

The structure, virulence and molecular characterization of *Puccinia triticina* and *Puccinia graminis* populations in various regions of Russia in 2001-2008

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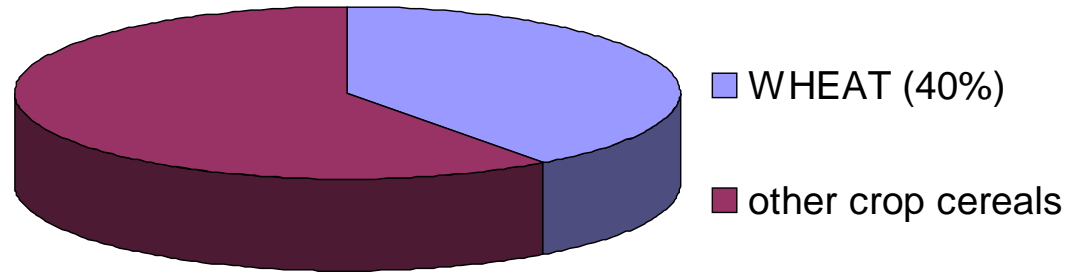


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St. Petersburg

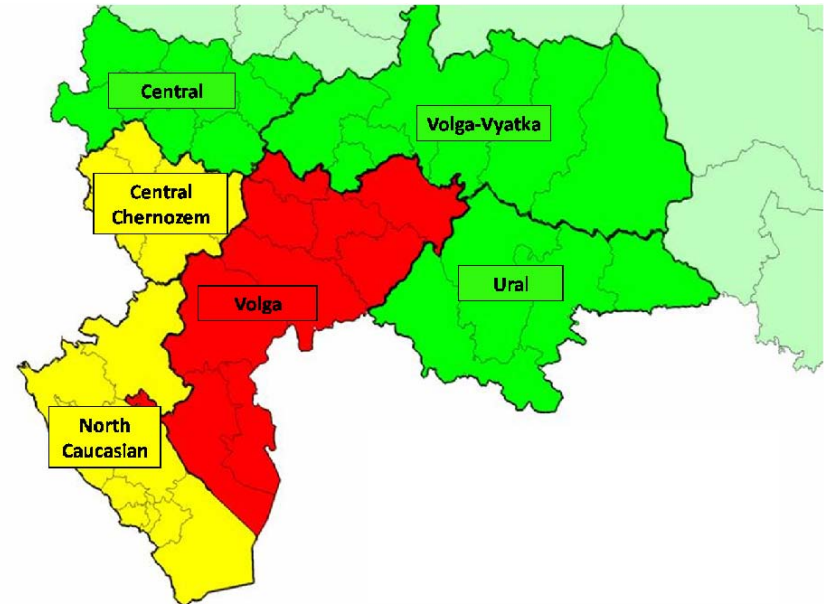


³ Lomonosov Moscow State University

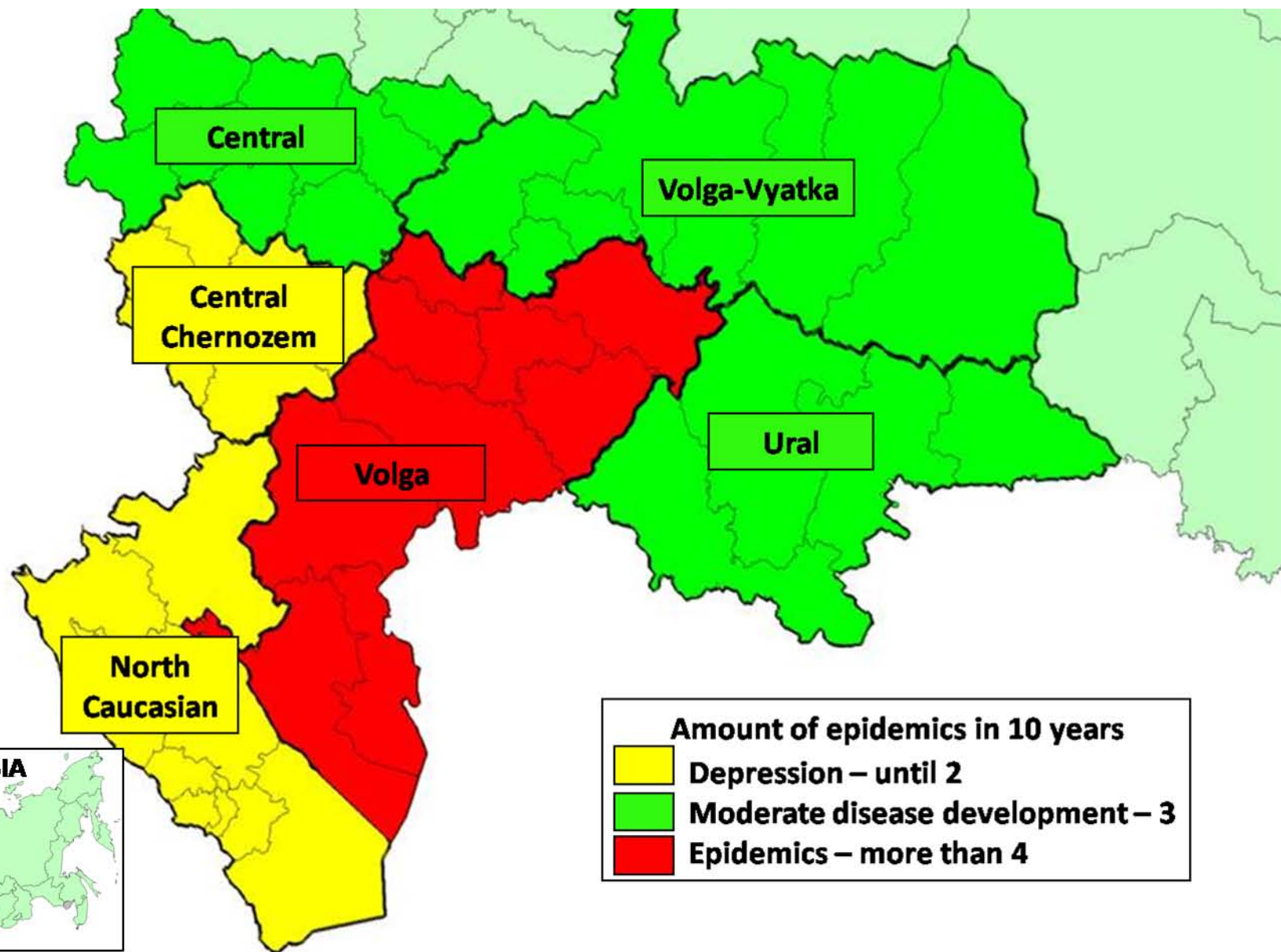
Wheat is a major field crop in Russia constituting 40% of total cereal production

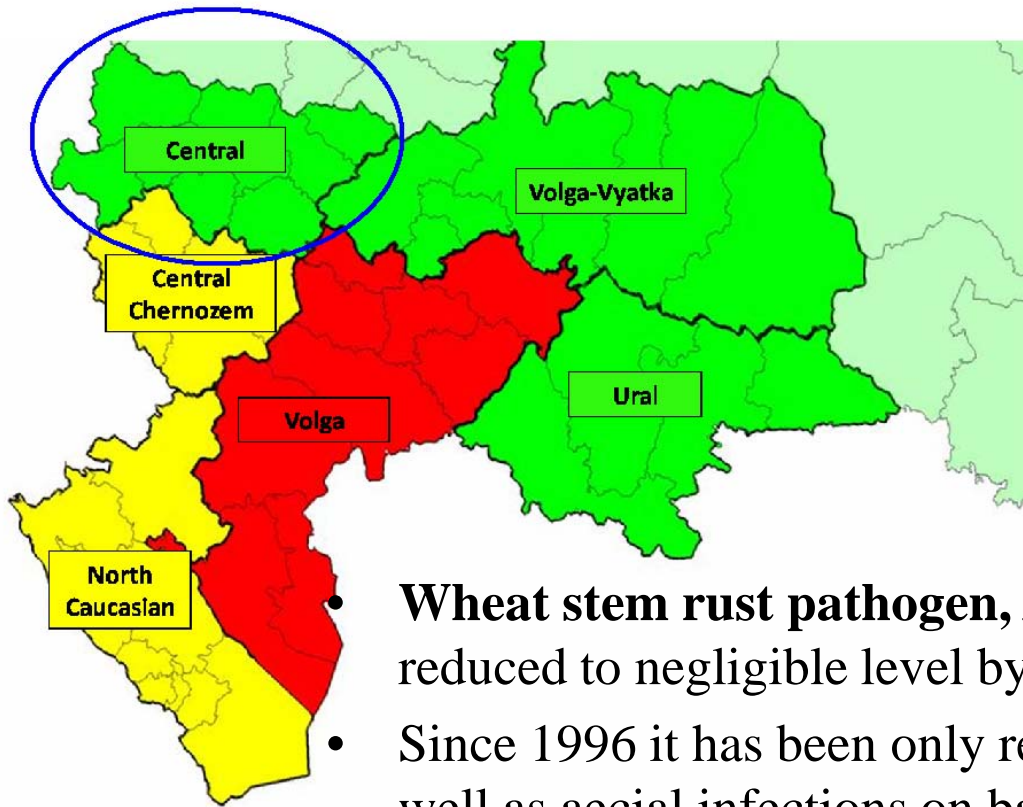


The main grain producing regions in Russia are the Central-Chernozem, North Caucasus, Central, and Volga regions. They account for 75% of the wheat area.



Development of leaf rust pathogen, *P. triticina*, in various areas of the Russian Federation, 1997-2007 (corresponding to Plant Pathologist Dr. Sanin A.)





- **Wheat stem rust pathogen, *Puccinia graminis* f.sp.*tritici*** has been reduced to negligible level by resistant cultivars in Central Russia.
- Since 1996 it has been only reported on barley and wild grasses as well as aecial infections on barberry (Lekomtseva et al. 2004; Skolotneva and Lekomtseva 2009).



Hordeum



Agropyron

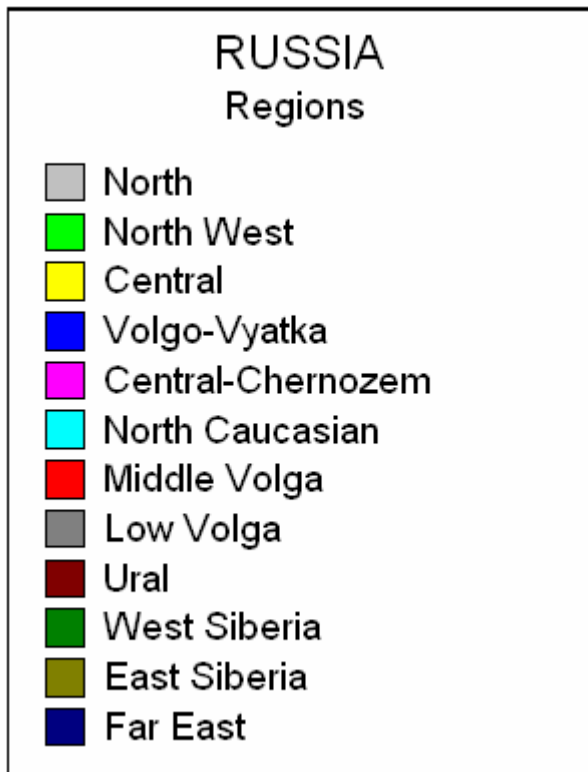


Dactylis



Berberis

- Disease control of the cereal rusts has been successfully realized through the use of resistant cultivars worldwide including the Russian Federation.
- There are many agro-ecosystems in Russia and resistance may become ineffective after a period somewhere.
- Pathogen samples should be obtained from different cultivars and regions.



Leaf rust isolates

Stem rust isolates

susceptible wheat cultivar
Khakasskaya

near-isogenic Thatcher wheat lines with the following genes *Lr1, Lr2a, Lr2b, Lr2c, Lr3a, Lr3bg, Lr3ka, Lr9, Lr10, Lr11, Lr14a, Lr14b, Lr15, Lr16, Lr17, Lr18, Lr19, Lr20, Lr21, Lr23, Lr24, Lr25, Lr26, Lr27+Lr31, Lr28, Lr29, Lr30, Lr32, Lr33, Lr36, Lr38, Lr39, Lr40, Lr41, Lr42, Lr44, Lr45, Lr46, Lr47* and *LrB*

pathotypes

differential lines (Long & Kolmer, 1989)

SET 1	<i>Lr1, Lr2a, Lr2c, Lr3</i>	SET 4	<i>Lr10, Lr18, Lr14a, LrB</i>
SET 2	<i>Lr9, Lr16, Lr24, Lr26</i>	SET 5	<i>Lr2e, Lr3bg, Lr14b, Lr15</i>
SET 3	<i>Lr3ka, Lr11, Lr17, Lr30</i>	SET 6	<i>Lr19, Lr20, Lr21, Lr28</i>

differential lines (Roelfs & Martens 1998; Jin et al., 2008)

SET 1	<i>Sr5, Sr21, Sr9e, Sr7b</i>	SET 4	<i>Sr9a, Sr9d, Sr10, SrTmp</i>
SET 2	<i>Sr11, Sr6, Sr8a, Sr9g</i>	SET 5	<i>Sr24, Sr31, Sr38, SrWld I</i>
SET 3	<i>Sr36, Sr9b, Sr30, Sr17</i>		

racess
assigned following the NA (North America) nomenclature system.

infection types were estimated for 10 days after inoculation

by Mains and Jackson (1926)

by Roelfs (1985)

Leaf rust isolates



Stem rust isolates



- All experiments were conducted in the growth chambers at $t=20^{\circ}\text{C}$ with humidity 60-70%, illumination 10 thousand lux, light period 16 h; for leaf rust using the techniques described by Browder (1971), and by Stubbs et al (1986) for stem rust.

Primer sets used in molecular analysis of *P. triticina* and *P. graminis tritici* isolates from different regions of Russia

Pathogen	Region	RAPD primers	PCR-conditions	References	Laboratory
<i>P. triticina</i>	Central Volga- Vyatka Western Siberia Eastern Siberia	CRL-07, 450, 538, 556, 489, 521, 517, 402	corresponding to references	Kolmer et al. 1995	Moscow State University Lomonosov
<i>P. triticina</i>	North-West	450, 490, 538, 280.02, 270.09, 480.09 and UP- PCR primer AS4	corresponding to references	Park et.al. 2000; Mironenko and Bulat 2004: Kolmer et al., 1995	All- Russian Institute for Plant Protection
<i>P. graminis tritici</i>	Central, North Caucasus, Western Siberia	CRL-1, CRL-7, CRL-9, CRL-11, CRL- 13	corresponding to references	Kubelik and Szabo 1995	Moscow State University Lomonosov

Leaf rust studies



- Leaf rust pathogen from different agro-climatic regions of Russia (Central, Central-Chernozem, North Caucasus, Middle and Low Volga, Volga-Vyatka, Western and Eastern Siberia) was studied in All-Russian Research Institute of Phytopathology in 2001-2008.





Virulence spectra of *P. triticina* populations in different regions of Russia, 2001 – 2008

Region	Weakly virulent pathotypes (%) 1-13 virulence genes								Moderately virulent pathotypes (%) 14-17 virulence genes								High virulent pathotypes (%) 18 virulence genes, from above							
	2 0 0 1	2 0 0 2	2 0 0 3	2 0 0 4	2 0 0 5	2 0 0 6	2 0 0 7	2 0 0 8	2 0 0 1	2 0 0 2	2 0 0 3	2 0 0 4	2 0 0 5	2 0 0 6	2 0 0 7	2 0 0 8	2 0 0 1	2 0 0 2	2 0 0 3	2 0 0 4	2 0 0 5	2 0 0 6	2 0 0 7	2 0 0 8
Central	7	10	7	0	0	0	3	0	57	54	64	45	21	0	7	14	35	36	28	55	79	100	89	86
Ce-Ch	53	13	1	9	-	0	0	3	47	52	50	33	-	14	50	13	0	35	49	57	-	86	50	84
N C	4	8	0	0	0	0	7	3	59	74	52	39	7	3	28	13	37	18	48	61	93	97	64	84
Middle Volga	-	1	0	-	-	0	-	0	-	43	33	-	-	10	-	18	-	56	67	-	-	90	-	82
Low Volga	6	2	-	-	0	0	0	0	36	85	-	-	13	6	0	25	58	7	-	-	87	94	100	75
Western Siberia	9	11	0	-	-	0	0	0	63	73	50	-	-	0	12	56	28	16	50	-	-	100	87	44

Ce-Ch – Central-Chernozem region

N C – North Caucasus region



Virulence has been detected to 28 leaf rust resistance genes.

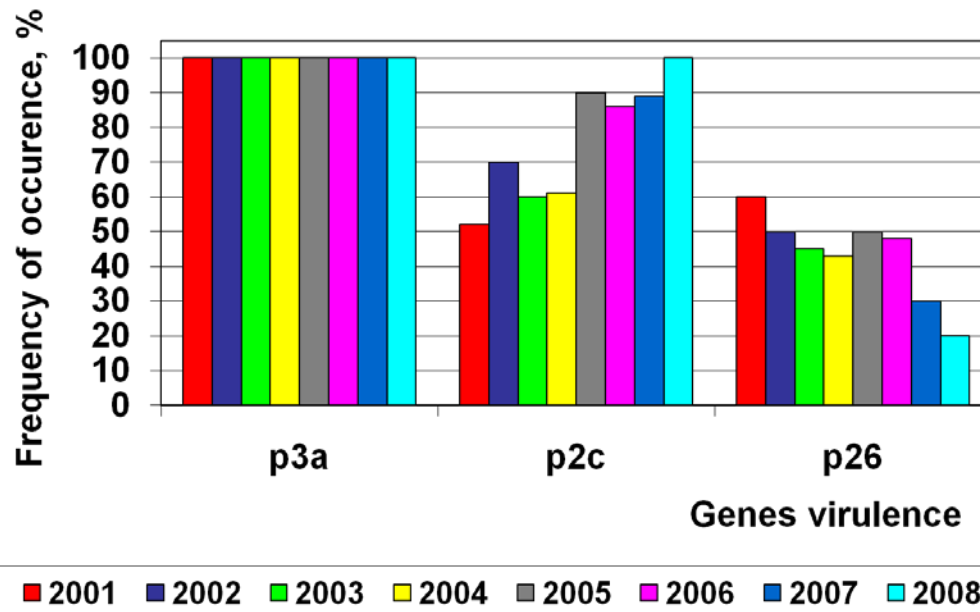
- Greater than 80% pathotypes were reported to *Lr1, 2c, 3a, 3bg, 10, 11, 14a, 17, 18, 21, 30, 33*, and *B*.
- At the rate of 20-75% virulence occurred to *Lr2a 2b, 3ka, 14b, 15, 16, 19, 20, 23, 25, 26, 28, 32, 36*.
- Pathotypes to genes *Lr9, 24, 29, 38, 41, 42, 45* have not been detected in Russian *P. triticina* populations.

Virulence genes were divided into the groups by dependence on *selection pressure*



- 1) Genes occurring at permanent high frequencies (80-100%); e.g. to ***Lr 3a, 3bg, 11, 17, 18, 30, 33, B***
- 2) Genes with increasing frequency; e.g. increase in virulence to ***2c, 16, 27+31, and 32*** occurred throughout the country
- 3) Genes with decreasing frequency: in Central region to ***Lr26***; Central-Chernozem for ***Lr23***; in North Caucasus to ***Lr14b*** and ***21***; in Middle Volga to ***Lr28*** and ***36***; in Low Volga to ***Lr2a, 2b, 3ka, 14b, and 28***; and in Western Siberia to ***Lr2b, 3ka, 14b, and 15***.

Frequency of occurrence of genes virulence in the Central region in 2001-2008

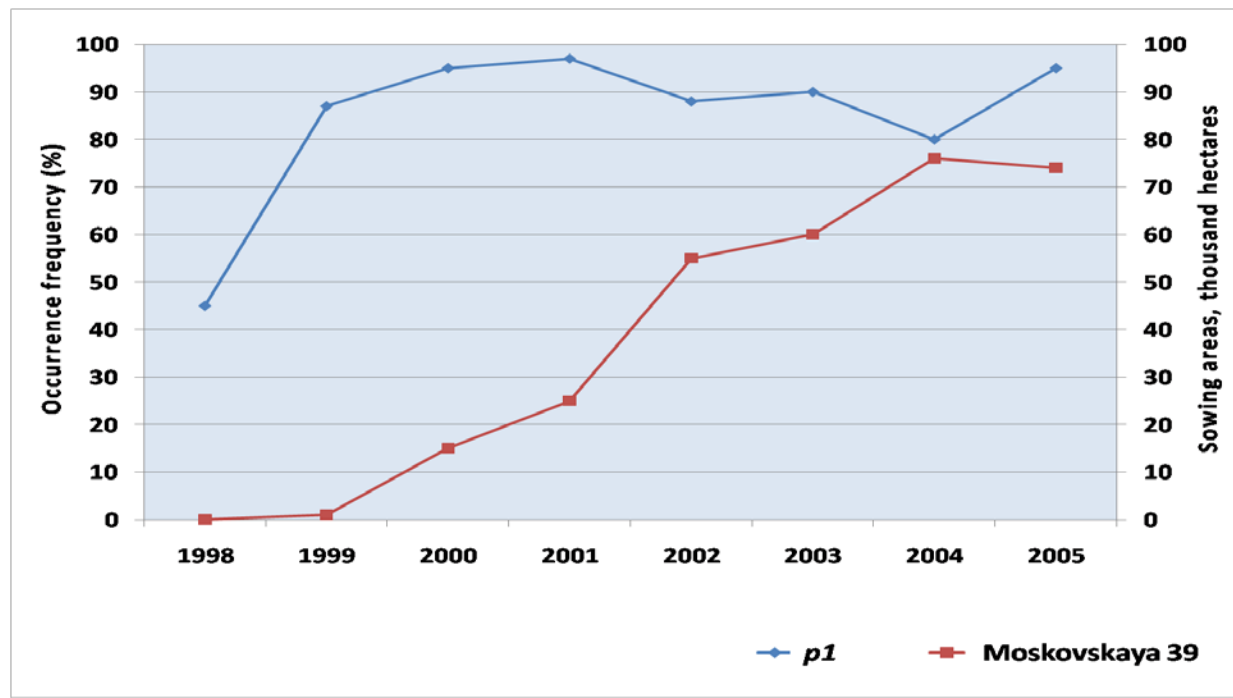




It was clearly observed that virulence frequency depended on harvested cultivars especially on varieties with race-specific resistance:

- When they were released long and cover large areas there were new pathotypes detected or increasing frequency of prevailing races was indicated (as it was with *winter wheat Moskovskaya 39 carrying gene Lr1*)
- After they were removed from production there were reducing or eliminating of corresponding pathotypes

Sowing areas under winter wheat Moskovskaya 39 and dynamics of virulence to *Lr1* in *Puccinia triticina* populations in the Central region





Occurrence of *P. triticina* isolates with gene *p19* in Russia in 1997-2007

RUSSIA

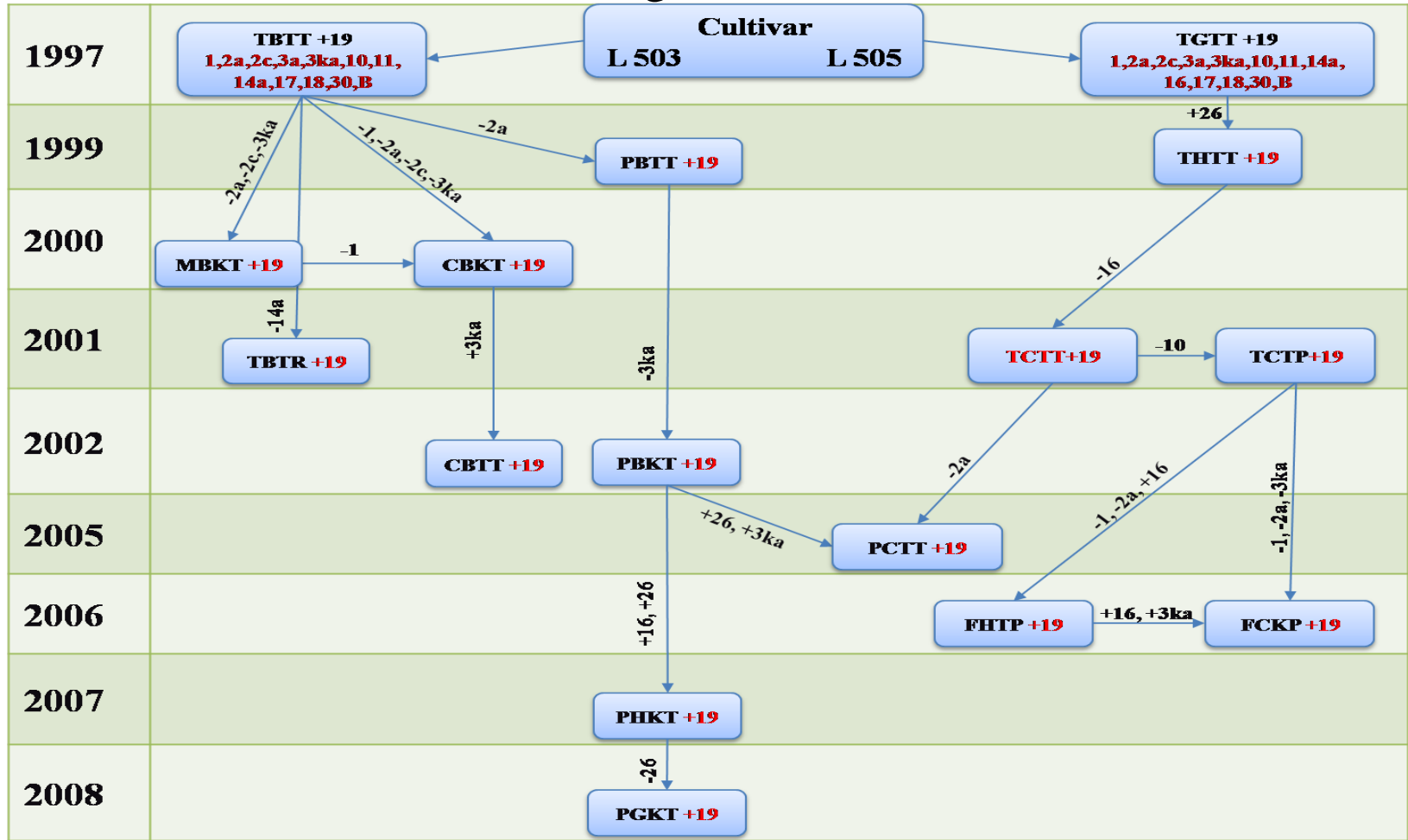
Regions

- North
- North West
- Central
- Volgo-Vyatka
- Central-Chernozem
- North Caucasian
- Middle Volga
- Low Volga
- Ural
- West Siberia
- East Siberia
- Far East





Evolution of *P. triticina* races with virulence gene *p19* in Low Volga, 1997-2008



Cultivars with overcome resistance: **Mironovskaya 808, Donskaya bezostaya, Saratovskaya 29, Saratovskaya 42, Saratovskaya 90, Albidum 31**

A total of 1740 leaf rust isolates were analyzed during 2001 to 2008 using 16 differential lines (Long, Kolmer, 1989) and divided into 8 race groups



Race groups	Central	Central-Chernozem	North Caucasus	Middle Volga	Low Volga	Western Siberia
C- (to Lr 3)	5.5	14.6	14.7	8.5	3.6	1.2
F- (to Lr 2c, Lr 3)	12.1	13.7	26.7	7.7	5.6	3.7
H- (to Lr 2a, Lr 3)	0.5	0.2	0	0	0	0
K- (to Lr 2a, Lr 2c, Lr 3)	0.5	2.4	0.2	1.5	2.0	2.4
M- (to Lr 1, Lr 3)	20.7	16.2	18.2	6.9	1.7	8.0
P- (to Lr 1, Lr 2c, Lr 3)	37.7	27.4	20.7	7.7	40.3	34.3
R- (to Lr 1, Lr 2a, Lr 3)	1.8	2.8	0.5	0.8	2.5	1.2
T- (to Lr 1, Lr 2a, Lr 2c, Lr 3)	22.8	21.8	18.5	66.6	34.1	46.6
Number of isolates	455	347	393	140	222	183

We used “race groups” according to Long, Leonard, Roberts, 1998, PL. Deas.

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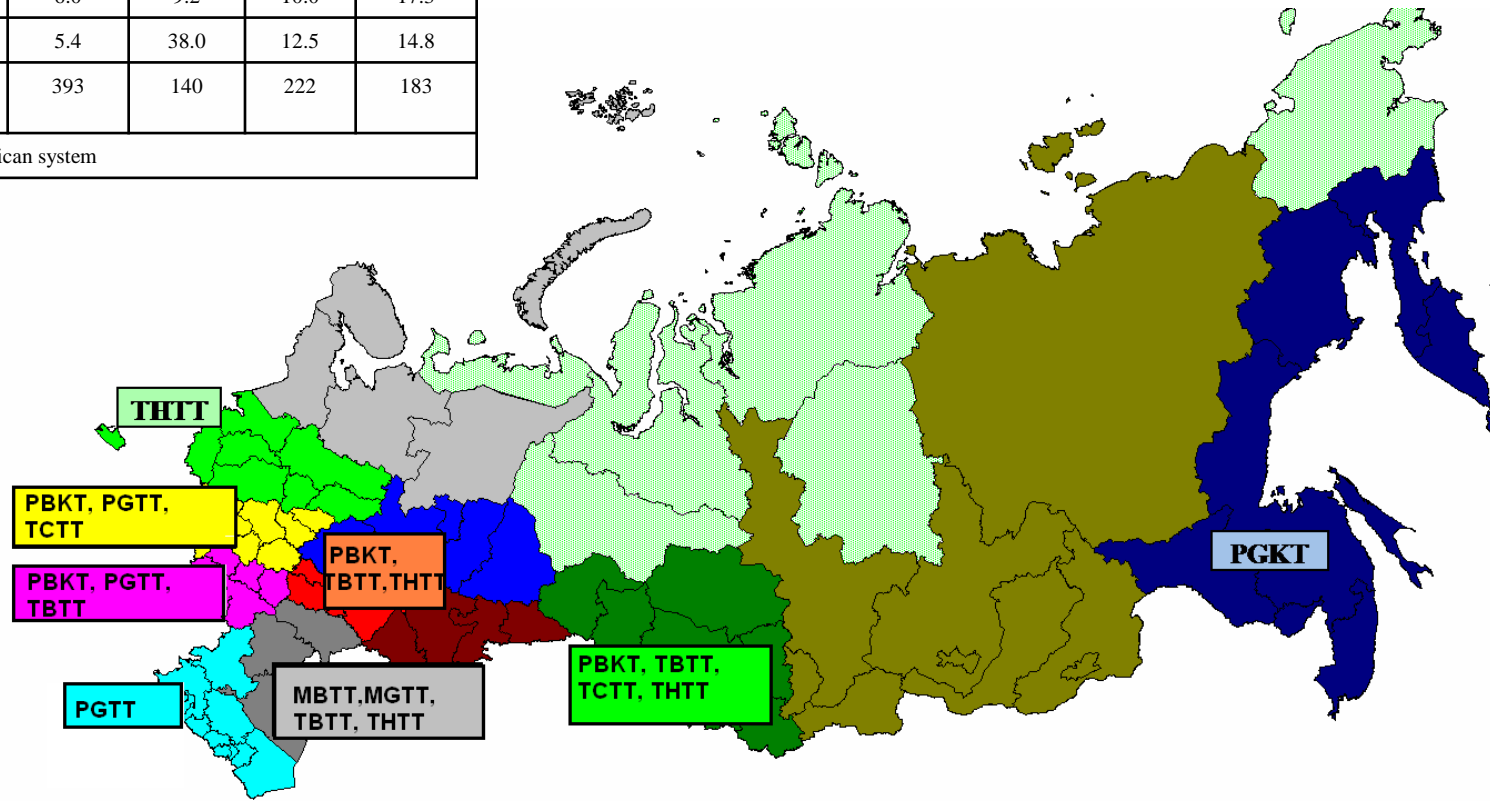
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H- (to Lr 2a, Lr 3)	0.5	0.2	0	0	0	0
K- (to Lr 2a, Lr 2c, Lr 3)	0.5	2.4	0.2	1.5	2.0	2.4
M- (to Lr 1, Lr 3)	20.7	16.2	18.2	6.9	1.7	8.0
P- (to Lr 1, Lr 2c, Lr 3)	37.7	27.4	20.7	7.7	40.3	34.3
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Frequencies of widely distributed *P. triticina* races of in Russia, 2001 - 2008

Race	Central	Central-Chernozem	North Caucasus	Middle Volga	Low Volga	Western Siberia
MBTT	6.0	5.6	7.0	25.0	5.8	9.1
MGTT	5.0	8.0	4.0	12.5	1.2	4.8
PBKT	12.0	17.0	4.7	1.2	13.6	12.2
PBTT	9.8	4.0	4.0	1.2	0.2	8.5
PGTT	17.4	13.3	14.2	2.4	10.3	0.3
TBTT	5.6	11.3	4.1	48.1	16.0	23.4
TCTT	11.0	10.0	6.0	9.2	10.0	17.3
THTT	6.0	7.0	5.4	38.0	12.5	14.8
Number of isolates	455	347	393	140	222	183
Races named according to the North American system						





Leaf rust pathogen from the North-West region (Pskov, Novgorod and Leningrad areas) in 2001-2009 was studied in All- Russian Institute of Plant Protection (VIZR).





Effectiveness of *Lr*-genes at the adult plant stage (North-West region of RF, 2001-2009)



Disease severity
0%

Lr9
Lr19
Lr24
Lr25
Lr28
Lr29
Lr38
Lr41
Lr42
Lr43
Lr47
Lr49

**Highly
resistant**

Disease severity
0-5%

Lr23
Lr27+31
Lr37
Lr48
Lr50

Resistant

Disease severity
5-30%

Lr12
Lr13
Lr21
Lr22
Lr39
Lr40
Lr46
LrW

**Middle
resistant**

Disease severity
30-60%

Lr14b
Lr18
Lr21
Lr22
Lr32
Lr34
Lr39
Lr44
Lr46

**Middle
susceptible**

Disease severity
60-100%

Lr1, Lr2a,
Lr2b, Lr2c,
Lr3a, Lr3bg,
Lr3ka, Lr10,
Lr 11, Lr14a,
Lr15, Lr16,
Lr17, Lr30,
Lr35, LrB

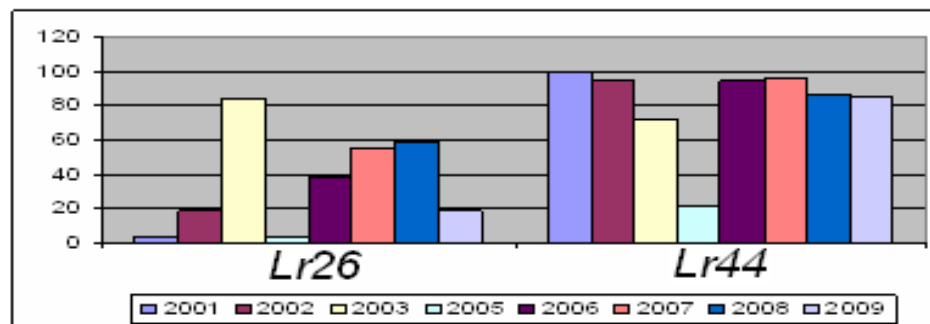
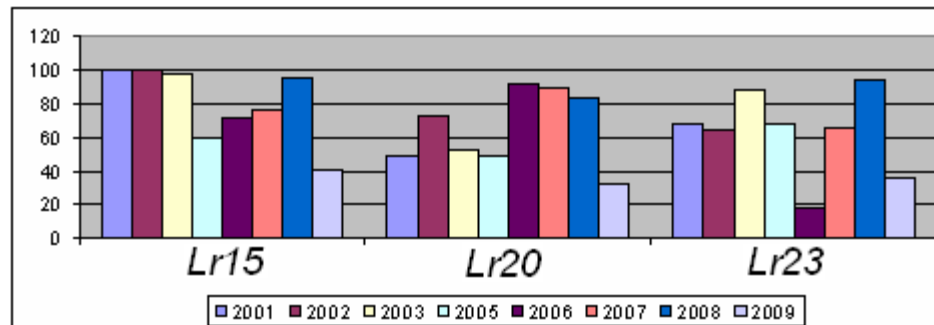
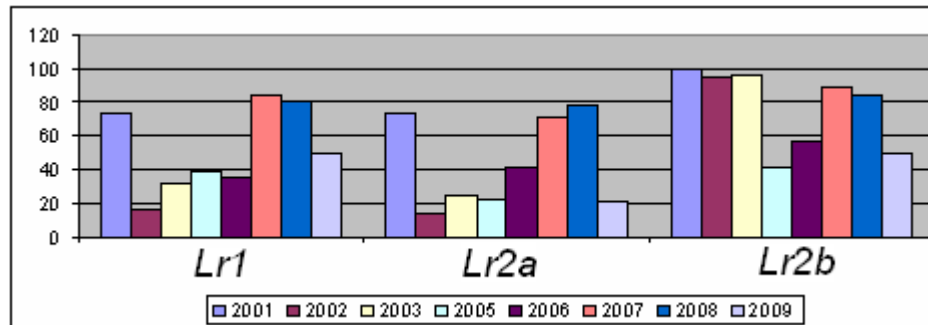
**Highly
susceptible**

VIRULENCE TO *Lr*-GENES AT THE SEEDLING STAGE (North-West region of RF, 2001-2009)

No virulent isolates

- Lr9*
- Lr24*
- Lr28*
- Lr29*
- Lr38*
- Lr41*
- Lr42*
- Lr43*
- Lr47*

Changing virulence rate



Virulence 80-100%

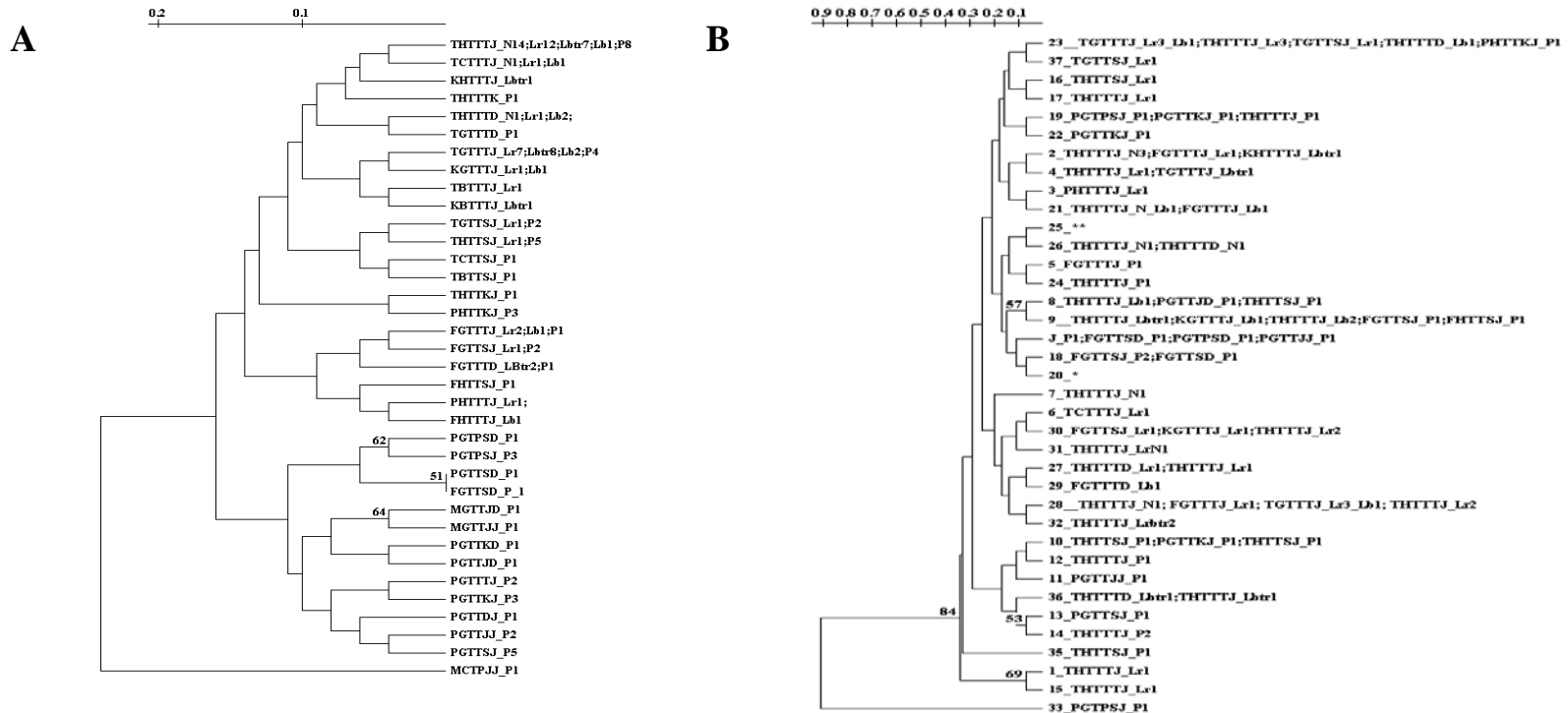
- Lr2c*
- Lr3a*
- Lr3ka*
- Lr3bg*
- Lr10*
- Lr11*
- Lr14a*
- Lr14b*
- Lr16*
- Lr17*
- Lr18*
- Lr21*
- Lr30*
- LrB*
- LrW*

Virulence <5%

- Lr19*
- Lr25*



In 2007 thirty six pathotypes and 37 DNA phenotypes were obtained from 139 isolates collected from state cultivar testing stations and commercial fields in the North-West region



Dendrograms of similarity between North-West isolates of *P. triticina* generated by UPGMA using: **virulence markers (A)** and **RAPD-markers (B)**

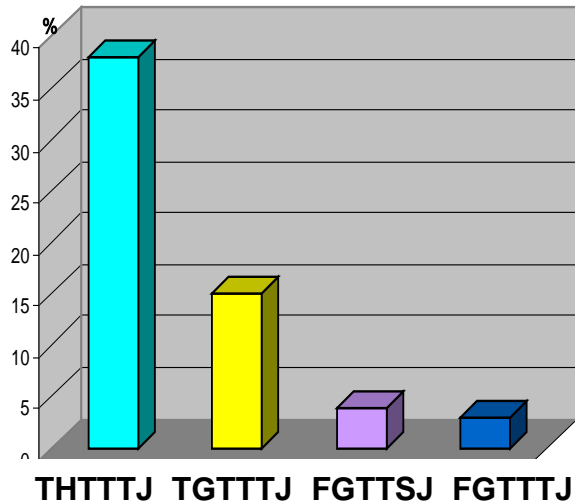
The Mantel-index of similarity between virulence and molecular matrixes: **$r = 0.08367$** .



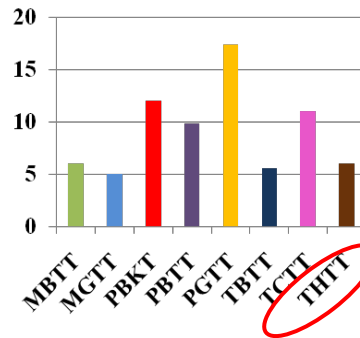
Wide spread races of *P. triticina* in different regions of the Russian Federation, 2001-2008



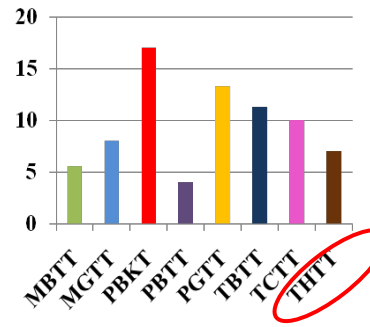
North-West region



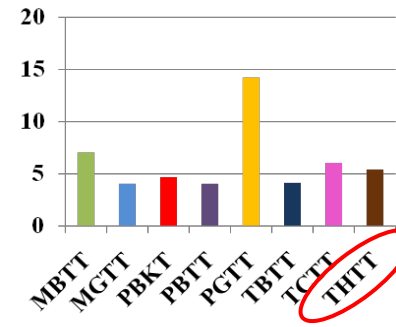
Central region



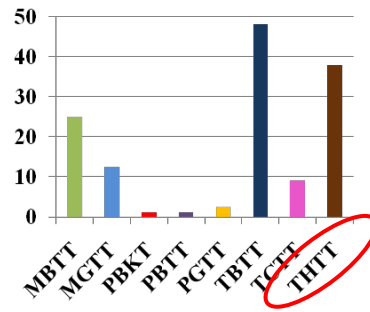
Central-Chernozem



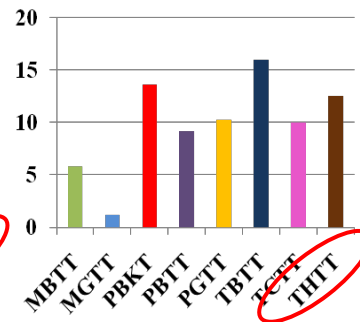
North Caucasus



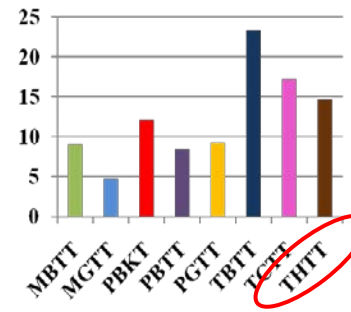
Middle Volga



Low Volga



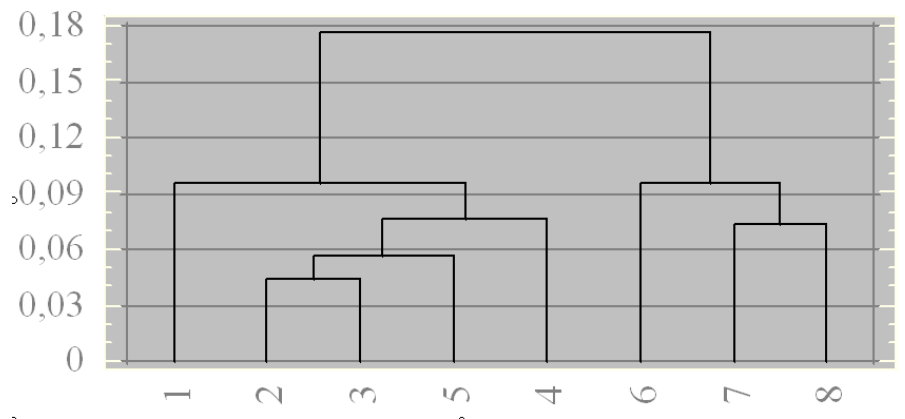
Western Siberia





The high degree of similarity between leaf rust isolates from the western part of Russia and those from the eastern part supports the hypothesis by Plant Pathologist Dr Mikhailova L.A. that there are two main populations of *P. triticina* in the Russian Federation:

European and Asian



Dendrogram of **Rodger's index** similarity between virulence spectrum of leaf rust populations from some regions of the Russian Federation: 1 – North-West; 2 – Central; 3 – Volga-Vyatka; 4 – Central-Chernozem; 5 – North Caucasus; 6 – Low Volga; 7 – Western Siberia; 8 – Eastern Siberia



- The structure of *P. triticina* populations has been studied in All-Russian Research Institute of Phytopathology for more than 40 years. This resulted in a great collection of pathogen samples (about 480) from spring and winter crops, advanced accessions of wheat, and triticale from different region of the Russian Federation.

Stem rust study



- Race analyses of stem rust population of Central Russia were conducted annually at Lomonosov Moscow State University.
- The 16 differentials were used in 1996-2005 (Roelfs & Martens 1998). Additional subset of Sr genes were included in 2006-2008 : *Sr24*, *Sr31*, *Sr38*, *Sr Wld I* (Jin et al., 2008).

Current stem rust pathogen of the central Russian population:



- full disease cycle with sexual process on **barberry** which widely distributed in the region
- depression on wheat, but supporting by **barley and wild grasses**

Races of aecio- and uredinio-populations identified in Central Region of the Russian Federation in 2001-08

Year	Plant hosts	Races	Year	Plant hosts	Races
2001	wheat	TKNP	2005	wheat	TKNT, TTNT,
	Hordeum	TKNT, PKJG, FKNL		Hordeum	TKNT, TKST
	wild grasses	TKNT		wild grasses	TKNT, TTNT
	Berberis	MKBT, TKFT		Berberis	TKNT, TTNT
		MKBT, RKDT			
2002	Hordeum	MKBT	2006	wheat	TKNT, TKPT, TKST
	wild grasses	MKBT, MKLT, MKBP		Hordeum	TKNT
	Berberis	TKNT		wild grasses	TKNT, TKST, TKNS, TTNT
2003	Hordeum	TKNT	2007	wild grasses	TKNT, KJNT, RKNT, RKNS
	wild grasses	TTNT, TTNS, MKNS		Berberis	TKNTF
	Berberis	TTNT, TKNT, PKNT, MKNS		Berberis	TKNTF, TKSTF
2004	Hordeum	TKNT	2008	Hordeum	TKNTF
	wild grasses	TKNT		wild grasses	TKNTF, TKSTF, TKNTC
	Berberis	TKNT, TTNT, TKST, PKST		Berberis	TKNTF, TKSTF, TKNTC

Current stem rust pathogen of the central Russian population:

➤ In favorable years (2001, 2005, and 2006) with hot and wet May-June pathogen infected also **wheat but just in trace**



Races of aecio- and uredinio-populations identified in the Central Region of the Russian Federation in 2001-08

Year	Plant hosts	Races	Year	Plant hosts	Races
2001	wheat	TKNP	2005	wheat	TKNT, TTNT,
		TKNT, PKJG, FKNL		<i>Hordeum</i>	TKNT, TKST
	<i>Hordeum</i>	TKNT		wild grasses	TKNT, TTNT
	wild grasses	MKBT, TKFT		<i>Berberis</i>	TKNT, TTNT
	<i>Berberis</i>	MKBT, RKDT			
2002	<i>Hordeum</i>	MKBT	2006	wheat	TKNT, TKPT, TKST
	wild grasses	MKBT, MKLT, MKBP		<i>Hordeum</i>	TKNT
	<i>Berberis</i>	TKNT		wild grasses	TKNT, TKST, TKNS, TTNT
		<i>Berberis</i>		TKNT, KJNT, RKNT, RKNS	
2003	<i>Hordeum</i>	TKNT	2007	wild grasses	TKNTF
	wild grasses	TTNT, TTNS, MKNS		<i>Berberis</i>	TKNTF, TKSTF
	<i>Berberis</i>	TTNT, TKNT, PKNT, MKNS			
2004	<i>Hordeum</i>	TKNT	2008	<i>Hordeum</i>	TKNTF
	wild grasses	TKNT		wild grasses	TKNTF, TKSTF, TKNTC
	<i>Berberis</i>	TKNT, TTNT, TKST, PKST		<i>Berberis</i>	TKNTF, TKSTF, TKNTC

Current stem rust pathogen of the central Russian population:

➤ Since 2003 the prevalent race is **TKNT (TKNTF)** through aecio- and uredinio- populations



Races of aecio- and uredinio-populations identified in the Central Region of the Russian Federation in 2001-08

Year	Plant hosts	Races	Year	Plant hosts	Races
2001	wheat	TKNP	2005	wheat	TKNT , TTNT,
	<i>Hordeum</i>	TKNT, PKJG, FKNL		<i>Hordeum</i>	TKNT , TKST
	wild grasses	TKNT		wild grasses	TKNT , TTNT
	<i>Berberis</i>	MKBT, TKFT		<i>Berberis</i>	TKNT , TTNT
2002	<i>Berberis</i>	MKBT, RKDT	2006	wheat	TKNT , TKPT, TKST
	<i>Hordeum</i>	MKBT		<i>Hordeum</i>	TKNT
	wild grasses	MKBT, MKLT, MKBP		wild grasses	TKNT , TKST, TKNS, TTNT
<i>Berberis</i>	TKNT	<i>Berberis</i>		TKNT , KJNT, RKNT, RKNS	
2003	<i>Hordeum</i>	TKNT	2007	wild grasses	TKNTF
	wild grasses	TTNT, TTNS, MKNS		<i>Berberis</i>	TKNTF , TKSTF
	<i>Berberis</i>	TTNT, TKNT , PKNT, MKNS	2008	<i>Hordeum</i>	TKNTF
<i>Hordeum</i>	TKNT	wild grasses		TKNTF , TKSTF, TKNTC	
wild grasses	TKNT	<i>Berberis</i>		TKNTF , TKSTF, TKNTC	
2004	<i>Berberis</i>	TKNT , TTNT, TKST, PKST			

➤ Pathogen surveys have detected some changes in stem rust population in the Central region for the period 2001 - 2008 (Lekomtseva et al. 2004; Skolotneva and Lekomtseva 2009).



➤ Up to 2003, races from **M**- and **T**- groups (virulent to *Sr5*, *Sr7b*; and *Sr5*, *Sr21*, *Sr9e*, *Sr7b*, respectively) were equally important in pathogen population

➤ Since 2004 high virulent races (TKNT, TTNT, and TKST) started to dominate with increasing frequency of virulence to *Sr21* and *Sr9e*.

Races of *P. graminis* f.sp. *tritici* identified in the Central Region of the Russian Federation in 2001-08

Year	Dominate races (%)	Number of rare races*	Number of races	Number of clones
2001	M KBT (29), T KNT (25), T KNP (12.5), T KFT (12.5)	3	7	24
2002	M KLT (33), M KBT (33), M KBP (16.7), T KNT (16.7)	-	4	18
2003	T TNT (53.8), M KNS (23), T KNT (10)	2	5	39
2004	T KNT (66.7), T KST (16.7)	2	4	36
2005	T KNT (69), T TNT (22)	1	3	36
2006	T KNT (64), T KPT (9.5), T KNS (9.5)	5	8	42
2007	T KNTF (96)	1	2	78
2008	T KNTF (58), T KSTF (25), T KNTC (16.7)	-	3	36

* rare races- with frequency less than 8%

➤ Using 20 differential lines of North America (Roelfs & Martens 1998, Jin et al., 2008) we were able to name the prevalent races TKNTF, TKSTF and TKNTC.

➤ In 2007-2008 virulent races to *Sr11*, *Sr17*, *Sr24*, and *Sr31* were not identified in current stem rust population.

➤ TTKSK has not been detected in the Central region and *Sr24*, *Sr31* remain effective.



Sr6, *Sr36* and *SrTmp* have lost their effectiveness in the Central region by the end of last century (Lekomtseva et al. 1996, 2002 in Russian).

**Races of *P. graminis* f.sp. *tritici* identified in the Central Region
of the Russian Federation in 2001-08**

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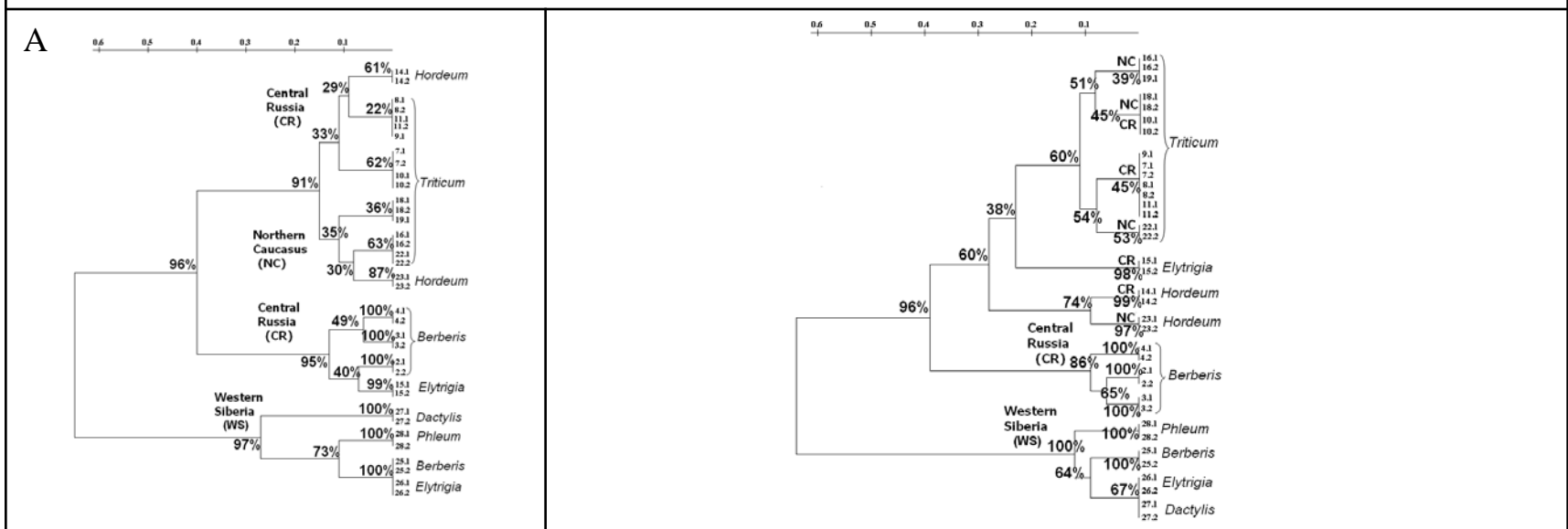
➤ To analyze molecular diversity of stem rust population we screened five high-GC arbitrary primers (Kubelik and Szabo 1995) on DNA from 36 stem isolates, collected in 2005 from different geographic regions and host plants.



➤ The dendrograms were characterized by similar topologies, but different levels of cluster stability.

➤ There was a clear host plant grouping of isolates according to barberry, wheat, barley, and grasses. Isolates from wheat were constantly clustered (by bootstrap index up to 91%).

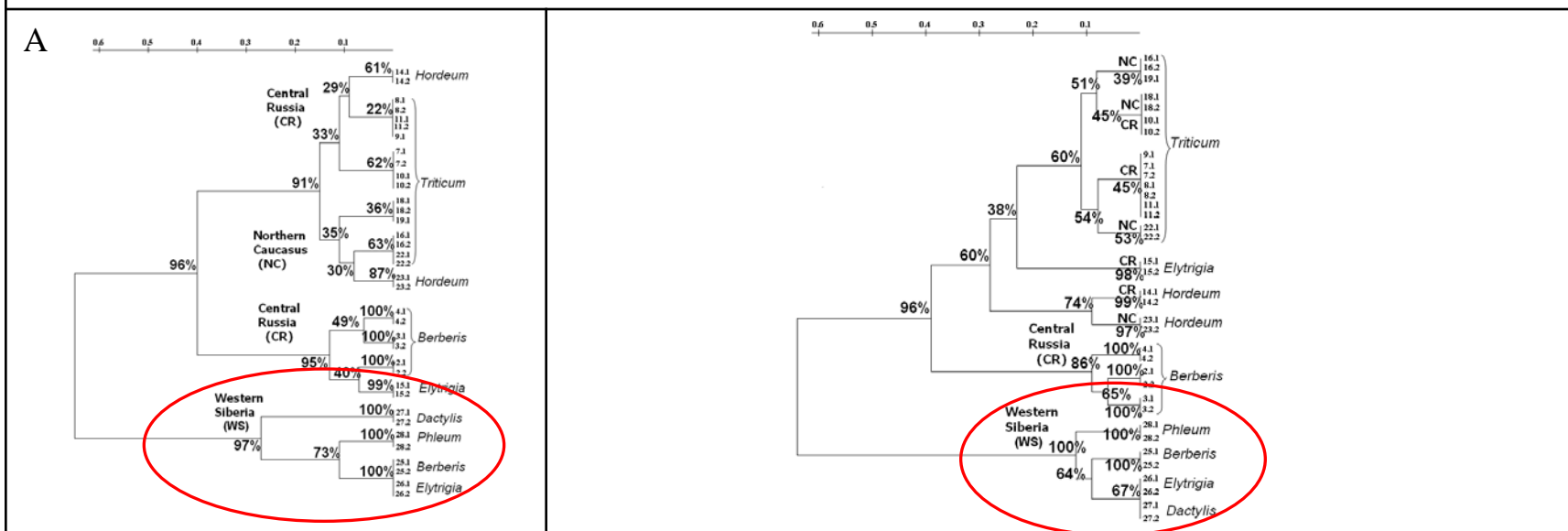
RAPD-diversity of 36 Russian isolates of *P. graminis* f.sp. *tritici*. **A:** by the high-GC primer **CRL-11**; **B:** by the high-GC primer **CRL-9**





➤ There was also a separable cluster of isolates with geographical (from Western Siberia, Tomsk area) and molecular (on RAPD-data) differences. However comparable virulence analysis of stem rust populations from European Russia and Western Siberia has not demonstrated their isolation (Lekomtseva et al. 2007).

RAPD-diversity of 36 Russian isolates of *P. graminis* f.sp. *tritici*. **A:** by the high-GC primer **CRL-11**; **B:** by the high-GC primer **CRL-9**





Stem rust pathogen in the Central region could be characterized by the following descriptions:

- 1) disease cycle with sexual process on barberry which widely distributed in the region
- 2) depression on wheat, but supporting by barley and wild grasses
- 3) relatively low virulence variety with prevalent pathotypes from the T race group (virulent to *Sr5*, *21,9e* and *7b*)
- 4) high RAPD-diversity based on host plant origin.

Data of leaf and stem rust pathogens in most of Russia were summarized here. It could be provide a basis for breeders to identify suitable resistance and to devise breeding strategies to improving genetic control of cereal rust diseases in the European part of the Russian Federation and Siberia.



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Thank you for attention!



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