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ORIGINAL RESEARCH PAPER in PLANT PATHOLOGY

First Report of *Bipolaris oryzae* on *Typha latifolia* and the Pathogenicity of Its Isolates on Different Rice Varieties

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Abstract

Rice (*Oryza sativa* L.) is the staple food of more than half of the world population. However, its production is facing several biotic constraints. Among serious biotic factors that harm rice crops, the Helminthosporium disease has severe adverse impacts on rice yield, generating heavy losses of up to 90%. Four *Bipolaris oryzae* isolates were recovered for the first time from leaf lesions in the weed species *Typha latifolia*, and then subjected to pathogenicity tests on several rice varieties. The results indicated that Moroccan isolates of *B. oryzae* altered the leaf surface of five rice varieties tested. Among four isolates, Hor4 was the most pathogenic, showing high aggressiveness on the Cererrer and Elio varieties, with disease severity of 92.59%, followed by the Hor1, Hor2, and Hor3 isolates. The Arpa variety showed higher resistance to the Hor1 isolate, with a severity index of 35.18%. Through mycelial cutting or conidial suspension, *B. oryzae* isolated from *T. latifolia* was able to produce conidia on the leaves of this weed species.

Keywords

Morocco; weeds; phytopathology; diseases

1. Introduction

Rice (*Oryza sativa* L.) is a major cereal crop that serves as a staple food for 60% of the world population (Nahar et al., 2016). Worldwide rice production in 2020 was as high as 510.6 million tons, which is an increase of 1.8% compared to that in 2019 and an all-time high. This is mainly due to increased yields in China (mainland), the Philippines, Guinea, the Democratic Republic of Congo, and the Bolivarian Republic of Venezuela (FAO, 2021).

In Morocco, the total area of rice crop reached 7,973 hectares with a production of 64,598 tons (FAOSTAT, 2020). Nevertheless, rice domestic consumption is considered to be one of the lowest in the world (1.2 kg of rice per capita), which represents a major constraint to rice production in the country (Food and Agriculture Organization of the United Nations, 2003).

Rice crop is facing several threats, such as diseases caused by fungi, bacteria, and viruses, which affect grain production and quality (Ou, 1985). Helminthosporium disease, caused by *Bipolaris oryzae* (Breda de Haan) Shoemacker, has been reported in all rice-producing countries (Ou, 1985). The disease is responsible for heavy losses in grain yield (up to 90%) (Sunder et al., 2014). In Morocco, it was first reported in 1996 by Bouslim (1996).

Many studies have reported the presence of *B. oryzae* in rice. It was detected on rice leaves, stems, and grains (Bahous et al., 2003; Benkirane, 1995; Benkirane et al., 1998, 2000; Gnancadji-Andre et al., 2005; Serghat, Mradmi, et al., 2005; Tajani et al., 2001). The disease symptoms appear on the coleoptiles, leaf sheath, and blade as brown spots with gray or whitish centers, cylindrical or oval in shape (Chauhan et al., 2017).

The optimum temperature for *B. oryzae* growth and its conidial germination ranges from 27 to 30 °C and 25 to 30 °C, respectively (Bouslim et al., 1997; Lucas et al., 1992; Ou, 1972, 1985). Infection requires a relative humidity of above 89% at 25 °C (Angladette, 1966; Ou, 1985). The conidia of *B. oryzae* can remain viable for 4 years on infected kernels as well as on healthy-looking kernels (Ou, 1985). This parasite is also conserved in soil (Lucas et al., 1992) and on crop remains (Nyvall et al., 1995).

According to Lage (1997), the presence of *Pyricularia* infection, Helminthosporium disease, and weeds (*Echinochloa crus-galli*, *Panicums* spp., *Typha* spp., and *Cyperus* spp.) could slow down rice production (Boulet & Bouhache, 1990). The Food and Agriculture Organization of the United Nations (FAO) stated in 2003 that the most common weed species affecting rice in the Mediterranean region belong to Poaceae and Cyperaceae. In the Gharb region, the most common weeds are *Panicum* (*P. repens*, *Ligustrum obtusifolium* Del.), *Typha* (*T. latifolia* L., *T. marsii* Bat.), *Scirpus* spp., *Cyperus* spp., and *Echinochloa* spp. (Miège, 1951). These species are well adapted to the different agroecosystems where rice is cultivated and can promote the conservation and multiplication of pathogenic species (Pugh & Mulder, 1971; Singh et al., 2008).

Benkirane et al. (2000) observed that Moroccan isolates of *Pyricularia oryzae*, originating from *Stenotaphrum secundatum*, are pathogenic on rice. Likewise, Serghat, Mradmi, et al. (2005) found that the fungal pathogen *Pyricularia oryzae*, isolated from *Echinochloa phyllopogon* and *Phragmites australis*, induced leaf lesions and sporulate on the foliage of certain rice varieties.

The leaves of *Typha latifolia*, a perennial plant species found around rice fields, often show similar leaf lesions to those observed in rice plants infected with *B. oryzae*. In this study, *B. oryzae* isolates obtained from leaf lesions in *T. latifolia* were subjected to pathogenicity tests on the leaves of five rice varieties. Indeed, the objective of this study was to highlight the role of an infectious reservoir, such as *T. latifolia*, in harboring and spreading leaf pathogens, particularly *B. oryzae*, to neighboring rice fields, which will cause significant damage to the rice fields.

2. Material and Methods

2.1. Sampling

The study was carried out during 2014–2016 in the Moroccan North-West (Gharb), including the Souk Tlet region (latitude 34°36′6.153″ N, longitude 6°10′27.687″ W) and the Merjas of Kenitra (latitude 34°30′54.838″ N, longitude 5°52′59.243″ W). *Typha latifolia* samples were randomly taken from different plots using the diagonal sampling technique (five samples per plot). White plastic bags (70 cm × 50 cm) containing the samples were transferred to the laboratory for analysis.

2.2. Mycological Analysis

Infected *T. latifolia* plants showing leaf symptoms were transferred to the laboratory for microscopic examination, isolation, purification, and pathogenicity test. The blotter method was used for the isolation of *B. oryzae*. Infected leaf tissues of *T. latifolia* were collected, cut into small pieces, sterilized by immersion in 0.5% sodium hypochlorite for 1–5 min, and then washed three times with sterile distilled water. The leaf fragments (1 cm in diameter) were placed in sterile Petri dishes containing two discs of filter paper moistened with sterile distilled water. The dishes were then incubated at 22 °C in alternating 12-hr light and darkness. After 48 hr, the leaf fragments were examined under an optical microscope at magnification ×100 for observation of the presence of fungal spores. The fungal species was determined using identification keys (Ou, 1985; Tarr, 1962).

Bipolaris spores were then single-spored with a capillary tube, placed on agar medium (agar-agar: 15 g, distilled water: 1,000 mL), and subsequently transferred using a sterilized needle to the surface of a rice flour-based medium (rice flour: 14 g, agar-agar: 15 g, yeast extract: 4 g, distilled water: 1,000 mL). Four isolates of *B. oryzae* were cultured.

2.3. Pathogenicity Test

The seeds of five rice varieties, namely, Elio, Taibonet, Arpa, Eurano, and Cererrer, were disinfected by immersion in 5% hypochlorite sodium solution for 2 min, followed by three rinses with sterile distilled water. They were then dried on a sterile filter paper and pre-germinated on Petri dishes 90 mm in diameter containing sterile cotton soaked with sterile distilled water. After 72 hr of incubation in the dark at 28 °C, the obtained plantlets were transplanted into pots filled with Mamora soil and then placed in a greenhouse. The seedlings were watered with tap water until they reached the stage required for inoculation, that is, when they had grown four to five true leaves.

2.4. Inoculum Preparation

Inoculum was prepared by independently growing each of the four isolates of *B. oryzae* (Hor1, Hor2, Hor3, Hor4) on a rice flour-based medium (rice flour: 15 g; Agar-agar: 15 g; yeast extract: 4 g and 1,000 mL of distilled water), which is favorable for the sporulation of *B. oryzae*, for 15 days (continuous photoperiod, 25 °C). After incubation, the cultures were flooded with 15 mL of distilled water, and spores were dislodged using a sterile spreader.

Afterward, the fungal suspension was filtered through a fine mesh cloth to separate the spores from the mycelial fragments. The concentration of the conidia was adjusted to 10^5 conidia/mL using Malassez slide by adding sterile distilled water supplemented with a drop of Tween 20 and 0.5% gelatin.

2.5. Inoculation of *Typha latifolia* With Spore Suspension

Healthy leaves were soaked in the conidial suspension of each *B. oryzae* isolate. Control leaves were soaked in distilled water containing a drop of Tween 20 and gelatin. The leaves were placed in 9-cm-diameter Petri dishes containing glass beads moistened with sterile distilled water and then incubated under continuous white light at room temperature for 7 days.

2.6. Inoculation of *Typha latifolia* With Mycelial Discs

Healthy leaves were placed in 9-cm-diameter Petri dishes containing glass beads in the presence of sterile distilled water. They were then inoculated with the mycelial plug (5 mm in diameter) of each isolate: one mycelial plug was placed on the central part of leaf segment, and another mycelial plug was deposited near the leaf apex. Noninoculated leaves (treated with water agar discs only) served as a control. In both detached leaf assays, controls were treated with sterile distilled water containing 0.01% Tween 20. Inoculated and control leaves were kept at ambient temperature under a natural light/dark cycle in the laboratory.

2.7. Inoculation of Rice Varieties

Rice seedlings at the stage of five to six leaves were inoculated by foliar spraying of 60 mL of spore suspension at 10^5 conidia/mL concentration using a hand compressed spray. Control plants were sprayed with sterile distilled water containing Tween 20 and gelatin. After spraying, all plants were covered with a black plastic bag and placed in a greenhouse for the development of symptoms. The plastic bag was used for the first 48 hr to ensure 100% relative humidity during conidium germination and fungal penetration. The replication consisted of three pots with three plants per pot for each rice variety. The experiment was repeated three times.

2.8. Assessment of Infection Severity

The degree of leaf necrosis was evaluated on the seventh day after inoculation for the four rice varieties artificially inoculated with conidial suspension, and at 2 days later for the rice inoculated with mycelial plugs. Disease severity was assessed as the proportion of infected leaf area on randomly selected rice plants. It was estimated using a disease rating scale of 1–9, as suggested by Notteghem et al. (1980), on the last two leaves of each infected rice plant. The results are described in Table 1.

Table 1 Disease rating scale (Notteghem et al., 1980).

Note	Diseased leaf area (%)
0	0.00
1	0.05
2	0.50
3	1.50
4	3.50
5	7.50
6	17.50
7	37.50
8	62.50
9	87.50

For analysis, the severity scale was converted into Percentage Severity Index (IS) using the following formula:

$$IS = \frac{\sum x_i n_i}{9N_t} \times 100,$$

where x_i: disease severity scale; n_i: number of infected plants (or leaves) with a rating of i; N_t: total number of plants observed; 9: maximum disease severity scale.

2.9. Sporulation on the Host Plant

Sporulation was determined according to the technique of Hill and Nelson (1983) by estimating the average number of conidia produced per unit area of the infected leaves (expressed in number of spores/cm^2).

At 7 days after inoculation, rice leaves that showed lesions were removed, cut into four–five fragments, and then placed in Petri dishes containing filter paper moistened with sterile distilled water (one sheet per dish). The dishes were placed under continuous fluorescent light for 72 hr at 28 °C.

Each leaf segment was then collected in a test tube containing 1 mL of sterile distilled water. After that, the tubes were agitated in a vortex mixer for 2 min to detach the conidia from the mycelium. The conidia of the pathogen were counted using a Malassez slide under an optical microscope, with 10 counting for each sample.

2.10. Statistical Analysis

The statistical analysis of data was conducted using analysis of variance after transformation of percentages to arcsin √P. Comparison between means was performed using the least significant difference (LSD) test at $p < 0.05$.

3. Results

3.1. Morphological Characteristics

On the rice flour-based medium, fungus isolates formed fluffy and cottony aerial mycelia. The colony was gray to dark greenish gray in color (Figure 1);

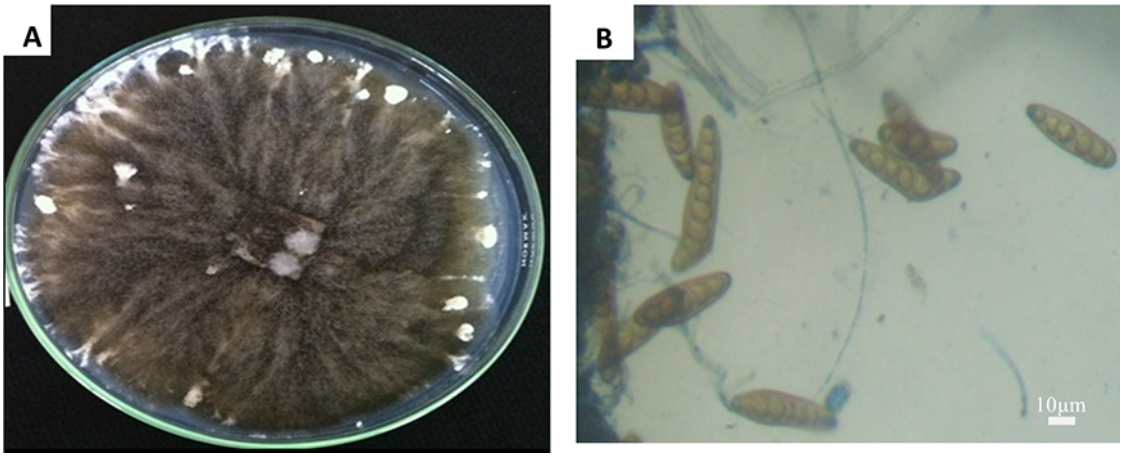

Figure 1 (**A**) Seven-day-old pure culture of *Bipolaris oryzae* isolate on rice flour-based medium. (**B**) Conidia of *B. oryzae* (×400).

the conidiophores grew in singles or in groups, branched or simple, multiseptate, flexuous, sometimes with geniculate upper part, brown to black. The conidia of the fungal species were straight, cylindrical, usually curved, light brown to golden brown, with six to 14 transverse partition. The conidial size ranged from 63 to 153 (avg. 109) µm × 14 to 22 (avg. 17) µm (Figure 1B). Morphologically, this fungus was therefore identified as *B. oryzae* (Ellis, 1971; Ou, 1985; Tarr, 1962).

3.2. Pathogenicity

3.2.1. Inoculation of Typha latifolia *Leaves With Spore Suspension*

The size of the lesions induced by *B. oryzae* varied depending on the isolate. The four isolates of *B. oryzae* (Hor1, Hor2, Hor3, and Hor4) caused lesions of different sizes on *T. latifolia* (14.4, 12.3, 15.8, and 13.8 mm, respectively) (Table 2, Figure 2).

Table 2 The degree of lesion on *Typha latifolia* leaves at 7 days after inoculation with the conidial suspension of each isolate of *Bipolaris oryzae* (mm).

***Bipolaris oryzae* isolates**	**Hor1**	**Hor2**	**Hor3**	**Hor4**
Lesion size	14.4 ab	12.3 b	15.8 a	13.8 ab

Two results on the same line are not significantly different at a 5% level (p.p.d.s test) if assigned with a common letter.

3.2.2. Inoculation of Typha latifolia *Leaves With Mycelial Discs*

Table 3 shows that *B. oryzae* isolates Hor4 and Hor2 induced fairly large lesions (20.8 and 16.7 mm, respectively) on leaves of *T. latifolia*, compared to those induced by Hor3 (11 mm) and Hor1 (9.2 mm) (Figure 3).

Table 3 The degree of lesion on *Typha latifolia* leaves at 7 days after inoculation with the mycelial discs of each isolate of *Bipolaris oryzae* (mm).

***Bipolaris oryzae* isolates**	**Hor1**	**Hor2**	**Hor3**	**Hor4**
Lesion size	9.2 b	16.7 a	11.0 b	20.8 a

Two results on the same line are not significantly different at a 5% level (p.p.d.s test) if assigned with a common letter.

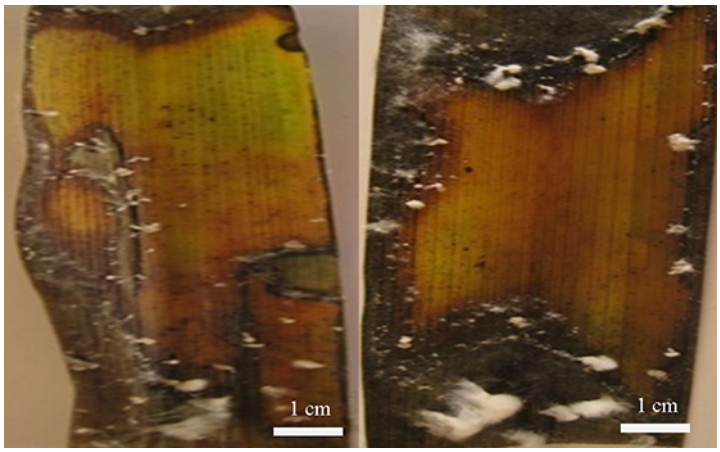

Figure 2 Symptoms developed on detached leaves of *Typha latifolia* artificially inoculated with a spore suspension of *Bipolaris oryzae*.

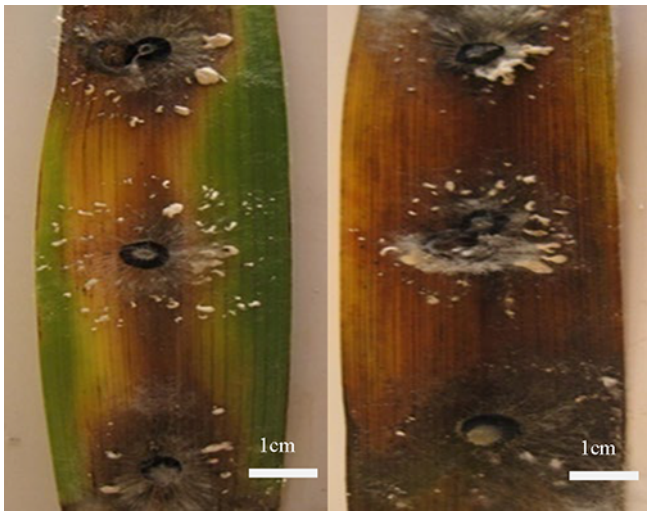

Figure 3 Symptoms of reddish-brown lesions developed on the leaves of *Typha latifolia* artificially inoculated with mycelial discs of *Bipolaris oryzae*.

3.2.3. *Sporulation of* Bipolaris oryzae *Isolates*

Through inoculation with either mycelial discs or spore suspensions, *B. oryzae* was able to sporulate on *T. latifolia* leaves, and no significant difference in intensity between the isolates tested was observed. Indeed, the average number of conidia ranged between 0.13×10^5 conidia/cm^2 and 0.83×10^5 conidia/cm^2 for the first technique (Table 4), and from 0.1×10^5 conidia/cm^2 to 0.6×10^5 conidia/cm^2 for the second technique (Table 5).

Table 4 Sporulation of *Bipolaris oryzae* on *Typha latifolia* leaves after inoculation with mycelial discs ($\times 10^5$ spores/cm^2).

***Bipolaris oryzae* isolates**	**Hor1**	**Hor2**	**Hor3**	**Hor4**
Number of spores	0.76 a	0.83 a	0.13 a	0.2 a

Two results on the same line are not significantly different at a 5% level (p.p.d.s test) if assigned with a common letter.

Table 5 Sporulation of *Bipolaris oryzae* on *Typha latifolia* leaves after inoculation with spore suspensions ($\times 10^5$ spores/cm^2).

***Bipolaris oryzae* isolates**	**Hor1**	**Hor2**	**Hor3**	**Hor4**
Number of spores	0.43 a	0.1 a	0.6 a	0.2 a

Two results on the same line are not significantly different at a 5% level (p.p.d.s test) if assigned with a common letter.

3.2.4. Inoculation of Rice Varieties With Conidial Suspension

After 7 days of inoculation, all the rice varieties tested displayed high sensitivity towards the four isolates of *B. oryzae* studied, as reflected by necrotic areas on the inoculated leaf surfaces. Thus, similar symptoms were observed in the five varieties of rice; they were generally of reddish-brown color, oval shape surrounded by a pale yellow halo, and were relatively uniform and regularly distributed (Figure 4).

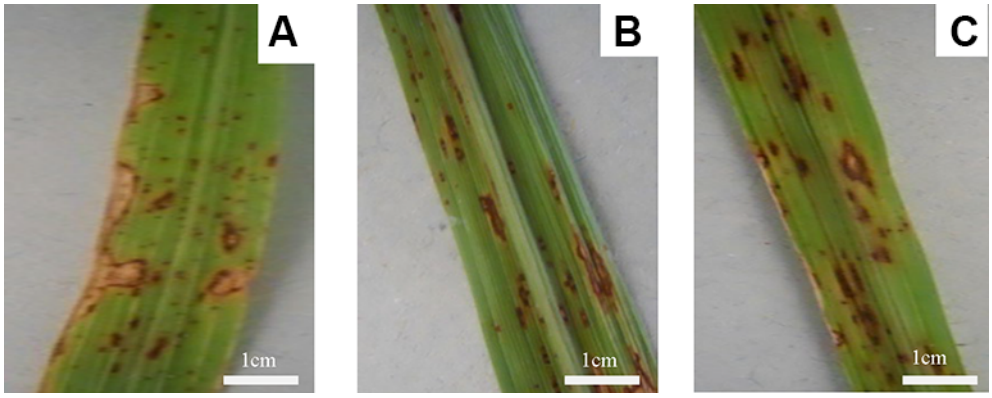

Figure 4 The symptoms of rice leaves artificially inoculated with spore suspensions of *Bipolaris oryzae*; (**A**) Taibonet variety, (**B**) Eurano variety, and (**C**) Cererrer variety.

As shown in Table 6, among the five rice varieties tested, Elio was the most sensitive to the Hor1 isolate, with a severity index of 90.73%, followed by the varieties Taibonet, Cererrer, and Eurano, whose severity indexes reached 85.18%, 79.63%, and 79.62%, respectively. In comparison, the Arpa variety was less sensitive, showing a severity index of 35.18% against the same isolate.

Table 6 Mean percentages of disease severity induced by *Bipolaris oryzae* isolates on rice varieties.

***Bipolaris oryzae* isolates**	**Rice varieties**				
	Eurano	**Taibonet**	**Arpa**	**Cererrer**	**Elio**
Hor1	79.62 a	85.18 a	35.18 b	79.63 a	90.73 a
Hor2	62.96 b	74.99 a	48.14 ab	90.74 a	81.47 a
Hor3	74.07 ab	69.44 a	62.03 a	90.73 a	79.63 a
Hor4	84.25 a	87.03 a	59.26 a	92.59 a	92.59 a

Two results on the same column do not differ significantly at a 5% level (p.p.d.s test) if assigned with a common letter.

The Cererrer and Elio varieties were more sensitive to the Hor2 isolate, with a severity index of 90.74% and 81.47%, respectively, which was superior to those of the Taibonet, Eurano, and Arpa varieties, which did not exceed 74.99%. The sensitivity of the Cererrer variety to the Hor3 isolate was marked by a high severity index of 90.73%, followed by the Elio, Eurano, Taibonet, and Arpa varieties, with respective indexes of 79.63%, 74.07%, 69.44%, and 62.03%. The Cererrer and Elio varieties were the most sensitive to the Hor4 isolate, showing the highest severity index (92.59%).

Table 7 Mean percentages of disease severity induced by *Bipolaris oryzae* isolates in rice varieties.

Rice varieties	*Bipolaris oryzae* isolates			
	Hor1	Hor2	Hor3	Hor4
Eurano	79.62 a	62.96 bc	74.07 ab	84.25 a
Taibonet	85.18 a	74.99 ab	69.44b	87.03 a
Arpa	35.18 b	48.14 c	62.03b	59.26 b
Cererrer	79.63 a	90.74 a	90.73 a	92.59 a
Elio	90.73 a	81.47 ab	79.63 ab	92.59 a

Two results on the same column do not differ significantly at a 5% level (p.p.d.s test) if assigned with a common letter.

However, the severity varied between 87.03% and 59.26% for the varieties Taibonet, Eurano, and Arpa.

Table 7 shows that for a given isolate, the severity varies depending on the inoculated rice variety. The Hor4 isolate was found to be most pathogenic to the Cererrer and Elio rice varieties, whose disease severity index reached 92.59%, followed by the Taibone (87.03%) and Eurano (84.25%) varieties.

The Hor3 isolate was highly pathogenic to the Cererrer variety (90.73%) and less pathogenic on Elio and Eurano varieties, with respective disease indexes of 79.63% and 74.07%. The severity indexes for the Hor2 isolate were the highest in the Cererrer and Elio rice varieties (90.74% and 81.74%, respectively), but did not exceed 74.99% in the Eurano, Taibonet, and Arpa varieties. Hor1 was more pathogenic to the Elio and Taibonet varieties, with respective indexes of 90.73% and 85.18%.

The sporulation ability of *B. oryzae* isolates from *T. latifolia* on the leaves of five varieties of rice showed pronounced variability between isolates. The highest spore number of the Hor1 isolate was observed in the Eurano variety, with 1.16×10^5 spores/cm^2, followed by the Arpa and Taibonet varieties, with 0.26×10^5 and 0.16×10^5 spores/cm^2, respectively. However, the sporulation was very low in the Elio and Cererrer varieties, in which the number of spores was reduced to 0.06×10^5 spores/cm^2 (Table 8).

The highest number of spores of the Hor2 isolate was 1.06×10^5 and 1.03×10^5 spores/cm^2 in the varieties Elio and Arpa, respectively, followed by that in the Cererrer and Eurano varieties, with 0.23×10^5 and 0.13×10^5 spores/cm^2, respectively. The Taibonet variety showed the lowest spore density, equal to 0.06×10^5 spores/cm^2 (Table 8).

The sporulation intensity of the Hor3 isolate on the leaves of the Arpa and Cererrer varieties was high (1.43×10^5 and 1.33×10^5 spores/cm^2, respectively).
In comparison, on the leaves of the Eurano and Elio varieties, the spore number was 0.7×10^5 and 0.63×10^5 spores/cm^2, respectively. Moreover, no spore was found on the leaves of the Taibonet variety (Table 8).

Table 8 Sporulation of the four isolates of *Bipolaris oryzae* on the leaves of the five varieties of rice ($\times 10^5$ spores/mL).

Bipolaris oryzae isolates	Rice varieties				
	Eurano	Taibonet	Arpa	Cererrer	Elio
Hor1	1.16 ab	0.16 a	0.26 b	0.06 b	0.06 b
Hor2	0.13 b	0.06 ab	1.03 a	0.23 b	1.06 a
Hor3	0.70 b	0.00 b	1.43 a	1.33 a	0.63 c
Hor4	2.26 a	0.00 b	0.00 b	0.00 b	0.00 b

Two results on the same column do not differ significantly at a 5% level (p.p.d.s test) if assigned with a common letter.

The maximum spore production of the Hor4 isolate was observed on the leaves of the Eurano variety, with 2.26×10^5 spores/cm^2. However, no spore was found in the other varieties (Taibonet, Arpa, Cererrer, and Elio).

4. Discussion

In the pathogenicity tests, Moroccan isolates of *B. oryzae*, which were isolated for the first time from the leaves of *T. latifolia*, were revealed to be pathogenic on rice seedlings, as indicated by necroses and discoloration on a large portion of the leaf blades of the five studied varieties. We can consider *T. latifolia* an alternative host of *B. oryzae*. Our result is in agreement with previous research results indicating that *B. oryzae* had a wide range of hosts, including *Oryza sativa*, *Triticum aestivum*, *Panicum virgatum*, *Zea mays*, *Brachypodium distachyon* (Farr & Rossman, 2017; Kaspary et al., 2018; Manamgoda et al., 2014), and *Typha orientalis* (Wang et al., 2019). Based on the severity index, *B. oryzae* isolates showed variable pathogenic capacity to the five tested rice varieties. The high pathogenicity of *B. oryzae* on rice varieties is supported by Monira et al. (2021), who reported that *B. oryzae* can attack rice seedlings and seeds and can remain viable for up to 5 years on Heera 2 hybrid rice seeds under suitable storage conditions. Based on their response to *B. oryzae* inoculum, the tested varieties were classified as moderately sensitive to highly sensitive according to the severity scores defined by Boka et al. (2018).

Regarding the sporulation ability, the tested isolates of *B. oryzae* succeeded in producing abundant spores on the leaf lesions, suggesting successful infectivity irrespective of rice variety and host. The attack of a plant by a pathogen depends on the pathogen itself (Notteghem et al., 1980) or on the host plant genotype (Marchetti & Bonman, 1989). However, the severity scores can vary depending on the isolates and rice variety. Similar observation was made by Ouazzani Touhami et al. (2000), who found different pathogenicity levels among isolates of *Helminthosporium spiciferum* and *H. australiensis*, whose capacity to sporulate on host plants also depends on the rice variety.

Helminthosporium can be isolated from a wide range of hosts, including corn and grasses (Nelson & Kline, 1961, 1962; Nelson et al., 1963). The parasitism of *B. oryzae* on corn was reported by Ou (1972) and Vidhyasekaran et al. (1986). The lesions appeared at approximately 18 hr after the inoculation of *B. oryzae* on the rice plant (Dallagnol et al., 2009). The results showed that the pathogenicity of *B. oryzae* was not specific to the rice from which it was isolated. This fungus can, without doubt, extend to other species widely cultivated in the vicinity of rice fields. Serghat, Ouazzani Touhami, & Douira (2005) have, in fact, shown that *B. oryzae* can be isolated from grasses, such as wheat, corn, and barley, that are located close to rice fields. The fungi present on leaf lesions in weeds can constitute a potential source of inoculum for rice plants. The presence of these weeds in and around rice fields helps maintain a high level of contaminating inoculum, promoting the progression of the epidemic. Indeed, the production of secondary inoculum via multiplication of infectious elements on the leaf lesions generates new contaminations and allows the disease to progress in rice fields (Serghat, Mradmi, et al., 2005; Serghat, Ouazzani Touhami, & Douira, 2005). According to Boulet and Bouhache (1990), the presence of an adventitious flora adapted to the conditions of rice fields, such as *Echinochloa crus-galli* and *E. phyllopogon*, greatly compromises the health of rice fields. Likewise, this flora harbors the same fungi that are found on rice leaf lesions. In the same context, the studied mycoflora on *Echinochloa phyllopogon* and *Phragmites australis* (two weeds adapted to rice fields) showed two types of fungi: true rice pathogens (*Pyricularia grisea*, *Helminthosporium oryzae*, *H. sativum*, *H. australiensis*, *H. spiciferum*, and *Curvularia lunata*) and saprophytes that cause rice discoloration (*Trichoderma harzianum*, *Alternaria alternata*, *Nigrospora oryzae*, *Epicoccum nigrum*, *Fusarium moniliforme*, *Cladosporium herbarumand*, and *Trichothecium roseum*) (Serghat, Mradmi, et al., 2005). Weeds in rice fields can also harbor other pests, including viruses, bacteria, and insects. Thus, in the presence of *Typha* sp., the development of *Sesamia* (*Sesamia nonagrioides*) in rice fields is much faster because this weed species is a plant host for the first larval stages of this predatory rice insect (Fazeli, 1992). *Echinochloa crus-galli* was identified as capable of hosting and

transmitting the southern rice black-streaked dwarf virus in South China (Li et al., 2012). In addition, *E. crus-galli* has proven to be an important reservoir of Aphid and Barley yellow dwarf luteovirus (BYDV) (Geissler & Karl, 1989). According to Bouhache et al. (1989), weeding rice fields and eradicating the surrounding weeds may protect rice plants from infection by the inoculum produced on these weeds. Our data provide important information on the novel isolates of *B. oryzae* and on the possibility of latent inoculum transmission that leads to disease in crops and weeds surrounding rice fields. However, the development of pathogen control strategies based on the genetic structure of the pathogen populations would certainly be effective (Nagaty & El Assal, 2011), and more knowledge is needed on host shifting and host expansion in fungal plant pathogens.

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