Allelic Variation Analysis at the Vernalization Response and Photoperiod Genes in Russian Wheat Varieties Identified Two Novel Alleles of Vrn-B3

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Abstract: Heading time is an important agronomic trait affecting the adaptability and productivity of common wheat. In this study, 95 common wheat varieties from Russia and the late-maturing breeding line ‘Velut’ were tested for allelic diversity of genes having the strongest effect on heading. In this research, allelic variation at the Ppd-D1, Vrn-A1, Vrn-B1, Vrn-D1, and Vrn-B3 loci was tested. The Vrn-B1 and Vrn-B3 loci provided the largest contribution to genetic diversity. We found two novel allelic variants of the Vrn-B3 gene in the studied varieties. Ten varieties carried a 160 bp insertion in the promoter region, and the breeding line ‘Velut’ carried a 1617 bp insertion. These alleles were designated Vrn-B3c and Vrn-B3d, respectively. The analysis of the sequences showed the recent insertion of a retrotransposon homologous to the LTR retrotransposon (RLX_Hvul_Dacia_RND-1) in the Vrn-B3d allele. Plants with the Vrn-B3c and the ‘Velut’ line with the Vrn-B3d allele headed later than the plants with the wild-type allele; among these plants, ‘Velut’ is the latest maturing wheat variety. Analysis of the gene expression of two groups of lines differing by the Vrn-B3 alleles (Vrn-B3d or vrn-B3) from the F2 population with ‘Velut’ as a parental line did not reveal a significant difference in the expression level between the groups. Additional research is required to study the reasons for the late maturation of the ‘Velut’ line. However, the studied wheat varieties could be used as a potential source of natural variation in genes controlling heading times.

Keywords: common wheat; Vrn and Ppd genes; heading time; flowering locus T

1. Introduction

The yield potential of wheat largely depends on the duration of each of the developmental phases. The heading time of wheat is mainly determined by the allelic composition of vernalization (Vrn) and photoperiod response (Ppd-1) genes [1]. The Vrn-B3 gene (also known as TaFT1) is the central integrator of the vernalization and photoperiod pathways [2]. Under the control of the Vrn-1, Vrn-2, and Ppd-1 genes, Vrn-B3 is expressed in leaves and then moves to the shoot apical meristem and promotes flowering [2–4].

The Vrn-1 genes controlling the wheat vernalization requirement were mapped to homologous chromosome group 5 [5]. The presence of at least one dominant Vrn-1 allele in hexaploid wheat results in spring growth habit. The dominant Vrn-A1 is epistatic to Vrn-B1 and Vrn-D1, which are associated with a slight vernalization response [6]. Genetic variation at the Vrn-1 loci is one of the most important factors affecting heading time in wheat. Plants with the dominant Vrn-A1 allele flower earlier than those with dominant Vrn-D1 or Vrn-B1 [7]. The dominant alleles at the Vrn-A1 locus are characterized by molecular variations (insertions and/or deletions) within the promoter region or by large intron deletions [8,9]. The dominant Vrn-B1 and Vrn-D1 alleles are also associated with deletions.
within the first intron [9]. Studies of the Vrn-1 composition of Russian spring wheat showed that the combination of the vernalization-insensitive allele Vrn-A1a with the dominant Vrn-B1a or Vrn-B1c allele was the most frequent [10]. These two dominant alleles were found to be equally frequent: 47.6% of the examined varieties had the Vrn-B1a allele, and 40.4% had the Vrn-B1c allele. In contrast, in the analysis of 245 European spring wheat varieties, the Vrn-B1c allele was detected only in 16 varieties, mainly from Eastern European countries (Shcherban et al. 2015).

The sensitivity of wheat to photoperiod is mainly affected by Ppd-1 genes, localized on 2A (Ppd-A1), 2B (Ppd-B1), and 2D chromosomes (Ppd-D1) [11]. Plants with the dominant Ppd-1 are insensitive to the day length, which results in early heading under conditions of both short and long days. Worland (1998) reported that the Ppd-D1 gene has the strongest effect. The dominant Ppd-D1a allele with a 2 kb deletion upstream of the coding region is associated with photoperiod insensitivity. Photoperiod-insensitive alleles predominate at southern latitudes, while wheat varieties grown in northern regions are usually sensitive to the photoperiod [12,13]. The presence of photoperiod-sensitive alleles significantly delays heading, especially under short-day conditions [14].

The Vrn-B3 gene (TaFT1) is a key gene acting as an integrator of several autonomous pathways. The common wheat Vrn-B3 gene mapped on chromosome 7B is homologous to the Arabidopsis FT1 gene [2]. FT-like genes belong to the PEBP gene family and control flowering time in dicot and monocot plants [15]. There are four allelic variations that have been characterized, including the recessive vrn-B3 allele. The dominant Vrn-B3a allele contains a 5295 bp retrotransposon insertion 591 bp upstream of the coding sequence [2]. The Vrn-B3c allele differs from Vrn-B3a by two small deletions (4 bp and 20 bp) inside the retrotransposon region [16]. Both alleles have similar levels of expression. The other dominant allele, designated Vrn-B3b, contains an insertion of 890 bp and is characterized by a lower expression level compared with the wild-type allele, associated with a delay in heading. At present, the natural variation of the Vrn-B3 gene among Russian common wheat has not been sufficiently investigated, as most of the studies have focused on allelic variation at the Vrn-1 and Ppd-1 genes.

In this research, 95 spring common wheat varieties adapted to Siberian environments were tested for their allelic composition at the Ppd-D1, Vrn-A1, Vrn-B1, Vrn-D1, and Vrn-B3 loci, which presumably are the main genetic factors involved in heading time development.

2. Materials and Methods

2.1. Plant Materials

Ninety-five Russian common wheat varieties adapted to the Ural and Siberian region growing conditions were used (Table S1). Phenotyping was conducted in 2016 and 2017 at the experimental field of the Federal Research Centre Institute of Cytology and Genetics SB RAS in Novosibirsk. The late-ripening breeding line ‘Velut’ was also included in the analysis. The origin of the ‘Velut’ line is shown in the Figure S1 [17–20]. The F2 population used in this study was developed from a cross between wheat varieties with different Vrn-B3 alleles (‘Velut’ and Tobolskaya).

Varieties were sown in accordance with the systematic method of sample location in two replicates on plots of 1 m², 60 grains per replicate, and between-row spacing 25 cm. The soil in the field was leached chernozem. Heading time was measured as the number of days from seedling emergence to the date when 1/2 of spike emerged from the flag leaf.

2.2. PCR Amplification

Genomic DNA was extracted from 3 to 4 seedlings following a modified protocol described by Plaschke et al. [21].

Allelic variation at the Ppd-D1, Vrn-A1, Vrn-B1, Vrn-B3, and Vrn-D1 loci was determined using previously reported specific primers (Table 1). PCR amplification was performed as described in these papers. The 20 µL PCR mixture contained 2X HS-Taq
PCR-Color (Biolabmix, Novosibirsk, Russia), 0.25 μmoles each of the reverse and forward primers, DNA at a concentration of 5 ng/μL, and sterile water.

| Primers   | Sequence                                      | Gene Region         | Annealing Temp. °C | Allelic Variant | Product Size (bp) | Source |
|-----------|-----------------------------------------------|---------------------|---------------------|-----------------|-------------------|--------|
| Vrn1AF    | gaagggaaaaatctgctcg gaggaaaagcgaatggaag      | VRN-A1 promoter     | 60                  | vrn-A1          | 734               | [8]    |
| Vrn1-Int1R| gcaggaaatcgaatggaag                            |                     |                     | vrn-A1a         | 876 and 965       |        |
|           |                                               |                     |                     | vrn-A1b         | 714               |        |
| Ex1/C/F   | gttcacaaggctatggt tcatagccaaaaaaaagatgaga     | VRN-B1 intron-1     | 58                  | vrn-B1a         | 1091              | [9]    |
| Intr1/B/R3|                                               |                     |                     | vrn-B1b         | 1055              |        |
| Intr1/C/F |                                               |                     |                     | vrn-B1c         | 705               |        |
| Intr1/D/R3|                                               |                     |                     |                 |                   |        |
| Intr1/D/R4|                                               |                     |                     |                 |                   |        |
| Ppd-D1_F  | aqcttcctcactacagt gttgtttcacaacgaagagcc      | VRN-D1 intron-1     | 61                  | vrn-D1          | 997               | [9]    |
| Ppd-D1_R1 |                                               | cactggtgctgctgaatt  |                     |                 | 1671              |        |
| Ppd-D1_R2 |                                               |                     |                     |                 |                   |        |
| FT-B-INS-F| ctaatagccaaagcgggtgagctcctgtaaggctgactg      | VRN-B3 promoter     | 57                  | vrn-B3a         | 1200              | [2]    |
| FT-B-INS-R|                                               |                     |                     |                 |                   |        |
| FT-B-NOINS-F|                                             |                     |                     |                 |                   |        |
| FT-B-NOINS-R|                                            |                     |                     |                 |                   |        |
| TaFF-F3   | cagcagcaggggtgtag aagggttctcatacgctgtagag     | VRN-B3 promoter     | 57                  | vrn-B3          | 1140              | [2]    |
| TaFF-R3   |                                               | cctcctcgcgccgctag   |                     |                 |                   |        |
| TaFF-B1-F | gttctgctgctgctgctgaag gcgggttctcatacgctgtag | VRN-B3 exons 1 and 2| 60                  |                 | 72                | [2]    |
| TaFF-B1-R |                                               |                     |                     |                 |                   |        |
| FTPr-F    | cgaagcgggggttatattgaac gcaacatcgaagcagctag   | VRN-B3 promoter     | 60                  | vrn-B3          | 1384              | [23]   |
| FTPr-R    |                                               |                     |                     |                 | 1544              |        |
| Vrn-B3d-F |                                               |                     |                     |                 | 3001              |        |
| Vrn-B3d-R |                                               |                     |                     |                 |                   |        |

The PCR amplification conditions with the primers designed in this study (Table 1) were as follows:

1. FTPr-F//R: 94 °C for 4 min to denature the DNA, 35 cycles at 94 °C for 10–40 s, 61 °C for 30 s, and 68 °C for 2.5 min, followed by a final extension at 72 °C for 10 min. The reaction was performed with 2X LR HS-PCR-Color (Biolabmix, Novosibirsk, Russia);
2. Vrn-B3d-F//R: 95 °C for 5 min; 35 cycles of 95 °C for 45 s, 62 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 10 min;
3. Vrn-B3e-F//R: 95 °C for 5 min, 35 cycles of 95 °C for 15 s, 59 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 5 min.

2.3. DNA Sequencing and Analysis

PCR products were isolated from the 2% agarose gel using a Zymoclean™ Gel DNA Recovery Kit (ZymoResearch, Irvine, CA, USA) and directly sequenced using a BigDye Terminator v.3.1 kit (Applied Biosystems, Forster City, CA, USA). Sequencing products were analyzed using an ABI 3130XL Genetic Analyser at the SB RAS Genomics Core Facility. The sequence results were analyzed by Chromas Version 2.6.6. The sequences were aligned by BioEdit version 7.2 software. Annotation was performed using Unipro UGENE version 35, BlastN analysis, and the Transposable Elements Platform (TREP) database [24].
2.4. Gene Expression Analysis

Total RNA was extracted during two growth stages, GS15 (5 leaves unfolded) and GS31 (1st node) [25], from the leaf apices of the plants using an RNAasy Plant Mini Kit (QIAGEN, Hilden, Germany). RNA samples were then treated with an RNase-free DNase Set (QIAGEN, Germany). cDNA was synthesized using M-MuLV–RH Reverse Transcriptase (Biolabmix, Novosibirsk, Russia). Two previously reported primer sets (Table 1) were used for qRT-PCR analysis: TaFT-F3//R3 and TaFT-B1F//R. Amplification with elongation factor 1-alpha [26] and RNase L inhibitor-like protein [27] primers were used as internal controls. qRT-PCR was carried out using an Applied Biosystems Real-Time PCR System 7500 Fast (Applied Biosystems, Foster City, USA), and the reactions were performed with HS-qPCR Lo-ROX SYBR (2X) (Biolabmix, Novosibirsk, Russia), including three technical replicates per reaction.

3. Results

3.1. Allelic Composition of Vrn-1, Vrn-3, and Ppd-D1 Alleles in Common Russian Wheat Varieties

Ninety-five common wheat varieties and the ‘Velut’ line were characterized for the allelic composition of vernalization and photoperiod alleles (Table S1). Amplification of genomic DNA using Vrn1AF and Vrn1-INT1R primers (see Table 1) showed that only five varieties harbored the recessive vrn-A1 allele, and one variety had the Vrn-A1b allele. The remaining 90 varieties gave an 876 bp and 965 bp PCR product, indicating the presence of the Vrn-A1a allele.

Analysis of the Vrn-B1 genes showed that among the analyzed genotypes, recessive vrn-B1 was present in 23 varieties, 39 had the Vrn-B1a allele, and Vrn-B1c was found in 23 varieties. Additionally, six varieties were heterozygous vrn-B1/Vrn-B1a, two had Vrn-B1a/Vrn-B1c alleles, and three had vrn-B1/Vrn-B1c. The dominant Vrn-D1a allele was found only in two varieties, with one of them being heterozygous. The rest of the varieties carry the recessive vrn-D1 allele. Analysis of the Ppd-D1 genes showed that only one variety had the Ppd-D1a (photoperiod-insensitive) allele, and the remaining varieties had Ppd-D1b.

Genotyping with primer sets FT-B-INS-F//R indicated that only 2 out of 96 spring wheat varieties had the dominant Vrn-B3a allele (Kuibyshevskaya 2, Kazachka) (Table S1). PCR screening with the primer set FT-B-NOINS-F//R indicated that 82 wheat varieties had the recessive vrn-B3 allele (PCR fragment length 1140 bp), and 10 varieties (Altaiskaya 99, Novosibirskaya 29, Novosibirskaya 67, Novosibirskaya 89, Obskaya 14, Kantegirskaya 89, Aleksandrina, Mana 2, Maria, Omskaya 23) had a novel allele (PCR fragment length 1300 bp). Primer sets FT-B-NOINS-F//R failed to amplify a PCR product in the ‘Velut’ line.

Therefore, the newly specific primer set FTpr-F//R was designed based on sequences of DQ890165.1 (complete sequence of the Vrn-B3 gene, including the coding sequence and the promoter region of Triticum aestivum variety CS (Hope7B)), which covered a greater part of the promoter region and the first intron. Using these primers, a PCR product of approximately 3000 bp was obtained for the ‘Velut’ line and nearly 1500 bp for ten varieties with the Vrn-B3c allele (Figure 1). Varieties with the recessive vrn-B3 allele had a PCR product of approximately 1380 bp.

**Figure 1.** (A) PCR amplification using FTpr-F//R primers differentiating vrn-B3 (1384 bp) and Vrn-B3c (1500 bp) alleles; (B) PCR amplification with FTpr-F//R and the ‘Velut’ line.
3.2. Sequence Analysis of the Two Novel Vrn-B3 Alleles

The PCR fragment of approximately 3000 bp length obtained in the ‘Velut’ line was sequenced from both directions using an extra set of allele-specific primers described in Supplementary Table S2. Sequence analysis of the Vrn-B3 variant of ‘Velut’ indicated that a 1617 bp fragment was inserted 85 bp upstream of the start codon (Figure 2). The new Vrn-3 allele was designated Vrn-B3d (submitted to NCBI No: MZ576251). Analysis of the sequence was performed using the UniPro UGENE toolkit. It was found that the fragment inserted in the promoter has identical direct LTRs that are 316 bp in length. The Transposable Elements Platform (TREP) search revealed that the closest homolog was the unannotated *Hordeum vulgare* L. LTR retrotransposon RLX_Hvul_Dacia_RND-1.

![Figure 2](image-url)

**Figure 2.** Allelic variations of the Vrn-B3 gene identified in wheat varieties in this study. The white triangles represent insertions, the white rectangles represent exons, and the black arrows represent the transcriptional start.

The products of approximately 1500 bp obtained from varieties Altaiskaya 99, Novosibirskaya 29, Novosibirskaya 67, Novosibirskaya 89, Obskaya 14, Kantegirskaya 89, Aleksandrina, Mana 2, Maria, and Omskaya 23 were sequenced using the FTpr-F primer. Sequencing confirmed that all of these varieties contained an identical insertion of 160 bp located in the promoter region of the Vrn-B3 gene 881 bp upstream of the start codon (Figure 2). This allele was designated Vrn-B3e (submitted to NCBI No: MZ576252). The insertion sequence shows high homology to a number of regions of the wheat genome (BLASTN analysis relative to the IWGSC 2.0 annotation). These regions are currently not annotated.

3.3. Development of Specific Markers for Detecting Novel Vrn-B3 Alleles

Based on the sequences of Vrn-B3d (NCBI No. MZ576251) and Vrn-B3e (MZ576252), (Table 1), the Vrn-B3e allele was identified by a 302 bp product amplified using the primers Vrn-B3e-F/R, whereas the primer pair Vrn-B3d-F//R with an expected product size of 243 bp was used to detect the Vrn-B3d allele (Figure 3). These primer sets for novel alleles can be used to facilitate the detection of Vrn-B3 alleles and homozygous/heterozygous plants during marker-assisted selection.
The studied population varied from 42.1 to 54.0 days.

Varieties with the earliest heading were Provinciya, Polushko, and Tarskaya. The latest heading varieties were ‘Velut’, Kinelskaya 60, Novosibirskaya 91, and Turinskaya. On average, the plants headed 46.3 days from sprouting.

Figure 3. PCR products obtained with the Vrn-B3d-F//R (A) and Vrn-B3e-F//R (B) markers.

3.4. The Effects of the Vrn-B3d and Vrn-B3e Alleles on Wheat Heading Time

The results of the phenotypic analysis are presented in Figure 4. The mean heading time estimated over two years in the studied population varied from 42.1 to 54.0 days. Varieties with the earliest heading were Provinciya, Polushko, and Tarskaya. The latest-heading varieties were ‘Velut’, Kinelskaya 60, Novosibirskaya 91, and Turinskaya. On average, the plants headed 46.3 days from sprouting.

Phenotypic data were used to measure the differences among days to heading between two groups with certain combinations of vernalization and photoperiod alleles (Ppd-D1b/Vrn-A1a/vrn-D1/vrn-B3 and Ppd-D1b/Vrn-A1a/vrn-D1/Vrn-B3e). Three of ten varieties with vrn-B3e had the recessive vrn-B1 allele, three had Vrn-B1a, three had Vrn-B1c, and one had a vrn-B1/Vrn-B1a allelic combination.

We found that varieties with the combination of Ppd-D1b/Vrn-A1a/vrn-D1/Vrn-B3e headed, on average, 1.5 days later than varieties with the combination of Ppd-D1b/Vrn-A1a/vrn-D1/vrn-B3 under long-day conditions (Figure 5). Therefore, it can be assumed that the new Vrn-B3e allele delays the heading time even under long-day conditions. However, further investigation is required to assess the effect of a given allele on the heading time.
3.5. The Vrn-B3d Allele: Expression-Level Analysis

In the quantitative PCR experiment, we used the F2 population from the cross ‘Velut’ (the new Vrn-B3d allele) × Tobolskaya (wild-type vrn-B3). These varieties were tested for allelic variations at the Vrn-A1, Vrn-B1, Vrn-D1, and Ppd-D1 loci (the major determinants of flowering time). The ‘Velut’ and Tobolskaya varieties have the same Ppd-D1b, Vrn-B1c, and vrn-D1 alleles, but they differ in their Vrn-A1 alleles (Vrn-A1a and Vrn-A1b, respectively) (Table S1).

We analyzed F2 hybrids using sequence-specific markers to select homozygous plants carrying the Vrn-B3d or vrn-B3 allele (we used Vrn-B3d-F/>R (this study) and FT-B-NOINS [2] primers for screening). Ten biological replicates were sampled, and each plant represented an individual replicate. All selected plants were heterozygous for the Vrn-A1 loci (initially, we planned to use homozygous plants with the dominant Vrn-A1 allele, but there were not enough such plants in our sample). Expression was investigated in leaf tissue sampled at two growth stages (five leaves unfolded and the first node) under short-day conditions (9 h of light). The genes we used as an internal control showed specific and efficient amplification, while the expression level of the target gene turned out to be both weak and nonspecific. This implies that there is no difference in the expression level or heading time between plants with vrn-B3 and Vrn-B3e alleles.

4. Discussion

Allelic diversity within genes controlling flowering time is one of the most important factors contributing to the adaptability and productivity of wheat. In the present study, allelic variation at these loci was tested for 95 spring wheat varieties grown in Siberia.

The genetic diversity within the studied genotypes is mainly based on the Vrn-B1 and Vrn-B3 loci. The dominant alleles of the Vrn-B1 gene (Vrn-B1a or Vrn-B1c) predominated. In the current study, the dominant Vrn-B1 was found in 76% of varieties. Low variation was observed at the Vrn-A1, Vrn-D1, and Ppd-D1 loci. This agrees with a previously conducted study [28] of 48 Siberian spring common wheat varieties, where varieties with the dominant Vrn-B1 haplotypes were the most frequent. Scherban et al. (2015) reported screening 245 predominantly spring wheat varieties for the photoperiod and vernalization response genes in common wheat varieties from different regions of Europe [29]. As expected, insensitivity to the photoperiod Ppd-D1a allele was mostly found in wheat from southern regions (32%), while in central, western, and northern regions, the Ppd-D1a allele was more frequent (98.8% and 95.6%, respectively). In the same study, Vrn-A1a together with the dominant
alleles of Vrn-B1 (mostly Vrn-B1a) and recessive vrn-D1 were found in nearly half of the varieties. The Vrn-A1a allele, either alone or with the dominant Vrn-B1, also predominated in wheat genotypes from Canada and the USA [30,31]. The strong presence of the dominant Vrn-A1 and/or Vrn-B1 in high latitude spring wheat varieties has been reported multiple times. Under these growing conditions, this genetic background causes an earlier heading time, increasing the adaptability of plants in areas with a short growing season.

The presence of TEs can influence plant gene expression through several potential mechanisms. TE insertions into the promoter or nearby regulatory regions may disrupt the existing regulation of genes, resulting in changes in the expression patterns. Several studies have found such dominant alleles of the Vrn-B3 gene in wheat.

Dominant Vrn-B3 alleles are not common in wheat germplasm, although the Vrn-B3a allele was detected widely in the cultivated varieties of T. durum from Ukraine and Russia [32]. This allele was first identified in the common wheat variety Hope, which was derived from a cross between the emmer wheat variety ‘Yaroslav’, the donor of Vrn-B3a in Hope, and the Canadian T. aestivum variety ‘Marquis’ [2,33,34]. Subsequently, this allele was found in three varieties of T. aestivum from China [16,35]. Although the Vrn-B3a allele is rare in common wheat, the analysis of 214 varieties from different ecogeographical areas showed that the dominant allele was abundant among tetraploid wheat T. durum and T. dicoccum originating from Ukraine and Russia. It was demonstrated that the Vrn-B3a allele is associated with early flowering under both long-day and short-day conditions [2]. The Vrn-B3c allele has two small deletions (4 bp and 20 bp) inside of a retrotransposon detected in Hope [16]. The Vrn-B3b allele is characterized by an insertion of 890 bp within the promoter, resulting in delayed heading. It was assumed that the Vrn-B3b allele has a more recent origin (compared to the Vrn-B3a allele) in the T. dicoccum lineage [34]. Additionally, two novel 1300 bp and 2000 bp fragments were observed from amplification using primers FT-B-NOINS-F and FT-B-NOINS-R (an amplification product of 1140 bp indicates the recessive vrn-B3 allele) in Iranian wheat landraces [36]. Molecular characterization of these alleles was not performed.

In the present study, marker analysis revealed that only two varieties carried the Vrn-B3a allele, and the great majority (86%) of varieties carried the recessive vrn-B3 allele. During the investigation, two novel Vrn-B3 allelic variants carrying mutations within the promoter region were found. These varieties were characterized by 160 bp and 1617 bp insertions and were designated Vrn-B3e and Vrn-B3d, respectively.

The primary structure analysis showed that the Vrn-B3d allele has an insertion of a retroelement flanked by identical 316 bp LTRs. The fact that the LTRs are completely identical implies that the insertion occurred recently. Several studies utilizing an approach of comparison of long terminal repeat (LTR) divergences in plant genomes, including hexaploid wheat, showed that the proliferation of retroelements specific to certain genomes occurred in the diploid progenitors of hexaploid wheat [37–39]. Thus, the proliferation of the TEs in the genomes of tetraploid and hexaploid wheat is suppressed. According to the scheme of origin of the ‘Velut’ line (Figure S1), insertion of the transposon could have occurred during pollination of the Rodina variety with gamma-irradiated pollen Ae. speltoides.

Interestingly, the similarity search with BLASTN against available TE databases found a strong hit to a Hordeum vulgare L. LTR retrotransposon (RLX_Hvul_Dacia_RND-1). The retrotransposon was not classified into any known class because no structural motifs were found. These transposons have high DNA sequence similarity in their coding internal domain (identity > 80%) but not in the terminal repeat region. Both of them are presumably nonautonomous because no structural motifs were found in their internal domains. Unlike the previously identified allelic variations of the Vrn-B3 gene, the one found in the ‘Velut’ line did not have any significant effect on flowering time. Presumably, the insertion region does not include any important regulatory sequences within the promoter.

Ten wheat varieties possessed the novel Vrn-B3e allele, which had a smaller insertion within the promoter. Our field data suggest that this allele might be associated
with delayed heading. Pedigree analyses performed using the database GRIS (http://www.wheatpedigree.net, accessed on 16 November 2021) for the ten varieties from this study with *Vrn-B3e* indicated that seven of them (Novosibirskaya 67, Novosibirskaya 29, Novosibirskaya 89, Kantegirskaya 89, Obskaya 14, Omskaya 23, Aleksandrina) were derived from a cross of Lutescens 55-11 and Albidum 24 originating in the Volga region (Supplementary Figure S2). Based on the length of the PCR fragment, we can assume that this allele was also found among Iranian wheat landraces [40]. Among 395 wheat landraces studied, Derakhshan et al. (2013) found this allele in 9, both winter and spring genotypes. We suggest that this allele must be widespread in common wheat from different cultivation centers. Overall, our results demonstrate that Russian wheat germplasm could be used as a potential source of genetic diversity for controlling heading times in wheat.

5. Conclusions

In this study, we examined allelic composition at the *Ppd-D1, Vrn-A1, Vrn-B1, Vrn-D1*, and *Vrn-B3* loci among Siberian common wheat. Two novel alleles of the *Vrn-B3* gene were found. We showed that *Vrn-B3d* and *Vrn-B3e* alleles carrying 1617 bp and 160 bp insertions, respectively, within the promoter region, were associated with a delay of heading under long-day conditions.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/biom11121897/s1, Table S1: Heading date and allelic composition of the *Vrn-1*, *Vrn-3*, and *Ppd-1* genes in the studied Russian common wheat varieties. Phenotyping was performed in 2016–2017, Table S2: Primer sets used for sequencing of the *Vrn-B3d* allele promoter, Figure S1: The origin of the ‘Velut’ line with a delayed heading time, Figure S2: The phylogenetic relationships and origins of the varieties carrying the novel *Vrn-B3e* allele (the varieties are given in bold).

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Data Availability Statement: The data presented in this study are available in Supplementary Materials. The sequencing data have been submitted to the NCBI database (accession numbers MZ576251 and MZ576252).

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