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ORIGINAL ARTICLE

Report on emerging foliar soft rot disease on ginseng *Panax vietnamensis* and the identification of *Neocosmospora ipomoeae* and *Fusarium miscanthi* as the causal pathogens

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Abstract

In the spring-summer season of 2022, a disease outbreak occurred in Kon Tum province, the native habitat of *Panax vietnamensis* var. *vietnamensis* (Ngoc Linh ginseng), affecting (scattered) a farming area of more than 50,000 ginseng plants. In relation to this a survey was conducted on a total area of 15 ha, covering ginseng farms in Kon Tum and some other main ginseng planting areas in Vietnam. Two main foliar diseases were recorded in the survey: a previously reported anthracnose disease and a new foliar soft rot disease that occurred with a disease incidence of 1–30% and 2–26%, respectively. The latter was not reported for other Panax ginseng varieties, but only for *P. vietnamensis*. Among 79 fungal strains isolated from infected leaves, two strains NL5 and KT2.1.2 were identified as true pathogens causing foliar soft rot disease according to the results of pathogenicity tests (following Koch's postulates), which confirmed a disease index of 12% for both strains. Based on microscopic morphology and comparative analyses of ITS, TEF1, and BT2 sequences, the two strains were identified as *Neocosmospora ipomoeae* NL5 and *Fusarium miscanthi* KT2.1.2. The newly discovered foliar soft rot disease on ginseng *P. vietnamensis* and the associated pathogens *N. ipomoeae* and *F. miscanthi* reported in this study raised concern about measures that should be taken to control this disease on Panax ginseng.

Keywords: foliar soft rot disease, *Fusarium miscanthi*, *Neocosmospora ipomoeae*, *Panax vietnamensis*

Introduction

Panax ginseng is a valuable medicinal plant widely grown in Asia and America. The *Panax* genus comprises a total of 17 species, of which several species are well known for their use as functional food and medicine, such as *P. ginseng* (Korean ginseng), *P. quinquefolius* (American ginseng), *P. notoginseng* (Chinese ginseng), and *P. japonicus* (Japanese ginseng) (Zhang *et al*. 2020). A recent member *P. vietnamensis,* which is intensively utilized as a medicinal herbal resource in Vietnam, was added to the *Panax* genus. Two varieties of the Vietnamese Panax ginseng have been reported that differ in ginsenoside compositions: the *P. vietnamensis* var. *vietnamensis* (Ngoc Linh ginseng) which mainly grows on Ngoc Linh mountain in Quang Nam and Kon Tum Provinces (southwestern Vietnam), and the *P. vietnamensis* var. *fuscidiscus* (Lai Chau ginseng) which mainly grows in Lai Chau Province (northwestern Vietnam) (Nguyen *et al*. 2023).

Panax vietnamensis grows on high mountains of 1500 m above sea level, loves shady conditions with a coverage of 70–90%, and requires high humidity of 85–90% (Nguyen *et al*. 2023). Generally, the harvest time for ginseng plants is at 5 to 6 years, at which time they produce a good quantity of medicinal compounds.

Having a long cultivation time, ginseng plants growing either under natural forest canopy or under farm conditions confront infectious diseases that seriously threaten the harvest. Diseases of ginseng plants are most notably mediated by microorganisms, especially fungi (Hill and Hausbeck 2009).

The major foliar diseases of ginseng include leaf blight caused by *Alternaria panax*, leaf spot caused by *A. alternata* and *Boeremia linicola*, anthracnose caused by *Colletotrichum* spp., and gray mold caused by *Botrytis cinerea* (Li *et al*. 2020; Guan *et al*. 2021a, b; Wang *et al*. 2024). The reported foliar diseases on ginseng generally have symptoms of dark lesions on the leaves that can become enlarged (in the case of leaf blight or anthracnose) or multiply (in the case of leaf spots). The diseases are common in various ginseng varieties throughout the world and may cause rapid defoliation and plant death (Hill and Hausbeck 2009).

In spring-summer 2022 there was a disease outbreak in Kon Tum Province, threatening a planting area of more than 50,000 Ngoc Linh ginseng plants (Department of Agriculture and Rural Development of Kon Tum Province). Related to this issue, a survey was conducted in planting areas of the two important varieties Ngoc Linh and Lai Chau ginseng. Of special attention was the foliar soft rot disease recorded for the first time on the surveyed ginseng farms with a disease incidence of 2–26%. Here we report symptoms of the disease and the identification of causal pathogens following Koch's postulates. Further, the phylogenetic affiliation of the pathogens was identified based on their morphological traits together with a comparative analysis of the internal transcribed space (ITS), the translocation elongation factor 1 (TEF1), and beta-tubulin 2 (BT2) sequences.

Materials and Methods

Field survey and sampling

The survey was conducted during the ginseng vegetative season from June to September 2022. The first survey and sampling site was in southwestern Vietnam, i.e., in Nam Tra My district (15°01'36"N 107°58'53"E and 15°00'607"N 108°01'660") of Quang Nam province and Tumorong district (14°59'30"N 108°01'16"E and 14°59'38"N 108°01'10"E) of Kon Tum province, the native habitat of the Ngoc Linh ginseng variety. The second survey and sampling site was in northwestern Vietnam, i.e., Sapa town of Lao Cai province (22°21'225"N 103°51'476"E) where both Ngoc Linh and Lai Chau ginseng varieties have been acclimatized in net houses, and Muong Te (22°33'42"N 102°49'49"E), and Sin Ho (22°33'42"N 103°14'4"E) districts of Lai Chau province, the native habitat of the Lai Chau ginseng variety. The farms selected for the survey covered 2–3 ha, including farms under a natural forest canopy and the acclimatized net house farms. Ginseng plants on the farms were of different ages, from 1 to 6 years old. The survey was performed following the Handbook of Plant Disease Diagnosis in Vietnam by Burgess *et al*. (2009). In each area, four ginseng farms (2–3 ha each) were surveyed, and on each farm, 10 random points lying diagonally across the farm were selected, and at each point, 10 random plants were subjected to disease observation (taking notes on symptoms of roots, stems, and leaves).

Diseased ginseng plants were collected, placed in paper bags and information about samples and collection locations was recorded. They were then put in plastic trays and transferred to the laboratory for the isolation of microbial pathogens.

Isolation of fungal pathogens

The diseased leaf samples collected from ginseng farms were used for the isolation of pathogens. The samples were surface sterilized by immersing in 70% ethanol for 2 min, then rinsing thoroughly in distilled water. Small 2 × 2 mm pieces of the infected tissues were taken with sterile scalpels and placed on Potato Dextrose Agar (PDA) (20 g dextrose, 4 g potato extract, 16 g agar) and Lignocellulose Agar (LCA) (1 g glucose, 1 g KH_2PO_4, 0.2 g $MgSO_4.7H_2O$, 0.2 g KCl, 2 g $NaNO_3$, 0.2 g yeast extract, 16 g agar, pH 7) plates, and incubated at 25°C for 7–10 days in the dark. Mature fungal mycelia were subcultured on LCA plates until their purity was ensured. Finally, the pure strains were transferred to PDA plates and incubated for another 7 days at 25°C to get maximal growth.

Pathogenicity test

The pathogenicity of the fungal isolates was confirmed via artificial infection on the ginseng host following Koch's postulates. The test was comprised of two rounds. The first one was carried out on individual ginseng leaves in a microclimate chamber. Leaves showing clear infection were tested in the second round using 2-year-old ginseng plants in a net house.

In the first round of the pathogenicity test, leaves of *P. vietnamensis* ginseng, free of microbial infection were selected, surface sterilized with 70% ethanol, then rinsed in distilled water, and air-dried. The suspected fungal pathogens were pre-grown on PDA plates for 5–7 days, then agar discs (5 mm diameter) containing fungal mycelia were dug using sterile plastic straws and transferred to the leaf surface (Ara *et al*. 2012). As a control, 2 × 2 mm pieces of PDA were used. The leaves were then placed in a microclimate chamber at 25°C, 90% humidity under light conditions of

1000 lux/dark periods of 16 h/8 h. The disease symptoms were recorded every day for up to 15 days.

The second round of the pathogenicity test was performed on healthy 2-year-old *P. vietnamensis* ginseng plants. Before infecting with fungal isolates, leaves of the ginseng plants were surface sterilized with 70% ethanol, then rinsed in distilled water, and air-dried. The suspected fungal pathogens were pregrown on PDA plates for 5–7 days, and a suspension of fungal mycelia and spores was prepared in 1X PBS buffer to the OD_{600} of 0.1 (equal to a cell density of 10^6 CFU · ml^{-1}), then 10 µl of that was dropped onto the leaf surface (Ara *et al*. 2012). In the control, sterile 1X PBS without fungal mycelia was used. The plants were grown in a net house of a ginseng farm. The disease symptoms were recorded every day for up to 15 days.

Morphology observation

For microscopic examination, the fungal strains were cultured on PDA plates, each containing a slanted glass cover-slide, and incubated at 25°C for 5–14 days. The mycelia, conidiophores, macro- and microconidia, and ascospora were observed and photographed using a differential interference contrast (DIC) microscope Axio Imager A2 (Zeiss, Germany) with 400× objective.

DNA extraction, PCR, and sequencing

The fungal strains were cultured in Potato Dextrose Broth (PDB) for 2 days at 25°C, shaking at 180 rpm. The fungal biomass was obtained by centrifugation, and the genomic DNA was extracted by using E.Z.N.A.® DNA Kit (Omega, USA). The internal transcribed space (ITS) was amplified with ITS-specific primers, the ITS1 (forward) 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4 (reverse) 5'-TCCTCCGCTTATTGATATGC-3' (White *et al*. 1990) using Promega PCR reaction kit (USA). In addition, two other gene fragment sequences were used for taxonomic identification, i.e., the translocation elongation factor 1 (TEF1) and beta-tubulin 2 (BT2). The TEF1 gene fragment was amplified using specific primers EF-1 (forward) 5'-ATGGGTAAGGARGACAAGAC-3' and EF-2 (reverse) 5'-GGARGTACCAGTSATCATG-3' (O'Donnell *et al*. 2022), and the BT2 gene fragment was amplified using the primers Bt2a (forward) 5'-GGTAACCAAATCGGTGCTGCTTTC-3' and Bt2b (reverse) 5'-ACCCTCAGTGTAGT-GACCCTTGGC-3' (Glass and Donaldson 1995). The reaction mixture (50 µl) contained 10 µl of 5× reaction buffer, 1.25 mM $MgCl_2$, 0.25 mM of each deoxynucleoside triphosphate, 40 pmol of each primer, 1.25 U of *Taq* DNA polymerase (Promega), and 2 µl of template DNA (10 ng · μl^{-1}). The PCR process included an initial denaturation stage at 96°C for 3 min, followed by 35 cycles containing a second denaturation stage at 94°C for 35 s, an annealing step at 55°C for 45 s (ITS)/ at 53°C for 55 s (TEF1) or at 58°C for 45 s (BT2), and an extension step at 72°C for 2 min, and a final extension step at 72°C for 7 min. The PCR products were checked by agarose gel electrophoresis (1.5% w/v) containing RedSafe, and then purified with AccuPrep® PCR/Gel Purification Kit (Bioneer, S. Korea), and sequenced on the ABI 3730 Avant Applied Biosystems sequencer (ABI, USA). The ITS, TEF1, and BT2 sequences were compared with the related sequences available on the GenBank and MycoBank databases by using the BLAST Search tool. Alignment of the sequences was performed using the CLUSTAL_X program, version 1.8 (Thompson *et al*. 1997), and phylogenetic trees were reconstructed using the neighbor-joining method (Saitou and Nei 1987). The topography of the reconstructed trees was evaluated by bootstrap analysis with 1000 replicates (Felsenstein 1985).

Statistical analysis

All experiments were performed in triplicate unless otherwise stated. The collected experimental data were processed using Microsoft Excel and graphed using SigmaPlot software. The statistical test was performed by using the SAS software, and the $p \leq 0.05$ was accepted as statistically significant.

Results and Discussion

Survey of ginseng disease

The field survey was carried out in the two largest Panax ginseng cultivating areas in Vietnam, i.e., Quang Nam and Kon Tum provinces, the native habitats of Ngoc Linh ginseng, and Lai Chau and Lao Cai provinces, the native habitats of Lai Chau ginseng (Fig. 1A). Two main foliar diseases were noted at significant incidences on all surveyed farms: the known anthracnose disease with a variable incidence of 1–30%, and a new foliar soft rot disease with a significant incidence of 2–26% (Fig. 1B). On the other hand, tuber-associated diseases, which were generally not recognizable until the diseases were very advanced, affected the phylospheric compartments, and killed the plants. Therefore, a survey of tuber diseases was not realistic, though root rot symptoms were also detected.

Foliar soft rot disease on ginseng is reported for the first time in this study. The disease symptoms were irregular water-soaked rotten lesions of 5 to 10 mm in diameter with a dark gray to black spot in the center (Fig. 2). As the disease progressed, the lesions

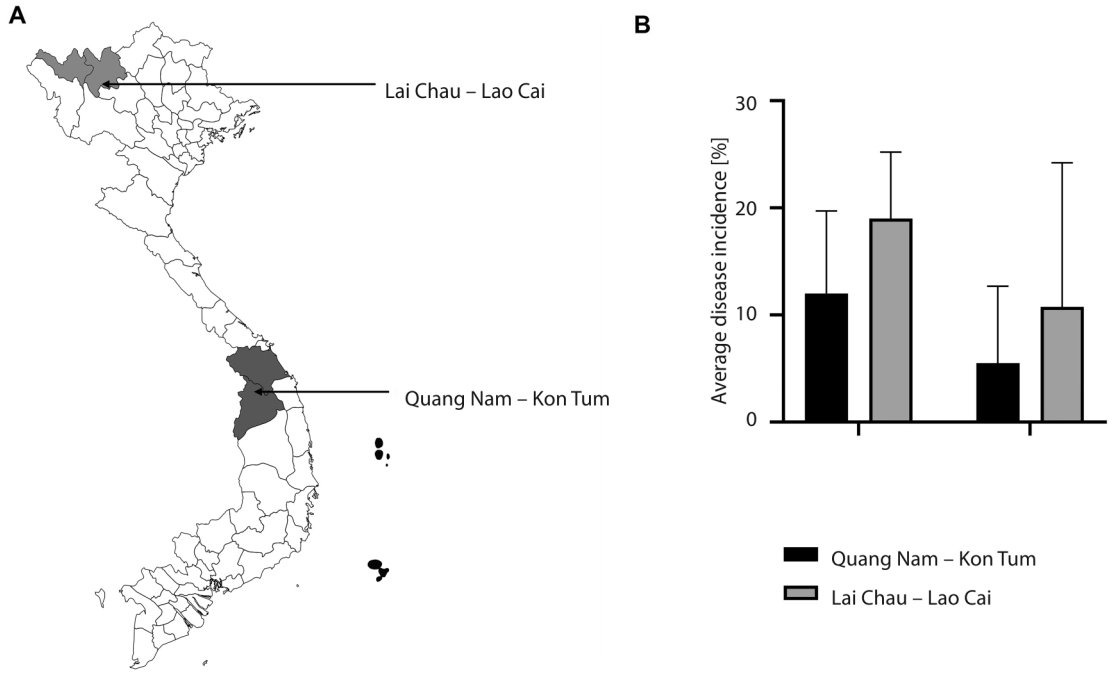

Fig. 1. Survey areas (A) and disease incidence (B) of two main foliar diseases detected on *Panax vietnamensis*

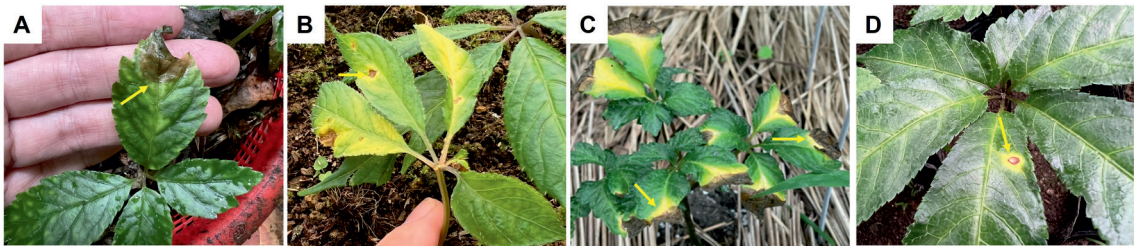

Fig. 2. Symptoms of the foliar soft rot disease (A – on Ngoc Linh variety; C – on Lai Chau variety) and anthracnose disease (B – on Ngoc Linh variety; D – on Lai Chau variety) on *Panax vietnamensis*

enlarged, and infected leaves turned black, easily falling out. The disease was observed in ginseng plants of all ages, however, it was more severe in 2- and 3-year-old plants (with an incidence of >20%) than 5–6-year-old plants (with an incidence of 5–15%) (Table S1). The Lai Chau variety was more prone to the disease than the Ngoc Linh variety (Table S1).

Leaves from 34 diseased plants were collected and used for the isolation of the associated pathogens. A total of 79 fungal strains were obtained in pure cultures and were subjected to pathogenicity tests. The first round of the pathogenicity test performed on ginseng leaves in the microclimate chamber revealed two pathogenic candidates, strain LN5 (from the Lai Chau variety) and strain KT2.1.2 (from the Ngoc Linh variety). These two strains caused clear disease symptoms resembling the original diseased samples after 5–7 days of infection (Fig. 3A–D). In the second round of the pathogenicity test which was carried out on 2-year-old ginseng plants under net house conditions, both strains developed rotten zones of 3–5 mm in diameter just after 3 days of contact, and after 12 days the lesions enlarged significantly, causing leaf wilting (Table S2; Fig. 3F–I). The same fungal strains were re-isolated from the diseased leaves of each test plant, confirming that they were true pathogens causing the foliar rot disease in *P. vietnamensis* ginseng.

Taxonomical identification of the two pathogenic fungal strains LN5 and KT2.1.2 was achieved by observation of microscopic morphology and comparative analyses of the ITS, TEF1, and BT2 sequences. Strain LN5 actively grew on the PDA medium, giving white colonies with a maximal diameter of 6070 mm (Fig. 4A), whereas it did not grow well on the LCA medium (Fig. 4B). On the PDA medium, the strain produced septate mycelia (Fig. 4C) with unbranched

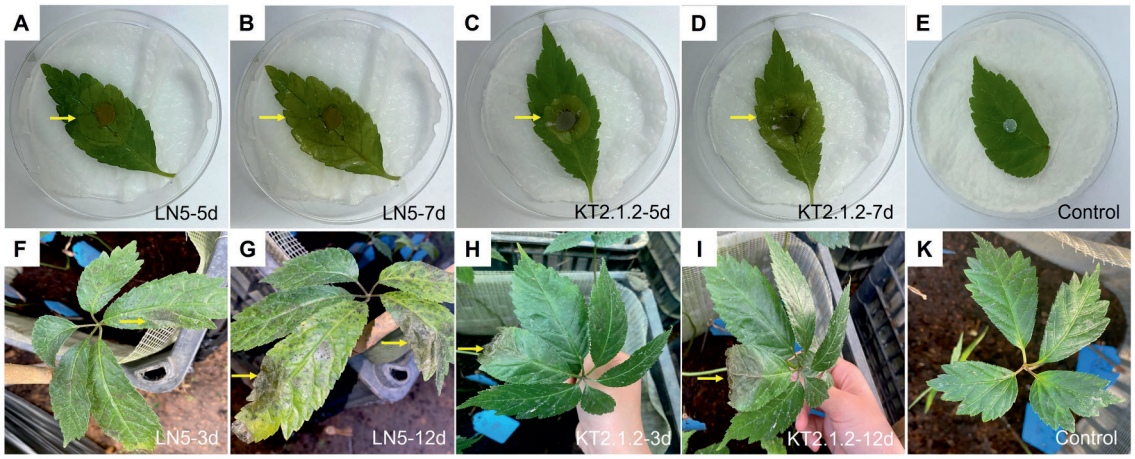

Fig. 3. Infection tests to prove the pathogenicity of the two isolates LN5 and KT2.1.2. A–D –the first round performed on ginseng leaves in a microclimate chamber; F–I – the second round performed on 2-year-old ginseng plants in a net house; E, K – negative controls (ginseng leaves or plants treated with a sterile medium only). Lesions are indicated by arrows

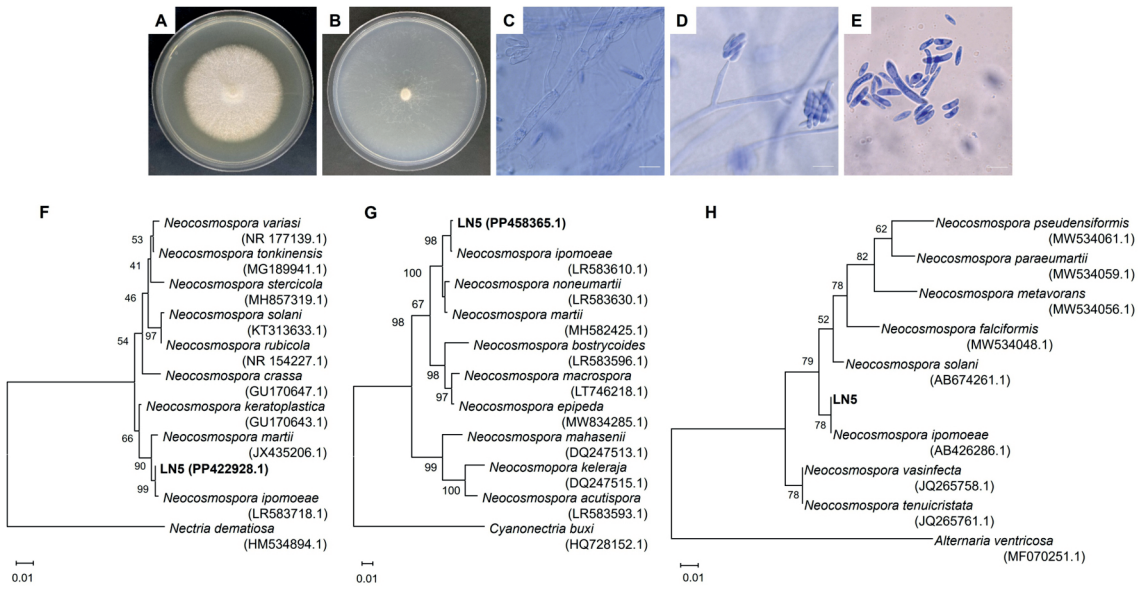

Fig. 4. Morphology and taxonomic position of strain LN5. A – colony on PDA; B – colony on LCA; C – septate mycelia; D – unbranched conidiophore; E – macro- and microconidia. Scale bars 10 µm. F, G, H – phylogenetic trees based on comparative analyses of the ITS, TEF1, and BT2 sequences, respectively (accession numbers of these sequences are indicated in parentheses next to the strain name)

conidiophores (Fig. 4D). Macroconidia were cylindrical to crescentic, 25–40 × 3–5 µm in size, whereas microconidia were ellipsoidal to cylindrical, 7–10 × 1–3 µm in size (Fig. 4E). Comparative analyses of three sequences ITS (Fig. 4F), TEF1 (Fig. 4G), and BT2 (Fig. 4H) indicated that strain LN5 was most closely related to *N. ipomoeae* with sequence homology of 100% for ITS (484 bp), 99.51% for TEF1 (651 bp), and 100% for BT2 (323 bp).

Strain KT2.1.2 grew well on the PDA medium, giving pinkish colonies of 60–70 mm in diameter (Fig. 5A), whereas on the LCA medium, the growth was significantly attenuated (Fig. 5B). On the PDA medium, the strain possessed septate mycelia (Fig. 5C) bearing unbranched conidiophores (Fig. 5D). Macroconidia were long diamond shaped 12–18 × 1–3 µm in size, and microconidia were cylindrical 2–5 × 0.5–1 µm in size. Asci and ascospora were not observed. Comparative analyses of ITS, TEF1, and BT2 sequences showed that strain KT2.1.2 was most closely related to *Fusarium miscanthi*, with a sequence homology of 99.79% for the ITS (470 bp), 97.67% for the TEF1 (601 bp), and 99.68% for the BT2 (315 bp).

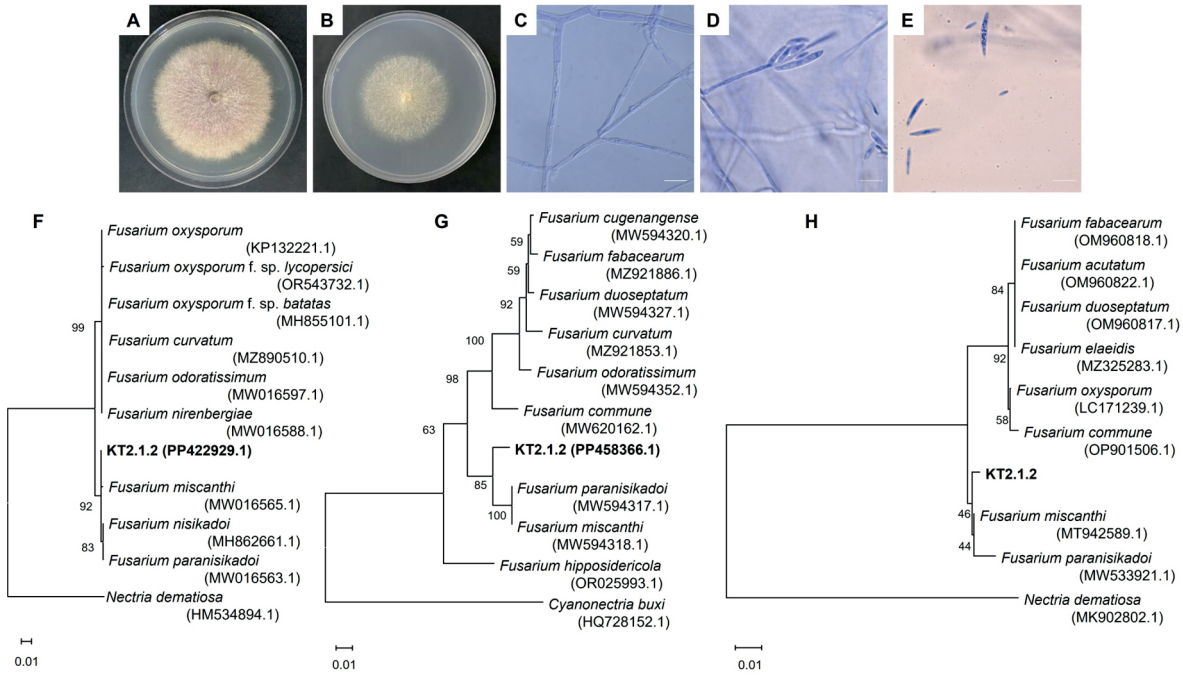

Fig. 5. Morphology and taxonomic position of strain KT2.1.2. A – colony on PDA; B – colony on LCA; C – septate mycelia; D – unbranched conidiophore; E – macro- and microconidia. Scale bars: 10 µm. F, G, H – phylogenetic trees based on comparative analyses of the ITS, TEF1, and BT2 sequences, respectively (accession numbers of these sequences are indicated in parentheses next to the strain name)

Discussion

Foliar soft rot disease was first reported in this study on *P. vietnamensis* with an incident rate of 2–26%, comparable to other reported foliar diseases, i.e., alternaria blight (caused by *A. panax*) at 20–30% (Putnam and Du Toit 2003; Wei *et al.* 2018), leaf spot (caused by *A. alternata* and *Boeremia exigua*) at 10–15% (Li *et al.* 2020; Wang *et al.* 2024), anthracnose (caused by *Coletotrichum* spp.) at 20% (Han *et al.* 2004; Guan *et al.* 2021b), or gray mold (caused by *B. cinerea*) at 15–30% (Sun *et al.* 2019) (Tab. S3). So far the disease has not been reported on other Panax ginseng cultivars, however, it seems to seriously affect ginseng farms in Vietnam. Growing in high humidity and shadowed light, *P. vietnamensis* is prone to foliar soft rot disease (Fig. 1B). Although, the yield loss has not been evaluated it is expected to be significant.

In this study, the two fungal species *N. ipomoeae* and *F. miscanthi* were first documented as pathogens associated with the foliar soft rot disease on Vietnamese Panax ginseng. Previously assigned to the *Fusarium solani* species complex (O'Donnell *et al.* 2022), the genus *Neocosmospora* is among the most important groups of phytopathogenic fungi. Later, based on sequence data from several marker sequences such as internal transcribed spacers (ITS), RNA polymerase subunit I (RPB1), translation elongation factor 1-alpha (TEF1), and beta-tubulin (BT) in combination with morphological characterizations, this group was reclassified as genus *Neocosmospora* in the family *Nectriaceae* (Sandoval-Denis *et al.* 2019). *Neocosmospora* spp. are widely distributed, being infective to nearly 500 different plant hosts, and generally cause rot diseases affecting different plant compartments (Farr and Rossman, updated on May 13, 2024). The most notable *Neocosmospora* diseases include stem rot in potatoes caused by *N. rubicola* (Riaz *et al.* 2022), stem rot in soybeans caused by *N. vasinfecta* (Greer *et al.* 2015), root and pod rot in peanuts caused by *N. vasinfecta* and *N. striata* (Sun *et al.* 2012), and root rot in succulent plants caused by *N. falciformis* (Kamali-Sarvestani *et al.* 2022). Specifically, *N. ipomoeae* is known as a soil-born fungus causing stem rot on various plant hosts such as tomato and paprika (Kwon *et al.* 2017; Tomita *et al.* 2021). Recently it has been reported to cause basal stem rot on desert rose *Adenium obesum* (Custódio and Pereira 2023). Interestingly, *Neocosmospora* spp. in general, have not been reported to attack plant leaves except the *N. ipomoeae* causing foliar soft rot as shown in this study. *Neocosmospora* spp. can infect host plants through lenticels without wounds. Their hyphae grow intracellularly at the early stage of infection and then expand intercellularly causing the death of the host cells (Riaz *et al.* 2020). The same infection route likely occurred with *N. ipomoeae* as the fungus successfully infected ginseng leaves via contact

with the leaf surface, without any injury (as shown in the artificial infection experiments).

Little is known about the ecology and pathogenicity of the fungus *F. miscanthi*. The species was first discovered from a litter of Japanese silver grass *Miscanthus sinensis* buried in a Danish field. Taxonomically, *F. miscanthi* falls with *Fusarium nishikadoi* in a putative sister group to the *Fusarium oxysporum* complex, and the two can be separated only via comparative analyses of mtSSU rDNA and beta-tubulin gene sequences (Okungbowa and Shittu 2012). Indeed, the taxonomic identification of strain KT2.1.2 in this study was achieved only when multiple gene sequences were compared, i.e., ITS, TEF1, and BT2 (Fig. 5). *Fusarium* spp. are known as important wilt pathogens with a high level of host specificity. The most notable pathogenic fusaria include *F. eumartii*, *F. oxysporum*, *F. avenaceum*, *F. solani, F. sulphureum* and *F. tabacinum* (Okungbowa and Shittu 2012). For decades, *F. miscanthi* was not considered to be pathogenic and of minor quarantine importance (Summerell *et al*. 2011). Recently it has been reported as a pathogen causing ear rot disease in maize (Shang *et al*. 2021), and wilt disease in chickpea (Younesi *et al*. 2021). The finding of *F. miscanthi* as the causal agent of foliar soft rot disease has expanded the host range of this pathogen, and at the same time, raises concern about the infection possibility of this fungus on other Panax ginseng varieties.

In conclusion, the foliar soft rot disease on ginseng *P. vietnamensis* and the associated pathogens *N. ipomoeae* and *F. miscanthi* reported in this study raised concern about ginseng cultivation in Vietnam, and to a certain extent, for the cultivation of other Panax ginseng varieties worldwide, especially in neighboring countries. Further studies on the infection routes and measures to control the disease are urgent. Biological control is a promising approach to be focused on.

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