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Characterization of the *VRN-A1* allele introgressed from *T. aestivum* ssp. *petropavlovskyi* that influences the heading time in bread wheat

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Abstract The diverse timing of the heading stage, mainly determined by the *VRN* genes, contributes to the wide spread of bread wheat and the realization of adaptive and breeding potential. Wild wheat species are valuable sources for expanding the bread wheat genetic diversity by the introgression of new gene alleles, including *VRN* genes. In this study, a near-isogenic line of the winter wheat cultivar Bezostaya 1 with a *VRN-A1* dominant allele was obtained with a *Triticum aestivum* ssp. *petropavlovskyi* accession as the donor. Using known PCR markers for the promoter and first intron sequences of the *VRN-1* gene and subsequent sequencing of PCR fragments, the presence of the Langdon deletion was revealed in the first intron (*Vrn-A1L* allele), previously described only in tetraploid wheat. The allele composition of *VRN* genes was determined in *T. aestivum* ssp. *petropavlovskyi* accessions and the presence of the *Vrn-A1L* allele was established in all accessions. It was shown that the *Vrn-A1L* dominant allele increased the shoots-heading period under long- and short-day conditions, which is associated with a prolongation period before the first node formation. The comparative study of productivity characteristics of near-isogenic lines with *Vrn-A1a* and *Vrn-A1L* alleles on spike and plant productivity is presented.

Keywords Bread wheat · *T. aestivum* ssp. *petropavlovskyi* · Near-isogenic lines · *Vrn-A1L* · Heading time · Productivity

Introduction

For cereals, including bread wheat, the growth season duration and, in particular, the onset time and duration of certain stages of organogenesis, is an important trait that allows plants to adapt to certain natural and climatic conditions, being in close relationship with the resistance to adverse biotic and abiotic factors and, ultimately, productivity (Worland 1996; Snape et al. 2001; Cockram et al. 2007; Kamran et al. 2014). To date, data has been complied on the genetic control of growth season length and molecular mechanisms of flowering regulation in wheat and other cereal species (Distelfeld et al. 2009). It is known that the duration of the bread wheat growth season and the transition from the vegetative to the generative stage are controlled by complex interaction mechanisms between several genetic systems, among which the vernalization response genes (*VRN*) and photoperiod sensitivity genes (*PPD*) play the leading role (Snape et al. 2001; Kamran et al. 2014). One of the most

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effective mechanisms underlying the regulation of the wheat transition from the vegetative stage to the generative stage is the need for vernalization, which distinguishes spring wheat from winter wheat (Loukoianov et al. 2005). The need to vernalize winter crops is an important adaptive mechanism to prevent low temperature damage to the sensitive apical meristem of the growth apex (Yan et al. 2003; Trevaskis et al. 2007).

The main genes determining the wheat genetic diversity in terms of heading time were cloned: *VRN-1* (Yan et al. 2003), *VRN-2* (Yan et al. 2004a), *VRN-3* (Yan et al. 2006), and *VRN-D4* (Kippes et al. 2015). The *VRN-1* locus encodes the MADS-box transcription factor, the homolog of the *Arabidopsis* meristem identity gene *APETALA1* (Yan et al. 2003), which controls the transition of plants from the vegetative stage to the generative stage. For the beginning of wheat flowering, the *VRN-1* transcription must exceed a certain threshold value (Loukoianov et al. 2005). The *VRN-2* gene encodes two proteins: ZCCT1 and ZCCT2, the products of which contain the putative domains: zinc finger and CCT and acts as a dominant repressor of *VRN-3* and flowering (Yan et al. 2004a). The *VRN-3* (*TaFT1*) locus is a homolog of the *Arabidopsis FT* (*FLOWERING LOCUS T*) gene. It is an integrator of various pathways involved in the formation of heading time, up-regulated by a long day, which is a regulator of the *VRN-1* expression. The bread wheat *VRN-B3* gene is mapped in the short arm of the 7B chromosome (Yan et al. 2006). The *VRN-4* gene is a *VRN-A1* gene paralog resulting from the insertion of the 290 kb region of the 5A chromosome long arm, with the *VRN-A1* gene, into the short arm of chromosome 5D (Kippes et al. 2015). Low positive temperatures activate the expression of *VRN-1*, which initiates the apical meristem transition to the generative stage of development, and suppresses the action of *VRN-2*, which is a *TaFT1* repressor (Yan et al. 2004b; Trevaskis et al. 2007). The *VRN-1* locus is represented by three homeologous genes: *VRN-A1, VRN-B1,* and *VRN-D1* localized in the long arms of 5A, 5B, and 5D chromosomes, respectively, which mainly determine the need for vernalization in bread wheat (Snape et al. 2001; Yan et al. 2003). In wheat, winter, spring, and facultative types are known, which are distinguished according to allelic variations in the *VRN-1* locus. The *VRN-A1, VRN-B1*, and *VRN-D1* dominant alleles determine the spring type of development with a varying degree of genotype sensitivity to vernalization, and recessive ones determine the winter type. The lowest plant sensitivity to vernalization is determined by the *VRN-A1* gene, while genotypes with *VRN-B1* and *VRN-D1* dominant genes are more sensitive to vernalization and determine later heading (Stelmakh 1992), correlating with the relative expression of these genes and the effect of transcripts on reducing the *VRN-2* expression (Loukoianov et al. 2005).

It was shown that the known allelic variation in the *VRN-1* locus associated with the need or lack of vernalization was mainly determined by mobile element insertions, deletions, and duplications in two regulatory regions: the promoter and the first intron (Distelfeld et al. 2009). Three *Vrn-A1a* alleles are described: *Vrn-A1a.1*, *Vrn-A1a.3* (syn. *Vrn-A1a,* described for hexaploid and tetraploid wheat) and *Vrn-A1a.2*, which have MITE insertion and duplication in the promoter region. *Vrn-A1a.2* contains a 16 bp deletion and four single nucleotide deletions within the MITE insertion compared to *Vrn-A1a.1* (Yan et al. 2004b; Muterko et al. 2016a). Seven *Vrn-A1b* allele variants with 20 bp deletion, with different polymorphisms in A-tract and C-rich fragment within the VRN-box were detected (Konopatskaia et al. 2016; Muterko et al. 2016a). *Vrn-A1d*, *Vrn-A1e, Vrn-A1f, Vrn-A1g, Vrn-A1h,* and *Vrn-A1k* are characterized by small deletions in the promoter region in diploids and tetraploids (Yan et al. 2004b; Golovnina et al. 2010; Muterko and Salina 2017). *Vrn-A1i*, described in *T. turgidum* and *T. durum*, has a point mutation in the VRN-box (Muterko et al. 2016a). In the *T. compactum* hexaploid wheat, the *Vrn-A1j* allele with a deletion in the promoter region was detected (Muterko et al. 2016b). The *vrn-A1u* and *Vrn-A1ins* alleles characterized by a 1.4 kb deletion or 0.5 kb insertion in the first intron were described for diploid species (Dubcovsky et al. 2006; Shcherban et al. 2015). The *Vrn-A1c* and *Vrn-A1L* alleles found in tetraploids (*Vrn-A1c* and *Vrn-A1L*) and bread wheat (*Vrn-A1c*) are characterized by extended deletions of 5.5 kb and 7.2 kb in the first intron, respectively (Fu et al. 2005; Shcherban et al. 2015). Sehgal et al. (2015) identified the allele with 6 kb deletion the first intron marked as *Vrn-A1f* in *T. aestivum* landraces. *T. araraticum* and *T. timopheevii* are characterized by *Vrn-A1f-del* and *Vrn-A1f-ins* alleles containing the *Vrn-A1f* promoter

(50 bp deletion) with 2.7 kb deletion and 0.4 kb insertion in the first intron (Shcherban et al. 2016), and *Vrn-A1f-like* with additional 8 bp deletion, 0.4 kb insertion, 2.7 kb deletion in the first intron and SNP in the coding region (Ivaničová et al. 2016).

The dominant alleles of the *VRN-B1* and *VRN-D1* loci differ from recessive alleles mainly by the presence of insertions/deletions in the first intron (Fu et al. 2005; Shcherban and Salina 2017; Shi et al. 2019). Tetra- and hexaploid wheat species are characterized by allelic variants with mutations in the first intron: *Vrn-B1a* (6.8 kb deletion) (Fu et al. 2005), *Vrn-B1b* (6.8 kb and 36 bp deletions) (Santra et al. 2009), *Vrn-B1c* (6.8 kb and 0.8 kb deletions and 0.4 kb duplication) (Milec et al. 2012; Shcherban et al. 2012) and *Vrn-B1d* (6.8 kb and 187 bp deletions, one SNP and one 4 bp mutation) (Zhang et al. 2018). Mutant alleles with changes in the promoter region are represented by the *Vrn-B1a* allele with 127 bp insertion (Golovnina et al. 2010), *Vrn-B1ins* (5.4 kb insertion) (Chu et al. 2011), *Vrn-B1dic* (11% dissimilarity vs. *vrn-B1*) (Konopatskaia et al. 2016). The *Vrn-D1a* and *Vrn-D1b* dominant alleles of spring bread wheat have a 4.2 kb deletion in the first intron differing from each other by a single nucleotide mutation (Fu et al. 2005; Zhang et al. 2012). *Vrn-D1s* (0.8 kb insertion) and *Vrn-D(t)1* (5.4 kb deletion) alleles are described in *T. spelta* and *Ae. tauschii* (Takumi et al. 2011; Muterko et al. 2015). The *Vrn-D1c* allele in bread wheat is associated with a 174 bp insertion in the promoter region (Zhang et al. 2015). Of the described allelic diversity of the *VRN-1* locus in bread wheat, the following *Vrn-A1a*, *Vrn-A1b*, *Vrn-A1c*, *Vrn-A1f* (6 kb deletion in the first intron), *Vrn-B1a*, *Vrn-B1b*, *Vrn-B1c*, *Vrn-D1a*, *Vrn-D1b*, *Vrn-D1c*, and *Vrn-D1s* alleles were founded.

The *VRN-B3* locus is represented by five dominant alleles found in bread wheat and tetraploid species associated with structural changes in the promoter. *Vrn-B3a* in the Chinese Spring/Hope 7B substitution line contains a retrotransposon insertion of 5.3 kb (Yan et al. 2006). The *Vrn-B3c* allele differs from *Vrn-B3a* one by two deletions (4 bp and 20 bp) within the retrotransposon region (Chen et al. 2013). The *Vrn-B3b* allele has 890 bp insertion (Chen et al. 2013). The *Vrn-B3d* and *Vrn-B3e* alleles have 1615 bp and 160 bp insertions, respectively (Berezhnaya et al. 2021).

It is important to study the effect of different alleles associated with structural changes in the regulatory regions of the *VRN-1* genes on the beginning of heading and flowering of bread wheat. Near-isogenic lines (NILs) of bread wheat are the most suitable object for studying the *VRN-1* allele polymorphism in heading time control. It was found that genotypes with the *Vrn-A1a* dominant allele were the fastest growing. Cultivars with the dominant *Vrn-A1b* allele have a few days later heading than ones with the dominant *Vrn-A1a* allele (Koval and Goncharov 1998). The *Vrn-B1a* dominant allele increases the duration of the shoots-heading period relative to the *Vrn-B1c* allele (Efremova et al. 2011).

Since, as mentioned above, the currently known genetic diversity of bread wheat is described by a small number of *VRN* gene alleles found in diploid and tetraploid cereals. Therefore, the transfer of *VRN* gene alleles from wild and cultivated wheat species will make it possible to study the effect of alleles on heading time and obtain new genotypes for breeding. In this study, the research team obtained and studied a NIL of the Bezostaya 1 (Bez1) winter cultivar with a *Vrn-A1*^{pet} dominant allele whose donor was a *T. aestivum* ssp. *petropavlovskyi* (Udacz. et Migusch.) N.P. Gontsch., hexaploid wheat (2n = 6x = 42, BBA^{u}A^{u}DD) (endemic to Xinjiang and Tibet) accession that has a species-specific *P1* gene determining the elongated external glumes (Xiao et al. 2021). Previous studies contain limited information on the genetic control of the growth season duration of *T. aestivum* ssp. *petropavlovskyi* (Efremova et al. 2000; Goncharov 2012; Dragovich et al. 2021). Therefore, the purpose of this work was to identify the allelic variants of the *VRN* genes in NIL and *T. aestivum* ssp. *petropavlovskyi* accessions and to evaluate the influence of the *VRN-A1* gene structural features on the developmental phase duration and productivity traits.

Materials and methods

Plant material

The material for the study was the NIL of the winter cultivar Bez1 with the *Vrn-A1*^{pet} dominant allele, whose donor was an accession of *T. aestivum* ssp. *petropavlovskyi* (Udacz. et Migusch.) N.P. Gontsch (catalog number unknown), designated "KIZ", from

the Federal Research Center N.I. Vavilov Institute of Plant Genetic Resources (VIR, Russian Federation). i:Bez1*Vrn-A1*^{pet} was obtained by crossing of spring accession *T. aestivum* ssp. *petropavlovskyi* (KIZ) with recurrent winter cultivar Bez1. In eight backcrosses, spring heterozygotes tagged with the *Vrn-A1*^{pet} dominant allele were used as fathers. After selfing BC_8F_1 plants, homozygotes were isolated in generation BC_8F_2. In addition, the i:Bez1*Vrn-A1a*, obtained earlier by the authors, as well as seven accessions of *T. aestivum* ssp. *petropavlovskyi* from the VIR collection, were used in the work.

DNA extraction, PCR amplification and sequencing

Total DNA was isolated from 100 mg of leaves using the DNeasy Plant Mini Kit (QIAGEN) according to the manufacturer's protocol. Polymerase chain reactions (PCR) were performed in a 20 µl volume with 1 × reaction buffer (10 mM TrisHCl, pH 8.8; 4 mM $MgCl_2$; 1 mM $(NH_4)_2SO_4$), 200 µM of each dNTP, 0.5 µM of each primer and 1 unit of *Taq* DNA polymerase and 0.1 µg of genomic DNA. The structure of the used primers for amplifying the promoter and first intron sequences of *VRN-A1*, *VRN-B1, VRN-D1,* and *VRN-B3* genes and PCR conditions were consistent with the published protocols (Table 1). The reaction was run on a Bio Rad T100 Thermal Cycler (USA). The amplification products were separated by electrophoresis on a 1.5% agarose gel in 1 × TAE buffer with the addition of ethidium bromide. After electrophoresis, the gel was photographed in ultraviolet light using the Doc-Print II gel documentation system (Vilber Lourmat, France).

PCR products were separated by agarose gel electrophoresis and purified using a QIAquick Gel Extraction Kit (QIAGEN, Germany) according to the manufacturer's protocol. Sequencing reactions were performed with the ABI BigDye Terminator Kit and corresponding specific primers on an ABI 3130XL Genetic Analyser (Applied Biosystems) in the SB RAS Genomics Core Facility (http://www.niboch.nsc.ru/doku.php/corefacility) following the manufacturer's protocol. The search for nucleotide sequence homology was performed using the FASTA and BLAST algorithms in the NCBI databases (www.ncbi.nlm.nih.gov/Database/).

Table 1 Set of primers used in the present study

Primers name	Sequence (5' to 3')	Target sequence	Alleles	Expected size of product (bp)	Reference
Intr1/C/F Intr1/AB/R	GCACTCCTAACCCACTAACC TCATCCATCATCAAGGCAAA	*VRN-A1* intron 1	*vrn-A1*	1068	Fu et al. (2005)
Ex1/C/F Intr1/A/R3	GTTCTCCACCGAGTCATGGT AAGTAAGACAACACGAATGTGAGA		*Vrn-A1L*	522	
Intr1/A/F2 Intr1/A/R3	AGCCTCCACGGTTTGAAAGTAA AAGTAAGACAACACGAATGTGAGA		*Vrn-A1c*	1170	
VRN1AF VRN1-INT1R	GAAAGGAAAAATTCTGCTCG GCAGGAAATCGAAATCGAAG	*VRN-A1* promoter	*vrn-A1*	713	Yan et al. (2004b)
Ex1/B/F3 Intr1/B/F Intr1/B/R3 Intr1/B/R4	GAAGCGGATCGAGAACAAGA CAAGTGGAACGGTTAGGACA CTCATGCCAAAAATTGAAGATGA CAAATGAAAAGGAATGAGAGCA	*VRN-B1* intron 1	*Vrn-B1a* *vrn-B1*	709 + 1235 1149	Milec, et al. (2012)
Intr1/D/F Intr1/D/R3	GTTGTCTGCCTCATCAAATCC GGTCACTGGTGGTCTGTGC	*VRN-D1* intron 1	*Vrn-D1a*	1671	Fu et al. (2005)
Intr1/D/F Intr1/D/R4	GTTGTCTGCCTCATCAAATCC AAATGAAAAGGAACGAGAGCG		*vrn-D1*	997	
VRN4-B-INS-F VRN4-B-INS-R	CATAATGCCAAGCCGGTGAGTAC ATGTCTGCCAATTAGCTAGC	*VRN-B3* promoter	*Vrn-B3a*	1200	Yan et al. (2006)
VRN4-B-NOINS-F VRN4-B-NOINS-R	ATGCTTTCGCTTGCCATCC CTATCCCTACCGGCCATTAG		*vrn-B3*	1140	

Analysis of the heading time, the developmental phases duration and the productivity traits

NILs with dominant alleles of *VRN-A1* loci and accessions of *T. aestivum* ssp. *petropavlovskyi* were grown in the experimental field of the Breeding and Genetics Laboratory of the Institute of Cytology and Genetics (ICG SB RAS) under a natural long photoperiod (55° 2,49' N, 82° 56,80' E; day length for the May–August period, 17 h) in 2017–2019 and 2021. Seed sowing was on May 15 in 2017, May 22 in 2018, May 13 in 2019 and May 17 in 2021, respectively. Also, NILs and *T. aestivum* ssp. *petropavlovskyi* (KIZ) were grown in the greenhouse of the Laboratory of Artificial Plant Growth at ICG SB RAS at 20–25 °C under a long (16–18 h light) and short (12–14 h light) photoperiod, with or without vernalization treatment. The following developmental phases were studied: shoots, third leaf, first node, flag leaf, heading, and ripening. The first node phase was recorded when the first node appeared on the main shoot at a height of 1 cm above the soil surface. The heading was recorded when the head had fully emerged from the flag leaf. A total of 20–30 plants of each line and accessions of *T. aestivum* ssp. *petropavlovskyi* were examined. Also, the duration of the shoots-heading phase of F_1 and F_2 hybrids of NILs Bez1*Vrn-A1a* and Bez1*Vrn-A1*^{pet} was studied**.**

For a comparative productivity study, the best 25–30 samples from each NIL were selected. The productivity components of the main spike (spike length, number of spikelets, grain number and weight) and plant (spike number, grain number and weight), the weight of 1000 grains, and plant height were examined.

Statistical treatment of the data

Statistical treatment of the obtained data was carried out using Microsoft Excel 2013. To assess the statistical significance of the differences between the mean values, Student's test was used (t-test). A two-factor ANOVA was used to investigate the effect of *VRN-A1* dominant alleles on spike and plant productivity.

Meteorological conditions

The four field experiments performed in the 2017, 2018, 2019 and 2021 seasons had different climatic conditions (Fig. S1). In our studies, groundbased observations of the local weather station were used. June and July 2017 were characterized by higher temperatures compared to the annual average (deviation was 2.4 °C). In the remaining months the values corresponded to the annual average. Precipitation in May was 73% of the normal, while June, July and September were, on the contrary, wetter (precipitation was 30–63% higher than the annual average). In 2018, the average temperature in May was 4 °C below annual average. June and September, on the contrary, were on average warmer by 2 °C. May precipitation was 200% and June 130% of annual average; August and September precipitation totals were only 49% and 11% of normal, respectively. In 2019, August was warmer by 2.6 °C. Temperatures in the other months were no different than annual average. In 2019, there was a significant deficit in precipitation in all summer months, amounting to only 17% and 11% of normal in June and August, and not exceeding 60% in May, July, and September. In 2021, temperatures were 3.3 °C higher in May and 1.9 °C higher in August. May and July were more dry (68% and 37% of annual average precipitation, respectively), in contrast, in June, the amount of precipitation was 30% higher than annual average.

Results

Determination of allelic variants of *VRN* genes in NIL by the Bez1 cultivar

The goal was to determine the allelic variants of *VRN* genes in NIL by the Bez1 cultivar with an unidentified allele from *T. aestivum* ssp. *petropavlovskyi* Udacz. et Migusch. (*Vrn-A1*^{pet}), which is non-allelic to the known *Vrn-A1a* and *Vrn-A1b* loci. Using a pair of VRN1AF and VRN1-INT1R primers to determine allelic variants of the *VRN-A1* promoter in i:Bez1*Vrn-A1*^{pet} and *T. aestivum* ssp. *petropavlovskyi* (KIZ), a PCR product of about 700 bp was amplified and then sequenced (Fig. 1a). The sequenced regions were found to be completely identical to the sequences of the intact promoter *vrn-A1 T. aestivum* NIL Triple Dirk C. The first intron of the *VRN-A1* gene was analysed using three primer pairs. When Intr1/C/F and Intr1/AB/R primers were used, which amplify the intact sequences of the first intron, there was no

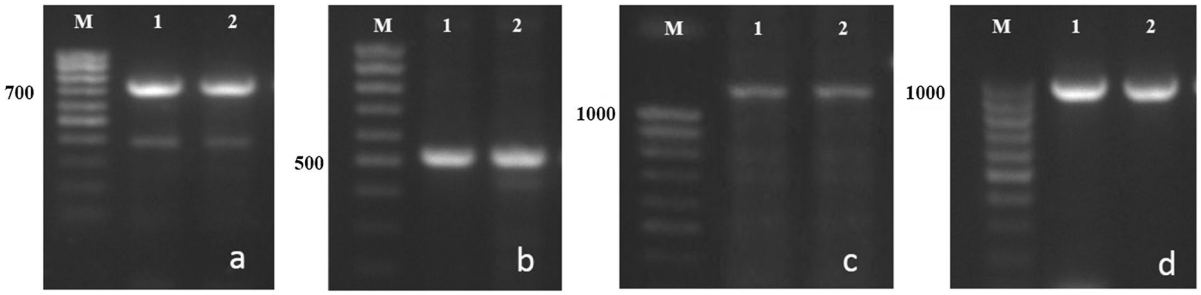

Fig. 1 Amplification products with primers: VRN1AF and VRN1-INT1R (**a**), Ex1/C/F and Intr1/A/R3 (**b**), Ex1/B/F3, Intr1/B/F, Intr1/B/R3, and Intr1/B/R4 (**c**), Intr1/D/F and Intr1/D/R4 (**d**) for the regions of the promoter and the first intron of *VRN-A1, VRN-B1,* and *VRN-D1*: 1—i:Bez1*Vrn-A1L,* 2—*T. aestivum* ssp. *petropavlovskyi* (KIZ). M—100 bp Ladder

PCR product of 1068 bp. No PCR products of 5.5 kb deletion IL369 identified in hexaploid Afghanistan landrace IL369 were detected. Using the Ex1/C/F and Intr1/A/R3 primer pair in i:Bez1*Vrn-A1*^{pet} and *T. aestivum* ssp. *petropavlovskyi* (KIZ), a 522 bp product was amplified (Fig. 1b), indicating the 7.2 kb deletion (391 bp to 7612 bp) in the first intron. Such deletion was first described for the tetraploid cultivar Langdon. The recessive alleles *vrn-B1* and *vrn-D1* were also detected (Fig. 1c, d).

Thus, the isogenic line identified is designated as i:Bez1*Vrn-A1L*. Since the *Vrn-A1L* dominant allele has not been previously detected in bread wheat accessions, it is of interest to establish its effect on the growth season duration.

The analysis of heading time and the developmental phase duration of NILs with *VRN-1* gene dominant alleles

During the experimental years, the duration of the period from shoots to heading in i:Bez1*Vrn-A1a* under field conditions varied within 42–50 days. NILs with the *Vrn-A1L* dominant allele, as well as the parent of this allele, *T. aestivum* ssp. *petropavlovskyi* (KIZ), were later mature than the i:Bez1*Vrn-A1a* line. The duration of the period from shoots to heading of i:Bez1*Vrn-A1L* was 49 to 61 days, 6–17 days later than that of i:Bez1*Vrn-A1a*) ($P < 0.001$) (Table 2).

In the analysis of Table 3, we can note a response to the day length of the i:Bez1*Vrn-A1L*. Thus, the increase in the average number of days before heading under short-day (12–14 h) relative to long-day (16–18 h) conditions was about 5 days. In i:Bez1*Vrn-A1L* compared with i:Bez1*Vrn-A1a*, heading came later by 18–29 and 17–19 days under short- and long-day conditions, respectively ($P < 0.001$). A similar trend was showed in *T. aestivum* ssp. *petropavlovskyi* (KIZ).

When analyzing the duration of individual phases of development (Figs. 2 and 3), we can note that the increasing shoots – heading period in i:Bez1*Vrn-A1L* versus i:Bez1*Vrn-A1a* is associated with an increasing period to form the first node. This was established in the experiments under both field and greenhouse conditions. The duration of the first node – flag leaf and flag leaf – heading periods varied insignificantly. Under field conditions, i:Bez1*Vrn-A1L* versus

Table 2 Days to heading of the NILs Bez1*Vrn-A1a*, Bez1*Vrn-A1L* and *T. aestivum* ssp. *petropavlovskyi* (KIZ) in field

Genotype	2017	2018	2019	2021
i:Bez1*Vrn-A1L*	50.20 ± 3.87***[1] **[2]	49.14 ± 3.82***[1]	58.94 ± 4.66***[1] ***[2]	60.76 ± 2.45***[1] **[2]
T. aestivum ssp. *petropavlovskyi* (KIZ)	53.03 ± 3.75		65.33 ± 4.72	59.19 ± 1.48
i:Bez1*Vrn-A1a*	41.79 ± 2.33	43.11 ± 2.21	50.07 ± 2.11	43.63 ± 1.88

Significant differences: [1]from i:Bez1*Vrn-A1a*, [2]from *T. aestivum* ssp. *petropavlovskyi* (KIZ)

**$P < 0.01$, ***$P < 0.001$. Values are means ± S.D

Table 3 Days to heading of the NILs Bez1*Vrn-A1a*, Bez1*Vrn-A1L* and *T. aestivum* ssp. *petropavlovskyi* (KIZ) in greenhouse

Genotype	Long photoperiod		Short photoperiod	
	16 h	18 h	12 h	14 h
i:Bez1*Vrn-A1L*	62.71 ± 2.32***[1]	62.80 ± 1.10***[1] ***[2]	68.47 ± 3.69***[1] ***[2]	67.43 ± 1.99***[1]
T. aestivum ssp. *petropavlovskyi* (KIZ)	63.20 ± 1.93	58.54 ± 1.13	76.40 ± 1.67	67.50 ± 1.43
i:Bez1*Vrn-A1a*	46.21 ± 2.72	43.95 ± 1.35	39.40 ± 1.39	49.17 ± 1.95

Significant differences: [1]from i:Bez1*Vrn-A1a*, [2]from *T. aestivum* ssp. *petropavlovskyi* (KIZ)

***$P < 0.001$. Values are means ± S.D

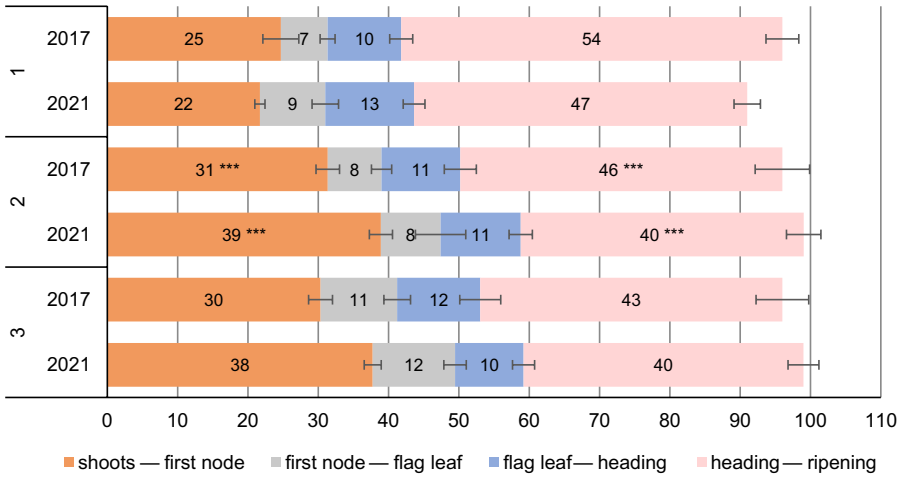

Fig. 2 Developmental phase duration of NILs with dominant alleles of *VRN-A1* loci: i:Bez1*Vrn-A1a* (1) and i:Bez1*Vrn-A1L* (2), and *T. aestivum* ssp. *petropavlovskyi* (KIZ) (3) (field, 2017 and 2021). ***—significant differences between i:Bez1*Vrn-A1a* and i:Bez1*Vrn-A1L* at $P < 0.001$

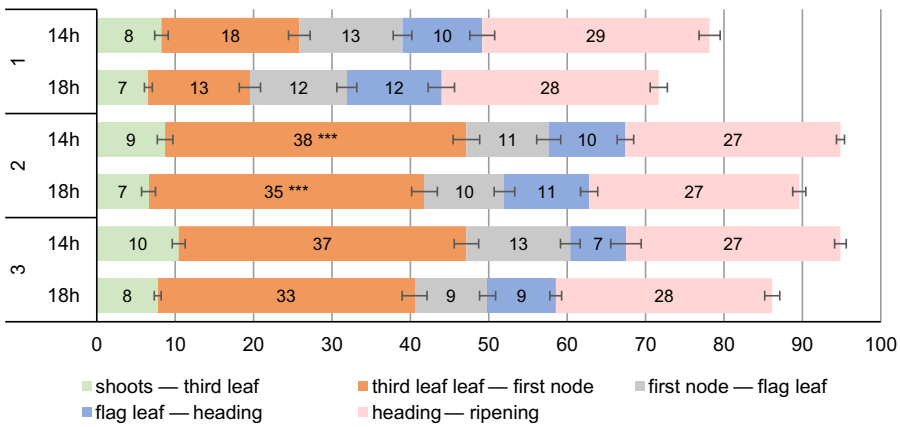

Fig. 3 Developmental phase duration of NILs with dominant alleles of *VRN-A1* loci: i:Bez1*Vrn-A1a* (1) and i:Bez1*Vrn-A1L* (2), and *T. aestivum* ssp. *petropavlovskyi* (KIZ) (3) (greenhouse, 14 h and 18 h photoperiod). ***—significant differences between i:Bez1*Vrn-A1a* and i:Bez1*Vrn-A1L* at $P < 0.001$

i:Bez1*Vrn-A1a* was characterized by an acceleration of the period from heading to ripening, while under greenhouse conditions, this trend was not confirmed. The specifics of individual phases of *T. aestivum* ssp. *petropavlovskyi* development were similar to i:Bez1*Vrn-A1L*. In short-day conditions, an increase in the total duration of the growing season due to the vegetative phase lengthening was also confirmed. The increase in the duration of the studied phases of development in NILs with a decrease in day length is explained by the Bez1 cultivar characteristic response to day length.

Thus, it was found that the *Vrn-A1L* dominant allele caused later heading compared to the *Vrn-A1a* allele, which is mainly due to the prolongation of the period before the first node formation.

The effect of vernalization duration on the heading time of the i:Bez1*Vrn-A1L* and *T. aestivum* ssp. *petropavlovskyi* (KIZ)

The i:Bez1*Vrn-A1a* is known to be insensitive to vernalization. Without vernalization, the duration of the period from shoots to heading in the i:Bez1*Vrn-A1L* and *T. aestivum* ssp. *petropavlovskyi* (KIZ) was 63 days under 16 h photoperoid (Fig. 4), while when vernalized from 14 to 49 days, both genotypes responded sensitively by accelerating heading. The duration of the period before heading in i:Bez1*Vrn-A1L* plants at a vernalization duration of 14, 35 and 49 days decreased by 5, 16 and 22 days, respectively compared to the non-vernalized plants. After 49 days of vernalization, the plants formed ears in 42 days, which coincides with the time of heading of non-vernalized i:Bez1*Vrn-A1a* plants.

The duration of the shoots-heading period in F_1 and F_2 hybrids obtained by crossing NILs carrying the dominant alleles ***Vrn-A1a*** and ***Vrn-A1L***

The duration of the shoots-heading period in the NILs Bez1*Vrn-A1a* and Bez1*Vrn-A1L* was 39 and 62 days, respectively. In the F_1 hybrids between these lines, the value was intermediate but closer to the early-ripening parental form and was 48 days. Among the F_2 hybrids, not a single winter plant was segregated, indicating the allelism of the *Vrn-A1a* and *Vrn-A1L* loci. F_2 population segregation shows that the plants are divided into early heading (37–45 days as in i:Bez1*Vrn-A1a*) and late heading (58–72 days as in i:Bez1*Vrn-A1L*), as well as an intermediate class (46–57 days), corresponding in heading time to F_1 hybrids (Fig. 5).

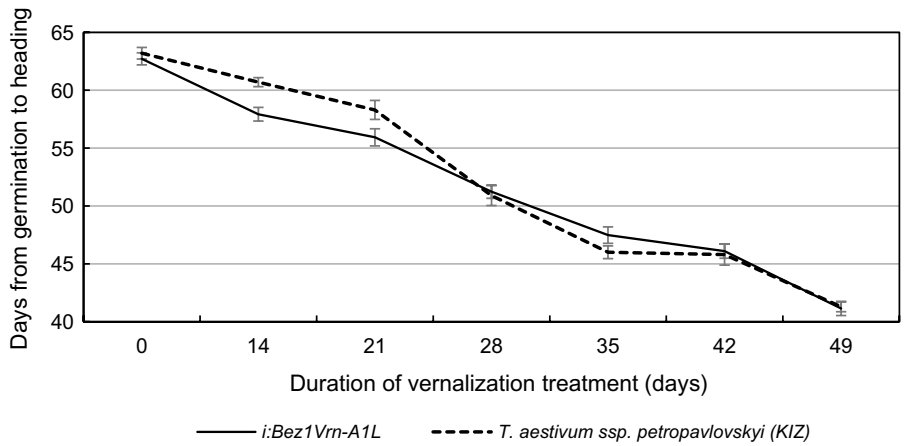

Fig. 4 Effect of vernalization duration on the heading time of the i:Bez1*Vrn-A1L* and *T. aestivum* ssp. *petropavlovskyi* (KIZ) in greenhouse conditions under a long photoperiod (16 h). Error bars are standard errors of the means

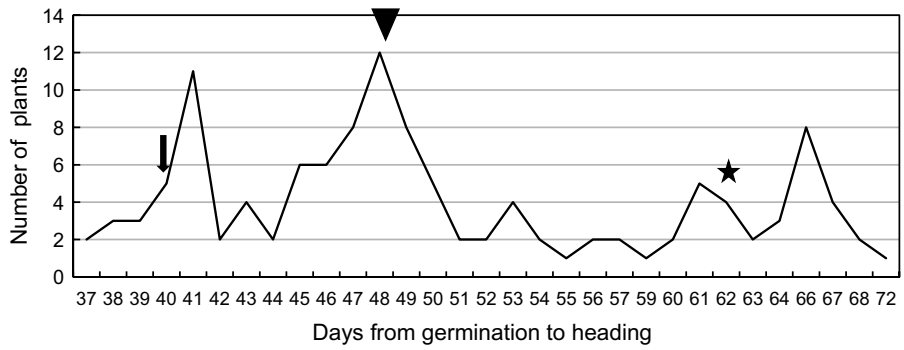

Fig. 5 Frequency distributions of plants for heading time in F_2 population of i:Bez1*Vrn-A1a*×i:Bez1*Vrn-A1L*. Arrow indicates i:Bez1*Vrn-A1a*, star indicates i:Bez1*Vrn-A1L*, and triangle indicates F_1 hybrids

A comparative analysis of spike and plant productivity of the NILs Bez1*Vrn-A1a* and Bez1*Vrn-A1L*

A comparative analysis of the spike and plant productivity of the NILs Bez1*Vrn-A1a* and Bez1*Vrn-A1L* was carried out under field conditions for 4 years (Table 4). The influence of different alleles on production performance was found to be unequal in different years. Thus, i:Bez1*Vrn-A1L* was more productive compared to i:Bez1*Vrn-A1a* in 2017–2018, while the opposite was observed in 2019 and 2021. In i:Bez1*Vrn-A1L*, the number of spikelets in the spike was higher than in i:Bez1*Vrn-A1a* in all experiments. i:Bez1*VRN-A1L* had a slightly long stem than i:Bez1*Vrn-A1a.*

Two-factor ANOVA revealed a significant effect of the *Vrn-A1a* and *Vrn-A1L* alleles on the number of spikelets per spike, plant productivity traits (the number of spikes, grains, and grain weight), and plant height. The vegetation conditions had an effect on all studied traits. The genotype × environment interaction influenced the variability of spike length, the weight of grains per spike, the weight of 1000 grains, and plant productivity traits (Table S1).

Allelic diversity of *VRN* genes in *T. aestivum* ssp. *petropavlovskyi* accessions from the VIR collection

Since the *Vrn-A1L* dominant allele was detected in *T. aestivum* ssp. *petropavlovskyi* (KIZ) accession, it's necessary to determine the allelic diversity of the *VRN* genes in other *T. aestivum* ssp. *petropavlovskyi* accessions from the VIR collection (Table 5, Fig. S1). These *T. aestivum* ssp. *petropavlovskyi* accessions have a spring type of development; the shoots-heading period in the field conditions varied from 46 to 59 days. All the accessions studied using the VRN1AF and VRN1-INT1R primers amplified a 713 bp PCR product characteristic of the *vrn-A1* allele, which characterizes the intact promoter, while the Intr1/A/F2 and Intr1/A/R3 primers detected a 522 bp fragment of the *Vrn-A1L* allele. Four *T. aestivum* ssp. *petropavlovskyi* accessions had the *Vrn-B1a* dominant allele, and three had the *vrn-B1* recessive allele. The *Vrn-D1a* dominant allele was detected in five samples when amplified with Intr1/D/F and Intr1/D/R3 primers (1671 bp PCR product); the remaining accessions showed a *vrn-D1* recessive allele with Intr1/D/F and Intr1/D/R4 primers (PCR product of about 1000 bp). A recessive allele *vrn-B3* was detected in the accessions studied. Thus, differences were revealed in the allelic state of the *VRN-B1* and *VRN-D1* loci.

The spring type of development of K-51763, K-43351, and K-43376 T. *aestivum* ssp. *petropavlovskyi* accessions is determined by three dominant alleles: *Vrn-A1L, Vrn-B1a,* and *Vrn-D1a.* K-44126 and K-51766 accessions carry two dominant alleles: *Vrn-A1L Vrn-B1a* and *Vrn-A1L VRN-D1a*. K-51764 and KIZ are monogenic carriers; their spring type of development is due to the *Vrn-A1L* allele. This may explain the longer shoots-heading period of these accessions compared to accessions with two or three dominant genes.

Discussion

In terms of expanding the breeding and adaptive potential of bread wheat, wild and cultivated wheat species are of special interest because they carry valuable genetic variants not represented in the bread wheat genome. Examples of introgression of biotic resistance genes are the most common. However, the interspecific or intergeneric introgression of gene alleles, which determine different heading initiation times, provide an important opportunity to control the duration of the growing season, resulting in increased adaptive capacity of bread wheat, especially in changing climate conditions. However, there are only a few known examples of such introgressions. For example, Ivaničová et al. (2016) introgressed the first discovered *Vrn-A1f-like* allele from *T. militinae* into the bread wheat genome. In the research by Takumi et al. (2020), segments of *Ae. tauschii* chromosomes with early heading date genes were introgressed into Japanese elite cultivars through synthetic hexaploid wheat lines. In our present work, in winter cultivars Filatovka and Ul'ynovka, the recessive *vrn-A1* allele of wheat was replaced with the dominant rye *VRN-R1* gene, which led to a change in the winter type of development to the spring type (Efremova et al. 2022). In this study, *T. aestivum* ssp. *petropavlovskyi* hexaploid wheat was used for this purpose to increase the bread wheat genetic diversity by allelic variants of the *VRN-A1* gene. According to the latest data, *T. aestivum* ssp. *petropavlovskyi,* also known as

Table 4 Productivity of the NILs Bez1*Vrn-A1a* and Bez1*Vrn-A1L* in field

Line	Year	Productivity of the main spike				Plant productivity			Weight of 1000 grains, g	Plant height, cm
		Spike length, cm	Spikelet number, pcs	Grain number, pcs	Grain weight, g	Spike number, pcs	Grain number, pcs	Grain weight, g		
i:Bez1*Vrn-A1L*	2017	8.76 ± 0.51	18.92 ± 1.32	32.60 ± 7.14	1.14 ± 0.36	9.16 ± 3.13	178.72 ± 781.78	5.80 ± 2.63	32.38 ± 7.87	
i:Bez1*Vrn-A1a*		8.27 ± 0.46	15.58 ± 0.97	35.29 ± 7.15	1.12 ± 0.20	6.92 ± 1.91	149.83 ± 43.72	4.15 ± 1.42	27.76 ± 4.76	
Differences		+ 0.49 **	+ 3.34 ***	− 2.69	+ 0.02	+ 2.24 **	+ 28.89	+ 1.65 **	+ 4.62 *	
i:Bez1*Vrn-A1L*	2018	9.77 ± 0.39	19.96 ± 1.17	42.84 ± 4.62	2.02 ± 0.32	6.56 ± 2.96	223.90 ± 43.72	8.99 ± 2.02	40.98 ± 5.24	74.12 ± 4.95
i:Bez1*Vrn-A1a*		9.36 ± 0.09	18.68 ± 0.22	39.36 ± 1.13	1.56 ± 0.06	6.32 ± 0.34	191.96 ± 10.45	7.00 ± 0.45	36.31 ± 4.63	64.16 ± 3.92
Differences		+ 0.41 **	+ 1.28 ***	+ 3.48 *	+ 0.46 ***	+ 0.24	+ 31.94 *	+ 1.99 **	+ 4.67 **	+ 9.96 ***
i:Bez1*Vrn-A1L*	2019	8.70 ± 0.91	18.28 ± 0.98	38.92 ± 7.05	1.69 ± 0.40	3.20 ± 0.96	111.16 ± 42.45	4.80 ± 1.91	42.96 ± 3.40	56.48 ± 4.88
i:Bez1*Vrn-A1a*		8.77 ± 0.57	16.71 ± 1.51	43.82 ± 4.97	1.95 ± 0.28	4.14 ± 1.43	161.96 ± 62.37	6.85 ± 2.23	42.76 ± 2.89	52.14 ± 3.50
Differences		-0.07	+ 1.57 ***	− 4.90 **	− 0.26 **	− 0.94 **	− 50.80 **	− 2.05 ***	+ 0.20	+ 4.34 ***
i:Bez1*Vrn-A1L*	2021	8.92 ± 0.65	19.40 ± 1.40	42.27 ± 5.98	1.78 ± 0.35	7.60 ± 2.06	246.40 ± 81.73	9.04 ± 2.92	37.07 ± 4.20	76.90 ± 7.52
i:Bez1*Vrn-A1a*		9.63 ± 0.53	18.57 ± 1.57	43.53 ± 4.33	1.88 ± 0.25	11.43 ± 3.10	419.07 ± 132.84	17.10 ± 5.48	40.81 ± 4.00	73.00 ± 2.24
Differences		-0.71 ***	+ 0.83 *	− 1.26	− 0.10	− 3.83 ***	− 172.67 ***	− 8.06 ***	− 3.74 ***	+ 3.90 **

Significant differences between NILs Bez1*Vrn-A1a* and Bez1*Vrn-A1L*: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Values are means ± S.D

Table 5 Allelic variants of *VRN* genes in *T. aestivum* ssp. *petropavlovskyi* accessions from the VIR collection

Accession	Days to heading (field 2021)	Allelic variants of *VRN* genes				
		VRN-A1 promoter	*VRN-A1* intron 1	*VRN-B1* intron 1	*VRN-D1* intron 1	*VRN-B3* promoter
K-44126	48.11 ± 2.45	*vrn-A1*	*Vrn-A1L*	*Vrn-B1a*	*vrn-D1*	*vrn-B3*
K-51763	45.71 ± 1.41	*vrn-A1*	*Vrn-A1L*	*Vrn-B1a*	*Vrn-D1a*	*vrn-B3*
K-43351	48.27 ± 1.48	*vrn-A1*	*Vrn-A1L*	*Vrn-B1a*	*Vrn-D1a*	*vrn-B3*
K-51764	51.97 ± 2.65	*vrn-A1*	*Vrn-A1L*	*vrn-B1*	*vrn-D1*	*vrn-B3*
KIZ	59.19 ± 2.20	*vrn-A1*	*Vrn-A1L*	*vrn-B1*	*vrn-D1*	*vrn-B3*
K-51766	45.82 ± 1.52	*vrn-A1*	*Vrn-A1L*	*vrn-B1*	*Vrn-D1a*	*vrn-B3*
K-43376	47.24 ± 1.25	*vrn-A1*	*Vrn-A1L*	*Vrn-B1a*	*Vrn-D1a*	*vrn-B3*

"Daosuimai" or rice-head wheat endemic, is a subspecies of bread wheat, which has a limited range of growing in the wild in Xinjiang and Tibet.

In this work, we studied the genetic control of heading time of *T. aestivum* ssp. *petropavlovskyi* accessions. It was shown that the spring type of development in *T. aestivum* ssp. *petropavlovskyi* accessions is determined by one to three dominant alleles: *Vrn-A1L, Vrn-B1a,* and *Vrn-D1a*, associated with deletions in the first intron. The *Vrn-B1a* and *Vrn-D1a* alleles are common among bread wheat cultivars, while *Vrn-A1L* has not been found in wheat hexaploid forms including *T. aestivum*. Previously, the *Vrn-A1L* was described only in tetraploid wheat belonging to the *Dicoccoides* section: *T. aethiopicum, T. carthlicum, T. dicoccoides, T. dicoccum*, *T. durum, T. polonicum*, and *T. turgidum* (Fu et al. 2005; Oliveira et al. 2012; Shcherban et al. 2015; Konopatskaia et al. 2016; Muterko et al. 2016a). Phylogenetic analysis indicates that *T. aestivum* ssp. *petropavlovskyi* originated in Xinjiang as a result of divergence between *T. polonicum* tetraploid wheat and unknown hexaploid landrace either by spontaneous introgression or by a breeding effort (Liu et al. 2022). It can be assumed that the introgression of *Vrn-A1L* into the genome of *T. aestivum* ssp. *petropavlovskyi* from *T. polonicum* which participated in the origin as one of the presumed ancestors (Liu et al. 2022). The detection of the *Vrn-A1L* dominant allele in all analyzed *T. aestivum* ssp. *petropavlovskyi* accessions in the present research suggests a wide distribution of this allele in *T. aestivum* ssp. *petropavlovskyi* along with tetraploid species. The presence of the *Vrn-D1a* dominant allele in *T. aestivum* ssp. *petropavlovskyi* is explained by the distribution of the *VRN-D1* gene among local and commercial Chinese bread wheat cultivars (Zhang et al. 2010; Goncharov 2012; Chen et al. 2013; Guo et al. 2015).

It is well known that the differences between spring and winter forms of wheat are associated with mutations in the regulatory regions of the *VRN-1* gene, which are the promoter and the first intron. Therefore, the analysis of the structure of these gene regions is extremely important for understanding the mechanisms and features underlying the regulation of heading time. An important task is to investigate the role of first intron structural features in the regulation of plant transition from the vegetative stage to the generative stage. Earlier results confirm the importance of first intron in the sensitivity of plants to vernalization and in determining the heading time. Mutations described above typically affect a putative 2.8 kb vernalization critical region located near the left side of first intron (Fu et al. 2005), where insertions or deletions (including the Langdon mutation characterized by 7222 bp deletion from 391 to 7612 bp) affect changes in *VRN-1* expression (Fu et al. 2005; Shcherban et al. 2013).

It was shown that the *Vrn-A1L* dominant allele in the NIL caused significantly later heading versus the *Vrn-A1a* allele. The main differences in the duration of individual phases, which ultimately determine the duration of the growth season, were observed in the period before the formation of the first node, which reached 20–22 days in a greenhouse. The range of differences in the field under the possible influence of environmental factors decreased slightly, but this trend persisted. The results confirm the data that the differences in the duration of the shoots-heading period are largely due to changes in the length of the tillering – first node period, which is the key stage determining the differences in the duration of the

growing season among the genotypes (Pánková and Košner 2004; Emtseva et al. 2013; Chumanova et al. 2020).

In addition, in the discussed experiments, i:Bez1*Vrn-A1L* was late ripening compared to NILs carrying alleles of the *VRN-B1* locus, so, the longer shoots-heading period of two *T. aestivum* ssp. *petropavlovskyi* accessions compared to other studied accessions is associated with the one *Vrn-A1L* dominant allele in these genotypes. However, the four accessions with different combinations of *Vrn-B1a/vrn-B1* and *Vrn-D1a/vrn-D1* were characterized by almost the same duration of time to heading. This may be due to the different allelic states of other loci involved in the formation of differences in the time of heading in these accessions, as well as differences in the time of heading in monogenic carriers of the *Vrn-A1L* dominant allele. The possible allelic differences in the *PPD* genes confirm the presence of a response to the length of day in the *T. aestivum* ssp. *petropavlovskyi* (KIZ) accession, which requires additional research.

It is known that the variability of wheat flowering timing, determined to a greater extent by *VRN* genes, is of great practical importance, since, being in close relationship with resistance to biotic and abiotic factors, it affects yield components (Worland 1996; Snape et al. 2001; Cockram et al. 2007; Kamran et al. 2014). On the one hand, under the Western Siberia conditions, fast-growing and midseason-ripening cultivars of spring wheat are more productive due to the faster initial organogenesis when the laying of spike productivity traits takes place, which allows them to avoid damage by possible late spring frosts and to form a productive spike with limited precipitation. On the other hand, the longer tillering – first node phase in late maturing cultivars increases the productive tilling capacity and the number of grains under favorable conditions. In different years the influence of different alleles on production performance was found to be unequal. Thus, in 2017–2018, i:Bez1*Vrn-A1L* was more productive compared with i:Bez1*Vrn-A1a*, while in 2019 and 2021, the opposite was observed, which is probably due to less favorable conditions for the vegetative phase of plants, in particular, the drier period in early spring and May–June when the elements of yield structure were laid. However, in all years of observations, i:Bez1*Vrn-A1L* was characterized by a large number of spikelets per spike.

Conclusions

The authors introgressed the *Vrn-A1L* dominant allele from *T. aestivum* ssp. *petropavlovskyi*, characterized by the presence of the Langdon deletion in the first intron, into the bread wheat genome by producing a NIL of the Bez1 winter cultivar. An analysis of the allele composition of *VRN* genes in *T. aestivum* ssp. *petropavlovskyi* accessions revealed that the spring type of development was determined by one to three dominant alleles: *Vrn-A1L, Vrn-B1a,* and *Vrn-D1a*, suggesting a wide distribution of the *Vrn-A1L* allele in *T. aestivum* ssp. *petropavlovskyi* along with tetraploid species. It was shown that the *Vrn-A1L* allele, compared with the *Vrn-A1a* allele, increases the heading time of NIL plants, with the maximum differences associated with the time from seedlings to the first node formation. No unambiguous effect of *VRN-A1* alleles on spike and plant production performance could be detected in a comparative study of NILs. In summary, the new scientific information can be valuable for breeding and further research the mechanisms of regulation of heading time.

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Authors contribution EC: wrote and edited the article, performed PCR, field and greenhouse experiments, and data analysis. TE: conceived and designed research, edited the article, performed field and greenhouse experiments. VV: performed PCR, sequencing samples.

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Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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