Moreover, tan spot also causes significant losses in grain quality by grain shriveling, red smudge, and black point. In recent years, this disease more often appear in the plantings of spring bread wheat in the Lower Volga Region of the Russian Federation. To screen for resistance to this pathogen, we evaluated 140 introgression lines of spring bread wheat in a nursery. Among the lines, 35 were resistant (% infection = 0) to the local tan spot population. NIL L400R, with a *Thinopyrum intermedium* 6Agi (6D) substitution was more resistant to tan spot than its sib lines L400S that lack 6Agi (Table 5). The combination of translocations T7DS·7DL-7Ae#1L (*T. elongatum*) and T6BS·6BL-6U#1L (*Ae. umbellulata*) and T7DS-7DL-7Ae#1L and 4BS·4BL-2R#1L (*S. cereale*) increase the resistance of the spring bread wheat lines to the tan spot, however, the combination T7DS·7DL-7Ae#1L and T1BL·1R#1S significantly increased susceptibility. NILs with a combination of T7DS·7DL-7Ae#1L and T3DS·3DL-3Ae#1L (*T. elongatum*) did not significantly differ on the extent of damage with respect to the cultivar (Table 5).

Table 5. The reaction of cultivars and NILs of spring bread wheat to *Pyrenophora tritici-repentis*.

Cultivar / NIL	Translocation	% infection
L 400 R	6Agi (6D)	5
L 400 S	—	30
L 503	T7DS·7DL-7Ae#1L	0
L 503 (*Lr26+Lr19*)	T7DS·7DL-7Ae#1L	0
L 503 (*Lr26+Lr19*)	T7DS·7DL-7Ae#1L + T1BL·1R#1S	5–10
L 505//L503 (*Lr26+Lr19*)	T7DS·7DL-7Ae#1L + T1BL·1R#1S	5
Dobrynya	T7DS·7DL-7Ae#1L	30
Dobrynya (*Lr19+Lr9*)	T7DS·7DL-7Ae#1L + T6BS·6BL-6U#1L	3
Dobrynya (*Lr19+Lr24*)	T7DS·7DL-7Ae#1L +T3DS·3DL-3Ae#1L	30
Dobrynya (*Lr19+Lr25*)	T7DS·7DL-7Ae#1L + 4BS·4BL-2R#1L	10

Department of Biotechnology, Laboratory of Cell Breeding, 7 Tulaikov Street, Saratov, 410010, Russian Federation.

The microclonal propagation of sterile triticale plants.

V.N. Akinina, T.I. Dyatchouk, and A.V. Pominov.

Applying biotechnology to crop improvement induces nonconventional plant breeding methodology. In biotechnology and plant breeding, preserving and propagating sterile plants, undoubled haploids, and remote hybrids for future use is important. The most frequent explant sources for morphogenetic callus induction in cereals are mature and immature embryos. To establish tissue culture systems in sterile plants, it is necessary to select the explant source.

Wheat–triticale hybrids (three genotypes) and haploids (nine genotypes) were grown in a phythotron. Somatic embryogenesis was used to produce the largest number of plantlets. Segments of immature inflorescence 2–3 cm and completely covered by leaves served as explants to establish callus cultures. A solid MS nutrient medium, supplemented with 2 mg/L 2,4-D and 2% sucrose was optimum for callus induction. Callus cultures were grown in the dark, with subculturing on fresh culture medium every 3–4 weeks. The subsequent elimination of 2,4-D from the culture medium and the addition of IAA (1 mg/L) allowed for embryogenesis. Centers of morphogenesis were milky white, compact, and modulary. The start of regeneration was observed within 7–10 days. The frequency of callus induction ranged from 76.5 to 84.0% and the frequency of morphogenetic callus was from 39.1 to 46.6%.

The microclonal propagation of sterile triticale sterile was elaborated using segments of immature inflorescence as source of totipotent cultures. Embryogenic callus and plant regeneration were formed. This technology allows one to obtain clones of each genotype in an unlimited amount for use in breeding work.

Identification of leaf rust resistance genes of lines of soft spring and winter wheats.

T.S. Markelova and O.V. Ivanova.

Leaf rust resistance genes were identified in 14 introgressed lines of soft wheat carrying noncompensating translocations from *Aegilops speltoides*, *Triticum turgidum* subsps. *dicoccum* and *persicum*, and wheat cultivars from the collection of the All-Russian Institute of Plant Breeding using phytopathological and molecular methods.

After molecular screening of wheat cultivars, we discovered three with gene *Lr9* and one with *Lr19* (Table 6, p. 197). The identification of *Lr9* used SCAR marker SCS5 (Gupta et al. 2005). A characteristic 550-bp fragment was found among breeding lines 6 and 7, Clez Alta, and a control line Tc *Lr9*. The results from the molecular screening of these species correspond with the phytopathological test. Gene *Lr9* in line 11 is unidentified despite the fact that it was used when the line was created. In addition to leaf rust resistance, this line also has mildew resistance from *T. turgidum* subsp. *persicum*.

Table 6. Leaf rust (*Lr*) genes identified using DNA markers.

#	Description	*Lr9*	*Lr10*	*Lr19*	*Lr20*	*Lr26 iag95*	*Lr26 SCM9*	*Lr34*	*Lr35*	*Lr37*
1	Saratovskaya 29/*T. persicum*//*T. dicoccum*				+				+	
2	Saratovskaya 55//*T. dicoccum*/*Ae. speltoides*								+	
3	Saratovskaya 55// *T. dicoccum*/ *Ae. speltoides*		+						+	
4	Saratovskaya 29//*T. persicum*/3/ *T. dicoccum*/*Ae. speltoides*								+	
5	Saratovskaya 29// *T. dicoccum*/*Ae. speltoides*								+	
6	Saratovskaya 29/*T. persicum*//*Lr9*	+								
7	Saratovskaya 29/*T. persicum*/*Lr9*	+								
8	Saratovskaya 29/*T. persicum*//*T. dicoccum*/*Ae. speltoides*								+	
9	Saratovskaya 55//*T. dicoccum*/*Ae. speltoides*								+	
10	Saratovskaya 55//*T. dicoccum*/*Ae. speltoides*								+	
11	Saratovskaya 29/*T. persicum*//*Lr9*								+	
12	Saratovskaya 29/*T. persicum*// *T. dicoccum*/*Ae. speltoides*								+	
13	Saratovskaya 55//*T. dicoccum*/*Ae. speltoides*								+	
14	Saratovskaya 29/*T. persicum*// *T. dicoccum*/*Ae. speltoides*								+	
15	Clez Alta	+								
16	K-34612			+						
17	W464//VEE/KOEL/3/PEG//MRL/BUC		+			+	+	+		
18	CROC-1/*Ae. tauschii* (205)//KAUZ/3/ATTILA					+	+			

A characteristic 130-bp fragment was found in sample 16 with the help of STS marker Gb *Lr19* (Prins et al. 2001). A 487-bp fragment was discovered in lines 1–5 and 8–14 with the help of marker Sr39#22 for gene *Lr35*. Gene *Lr35* is located on the short arm of chromosome 2B and is situated in the same translocation with seedling gene *Sr39*, which defends against *Puccinia graminis* race Ug99 (TTKS) (Singh et al. 2008). Diploid *Ae. speltoides* is the source of this translocation.

When marker PS10 for gene *Lr47* from *Ae. speltoides* was being applied its carriers were not discovered. Although gene *Lr35* has the potential for leaf rust resistance, examples of its application have not been given in the literature to date (McIntosh et al. 1995; Mago et al. 2009; Serfling et al. 2011). A good example of introgressing *Lr*/*Sr* genes from *Ae. speltoides* in Russia is the set of amphidiploid lines ((*T. dicoccum* x *Ae. speltoides*)*5//Saratovskaya 29) created at the ARJPB (Odintsova et al. 1991). These lines were widely used in many selection centres in the USSR, including SRJA of South-East. Thus, the existence of the given translocation in lines 1–5 and 8–14 is logical. Their resistance to

race *P. graminis* Ug99+*Lr24* (TTKST) (Sibikeev et al. 2011) is important to characteristics of these lines. Leaf and stem rust resistance, possibly caused by Lr and Sr cohesion, supports the existence of *Lr35*/*Sr39* genes in lines 1–5 and 8–14.

Although *Lr10* was identified with the primers F 12245 and *Lr10*-6/r2 (Schachermayr et al. 1997), an amplicon with a molecular weight of 310 bp was discovered in lines 3 and 17.

Using the primers STS638-L and STS638-R for gene *Lr20* (Neu et al. 2002), a marker of 540 bp was found in line 1 and in the control Thatcher with *Lr20;* line 1 includes wild *T. persicum* and *T. dicoccum* in the pedigree. Today, *Lr20* has lost its efficiency practically everywhere (McIntosh et al. 1995; Khan et al. 2005).

To identify gene *Lr26*, two markers, SCM9 (Mago et al. 2002) and iag95 (Weng et al. 2007), were used. A characteristic 207-bp component of SCM9 and an ~1,000-bp iag95 marker were identified in lines 17 and 18, which corresponds to their gender lines.

By applying phytopathological and molecular methods and also analyzing pedigrees, we discovered *Lr* genes and their combinations in 14 introgressive lines and in four soft spring wheats. These results are very important for programs that use the application of certain *Lr* genes and their combinations in practical selection.

References.

Gupta SK, Charpe A, Koul S, et al. 2005. Development and validation of molecular markers linked to an *Aegilops umbellulata*-derived leaf rust- resistance gene, *Lr9*, for marker-assisted selection in bread wheat. Genome **48**(5):823-830.

Khan RR, Bariana HS, Dholakia BB, et al. 2005. Molecular mapping of stem and leaf rust resistance in wheat. Theor Appl Genet 111(5):846-850.

Mago R, Spielmeyer W, Lawrence G, et al. 2002. Identification and mapping of molecular markers linked to rust resistance genes located on chromosome 1RS of rye using wheat-rye translocation lines. Theor Appl Genet 104:131-134.

Mago R, Zhang P, Bariana HS, et al. 2009. Development of wheat lines carrying stem rust resistance gene *Sr39* with reduced *Aegilops speltoides* chromatin and simple PCR markers for marker-assisted selection. Theor Appl Genet 124:65-70.

McIntosh RA, Wellings CR, and Park RF. 1995. Wheat rusts: an atlas of resistance genes. CSIRO, Australia, and Kluwer Academic Publishers, Dortrecht, the Netherlands. 200 pp.

Odintsova IG, Agafonova NA, and Boguslavskyi RL. 1991. The spring bread wheat introgression lines, carrying resistance to le af rust transferred from *Aegilops speltoides*. *In:* Proc Appl Bot, Genet and Breed, Vavilov Inst Res 142:106-110

Prins R, Groenewald J, Marais G, et al. 2001. AFLP and STS tagging of *Lr19*, a gene conferring resistance to leaf rust in wheat. Theor Appl Genet 103(6):618-624.

Schachermayr G, Feuillet C, and Keller B. 1997. Molecular markers or the detection of the wheat leaf rust resistance gene *Lr10* in diverse genetic backgrounds. Mol Breed 3:65-74.

Serfling A, Krämer I, Lind V, et al. 2011. Diagnostic value of molecular markers for *Lr* genes and characterization of leaf rust resistance of German winter wheat cultivars with regard to the stability of vertical resistance. Eur J Plant Path 130(4):559-575.

Sibikeev SN, Markelova TS, Druzhin AE, Vedeneeva ML, and Singh D. 2011. Evaluation of the introgression spring bread wheat lines of Agricultural Research Institute for South-East Regions bred for resistance to race of *Puccinia graminis* f. sp. *tritici* Ug99 + *Sr24* (TTKST). Rus Agric Sci 2:3-5.

Singh RP, Hodson DP, Huerta-Espino J, et al. 2008. Will stem rust destroy the world's wheat crop? Adv Agron 98:310.

Weng Y, Azhaguvel P, Devkota RN, and Rudd JC. 2007. PCR-based markers for detection of different sources 1AL·1RS and 1BL·1RS wheat-rye translocations in wheat background. Plant Breed 126:482-486.