FIRST REPORT OF LEAF BLIGHT DISEASE OF WATERMELON CAUSED BY *ALTERNARIA ALTERNATA* F. SP. *CUCURBITAE* IN BANGLADESH

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ABSTRACT

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Watermelons (*Citrullus lanatus*) were severely affected by leaf blight disease in different parts of Nayabazar, Gorudaspur upazila, and Natore district of Bangladesh. The watermelon leaves those were affected by the leaf blight disease had numerous irregularly shaped dark brown patches that eventually blighted the leaves. The morphological, microscopic, and rDNA sequencing analyses supported the association of *Alternaria alternata* f. sp. *cucurbitae* with the leaf blight disease of watermelon. The pathogenicity of *Alternaria alternata* in watermelons was also proven by Koch's postulates. The pathogen *Alternaria alternata* causing leaf blight of watermelon was seemed to be the first time in Bangladesh.

Keywords: Leaf blight, *Alternaria alternate,* watermelon.

Watermelon (*Citrullus lanatus*) is a significant high-value crop for medium and small-scale farmers who used the land as sharecroppers and ran the landowners' properties as a rent or through other means in Bangladesh. Generally, watermelon is grown in summer for commercial purposes in Bangladesh until the year-round hybrid variety is introduced recently. However, watermelon farming in Bangladesh is a source of income for the farmers of coastal regions though the crop become vulnerable to many constraints due to climate change. The Bangladeshi watermelon industry is severely constrained by biotic pressures. The watermelon sown in late March to the first few weeks of April was affected by leaf blight disease, with an incidence of 80 to 100% in Gurudaspur (24.36148412°N, 89.25533295°E) of Natore district of Bangladesh. The symptoms were about 1 mm wide dark brown circular patches that merged into a larger necrotic area and eventually led to entirely blighted leaves (Fig. 1a and 1b). Pieces of the diseased watermelon leaves were sterilized by 0.5% NaOCl for 2-3 minutes, followed by several rinses with sterile distilled water, dried up, and placed onto the water agar (WA). After the following day, single germinated conidia were collected using a sterilized glass needle under a dissecting microscope, transferred to petri plates containing Potato Dextrose Agar (PDA), and incubated at 25±5°C for twelve days.

(a) (b)

Figure 1. (a) Initial and (b) severe leaf blight symptom by *Alternaria alternata* f. sp. *cucurbitae* in watermelon

Twenty isolates were found in the infected symptomatic leaf samples, and based on cultural and microscopic examination, they were tentatively identified as *Alternaria* sp. The fungal growth on PDA plates had a circular shape, a grey centre that was black, and a light greenish periphery (Fig. 2a); the reverse side of the plate had a brownish periphery and a black centre (Fig. 2b). The fungi isolates were characterized by small, short-beaked, multicellular conidia with an average length of 39 ùm (range, 17 to 80 ùm) and a breadth of 14 ùm (range, 7 to 20 ùm). Conidia were generated in a chain on short conidiophores and were ovoid, obclavate, and occasionally ellipsoidal. The beaks were small, conical or cylindrical, and frequently less than one-third the length of the body (Fig. 2c and d).

Three of the twenty identical isolates, designated as AL1, AL2, and AL3, were added to the culture collection of the Plant Pathology Division, BARI with the accession numbers BAPC051, BAPC052, and BAPC053, and further molecular characterization was carried out using rDNA sequencing of the ITS region. For molecular characterization total genomic DNA of the isolated fungi was extracted from twelve-day-old mycelium using Wizard® Genomic DNA Purification Kit (Promega, USA). According to White (1990), the rDNA-ITS region was amplified by PCR using the universal primer pairs ITS1 (5/CGGATCTCTTGGTTCTGGCA3/) and ITS4 (5/ GACGCTCGAACAGGCATGCC 3/). By using a 1% agarose gel in Tris-EDTA (TAE), the size of the PCR product was confirmed (Fig. 3a and b).

The Wizard® SV Gel and PCR Clean-Up System (Promega, USA) was used to purify the PCR product before it was sent to the National Institute of Biotechnology, Savar, Bangladesh for sequencing. The Cap3 (http://doua.prabi.fr/software/cap3) was used to assemble the sequencing products. The resulting sequences were submitted to GenBank under the accession numbers MT107082, MT107083, and MT107084, respectively, compared with the sequences in GenBank using BLASTn. The phylogenetic analysis was conducted in MEGA11 (Tamura and Kumar 2021). The blast search result showed that all tested three isolates were 99.82% similar to *Alternaria alternata*. On the other hand, the phylogenetic analysis revealed that all three isolates were clustered in the same clade with *Alternaria alternata* (Fig 4).

According to Akhter *et al*. (2009), a symptomless detached leaf assay was used for the pathogenicity test. One isolate (AL2)'s mycelial plug (6mm) was applied to a watermelon leaf that was previously cleaned with distilled water. The porcelain desiccator plate was used to store the inoculated leaves. A total of 9 watermelon leaves were inoculated with the mycelial plug (6mm) of *Alternaria alternata* while the PDA plug (6mm) without fungal fake was used to inoculate additional 3 leaves as check. After

inoculation, the desiccator was incubated at 25°C with 90% relative humidity and 12 hours of light followed by 12 hours of darkness. All inoculated leaves manifested leaf blight symptom after 4 days of inoculation (Fig. 5a and b).

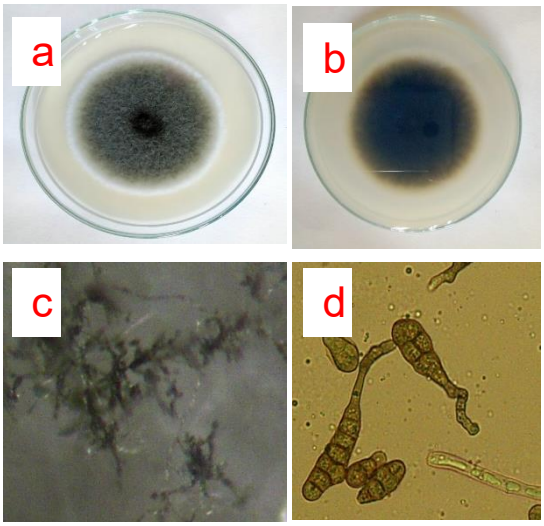

Figure 2. Cultural and morphological characteristics of *Alternaria alternata* f. sp. cucurbitae on PDA. The *Alternaria alternata* f. sp. cucurbitae growth on PDA upper and reverse side (2a-b). Fungal structure in stereomicroscope (Olympus SZ61) and conidia in compound microscope Olympus BX41 (2c-d).

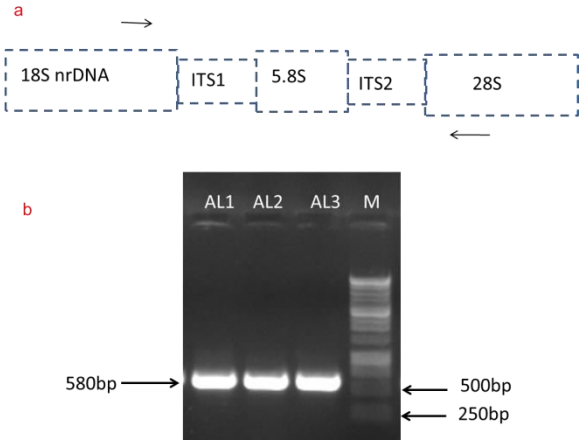

Figure 3. Diagram of primer position in *Alternaria alternata* f. sp. cucurbitae genome and gel electophersis of amplified PCR product (3a-b)

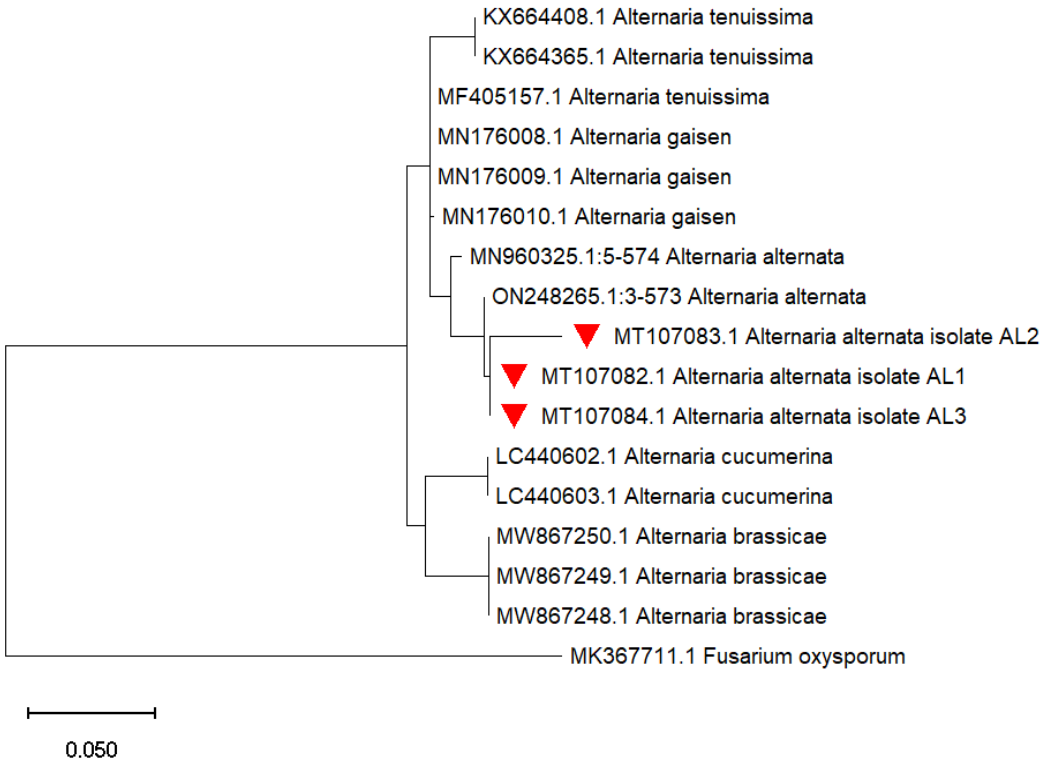

Figure 4. Phylogenetic analysis using the rDNA sequencing of *Alternaria alternata* f. sp. cucurbitae and related *Alternaria* sp retrieving from NCBI

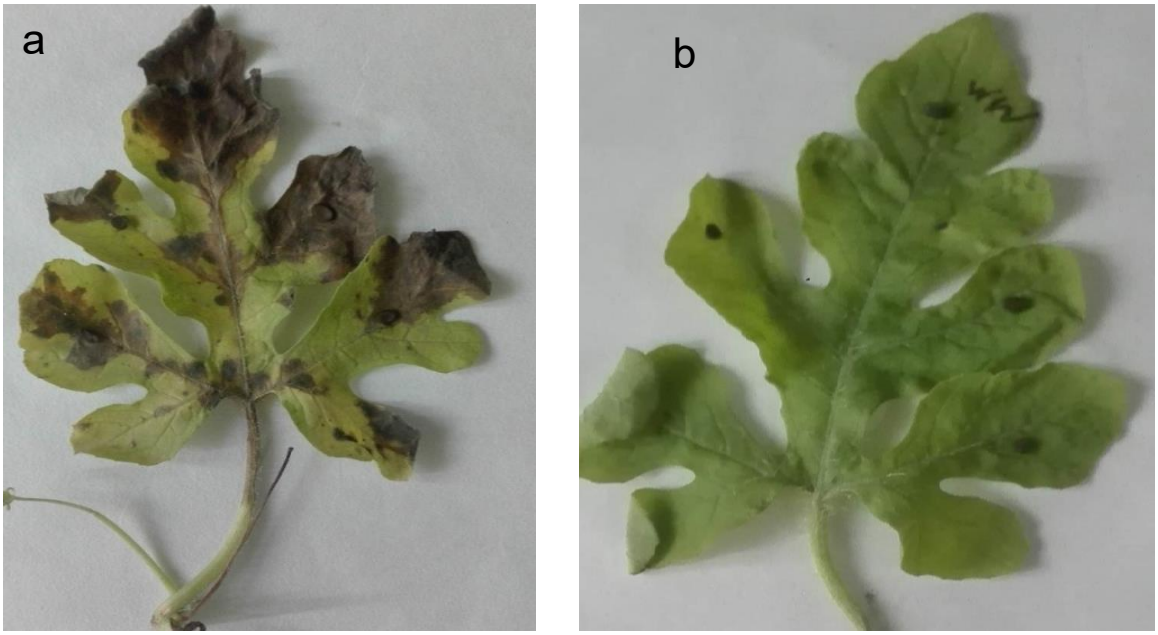

Figure 5. The pathogenicity test; inoculated (5a) and not inoculated (5b) watermelon leaf.

The isolates were identified as *Alternaria alternata* f.sp *cucurbitae* based on morphological, microscopic, and molecular examination. Koch's pustulates provided conclusive evidence that the watermelon leaf blight disease in Bangladesh was caused by *Alternaria alternata* f.sp. *cucurbitae*. Watermelon leaf blight disease caused by *Alternaria alternata* f.sp. *cucurbitae* was documented from China and South Korea (Zhao *et al*. 2016, Ma *et al*. 2021, Kwon *et al*. 2021). To develop strategies for management for the watermelon leaf blight disease in Bangladesh, additional epidemiology and control measure investigations were required. The ITS sequences of *Alternaria alternata* f. sp. *cucurbitae* were available under the accession number MT107082, MT107083, and MT107084.

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