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Novel Genotypes in *Phytophthora infestans* Populations in Hungary

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Twenty-seven isolates of *Phytophthora infestans* collected in Hungary in 2001 were tested for mating type, response to metalaxyl, isozyme genotype at glucose-6-phosphate isomerase *(Gpi)* and peptidase A *(Pep)* loci and nuclear DNA fingerprints with probe RG57. The ratios of the mating types A1 to A2 were 5:6 and 9:7 among isolates from potato and tomato, respectively. Seventeen isolates were sensitive to metalaxyl, 1 isolate responded intermediately and 9 isolates were resistant. No novel combinations of isozyme alleles were found; all isolates were *Gpi* 100/100, and genotypes at the *Pep* locus were 96/96 (63%), 83/96 (11%) and 100/100 (26%). In contrast, all of the 22 RG57 fingerprints exhibited patterns that have not been reported in Hungary before. On the basis of combined traits, 22 multilocus genotypes, unnoted elsewhere in Europe, were constructed among the 27 isolates analysed. These results indicate that variability in the Hungarian *P. infestans* populations is likely due to local events (asexual and sexual interactions) rather than migration from other countries.

Keywords: Isozymes, mating type, metalaxyl sensitivity, RFLP-DNA fingerprints, multilocus genotypes, potato late blight.

Phytophthora infestans (Mont.) de Bary is one of the most devastating and economically important pathogen of potato and tomato in the world including Hungary.

The pathogen is heterothallic, i.e. requires compatible mating types A1 and A2 for sexual reproduction. Until the 80s, only one clonal lineage (US-1) representing the A1 mating type was known to occur worldwide outside Central Mexico (Fry and Goodwin, 1995). Following the first detection of the A2 mating type outside Mexico (Hohl and Iselin, 1984), new genotypes of *P. infestans* occurred and displaced the old population in many parts of the world. These new populations harbour isolates of both mating types with increased virulence and/or aggressiveness, resistance to the specific fungicide metalaxyl and novel isozyme genotypes or RG57 fingerprints (Spielman et al., 1991; Fry et al., 1993; Koh et al., 1994). Interaction between the opposite mating types results in the formation of oospores that may survive in soil for a long period of time, thus affecting the epidemiological dynamics of the disease. Additionally, sexual reproduction may greatly enhance the genetic diversity of the pathogen. For instance, sexual events resulted in the occurrence of new RG57 genotypes (Drenth et al., 1993) and an aggressive clonal lineage (Gavino et al., 2000).

In Hungary, the first study on a regional population of *P. infestans* regarding its racial composition, was published by Mudich (1965). Most recently, Bakonyi et al. (1998; 2002) investigated the population structure of the pathogen using phenetic and genetic markers.

The aim of this study was to conduct further studies on the population structure of *P. infestans* in various regions of Hungary.

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Materials and Methods

Sources of isolates

Isolates were collected from young single lesions of potato or tomato plants and were purified from contaminants on a selective pea-broth agar (PBA) (Érsek and Bakonyi, 1997). Where more than one isolate were obtained from one field, the infected plants were selected randomly. Pure cultures were grown on non-amended PBA medium at 20 °C in the dark.

Standard isolates used in genotypic analysis were US940501, US930287, PO880033 and 1568 kindly provided by W. E. Fry, Cornell University Ithaca NY, USA, and G. A. Forbes, International Potato Center, Quito, Ecuador, respectively.

Mating type analysis

Mating type was determined by pairing each sample isolate with known A1 (US940501) and A2 (US930287) testers on PBA plates at 20 °C in the dark. Formation of sexual structures was recorded on the 14th day. An unknown isolate was designated A1 if it produced oospores with the A2 mating type; an isolate forming oospores with the A1 mating type was designated A2.

Response to metalaxyl

Agar plugs (5-mm) were cut from the edge of actively growing colonies and placed into 60-mm-diameter plastic Petri plates containing 7 ml PBA amended with metalaxyl at final concentrations of 100 or 5 mg L^{-1} active ingredient. Growth on amended medium was compared to growth on unamended control plates. Categories in response to the drug were determined according to Koh et al. (1994). The assays were conducted in two replicates.

Isozyme analysis

Mycelium was grown on pea-broth medium for 7–10 days. Liquid cultures were then harvested by filtration, washed with distilled water and lyophilised. Proteins for electrophoresis were extracted from 25 mg ground and lyophilised mycelia in 300 µl extraction buffer according to Láday et al. (2000). Cellulose acetate gel electrophoresis was performed at 180 V for 25 minutes as described by Goodwin et al. (1995). Isozyme patterns were visualized using agar overlays according to the manufacturer's directions (Hebert and Beaton, 1993). Chemicals were supplied by Sigma-Aldrich Ltd., Budapest, Hungary.

RG57 hybridization

Total genomic DNA for fingerprinting was extracted with phenol–chlorophorm from 160 mg of mycelium powder suspended in an extraction buffer (Goodwin et al., 1992). Thirty µg of DNA was digested at 37 °C with *Eco*RI, and restriction fragments were separated by electophoresis in 0.8% agarose gel. DNA was then electroblotted onto Hybond N^+ membrane (Amersham Biosciences Trading GmbH, Vienna, Austria) at 1.5A

for 4 hours in 1×TBE buffer (Sambrook et al., 1989). Probe RG57 was randomly labelled with DIG-11-dUTP according to the manufacturer's directions and hybridised to the blot at 68 °C overnight (Anonym, 1995). Chemicals were supplied by Roche Diagnostics GmbH, Mannheim, Germany.

Data analysis

Mating type, isozyme genotypes and RG57 fingerprint data were combined to form multilocus genotypes according to Forbes et al. (1998).

Results and Discussion

Twenty-seven single-lesion isolates, 11 from potato and 16 from tomato were collected in 16 fields at 12 locations (mainly in the north-eastern region of the country) in 2001. Conventionally, these isolates were characterised for response to metalaxyl and multilocus genotypes *(Table 1).*

Regarding the response to metalaxyl *in vitro,* 17 sensitive (63%), 1 intermediate and 9 resistant (33%) isolates were found. Sensitive and intermediate isolates occurred only in fields where metalaxyl was not used at all. Resistance to the fungicide developed in isolates derived from either tomato or potato and appeared to be irrespective of the mating type.

All isolates were homozygous (100/100) at the locus for glucose-6-phosphate isomerase. This is the only allele at the *Gpi* locus ever found in Hungary (Bakonyi et al., 1998; 2002). The most frequent allele at the locus for peptidase was 96; allele combinations determined for *Pep* were 96/96 (63%), 83/96 (11%) and 100/100 (26%). Since Bakonyi et al. (2002) earlier reported on similar alleles and allele frequencies, the *Pep* 96 allele is likely to be the predominant one in Hungary.

The RG57 patterns seem to be unique to Hungary, and all locations harbour isolates of novel and different RG57 genotypes. Most of the marker bands were polymorphic among isolates. Monomorphic bands, i.e. those that were either present in all isolates or none of the isolates contained them, were as follows: 1, 5, 10–15, 24 and 25.

In the two host-based subpopulations of the pathogen, the ratios of the mating types A1 to A2 were 5:6 and 9:7 among isolates from potato and tomato, respectively. We found an isolate at Nagykálló that produced oospores in culture. Since hyphal tip subcultures of this isolate formed no oospores within a single colony, the possibility of selfing can be precluded. Although we failed to re-isolate both of the mating types from these subcultures (A2 was regained), the occurrence of variable multilocus genotypes in the region is possibly due to the sexual outcross of naturally co-existing A1 and A2 mating types *(Table 1).* In contrast, at location of Debrecen II where only mating type A1 was found, each isolate had the same multilocus genotype. At location of Budapest II, two isolates derived from the lower stem and the fruit of a single tomato plant exhibited identical multilocus genotype.

On the basis of combined traits, 22 multilocus genotypes, unnoted in Hungary and elsewhere before, were constructed among the 27 isolates analysed. Our results suggest that diversity of the *P. infestans* populations in Hungary is likely due to local events (asexual and

Table 1

Response to metalaxyl and multilocus genotypes of Hungarian *P. infestans* isolates*

Location	Mating type	Host[1]	Response to metalaxyl	*Pep*[2]	RG57[3]
Nagykálló	A2	P	R	100/100	110_1-10001-00110-11001-10011
	A2	P	R	100/100	110_1-10001-00110-10101-01111
	A2	P	I	100/100	110_1-10001-00110-10101-01111
	A2	P	S	100/100	110_1-10001-00110-10101-01111
	A2	P	S	100/100	110_1-00001-00110-10001-01011
Debrecen I	A1	P	S	100/100	101_1-11001-00110-00101-10011
	A1	P	S	100/100	100_1-10001-00110-00101-10011
	A2	P	S	96/96	111_1-01101-00110-00111-00011
Debrecen II (5)	A1	T	S	96/96	110_1-10101-00110-00101-10111
Szomolya	A2	T	S	96/96	100_1-10101-00110-01010-10011
Heves	A2	T	S	96/96	110_1-10101-00110-00101-1….[4]
Hajdúböszörmény	A1	T	R	96/96	110_1-10101-00110-00111-10011
	A1	T	R	96/96	110_1-00101-00110-01001-10011
Berkesz	A1	T	S	96/96	110_1-10101-00110-01010-01011
Budapest I	A2	T	S	96/96	111_1-10001-00110-10001-10011
Budapest II (2)	A2	T	S	96/96	111_1-11001-00110-11011-01011
Érd	A2	T	R	96/96	111_1-11001-00110-10011-10011
Kecskemét	A1	T	S	96/96	111_1-11111-00110-10001-11011
Monorierdő	A2	T	R	96/96	111_1-11001-00110-11011-10011
Keszthely	A1	P	R	83/96	111_1-01101-00110-00011-10011
	A1	P	R	83/96	101_1-11111-00110-10011-10011
	A1	P	R	83/96	111_1-11001-00110-10001-11011

[1] P: potato, T: tomato.
[2] All isolates were 100/100 at the *Gpi* locus.
[3] RG57 data are ordered from left to right for bands 1 to 25; 1 means the presence of the band whereas 0 stands for its absence. Band 4 was excluded (_).
[4] Bands 22–25 were not resolvable in any manner.
* Each row in the table represents data for individual isolates from separate fields. Number in parentheses next to a location indicates the number of isolates from that location.

sexual interactions) rather than migration from other countries. A comparable situation has been recorded in Poland (Sujkowski et al., 1994), that is reminiscent of the case in Central Mexico, the centre of diversity and presumed centre of origin of *P. infestans*.

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