Australasian Plant Dis. Notes (2013) 8:115–116
DOI 10.1007/s13314-013-0110-1

Pseudocercospora cruenta on *Vigna unguiculata* in Mexico

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Received: 22 March 2013 / Accepted: 10 July 2013 / Published online: 23 August 2013

Abstract Cowpea is one of the most important grain legume crops worldwide. Although *Pseudocercospora cruenta* on cowpea was first observed in Mexico in 1976, this is the first documented report of this pathogen in Mexico. The fungus was isolated from symptomatic cowpea leaves collected from Nayarit state, Mexico and identified on the basis of morphology and sequence analysis of the internal transcribed spacer (ITS). Pathogenicity was verified on cowpea plants.

Keywords Cowpea · Foliar disease · Morphology · Mexico

Cowpea (*Vigna unguiculata* (L.) Walp.) is grown worldwide and is one of the most important legume crops in Central and South America. The majority of cowpea production in Mexico is exported to Asia and North America (Diaz and Leal 1992). During 2011, a fungal disease was observed on leaves of cowpea in Nayarit state, Mexico. The disease was affecting lower leaves of all surveyed plants with an average severity of 5 % (Albert et al. 2008). Small (1–10 mm) circular to irregular spots that were greenish-gray in color were observed on the adaxial part of the leaves (Fig. 1a).

To isolate the fungus, fungal sporulation was induced by placing symptomatic leaf tissue in a moisture chamber for 24 h at room temperature. Five single-spore cultures were obtained by transferring conidia onto unclarified V8 medium (200 ml V8; 1.5 g $CaC0_3$; 800 ml distilled water) and incubating the plates at 24 °C under continuous near UV-light for 2 weeks.

Pathogenicity tests were performed with five single-spore isolates by spraying 30-day-old cowpea seedlings with 1.2×10^4 spores/ml until run-off (Sinsiri and Laohasiriwong 2008). Fifteen pots containing three seedlings each were inoculated with the five isolates (three pots per isolate). Seedlings were placed at high relative humidity (~95 %) for 24 h and then transferred to the greenhouse (24–26 °C). Untreated control plants were sprayed with sterile distilled water. Koch's postulates were fulfilled by re-isolation of the pathogen from the leaves of cowpea showing symptoms similar to those observed in the field after 20 days in the greenhouse.

Fungal DNA was extracted from actively growing mycelia of 10-day old single-spore isolates using a DNeasy Plant Mini Kit (Qiagen, Valencia C.A.). ITS region was amplified (White *et al.* 1990) using ITS5 and ITS4 primers and sequenced. The consensus sequence of one isolate was deposited in GeneBank (accession No. JQ717056) and compared with other sequences using the Blast search.

Naturally infected leaves of cowpea showing typical symptoms of the disease and slides that contain the structures of the fungus were deposited at the Colección Micológica del Herbario de la Escuela Nacional de Ciencias Biológicas del Instituto Politecnico Nacional, Prolongación de Carpio y Plan de Ayala s/n, Santo Tomás, Miguel Hidalgo, México.

Molecular and morphological characteristics (Chupp 1954; Mulder and Holliday 1975; Sivanesan 1990) provided unambiguous identification of five single-spore isolates as *Pseudocercospora cruenta* (Sacc.) Deighton (=*Cercospora*

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Fig. 1 *Pseudocercospora cruenta* (Sacc.) Deighton. **a** Leaf spot of cowpea, the result of natural infection. **b** Conidia and conidiophores. *Bar*=25 μm

cruenta Saccardo). ITS region of the isolates from Nayarit were 99 % similar to *Pseudocercospora cruenta* strain CPC 10846 (Accesion GU214673; Crous *et al.* 2009). The fungus produced stromata. Conidiophores in fascicles, subhyaline to brown straight to sinuous 4–6×21–40 μm, 1–3 septate, rarely branched, conic tip that show a minute spore scar. Conidia subhyaline to olivaceous brown, obclavate-cylindric, straight to mildly curved, obconic base, tip obtuse, 3–12 septa, 4–6× 49–101 μm (Fig. 1b).

This pathogen has been reported from Bangladesh, Barbados, Bolivia, Brazil, Brunei, Burma, China, Cuba, Fiji, Granada, Guatemala, Guyana, Haiti, Hong Kong, India, Indonesia, Iran, Egypt, Ghana, Jamaica, Liberia, Malaysia, Malawi, Niger, Nigeria, Pakistan, Papua New Guinea, Puerto Rico, Rwanda, San Vicente, Santa Lucia, Saudi Arabia, Sierra Leone, Sudan, Taiwan, Togo, Trinidad, Venezuela, Uganda and Zambia (Sivanesan 1990). In North America, the pathogen was recorded in The United States of America and Canada (Sivanesan 1990). We believe that the lack of scientific evidence of the presence of *P. cruenta* in Mexico (Alvarez 1976) resulted in exclusion of Mexico from global geographic distribution of the pathogen (Sivanesan 1990; Mycobank data base). This study represents the first formal report of *P. cruenta* on cowpea in Mexico.

Acknowledgments The authors would like to thank Dr Melanie L. Lewis Ivey and Mr Jhony Roberto Mera Macias, for their important inputs and guidance during the preparation of the present paper.

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