



Incidence of the potato late blight pathogen, *Phytophthora infestans*, in Syria and its mating type

Walid Naffaa¹ · Soulima Ibrahim² · Taissir Abou Alfadil³ · Antonious Al-Daoude⁴

Received: 14 January 2017 / Accepted: 26 September 2017 / Published online: 14 October 2017
© Deutsche Phytomedizinische Gesellschaft 2017

Abstract Late blight, caused by *Phytophthora infestans* (Mont.) de Bary, is a devastating disease found worldwide. During 2011, 2013 and 2014 cropping seasons, prevalence of potato late blight in all survived areas throughout the interior of Syria was examined and the disease incidence varied and ranged from 0 to 85.71%. Potato leave samples showing late blight symptoms were collected from five different regions known to grow potato intensively. Based on their morphological characteristics, 22 isolates were distinguished as *P. infestans*. Using two fungal specific primers, ITS3 and PINF2 in a PCR assay, all isolates were confirmed as *P. infestans*, while they produce ~ 450 bp band. Similarly, all confirmed *P. infestans* isolates were able to produce a 170-bp band when INF-1 and INF-2 primers were deployed indicating that studied isolates had merely mating type A1 and no isolate with mating type A2

was found which might explain the absence of oospores in considered areas.

Keywords *P. infestans* · Disease incidence · Mating type · Potato · Syria

Introduction

Late blight disease (LBD) caused by *Phytophthora infestans* Mont. de Bary is a major disease of potato and tomato worldwide and can cause up to 100% yield losses (Nowicki 2012). The oomycete pathogen *P. infestans* initially emerged in the early 1840s in the USA and Europe and rapidly spread across other potato-growing regions, leading to the well-known Irish famine that resulted in the death of over one million people and the immigration of 1.5 million people to further parts of Europe or North America (Haverkort et al. 2009).

Phytophthora infestans is considered as a specialized pathogen, primarily causing disease on the foliage and fruits of a range of Solanaceous species (Erwin and Ribero 1998). Infections may be initiated asexually when sporangia land on the surface of a leaf and release zoospores that rapidly encyst and produce a germ tube where its tip develops into an appressorium and produces an infection peg that penetrates plant tissues. Sporangioophores emerge from the stomata on the underside of the leaf which release sporangia to permit aerial spreading of the pathogen (Jmour and Hamada 2006). For sexual reproduction, *P. infestans* requires both A1 and A2 mating types to produce oospores (Fry and Goodwin 1997). The center of diversity of this oomycete is located in the highlands of Mexico, where both mating types originate. At least two different migration events from Mexico have occurred, the first one

✉ Walid Naffaa
walid1851966@yahoo.com

Soulima Ibrahim
soulima222@gmail.com

Taissir Abou Alfadil
tafadil@yahoo.com

Antonious Al-Daoude
aaldaoude@gmail.com

¹ Plant Protection Department, Faculty of Agriculture, Damascus University, 30621 Damascus, Syria
² General Commission for Scientific Agricultural Research (GCSAR), Damascus, Syria
³ Al Rasheed International Private University for Science and Technology, Daraa, Syria
⁴ Department of Molecular Biology and Biotechnology, Atomic Energy Commission of Syria, PO Box 6091, Damascus, Syria

is postulated to have taken place before 1845, after which *P. infestans* swept through Europe and Ireland (Drenth et al. 1994). The second migration happened in the 1970s to 1990s, bringing the A2 mating type out of Mexico resulting in the formation of oospores in many regions in the world (Fry et al. 1993; Runno-Paurson et al. 2010). However, only few efforts have been made to clarify the role of oospores in initiating and in the epidemiology of late blight (Andrison 1995; Drenth et al. 1995; Andersson et al. 1998; Pittis and Shattock 1994). A PCR assay was developed for the detection of late blight of potato and determination of the pathogen mating type in many parts of the world which is based on small nucleotide differences in the internal transcribed spacer sequences between 18S, 5.8S and 28S ribosomal DNAs (Hussain et al. 2005; Jyan et al. 2002; Judelson and Tooley 2000).

The aims of this study were to determine the distribution of late blight disease on potatoes in Syria and to detect the mating type of obtained *P. infestans* isolates deploying a PCR assay.

Materials and methods

Field survey and sampling

During 2011, 2013 and 2014, a field survey was conducted in order to examine all major potato-growing regions in Syria for the prevalence of potato late blight disease. The frequency of *P. infestans* on symptomatic potato plants was recorded, and samples of potato leaves showing late blight symptoms were collected randomly from 12 fields in five districts (Daraa, Hama, Al Ghab, Latakia and Tartous).

Pathogen isolation

Infected leaves were superficially sterilized for 2 min in 3% sodium hypochlorite, washed 3 times with sterile water, cut into small pieces before they were placed under potato slices in Petri dishes. Dishes were incubated at 20 ± 2 °C for 7 days until abundant sporulation appeared on the upper side of the slice. Clean and pure mycelium was placed on Rye Agar medium (RAM) (60 g of rye grain, 15 g of agar and 20 g of dextrose in 1 l of distilled water) amended with 12 mg/l of rifampicin in Petri dishes. Incubation was carried out for at least 2 weeks at 20 °C (Jmour and Hamada 2006). Fungus identification was based on colony, sporangiophores and sporangia morphological characteristics.

DNA extraction

Genomic DNA was extracted from the mycelium of 22 *P. infestans* isolates according to the cetyltrimethylammonium bromide (CTAB) procedure (Murray and Thompson

1980). 150 mg of fresh mycelium was ground in liquid nitrogen in an Eppendorf tube of 2 ml to which 850 µl of extraction buffer CTAB (0.1 M Tris HCl at pH 8.0, 1.4 M NaCl, 20 mM EDTA at pH 8.0, 2% (w/v) CTAB and 3% 2-mercapto-ethanol) was added. The suspension was incubated for 30 min at 65 °C, and the content was gently mixed by inversion of the tubes once every 10 min. A volume of 700 µl of chloroform/isoamyl alcohol (24 v:1 v) was then added, and the resulting mix was centrifuged for 10 min at 10,000 rpm/min at 4 °C. 500 µl of the supernatant was transferred to another Eppendorf tube to which 500 µl of chloroform/isoamyl alcohol (24 v:1 v) was added and centrifuged as previously. 500 µl of the supernatant was again transferred to a fresh Eppendorf tube and placed on ice for 20–30 min. DNA was precipitated by adding two volumes of ethanol and centrifugation at 10,000 rpm for 10 min at 4 °C. DNA pellets were separated from supernatants, washed twice with 70% ethanol, re-centrifuged at 10,000 rpm for 10 min and dried at room temperature. Finally, pellets were dissolved in TE buffer (10 mM Tris HCl, 1 mM EDTA at pH 8.0) and stored at –20 °C. DNA yield and purity were determined by a spectrophotometer.

P. infestans identification by a specific PCR assay

PCR amplification was conducted in a 30-µl volume containing: 300 ng of fungal DNA, 1.5 mM MgSO₄, 2 µM dNTPs, 1× PCR buffer (3 µl), 1.5 unit of *Taq* DNA polymerase, 2 µM of each primer, ITS3 (5'-GCATC-GATGAAGAACGCAGC-3') and PINF2 (5'-CGATT-CAAATGCCAA GCTAAAG-3') and distilled water (Tooley et al. 1997). Another pair of primers, ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and PERY2 (5'-CTGTTCCGGCGTAAGCTG-3') described for *P. erythrospora*, was also tested. The amplification was initiated by a denaturation at 94 °C for 5 min, followed by 30 cycles, each consists of (a 10-s denaturation at 94 °C, a 30-s annealing at 55 °C, a 40-s elongation at 72 °C) and a final elongation at 72 °C for 5 min in a thermocycler. PCR amplicons were analyzed after fractionation by 1% Agarose gel electrophoresis in 1× TBE buffer, stained with ethidium bromide.

Mating type determination by PCR

The mating type of *P. infestans* isolates was determined by a specific PCR assay in a 25-µl reaction mixture containing 300 ng of fungal DNA, 1 mM MgSO₄, 2 µM dNTPs, 1× PCR buffer, 1.5 unit of *Taq* DNA polymerase, 2.25 µM of each primer (specific for detecting mating type A1), INF-1 (5'-AAGCTATACTGGGACAGGGT-3') and INF-2 (5'-GCGTTCTTTCGTATTACCAC-3') and distilled water.

The amplification was initiated by denaturation at 95 °C for 4 min, followed by 30 cycles of (95 °C/35 s, 56 °C/35 s, 70 °C/35 s), and a 5-min extension at 70 °C in a thermocycler. PCR amplicons were analyzed after fractionation by 1% Agarose gel electrophoresis in 1× TBE buffer and stained with ethidium bromide.

Results and discussion

Occurrence of late blight diseases

The incidence of late blight on potato varied among geographical locations where potatoes are mainly and heavily grown in Syria. Late blight prevalence rate ranged from 0 to 85.71%. The highest disease frequency was recorded in Hama (85.71%) and Al Ghab (71.42%), while it was relatively low in Latakia (16%) and Tartous (11.11%). However, no infection was observed in the unique studied site in Daraa (Table 1).

Variability in disease frequency on potato and tomato has been recorded in many locations and countries worldwide. In Uganda, studies conducted by Mukalazi et al. (2000) showed that late blight incidence in various countries ranged from 5 to 85.4%. The variation of disease incidence and severity may be accounted for differences in rainfall patterns between seasons and years. Furthermore, variation has been equally attributed to susceptibility and resistance of diverse varieties grown in many areas, different planting dates (disease escape) and various late blight management practices (Olanya et al. 2009).

Fungal isolation

Samples of blighted potato leaves were obtained from 12 fields distributed over five different geographical areas where potatoes are grown in Syria. After isolation which was based on morphological characteristics of mycelium, sporangiophores and sporangia, 22 isolates out of 83 (27.71%) were morphological classified *P. infestans* (Kirk et al. 2009; Lepoivre 2003).

PCR amplification of *P. infestans* DNA

Genomic DNA of the 22 isolates morphologically determined to be *P. infestans* was extracted and amplified using ITS3 and PINF2 primer combination in a PCR assay. All isolates showed a ~ 450 bp band (Fig. 1), while no band was detected when ITS4 and PERY2 primers described for *P. erythrosetpica* were used (data not shown). These results confirmed the morphological classification and further confirmed the identity of obtained isolates as *P. infestans*. In fact, many primer combinations designed for specific detection of *P. infestans* are available. The use of PINF and ITS5 primers resulted in a PCR amplification of an approximately 600 base pair product with barely isolates of *P. infestans* from potato and tomato (Trout et al. 1997). Jyan et al. (2002) demonstrated that a PCR analysis with Pi1S-1/Pi2A-1 primers gave rise to amplified products of approximately 350 bp from *P. infestans* as well as other *Phytophthora* spp., while Pi1S-1/Pi2A-2 primers generated a DNA band only from *P. infestans* but not from other fungal species.

Identifying the mating type of *P. infestans* isolates

The mating type of the 22 isolates of *P. infestans* was identified by a PCR assay with INF-1 and INF-2 primers that are specific for mating type A1 (Mat-A1). DNA fragment of 170 bp was amplified (Fig. 2) indicating that all tested *P. infestans* isolates are proved to be of Mat-A1. This suggests that the Syrian *P. infestans* population may belong to the “old” *P. infestans* type (A1), which is the most common mating type of *P. infestans* described all over the world. Our results were similar to those of previous studies, where the same pair of primers (INF-1/INF-2) was designed and used to differentiate *P. infestans* Mat-A1 from Mat-A2 strains (Runno-Paurson et al. 2010). The Mat-A2 of *P. infestans* was later detected in Tunisia (Jmour and Hamada 2006), in Turkey (Tosun et al. 2007) and in Czech Republic (Mazáková et al. 2006). There have been reports of a lower A1:A2 ratio from several European countries (Hermansen et al. 2000; Bakonyi et al.

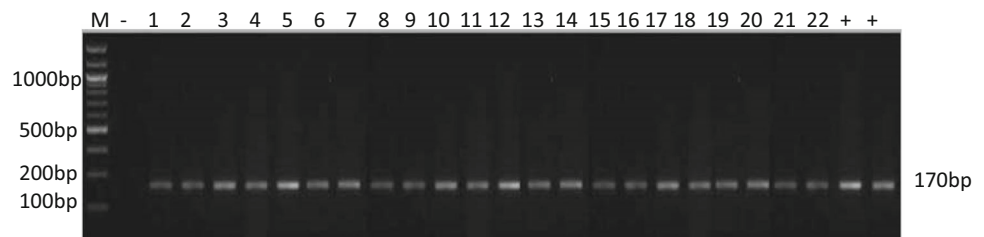
Table 1 Incidence percentage of late blight disease at the main Syrian potato-growing areas

Place of collection	Date of collection	Number of samples	Number of infected samples by <i>P. infestans</i>	Disease incidence (%)
Daraa	25/11/2011	10	0	0
Hama	05/11/2014	7	6	85.71
Al Ghab	18/11/2013	14	10	71.42
Latakia	12/11/2014	25	4	16
Tartous	10/11/2014	27	3	11.11
		83	23	27.71



Fig. 1 Agarose gel electrophoresis of a PCR assay using ITS3/PINF2 specific primers for detecting *P. infestans*. M: molecular marker, numbers from 1 to 22 are fungal isolates, +: positive control

Fig. 2 PCR profile using INF-1 and INF-2 specific primers for A1 mating type of *P. infestans*. M: molecular marker, numbers from 1 to 22 are fungal isolates, +: positive controls



2002; Cooke et al. 2007; Lehtinen et al. 2007). A higher proportion of A2 mating type has been found in Austria, Netherlands and Poland (Avenidaño Córcoles 2007; Zwankhuizen et al. 2000; Sliwka et al. 2006). In contrast, all obtained isolates between 1994 and 2000 in Antioquia and Colombia were of the Mat-A1 (Ramelli et al. 2009). In southern Germany, the majority of isolates collected in 1995 and 1999 were characterized as Mat-A1 (Moller et al. 2009), and the majority of Polish isolates of *P. infestans* were also of the A1 mating type (Chmielarz et al. 2014). The frequency of A1 and A2 mating type may change according to the region, the host plant and the year. For instance, there was a mating type shift in the population from predominantly A2 to completely A1 in the period 1994–1997 and the co-occurrence of mating type was restricted to very few fields in the area around Los Mochis where tomato and potato crops are grown (Jaime-Garcia et al. 2001). The A1 and A2 mating type ratio is important to aid in the prediction of the extent of sexual recombination and thus the risk of long-lived oospores serving as primary inoculum sources. Our results showed the only presence of Mat-A1 of all *P. infestans* isolates that suggests no sexual reproduction, and formation of oospores can occur in Syria, and the primary inoculum source of late blight infection under local conditions is infected tubers. So, it is highly recommended that farmers select only healthy tubers and eliminate the infected ones in order to reduce the risk of consecutive *P. infestans* infections.

Acknowledgements The authors would like to thank the Director General of the Atomic Energy Commission of Syria and the head of the Molecular Biology and Biotechnology department for their continuous support throughout this work.

Compliance with ethical standards

Conflict of interest Walid Naffaa, on behalf of the co-authors, we have no conflict of interest.

References

- Andersson B, Sandström M, Stroëmberg A (1998) Indications of soil borne inoculum of *Phytophthora infestans*. *Potato Res* 41:305–310
- Andrion D (1995) Biology, ecology and epidemiology of the potato late blight pathogen *Phytophthora infestans* in soil. *Phytopathology* 85:1053–1056
- Avenidaño Córcoles J (2007) Survey of *Phytophthora infestans* population in Austria based on phenotypic and molecular markers. Ph.D. Thesis, University of Natural Resources and Applied Life Sciences, Vienna, Austria
- Bakonyi J, Ládai M, Dula T, Érsek T (2002) Characterization of isolates of *Phytophthora infestans* from Hungary. *Eur J Plant Pathol* 108:139–146
- Chmielarz M, Sobkowiak S, Debski K, Cooke DEL, Brurberg MB, Sliwka J (2014) Diversity of *Phytophthora infestans* from Poland. *Plant Pathol* 63:203–211
- Cooke DEL, Schena L, Cacciola SO (2007) Tools to detect, identify and monitor *Phytophthora* species in natural ecosystems. *J Plant Pathol* 89:13–28
- Drenth A, Tas ICQ, Govers F (1994) DNA fingerprinting uncovers a new sexually reproducing population of *Phytophthora infestans* in the Netherlands. *Eur J Plant Pathol* 100:97–107
- Drenth A, Janssen EM, Govers F (1995) Formation and survival of oospores of *Phytophthora infestans* under natural conditions. *Plant Pathol* 44:86–94
- Erwin DC, Ribero OK (1998) *Phytophthora* diseases worldwide. *Plant Pathol* 47:224–226
- Fry WE, Goodwin SB (1997) Re-emergence of potato and tomato late blight in the United States and Canada. *Plant Dis* 81:1349–1357
- Fry WE, Goodwin SB, Dyer AT, Matuszak JM, Drenth A, Tooley PW, Sujkowski LS, Koh YJ, Cohen BA, Spielman LJ, Deahl KL, Inglis DA, Sandlan KP (1993) Historical and recent migrations

- of *Phytophthora infestans*: chronology, pathways and implications. *Plant Dis* 77:653–661
- Haverkort AJ, Struik PC, Visser RGF, Jacobsen E (2009) Applied biotechnology to combat late blight in potato caused by *Phytophthora infestans*. *Potato Res* 52:249–264
- Hermansen A, Hannukkala A, Naerstad RH, Brurberg MB (2000) Variation in populations of *Phytophthora infestans* in Finland and Norway: mating type, metalaxyl resistance and virulence phenotype. *Plant Pathol* 49:11–22
- Hussain S, Lees AK, Duncan JM, Cooke DEL (2005) Development of a species-specific and sensitive detection assay for *Phytophthora infestans* and its application for monitoring of inoculum in tubers and soil. *Plant Pathol* 54:373–382
- Jaime-García R, Orum TV, Felix-Gastelum R, Trinidad-Correa R, Vanetten HD, Nelson MR (2001) Spatial analysis of *Phytophthora infestans* genotypes and late blight severity on tomato and potato in the Del Fuerte Valley using geostatistics and geographic information systems. *Phytopathology* 91:1156–1165
- Jmour W, Hamada W (2006) First report of A2 mating type of *Phytophthora infestans* in Tunisia using molecular markers and some observations on its metalaxyl resistance. *Tunis J Plant Prot* 1:85–91
- Judelson HS, Tooley PW (2000) Enhanced polymerase chain reaction methods for detecting and quantifying *Phytophthora infestans* in plants. *Phytopathology* 90:1112–1119
- Jyan MH, Huang LC, Ann PJ, Liou RF (2002) Rapid detection of *Phytophthora infestans* by PCR. *Plant Pathol Bull* 11:25–32
- Kirk WW, Samen F, Abu El, Wharton P, Douches D, Tumbalam P, Thill C, Thompson A (2009) Impact of different US genotypes of *Phytophthora infestans* on potato seed tuber rot and plant emergence in a range of cultivars and advanced breeding lines. *Potato Res* 51:121–140
- Lehtinen A, Hannukkala A, Andersson B, Hermansen A, Le VH, Naerstad R, Brurberg MB, Nielsen BJ, Hansen JG, Yuen J (2007) Phenotypic variation in Nordic populations of *Phytophthora infestans* in 2003. *Plant Pathol* 57:227–234
- Lepoivre P (2003) *Phytopathology*. De Boeck, Bruxelles
- Mazáková J, Táborický V, Zouhar M, Ryšánek P, Hausvater E, Doležal P (2006) Occurrence and distribution of mating types A1 and A2 of *Phytophthora infestans* (Mont.) de Bary in the Czech Republic. *Plant Prot Sci* 42:41–48
- Moller K, Dilger M, Habermeyer J, Zinkernagel V, Flier WG, Hausladen H (2009) Population studies on *Phytophthora infestans* on potatoes and tomatoes in southern Germany. *Eur J Plant Pathol* 124:659–672
- Mukalazi J, Adipala E, Sengooba T (2000) Population structure of *Phytophthora infestans* in Uganda. *Crop Prot (Natrass RM, Ryan M (1951) New hosts of *Phytophthora infestans* in Kenya. *Nature* 168:85–86)*
- Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res* 10:4321–4325
- Nowicki N (2012) Potato and tomato late blight caused by *Phytophthora infestans*: an overview of pathology and resistance breeding. *Plant Dis* 96:4–17
- Olanya MO, Ojiambo PS, Nyankanga RO, Honeycutt CW, Kirk WW (2009) Recent developments in managing tuber blight of potato (*Solanum tuberosum*) caused by *Phytophthora infestans*. *Can J Plant Path* 31:280–289
- Pittis JE, Shattock RC (1994) Viability germination and infection potential of oospores of *Phytophthora infestans*. *Plant Pathol* 43:387–396
- Ramelli EG, Villegas JS, Reynaldi S (2009) Efecto sobre la sarna polvosa de cuatro aislamientos del hongo *Trichoderma asperellum* en tres tipos de suelo. En: Colombia Revista Facultad Nacional De Agronomía - Medellín ISSN: 0304-2847, ed: Universidad Nacional de Colombia v.62 fasc.1, pp 4783–4792
- Runno-Paurson E, Rimmel T, Koppel M, Tähtjärv T (2010) Occurrence and distribution mating types A1 and A2 of *Phytophthora infestans* in eastern Estonia. *Agron Res* 8(Special Issue II):471–474
- Śliwka J, Sobkowiak S, Lebecka R, Avendaño-Córcoles J, Zimnoch-Guzowska E (2006) Mating type, virulence, aggressiveness and metalaxyl resistance of isolates of *Phytophthora infestans* in Poland. *Potato Res* 49:155–166
- Tooley PW, Bunyard BA, Carras MM, Hatziloukas E (1997) Development of PCR primers from internal transcribed spacer region 2 for the detection of *Phytophthora* species infecting potatoes. *Appl Environ Microbiol* 63:1467–1475
- Tosun N, Yildirim A, Turkusay H, Tanyolac B (2007) Genetic variation among *Phytophthora infestans* (tomato blight) isolates from western Turkey revealed by inter simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) markers. *Pak J Bot* 39(3):897–902
- Trout CL, Ristaino JB, Madritch M, Wangsomboondee T (1997) Rapid detection of *Phytophthora infestans* in late blight-infected potato and tomato using PCR. *Plant Dis* 81:1042–1048
- Zwankhuizen MJ, Govers F, Zadoks JC (2000) Inoculum sources and genotypic diversity of *Phytophthora infestans* in Southern Flevoland, the Netherlands. *Eur J Plant Pathol* 106:667–680