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Races of Puccinia graminis f. sp. tritici in Russian Federation in 2006.

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Monitoring the race composition of the wheat stem rust pathogen is done annually in the same fields of three regions of the Russian Federation; Central Russia (Moscow Region), the Northern Caucasus (Rostov Region), and Western Siberia (Tomsk Region). Development of disease may vary according to climatic conditions and the source of infection. Long-term observations indicated that infection on wheat and other grains generally appeared in the fields as separate groups of plants infected by stem rust. Under conditions unfavorable for development of the fungus, plants infected by rust were usually found on separate wheat cultivars with high susceptibility to pathogen. For example, the stem rust pathogen is found yearly in Rostov Region on wheat cultivars Albidum 28 and Albidum 43. However, considering the high infection potential of the pathogen, monitoring virulence and race composition of the fungus is extremely important to predict the appearance of new pathogen races in order to control development of stem rust epidemics.

The 2006 growing season was relatively favorable for development of wheat stem rust. Separate hotbeds of the pathogen were found on many wheat and barley cultivars in Central Russia and the Northern Caucasus. In Northern Siberia, only aeciospores were found on barberry. No large infection was observed, possibly explained by an insufficient amount of inoculum.

Races were determined by infecting 16 wheat lines with known resistance genes with monouredinial fungal isolates (Roelfs and Martens 1988). Fourteen races of *P. graminis* f.sp. *tritici* were identified in populations of the fungus from different regions of the Russian Federation. Races that occurred with a frequency of 8% or higher were referred to as dominant and those with lesser frequency as rare (Lekomtseva et al. 2007). Races TKNT (46%), TKNS (11%), and TKPT (8%) dominated in 2006. Races TKST and TTNT had occurrence frequencies of 7% (Table 1).

Table 1. Races of *Puccinia graminis* f. sp. *tritici* in the Russian Federation in 2006.

Race	Susceptibility of *Sr* genes	Number of isolates	%
KJNT	21, 9e, 7b, 6, 8a, 36, 30, , 9a, 9d, 10, Tmp	1	1
RKNT	5, 21, 7b, 6, 8a, 9g, 36, 30, 9a, 9d, 10, Tmp	3	4
TKNT	**5, 21, 9e, 7b, 6, 8a, 9g, 36, 30, 9a, 9d, 10, Tmp**	**34**	**46**
TKNS	**5, 21, 9e, 7b, 6, 8a, 9g, 36, 30, 9a, 9d, 10**	**8**	**11**
TKPT	**5, 21, 9e, 7b, 6, 8a, 9g, 36, 30, 13, 9a, 9d, 10, Tmp**	**6**	**8**
TKST	**5, 21, 9e, 7b, 6, 8a, 9g, 36, 30, 9a, 9d, 10, Tmp**	**5**	**7**
TKKT	5, 21, 9e, 7b, 6, 8a, 9g, 36, 30,13, 9a, 9d, 10, Tmp	3	4
TFNT	5, 21, 9e, 7b, 8a, 9g, 36, 30, 9a, 9d, 10, Tmp	1	1
TTNT	**5, 21, 9e, 7b, 11, 6, 8a, 9g, 36, 30, 9a, 9d, 10, Tmp**	**5**	**7**
TTPT	5, 21, 9e, 7b, 11, 6, 8a, 9g, 36, 30,13, 9a, 9d, 10, Tmp	4	5
RTPT	5, 21, 7b, 11, 6, 8a, 9g, 36, 30,13, 9a, 9d, 10, Tmp	1	1
PKQT	5, 9e, 7b, 6, 8a, 9g, 9a, 9d, 10, Tmp	2	3
TTST	5, 21, 9e, 7b, 11, 6, 8a, 9g, 36, 9b, 30, 9a, 9d, 10, Tmp	1	1
TJST	5, 21, 9e, 7b, 6, 8a, 36, 9b, 30, 9a, 9d, 10, Tmp	1	1
Total		75	100

The frequency of rare races in populations of the stem rust pathogen may indicate instability in the composition of the fungal population, connected with the different susceptibility of host plant cultivars, the different adaptation properties of various pathogen races to the environment, or the sensitivity of individual virulence genes to temperature or illumination.

Composition analysis of races in different regions and on various host plants indicated that the percent of rare races was similar for barberry, wheat, and barley (22–23%) in Central Russia and in the Northern Caucasus in 2006; the frequency of dominant races was less than that of rare races (43% and 57%, respectively). The proportion of dominant and rare races was similar (66% in Central Russia and 34% in the Northern Caucasus) for Gramineous plants in these regions. Only dominant races were registered on barberry in Western Siberia (Table 2).

Table 2. Dominant and rare races of *Puccinia graminis* f.sp. *tritici* in various regions of the Russia Federation in 2006.

Region	Host plant	Dominant races (%)	Rare races (%)
Central Russia	Barberry	77	23
	Wheat	78	22
	Barley	77	23
	Gramineous plants	66	34
Northern Caucasus	Wheat	43	57
	Gramineous plants	66	34
Western Siberia	Barberry	100	0

The high variability of race composition of wheat in Northern Caucasus also may indicate the presence of infective sources from places where barberry grows in the mountainous regions of this zone. Evaluating tolerance of wheat lines indicated that, in 2006, most were found susceptible to stem rust, with the exclusion of *Sr11*, *Sr9b*, and *Sr13* (Table 3).

Table 3. Number of monouredinial isolates virulent to wheat lines with *Sr* genes.

Sr gene	%
5	98
6	98
7b	100
8a	98
9a	75
9b	**12**
9d	97
9e	97
9g	97
10	98
11	**16**
13	20
21	97
30	98
36	100
Tmp	89

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RAPD distribution of Russian isolates of Puccinia graminis f.sp. tritici by the high-GC primers.

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To minimize wheat loss due to rust in the Russian Federation, annual control of the molecular and virulence variation in the *P. graminis* f.sp. *tritici* population is essential. Although the variation in virulence is under strong race selection, the molecular polymorphism of isolates could reflect any neutral trend in the current pathogen population (Roelfs et al. 1997; McCallum et al. 1999).

For the genetic analysis of fungal DNA polymorphism, we used RAPD–PCR improved by the high-GC 10-nt primers (Kubelik and Szabo 1995). These primers generated much higher numbers of both amplification products per primer and polymorphism among isolates in comparison with the 15-nt primers we used previously (Table 1).

Table 1. Comparison of 15-nt arbitrary primers with 10-nt high-GC primers from RAPD analysis of DNAs from 36 Russian isolates of *Puccinia graminis* f.sp. *tritici*.

Item	PR3 (GTG)5	Core GAGGGTGGXGGXTCT	CRL-11 CCACCGCGCC	CRL-9 CAGCCGCCCC	CRL-7 GCCCGCCGCC
Segment/primer	5.7	8.6	10.25	11.9	14.3
% Polymorphism detected/primer	42	50	82	75	65

The high-GC primers increased the efficiency of DNA-fingerprinting for genetic analysis of *P. graminis* f.sp. *tritici* (Pgt). We screened five arbitrary primers on DNAs from 36 Russian *Pgt*-isolates with different geographic and host-plant origin collected in 2005 (Table 2). All primers gave scorable DNA segments (strong bands on the gel), and the high-GC primers yielded an average of 12.15 amplification products per primer with an average of 74% of polymorphisms. To estimate genetic variation of *Pgt*-isolates, we used a clustered method to create phylogenetic trees (Treecon for Windows). All dendrograms were characterized by similar topologies but different levels of cluster stability. The higher indexes confirmed the RAPD-distribution of isolates by the high-GC primers (Fig. 1, p. 121). Because it had been already fixed by annual analysis since 2001 (Skolotneva et al. 2005, 2007), there was a clear host-plant grouping of *Pgt*-isolates in the barberry cluster, wheat, barley, and some cereal grasses.

Table 2. Isolates of *Puccinia graminis* f.sp.*tritici* in different regions of the Russian Federation in 2005.

Isolate No.	Region	Host plant
2.1/2.2	Central Russia, Moscow area	*Berberis vulgaris*
3.1/3.2	Central Russia, Moscow area	*Berberis vulgaris*
4.1/4.2	Central Russia, Moscow area	*Berberis vulgaris*
7.1/7.2	Central Russia, Moscow area	*Triticum aestivum*
8.1/8.2	Central Russia, Moscow area	*Triticum aestivum*
9.1	Central Russia, Moscow area	*Triticum aestivum*
10.1/10.2	Central Russia, Moscow area	*Triticum aestivum*
11.1/11.2	Central Russia, Moscow area	*Triticum aestivum*
14.1/14.2	Central Russia, Moscow area	*Hordeum distichum*
15.1/15.2	Central Russia, Moscow area	*Elytrigia repens*
16.1/16.2	Northern Caucasus, Rostov area	*Triticum aestivum*
18.1/18.2	Northern Caucasus, Rostov area	*Triticum aestivum*
19.1	Northern Caucasus, Rostov area	*Triticum aestivum*
22.1/22.2	Northern Caucasus, Rostov area	*Triticum aestivum*
23.1/23.2	Northern Caucasus, Rostov area	*Hordeum distichum*
25.1/25.2	Western Siberia, Tomsk area	*Berberis vulgaris*
26.1/26.2	Western Siberia, Tomsk area	*Elytrigia repens*
27.1/27.2	Western Siberia, Tomsk area	*Dactylis glomerata*
28.1/28.2	Western Siberia, Tomsk area	*Phleum pratense*

Isolate genotypes from wheat were constantly clustered (by index bootstrap up to 91%). Only traces of the geographical differences among them were found on the subcluster level. Therefore, we suggested the wheat cultivars provided the same pressure on the molecular polymorphism of the wheat stem rust pathogen throughout the European Russia.

Table 3. The index description of the separated clusters with Western Siberian isolates of *Puccinia graminis* f.sp. *tritici* created by RAPD-analysis with five arbitrary primers.

Item	PR3	Core	CRL-11	CRL-9	CRL-7
Average percentage of the dendrogramm groups	50.2%	54,25%	70.75%	71.44%	64%
Bootsrap values of cluster reliability	76%	36%	97%	100%	57%
Divergence of the cluster from other groups in genetic scale	0.31	0.28	0.63	0.62	0.18

The sexual process on barberry is the greatest source of the race variability in the pathogen population. However, we observed that the RAPD polymorphism of *Pgt*-isolates was independent of recombination forces, combining their genotypes into the stable cluster (by index bootstrap up to 86%). Having more information available about genotypes by this improved RAPD method suggested an important role of the host-plant type with a special set of the biochemical and physiological characters for the molecular variation of wheat stem rust pathogen. We also could describe a separate cluster of *Pgt*-isolates with geographical (from Western Siberia, Tomsk area) and molecular (on RAPD data) differences (Table 3, p. 120).

Comparable virulence analysis of the spore collections from European Russia and Western Siberia had not demonstrated their isolation (Lekomtseva et al. 2007). We predicted that new local population could be formed by the accumulation of the neutral DNA-polymorphisms in the distinct region.

We suggest a relatively stable RAPD distribution of *P. graminis* f.sp. *tritici* isolates in European Russia by host-plant origin during last 5 years and that the molecular outlines of the pathogen group from the Western Siberia obtained by using the high-GC primers.

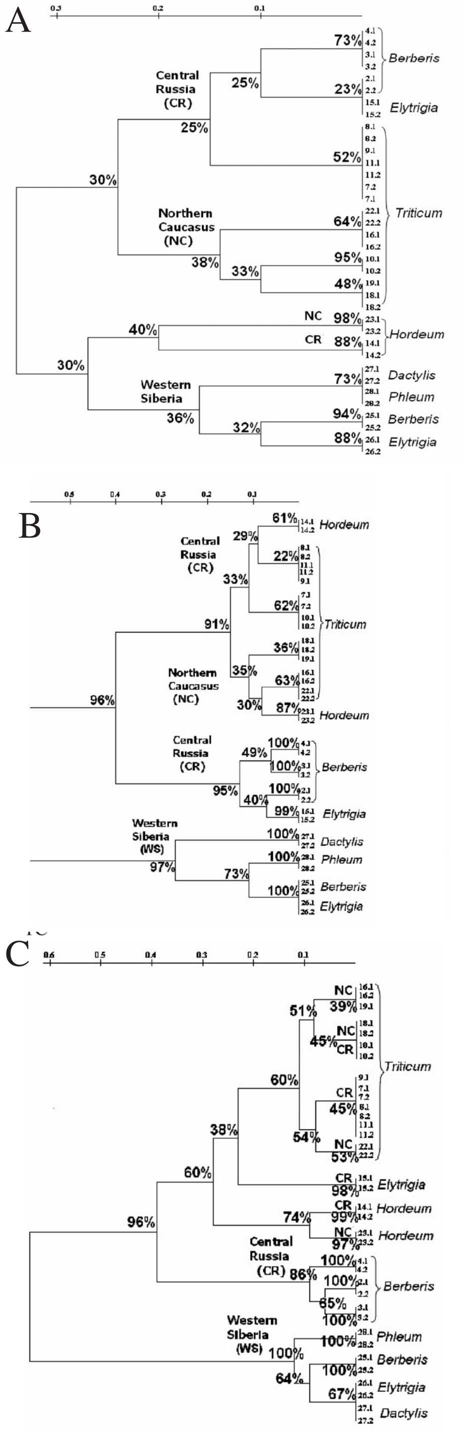

Fig. 1. RAPD-diversity of 36 Russian isolates of *Puccinia graminis* f.sp. *tritici*. A: by primer CORE; B: by the high-GC primer CRL-11; and C: by the high-GC primer CRL-9.

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