

# Acrophialophora Wilt of Gram in India

R. P. PURKAYASTHA, Reader-in-Charge, Plant Pathology Laboratory, Department of Botany; and B. N. CHAKRABORTY, Junior Research Fellow, Department of Botany, University of Calcutta, Calcutta-700 019, India

## ABSTRACT

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*Acrophialophora* wilt of gram (*Cicer arietinum*) and its characteristic features have been observed in India. *A. fusispora* and *Fusarium oxysporum* f. sp. *ciceris* cause a wilt disease of the *Fusarium*-susceptible gram cultivar JG-62 but not of the resistant cultivar BG-212. The presence of both pathogens caused wilt to develop in BG-212, however.

*Fusarium* wilt of gram (*Cicer arietinum* L.) is common in India. Recently, the *Fusarium* wilt-resistant cultivar BG-212 has showed symptoms of wilt disease in some field plots in Berhampore, West Bengal. We investigated whether a new virulent strain of *Fusarium* or other pathogen was the causal agent.

## MATERIALS AND METHODS

**Plants and growing conditions.** Seeds of gram cultivars BG-212 (wilt resistant) and JG-62 (wilt susceptible) were obtained from the Indian Agricultural Research Institute, New Delhi. Soil samples and infected plants were collected from Berhampore, West Bengal.

Seeds were surface-sterilized with 0.1%  $\text{HgCl}_2$ , washed with sterile distilled water, and sown in 10-cm-diameter earthenware pots (10 seeds per pot) containing sterilized soil. The plants were grown at 26–34 C in the department's Experimental Garden in Calcutta.

**Koch's postulates.** For isolation of fungi, pieces of infected gram roots were washed with water, surface-disinfested with 0.1%  $\text{HgCl}_2$ , rinsed with sterile distilled water, and plated on potato-dextrose agar (PDA) and Reischer agar (0.41 g sucrose, 0.12 g L-asparagine, 30 mg  $\text{KH}_2\text{PO}_4$ , 20 mg  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.56 mg  $\text{CaCl}_2$ , 2.88 mg  $\text{MnCl}_2$ , 1.67 mg  $\text{ZnCl}_2$ , 0.10 mg  $\text{FeCl}_2$ , 11.6 mg EDTA, 0.04 mg thiamine hydrochloride, 50 mg endomycin, 50 mg streptomycin sulfate,

20 g Difco agar, and 1,000 ml distilled water).

Standard dilution technique was used to isolate the pathogen from soil. Plates were incubated at 28–30 C in diffused light. *Acrophialophora fusispora* (Saksena) Samson and *F. oxysporum* f. sp. *ciceris* (Padw.) Matuo & Sato were most consistently isolated from both soil and infected plants.

Fifteen-day-old plants were inoculated with a conidial suspension of *A. fusispora* and/or *F. oxysporum* f. sp. *ciceris* ( $1 \times 10^6$  conidia/ml) prepared from 15-day-old cultures grown on PDA. Usually 1 ml of conidial suspension was applied to the roots of each plant, but in mixed inoculations, 0.5 ml of conidial suspension of each pathogen was used.

Disease intensity (wilting index) was evaluated after 14 and 28 days by using a 0–6 scale (Table 1). The total values divided by the number of plants gave the wilting index of a plant.

Reisolation from root tissue consistently yielded *A. fusispora* from plants inoculated with *A. fusispora*.

## RESULTS AND DISCUSSION

Wilting developed within 2 wk after inoculation and increased rapidly up to the third week. Drooping and yellowing affected lower leaves initially and upper leaves later. When the disease was severe, complete defoliation of leaves sometimes occurred.

Pathogenicity tests of *A. fusispora* and *F. oxysporum* f. sp. *ciceris* on gram cultivars were repeated three times (Table 1). In all cases, either pathogen could cause wilt disease of the cultivar susceptible to *F. oxysporum* f. sp. *ciceris* but not to the resistant cultivar. When both organisms were inoculated together,

**Table 1.** Pathogenicity of *Acrophialophora fusispora* and *Fusarium oxysporum* f. sp. *ciceris* on gram cultivars

Pathogen	Wilting index <sup>a</sup> /plant <sup>b</sup>			
	JG-62		BG-212	
	14	28	14	28
<i>A. fusispora</i>	1.5	4.5	0	0
<i>F. oxysporum</i> f. sp. <i>ciceris</i>	3.0	6.0	0	0
<i>A. fusispora</i> and <i>F. oxysporum</i> f. sp. <i>ciceris</i>	4.0	6.0	3.0	5.0
Control (uninoculated)	0	0	0	0

<sup>a</sup> Wilting index: 0 = no wilting, 1 = 1 or 2 wilted leaves, 2 = 3 or 4 leaves, 3 = 5 or 6 leaves, 4 = 7 or 8 leaves, 5 = 9 or 10 leaves, and 6 = all leaves wilted.

<sup>b</sup> 50 plants per treatment.

they caused disease in the resistant cultivar, probably because of a synergistic reaction of the pathogens. Kerr (1), working with pea wilt, and Pieczarka and Abawi (2) studying root rot of snap bean, made similar observations and noted synergistic reactions of the pathogens.

In our study of *Acrophialophora* wilt of gram, the characteristic features of the pathogen are as follows: Mycelia were usually hyaline and subhyaline, white when young but grayish brown to dark brown with age. Hyphae were branched and septate, conidiophores tall, slender, unbranched, hyaline to subhyaline, and  $24\text{--}35 \times 1\text{--}2 \mu$ , with flask-shaped phialides usually produced directly on mycelia. Conidia were one-celled, hyaline or subhyaline, ovoid to broadly fusoid, and cutenulate,  $6\text{--}9 \times 3\text{--}5 \mu$ .

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