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NOVEL REPORT OF *BOTRYTIS CINEREA* CAUSING GREY MOLD DISEASE OF WATERMELON (*CITRULLUS LANATUS L.*) IN PAKISTAN

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ABSTRACT

Watermelon (*Citrullus lanatus L.*) is a commercially valuable and highly nutritious vegetable around the world. Despite its importance, In Pakistan, grey mold disease is an emerging threat to watermelon cultivation. For this purpose, in May, 2021 symptoms of grey mold were observed included greyish to brown lesions with fuzzy-like appearance on foliar tissues of watermelon in vegetable research station Sahiwal, Punjab, Pakistan. Morph-molecular characterization and pathogenicity test concluded that the causal agent of disease was *Botrytis cinerea*. Thus, this study signifies the first time report of *Botrytis cinerea* causing grey mold disease of watermelon in Pakistan.

Keywords: *Botrytis cinerea*, Grey mold, Genotype (G.type), ITS, Pathogenicity, Watermelon.

INTRODUCTION

Watermelon (*Citrullus lanatus L.*) is an important vegetable of Pakistan, available in the market for fresh consumption. This vegetable faces various biotic stresses during their growing season but in recent years' grey mold has been emerged as the most epidemic fungal disease throughout the world due to its wide host range (Williamson *et al.*, 2007; Borgatta *et al.*, 2018). For this purpose, this research work aimed to identify the pathogen causing grey mold disease of watermelon in Pakistan. Therefore, a comprehensive survey was conducted in the month of May, 2021 for the collection of infected leaves of watermelon (Genotypes. Global, Sultan and Shinka) at Vegetable Research Station, Sahiwal (VRSS) Punjab (30°63'91.1" N, 73°11'91.5" E). Symptoms observed *viz.* grayish to brown lesions on foliar tissues causing disease incidence up to 20% (Figure 1a). Infected tissues were excised and surface

sterilized by immersion in 1% sodium hypochlorite for 60 second, placed on Potato Dextrose Agar media (PDA) and incubated at 25± 2°C. After 7 days of incubation fungal colonies found dark brown in color with fluffy and arial mycelium. Moreover, after three weeks of post inoculation black, rounded sclerotia (0.4- 2.0 mm) were observed throughout the colonies (Figure 1b & c). During microscopic observation, conidia was found elliptical, measuring (L x W) 6.3 ± 1.3 to 13 ± 0.8 µm x 6.7 ± 2.1 to 9.6 ± 0.9 µm (n = 50), producing tree-like conidiophores (Figure 1d). On the basis of morphological studies, all isolates were presumptively identified as *B. cinerea* Pers. (Ellis, 1971). After morphological conformation two isolates: VRSBOT1 and VRSBOT2, were selected for further molecular studies. The DNA was initially extracted using Prem Man® Ultra sample preparation Reagent following manufacturer instructions. The extracted DNA was amplified with internal transcribed spacer region (ITS gene) by using Thermo-cycler PCR machine (Model PCT-100; Waltham MA). A total of 50 µL mixture was prepared for polymerase chain reaction (PCR) contains MgCl₂ (4 mM), Promegma buffer (10 µL), dNTPs (0.2 mM), ITS1 & ITS4 primers (0.75 µM), Taq polymerase (1.25 units) and

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quantity of DNA template was used 2 µl respectively (white *et al.*, 1990). Following conditions for PCR reaction were arranged viz. 2 min at 95°C, followed by 30 cycles of 95°C for 1 min, 56°C at 1 min, 72°C at 1min and 5 min at 72°C. After amplification, DNA was verified by running 6 µl of the PCR product in a 1% agarose gel and visualized by an ultraviolet imaging system. All The PCR products were purified with Gel Band Purification Kit (GE Healthcare Bio-sciences, Pittsburgh, Pennsylvania), sequenced by using DNA analyzer (Model 3730 xl; Applied Biosystems) and software (BioEdit v. 7.0.5.2) was used to edit the sequences manually (Hall, 1999). Furthermore, Gen-Bank database NCBI program was used for BLAST searches of acquired sequences to obtain the closest relatives

ITS sequences and included in the ITS phylogenetic analysis. According to the result of BLAST searches revealed 99-100% genetic homology of Accession Nos. (MZ268632.1 and MZ268633.1) with previous sequences of *Botrytis cinerea*. Finally, by using software (MEGA v. 7) sequences were aligned in the CLUSTAL-X v. 1.81 and the Neighbor-Joining analysis was performed on ITS sequence of two isolates VRSBOT1 and VRSBOT2 from watermelon with closely related sequences of *Botrytis cinerea* isolated from other hosts available in Genbank (Thompson *et al.*, 1997; Kumar *et al.*, 2016). Neighbour-Joining (NJ) analysis showed that isolates VRSBOT1 and VRSBOT2 obtained from the watermelon clustered with authentic isolates of *Botrytis cinerea* (Figure 2).

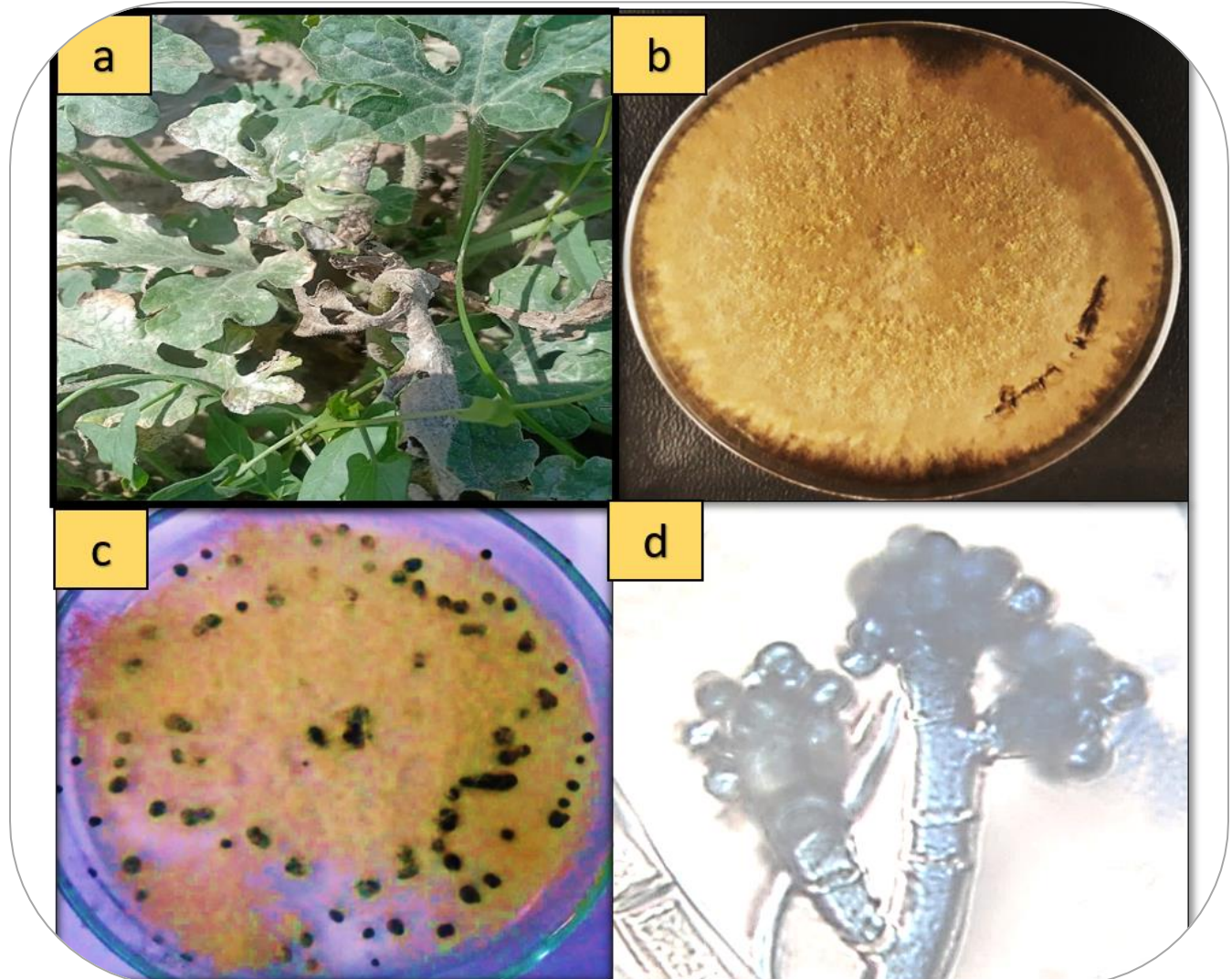


Figure 1. (a) Symptomology (b & C) Cultural characteristics of *Botrytis cinerea* (d) Microscopic characters.

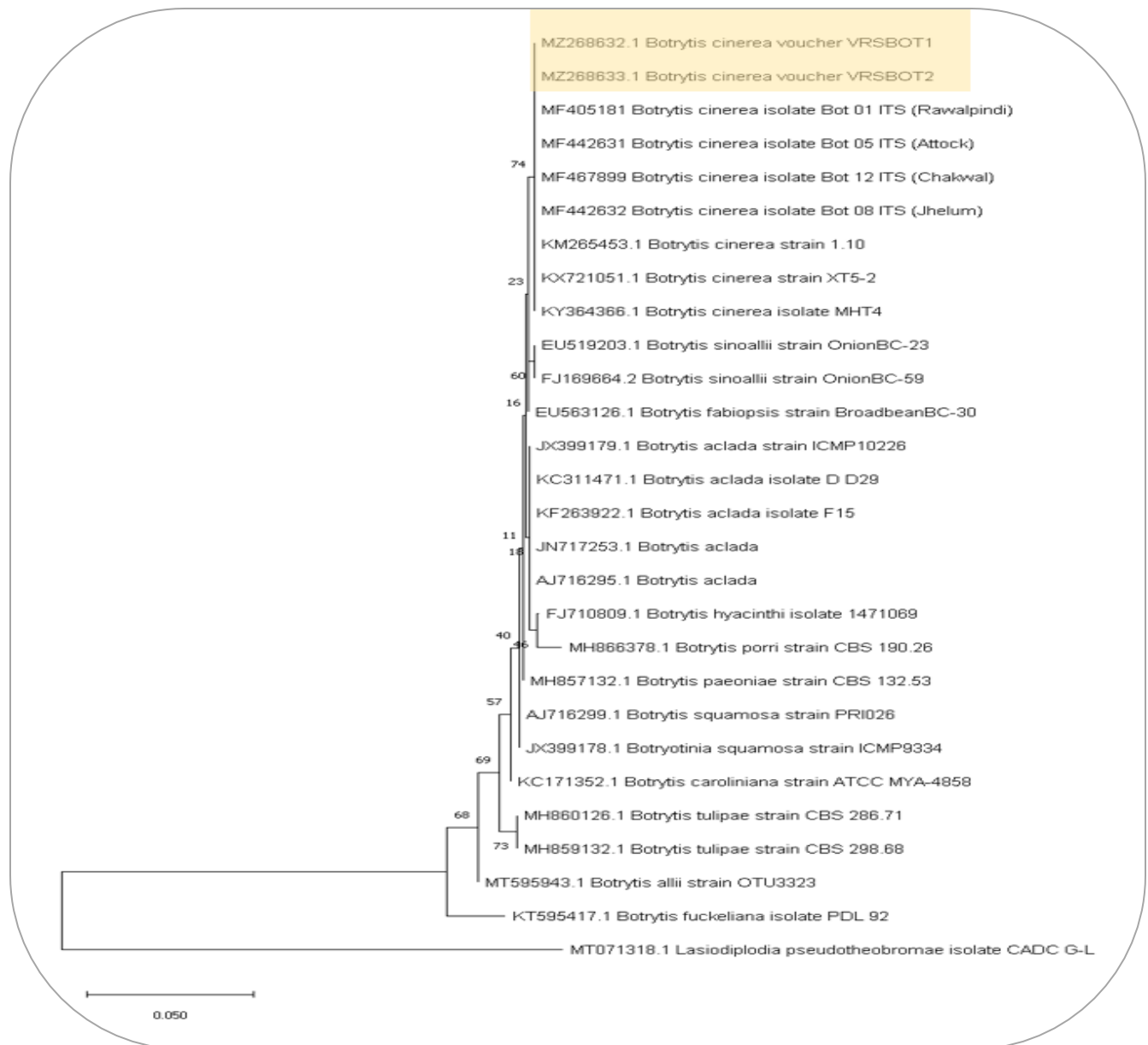


Figure 2. Showing Phylogenetic analysis of internal transcribed spacer sequences by using neighbor-joining method and also described the closest known relatives of *Botrytis cinerea*.

A total of twenty healthy plants of watermelon (Global Genotype) were used for confirming the pathogenicity, ten healthy plants were sprayed with conidial suspension (1×10^5 conidia/mL) of both isolates in a condition of two true leaves emergence in a control environment. While other ten plants were inoculated with sterile water set as a control. After 7 days, the symptoms on the leaves of all artificial infected plants were similar to those observed in the field while, no symptoms have been developed in the plants inoculated with sterile water. For fulfilling Koch's postulates same techniques has been applied for successfully recovered

the original isolate. *Botrytis cinerea* has been reported previously to cause grey mold disease on vegetables belongs to Cucurbitaceous family viz. Cucumber (Soliman *et al.*, 2014) and Bottle Gourd (Aktaruzzaman *et al.*, 2019) in different regions of the world. Based on research work it is concluded that *Botrytis cinerea* is responsible to cause Grey mold disease of watermelon and first time reported in Pakistan. Moreover, this disease poses a significant threat to sustainability of watermelon producers. Meanwhile, a management strategy to minimize gray mold should be developed to protect watermelon from this disease.

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Contribution of Authors:

Salman Ghuffar	:	Conceived the idea and conducted the research
Kashif Rashid	:	Methodology setting
Muhammad Najeebullah	:	Technical Review
Muhammad Z. Ahmed	:	Help in conducting molecular studies
Waseem Abbas	:	Help in conducting morphological studies
Mudassar Iqbal	:	Review and proof reading
Muhammad S. Saeed	:	Help in data analysis
Muhammad U. Khalid	:	Help in paper setting