

Genetic Diversity of the Population of *Phytophthora infestans* in Hungary

Z. Á. NAGY¹, J. BAKONYI¹, VIRÁG SOM^{1, 2} and T. ÉRSEK¹

¹Plant Protection Institute, Hungarian Academy of Sciences,
H-1525 Budapest, P. O. Box 102, Hungary

²Department of Plant Protection, Faculty of Agricultural and Food Sciences, University of West Hungary,
H-9200 Mosonmagyaróvár, Kolbai K. u. 8, Hungary

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A total of 123 isolates of *Phytophthora infestans* were recovered from 43 commercial fields and allotments of potato and tomato in Hungary. Isolates were characterised for mating type, response to metalaxyl, isozyme genotype at glucose-6-phosphate isomerase (*Gpi*) and peptidase A (*Pep*) loci, nuclear DNA fingerprint with probe RG57 and mitochondrial haplotype. Both mating types were detected in 8 potato and 3 tomato fields. The frequency of A1 and A2 were 47.5% and 52.5% on potato and 60.5% and 39.5% on tomato. More than sixty per cent of the whole population were resistant (31.1%) or responded intermediately (30.2%) to the fungicide metalaxyl *in vitro*. All isolates were monomorphic at the *Gpi* locus (100/100), but four allele combinations 100/100 (33%), 96/100 (28%), 96/96 (31%) and 83/96 (8%) were found at the *Pep* locus. Genotype *Pep* 83/96 is rare in Europe and all isolates with this allele combination were of A1 mating type and Ia mitochondrial haplotype. As in other European populations, only two of the four mitochondrial haplotypes (Ia, 32.5% and IIa, 67.5%) were detected. Diversity of RG57 fingerprints was high. Of the 79 different fingerprints found 67 were not detected previously.

Keywords: late blight, multilocus genotypes, *Phytophthora infestans*, population diversity.

Phytophthora infestans (Mont.) de Bary is one of the most serious and economically important pathogens in potato and tomato fields worldwide including Hungary. Being a heterothallic organism, *P. infestans* requires both A1 and A2 mating types to be present to allow sexual oospores to form. This feature, connected with high genetic variability had been restricted only to Central Mexico before 1980s where the pathogen had evolved (Niederhauser, 1991; Fry and Goodwin, 1995). In contrast only one clonal lineage (US-1) had predominated in the rest of the world before the first report of A2 mating type outside Mexico (Hohl and Iselin, 1984). Following a second migration from Central Mexico, both A1 and A2 mating types spread to many countries in the last two decades (Goodwin, 1997). These new lines of *P. infestans*, having new isozyme genotypes and RG57 fingerprint patterns, were more variable and aggressive and displaced the old populations of the US-1 clonal lineage (Spielman et al., 1991; Fry et al., 1993; Koh et al., 1994).

Detection of the population change stimulated further work on diversity in other countries in Europe and elsewhere. Numerous data were presented describing the struc-

ture of the pathogen population from Europe (e.g. Sujkowski et al., 1994; Zwankhuizen et al., 2000) and from other continents (e.g. Jaime-Garcia et al., 2000; Sedegui et al., 2000; McLeod et al., 2001).

Early work in Hungary detected diversity in virulence to specific resistance genes (Mudich, 1965). Most recently Bakonyi et al. (1998; 2002b) and Nagy et al. (2003) used mating type, isozyme and RG57 data to detect the presence of new genotypes in Hungary; this was confirmed with mitochondrial markers (Som et al., 2004). These results revealed high diversity in the populations of *P. infestans* in Hungary even in small sample sizes. Our aim was therefore to assess the level of diversity in larger collections of *P. infestans* on a countrywide scale as well as within multiple isolates from the same field.

Materials and Methods

Sources of isolates

Isolates were collected throughout two growing seasons in 2001–2002 from young, single lesions on potato or tomato leaves and were purified from contaminants on a selective pea-broth agar (PBA) (Érsek and Bakonyi, 1997). Additionally, five isolates from four different sites, collected in 1993–1998 were also included in the analysis. If more than one lesion were sampled from the same field, the infected plants were selected randomly. Some isolates were taken from lesions on tomato fruits and potato tubers. Pure cultures were stored on antibiotic-free PBA at 20 °C in the dark.

Standard isolates used in genotypic analysis were US940501, US930287 and Pi1568 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 oospores was recorded after two weeks. If an unknown isolate produced oospores with the A2 mating type tester it was designated A1, whereas an isolate which formed oospores with the tester of A1 mating type was designated A2.

Response to metalaxyl

Response to metalaxyl was determined *in vitro*. Agar plugs (5-mm-diameter) were cut from the edge of actively growing colonies and placed into 6-cm-diameter plastic Petri dishes containing 7 ml PBA amended with metalaxyl at final concentrations of 100 and 5 µg/ml active ingredient. Radial growth on amended medium was compared to growth on unamended control plates after one week provided that the diameter of the control plate was at least 2 cm. All measurements were means from two replicate plates. An isolate was sensitive to metalaxyl if the relative growth of the colony was less than 40% of the control on both 5 and 100 ppm. An isolate had intermediate resistance if the relative growth on 5

ppm was higher than 40% of the control but less than 40% on 100 ppm. Those isolates which exhibited more growth than 40% of the control on both concentrations were determined to be resistant to metalaxyl (Daggett et al., 1993; Koh et al., 1994).

Isozyme analysis

Mycelium was grown on pea-broth for 7–10 days. Liquid cultures were then harvested by filtration, washed with deionised water and lyophilised. Proteins for electrophoresis were extracted from 25 mg ground mycelia in 300 µl extraction buffer according to Ládai et al. (2000). Cellulose acetate gel electrophoresis was performed at 180V 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 hybridisation

Two methods were used for DNA extraction. Total genomic DNA was extracted with phenol–chlorophorm from 80 mg lyophilised mycelium grown on pea-broth cultures suspended in an extraction buffer according to Goodwin et al. (1992a). The quality of the DNA recovered from one-third of the isolates was poor for digestion with restriction endonuclease therefore an alternative method was used with slight modification (Murray and Thompson, 1980). DNAs were extracted from 50 mg of lyophilised mycelium powder into hexadecyltrimethylammonium bromide (CTAB) buffer. Extraction buffer contained 1% CTAB, 0.7 M NaCl, 50 mM Tris-HCl (pH=8.0), 250 mM EDTA (pH=8.0). After extraction with phenol–chloroform the DNA was precipitated with the addition of one volume of 1% CTAB, 50 mM Tris-HCl (pH=8.0), 10 mM EDTA (pH=8.0), washed, dissolved in 1 M NaCl, 10 mM Tris-HCl (pH=8.0), 0.1 mM EDTA (pH=8.0) then precipitated again with 0.54 volume of isopropanol. At the end of both extraction methods DNA from each isolate was dissolved in 50 µl 10 mM Tris-HCl (pH=8.0). Chemicals were supplied by Sigma–Aldrich Ltd., Budapest, Hungary and Reanal Finechemical Co. Budapest, Hungary.

Six to 10 µl of DNA solution were then digested at 37 °C with *EcoRI* overnight and restriction fragments were separated by electrophoresis in 0.8% agarose gel for 16 hours. DNA was then electroblotted onto Hybond N⁺ membrane (Amersham Biosciences Trading GmbH, Vienna, Austria) at 1.5A for 3.5 hours in 0.5 × TBE buffer (Sambrook et al., 1989). Probe RG57 (Goodwin et al., 1992a) was randomly labelled with DIG-11-dUTP according to the manufacturer's directions (Roche Diagnostics GmbH, Mannheim, Germany) and hybridised to the blot at 68 °C overnight (Anonym, 1995). Membranes were then scored for presence of hybridising bands at the 25 known loci. Band 4 was excluded because the probe available hybridises to this fragment inconsistently (e.g. Forbes et al., 1998).

Mitochondrial haplotype analysis

Sequences from the mitochondrial (mt) genome were amplified by the polymerase chain reaction (PCR) in a PTC 150 Mini Cycler™ (MJ Reserach Inc, USA). The final reac-

tion volume of 25 µl contained 5 µl template DNA which was a hundredfold dilution of the DNA used in RG57 analysis. PCR conditions and sequences of primers were as recommended by Griffith and Shaw (1998), with the exception of annealing temperature which was 62 °C. Five to ten µl of PCR product were digested overnight with 10 U of restriction endonuclease *EcoRI* and *MspI* in a final volume of 30 µl; restriction fragments were separated in 2% agarose gel (Invitrogen Ltd, Paisley, Scotland) and stained with ethidium bromide.

Data analysis

For assessing the structure of *P. infestans* populations in Hungary, gene diversity analyses (Nei, 1973) were carried out between subpopulations on the basis of hosts and mating types. Nei's genetic differentiation coefficient (G_{ST}) were calculated from the average gene diversity within subpopulations ($\bar{H}_S$) as a proportion of total gene diversity ($\bar{H}_T$) in the whole population with correction of bias in sampling (Nei and Chesser, 1983). Allele frequencies for this analysis were calculated on both isozyme loci. Data from RG57 fingerprints were not incorporated into this analysis because homozygosity and heterozygosity at each locus cannot be distinguished. The whole analysis was repeated with clone-corrected data where each clonal lineage was determined by their identical multilocus genotypes (mating type, isozyme genotype, RG57 fingerprint and mitochondrial haplotype). Nei's unbiased genetic distance (Nei, 1978) between the subdivisions were also calculated with Popgene 1.31 program (Yeh et al., 1997). Using these genotypes, a Shannon diversity index was calculated as a measure of genetic diversity defined as $H = -\sum_{i=1}^k p_i \ln p_i$. Where p_i is the frequency of isolates with the i th genotype and k is the number of genotypes in the sample. A t -test was used for assessing significant differences of H values (Hutcheson, 1970; Sigler and Zeyer, 2002; Day et al., 2004).

Results

During the growing seasons in 2001 and 2002 a total of 118 isolates were collected from Hungary covering 18 potato and 22 tomato sites (Fig. 1, Table 1). A majority of the isolates were collected in 2002 from sites close to each other either in Northern Hungary (Ludányhalászi) or Northern Transdanubia (Borzavár, Porva, Olaszfa, Tündérmajor). Additional five isolates collected from potato at 4 different sites between 1993 and 1998 were also included. The host plant was tomato in thirteen of the 17 locations where only one or two isolates were collected.

Out of the 123 isolates tested, 80 originated from potato and 43 from tomato. The frequency of A1 and A2 mating types was similar on both hosts. The number of isolates of A1 and A2 mating types were 38 and 42 on potato and 26 and 17 on tomato, respectively. Thirty eight isolates collected from potato had A1 mating type and 42 were A2. A1 mating type was detected at 10 of the 21 potato sites and A2 mating type was detected at 3 sites. Both mating types were present at 8 sites. On the other hand, 11 and 8 of the 22 tomato fields sampled had isolates with only A1 and A2 mating types, respectively, but only 3 fields had A1 and A2 mating types.



Fig. 1. Sampling locations of *Phytophthora infestans* isolates in Hungary. Numbers in parentheses indicate the number of isolates from one location. Locations where isolates originated only from potato or tomato are indicated with a triangle and a square, respectively. A circle indicates the locations where isolates were sampled from both hosts

All the isolates had 100/100 genotype at the *Gpi* locus. At the *Pep* locus, however, four genotypes were resolved: 100/100 (33% of the isolates tested), 96/100 (28%), 96/96 (31%) and 83/96 (8%).

Five of the 25 RG57 bands did not show polymorphism. Bands 11, 12 and 15 were not present in any of the isolates and bands 13 and 25 were present in all isolates. All other bands were polymorphic. Forty-seven different RG57 fingerprints were recovered among the 80 isolates originating from potato and 28 fingerprints were recovered among 43 isolates from tomato. Only four RG57 fingerprints were detected on both hosts. The most frequent RG57 genotype (XXVII) occurred in 9 isolates originating from 5 different sites (Table 1). Comparing isolates from different hosts the mean of gene diversities between subpopulations was $H_S = 0.259$ whereas in a mating type comparison this value was $\bar{H}_S = 0.255$. Total gene diversities in the population were of $\bar{H}_T = 0.265$ and $\bar{H}_T = 0.269$ in the two comparisons, respectively. Differences between subdivisions remained at a low level according to the genetic differentiation coefficient (G_{ST}), which were 0.020 and 0.050 in the comparisons of subdivisions from the two hosts and of the different mating types, respectively (Table 2a). By using clone-corrected data no difference was revealed in the population structure (Table 2b). In both analyses the low level of genetic difference was in agreement with Nei's unbiased genetic distance of the subdivisions.

Table 1Features of *P. infestans* isolates from various locations in Hungary

Isolate	Location ¹	MT ²	Host ³	Response to meta- laxyl ⁴	Mt type	Pep	RG57 fingerprint ⁵	RG57 genotype ⁶
H3.1/93	Monor	A1	P	I	Ia	83/96	111.1-01001-00110-11011-11011	IV
H-1/2/98	Inke	A2	P	S	IIa	96/100	110.1-00001-00110-01011-11011	I
H-3/2/98	Inke	A1	P	R	IIa	100/100	100.1-00011-00110-10001-11011	IX
H-3/3/98	Inke	A2	P	S	IIa	96/100	110.1-00001-00110-10011-11011	II
H-2a/98	Forráskút	A1	P	S	Ia	83/96	110.1-01001-00110-11011-11011	XVII
H-1/1/01	Nagykálló	A2	P	R	IIa	100/100	110.1-10001-00110-11001-10011	LXV
H-1/2/01	Nagykálló	A2	P	R	Ia	100/100	110.1-10001-00110-10101-01111	LXXIV
H-1/3/01	Nagykálló	A2	P	I	IIa	100/100	110.1-10001-00110-10101-01111	LXXIV
H-1/4/01	Nagykálló	A2	P	S	IIa	100/100	110.1-10001-00110-10101-01111	LXXIV
H-1/5/01	Nagykálló	A2	P	S	IIa	100/100	110.1-00001-00110-10001-01011	LXXV
H-2/1/01	Debrecen I	A1	P	S	Ia	100/100	101.1-11001-00110-00101-10011	LXXXVI
H-3/1/01	Debrecen I	A1	P	S	IIa	100/100	100.1-10001-00110-00101-10011	LXXXVII
H-4/1/01	Debrecen I	A2	P	S	IIa	96/96	111.1-01101-00110-00111-00011	LXXXVIII
H-21/3/01	Keszthely IV	A1	P	R	Ia	83/96	111.1-01101-00110-00011-10011	LXXXVIII
H-22/1/01	Keszthely V	A1	P	R	Ia	83/96	101.1-11111-00110-10011-10011	LXXXIX
H-22/2/01	Keszthely V	A1	P	R	Ia	83/96	111.1-11001-00110-10001-11011	XM
H-2/1/02	Ludányhalászi I	A1	P	R	IIa	100/100	100.1-00011-00110-10001-10011	XX
H-2/4/02	Ludányhalászi I	A1	P	S	Ia	83/96	111.1-01001-00110-11001-10011	XXI
H-3/1/02	Ludányhalászi II	A1	P	I	Ia	96/100	101.1-11011-00110-11001-10011	XXII
H-3/2/02	Ludányhalászi II	A1	P	I	Ia	96/96	110.1-10011-00110-11001-10011	XXIII
H-3/4/02	Ludányhalászi II	A1	P	R	IIa	100/100	100.1-00011-00110-10001-10011	XX
H-4/1A/02	Ludányhalászi III	A1	P	R	Ia	83/96	101.1-11101-00110-10011-10011	XXIV
H-4/1B/02	Ludányhalászi III	A1	P	R	Ia	96/100	111.1-01001-00110-00011-01011	XXV
H-4/1C/02	Ludányhalászi III	A1	P	R	IIa	100/100	110.1-00001-00110-10001-10011	XXVI
H-4/3B/02	Ludányhalászi III	A1	P	R	IIa	100/100	100.1-00011-00110-10001-10011	XX
H-4/4/02	Ludányhalászi III	A2	P	I	IIa	96/100	111.1-11011-00110-11001-10011	XXVII
H-4/5/02	Ludányhalászi III	A1	P	I	Ia	96/100	110.1-10101-00110-11001-10011	XXVIII
H-5/1/02	Ludányhalászi IV	A1	P	I	IIa	96/96	111.1-01011-00110-10011-10011	XXIX
H-5/2/02	Ludányhalászi IV	A1	P	R	Ia	83/96	101.1-11101-00110-11011-10011	XXX
H-5/3/02	Ludányhalászi IV	A1	P	R	IIa	100/100	110.1-00011-00110-10001-10011	XXXI
H-5/4/02	Ludányhalászi IV	A1	P	R	IIa	100/100	100.1-00011-00110-10001-11011	IX
H-5/5/02	Ludányhalászi IV	A1	P	I	Ia	83/96	101.1-01001-00110-10001-10011	XXXII
H-6/1/02	Ludányhalászi V	A1	P	I	Ia	100/100	101.1-01011-00110-10001-10011	XXXIII
H-6/3/02	Ludányhalászi V	A1	P	I	Ia	83/96	101.1-11001-00110-10001-10011	XXXIV
H-6/4/02	Ludányhalászi V	A1	P	R	IIa	100/100	100.1-00011-00110-10001-10011	XX
H-6/5/02	Ludányhalászi V	A1	P	R	IIa	100/100	100.1-00011-00110-01001-10011	XXXV
H-10/1/02	Borzavár I	A2	P	I	IIa	96/96	111.1-01011-00110-01011-01011	XXXVII
H-11/1/02	Borzavár I	A2	P	I	IIa	96/96	111.1-01011-00110-01001-10011	XXXVIII
H-11/2/02	Borzavár I	A2	P	I	IIa	96/96	111.1-01011-00110-01001-10011	XXXVIII
H-11/3/02	Borzavár I	A2	P	R	IIa	96/96	111.1-11011-00110-01001-10011	XXXIX
H-11/5/02	Borzavár I	A2	P	I	IIa	96/96	111.1-11011-00110-01001-10011	XXXIX
H-11/9/02	Borzavár I	A2	P	I	IIa	96/96	111.1-11011-00110-01001-10011	XXXIX
H-11/6/02	Borzavár I	A2	P	R	IIa	100/100	111.1-01011-00110-01011-10011	XL
H-11/8/02	Borzavár I	A2	P	S	Ia	100/100	111.1-01011-00110-01001-00011	XLI
H-11/11/02	Borzavár I	A2	P	R	IIa	96/100	111.1-11011-00110-11001-10011	XXVII

Table 1 (cont.)

Isolate	Location ¹	MT ²	Host ³	Response to meta- laxyl ⁴	Mt type	Pep	RG57 fingerprint ⁵	RG57 genotype ⁶
H-11/12/02	Borzavár I	A2	P	I	IIa	96/100	111.1-11011-00110-11001-10011	XXVII
H-12/1/02	Borzavár II	A2	P	R	IIa	96/96	111.1-01011-00110-01001-10011	XXXVIII
H-12/8/02	Borzavár II	A2	P	R	IIa	96/96	111.1-01011-00110-01001-10011	XXXVIII
H-12/2/02	Borzavár II	A2	P	I	IIa	96/100	111.1-11011-00110-01011-10011	XLII
H-12/14/02	Borzavár II	A2	P	I	IIa	96/100	111.1-11011-00110-01011-10011	XLII
H-12/3/02	Borzavár II	A2	P	I	IIa	96/96	111.1-11011-00110-01011-10011	XLII
H-12/6/02	Borzavár II	A2	P	I	IIa	96/96	111.1-11011-00110-01011-10011	XLII
H-12/7/02	Borzavár II	A2	P	I	IIa	96/96	111.1-11011-00110-01011-10011	XLII
H-12/4/02	Borzavár II	A2	P	R	IIa	96/100	111.1-11011-00110-01001-01011	XLIII
H-12/5/02	Borzavár II	A1	P	I	Ia	100/100	110.0-00001-00110-10001-11011	XLIV
H-12/9/02	Borzavár II	A2	P	R	IIa	96/100	111.1-11011-00110-01001-10011	XXXIX
H-12/10/02	Borzavár II	A1	P	I	Ia	100/100	110.0-00001-00110-10001-10011	XLV
H-12/11/02	Borzavár II	A2	P	R	IIa	96/100	111.1-01011-00110-11011-10011	XLVI
H-12/13/02	Borzavár II	A2	P	R	IIa	96/100	111.1-01011-00110-11000-10011	XLVII
H-13/2/02	Borzavár II	A2	P	I	IIa	96/100	111.1-01011-00110-01011-10011	XL
H-13/3/02	Borzavár II	A1	P	I	IIa	100/100	111.1-01011-00110-01001-10011	XXXVIII
H-13/4/02	Borzavár II	A2	P	I	Ia	100/100	111.1-01011-00110-01001-10011	XLVIII
H-19/1/02	Borzavár III	A2	P	R	IIa	96/100	111.1-11011-00110-11001-10011	XXVII
H-19/2/02	Borzavár III	A1	P	I	Ia	100/100	110.0-00001-00110-10001-10011	XLV
H-14/3/02	Porva	A2	P	I	IIa	96/96	111.1-11011-00110-11001-10011	XXVII
H-14/5/02	Porva	A2	P	R	IIa	96/96	111.1-11011-00110-11001-10011	XXVII
H-14/6/02	Porva	A1	P	I	Ia	100/100	110.0-00001-00110-11001-11111	XLIX
H-14/7/02	Porva	A1	P	I	Ia	100/100	111.1-11011-00110-11001-10011	XXVII
H-15/1/02	Tündérmajor	A2	P	S	Ia	96/96	011.1-01001-00110-00001-10011	L
H-15/2/02	Tündérmajor	A2	P	I	IIa	100/100	111.1-11001-00110-11001-10011	LI
H-15/4/02	Tündérmajor	A2	P	I	IIa	96/100	111.1-11011-00110-11011-10011	LII
H-15/5/02	Tündérmajor	A2	P	I	Ia	96/100	111.1-11011-00110-11001-10011	XXVII
H-15/8/02	Tündérmajor	A2	P	I	IIa	96/100	111.1-11011-00110-11001-10011	XXVII
H-15/6/02	Tündérmajor	A1	P	S	Ia	100/100	110.1-10001-00110-10001-10011	LIII
H-18/1/02	Olaszfalú	A1	P	S	Ia	100/100	110.0-00001-00110-11001-10011	LV
H-33/1/02	Röjtökmuzsaj	A1	P	S	IIa	96/100	100.1-10000-00110-01001-10011	LXIV
H-33/2/02	Röjtökmuzsaj	A2	P	I	IIa	96/100	111.1-11011-00110-01001-10011	XXXIX
H-37/2/02	Keszthely II	A2	P	S	Ia	96/100	111.1-01001-00110-11011-10011	LXXII
H-38/1/02	Keszthely III	A1	P	S	IIa	96/100	100.1-00000-00110-01011-10011	LXXIII
H-38/2/02	Keszthely III	A1	P	S	IIa	96/100	100.1-00000-00110-01011-10011	LXXIII
H-16/1/01	Budapest I	A2	T	S	IIa	96/96	111.1-10001-00110-10001-10011	LXXXIII
H-18/1/01	Budapest II	A2	T	S	IIa	96/96	111.1-11001-00110-11011-01011	LXXXV
H-18/2/01	Budapest II	A2	T	S	IIa	96/96	111.1-11001-00110-11011-01011	LXXXV
H-32/1/02	Budapest VIII	A1	T	S	Ia	96/100	110.0-10001-00110-11001-01011	LXI
H-32/2/02	Budapest VIII	A1	T	S	Ia	96/100	110.1-10001-00110-11001-01011	LXII
H-32/5/02	Budapest VIII	A1	T	R	IIa	100/100	110.1-10100-00110-11011-10011	LXIII
H-32/4/02	Budapest VIII	A1	T	I	IIa	100/100	110.1-10100-00110-10001-10011	XIX
H-31/2/02	Budapest XVII	A1	T	S	Ia	96/100	110.0-10001-00110-11011-10011	LX
H-6/1/01	Heves	A2	T	S	IIa	96/96	110.1-10101-00110-00101-11011	XXII
H-35/4/02	Heves	A2	T	n.t.	Ia	96/96	110.1-10111-00110-01001-10011	LXIX
H-35/5/02	Heves	A1	T	n.t.	Ia	96/96	100.1-00101-00110-01001-10011	LXX
H-5/1/01	Szomolya	A2	T	S	Ia	96/96	100.1-10101-00110-01010-10011	XMI

Table 1 (cont.)

Isolate	Location ¹	MT ²	Host ³	Response to meta- laxyl ⁴	Mt type	Pep	RG57 fingerprint ⁵	RG57 genotype ⁶
H-7/1/01	Debrecen II	A1	T	S	Ila	96/96	110.1-10101-00110-00101-10111	LXXIX
H-7/2/01	Debrecen II	A1	T	S	Ila	96/96	110.1-10101-00110-00101-10111	LXXIX
H-7/3/01	Debrecen II	A1	T	S	Ila	96/96	110.1-10101-00110-00101-10111	LXXIX
H-7/4/01	Debrecen II	A1	T	S	Ila	96/96	110.1-10101-00110-00101-10111	LXXIX
H-7/5/01	Debrecen II	A1	T	S	Ila	96/96	110.1-10101-00110-00101-10111	LXXIX
H-8/1/01	Hajdúböszörmény	A1	T	R	Ila	96/96	110.1-10101-00110-00111-10011	LXXX
H-8/2/01	Hajdúböszörmény	A1	T	R	Ila	96/96	110.1-00101-00110-01001-10011	LXXXI
H-15/1/01	Berkesz	A1	T	S	Ila	96/96	110.1-10101-00110-01010-01011	LXXXII
H-17/1/01	Érd	A2	T	R	Ila	96/96	111.1-11001-00110-10011-10011	LXXXIV
H-19/1/01	Kecskemét	A1	T	S	Ila	96/96	111.1-11111-00110-10001-11011	LXXXVI
H-20/1/01	Monorierdő	A2	T	R	Ila	96/96	111.1-11001-00110-11011-10011	LXXXVII
H-1/2/02	Pápa	A1	T	S	Ila	100/100	110.1-10100-00110-10001-10011	XIX
H-1/3/02	Pápa	A1	T	S	Ila	100/100	110.1-10100-00110-10001-10011	XIX
H-1/4/02	Pápa	A1	T	R	Ila	100/100	110.1-10100-00110-10001-10011	XIX
H-24/1/02	Sopron	A1	T	S	Ila	100/100	110.1-10100-00110-10001-10011	XIX
H-7/1/02	Fertőd	A1	T	R	Ila	100/100	110.1-10100-00110-10001-11011	XXXVI
H-17/1/02	Monorierdő	A2	T	n.t.	Ia	96/96	101.1-11001-00110-11001-10011	LIV
H-21/4/02	Ócsa II	A2	T	S	Ila	96/100	101.1-11101-00110-11011-10011	XXX
H-20/7/02	Ócsa I	A2	T	S	Ila	96/96	011.1-01001-00110-00001-10011	L
H-21/1/02	Ócsa II	A1	T	S	Ia	100/100	110.0-00001-00110-10001-10011	XLV
H-21/5/02	Ócsa II	A2	T	S	Ila	96/100	011.1-01001-00110-00001-10011	L
H-21/2/02	Ócsa II	A1	T	S	Ila	100/100	101.1-11001-00110-11001-10011	LIV
H-21/6/02	Ócsa II	A2	T	S	Ila	100/100	101.1-01000-00110-10001-10011	LVI
H-22/1/02	Ócsa III	A2	T	S	Ila	96/100	011.1-01001-00110-10001-10011	LVII
H-27/1/02	Keszthely I	A1	T	S	Ila	96/100	110.1-10110-00110-10001-11011	LVIII
H-28/1/02	Kápolnásnyék	A1	T	S	Ila	100/100	110.1-11100-00110-00001-10011	LIX
H-34/1/02	Kótaj	A1	T	S	Ia	96/100	110.1-10001-00110-11001-10011	LXV
H-34/2/02	Kótaj	A2	T	S	Ila	100/100	110.0-10101-00110-00100-10011	LXVI
H-34/3/02	Kótaj	A1	T	R	Ia	96/100	110.1-00001-00110-10011-11001	LXVII
H-34/4/02	Kótaj	A2	T	R	Ila	96/100	110.1-10001-00100-00000-01011	LXVIII
H-36/1/02	Hatvan	A2	T	n.t.	Ia	96/96	111.1-11000-00110-10001-10011	LXXI

¹ Roman figures in the column of location distinguish separate fields from the same location.

² MT: mating type.

³ P: potato, T: tomato.

⁴ n.t.: not tested; R: resistant, I: intermediate, S: sensitive.

⁵ RG57 fingerprint 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 from analyses (.).

⁶ RG57 genotypes are numbered with Roman figures continuously according to Bakonyi et al. (2002b).

On the basis of combined traits of mating type, isozyme genotypes and RG57 patterns the population were even more diverse. The most frequent multilocus genotype occurred only six times from five different sites. Two mitochondrial haplotypes, Ia (40 isolates) and Ila (83 isolates) were identified in the whole *P. infestans* population. Details of genotypic and phenotypic features of the *P. infestans* populations on the basis of the

Table 2a

Gene diversity¹ and genetic distance² analysis of the two isozyme loci between subdivisions of *P. infestans* on the basis of the host and different mating types

Sampling units	$\bar{H}_T$	$\bar{H}_S$	G_{ST}	Genetic distance
Host comparison	0.259	0.265	0.020	0.0151
Mating type comparison	0.255	0.269	0.050	0.0385

¹ Gene differentiation coefficient (G_{ST}) were computed from average gene diversity of the two isozyme loci in the whole population ($\bar{H}_T$) and within its subdivisions ($\bar{H}_S$) (Nei and Chesser, 1983).

² Unbiased genetic distance (Nei, 1978).

Table 2b

Gene diversity¹ and genetic distance² analysis of the two isozyme loci between subdivisions of *P. infestans* on the basis of the host and different mating types. Each multilocus genotype were represented by one isolate

Sampling units	$\bar{H}_T$	$\bar{H}_S$	G_{ST}	Genetic distance
Host comparison	0.260	0.271	0.040	0.0305
Mating type comparison	0.267	0.275	0.028	0.0245

¹ Gene differentiation coefficient (G_{ST}) were computed from average gene diversity of the two isozyme loci in the whole population ($\bar{H}_T$) and within its subdivisions ($\bar{H}_S$) (Nei and Chesser, 1983).

² Unbiased genetic distance (Nei, 1978).

Table 3

Distribution of various features of *P. infestans* isolates on the basis of the host

Characters		Isolates from potato	Isolates from tomato	Total
No. of isolates		80 (100%)	43 (100%)	123 (100%)
Mating type:	A1	38 (47.5%)	26 (60.5%)	64 (52.0%)
	A2	42 (52.5%)	17 (39.5%)	59 (48.0%)
<i>Gpi</i>	100/100		123 (100%)	
<i>Pep</i>	100/100	29 (36.2%)	12 (28.0%)	41 (33.4%)
	96/100	24 (30.0%)	10 (23.2%)	34 (27.6%)
	96/96	17 (21.2%)	21 (48.8%)	38 (30.9%)
	83/96	10 (12.6%)	0	10 (8.1%)
<i>RG57</i> genotypes		47/80	28/43	79/123 ²
Mitochondrial	Ia	29 (36.2%)	11 (25.6%)	40 (32.5%)
haplotype:	IIa	51 (63.8%)	32 (74.4%)	83 (67.5%)
Response	R	28 (35.0%)	9 (23.0%)	37 (31.1%)
to metalaxyl ¹ :	I	35 (43.7%)	1 (2.6%)	36 (30.2%)
	S	17 (21.3%)	29 (74.4%)	46 (38.7%)

¹ Overall 119 isolates were tested for response to metalaxyl (R: resistant, I: intermedier, S: sensitive).

² Four additional *RG57* genotypes occurred on both potato and tomato.

host are summarised in Table 3. Table 4 shows these features when isolates are grouped on the basis of mating type.

The Shannon diversity index for A1 isolates was $H = 3.748$ and for A2 was $H = 3.459$, but for isolates originating from potato and tomato these values were $H = 3.899$ and $H = 3.355$, respectively. *T*-test did not show any significant differences between either pair of subdivisions at a level of $P = 0.05$.

In spite of the fact that there was no significant difference between any subdivisions of *P. infestans* in Hungary, this homogeneity is not in agreement with phenotypic features such as response to metalaxyl. In this case outstanding differences were observed

Table 4

Distribution of various features of *P. infestans* isolates on the basis of mating type

Characters		A1	A2	Total
No. of isolates		64 (100%)	59 (100%)	123 (100%)
<i>Gpi</i>	100/100		123 (100%)	
<i>Pep</i>	100/100	30 (46.9%)	11 (18.6%)	41 (33.3%)
	96/100	12 (18.8%)	22 (37.3%)	34 (27.7%)
	96/96	12 (18.8%)	26 (44.1%)	38 (30.9%)
	83/96	10 (15.5%)	0	10 (8.1%)
<i>RG57</i> genotypes		44/64	30/59	79/123 ²
Mitochondrial	Ia	30 (46.9%)	10 (16.9%)	40 (32.5%)
haplotypes:	IIa	34 (53.1%)	49 (83.1%)	83 (67.5%)
Response	R	21 (33.3%)	16 (28.6%)	37 (31.1%)
to metalaxyl ¹ :	I	15 (23.8%)	21 (37.5%)	36 (30.2%)
	S	27 (42.9%)	19 (33.9%)	46 (38.7%)

¹ Overall 119 isolates were tested for response to metalaxyl (R: resistant, I: intermedier, S: sensitive).

² Five additional *RG57* genotypes were identified on both A1 and A2 mating type.

Table 5

Characterisation of 59 isolates collected from Northern Hungary and Northern Transdanubia in 2002

Characters		A1		A2		Total	
		N Hung ¹	N TrD ²	N Hung ¹	N TrD ²	N Hung ¹	N TrD ²
<i>Pep</i>	100/100	9 (47.4%)	8 (100.0%)	0	4 (12.9%)	9 (45.0%)	12 (30.8%)
	96/100	3 (15.8%)	0	1 (100.0%)	12 (38.7%)	4 (20.0%)	12 (30.8%)
	96/96	2 (10.5%)	0	0	15 (48.4%)	2 (10.0%)	15 (38.4%)
	83/96	5 (26.3%)	0	0	0	5 (25.0%)	0
Response to	R	11 (57.9%)	0	0	11 (35.5%)	11 (55.0%)	11 (28.2%)
metalaxyl ³ :	I	7 (36.8%)	6 (75.0%)	1 (100.0%)	18 (58.1%)	8 (40.0%)	24 (61.5%)
	S	1 (5.3%)	2 (25.0%)	0	2 (6.4%)	1 (5.0%)	4 (10.3%)
<i>RG57</i> genotypes		16	4	1	12	17	18 ⁴
Mt haplotypes:	Ia	10 (52.6%)	7 (87.5%)	0	4 (12.9%)	10 (50.0%)	11 (28.2%)
	IIa	9 (47.4%)	1 (12.5%)	1 (100.0%)	27 (87.1%)	10 (50.0%)	28 (71.8%)

¹ Northern Hungary region.

² Northern Transdanubia region.

³ R: resistant, I: intermedier, S: sensitive.

⁴ Two additional *RG57* genotypes were found on both mating types.

and the proportion of isolates sensitive to this fungicide were higher (74.4%) on tomato than on potato (21.3%).

In order to increase the resolution of the recovery of different genotypes, multiple samples were collected from potato fields in two smaller areas in Hungary. Thirty-nine isolates originated from sites in Northern Transdanubia (Borzavár, Porva, Olaszfalu and Tündérmajor) and 20 isolates were collected from Northern Hungary (Ludányhalászi). The ratio of *Pep* genotypes in the Northern Transdanubian region were not different from their ratios in the whole population in Hungary (the distribution was close to 1/3:1/3:1/3), but all the 8 isolates with A1 mating type from that region were of *Pep* 100/100 genotype. Moreover these eight isolates did not show resistance to metalaxyl in spite of the fact that the host was potato (Table 5).

Discussion

The presence of both mating types at the same site gives an opportunity for *P. infestans* to propagate sexually, generating genotypic diversity by means of meiotic recombination. Therefore in those areas where sexual reproduction is present, genetic variability may become as high as that in Central Mexico, the place of origin of the pathogen (Goodwin et al., 1992b).

Isolates of *P. infestans* with A2 mating type were detected in Hungary in 1996 (Bakonyi and Érsek, 1997). Bakonyi et al. (2002b) identified 20 different multilocus genotypes in a relatively small sample of 36 isolates. In this present study, results of analysis of one hundred and eighteen isolates from 40 sites collected during two growing seasons are presented. An additional five isolates were also included from 1993–1998 as a representative set in those years.

Four combinations of 3 alleles were observed at the *Pep* locus. Moreover two of them which are common in Hungary (*Pep* 96/96, and 96/100) proved to be rare outside the country. The *Pep* 83/96 genotype was identified first in Hungary (Bakonyi et al., 2002b) and later in Belgium (Bakonyi et al., 2002a). The high frequency of the *Pep* 96 allele is also typical of the population of *P. infestans* from Hungary. In contrast, only one genotype was detected at the *Gpi* locus (100/100) in this study and in the previous studies (Bakonyi et al., 1998; 2002b). *Gpi* 100/100 has also been the only new genotype detected in UK (Shattock et al., 1990; Carlisle et al., 2001) and has been the predominant genotype in Poland, France (Sujkowski et al., 1994; Lebreton and Andrivon, 1998; Bakonyi et al., 2002a).

Both mating types were detected in eleven of 43 fields (Table 1) facilitating the recombination. Our work uncovered several novel RG57 genotypes of *P. infestans* which had never been reported before. Among the numerous RG57 fingerprints observed in earlier studies only two occurred in 2002 again. When multiple samples were collected from some fields in smaller areas, the diversity remained high. Sites sampled with 5 to 13 isolates did not have dominant genotype (Table 5).

Combining the pheno- and genotypic data into one multilocus genotype, diversity in the Hungarian *P. infestans* populations was even greater. Generally, half of the isolates

had unique multilocus genotypes which had never been observed before although this high diversity itself is not an outstanding event. Some populations in other European countries were similar, but had one or a few genotypes which appeared as a significant proportion of the population (Sujkowski et al., 1994; Brurberg et al., 1999; Zwankhuizen et al., 2000; Day et al., 2004). In contrast to these results, the *P. infestans* population in Hungary does not have any dominant genotype. The most frequent RG57 fingerprint (XXVII) occurred nine times at 5 sites, but the most frequent multilocus genotype occurred as few as six times (at 4 sites) in the whole population. Using isozyme and RG57 markers Goodwin et al. (1992b) found high diversity in Central Mexico, where the sexual recombination is believed to be an integrated part of life cycle of *P. infestans*. The high diversity observed in Hungary also implies that sexual recombination does take place.

According to the great number of different multilocus genotypes in all subpopulation of *P. infestans* (on the basis of host or mating type) the Shannon diversity index was large in each subdivision. Using a normalised Shannon index, ranging between 0 and 1, which allows comparisons between populations of different size, the diversity within any of the four subdivisions analysed ranged from 0.848 to 0.901 (unpublished data). The lack of any significant difference between any subdivisions of the population described by the Shannon indices indicates that the population is not substructured.

The RG57 probe is a useful tool for identifying clonal lineages but the heterozygous state is not easily distinguished from homozygosity and therefore allele frequency cannot be calculated. Only the isozyme genotypes can be used for statistical analysis with calculation of allele frequencies. On the basis of these two markers, Nei's gene diversity analysis (Nei, 1973; Nei and Chesser, 1983) revealed small differences if the isolates were divided into two subdivisions according to their mating type or host. The great diversity observed in the whole population of *P. infestans* is due to the diversity within these subdivisions rather than between them. Therefore no subpopulations can be detected in Hungary based on isozyme genotypes. Although genetic differentiation based on gene diversity can be measured in a fairly straightforward way, it is not clear what G_{ST} value indicates significant differentiation. But only 5 and 2% of the total genetic diversity is due to differences between groups of isolates on the basis of mating type and host, respectively.

These results are in contradiction of others. The similarity in samples from potato and from tomato supports the hypothesis that the samples belong to a simple population. Studies in France, however, indicated that separate potato-specific and tomato-specific subpopulations existed (Lebreton and Andrivon, 1998). In Hungary, according to our results with isozyme markers, the populations of *P. infestans* cannot be substructured on the basis of host possibly because of the massive proportion of sexual reproduction in the life cycle of the pathogen.

Response to metalaxyl is the only one outstanding difference within the *P. infestans* population in Hungary. Samples from tomato mainly originated from gardens/allotments where chemical control is less used. Therefore this selection factor may be less strong in gardens allowing growth of such genotypes which are unable to propagate under conditions of a chemically controlled field.

Oospores are a greater source of genetic diversity than overwintering mycelia. The role of the oospores in the transmission of *P. infestans* populations is not yet well understood. Oospore formation depends on many factors. Solanaceous weeds may also be a significant source of inoculum. Cooke et al. (2002) found oospores in naturally infected woody nightshade, a common weed in agricultural ecosystems. But serious infection may occur under optimal condition in the field and these infections are caused by asexually formed sporangia. It is difficult to estimate the ratio of these two methods of propagation within the population. Assuming that individuals of the same multilocus genotypes originate from asexual reproduction, excluding the clones eliminates one blurring factor from the gene difference analysis. After this simplification the result was similar (*Table 2b*) to those obtained without this correction (*Table 2a*). This also revealed that the role of sexual reproduction may be fundamental in the populations of late blight pathogen in Hungary.

Results of any population genetic statistics, however, are as accurate as many loci are incorporated into the analysis. More markers (e.g. microsatellite) would detect more variation. However small sample sizes from many site can bias the result, because the rare genotypes/phenotypes would often not be detected.

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