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## Tropical phytopathogens 1: *Pseudocercospora punicae*

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The present paper is the first in a series, which aims to describe important tropical plant pathogens, with illustrations and description and notes on symptoms. Leaf and fruit spot/blotch disease of *Punica granatum* is caused by *Pseudocercospora punicae* and the fungus and symptoms of the disease are illustrated and described in this paper. *Punica granatum* (pomegranate) has worldwide importance due to consumer demand for this high nutritional, therapeutic and medicinal fruit. The host, however, suffers from a cercosporoid disease that causes spotting on leaves, blotching of fruits and premature fruit drop. *Pseudocercospora punicae* leaf and fruit spots appear as subcircular to irregular brown to dark brown or black blotches which are 1–12 mm in diameter for fruit spots and 1–4 mm in diameter for leaf spots. Conidophores are 7–22 × 3–4 µm and some are distinct in having geniculate characters, whereas the conidia are 42–72 × 2–4 µm. The partial sequence (771 bp) of the large subunit (LSU) rRNA gene of this species is also provided.

**Key words** – diversity – hyphomycetes, leaf spot – resistance – systematic fungicide – taxonomy – Thailand.

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### Introduction

*Cercospora s. lat.* can be classified into several genera based on their distinct morphological characteristics (Deighton 1976, Braun 1995, Crous & Braun 2003). Mycologists have frequently studied cercosporoid fungi due to their importance in plant pathology and the number of reported species belonging to this fungal group has increased yearly (Meeboon et al. 2007, Nakashima et al. 2007, Meeboon 2009, To-anun et al. 2009, Nuandee et al. 2010, Phengsintham et al. 2010a,b, To-anun et al. 2010). *Pseudocercospora* is one of the genera in this group and a single species causing disease of pomegranate is dealt with in this

paper.

This is the first of a special paper series aiming to describe important tropical plant pathogens, with illustrations and description and notes on host symptoms. Here we introduce the infection of *Pseudocercospora punicae* on *Punica granatum* L.

### Host

*Punica granatum* is a small fruit-bearing deciduous tree growing between five to eight meters tall (Ho 1991). The fruits are known as pomegranate, the tree is native to the Iranian Plateau, and has been cultivated in Asia since ancient times. The importance of the crop is



**Fig. 1** – Leaf and fruit spots caused by *Pseudocercospora punicae* on *Punica granatum*.

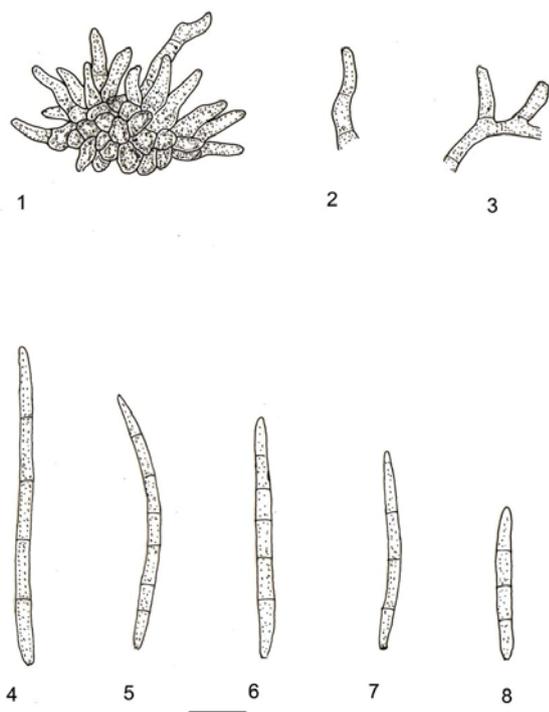
rising due to worldwide increasing consumer demand for its high nutrition and use as therapeutic foods (Palou et al. 2009). It is also used in traditional medicine (<http://en.wikipedia.org/wiki/Pomegranate>).

Pala et al. (2009) studied fungal pathogens causing postharvest decay of pomegranate and concluded that *Alternaria alternata*, *Coniella granati* and *Aspergillus niger* were the major biotic diseases. Fruit cracks, sunburn and hail damage were the most commonly detected abiotic diseases. Pomegranate's leaf and fruit spots can be caused by various organisms such as: *Colletotrichum gloeosporioides*, *Sphaceloma punicae*, *Cercospora punicae*, *Drechslera* sp. and *Phomopsis* sp., which take a heavy toll on the crop yield and quality (Jamadar & Patil 2007).

*Cercospora punicae* was first recorded in Japan by Hennings in 1906 (Chupp, 1954), while Petcharat and Kanjanamaneesathian (1989) recorded *Cercospora punicae* in southern Thailand, but without any description. Deighton (1976) redescribed this species and transferred it to the genus *Pseudocercospora*.

### Symptoms

These are rather variable (Fig. 1). The affected fruits show small irregular black spots, which later coalesce into large spots measuring 1–12 mm diam. Leaf spots/lesions are subcircular to irregular, 1–4 mm diam., at first brown, dingy gray to pale tan and eventually brown to dark brown at the margin.



**Fig. 2** – *Pseudocercospora punicae* on *Punica granatum*: **1.** Stroma with attached conidiophores. **2.** Conidiophore. **3.** External hypha with attached conidiophores. **4–8.** Conidia. Bars: = 10 µm.

### Taxonomy

*Pseudocercospora punicae* (Henn.) Deighton, Mycol. Pap. 140: 151 (1976). Figs 2–3  
 = *Cercospora punicae* Henn., Bot. Jahrb. genous Syst. 37: 165. 1906.

Description – Caespituli/colonies amphigenous, conspicuous. Mycelium internal and external; internal hyphae branched, 2–3 µm wide ( $\bar{x}$  = 2.2 µm), septate, constricted at the septa, distance between septa 6–10 µm ( $\bar{x}$  = 8.8 µm), brownish or subhyaline, wall 0.3–0.5 µm wide ( $\bar{x}$  = 0.34 µm), smooth, forming plate-like plectenchymatous stromatic hyphal aggregations; external hyphae branched, 2–4 µm wide ( $\bar{x}$  = 3 µm), septate, constricted at the septa, distance between septa 4–8 µm ( $\bar{x}$  = 4.7 µm), brownish or subhyaline, wall 0.3–0.5 µm wide ( $\bar{x}$  = 0.43 µm), smooth. Stromata oval to ellipsoidal, 10–35 µm diam. ( $\bar{x}$  = 22.5 µm), brown to dark brown, stroma cells oval, ellipsoidal to angular, 3–9 µm wide ( $\bar{x}$  = 6.3 µm), dark brown, wall 0.3–0.5 µm wide ( $\bar{x}$  = 0.45 µm), smooth. Conidiophores fasciculate or

solitary, arising from stromata (4–40 per fascicle) and external mycelium, geniculate, branched, 7–22 × 3–4 µm ( $\bar{x}$  = 13.8 × 3.28 µm), 0–1-septate, slightly constricted at the septa, distance between septa 5–17 µm long ( $\bar{x}$  = 8.33 µm), uniformly pale to medium brown, paler and narrower towards the tip, wall 0.3–0.5 µm ( $\bar{x}$  = 0.48 µm), smooth. Conidiogenous cells integrated, terminal, 7–17 × 3–4 µm ( $\bar{x}$  = 10 × 3.11 µm), apex obtuse; conidiogenous loci inconspicuous, unthickened, not darkened. Conidia solitary, obclavate, straight to slightly curved, 42–72 × 2–4 µm ( $\bar{x}$  = 54.23 × 2.46 µm), 2–6-septate, pale olivaceous–brown, wall 0.3–0.5 µm wide ( $\bar{x}$  = 0.36 µm), smooth, tip subacute, base truncate, hila 1.5–2 µm wide ( $\bar{x}$  = 1.55 µm).

Known hosts – *Punica granatum* L. (Lythraceae).

Known distribution – Africa: Egypt, Ethiopia, Kenya, Mauritius, Mozambique, Sudan, Tanzania, Uganda, Zambia; Asia: Afghanistan; Cambodia, China, Hong Kong, India, Indonesia, Iran, Japan, Laos, Malaysia, Maldives, Nepal, Pakistan, Philippines, Saudi Arabia, Singapore, Taiwan, Thailand; Europe (Caucasus): Azerbaijan, Georgia; North America: Cuba, Dominican Rep., Guatemala, Jamaica, Panama, Puerto Rico, USA (FL, HI, TX), Virgin islands; South America: Brazil, Colombia, Venezuela (Crous & Braun 2003).

Cultural characteristics – Mycelial colonies on PDA after three weeks at 25°C gray to dark gray, reaching 16–18 mm diam., hyphae 2–9 µm wide ( $\bar{x}$  = 5 µm), septate, constricted at the septa, distance between septa 5–26 µm ( $\bar{x}$  = 11.37 µm), brownish or subhyaline, wall 0.3–1 µm wide ( $\bar{x}$  = 0.61 µm), smooth. Conidiophores and conidia not formed in culture.

Material examined – Thailand, Chiang Rai Province, Muang District, Ban Du Village, on leaf of *Punica granatum* (Lythraceae), 1 July 2010, P. Phengsintham (MFLU10-0323). Culture (MFLUCC 11-0284), GenBank accession no JN107998; Laos, Xiangkhouang Province, Phonsavan Village, 26 April 2011, P. Phengsintham (NUOL P623).

Remarks – The collection from Thailand differs from *Pseudocercospora punicae* as described by Hsieh & Goh (1990) in having geniculate conidiophores.



**Fig. 3** – *Pseudocercospora punicae* on *Punica granatum* from leaf spots: **1–2**. Lesions on host leaves (**1**. upper surface and **2**. lower surface). **3**. Caespituli. **4**. Stromata with attached conidiophores. **5–8**. Conidia. **9**. Culture. Bars: 1, 2, 9 = 10 mm. 4–8 = 10  $\mu$ m.

**Table 1** Conidial size and septation of *Pseudocercospora punicae* isolates/strains.

Species	Conidia		References
	Septa	Size ( $\mu$ m)	
<i>Cercospora punicae</i> = <i>Pseudocercospora punicae</i>	2–8-septate	25–85 $\times$ 2.5–5	Chupp (1954)
<i>Pseudocercospora punicae</i>	2–8-septate	18–85 $\times$ 2.5–5	Hsieh & Goh (1990)
<i>Pseudocercospora punicae</i>	2–6-septate	42–72 $\times$ 2–4	Present study

The leaf spots and fruits caused by this fungal species lead to reduced harvest and quality. The conidial septation and size of *Pseudocercospora punicae* are shown in Table 1.

Additionally, we describe here a partial sequence of the large subunit LSU (rRNA gene of *P. punicae*. For this, the genomic DNA from fungal mycelium was isolated using the pro-

ocol based on Cai et al. (1999). The incomplete sequence of the LSU rRNA operon was then amplified using the primers LROR and LR5 and sequenced using the same primers by SinoGenoMax Company limited, China for sequencing. The sequence obtained was 771 bp and is deposited in GenBank (JN107998). This sequence information can be useful for phylogenetic study in future work.

### Management of the Disease

Mancozeb 0.25 % sprayed at 15 days interval gives good control of the disease on leaves ([http://agritech.tnau.ac.in/crop\\_protection/crop\\_prot\\_crop%20diseases\\_fruits\\_pomegranate.html](http://agritech.tnau.ac.in/crop_protection/crop_prot_crop%20diseases_fruits_pomegranate.html)).

Two to three sprayings at 15 days interval of Dithane M-45 or Captan @ 2.5 g in one litre of water after fruit formation gives good control of the disease on fruit. ([http://nhb.gov.in/bulletin\\_files/fruits/pomegranate/pom002.pdf](http://nhb.gov.in/bulletin_files/fruits/pomegranate/pom002.pdf)). Diseased fruits should be collected and destroyed.

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