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Editorial

Alternaria alternata Causing Disease on Sugar Beet (*Beta vulgaris*) Steckling in Arizona, USA

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Editorial

Sugar beet (*Beta vulgaris*. L) is an economically important crop which is contributing 55% of the total sugar in the USA. In April 2018, irregular dark brown symptoms were observed in sugar beet stecklings in Arizona where different sugar beet cultivars were grown for seed production (Figure 1). The symptoms covered approximately 5% on the sugar beet root surface. Symptomatic beet root tissue were excised from the junction of diseased and healthy tissue. Small pieces (5mm^2) were surface sterilized with 10% sodium hypochlorite for 1min, rinsed thrice with sterile distilled water, air dried and transferred to clarified V8 (CV8), and Potato Dextrose Agar (PDA), and incubated at 24°C with a 12h photoperiod for 5 days. White-dark green velvety colony appeared on both media (Figure 2). Isolates were developed by the single spore isolation technique. Conidia were obclavate or oviod, two to four transverse septa, and pale brown, often in chains (4 to 8 conidia) and or solitary (Figure 3). The dimension of conidia varied from 20.21-40.15 x 7.50-14.12 µm [1-3]. Based on the morphological characters, the fungus was tentatively identified as *Alternaria species*. Genomic DNA were extracted *via* Qiagen kit. A total of 40ng genomic DNA was used to generate a library using NEBNext® Fast DNA Fragmentation & Library Prep Set for Ion Torrent™ for sequencing in Ion Torrent™ Personal Genome Machine™ System. The sequencing data were BlastX searched against an updated NCBI non-redundant database utilizing the high-throughput alignment program DIAMOND. A microbiome analysis tool MEGAN Community Edition v6.12.3 was then used to assess the blastX results and this confirmed to be *A. alternata*

Figure 1: Infected sugar beet root collected from the field in Arizona.

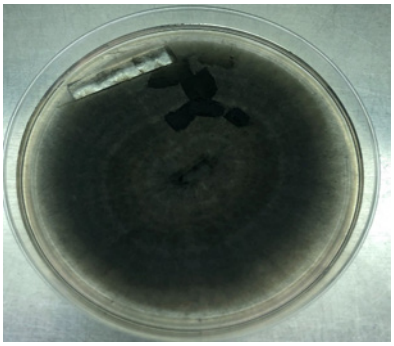

Figure 2: Appearance of *A. alternata* grown on potato dextrose agar.

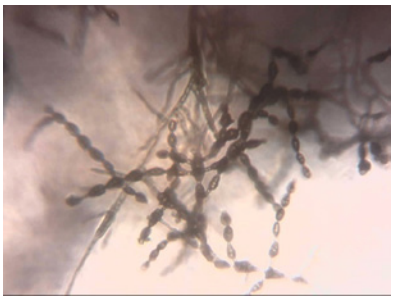

Figure 3: Morphological characteristics of conidia of *A. alternata*.

Figure 4: Symptoms on sugar beet roots inoculated artificially with *A. alternata*.

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(Genbank reference # MT482506.1). In addition, primers 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 (GenScript, Piscataway, NJ). The sequences from GenScript were congruence to the previous reference sequence ID MT482506.1. The ITS sequences showed 100% homology to *A. alternata*, with 0.0 E-value and the sequences were deposited at NCBI (GenBank accession nos. MK441720). Koch postulates were followed by soaking seeds in conidia suspension (5×10^5 conidia/ml) and kept at 24°C and 75-80% relative humidity *in vitro* for 1 day. The sprouted seeds (40 biological replicates) were sown in the greenhouse at 28-30°C and humidity 80-85%. Mock-inoculated seeds were also sown as a control. Eight weeks of post inoculation, sugar beet steckling were harvested and the similar irregular dark brown symptoms found on the surface of beet root, it appeared in 5% of the harvested beet (Figure 4). No symptoms were observed in the mock. The experiment was conducted twice. The fungus was re-isolated from the diseased beet root tissue, as described above. Macroscopic and microscopic analysis indicated the similar white-green colony morphology and conidial structure, respectively. Genomic DNA extracted from the three isolates as described above. Molecular detection performed using the same ITS primers and sent for Sanger sequencing by GenScript, it further confirmed to be *A. alternata*. Another close species of Alternaria has been recently reported in sugar beet to cause leaf spot in North Dakota [4,5]. Based on all tests, the causal agent on sugar beet root was identified as *A. alternata*. To our best knowledge, this is the first report of *A. alternata* causing disease on sugar beet steckling in Arizona, USA.

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