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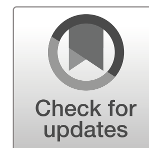

Stagonosporopsis cucurbitacearum the causal agent of gummy stem blight of watermelon in India

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Abstract

Gummy stem blight is a major disease affecting cultivation of cucurbitaceous vegetable crops all over the world. In India, this disease has been reported in several cucurbits such as ash gourd, bitter gourd, chow-chow, cucumber, muskmelon and ridge gourd. Our report represents first time identification, isolation and molecular characterisation of gummy stem blight fungus in watermelon at Indian Institute of Horticultural Research, Bangalore, India. As it infects several curcurbits and is a seed borne pathogen, seed importation may have been the source of introduction to the region. To our knowledge, this is the first report of gummy stem blight affecting watermelon in India.

Keywords Gummy stem blight · Watermelon · Molecular characterisation · India

Gummy stem blight (GSB) is a major production constraint in growing cucurbitaceous vegetable crops worldwide. In India, this disease was first reported on chow-chow (Sohi and Prakash 1972) in Mysore and then subsequently on other cucurbit crops viz., cucumber (Kumar and Khan 1984), bitter gourd (Kulwant and Shetty 1996), ash gourd (Pandey and Pandey 2003), muskmelon (Sudisha et al. 2004), and ridge gourd (Bhat et al. 2018) which represent a major share of vegetable production in India. All these reports indicate that *Didymella bryoniae* was the causal organism of this disease. Aveskamp et al. (2010) described the new species *Stagonosporopsis cucurbitacearum* as a synonym of *D. bryoniae* infecting cucurbits. Though the reports on occurrence of gummy stem blight in watermelon in other countries are available, there has been no report of this disease in watermelon from India.

Watermelon (*Citrullus lanatus*) is an important commercial, cucurbitaceous vegetable cultivated throughout the year in Bengaluru, India. Recently, GSB has become a threat for the production of watermelon that has not been reported earlier in this region. Disease symptoms initially start with foliar marginal brown discolouration, spreading towards the inside leaf lamina. Later, the pathogen spreads to cover complete leaf, turning it dark brown and dry affecting photosynthesis and yield. In severe cases, the pathogen enters into the vascular bundles of plant and clogs the phloem tissue and later damaging the xylem. It causes complete wilting of plant with an estimated yield loss up to 100% (Bala and Hosein 1986). We attempted to isolate and characterise the gummy stem blight pathogen of watermelon at the Indian Institute of Horticultural Research, Bengaluru, India from diseased plants observed in our research farm. Samples were collected from the infected watermelon plant from the symptomatic region of stem. These bits of 5 mm length and 2 mm width were washed in normal water followed by surface sterilisation with 0.1% NaOCl for 2 min and rinsed twice with sterile distilled water to make it free from any surface contamination. Samples were kept on pre-sterilised blotting paper for 5 min for air-drying. The dried pieces of infected stems were placed on potato dextrose agar (PDA) and kept incubated at ambient conditions. After two days when growth was observed on plates, the growing tip of the fungal colony was transferred to another freshly prepared PDA plate for purification. A thick layer of mycelium appeared just seven days after transfer without any sporulation. Initially mycelium was bright white in colour later turning into light olive-green colour as reported by Keinath et al. (1995). The fungus has been characterised molecularly using ITS primers viz., ITS 1 and ITS 4 (White et al. 1990). Genomic DNA was extracted by CTAB method (Mishra et al. 2014) and PCR reactions were carried out in a total volume of 25 µL. Reaction components consisted of 2.5 µL of 10X PCR buffer with 25 mM $MgCl_2$ (GeNei, India), 0.5 µL10 mM dNTPs (GeNei, India), 0.5 µL of10 µM forward and reverse primers, 0.3 µL 3 U/µL.

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Taq (GeNei, India), and one µL DNA template. PCR conditions included an initial denaturation step at 94 °C for 2 min followed by 35 cycles of denaturation at 94 °C for 30 s., annealing at 52 °C for 30 s., extension at 72 °C for 30 s., followed by a final extension at 72 °C for 5 min. PCR product of 5 µL volume was loaded on to 1% (*w*/*v*) agarose gel and electrophoresed at 75 V for 1.5 h. The ethidium bromide stained agarose gel was visualised under UV light (UVITEC FIREREADER V.10, Cambridge, U.K). Remaining PCR product was purified and sequencing was performed in ABI-3710 Prison automated DNA analyser (Eurofins, India). The sequence of the isolate was compared with the NCBI – GenBank database using the blast algorithm (www.ncbi.nlm.nih.gov). Results confirmed that the fungal isolate was sharing 99% homology with the *S. cucurbitacearum* isolate (NCBI accession no.KC460840.1) available in the database submitted by Liu et al. (2013). Isolated DNA was also used to amplify with SCAR primers (RG-I-F 5'-TGTCGTTGACATCATTCCAGC-3′ and RG-I-R 5'-ACCACTCTGCTTAGTATCTGC-3′) which are specific to *S. cucurbitacearum* (Babu et al. 2015). The amplicon obtained with this primer was of 735 bp size. The sequence of the amplified product was deposited at NCBI with accession No. MN396362. The live culture has been deposited at National Agriculturally Important Microbial Culture Collection (NAIMCC), Mau, Uttar Pradesh, India, which is one of the culture collections registered by the World Federation for Culture Collections (http://www.wfcc.info/collections/). The accession number of the culture is NAIMCC-TF 2438 (Figs. 1 and 2).

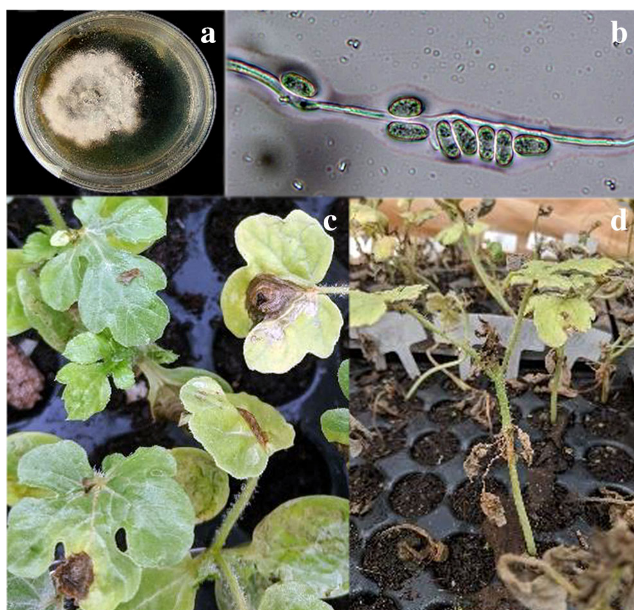

Fig. 1 Characterisation of gummy stem blight pathogen *Stagnosporsis cucurbitacearum* infecting water melon. a. Mycelia colony of *S. cucurbitacearum*. b. Spores of *S. cucurbitacearum*. c. Gummy Stem Blight symptoms on leaf. d. Seedling infection after 14th day of inoculation

The same fungal isolate was used to re-inoculate watermelon seedlings to confirm pathogenicity. The mycelium mat of a seven-day-old culture was used for this purpose. Four mm round discs using a cork borer were placed on leaf lamina of 17-day-old seedlings at 2 true leaf stage. The inoculated seedlings were kept under constant moist condition (>80% relative humidity) to create conducive environment for disease development. Two days after inoculation, dark brown, water soaked and necrotic lesions spreading around the place of inoculation could be observed. No changes were detected in the control seedlings. The same fungus could be successfully re-isolated from the infected seedling, fulfilling Koch's postulates.

Gummy stem blight has been reported in watermelon worldwide in several places viz., southern United States (Sherbakoff 1917; Schenck 1962; Power 1992), South Carolina (Keinath et al. 1995), Antalya and Turkey (Basım et al. 2016). *S. cucurbitacearum* has been reported in several cucurbitaceous vegetables viz., cucumber and watermelon in France (Chester 1891), Italy (Chiu and Walker 1949) and United States (Farr and Rossman 2010); melon in Brazil (Dalcin et al. 2017) and *Cucurbita maxima* in northeast China (Zhao et al. 2018). With the emergence of free trade order over the last two decades, there is an increased movement and exchange of seed material. This has probably led to the introduction of this disease in Bengaluru, India where the vegetable seed industry is active. Though occurrence of gummy stem blight by *S. cucurbitacearum* on other cucurbitaceous crops has already been reported, this is the first report of gummy stem blight by *S. cucurbitacearum* on watermelon in India.

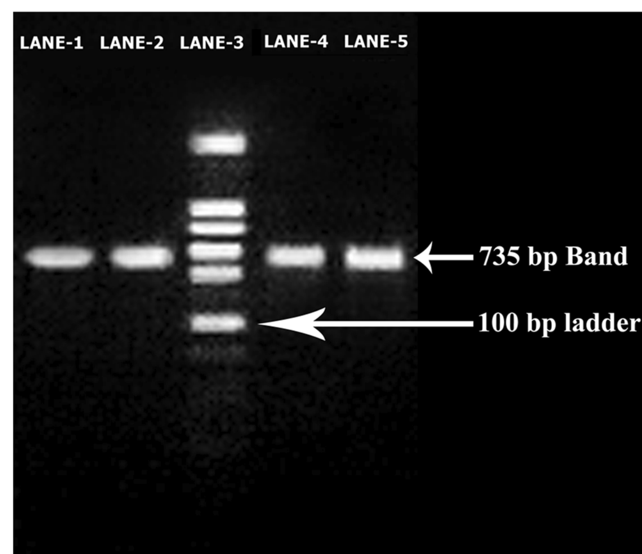

Fig. 2 Agarose gel of PCR products of *D. bryoniae* using two primers. Lane 1 and 2 = 8 amplified RG primer; 3 = 100 bp DNA ladder; 4 and 5 = Repeated RG primer

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