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Fusarium wilt of basil caused by *Fusarium oxysporum* f. sp. *basilici* in Taiwan

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ABSTRACT

Chen, Y.-J., Lin, Y.-S., and Chung, W.-H. 2017. Fusarium wilt of basil caused by *Fusarium oxysporum* f. sp. *basilici* in Taiwan. J. Plant Med. 59(1_2):1-6.

During 2000's, a new-wilting disease has impaired 20 - 40 % of yields of basil (*Ocimum basilicum* L.) in Taiwan. Infected plants showed stunting, root/stem rot, defoliation, dieback, vascular discoloration and wilting. Twenty-four fungal isolates (FOB01-24) were isolated from diseased basil plants in fields. Pathogenicity tests based on Koch's postulates indicated that all of 24 isolates caused above-mentioned symptoms in basil but no symptoms in another 5 Lamiaceae and 6 other Family plants after inoculation. Moreover, these isolates were identified as *Fusarium oxysporum* based on morphological characteristics and showed the same sequence of *elongation factor 1-alpha* gene (EF-1) with forma specialis "*basilici*". The results demonstrated that the wilting disease of basil in Taiwan is a new disease and can be caused by *F. oxysporum* f. sp. *basilici*.

Keywords: basil、Fusarium wilt、*Fusarium oxysporum* f. sp. *basilici*

Basil (*Ocimum* spp.) is a member of the Lamiaceae which comprises about 100 species and distributes over the tropical and subtropical areas in the world [4,9]. Among these species, common basil (*O. bacillicum* L.), an annual crop, is commercially cultivated and has been utilized in food, perfumery, and medical industries in South Europe, Southeast Asian and America [2]. In Taiwan, common basil contains several local ecotypes, widely cultivated as vegetable or spice at countryside, and there are approximate 100 ha of commercial fields located at south and central Taiwan [14].

During summers in 2000's, a wilting disease caused severe losses (20 - 40 %) of a local ecotype of basil "White stem" occurred in several commercial fields in central Taiwan and the disease extended to other production areas. The diseased plants showed stunting symptom first, followed with the discoloration of the vascular bundles (Fig. 1a). Then the tender shoots developed dieback, and roots would rot (Fig. 1b and c). Infected plants remained

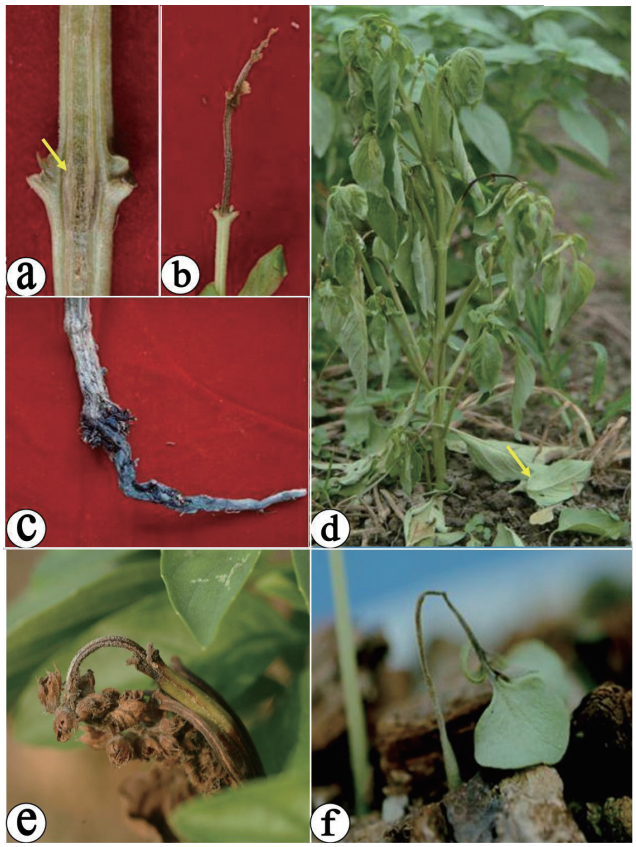

Fig. 1. Symptoms of the wilting disease on basil (*Ocimum bacillicum* L.) caused by *Fusarium oxysporum* in fields. The vascular bundles showed discoloration (yellow arrow) (a). Tender shoots developed dieback (b). Roots rot (c). Infected plants remained defoliated (yellow arrow) and lateral wilt (d). Flowers rot (e). For seedlings, the diseased basil developed damping-off within a few days (f).

defoliated, lateral wilt, flower rot (Fig. 1d and e) and followed by eventual death of plants. For diseased basil seedlings, damping-off occurred within a few days (Fig. 1f). Sometimes white mycelia and spore masses covered rotted stems buds or flowers. The aim of this study is to identify the causal agent that caused the wilting disease in basil in Taiwan.

For pathogen isolation, the basil plants exhibiting wilting and yellowing symptoms were inspected for vascular discoloration. They were collected from Dali in Taichung city, Silou and Erlun in Yunlin county, and Wandan in Pingtung county in Taiwan. The symptomatic stems were cut into 1-cm-long pieces and washed with tap water followed by surface sterilization (2 % sodium hypochlorite/95 % ethanol, 1/1, v/v) for 15 sec. The samples were then rinsed in sterile distilled water (SDW) before being placed onto 2 % water agar (WA) or Komada medium[6] in Petri plates (9 cm of diameter) and then incubated at 25 ˚C with 12 h light period per day. After 2 - 4 days incubation, the single fungal conidium from colonies developed around the incisions of samples on WA were transferred to potato dextrose agar (PDA) and incubated as mentioned above. A total of 24 isolates (FOB01 – 24; Table 1) were cultured on PDA and carnation leaf-piece agar (CLA), incubated at 25 ˚C with 12 h light period per day. All 24 isolates produced abundant aerial, white, cottony mycelia on PDA with limited light orange/purple pigment (Fig. 2a), and 3 kinds of asexual spores were developed on CLA. Macroconidia were the type "Fusarium" spores, with hyaline; 1 - 4 septates; 19.8 - 49.5 × 3.9 - 5.4 μm (Fig. 2b). Abundant microconidia were produced from monophialides; single-celled, hyaline, elliptic, or ovoid; 5.9 - 16.3 × 2.4 - 4.9 μm (Fig. 2c). Chlamydospores were spherical to ovoid, smooth-surfaced, intercalary or terminal; 7.4 - 12.3 μm of diameter (Fig. 2d). The morphological characterization indicated that all isolates were ***Fusarium oxysporum*** Schlechtend: Fr. (Fo) based on Snyder and Hansen's system [11,12]. All cultures were maintained at 4 ˚C on PDA for the following experiments.

Pathogenicity tests of Fo isolates from basil (FOB01 - 24) and from other crops (*F. oxysporum* f. sp. *lactucum* Fol-10, f. sp. *raphani* For981, f. sp. *luffae* Fol226, f. sp. *cucumerium* Foc100, f. sp. *pisi* F45, and f. sp. lycopersici Fot99) were conducted on 3 species of basil [common basil (*Ocimum basilicum* L.) ecotype "white stem", lemon basil (*Ocimum* × *citriodorum* Vis.), and santa basil, (*O. tenuiflorum* L.)] and other crops {Lamiaceae: majoran (*Origanum majorana* L.), Indian borage [*Plectranthus amboinicus* (Lour.) Spreng.], and coleus [*Plectranthus scutellarioides* (L.) R. Br.]; Asterales: lettuce (*Lactuca sativa* L.); Brassicaceae: radish (*Raphanus sativus* L.); Cucurbitaceae: luffa (*Luffa aegyptiaca* Mill), and cucumber (*Cucumis sativus* L.); Fabaceae: pea (*Pisum sativum* L.); Solanaceae: tomato (*Solanum lycopersicum* L.)}. All

TABLE 1. The *Fusarium oxysporum* (Fo) isolates isolated from wilting basils in this study

Isolates	Origination[1]	Location	Fields (coordinates)[2]	Isolation Date
FOB01	WS	Dali, Taichung (大里)	1 (24˚06'06.8"N 120˚39'31.2"E)	Jun. 2003
FOB02				
FOB03				
FOB04				
FOB05	WS	Dali, Taichung	2 (24˚06'07.6"N 120˚39'32.3"E)	Jul. 2003
FOB06				
FOB07				
FOB08				
FOB09				
FOB10				
FOB11	WS	Erlun, Yunlin (二崙)	1 (23˚47'02.0"N 120˚23'58.5"E)	Jul. 2003
FOB12				
FOB13	WS	Erlun, Yunlin	2 (23˚47'43.2"N 120˚23'46.5"E)	Jul. 2003
FOB14				
FOB15	Bush	Siluo, Yunlin (西螺)	1 (23˚47'31.5"N 120˚26'26.1"E)	Feb. 2004
FOB16	RS	Dali, Taichung	1	Feb. 2004
FOB17				Mar. 2004
FOB18	WS	Erlun, Yunlin	1	Apr. 2004
FOB19				
FOB20	WS	Erlun, Yunlin	2	Apr. 2004
FOB21	WS	Dali, Taichung	1	Mar. 2005
FOB22	WS	Wandan, Pingtung (萬丹)	1 (22˚35'32.3"N 120˚28'04.4"E)	Mar. 2009
FOB23				
FOB24				

[1] WS = *Ocimum basilicum* ecotype "white stem"; Bush = *O. basilicum* cv. Bush; RS = *O. basilicum* ecotype "red stem".

[2] The latitudes and longitudes were recorded for each sampled field using the GPS car navigation Mio® CLASSIC 500 (MiTAC Intl. Taiwan), and confirmed via Google Map (https://www.google.com.tw/maps).

Fo isolates were grown on PDA plates for 7-14 days at 28 ˚C with 12 h light period per day. After incubation, conidia from each isolate were collected in SDW and adjusted to 1^{05} conidia/ml of SDW as inocula. Three-wks- old seedlings (2 to 3 leaves) grown in plastic pot (20 cm in diameter, 15 cm in height) with peat moss (T059, Kekkilä Garden, Finland) were uprooted from pots and dipped in inoculum suspensions for 10 min, then transplanted into new pots with peat moss. Each isolate was tested in seven seedlings of each crop with three repeats. The seedlings inoculated with SDW were used as controls. All of the inoculated seedlings were maintained in the greenhouse (25 - 35 ˚C under natural light). The disease severities were evaluated 4-wks after inoculation. The disease index was modified from Pastor-Corrales and Abawi (1987)[8]: 0 = no

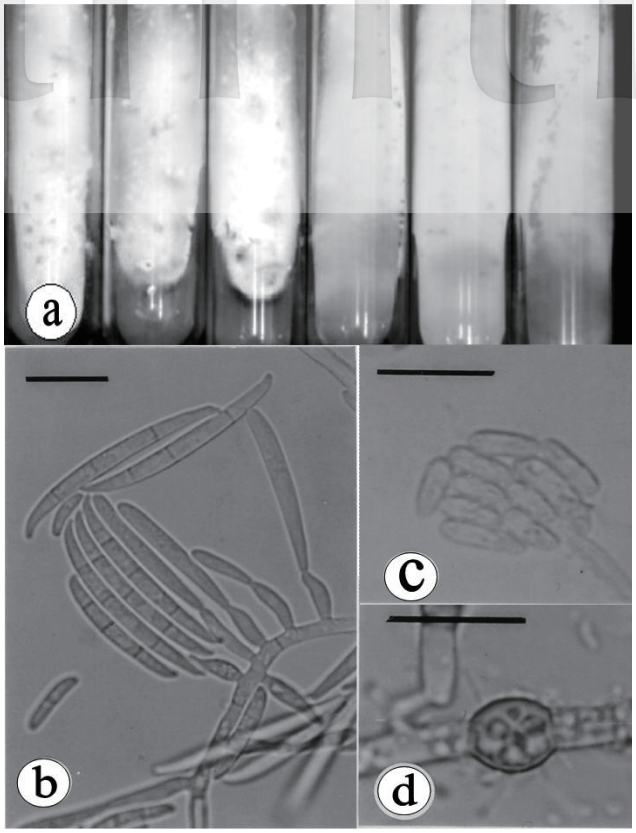

Fig. 2. Morphology of *Fusarium oxysporum* obtained from *Ocimum bacillicum* L. Colony characteristics cultured on potato dextrose agar (a), and conidial morphology cultured on carnation leaf-piece agar: (b) macroconidia; (c) microconidia; (d) chlamydospores. Bar = 10 μm.

symptoms; 1 = plant stunting or one leaf wilted; 2 = two or three leaves wilted; 3 = more than half of leaves wilted and yellowing; 4 = whole plant wilted and die. Results showed that each *Fo* isolate was pathogenic to their original host, but was unable to cause disease on other crops (Table 2). The same *Fo* isolates could be re-isolated from the inoculated diseased plants, and the experiments were conducted twice. Among these isolates, FOB01, 08, 09 and 10 showed high virulence (mean disease index = 4.0) to common basil.

For molecular phylogenetic analysis of *elongation factor*-1 *alpha* (EF1) gene, total DNAs from mycelial mats of 24 isolates from basil were extracted according to Sambrook and Russell (2001) [10]. Fragments of EF1 gene were amplified by PCR with the primer pair ef1/2 (5'-ATGGGTAAGGAAGACAAGAC-3' / 5'-GGAGGTACCAGTGATCATGTT-3') [7] using the conditions described by Geiser et al. (2004) [3]. The primers amplify an ~700 bp region of EF1, and these aligned EF1 gene sequence data were analyzed with a BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and compared with existing sequences of *Fo* isolates in GenBank database. BLAST analysis showed that all basil *Fo* isolates shared 99 % sequence identities with EF1 gene of *F. oxysporum* f. sp. *basilici* (FJ985381). Further, these aligned EF1 gene were analyzed using sequences of *F. proliferatum* (DQ837697) and *F. fujikuroi* (KJ025039) as the out-group. Sequences from different f. sp. of *Fo*, to wit, f. sp. *allii* (FJ985288), *asparagi* (DQ837691), *batatas* (FJ985368), *cepae* (GU165966), *cubenese* (FJ985329), *lycopersici* (FJ790388), *niveum* (FJ985410), *radicis-lycopersici*

TABLE 2. Pathogenicity tests of Taiwanese *Fusarium oxysporum* (*Fo*) isolates from basils on basil and other crops

Isolates (Origination)	Mean disease index[1]											
	Labiatae						Asterales	Brassicaceae	Cucurbitaceae		Fabaceae	Solanaceae
	Ob[2]	Oc	Ot	ORm	Pa	Ps	Ls	Rs	La	Cs	PIs	Sl
F. oxysporum isolates Fob01-24 (basil)	0.2 - 4.0	0	0	0	0	0	0	0	0	0	0	0
F. oxysporum f. sp. l*actucum* Fol-10 (lettuce)	0	0	0	0	0	0	3.8	0	0	0	0	0
F. oxysporum f. sp. *raphani* For981 (radish)	0	0	0	0	0	0	0	2.4	0	0	0	0
F. oxysporum f. sp. *luffae* Fol226 (luffa)	0	0	0	0	0	0	0	0	4.0	0	0	0
F. oxysporum f. sp. *cucumerium* Foc100 (cucumber)	0	0	0	0	0	0	0	0	0	4.0	0	0
F. oxysporum f. sp. *pisi* F45 (Pea)	0	0	0	0	0	0	0	0	0	0	3.6	0
F. oxysporum f. sp. *lycopersici* Fot99 (tomato)	0	0	0	0	0	0	0	0	0	0	0	3.2

[1] Disease index at 4-week after inoculation was rated: 0, no symptoms; 1, plant stunting or one leaf wilted; 2, two or three leaves wilted; 3, more than half of leaves wilted and yellowing; 4, whole plant wilted and die.

[2] Ob = *Ocimum basilicum* L. (basil); Oc = *Ocimum* × *citriodorum* Vis. (lemon basil); Ot = *Ocimum tenuiflorum* L. (Santa basil); ORm = *Origanum majorana* L. (majoran); Pa = *Plectranthus amboinicus* (Lour.) Spreng. (Indian borage); Ps = *Plectranthus scutellarioides* (L.) R. Br. (coleus); Ls = *Lactuca sativa* L. (lettuce); Rs = *Raphanus sativus* L. (radish); La = *Luffa aegyptiaca* Mill (luffa); Cs = *Cucumis sativus* L. (cucumber); PIs = *Pisum sativum* L. (pea); Sl = *Solanum lycopersicum* L. (tomato).

(FJ790405), *tracheiphilum* (FJ985364), *tulipae* (FJ985275), and *vasinfectum* (DQ837695), were obtained from GenBank and included for comparison with the 4 selected high virulence Fo isolates from basil in Taiwan (FOB01, 08, 09, and 10). Phylogenetic analyses were perused with the programme Molecular Evolutionary Genetics Analysis Version 6.0 (MEGA 6.0) [13]. Phylogenetic trees were generated with neighbor-joining (NJ) method and viewed with the NJ plot program [5]. Reliability of the inferred trees was estimated by 1000 bootstrap resampling using the same program. The analysis indicated that all the selected *Fo* isolates from basil in Taiwan clustered together and formed one group with *F. oxysporum* f. sp. *basilici* (FJ985381), while the other f. sp. clustered separately or formed a separate group (Fig. 3). The Fo isolates from basil in Taiwan had the closest relationships with f. sp. *basilici*, and the partial sequence of isolates FOB08's EF1 gene has been submitted to GenBank (accession number = MF042165) for searching and studying.

Fusarium oxysporum produced the type "Fusarium" of macrocondia, is a soil-born phytopathogen causing more than 100 crops wilting diseases[1,11]. Among these diseases, the pathogen has been divided to many different formae speciales (f. sp.) based on their host specificity [1]. In this study, all *F. oxysporum* isolates from diseased basil showed specific pathogenisity to *Ocimum basilicum* L. and were classified as f. sp. *basicili* based on the pathogenic and phylogenetic analyses. The results strongly suggested that the new wilting disease is the Fusarium wilt of basil caused by *F. oxysporum* f. sp. *basilici* in Taiwan.

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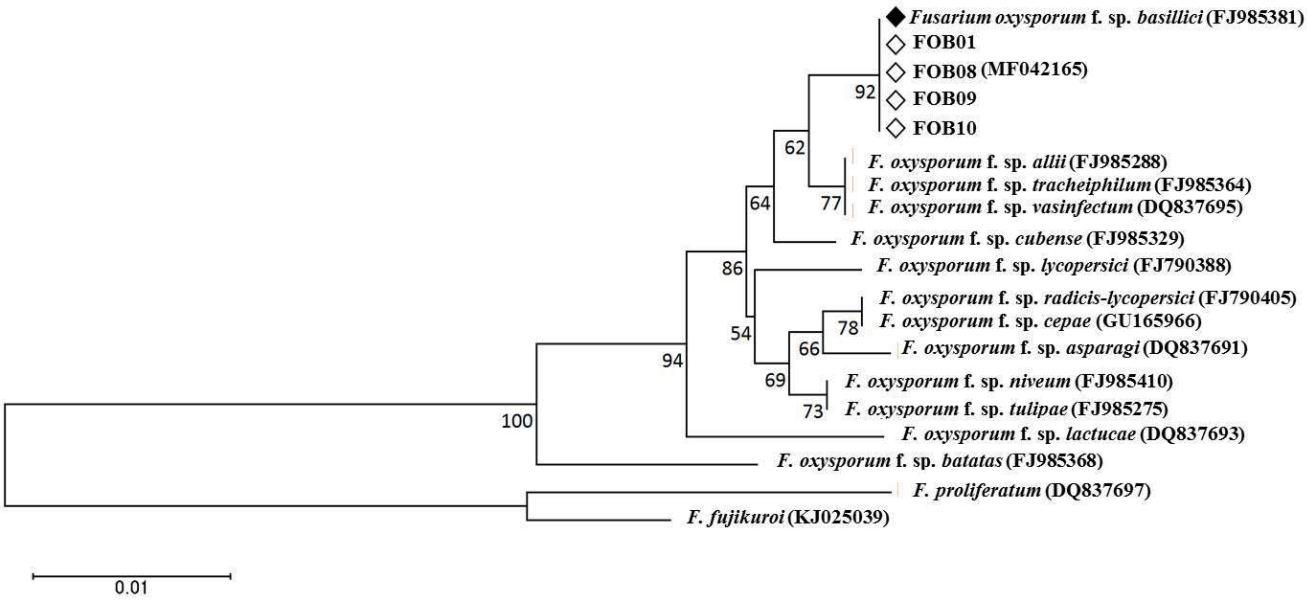

Fig. 3. Position of *Fusarium oxysporum* isolates from wilting basil plants in Taiwan [FOB01, 08 (MF042165), 09 and 10] (hollow diamond ◇) and *F. oxysporum* f. sp. *basillici* (FJ985381) (black diamond ◆) in a phylogenetic tree constructed based on the *elongation factor 1-alpha* gene. The figure was drawn by MEGA 6.06 with neighbor-joining model. *Fusarium proliferatum* (DQ837697) and *F. fujikuroi* (KJ025039) were used as out-groups to root the tree. Bootstrap values are calculated from 1,000 repetitions. Scale bar represents phylogenetic distance between nucleotides.

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摘　要

陳以錚、林益昇、鍾文鑫。2017。由尖鐮胞菌***Fusarium oxysporum*** f. sp. ***basilici***引起之羅勒萎凋病。植物醫學59(1_2):1-6。

2000年間，台灣中部及南部地區羅勒 (***Ocimum basilicum*** L.) 普遍發生一種不明病因之萎凋型病害，造成田間20-40 %的產量損失。罹病植株首先矮化及生長不良，後續發生根莖腐敗、落葉及梢枯等病徵，切開組織可見維管束褐化，嚴重時全株萎凋死亡。自受感染組織中分離得到24株真菌分離株(FOB01-24)，經柯霍氏法則證實FOB01-24皆對羅勒具有病原性，但對其他5種唇型科及6種不同科植物則無。進一步根據FOB01-24的孢子及菌落形態，佐以*elongation factor 1-alpha* gene (EF-1) 基因序列分析，證實臺灣羅勒萎凋病是由***Fusarium oxysporum*** f. sp. ***basilici***所引起，為羅勒新記錄病害。

關鍵詞：羅勒 (***Ocimum basilicum*** L.)、萎凋病、***Fusarium oxysporum*** f. sp. ***basilici***。