Characterization of Russian Phytophthora infestans populations: DNA fingerprinting and SSR analysis

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Summary

A collection of Phytophthora infestans isolates collected in 2008-2011 from potato and tomato fields of six regions of Russian Federation (Moscow, Nizhni Novgorod, Leningrad, Kostroma, Smolensk, and Astrakhan) has been characterized by the RG57 fingerprinting (75 isolates); the mtDNA haplotype, Pep1 and Gpi loci, mating type, and metalaxyl resistance of these isolates have been also determined. In addition, the SSR fingerprinting of 87 isolates from the same collection has been first carried out. The number of revealed RG57 genotypes is 26 varying from 1 to 11 per population; their comparison with the data obtained for the Russian populations of 1997-1998 did not reveal any common genotypes. The total number of RG57-based multilocus genotypes (MLGs) determined using additional markers makes 30. The total number of SSR MLGs makes 47 varying from 2 to 16 per population. In both cases, the Moscow "potato" population was the most diverse (14 and 16 RG57- and SSR-based MLGs, respectively). According to the SSR study, both "tomato" populations are the most different to other populations; populations from the central Russia are the most similar. The obtained results revise our earlier ideas about the uniformity of some of the populations studied. These new data are planned to be added to the Eucablight database.

Materials and methods

***P. infestans* isolates** were collected during a period of 2008-2011 and stored at the State Collection of Phytopathogenic Microorganisms of the ARRIP. Within the framework of the joint research project, a set of 100 isolates was sent to GIFVL and HCRL. 75 and 87 isolates were successfully restored in these labs, respectively, and analyzed by two visiting ARRIP scientists. The SSR study was performed at the HCLR, whereas the RG57 fingerprinting and determination of the mating type, mtDNA haplotype, genotypes at two allozyme loci, and metalaxyl sensitivity was performed at the GIFVL.

Mating type and metalaxyl sensitivity assessment, allozyme analysis, mtDNA haplotyping, and RG57 fingerprinting were carried out according to the common procedures. **SSR analysis** was conducted using the standard EUCABLIGHT protocol and 12 previously published primers (D13, PiG11, Pi04, Pi4B, Pi63, Pi70, SSR2, SSR3, SSR4, SSR6b, SSR8, and SSR11). Genotypes were determined by comparing of fragment sizes with isolates previously genotyped. Reproducibility of novel allele sizes was confirmed.

Data analysis. Population structure was studied basing on allele frequencies observed for populations. Trees were constructed using the UPGMA algorithm from Nei's unbiased genetic distance matrix; statistical support was obtained using 1,000 bootstrapped samples using a TFPGA program. Discriminant analysis of principal components (DAPC) was performed using the adegenet 1.3-4 package.

Results

Mating type and metalaxyl resistance. The results of the mating type determination are presented in Tables 1 and 2. The data on the metalaxyl resistance of the examined populations are shown on Fig. 1.

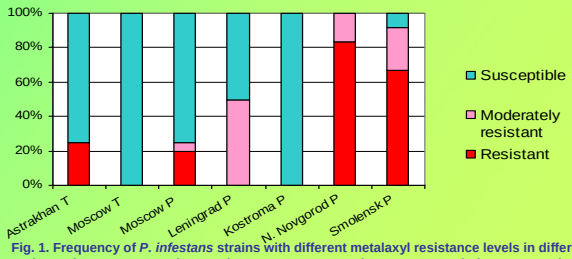

Fig. 1. Frequency of *P. infestans* strains with different metalaxyl resistance levels in different regions of European Russia. *P* and *T* mean "potato" and "tomato" populations, respectively.

RG57 fingerprinting. A total of 25 bands can be detected by the RG57 analysis; in our case, the bands ***4, 11, 12, 15***, and ***18*** were not detected, and the bands ***1, 13, 14, 24***, and ***25*** presented in all isolates. The remaining 15 bands (bands ***2, 3, 5, 6, 7, 8, 9, 10, 16, 17, 19, 20, 21, 22***, and ***23***) were polymorphic.
The total number of different RG57 genotypes revealed among 75 samples was 26. The total number of RG57-based multilocal genotypes was 30 (Table 1).
Each revealed RG57-MLG genotype was designated by a code, using ***R*** for Russia followed by two letters for the region of collection, one letter for the host plant (***P*** and ***T*** for potato and tomato, respectively) and then a genotype number.
The comparison of the obtained data with the results of the earlier study of Russian *P. infestans* populations of 1997-1998 did not reveal any coinciding genotypes.

Table 1. RG57-based multilocal genotypes (RG57-MLGs) and the origin of Russian *P. infestans* populations

RG57-MLG name	Number of isolates	RG57 fingerprint	Mating type	mtDNA haplotype	PEP genotype	Metalaxyl sensitivity	Region of origin / Total number of isolates	Year of collection
RMO-P1	3	110 000 000 100 110 100 011 001 1	A1	Ia	100/100	S	Moscow / 20	2008
RMO-P1_1	1	110 000 000 100 110 100 011 001 1	A1	IIa	100/100	S		
RMO-P2	2	101 010 001 100 110 100 011 001 1	A2	Ia	100/100	R		
RMO-P2_1	1	101 010 001 100 110 100 011 001 1	A2	IIa	100/100	R		
RMO-P3	1	111 000 100 100 110 100 011 001 1	A1	Ia	92/100	S		
RMO-P4	1	101 010 101 100 110 000 011 001 1	A1	IIa	100/100	R		
RMO-P5	1	111 000 101 100 110 100 011 001 1	A2	Ia	100/100	S		
RMO-P6	2	101 010 101 100 110 010 011 001 1	A1	Ia	100/100	S		2009
RMO-P7	1	111 011 010 100 110 100 011 001 1	A1	IIa	100/100	S		
RMO-P8	1	110 011 010 100 110 100 001 101 1	A1	IIa	100/100	MR		
RMO-P9	1	111 010 100 100 110 000 011 001 1	A2	Ia	100/100	S		
RMO-P10*	1	110 010 000 100 110 100 011 001 1	A1	Ia	92/100	S		2010
RMO-P10_1	2	110 010 000 100 110 100 011 001 1	A1	Ia	100/100	S		
RMO-P11	2	110 010 011 100 110 100 011 001 1	A2	Ia	100/100	S		2011
RMO-T1	9	110 011 000 000 110 000 001 001 1	A2	Ia	100/100	S	Moscow / 9	2010
RKO-P1	1	111 011 101 100 110 100 011 001 1	A1	IIa	100/100	S	Kostroma / 6	2008
RKO-P2	2	110 010 001 100 110 000 011 011 1	A1	Ia	100/100	S		
RKO-P3	2	110 010 100 100 110 100 011 001 1	A1	IIa	100/100	S		2009
RKO-P4	1	110 010 000 100 110 100 011 001 1	A1	Ia	100/100	S		
RLE-P1	8	110 011 000 100 110 000 011 001 1	A1	Ia	100/100	S	Leningrad / 13	2008
RLE-P2	5	100 010 100 100 110 000 111 001 1	A1	Ia	100/100	S, MR		
RNN-P1	2	100 011 000 100 110 100 111 001 1	A1	Ia	100/100	MR, R	N. Novgorod / 6	2008
RNN-P1_1	2	100 011 000 100 110 100 111 001 1	A2	Ia	100/100	R		
RNN-P2	1	110 010 001 100 110 100 010 001 1	A1	IIa	100/100	R		
RNN-P3	1	100 010 001 100 110 100 001 001 1	A2	IIa	100/100	R		
RSM-P1	10	111 011 100 100 110 100 001 001 1	A2	Ia	100/100	MR, R	Smolensk / 12	2009
RSM-P2	1	111 011 101 100 110 100 001 101 1	A2	Ia	100/100	R		
RSM-P3	1	100 010 000 100 110 100 011 001 1	A1	Ia	100/100	R		
RAS-T1	6	110 011 000 100 110 100 011 001 1	A1	Ia	100/100	S	Astrakhan / 8	2008
RAS-T2	2	110 011 000 100 110 100 011 001 1	A1	Ia	100/100	R		

*This genotype is also characterized by the *Gpi* 86/86 genotype; all other genotypes have the *Gpi* 100/100 genotype.

Allozyme analysis and mtDNA genotypes. All isolates tested were homozygous at the *Gpi* locus (100/100), excepting one isolate collected in 2010 in the Moscow region (86/86) that confirms the earlier data on a very low variability of this marker in Russian *P. infestans* populations. The vast majority of isolates were also homozygous at the *Pep1* locus (100/100), excepting two 92/100 isolates collected in the Moscow region in 2008 and 2010, that also corresponds to our earlier studies. Like in the previous studies, only two mtDNA haplotypes (Ia and IIa) were revealed among the tested isolates (Table 1). Haplotype Ia predominated for the most of the studied populations.

SSR genotyping. The total number of revealed SSR genotypes was 47. The studied *P. infestans* populations are moderately diverse showing 2-16 genotypes per a region (Table 2). Both "tomato" populations are most different to the "potato" ones; populations in central Russia are most similar based on distance analysis (Fig. 2). Populations were well differentiated by region and crop; the tomato populations clustered separately (Figs. 2 and 3).

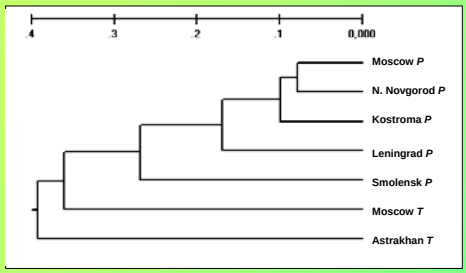

Figure 2. Dendrogram of Nei's unbiased genetic distance for Russian populations of *Phytophthora infestans*.

Table 2. Number of SSR-based MLGs in *P. infestans populations* sampled in different regions of Russia

Region	Host	Sample size	MLG (SSR)	A1:A2	$\hat{h}$
Astrakhan	Tomato	8	8	9:0	0.405
Kostroma	Potato	6	6	10:1	0.561
Leningrad	Potato	14	7	15:0	0.493
Moscow	Potato Tomato	24 14	16 2	15:9 0:14	0.513
N. Novgorod	Potato	8	6	9:3	0.589
Smolensk	Potato	13	2	1:13	0.291

MLG = multilocus genotype based on SSR analysis. $\hat{h}$ = unbiased average heterozygosity based on SSR analysis.

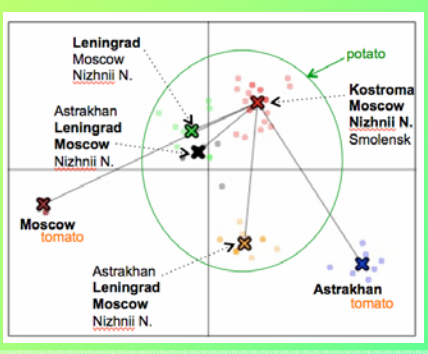

Figure 3. A scatter plot showing the first two principal components of a discriminant analysis of principal components (DAPC) used to infer population structure. The plot shows individual strains based on multilocus genotypes determined by SSR analysis. The crosses show the center of a cluster and the lines denote distances of a minimum spanning tree based on the squared distances between populations within the space.

The maximum number of alleles (6) was detected for the D13, G11, and SSR4 loci. The minimum number of alleles (2) was detected for the SSR2, SSR6b, and Pi70 loci; the last one was the most monomorphic locus, since 82 out of 87 isolates tested had the same genotype.

The further analysis of the obtained results is planned; all obtained data will be added to the Eucablight database.

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