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Short Communication

First Report of *Alternaria alternata* Causing Leaf Spot on *Beta vulgaris* in North Dakota, USA

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Received: February 22, 2021; Accepted: February 26, 2021; Published: March 05, 2021

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Sugar beet is an economically important crop which is contributing 55% of the total sugar in the USA. In June 2018, irregular dark brown somewhat circular spots were observed on sugar beet leaves in Hickson, North Dakota. The symptoms covered approximately 5% on the lower leaves. Symptomatic leaf tissue were excised from the junction of diseased and healthy tissue. Small pieces (5 mm²) were surface sterilized with 10% sodium hypochlorite for 1 min, rinsed thrice with sterile distilled water, air dried and transferred to Potato Dextrose Agar (PDA), and incubated at 24°C with a 12-h photoperiod for 5 days. Dark-green velvety colony appeared in two weeks. Three isolates were developed by the single spore isolation technique. Conidia were club-shaped, two to four transverse septa, and pale brown, without any beak, often in chains (4 to 8 conidia) and or solitary. The dimension of conidia varied from $25-40x7-14 \mu m$ [1,2]. Based on the morphological characters, the fungus was tentatively identified as Alternaria species. Genomic Deoxyribonucleic Acids (gDNAs) were extracted from the culture generated from a single spore using Qiagen kit. ITS4/ITS5 were used to amplify the fragments of the Internal Transcribed Spacer (ITS) region. The amplified PCR products were cleaned and sent for Sanger sequencing by GenScript (GenScript, Piscataway, NJ). The sequences from GenScript were

congruence to the reference sequence ID MT126620.1. The entire sequences were deposited at NCBI (GenBank accession nos. MK441717). Koch postulates were followed by spraying conidia suspension (5×10⁵ conidia/ml) to 8-week age of 20 plants of and kept in humidity chamber at 28-30 °C, 80-85 % RH. Mock-inoculated seeds were also sown as a control. Three weeks of post inoculation, the similar irregular dark brown symptoms observed in twelve plants. No symptoms were found in the mock. The experiment was conducted twice. The fungus was reisolated from the diseased leaf tissue, as described above. Macroscopic and microscopic analysis indicated the similar dark-green colony and morphology, respectively. Molecular detection performed using the same ITS primers and sent for Sanger sequencing by GenScript, this study further confirmed that the isolate was similar to A. alternata [3]. Another close species of Alternaria was recently reported in sugar beet to cause leaf spot in Minnesota [4,5]. To our best knowledge, this is the first report of A. alternata causing leaf spot on sugar beet in North Dakota.

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Austin J Plant Biol - Volume 7 Issue 1 - 2021 **ISSN: 2472-3738** | www.austinpublishinggroup.com Parvin et al. © All rights are reserved

Citation: Haque E, Parvin MS. First Report of *Alternaria alternata* Causing Leaf Spot on *Beta vulgaris* in North Dakota, USA. Austin J Plant Biol. 2021; 7(1): 1026.