Morpho-molecular identification of *Alternaria alternata* causing leaf spot on loquat in Pakistan

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Loquat (*Eriobotrya japonica*) is an important sub-tropical fruit of Pakistan. During the year 2014–15, circular reddish brown leaf spots with concentric rings surrounded by yellow halo were observed in Taxila (33.74° N, 72.78° E), Wah Cantt (33.77° N, 72.75° E), Khanpur (33.80° N, 72.93° E), Tret (33.83° N, 73.30° E), Chatar (33.77° N, 73.24° E), Murree (33.90° N, 73.39° E), Kalar Kahar (32.78° N, 72.70° E) and Choa Saidan Shah (32.71° N, 72.98° E). The leaves (3 cm²) were sterilized by 75% ethanol, rinsed twice in sterile distilled water, blotted dry and transferred in PDA. The samples were incubated at 25 ± 2°C for 7 days and golden brown colonies which later turned black were observed. The conidia were single or in short chains, obpyriform, obclavate measuring ranged from 11 to 41 × 6.4 to 18 µm with 0 to 3 longitudinal and 2 to 6 transverse septa. Spore suspensions (10⁶ conidia ml⁻¹) of PAK38 and PAK39 isolates were sprayed on three healthy asymptomatic detached leaves and sterile distilled water was used as negative control. The experiment was repeated twice, leaves were incubated at 25 ± 2°C in sterile glass chambers. Symptoms were recorded 5 days after inoculation, while no symptoms were recorded on negative control. Fungal isolates were re-isolated from the diseased portions and isolated fungi were identified as *Alternaria alternata*. For the purpose of molecular identification, the sequences from internal

transcribed spacer regions (ITS) and Beta tubulin (BT) gene of PAK38 (GenBank accession Nos. KT280011 and KY126829) and PAK39 (KT312818 and KY113113) were submitted to National Center for Biotechnology Information (NCBI). Both isolates were exhibiting 99% genetic homology with previously reported ITS region (KT280009, KF039678 and KT280010) and BT (KU512287 and KT345697) gene sequences of *A. alternata* in the public database of NCBI. *A. alternata* has been reported to cause leaf spots and fruit rot of loquat in Taiwan, Greece and Iran (Tziros 2013; Mirhosseini et al. 2015) but to our knowledge this is the first report of *A. alternata* causing leaf spots and fruit rot on loquat in Pakistan.

References

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