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Reevaluation of the report of the A2 mating type of *Phytophthora infestans* on tomato in Taiwan

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ABSTRACT. In June 2008, Deahl et al. reported the first detection of two isolates of the A2 mating type of *Phytophthora infestans* on tomato from two locations in Taiwan based on the tests performed at USDA, Beltsville, using A1 and A2 mating types of *P. infestans* as testers. However, the third and fourth authors of the paper showed that these two isolates (Pi 214 and Pi 566) behaved as A1 mating type when paired with A1 and A2 testers of *P. nicotianae* (= *P. parasitica*) at Asian Vegetable Research and Development Center, Shanhua, Taiwan. This information was not included in the report. These two isolates along with two other isolates of *P. infestans* (Pi 215 and Pi 564) isolated from the same locations on the same dates were re-tested independently in three laboratories using A1 and A2 mating types of *P. infestans*, *P. nicotianae* and *P. capsici* as testers. All four isolates displayed oospore formation when paired with A2 but not A1 mating type regardless of species used as the testers, indicating that all of them are of the A1 mating type. New isolation of *P. infestans* from diseased tomato plants from the same locations also showed the presence of only the A1 mating type. These results refute the claim by Deahl et al. of the discovery of the A2 mating type of *P. infestans* from Taiwan.

Keywords: Late blight; Mating type; *Phytophthora infestans*; Tomato.

INTRODUCTION

Late blight of potato and tomato caused by *Phytophthora infestans* (Mont.) de Bary is one of the most devastating plant diseases worldwide. The disease was reported from Taiwan in 1908 by Kawakami and Suzuki and in 1919 by Sawada. It had not received much attention until 1995 when Hartman and Huang reported the isolation of 13 isolates of *P. infestans* from tomato and the discovery that all isolates were of the A1 mating type. The presence of only the A1 mating type of *P. infestans* in Taiwan was confirmed in 1998 by Ann et al. (1998) who reported that 68 isolates from tomato and 2 isolates from potato, were all of the A1 mating type. In 2004, Jyan et al. reported collection of 94 tomato and potato isolates of *P. infestans* throughout Taiwan from 1992 to 2002 and found that all were of the A1 mating type. Since 1998, the senior author has been surveying the mating type distribution of *P. infestans* in Taiwan yearly. As of 2008, 409 potato isolates and 1172 tomato isolates were collected and all have been of the A1 mating type (Ann, unpublished data).

In 2008, Deahl et al. reported the detection in Taiwan of one A2 isolate of *P. infestans* (Pi 214) out of 200 isolates tested in 2004 and another A2 isolate (Pi 566) out of 102 isolates tested in 2006. Due to the importance of the A2 mating type of *P. infestans* for quarantine status in Taiwan, it is essential to confirm this purported A2 mating type. We, therefore, wrote to Deahl to request cultures of these two A2 isolates (Pi 214 and Pi 566). He replied that the quarantine permit only allowed the receipt of cultures but no redistribution to others, and suggested that Wang the fourth author be contacted for cultures (W. H. Ko, *personal communication*). Wang shared the two putative A2 cultures (Pi 214 and Pi 566) along with two A1 cultures (Pi 215 and Pi 564) obtained from the same locations in 2004 and 2006 (Table 1). He mentioned that he and Black, the third author, informed Deahl that Pi 214 and Pi 566 were A1 based on the result of pairing with the A1 and A2 testers of *Phytophthora nicotianae* van Breda de Haan (= *Phytophthora parasitica* Dastur) and requested that this result be added to the article. However, the request was ignored because it was not based on the pairing with the A1 and A2 testers of *P. infestans*, a procedure used at USDA, Beltsville (T. C. Wang, *personal communication*). This rejection is not logical because sexual reproduction of heterothallic *Phytophthora* is unique with normal oospores produced readily when opposite mating types of different species are paired (Shen et al., 1983; Ko, 2007). Moreover, all 302 *P. infestans* isolates were tested using A1 and A2 testers of *P. nicotianae* in Taiwan and all were found to be of the A1 mating type. Therefore, the mating

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type of the four isolates of *P. infestans* mentioned above were reevaluated by performing independent experiments in three different laboratories using A1 and A2 testers of *P. infestans*, *P. nicotianae* and *P. capsici* Leonian and by obtaining quantitative data from each test. In addition, new isolates were collected from diseased leaves, fruit and stems of infected tomato plants from Nantou and Hualien counties where the two purported A2 isolates were collected. Details of these studies are reported herein.

MATERIALS AND METHODS

Sources of isolates

The origins of *P. infestans* isolates included in the report of Deahl et al. (2008) and used in this study for reevaluation of mating type are described in Table 1. New *P. infestans* from diseased tomato leaves, stems and fruit were collected from Hsinyi of Nantou county in June and August 2008, and from Shoufeng of Hualein county in February and April 2009. These are the sites for the isolation of Pi 214 and Pi 566, the purported A2 isolates. Pieces of diseased leaves (10 × 10 mm), stems (5 × 5 mm) and fruit (5 × 10 × 10 mm) each with a single lesion were surface-sterilized with 0.5% sodium hypochlorite for 1 min and placed on rye A agar (Caten and Jinks, 1968) supplemented with 100 ppm ampicillin, 50 ppm nystatin and 10 ppm pentachloronitrobenzene to inhibit bacteria and non-pythiaceous fungi (Ko et al., 2006). Plates were incubated at 20°C and observed daily. Hyphae of *P. infestans* emerging from a tissue were transferred to a plate of rye A agar. Cultures were maintained on rye agar and stored in culture slants covered with mineral oil in test tubes at 16°C.

Determination of mating type

The sources of mating type testers of *Phytophthora* used in this study are listed in Table 2. At the Taiwan Agricultural Research Institute (TARI), rye broth used to prepared rye agar was prepared as described by Caten and Jinks (1968), while at the Asian Vegetable Research and Development Center (AVRDC) and the National Chung Hsing University (NCHU), it was modified by grinding the rye grain preparation in an Omni mixes at 4,000 rpm for 1 min before filtration through three layers of cheesecloth. The modified rye agar was used for pairing with *P. nicotianae* testers, and rye agar supplemented with 10% V-8 juice (V-8 rye agar) (Ho and Ko, 1999) was used for pairing with *P. capsici* or *P. infestans*.

At AVRDC, 5-mm culture discs of *P. infestans* and the A1 or A2 tester of *P. nicotianae* were paired 5 mm apart on the center of a rye agar plate (9 cm), and incubated at 20°C in darkness. After 12 days, the paired culture was mixed with 100 ml water in a blender at high speed for 1 min and oospore concentration was determined with a haematocytometer. At TARI and NCHU, a small piece (3 × 3 × 3 mm) of *P. infestans* culture was paired with the same size culture of a tester on a rye or V-8 rye agar block (20 × 15 × 3 mm). Ten blocks were placed in a 9-cm plate at equal distance from each other. After incubation at 20°C for 21 days at TARI or 16°C for 21 days at NCHU, all the blocks were examined under a microscope for oospore formation. The total number of oospores was counted for the blocks producing less than 500 oospores each. For those forming large amount of oospores, each block was mixed with 100 ml water in an Omni mixer at 4,500 rpm for 1 min, and oospore concentration was determined with a Pipetman microliter pipette (West Coast Scientific Inc., Oakland,

Table 1. Sources of isolates of *Phytophthora infestans* from tomato in Taiwan used in the reevaluation of mating type.

Isolate No.	Location	Reported mating type	Source	Reference
Pi 214	Shoufeng, Hualien	A2	T. C. Wang	Deahl et al., 2008
Pi 566	Hsinyi, Nantou	A2	T. C. Wang	Deahl et al., 2008
Pi 215	Shoufeng, Hualien	A1	T. C. Wang	Deahl et al., 2008
Pi 564	Hsinyi, Nantou	A1	T. C. Wang	Deahl et al., 2008

Table 2. Sources and habitats of mating type testers of *Phytophthora* used in the study.

Species and isolate	Mating type	Habitat	Origin	Source
P. nicotianae				
P 991(ATCC 38607)	A1	Soil	U.S.A.	G. A. Zentmyer
P 731(ATCC 38606)	A2	Soil	U.S.A.	G. A. Zentmyer
P. infestans				
P 95039	A1	Potato	Taiwan	P. J. Ann
DN 103	A2	Potato	Japan	A. Ogoshi
P. capsici				
P 28120	A1	Tomato	Taiwan	P. J. Ann
P 28117	A2	Tomato	Taiwan	P. J. Ann

California) (Ko et al., 1973). Three replicates were used and all the experiments were repeated at least once.

RESULTS

When the four isolates of *P. infestans* used in the report of Deahl et al. (2008) including the two purported A2 isolates (Table 1) were paired with the A1 tester of *P. nicotianae* at AVRDC, oospores were not produced in any pairing test. However, when paired with the A2 tester of *P. nicotianae* abundant oospores ranging from 14,200 to 85,600/cm^3 were produced by every pair (Table 3). Similar result was obtained at NCHU when *P. nicotianae* testers were used. Pairing of the same *P. infestans* isolates. with the A1 tester did not result in oospore formation, but oospores ranging from 7,200 to 23,400/cm^3 were produced when paired with the A2 tester (Table 4).

When Deahl's A2 isolates of *P. infestans* were paired with *P. capsici* testers at NCHU, oospores were not produced in the pairings with the A1 tester, but oospores ranging from 13,900 to 42,400/cm^3 were produced with the A2 tester (Table 4). Similar result was obtained at TARI where oospores ranging from 6,300 to 22,800/cm^3 were produced when paired with the A2 *P. capsici* tester (Table 5).

Table 3. Oospore formation resulting from pairings at AVRDC between *Phytophthora infestans* isolates from Taiwan and the A1 or A2 tester of *P. nicotianae*.

P. infestans isolates	Oospores produced (no. ± SD/cm^3)[a]	
	P. nicotianae tester	
	A1	A2
Pi 214	0	15,000 ± 3,300
Pi 566	0	14,200 ± 9,100
Pi 215	0	85,600 ± 16,500
Pi 564	0	60,700 ± 6,900

[a]Data were subjected to ANOVA. Numbers are the mean ± standard deviation.

Table 4. Oospore formation resulting from pairings at NCHU between *Phytophthora infestans* isolates from Taiwan and the A1 or A2 tester of *P. nicotianae* or *P. capsici.*

P. infestans isolates	Oospores produced (no. ± SD/cm^3)[a]			
	P. nicotianae tester		*P. capsici* tester	
	A1	A2	A1	A2
Pi 214	0	8,300 ± 2,200	0	42,400 ± 5,300
Pi 566	0	7,200 ± 3,500	0	37,500 ± 10,400
Pi 215	0	10,200 ± 200	0	13,900 ± 5,600
Pi 564	0	23,400 ± 17,300	0	16,000 ± 5,000

[a]Data were subjected to ANOVA. Numbers are the mean ± standard deviation.

Table 5. Oospore formation resulting from pairings at TARI between *Phytophthora infestans* isolates from Taiwan and A1 or A2 tester of *P. capsici*. or *P. infestans*.

P. infestans isolates	Oospores produced (no. ± SD/cm^3)[a]			
	P. capsici tester		*P. infestans* tester	
	A1	A2	A1	A2
Pi 214	0	6,300 ± 1,200	0	38,500 ± 3,200
Pi 566	0	22,800 ± 900	0	40,100 ± 13,700
Pi 215	0	12,900 ± 1,500	0	29,000 ± 3,900
Pi 564	0	19,700 ± 2,900	0	53,400 ± 12,200

[a]Data were subjected to ANOVA. Numbers are the mean ± standard deviation.

The four isolates of *P. infestans* behaved similarly when paired with *P. infestans* testers at TARI. No oospores were produced when Deahl's purported A2 isolates were paired with the A1 tester, but oospores ranging from 38,500 to 40,100/cm^3 were produced when paired with the A2 *P. infestans* tester (Table 5).

At TARI, 41 and 40 new isolates of *P. infestans* were obtained from Nantou and Hualien, respectively. *Phytophthora infestans* testers were used for mating type determination and all new isolates were of the A1 mating type.

DISCUSSION

Data from tests performed in the three laboratories are consistent in showing that all the four isolates of *P. infestans* including the two purported A2 isolates from Deahl et al. (2008) displayed oospore formation only when paired with A2 but not A1 testers. This indicates that all of them are of the A1 mating type. Our results unequivocally refute the claim by Deahl et al. (2008) of the discovery of A2 mating type in Taiwan. This study also shows that the initial designation of Pi 214 and Pi 566 as A1 mating type in Taiwan based on results using A1 and A2 mating types of *P. nicotianae* as testers was sound. These two isolates behaved as A1 type regardless of pairing with *P. nicotianae* (Tables 3 and 4), *P. capsici* (Tables 4 and 5) or *P. infestans* (Table 5). This is consistent with the previous reports that in heterothallic *Phytophthora* oospore production occurs when opposite mating types are paired regardless of whether they are the same or different species (Ko, 2007). Our recent collection of *P. infestans* isolates also show the presence of only the A1 mating type of this species in the Nantou area in 2008 and in the Hualien area in 2009.

The reason for Pi 214 and Pi 566 to behave as the A2 mating type when tested previously is not known. Mating type change in *Phytophthora* has been reported to occur as a result of aging (Ko, 1981), germination of selfed oospores (Ann and Ko, 1988; Ko, 1994) or exposure to fungicides (Ko, 1981; Chang and Ko, 1990; Groves and Ristaino, 2000). It is not known if any of these events occurred. In this study, not unexpectantly, environmental

conditions such as temperature affect oospore production. When Pi 215 was paired with *P. nicotianae* A2 tester on rye agar plate at 20°C at AVRDC, 85,600 oospores/cm^3 were produced (Table 3), but only 10,200 oospores/cm^3 were produced when the same two isolates were paired on the same rye agar block at 16°C at NCHU (Table 4). When Pi 215 was paired with *P. infestans* A2 tester on V-8 rye agar block at 20°C at TARI, 29,000 oospores/cm^3 were produced and other *P. infestans* isolates formed between 38,500 and 53,400 oospore/cm^3. This indicates that pairing of A1 and A2 mating types between the same species is not necessary better than that between different species. Similar results were obtained when different combination of mating types and species of *Phytophthora* were paired on the opposite sides of polycarbonate membranes (Ko, 1978; Ko and Kunimoto, 1981). This is because sexual reproduction in *Phytophthora* is regulated by mating type specific α hormones (Ko, 2007), and different isolates of the same or different species of *Phytophthora* vary in ability to produce and receive hormones (Ko and Kunimoto, 1981).

In conclusion, research at TARI, AVRDC and NCHU all indicates that there is no evidence of the presence of the A2 mating type of *P. infestans* in Taiwan. It is, therefore, recommended that the quarantine regulations are enforced continuously in Taiwan to prevent the introduction of the A2 *P. infestans* from foreign countries.

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重新評審在台灣番茄上發現 *Phytophthora infestans* A2 配對型的報告

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Deahl 等於 2008 年 6 月報告說，他們在美國農部，以 *Phytophthora infestans* A1 及 A2 配對型當測試菌株 , 第一次在台灣二個地方的番茄上，各測到一株 *P. infestans* 的 A2 配對型。但是該論文的第三及第四作者，在台灣亞洲蔬菜研究中心，以 *P. nicotianae* 的 A1 及 A2 配對型當測試菌株時，那二株菌是 A1 配對型，他們的試驗結果，在報告裡卻沒有被提及。我們將這兩株菌同另外二株同時在同地點分離到的，以 *P. nicotianae*, *P. capsici* 及 *P. infestans* 之 A1 同 A2 配對型當測試株，重新在三個不同試驗室，進行配對型的測試。這四株菌同 A2 配對型，不管是那一個種，配對時均會產生卵孢子，但同 A1 配對型配對時則不會，顯示這四株菌全屬 A1 配對型。從那二個地方採番茄病組織回來分離的結果，所得的菌株也全屬於 A1 配對型。我們的結果顯示 Deahl 等的報告說在台灣發現 *P. infestans* A2 配對型是錯誤的。

關鍵詞：晚疫病；配對型；*Phytophthora infestans*；番茄。