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Phytophthora Blight of Peace Lily in Taiwan

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ABSTRACT

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Phytophthora parasitica (= *P. nicotianae*) has been detected from the diseased tissues of peace lily (*Spathiphyllum kochii*) since 1992 in Taiwan. The pathogen caused severe leaf blight, root rot and death of the whole plants of the ornamental plant in many fields. A total of 19 A^1 and 8A^2 isolates of *P. parasitica* were obtained from 7 fields distributing in Taipei, Nantow, Changhua, Chiayi and Tainan. After stored for 3-8 years, 8 of 16 A^1 isolates changed to A^2 type and 2 to A^1A^2 type. However, none of A^2 isolates changed. Disease symptoms similar to appearing in the fields were reproduced when plantlets of peace lily were inoculated with zoospore suspension of *P. parasitica* in pathogenicity tests. Three-month-old plantlets of peace lily showed high susceptibility to the pathogen and more than 80% inoculated plantings were killed within one month. *Phytophhtora parasitica* was reisolated from all artificially infected tissues. The peace lily isolates of *P. parasitica* were pathogenic to three of four members of aroids, including *Anthurium andreanum*, *Epiprenmum aureum*, *Philodendron scandens* subsp. *Oxycardium*, which have been record as hosts of the pathogen in Taiwan and *vice versa*. The pathogen did not caused disease symptoms on *Dieffenbachia amoena*. *Phytophthora* disease of peace lily is reported for the first time in Taiwan.

Key words: New disease record, *Phytophthora parasitica*, mating type, peace lily (*Spathiphyllum kochii*)

INTRODUCTION

Ornamental plants have become important economic commodities in Taiwan in recent years and many famous varieties of ornamental plants were introduced from foreign countries. Peace lilies (*Spathiphyllum* spp.), which are tropical perennial plants native to tropical America, are important ornamental plants grown wildly in Taiwan. The plants are good indoor ornamental plants, which could be grown in single-pots or mass planted in the soil in office buildings [8]. Both flowers and leaves of peace lilies were used for ornamental purpose. Although these ornamental plants have been cultivated in Taiwan for very long times, non-diseases have been reported [22]. Based on records in other countries [8], a few infectious diseases, including Dasheen mosaic, Erwinia rot, cylindrocladium rot, myrothecium spot, southern blight, and Phytophthora blight, were reported to occasionally occur in the fields. I reported herein new Phytophthora diseases of peace lily occurred in Taiwan.

MATERIALS AND METHODS

Isolation, maintenance and identification of pathogen

Diseased tissues of affected peace lilies were collected from fields. Pieces of tissues taken from roots (ca. 5-10 mm long), leaves and petioles (ca. 7 X 7 mm^2) with advanced disease symptoms were disinfested with 0.5% NaClO for 3 min. The treated tissues were plated onto selective medium consisting of 5% clarified V-8 juice agar and 2% Bacto agar supplemented with 200 ppm ampicillin, 50 ppm mycostatin, and 10 ppm pentachloro-nitrobenzene [16]. V-8 juice plus 0.2% $CaCO_3$ was clarified by centrifugation at 1500 rpm for 5 min. After incubation at 24℃ for 1-3 days, mycelial mats of *Phytophthora* growing out of diseased tissues on selective medium were transferred to 5% V-8 juice agar. Single-zoospore cultures were obtained using the method described by Ko [15]. Cultures were maintained on 5% V-8 juice agar blocks in sterile water in test tubes at 24℃. Classification Keys described by Stamps *et al.* [19] and Waterhouse [24] were used for identification of the *Phytophthora* isolates obtained.

Production of sporangia and zoospores

The method described by Hwang *et al.* [11] was used to produce large amount of sporangia for morphological studies and pathogenicity tests. Zoospore suspension was prepared by chilling the mycelial mats with sporangia at 15℃ for 30 min and immediately moving back to 24℃ for another 30 min. Concentration of zoospore suspension was counted and adjusted with aid of a micropipette [17].

Determination of mating types and production of oospores

Cultures of *Phytophthora* isolates were grown on 10% V-8 juice agar (10% V-8 juice, 0.02% $CaCO_3$, 2% Bacto agar) at 24℃ in darkness for 10 days. Isolates, which did not form oospores in single cultures, were paired with the standard A^1 (p991) and A^2 (p731) mating type of *Phytophthora parasitica* for determination of mating types [2]. Those forming oospores when paired with the A^2 tester were A^1; while those forming oospores with A^1 tester were A^2. The polycarbonate membrane method described by Ko [13] was used for study of sexual reproduction of heterothallic species of *Phytophthora*, and for determination of their sexuality types [14].

Growth of Phytophthora

Phytophthora isolates were grown on 5% V-8 juice agar for 3-5 days. Agar discs (5 mm diam.) cut from the periphery of the colonies with a sterile cork borer were each placed on the edge of a V-8 agar plate and incubated at 8, 10, 12, 15, 18, 21, 24, 27, 30, 33, 36 or 38℃ in darkness. Colonies were measured daily until the mycelia reached the opposite edge of the plates or 10 days after inoculation. Four plates were used for each temperature and the experiment was repeated twice.

Pathogenicity tests

One isolate of *Phytophthora* from each field was selected for pathogenicity tests. Concentration of zoospore suspension used for inoculation was 10^5-10^6 zoospores per milliliter. Plantlets of peace lily (*Spathiphyllum kochii*) from tissue cultures grown in disinfested soils in 9- cm-diameter pots for 3 months were used as inoculated plants. For inoculation, the whole tested plants were sprayed with zoospore suspension until aerial leaves and petioles were completely wet. Each inoculated plantlet was put in a plastic bag to keep high moist conditions for 24 hours. Five plantlets were inoculated and tests were repeated twice. Controls were similarly treated with distilled water. Disease incidence was rated every 3 days. Diseased tissues were taken from artificially infected plants for pathogen reisolation following the procedures described above. Disease incidence was classified as 4 grades, including +++ (50-100% of inoculated plants died), ++ (50-100% inoculated portions rotted completely), + (inoculated portions necrotic or blight slightly), and non-pathogenic (no disease symptom appeared).

Pathogenicity of peace lily isolates to other aroids was also tested. Members in Araceae, including anthurium (*Anthurium andreanum* 火鶴花), Dieffenbachia (*Dieffenbachia amoena* 夏雪黛粉葉), pothos vine (*Epiprenmum aureum* 黃金葛), Philodendron (*Philodendron scandens* subsp. *oxycardium* 心葉蔓綠絨), were inoculated. One- to three-month old of plantlets of anthurium and cuttings of other aroids were sprayed with zoospore suspension of peace isolates. Inoculated plants were treated as description above. Disease incidence was rated and pathogen reisolatation from infected tissues was conducted.

RESULTS

Isolation of Phytophthora and disease symptoms

Phytophthora parasitica Dastur (= *P. nicotianae* Breda de Haan) on diseased peace lily was first found in Chiayi at the end of 1992. Subsequently, Phytophthora blight of the ornamental plants frequently appeared in many nurseries and fields, and the same species of *Phytophthora* was isolated. The fungus was able to affect the whole host plants in the fields. Mostly the infection was start from leaf margins or occasionally from center. The affected leaves and petioles appeared black discolored spots initially. Under moist conditions, the diseased areas expended continuously and eventually the whole plants wilted, drooped and died (Fig.1). Infected root tissues were necrosis and eventually rot and dead. A total of 27 isolates of *P. parasitica* were obtained from 7 nurseries distributing in Taipei, Nantow, Changhua, Chiayi, and Tainan (Table 1).

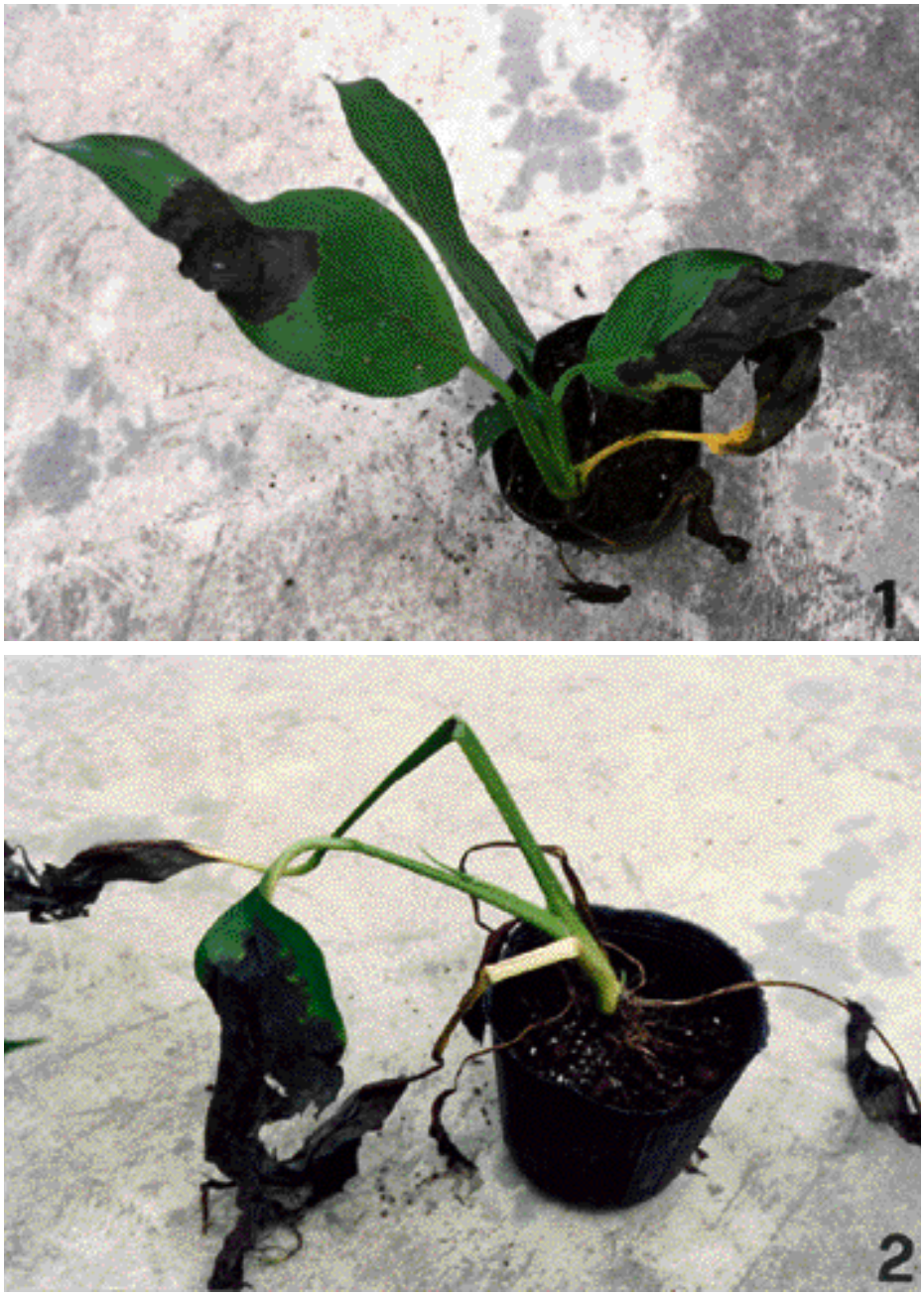
Characteristics of *P. parasitica* isolated from peace lilies

Colonies of the peace lily isolates showed the pattern of mosaic spots with a few scantly aerial mycelia on 5%V-8 agar plates. These isolates produced abundant sporangia in water and a few chlamydospores on agar plates. Sporangia were unsymmetrical, spherical to oval, papillate and non-deciduous. Average sizes of sporangia ranged 48.3-66.5 μm X 38.3-49.1 μm (Table 2). Mean diameter of chlamydospores ranged 32.9-37.8 μm (Table 2). All of the isolates of *P. parasitica* obtained from peace lily belong to cross-inducing (Heterothallic) type, which do not form oospores when cultured individually. Among the 27 isolates obtained, nineteen isolates belonged to the A^1 mating type and eight isolates belonged to A^2 type (Table 1). All the A^1 isolates can be stimulated by the A^2 and induce the A^2 isolate (p731) of *P. parasitica* to form oospores. Therefore, they belonged to the sexuality type S4 [13,14]. Meanwhile, all A^2 isolates, which can be stimulated by A^1 and induce A^1 isolate (p991) of *P. parasitica* to form oospores, belonged to S^1 type. Average sizes of oogonia, oospores, and antheridia ranged 22.5-27.9 μm, 19.1-22.7 μm, and 9.2-10.9 μm X 10.6-14.3 μm, respectively

Table 1. Isolation of *Phytophthora parasitica* from diseased tissues of peace lilies (*Spathiphyllum kochii*) in Taiwan

Isolation year	No of isolates & mating type	Location	No of isolates survived in[1] 2000 & mating type
1992	$2A^2$	Chiayi	$2A^2$
1992	$6A^1$	Puli, Nantow	**$4A^2$**
1992	$3A^1$	Puli, Nantow	$1A^1$, **$2A^2$**
1993	$6A^2$	Chungpu, Chiayi	$6A^2$
1993	$3A^1$	Tianwei, Changhua	$1A^1$, **$1A^2$**
1995	$3A^1$	Shanshang, Tainan	**$1A^2$, $2A^1A^2$**
1997	$4A^1$	Yang-min shan, Taipei	$4\ A^1$
Total	**$19A^1$:$8A^2$**		**$2A^1$:$16A^2$:$2A^1A^2$**

1. Broad letters: indication of mating type change of isolates after storage.



Figures 1&2. Disease symptoms of peace lily incited by *Phytophthora parasitica*.

(Table 3). All tested isolates were able to grown on 5% CV-8 agar from 12 to 36-37℃ and the optimal growth temperatures were 24-32℃ (Table 2).

After stored in sterile distilled water at 24℃ for 3 to 8 years (from 1992 to 2000) , all the survived isolates were subcultured in 2000. Sporangia were produced and measured. Average sizes of sporangia (43.8-52.8 μm X 34.0-41.2 μm) of each tested isolates were smaller than that measured in the year of isolation (Table 2). Meanwhile, of the 16 isolates originally belonging to A^1 mating type, 8 isolates changed to A^2 type and 2 isolates changed to A^1A^2 type after long-term storage (Table 1). The single-zoospore cultures of the two A^1A^2 isolates, like homothallic *Phytophthora*, produced oospores when cultured individually. Whereas, the mating type of all the 8 survived isolates originally belonging A^2 type were not changed.

Pathogenicity test

Peace lily showed highly susceptible to the fungus in pathogenicity test. Disease symptoms similar to those appearing in natural conditions were reproduced within 2-7 days when 3-mo-old plantlets were sprayed with zoospore suspension (about 10^6 zoospores/ml) of *P. parasitica* under moist conditions. More than 80% inoculated plants were

Table 2. Size of sporangia and growth reactions to temperatures of isolates of *Phytophthora parasitica* obtained from peace lily

Isolate no. & mating type	Location	Sporangia			Diameter of chlamydospores (μm)	Growth reaction to temperatures (℃)
			Length X width (μm)	Length/width		
PPSp1 (A^2)	Chiayi	A[1]	22.5-(66.5)[2]-81.5 X 20-(49.1)-70	1.06-(1.29)-1.56	25-(37.8)-45	10-(24-32)-36[3]
		B	40-(52.8)-62.2.X 32.5-(41.2)-50	1.0-(1.35)-2.0		
PPSp2 ($A^1 \rightarrow A^2$)[4]	Nantow	A	36-(48.3)-62 X 28-(38.0)-44	1.0-(1.23)-1.58	30-(37.5)-42.5	10-(28-32)-36
		B	40-(46.8)-55 X 30-(38.3)-45	1.12-(1.27)-1.63		
PPSp3 (A^1)	Chiayi	A	40-(51.7)-70 X 25-(40.5)-44.5	1.0-(1.30)-1.71	27.5-(37.4)-45	10-(28-32)-36
		B	NT[5]	NT		
PPSp4 (A^2)	Changhua	A	42.5-(52.3)-60 X 30-(39.8)-50	1.11-(1.29)-1.57	30-(39.8)-50	10-(24-28)-36
		B	31-(49.5)-65 X 25.5-(37.7)-45	1.03-(1.32)-1.64		
PPSp5-1 ($A^1 \rightarrow A^2$)	Tainan	A	32-(55.0)-70 X 24-(41.8)-52	1.13-(1.31)-1.48	30-(37.3)-45	10-(24-32)-36
		B	35-(45.6)-60 X 27.5-(34.9)-40	1.11-(1.32)-1.64		
PPSp5-2 (A^1)	Tainan	A	NT	NT	NT	NT
		B	45-(48.4)-55 X 30-(38)-45	1.06-(1.28)-1.50		
PPSp6 ($A^1 \rightarrow A^2$)	Taipei	A	27.5-(52.0)-65 X 20-(42.7)-57.5	1.02-(1.23)-1.86	25-(32.9)-42.5	110-(24-32)-36
		B	37.5-(43.8)-55 X 25-(34.0)-45	1.13-(1.32)-1.7		

1. Sporangia were measured in the year of isolation (A) and in 2000 (B).
2. Data in parenthesis are in average.
3. Mycelial growth reactions to temperatures on V-8 agar are indicated as minimum -(optimum)-maximum.
4. $A^1 \rightarrow A^2$: mating type changed from A^1 to A^2
5. NT: not tested.

Table 3. Size of sexual organs of isolates of *Phytophthora parasitica* obtained from peace lily

Isolate no. & mating type	Location	Diamter (μm)[1]		Antheridia (μm)	
		Oogonia	Oospores	Length	Width
PPSp1 (A^2)	Chiayi	22-(27.9)[1]-36	18-(22.4)-26	8-(10.4)-14	10-(14.3)-20
PPSp2 (A^1)	Nantow	24-(26.8)-32	20-(22.7)-26	6-(9.6)-12	10-(12.5)-16
PPSp3 (A^1)	Puli	22-(25.6)-30	18-(21.3)-24	6-(9.2)-12	8-(11.8)-16
PPSp4 (A^2)	Changhua	22-(25.4)-30	18-(21.2)-26	6-(10.5)-14	10-(12.9)-16
PPSp5-1 (A^1)	Tainan	20-(24.9)-30	16-(19.8)-24	6-(10.9)-16	6-(12.0)-14
PPSp6 (A^1)	Taipei	14-(22.5)-26	16-(19.1)-24	8-(10.5)-16	8-(10.6)-14

1. Data in parenthesis are in average.

killed within one month. The same fungus was reisolated from all affected tissues. Whereas, all plants inoculated with distilled water remained healthy during the test periods.

Pathogenicity of an A^1 isolate of *P. parasitica* from peace lily to other aroids was also tested. The isolate was highly pathogenic to Philodendron (*P. scandens* subsp. *oxycardium* 心葉蔓綠絨) and killed all the inoculated cuttings within one month. The tested pathogen was moderately pathogenic to plantlets of anthurium (*A. andreanum* 火鶴花) and young cuttings of pothos vine (*E. aureum* 黃金葛), and all inoculated plants appeared black spots. *Phytophthora parasitica* from peace lily was not pathogenic to Dieffenbachia (*D. amoena* 夏雪黛粉葉).

DISCUSSION

Phytophthora parasitica was reported to cause serious aerial blight of peace lily in Hawaii in 1989 [23]. In my previous report [1], *Phytophthora* was not detected on peace lily in Taiwan before 1992. However, the disease was frequently observed in the fields and it has already become the critical factor for culfivation of the plants today. Variety susceptibility, favorable climatic conditions and diverse sources of inocula may be the principal factors contributing to the seriousness of *Phytophthora* diseases of peace lily in Taiwan. The tested new variety showed high susceptibility to the fungi in pathogenicity tests. The climate factors, such as high relative humidity and temperature in Taiwan are favorable to the development of Phytophthora diseases [5]. New *Phytophthora* isolates may arrive with the imported plants [1]. According to previous reports, the host ranges of *P. parasitica* are wide [9,11]. Moreover, *P. parasitic*a isolates obtained from different host plants can cause severe diseases on other hosts in cross-inoculation studies (1,4 & unpublished data). In this study, the peace lily isolate of *P. parasitica* also showed pathogenicity to some members in Araceae.

The major morphological and physiological characteristics of all tested isolates of *P. parasitica* from peace lily were similar each other (Table 2,3). All isolates formed unsymmetrically spherical to ovoid, papillate and non-deciduous sporangia with a shorter L/W (length/width) ratio of about 1.2-1.4. All isolates belong to cross-inducing (Heterothallic) type. They form oospores when dual-cultured with isolates of different mating types, but not in single cultures. Therefore, all Taiwanese cultures of *P. parasitica* from peace lily have essentially all the characteristics of typical *P. parasitica* (Table 2) [19] based on Tucker's descriptions in 1931y [20].

Generally heterothallic *Phytophthora* species do not form oospores in single cultures. However, some reports indicated that certain cultures of *P. capsici* leonian [12], *P. palmivora* (Butler) Butler [6] and *P. parasitica* [21] formed oospores in sectors or around the initial inocula in single cultures after long-tern storage. Subsequently Ko [15] reported that oospores formation by cultures of *P. parasitica* after storage was result from the changes of certain individuals to an opposite mating type during storage period. Moreover, certain chemicals such as chloroneb [3,18], ethazol [3,18], and metalaxyl [7] were found to have the effect of inducing mating-type changes of *P. parasitica* [3,18], *P. cannamoni* Rands [3], and *P. infestans* (Mont.) de Bary [7]. In this study, we found that the mating type of certain isolates of *P. parasitca* of peace lily changed after stored for 3 to 8 years. However, only isolates which original belonged to A^1 type, but not A^2 type, changed to A^2 or A^1A^2 mating type (Table 1).

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摘 要

安寶貞[1,2]、蔡志濃[1]. 2000. 台灣白鶴芋疫病. 植病會刊 9:145-150. ([1.]台中縣霧峰鄉 農業試驗所植物病理系，[2.]聯絡作者，電子郵件: pjann@wufeng.tari.gov.tw; 傳真: 04-3338162)

在台灣，白鶴芋罹患疫病首次於 1992 年在嘉義發現，病原菌為 *Phytophthora parasitica*。該菌危害白鶴芋時，造成葉枯與根腐，嚴重時導致全株死亡。自 1992 年至 1997 年，於台北、南投、彰化、嘉義、台南等地之七處花園中共分離的到 27 菌株，包括 19 株 A^1 與 8 株 A^2，經鑑定均為標準型的 *P. parasitica*。菌絲塊經過 3-8 年不同時間於 24℃ 下保存於無菌水中貯藏，存活的 16 株 A^1 菌株中，有 8 株的配對型改變成 A^2，2 株變成 A^1A^2，只有 6 菌株仍然為 A^1 配對型。然而，存活的 8 株 A^2 菌株均仍然為 A^2，配對型未曾改變。白鶴芋幼苗在接種分離得到的 *P. parasitica* 疫病菌遊走子懸浮液 (10^{5-6}zoospores/ml) 後，都出現與田間自然發病時相同的病徵。由於白鶴芋幼苗十分罹病，在接種一個月後，80% 以上之植株均會死亡，而且相同的疫病菌均可自發病組織回分得到。在病原性測定試驗中，自白鶴芋分離的 *P. parasitica* 亦同時可感染火鶴花 (*Anthurium andreanum*)、心葉蔓綠絨 (*Philodendron scandens* subsp. *Oxycardium*)、及黃金葛 (*Epiprenmum aureum*) 等三種天南星科觀賞植物，反之亦然。但該菌不危害夏雪黛粉葉 (*Dieffenbachia amoena*)。白鶴芋疫病為首次在台灣報導。

關鍵詞：白鶴芋、疫病、*Phytophthora parasitica*、配對型、新紀錄。