First report of *Botrytis cinerea* on golden berry

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Abstract *Botrytis cinerea* infection of golden berry was reported for the first time from Turkey. The pathogen caused grey mould disease. The disease was widely observed in a golden berry orchard in Samsun province (Eastern Black Sea region), Turkey. The fungus was identified as *B. cinerea* based on morphological characteristics and sequence analysis.

Keywords *Botrytis cinerea* · *Physalis peruviana* · Golden berry · Turkey

Cape gooseberry known as golden berry (*Physalis peruviana*) is grown naturally in South America. This species was cultivated firstly in 1995 in Turkey but the demand of growers and consumers has grown since 2009 (Besirli et al. 2011). In autumn 2011, disease symptoms consisting of wrinkles and depression of golden berry fruit surfaces were observed in a golden berry orchard in Samsun province (Eastern Black Sea region), Turkey. The heavily infected fruits were rotted. To isolate the causal agent, conidia and conidiophores were scraped aseptically from freshly infected golden berries, suspended in sterile distilled water, and streaked onto the surface of potato dextrose agar (PDA) (Oxoid Ltd., Basingstoke,

Hampshire, England) plates, and incubated at 23 °C. Fungal cultures from single-spores were firstly colorless and later turned grey to brown when the conidiophores and conidia formed. The average size of the conidia produced on PDA ranged from 5.5–9.4×7.0–13.0 µm (average 7.5×9.2 µm) based on 50 spore measurements. The conidia were single celled, ellipsoid or ovoid in shape, colorless or pale brown. Conidiophores were brown in color, hyaline near the apex, branched, and septate. Sclerotia formed about 2 weeks later, were black-brown, hard and irregular shaped and measured 1–4 mm. Based on these morphological features, the fungus was identified as *Botrytis cinerea*. Also, for molecular characterization of *B. cinerea* isolates, fungal genomic DNA was extracted with DNeasy plant mini kit (Qiagen, Germany) according to the manufacturer's instructions. Polymerase chain reaction (PCR) analysis was performed according to the method of Rigotti et al. (2002) with species-specific primers C729+ (5'-AGCTCGAGAGAGATCTCTGA-3') and C729- (5'-CTGCAATGTTCTGCGTGGA-3'). The PCR product was separated by electrophoresis on 1 % agarose gel in 1XTBE (Tris-Borate-EDTA) buffer and stained with GelRed and were visualized with the transilluminator. As a result, *B. cinerea* species-specific PCR using C729± primer set amplified the expected 700-bp DNA fragment from the isolates tested. Subsequent to the PCR products were sequenced both directions with the same primers used for the PCR amplifications by Macrogen Inc., Korea, using the ABI 3730 XL DNA sequencer. The sequences were analyzed using BLAST algorithm against the NCBI GenBank database. The result of blast analysis, the C729± sequences of *B. cinerea* in this study were 99 % identical to other *B. cinerea* isolate on GenBank. The resulting sequences were deposited in the GenBank (EMBL) database under accession numbers LN866604 and LN866605. Eventually all these results confirmed morphological diagnosis of *B. cinerea* isolates. The isolates were

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Fig. 1 Grey mould symptoms caused by *Botrytis cinerea* on golden berry fruits

maintained on PDA slants at +4 °C and in 15 % glycerol at –80 °C and deposited in the culture collection of the Mycology Laboratory of the Plant Protection Department, Faculty of Agriculture, Ondokuz Mayıs University (accession numbers: OMU-ZRT 2011-0055). A pathogenicity tests was conducted using GBc-78 isolate of *B. cinerea* and performed twice. Twenty five golden berry fruits were surface-sterilized with 1 % NaOCl, rinsed in sterile distilled water three times, and allowed to dry. Berries were then placed on sterile petri dishes on moist filter papers in a plastic box (30×20×15 cm). The isolate was cultivated on PDA medium for 10 days at 23±1 °C. Conidia were harvested from this cultures grown on PDA with a small paint brush, suspending the conidia in sterile distilled water and filtering through cheesecloth. Conidia were then diluted in sterile distilled water plus 0.1 % polysorbate 20 to a concentration of 1×10^5 conidia/mL and were sprayed on the surface of the berries. Sterilized distilled water was sprayed on control the berries. Both inoculated and non-inoculated the berries were incubated at 100 % relative humidity at 20 °C and after 24 h, the berries were moved to a greenhouse bench. The disease symptoms appeared on the berries with mycelia and conidia after 7–10 days of inoculation, whereas these symptoms did not develop on the control berries (Fig. 1). The pathogen was reisolated from inoculated

fruits exhibiting grey mould symptoms, successfully completing Koch's postulates. The pathogen was previously isolated from leaves of kiwifruit in the Black Sea region of Turkey (Karakaya and Bayraktar 2009) and is a common pathogen on a wide range of host plant species world wide (Ellis and Waller 1974). To our knowledge, this is the first report of *Botrytis cinerea* causing grey mould on golden berry in Turkey. In the future, studies are needed to determine the distribution and economic importance of the disease on golden berry.

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