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HELMINTHOSPORIUM ROSTRATA, FOLIAR PARASITE OF RICE IN MOROCCO

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Abstract

The plants of rice varieties (Arco, Thaibonnet and Elio) were inoculated with three isolates of *Helminthosporium rostrata* (HR1 HR2 and HR3), isolated for the first time in Morocco from the rice seed of Taibonnet variety at the end of the growing season.

The results obtained showed that all the isolates are able to induce the disease on rice plants and sporulate on the foliar lesions. HR1, HR2 and HR3 was respectively the most pathogenic on Elio (I.C = 113), Arco (I.C = 212.5), and Taibonnet (130.48).

The symptoms induced by the isolates are similar to those induced by *Helminthosporium sativum* on rice.

Key words *:* Rice, *Helminthosporium rostrata*, symptoms, pathogenicity.

Introduction

The helminthosporium disease is caused by fungi of the genus *Helminthosporium*. These fungi spend unfavorable season, as mycelium on infected plants and plant residues (Zambettakis, 1967; Lucas *et al.*, 1985). The inoculum is also carried by the seeds (Wells and Winstead 1965, Wilson *et al*., 1993). The host range of these fungi is not limited only to the plants, particularly cereals (Paul, 1926; Serghat *et al.,* 2005), but also extends to animals and humans (Pritchard *et al*., 1977).

The helminthosporium disease of rice is widespread in Moroccan rice fields. It is caused by a large number of species: *Helminthosporium oryzae* (Bouslim *et al*., 1997), *H. spiciferum* (Ennaffah *et al*., 1997), *H. australiensis*, *H. sativum* (Ouzzani Touhami *et al*., 2000), *H. cynodontis* (Zehhar *et al*., 2008) et *H. bicolor* (Kadri *et al*., 2013). Sometimes, rice foliar lesions can accommodate one or two *Helminthosporium* species, in addition to *Pyricularia grisea,* all these *Helminthosporium* may be encountered on the lesions of a single foot (Ouazzani Touhami *et al.,* 2000). This involves the estimation of losses due to these pathogens and the share due to each of them (Hannin, 2003). The main thing is that each species produces sporulating lesions on rice leaves (Bahous *et al.,* 2003).

In recent years, it was noted the presence of *Helminthosporium rostrata* on the rice grains harvested at the end of the vegetative cycle. This fungus has never been reported among rice mycoflora.

In this study, the symptoms of *Helminthosporium rostrata* are described and its pathogenicity was studied on three rice varieties.

Material and Methods

Plant material

The grains of three varieties of rice plants (Arco, Thaibonnet and Elio) are disinfected by soaking in the hypochlorite of sodium in 0, 6 % during ten minutes, then rinsed strictly in the sterile distilled water. After 24h of drying, grains are put in Petri dishes containing some sterile cotton soaked with distilled water. After 75h of incubation in the darkness and in 28°C, the obtained seedlings are planted in jars containing the soil of the forest Mamora. Then, they are watered with the tap water until the stage required for the inoculation (plant

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with 3 or 4 leaves).

Fungal material

The isolates of *Helminthosporium rostrata* (HR1, HR2 and HR3) studied are obtained from the rice grains of Taibonnet variety at the end of the growing season. These three isolates were previously transplanted from single conidia.

Inoculum preparation

H. rostrata isolates were pricked out on a rice flour medium (14 g of rice flour, 15 g agar-agar, 4g of yeast extract and 1000 ml of distilled water). The cultures were incubated for ten days at a temperature of 28°C in the dark. The surface charged with spores is scraped aseptically using a metal spatula. Conidial suspension obtained was then adjusted with sterile distilled water containing 0.05% of Tween 20 and 0.5% of gelatin, in order to have a final concentration of 10^6 spores/ml.

Inoculation and Result's evaluation

The plants were inoculated at the stage of 4 to 5 leaves by spraying the conidial suspension above the rice leaves using a charging pulverized. The inoculated plants are placed for 48 hours under a black plastic bag to maintain a high relative humidity. Control plants are sprayed with sterile distilled water containing 0.05% of Tween 20 and 0.5% of gelatin. The inoculated and control plants are then placed in the greenhouse for development of symptoms. Seven days after inoculation, the disease severity (S) is estimated using the scale of Notteghem *et al*., (1980), the disease incidence (I) represents the number of infected leaves. The infection coefficient (I.C) is calculated by multiplying I x S.

The disease severity index is determined by the percentage of the diseased leaf area estimated by the rating scale Notteghem *et al*., (1980).

Note	0	1	2	3	4	5	6	7	8	9
diseased leaf area	0	0.05	0.5	1.5	3.5	7.5	17.5	37.5	62.5	87.5

The statistical treatment of data focused on the variance analysis and p.p.d.s test at the threshold of 5%.

Sporulation on the host

Sporulation on host is estimated by the method of Hill and Nelson (1983) by counting the average number of spores produced per unit area of the host leaves carrying lesions (number of spores / ml).

Ten days after inoculation, leaves showing lesions are taken from inoculated plants of rice, cut into pieces of 1 cm and then placed in Petri plates containing two discs of filter paper soaked with sterile distilled water (1 leaf per plate). The plates are placed at 30 cm in continues light to 28ºC.

After 48 h, the fragments of each leaf were placed in a test tube containing 1ml of sterile distilled water and stirred by vortexing for 2 minutes in order to detach the conidia from the mycelium.

The spore's number in the suspension was determined using a Malassez blade (10 counts per sample). The observation is made at magnification factor × 100.

Results

The leaves of rice three varieties, inoculated with *H. rostrata,* have tapered brown spots, and in the center, a dark brown aureole corresponding to the fungus penetration area on the leaves. Lesion size is variable and may be up to 5 mm length on certain varieties (Arco) (Fig. 1).

The results of table 1 indicate that *H. rostrata* isolates showed pathogenicity, as estimated by the infection coefficient, varying depending on the tested rice varieties; HR1, HR2 and HR3 was respectively the most pathogenic on Elio (I.C = 113), Arco (I.C = 212.5), and Taibonnet (130,48).

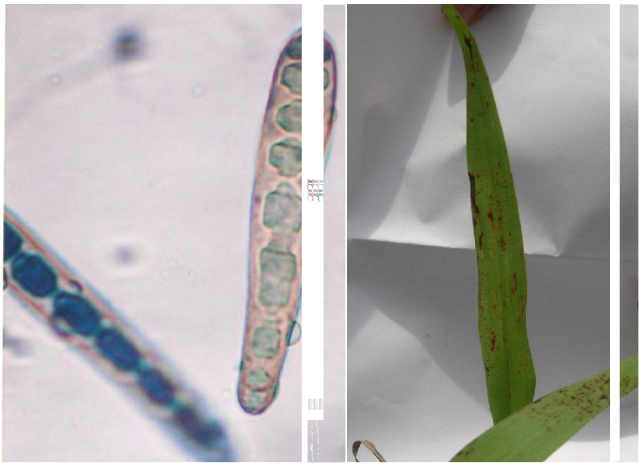

Fig. 1: *Helminthosporium rostrata* : Conidia (A) and symptoms on rice leaves (B).

The results given in table 2 show that all fungal species tested are able to sporulate on the leaves of rice varieties. Sporulation of HR2 isolate is very important on the three varieties. It is in the order of 17.33 10^5 conidia/cm^2 on Arco, 15.33 10^5/cm^2 on Taibonnet and 13.33 10^5 conidia/cm^2 on Elio. The isolate HR1 sporulates well on Arco variety (1710^5 conidia/cm^2), however sporulation is about 11.66 10^5 conidia /cm^2 on Taibonnet and decreases in the variety Elio (9 10^5 conidia/cm^2).

The isolate HR3 sporulates less on Arco varieties (2.33 10^5 conidia/cm^2) and Elio (4.3310^5 conidia/cm^2).

Table 1: Disease Incidence, severity and infection coefficient of rice varieties inoculated with *H. rostrata* isolates.

Isolates		Varieties								
		Arco			Taibonnet			Elio		
		I	S	I.C	I	S	I.C	I	S	I.C
Helminthosporium rostrata	HR1	28	6.8	191.33^{b}	37	5.74	117.67^{b}	16	5.16	113^{a}
	HR2	19	6.1	212.5^{a}	25	4.8	120^{b}	23	5.67	84.66^{b}
	HR3	17	6.68	82.61^{c}	20	4.23	130.48^{a}	15	5.37	80.6^{b}

Two results on the same column differ significantly at 5% level (p.p.d.s test) if they are not assigned by any letter in common, insignificant in the contrary case.

Table 2: Sporulation of *Helminthosporium rostrata* isolates on rice varieties (10^5 conidia/cm^2).

Varieties	Isolates sporulation (10^5 conidia/cm^2)		
	HR1	HR2	HR3
Arco	17.00^{a}	17.33^{a}	2.33^{ab}
Taibonnet	11.66^{b}	15.33^{a}	10.00^{b}
Elio	9,00^{b}	13.33^{a}	4.33^{c}

Two results on the same column differ significantly at 5% level (p.p.d.s test) if they are not assigned by any letter in common, insignificant in the contrary case.

Discussion & Conclusion

The results obtained showed that *H. rostrata*, represented by the three isolates tested, is capable of altering the rice plants foliage. This pathogen encountered for the first time on the rice grains of Taibonnet variety, can induce the disease on the leaves of other varieties of rice (Arco and Elio).

Symptoms developed on the rice plants leaves varies from variety to another. They are similar morphologically to those induced by *Helminthosporium sativum* on Rice (Ouazzani *et al*., 2000), but different from those caused by *H. oryzae* (Bouslim *et al*., 1997), *H. spiciferum* (Ennaffah *et al*., 1997), *H. australiensis* (Ouazzani *et al*., 2000) and *H. bicolor* (Leopold, 2005).

All developed leaf lesions are sporulating, that is to say, that *H. rostrata* is capable, once inoculated to the rice plants leaves to produce secondary inoculum that can infect other healthy rice leaves and therefore to participate in the progression of the disease.

All these observations allow to incriminate *H. rostrata*, isolated from seeds, as a new rice leaf pathogen.

The introduction of some sensitive varieties, case of Arco variety, will favor the multiplication of this pathogen which probably finds in the Moroccan rice all favorable conditions for its development.

Furthermore, on other host plants, *H. rostrata* attack all aerial parts of corn plant, sorghum, and some plants such as pearled millet, which is an annual herb of the hot season (Mathur *et al*., 1973).

But according to some authors (Kadir and Ahmed, 2004), *H. rostrata* is unable to attack the bean. These authors suggested introducing in rotation this plant species to reduce the inoculum of this pathogen.

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