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Potential Threat of *Phytophthora infestans* A2 Mating Type Detected on Sri Lankan Potato Cultivation

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ABSTRACT

An experiment was carried out to detect the threat of A2 mating type of *Phytophthora infestans* in Sri Lankan potato cultivation. Leaf, soil and tuber samples were collected from infected farmer fields of Nuwara Eliya, Welimada and Bandarawela areas while seed potatoes were collected from National Plant Quarantine Service. The pathogen, *Phytophthora infestans* was able to isolate from leaves and stem parts only while isolation from soil and tubers were not successful. Then isolates were subjected to Metalaxyl sensitivity test for differentiating mating types. Results revealed that metalaxyl sensitive mating type (A1) was more prominent among isolates and metalaxyl resistant genotype (A2) was also detected in this study. A1 and A2 were 100% and 42% from total tested population respectively. A2 type was detected from five locations and A1 type was detected from all 12 tested locations. Hence, the ratio of A1: (A2+A1) was 7:5. A1 and A2 isolates were mated to form oospores on Rye Agar B media and tested for metalaxyl sensitivity. All the resultant F1 generation showed resistant to metalaxyl. Presence of both mating types may permit genetic recombination with emergence of new, more aggressive or fungicide resistant types of *P. infestans*.

KEYWORDS: Late blight, Mating types, *Phytophthora infestans*, Potato

INTRODUCTION

Potato (*Solanum tuberosum*), also called the hidden treasure, belongs to family Solanaceae. It is native to South American Andes Mountains.

Potato is the third most important food crop in the world after rice and wheat in terms of human consumption. It is a versatile energy rich, notorious food which makes it to become staple food in many European and Asian countries (Anon, 2010).

More than a billion people consume potato in many ways and it is cultivated in more than 100 countries worldwide. China is the world largest potato producer, and nearly one third of world potato production is harvested from China and India (Anon, 2010). Since the early 1960s, the growth of potato producing areas has rapidly overtaken all other food crops in developing countries, particularly in Asia.

Potato is a global food, which ensures food security of present and future generations of the people, becoming the "food for the future" that produced more nutritious food more quickly, on less land and in harsh climate than any other major crop (Anon, 2008).

In 1948, the Department of Agriculture has introduced potato in to Sri Lanka, and larger scale potato cultivation was started in 1957 in Nuwara Eliya and Badulla districts where climatic conditions are optimum for the plant (Sathiamoorthy *et al.*, 1985). Commercial level cultivation is now successfully established in four regions; Nuwara Eliya, Badulla, Jaffna and Puttlam, approximately in 8,000 ha. According to the Censes and Statistics Department (2010), the total national potato production was 51,294 metric tons and 101,360 metric tons of potatoes were imported to meet the local consumption demand.

Potato production is confined with several constrains, among them diseases are the most often occurring problem. Late blight of potato is the most devastating disease and it led to the Irish potato famine in 1945 (Drenth *et al.*, 1993). The disease is most destructive in cool and moist weather. Dieback of the growing point and blighting of the foliage as well as discoloration of tubers are symptoms of the disease. White downy mildew of velvety growth on lesions distinguishes late blight from other foliar diseases (Barnes, 1979).

This disease is widely recognized as the single worst disease of food crops due to yield losses, cost of control measures and threat to food security (Anon, 2010). In developing countries approximately ten billion dollars spent annually to control the disease.

There are several methods to manage the late blight, such as; planting resistance varieties, avoid sources of inoculum, planting of healthy seeds, and chemical control. Among them, chemical control is the most effective method.

In Sri Lanka, Propineb, and Mancozeb are used as protective fungicide to control the disease. Besides Metalaxyl+Mancozeb, Oxisdixyl+Propineb are protective, systemic and eradicative fungicides that are used to control late blight. Sri Lankan farmers spent

about 40,500-54,000 of rupees during rainy season on fungicide per hectare to control the damage caused by the disease (Kelaniyangoda and Somachandra, 2001).

Late Blight is caused by *Phytophthora infestans;* it is not a true fungus but a water mold belonging to phylum oomycota, which is diploid heterothallic fungus with two mating types as A1 and A2 (Flier *et al.*, 2001). There are two reproduction cycles in *Phytophthora infestans* as sexual and asexual. During asexual reproduction fungus produce zoosopres and sporangia act as vehicles of asexual reproduction (Singh and Bhattacharyya, 1998).

Prior to early 1980s, the sexual reproduction was detected only in Mexico. But, now it has been observed in different parts of the world. A1 and A2 mating types are believed to represent compatibility mating types (Jmour and Hamada, 2006). Interaction between hyphae of opposite mating types result in the formation of oospores, which are developed thick wall structure that survive for many years in soil even in the absence of the host plant (Mazakova *et al.*, 2006). During sexual reproduction, development of antheridium and oogonium are stimulated by hormones at the contact zone (Jmour and Hamada, 2006).

The Phenylamide fungicide and Metalaxyl has significant contribution to control of *Phytophthora infestans.* In the 1980s, Phenylamide resistant isolate (A2) was detected in potato grown fields, since control of late blight from chemicals is speculative (Davidse *et al.,* 1981). Earlier, A2 mating type was confined to Mexico, and only A1 mating type was detected in other countries (Davidse *et al.*, 1981; Singh *et al.*, 1994). But currently, A2 type present all over the potato growing areas and in a field survey conducted in 2004, both A1 and A2 types were identified in imported seed potato consignment (Kelaniyangoda, 2011), therefore, there is a higher possibility of occurrence of A2 type in Sri Lankan potato field.

Therefore, this study was conducted with the objectives of identifying of existence of exotic (A2) type in Sri Lanka and to identify the pathway of dissemination of the A2 type in Sri Lanka.

MATERIALS AND METHODS

Location

This study was conducted at the Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila during the period from January to April 2013.

Sample Collection

The diseased leaf samples, seed potatoes and soil samples were collected from farmer fields in the major potato growing areas of Nuwara Eliya, Walimada and Bandarawela. Imported seed potatoes from different varieties were collected from National Plant Quarantine Service. The leaves with typical symptom (small lesions with white mildew) were selected.

Isolation of Phytophthora infestans

Each leaf sample was washed under running tap water. Then infected plant tissues were separated using a sterile fine scalpel. Next they were washed in sterilized water. The lesions were cut into small pieces with healthy tissue.

Tubers were washed under running tap water. Then they were surface sterilized by dipping them in 5% Clorox for 2-3 seconds. Next they were washed in autoclaved distilled water three times. Small tuber and leaf pieces and stem parts were cultured on Potato Dextrose Agar and kept in the incubator for 7–10 days at 15-18° C until formation of Sporangia.

Serial dilution was done to isolate *Phytophthora infestans* from soil.

Identification of Phytophthora infestans

Phytophthora infestans was identified by using light microscope from there morphological characters that are, large, clear lemon shaped sporangia on stalks (Krik *et al.,* 2004) as shown in Figure 1a.

Identified white colony of late blight pathogen were removed from Potato Dextrose Agar (PDA) medium and then inoculated on plate medium of Rye Agar B. Every tenth day the mycelium was re-inoculated to fresh Petri dishes to maintain pure culture. Thirty six isolates were maintained in pure culture.

Identification of A1 and A2 Types by Metalaxyl Sensitivity Test

Pure cultures of *Phytophthora infestans* were inoculated into PDA medium with 8% metalaxyl (0.0025g/ml). After identification of A1 and A2 types by using their sensitivity to metalaxyl, pure cultures of A1 and A2 were maintained.

Confirmation Test

Known sample (A1 or A2) were cultured with unknown *Phytophthora infestans* sample in same Petri dish on Rye agar B. The Petri dishes were incubated in the dark at 18° C for 14-21 days or until formation of oospores (Zhang *et al.*, 2001) (Figure 1b). Mycelia of

contact zone were observed under microscope for oospores; furthermore, oospores were subjected to metalaxyl test.

(a) Zoospore of *P. infestans*

(b) Oospores of *P. infestans*

Figure 1. Spore shapes of *Phytophthora infestans*

RESULTS AND DISSCUSSION

Isolation of Phytophthora infestans

Most isolates of *P. infestans* were originated from leaves and fewer from stem parts. Isolation from tubers and soil were not succeeded because it could be latent (Yuen *et al.*, 2006) (Table 1).

Table 1. Presence of *Phytophthora infestans* in different inocular

Inocular	Presence
Leaves	+
Stem	+
Tubers	-
Soil	-

(+)Presence; (-) Absence

Phytophthora infestans is not a true fungus but a water mold belonging to the phylum Oomycota that requires high moisture for survival. Zoospores, sporangia and free living mycelia are produced as vegetative structures in asexual reproduction. These structures are considered to be short lived in the soil, while in some cases pathogen is required to survive as living mycelia in tubers (Anna, 2010).

According to Table 2, *Phytophthora infestans* was not detected in imported seed potato consignment, which was cultured in *in-vitro*. According to Kelaniyangoda (2011) *Phytophthora infestans* was detected in seed tubers which were cultivated on field.

After planting of infected tubers with *Phytophthora infestans,* zoosporangia are formed on their surface which then generates zoospores infecting the underground parts of a plant. As a result of zoospore migration through soil capillary bottom leaves, which contact with the ground, show the symptoms.

Table 2. Occurrence of *Phytophthora infestans* mating types in imported seeds

Variety	Location	*P. infestans*
Belmondo	Germany	-
Granola	Netherland	-
Desiree	Netherland	-
Atlas	France	-
Red Lady	Germany	-
Granola	Netherland	-
El mundo	Netherland	-
Mariska	Netherland	-
Mahageliya	U. S.A	-
Arnova	Netherland	-
Conneet	Netherland	-

(+)Presence; (-) Absence

Identification of Mating Types by Metalaxyl Sensitivity Test

Table 3. Occurrence of *Phytophthora infestans* mating types in Upcountry

Location	Research results in 2013		Surveyed in 2004	
	A1	A2	A1	A2
Seetha Eliya	+	-	+	-
Meepilimana	+	-	+	-
Black pool	+	-	+	-
Kalu kale	+	-	#	#
Santhipura	+	+	#	#
Keppetipola	+	+	#	#
Uva paranagama	+	+	#	#
Hevenakumbura	+	-	#	#
Thennakoonewela	+	+	#	#
Nugathalawa	+	+	#	#
Bandarawela	+	-	+	-
Kahagolla	+	-	#	#

(#)Not Studied; (+) Presence; (-) Absence

The results revealed that the old Sri Lankan genotype (A1, Metalaxyl sensitive) was more prominent among isolates than A2 genotype (Table 3). A1 is sensitive to metalaxyl while A2 is not sensitive to metalaxyl. A1 mating type recorded from all the 12 tested locations, but A2 mating type was limited only to 5 locations, which are 100% and 42% respectively from total tested population. Presence of A2 mating type was more prominent in Welimada area than Nuwara Eliya area.

A1: (A1+A2) mating type ratios were 7:5. Monitoring of A1 and A2 mating type ratios are important to aid in the prediction of the extent of sexual recombination and thus the

risk of long-lived oospores serving as primary inoculums sources (Jmour and Hamada, 2006).

Confirmation Test

Table 4. Pairing of mating types and Metalaxyl sensitivity test

Test	Mating type		Metalaxyl sensitivity test
	A1	A2	
1	Hevenakum bura	Santhipura	-
2	Seetha Eliya	Keppetipola	-
3	Seetha Eliya	Uva paranagama	-

(+) Sensitive: (-) Resistance

Every germinated oospore generates several new and unique recombinant genotypes of the pathogen (Shaw *et al.*, 2006). Germination of oospores release progeny of A1, A2 or altered type that are able to infect newly planted crop (Drenth *et al.*, 1993).

Results of pairing test showed that F1 generation produced by diffusion of mating types were not sensitive to Metalaxyl hence; F1 generation was more or less similar to A2 type or altered as a novel type (Table 4).

When consider about the detection of exotic mating type (A2) in Sri Lanka, it can be assumed that it might have migrated to Sri Lanka through importation of seed potatoes.

The four most likely hypotheses to explain the occurrence of the A2 mating type outside Mexico are that it (i) was always present, but undetected; (ii) was introduced by migration; (iii) arose by mutation or mitotic recombination; or (iv) arose by mating type change, either from exposure to fungicides or by induced selfing (Stephen *et al.*, 1997). Among these, the migration hypothesis is the only one that shows strong scientific evidence.

Therefore, introduction by migration strongly proves as the pathway of dissemination A2 mating type to Sri Lanka.

Sexual recombination will result in an increased genetic variation. The combination of sexual recombination (new genotypes) and clonal propagation (maintain and spread of successful genotypes) will further enhance the evolutionary potency of creating novel types of *Phytophthora infestans.*

This study was only focused on identification of A2 mating type in upcountry of Sri Lanka, but it is necessary to have further study on biology and epidemics of oospores, molecular level study using molecular markers on mating types will provide more accurate facts on both mating types in their genetics. The numbers of studies documenting the occurrence of mating types of *Phytophthora infestans* are rare in Sri Lanka, therefore continuous monitoring programme of mating types is a vital need for commercial level potato production.

CONCLUSIONS

In this study A2 mating type of *Phytophtora infestans* were detected from field grown potatoes of Nuwara Eliya and Welimada areas while A1 type was identified in all the tested locations. The occurrence of a sexual cycle diffused with A1 and A2 types has resulted in increased virulence, gene transfer, and genetic variation in populations of *Phytophthora infestans*, creating new challenges for late blight management. Oospores production and survival in the soil provides a new source of inoculums for the initiation of epidemics. However, in contrast to the asexually derived spores, the sexually produced oospores are more robust and can survive in soil at least two years. Hence, Sri Lankan potato cultivation would encounter devastation in future resulting loss in local potato production if Quarantine measures are not adheres to proper standards.

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