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Short Communication

First report of *Fusarium oxysporum* causing root rot of garlic in China

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In the springs of 2020 and 2021, with a temperature of 15°C, root rot on garlic were widespread in Enshi, Hubei Province, China. Based on micro-morphological and cultural characteristics, the pathogen was identified as a *Fusarium* sp. Further, based on multilocus (ITS, *EF-1α*) phylogenic data, the strains were identified as *Fusarium oxysporum*. Koch's postulates were thus fulfilled by pathogenicity tests on garlic seedlings cultured in vitro.

Key words: Garlic, root rot, *Fusarium oxysporum.*

INTRODUCTION

Garlic (*Allium sativum* L.) is both flavorful and rich in nutrients, and has important medicinal value compared to other vegetables. It has bactericidal, anti-cancer, anti-corrosion and anti-aging properties, among others (Yayeh et al., 2021; Oosthuizen et al., 2018). Currently, the garlic planting area in the world exceeds 1.2 million hectares. China accounts for nearly 70% of the global garlic production (Seth et al., 2018). In recent years, root rot of garlic occurred more frequently in China, which leads to about 20-30% production loss rate and more than 50% in serious area (Xie et al., 2015). Garlic root rot symptoms are yellowing leaves, basal stem discoloration and rotten roots found in various provinces in China, caused by fungi such as *Pythium* sp. in Shandong (Zhang et al., 2021) and *Ceratobasidium* sp. in Jinxiang, Shandong, and Feng Counties, Jiangsu (Yin et al., 2020).

MATERIALS AND METHODS

The investigation of Local Agricultural Technology Extension Department indicated that the root rot incidence reached up to 35-40% in the garlic cultivation bases in Enshi Tujia Autonomous Prefecture, Hubei Province in China in the springs of 2020 and 2021. In order to isolate and identify the causal agent of this disease, symptomatic plants were collected and the infected roots were cut into small root segments. These root segments were washed thoroughly with 75% ethanol followed by surface sterilization with 0.1% $HgCl_2$ for 1 min and rinsed with sterilized distilled water three times. The sterilized root segments were put on potato dextrose agar (PDA) plates and penta chloro nitrobenzene agar medium (PPA) plates (Fusarium selective medium) (Nash

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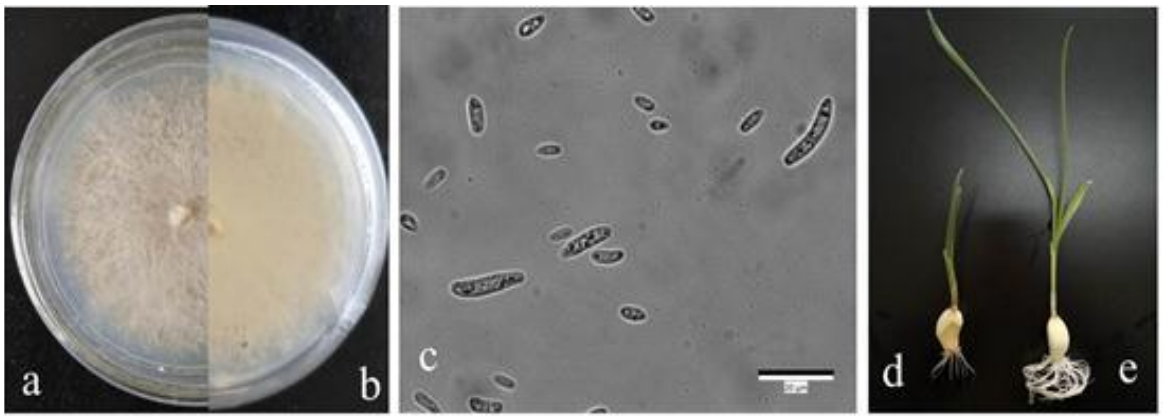

Figure 1. Symptoms and pathogen morphology of root rot on garlic caused by *Fusarium oxysporum. (*a & b) Front and back view of colony after 7 days at 25°C on PDA plates in the dark. (c) Conidiogenous cells and developing conidia scale bar=20 µm apparatus. (d) Lesion on garlic at 3 weeks after inoculation with conidial of *Fusarium oxysporum*. e) After inoculation with water as control.

and Snyder, 1962), at 25°C for 24 h in the dark. The isolated single-spore *Fusarium* colonies were inoculated on carnation leaf agar (CLA) medium and *Fusarium* species were identified through their morphological characteristics (Awere et al., 2021).

RESULTS

The colonies on potato dextrose agar medium (PDA) exhibited typical *Fusarium* characteristics, viz; *Fusarium*-like, floccose, and pale orange with aerial mycelia (Figures 1a and b). The colony had a diameter of 4.5 cm after 4-day culture at 25°C. The colony microconidia were in ovate or reniform shape, 0~1 septate, 4.8~10.1 µm×2.0~4.6 µm, whereas colony macroconidia were in falcate shape, 3~5 septate, and 20.2~36.6 µm × 3.3~4.5 µm (Figure 1c). Our observation of morphological characteristics was similar to those of *Fusarium oxysporum* (Leslie and Summerell, 2006). We obtained a total of 26 *Fusarium* isolates from 48 garlic rots collected in 2020 and 2021 in Hubei province. Since the morphological observation was consistent for all isolates, one isolated colony was selected for molecular identification. CTAB method was applied to extract pathogen genome DNA from isolate (GRR-1) (Wu et al., 2001). The primers EF1/EF2 were used to amplify the DNA sequence of translation elongation factor 1 alpha (*EF-1α*, a marker gene of *F. oxysporum*) (Geiser et al., 2004). The resultant *EF-1α* sequence (MW660368) of GRR-1 exhibited 99% identity with that of *F. oxysporum* (MK560296). Also, the results of ITS sequence (MW644753) analysis displayed a 99% match to one accession of *F. oxysporum* (MK560296) through BLAST against the NCBI nucleotide database (https://www.ncbi.nlm.nih.gov/). The phylogenetic tree of GRR-1 and the other *Fusarium* species in Figure 2 shows that GRR-1 and *F. oxysporum* comprise the same cluster.

Pathogenic examination of GRR-1 strain

The two-week-old garlic plants were used as materials for the pathogenicity tests. The root irrigation method was employed to inoculate the healthy garlic plants with 100 ml of conidial suspension (3.0×10^7 conidia/ml), and the same volume of sterile water served as a control. Three weeks after inoculation with conidial suspension, all the garlic leaves gradually turned yellow, which was consistent with the symptoms observed in the field. Four weeks after inoculation with conidial suspension, all the garlic root systems exhibited typical rotten symptoms (Figure 1d), followed by eventual plants withering and death, whereas the control plants stayed healthy (Figure 1e). We further isolated the same fungus colonies and confirmed Koch's hypothesis (Silva et al., 2013).

Conclusion

In this study, the root rot of garlic caused by *F. oxysporum* in Southern China was reported for the first time. Thus, the identification of *F. oxysporum* as the causal agent of the observed root rot on garlic is critical to the prevention and control of this disease in the future.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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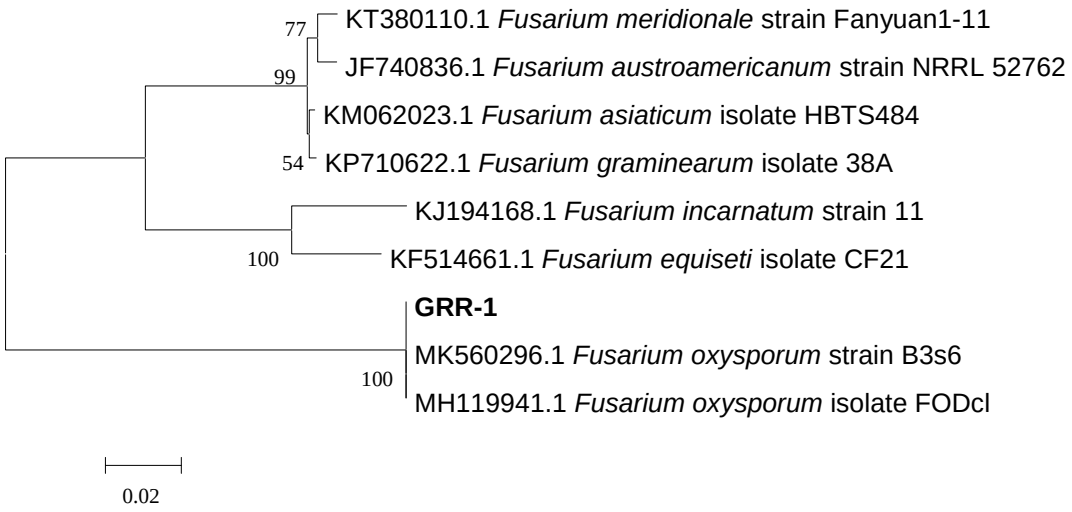

Figure 2. Phylogram generated from neighbor joining analysis based on alignment of ITS and *EF-1α* gene sequences. Values above the branches are parsimony bootstrap (equal or above 50%).

Vegetable Industry Technology System of China (CARS-24-G-14).

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