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Molecular identity and cultural variability of *Athelia rolfsii* TMSR-001 and its relative pathogenicity to tomato genotypes

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Abstract

Athelia rolfsii (syn. *Sclerotium rolfsii*), is a destructive soilborne fungal pathogen that causes massive yield losses and plant mortality in tropical countries. In this study, we characterized a Philippine isolate of *Athelia rolfsii* (TMSR-001) that causes southern blight in tomatoes and evaluated the response of 20 tomato genotypes to southern blight in screen house trials. *Athelia rolfsii* TMSR-001 was pathogenic to tomato, and its optimal radial growth was recorded in the V8 juice agar medium and oatmeal agar (OA) medium with a 90.00 mm colony growth three days post-incubation (dpi). Sclerotial masses were observed on media, where *A. rolfsii* (TMSR-001) had poor and moderate mycelial density. The highest number of sclerotial masses was observed in Quarter Strength Potato Dextrose Agar and V8 medium. All tomato genotypes were susceptible to *A. rolfsii* infection. However, disease severity (% DS) varied significantly ($p \leq 0.05$) among the tested tomato genotypes. The USDA 358811 and Marimax showed relatively low susceptibility to the pathogen (DS of 51.85%). The PH 8679 and Cherry were the most susceptible genotypes, with DS of 94.46% and 92.59%, respectively. This study identified *Athelia rolfsii* TMSR-001 as a tomato southern blight pathogen. The fungus exhibited variable responses to culture media, highlighting the significant role of nutrition in the growth and survival of *A. rolfsii*. Further selection of disease-resistant tomatoes is warranted. To our knowledge, this is the first screening study conducted on tomato plants in response to the infection of *A. rolfsii* in the Philippines.

Keywords – *Solanum lycopersicon* – *Sclerotium rolfsii* – sclerotial bodies – tomato disease

Introduction

Tomato (*Solanum lycopersicon* L.), a nightshade family member, is one of the most important vegetables cultivated globally. Tomato is a good source of vitamins, minerals, carotenoids, and antioxidants (Hadizadeh et al. 2009). In 2020, among Southeast Asian countries, the Philippines ranked 4th in tomato production, with an average annual production quantity of 134,972 kg/ha (FAOSTAT 2019). Tomato also ranked fourth most produced vegetables grown in the Philippines (PSA 2021), with the Ilocos (73, 435.34 MT), Northern Mindanao (47, 205.57 MT), Central Luzon (31, 802.44 MT), CALABARZON (13, 902.02 MT), and Western Visayas (11, 813.34 MT) as top producing regions. Tomatoes, a relatively short-term crop with high yield

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potential, have a good market value in both local and international markets, significantly contributing to the income of growers and farmers. However, several abiotic and biotic factors threaten the cultivation of tomatoes, resulting in considerable losses during the vegetative and postharvest stages. Biotic factors include plant-pathogenic organisms that attack tomatoes, greatly affecting the production quantity and quality of the produce; an example is the destructive soilborne fungal pathogen *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr (Punja 1988, Bhagat 2022, Kwon & Park 2022, Adam Dade et al. 2023).

Athelia rolfsii is a soil-inhabiting pathogen that causes different diseases such as damping-off in seedlings, stem canker, collar rot, crown blight, and rotting in roots, crowns, bulbs, tubers, and fruits (Kwon & Park 2002) in a wide variety of plant hosts including vegetables, legumes, cereals, forage plants, and even several weed species in tropical and sub-tropical regions (Punja 1985, Agrios 1998). Mycelia and 'sclerotial bodies' are the distinguishing structures that serve as specialized overwintering structures in the soil, acting as future inoculum sources through germination in response to alcohols and volatile compounds released from decomposing materials (Punja 1985). For example, in tomatoes, *A. rolfsii* causes damping-off in seedlings and southern blight disease in mature plants (Mullen 2001). However, any susceptible part in contact with infested soil, like the herbaceous stem or vegetative tissues, is also susceptible to infection through direct pathogen penetration (Mullen 2001, Kator et al. 2015, Nugruho et al. 2019). Initial symptoms of the disease appear as dark water-soaked lesions on the basal stem, which expand rapidly and girdle the stem, eventually leading to plant wilt 2–4 days upon infection (Mullen 2001). In older plants, when lower stems (crowns/collars) are infected, these may dry lower foliage and eventually the whole plant, giving a distinct symptom of wilting (Banyal et al. 2008).

Diseases caused by *A. rolfsii* can result in severe losses to producers (Martinez-Ramirez et al. 2015). Management measures have been explored, from utilizing biological control agents' antagonistic properties to integrated cultural practices and examining various chemical control measures. Nevertheless, planting resistant cultivars remains the most economical and durable approach to managing diseases caused by *A. rolfsii* (Bennett 2020). Studies on tomato-resistant genotypes of *A. rolfsii* in the Philippines are limited. This study was conducted to determine and evaluate the resistance of several tomato lines and accessions to infection by *A. rolfsii*. Molecular characterization was done to determine the phylogenetic placement of the fungal isolate from the Philippines. Physiological studies were also performed to confirm which culture media suit the maximum mycelial growth and sclerotial production of the Philippine *A. rolfsii* isolate from tomato plants.

Materials & Methods

Disease occurrence

Dead and wilted plants were observed at Los Baños, Laguna, Philippines (14.153298° N, 121.264117° E) in May 2021. The high temperatures and frequent rains during this time provided high moisture, which could have predisposed the tomato plants to *A. rolfsii* infection. A cottony, white mass of fungal mycelia covered the lesions of the basal stem of the tomato plants. Some plants wilted, and defoliation was also observed. The typical sclerotia structures were also observed at the bases of some plants. The disease occurrence rate in the field was 25.75%. Randomly collected samples were brought to the laboratory for pathogen isolation and further characterization.

Fungal isolation

Infected basal stems of tomato plants (cv. 'Rica') showing signs of *A. rolfsii* were used for fungal isolation using tissue planting. Infected 3–5 mm^2 tissues were cut and subjected to surface sterilization by soaking the tissues in 10% sodium hypochlorite solution (NaOCl) (v/v, Zonrox, Philippines) for 3 minutes. Tissues were then rinsed twice with sterilized distilled water (sdH_2O) for 2 minutes in each rinse. Surface-sterilized tissues were air-dried inside a laminar flow hood.

They were then planted in a potato dextrose agar (PDA) medium (Himedia Laboratories Pvt. Ltd., India). Plates were sealed, labeled, and incubated at room temperature in an alternating cycle of 12 hours of light and 12 hours of dark. The fungus was purified following the hyphal tip technique (Tuite 1996). Isolated fungus was designated as isolate TMSR-001, and stock cultures were maintained and deposited at the Fungal Repository of the Plant Pathology Laboratory, Institute of Plant Breeding, College of Agriculture and Food Science, University of the Philippines, Los Baños.

Pathogenicity testing on tomato plants

Two-month-old tomato plants (var. 'Yellow Plum') were used in the pathogenicity testing. 5-mm agar plugs were obtained from the advancing colony of a 7-day-old representative isolate *A. rolfsii* TMSR-001 and inoculated at the basal stem of each test plant, conducted in needle-pricked (lightly) wounded (around the base) and unwounded conditions. Plants inoculated with a 5 mm agar plug alone were the negative control. Plants were placed and incubated in a moisture chamber (>95% relative humidity) for three days, during which time the infection became evident. Three replicates per treatment were done. The pathogenicity testing was conducted twice.

DNA isolation, PCR assay, and sequencing

Fungal genomic DNA (gDNA) from isolate TMSR-001 was extracted using the Cetyl Trimethylammonium Bromide (CTAB) DNA Extraction method developed by Doyle & Doyle (1987). Fungal DNA quality was checked through gel electrophoresis using a 1.5% agarose gel (Vivantis) amended with 1.2 µL of GelRed (Biotium), run in 0.5X TAE Buffer. The nuclear ribosomal RNA targeting the internal transcribed spacer (ITS) and large subunit ribosomal (LSU) regions were amplified through a polymerase chain reaction assay (PCR) using the primer pairs ITS5 (5'-GGA AGT AAA AGT CGT AAC AAG G-3')/ ITS 4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (White et al. 1990), and LR0R (5'-ACC CGC TGA ACT TAA GC-3')/ LR5 (5'-TCC TGA GGG AAA CTT CG-3') (Vilgalys & Hester 1990), respectively. The thermal profile for ITS followed at 94 ˚C for 5 min (initial denaturation), followed by 24 cycles at 94 ˚C for 45 s (denaturation), 55 ˚C for 45 s (annealing), 72 ˚C for 1 min (extension), and a final extension at 72 ˚C for 7 min. For LSU, the cycle followed 94 ˚C for 4 min (initial denaturation), 94 ˚C for 30 s (denaturation), 55 ˚C for 30 s (annealing), 72 ˚C for 30 s (extension), and 72 ˚C for 10 min (final extension). Amplified products were sent to Apical Scientific Sdn. Bhd. for DNA sequencing.

Phylogenetic analysis

The consensus DNA sequence was obtained by combining the forward and reverse DNA sequences, which were edited using Geneious R9 software (Biomatters, New Zealand). The resulting sequences for the ITS and LSU genes of isolate TMSR-001 were compared with sequences of other *Athelia* species using the Basic Local Alignment Search Tool (BLASTn) available at NCBI GenBank (https://blast.ncbi.nlm.nih.gov). Generated ITS and LSU DNA sequences of TMSR-001 were compared and aligned with authentic sequences of other *Sclerotium* species retrieved from the NCBI Nucleotide database (Table 1). The phylogenetic tree was constructed by aligning sequences in Clustal W, then constructing a Maximum Likelihood (ML) tree with 1,000 bootstrap replications using the Tamura-Nei substitution model with gamma distribution (T93+G) as the best-fitting model (according to the Bayesian Information Criterion (BIC)) in Molecular Evolutionary Genetic Analysis (MEGA X) software (Kumar et al. 2017).

Effect of culture media on *A. rolfsii* growth

Different strengths of PDA medium were tested (quarter strength, half strength, and full-strength), as well as other culture media (synthetic Potato Dextrose Agar (sPDA), Nutrient Agar (NA), Oat Meal Agar (OAT), Malt Yeast Extract Agar (MYEA), Yeast Extract Agar (YEA), V8 Agar, Sabouraud Dextrose Agar (SDA), and Water Agar (WA)) to identify appropriate and conducive media for optimal colony growth and production of sclerotial bodies for isolate TMSR-001. Petri plates with the corresponding culture media were inoculated with 5-mm mycelial plugs

cut from actively growing colonies of 7-day-old *A. rolfsii* colony previously maintained on PDA medium. Each treatment had ten replicate plates. Plates were incubated under uniform environmental conditions (in a laboratory room), maintained at a constant room temperature (28–30 ^{o}C), and subjected to alternating light (10 hours) and dark (14 hours) conditions. Radial growth rate (mm/day), number of sclerotial bodies (at 30 dpi), and mycelial growth characteristics (mycelial density and mycelial texture) were noted.

Table 1. List of *Sclerotium* species used for phylogenetic comparison with fungal isolate TMSR-001, along with their respective ITS and LSU GenBank accession numbers.

Species	Strain[a]	Country	GenBank Accession Numbers[b]	
			ITS	LSU
Athelia rolfsii	SPL16001	Korea	KY446387	KY446374
Athelia rolfsii	AFTOL-ID 664	USA	DQ484062	AY635773
Athelia rolfsii	BCRC FU30952	Taiwan	MN380239	MN368290
Athelia rolfsii	SPL15002	Korea	KY446393	KY446369
Sclerotium cepivorum	CBS 189.82	Egypt	FJ231398	FJ212339
Sclerotium denigrans	CBS 118.43	Germany	FJ231404	FJ212347
Sclerotium perniciosum	CBS 268.30	Netherlands	FJ231406	FJ212354
Sclerotium rhizodes	CBS 126.13	Netherlands	FJ231394	FJ212358
Sclerotium hydrophilum	CBS 201.57	Netherlands	FJ231390	FJ212349
Chloroscypha enterochroma	AFTOL-ID 67	USA	U92312	AY544735

[a]***SPL***: Sweet Potato Lab., Muan, Korea; ***AFTOL***: Assembling the Fungal Tree of Life, Germany; ***BCRC***: Bioresource Collection and Research Center, Taiwan; ***CBS***-Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands.

Response of tomato genotypes to *A. rolfsii*

The response of 20 tomato genotypes to southern blight caused by *A. rolfsii* was assessed in the screen house at the Institute of Plant Breeding, College of Agriculture and Food Science, University of the Philippines, Los Baños. Tomato seeds were sown in sterilized soil on plastic trays for germination until they reached the two-leaf stage. Plants were later transferred to 6 × 6 × 10 cm PE plastic pots containing sterilized sandy-loam soil and carbonized rice hull at a 1:1 ratio, and seedlings were grown for 60 days. *Athelia rolfsii* inoculum was prepared following the methods of Sennoi et al. (2010), Junsopa et al. (2016), and Mahato et al. (2017), with modifications. Sorghum grains were placed in 200 mL Erlenmeyer flasks, each containing 50 g of sorghum grain. Flasks were tightly plugged and sterilized by autoclaving at 15 psi at 121 ^{o}C for 30 minutes. After sterilization and cooling, sorghum grains in flasks were inoculated with five 5 mm mycelial discs obtained from a 7-day-old pure culture of *A. rolfsii* TMSR-001 isolate. Sorghum seed medium was incubated at room temperature (30 ± 2 ^{o}C) for 14 days.

Sixth-day tomato plants were inoculated using the method of Bonsi et al. (1986) and Sennoi et al. (2010). Inoculation was performed by spreading 30 seeds (~0.7 g) of sorghum-based inoculum around the basal stems and covering them lightly with soil. Plants receiving sorghum seeds without the fungus served as the negative control setup. There were nine technical replicates per genotype. Disease incidence (DI) and severity (DS) were recorded for each line. Data for disease incidence were recorded on the sixth day post-inoculation (dpi) and were converted to the percent infected plants using the formula as follows (Sennoi et al. 2010): % DI = number of infected plants over the total number of plants assessed multiplied by 100. For disease severity, the rating score was made using the scale used by Paparu et al. (2020), with modifications: 1 - plant remains healthy, 2 - water-soaked lesion prominent on stem above the soil line (stem base) with no

visible sign of fungal growth, 3 - silky-white mycelia prominent on stem base or presence of sclerotial bodies, 4 - younger leaves start to wilt with stem begins to shrivel and initial plant wilting, 5 - desiccation of leaves and stem and complete plant wilting, and 6 - plant senesced. Disease severity (DS) was assessed at six days post-inoculation. The severity scores for each tomato line were converted to percentages following the formula by Junsopa et al. (2016): % DS = Σ(Class Frequency × Score of Rating Scale)/(Total Number of Observations) × (Maximal Disease Index) × 100].

Statistical analysis

Data were analyzed using the analysis of variance (ANOVA) in a Completely Randomized Design (CRD) for the *in vitro* tests and a Randomized Complete Block Design (RCBD) for the glasshouse test. Duncan's Multiple Range Test (DMRT) was used to compare the mean differences. Statistical analyses were done using SAS V9.4 (SAS Institute 2016).

Results

Pathogenicity of *A. rolfsii*

Inoculated tomato plants showed lesions that were evident at the inoculation sites. Lesions were covered with a white mycelial mass and had an average length of 3.5 cm above the soil line. Generally, infected plants show overall wilting (Figure 1) in the unwounded and wounded inoculated setups. The symptoms and signs observed in the inoculated plants strongly suggest that isolate TMSR-001 has successfully infected the tomato plants. Infection was observed as early as 2 days from inoculation. The same fungal isolate was obtained upon re-isolation. Thus, Koch's postulates were satisfied.

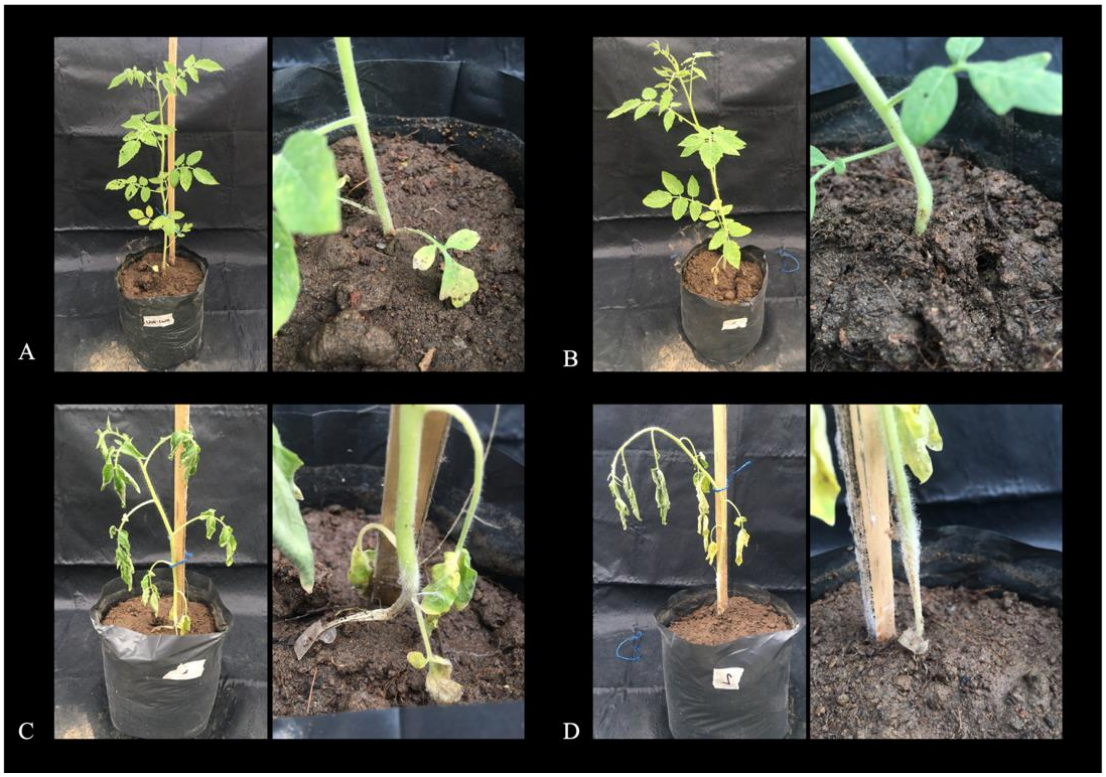

Fig. 1 – Pathogenicity Testing in tomato var 'Yellow Plum' showed infection of *A. rolfsii* TMSR-001 seven days post-inoculation. A (uninoculated, unwounded). B (uninoculated, wounded). C (inoculated, unwounded) and D (inoculated, wounded).

Phylogenetic analyses

The edited and trimmed sequences of the ITS and LSU genes were 742 bp and 1,271 bp, respectively. The BLASTn searches for both genes revealed high similarity (>99%) to *Athelia rolfsii*. The concatenated ITS and LSU gene sequences follow a multigene dataset containing 1,034 characters, including alignment gaps. The gene boundaries of the dataset for ITS were 1 to 495 bp, and for LSU, they were 496 to 1,034 bp. The combined gene analyses resulted in the fungal isolate TMSR-001 clustering with *Athelia rolfsii* strains SPL16001, AFTOL-ID 664, SPL 15002, and BCRC FU30952, with a high bootstrap value of 100%, confirming the isolate's identity (Fig. 2).

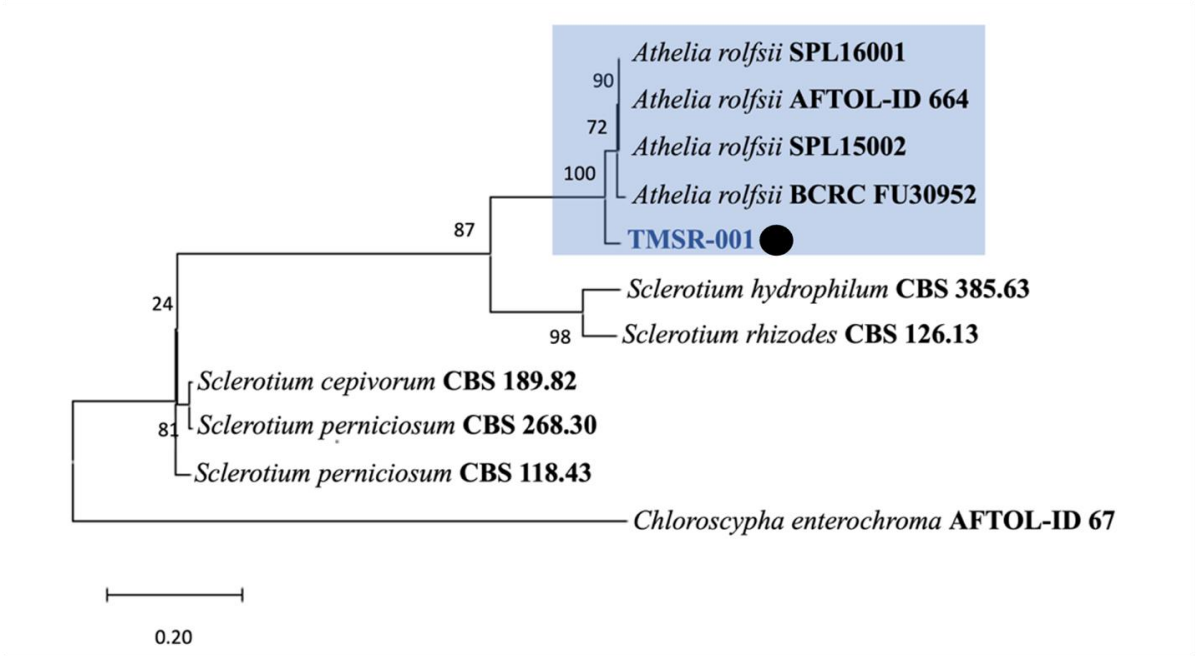

Fig. 2 – The maximum likelihood tree was generated using the concatenated ITS and LSU genes of *Sclerotium* species used in this study, compared to fungal isolate TMSR-001. Bootstrap support values (based on 1,000 replications) are indicated in the branches. The tree is rooted with *Chloroscypha enterochroma* strain AFTOL-ID 67 as the outgroup.

Cultural variability of *A. rolfsii*

Athelia rolfsii TMSR-001 grew on all tested culture media (Fig. 3). However, the growth rate recorded three days post-inoculation at 28 °C varied significantly ($p \leq 0.05$). Among all the culture media, the optimal radial growth of *A. rolfsii* isolate TMSR-001 occurred on V8 Agar, Oatmeal Agar, and YE agar medium, with a mean colony diameter of 90.00 mm for both V8 and OA media and 89.90 mm for YEA medium. This is followed by Nutrient Agar, hsPD, MYE, fsPD, qsPD, SD, and sPD agar medium (Table 2). The poorest mycelial growth was observed on WA medium, with a measured colony growth of 57.68 mm. Morphological differences were also observed in different culture media, and their relationship to the production of a sclerotial mass by the *A. rolfsii* isolate TMSR-001 (Figure 5). Mycelial growth was abundant and characterized by cottony, dense growth when grown on OA, MYE, fsPD, SD, and sPD. However, no signs of the formation of sclerotial mass were observed on these culture media on the 30th day of incubation. For culture media V8, YE, NA, hsPD, qsPD, and WA, where the growth of *A. rolfsii* isolate TMSR-001 was sparse to moderate, the formation of sclerotial mass was evident. A greater sclerotial mass was observed on the hsPD medium and V8 medium, with 50 and 40 sclerotial masses counted from all the replicate plates, respectively (Table 3). Regardless of the tested culture media, the sclerotial mass observed from *A. rolfsii* isolate TMSR-001 was irregularly shaped and not of the observed brown to tan, round, mustard seed-sized bodies typically observed from other isolates of *A. rolfsii* published in other literature (Kwon & Park et al. 2002).

Table 2. Radial mycelial growth and sclerotial mass production of *A. rolfsii* TMSR-001 on eleven culture media incubated at room temperature.

Culture Media	Colony Morphology		Mean Colony Diameter (mm)[1]	No. of Sclerotial Mass[2]
	Mycelial Density	Mycelial Texture		
V8 Agar (V8)	Moderate	Flat to Semi-Cottony	90.00^{a}	40^{ab}
Oatmeal Agar (OA)	Moderate to Abundant	Cottony	90.00^{a}	0^{d}
Yeast Extract Agar (YE)	Slightly Moderate	Flat to Semi-Cottony	89.90^{a}	10^{cd}
Nutrient Agar (NA)	Moderate	Flat to Semi-Cottony	88.45^{ab}	13^{cd}
Half Strength Potato Dextrose Agar (hsPD)	Slightly Moderate	Flat to Semi-Cottony	83.50^{ab}	50^{a}
Malt Yeast Extract Agar (MYE)	Abundant	Cottony	81.88^{abc}	0^{d}
Full Strength Potato Dextrose Agar (fsPD)	Abundant	Cottony	80.83^{bc}	0^{d}
Quarter Strength Potato Dextrose Agar (qsPD)	Moderate	Flat to Semi-Cottony	75.30^{c}	30^{bc}
Saboraud Dextrose Agar (SD)	Abundant	Cottony	75.03^{c}	0^{d}
Synthetic Potato Dextrose Agar (sPD)	Abundant	Cottony	73.90^{c}	0^{d}
Water Agar (WA)	Sparse	Flat	57.68^{d}	12^{cd}

[1] Recorded three days after incubation
[2] Recorded 30 days after incubation.
*Average of 10 replications observed per culture media.
*Values followed by the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$.

Response of tomato genotypes to *A. rolfsii*

All tomato lines used in this study exhibited susceptibility to the *A. rolfsii* isolate TMSR-001, as indicated by a calculated disease incidence (%DI) of 100% among all lines tested (Figure 4, Table 3). However, the response of the different tomato lines to infection varied significantly at $p \leq 0.05$. Tomato line USDA 358811 and Marimax were the least susceptible accessions/lines among all the lines tested in this study, with disease severity (%DS) of 51.85 and a mean severity score of 3.11 (Table 3 and Figure 6). The plants from these lines remained relatively healthy with no severe lesions. Minimal white mats formed on the stem base of each replicate plant. Furthermore, PH 8679 and Cherry accessions were the most susceptible, with a %DS of 94.46 and 92.59 and mean severity scores of 5.67 and 5.56, respectively. These highly susceptible accessions were observed to have already succumbed due to excessive wilting. Furthermore, severe lesions (characterized by evident drying) were observed on the inoculation sites, extending from the basal stem to the plant canopy, resulting in desiccated and dry leaves. Tomato lines PH 8679 and Cherry %DS values were close to those of the positive (susceptible) check variety used in this study, Yellow Plum, with a %DS value of 83.33 and a mean severity score of 5.11 (Table 3).

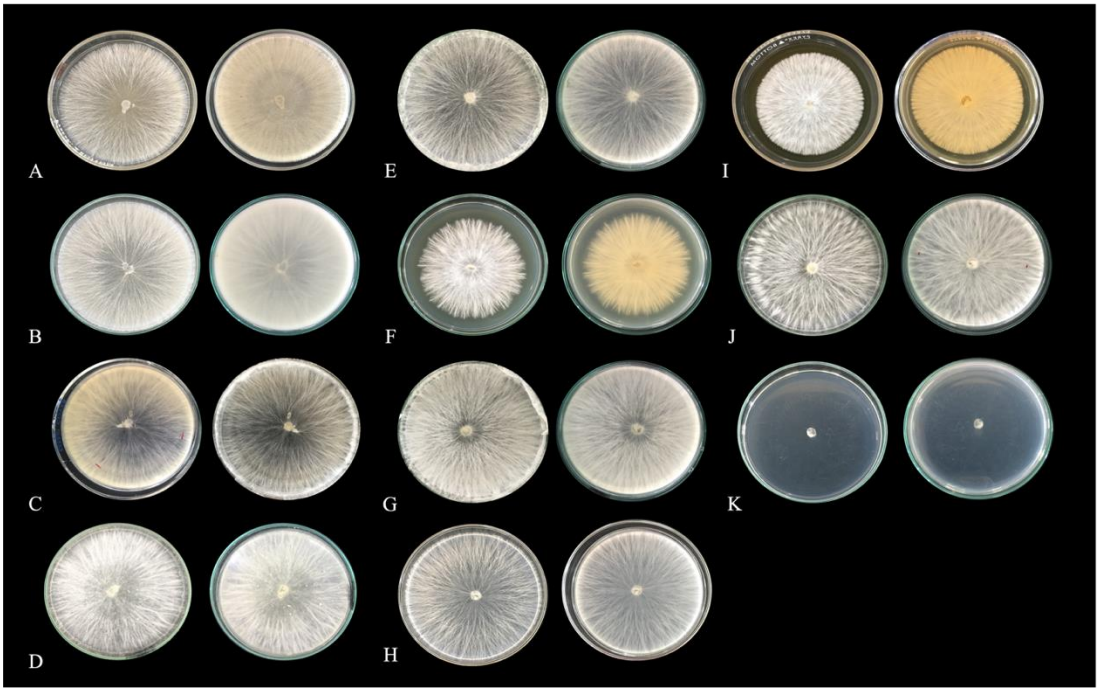

Fig. 3 – Colony growth of *Athelia rolfsii* isolate TMSR-001 on different culture media: (A) V8 Agar, (B) Oatmeal Agar, (C) Yeast Extract Agar, (D) Nutrient Agar, (E) Half-strength Potato Dextrose Agar, (F) Malt Yeast Extract Agar, (G) Full-strength Potato Dextrose Agar, (H) Quarter-strength Potato Dextrose Agar, (I) Sabouraud Dextrose Agar, (J) Synthetic Potato Dextrose Agar, and (K) Water Agar grew three days after incubation at 28 ºC.

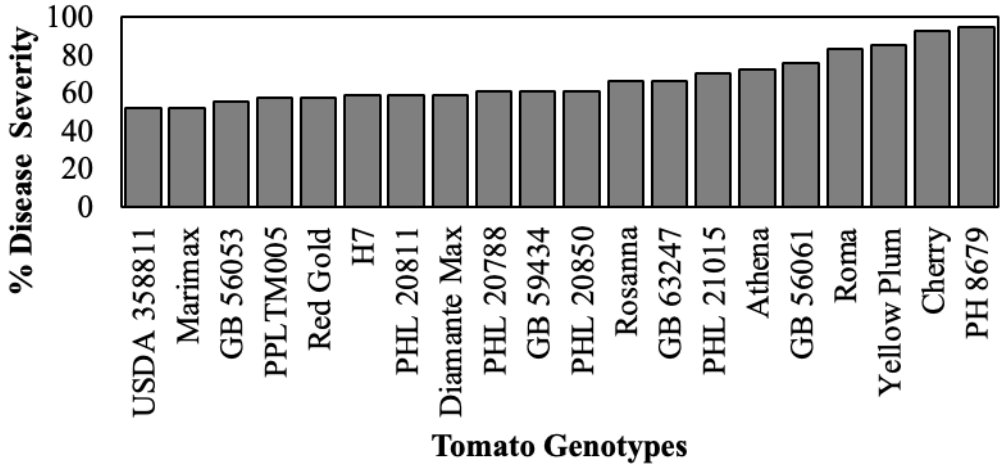

Fig. 4. Disease severity percentage of different tomato lines in response to the infection of *A. rolfsii* TMSR-001, causing Southern Blight six days post-incubation

Discussion

Athelia rolfsii is a soil-inhabiting fungal pathogen that causes severe problems for many host plants, including those of high economic importance. This pathogen can infect the hosts at any stage throughout the life cycle of the host plant (Mullen 2001, CABI 2021). The *A. rolfsii* infection results in a brown, dry lesion that girdles the stem beneath the soil line in the Solanaceae family. This leads to the plant's rapid wilting and chlorosis (Roberts et al. 2014). Numerous investigations have been reported describing the morphological and pathogenic characteristics of *A. rolfsii* infecting other host crops worldwide. However, in the Philippines, studies on the phylogenetic

placement and the effect of environmental factors on the growth and survival of *A. rolfsii* have been limited.

Analysis of the concatenated ITS and LSU sequences of isolate TMSR-001 and the reference sequences revealed that *A. rolfsii, S. hydrophilum,* and *S. rhizodes* clustered together, implying their close relatedness. However, the latter two species formed a subgroup within the same cluster, which separates them from *A. rolfsii.* This study's ITS and LSU phylogeny was similar to that of previous studies by Xu et al. (2010) and Paul et al. (2017). This further validates the fungus's identity in this study as *A. rolfsii* based on combined ITS and LSU sequence analyses.

The current study also investigated the suitability of 11 fungal culture media for mycelial growth and sclerotial production of *A. rolfsii* TMSR-001. Optimal radial growth was observed with the V8 and OA medium and, while the majority of the culture media used also supported good radial growth except for WA medium, having the poorest growth for isolate TMSR-001, agreeing with previous studies (Punja & Damiani 1996, Basamma et al. 2012, Ayed et al. 2018). This study demonstrates how the culture media affect the cultural growth of *A. rolfsii* TMSR-001, specifically in terms of mycelial density, texture, and sclerotial mass production, for each medium used. Mycelium growth can vary based on the availability of nutrients, but the response may differ among pathogen strains (Sab et al. 2014, Ayed et al. 2018). The observed sclerotial masses of *A. rolfsii* TMSR-001 were generally irregular in shape, regardless of the media. Previous studies reported that sclerotial bodies produced from agar media differ morphologically and physiologically from sclerotia produced in natural infections (Punja 1985, Smith et al. 1989). The low sclerotial mass observed in each culture medium may be attributed to other external factors during incubation, such as the availability of nutrients (e.g., carbohydrate and protein sources) and oxygen concentrations. In another study (Griffin & Nair 1968), sclerotia production by the aerial mycelium requires oxygen concentrations higher than 15%, which are not attained inside the agar medium. However, low oxygen concentrations inside the plate do not inhibit hyphal growth. Additionally, sclerotial initiation and the growth of the vegetative mycelium competed for metabolites required for sclerotium production (Wheeler & Waller 1965).

Tomato lines from the Philippines used in this study were susceptible to infection caused by *A. rolfsii* TMSR-001. However, susceptibility levels varied among the lines. The mean severity score and %DI were the highest, with PH 8679 and Cherry, with values close to the susceptible check, tomato cv. Yellow Plum, and the lowest severity score and %DI observed from USDA 358811 and Marimax. The success of *A. rolfsii* infection is attributed to the pathogen's ability to produce large quantities of polygalacturonases and oxalic acid, which are responsible for degrading the host's outer cell layer, facilitating successful penetration. Thus, infection eventually leads to the death of the infected plants (Punja 1985, Punja & Damiani 1996, Fery & Dukes 2002.) The combined physical, biochemical, physiological, and genetic properties may be responsible for the comparative disease tolerance observed in USDA 358811 and Marimax lines and warrant further study for verification. To the best of our knowledge, this is the first screening study conducted on various tomato genotypes in response to *A. rolfsii* infection in the Philippines. This fungus has also been studied in Korea (Kwon & Park et al. 2002), where it was found to cause stem, crown rot, and blight of tomatoes, and in India (Bhagat 2022). In Mexico, 42 wild tomato accessions have also been screened for their susceptibility to the pathogen, and five accessions (8, 9, 10, 11, and 12) exhibited resistance (Martinez-Ramirez et al. 2015).

Conclusion

The identity of *A. rolfsii* TMSR-001 was confirmed by combining the fungal isolate's morpho-cultural, pathogenicity, and molecular characteristics. The *in vitro* study investigated the relationship between nutrition and its role as a factor affecting the critical stages (growth and survival) of *A. rolfsii*, specifically in terms of mycelial growth and sclerotial production. However, further investigations are warranted to examine the effects and interactions of different selected environmental factors (e.g., temperature, relative humidity, pH) and nutritional factors. The results will enhance our understanding of the population dynamics of the pathogen and the role of these

factors in the growth of *A. rolfsii*, providing significant information for formulating effective disease management strategies. Despite the limitations of discerning tomato lines with superior resistance to *A. rolfsii*, this study provided valuable information in discriminating tomato lines with a relatively low and highly susceptible reaction to *A. rolfsii* infection. Additional tomato lines should be screened or assessed for tolerance or resistance to *A. rolfsii* to identify sources of disease resistance.

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Accessibility of data

Raw data will be made available on request.

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