Volume: 5, Issue: 2, (December, 2023), pp 4-13
http://agri.rjt.ac.lk/sljae

SRI LANKAN JOURNAL OF AGRICULTURE AND ECOSYSTEMS *e*ISSN: 2673-1401

SLJAE

ORIGINAL ARTICLE

Studies on the Presence of Different Mating Types of *Phytophthora infestans* in Nuwara Eliya Sri Lanka

W. M. A. M. Ranasinghe[1], A. S. A. Salgadoe[1] and C. Ranasinghe[2]

[1]*Department of Horticulture and Landscape Gardening, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP), 60170, Sri Lanka.*

[2]*Agriculture Research Station, Seetha Eliya, Sri Lanka.*

Correspondence:
ranasingheasitha7@gmail.com

https://orcid.org/0000-0002-5253-7518

DOI: https://doi.org/10.4038/sljae.v5i2.91

Abstract

Late blight, caused by *Phytophthora infestans* is one of the most devastating diseases in potato cultivation. It results in direct crop losses but also causes a considerable amount of cost for disease management. The primary objective of this study was to investigate *P. infestans* mating types in selected areas in Sri Lanka and evaluate the risks associated with mating types. In this experiment potato leaves from a range of late blight severity levels were sampled from infected farmer fields. The pathogen was isolated by leaf culture method and confirmed by microscopic observations. The mating types of each isolate were studied by a Metalaxyl sensitivity test. Mating type A1 has been previously reported in Sri Lanka, but mating type A2 was not reported since 2013. Results of this study reveal that mating type A1 was more prominent than A2 (75% and 50% respectively). A2 was reported only from two fields. These observations prove the presence and emergence of mating type A2 in Sri Lankan potato fields. Gradual increase of detectable A2 mating types suggests a high possibility of pathogen inoculum movement between the locations over time. The presence of both mating types raises the possibility of sexual reproduction and generation of oospores. The ratio of A1: A2: (A1+A2) in the current study was 2:1:1. This could permit genetic recombination with the biological evolution of fungicide-resistant types of *P. infestans* species. This study emphasizes the importance of investigations on the mating types to manage late blight disease in Sri Lankan potato fields.

Keywords*: Fungicide resistance, Mating type, Oospores, Phytophthora infestans, Potato Late blight*

Date of Submission: 05.11.2023 Date of Acceptance: 31.12.2023

1. Introduction

Potato (*Solanum tuberosum L.*) is a vital tuberous crop. Billions of people are consuming potatoes in several ways because it stands as an energy-rich nutritious food (Kelaniyangoda et al. 2013). Potato late blight (PLB) is one of the most aggressive diseases in potato cultivation. According to the latest studies done by Fry (2008) *Phytophthora infestans (P. infestans)* is primarily a foliage plant pathogen and not a true fungus. It belongs to the group oomycete which is water molds. This pathogen has the capability to infect and spread within the potato plant through its roots, when the soil is moistened and spread into vegetative parts by mycelial development (Fry et al. 1993). When the plant is infected by *P. infestans* it shows symptoms of water-soaked gray lesions or patches on the leaf surface (Figure 1a) with white molds underside of the leaves (Figure 1b). In the early stages, it can be easily observed as misted dark blotches on the leaf blade. When tubers are infected dark or gray color patches with reddish-brown beneath the skin of the tubers are apparent. As a result of secondary infections of fungi and bacteria emerging in tubers with the development of PLB, tubers may be subjected to rotting. Therefore, it results in a great loss during the stages of harvesting, storage, and marketing (Kelaniyangoda and Somachandra 2001). PLB management requires a continuous study of the pathogen behavior and correct assessment of the disease severity in field conditions (Duarte-Carvajalino et al. 2018).

Figure 1: Potato Late Blight symptoms in a potato leaf; (a) water-soaked grey patches on the adaxial leaf surface, (b) White molds on the abaxial leaf surface

Phytophthora infestans is capable of multiplying both sexually and asexually. According to Bengtsson (2013), sporangia structures are produced asexually, and they contain zoospores that swim in the water until a host tissue is found. When conditions are favorable, germinate and develop the mycelium. (Singh et al. 1998; Singh and Bhattacharyya 1990). Sexual reproduction happens when opposite mating types of A1 and A2 are met (Gallegly and Glindo 1958). Bengtsson (2013) has proven that the antheridium and oogonium are stimulated by hormones that are in the contact zone during the period of sexual reproduction (Jmour and Hamada 2006). These two mating types (A1 and A2) are having biologically differentiated structures due to their ability to produce and respond to specific sexual compatibility substances (Kwon and Lee 2002).

As early as 1850, English planters in Nuwara Eliya attempted to grow potatoes in their home gardens. However, the exact period of introduction of this crop to Sri Lanka is not known. In 1948 the Department of Agriculture began working on potatoes, dedicating the first few years to investigating suitable cultivars, cultural practices,

fertilizer applications, and pest and disease control. Eventually, commercial-level potato cultivations were successfully established in the central province; Seetha Eliya, Kandapola, Black Pool, and Mepilimanna areas. These areas were also considered the geographical hotspots for PLB caused by *P. infestans* due to favorable climatic conditions prevailing in those areas (Sathiamoorthy et al. 1985).

In 1996, Physiological races of *P. infestans* were first confirmed in Sri Lanka by Kelaniyangoda and Somachandra (2001) and later mating types were also confirmed in Sri Lanka in 2005 (Kelaniyangoda 2011). Previous studies conducted in 2013 reported the presence of both A1 and A2 mating types from the areas of Santhipura, Keppetipola, Uva Paranagama, Thennakoonewela, and Nugathalawa (Kelaniyangoda et al. 2013). However, since 2013 there has been limited, recorded evidence found on the progression of *P. infestans* mating types in Sri Lanka.

The availability of different sexual mating types in potato fields is a huge threat. Because this can develop virulent strains in the field through the process of sexual reproduction (Rajapaksha et al. 2015; Kelaniyangoda 2011). Not only that A1 and A2 mating types are supposed to characterize the compatibility mating types (Jmour and Hamada, 2006). Interaction between hyphae of opposite mating types results in the creation of oospores, which are capable of developing a thick wall structure that will allow survival for many years in the soil even in the absence of the host plant (Mazakova et al. 2006). Therefore, this study was led with the objective of performing a reinvestigation on the progression of *P. infestans* mating types in the geographical hotspots in the central province of Sri Lanka.

2. Materials and Methods

Study fields

Several fields were randomly selected from late blight geographical hotspots listed by Kelaniyangoda et al. (2013) and Kelaniyangoda (2011) in Nuwara Eliya district (6055'26.2" N, 80046'18.0" E) Central Province, Sri Lanka. Open and Protected houses in Agricultural Research Station, Seetha Eliya, farmer fields where located at the Seetha Eliya, Black Pool, Meepilimana and Kandapola were studied through this study. The research was based on the popular potato variety "Granola". The study was conducted from August to November 2021.

Leaf sample collection

More than 200 samples of potato leaves showing PLB symptoms were randomly collected from the selected fields. They were stored in sealed transparent breathable polythene bags and stored in a cool box until transported to the laboratory located at the Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP), Sri Lanka.

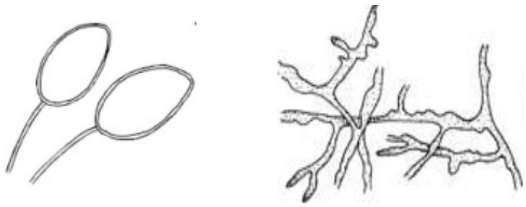
Isolation of pathogen by leaf culture method

For pathogen isolation the procedure suggested by Kelaniyangoda et al. (2013) was followed. Infected leaf samples were initially washed under running tap water to remove any inert matter, and then were transferred into the laminar floor to isolate the pathogen by leaf culture method. Leaves were surface sterilized by dipping in 5% Clorox for 2-3 seconds and washing three times with deionized

autoclaved distilled water. Then they were blot dried with clean dry paper towels before small pieces (1 cm) of the leaves including fresh and infected parts (50%) were dissected using a sterilized scalpel. Tissue pieces were placed on sterilized culture plates suspended with Potato Dextrose Agar (PDA) and incubated for 7-10 days at 15-18 ^{0}C until the formation of sporangia. 222 samples were collected from the host while only 66 isolates were successfully purified, maintained, and stored for further studies.

Confirmation of Phytophthora infestans

The mating type was determined for the subset of 66 isolates. To confirm the isolated pathogen, a loop full of fresh mycelial was extracted from the majority of grown mycelial colonies using an inoculation loop and their morphological mycelial characteristics were observed under the light microscope. Key characteristic features of large, clear, lemon-shaped sporangia on stalks and hyphal swellings in the mycelia as described by Krik et al. (2004) were used to confirm the pathogen *P. infestans* (Figure 2).



a b

Figure 2: Key morphological characteristic features of *P. infestans* a) lemon-shaped sporangia b) hyphal swellings Krik et al, (2004)

Maintaining pure cultures

After the pathogen was confirmed, a loopful of inoculum was transferred into rye agar B media plates and incubated in the dark at 18^0 C to maintain a stock of pure culture for further laboratory tests (Zhang et al. 2001).

Identification of A1 and A2 Types by Metalaxyl Sensitivity Test

A loopful of *P. infestans* was inoculated into culture plates of PDA media suspended with with 8% Metalaxyl (0.0025 g/ml).. A1 and A2 types were identified according to sensitivity to Metalaxyl.

Cross Test of Mating-types A1 and A2

Samples confirmed for type A1 and type A2 from the Metalaxyl sensitivity were further tested by crossing opposite mating types. Mycelia plugs were placed on the ends of fresh rye agar B media plates and incubated in dark at 18 ^{0}C for 14 - 21 days or until the formation of the contact zone (Zhang et al. 2001). The presence of oospores in the contact zone was observed under the light microscope.

3. Results and Discussion

Confirmation of Phytophthora infestans

a b

Figure 3: a) Mycelia, b) Early stages of swelling and sporangia development

After eight days of incubation, white colonies were visible on PDA plates cultured with diseased potato leaves. They were confirmed to be *P. infestans* based

on the microscopic observation of morphological characteristics of the mycelia (Figure 3-a), mycelial swelling and sporangia (Figure 3-b) (Krik et al. 2004). Coincidently, the sampled potato fields were proven to be associated with PLB disease except few fields at Seetha Eliya (Table 1).

Table 1: Presence of *P. infestans* in different locations after seven days of incubation period.

Location		Presence
Seetha Eliya	S	-
	SF1	-
	SF2	+
Black pool	B1	+
	B2	+
	B3	+
Meepilimana	M1	+
	M2	+
	M3	+
Kandapola	K1	+
	K2	+
	K3	+

(S) Agricultural Research Station Seetha Eliya; (SF1) Seetha Eliya field 1; (SF2) Seetha Eliya field 2; (B1) Black pool 1; (B2) Black pool 2; (B3) Black pool 3; (M1) Meepilimana field 1; (M2) Meepilimana field 2; (M3) Meepilimana field 3 (K1) Kandapola field 1;(K2) Kandapola field 2; (K3) Kandapola field 3; *P. infestans* (+) Present; (-) Absent.

Identification of A1 and A2 Mating Types

The mating type of *P. infestans* sampled from each location was recorded in Table 2. According to the results from the current work (Table 2) Seetha Eliya and Meepilimana reported only A1, the Black pool only A2 and Kandapola both A1 and A2 mating types. Interestingly, the current results for Seetha Eliya and Meepilimana are consistent with previous work conducted in in 2001where only A1 mating type has been prominent (Table 2). However, Black Pool and Kandapola reported A2 for the first time (Table 2).

Cross Test of A1 and A2 mating types

The above results were further confirmed by the cross-test (Table 3). Contact zones were developed when isolates were crossed between Seetha Eliya – Kandapola, Kandapola – Blackpool, and Kandapola-Kandapola (Table 3). This may be due to the reason that Kandapola had both A1 and A2 mating (Table 2) and it could possibly cross with A1 isolates from Seetha Eliya as well as A2 isolates from the Black pool and its own isolates with compatible opposite types.

Table 2: Mating type of *Phytophthora infestans* confirmed by Metalaxyl sensitivity test. (#) Not Studied; (+) Presence; (-) Absence

Location	Research results in 2021 (Current Study)		Research results in 2013		Research results in 2004		Research results in 2001	
	A1	A2	A1	A2	A1	A2	A1	A2
Seetha Eliya (SF1, SF2)	+	-	+	-	+	-	+	-
Black pool (B1, B2, B3)	-	+	+	-	+	-	+	-
Meepilimana (M1, M2, M3)	+	-	+	-	+	-	+	-
Kandapola (K1, K2, K3)	+	+	#	#	#	#	#	#

The results suggest that *P. infestans* isolation was successful for most of the sampled areas except for samples from Agriculture Research Station Seetha Eliya which were the only samples collected from a protected house environment. The absence of the pathogen must be due to the durability of the fungicide effect in an enclosed protected house environment than in an open field environment.

Table 3: Test for contact zone development by cross-test of A1 and A2 mating types. (+) presence; (-) absence.

Paired mating-types		Presence of mycelia growth in the contact zone
A1	A2	
SF2	B1	-
SF2	B2	-
SF2	B3	-
SF2	K1	+
M1	B1	-
M1	B2	-
M1	B3	-
M1	K1	-
K2	B1	-
K2	B2	+
K2	B3	-
K2	K1	+
K3	B1	-
K3	B2	-
K3	B3	-
K3	K1	-

Screening of mating types was possible on Metalaxyl fungicide. Gallegly and Galindo (1958) also stated that the A1 mating type is Metalaxyl sensitive while A2 is Metalaxyl resistant. This is consistent even with work carried out by Kelaniyangoda and Somachandra (2001) on screening mating types. In the current study, the average percentage of results in A1 and A2 mating types from the total sample was 75% and 50% respectively. In 2013, it was 100% and 42% respectively, whereas in both 2004 and 2001, it was 100% and 0% respectively. The gradual increase of detectable A2 mating type suggests a high possibility of pathogen inoculum movement between the locations over time. This may be due to the sharing of planting

material from other field locations which were initially mixed with A2 mating type or directly introduced through imported planting seed material.

In 2001, 2004, and 2013, the areas of Seetha Eliya, Black Pool and Meepilimana resulted in only A1 type and A2 mating type was not recorded. However, according to this research work in 2021 in the Black Pool area A2 type was recorded while A1 was not recorded. It was a remarkable change of pathotypes in *P. infestans.* It was proved that getting seed materials from other countries had dangerous effect according to the survey results of Kelaniyangoda and Somachandra (2001). If the area of Kandapola was not tested within the previous 20 years, the results of the year 2021 highlight that, there were both A1 and A2 mating types in farmer fields of the Kandapola area.

The ratio of A1: A2: (A1+A2) in the current study was 2:1:1. It is more important to monitor the ratio of mating types to make predictions regarding the range of sexual recombination and this assist in identifying the risk of long-life oospores which serves as primary inoculum sources (Jmour and Hamada 2006). When comparing the research results of 2001, 2004 and, 2013, with 2021 there was an increase in the percentage of2 type in Sri Lankan upcountry cultivations within twenty years period.

When germinating the oospores, every germination initiates a diverse amount of novel and different genotypes of this pathogen (Shaw et al. 2006).In each year it was identified with a unique genotype and suggesting that every year PLB was caused by varying genotypes (Drenth et al. 1993). The production of these germinations may pose a risk of infection to newly cultivated plants, as they can be affected by different strains of the pathogen, including type A1, type A2. These strains can potentially cause disease and impact the health and productivity of the cultivated plants. It is crucial to consider the potential presence of these pathogen strains and take appropriate measures to prevent or manage their spread in order to safeguard the overall crop production. (Drenth et al. 1993). According to the results of pairing mating types of A1 and A2 (Table 3), results of mated types divulge 12.5% created a contact zone while 87.5% were not created. This means most of the isolates were in the same mating type while the lesser were in opposite mating types. It was proved that sexual reproduction resulted in genetically more diverse *P. infestans* generations (Drenth et al. 1995). Sexual reproduction will not only facilitate the creation of oospores but also enable them to develop a thick wall structure, allowing pathogens to survive in the soil for many years. Not only sexual reproduction will facilitate the creation of oospores, which are capable to develop a thick wall structure that allows the pathogens to survive for many years in the soil. However, there is a higher possibility from those areas identified with positive mating types, such as Seetha Eliya and Kandapola. However, there is a higher possibility from those areas got positive for identified mating

types i.e., Seetha Eliya and Kandapola. New strains of these mating types have emerged, exhibiting genetic variations and the potential for gene transmission through sexual germination. These strains can disperse to other locations through the transportation of potatoes or planting materials. This movement of infected materials can introduce these novel strains to previously unaffected areas, potentially leading to the establishment of new populations and the spread of disease. It is crucial to closely monitor and regulate the transportation and distribution of potatoes and planting materials to mitigate the risk of introducing and disseminating these new strains, thereby safeguarding the health and productivity of agricultural systems. (Kelaniyangoda and Somachandra 2001).

This investigation produced encouraging results that in open fields' the presence of *P. infestans* is abundant. The mating-type A1 was more prominent in the Nuwara Eliya area when compared with mating-type A2, but there was a considerable increment in type A2 within the last few years according to the results of research work in 2021 and the previous research findings since 2000. (Kelaniyangoda et al. 2013: Kelaniyangoda and Somachandra 2001). Not only that this higher usage of chemicals can cause higher production costs and environmental degradation.

To manage fungicide resistance development in *P. infestans* the causes of resistance induction need to be reviewed clearly. Understanding the possible root causes for resistance development can be achieved through surveys, sensitivity tests of isolates, and mitochondrial haplotyping, using molecular and genetic methods. If the disease aggressively spreads in the farmer fields combination of different classes of fungicides may be useful in the management of PLB. Reducing the spray frequencies and enhancing the spray intervals, substituting the application of a diverse group of fungicides in a growing period can manage the risk (Vincelli and Dixon 2002). Upgradation of existing fungicides or reformulation of new chemicals based on resistance risk surveys generally are helpful tactics to overcome the resistance development in *P. infestans.*

Employing diverse disease management strategies alongside chemical applications can yield favorable outcomes characterized by reduced production costs and diminished ecological degradation. The integration of potato and tomato cultivation presents a potential avenue for mitigating the elevated risks associated with fungicide resistance incidents. Utilizing cultivars endowed with resistance to late blight and implementing strategies to minimize sources of inoculum represent viable and sustainable solutions. (Kelaniyangoda et al. 2013). Awareness of the farmers regarding disease identification and disease management can be considered vital points.

The rise of novel variants of *P. infestans* continuously poses a high risk of fungicide resistance (Kelaniyangoda et al. 2013). In mitigating this risk, the adoption of

sustainable practices alongside consistent disease monitoring and assessment of mating type dynamics are imperative. Comprehensive surveys and risk evaluations are essential across various regions to gauge the prevalence of P. infestans isolates resistant to specific fungicide groups. Drawing from these surveys and assessments, coordinated national strategies should be enacted to preemptively address the potential outbreak of potato late blight.

4. Conclusions

In summary, it can be concluded that there is a gradual increase in detectable A2 mating type in Central Province, Sri Lanka. Oospores formed by sexual reproduction will make this situation much worse since they could survive for a long time under harsh environmental conditions. Hence, it is important to conduct regular surveys and risk assessments to control PLB. Based on the data relevant actions should be taken to manage the PLB outbreak in Srilankan potato fields.

Conflicts of Interest: The authors have no conflicts of interest regarding this publication

5. References

Bengtsson T (2013). Boosting Potato Defense against Late Blight; A Study from Field to Molecule. Doctoral Thesis, Swedish University of Agricultural Sciences, Alnarp.

Drenthc A, Turkensteen LJ, and Govers F (1993). The occurrence of the A2 mating type of *Phytophthora infestans* in the Netherlands; significance and consequences. Netherlands Journal of Plant Pathology, 99, (3), 57-67. https://doi.org/10.1007/BF03041396

Duarte-Carvajalino J, Alzate DF, Ramirez AA, Sepulveda JDS, Rojas AEF, Suarez MS (2018). Evaluating late blight severity in potato crops using unmanned aerial vehicles and machine learning algorithms. Remote Sensing, 10, (10), 1513. https://doi.org/10.3390/rs10101513

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 diseases, 77, 653-661. http://dx.doi.org/10.1094/PD-77-0653

Fry W (2008). *Phytophthora infestans*: the plant (and R gene) destroyer. Molecular Plant Pathology, 9, (3), 385-402. https://doi.org/10.1111/j.1364-3703.2007.00465.x

Gallegly ME, Galindo J (1958). Mating types and oosporse of *Phytophthora infestans* in nature. Phytophathol, 48, 274- 277.

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. Tunisian Journal of Plant Protection, 1, 85-91.

Kelaniyangoda DB, Somachandra KP (2001). Present status and prospect for management of late blight in Sri Lanka. Annals of the Department of Agriculture, 3, 87–94.

Kelaniyangoda DB (2011). Exotic strains of *Phytophthora infestans* in Sri Lanka. Potato Journal of India, 38, (2), 185–187.

Kelaniyangoda DB, Salgadoe ASA, Dissawa DMDM (2013). Threat of *Phytophthora Infestans* a2 mating type on Sri Lankan potato. Potato Journal, 40, (2), 190–193.

Krik W, Wharton P, Hammerschmidt R, Abu-El Samen F, Douches D (2004). Potato Diseases-Late Blight, Extension Bulletin, E-2945.

Kwon KJ, Lee YS (2002). Genetic DNA Marker for A2 mating type in *Phytophthora Infestans.* The journal of microbiology, The microbiological society of korea, 254-259

Mazakova J, Taborsky V, Zouhar M, Rysanek P, Hausvater E, and Dolezal P (2006). Occurrence and Distribution of Mating Types A1 and A2 of Phytophthora infestans (Mont.) de Bary in the Czech Republic. Journal of Plant Protection Science. 42 (2), 41–45 http://dx.doi.org/10.17221/2697-PPS

Rajapaksha RMAOA, Salgadoe ASA, Attanayake RN, Amirthagunanathan S, Attanayaka DPSTG (2015). Phenotypic and genetic variations of *Phytophthora infestans*; causal organism of potato late blight. Proceedings of the 14th Agricultural Research Symposium, 30-34.

Sathiamoorthy K, Prange R, Mapplebeck L, Hiburton T (1985). Potato Production in Sri Lanka. American Potato Journal, 62, 555-564. https://doi.org/10.1007/BF02854402

Singh BP, Bhattacharyya SK (1990). Appearance, build-up and spread of Late Blight in relation to source of inoculum. Indian phytopathol, 43, 393-400.

Singh BP, Roy S, Bhattacharyya SK (1998). Pathological Problems of Economic Crop Plants and their Management, 465-487.

Vincelli P, Dixon E. 2002. Resistance to QoI (strobiluri-like) fungicides in isolates of Pyricularia grisea from perennial ryegrass. Plant Dis., 86(3): 235-240.

Zhang Z, Zhu J, Song BLIY, Tian S, Jiang H (2001). Further investigation on A2 mating-type of *Phytophthora infestans* in China. Journal of Hebei Agricultural University