Вавиловский журнал генетики и селекции. 2018;22(8):951-956
DOI 10.18699/VJ18.437

Development and investigation of common wheat lines of winter cultivar Bezostaya 1 with combinations of dominant alleles of *VRN-1* loci

E.V. Chumanova[1] ✉, T.T. Efremova[1], Y.V. Kruchinina[1, 2], L.A. Pershina[1]

[1] Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia
[2] Novosibirsk State Agrarian University, Novosibirsk, Russia

VRN genes, determining wheat sensitivity to vernalization, are the main genetic system that defines the duration of the entire growing period and the durations of the main organogenesis phases. To date, several alleles have been described for *VRN-1* loci, and allele-specific primers have been developed that allow rapid identification of allelic spectra in common wheat varieties and lines. The unequal influence of different alleles of *VRN-1* loci on the duration of the growing period has also been shown; however, there is little information on the effect of the combination of different alleles on heading time. In developing genotypes having different alleles of dominant *VRN* genes on the base of the same genetic background, it is necessary to study the genetic effects of *VRN* genes on the duration of the growing season and the individual developmental phases, as well as on productivity. Most varieties presently grown in Russia carry the dominant alleles of two *VRN-1* genes: *Vrn-A1a* and *Vrn-B1a* or *Vrn-B1c*; thus, the task was to create lines combining the dominant alleles of *Vrn-A1a* with *Vrn-B1a* and *Vrn-B1c* against the genetic background of the winter variety Bezostaya 1 (Bez1 *Vrn-A1a*/*Vrn-B1a* and Bez1 *Vrn-A1a*/*Vrn-B1c*). Homozygous plants were isolated in the F_2 generation by using known allele-specific primers for the *Vrn-A1* and *Vrn-B1* loci. The durations of the tillering–first node period, which is the key stage determining growing duration, and the period from shoots to heading were significantly reduced in lines with a combination of two dominant alleles of *VRN-1* loci compared to isogenic lines of Bezostaya 1 with the dominant alleles *Vrn-B1a* and *Vrn-B1c*. The duration of these developmental phases also decreased in the obtained lines as compared to the isogenic line containing the dominant *Vrn-A1a* allele, but the differences were not significant. No substantial differences were found in the duration of other growing phases in lines with two dominant alleles of the *VRN-1* loci as compared to isogenic lines of Bezostaya 1.

Key words: common wheat lines; duration of growing phases; alleles of the *VRN-1* loci; allele-specific primers.

Received August 18, 2018
Revised October 5, 2018
Accepted October 8, 2018

✉ e-mail: chumanova@bionet.nsc.ru

Получение и изучение линий мягкой пшеницы по озимому сорту Безостая 1 с комбинацией доминантных аллелей локусов *VRN-1*

Е.В. Чуманова[1] ✉, Т.Т. Ефремова[1], Ю.В. Кручинина[1, 2], Л.А. Першина[1]

[1] Федеральный исследовательский центр Институт цитологии и генетики Сибирского отделения Российской академии наук, Новосибирск, Россия
[2] Новосибирский государственный аграрный университет, Новосибирск, Россия

Гены чувствительности к яровизации (*VRN*) являются основными генетическими системами, определяющими продолжительность вегетационного периода в целом, а также длительность основных этапов органогенеза. К настоящему времени для локусов *VRN-1* описан ряд аллелей и разработаны аллель-специфичные праймеры, позволяющие проводить быструю идентификацию аллельного состава у сортов и линий мягкой пшеницы. Установлено неодинаковое влияние различных аллелей локусов *VRN-1* на продолжительность вегетационного периода, однако исследований, касающихся влияния комбинации различных аллелей на время колошения, недостаточно. Получение полных наборов всех возможных генотипов по разным аллелям доминантных генов *VRN* на одном генетическом фоне необходимо для более глубокого изучения генетических эффектов генов *VRN* на продолжительность вегетационного периода и отдельных фаз развития, а также на продуктивность. Поскольку большинство современных сортов России несет доминантные аллели двух генов *VRN* (*Vrn-A1a* и *Vrn-B1a* или *Vrn-B1c*), нами была поставлена задача – получить линии, сочетающие доминантные аллели *Vrn-A1a* с *Vrn-B1a* и *Vrn-B1c* на генетическом фоне озимого сорта Безостая 1 (Без1 *Vrn-A1a*/*Vrn-B1a* и Без1 *Vrn-A1a*/*Vrn-B1c*). С использованием известных аллель-специфичных праймеров для локусов *Vrn-A1* и *Vrn-B1* в поколении F_2 были выделены гомозиготные растения. У полученных линий с комбинацией двух доминантных аллелей локусов *VRN-1* достоверно уменьшалась продолжительность периода «кущение–первый узел», который представляет собой ключевой этап, определяющий продолжительность вегетационного периода и периода от всходов до колошения по сравнению с изогенными линиями по сорту Безостая 1 с доминантными аллелями *Vrn-B1a* и *Vrn-B1c*. По сравнению с изогенной линией с доминантным аллелем *Vrn-A1a* у полученных линий также произошло уменьшение продолжительности этих фаз развития, однако различия были недостоверны. Достоверных различий по продолжительности

остальных фаз развития у линий с двумя доминантными аллелями локусов *VRN-1* относительно изогенных линий по сорту Безостая 1 выявлено не было.

Ключевые слова: линии мягкой пшеницы; продолжительность фаз развития; аллели локуса *VRN-1*; аллель-специфичные праймеры.

HOW TO CITE THIS ARTICLE:
Chumanova E.V., Efremova T.T., Kruchinina Y.V., Pershina L.A. Development and investigation of common wheat lines of winter cultivar Bezostaya 1 with combinations of dominant alleles of *VRN-1* loci. Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov Journal of Genetics and Breeding. 2018;22(8):951-956. DOI 10.18699/VJ18.437

The duration of the growing period in common wheat is an important adaptive trait, which determines plant productivity and resistance to biotic and abiotic stress factors: drought, low temperatures, diseases, and pests (Stelmakh, 1990; Worland, 1996; Snape et al., 2001; Cockram et al., 2007; Kamran et al., 2014). The main genetic systems that initiate the transition of wheat plants from the vegetative to the generative stage of development are the genes for vernalization (*VRN*) and photoperiod sensitivity (*PPD*) (Snape et al., 2001; Kamran et al., 2014).

The main loci that determine sensitivity to the photoperiod (*PPD-1*) in wheat were mapped on the short arms of the second homoeologous group chromosomes: 2D, 2B, and 2A (Worland et al., 1998; Snape et al., 2001).

The duration of the wheat growing period is determined by three main *VRN* genes: *VRN-1*, *VRN-2*, and *VRN-3*. The genes *VRN-1* and *VRN-3* were mapped on chromosomes of groups 5 and 7, respectively (Yan et al., 2003, 2006).

Common wheat has three homoeologous *VRN-1* loci: *VRN-A1*, *VRN-B1*, and *VRN-D1*. They are located on the long arms of chromosomes 5A, 5B and 5D, respectively (Snape et al., 2001; Yan et al., 2003). The spring habit of development is determined by the presence of at least one dominant allele; the presence of all the three recessive *vrn* alleles defines the winter habit of wheat varieties. Cultivars bearing the dominant *VRN-A1* allele are completely insensitive to vernalization, whereas cultivars with the dominant alleles *VRN-B1* and *VRN-D1* are slightly sensitive to vernalization (Pugsley, 1971).

For *VRN-1* loci (*VRN-A1*, *VRN-B1*, *VRN-D1*), a series of different alleles was described, mutations resulting in changes in the structure of these alleles were cloned and characterized, and allele-specific primers were designed for rapid genotyping of common wheat varieties and lines (Yan et al., 2004; Fu et al., 2005; Milec et al., 2012; Shcherban et al., 2012). It was found that the dominant alleles of these loci had insertions or deletions in the promoter and the first intron.

It is known that wheat cultivars bearing the dominant *Vrn-A1a* allele are the earliest ripening. The *Vrn-A1b* allele, on the contrary, increases the duration of the heading period (Koval, Goncharov, 1998; Kamran et al., 2014). Genotypes with the dominant *Vrn-B1c* allele ripe earlier than genotypes with *Vrn-B1a* allele (Efremova et al., 2011).

It has been shown that various combinations of alleles of the *VRN* and *PPD* loci differently affect the lengths of the growing period and phases of plant development, as well as productivity. The earliest ripening genotypes are those with three dominant genes (*VRN-A1*, *VRN-B1*, *VRN-D1*); however, they tend to have lower yields, and, for this reason, they are seldom used in breeding. The habit of most Russian varieties (including Siberia), Europe, North America and Australia is determined by two dominant genes *VRN-A1* and *VRN-B1*. These varieties ripe earlier, and they are more productive than varieties with one *VRN* gene, because such allelic composition of the *VRN-1* genes allows the plants to sustain late spring and early autumn frosts (Stelmakh, 1998; Goncharov, 2004; Efremova et al., 2016). Genotypes with the dominant *VRN-D1* gene are advantageous in regions with extreme conditions such as drought and high temperature during grain filling (Stelmakh, 1993).

In particular, the varieties predominant in Russia and West Siberia have two dominant alleles: *Vrn-A1a* and *Vrn-B1c* or *Vrn-B1a* (Shcherban et al., 2012; Likhenko et al., 2014; Efremova et al., 2016). Thus, according to (Efremova et al., 2016), the *Vrn-A1a* allele, which has the greatest effect on the length of the growing period, occurs in about 80 % in the investigated varieties of West Siberia. The frequencies of the *Vrn-B1c* and *Vrn-B1a* alleles vary among accessions within 40–50 % and 30–50 %, respectively. It was also found that the *Vrn-A1a* allele, along with *Vrn-B1a* and *Vrn-B1c*, was typical for spring varieties in Europe (Milec et al., 2012; Shcherban et al., 2014). The *Vrn-A1a* allele is widely distributed among the spring varieties of Canada, USA, Argentina, and China (Yan et al., 2004; Iqbal et al., 2007; Zhang et al., 2008; Santra et al., 2009).

Isogenic lines created on the basis of winter varieties with known alleles of *PPD* genes play an important role in the detailed analysis of the *VRN* genes, which control the duration of the growing period (Stelmakh, Avsenin, 1983; Voronin, Stelmakh, 1985; Koval et al., 2001; Efremova et al., 2011). Study of such lines permits one to single out the effect of alleles of each of the *VRN* genes or different alleles of the same gene, as well as of their combinations, on the duration of the heading period (Merezhko, 1994; Koval, Goncharov, 1998; Stelmakh et al., 2000; Efremova et al., 2011), the duration of the main stages of organogenesis (Voronin, Stelmakh, 1985; Emtseva et al., 2013), and productivity and fitness (Voronin, 1988). In addition, isogenic lines are suitable models for studying the primary structure and expression of *VRN* alleles (Loukoianov et al., 2005; Shcherban et al., 2013).

A series of near-isogenic lines of winter Bezostaya 1 cv. (Bez1) possessing one dominant *Ppd-D1a* gene (determining weak sensitivity to day length (Worland et al., 1998)), with two different alleles of the *VRN-B1* gene (Efremova et al., 2011), as well as on the *VRN-A1a* and *VRN-D4* loci were raised at the Institute of Cytology and Genetics, Novosibirsk. In these lines, different alleles of the dominant *VRN-B1* gene (*Vrn-B1a* and *Vrn-B1c*) were in the same genetic background, and this fact allowed more accurate identification of differences in the duration of the period from shoots to heading. With these lines, it was shown that the *Vrn-A1a* allele determined earlier heading than the *Vrn-B1c* and *Vrn-B1a* alleles, and the *Vrn-B1c*

Table 1. Isogenic lines of Bez1 cultivar used to obtain lines with two dominant alleles of the *VRN-1* loci

Isogenic line	Haploid genotype for *VRN* genes	Donor of the dominant *VRN* gene	References
i: Bez1*Vrn-A1a*	*Vrn-A1a vrn-B1 vrn-D1*	Triple Dirk D	Efremova (unpublished)
i: Bez1*Vrn-B1a*	*vrn-A1 Vrn-B1a vrn-D1*	Diamant II	Efremova et al., 2011; Shcherban et al., 2012
i: Bez1*Vrn-B1c*	*vrn-A1 Vrn-B1c vrn-D1*	Saratovskaya 29	»

Table 2. Primers for identifying alleles of the *VRN-A1* and *VRN-B1* genes in common wheat lines

Allele	Allele-specific primers		Amplicon size, bp	References
Vrn-A1a	VRN1AF	GAAAGGAAAAATTCTGCTCG	650 + 750	Yan et al., 2004
Vrn-A1b	VRN1R	TGCACCTTCCC(C/G)CGCCCCAT	480	
vrn-A1			500	
Vrn-B1a	Ex1/B/F3	GAAGCGGATCGAGAACAAGA	709 + 1235	Milec et al., 2012
Vrn-B1c	Intr1/B/F	CAAGTGGAACGGTTAGGACA	849	
vrn-B1	Intr1/B/R3	CTCATGCCAAAAATTGAAGATGA	1149	
	Intr1/B/R4	CAAATGAAAAGGAATGAGAGCA		

Table 3. PCR schedules with allele-specific primers for the *VRN-A1* and *VRN-B1* genes

Allele	PCR conditions					
	Predenaturation, t^o (min)	Number of cycles	Denaturation, t^o (s)	Annealing, t^o (s)	Elongation, t^o (s)	Final elongation, t^o (min)
Vrn-A1a *Vrn-A1b* *vrn-A1*	94 (10)	40	95 (30)	55 (30)	72 (60)	72 (10)
Vrn-B1a *Vrn-B1c* *vrn-B1*	94 (2)	35	94 (30)	52 (30)	72 (90)	72 (5)

allele, in turn, reduced the duration of the period from shoots to heading as compared to the *Vrn-B1a* allele (Emtseva et al., 2013). However, since most modern commercial varieties in Russia carry the dominant alleles of two genes, *Vrn-A1a* and either *Vrn-B1a* or *Vrn-B1c*, it should be found out how the combination of different alleles of the two genes can affect the time before heading. Therefore, it is advisable to obtain lines with two alleles of these genes against the genetic background of Bez1 cv. This would allow a more detailed study of the contribution of *VRN* alleles to early maturity and productivity. It should be noted that all previously created isogenic lines and lines with two or three dominant *VRN* genes were obtained without taking into account the presence of alleles of the *VRN* genes and their role in controlling heading time. Therefore, the raise of complete sets of all possible genotypes for different alleles of the *VRN-1* loci against the same genetic background is necessary for a more comprehensive study of the genetic effects of the *VRN* loci.

The objectives of this work were (1) to obtain lines combining two different *VRN-1* loci (*Vrn-A1a*/*Vrn-B1a* and *Vrn-A1a*/*Vrn-B1c*) in one genotype on the base of previously obtained isogenic common wheat lines with dominant *Vrn-A1a*, *Vrn-B1a* and *Vrn-B1c* for the winter variety Bez1 (Efremova et al., 2011) and (2) to determine the effect of the combination of alleles on the duration of individual development phases under the conditions of the forest-steppe zone of the Novosibirsk region.

Materials and methods

Plant material. Experiments were performed with (1) lines of winter cultivar Bez1 with combinations of two alleles of the *VRN-1* loci: Bez1 *Vrn-A1a*/*Vrn-B1a* and Bez1 *Vrn-A1a*/*Vrn-B1c* and (2) the parental isogenic lines of Bez1 with dominant alleles *Vrn-A1a*, *Vrn-B1a*, and *Vrn-B1c* (Table 1). The design of the raise of isogenic lines carrying the *Vrn-B1a* and *Vrn-B1c* alleles from the Saratovskaya 29 and Diamant II varieties, respectively, was described in (Efremova et al., 2011). The isogenic lines of Bez1 with the dominant *Vrn-A1a* allele were obtained in a similar manner (Efremova, unpublished). The donor of the dominant *Vrn-A1a* allele for isogenic line i:Bez1*Vrn-A1a* was a Triple Dirk D isogenic line (Pugsley, 1971).

DNA isolation and PCR. Genomic DNA was isolated from leaves of adult plants after digestion with proteinase K according to a previously described method (Edwards et al., 1991). All amplification reactions were carried out in a 25 μl volume containing 50–100 ng genomic DNA, reaction buffer (67 mM Tris HCl pH 8.8, 1.5 mM $MgCl_2$, 18 mM $(NH_4)_2SO_4$, 0.01 % Tween 20), 0.2 mM each dNTP, 0.25 μM primer pair, and 1 unit of *Taq* polymerase.

Amplification of DNA was carried out according to our domestic protocols. The nucleotide sequences of the primers and PCR conditions are shown in Tables 2 and 3. Amplification products were resolved by electrophoresis in 1.5 % agarose gel in 1×TAE buffer with the presence of ethidium bromide.

The gels were photographed under UV illumination with the Doc-Print II documentation system for gels (Vilber Lourmat).

Study of the duration of developmental phases. The durations of individual phases of development in the lines of common wheat with dominant alleles of the *VRN-1* loci were studied in an experimental field of the Institute of Cytology and Genetics SB RAS at natural daylength (At 55°2′ N, 82°56′ E, the daylength in May-August is 17 h.) in 2017. The following phases of development were recorded: shooting, the emergence of the third leaf, tillering, the emergence of the first node, stem elongation, and heading. Tillering was recorded when the second shoot began to depart from the main shoot. The first node phase was recorded when the first node was palpable on the main shoot at a height of 1 cm above the soil surface. The stem elongation phase was recorded when the first node rose to a height of about 5 cm and the second node began to separate from it. Heading was recorded when the spike was completely out of the flag leaf. The dates of developmental stages were recorded for each plant individually, and the mean values were calculated. Twenty-five plants of each line were studied.

The statistical evaluation of the data was carried out with Microsoft Excel 2013. The statistical significance of the differences between mean values was assessed by Student's *t*-test (Rokitskii, 1974).

Results and discussion

Raise of common wheat lines of winter variety Bez1 with a combination of dominant alleles of the *VRN-1* loci. Two near-isogenic lines, i: Bez1*Vrn-B1a* and i: Bez1*Vrn-B1c*, were crossed to the isogenic line i: Bez1*Vrn-A1a*. The resulting F_1 hybrids were self-pollinated. Homozygous plants with two dominant alleles of the *VRN* genes, *Vrn-A1a/Vrn-B1a* and *Vrn-A1a/Vrn-B1c*, were selected with known allele-specific primers for the *VRN-A1* and *VRN-B1* genes presented in Table 2 in the F_2 generation. Amplification of the dominant allele *Vrn-A1a* with the allele-specific primers VRN1AF and VRN1R revealed two fragments approximately 650 bp and 750 bp in size, and in the PCR of the recessive *vrn-A1* allele one fragment of about 500 bp was obtained. Correspondingly, three fragments were amplified in heterozygous plants.

Multiplex PCR with four primers Ex1/B/F3, Intr1/B/F, Intr1/B/R3, and Intr1/B/R4 was used to detect three alleles of the *VRN-B1* locus: *Vrn-B1a*, *Vrn-B1c* and *vrn-B1*. In case of the dominant *Vrn-B1a* allele, the PCR products included two fragments, 709 bp and 1235 bp. For the dominant *Vrn-B1c* allele, a fragment of 849 bp was typical. The presence of the recessive *vrn-B1* allele was judged from the presence of a 1149 bp fragment. In heterozygous *Vrn-B1a/vrn-B1* and *Vrn-B1c/vrn-B1* plants, fragments characteristic of both the dominant and recessive alleles were present (Fig. 1 and 2).

Twenty-eight plants were analyzed in the *Vrn-A1a/Vrn-B1a* combination and nineteen, in *Vrn-A1a/Vrn-B1c*. In the *Vrn-A1a*/*Vrn-B1a* combination, fourteen plants with two dominant alleles were isolated, five of them being homozygous, two with the dominant *Vrn-A1a* allele and recessive *vrn-B1*, ten plants with the dominant *Vrn-B1a* allele and recessive *vrn-A1*, and two winter plants with recessive alleles *vrn-A1* and *vrn-B1*. In the *Vrn-A1a/Vrn-B1c* combination, nine plants with two dominant alleles were isolated. Four of them were homozygous, one plant had the dominant *Vrn-A1a* and the recessive *vrn-B1* allele, eight plants had the dominant *Vrn-B1a* allele and recessive *vrn-A1*, and one plant showed the winter habit.

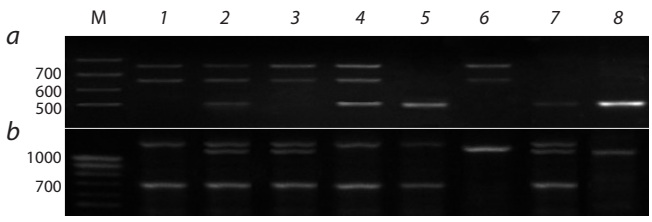

Fig. 1. Identification of alleles of *VRN-1* loci in F_2 hybrids in the raise of the Bez1 *Vrn-A1a/Vrn-B1a* line with allele-specific primers.

a – VRN-A1; *b – VRN-B1*. Lanes: M, 100-bp ladder; *1–8* – plant genotypes: *1 – Vrn-A1a Vrn-A1a Vrn-B1a Vrn-B1a*; *2 – Vrn-A1a vrn-A1 Vrn-B1a vrn-B1*; *3 – Vrn-A1a Vrn-A1a Vrn-B1a vrn-B1*; *4 – Vrn-A1a vrn-A1 Vrn-B1a Vrn-B1a*; *5 – vrn-A1 vrn-A1 Vrn-B1a Vrn-B1a*; *6 – Vrn-A1a Vrn-A1a vrn-B1 vrn-B1*; *7 – vrn-A1 vrn-A1 Vrn-B1a vrn-B1*; *8 – vrn-A1 vrn-A1 vrn-B1 vrn-B1*.

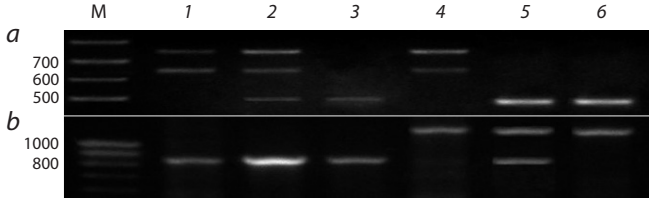

Fig. 2. Identification of alleles of the *VRN-1* loci in F_2 hybrids in the raise of the Bez1 *Vrn-A1a/Vrn-B1c* line with allele-specific primers.

a – VRN-A1; *b – VRN-B1*. Lanes: M, 100-bp ladder; *1–6* – plant genotypes: *1 – Vrn-A1a Vrn-A1a Vrn-B1c Vrn-B1c*; *2 – Vrn-A1a vrn-A1 Vrn-B1c Vrn-B1c*; *3 – vrn-A1 vrn-A1 Vrn-B1c Vrn-B1c*; *4 – Vrn-A1a Vrn-A1a vrn-B1 vrn-B1*; *5 – vrn-A1 vrn-A1 Vrn-B1c vrn-B1*; *6 – vrn-A1 vrn-A1 vrn-B1 vrn-B1*.

Thus, by using molecular markers already in the F_2 generation, we managed to isolate homozygous plants with two dominant alleles of the *VRN-A1* and *VRN-B1* genes. This approach is the most effective in isolating genotypes for a target gene, as it shortens the time for selecting the desired genotype significantly and permits one to determine the presence of *VRN* loci at early stages of plant development. At present, marker-assisted selection complements traditional methods, and it is widely used to introgress target genes and create near-isogenic lines, especially in the breeding of lines with genes that control the resistance to various types of stress (Leonova, 2013).

Determination the effect of the combination of dominant alleles in the *VRN-1* loci on the duration of individual developmental phases in the forest-steppe zone of the Novosibirsk region. We studied the duration of individual developmental phases of the common wheat lines with two dominant alleles of the *VRN-1* loci obtained in the present work, Bez1 *Vrn-A1a/Vrn-B1a* and Bez1 *Vrn-A1a/Vrn-B1c*, in an experimental field of the Institute of Cytology and Genetics with natural daylength in the spring of 2017. Isogenic lines of Bez1 with dominant alleles *Vrn-A1a*, *Vrn-B1a*, and *Vrn-B1c* were used as controls. The results are presented in Table 4 and Fig. 3.

Isogenic line i: Bez 1*Vrn-A1a* headed on day 42. Plants of isogenic lines with dominant alleles in the *VRN-B1* locus

Table 4. Duration of developmental phases in common wheat lines with dominant alleles of the *VRN-1* loci (field, 2017)

Line	Duration of individual phases of development, days						
	shoots–third leaf	third leaf–tillering	tillering–first node	first node–stem elongation	stem elongation–flag leaf	flag leaf–heading	shoots–heading
i: Bez1*Vrn-A1a*	5.2 ± 0.7	6.6 ± 1.4	12.9 ± 2.4	3.7 ± 1.0	2.9 ± 1.2	10.5 ± 1.6	41.8 ± 2.2
i: Bez1*Vrn-B1a*	6.3 ± 2.4	5.6 ± 1.8	20.3 ± 1.7	3.6 ± 1.2	2.1 ± 1.0	10.6 ± 1.4	48.5 ± 2.9
i: Bez1*Vrn-B1c*	5.3 ± 1.1	5.7 ± 1.3	17.8 ± 2.0	3.6 ± 1.1	3.0 ± 1.3	9.5 ± 1.8	44.8 ± 2.0
Bez1 *Vrn-A1a/Vrn-B1a*	5.1 ± 1.1	5.5 ± 0.8	11.0 ± 1.1^{***1}	4.1 ± 0.4	3.6 ± 1.5	10.6 ± 1.2	39.9 ± 1.1^{**1}
Bez1 *Vrn-A1a/Vrn-B1c*	6.7 ± 1.5	5.3 ± 2.2	10.2 ± 1.5^{***2}	4.0 ± 0.4	2.6 ± 1.5	11.0 ± 1.5	39.8 ± 1.4^{*2}

Significant differences: [1] – from i: Bez1*Vrn-B1a*; [2] – from i: Bez1*Vrn-B1c*. $^{*}p \le 0.05$, $^{**}p \le 0.01$, $^{***}p \le 0.001$.

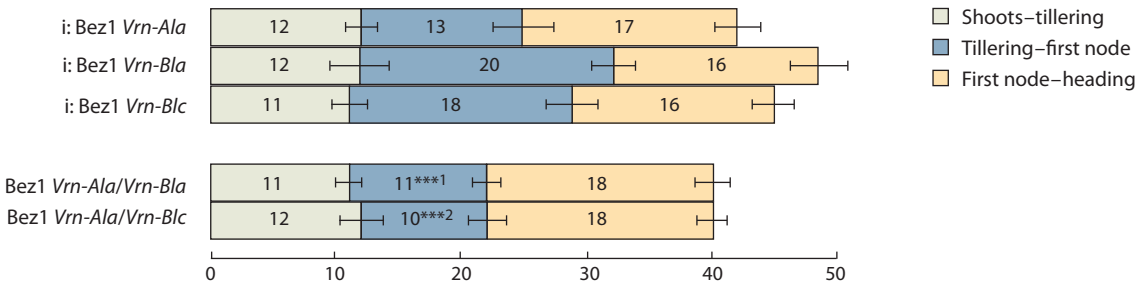

Fig. 3. Durations of developmental phases in common wheat lines with dominant alleles of *VRN-1* loci (field, 2017).
Significant differences: [1] – from i: Bez1*Vrn-B1a*; [2] – from i: Bez1*Vrn-B1c*. $^{***}p \le 0.001$.

headed later than the isogenic line with *Vrn-A1a.* The difference between the isogenic lines with the *Vrn-B1a* and *Vrn-B1c* alleles in this experiment was four days. The isogenic line with the *Vrn-B1a* allele headed on day 49, and the line with *Vrn-B1c*, on day 45. This result is consistent with the previous work (Emtseva et al., 2013). In the lines obtained in this work with the combination of two alleles of the *VRN-1* loci (Bez1 *Vrn-A1a/Vrn-B1a* and Bez1 *Vrn-A1a/Vrn-B1c*), the period from shooting to heading was forty days, which was two days less than in the isogenic line with the *Vrn-A1a* allele (differences are not significant), and significantly shorter than in the isogenic lines i: Bez1 *Vrn-B1a* (nine days shorter) ($p \le 0.01$) and i: Bez1 *Vrn-B1c* (five days shorter) ($p \le 0.05$). It was shown that varieties with two alleles, *Vrn-A1a* and *Vrn-B1c*, headed earlier than varieties with one dominant allele, *Vrn-A1a* or *Vrn-B1c* (Efremova et al., 2016).

The durations of the main phases of development, which ultimately determine the duration of the period from shooting to heading, are shown in Fig. 3. The duration of the shoots–tillering period was approximately the same in all lines examined, 11–12 days. The length of the first node–heading period, which was 16–18 days, did not vary significantly either. Significant differences were observed only in the tillering–first node period. For lines with two dominant alleles of *VRN-1* loci (Bez1 *Vrn-A1a/Vrn-B1a* and Bez1 *Vrn-A1a/Vrn-B1c*), the tillering–first node phases were 11 and 10 days, respectively. In comparison to the isogenic line with the *Vrn-A1a* allele, for which this phase lasted 13 days, the differences were insignificant. For isogenic lines i: Bez1*Vrn-B1a* and i: Bez1*Vrn-B1c*, the durations were 20 and 18 days, respectively. The combination of the dominant *Vrn-A1a* allele with *Vrn-B1a* and *Vrn-B1c* in the genotype resulted in a decrease of the tillering–first node phase compared to the isogenic lines i: Bez1*Vrn-B1a* and i: Bez1*Vrn-B1c*: nine and eight days respectively ($p \le 0.001$). Several studies of the duration of developmental phases of substituted and isogenic common wheat lines with different alleles of the *VRN-1* loci show that the key stage determining the duration of the vegetation period is just the length of the tillering–first node period (Voronin, Stelmakh, 1985; Pánková, Košner, 2004; Emtseva et al., 2013).

Thus, lines derived from the Bez1 cultivar with two dominant alleles of *VRN-1* loci (Bez1 *Vrn-A1a*/*Vrn-B1a* and Bez1 *Vrn-A1a*/*Vrn-B1c*) turned out to be earlier ripening than the isogenic lines i: Bez1*Vrn-B1a* and i: Bez1*Vrn-B1c* and slightly earlier than the isogenic line i: Bez1*Vrn-A1a* (difference about two days). Differences in the duration the shoots–heading period were associated with a decrease in the duration of the tillering–first node period.

Acknowledgements
The study is supported by the Russian Foundation for Basic Research, project No. 18-34-00146 mol_a; and State Budgeted Project No. 0324-2018-0018.

Conflict of interest
The authors declare no conflict of interest.

References
Cockram J., Jones H., Leigh F.J., O'Sullivan D., Powell W., Laurie D.A., Greenland A.J. Control of flowering time in temperate cereals: Genes, domestication, and sustainable productivity. J. Exp. Bot. 2007;58(6):1231-1244. DOI 10.1093/jxb/erm042.

Edwards K., Johnstone C., Thompson C. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucl. Acids Res. 1991;19:1349.

Efremova T.T., Arbuzova V.S., Leonova I.N., Makhmudova K. Multiple allelism in the *Vrn-B1* locus of common wheat. Cereal Res. Commun. 2011;39(1):12-21. DOI 10.1556/CRC.39.2011.1.2.

Efremova T.T., Chumanova E.V., Trubacheeva N.V., Arbuzova V.S., Belan I.A., Pershina L.A. Prevalence of *VRN1* locus alleles among spring common wheat cultivars cultivated in Western Siberia. Russ. J. Genet. 2016;52(2):146-153. DOI 10.1134/S102279541601004X.

Emtseva M.V., Efremova T.T., Arbuzova V.S. The influence of *Vrn-B1a* and *Vrn-B1c* alleles on the length of developmental phases of substitution and near isogenic lines of common wheat. Russ. J. Genet. 2013;49(5):545-552. DOI 10.1134/S1022795413050050.

Fu D., Szucs P., Yan L., Helguera M., Skinner J.S., von Zitzewitz J., Hayes P.M., Dubcovsky J. Large deletions within the first intron in *VRN-1* are associated with spring growth habit in barley and wheat. Mol. Genet. Genomics. 2005;273:54-65.

Goncharov N.P. Response to vernalization in wheat: its quantitative or qualitative nature. Cereal Res. Commun. 2004;32:323-330.

Iqbal M., Navabi A., Yang R.C., Salmon D.F., Spaner D. Molecular characterization of vernalization response genes in Canadian spring wheat. Genome. 2007;50:511-516. DOI 10.1139/G07-028.

Kamran A., Iqbal M., Spaner D. Flowering time in wheat (*Triticum aestivum* L.): a key factor for global adaptability. Euphytica. 2014; 197:1-26. DOI 10.1007/s10681-014-1075.

Koval S.F., Goncharov N.P. Multiple allelism at the *VRN1* locus of common wheat. Acta Agron. Hung. 1998;46(2):113-119.

Koval S.F., Koval V.S., Shamanin V.P. Wheat near-isogenic lines. Omsk, 2001. (in Russian).

Leonova I.N. Molecular markers: Implementation in crop plant breeding for identification, introgression and gene pyramiding. Russ. J. Genet.: Appl. Res. 2013;3(6):464-473. DOI 10.1134/S2079059713060051.

Likhenko I.E., Stasyuk A.I., Shcherban A.B., Zyryanova A.F., Likhenko N.I., Salina E.A. Analysis of the allelic variation of the *Vrn-1* and *Ppd-1* genes in siberian early and medium early varieties of spring wheat. Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov Journal of Genetics and Breeding. 2014;18(4/1):691-703. (in Russian).

Loukoianov A., Yan L., Blechl A., Sanchez A., Dubcovsky J. Regulation of *VRN-1* vernalization genes in normal and transgenic polyploid wheat. Plant Physiol. 2005;138:2364-2373. DOI 10.1104/pp.105.064287.

Merezhko A.F. The Problem of Donors in Plant Breeding. St. Petersburg: VIR, 1994. (in Russian)

Milec Z., Tomková L., Sumíková T., Pánková K. A new multiplex PCR test for the determination of *Vrn-B1* alleles in bread wheat (*Triticum aestivum* L.). Mol. Breed. 2012;30:317-323. DOI 10.1007/s11032-011-9621-7.

Pánková K., Košner J. Chromosome substitutions with dominant loci *Vrn-1* and their effect on developmental stages of wheat. Czech J. Genet. Plant Breed. 2004;40(2):37-44.

Pugsley A.T. A genetic analysis of the spring-wheat habit of growth in wheat. Aust. J. Agric. Res. 1971;22:23-31.

Rokitskii P.F. Introduction to Statistical Genetics. Minsk, 1974. (in Russian).

Santra D.K., Santra M., Allan R.E., Campbell K.G., Kidwell K.K. Genetic and molecular characterization of vernalization genes *Vrn-A1*, *Vrn-B1* and *Vrn-D1* in spring wheat germplasm from the Pacific Northwest Region of the USA. Plant Breed. 2009;128:576-584. DOI 10.1111/j.1439-0523.2009.01681.x.

Shcherban A.B., Borner A., Salina E.A. Effect of *VRN-1* and *PPD-1* genes on heading time in European bread wheat cultivars. Plant Breed. 2014. DOI 10.1111/pbr.12223.

Shcherban A.B., Emtseva M.V., Efremova T.T. Molecular genetical characterization of vernalization genes *Vrn-A1*, *Vrn-B1* and *Vrn-D1* in spring wheat germplasm from Russia and adjacent regions. Cereal Res. Commun. 2012;40(3):425-435. DOI 10.1556/CRC.40.2012.3.4.

Shcherban A.B., Khlestkina E.K., Efremova T.T., Salina E.A. The effect of two differentially expressed wheat *VRN-B1* alleles on the heading time is associated with structural variation in the first intron. Genetica. 2013;141:133-141.

Snape J.W., Butterworth K., Whitechurch E., Worland A.J. Waiting for fine times: genetics of flowering time in wheat. Euphytica. 2001; 119:185-190.

Stelmakh A.F. Geographic distribution of *Vrn* genes in landraces and improved varieties of spring bread wheat. Euphytica. 1990;45: 113-118.

Stelmakh A.F. Genetic effects of *Vrn* genes on heading date and agronomic traits in bread wheat. Euphytica. 1993;65:53-60.

Stelmakh A.F. Genetic systems regulating flowering response in wheat. Euphytica. 1998;100:359-369.

Stelmakh A.F., Avsenin V.I. Creation of sets of near isogenic lines at *Vrn 1-3* loci. Nauch.-Techn. Bull. VSGI = Scientific and Technical Bulletin VSGI. 1983;48(2):24-28. (in Russian).

Stelmakh A.F., Fait V.I., Martyniuk V.R. Genetic system of the type and speed of development of common wheat. Cytologia i Genetica = Cytology and Genetics. 2000;34(2):39-45. (in Russian).

Voronin V.I. The role of the loci *Vrn 1-3* in determining the differences in productivity in comon wheat. Prikladnye Aspecty Genetiki, Tsytologii i Biotechnologii Selskochozyaistvennych Rasteniy = Applied Aspects of Genetics, Cytology and Biotechnology of Agricultural Plants. Odessa: VSGI, 1988;13-20. (in Russian).

Voronin A.N., Stelmakh A.F. Stages of organogenesis in near isogenic *Vrn 1-3* comon wheat lines. Nauch.-Techn. Bull. VSGI = Scientific and Technical Bulletin VSGI. 1985;55(1):19-23. (in Russian).

Worland A.J. The influence of flowering time genes on environmental adaptability in European wheats. Euphytica. 1996;89:49-57. DOI 10.1007/BF00015718.

Worland A.J., Börner A., Korzun V., Li W.M., Petrovic S., Sayers E.J. The influence of photoperiod genes on the adaptability of European winter wheats. Euphytica. 1998;100:385-394.

Yan L., Fu D., Li C., Blechl A., Tranquilli G., Bonafede M., Sanchez A., Valarik M., Dubcovsky J. The wheat and barley vernalization gene *VRN3* is an orthologue of *FT*. Proc. Natl. Acad. Sci. USA. 2006; 103;19581-19586. DOI 10.1073/pnas.0607142103.

Yan L., Helguera M., Kato K., Fukuyama S., Sherman J., Dubcovsky J. Allelic variation at the *VRN-1* promoter region in polyploid wheat. Theor. Appl. Genet. 2004;109:1677-1686.

Yan L., Loukoianov A., Tranquilli G., Helguera M., Fahima T., Dubcovsky J. Positional cloning of wheat vernalization gene *VRN1*. Proc. Natl. Acad. Sci. USA. 2003;100:6263-6268. DOI 10.1073/pnas.0937399100.

Zhang X.K., Xiao Y.G., Zhang Y., Xia X.C., Dubcovsky J., He Z.H. Allelic variation at the vernalization genes *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, and *Vrn-B3* in Chinese wheat cultivars and their association with growth habit. Crop Sci. 2008;48:458-470. DOI 10.2135/cropsci2007.06.0355.

ORCID ID

E.V. Chumanova orcid.org/0000-0002-2746-2307

T.T. Efremova orcid.org/0000-0002-2865-5418

Y.V. Kruchinina orcid.org/0000-0002-1084-9521

L.A. Pershina orcid.org/0000-0002-9941-2026