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FIRST REPORT OF *FUSARIUM PROLIFERATUM* CAUSING FRUIT ROT OF GRAPES (*VITIS VINIFERA*) IN PAKISTAN

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ABSTRACT

Grapes (Vitis vinifera) are the important fruit crop in Pakistan, mostly cultivated for edible purpose. In September 2016, unusual fruit rot symptoms were observed 3-5 days after harvesting on grapes cv. Kishmishi in post-harvest packing houses in Jehlum district (32°56'22.3"N 73°43'31.4"E) of Punjab province. To determine the disease incidence, a total of 10 boxes of grapes from 5 different locations were selected randomly. Each box contained average 12 bunches and 30 bunches out of 120 inspected bunches displayed typical symptoms of the disease. The initial Symptoms were small, round, water-soaked lesions that rapidly developed into soft, white to light pink mycelium near the centre of infected fruits (Figure 1). A total of 186 symptomatic berries were surface sterilized with 1% sodium hypochlorite, rinsed three times with sterile distilled water and dried by placing on filter paper for 45 sec. Sterilized tissues (approximately 4 mm³) were excised and incubated on potato dextrose agar (PDA) medium at 25 ± 4°C. One week after incubation, colonies with abundant aerial mycelium were initially white, cottony and turned to violet and dark purple with age (Figure 2). A total of 25 isolates were examined morphologically. Macroconidia were slender, thin-walled, 3 to 5 septate, curved apical cell, with 20.9 to 45.2×3.2 to 7.1 μ m and Microconidia were thin-walled, aseptate, club-shaped with 4.5 to 11.2×2.3 to 4.1 µm (Figure 3). These characteristics best fit for the description of Fusarium proliferatum (Leslie and Summerell, 2006). Portions of the internal transcribed spacer (ITS) region were sequenced (White *et al.*, 1990). Sequences of two isolates Fus 07 and Fus 09 (GenBank Accessions; MH444366 and MH464139) showed 100% identity to the corresponding gene sequences of Fusarium proliferatum (GenBank Accessions; MH368119, MF033172 and KU939071) (Figure 4). Pathogenicity test was performed by inoculation with $50-\mu l$ conidial suspension (1 × 10^6 conidia/ml) of two isolates onto three non-wounded and four wounded asymptomatic grapes berries. Sterile distilled water was used for a negative control (Figure 5). The experiment was conducted twice and berries were incubated at 25 ± 2°C in sterile moisture chambers (Ghuffar et al., 2018). White to light pink mycelium in appearance with the original symptoms were observed on both wounded and non-wounded inoculated berries after 3 days, whereas no symptoms were observed on the negative control. The morphology of the fungus that was re-isolated from each of the inoculated berries was identical to that of the original cultures. Fusarium proliferatum, one of the destructive species, causes diseases like foot-rot of corn (Farr et al., 1990), root rot of soybean (Díaz Arias et al., 2011), bakanae of rice (Zainudin et al., 2008), wilt of date palm (Khudhair et al., 2014), tomato wilt (Chehri, 2016) and tomato fruit rot (Murad et al., 2016). To our knowledge, this is the first report of Fusarium proliferatum causing fruit rot of grapes in Pakistan, where the disease poses a significant threat to the sustainability of this major fruit crop.

Keywords: Fruit Rot of Grapes, Fusarium proliferatum, Vitis vinifera, ITS.

Running Title: Fusarium proliferatum causing fruit rot of grapes.



Figure 1. White to light pink mycelium on the infected fruit berries.



Figure 2. Fungal colony showing white, cottony mycelium that later turned to violet and dark purple on PDA media.



Figure 3. Microscopic characterization of Fusarium proliferatum.

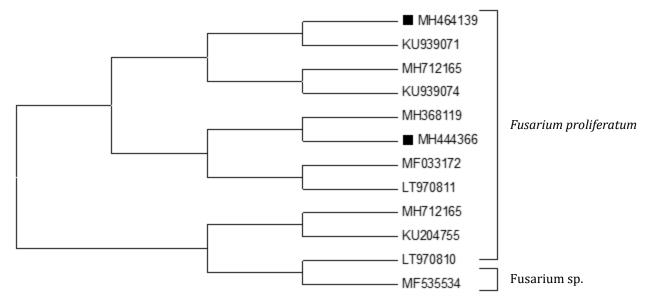


Figure 4. Phylogenetic tree based on Maximum Likelihood analysis generated from the ITS gene sequences of 12 Fusarium isolates along with their Genkbank accessions.



Figure 5. Pathogenicity testing showing white to light pink mycelium on the inoculated fruit berries.

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