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First report of *Bipolaris cynodontis* on *Oryza sativa* in Morocco

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Summary. *Bipolaris cynodontis* was isolated for the first time from *Oryza sativa* leaves in northwestern Morocco. The pathogenicity of three isolates of *B. cynodontis* was compared by inoculating the isolates on the leaves of three rice varieties. All the isolates developed similar foliar lesions that differed from those caused by other species of *Bipolaris* pathogenic on rice.

Key words: pathogenicity, rice, brown spot.

Introduction

Rice is subject to many parasitic diseases. Among them, blast and brown spot, caused by *Pyricularia oryzae* and *Drechslera oryzae* respectively, are well known and have a wide distribution, with a significant economic impact. Other pathogenic fungi have also become widespread in Morocco, such as *D. sativa*, *D. specifera*, *D. australiensis* and *Curvularia lunata* (Hassikou *et al.*, 2001; Ouazzani Touhami *et al.*, 2000). Brown spot caused by *D. oryzae* is regarded as the second most important disease of rice (Vindhyasekaran *et al.*, 1986). When brown spot is severe, harvest losses can reach up to 75% (Kohls *et al.*, 1987). This disease was firstly reported in Morocco in 1996 (Bouslim, 1996). *Bipolaris cynodontis* also causing brown spot was first isolated in pure culture and identified during the monitoring of the mycoflora of rice in the fields of the Gharb region in northwestern Morocco. This work reports on brown spot and the pathogenicity of three isolates of *B. cynodontis* on three varieties of rice commonly grown in Morocco.

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Materials and methods

Pathogen isolation

Brown spot infected leaves of rice were washed in tap water, disinfected with alcohol, placed in Petri dishes on filter paper moistened with sterile distilled water and incubated at 22°C under continuous lighting for 3–5 days. The conidia emerging from the lesions were suspended in sterile water and transferred to an agar medium by a capillary tube. The colonies developing from a single spore were retained, as representing a pure culture, and maintained on rice flour medium (rice flour 14 g, yeast extract 4 g, agar-agar 15 g, distilled water 1000 ml) in the dark for 10 days at 28°C for species determination, according to the method of Ellis (1971).

Host plants

The seeds of three rice varieties (Arco, Elio and Thaibonet) were surface-sterilised by immersing in a 0.6% sodium hypochlorite for 10 min. and then rinsed vigorously with distilled water. After drying for 24 h on filter paper, the seeds were pre-germinated in the dark at 28° C for 75 hours in Petri dishes containing sterile water. Seedlings were potted in soil (3 per pot), watered and grown until the inoculation stage (4 to 6 leaves).

Inoculation tests

Strains BC1, BC2 and BC3 of *B. cynodontis*, were grown on rice flour medium at 28°C in the dark. After 10 days of incubation, sterile water was added to each Petri dish, the culture surfaces were scraped off, and the resulting spore suspensions were filtered on muslin tissue to eliminate mycelium fragments, and diluted to 10^5 spores ml^{-1} in sterile distilled water containing 0.05% Tween 20 and 0.5% gelatine.

The spore suspensions were sprayed on the plant leaves at a rate of 60 ml per pot. The controls were sprayed with a water suspension containing 0.05% Tween 20 and 0.5% gelatine. The plants were incubated for 48 h at 100% relative humidity and were then transferred to a greenhouse.

The symptoms were evaluated after 7 days in terms of both disease severity (extent of diseased leaf area) using the scale of Nottegham (1980) and disease incidence (number of leaves with symptoms). The coefficient of infection (CI) was calculated by multiplying the incidence by the disease severity (Leogering, 1959).

The sporulating ability of the 3 isolates on the rice leaves was estimated according to the technique of Hill and Nelson (1983) as the number of spores per cm^2 of infected tissue.

Results were tested for statistical significance using variance analysis and the LSD test.

Results

Pathogen description

Conidiophores arose singly or in small groups and were pale to mid-brown in colour. Conidia were 29–70×8–15 μm, mostly slightly curved, sometimes almost cylindrical, but usually broadest in the middle, tapering towards the rounded ends, pale to mid golden brown and smooth, and had walls with 3–9 (usually 5–8) pseudosepta (Fig. 1).

Inoculation tests

All *B. cynodontis* strains were pathogenic on the rice varieties, causing similar symptoms (Fig. 2): lesions that initially had brown centres and faded margins, then became generally elliptic (from 2 to 4 mm) with faded centres and dark-brown margins.

Brown spot symptoms similar to those in the field appeared after seven days, and only on ino-

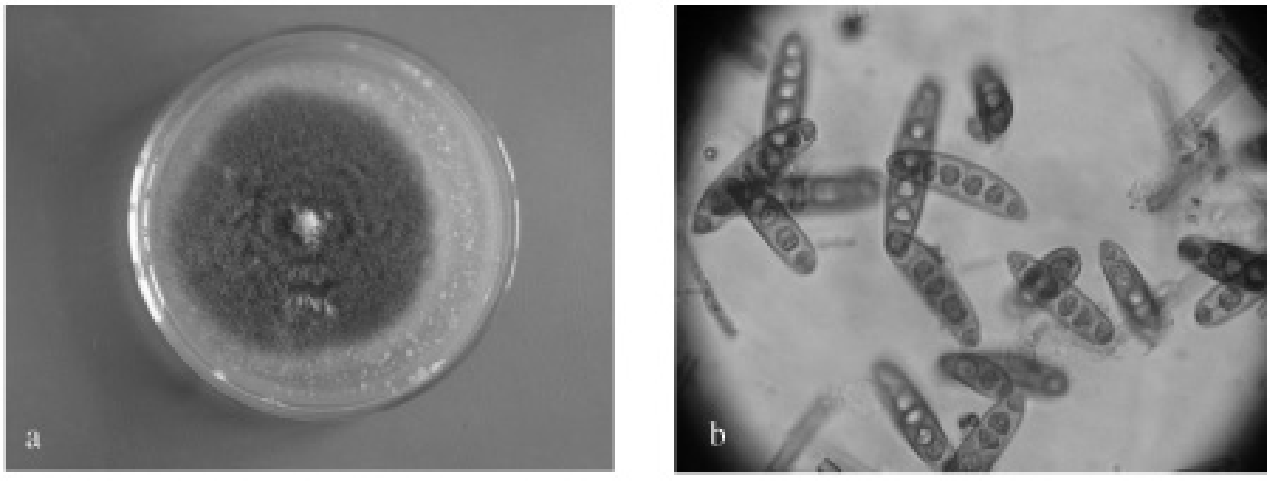

Fig. 1. *Bipolaris cynodontis*, (a) macroscopic aspect on rice flour medium; (b) conidia.

Fig 2. Brown spot symptoms on leaves of *Oryza sativa* inoculated with a conidial suspension of *Bipolaris cynodontis*

culated plants. *B. cynodontis* was the only fungus reisolated from the diseased tissues.

BC2 was the most virulent isolate, causing the highest brown spot incidence and severity on all the rice varieties with a CI ranging from 237.78 on 'Arco' to 189.65 on 'Thaibonet'. Isolate BC1 was the least virulent, with a CI not exceeding 82.75 on 'Elio', whereas isolate BC3 showed intermediate virulence (Table 1).

The three strains sporulated on all the rice varieties, producing between 3.13×10^5 and 19.33×10^5 conidia per cm^2. The highest number of conidia was produced by isolate BC2 (Fig. 3) reaching 19×10^5 conidia per cm^2 on 'Elio'.

Table 1. Virulence of three strains of *Bipolaris cynodontis* on three rice varieties, estimated as the coefficient of infection (CI) seven days after inoculation.

Rice variety	*B. cynodontis* strain [a]								
	BC_1			BC_2			BC_3		
	Incidence	Severity	CI	Incidence	Severity	CI	Incidence	Severity	CI
Arco	9	1.62 0.62 1.87 3.66	12.33[f]	37	6.6 6.6 6.08 5.62	237.78[a]	24	5.15 4.55 5.66 5.81	122.88[d]
Elio	21	3.4 4.78 2.62	82.75[e]	37	6.3 5.5 5.69	214.84[ab]	27	5.33 5.44 4.91	149.22[c]
Thaibonet	27	2.23 1.61	58.14[e]	32	6.16 5.93	189.65[b]	27	6.67 4.44	144.18[cd]

[a] Data accompanied by the same letter do not differ according to the LSD test (P=0.05).

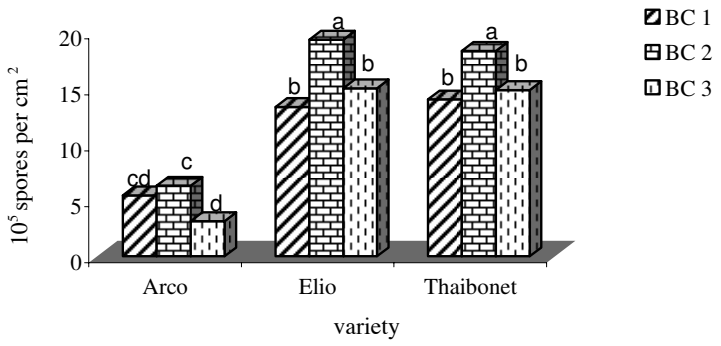

Fig. 3. Sporulation of the strains of *Bipolaris cynodontis* assessed as number of spores per cm^2 of diseased leaves seven days after inoculation on three rice varieties. Results accompanied by the same letter do not differ according to the LSD test (P=0.05).

Discussion and conclusion

Bipolaris cynodontis is the causal agent of bermudagrass (*Cynodon dactylon*) blight, characterised by round or oblong spots, usually brown or crimson, with their longitudinal axis parallel to the blade of the leaf (Pratt, 2000). *B. cynodontis* has been reported on grasses, on *Triticum durum*, and it has also been isolated from apple leaves, pine and ipomoea (Ellis, 1971; Carranza, 1979; Puttoo and Chaudhry, 1982). More recently, it has been isolated from rice seeds (Barrios and Perez, 2005) and *Brachiaria brizantha* (Macedo and Barreto, 2007), showing leaf blight symptoms.

In the last number of years, on rice grown in the Gharb region of Morocco, a blight was seen whose symptoms differed from those caused by *Drechslera oryzae*. This particular pathogen causes oval chestnut spots with a grey or white contour (Bouslim, 1996) and is regarded as potentially the most harmful pathogenic agent of rice crops (Vindhyasekaran *et al*., 1986).

The disease we observed for the first time in Morocco, on the other hand, was constantly associated with *B. cynodontis,* which reproduced the disease symptoms on those rice varieties that were inoculated with it. All tested *B. cynodontis* strains were pathogenic on rice, but they differed in their virulence, and this difference also depended in part on the rice variety. Among the rice varieties, 'Arco' was the most tolerant. All *B. cynodontis* strains sporulated on rice plants inoculated with them, producing a number of conidia per cm^2 of infected leaf tissue depending on the host susceptibility according to the postulates of Rotem (1978). *B. cynodontis* must be considered a foliar parasite of *Oryza sativa*, that may in future pose a threat to rice crops.

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