CHARACTERISTICS OF THE *PHYTOPHTHORA INFESTANS* POPULATION IN RUSSIA

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Summary

Phytophthora infestans strains, collected in 2007-2009 in different regions of the European part of Russia, were analyzed for several genotypic and phenotypic characteristics, such as mtDNA haplotype, Pep1 and Pep2 loci, mating type, virulence, and sensitivity to metalaxyl.

Fig. 1. Location of sampling sites.

Materials and methods

***P. infestans* isolates** were collected from commercial potato and tomato fields, located in the following regions of the European Russia (Fig. 1): Leningrad (21 isolate), Moscow (100 isolates, including 20 from tomato plants), Nizhni Novgorod (13 isolates), Astrakhan (31 isolates from tomato plants), Kostroma (105 isolates), Smolensk (49 isolates), and the Mariy El Republic (115 isolates, including 93 from tomato plants). The total number of studied isolates was 434.

Allozyme analysis. Genotypes at two peptidase loci (Pep1 and Pep2) were analyzed using a cellulose acetate gel electrophoresis according to (1) with some modifications (2). The genetic diversity for the Pep1 locus in Russian *P. infestans* populations is significantly lower than that of the Pep2 locus, represented by two alleles (100 and 112) and stained simultaneously with the Pep1 locus (Fig. 2), so we used both these markers.

The mtDNA haplotype identification was carried out according to (3) with some modifications.

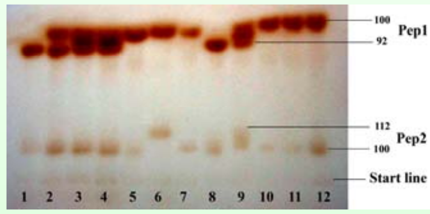

Fig. 2. Cellulose acetate plate showing typical allozyme banding patterns of two peptidase loci for *P. infestans* isolates. **Pep1 locus**: 92/92 (lines *1* and *8*); 100/100 (lines *5-7, 10-12*); 92/100 (lines *3*, *4*, *9*). **Pep2 locus**: 100/100 (lines *1-5*, *7*, *8*, *10-12*); 112/112 (line *6*); 100/112 (line *9*).

The mating type was tested by the growing isolates on rye agar with the known reference strains of the A1 and A2 mating types.

Metalaxyl resistance. The sensitivity of isolates to metalaxyl-containing fungicides was determined by the inoculation of fungicide-treated tuber discs (4) or fungicide-containing medium (5) with the tested isolates at different fungicide concentrations. According to the obtained results, isolates were considered as sensitive (S), moderately resistant (MR), or resistant (R) .

Virulence. To study the virulence of isolates, we used a set of differentiator cultivars, obtained from the International Potato Center (CIP, Peru) and containing 22 genotypes, including all known resistance genes in different combinations. We also used the test set, containing R0-R11 genotypes and obtained from the Institute of Plant Cultivation and Acclimatization (IHAR, Poland). The analysis was carried out under laboratory conditions using detached potato leaves.

Results

Allozyme analysis. The results of the allozyme analysis for Pep1 and Pep2 loci are shown on Fig. 3.

In all populations the predominant genotype of the Pep1 locus was 100/100; all "tomato" populations were represented by only this genotype. The presence of all possible variants (92/92, 92/100, and 100/100) was revealed only in the Moscow "potato" population. For Pep2 locus, the genetic diversity was higher. All three possible variants were revealed in 4 populations at different proportions. The most frequent genotype was 100/100, excepting 3 populations. This genotype was predominant for all tomato populations.

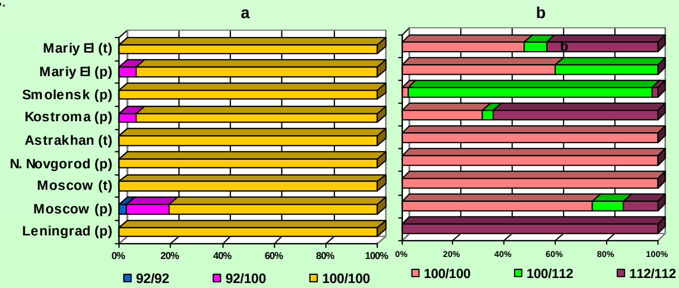

Fig. 3. Frequency of Pep1 (a) and Pep2 (b) genotypes of *P. infestans* samples from different regions of European Russia; (p) and (t) mean "potato" and "tomato" populations, respectively.

Mitochondrial DNA haplotype. The results of the mtDNA analysis are shown on Fig. 4. We revealed only two mtDNA haplotypes (Ia and IIa). The Ia genotype was predominant for all populations, excepting the Mariy El "tomato" population; two populations (Leningrad and Astrakhan) were presented by the only this genotype.

Mating type. Mating type frequencies, determined for all studied populations, are shown on Table 1.

The A1 mating type predominated in the most of examined populations; in the case of Leningrad and Astrakhan (tomato) populations, it was the only variant revealed. Populations from the Moscow and Kostroma regions included a small percent of A1A2 strains, able to generate oospores with both reference strains.

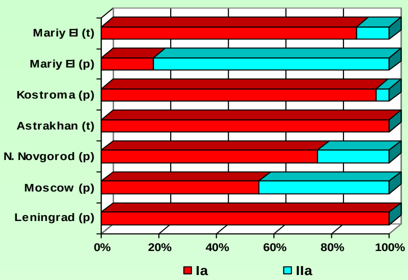

Fig. 4. Mitochondrial DNA haplotypes of *P. infestans* samples; (p) and (t) mean "potato" and "tomato" populations, respectively.

Table 1

Occurrence of A1 and A2 mating types in Russian *P. infestans* populations

Region	Mating type (%)		
	A1	A2	A1A2
Leningrad (potato)	100	0	0
Moscow (potato)	61	35.4	3.6
Moscow (tomato)	65	35	0
Nizhni Novgorod (potato)	0	100	0
Astrakhan (tomato)	100	0	0
Kostroma (potato)	80.1	19.4	0.5
Smolensk (potato)	5.6	94.4	0
Mariy El (potato)	35.7	64.3	0
Mariy El (tomato)	77.8	22.2	0

Virulence. The results of our virulence study are shown in Fig. 5.

According to our data, populations from the Kostroma and Leningrad regions were similar to each other. The most frequent races in both populations included 8-10 virulence genes.

Both tomato and potato populations of *P. infestans* from the Mariy El Republic were represented by complex races and had the similar structure. The most frequent races were 1.2.3.4.6.7.8.10.11 (28.2%) and 1.2.3.4.5.6.7.8.10.11 (50%).

The Moscow population included all virulence genes and was presented mainly by complex races, containing 7-11 genes (including gene 9, which was absent in the most of the studied populations).

The pathogen population from the Nizhnii Novgorod region consisted mainly of complex races, containing 6-11 virulence genes (57.9%). Like the Moscow population, this population also contained the rare gene 9; it is also interesting that the frequency of gene 8 was unusually low. The FV value (6.3) was lower than for the above-mentioned populations (7.2-10.0).

In the case of the Astrakhan population, we revealed a significant predominance of virulence genes 1, 3, 4, 7. Genes 5, 8, 10, and 11 were not revealed; the frequencies of genes 2 and 9 were very low. The FV value for this region was very low (3.8), and the most frequent race was the race 1.3.4.7 (47%). Thus, this population significantly differed from other ones.

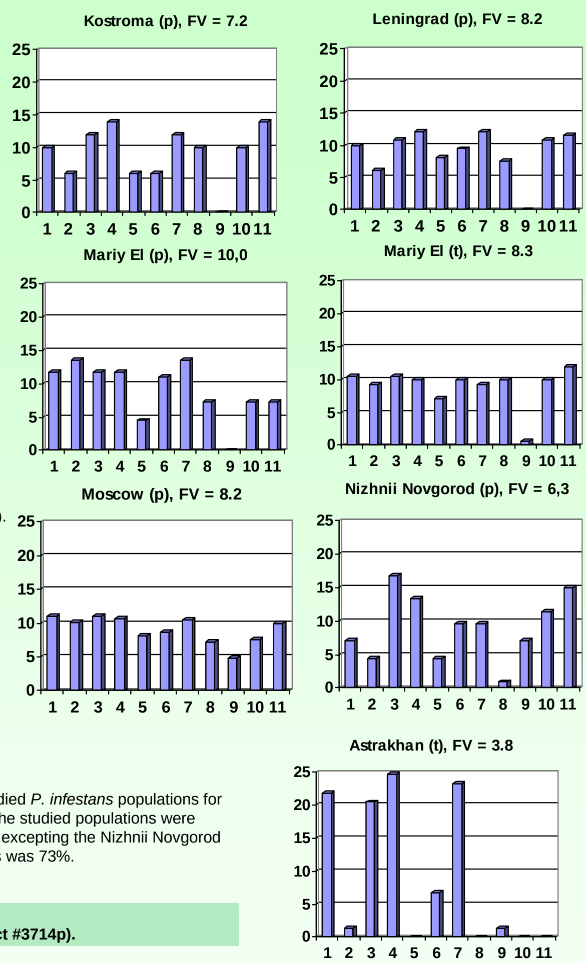

Fig. 5. Frequencies of separate virulence genes in the studied *P. infestans* populations. *FV*, factor of virulence; (p) and (t) mean "potato" and "tomato" populations, respectively.

Metalaxyl resistance. The results of the analysis of the studied *P. infestans* populations for their metalaxyl resistance are shown in Fig. 6. The most of the studied populations were represented by susceptible or moderately resistant isolates, excepting the Nizhnii Novgorod population, where the number of metalaxyl-resistant isolates was 73%.

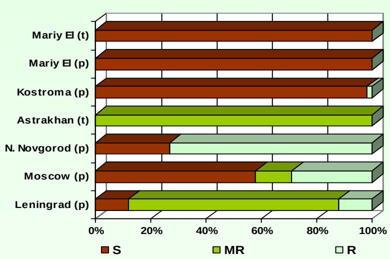

Fig. 6. Metalaxyl resistance of *P. infestans* populations from different regions of Russia. *S*, susceptible strains, *MR*, moderately resistant strains, *R*, resistant strains; (p) and (t) mean "potato" and "tomato" populations, respectively.

ACKNOWLEDGMENTS

This study was supported by the ISTC-USDA-ARS (project #3714p).

References

1. Hebert, P.D.N. and Beaton, M.J. (1993) Methodologies for allozyme analysis using cellulose acetate electrophoresis. A practical handbook. – Guelph, Ontario.
2. Elansky, S.N. and Smirnov, A.N. (2003) Second locus of peptidase as a marker for genetic investigations of *Phytophthora infestans*. // Botanica Lithuanica, 9(3): 275-283.
3. Griffith, G.W. and Shaw, D.S. (1998) Polymorphisms in *Phytophthora infestans*: four mitochondrial haplotypes are detected after amplification of DNA from pure cultures or from host lesions. // Envir. Microbiol., 64, 4007-4014.
4. Cohen, Y. and Reuveni (1983) Occurrence of metalaxyl-resistant isolates of *Phytophthora infestans* in potato fields in Israel. // Phytopathology, 73, 925-927.
5. Davidse, L.C., Gerritsma, O.C.M., and Veltuis, G.C.M. (1984) A differential basis of antifungal activity of acylalanine fungicides and structurally related chloroacetanilide herbicides in *Phytophthora megasperma* f.sp. *medicaginis*. // Pesticide Biochem.Physiol., 21, 301-308.