Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Panax notoginseng* Wilt Disease Caused by *Fusarium graminearum* in China

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Panax notoginseng is one of the important economic crops grown under the forest canopy and is widely planted in Yunnan Province, China. In August of 2022, a survey in Xundian County (25°26′N, 103°7′E) was accomplished to verify the occurrence of wilt disease in *P. notoginseng* and understand its etiology. The site is an underforest area of organic *P. notoginseng* cultivation, covering over 40 ha. Disease symptoms included severe stunting, leaf chlorosis, red or yellow stalks, and root rot. The entire plant gradually wilted and died with disease progression. To identify the causal agent, we collected more than 30 wilted *P. notoginseng* plants and dissected the plant tissues from the symptomatic leaves, stalks, and roots. The tissues were surface sterilized with 0.5% sodium hypochlorite for 2 min, followed by 75% alcohol for 1 min, and rinsed three times in sterilized water. Upon drying, the samples were placed onto potato dextrose agar (PDA) and incubated in the dark at 25°C. Isolates were then transferred to carnation leaf agar (CLA) to induce sporulation. Colonies on PDA were yellow, orange to red, with abundant fluffy aerial mycelia with a dark red pigment on the reverse side. Colonies on CLA were orange to yellow. Fusiform macroconidia and bottle-shaped conidiogenous cells were visible under a microscope. Microconidia were not observed. Macroconidia were measured as 18.5 to 40.5 × 3 to 4.7 µm (n = 60) and possessed 2 to 6 septa. These characteristics were similar to previously reported morphological characteristics of *Fusarium graminearum* (Martinez et al. 2019; Shikur et al. 2018). The cetyltrimethylammonium bromide rapid plant genome extraction kit (DN14, Aidlab Biotechnologies, Beijing, China) was used to obtain genomic DNA from two representative isolates. The internal transcribed spacer (ITS), translation elongation factor 1-alpha (TEF1), and RNA polymerase second largest subunit (RPB2) genes were amplified by polymerase chain reaction using the primers ITS5/ITS4 (White et al. 1990), EF1-983F/EF1-2218R (Rehner et al. 2005), and bRPB2-6F/bRPB2-7.1R (Matheny et al. 2002), respectively. A BLAST homology search for nucleotide sequences revealed >99% similarity to *F. graminearum* ITS (550 bp; MG274308 and KU847854), TEF1 (1,000 bp; MH572248 and MH572252), and RPB2 (1,000 bp; KT855203 and KT855206) sequences. All sequences generated from this study were deposited in GenBank (OP617343 and OP617344 for ITS; OP930951 and OP930952 for TEF1; and OP930953 and OP930954 for RPB2). In the phylogenetic tree, the isolates (SWFU 0000116 and SWFU 0000117) clustered with the representative strains of *F. graminearum*. The morphology and multigene phylogenetic analysis indicated that the new isolate was *F. graminearum*. Koch's postulates were used to confirm that the symptoms in wilted *P. notoginseng* were attributable to *F. graminearum*. First, healthy leaves were gently wounded with a needle and sprayed with spore suspension (1.0×10^6 spores/ml) using a hand sprayer (Martinez et al. 2019). All *P. notoginseng* plants were then replanted in pots with a diameter of 20 cm (one plant per pot) filled with a mixture of sterilized soil and incubated at 25 to 27°C. The blank control comprised sterile cotton soaked in sterile water and inactivated mycelia sprayed on the leaves. After 7 days of incubation, all inoculated leaves and stalks developed necrosis with pale red mycelia, whereas the control plants remained symptomless. The pathogen was successfully isolated from the inoculated plants and identified as *F. graminearum*. Koch's postulates were fulfilled. To the best of our knowledge, this is the first report from China with evidence of *F. graminearum* infecting *P. notoginseng*.

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