale recombinant lines derived from cross Valentin90 x Dublet based on 2018–2019 field experiment, one-way analysis of variance and Tukey’s test; Table S2: Mean values of plant traits measured in spring triticale recombinant lines derived from cross Valentin90 x Dublet based on 2018-2019 field experiment, two-way analysis of variance and Tukey’s test.

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

Funding: This research was funded by Russian Foundation for Basic Research grant № 20-316-90046 and Russian State Task 0431-2022-0007.

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. Oettler, G. The fortune of a botanical curiosity—Triticale: Past, present and future. J. Agric. Sci. 2005, 143, 329–346. [CrossRef]
2. FAO. Triticale Improvement and Production. Available online: https://www.fao.org/3/y5553e/y5553e00.htm (accessed on 8 August 2022).
3. Leonova, S.; Badamshina, E.; Koschchina, E.; Kalugina, O.; Gareeva, I.; Leshchenko, N. Triticale flour in bakery and rusk products. Food Sci. Technol. Int. 2021, 28, 524–534. [CrossRef]
4. Bielski, S.; Romanekas, K.; Novikova, A.; Sarasusks, E. Are Higher Input Levels to Triticale Growing Technologies Effective in Biofuel Production System? Sustainability 2019, 11, 5915. [CrossRef]
5. Zhu, F. Triticale: Nutritional composition and food uses. Food Chem. 2018, 241, 468–479. [CrossRef]
6. Dreccer, M.; Condon, A.; Macdonald, B.; Rebetzke, G.; Awasi, M.; Borgognone, M.; Peake, A.; Piñera-Chavez, F.; Hundt, A.; Jackway, P.; et al. Genotypic variation for lodging tolerance in spring wheat: Wider and deeper root plates, a feature of low lodging, high yielding germplasm. *Field Crops Res.* 2020, 236, 107942. [CrossRef]

7. Hura, T.; Dziurka, M.; Hura, K.; Óstrowska, A.; Dziurka, K.; Gadzinowska, J. Wheat and rye genome confer specific phytohormone profile features and interplay under water stress in two phenotypes of triticale. *Plant Physiol. Biochem.* 2017, 118, 494–509. [CrossRef]

8. Trini, J.; Maurer, H.; Neuwieder, J.; Würschum, T. Identification and Fine-Mapping of Quantitative Trait Loci Controlling Plant Height in Central European Winter Triticale (*Triticosecale Wittmack*). *Plants* 2021, 10, 1592. [CrossRef]

9. Jatayev, S.; Sukhikh, I.; Vavilova, V.; Smolenskaya, S.; Goncharov, N.; Kurishbayev, A.; Zotova, L.; Absattarova, A.; Serikbay, D.; Hu, Y.; 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]

10. Sukhikh, I.; Vavilova, V.; Binov, A.; Goncharov, N. Diversity and Phenotypical Effect of Allelic Variants of *Rht* Dwarfing Genes in Wheat. *Russ. J. Genet.* 2021, 57, 127–138. [CrossRef]

11. Dowla, M.; Islam, S.; Stefanova, K.; Hara, G.; Ma, W.; Edwards, I. Phenology and Dwarfing Gene Interaction Effects on the Adaptation of Selected Wheat (*Triticum aestivum* L.) Advanced Lines across Diverse Water-Limited Environments of Western Australia. *Agriculture* 2020, 10, 470. [CrossRef]

12. McIntosh, R.A.; Yamazaki, Y.; Dubcovsky, J.; Rogers, J.; Morris, C.; Appels, R. Catalogue of Gene Symbols for Wheat. In *Proceedings of the 12th International Wheat Genetics Symposium, Yokohama, Japan, 8–13 September 2013*; pp. 1–31.

13. McIntosh, R.A.; Yamazaki, Y.; Dubcovsky, J.; Rogers, J.; Morris, C.; Appels, R. Catalogue of Gene Symbols for Wheat. Available online: https://Shigen.Nig.Ac.jp/Wheat/Komugi/Genes/Macgene/Supplement2017.Pdf (accessed on 6 June 2019).

14. Ganeva, G.; Keszun, V.; Landjeva, S.; Tsenov, N.; Atanassova, M. Identification, distribution and effects on agronomic traits of the semi-dwarfing *Rht* alleles in Bulgarian common wheat cultivars. *Euphytica* 2005, 145, 305–315. [CrossRef]

15. Chapman, S.; Mathews, K.; Trehthowan, R.; Singh, R. Relationships between height and yield in near-isogenic spring wheats that contrast for major reduced height genes. *Euphytica* 2006, 157, 391–397. [CrossRef]

16. Butler, J.; Byrne, P.; Mohammadi, V.; Chapman, P.; Haley, S. Agronomic Performance of *Rht* Alleles in a Spring Wheat Population across a Range of Moisture Levels. *Crop Sci.* 2005, 45, 939–947. [CrossRef]

17. Li, X.; Lan, S.; Liu, Y.; Gale, M.; Worland, T. Effects of different *Rht-B1b*, *Rht-D1b* and *Rht-B1c* dwarfing genes on agronomic characteristics in wheat. *Cereal Res. Commun.* 2006, 34, 919–924. [CrossRef]

18. Mathews, K.; Chapman, S.; Trehthowan, R.; Singh, R.; Crossa, J.; Pfeiffer, W.; Ginkel, M.; DeLacy, I. Global Adaptation of Spring Bread and Durum Wheat Lines Near-isogenic for Major Reduced Height Genes. *Crop Sci.* 2006, 46, 603–613. [CrossRef]

19. Rebetzke, G.; Bonnett, D.; Ellis, M. Combining gibberellin acid-sensitive and insensitive dwarfing genes in breeding of higher-yielding, sesqui-dwarf wheats. *Field Crops Res.* 2012, 127, 17–25. [CrossRef]

20. Rebetzke, G.; Ellis, M.; Bonnett, D.; Mickelson, B.; Condon, A.; Richards, R. Height reduction and agronomic performance for selected gibberellin-responsive dwarfing genes in bread wheat (*Triticum aestivum* L.). *Field Crops Res.* 2012, 126, 87–96. [CrossRef]

21. Liu, Y.; Zhang, J.; Hu, Y.; Chen, J. Dwarfing genes *Rht4* and *Rht-B1B* affect plant height and key agronomic traits in common wheat under two water regimes. *Field Crops Res.* 2017, 204, 242–248. [CrossRef]

22. Gadzieliewska, A.; Milczarski, P.; Molik, K.; Pawlowska, E. Identification and mapping of a new recessive dwarfing gene *dw9* on the 6RL rye chromosome and its phenotypic effects. *PLoS ONE* 2020, 15, e0229564. [CrossRef]

23. Divashuk, M.; Litvinov, D.; Chernook, A.; Karlov, G.; Bashenov, M. Effect of allelic forms of GRF genes on the development of common wheat under different conditions of nitrogen supplementation. *Plant Genet. Genom. Bioinform. Biotechnol.* 2021, 53. [CrossRef]

24. Kroupin, P.; Chernook, A.; Karlov, G.; Soloviev, A.; Divashuk, M. Effect of Dwarfing Gene *Ddvd1* on Height and Agronomic Traits in Spring Triticale in Greenhouse and Field Experiments in a Non-Black Earth Region of Russia. *Plants* 2019, 8, 131. [CrossRef] [PubMed]

25. Kroupin, P.; Chernook, A.; Karlov, G.; Solovev, A.; Korshunova, A.; Divashuk, M. Effects of Dwarfing Wheat (*Triticum aestivum* L.) And Rye (*Secale cereale* L.) Genes in Spring Triticale Segregating Population as Studied in Pot Trials. *Sel’skokhozyaistvennaya Biol.* 2019, 54, 920–933. [CrossRef]

26. Kroupin, P.; Chernook, A.; Bazhenov, M.; Karlov, G.; Goncharov, N.; Chikida, N.; Divashuk, M. Allele mining of *TaGRF-2D* gene 5'-UTR in *Triticum aestivum* and *Aegilops tauschii* genotypes. *PLoS ONE* 2020, 15, e0231704. [CrossRef] [PubMed]

27. Chernook, A.; Kroupin, P.; Karlov, G.; Soloviev, A.; Korshunova, A.; Rubets, V.; Ignin, V.; Divashuk, M. Effects of *Rht-B1B* and *Ddvd1* Dwarfing Genes in Two Connecting Populations of Spring Triticale under Greenhouse Experiment Conditions. *Agriculture* 2019, 9, 119. [CrossRef]

28. Tikhonenko, N.; Tsivetkova, N.; Voylokov, A. The Effect of Parental Genotypes of Rye Lines on the Development of Quantitative Traits in Primary Octoploid Triticale: Plant Height. *Russ. J. Genet.* 2003, 39, 52–56. [CrossRef]

29. Khlestkina, E.; Shvachko, N.; Zavzarzin, A.; Börner, A. Vavilov’s Series of the “Green Revolution” Genes. *Russ. J. Genet.* 2020, 56, 1371–1380. [CrossRef]

30. Banaszk, Z. Breeding of Triticale in DANKO. Materials of the 61st Meeting Association of Breeders and Seed Growers Austria. *Raumberg Gumpenstein* 2011, 61, 65–68.
Plants 2022, 11, 3032

31. Ittu, G.; Saulescu, N.; Ittu, M.; Mustatea, P. Present and Perspectives in Romanian Triticale Breeding Program. Commun. Agric. Appl. Sci. 2014, 79, 181–191. [PubMed]

32. van der Knaap, E.; Kim, J.; Kende, H. A Novel Gibberellin-Induced Gene from Rice and Its Potential Regulatory Role in Stem Growth. Plant Physiol. 2000, 122, 695–704. [CrossRef]

33. Kim, J.; Choi, D.; Kende, H. The AtGRF family of putative transcription factors is involved in leaf and cotyledon growth in Arabidopsis. Plant J. 2003, 36, 94–104. [CrossRef]

34. Lee, G.; Lee, B.; Jung, J.; Lee, S.; Mai, T.; Kim, J. Systematic Assessment of the Positive Role of Arabidopsis thaliana growth-regulating factors in Regulation of Cell Proliferation During Leaf Growth. J. Plant Biol. 2022. [CrossRef]

35. Zhang, D.; Li, B.; Jia, G.; Zhang, T.; Dai, J.; Li, J.; Wang, S. Isolation and characterization of genes encoding GRF transcription factors and GRF transcriptional coactivators in Maize (Zea mays L.). Plant Sci. 2008, 175, 809–817. [CrossRef]

36. Choi, D.; Kim, J.; Kende, H. Whole Genome Analysis of the OsGRF Gene Family Encoding Plant-Specific Putative Transcription Activators in Rice (Oryza sativa L.). Plant Cell Physiol. 2004, 45, 897–904. [CrossRef] [PubMed]

37. Debernardi, J.; Meccia, M.; Vercruysse, L.; Smaczniak, C.; Kaufmann, K.; Inze, D.; Rodriguez, R.; Palatnik, J. Post-transcriptional control of GRF transcription factors by microRNA miR396 and GIG co-activator affects leaf size and longevity. Plant J. 2014, 79, 413–426. [CrossRef]

38. Horiguchi, G.; Kim, G.; Tsukaya, H. The transcription factor AtGRF5 and the transcription coactivator AN3 regulate cell proliferation in leaf primordia of Arabidopsis thaliana. Plant J. 2005, 43, 68–78. [CrossRef] [PubMed]

39. Kuijt, S.; Greco, R.; Agalou, A.; Shao, J.; ‘t Hoen, C.; Overmàs, E.; Osnato, M.; Curiale, S.; Meynard, D.; van Gulik, R.; et al. Interaction between the growth-regulating factor and knotted1-like homeobox families of transcription factors. Plant Physiol. 2014, 164, 1952–1966. [CrossRef] [PubMed]

40. Che, R.; Tong, H.; Shi, B.; Liu, Y.; Fang, S.; Liu, D.; Xiao, Y.; Hu, B.; Liu, L.; Wang, H.; et al. Control of grain size and rice yield by GL2-mediated brassinosteroid responses. Nat. Plants 2015, 2, 1–8. [CrossRef]

41. Duan, P.; Ni, S.; Wang, J.; Zhang, B.; Xu, R.; Wang, Y.; Chen, H.; Zhu, X.; Li, Y. Regulation of OsGRF4 by OsmiR396 controls grain size and yield in rice. Nat. Plants 2015, 2, 15203. [CrossRef]

42. Li, S.; Gao, F.; Xie, K.; Zeng, X.; Cao, Y.; Zeng, H.; He, Z.; Ren, Y.; Li, W.; Deng, Q.; et al. The OsmiR396c-OsGRF4-OsGIF1 regulatory module determines grain size and yield in rice. Plant Biotechnol. J. 2016, 14, 2134–2146. [CrossRef]

43. Sun, P.; Zhang, W.; Wang, Y.; He, Q.; Shu, F.; Liu, H.; Wang, J.; Wang, J.; Yuan, L.; Deng, H. OsGRF4 controls grain shape, panicle length and seed shattering in rice. J. Integr. Plant Biol. 2016, 58, 836–847. [CrossRef]

44. Nelissen, H.; Eeckhout, D.; Demuyrrck, K.; Persiau, G.; Walton, A.; van Bel, M.; Vervoort, M.; Candaeele, J.; De Block, J.; Aesaert, S.; et al. Dynamic changes in angustifolia3 complex composition reveal a growth regulatory mechanism in the maize leaf. Plant Cell 2015, 27, 1605–1619. [CrossRef] [PubMed]

45. Zhang, J.; Zhou, Z.; Bai, J.; Tao, X.; Wang, L.; Zhang, H.; Zhu, J. Disruption of MIR396e and MIR396f improves rice yield under nitrogen-deficient conditions. Natl. Sci. Rev. 2019, 7, 102–112. [CrossRef] [PubMed]

46. Huang, W.; He, Y.; Yang, L.; Lu, C.; Zhu, Y.; Sun, C.; Ma, D.; Yin, J. Genome-wide analysis of growth-regulating factors (GRFs) in Triticum aestivum. PeerJ 2021, 9, e10701. [CrossRef]

47. Divashuk, M.; Chernook, A.; Kroupina, A.; Vukovic, M.; Karlov, G.; Ermolaev, A.; Shrinin, S.; Avdeev, S.; Igonin, V.; Pylnev, V.; et al. TaGRF3-2A Improves Some Agronomically Valuable Traits in Semi-Dwarf Spring Triticale. Plants 2021, 10, 2012. [CrossRef]

48. Bazhenov, M.; Chernook, A.; Bespalova, L.; Gritsay, T.; Polevikhova, N.; Karlov, G.; Nazarova, L.; Divashuk, M. Alleles of the GRF3-2A Gene in Wheat and Their Agronomic Value. Int. J. Mol. Sci. 2021, 22, 12376. [CrossRef]

49. Cannarozzi, G.; Schraudolph, N.; Faty, M.; von Rohr, P.; Friberg, M.; Roth, A.; Gonnet, P.; Gonnet, G.; Barral, Y. A Role for Codon Order in Translation Dynamics. Cell 2010, 141, 355–367. [CrossRef]

50. Morello, L.; Breviario, D. Plant Spliceosomal Introns: Not Only Cut and Paste. Curr. Genom. 2008, 9, 227–238. [CrossRef]

51. Kalih, R.; Maurer, H.; Hackauf, B.; Miedaner, T. Effect of a rye dwarfing gene on plant height, heading stage, and Fusarium head blight in triticale (× Triticeae Wittmack). Theor. Appl. Genet. 2014, 127, 1527–1536. [CrossRef]

52. Zhang, J.; Li, Z.; Jin, J.; Xie, X.; Zhang, H.; Chen, Q.; Luo, Z.; Yang, J. Genome-wide identification and analysis of the growth-regulating factor family in tobacco (Nicotiana tabacum). Gene 2018, 639, 117–127. [CrossRef]

53. Du, W.; Yang, J.; Li, Q.; Su, Q.; Yi, D.; Pang, Y. Genome-Wide Identification and Characterization of Growth Regulatory Factor Family Genes in Medicago. Int. J. Mol. Sci. 2022, 23, 6905. [CrossRef]

54. Postma-Haarsma, A.D.; Rueb, S.; Scarpetta, E.; den Besten, W.; Hoge, J.C.; Meijer, A.H. Developmental Regulation and Downstream Effects of the Knox Class Homeobox Genes OsK2n and OsK3n from Rice. Plant Mol. Biol. 2002, 48, 423–441. [CrossRef] [PubMed]

55. Ortolan, F.; Fonini, L.; Pastori, T.; Mariath, J.; Saibo, N.; Margis-Pinheiro, M.; Lazzarotto, F. Tightly controlled expression of OsHBH3 is critical for ant her development in rice. Plant Sci. 2021, 302, 110716. [CrossRef] [PubMed]

56. Braun, E.-M.; Tsvetkova, N.; Rotter, B.; Siekmann, D.; Schwefel, K.; Krezdorn, N.; Plieske, J.; Winter, P.; Melz, G.; Volyokov, A.V.; et al. Gene Expression Profiling and Fine Mapping Identifies a Gibberellin 2-Oxidase Gene Co-segregating With the Dominant Dwarfing Gene Dd1 in Rye (Secale cereale L.). Front. Plant Sci. 2019, 10, 857. [CrossRef] [PubMed]

57. Korzun, V.; Röder, M.; Worland, A.J.; Börner, A.J.P.B. Intrachromosomal mapping of genes for dwarfing (Rht12) and vernalization response (Vrn1) in wheat by using RFLP and microsatellite markers. Plant Breed. 1997, 116, 227–232. [CrossRef]
58. Buss, W.; Ford, B.A.; Foo, E.; Schnippenkoetter, W.; Borrill, P.; Brooks, B.; Ashton, A.R.; Chandler, P.M.; Spielmeyer, W. Overgrowth mutants determine the causal role of gibberellin GA2oxidaseA13 in Rht12 dwarfism of wheat. *J. Exp. Bot.* 2020, 71, 7171–7178. [CrossRef] [PubMed]

59. Hao, J.; Zhao, Z.; Sun, N.; Zhi, L.; Qiao, P.; Amo, A.; Zotova, L.; Hu, Y.G.; Chen, L. Wheat dwarf genes Rht12 and Rht-B1b affected the performance of agronomic traits in hexaploid triticale. *Agron. J.* 2022, 114, 2147–2158. [CrossRef]

60. Weather Online UK–Current Weather and Weather Forecast Worldwide. Available online: https://www.weatheronline.co.uk/ (accessed on 8 August 2022).

61. Balyukova, K.S.; Psalom, P.I.; Sokolova, I.V. Diseases of Grain Crops. Available online: https://kubsau.ru/upload/iblock/43c/43c58f3dba16678882fc12245f92adab.pdf#page=85 (accessed on 1 June 2022).

62. Doyle, J. *DNA Protocols for Plants //Molecular Techniques in Taxonomy*; Springer: Berlin/Heidelberg, Germany, 1991; pp. 283–293. ISBN 978-3-642-83964-1.

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

64. Rabanus-Wallace, M.; Hackauf, B.; Mascher, M.; Lux, T.; Wicker, T.; Gundlach, H.; Baez, M.; Houben, A.; Mayer, K.; Guo, L.; et al. Chromosome-scale genome assembly provides insights into rye biology, evolution and agronomic potential. *Nat. Genet.* 2021, 53, 564–573. [CrossRef]

65. Stanke, M.; Morgenstern, B. AUGUSTUS: A web server for gene prediction in eukaryotes that allows user-defined constraints. *Nucleic Acids Res.* 2005, 33, W465–W467. [CrossRef]

66. Bazhenov, M.; Chernook, A.; Goncharov, N.; Chikida, N.; Belousova, M.; Karlov, G.; Divashuk, M. The Allelic Diversity of the Gibberellin Signaling Pathway Genes in *Aegilops tauschii* Coss. *Plants* 2020, 9, 1696. [CrossRef]

67. Robinson, J.; Thorvaldsdóttir, H.; Winckler, W.; Guttman, M.; Lander, E.; Getz, G.; Mesirov, J. Integrative genomics viewer. *Nat. Biotechnol.* 2011, 29, 24–26. [CrossRef] [PubMed]

68. Nicholas, K.B. GeneDoc: Analysis and Visualization of Genetic Variation. *Embnew News* 1997, 4, 14.

69. Feng, J.; Liu, T.; Qin, B.; Zhang, Y.; Liu, X. Identifying ChIP-seq enrichment using MACS. *Nat. Protoc.* 2012, 7, 1728–1740. [CrossRef] [PubMed]

70. Marinova, K.; Pourcel, L.; Weder, B.; Schwarz, M.; Barron, D.; Routaboul, J.; Debeaujon, I.; Klein, M. The *Arabidopsis* MATE Transporter TT12 Acts as a Vacuolar Flavonoid/H+-Antiporter Active in Proanthocyanidin-Accumulating Cells of the Seed Coat. *Plant Cell* 2007, 19, 2023–2038. [CrossRef] [PubMed]

71. Tenhola-Roininen, T.; Tanhuapää, P. Tagging the dwarfing gene *Ddw1* in a rye population derived from doubled haploid parents. *Euphytica* 2009, 172, 303–312. [CrossRef]

72. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2021; Available online: https://www.semanticscholar.org/paper/R%3A-A-language-and-environment-for-statistical-Team/659408b243ec55de8d1a3bc51b81173007a89b (accessed on 8 July 2022).

73. Fox, J.; Weisberg, S. *An R Companion to Applied Regression, 3rd ed*; Sage: Thousand Oaks, CA, USA, 2021; Available online: https://Socialsciences.Mcmaster.ca/Jfox/Books/Companion/ (accessed on 8 July 2022).

74. De Mendiburu, F. *Agricolae: Statistical Procedures for Agricultural Research*; R Package Version 1.3-5; R Foundation for Statistical Computing: Vienna, Austria, 2021; Available online: https://CRAN.R-Project.Org/Package=agricolae (accessed on 8 July 2022).

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