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ORIGINAL PAPER

Marker-assisted development of wheat lines of the winter cultivar Bezostaya 1 and the effects of interaction between alleles of *Vrn-A1L* and *Vrn-B1* loci on heading time

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Abstract
The main genetic factor which initiates the flowering is the *Vrn* gene system, which determines the rate of many of the plant's growth and development processes. Introgression of new *Vrn* gene alleles from its relatives into bread wheat makes it possible to increase genetic variables connected to the duration of the growing season parameters and individual developmental phases. Two lines of the winter cultivar of bread wheat Bezostaya 1 (Bez1) with a combination of the dominant alleles *Vrn-A1L Vrn-B1a* and *Vrn-A1L Vrn-B1c* were created which included the introgression of the *Vrn-A1L* (or *Vrn-A1c* Langdon-type deletion) allele from *Triticum petropavlovskyi* Udacz. et Migusch. (or *T. aestivum* ssp. *petropavlovskyi* (Udacz. et Migusch.) N.P. Gontsch). Homozygous lines were isolated from F_3 hybrids by using marker-assisted selection. This lines matured earlier in relation to the original near-isogenic lines which contained the *Vrn-A1L, Vrn-B1a,* and *Vrn-B1c* alleles. The Bez1 *Vrn-A1L Vrn-B1c* line had a shorter germination-first node and germination-heading periods compared to Bez1 *Vrn-A1L Vrn-B1a*, practically showing no difference, in terms of heading, with the early-maturing line i:Bez1 *Vrn-A1a*. In the current paper the results of research into the productivity of the lines using different combinations of *VRN-1* alleles are presented. Thus, the obtained results indicate the possibility of using the *Vrn-A1L* allele carrying out modification for earlier maturity arising as a result of combinations with other dominant *Vrn-B1* alleles.

Keywords *Vrn-A1L* · *Vrn-B1* · PCR · Heading time · Developmental phases · Productivity

Introduction

The adaptability of wheat to a wide range of environment conditions is mainly controlled by genes, determining the vernalization requirement (*Vrn*) and photoperiod sensitivity (*Ppd*). The vernalization pathway is responsible for the prevention of flowering before or during periods of low temperature which are detrimental to the apical meristem of the growth apex. Sensitivity to the photoperiod (day length) in wheat is controlled by the *PPD-1* genes: *Ppd-D1*, *Ppd-B1,* and *Ppd-A1*, localized in the 2D, 2B, and 2A chromosomes, respectively, and allelic differences between dominant and recessive alleles are determined by deletions or insertions in the promoter region (Beales et al. 2007; Nishida et al. 2013). *PPD-1* is the main activator of *TaFT1* (*VRN-3*).

The response to vernalization is controlled by *VRN-1*, *VRN-2*, *VRN-3*, and *VRN-4* genes. *VRN-1* encode MADS-box proteins with high similarity to *Arabidopsis thaliana* meristem identity *APETALA1*. *VRN-1* are three orthologous genes: *Vrn-A1*, *Vrn-B1*, and *Vrn-D1*, located in the 5A, 5B, and 5D chromosomes, respectively (Yan et al. 2003). The presence of at least one *VRN-1* dominant allele determines the spring growth habit in wheat. Plants with *Vrn-A1* mature earlier than plants with *Vrn-B1* or *Vrn-D1* (Goncharov 2004), which correlates with the relative level of expression of these genes (Loukoianov et al. 2005). *VRN-1* is dominant for spring growth habit, whereas *VRN-2* is dominant for winter growth habit (Yan et al. 2004b). Although *VRN-3* in wheat was originally identified as a vernalization gene, the *VRN-3* (*TaFT1*) gene is an ortholog of the *Arabidopsis FT* gene, and an integrator of various pathways, involved in the determination of flowering time

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(Yan et al. 2006). *Vrn-D4* is a *VRN-1* paralog on chromosome 5DS (Kippes et al. 2015). In a simplified form, the process of the interaction of *VRN* genes looks like this: prior to the onset of vernalization, the expression level of *VRN-1* and *VRN-3* is low, which prevents plants from progressing to the stage of generative development. Due to the effects brought about by the influence of vernalization, *VRN-1* expression increases in the cells of the apical meristem, which when reaching a certain threshold value, initiates its transition from the vegetative to the generative stage (Loukoianov et al. 2005). In leaves, the product of the *VRN-1* gene suppresses transcription of the flowering repressor gene *VRN-2,* which in turn suppresses transcription of *VRN-3*. The product of the *VRN-3* gene, whose expression is induced by *PPD-1*, binds to the promoter region of the *VRN-1* gene in apical meristem cells. *VRN-1* expression is then activated, which leads to the formation of a positive feedback loop, and thus the initiation of flowering (Chen and Dubcovsky 2012).

A polymorphism in the promoter and the first intron of *VRN-1* genes causes the known allelic diversity of wheat in heading time. The most common allele of the *Vrn-A1* locus in bread wheat, *Vrn-A1a,* contain a 222 bp insertion and a duplication in the promoter (Yan et al. 2004a). In addition *to Vrn-A1a*, several other alleles were described in bread wheat: *Vrn-A1b* (*Vrn-A1b.1*) and *Vrn-A1c* with a 20 bp and 5.5 kb deletion in the promoter (Yan et al. 2004a) and in the first intron (Fu et al. 2005), respectively. Zhang et al. (2022) identified a new dominant *Vrn-A1o* and resessive *vrn-A1n* alleles with 54 bp and 11 bp deletions in the promoter region, respectively. In the first intron of bread wheat, large deletions are described, that distinguish the dominant alleles *Vrn-B1a* (6.8 kb deletion) (Fu et al. 2005), *Vrn-B1b* (6.8 kb and 36 bp deletions and SNP) (Santra et al. 2009), *Vrn-B1c* (7.6 kb deletion and 400 bp duplication) (Milec et al. 2012; Shcherban et al. 2012b), and *Vrn-B1d* (6.8 kb and 187 bp deletions, 4 bp mutation and SNP) (Zhang et al. 2018) from the recessive ones. A 4.2 kb deletion and 0.8 kb insertion in the first intron is characteristic of the *Vrn-D1a* and *Vrn-D1s* alleles, respectively (Fu et al. 2005; Muterko et al. 2015). *Vrn-D1b* and *Vrn-D1c* are characterized an SNP and a 174 bp insertion in the promoter region, respectively (Zhang et al. 2012, 2015).

The influence of different mutations of the *VRN-1* locus on the timing of the onset of flowering and heading of bread wheat has been established. The most early to mature are the genotypes carrying the *Vrn-A1a*. Genotypes carrying *Vrn-B1c*, in turn, are faster to mature compared to carriers of *Vrn-B1a* (Emtseva et al. 2013). Plants whose development type is controlled by more than one dominant allele (*Vrn-A1a, Vrn-B1a, Vrn-B1c* or *Vrn-D1a*) tend to be faster maturing than those containing only one allele (Kiss et al. 2014; Efremova et al. 2016).

The determined distribution of allelic variants of *Vrn* and *Ppd* differs by geographical region, and different ecological and geographical zones, with different combinations of alleles possessing an advantage and providing a wide level of ecological plasticity in bread wheat (Shcherban et al. 2012a, 2015; Milec et al. 2013; Zhang et al. 2015, 2022; Efremova et al. 2016; Whittal et al. 2018; Shi et al. 2019). The majority of spring cultivars in Russia (including Western Siberia), as well as a number of temperate regions, is characterized by the presence of the two dominant genes *Vrn-A1* (*Vrn-A1a*) and *Vrn-B1* (*Vrn-B1a, Vrn-B1c*) which are faster in maturing and more productive than cultivars with a single *Vrn* gene (Potokina et al. 2012; Likhenko et al. 2015; Efremova et al. 2016; Smolenskaya et al. 2022).

To increase the genetic diversity of common wheat, dominant alleles found in the *Vrn* genes of its wild relatives can be introgressed into bread wheat genome (Stelmakh and Avsenin 1996; Zhang et al. 2008; Ivaničová et al. 2016). We obtained the near-isogenic line (NIL) of the winter cultivar Bezostaya 1 (Bez1) with the dominant *Vrn-A1L* allele (or *Vrn-A1c* Langdon-type deletion) introgressed from hexaploid wheat *Triticum petropavlovskyi* (Udacz. et Migusch.) (or *T. aestivum* ssp. *petropavlovskyi* (Udacz. et Migusch.) N.P. Gontsch), the effect of which is connected to a significant increase in heading time when compared to the *Vrn-A1a* allele (Chumanova et al. 2023). *Vrn-A1L* occurs in the tetraploid wheat species (Shcherban and Salina 2017; Shi et al. 2019). However, as mentioned above, modern cultivars of spring bread wheat in the world mainly carry alleles of two dominant genes, *Vrn-A1* and *Vrn-B1*. It would therefore be of interest to study the phenotypic effects of the interaction of the *Vrn-A1L* allele with common alleles of the *Vrn-B1* locus (*Vrn-B1a* и *Vrn-B1c*) present among modern commercial bread wheat cultivars in Russia (including Western Siberia) and a number of temperate countries. For these reasons this work is devoted to obtaining and studying lines possessing a combination of these alleles in the genetic background of the winter cultivar Bez1 and studying their influence on the duration of individual developmental phases and productivity.

Materials and methods

Plant material

In the current paper, the lines of the winter cultivar Bez1 with a combination of dominant alleles were obtained by crossing a i:Bez1 *Vrn-B1a* and i:Bez1 *Vrn-B1c* with the i:Bez1 *Vrn-A1L* (Table 1). PCR markers were used to identify homozygous plants among F_2 and F_3 hybrids (Table 2). The lines presented in Table 1 were also used as research material.

Table 1 Lines of the Bez1 cultivar with different *Vrn* alleles used in the study

Lines	Haploid *Vrn* genotype	Donor of dominant *Vrn* gene	References
i:Bez1 *Vrn-A1a*	*Vrn-A1a vrn-B1 vrn-D1*	Triple Dirk D	Efremova (unpublished)
i:Bez1 *Vrn-A1L*	*Vrn-A1L vrn-B1 vrn-D1*	*T. aestivum* ssp. *petropavlovskyi* (KIZ)	(Chumanova et al. 2023)
i:Bez1 *Vrn-B1a*	*vrn-A1 Vrn-B1a vrn-D1*	cv. Diamant II	(Efremova et al. 2011; Shcherban et al. 2012b)
i:Bez1 *Vrn-B1c*	*vrn-A1 Vrn-B1c vrn-D1*	cv. Saratovskaya 29	(Efremova et al. 2011; Shcherban et al. 2012b)
Bez1 *Vrn-A1a Vrn-B1a*	*Vrn-A1a Vrn-B1a vrn-D1*	i:Bez1 *Vrn-A1a* i:Bez1 *Vrn-B1a*	(Chumanova et al. 2018)
Bez1 *Vrn-A1a Vrn-B1c*	*Vrn-A1a Vrn-B1c vrn-D1*	i:Bez1 *Vrn-A1a* i:Bez1 *Vrn-B1c*	(Chumanova et al. 2018)
Bez1 *Vrn-A1L Vrn-B1a*	*Vrn-A1L Vrn-B1a vrn-D1*	i:Bez1 *Vrn-A1L* i:Bez1 *Vrn-B1a*	Present study
Bez1 *Vrn-A1L Vrn-B1c*	*Vrn-A1L Vrn-B1c vrn-D1*	i:Bez1 *Vrn-A1L* i:Bez1 *Vrn-B1c*	Present study

Table 2 Set of primers used in the present study

Primers name	Sequence (5′ to 3′)	Alleles	Annealing temperature (°C)	Expected size of product (bp)	References
Intr1/C/F Intr1/AB/R	GCACTCCTAACCCACTAACC TCATCCATCATCAAGGCAAA	*vrn-A1*	56	1068	Fu et al. (2005)
Ex1/C/F Intr1/A/R3	GTTCTCCACCGAGTCATGGT AAGTAAGACAACACGAATGTGAGA	*Vrn-A1L*	56	522	
Intr1 Intr1/B/R3	ATCATCTTCTCCACCAAGGG CTCATGCCAAAAATTGAAGATGA	*Vrn-B1a* *Vrn-B1c*	58	1124 737	Shcherban et al. (2012b)
Intr1/B/F Intr1/B/R4	CAAGTGGAACGGTTAGGACA CAAATGAAAAGGAATGAGAGCA	*vrn-B1*	56	1149	Fu et al. (2005)

DNA extraction and PCR amplification

Genomic DNA was extracted from leaves following Sharp et al. (1988). PCR was performed in a 25 µL volume with 12.5 µl BioMaster HS-Taq PCR (2×) (Biolabmix, Russia) (100 mM Tris–HCl (pH 8.5 at 25 °C) 100 mM KCl, 0.4 mM of each dNTP, 4 mM $MgCl_2$, 0.06 U/µl Taq DNA polymerase, 0.2% Tween 20, stabilizers of HS-Taq DNA polymerase), 0.5 µM of each primer, 100 ng of genomic DNA and H_2O, up to 25 µL. The structure of the used primers and PCR conditions were consistent with the published protocols (Table 2). The reactions were run on a BIO-RAD T100 Thermal Cycler (Bio-Rad, USA). The amplification products were separated by electrophoresis on a 1.5% agarose gel in 1×TAE buffer stained with ethidium bromide and visualized using the Doc-Print II gel documentation system (Vilber Lourmat, France).

Growth conditions and data analysis

The study of the duration of the individual developmental phases in the lines presented in Table 1 was carried out during spring sowing in 2022 in the experimental field of the Institute of Cytology and Genetics (IC&G SB RAS) (Novosibirsk) (55°N, 82°E) under natural long day (LD) (day length for the May–August period, 17 h). Seed sowing was on May 17 in 2021 and May 31 in 2022. The experiment was also conducted in a hydroponic greenhouse of the Laboratory of Artificial Plant Growth of IC&G under conditions with controlled temperature (20–25 °C) and illumination. The experiment was conducted under both LD (18 h light) and short day (SD, 14 h light) conditions. Plants were grown in pots with volume of 5 L with ten plants per pot. F_1 and F_3 hybrids were grown in the greenhouse under SD, while F_2 hybrids were grown under LD conditions. Phenological stages were recorded following the Zadoks scale (Tottman et al. 1979). The following developmental phases were noted both in the field and in the greenhouse: Z10 (emergence), Z31 (first node), Z39 (flag leaf) and Z60 (full heading). At least 30 plants from each line were studied in each experiment. To assess the significance of the differences between the mean values, Student's test was used. The productivity of the plants from the new lines was evaluated during cultivation in the field in 2022. Bez1 *Vrn-A1a Vrn-B1a* and Bez1 *Vrn-A1a Vrn-B1c* lines were grown in 2021. The productivity components of the main spike and plant, the weight of 1000 grains, and plant height were examined. 25 plants from each line were studied.

Overall, 2021 was quite favorable in the field of plant growth and development (Fig. 1). May was characterized by high temperatures and a strong lack of precipitation. The average daily temperature was 14.2 °C, which was 3.3 °C higher than annual average. May and July were characterized by below-normal precipitation (70% and 40% of annual average, respectively). Precipitation in June was 130% of the annual average. The meteorological conditions of the 2022 growing season, were unfavorable for the growth of plants. May also was characterized by high temperatures and a strong lack of precipitation. The average daily temperature was 15.4 °C, which was 4.5 °C higher than annual average. The total amount of precipitation for the month (3 mm) was only 8% of the annual average. Before sowing, there was a significant lack of moisture in the soil, which, together with the high temperatures, had a negative impact on the initial periods of plant growth. In June, on the contrary, the first ten days turned out to be cold with an average temperature of about 12 °C. Also, the second ten days of July turned out to be dry, during which only 1 mm of precipitation fell, which could have affected the passage of the phases starting from the appearance of the first node until heading. In August, the first and third ten day periods were characterized by below-normal precipitation (40% of annual average). In general, 2022 was characterized by a significant lack of precipitation during the growing season.

Results

Production of Bez1 winter wheat lines with a combination of dominant alleles *Vrn-A1L Vrn-B1a* and *Vrn-A1L Vrn-B1c* using molecular markers

Using PCR markers (Table 1) in plants F_1: i:Bez1 *Vrn-A1L*×i:Bez1 *Vrn-B1a* and i:Bez1 *Vrn-A1L*×i:Bez1 *Vrn-B1c*, amplification fragments possessing the expected size characteristic of heterozygous genotypes were identified (Fig. 2). In the F_1 hybrids late maturation prevailed. The duration of the period before the heading of plants in both hybrid combinations was 69 days, which corresponded to the heading time of plants i:Bez1 *Vrn-A1L.*

Analysis was carried out on 38 plants possessing the combination F_2 i:Bez1 *Vrn-A1L*×i:Bez1 *Vrn-B1a*, and 37 of those with the combination F_2 i:Bez1 *Vrn-A1L*×i:Bez1 *Vrn-B1c*. In Fig. 3 and Fig. 4 electrophoregrams of different plant genotypes that were detected in the analysis of F_2 hybrids are shown. In the first combination, 27 plants (71.0%) with two dominant alleles were isolated and in the second combination there were 20 plants (54.0%), among which the number of plants with two *Vrn* genes homozygous for one of the alleles was 32–50%. Homozygous

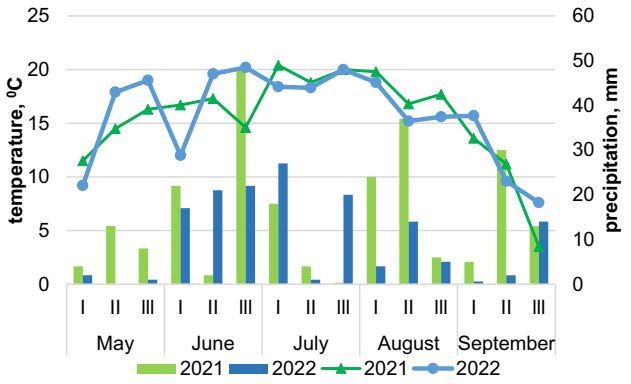

Fig. 1 Temperature and precipitation in the 2021 and 2022. Temperature is represented as a line, precipitation as a histogram

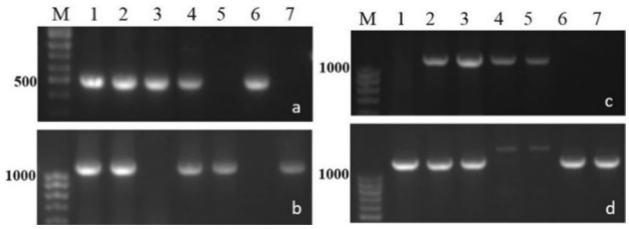

Fig. 3 Identification of dominant and recessive alleles of *Vrn-A1* and *Vrn-B1* loci in F_2 hybrids: i:Bez1 *Vrn-A1L*×i:Bez1 *Vrn-B1a* using PCR markers: **a** *Vrn-A1L,* **b** *vrn-A1,* **c** *Vrn-B1a,* **d** *vrn-B1.* M – 100 bp Ladder. 1 to 7—different genotypes: 1—*Vrn-A1L vrn-A1 vrn-B1 vrn-B1;* 2—*Vrn-A1L vrn-A1 Vrn-B1a vrn-B1;* 3—*Vrn-A1L Vrn-A1L Vrn-B1a vrn-B1;* 4—*Vrn-A1L vrn-A1 Vrn-B1a Vrn-B1a;* 5—*vrn-A1 vrn-A1 Vrn-B1a Vrn-B1a*; 6—*Vrn-A1L Vrn-A1L vrn-B1 vrn-B1*; 7—*vrn-A1 vrn-A1 vrn-B1 vrn-B1*

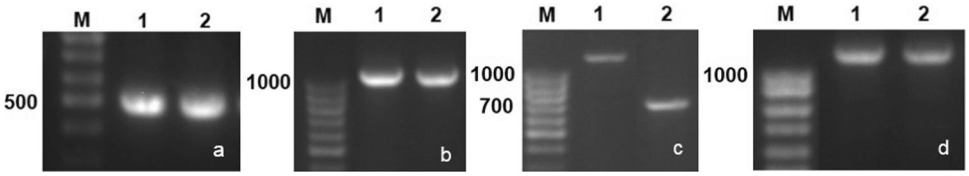

Fig. 2 Identification of dominant and recessive alleles of *Vrn-A1* and *Vrn-B1* loci in F_1 hybrids: i:Bez1 *Vrn-A1L*×i:Bez1 *Vrn-B1a* (1) and i:Bez1 *Vrn-A1L*×i:Bez1*Vrn-B1c* (2) using PCR markers: **a** *Vrn-A1L,* **b** *vrn-A1,* **c** *Vrn-B1a* and *Vrn-B1c,* **d** *vrn-B1.* M – 100 bp Ladder

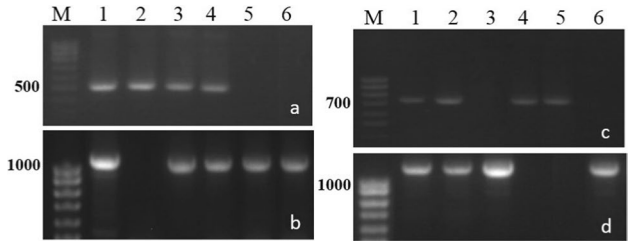

Fig. 4 Identification of dominant and recessive alleles of *Vrn-A1* and *Vrn-B1* loci in F_2 hybrids: i:Bez1 *Vrn-A1L*×i:Bez1 *Vrn-B1c* using PCR markers: **a** *Vrn-A1L,* **b** *vrn-A1,* **c** *Vrn-B1c,* **d** *vrn-B1.* M—100 bp Ladder. 1 to 6—different genotypes: 1—*Vrn-A1L vrn-A1 Vrn-B1c vrn-B1;* 2—*Vrn-A1L Vrn-A1L Vrn-B1c vrn-B1;* 3—*Vrn-A1L vrn-A1 vrn-B1 vrn-B1;* 4—*Vrn-A1L vrn-A1 Vrn-B1c Vrn-B1c;* 5—*vrn-A1 vrn-A1 Vrn-B1c Vrn-B1c*; 6—*vrn-A1 vrn-A1 vrn-B1 vrn-B1*

plants with two dominant alleles in the F_2 generation$_2$ were not isolated (Tables 3 and 4).

The heading time of plants with the combination of F_2: i:Bez1 *Vrn-A1L*×i:Bez1 *Vrn-B1a,* carrying the *Vrn-B1a* allele varied from 48.3 to 53.5 days, and those with the combination F_2: i:Bez1 *Vrn-A1L*×i:Bez1 *Vrn-B1c,* carrying the *Vrn-B1c* allele ranged from 44.8 to 53.9 days. The lowest number of days before heading in both combinations was typical for plants of the *Vrn-B1 Vrn-B1 Vrn-A1L vrn-A1* genotype. The highest number of days before heading was observed in plants of the *Vrn-A1L vrn-A1 vrn-B1 vrn-B1* genotype (69.0 and 65.4 days, respectively).

Further, during the self-pollination of plants with the *Vrn-A1L Vrn-A1L Vrn-B1a vrn-B1* and *Vrn-A1L Vrn-A1L Vrn-B1c vrn-B1* genotypes of generation F_3, homozygous plants were isolated. In the first combination there were 4 such plants out of the 38 that were analyzed and in the second 13 out of 39 (Table 5 and 6, Fig. 5).

Table 3 The average value of the number of days before the heading of the F_2 hybrid plants: i:Bez1 *Vrn-A1L*×i:Bez1 *Vrn-B1a*

Combination of genotype	Number of plants	Average heading time (days)
Vrn-A1L vrn-A1 Vrn-B1a Vrn-B1a	4 (10.5%)	48.3
Vrn-A1L Vrn-A1L Vrn-B1a vrn-B1	11 (28.9%)	51.0
Vrn-A1L vrn-A1 Vrn-B1a vrn-B1	12 (31.6%)	52.6
vrn-A1 vrn-A1 Vrn-B1a Vrn-B1a	2 (5.3%)	53.5
Vrn-A1L Vrn-A1L vrn-B1 vrn-B1	2 (5.3%)	65.0
Vrn-A1L vrn-A1 vrn-B1 vrn-B1	1 (2.6%)	69.0
vrn-A1 vrn-A1 vrn-B1 vrn-B1	6 (15.8%)	–
Total	38 (100%)	

– Indicates that values were not obtained before the experiment stopped (after 120 days from shoots)

Table 4 The average value of the number of days before the heading of the F_2 hybrid plants: i:Bez1 *Vrn-A1L*×i:Bez1 *Vrn-B1c*

Combination of genotype	Number of plants	Average heading time (days)
Vrn-A1L vrn-A1 Vrn-B1c Vrn-B1c	6 (16.2%)	44.8
vrn-A1 vrn-A1 Vrn-B1c Vrn-B1c	3 (8.1%)	46.0
Vrn-A1L Vrn-A1L Vrn-B1c vrn-B1	3 (8.1%)	47.0
Vrn-A1L vrn-A1 Vrn-B1c vrn-B1	11 (29.7%)	53.9
Vrn-A1L vrn-A1 vrn-B1 vrn-B1	7 (18.9%)	65.4
vrn-A1 vrn-A1 vrn-B1 vrn-B1	7 (18.9%)	–
Total	37 (100%)	

– Indicates that values were not obtained before the experiment stopped (after 120 days from shoots)

There was a clear trend in heading time differences depending on the allelic state *Vrn-B1a* and *Vrn-B1c* locus in F_3 hybrids since *Vrn-A1L* was in the homozygous state in all plants. The effect of *Vrn-B1a* and *Vrn-B1c* on heading time was 10–11 days in the presence of the *Vrn-A1L* allele in homozygous state (Tables 5 and 6). The difference between homozygotes for dominant and recessive alleles was 24 and 38 days in the hybrid combination F_3: i:Bez1 *Vrn-A1L*×i:Bez1 *Vrn-B1a* and i:Bez1 *Vrn-A1L*×i:Bez1 *Vrn-B1c*, respectively. Plants with *Vrn-B1c* headed earlier than those with *Vrn-B1a*, which corresponds to the previously obtained data concerning the differences in the timing of heading of isogenic lines i:Bez1 *Vrn-B1c* and i:Bez1 *Vrn-B1a* (Emtseva et al. 2013; Chumanova et al. 2020).

Thus, the use of molecular markers has proven to be effective in isolating plants, homozygous for *Vrn* loci targetting in F_3 generation hybrids.

Determination of the influence of combinations of dominant alleles of *VRN-1* loci on the heading time and the duration of individual developmental phases

The earliest maturing lines in the field and in the greenhouse were Bez1 *Vrn-A1L Vrn-B1a* and Bez1 *Vrn-A1L Vrn-B1c* (Table 7, Figs. 6 and 7) which practically did not differ from each other with regard to the duration up to Z60 period (38.7 and 38.1 days in the field and 40.9 and 42.2 in the greenhouse). In addition, these lines differed significantly in heading time from NIL with the allele *Vrn-A1a* associated with faster maturation, which possessed a duration of the up to Z60 period which varied from 44.5 to 49.2 days. On the contrary the *Vrn-A1L* allele is associated with late maturation. NIL was headed in 62.8–69.7 days. The heading time of i:Bez1 *Vrn-B1c* varied from 47.8 to 59.4 days, and i:Bez1 *Vrn-B1a* from 48.6 to 59.3 days. Plants of lines Bez1

Table 5 The average value of the number of days before the heading of the F_3 offspring from the self-pollination of plants of genotype *Vrn-A1L Vrn-A1L Vrn-B1a vrn-B1*

Combination of genotype	Number of plants	Average heading time (days)	Min–max days to heading
Vrn-A1L Vrn-A1L Vrn-B1a Vrn-B1a	4	54.3	51–58
Vrn-A1L Vrn-A1L Vrn-B1a vrn-B1	18	65.1	58–71
Vrn-A1L Vrn-A1L vrn-B1 vrn-B1	16	78.4	71–88
Total	38		

Table 6 The average value of the number of days before the heading of the F3 offspring from the self-pollination of plants of genotype *Vrn-A1L Vrn-A1L Vrn-B1c vrn-B1*

Combination of genotype	Number of plants	Average heading time (days)	Min–max days to heading
Vrn-A1L Vrn-A1L Vrn-B1c Vrn-B1c	13	42.9	41–43
Vrn-A1L Vrn-A1L Vrn-B1c vrn-B1	14	53.2	42–62
Vrn-A1L Vrn-A1L vrn-B1 vrn-B1	12	80.7	66–88
Total	39		

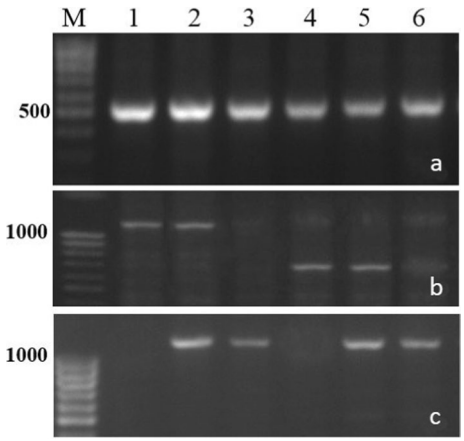

Fig. 5 Identification of dominant and recessive alleles of *Vrn-A1* and *Vrn-B1* loci in F_3 hybrids: i:Bez1 *Vrn-A1L*×i:Bez1 *Vrn-B1a* (1–3) and i:Bez1 *Vrn-A1L*×i:Bez1 *Vrn-B1c* (4–6) using PCR markers: **a** *Vrn-A1L,* **b** *Vrn-B1a* or *Vrn-B1c,* **c** *vrn-B1.* M—100 bp Ladder

Vrn-A1L Vrn-B1a and Bez1 *Vrn-A1L Vrn-B1c* had faster maturation rates than the original NILs with the alleles *Vrn-A1L*, *Vrn-B1a* and *Vrn-B1c,* respectively ($P < 0.05$), although, initially no differences were assumed with respect to carriers of the *Vrn-B1*. Thus, the difference in heading time between Bez1 *Vrn-A1L Vrn-B1a* and NILs with *Vrn-A1L* and *Vrn-B1a* alleles was 11.6–25.3 and 3.7–8.7 days depending on the vegetation, respectively ($P < 0.05$), and the difference between Bez1 *Vrn-A1L Vrn-B1c* and NILs with alleles *Vrn-A1L* and *Vrn-B1c* were 16.2–28.0 and 6.1–8.2 days, respectively ($P < 0.05$). At the same time, under LD conditions in the greenhouse, there were no differences in heading time between Bez1 *Vrn-A1L Vrn-B1a* and Bez1 *Vrn-A1L Vrn-B1c*, in the field or in the greenhouse under SD conditions, the line with the *Vrn-B1c* allele had faster heading than with the *Vrn-B1a* allele and the difference reached a period of 6.6 days ($P < 0.05$). In addition, it turned out that in terms of heading time Bez1 *Vrn-A1L Vrn-B1c* practically did not differ from the faster maturation line i:Bez1 *Vrn-A1a*. The observed differences between the genotypes with regard to the heading time, both in the field and in the greenhouse, are determined by differences in the duration of the up to Z31 period, which mainly determines the differences in the duration of the germination-heading period (Emtseva et al. 2013; Chumanova et al. 2020) (Figs. 6 and 7). For the duration of the periods Z31–Z39 and Z39–Z60, there was no unambiguous trend. It should be noted that for all the studied lines, a reaction to the length of the day associated with the *Ppd-D1a* allele was characteristic and caused moderate photoperiodic sensitivity of the Bez1 cultivar, which was expressed in an increase in the duration of the period before heading (by 4–11 days) under SD conditions mainly due to an increase in the duration of the up to Z31 period (by 4–14 days) while the duration of the periods Z31–Z39 and Z39–Z60 either showed almost no practical change or only decreased slightly.

Thus, despite the fact that the *Vrn-A1L* allele causes late maturity in plants, it can be successfully used in breeding for faster maturation as a result of combination with the dominant alleles of the *Vrn-B1*.

Study of the productivity of lines in the Bez1 cultivar

Determining the influence of the various dominant alleles of *Vrn* loci and their combinations on productivity indicators is an important task that allows us to select valuable genotypes

Table 7 Student's test for days to heading of the Bez1 lines with different dominant alleles of *VRN* loci and their combinations in field and in greenhouse

Lines	Field, 2022	Greenhouse		
		SD1	SD2	LD
i:Bez1 *Vrn-A1a*	44.47 ± 4.46	47.40 ± 2.27	49.17 ± 1.95	43.95 ± 1.35
i:Bez1 *Vrn-A1L*	69.68 ± 3.44 *1	66.56 ± 1.99 *1	67.43 ± 1.99 *1	62.80 ± 1.10 *1
Difference	25.21	19.16	18.26	18.85
i:Bez1 *Vrn-B1a*	48.60 ± 3.08 *1	58.73 ± 1.44 *1	59.29 ± 2.89 *1	52.82 ± 1.13 *1
i:Bez1 *Vrn-B1c*	47.81 ± 3.02 *1	55.42 ± 3.13 *1 *3	59.40 ± 2.80 *1	50.00 ± 1.81 *1 *3
Difference	0.79	3.31	0.11	2.82
Bez1 *Vrn-A1L Vrn-B1a*	44.40 ± 2.54 *2 *3 *6	55.00 ± 3.22 *1 *3 *6	52.62 ± 2.89 *1 *2 *3	44.08 ± 1.13 *2 *3
Bez1 *Vrn-A1L Vrn-B1c*	41.71 ± 3.14 *1 *2 *4 *5 *7	48.41 ± 2.99 *4 *5 *7	51.23 ± 2.22 *1 *2 *4	43.88 ± 2.91 *2 *4
Difference	2.70	6.59	1.39	0.20
Bez1 *Vrn-A1a Vrn-B1a*	38.68 ± 2.64 *1 *3	40.93 ± 1.68 *1 *3		
Bez1 *Vrn-A1a Vrn-B1c*	38.13 ± 2.66 *1 *4	42.11 ± 1.91 *4		
Difference	0.55	1.18		

Significant differences: 1—from i:Bez1 *Vrn-A1a,* 2—from i:Bez1 *Vrn-A1L,* 3—from i:Bez1 *Vrn-B1a*, 4—from i:Bez1 *Vrn-B1c*, 5—from Bez1 *Vrn-A1L Vrn-B1a*, 6—from Bez1 *Vrn-A1a Vrn-B1a,* 7—from Bez1 *Vrn-A1a Vrn-B1c*. * $P < 0.05$. Values are means ± standard deviation. SD1 and SD2 – two experiments under short days conditions

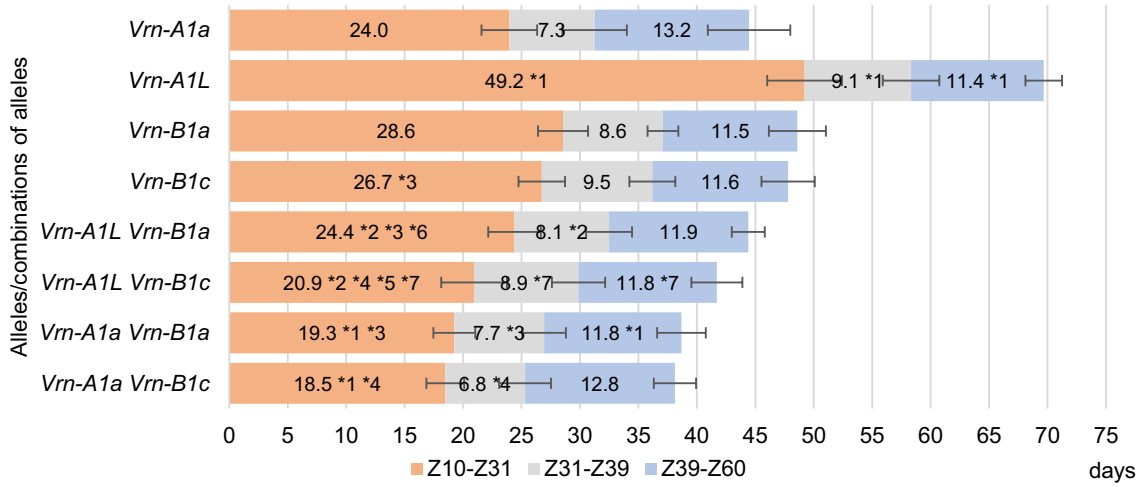

Fig. 6 The duration of developmental phases according to the Zadoks scale (Tottman et al. 1979) of Bez1 lines with dominant alleles of *VRN-1* in field 2022. Significant differences: 1—from i:Bez1 *Vrn-A1a,* 2—from i:Bez1 *Vrn-A1L,* 3—from i:Bez1 *Vrn-B1a*, 4—from i:Bez1 *Vrn-B1c*, 5—from Bez1 *Vrn-A1L Vrn-B1a*, 6—from Bez1 *Vrn-A1a Vrn-B1a*, 7—from Bez1 *Vrn-A1a Vrn-B1c*. *$P < 0.05$. Error bars are standard deviation of the means

for breeding purposes. A comparative study of the productivity of new lines revealed that the main spike and plant productivity of the line with a combination of alleles *Vrn-A1L* and *Vrn-B1a,* in all of the studied characters, exceeded those with a line possessing a combination of alleles *Vrn-A1L* and *Vrn-B1c* (Table 8). The greatest differences were observed in terms of the spikelet number, grain weight per spike and plant productivity (exceeding by 13–25%) ($P < 0.05$). Comparison of lines with a combination of *Vrn-A1a Vrn-B1a* and *Vrn-A1a Vrn-B1c* alleles showed that the first line was more productive than the second line, which is especially evident with respect to grain weight per spike and per plant and weight of 1000 grains (exceeding by 33–49%) ($P < 0.05$) (Table 8).

Discussion

DNA markers (marker-assisted selection) are widely used in genetic research and plant breeding to solve theoretical problems and accelerate and simplify the breeding process.

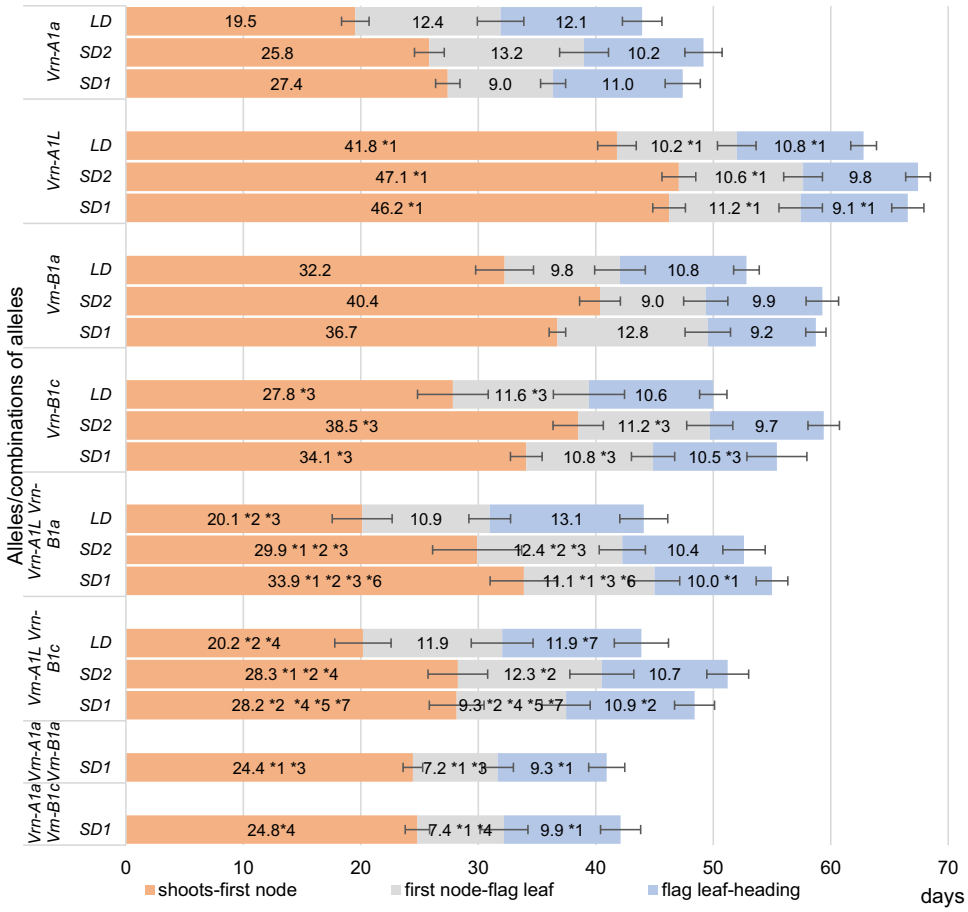

Fig. 7 The duration of developmental phases of Bez1 lines with dominant alleles of *VRN-1* in greenhouse under SD and LD conditions. Significant differences: 1—from i:Bez1 *Vrn-A1a,* 2—from i:Bez1 *Vrn-A1L,* 3—from i:Bez1 *Vrn-B1a*, 4—from i:Bez1 *Vrn-B1c*, 5—from Bez1 *Vrn-A1L Vrn-B1a*, 6—from Bez1 *Vrn-A1a Vrn-B1a*, 7—from Bez1 *Vrn-A1a Vrn-B1c*. * $P < 0.05$. Error bars are SD of the means. SD1 and SD2-experiments under short days in 2022 and in 2023, respectively

Markers are also used for the pyramiding of genes controlling the same trait. The use of markers allows for the identification of genotypes containing the targeted combinations of genes at the earliest stages of the creation of breeding material (Song et al. 2023). In this work, homozygous Bez1 cultivar lines were obtained among F_3 hybrids. The use of PCR markers developed for the regions of the promoter and the first intron of *VRN-1* makes it possible to increase the screening efficiency of alleles of *Vrn* genes and reduce the selection time of target gene combinations.

The use of different alleles or different combinations of allelic variants of *VRN-1* with different phenotypic manifestations at the heading time provides an opportunity for the manipulation of the length of the growing season inherent in bread wheat (Kamran et al. 2014; Shi et al. 2019). The genetic models we used include combinations of *Vrn-A1*a/*Vrn-A1L* alleles with *Vrn-B1aVrn-B1c*. The presence of different sets of genotypes makes it possible to study the genetic effects of different combinations of alleles within a specific targeted genetic background. The lines with the *Vrn-A1* locus: *Vrn-A1a* and *Vrn-A1L* differ from each other in heading time (Chumanova et al. 2023), and the differences between NILs with *Vrn-B1c* and *Vrn-B1*a are were about 4 days (Emtseva et al. 2013). Based on this, the dominant alleles of *VRN-1* in order to decrease the duration of the period before heading can be arranged as follows: *Vrn-A1a* > *Vrn-B1c* > *Vrn-B1a* > *Vrn-A1L*. The data presented and obtained earlier (Chumanova et al. 2020) show that the lines with the*Vrn-A1a Vrn-B1a* and *Vrn-A1a Vrn-B1c* alleles do not differ in the timing of heading or the duration of the developmental phases, and they are also faster in maturation compared to i:Bez1 *Vrn-A1a*. It is known that *Vrn-A1* has the strongest effect on the acceleration of heading among the three *VRN-1* genes (Goncharov et al. 2004) and that the *Vrn-A1a* allele is epistatic with respect to the alleles of the *Vrn-B1* (Li et al. 2017). However, plants bearing combinations of two or three dominant alleles tend to possess faster maturation than those with a single allele, including *Vrn-A1a*, which is associated with the additive effect of genes (Potokina et al. 2012; Kiss et al. 2014; Zhang et al. 2014; Shcherban et al. 2015; Efremova et al. 2016). Interesting results were obtained when studying the second group of lines possessing a combination of alleles. The introduction of the dominant *Vrn-A1L* allele into the genotype of the winter cultivar Bez1, which determines the late maturity of the plants, in combination with alleles of the *Vrn-B1* led to the production of faster maturing lines relative to the original NILs, carriers of these alleles. It is probable that there is also

Table 8 Productivity of lines with the combination of dominant alleles of the *VRN-1* loci

Lines	Year	Plant height, cm	Productivity of the main spike				Weight of 1000 grains (g)	Plant productivity		
			Spike length (cm)	Spikelet number (pcs)	Grain number (pcs)	Grain weight (g)		Spike number (pcs)	Grain number (pcs)	Grain weight (g)
Bez1 *Vrn-A1L Vrn-B1a*	2022	59.22 ± 0.96	8.48 ± 0.06	19.63 ± 0.39	39.89 ± 0.86	1.67 ± 0.05	38.64 ± 0.58	5.05 ± 0.30	172.89 ± 10.46	6.70 ± 0.42
Bez1 *Vrn-A1L Vrn-B1c*	2022	56.68 ± 1.34	7.89 ± 0.09	16.39 ± 0.26	36.96 ± 0.77	1.48 ± 0.06	37.18 ± 0.67	4.25 ± 0.26	143.61 ± 9.56	5.35 ± 0.38
Difference		2.54 (4.5%)	0.59 (7.5%) *	3.24 (19.8%) *	2.93 (7.9%) *	0.19 (12.8%) *	1.46 (3.9%)	0.80 (18.8%)	29.28 (20.4%)	1.35 (25.2%) *
Bez1 *Vrn-A1a Vrn-B1c*	2021	68.96 ± 0.78	9.34 ± 0.09	18.08 ± 0.31	39.88 ± 1.36	1.99 ± 0.07	50.08 ± 1.58	8.00 ± 0.49	249.28 ± 16.62	12.34 ± 0.77
Bez1 *Vrn-A1a Vrn-B1a*	2021	62.48 ± 0.58	8.88 ± 0.09	17.80 ± 0.20	38.60 ± 1.58	1.50 ± 0.08	35.78 ± 0.93	7.68 ± 0.43	229.12 ± 15.66	8.31 ± 0.69
Difference		6.48 (10.4%) *	0.46 (5.2%) *	0.28 (1.6%)	1.28 (3.3%)	0.49 (32.7%) *	14.3 (40.0%) *	0.32 (4.2%)	20.16 (8.8%)	4.03 (48.5%) *

* – Significant differences between lines at $P < 0.05$. Values are means ± SEM

an additive effect of homeologous *Vrn* genes, that is that in the presence of *Vrn-B1*, there is an increase in the expression of *Vrn-A1L*, which manifests itself in a decrease in the duration of the period from germination to heading, due to a decrease in the duration of the period before the appearance of the first node. We hope that experiments into the study of expression, which we plan to carry out in the future, will help to answer this question. In addition, the Bez1 *Vrn-A1L Vrn-B1c* line differed slightly from the NIL with the *Vrn-A1a*. Similar results were obtained in the work (Dowla et al. 2020). A synthetic cultivar of wheat with *Vrn-A1L* from Langdon durum wheat and *Vrn-B1a*, had a pre-heading period duration only 2 days longer than lines with *Vrn-A1a Vrn-B1a* alleles. In our work, the combination of *Vrn-A1L* and *Vrn-B1c* alleles leads to slightly earlier heading, as well as lessening the duration of the period before the appearance of the first node, than that of the combination of *Vrn-A1L* and *Vrn-B1a*, which may be explained by differences in the heading times of monogenic carriers with *Vrn-B1c* and *Vrn-B1a* as described Emtseva et al. (2013) as well as the genetic background of the lines.

It is well known that the heading time of bread wheat and the duration of the individual developmental phases are extremely important features that determine the ability of bread wheat cultivars to adapt to varied climatic conditions, thereby contributing to a consistently high productivity (Kamran et al. 2014). When choosing cultivars for cultivation that are most suitable for the climatic conditions of a particular region, it is necessary to take into account the maturation speed of different cultivars, so that the most sensitive phases of development, take place under the most optimal environmental conditions (Santra et al. 2009; Gomez et al. 2014; Kamran et al. 2014; Grogan et al. 2016; Amo et al. 2022). In Siberia the selection of early ripening cultivars is one of the important aspects in the breeding of spring bread wheat. Among modern widespread cultivars in Western Siberia, genotypes with a combination of *Vrn-A1a* and *Vrn-B1a* or *Vrn-B1c* alleles predominate, since it is this combination of alleles that ensures optimal heading times and yield potential when taking into account the specific features of the climatic conditions of the region (Likhenko et al. 2015; Efremova et al. 2016; Smolenskaya et al. 2022). The lines with a combination of *Vrn-A1L* and *Vrn-B1a or Vrn-B1c* alleles can be classified as medium-ripe in terms of heading time, which demonstrates the importance of the results obtained for the conditions of Western Siberia. Therefore, the *Vrn-A1L* allele, which was previously introgressed by us from *T. petropavlovskyi*, which had previously not yet been involved in the selection of bread wheat, can be used in breeding for faster maturation as a result of the combination with alleles of the *Vrn-B1*. Introgression of dominant alleles of *Vrn* genes from related cereal species, or the use of rare alleles already available in the gene pool but not yet seeing

wide distribution, is a popular direction for of fundamental and breeding research, since it allows for the increasing of the adaptive potential of bread wheat by increasing the existing allelic diversity in genes that determine the timing of key developmental phases, including flowering.

A comparative study of the productivity of new lines showed that a line combining *Vrn-A1L* and *Vrn-B1a* turned out to result in higher productivity than that containing *Vrn-A1L* and *Vrn-B1c*. However, a comparison of combinations of *Vrn-A1a Vrn-B1a* and *Vrn-A1a Vrn-B1c* alleles with each other, in fact showed the opposite to be true. It is known that a longer vegetative period allows the wheat plant to produce a greater number of productive shoots and consequently a high number of grains under favorable conditions (Royo et al. 2018). Probably, the observed differences in productivity between Bez1 *Vrn-A1L Vrn-B1a* and Bez1 *Vrn-A1L Vrn-B1c* lines are due to the longer period before first node formation of the first line (3 days difference), due to which we observe the formation of a greater number of productive shoots and, consequently, a higher grain number and grain weight per plant. However, the observed differences between Bez1 *Vrn-A1L Vrn-B1a* and Bez1 *Vrn-A1L Vrn-B1c* lines cannot be explained by differences in the duration of developmental phases. It is known that in Western Siberia cultivars with a combination of alleles *Vrn-A1a Vrn-B1c* are more common than with a combination of *Vrn-A1a Vrn-B1a* (Efremova et al. 2016; Smolenskaya et al. 2022), which is associated not only with a shorter period to earing, but also, probably, with greater adaptation to climatic conditions and productivity of such genotypes. Unfortunately, experiments conducted over the course of different years do not allow a comparison to be made between these four combinations, since the realization of productive potential occurs in close interaction with the genotype and the external conditions. Therefore, we plan to conduct repeated experiments in order to obtain unambiguous conclusions regarding the effect of different combinations of *VRN-1* on actual yield, which would allow us to identify genotypes capable of maximizing their yield potential in Western Siberia.

Thus, the possibility has been demonstrated for the manipulation of the length of the growing season of bread wheat by combining the *Vrn-A1L* allele, which determines late maturity, with alleles of the *Vrn-B1* locus, has been demonstrated to facilitate obtaining earlier ripening genotypes that can be used for breeding of bread wheat in Western Siberia.

Conclusion

In this work, lines with two dominant alleles: *Vrn-A1L Vrn-B1a* and *Vrn-A1L Vrn-B1c* were obtained within the genetic background of the winter cultivar of wheat Bez1. Isolation of homozygous plants was performed using marker-assisted selection. The obtained lines produced a shorter duration of germination-heading and germination-first node periods relative to the original NILs with alleles *Vrn-A1L, Vrn-B1a* and *Vrn-B1c*. At the same time, the Bez1 *Vrn-A1L Vrn-B1c* line possessed a shorter duration of the period before heading and the period of germination of the first node compared to Bez1 *Vrn-A1L Vrn-B1a* practically did not differ from the precocious NIL with the *Vrn-A1a* allele. In summary, *Vrn-A1L* allele can be successfully used in breeding for faster maturation in Western Siberia in combination with *Vrn-B1a* and *Vrn-B1c* alleles.

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Authors' contributions 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.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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