Journal of Agricultural Technology 2010 Vol.6(1): 117-125
Available online http://www.ijat-rmutto.com
ISSN 1686-9141

Characterization of *Phytophthora infestans* population in potato crops from Chiang Mai and Tak Provinces

Jaimasit, P. and Prakob, W.*

Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Thailand.

Jaimasit, P. and Prakob, W. (2010). Characterization of *Phytophthora infestans* population in potato crops from Chiang Mai and Tak Provinces. Journal of Agricultural Technology 6(1): 117-125.

A total of 117 isolates of *P. infestans* were isolated from blighted potato foliage. The isolates were obtained from major potato-growing areas in Chiang Mai and Tak provinces between 2006 and 2009. These isolates were analyzed for their mating type, resistance to metalaxyl and mtDNA haplotypes. The results showed that all of these isolates are mating type A1 and the most were susceptible to metalaxyl, with 27 isolates being metalaxyl intermediate and 11 isolates were isolates to metalaxyl. In addition, one of four mtDNA haplotypes, IIa, dominated the population. This finding suggests that limited diversity within the current studied field population of *P. infestans* in Chiang Mai and Tak provinces. However, to gain a better understanding of structure and biodiversity among *P. infestans* populations in Thailand, these isolates as well as isolates acquired from other areas should be further genotypically characterized by using additional molecular techniques.

Key words: Late blight, *Phytophthora infestans*, metalaxyl resistance, mating type and population genetics

Introduction

Phytophthora infestans is an Oomycetes responsible for the late blight disease found in potatoes and tomatoes. First appearing in the 1840s as the cause of the Irish potato famine, late blight has become a particularly devastating disease worldwide during the past few decades (Goodwin *et al*., 1994a; Goodwin *et al*., 1994b). The pathogen is heterothallic and forms oospores between A1 and A2 mating types (Galindo and Gallegly, 1960; Judelson, 1997). Until the 1980s, the global population of *P. infestans* outside of central Mexico was thought to be derived from a single A1-mating-type clonal lineage, the 'old' population known as the US-1 lineage (Goodwin *et al*., 1994b). However, since then, isolates of 'new' populations of both A1 and A2

*Corresponding author: Waraporn Prakob; e-mail: awarapornp@hotmail.com

mating types have been found in other areas around the world (Hohl and Iselin, 1984; Deahl *et al*., 1991). Some of these isolates were shown to express metalaxyl resistance and a broader range of virulence factors (Dowley and Sullivan, 1981). The genetic and phenotypic diversity of *P. infestans* populations has subsequently been investigated in many countries (Goodwin *et al*., 1994a; Goodwin *et al*., 1995c; Koh *et al*., 1994; Day *et al*., 2004). These studies suggested that new populations of *P. infestans* appear as a result of migration into regions and / or sexual recombination within them. A global marker database for *P. infestans*, containing information on RFLPs obtained using the RG57 probe, mitochondrial DNA (mtDNA), haplotypes (Carter *et al*., 1990; Goodwin, 1991) allozyme genotypes, sensitivity to metalaxyl, mating types and other factors, was compiled (Forbes *et al*., 1998) Following the construction of the database, some reports of Asian *P. infestans* populations were published. Isolates found in Korea, India, Taiwan, Indonesia, Thailand, Nepal and China during the period from 1992–1997 were also investigated and the results showed A2-mating-type isolates and Asian-specific allozyme genotypes. In Thailand, potato is usually grown in a single winter crop grown primarily from October to February in Northern and North-eastern part of the country. Growing areas of potato tend to increase every year to provide potato to food industry. Even though, the late blight epidemics have not been well recorded in Thailand, occurrence of the disease is found every year, often causing seriously economic losses. The growers have incurred to expense fungicide application to eliminate the disease. Phenylamide fungicides, especially metalaxyl, were the most effective and commonly used to control late blight. It was shown that isolates of 'new' populations of both A1 and A2 mating types, some of which were metalaxyl resistant (Dowley and Sullivan, 1981) and had a broader range of virulence factors (Smoot *et al*., 1958) that have been found in many parts of the world (Hohl and Iselin, 1984; Deahl *et al*., 1991). To establish the most effective control strategies for late blight, it is neccessary for up-to-date information on dispersal and variation within local *P. infestans* populations. Therefore, the appearance and rapid spread of metalaxyl-resistant strains of *P infestans* isolates found in Thailand should be thoroughly investigated. Moreover, studying the genetic and phenotypic structure of the *P. infestans* population in Thailand is required to provide an accurate understanding of the population structure and gene flow between *P. infestans* populations. The objective of this study was aims to characterize the population of *P. infestans* isolates, collected from various potato-cultivating areas in Chiang Mai and Tak provinces, by determining their mating type, mitochondrial DNA (mtDNA) haplotypes and resistance to the chemical Metalaxyl.

Materials and methods

Sampling of P. infestans isolates

Isolates of *P. infestans* in the infested areas were obtained from six major potato-growing areas of Chiang Mai and Tak provinces. The diseased samples were collected during potato growing seasons between 2006 and 2009 from the blighted potato leaves, with freshly sporulating lesions of *P. infestans*. The samples were kept in plastic bags brought to the laboratory.

Isolation of P. infestans

To stimulate sporulation, the infected potato leaflets were placed in a plastic box containing moist filter paper and incubated in darkness at 18°C for 24 hours. The leaflets, with freshly formed sporangia were pressed to selective media amended with antibiotics (ampicillin 50 µg/ml, nystatin 100 µg/ml, rifampicin 50 µg/ml and benomyl 10 µg/ml). The plates were then incubated at 18 ^{o}C for 5-7 days for mycelial growth. The hyphal tips were cut from the colony margins and transferred to amended rye A agar to get pure cultures. Pure cultures was maintained in unamended rye A agar for further study..

Mating type differentiation

Mating type was determined by placing an unknown isolate at a distance of 2.5 cm between two known A2 tester isolates of *P. infestans* (E13 and 618, which had been isolated from Egypt and Mexico, respectively) on Rye A agar media. Hyphal interaction zones were microscopically observed after 7 days incubation at 18°C in darkness. Oospores were produced in the margins of opposite mating types. Isolates that produced oospores with the known A2 tester isolates were designated as the A1 mating type and isolates that did not produce oospores with the known A2 tester isolates were designated as the A2 mating type. Self-fertile isolates were observed by examining 10 blocks of Rye A agar on which each isolate produced Oospore. Since there is no standard A1 used in this study, the isolates designated as mating A2 no Oospore formation were mated again with the mating type A1 isolates. Duplicate mating type tests were performed.

Metalaxyl resistance

All isolates of *P. infestans* were tested for phenylamide fungicide and metalaxyl resistance *in vitro*. The EC50 values were calculated on the basis of growth inhibition on rye A agar amended with four concentrations (0, 5 and

100 mg/l) of metalaxyl. The isolates were assigned to one of three groups: sensitive (EC50<5 mg/L), intermediate (5 mg/L<EC50< 100 mg/l) or resistant (EC50> 100 mg/l). Three replicates were used for each isolate.

MtDNA haplotypes

Mitochondrial DNA (mtDNA) haplotypes of 117 isolates were identified by using the method previously described by Griffith and Shaw (1998). DNA extraction was made by Nucleospin kit (Macherey-Nagel Inc. PA, USA). The primer pairs used to amplify region P2 and P4 were F2+R2 and F4+R4, respectively.

F2 (5'- TCCCTTTGTCCTCTACCGAT -3')
R2 (5'- TTACGGCGGTTTAGCACATACA -3')
F4 (5'-TGGTCATCCAGAGGTTTATGTT -3')
R4 (5'- CCGATACCGATACCAGCACCAA -3')

The polymerase chain reaction (PCR) products of P2 and P4 were digested with *Hpa*II instead of *Msp*I (Griffith and Shaw, 1998) in the same site, and *Eco*RI then, analysed by 2% agarose gel electrophoresis.

Results

Isolation of P. infestans

Due to unfavorable climate conditions during low temperature in December 2006 and February 2009, occurrence of late blight epidemic were extremely limited. A total of 117 isolates of *P. infestans* were iobtained from infected potato leaves that collected from two major potato-cultivating areas in Chiang Mai and Tak provinces. Pure cultures of all isolates were obtained by culturing sporangia on a selective medium amended with antibiotics. The colony morphology appears white and fluffy similar to all isolates, nonseptate sporangiophore. Sporangia were an average of 45 µm in length and 27 µm in width with a length/broadth ratio of 1.66. Sporangia were caducous and limoniform to ovoid in shape.

Mating type and sensitivity to metalaxyl

All isolates were proved to be A1 mating type as thick-walled oospores that produced during mating between isolates in both standard A2 isolates, E13 and 618. The thick-walled oospores were attached by antheridium around the oogonial stalk (amphigynous). No self-fertile isolates were found in this study. *In vitro* assays for metalaxyl sensitivity indicated that most isolates collected

between December 2006 and February 2009 showed 27 isolates were metalaxyl sensitive and 11 isolates were metalaxyl intermediate and resistant (Table 1).

Mitochondrial DNA haplotype

The amplification product sizes obtained from both regions (P2 and P4) of mitochondrial DNA were similarly with those obtained by Griffith and Shaw (1998). After digestion of PCR products with restriction enzymes, the size of restriction fragments were similar to those obtained by Griffith and Shaw (1998). Three restriction fragments (720, 203 and 147 bp) were obtained after cutting P2 amplified product with *Hpa*II (Fig. 1) whereas two restriction fragments (603 and 361 bp) were obtained after P4 amplified product that was cut with *Eco*RI (Fig. 2). This finding indicated that all the 117 isolates were IIa haplotype.

Table 1. Characteristics of *Phytophthora infestans* isolates collected from potato crops in Chiang Mai and Tak provinces between 2006 and 2009.

Districts	Sampling year	No. of fields	No. of isolates	Mating type		Metalaxyl[b] resistance			mtDNA haplotype			
				A1	A2	S	I	R	Ia	Ib	IIa	IIb
Sunsai, CM[a].	2006	5	61	61	0	58	3	0	0	0	61	0
Praow, CM.	2007	1	20	20	0	19	1	0	0	0	20	0
Sunsai, CM.	2009	1	15	15	0	2	11	2	0	0	15	0
Pobpra, Tak	2009	1	21	21	0	0	12	9	0	0	21	0

[a]Chiang Mai.
[b]Tak
[c]S, I, and R denote sensitive, intermediate and resistant to metalaxyl, respectively.

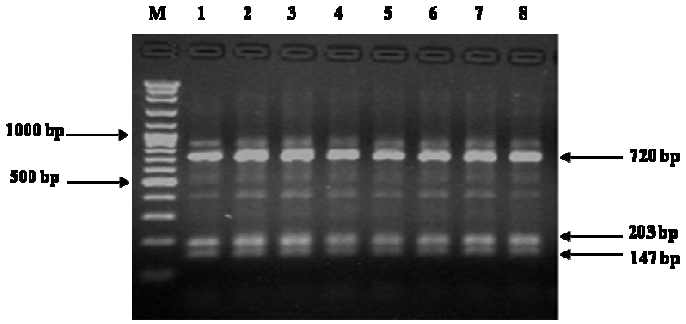

Fig. 1. Restriction enzyme digestions of PCR products amplified from *P. infestans*' mtDNA with primer pair F2-R2 (cut with *Hpa*II). Amplifications were conducted with DNA from a representative isolate of each field. Lane marked M contains 100-bp ladder (Vivantis Ltd.).

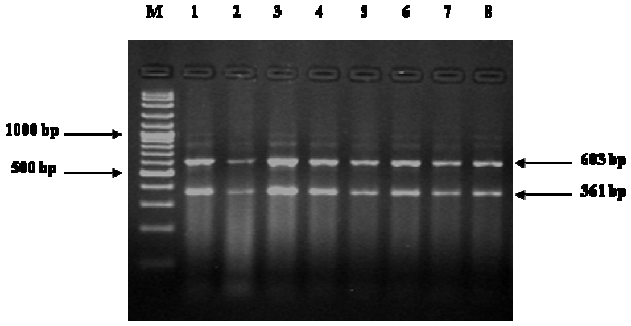

Fig.2. Restriction enzyme digestions of PCR products amplified from *P. infestans*' mtDNA with primer pair F4-R4 (cut with *Eco*RI). Amplifications were conducted with DNA from a representative isolate of each field. Lane marked M contains 100-bp ladder (Vivantis Ltd.).

Discussion

The presence of two mating types of heterothallic isolates of *Phytophthora infestans* is a prerequisite for their sexual reproduction and their mating type has been used as an indication of the origin (Galindo and Gallegly, 1960; Koh *et al*., 1994). Before 1984, the A2 mating type had only been found in Mexico, which was widely accepted as the possible origin of *P. infestans*. The A1 mating type prevailed throughout the rest of the world, including the United States, Canada, Western Europe, South Africa, and West India (Smoot *et al*., 1958). In recent years, the A2 mating type has been detected in many parts of the world. In 1984, it was first by reported in Switzerland and subsequently discovered throughout Europe, North America and Asia. This suggests that migration was the cause of the new occurrences of the A2 mating type. Long-distance migration of *P. infestans* frequently appears to be the inadvertent movement of infected plant materials (potato tubers, tomatoes), an unintended result of international trade. The appearance of new populations of *P. infestans* has often been accompanied by devastating results: loss of resistant varieties of hosts, the appearance of fungicide-resistant strains (Semal, 1995; Shaw, 1987; Sujkowski *et al*., 1994) and a broader range of virulence factors (Drenth *et al*., 1994). Potatoes are significant vegetable crops in Thailand, both of which are vulnerable to late blight disease. In the current study, a total of 117 isolates of *P. infestans* were obtained from infected potato leaves. The isolates of *P. infestans* tested in this study proved to be the A1 mating type. These results were similar to the study by Nishimura *et al.* (1999) and Petchaboon (2003). A1 mating type isolates were found in the districts of Mae-rim, Mae-Tang, Sunsai and Praow, which are located in Chiang Mai. However, the A2 mating isolates were only found in Chaiprakran and Fang

districts which are also located in Chiang Mai (Nishimura *et al*., 1999). This finding indicated there is no change in the mating types of the *P. infestans* populations in Sunsai and Praow districts. The isolates studied by Gotoh *et al* (2005), in which from a total of 44 isolates collected in Chiang Mai in 1994, 22 mating type A2 isolates were found, however the specific origin of these isolates was not confirmed (Gotoh *et al*., 2005). Most isolates in this finding were metalaxyl sensitive, with the exception of 27 isolates were metalaxyl intermediate, 11 isolates being resistant. In contrast, all isolates reported by Nishimura *et al*., 1999 and Gotoh *et al*. (2005) were sensitive to metalaxyl. Petchaboon (2003) also reported that most of isolates collected in Sunsai and Praow districts were resistant to metalaxyl. That is different from our research findings in that most of the isolates from the Sunsai and Praow districts were susceptible to metalaxyl. This might be due to different field collection. The application of metalaxyl in the areas where the isolates were collected in this study was less or these areas were newly cultivated to potato. Our result is similar to the findings by Petchaboon (2003) in that most of the Thai *P. infestans* that are resistant to metalaxyl. In this study the cultivated potato in Pobpra district, Tak province were heavy use of fungicides. It is possible that resistant to metalaxyl was a result (Dowley and Sullivan, 1981). It was also found that almost all 117 isolates have mtDNA haplotype IIa. Griffith and Shaw (1998) addressed that haplotype Ia was most often associated with A1, and haplotype IIa was most often associated with A2 mating type, however Ia haplotype was not found in this study. The results suggested that there was low diversity among *P. infestans* populations. However, this current study provides preliminary data of the *P. infestans* population in Thailand. Further work is needed to establish a complete structure of the entire population as well as gene flow between *P. infestans* populations. Moreover, to determine whether the Thai *P. infestans* population belongs to the old or new type and if there are any Thai and other Asian specific genotypes or recombinant genotypes among the populations, it will be necessary to characterize isolates used in this study and other isolates collected from potato fields from other areas. By using additional markers such as RFLP profiles, obtained by using the RG57 probe, mitochondrial DNA (mtDNA) haplotypes, allozyme genotypes and other factors, will be compiled.

Acknowledgements

This work has been financially supported by Chiang Mai University (awarded in 2009 for young investigators) and also the Graduate School, Chiang Mai University. We are grateful to Prof. Howard S Judelson, Dept. of Plant Pathology, University of California, Riverside, for providing helpful advice and the strains of A2 mating type. We also thank the Central lab,

Faculty of Agriculture, CMU for providing the lab facility. We would also like to thank Greg T Sholly for editing the manuscript.

References

Carter, D.A., Archer, S.A., Buck, K.W., Shaw, D.S. and Shattock, R.C. (1990). Restriction fragment length polymorphisms of mitochondrial DNA of *Phytophthora infestans*. Mycological Research 94: 1123–1128.

Day, J.P., Wattier, R.A.M., Shaw, D.S. and Shattock, R.C. (2004). Phenotypic and genotypic diversity in *Phytophthora infestans* on potato in Great Britiain, 1995–1998. Plant Pathology 53: 303–315.

Deahl, K.L., Goth, R.W., Young, R., Sinden, S.L. and Gallegly, M.E. (1991). Occurrence of the A2 mating type of *Phytophthora infestnas* in potato fields in the United States and Canada. American Potato Journal 68: 717–726.

Dowley, L.J. and E. O'Sullivan. (1981). Metalaxyl-resistant strains of *Phytophthora infestans* (Mont.) de Bary in Ireland. PotatoResearch 24: 417–421.

Drenth, A., Tas, I.C.Q. and Govers, F. (1994). DNA fingerprinting uncovers a new sexually reproducing population of *Phytophthora infestans* in the Netherlands. European Journal of Plant Pathology 100: 97–107.

Forbes, G.A., Goodwin, S.B., Drenth, A., Oyarzun, P., Ordonez, M.E. and Fry, W.E. (1998). A global marker database for *Phytophthora infestans*. Plant Disease 82: 811–818.

Galindo, J.A. and Gallegly, M.E. (1960). The nature of sexuality in *Phytophthora infestans*. Phytopathology 50: 123–128.

Goodwin, S.B. (1991). DNA polymorphisms in *Phytophthora infestans*: the Cornell experience. In: Lucas JA, Shattock RC, Shaw DS, Cooke LR, eds. Phytophthora. Cambridge, UK: Cambridge University Press : 256–271.

Goodwin, S.B., Cohen, B.A. and Fry, W.E. (1994b). Panglobal distribution of a single clonal lineage of the Irish potato famine fungus. Proceedings of the National Academy of Sciences,USA 91: 11591–11595.

Goodwin, S.B., Cohen, B.A., Deahl, K.L. and Fry, W.E. (1994a). Migration from northern Mexico as the probable cause of recent genetic changes in populations of *Phytophthora infestans* in the United States and Canada. Phytopathology 84: 553–558.

Goodwin, S.B., Sujkowski, L.S. and Fry, W.E. (1995c). Rapid evolution of pathogenicity within clonal lineages of the potato late blight disease fungus. Phytopathology 85: 669–676.

Gotoh, K., Akino, S., Kato, M., Kondo, N. and Naito, S. (2005). *Characterization of some Asian isolates of* Phytophthora infestans. Plant Pathology 54: 733–739.

Griffth, G.W. and Shaw, D.S. (1998). Polymorphisms in *Phytophthora infestans*: four mitochondrial haplotypes are detected after PCR amplication of DNA from pure cultures or from host lesions. Applied and Environmental Microbiology 64: 4007-4014.

Hohl, H.R. and Iselin, K. (1984). Strains of *Phytophthora infestans* from Switzerland with A2 mating type behavior. Transactions of the British Mycological Society 83: 529–530.

Judelson, H.S. (1997). The genetics and biology of *Phytophthora infestans*: modern approaches to a historical challenge. Fungal Genetics and Biology 22: 65–76.

Koh, Y.J., Goodwin, S.B., Dyer, A.T., Cohen, B.A., Ogoshi, A., Sato, N. and Fry, W.E. (1994). Migrations and displacements of *Phytophthora infestans* populations in East Asian countries. Phytopathology 84: 922–927.

Nishimura, R., Sato, K., Lee, W.H., Singh, U.P., Tun-tschu, C., Suryaningsih, E., Suwonakenee, S., Lumyong, P., Chamsawang, C., Wen-hua, T., Shrestha, S.K., Kato, M., Fujii, N., Akino, S., Kondo, N., Kobayashi, K. and Ogoshi, A. (1999). Distribution of *Phytophthora infestans* Populations in Seven Asian Countries. Ann. Phytopathology. Soc. Jpn. 56: 66-75

Petchaboon, K. (2003). Characterization of Population Structure of *Phytophthora infestans* from Tomato and Potato in Tak and Chiang Mai Provinces. Master Thesis, Department of Plant Pathology, Faculty of Agriculture, Kasedsart University, Thailand. 107 pages.

Semal, J. (1995). The epic of potato blight (1845-1995). Cahiers Agric. 4: 287-298.

Shaw, D.S. (1987). The breeding system of *Phytophthora infestans*: the role of the A2 mating type. In P.R. Day and G.J. Jellis (eds.), Genetics and Plant Pathogenesis. Blackwell Scientific Publications, Oxford, England, pp. 161-174.

Smoot, J.J., Gough, F.J., Lamey, H.A., Eichenmuller, J.J. and Gallegly, M.E. (1958). Production and germination of oospores of Phytophthora infestans. Phytopathology 48: 165-171.

Sujkowski, L.S., Goodwin, S.B., Dyer, A.T. and Fry, W.E. (1994). Increased genotypic diversity via migration and possible occurrence of sexual reproduction of *Phytophthora infestans* in Poland. Phytopathology 84: 201-207.

(Received 22 July 2009; accepted 3 November 2009)