Enhancement of Leaf Rust Resistance of Siberian Winter Wheat Varieties by Marker-assisted Selection

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Cultivation of winter wheat varieties in the West Siberian region of Russia has competitive advantages compared to spring varieties: utilization of spring-summer moisture, early maturation and harvest and a high yield potential. The poor resistance of winter varieties to foliar diseases results in significant yield losses and facilitates the spread of pathogens to the spring wheat cultivars. The present study was conducted to evaluate the effectiveness of molecular markers specific for *VRN-1* and *Lr* loci in selecting winter wheat genotypes resistant to leaf rust. The winter wheat cultivars Biyskaya ozymaya and Filatovka were crossed with spring wheat introgression lines 21-4 and 5366-180 and the spring wheat cultivar Tulaikovskaya 10 carrying *LrTt2*, *LrAsp5* and *Lr6Ai#2* loci from *Triticum timopheevii*, *Aegilops speltoides* and *Thynopyrum intermedium,* respectively. To identify winter wheat plants homozygous for target loci, F_2 populations were screened with functional markers to *VRN-1* genes and with markers specific for alien genetic material. Based on the genotyping analysis of 371 F_2 plants a total of 44 homozygous genotypes with winter habit was identified. There were eight genotypes containing *Lr* loci among them. Evaluation of F_2-derived F_{3-4} families for both seedling and adult resistance showed that only one F_{3-4} family had moderate susceptible reaction type to the field population of leaf rust. Others ranged from nearly immune to resistant with severity of 5%. The data also indicated the utility of the *VRN-1* allele-specific markers for detection of genotypes with winter habit without vernalization at early stages of plant breeding.

Keywords: winter and spring wheats, leaf rust, *VRN-1* genes, *Lr* genes, marker-assisted selection

Introduction

Western Siberia is among the leading grain-producing regions in the Russian Federation. Traditionally spring bread wheat is dominant in Western Siberia due to its cold climate and extreme winter temperatures. However, the climate changes in recent decades has expanded winter wheat production in Siberia. While spring wheat dominates in Siberia, winter wheat increased its acreage approximately 10 percent during last years. In general, winter wheat has some advantages over spring wheat: a higher yield potential, utilization of spring moisture, resistance to spring-summer droughts, and early harvest under more

favorable weather conditions at the end of July – the beginning of August, compared to spring wheats harvested in September–October. Cultivation of winter wheats in regions with short vegetation period, allows a reduction of the field work load during sowings in spring and in autumn during harvesting.

The modern varieties of winter wheat adapted to the Western Siberian steppe and mixed forest are characterized by high yield, good quality of grain and flour, frost tolerance and successful overwintering. However, most of them lack effective resistance to foliar diseases, which may result in significant crop losses and allowing diseases to spread to the spring wheat as well. Leaf rust caused by the fungus *Puccinia triticina* Eriks. is important disease for Western Siberia and all over the world. Annual yield losses are about 10–15% and can exceed 40–50% during the outbreaks when the weather conditions favor the pathogen (Sanin and Nasarova 2010). Thus, the development of durable genetic resistance to leaf rust should be among the priority objective of winter wheat breeding program in Western Siberia.

Growing resistant cultivars is the most efficient, cost-effective and environment-friendly approach for crop protection against fungal pathogens. More than 80 *Lr* (leaf rust resistance) genes assigned temporary or catalog symbols have been already identified in bread wheat and its relatives (McIntosh et al. 2013). However, only a few alien *Lr* genes are involved in wheat breeding for development of resistant varieties due to the negative effects of alien genetic material on agronomically important traits. The majority of Russian winter and spring cultivars are protected against leaf rust by relatively few *Lr* genes including *Lr9, Lr10, Lr19, Lr23, Lr24*, and *Lr26*. The resistance of Western Siberian spring wheat cultivars is provided by slightly effective genes *Lr3*, *Lr10, Lr13* and genes *Lr9*, *Lr19*, *Lr23*, and *Lr26* with resistant or moderate susceptible responses depending on the year (Morgounov et al. 2011; Gultyaeva et al. 2012). In contrast to the spring wheat, winter wheat varieties cultivated in the Siberian region, do not contain *Lr* genes that provide effective resistance to leaf rust.

The cultivation of genetically homogeneous cultivars enhances vulnerability to biotic stresses due to the selection of virulent races and accumulation of pathogen inoculum in the field. In the case of leaf rust, the data of race surveys in 2006–2014 and screening for resistance of cultivars released in the different regions of Russia showed that the efficiency of *Lr* genes used overall was decreasing or they were not effective at all (Gultyaeva et al. 2012; Kolmer et al. 2015). Virulence to *Lr9, Lr23* and *Lr19* genes important for Western Siberian germplasm was observed recently with an increase of *P. triticina* races virulent to *Lr19* (Gultyaeva et al. 2015). In Russian Federation winter wheat cultivars resistant to leaf rust are adapted to the Volga, Central Black Earth, North Caucasus regions and other areas with a mild climate but not to Western Siberia. To compensate the decreasing list of effective *Lr* genes it is necessary to search for new resistance genes and integrate them into breeding programs related to winter wheat varieties.

Conventional method for identification of resistant genes involves the evaluation of infection types on seedlings and adult plant inoculated with *P. triticina* isolates with known virulence to leaf rust resistance genes (Roelfs et al. 1992). All these procedures are time consuming and require laboratory support for fungal isolates and wheat lines. Now-

adays an alternative approach based on molecular technology is widely used for genotyping, and specific DNA molecular markers are developed for many resistant genes providing for the marker-assisted selection (MAS) and the introgression of targeted genes into the commercial cultivars and breeding lines (Xu 2010).

Here we presented the results of the introduction of new leaf rust resistance genes into the winter wheat cultivars adapted to Western Siberia environments. The spring bread wheat lines with *LrTt2* and *LrAsp5* from *Triticum timopheevii* (Timonova et al. 2013) and *Aegilops speltoides* (Petrash et al. 2016) and the spring bread wheat cultivar Tulaikovskaya 10 with *Lr6Ai#2* from *Thynopyrum intermedium* (Salina et al. 2015) were used as donor parents for resistance. The transfer of *Lr* genes into the recipient winter wheat cultivars was facilitated with specific markers for alien genetic material carrying the resistant genes. Finally, for identification of winter genotypes in crosses of winter and spring wheat lines it was essential to use the molecular markers developed for the different alleles of *VRN-1* (Yan et al. 2004; Fu et al. 2005; Shcherban et al. 2012).

Materials and Methods

Plant material and marker-assisted selection

The winter wheat varieties Filatovka and Biyskaya ozymaya adapted to Western Siberia were used as female parents to produce the hybrids. Together with productivity and high protein content, these varieties express resistance to abiotic stresses. However, they are sensitive to the most pathogen races of leaf rust. The following donor parents for resistance were used: 1) the spring common wheat introgression line 21-4 with translocation from *Ae. speltoides* (T5BS.5BL-5SL); 2) the spring common wheat introgression line 5366-180 containing translocation from *T. timopheevii* (T5BS.5BL-5GL); 3) the spring bread wheat cultivar Tulaikovskaya 10 with substitution of 6D chromosome on chromosome 6Ai#2 of wheatgrass *Thinopyrum intermedium*. They carry *LrAsp5*, *LrTt2,* and *Lr6Ai#2* loci protecting bread wheat against leaf rust (Timonova et al. 2013; Salina et al. 2015; Petrash et al. 2016). The total of 371 progenies in six F_2 populations were produced by crossing the recipient winter wheat cultivars with the leaf rust resistant donors. Populations of F_2 seedlings were simultaneously genotyped with molecular markers specific for different alleles of *VRN-1* loci and for alien genetic material containing *Lr* genes. F_2 plants were self-pollinated and F_{3-4} families were evaluated for growth habit, winter hardiness and leaf rust resistance in field trials during 2014–2016.

DNA extraction and PCR

Genomic DNA was isolated from 5–7-day-old seedlings as described by Kiseleva et al. (2016). PCR primers reported in Yan et al. (2004), Fu et al. (2005) and Shcherban et al. (2012) were used to detect the presence of dominant or recessive alleles of *Vrn-A1*, *Vrn-B1* and *Vrn-D1* genes. List of PCR primers for alien genetic material containing *Lr* loci from *Ae. speltoides*, *T. timopheevii* and *Th. intermedium* is presented in Table 1. PCR

Table 1. PCR primers for identification of alien fragments containing *Lr* resistance genes

Lr gene	Marker name	Marker structure	Annealing temp. (°C)	PCR fragment (bp)	Reference
LrAsp5	Pr1 Pr5	TACCCCTGCTACCAGTGCGC GGCCAACCCTACACCCCAAG	55	968 1100	Patent RU №2535985
LrTt2	*Xbarc232*	F-CGCATCCAACCATCCCCACCCAACA R-CGCAGTAGATCCACCACCCCGCCAGA	60	200, 240	Song et al. 2005
Lr6Ai#2	MF2/MR1r2 MF2/MR4	Available on request	55	347 328	Patent RU №2598275

conditions for SSR marker *Xbarc232* was as described in Song et al. (2005). PCR mixture and amplification protocols for other primers were as described in Shcherban et al. (2015). The PCR products were separated on 1.5% agarose gel, stained with ethidium bromide and visualized under ultraviolet light.

Phenotyping

The adult plant resistance to leaf rust and winter hardiness were evaluated on the experimental plots of Federal Research Center Institute of Cytology and Genetics SB RAS near Novosibirsk during the 2014/15 and 2015/16 growing seasons. The field experiment was carried out in a randomized design with three replicates: 25 g of seeds of each F_{3-4} family along with the parental winter wheat cultivars were sown in a plot of 1 m^2 in the first week of September. A 1 m border of highly susceptible winter wheat cultivars Albidum 12 and Dneprovskaya 521 surrounded the plots for disease spread. The spring wheat donors of the resistance genes were planted at the middle of May as additional control. Resistance to leaf rust was evaluated with natural infection of native population of *P. triticina* specific for Western Siberian region. Leaf rust severity was scored using 0–100% coverage of leaves with rust pustules according to modified Cobb's scale (Peterson et al. 1948).

Seedling response to leaf rust was tested at the greenhouse of the Institute under controlled environmental conditions. Eight-day-old seedlings were inoculated with urediniospores of the field population of *P. triticina* as described by Roelfs et al. (1992). Disease reaction was scored 12 days after inoculation using the scale of Mains and Jackson (1926). Plants with infection types 0, 1 and 2 were considered as immune, resistant and moderately resistant, while infection types 3 and 4 were considered moderately susceptible and susceptible. Winter hardiness was estimated at the beginning of the spring season (April–May) as the number of surviving plants (in percent).

Results

Genotyping of F$_2$ populations

F$_2$ populations derived from crosses between the winter recipient cultivars and spring donors of *Lr* genes were genotyped with allele-specific primers designed for detecting allelic variation of *VRN-1* genes *(Vrn-A1*, *Vrn-B1*, *Vrn-D1)*. The composition of the genes determines the growth habit of wheat plant. Genotypes with of all the recessive alleles *vrn-A1, vrn-B1,* and *vrn-D1* result in a winter habit and makes the plant sensitive to vernalization while even single dominant allele of any of these loci results in a spring growth habit (Pugsley 1971). For the recipient cultivars Filatovka and Biyskaya ozymaya the recessive alleles were confirmed at all loci (Fig. 1). Allelic combination of *Vrn-A1a* and *vrn-D1* was identified for all the donors of *Lr* resistance which differed for alleles of *Vrn-B1*. The breeding line 21-4 carried *vrn-B1*, the breeding line 5366-180 – *Vrn-B1c,* and the cultivar Tulaikovskaya 10 – *Vrn-B1a* (Fig. 1). A total of 132 individual F$_2$ plants with various combination of homozygous alleles for *VRN-1* loci were revealed from F$_2$ populations derived from different crosses. Among them 44 plants had the combination of all three recessive alleles (Table 2).

Table 2. Allelic composition of F$_2$ progenies homozygous for *VRN-1* genes, containing (*Lr+*) and not containing (*Lr–*) leaf rust resistance genes

Cross combination	Number of F$_2$ plants	Allelic composition of *VRN-1* loci	Number of *VRN-1* homozygo-us plants	Of these	
				Lr+*	Lr–
Filatovka × 5366-180	66	**Vrn-A1a Vrn-B1c vrn-D1**	7	3	
		Vrn-A1a vrn-B1 vrn-D1	2	0	
		vrn-A1 Vrn-B1c vrn-D1	7	6	
		vrn-A1 vrn-B1 vrn-D1	2	0	
Biyskaya ozimaya× 5366-180	56	**Vrn-A1a Vrn-B1c vrn-D1**	3	2	
		Vrn-A1a vrn-B1 vrn-D1	3	1	
		vrn-A1 Vrn-B1c vrn-D1	4	4	
		vrn-A1 vrn-B1 vrn-D1	3	0	
Filatovka × 21-4	69	**Vrn-A1a vrn-B1 vrn-D1**	18	6	12
		vrn-A1 vrn-B1 vrn-D1	17	4	13
Biyskaya ozimaya× 21-4	55	**Vrn-A1a vrn-B1 vrn-D1**	15	3	12
		vrn-A1 vrn-B1 vrn-D1	14	3	11
Filatovka × Tulaikovskaya 10	69	**Vrn-A1a Vrn-B1a vrn-D1**	2	0	2
		Vrn-A1a vrn-B1 vrn-D1	3	1	2
		vrn-A1 Vrn-B1a vrn-D1	10	4	6
		vrn-A1 vrn-B1 vrn-D1	2	0	2
Biyskaya ozymaya× Tulaikovskaya 10	56	**Vrn-A1a Vrn-B1a vrn-D1**	3	1	2
		Vrn-A1a vrn-B1 vrn-D1	4	1	3
		vrn-A1 Vrn-B1a vrn-D1	7	1	6
		vrn-A1 vrn-B1 vrn-D1	6	1	5
Total number of F$_2$	371				
Of these genotype		*vrn-A1 vrn-B1 vrn-D1*	44	8	31

Bold designates genotypes of donor lines for *VRN-1*.
*Presence/absence of *Lr* genes was postulated based on molecular marker analysis.
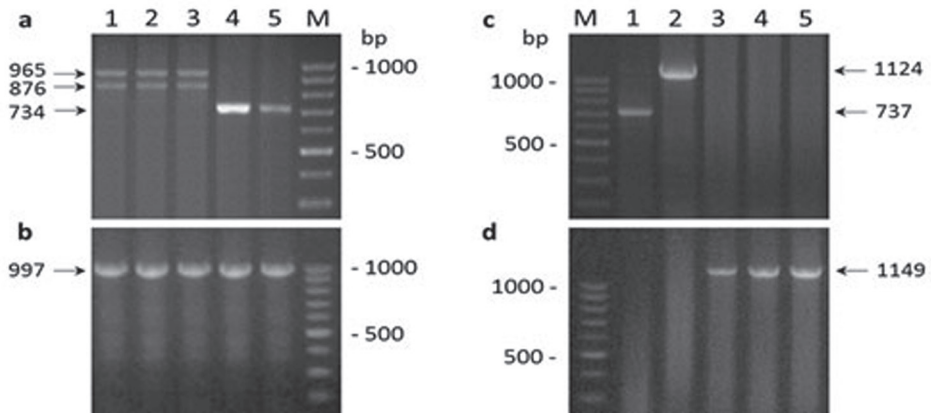
Figure 1. PCR profiles of primers for *VRN-1* genes in winter wheat varieties and in donors of *Lr* genes: (a) *vrn-A1* (734 bp) and *VRN-A1a* (965+876 bp); (b) *vrn-D1* (997 bp); (c) *VRN-B1a* (1124 bp) and *VRN-B1c* (737 bp); (d) *vrn-B1* (1149 bp). M – DNA ladder of molecular weight marker, lane1 – line 5366-180, lane 2 – Tulaikovskaya 10, lane 3 – line 21-4, lane 4 – Filatovka, lane 5 – Biyskaya ozimaya
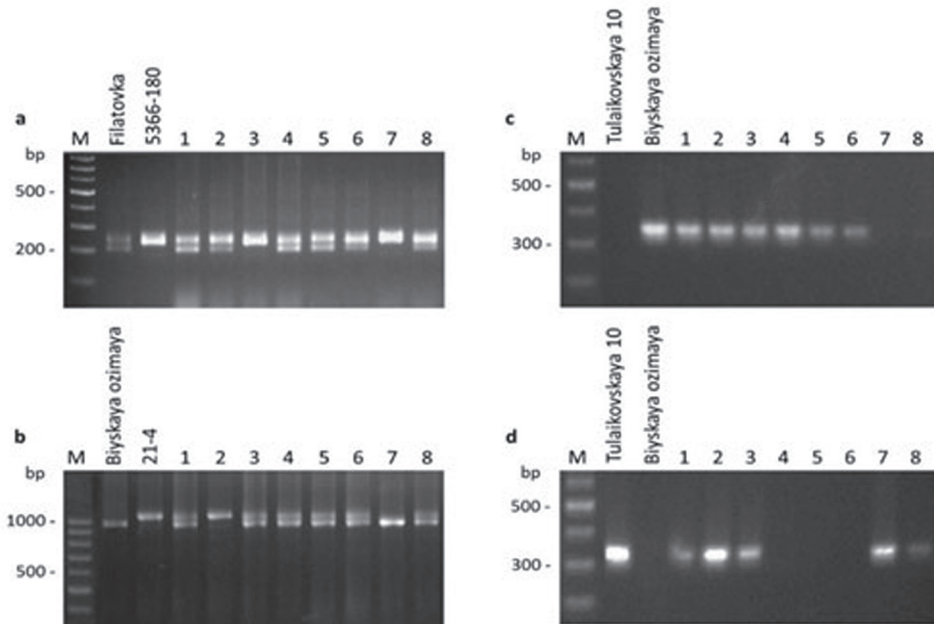

Figure 2. Examples of PCR patterns obtained amplifying genomic DNA of the winter wheat cultivars Filatovka and Biyskaya ozimaya and the donor of *Lr* genes with markers *Xbarc232* (a), Pr1/Pr5 (b), MF2/MR4 (c) and MF2/MR1r2 (d). Lane M is DNA ladder of molecular weight marker, lanes 1–8 – F$_2$ plants

To identify plants carrying *Lr* resistance genes F_2 populations were genotyped with primers specific to alien translocations (Table 1). The translocation T5BS.5BL-5GL containing *LrTt2* gene from *T. timopheevii* was detected with SSR marker *Xbarc232* posessing two alleles on 5AL and 5BL chromosomes (Somers et al. 2004). For the recipient cultivars both fragments specific to 5AL and 5BL chromosomes, 200 bp and 240 bp in size, were amplified with *Xbarc232* (Fig. 2*a*). There was no 200 bp fragment in the *Xbarc232* amplicon of the line 5366-180 carring the T5BS.5BL-5GL translocation. Neither genotypes heterozygous for *LrTt2* locus nor genotypes lacking this gene could be identified using the dominant marker *Xbarc232*. The following selections of individual plants were done after the PCR genotyping: nine spring genotypes homozygous for *LrTt2* locus from 66 progenies of F_2 population from Filatovka × 5366-180 cross, and seven spring genotypes with *T. timopheevii* translocation from 56 progenies of F_2 population of Biyskaya ozimaya × 5366-180 cross. But no winter genotypes carring the *T. timopheevii* introgressions were revealed among the progenies of both crosses.

The STS primers Pr1/Pr5 that cosegregated with leaf rust resistant gene *LrAsp5* from *Ae. speltoides* were developed to distinguish winter wheat cultivars Filatovka and Biyskaya ozimaya (968 bp fragment) and the donor line 21-4 (1100 pb fragment) (Fig. 2b). After molecular analyses of F_2 hybrids from the crosses between the winter wheat cultivars and the line 21-4 with Pr1/Pr5 primers the translocated fragment from *Ae. speltoides* was identified in 16 individual plants from 124 including 7 winter genotypes (Table 2).

The PLUG markers TNAC1752 and TNAC1674 were used before to identify in the cultivar Tulaikovskaya 10 the chromosome 6Ai#2 from *Th. intermedium* carrying the resistant gene *Lr6Ai#2* (Ishikawa et al. 2009). However, application of the PLUG markers required the treatment of PCR products with restriction endonucleases. To overcome this disadvantage we developed the STS primers MF2, MF4, and MR1r2 based on PLUG markers but more suitable for chromosome 6Ai#2 check. For the cultivar Tulaikovskaya 10 the primer combination MF2/MR1r2 generated the 347 bp fragment that was absent in winter genotypes. Meanwhile another combination MF2/MR4 discriminated the winter cultivars Filatovka and Biyskaya ozimaya amplifying the fragment of 328 bp (Fig. 2c, d). Homozygous or heterozygous plants for *Lr6Ai#2* locus could be identified in multiplex PCR with both primer combinations. Genotyping of F_2 populations from the crosses between recipient winter wheat cultivars with Tulaikovskaya 10 with MF2/MR1r2 and MF2/MR4 primers revealed 9 individual plants with alien introgression from *Th. intermedium* including one winter genotype (Table 2).

Thus, a total of 44 winter wheat genotypes from 371 progenies of F_2 populations were identified by molecular analysis. Among them there were eight plants carrying the alien genetic material from *Ae. speltoides* and *Th. intermedium* as homozygotes.

Evaluation of leaf rust resistance and winter hardiness

The F_{3-4} families from eight self-pollinated F_2 hybrids recessive for *VRN-1* gene and containing the *LrAsp5* and *Lr6Ai*#2 genes were evaluated for winter habit, winter hardiness

and leaf rust resistance using the recipient winter cultivars and the spring Lr donors as control genotypes.

The growing season of 2015/16 had more periods of freezing temperatures (up to −20 °C) without snow than the testing period of 2014/15 that affected the overwintering significantly. The cold hardiness of F_{3-4} hybrids and the parent cultivars Biyskaya ozimaya and Filatovka were similar but only 58% of plants survived in 2015/16. Independent of hybrid genotypes, there was approximately 95% survival of plants in 2014/15.

The seedling and adult resistance of F_{3-4} hybrid families to leaf rust are given in Table 3. For seedling plants, six hybrid families with alien translocations were resistant (ITs = 1 and 2). The same infection types were observed for the Lr donors Tulaikovskaya 10 and 21-4. Meanwhile the seedling score for plants of the recipient winter cultivars was 3 (moderate susceptible).

The adult plant resistance of F_{3-4} hybrid families was variable for infection types, which was the same for tested genotypes between growing seasons. Five F_{3-4} hybrid families (74-12, 83-8, 87-12, 87-1, 73-3) were immune or resistant to leaf rust in both field trials of 2014/15 and 2015/16. Two F_{3-4} families (76-6, 86-4) had moderate resistant reaction type. Only one sample 76-11 was evaluated as moderate susceptible after seedling and adult plant score (IT = 3) but the percentage of diseased leaf was lower than the controls and varied from 5 to 20% (Table 3).

Table 3. Response of winter wheat F_{3-4} populations, parental wheat varieties and donors of resistance genes to leaf rust at seedling and adult plant stages

Cross combination	No. F_{3-4} family	Infection type (IT)		
		Seedling	Adult plant	
			2014/15	2015/16
Biyskaya ozimaya × 21-4	74-12	1	0	TrR
	76-6	2	TrMR	5MR
	76-11	3	5MS	20MS
Filatovka × 21-4	83-8	1	0	5R
	86-4	2	TrMR	5MR
	87-1	2	0	5R
	87-12	1	0	5R
Biyskaya ozimaya × Tulaikovskaya 10	73-3	1	0	0
Biyskaya ozimaya		3	65S	80S
Filatovka		3	80S	80S
Tulaikovskaya 10		0-1	0	0
21-4		2	5MR	10MR

ITs of seedlings were scored as: 0 – no uredinia, 1 – small uredinia surrounded by necrosis, 2 – small to medium uredinia surrounded by necrosis or chlorosis, 3 – medium uredinia without necrosis and chlorosis, 4 – large uredinia without chlorosis. ITs of adult plant were scored as: R – resistance, MR – moderate resistance. MS – moderate susceptible, S – susceptible, 0 – immune, Tr – trace (<5%); numeric character means % of leaf coverage by uredinia.

Discussion

The objective of this investigation was to develop the breeding wheat lines with winter growth habit and resistance to leaf rust. A key approach in this study was marker-assisted selection (MAS) linked with target loci to assist genotyping selections in crop improvement. MAS has recently become a routine technique for early generation screening of wheat hybrids for genes conferring agronomically important traits rather than conducting the more labor intensive phenotypic tests. Moreover molecular markers and MAS are broadly applied to enhance the schemes of identification, introgression and pyramiding of fungal disease resistance genes including *Lr* genes against leaf rust (Xu 2010; Miedaner and Korzun 2012; Mallick et al. 2015).

For the last two decades the allelic composition of *VRN-1* genes has been studied by molecular genetic tools. Gene-specific markers and functional markers were proposed to develop isogenic lines and to use them in the genetic analysis of the World Germplasm diversity of wheat, barley, rye, and triticale (Cockram et al. 2009; Nowak et al. 2014). Moreover, the markers linked to the loci of different target traits, which are involved in frost resistance, spike architecture, and the others (Sutka 2001; Deng et al. 2015; Rizza et al. 2016), could also be used to selected germplasm with these traits. In this study, allele-specific markers to *VRN-1* were used to reveal the genotypes homozygous for recessive alleles from F_2 population, which were confirmed by following field trials for winter growth. Our results also demonstrate that functional markers diagnostic for *VRN-1* genes might be applied in preliminary breeding of winter wheat genotypes without vernalization treatment. This was one of the first attempt to exploit molecular markers to *VRN-1* loci for MAS breeding of winter wheat.

The predictive value of any markers across a set of breeding lines depends on linkage to the target loci, codominant nature and the presence of recombination events between recipient chromosome and translocated chromosome of donor line. In this study, the molecular markers specific for *Ae. speltoides*, *T. timopheevii* and *Th. intermedium* genetic material were used to detect the leaf rust resistance genes (Table 1). As it was shown earlier, translocated chromosomes of the donor lines have a reduced recombination with *T. aestivum* chromosomes (Salina et al. 2015; Petrash et al. 2016). Taking into account the partial homoeology between the common wheat chromosomes and the alien chromatin, we suggested the efficiency of the markers to track *Lr* genes in early generations. However, genotyping results indicated that only robust polymorphic markers developed for alien fragments of *Ae. speltoides* and *Th. intermedium* were able to identify the homozygous F_2 plants with rust resistance genes *LrAsp5* and *Lr6Ai#2*. The SSR markers *Xbarc232* used for detection of *LrTt2* gene from *T. timopheevii* amplified the products only from the winter wheat parents. Determination of homozygous and heterozygous genotypes using the marker *Xbarc232* is impossible among the plants from small array of the first generations until the development of the additional marker specific to 5GL chromosome from *T. timopheevii*.

The resistance of MAS genotypes of F_{3-4} populations from crosses Filatovka × 21-4 and Biyskaya ozimaya × Tulaikovskaya 10 was confirmed with the seedling tests (IT

from 0 to 2; Table 3) and field trials of adult plant resistance excluding the sample of the line 76-11, which expressed the moderate susceptible phenotype at seedling and adult plant stages. This could be result of reduction up to elimination of the introgressed segment after self-pollination of initial F_2 plant. Such instances were described in the literature (Olson et al. 2010; Timonova et al. 2013). The lines 83-8, 87-1, and 87-12 with similar responses at the adult plant stage had varying scores for seedlings (Table 3). The different genetic basis of juvenile and adult plant resistance may be assumed for the line 87-1. It expressed the moderate resistance at seedling stage (IT = 2) but immune or high resistance response at the adult stage (0 or 5R).

The sample size of experimental population depends on the number of analyzed loci and on the type of population. The more genes are investigated, the greater the population size is necessary to identify progenies that contain various gene combinations. The statistical requirement for multigene analysis of F_2 population providing by conventional phenotyping selection needed a large sample size for observation. Over several thousands of F_2 individual plants should be involved in searching for target genotype which homozygous for three or more unlinked loci controlling single trait (Witcombe and Virk 2001; Baenziger and DePauw 2009). Meanwhile in this study the sample size of 69 individual plants was enough to genotype the homozygous loci using the multiplex PCR with primers to various alleles of *VRN-1* genes together with codominant markers to *Lr* genes.

Hybridization between winter and spring cultivars is important breeding approach to enrich wheat genetic diversity for agronomically important traits. Introgression of the winter wheat gene pool into spring wheat is widely used to develop the spring varieties with high yielding multiflorous spikes and grain quality. From the other hand, the spring germplasm is valuable for breeding for drought tolerance and disease resistance (Kant et al. 2008; Sharma and Chaudhary 2009).

In Russia winter and spring bread wheat breeding programs for wide adaptation and high yield potential involving intra and interspecific hybridization have been conducted since the early 20th century up to the present day. A set of improved winter and spring cultivars originated from the hybridization of the winter cultivars Bezostaya 1, Avrora, Kavkaz with the high yielding spring cultivars with baking quality and drought tolerance (Morgounov et al. 2011). However there are disadvantages of conventional breeding for the winter genotypes from the crosses between the winter and spring cultivars: 1) assessment of the large sample of population to reveal individuals characterized by winter habit; 2) the use of labor-intensive methods to detect winter genotypes in winter × spring wheat crosses; 3) the long time of phenotypic assessment to differentiate spring and winter forms (Morgounov et al. 1996; Lapochkina et al. 2016). Considering our data of homozygous recessive alleles for *Vrn-1* genes obtaining from small sample, we could approve the application of the allele-specific markers to facilitate the genotyping and to reduce the time taken for selection. Additionally, winter habit for self-pollinated F_2 plants was confirmed during field trials.

According to our data, genes *LrAsp5* and *Lr6Ai#2* are still effective against Western Siberian population of leaf rust while many of resistant *Lr* genes in local germplasm are

overcome by new virulent races. Winter wheat derivatives carrying *LrAsp5* and *Lr6Ai#2* genes could be used as a source of resistance for development of new adapted wheat cultivars.

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References

Baenziger, P.S., DePauw, R.M. 2009. Wheat breeding: procedures and strategies. In: Carver, B.F. (ed.), Wheat Science and Trade. Wiley-Blackwell. Oxford, UK. pp. 275−308.

Cockram, J., Norris, C., Donal, M., O'Sullivan, D.M.O. 2009. PCR-based markers diagnostic for spring and winter seasonal growth habit in barley. Crop Sci. **49**:403−410.

Deng, W., Casao, M.C., Wang, P., Sato, K., Hayes, P.H., Finnegan, E.J., Trevaskis, B. 2015. Direct links between the vernalization response and other key traits of cereal crops. Nature Commun. **6**:5882.

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

Gultyaeva, E.I., Dmitriev, A.P., Kosman, E. 2012. Regional diversity of Russian populations of *Puccinia triticina* in 2007. Can. J. Plant Pathol. **34**:213−224.

Gultyaeva, E.I., Shaidayuk, E.L., Kazartsev, I.A., Aristova, M.K. 2015. Structure of Russian populations of *Puccinia triticina* Eriks. Plant Protection News **3**:5−10.

Ishikawa, G., Nakamura, T., Ashida, T., Saito, M., Nasuda, S., Endo, T.R., Wu, J., Matsumoto, T. 2009. Localization of anchor loci representing five hundred annotated rice genes to wheat chromosomes using PLUG markers. Theor. Appl. Genet. **118**:499−514.

Kant, L., Mani, V.P., Gupta, H.S. 2008. Winter × spring wheat hybridization – A promising avenue for yield enhancement. Plant Breed. **120**:255−258.

Kiseleva, A.A., Shcherban, A.B., Leonova, I.N., Frenkel, Z., Salina, E.A. 2016. Identification of new heading date determinants in wheat 5B chromosome. BMC Plant Biol. **16** (Suppl. 1):8.

Kolmer, J.A., Kabdulova, M.G., Mustafina, M.A., Zhemchuzhina, N.S., Dubovoy, V. 2015. Russian populations of *Puccinia triticina* in distant regions are not differentiated for virulence and molecular genotype. Plant Pathol. **64**:328−336.

Lapochkina, I.F., Baranova, O.A., Shamanin, V.P., Volkova, G.V., Gainullin, N.R., Anisimova, A.V., Galinger, D.N., Lazareva, E.N., Gladkova, E.V., Vaganova, O.F. 2016. The development of initial material of spring common wheat for breeding for resistance to stem rust (*Puccinia graminis* Pers. f. sp. *tritici*), including race Ug99, in Russia. Vavilov J. Genet. Breeding **20**:320–328.

Mains, E.B., Jackson, H.S. 1926. Physiological specialization in the leaf rust of wheat, *Puccinia triticina* Erikss. Phytopathol. **16**:89–120.

Mallick, N., Vinod, S.J.B., Sharma, J.B., Tomar, R.S., Sivasamy, M., Prabhu, K.V. 2015. Marker-assisted backcross breeding to combine multiple rust resistance in wheat. Plant Breed. **134**:172–177.

McIntosh, R.A., Yamazaki, Y., Dubcovsky, J., Rogers, J., Morris, C., Appels, R., Xia, X.C. 2013. Catalogue of Gene Symbols for Wheat. http://www.shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp

Miedaner, T., Korzun, V. 2012. Marker-assisted selection for disease resistance in wheat and barley breeding. Phytopathol. **102**:560–566.

Morgounov, A.I., Vlasenko, V.A., McNab, A., Braun, H.J. 1996. Wheat breeding: Objectives, methodology, and progress. http://repository.cimmyt.org/

Morgounov, A., Ablova, L., Babayants, O., Babayants, L., Bespalova, L., Khudokormov, Zh., Litvinenko, N., Shamanin, V., Syukov, V. 2011. Genetic protection of wheat rusts and development of resistant varieties in Russia and Ukraine. Euphytica **179**:297–311.

Nowak, M., Leśniowska-Nowak, J., Zapalska, M., Banaszak, Z., Kondracka, K., Dudziak, K., Kowalczyk, K. 2014. Analysis of *VRN1* gene in triticale and common wheat genetic background. Scientia Agricola **71**:345–355.

Olson, E., Brown-Guedira, G., Marshall, D., Stack, E., Bowden, R.L., Jin, Y., Rouse, M., Pumphrey, M.O. 2010. Development of lines with a small introgressed segment carrying stem rust resistance gene *Sr22*. Crop Sci. **50**:1823–1830.

Peterson, R.F., Campbell, A.B., Hannah, A.E. 1948. A diagrammatic scale for estimating rust intensity of leaves and stem of cereals. Can. J. Research Sect. C **26**:496–500.

Petrash, N.V., Leonova, I.N., Adonina, I.G., Salina, E.A. 2016. Effect of translocations from *Aegilops speltoides* Tausch on resistance to fungal diseases and productivity in common wheat. Rus. J. Genet. **52**:1253–1262.

Pugsley, A.T. 1971. A genetic analysis of spring-winter habit in wheat. Austr. J. Agric. Res. **22**:21–31.

Rizza, F., Karsai, L., Morcia, K., Badeck, F.W., Terzi, V., Pagani, D., Kiss, T., Stanca, A.M. 2016. Association between the allele compositions of major plant developmental genes and frost tolerance in barley (*Hordeum vulgare* L.) germplasm of different origin. Mol Breed. **36**:156–172.

Roelfs, A.P., Singh, R.P., Saari, E.E. 1992. Rust diseases of wheat: concepts and methods of disease management. CIMMYT. Mexico.

Salina, E.A, Adonina, I.G., Badaeva, E.D., Kroupin, P.Yu., Stasyuk, A.I., Leonova, I.N., Shishkina, A.A., Divashuk, M.G., Starikova, E.V., Khuat Thi Mai, L., Syukov, V.V., Karlov, G.I. 2015. A *Thinopyrum intermedium* chromosome in bread wheat cultivars as a source of genes conferring resistance to fungal diseases. Euphytica **204**:91–101.

Sanin, S.S., Nazarova, A.N. 2010. The phytosanitary situation in the wheat fields in the Russian Federation (1991–2008). Plant Protection and Quarantine **2**:70–78. (in Russian)

Sharma, S., Chaudhary, H.R. 2009. Combining ability and gene action studies for yield-contributing traits in crosses involving winter and spring wheat genotypes. Acta Agronomica Hungarica **57**:417-423.

Shcherban, A.B., Efremova, T.T., Salina, E.A. 2012. Identification of a new *Vrn-B1* allele using two near-isogenic wheat lines with difference in heading time. Mol. Breed. **29**:675–685.

Shcherban, A.B., Börner, A., Salina, E.A. 2015. Effect of *VRN-1* and *PPD-1* genes on heading time of European bread wheat cultivars. Plant Breed. **134**:49–55.

Somers, D.J., Isaac, P., Edwards, K. 2004. A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor. Appl. Genet. **109**:1105–1114.

Song, Q.J., Shi, J.R., Singh, S., Fickus, E.W., Costa, J.M., Lewis, J., Gill, B.S., Ward, R., Cregan, P.B. 2005. Development and mapping of microsatellite (SSR) markers in wheat. Theor. Appl. Genet. **110**:550–560.

Sutka, J. 2001. Genes for frost resistance in wheat. Euphytica **119**:167–172.

Timonova, E.M., Leonova, I.N., Röder, M.S., Salina, E.A. 2013. Marker-assisted development and characterization of a set of *Triticum aestivum* lines carrying different introgressions from the *T. timopheevii* genome. Mol. Breed. **31**:123–136.

Witcombe, J., Virk, D. 2001. Number of crosses and population size for participatory and classical plant breeding. Euphytica **122**:451–462.

Xu, Y. 2010. Molecular Plant Breeding. CAB International. Cambridge, MA, USA.

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