Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Fusarium incarnatum* Causing Spikelet Rot on Rice in China

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Rice (*Oryza sativa* L.) is the most important and widely grown crop, covering about 29.9 million hectares of total cultivation area in China. In the last decade, spikelet rot disease on rice has become much more frequent in the middle and lower reaches of the Yangtze River, China. *Fusarium proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg was reported to be a causal agent of spikelet rot on rice in Hangzhou, Zhejiang province (Huang et al. 2011). In September 2019, a survey was conducted to understand the etiology of the disease in the main rice growing regions of Jinshan District of Shanghai. Symptomatic panicles exhibiting reddish or brown discoloration on the glumes were collected from different rice fields, where disease incidence was estimated to be between 20 and 80%. Diseased glumes were cut into small sections (5 × 5 mm) from the boundary of necrotic and healthy tissues, surface sterilized with 75% ethanol for 30 s and 3% sodium hypochlorite for 90 s, rinsed twice with sterile distilled water, and then placed onto 1/5 strength potato dextrose agar (PDA). After 3 to 5 days of incubation at 28°C in the dark, fungal growth with *Fusarium*-like colonies was transferred to PDA and purified by the single-spore isolation method. A total of 12 isolates were obtained, and colonies showed loosely floccose, white mycelium and pale-yellow pigmentation on PDA. Microconidia were ovoid mostly with zero to one septum and measured 4.2 to 16.6 × 2.5 to 4.1 µm (*n* = 50). After 5 to 7 days of inoculation on carnation leaf agar, macroconidia produced usually had three to five septa, slightly curved at the apex, ranging from 15.7 to 39.1 × 3.3 to 5.0 µm (*n* = 50). Chlamydospores were produced in hyphae, most often solitary in short chains or in clumps, ellipsoidal or subglobose with thick and roughened walls. Molecular identification was performed on the representative isolates (JS3, JS9, and JS21). The rDNA internal transcribed spacer (ITS), translation elongation factor (TEF-1α), and

β-tubulin (β-TUB) genes were amplified and sequenced using the paired primers ITS1/ITS4 (White et al. 1990), EF1/EF2 (O'Donnell et al. 1998), and T1/T22 (O'Donnell and Cigelnik 1997), respectively. The obtained sequences were deposited in GenBank under accession numbers MT889972 to MT889974 (ITS), MT895844 to MT895846 (TEF-1α), and MT895841 to MT895843 (β-TUB), respectively. BLASTn search of the sequences revealed 99 to 100% identity with ITS (MF356578), TEF-1α (HM770725), and β-TUB (GQ915444) of *Fusarium incarnatum* isolates. *FUSARIUM*-ID (Geiser et al. 2004) analysis showed 99 to 100% similarity with sequences of the *F. incarnatum-equiseti* species complex (FD_01651 and FD_01628). In addition, a phylogenetic analysis based on the concatenated nucleotide sequences placed the isolates in the *F. incarnatum* clade at 100% bootstrap support. Thus, both morphological observations and molecular criteria supported identification of the isolates as *F. incarnatum* (Desm.) Sacc. (synonym: *Fusarium semitectum*) (Leslie and Summerell 2006; Nirenberg 1990). Pathogenicity tests were performed on susceptible rice cultivar 'Xiushui134'. At pollen cell maturity stage, a 2-ml conidial suspension (5 × 10⁵ microconidia/ml) of each isolate was injected into 10 rice panicles. Control plants were inoculated with sterile distilled water. Then, the pots were kept in a growth chamber at 28°C, 80% relative humidity, and 12-h/12-h light (10,000 lux)/dark. The experiment was repeated two times for each isolate. Two weeks post inoculation, all inoculated panicles showed similar symptoms with the original samples, whereas no symptoms were observed on the control. The pathogen was reisolated from inoculated panicles and identified by the method described above to fulfill Koch's postulates. Previous studies reported that *F. incarnatum* reproduced perithecia to overwinter on rice stubble as the inoculum of Fusarium head blight of wheat in southern China (Yang et al. 2018). To our knowledge, this is the first report of spikelet rot on rice caused by *F. incarnatum* in China. Further investigation is needed to gain a better understanding its potential geographic distribution of this new pathogen on rice crop.

References:

- Geiser, D. M., et al. 2004. Eur. J. Plant Pathol. 110:473.
- Huang, S. W., et al. 2011. Crop Prot. 30:10.
- Leslie, J. F., and Summerell, B. A. 2006. The *Fusarium* Laboratory Manual. Blackwell, Ames, IA.
- Nirenberg, H. I. 1990. Stud. Mycol. 32:91.
- O'Donnell, K., and Cigelnik, E. 1997. Mol. Phylogenet. Evol. 7:103.
- O'Donnell, K., et al. 1998. Proc. Natl. Acad. Sci. U.S.A. 95:2044.
- White, K., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.
- Yang, M. X., et al. 2018. Toxins (Basel) 10:115.

The author(s) declare no conflict of interest.

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