Russian populations of *Phytophthora infestans*

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Abstract

Two types of *Phytophthora infestans* populations have been registered in Russia: (1) Siberia and Far East populations, characterizing by a very low genotypic variability, and (2) greatly varying European populations. The possible reasons for the low variability of Siberian and Far East populations of *P. infestans* are the use of a local seed material for the potato and tomato reproduction and the inclement weather conditions, that do not allow the soil wintering of oospores. On the contrary, European populations are influenced by the active exchange of the seed material and a permanent import of potato tubers and tomato fruits from abroad. In this paper we discuss the role of different mechanisms (mutations, migrations, sexual and parasexual recombinations) in the variability of *P. infestans* populations in Russia and also the influence of a fungicide application and an intraspecific specialization on the structure of these populations.

Introduction

Phytophthora infestans (Mont.) de Bary, the causal agent of late blight of potato and tomato, attracts the fixed attention of scientists from different countries for more than 150 years. In the middle of XIX century, when this oomycete suddenly appeared in Europe, it caused the late blight epiphytoty, engraved on the memory of many generations. Even now this pathogen is often called as "Irish Famine Fungus". Almost 100 years after the first epiphytoties scientists found in Mexico some wild potato species, resistant to late blight and developed the techniques of their crossing with the cultivated potato (Muller, 1935), that resulted in the creation of new cultivars, resistant to late blight (Pushkarev, 1937). However, shortly after the start of the commercial growing of such cultivars, there have been revealed some virulent races of *P. infestans*, able to strike these resistant cultivars, so the new resistance genes, that have been introduced into cultivated potato from the wild Mexican potato, quickly lost their efficacy. Such failure in the use of the monogenic (vertical) resistance made potato breeders to seek more complex ways of the use of a nonspecific polygenic (horizontal) resistance.
The same problems arose in the case of any chemical protection of potato. The significant differences between the oomycetes and the true fungi, including their chemical composition, ultrastructure, and metabolism, cause many systemic fungicides, used for the protection of plants against the fungal diseases, to be inefficient in the case of the late blight. So, the chemical protection of potato against the late blight was based on the multiple spraying with contact fungicides. The development of phenylamide fungicides, toxic for oomycetes and systemically distributed in plants, became a revolution in the field of the chemical protection of potato against the late blight. However, the wide use of these preparations soon resulted in the accumulation of phenylamide-resistant strains in *P. infestans* populations (Davidse *et al.*, 1981), that complicated the use of plant protection techniques. In view of the aforesaid, one can easily understand such great attention of many scientists to the study of the structure of *P.*

infestans populations, mechanisms of changes in the number of different races and their genetic structure, and genetical mechanisms, providing the variability of this pathogen.

The structure of *P. infestans* populations in Russia

In the absence of a recombination or the insignificant contribution of this process into the population structure, the population consists of several clones, very rarely exchanging their genetic material. In such case the study of individual gene frequencies will be less informative, than the study of frequencies of genotypes, that have the common origin (clonal lineages) and differ only in the point mutations. The RFLP-RG57 method, developed in the early nineties, significantly accelerated population studies of the late blight agent and its clonal lineages. Along with this technique, scientists also use the mating type analysis, DNA fingerprinting, allozyme analysis for peptidase and glucose 6-phosphate isomerase, and the determination of the mitochondrial DNA (mtDNA) haplotype.

The US-1 clonal lineage was the predominant lineage in all *P. infestans* populations up to the late eighties, when it was replaced by other clones and finally vanished from Europe and North America. In Russia (Moscow and Ekaterinburg regions) this genotype was not registered since 1993 (Fry *et al*., 1992).

In Asiatic Russia, i.e. on the huge territory lying between Ekaterinburg and Sakhalin, the predominated clonal lineage is SIB-1, that can be characterized by the A1 mating type and IIa mtDNA haplotype. In 1993 this genotype was registered in the Moscow region, and some field populations consisted mainly from the metalaxyl-resistant strains of this clonal lineage. Since 1993 this genotype is not widespread. In 1997-1998 the SIB-1 lineage was widespread beyond the Ural Mountains, excepting the Khabarovsk Territory and the Jewish Autonomous Region, where the SIB-2 genotype predominated. The spatial separation of clones with different mating types excludes any sexual process in the Siberian and Far East populations of *P. infestans*. In contrast to Siberia, the population of the Moscow region is represented by many clonal lineages, and almost every isolate has its unique multilocus genotype (Elansky *et al*., 2001). One can not explain such diversity only by the fact of the "import" of new strains through the infected seed material, imported from different parts of the world. Since both mating types are presented in this population, then such diversity can be also conditioned by the recombination process. For example, it seems that BC-2, BC-3, and BC-4 genotypes arose in British Columbia because of a hybridization between BC-1 and US-6 clonal lineages (Goodwin *et al*., 1995). It is also possible, that the *P. infestans* populations of the Moscow region also contain some hybrid strains. For example, MO-4, MO-8, and MO-11 strains, heterozygous in the PEP locus, can be the hybrids between the MO-8 strain, which is homozygous in the one allele of the PEP locus and has A1 mating type, and MO-12, MO-21, and MO-22 strains, respectively, which have A2 mating type and are homozygous in another allele of the same locus. In this case there is a tendency to the strengthening of the role of the sexual process in the current *P. infestans* populations, and the value of information, obtained by the clonal lineage analysis, will reduce, as it was shown for the populations from the Moscow region.

Role of different mechanisms in the variability of *P. infestans* populations

Mutations

Since mutations represent a stochastic process with a low frequency, then their generation in any locus depends on the mutation frequency for this locus and the magnitude of population. A study of the mutation frequency of different *P. infestans* strains usually involves a determination of the number of colonies grown on the selective nutrient medium after a

treatment with chemical or physical mutagens. A high frequency of mutations, determining a metalaxyl resistance, can be one of the reasons of the accumulation of resistant strains in the nature. However, one-step mutations usually cause some genomic disbalance, and, along with the providing of a new property, reduce the total adaptation potential of the organism. The most of laboratory mutations provide the reduced aggressiveness and are not registered in the native populations. For example, the correlation coefficient between the level of phenylamide resistance of mutant *P. infestans* strains and their growth rate in artificial medium averages (-0.62). At the same time, correlation coefficient between the resistance to fungicides and the aggressiveness of these strains on potato leaves averages (-0.65) (Derevyagina *et al.*, 1993), that testifies the low adaptation level of mutant strains. Any mutations, affecting the level of dimethomorph resistance, were also accompanied by a sharp reduction in the strain viability (Bagirova et al., 2001).

The size of population plays the critical role in the appearance of spontaneous mutations. In the case of the moderate infection of a potato field (35 spots per one plant), each hectare of the field generates 8×10^{12} spores a day (Dyakov and Suprun, 1984). Apparently, such populations contain all mutations, permitted by the exchange type for each locus. Even any rare mutation with the frequency equal to 10^{-9} will be acquired by a thousand organisms from the billions, that live on the one hectare of this field. Therefore, it is quite possible that any resistant and competitive strains can be selected among the many thousands organisms, obtained any resistance mutation.

Migrations

Today we know two main types of migration of *P. infestans*: a short-distance migration (within the bounds of the same or neighboring fields) via the spreading of zoosporangia by air flows or rain drops, and a long-distance migration via the seed material or transported tomato fruits. The first way provides the nidus expansion, and the second one allows the appearance of new remote niduses. The spreading of infection with potato tubers or tomato fruits facilitates the appearance and development of the disease in new places and also represents the main source of the genetic diversity of populations. Agricultural enterprises of the Moscow region grow potato cultivars obtained from different regions of Russia and Western Europe. Tomato fruits are usually brought from the southern regions of Russia (Astrakhan region, the Krasnodar Territory, and North Caucasus). Tomato seeds, which also can serve as the source of infection (Rubin *et al.*, 2001), are brought from the Southern Russia, China, European and other countries. Apparently, this is one of the reasons of such high genetic diversity of *P. infestans* populations in the Moscow region.

Agricultural enterprises, located on the huge territory of Asiatic Russia (from Ekaterinburg to Sakhalin and Vladivostok), grow potato mainly from the local seed material; tomato is not widespread on this territory and also is grown from the local seed material. The genetic diversity of *P. infestans* populations on this territory is minimal; until now only three genotypes have been detected, and one of them (SIB-1) was registered in 79% of isolates (Elansky *et al.*, 2001). However, within Sib 1 and Sib 2 clonal lineages there are some differences between the tested isolates in the level of their metalaxyl resistance and the number of virulence genes. Such differences can be caused by any mutation processes.

Sexual recombination

To provide the contribution of a sexual recombination into the variability of a population, the presence of two mating types at a ratio of 1 : 1 and the initial variability of this population are necessary.

Almost all *P. infestans* populations from the European part of Russia contain strains of both mating types, but their proportion varies greatly in different populations and even in the same population in different years.

There are some indirect data, testifying the existence of the sexual process in certain years and in certain regions of Russia:

1) The study of *P. infestans* populations from the Moscow region showed that the total genetic diversity of 13 populations, where the A2 mating type was registered for less than 10% isolates, was 0.08 (calculated for three isozyme loci); for 14 populations, where the share of this mating type exceeded 30%, the genetic diversity was twice as much (0.15; Elansky *et al.*, 1999). So, the higher the sexual process probability, the greater the genetic diversity of the population.

2) The relation between the mating type ratio and the intensity of oospore formation was registered for the *P. infestans* populations of Israel (Cohen *et al.*, 1997) and Holland (Flier *et al.*, 2004). Our studies showed that for populations, containing 62, 17, 9, and 6% of isolates of A2 mating type, the presence of oospores on examined potato leaflets with two or more lesions was registered for 78, 50, 30, and 15% of leaflets, respectively. Samples with two or more lesions contained oospores more often, than those with only one lesion (32 and 14% from the total number of samples, respectively; Apryshko *et al.*, 2004).

3) The distribution of DNA fingerprint and isozyme loci in some populations from North Caucasus corresponds to the Hardy-Weinberg equation (Ahmatkhanova *et al.*, 2004), that can be evidence of a significant contribution of the sexual recombination into the population variability. The study of *P. infestans* populations from other regions of Russia did not show any correspondence to the Hardy-Weinberg distribution, but revealed the presence of a nonequilibrium linkage, testifying the prevalence of a clonal reproduction (Elansky *et al.*, 1999).

Parasexual recombination and mitotic gene conversion

The joint growing of *P. infestans* strains with mutations, affecting their resistance to different growth inhibitors, caused the appearance of isolates resistant to both inhibitors (Shattock and Shaw, 1975; Dyakov and Kuzovnikova, 1974; Kulish and Dyakov, 1979). Strains, resistant to both growth inhibitors, appeared as a result of a mycelium heterocaryotization; they were splitted in the case of their reproduction by uninuclear zoospores (Judelson and Ge Yang, 1998) and were not splitted in the monozoosporic progeny, since they have diploid (tetraploid, because the initial isolates were diploid) nuclei (Kulish and Dyakov, 1979). The segregation frequency of heterozygous diploids was very low because of the haploidization, nondisjunction of chromosomes, and mitotic crossingover (Poedinok *et al.*, 1982). The frequency of these processes can be increased through the certain impacts on heterozygous diploids (UV-treatment of germinating spores, mycelium treatment with *p*-phthorphenylalanine, etc.).

In spite of the unexpectedly high frequency of generation of heterocaryons and heterozygous diploids (tens of percents), this process occurs only in the case of the joint growing of mutant strains, originated from the same one. Because of a vegetative incompatibility, the heterocaryotization does not occur (or occurs with very low frequency) between differing native isolates (Poedinok and Dyakov, 1981; Anikina *et al.*, 1997; Cherepennikova-Anikina *et al.*, 2002). So, the role of the parasexual recombination can be reduced to the intraclonal recombination in heterozygous nuclei and the transition of certain genes into the homozygous state without any sexual process. This process can be epidemiologically important for the strains with recessive or semidominant mutations, affecting their resistance to fungicides. The transition of such mutation into the homozygous form due to the parasexual process increases the resistance of its carrier (Dolgova and Dyakov, 1986).

Use of fungicides

The use of fungicides not only decreases the magnitude of population of phytopathogenic fungi (i.e. influences on the quantitative characteristic of their populations), but also can change the frequencies of certain genotypes (i.e. influences on the quality composition of the populations). The changes in the aggressiveness, virulence, and resistance to fungicides are among the most important parameters of the populations, affecting by fungicides.

The level of such influence is determined mainly by the type of the fungicide used. All fungicides can be generally divided into three types: *poly-site*, *oligo-site*, and *single-site* ones. The **poly-site** group includes many contact fungicides. The resistance to such preparations (if it is really possible) is controlled by a large number of weakly expressing genes. Such properties determine the lack of any significant changes in the resistance of a population after the fungicidal treatment (though several experiments with different fungi, including *P. infestans*, demonstrated some increase in this resistance). The population, surviving after the treatment with contact fungicides, consists of two groups of strains: 1) strains, survived on the non-treated plant surfaces (since they did not contact with the fungicide, then their aggressiveness and resistance remain unchanged), 2) strains, contacted with a fungicide at a sublethal concentration. The resistance of this part of population does not change, but the general adaptation level and its parasitic component, i.e. an aggressiveness, decrease due to a partial damaging effect of a fungicide (even in the sublethal concentrations) on the fungal metabolism (Derevyagina and Dyakov, 1990). Thus, even survived part of the population, that contacted with the fungicide, has a low aggressiveness and can not generate any epiphytoty. Therefore, any careful treatment, reducing the frequency of that part of population, which will not contact with a fungicide, is a necessary condition of the successful protection activity.

The resistance of *P. infestans* to the **oligo-site** fungicides (some translaminar and systemic fungicides, for example, dimethomorph) is controlled by several additive genes. Each gene mutation results in a certain increase in resistance, and the total resistance is determined by the summation of such mutations. Therefore, such increase in resistance will be stepwise. For example, the dimethomorph resistance is polygenic and additive. The one-step mutation with frequency equal to $6,3 \times 10^{-7}$ slightly increase the resistance. Every additive mutation reduces the target size and, therefore, the frequency of any subsequent mutations (Bagirova *et al.*, 2001). The improvement of the average resistance of *P. infestans* after the multiple treatment with the oligo-site fungicide occurs gradually. The rate of this process is determined by at least three factors: (1) mutation frequency for resistance genes, (2) resistance coefficient (the ratio between the lethal doses for resistance and sensitive strains), and (3) effect of the resistance gene mutations on the adaptation ability of the organism.

The frequency of each subsequent mutation is lower, then for the previous one, so the process is of damped character (Bagirova *et al.*, 2001). However, in the case of any recombination (sexual or parasexual) processes, there is a possibility of the union of different parental mutations in the hybrid strain and the acceleration of the above-mentioned process. Therefore, panmictic populations acquire the heightened resistance more quickly, than agamic ones; the agamic populations, which do not have any vegetation incompatibility barriers, acquire this feature more quickly, than populations, separated by such barriers. As a result, the presence of strains with different mating types in a population accelerates the acquisition of the resistance to oligo-site fungicides.

The second and third factors do not promote any fast accumulation of dimethomorph-resistant strains in the population. Each subsequent mutation doubles the resistance, and, at the same time, reduces both growth rate on artificial medium and, especially, aggressiveness (Bagirova *et al.*, 2001; Stem, Kirk, 2004). Probably, this fact can explain almost complete lack of resistant strains among the native *P. infestans* strains, including those, that have been

collected from potato fields, treated with dimethomorph (Derevyagina *et al.*, 1999, Elansky *et al.*, 2007).

A population, treated with any oligo-site fungicide, also consists of two groups of strains. Strains from the first group did not contacted with the fungicide and, therefore, did not change their initial characteristics (if this group contains resistant strains, then they will not be accumulated because of the higher aggressiveness and competitive ability of sensitive strains). The second group includes strains, affected with the sublethal concentrations of the fungicide. The accumulation of resistant strains is possible in the second group, since in this case they have some advantages in comparison with the sensitive strains. Therefore, using oligo-site fungicides, one should remember, that the high concentration of the fungicide (several times more than the sublethal one) is more important factor, than the careful treatment, since in the case of the stepwise mutagenesis, the initial resistance of mutant strains is low.

Finally, mutations, affecting the resistance to the **mono-site fungicides**, are highly expressive, i.e. a single mutation can determine a high resistance level (up to the total lack of sensitivity). Therefore, the resistance of populations increases very quickly. Phenylamides, including the widespread Ridomil (the acting substance is methalaxyl) and Ridomil-Gold (the acting substance is mefenoxam), can serve as examples of such fungicides. Mutations, causing the resistance to these preparations, have a high frequency (6.9×10^{-6}), and the resistance level of such mutants is very high and exceeds that of a sensitive strain in one thousand times or even more (Derevyagina *et al.*, 1993). Though the growth rate and aggressiveness of the resistant mutants reduce (because of the lack of aggressive sensitive strains), the magnitude of resistant population quickly increases along with its aggressiveness. Thus, after the several years of the fungicide application, the aggressiveness of resistant strains can achieve the level of the sensitive ones and even exceed it (Derevyagina, Dyakov, 1990). The aggressiveness grows more quickly, if the sexual recombination is possible in the given population.

The study of metalaxyl resistance of *P.infestans* strains, isolated in 1988-2004 in different regions of Russia, showed that resistant strains are usually isolated from the infected leaves and stems of potato and tomato and also from potato tubers. However, the frequency of resistant strains, isolated from tomato fruits, was very low, even in the case when the leaves of the same plants were infected with resistant strains. It seems that the sensitive strains have any advantages regarding the process of the fruit infection.

After 1993-1994 we registered the reduction in the frequency of resistant strains in the most of the field *P. infestans* populations. It was possibly caused by some changes in the structure of a potato growing in Russia: in the beginning of nineties it was concentrated mainly on the small private gardens. The use of expensive fungicides, including metalaxyl, was practically ceased. According to our data, metalaxyl-sensitive *P. infestans* strains predominated on such private gardens, so we can consider these small gardens as the global reserve of sensitive isolates.

After 1994 the share of metalaxyl-resistant strains in almost all studied populations of the European part of Russia did not exceed 30%. A population usually contains a small number of resistant isolates even if metalaxyl-based fungicides did not used for a long period of time. In the case of the treatment of a potato field with such fungicides, the number of resistant strains sharply increased (Elansky *et al.*, 2007).

Intraspecific specialization

P. infestans can cause epiphytoties on two commercial crops - potato and tomato. In the case of potato the epiphytoties began shortly after this oomycete had spread to the new regions. The infection of tomato was also registered shortly after the appearance of the infection, but the first epiphytoties befell only one hundred years later - in the middle of XX century. In Russia the late blight of tomato was registered in XIX century. «During a long time scientists

did not pay attention to this disease, since it did not cause any significant crop losses, but in the sixties and seventies the late blight epiphytoties on tomato were registered in the Soviet Union, mainly in the Lower Volga, Ukraine, North Caucasus, and Moldavia…» (Balashova, 1979). Since then the late blight of tomato was registered every year and spread over the whole territory of the industrial and private vegetable cultivation, causing huge economical damage.

In 1993 the study of population markers of strains, isolated from the neighboring potato and tomato fields, showed that about ¼ of isolates, collected from tomato leaves, were transferred from the potato field (Dolgova *et al.*, 1997). In theory one can suppose that the divergence of these populations from different hosts will increase and result in the development of intraspecific specialized forms (f.sp. potato and f.sp. tomato), especially since oospores can survive in plant residues (Drenth *et al.*, 1995; Bagirova and Dyakov, 1998) and tomato seeds (Rubin *et al.*, 2001) and, therefore, tomato plants have a potato-independent source for the spring revival of *P. infestans*.

However, the real events did not correspond to this hypothesis. The wintering of oospores allowed the parasite to avoid the most narrow stage of its life cycle, i.e. a soil vegetation stage, when its parasitic abilities are reduced and then gradually recovered in summer during epiphytoty. Primary zoosporangia and zoospores, germinating from oospores, are highly active, especially if oospores were parthenogenetically generated under the influence of pheromones of the strain with the opposite mating type. Thus, the infection material, grown from tomato seeds, infected with *P. infestans* oospores, is highly pathogenic for both tomato and potato. These changes resulted in the new population rearrangement, expressed through the following important epidemiological changes:

1) Infected tomato sprouts became the important source of the primary infection of potato (Filippov *et al.*, 2004, Ivanyuk, personal communication).
2) Epiphytoties on potato fields arised one month earlier than usual (in June).
3) The percentage of T1 race on the potato fields increased, though earlier it was very low (Ulanova *et al.*, 2003).
4) The virulence of strains, isolated from tomato leaves and tested on the appropriate differenciator cultivars of potato, came up with that of the strains, isolated from potato; the aggressiveness of "tomato" strains exceeded that of "potato" strains on both tomato and potato plants (Lavrova *et al.*, 2003; Ulanova *et al.*, 2003).

Thus, the convergence of populations occurred instead of the divergence, i.e. the united population, characterizing by the high aggressiveness and virulence regarding to both tomato and potato, has developed on these host plants.

Conclusion

Two types of *Phytophthora infestans* populations have been registered in Russia: (1) Siberia and Far East populations, characterizing by a very low genotypic variability, and (2) greatly varying European populations. The possible reasons for the low variability of Siberian and Far East populations are the use of the local seed material for a potato and tomato reproduction and also the inclement weather conditions, that do not allow the soil wintering of oospores. On the contrary, European populations are influenced by an active exchange of the seed material and a permanent import of potato tubers and tomato fruits from abroad.

One of the most urgent questions for the potato and tomato growers concerns the future changes in *P. infestans* populations. In our opinion, the population structure of this pathogen will change in the following directions:

— increase in the genetic variety of all Russian populations, caused by the global warming, intensive exchange of the seed material, and import of tubers from other regions by processing enterprises.

— increase in the aggressiveness of strains, caused by the sexual recombination and the increase in the genetic variety.

— increase in the percentage of metalaxyl-resistant strains in *P. infestans* populations, caused by a predomination of large agricultural enterprises, practicing intensive chemical treatments, in the potato-growing sector of economics. Such global reserve of sensitive strains as the small private gardens is gradually vanishing. The mass appearance of strains resistant to oligo- and poly-site fungicides is highly unlikely.

— disappearance of the differences between *P. infestans* populations, developing on potato and tomato plants, and the prevalence of strains with the high aggressiveness towards both hosts.

Taking into account these possible changes, we can recommend growers to use cultivars with the heightened level of the nonspecific late blight resistance, to control the quality of the seed material, to observe the anti-resistant strategy, to use the forecast of the disease development during the application of chemical treatments, and to provide a spatial separation of potato and tomato fields.

Acknowledgements

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