International Journal of Agricultural Technology 2025 Vol. 21(5):1725-1744
DOI: https://doi.org/10.63369/ijat.2025.21.5.1725-1744
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Characterization of *Diaporthe batatas* Harter & E.C. Field causing leaf spot in water spinach

Garcia, T. A. P. and Montecalvo, M. P.*

National Crop Protection Center, College of Agriculture and Food Science, University of the Philippines Los Baños, College, Laguna, Philippines 4031.

Garcia, T. A. P. and Montecalvo, M. P. (2025). Characterization of *Diaporthe batatas* Harter & E.C. Field causing leaf spot in water spinach. International Journal of Agricultural Technology 21(5):1725-1744.

Abstract Water spinach (*Ipomoea aquatica* Forsk.) is important for its nutritional, economic, and environmental benefits. However, pathogen infections can impact its production. This study aimed to identify the fungal pathogen responsible for leaf spots on lowland water spinach. Infected leaves showed brown necrotic spots surrounded by yellow halos. Pathogenicity assays confirmed the virulence of the fungal pathogen WS1 in both lowland and upland water spinach. Through morphological, cultural, and molecular characterization, the pathogen was identified as *Diaporthe batatas* Harter & E.C. Field. Key characteristics of the fungal isolate included hyaline, septated, and branched hyphae, with conidia measuring 4.1 to 8.3 µm in length and 1.8 to 3.2 µm in width. The isolate displayed distinct growth patterns on various artificial media with transparent, flat, rhizoid mycelium on water agar; white, velvety mycelium with black pycnidia on malt extract agar and potato dextrose agar; and dark brown to black filamentous colonies on Sabouraud dextrose agar. Sequencing of the internal transcribed spacer (ITS) and β-tubulin genes matched reference sequences of *D. batatas*. The study identifies *D. batatas* as a fungal pathogen of water spinach.

Keywords: Etiology, Foliar disease, Pathogenicity, Polyphasic identification

Introduction

Ipomoea aquatica Forsk., more commonly known as water spinach, of the family Convolvulaceae is indigenous to Africa, Asia, and the Southwestern Pacific Islands (Austin, 2007). Known for its edible and nutritious leaves, this vegetable thrives in subtropical and tropical climates with temperatures between 25 to 30°C (Palada and Chang, 2003). It is a low-maintenance plant that can be propagated through seeds or stem cuttings. It is valued for its rich supply of vitamins, minerals, and antioxidants (Jiang *et al*., 2023; Singh *et al*., 2023). Its health benefits and culinary versatility have fueled commercial production globally. China, Belgium, and Spain are the leading exporters of water spinach, contributing 24.86%, 12.81%, and 11.94% of the global market, respectively

* **Corresponding Author**: Montecalvo, M.; **Email:** mpmontecalvo@up.edu.ph

(Tridge, 2023). The total global trade value for water spinach, encompassing both exports and imports, is $8.38 billion.

Water spinach is susceptible to diseases that can adversely affect its cultivation. Plant disease is a significant constraint on crop production (Gai and Wang, 2024). The presence of diseases can lead to substantial yield losses and reduced product quality. However, there are limited records of diseases affecting this crop. Commonly reported diseases of water spinach include white rusts and leaf spots (Hu *et al*., 2021). White rust caused by various species of *Albugo* is characterized by white or creamy-yellow pustules on leaves, stems, or flowers, predominantly on the lower leaf surfaces. *Albugo candida* has been reported in several Southeast Asian countries, including Thailand, Indonesia, Malaysia, Singapore, and Hong Kong (Dueñas-López, 2012). In the Philippines, *A. ipomoeae aquatica* Sawi was reported in 1978 by Gacutan and Quimio. Same symptoms were observed in the United States, where *A. ipomoeae-panduratae* has been identified as the causal agent (Quebral, 2000).

Liu *et al*. (2020) characterized leaf spot pathogens from several spinach production areas in the United States inciting anthracnose (*Colletotrichum spinaciae*) and *Stemphylium* leaf spot (*Stemphylium vesicarium* and *S. beticola*). Other pathogenic fungal species recovered in spinach were *Colletotrichum coccodes*, *C. truncatum*, *Cercospora beticola*, and *Myrothecium verrucaria*. On the other hand, Cerkauskas *et al*. (2006) recorded *Phyllosticta ipomoeae*, *Cercospora ipomoeae*, and *Pseudomonas syringae* pv. *syringae* as causal organisms of foliar diseases in *I. aquatica* in greenhouses in Ontario and California. More recently, Lee *et al*. (2022) documented the first case of leaf spot on water spinach caused by *Ectophoma multirostrata* in Korea. In the Philippines, *Pestalotia* sp. was the only recorded fungal pathogen inciting leaf spot in water spinach (Sasuman and Tangonan, 1995). Other reported pathogens causing diseases to *I. aquatica* are *Meloidogyne incognita* Chit causing root-knot (Valdez, 1968), Tobacco mosaic virus causing mosaic pattern development (Benigno, 1981), and *Xanthomonas campestris* and *X. perforans* causing bacterial leaf canker (Leksomboon *et al*., 1991; Hu *et al*., 2021).

Effective management of plant diseases requires accurate identification of the causal organism. Therefore, this research was conducted to identify the fungal pathogen associated with leaf spot symptoms in lowland water spinach. A polyphasic approach was employed to identify the causal organism based on its cultural, morphological, and molecular characteristics. The study also aimed to determine whether the fungal pathogen could cross-infect the upland water spinach.

Materials and methods

This research was conducted from January 2023 to April 2024 at the Mycology Laboratory at the National Crop Protection Center (NCPC) under the College of Agriculture and Food Science, University of the Philippines Los Baños, College, Laguna, Philippines.

Isolation and pathogenicity test

Leaves of water spinach showing brown necrotic lesions were collected and subsequently diagnosed at the Mycology Laboratory of NCPC. Sections of the leaf tissue from the advancing edge of the lesions were cut into pieces measuring approximately 3 x 3 mm. These sections were immersed in a 10% NaOCl solution for 1 minute, followed by three rinses in sterile distilled water for 30 seconds each. The surface-sterilized sections were air-dried inside a laminar flow hood and placed onto potato dextrose agar (PDA) plates and kept at room temperature for 3 days. Following the growth of fungal colonies, sections of the hyphal margins were selected for subculturing onto fresh PDA. The subcultures were then incubated at room temperature for 7 days.

Two methods of pathogenicity test were conducted to evaluate the susceptibility of two water spinach varieties, lowland and upland, to the fungal isolate. Each method utilized 20 plants per variety, divided equally into control and treated groups.

In the first method, a standardized conidial suspension served as the inoculum. Pycnidia from a 14-day-old culture were scraped and suspended in 0.1% Tween 80 solution. The resulting suspension was then filtered, and its concentration was determined and adjusted to 1 x 10^6 conidia/ml. Each plant of the treated group had three healthy leaves sprayed with 2 ml of the conidial suspension, while control plants were treated with sterile 0.1% Tween 80 solution. Daily monitoring was performed for 14 days and necrotic spots were assessed using the rating scale: 0 = no symptoms; 1 = dot-like necrotic spots with no yellow halo; 2 = < 0.5mm necrotic spots with yellow halo; 3 = 1mm < x < 0.5mm necrotic spots with yellow halo; and 4 = > 1mm necrotic spot with yellow halo.

In the second method, mycelial discs were inoculated in wounded and unwounded leaves. Using a flame-sterilized needle, leaves were pricked to create wounds. Five (5) mm mycelial discs were placed onto the healthy leaves of both lowland and upland water spinach varieties. These discs were secured at their respective inoculation points using adhesive tape. The plants were then covered with damp plastic for 24 hours. Disease progression was monitored. Necrotic lesions on the inoculation sites were measured after 14 days. Leaves showing

similar symptoms with the plant samples were subjected to re-isolation, which followed the same protocol as the initial isolation process.

Fungal identification

Polyphasic identification of the fungal pathogen was done by examining the cultural, morphological, and molecular characteristics of the fungal isolate.

Cultural characterization. The fungus was grown in various artificial media including water agar, malt extract agar (MEA), PDA, and Sabouraud dextrose agar (SDA) to determine its growth characteristics. The form, elevation, and margin of the fungal growth were described.

Morphological characterization. The fungal isolate was subjected to slide culture technique. Different morphological features of the hyphae, conidia, and conidiomata were examined using Zeiss Primo Star compound microscope. The data were referred to different taxonomic references including Illustrated Genera of Imperfect Fungi (Barnett and Hunter, 1972) and Simplified Fungi Identification Key (Williams-Woodward, 2001).

Molecular identification and phylogenetic analysis. Molecular characterization was also performed by DNA isolation and sequencing. The primers ITS5/ITS4 and BT2a/BT2b were used for the amplification of ITS and β-tubulin, respectively. BLASTn program was used to compare the DNA sequence of the fungal isolate with those in NCBI database (Table 1). The ITS gene sequences were aligned using MEGA X with phylogenetics inferred by using the maximum likelihood method and Kimura 2-parameter model with 1000 replicates. On the other hand, the β-tubulin gene sequences were aligned using MEGA X with phylogenetics constructed through maximum parsimony with 1000 bootstrap replications. The sequences were deposited in Genbank database.

Table 1. Reference sequences of *Diaporthe* species used in this study

Species	Isolate	Gene Sequence	Host*	Locality	GenBank Accession Number
Diaporthe batatas	*Diaporthe batatas* MS-1S-A	ITS	sweet potato	Miyazaki, Japan	LC543577.1
Diaporthe batatas	*Diaporthe batatas* isolate SP-d1	ITS	sweet potato	Seoul, South Korea	KU577616.1
Diaporthe batatas	*Diaporthe batatas* A11	ITS	sweet potato	Kagoshima, Japan	LC543586.1
Diaporthe batatas	*Diaporthe batatas* A5	ITS	sweet potato	Miyazaki, Japan	LC543582.1

Species	Isolate	Gene Sequence	Host*	Locality	GenBank Accession Number
Diaporthe batatas	*Diaporthe batatas* MS-3RJ-A1	ITS	sweet potato	Miyazaki, Japan	LC543576.1
Diaporthe batatas	*Diaporthe batatas*	ITS	sweet potato	Jiangsu, China	OK432549.1
Diaporthe batatas	*Diaporthe batatas* strain Y. H. Yeh I0608	ITS	*Ipomoea pes-caprae*	Taoyuan, Taiwan	MK336444.1
Diaporthe batatas	*Diaporthe batatas* CBS 122.21	ITS	*Ipomoea batatas*	Maryland, USA	NR_152456.1
Diaporthe batatas	*Diaporthe batatas* A10	ITS	sweet potato	Kagoshima, Japan	LC543585.1
Diaporthe batatas	*Diaporthe batatas* MOKM-3S-B	ITS	sweet potato	Miyazaki, Japan	LC543575.1
Diaporthe batatas	*Diaporthe batatas* MKM-1S1P-B	ITS	sweet potato	Miyazaki, Japan	LC543580.1
Diaporthe batatas	*Diaporthe batatas* A9	ITS	sweet potato	Kagoshima, Japan	LC543584.1
Diaporthe batatas	*Diaporthe batatas* MKM-1S1P-A	ITS	sweet potato	Miyazaki, Japan	LC543579.1
Diaporthe batatas	*Diaporthe batatas* A2	ITS	sweet potato	Kagoshima, Japan	LC543581.1
Diaporthe batatas	*Diaporthe batatas* MT-2L-A1	ITS	sweet potato	Miyazaki, Japan	LC543578.1
Diaporthe batatas	*Diaporthe batatas* A6	ITS	sweet potato	Kagoshima, Japan	LC543583.1
Diaporthe batatas	*Diaporthe batatas* strain K1	ITS	-	Nanchang, China	MW879521.1
Diaporthe batatas	*Diaporthe batatas* isolate WS	ITS	-	Uttar Pradesh, India	PP716519.1

Species	Isolate	Gene Sequence	Host*	Locality	GenBank Accession Number
Diaporthe phaseolorum	*Diaporthe phaseolorum* isolate 12	ITS	*Pilosella officinarum*	Poznan, Poland	MW580409.1
Diaporthe hordei	*Diaporthe hordei* strain CBS 481.92	ITS	*Hordeum vulgare*	Utrecht, Netherlands	KC343120.1
Diaporthe arezzoensis	*Diaporthe arezzoensis* MFLUCC 15-0127	ITS	*Cytisus sp.*	Italy	NR_171296.1
Diaporthe lusitanicae	*Diaporthe lusitanicae* strain GBC-Fungus16	ITS	*Ranunculus acris*	Taranaki, New Zealand	MN077420.1
Diaporthe cucurbitae	*Diaporthe cucurbitae* DAOM 42078	ITS	*Cucumis* sp.	Canada	NR_147563.1
Diaporthe stewartii	*Diaporthe stewartii* clone 072	ITS	*Phragmites australis*	New Orleans, USA	OM262255.1
Diaporthe unshiuensis	*Diaporthe unshiuensis* strain JFRL 03-1131	ITS	*Citrus sinensis*	Jiangxi, China	OQ691638.1
Diaporthe melonis	*Diaporthe melonis* isolate L5N151	ITS	*Solanum quitoense*	Pichincha, Ecuador	MF185355.2
Diaporthe guangdongensis	*Diaporthe guangdongensis* isolate MBELPIC63.1	ITS	*Solanum melongena*	Laguna, Philippines	OQ123527.1
Diaporthe endophytica	*Diaporthe endophytica*	ITS	*Cinchona calisaya*	Banten, Indonesia	AB899789.1
Diaporthe masirevicii	*Diaporthe masirevicii* voucher MFLUCC 17-1422	ITS	*Chromolaena odorata*	Chiang rai, Thailand	MH094275.1

Species	Isolate	Gene Sequence	Host*	Locality	GenBank Accession Number
Diaporthe chromolaenae	*Diaporthe chromolaenae* culture MFLUCC:17-1422	ITS	*Chromolaena odorata*	Chiang rai, Thailand	MT214362.1
Diaporthe batatas	*Diaporthe batatas* isolate K1	Beta-tubulin	-	Jiangxi, China	MW927510.1
Diaporthe batatas	*Diaporthe batatas* isolate AL32C	Beta-tubulin	sweet potato	Pernambuco, Brazil	OQ676832.1
Diaporthe batatas	*Diaporthe batatas* isolate TH34	Beta-tubulin	sweet potato	Pernambuco, Brazil	OQ676835.1
Diaporthe batatas	*Diaporthe batatas* isolate TH3	Beta-tubulin	sweet potato	Pernambuco, Brazil	OQ676833.1
Diaporthe batatas	*Diaporthe batatas* isolate TH47	Beta-tubulin	sweet potato	Pernambuco, Brazil	OQ676837.1
Diaporthe batatas	*Diaporthe batatas* isolate AL22A	Beta-tubulin	sweet potato	Pernambuco, Brazil	OQ676831.1
Diaporthe batatas	*Diaporthe batatas* isolate AL3A	Beta-tubulin	sweet potato	Pernambuco, Brazil	OQ676830.1
Diaporthe batatas	*Diaporthe batatas* strain ZJUDB07	Beta-tubulin	sweet potato	Zhejiang, China	KP973862.1
Diaporthe batatas	*Diaporthe batatas* strain ZJUDB18	Beta-tubulin	sweet potato	Zhejiang, China	KP973867.1
Diaporthe batatas	*Diaporthe batatas* isolate AL39B	Beta-tubulin	sweet potato	Seoul, South Korea	OQ676840.1
Diaporthe batatas	*Diaporthe batatas* isolate TH49	Beta-tubulin	sweet potato	Pernambuco, Brazil	OQ676838.1

Species	Isolate	Gene Sequence	Host*	Locality	GenBank Accession Number
Diaporthe batatas	*Diaporthe batatas* isolate SE85B	Beta-tubulin	sweet potato	Pernambuco, Brazil	OQ676829.1
Diaporthe batatas	*Diaporthe batatas* strain ZJUDB34	Beta-tubulin	sweet potato	Zhejiang, China	KP973871.1
Diaporthe batatas	*Diaporthe batatas* strain ZJUDB08	Beta-tubulin	sweet potato	Zhejiang, China	KP973863.1
Diaporthe batatas	*Diaporthe batatas* strain ZJUDB11	Beta-tubulin	sweet potato	Zhejiang, China	KP973865.1
Diaporthe batatas	*Diaporthe batatas* isolate SP-d1	Beta-tubulin	sweet potato	Seoul, South Korea	KU577614.1
Diaporthe batatas	*Diaporthe batatas* isolate AL48	Beta-tubulin	sweet potato	Pernambuco, Brazil	OQ676841.1
Diaporthe destruens	*Diaporthe destruens* MAFF:246953	Beta-tubulin	sweet potato	Kanagawa, Japan	LC624792.1
Diaporthe batatas	*Diaporthe batatas* isolate TH25	Beta-tubulin	sweet potato	Pernambuco, Brasil	OQ676834.1
Diaporthe ueckeri	*Diaporthe ueckeri* isolate MEMR0500	Beta-tubulin	*Glycine max*	São Paulo, Brazil	PP372870.1
Diaporthe passiflorae	*Diaporthe passiflorae* strain ZHKUCC20-0023	Beta-tubulin	*Citrus grandis* cv. Tomentosa	Guangdong, China	MT409301.1
Diaporthe breyniae	*Diaporthe breyniae* strain CBS 148910	Beta-tubulin	*Breynia oblongifolia*	Lower-Saxony, Germany	ON409186.1
Diaporthe moorei	*Diaporthe moorei strain BRIP 61500b*	Beta-tubulin	*Vigna radiata*	Queensland, Australia	OR039652.1

Species	Isolate	Gene Sequence	Host*	Locality	GenBank Accession Number
Diaporthe masirevicii	*Diaporthe masirevicii* strain ZHKUCC20-0009	Beta-tubulin	*Citrus grandis cv. Tomentosa*	Guangdong, China	MT409287.1
Diaporthe fructicola	*Diaporthe fructicola isolate NFIT-1-8*	Beta-tubulin	-	Hubei, China	MW208585.1
Diaporthe endophytica	*Diaporthe endophytica* isolate ZJUE0351	Beta-tubulin	*Citrus maxima*	Zhejiang, China	OQ719591.1
Diaporthe kochmanii	*Diaporthe kochmanii voucher HGUP192093*	Beta-tubulin	*Rosa roxburghii*	Guizhou, China	MZ724028.1
Diaporthe sojae	*Diaporthe sojae isolate GXN29*	Beta-tubulin	*Citrus maxima*	Fujian, China	PP516303.1
Diaporthe unshiuensis	*Diaporthe unshiuensis isolate NFFT-1-5*	Beta-tubulin	-	Hubei, China	MW208617.1
Diaporthe phaseolorum	*Diaporthe phaseolorum strain 202008-BM8*	Beta-tubulin	soybean	Jiangsu, China	MZ312835.1

*No host (-) was indicated in the GenBank database.

Statistical design and analysis

The experimental setup was arranged in CRD. The data were analyzed by performing one-way ANOVA. Pathogenicity tests in lowland and upland water spinach were compared using t-test ($P<0.05$). Means of colony growth of the fungal isolate on different artificial media were analyzed following Tukey's Honestly Significant Difference test ($P<0.05$).

Results

Pathogenicity of the fungal pathogen

Pathogenicity tests confirmed virulence of the fungal isolate WS1 in leaves of both lowland and upland water spinach (Figure 1). Necrotic lesions were surrounded with yellow halo. Spraying fungal conidial suspension caused 100% disease incidence (Table 2). Lesions on the lowland water spinach started coalescing after 7 days post-inoculation, resulting in larger spots that developed into blight symptoms. The upland variety had numerous spots with maximum size of 1 mm but did not coalesce even after 14 days of incubation. Severity of lesion was 51.44% and 43.48% in lowland and upland water spinach, respectively.

Inoculation of mycelial disc resulted in appearance of symptoms in upland variety apparent after 3 days of incubation for both wounded and unwounded setups. Conversely, symptoms in the lowland variety appeared at 4 days for wounded and 6 days for unwounded setups. In unwounded setup, higher infection (60.00%) was observed in lowland than upland (20.00%), however, the lesion length did not vary significantly ranging from 5.67-6.03mm. In wounded setup, the fungal pathogen caused similar degree of disease in both lowland and upland water spinach with 5.97-9.48mm lesions and 60.00-73.33% incidence.

Figure 1. Symptoms observed in upland and lowland water spinach leaves inoculated with conidia suspension and mycelial discs of *Diaporthe batatas* WS1

Table 2. Pathogenicity of *Diaporthe batatas* WS1 in lowland and upland water spinach

Water Spinach	Inoculated with Conidial Suspension*		Inoculated with Mycelial Disc			
			Unwounded		Wounded*	
	Severity (%)	Incidence (%)	Lesion Length* (mm)	Incidence (%)	Lesion Length (mm)	Incidence (%)
Lowland	51.44	100.00	6.03	60.00a	9.48	73.33
Upland	43.48	100.00	5.67	20.00b	5.97	60.00

*Means in columns with similar letters are not significantly different by t-test (P<0.05).

Identification of the fungal pathogen

Similar morphological and cultural characteristics were observed in the original and re-isolated fungal culture. Polyphasic identification was employed to confirm the identity of the fungal isolate.

Cultural characterization. Different artificial media with varying nutritional composition influenced colony characteristics of the fungal isolate (Figure 2). Among these artificial media, the highest average colony growth was evident in SDA with 84.00 mm and rapid growth rate of 23.15 mm/day (Table 3). SDA, with its high mycological peptone content, is naturally acidic, which effectively promotes fungal growth. Fungal colonies had alternating white and dark brown filamentous mycelia, devoid of any visible conidiomata even after 10 days of incubation (Figure 2D) and black pigmentation was evident in reverse (Figure 2H).

Growing the fungal isolate in PDA and MEA had similar effect on the average colony growth, with 69.40-73.40 mm and average colony growth rate of 16.37-16.50 mm/day. On these artificial media with high carbohydrate content, white, velvety, rhizoid mycelial growth was observed. However, several differences were observed between the two artificial media after 10 days of incubation. In PDA (pH = 5.6 ± 0.2), which is slightly less acidic than MEA (5.4 ± 0.2), the conidiomata were evenly distributed across the entire medium (Figure 2B), while on MEA, they were concentrated on the thick gummy mycelial growth near the center (Figure 2C). Moreover, in reverse, PDA displayed a pale-yellow pigmentation (Figure 2F), while MEA had a darker shade of yellow pigmentation (Figure 2G).

On the other hand, transparent, flat, rhizoid mycelial growth was observed in water agar (WA) (Figures 2A and 2E). The slow growth of the fungal isolate on this medium (21.60 mm colony growth at 4.31 mm/day), attributable

to its nutrient deficiency, hindered the development of other fungal structures such as conidiomata and conidial cells.

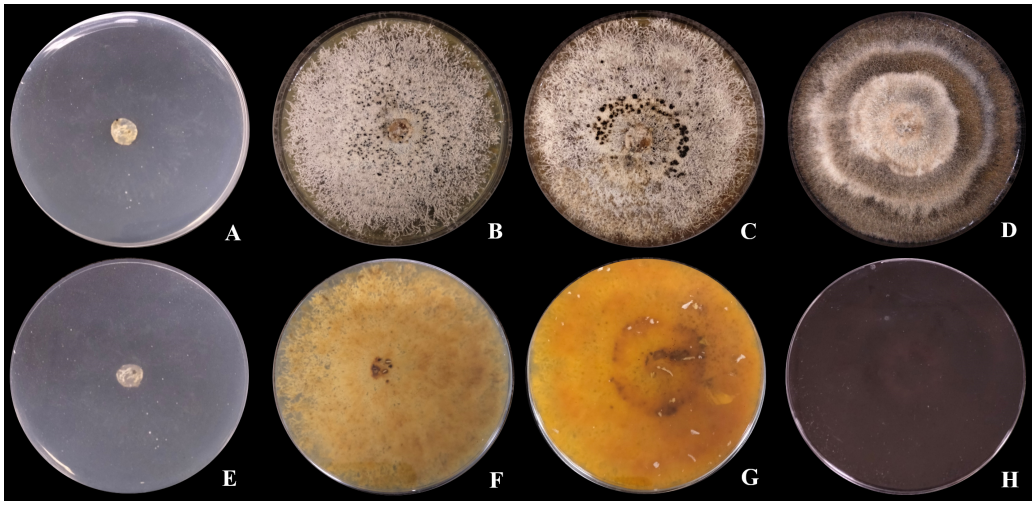

Figure 2. Obverse (A-D) and reverse (E-H) colony characteristics of the fungal isolate on different artificial media, Water Agar (A,E), Potato Dextrose Agar (B,F), Malt Extract Agar (C,G), and Sabouraud Dextrose Agar (D,H) after 10 days of incubation

Table 3. Average colony growth of the fungal isolate on different artificial media

Artificial Media	Average colony growth (mm)	Average colony growth rate (mm/day)
Water Agar	21.60c	4.31b
Potato Dextrose Agar	73.40b	16.50a
Malt Extract Agar	69.40b	16.37a
Sabouraud Dextrose Agar	84.00a	23.15a

*Means in columns with similar letters are not significantly different at Tukey's HSD ($P<0.05$).

Morphological characterization

The fungal isolate had pycnidial conidiomata that are sub-globose to irregular in shape, either solitary or clustered, black, erumpent, and thick-walled, with creamy to yellowish conidial cirrus exuding from the ostioles (Figure 3). The hyphae were hyaline, smooth, and septated. The alpha conidia were hyaline,

smooth, aseptate, ellipsoidal to fusoid, biguttulate, with a rounded base and a rounded or tapered apex, measuring 4.1-8.3 µm in length and 1.8-3.2 µm in width (mean = 6.37 x 2.82 µm, n = 100). Beta conidia were not observed.

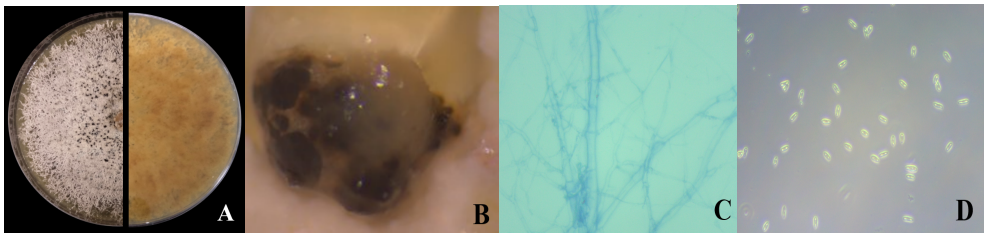

Figure 3. Morphological features of the fungal isolate, 14-day old culture on PDA (A), conidiomata (B), hyphae (C), and conidia (D)

Molecular identification and phylogenetic analysis

The comparison of DNA sequences based on ITS and β-tubulin demonstrated that the fungal isolate was similar to reference sequences in the Genbank database (Figures 4 and 5). The phylogenetic tree suggests that the fungal isolate was *Diaporthe batatas* Harter & E.C. Field conforming with the typical morphological and cultural characteristics. This fungal isolate from water spinach showed 100.00% homology with ITS sequence of *D. batatas* (GenBank Accession No. MW879521) and 99.44% homology with beta-tubulin sequence of *D. batatas* (GenBank Accession No. MW927510). The other *Diaporthe* species formed an outgroup including *D. phaseolorum*, *D. hordei, D. arezzoensis*, *D. lusitanicae*, *D. cucurbitae*, *D. stewartii*, *D. unshiuensis*, *D. melonis*, *D. guangdongensis*, *D. endophytica*, *D. masirevicii*, *D. chromolaenae, D. ueckeri, D. passiflorae, D. breyniae, D. moorei, D. fructicola, D. kochmanii,* and *D. sojae*.

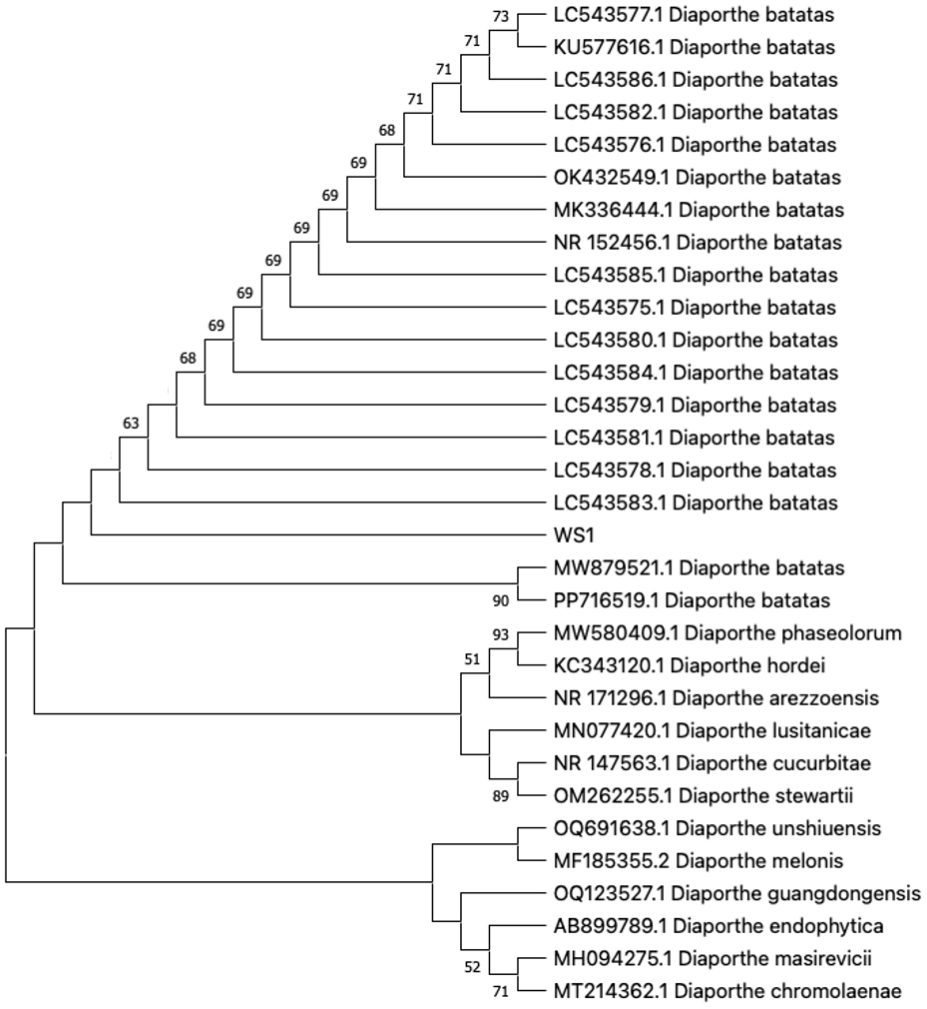

Figure 4. Phylogenetic tree of ITS gene sequences of fungal isolate WS1 forming a clade with *Diaporthe batatas* using maximum likelihood method and Kimura 2-parameter model with bootstrap consensus tree inferred from 1000 replicates

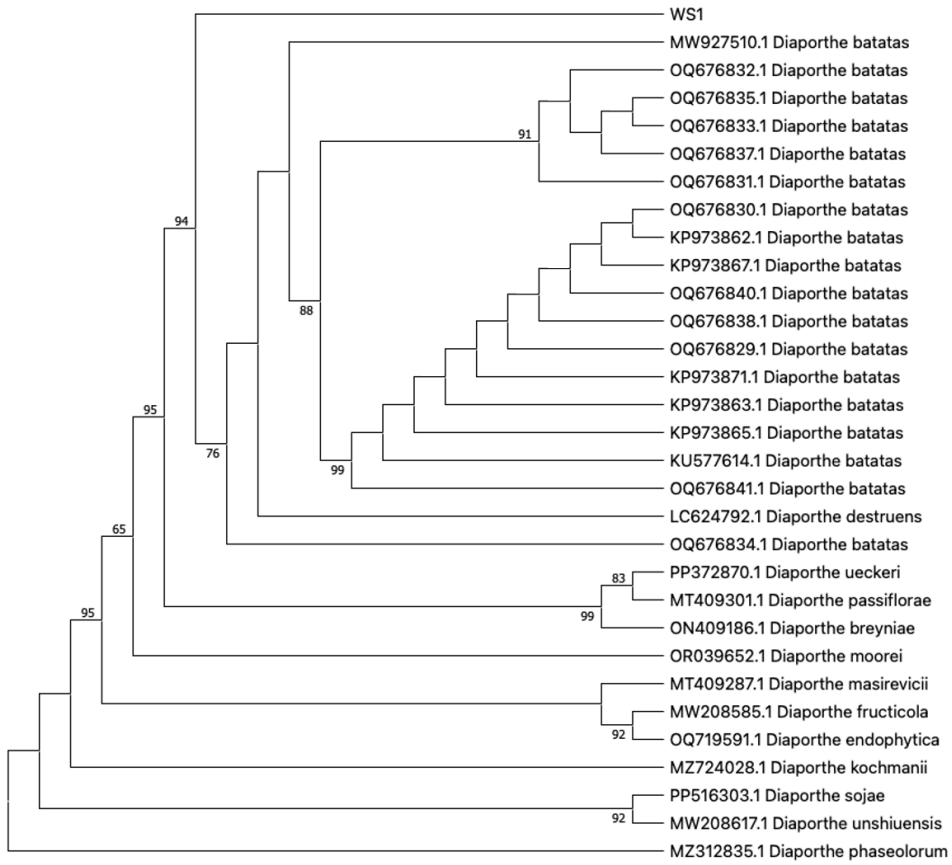

Figure 5. Phylogenetic tree of beta-tubulin gene sequences of fungal isolate WS1 as compared with other *Diaporthe* isolates in Genbank database using maximum parsimony method with bootstrap consensus tree inferred from 1000 replicates

Discussion

The identity of the fungal isolate WS1 from water spinach was found to be *Diaporthe batatas* Harter & E.C. Field based on host symptoms, and morphology, cultural, and molecular characteristics. Species of *Diaporthe* (*Phomopsis*) are commonly isolated plant pathogens, non-pathogenic endophytes, and saprophytes in several host plants (Gomes *et al*., 2013). This fungal genus has diverse ecological behavior, with plant pathogenic and endophytic species being strictly host specific, while others have many host plants co-colonising diseases or dead tissue. Gomes *et al*. (2013) suggested including at least ITS and HIS or TUB as molecular data in *Diaporthe* species

identification. *Diaporthe batatas* has taxon synonyms of *Phomopsis batatae* (Ellis & Halst.) Harter & E.C. Field, *Phoma batatas* Ellis & Halst., *Phomopsis batatas* (Ellis & Halst.) Harter & E.C. Field, *Phoma batatae* Ellis & Halst., and *Diaporthe phaseolorum* var. *batatae* (Harter & E.C. Field) Wehm. (Mycobank, 2024 and NCBI, 2024).

Diaporthe species cause a range of foliar symptoms across various crops. Infection due to *D. phaseolorum* on *Bacopa monnieri* is evident as brown spots that resulted into shot holes (Ghosh and Banerjee, 2015). Similarly, *D. humulicola* caused leaf spots and cone browning in common hop (Allan-Perkins *et al*., 2020). In China, *D. ueckeri* infection led to 100% disease incidence on cassava, with 80% of leaves developing necrotic spots (Du *et al*., 2024). *Diaporthe phoenicicola* incited severe defoliation and a 25% reduction in blueberry fruit yield (Lai *et al*., 2023). Multiple *Diaporthe* species have been linked to leaf spots on tea in Taiwan (Ariyawansa *et al*., 2021). Recent reports also include *D. phoenicicola* on *Pachira glabra* (Deng *et al*., 2024), *D. eres* on *Polygonatum sibiricum* (Tao *et al*., 2020), and *D. biconispora* on *Sapindus mukorossi* (Si *et al*., 2021).

Diaporthe batatas is a fungal plant pathogen recognized for causing dry rot in sweet potatoes. It was first reported in the USA by Harter and Field in 1912. In 2016, Lee *et al*. documented the pathogen for the first time in Korea. Additionally, *D. batatas* has been reported to cause foot rot disease in sweet potatoes in China (Tang *et al*., 2022). This fungus has also been recorded infecting *Ipomoea pes-caprae* in Taiwan (NCBI Accession: MK336444).

Several biotic and abiotic factors constrain the production and commercialization of sweet potatoes, with fungal diseases being particularly significant both on preharvest and postharvest stages (Scruggs and Quesada-Ocampo, 2016). The threat posed by fungal diseases highlights the need for effective disease management strategies. According to Tang *et al*. (2022), cultural management practices such as modifying the cultivation environment, adjusting soil pH, correct fertilizer application, and implementing crop rotation can help suppress the growth of *D. batatas*. However, due to limited research on *D. batatas*, knowledge regarding its management remains scarce. Further investigation is needed to explore additional strategies for controlling this pathogen.

The genus *Diaporthe* has been extensively studied, yet there are significant gaps in understanding its diversity and pathogenicity, especially in under-sampled regions like the Philippines. Ongoing research is essential to fill these gaps and reduce agricultural impacts. Therefore, it is recommended to test the pathogenicity of *D. batatas* isolated from lowland water spinach on other crops commonly grown or intercropped with water spinach. This will help identify potential alternate hosts and prevent future losses.

Intercropping water spinach is a valuable agricultural practice that enhances food security and boosts farmers' incomes. For example, intercropping water spinach with moringa and kale significantly raises farmers' earnings (Ardiansyah *et al*., 2023). Similarly, intercropping rice with water spinach improves land equivalent ratio, which increases farm productivity to 5.04 times that of rice monocropping (Liang *et al*., 2016). Improved disease detection in water spinach is crucial, as pathogens affecting this crop can spread to intercrops, posing a significant threat to overall crop health and productivity. When water spinach is infected with diseases, the causal pathogens can spread to other susceptible plants in the intercrops, potentially compromising plant health and yield. This cross-contamination can disrupt the benefits of intercropping, leading to decreased productivity and increased management challenges.

Acknowledgements

The authors acknowledge the host institution, University of the Philippines Los Baños and National Crop Protection Center Core Research. Likewise, they are thankful for the support of Mycology Laboratory staff particularly, Albert D. Sanfuego, Adrian M. Avila, Sylvia M. Calderon, Jerome D. Lescano, and Maricar O. Escote.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

References

Allan-Perkins, E., Li, D. W., Schultes, N., Yavuz, S. and LaMondia, J. (2020). The identification of a new species, *Diaporthe humulicola*, a pathogen causing *Diaporthe* leaf spot on common hop. Plant Disease, 104:2377-2390.

Ardiansyah, M., Halil, A., Syarif, A. and Anwar, A. R. (2023). Ketahanan pangan dan peningkatan pendapatan petani melalui tumpang sari kangkung dan kelor di kelurahan bontoramba kecamatan sombaopu kabupaten gowa. AgriMu, 3:1.

Ariyawansa, H. A., Tsai, I., Wang, J. Y., Withee, P., Tanjira, M., Lin, S. R., Suwannarach, N., Kumla, J., Elgorban, A. M. and Cheewangkoon, R. (2021). Molecular phylogenetic diversity and biological characterization of *Diaporthe* species associated with leaf spots of *Camellia sinensis* in Taiwan. Plants, 10:1434.

Austin, D. F. (2007). Water spinach (*Ipomoea aquatica*, Convolvulaceae): a food gone wild. Ethnobotany Research & Applications, 5:123-146.

Barnett, H. L. and Hunter, B. B. (1972). Illustrated genera of imperfect fungi. 3rd Edition, Burgess Publications Co., Minneapolis, 241.

Benigno, D. R. A. (1981). Philippine weeds and the viruses they harbor. NRCP Research Bulletin, 87:2-10.

Cerkauskas, R. F., Koike, S. T., Azad, H. R., Lowery, D. T. and Stobbs, L. W. (2006). Diseases, pests, and abiotic disorders of greenhouse-grown water spinach (*Ipomoea aquatica*) in Ontario and California. Canadian Journal of Plant Pathology, 28:63-70.

Deng, C., Wang, H., Wen, B., Gu, J., Peng, X. and Zhang, Z. F. (2024). First report of leaf brown spot caused by *Diaporthe phoenicicola* on *Pachira glabra* in China. Plant Disease, 108:2220.

Du, W., Chen, X. C., Zeng, X. M., Li, K. Y., Wu, Z. W. and Yi, R. H. (2024). Occurrence of cassava (*Manihot esculenta*) leaf spot disease caused by *Diaporthe ueckeri* in China. Plant Disease, 108:2917.

Dueñas-López, M. A. (2012). *Ipomoea aquatica* (swamp morning-glory). *CABI Compendium,* 28781. Retrieved from: cabidigitallibrary.org/doi /10.1079/cabicompendium.28781.

Gacutan, M. V. C. and Quimio, T. H. (1978). Studies on the white rust disease of kangkong (*Ipomoea aquatica* Forsk.). Philippine Phytopathology, 14:69-77.

Gai, Y. and Wang, H. (2024). Plant disease: A growing threat to global food security. Agronomy, 14:1615.

Ghosh, S. K. and Banerjee, S. (2015). First report of *Diaporthe phaseolorum* on *Bacopa monnieri* in India. Journal of Plant Pathology, 97:S68 ref. 3.

Gomes, R. R., Glienke, C., Videira, S. I. R., Lombard, L., Groenewald, J. Z. and Crous, P. W. (2013). *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. Persoonia-Molecular Phylogeny and Evolution of Fungi, 31:1-41.

Hu, M., Li, C., Zhou, X., Xue, Y., Wang, S., Hu, A., Chen, S., Mo, X. and Zhou, J. (2021). Microbial diversity analysis and genome sequencing identify *Xanthomonas perforans* as the pathogen of bacterial leaf canker of water spinach (*Ipomoea aquatic*). Frontiers in Microbiology, 12:752760.

Jiang, F., Wang, S., Wang, H., Xu, D., Wang, A., Yuan, L. and Fan, W. (2023). Improved chromosome-level genome and annotation data for a leafy vegetable water spinach (*Ipomoea aquatica*). Scientia Horticulturae, 320:112193.

Lai, J., Liu, B., Xiong, G., Liu, T., You, J. and Jiang, J. (2023). First report of *Diaporthe phoenicicola* causing leaf spot on blueberry (*Vaccinium virgatum*) in China. Plant Disease, 107:569.

Lee, Y. J., Mannaa, M., Jeong, J. J., Lee, H. U., Kim, W. and Kim, K. D. (2016). First report of dry rot of sweetpotato (*Ipomoea batatas*) caused by *Diaporthe batatas* in Korea. Plant Disease, 100:1786-1786.

Lee, G. B., Shim, H. S., Cho, W. D. and Kim, W. G. (2022). First report of leaf spot in water spinach caused by *Ectophoma multirostrata*. Korean Journal of Mycology, 50:367-372.

Leksomboon, C., Thaveechai, N. and Kositratana, W. (1991). "Bacterial leaf spot of Chinese convolvulus [*Ipomoea aquatica*]," in Proceedings of the 29th Kasetsart University: Plant Science (Bangkok: Kasetsart Univ.), 277-283.

Liang, K., Yang, T., Zhang, S., Zhang, J. E., Luo, M., Fu, L. and Zhao, B. (2016). Effects of intercropping rice and water spinach on net yields and pest control: an experiment in southern China. International Journal of Agricultural Sustainability, 14:448-465.

Liu, B., Stein, L., Cochran, K., du Toit, L. J., Feng, C., Dhillon, B. and Correll, J. C. (2020). Characterization of leaf spot pathogens from several spinach production areas in the United States. Plant Disease, 104:1994-2004.

Mycobank. (2024). *Diaporthe batatas*. Accessed at https://www.mycobank.org/Simple%20names%20search.

NCBI. (2024). *Diaporthe batatas*. Accessed at https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=748121.

Palada, M. C. and Chang, L. C. (2003). Suggested cultural practices for kangkong (No. AVRDC Extension Publication). AVRDC-The World Vegetable Center.

Quebral, F. (2000). White rust of kangkong (*Albugo ipomae-panduratae* [Schw.] Swing). Honolulu (HI): ADAP Project. 1 page. (*Agricultural Pests of the Pacific*; ADAP 2000-23).

Sasuman, J. and Tangonan, N. G. (1995). Leafspot of kangkong caused by *Pestalotia* sp.: short term research in PP 307 (Fungal Disease of Plants), USM, Kabacan, Cotabato.

Scruggs, A. C. and Quesada-Ocampo, L. M. (2016). Etiology and epidemiological conditions promoting *Fusarium* root rot in sweetpotato. Phytopathology, 106:909-919.

Si, Y. Z., Sun, J. W., Li, D. W., Huang, L., Ju, Y. and HuaZhu, L. (2021). Leaf spot of *Sapindus mukorossi* caused by *Diaporthe biconispora* in China. Australasian Plant Pathology, 50:193-202.

Singh, J., Chauhan, H., Kumari, V. and Singh, R. (2023). Aquatic vegetables for nutrition and entrepreneurship. In Vegetables for Nutrition and Entrepreneurship. Singapore: Springer Nature Singapore, pp.389-408.

Tang, W., Zhang, C. L., Wang, F., Yang, D. J., Ma, J. K., Chen, J. W., Xie Y. P. and Sun, H. J. (2022). Identification and growth characteristics determination of sweet potato foot rot pathogens. Journal of Southern Agriculture, 53:1917-1924.

Tao, H., Wang, H., Huang, S. X., Zhang, Y., Zhang, Z. H., Liu, W., Shi, N. X., Zhu, F., Ji Z. L. and Chen, X. R. (2020). Identification and characterization of *Diaporthe eres* causing leaf

blight disease on the medicinal herb *Polygonatum sibiricum*. Journal of General Plant Pathology, 86:468-476.

Tridge. (2023). Kangkong. Accessed at https://www.tridge.com/intelligences/kangkong.

Williams-Woodward, J. (2001). Simplified fungi identification key. Special Bulletin 37, The University of Georgia. Retrieved from: http://plantpath.caes.uga.edu/extension/documents/fungikey.pdf.

Valdez, R. B. (1968). Survey, identification, and host-parasite relationships of root-knot nematodes occurring in some parts of the Philippines. Philippine Agriculturist, 51:802-824.

(Received: 8 October 2024, Revised: 29 May 2025, Accepted: 29 July 2025)