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PATHOGENIC VARIABILITY OF *BIPOLARIS ORYZAE* CAUSING LEAF SPOT DISEASE OF RICE IN WEST AFRICA

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ABSTRACT

An assessment of rice crop resistance to *Bipolaris oryzae*, a causal agent of rice brown leaf spot or helminthosporium disease was carried out. Ten (10) irrigated and upland rice varieties were used in this study. Seven (7) isolates of *B. oryzae* from Burkina Faso, seven (7) from Côte d'Ivoire and six (6) from Togo were used. The ten (10) varieties were inoculated with *B. oryzae* strains to assess their effect and determine the resistance level of these varieties to rice leaf spot (*Helminthosporium*). The varieties Bouaké- AM, FKR60N, NIL130, V10, FKR62N and CY2 showed a good level of resistance against all the isolates. The incidence of the disease on leaves reached 62.22% of severity index. Results revealed also that the strain effects are not linked to their country of origin. The investigation on the pathogenicity of *Bipolaris oryzae* isolates displayed the existence of a pathogenic variability among the fungus populations. These findings suggest that the tested varieties possess resistance genes that can be used in varietal selection.

Keywords: *Helminthosporium*, rice, varietal resistance, pathogenicity.

INTRODUCTION

Rice (*Oryza sativa* L.) is the third cereal produced in the world after corn and wheat. It is the staple food of almost half the world population (Maclean *et al.*, 2002; Van Nguyen and Ferrero, 2006). In the Ivory Coast, rice is the main staple food of the population, with a per capita consumption of 70 kg/year. The national production of white rice was estimated at 2 118610 tons in 2016 and accounts 50,26 % of domestic demand (ONDR, 2018). To meet self-sufficiency in rice, the main agronomic constraints causing production losses should be overcome. Crop diseases are among the major constraints. *Helminthosporium* the causal agent of the rice leaf blight is becoming a serious threat to rice production for the past twelve years in the Ivory Coast (Bouet *et al.*, 2015). The mycosis in Burkina Faso, a neighboring country of Ivory Coast has also been a matter of concern for a long time. Other countries of the Economic Community of West African States (ECOWAS), particularly Togo, are facing the *Helminthosporium* disease. This fungal disease, due to *Bipolaris oryzae* (Shoemaker, 1959) is observed on rice leaves (Figure 1),

stems and grains (Benkirane, 1995; Benkirane *et al.*, 1998; Benkirane *et al.*, 2000; Tajani *et al.*, 2001; Bahous *et al.*, 2003; Serghat *et al.*, 2005; Gnancadja-Andre *et al.*, 2005). It causes bad germination, roots and stems decay (Van Nghiep and Gaur, 2004). *Helminthosporium* was responsible for the Bengal hunger in India in 1943 with a production loss evaluated between 50 and 95% (Padmanabhan *et al.*, 1948). Using rice varieties resistant to the disease is one of the recommended solutions. However, in order to manage sustainably crop resistance, the structure of the parasite population must be known. The objective of the present study was to discover the pathogenic diversity of *Bipolaris oryzae*. In that respect, a characterization survey of the pathogenic test of some isolates sample from the Ivory Coast, Burkina Faso, and Togo was carried out in a semi-controlled environment.

MATERIALS AND METHODS

Plant material: Ten (10) upland and irrigated rice varieties, certified or on-going certification in the Ivory Coast (BOUAKE-AM, IDSA10, NIL130, V10, and CY2) and Burkina Faso (WABC165, FKR62, FKR60, FKR45N,

FKR19) were used.

Fungal material: Twenty (20) *Bipolaris oryzae* isolates, of which seven (7) are from the Ivory Coast, seven (7) from Burkina Faso and six (6) from Togo, were used (Table 1). The isolates from the Ivory Coast were sampled from rice materials (leaves and grains) infected by

Helminthosporium in different agro-ecological zones, low-land, and up-land fields. The other isolates are from the “mycotheca” of the Phytopathology Laboratory of the Institute of Environment and Agricultural Research (INERA) of Farako-Bâ in Bobo-Dioulasso, Burkina Faso.



Figure 1. Field infected with brown spot in the lowland of Lébré, Ivory Coast (N=06°18.644, W= 06°20.405, alt= 227 m).

Table 1. *Bipolaris oryzae* isolates used for the pathogenicity test.

Code	Isolates name	Material sampled	Ecologies	Origins
BF3	Bagré 40 B02	grain	Low-land	Burkina Faso
BF14	N'Dorola BF8	grain	Low-land	Burkina Faso
BF16	Sahel 177 B04	grain	Low-land	Burkina Faso
BF17	Sahel 323	grain	Low-land	Burkina Faso
BF18	TCS 10 BZ B01	Leaf	Low-land	Burkina Faso
BF20	FKR 62N B03	grain	Low-land	Burkina Faso
BF21	FKR45N B01	grain	Low-land	Burkina Faso
CI1	Agboville B01	Leaf	Low-land	Côte d'Ivoire
CI31	Touresso B01	Leaf	Up-land	Côte d'Ivoire
CI4	Bondoukou B03	Leaf	Up-land	Côte d'Ivoire
CI7	Borotou B04	Leaf	Low-land	Côte d'Ivoire
CI11	Gbombaha B06	Leaf	Up-land	Côte d'Ivoire
CI26	Odienné B01	Leaf	Low-land	Côte d'Ivoire
CI27	Odienné B02	Leaf	Low-land	Côte d'Ivoire
TG1	Agbati B02	Leaf	Low-land	Togo
TG2	Gamabilé B03	Leaf	Low-land	Togo
TG5	Kanina B03	Leaf	Low-land	Togo
TG6	Kanina B04	Leaf	Low-land	Togo
TG17	Tantiégou B04	Leaf	Low-land	Togo
TG19	Timbo B04	Leaf	Up-land	Togo

Nursery preparation and seedlings: The nursery of each variety was established in two trays of 45cm x 25cm of dimension. Twenty (20) trays in total were used for the ten (10) varieties. They were filled with barren soil. Rice seed was disinfected with hypochlorite of sodium 5% before sowing. Two (2) weeks after nursery establishment, three (3) vigorous seedlings of each variety were randomly transplanted in one tray prepared as described above. In total, twenty (20) trays corresponding to the twenty (20) *Bipolaris oryzae* isolates were made. The seedlings were watered every morning with tap water till inoculation. The experiment was replicated three (3) times.

Inoculum production: The inoculum was produced in an Agar water medium (1.5% of gelatin) sterilized in an autoclave at 121°C for 30 minutes and poured in Petri dishes. Sterilized pieces of sorghum (*Sorghum sp.*) leaves 8 cm x 6 cm of dimension were placed in this media before solidification (Ouédraogo, 2008). Then, explants of about 3 mm of diameter, taken from a pure plant of each

Bipolaris oryzae isolates from 4 to 5 days (Figure 2), were placed in the Petri dishes. After fifteen (15) days of culture at 28°C under 12 hours photoperiod, the conidia on the surface of each culture were washed away using distilled water. The resulting solution was filtered with mosquito net of 1 x 1mm mesh size to separate the conidia from the mycelial fragments. The concentration of the conidia was fixed using a hematimeter, in a volume of 100 ml containing 1ml of Tween 20 at 10⁵ conidia/ml.

Inoculation of rice plants: Twenty-one days (21) after transplanting, rice seedlings from each tray were inoculated by spraying 100 ml of conidia suspension at a dose of 10⁵ conidia/ml of *Bipolaris oryzae* isolates (Figure 3). Inoculated seedlings were placed for 72 hours under a black plastic cover equipped with a humidifier containing distilled water. This measure enables to maintain a relative humidity up to 80%, which is favorable to germination and conidia development. After their stay under the black plastic cover, trays were put in a medium at room temperature (27° C) for 21 days.

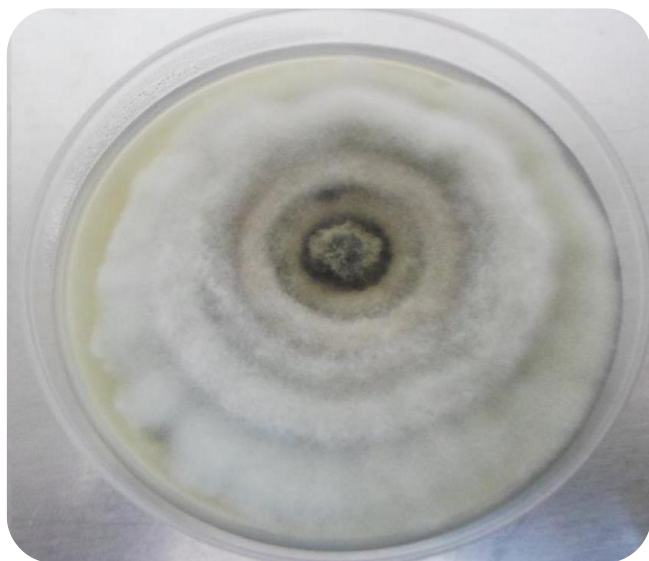


Figure 2. Five days old pure culture of *Bipolaris oryzae* isolate on Malt Agar medium.

Parameters studied

Index of severity: The index of severity of the disease was determined 14 days after inoculation. After the seedlings observation, severity grades of the disease were determined according to a grading scale of IRRI (2002) and the number of plants attacked was graded as well. The index was calculated according to the following equation:

$$IS = \frac{\sum Xi \cdot ni}{9Nt} \times 100$$



Figure 3. Conidia of *Bipolaris oryzae* isolates (x 400).

IS: Index of severity of the disease

X_i : severity of the disease (Note i)

n_i : number of plants showing the severity

Nt : total number of observed plants

9: the highest severity grade of the disease according to IRRI scale

Aggressiveness level of the isolates: To be able to categorize this parameter, three levels of aggressiveness were defined as follows: an isolate with an index of

severity (IS) greater than 50%, is qualified as “very aggressive”; “moderately aggressive” if the IS is between 25% and 50% and “non- aggressive” when the IS is less than 25%.

Resistance or sensitivity of rice varieties:

According to its response to the inoculum, the rice variety is said to be:

Resistant, if severity score varies from 1 to 3;

Moderately sensitive if the score varies from 4 to 5;

Sensitive if the score is greater than 5.

Statistical analyses: The different analyses were

carried out using the software GenStat 10.1. Dendrograms were designed according to a hierarchic bottom-up classification in rice genotype as well as *Bipolaris oryzae* isolates.

RESULTS

Response of rice genotypes to *Bipolaris oryzae*

inoculum: Results from table 2 showed that the majority of rice varieties was moderately sensitive to most of *B. oryzae* isolates. However, for a given variety, the response varies from an isolate to another. Severity scores were generally between 2 and 8.

According to these responses, the tested varieties could be classified as follow:

Varieties Bouaké-AM and FKR19. With a severity score of these varieties were sensitive to none of *Bipolaris oryzae* strains. These varieties seem to have a good level of high partial resistance.

Varieties V10, NIL130, CY2, FKR60N and FKR62N. Sensitive to one or two strains with a severity score of 6. Varieties IDSA10 and FKR45N, showed high levels of sensitivity to the isolates with severity scores of 6 and 7, respectively.

Table 2. Response of different rice varieties to *Bipolaris oryzae* isolates

<i>Bipolaris oryzae</i> strains	Rice varieties									
	Bouake-AM	IDSA 10	Nil 130	V10	CY2	WAB C 165	FKR62N	FKR60N	FKR45N	FKR19
Bagré40 B02	MS (5)	MS (4)	MS (5)	MS (4)	MS (4)	MS (5)	MS (4)	MS (4)	MS (5)	MS (4)
N'Dorola BF8	MS (4)	MS (4)	MS (4)	MS (4)	MS (4)	MS (4)	MS (4)	MS (5)	MS (4)	R (3)
Sahel177 B04	R (2)	R (3)	MS (4)	MS (4)	MS (5)	MS (5)	R (3)	MS (4)	MS (5)	MS (4)
Sahel 323	MS (4)	MS (4)	MS (4)	MS (4)	MS (4)	MS (5)	MS (5)	MS (4)	MS (5)	MS (4)
TCS10 BZB01	R (3)	MS (4)	MS (4)	R (3)	R (3)	MS (4)	R (3)	R (3)	MS (4)	R (3)
FKR62N B03	MS (4)	MS (4)	MS (4)	MS (4)	MS (4)	MS (4)	MS (4)	MS (4)	MS (4)	R (3)
FKR45N B01	R (3)	R (3)	MS (4)	MS (4)	MS (4)	R (3)	MS (4)	R (3)	MS (4)	MS (5)
Agboville B01	MS (4)	MS (4)	MS (4)	MS (4)	MS (4)	S (7)	MS (4)	MS (4)	MS (4)	MS (4)
Touresso B01	MS (4)	S (7)	MS (4)	MS (5)	MS (4)	MS (5)	MS (4)	MS (4)	MS (4)	MS (4)
Bondoukou B03	MS (4)	MS (5)	MS (5)	S (6)	MS (4)	MS (4)	MS (4)	MS (4)	MS (4)	MS (4)
Borotou B04	MS (4)	MS (4)	MS (4)	MS (5)	MS (5)	S (7)	MS (4)	MS (4)	MS (5)	MS (4)
Gbombaha B06	R (3)	MS (4)	MS (4)	MS (4)	MS (4)	MS (5)	MS (4)	MS (4)	MS (4)	MS (4)
Odienné B01	MS (5)	S (6)	S (6)	MS (5)	MS (5)	S (7)	MS (4)	MS (5)	S (7)	MS (5)
Odienné B02	MS (4)	MS (4)	MS (4)	MS (4)	MS (4)	MS (4)	MS (4)	R (3)	MS (4)	R(3)
Agbati B02	MS (4)	MS (4)	MS (4)	MS (4)	MS (4)	S (6)	MS (5)	MS (4)	S (7)	MS (4)
GamabiléB03	MS (4)	MS (5)	MS (4)	MS (4)	MS (4)	MS (4)	MS (4)	MS (4)	MS (5)	MS (4)
Kanina B03	MS (4)	MS (4)	MS (5)	MS (5)	S (6)	S (8)	S (6)	S (6)	S (6)	MS (5)
Kanina B04	MS (4)	S (6)	MS (5)	MS (5)	MS (4)	S (7)	S (6)	MS (5)	S (7)	MS (5)
TantiégouB04	MS (4)	MS (4)	MS (4)	MS (4)	R (3)	MS (5)	MS (5)	MS (4)	MS (5)	MS (4)
Timbo B04	MS (4)	MS (4)	MS (4)	MS (4)	MS (4)	S (6)	MS (4)	MS (4)	MS (5)	MS (4)

These varieties can be classified in the group of varieties having a moderately partial resistance level. Variety WAB C165, appeared moderately sensitive to twelve (12) of the isolates and sensitive to seven (7) of them. Concerning the sensitivity, severity scores varied from 6 to 8. This variety showed a rather low resistance level to *Bipolaris oryzae*.

The multivariate analysis based on the similarity according to responses of rice varieties to *Bipolaris oryzae* isolates identified four (4) varietal groups at 75% of similarity (Figure 4):

Group 1 comprises the variety WABC165 that is sensitive to almost all the isolates (19 isolates over 20);

Group 2 includes moderately sensitive varieties, FKR45N and IDSA10;

Group 3 comprises the variety FKR 19 moderately resistant to the disease (Figure 4)

Group 4 consist of the resistant varieties, Bouaké-AM, FKR60N, NIL130, V10, FKR62N, and CY2.

Pathogenicity of *Bipolaris oryzae* strains on ten rice genotypes: The results of *Bipolaris oryzae* isolates index of severity recorded in table 2 show that the aggressiveness of the strains varies according to rice varieties. However, some isolates were aggressive to almost all the rice varieties. Others were more or less aggressive, depending on the varieties. The dendrogram obtained from the severity indexes (Figure 5) displays the presence of three pathogroups according to the aggressiveness of strains on varieties at 80% of similarity.

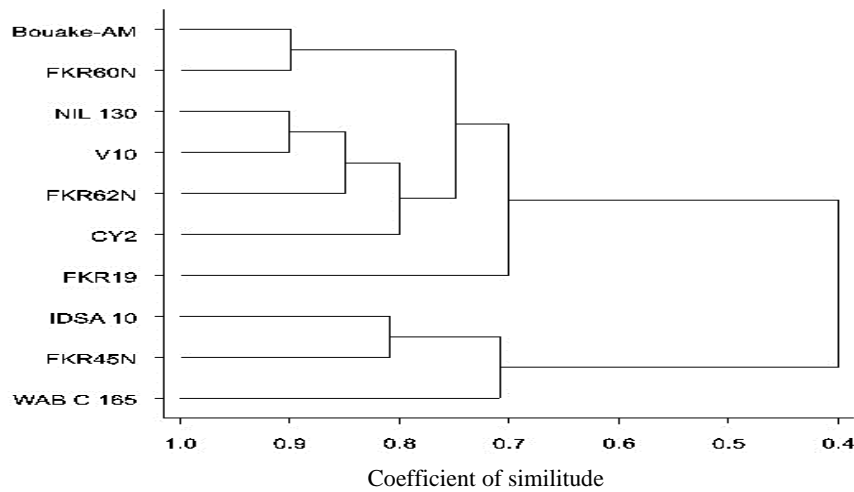


Figure 4. Rice varieties grouped according to the response profile to *Bipolaris oryzae* isolates.

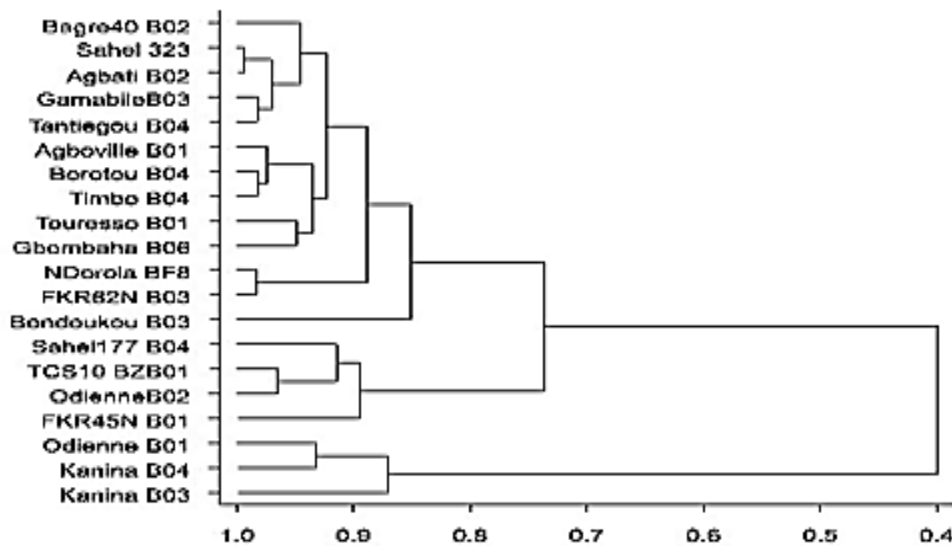


Figure 5. Dendrogram showing at 80% of similarity, the isolates of *Bipolaris oryzae* pathogroups tested on rice varieties.

The first pathogroup consists of very aggressive isolates on at least 80% to 100% of the investigated rice varieties. Isolates included in that pathogroup are Kanina B03 (IS = 62.22%), Kanina B04 (IS =

59.29%) from Togo and Odienné B01 (Côte d'Ivoire), of which severity index was assessed at 59.26% (Table 3).

The second pathogroup encompasses isolates that

showed no strong aggressiveness on any of the tested varieties (IS < 50%).

The third group represents strains that were very aggressive on 10 to 50% of rice varieties.

Table 3. Severity index (IS) of *Bipolaris oryzae* isolates on ten (10) rice varieties.

<i>Bipolaris oryzae</i> strains	Rice varieties										IS means
	Bouake-AM	IDSA 10	NIL 130	V10	CY2	WAB C 165	FKR62N	FKR60N	FKR45N	FKR19	
Bagré40 B02	51.85	48.15	51.85	44.44	40.74	51.85	44.44	40.74	59.26	40.74	47.40
N'Dorola BF8	48.15	44.44	44.44	48.15	48.15	48.15	40.74	55.56	44.44	33.33	45.55
Sahel177 B04	25.93	29.63	44.44	48.15	51.85	55.56	33.33	40.74	55.56	40.74	42.99
Sahel 323	44.44	48.15	44.44	48.15	44.44	55.56	51.85	48.15	59.26	48.15	49.26
TCS10 BZB01	33.33	40.74	40.74	37.04	37.04	44.44	33.33	33.33	40.74	37.04	37.77
FKR62N B03	44.44	44.44	48.15	51.85	44.44	51.85	44.44	48.15	44.44	37.04	45.92
FKR45N B01	37.04	33.33	40.74	40.74	44.44	37.04	40.74	33.33	44.44	55.56	40.74
Agboville B01	44.44	55.56	44.44	44.44	44.44	74.07	51.85	44.44	51.85	44.44	49.99
Touresso B01	44.44	66.67	48.15	55.56	44.44	55.56	44.44	48.15	48.15	44.44	50
Bondoukou B03	40.74	51.85	55.56	70.37	48.15	62.96	44.44	55.56	40.74	44.44	51.48
Borotou B04	44.44	48.15	44.44	51.85	51.85	77.78	44.44	48.15	59.26	44.44	51.48
Gbombaha B06	51.86	66.67	62.96	51.85	51.85	77.78	48.15	51.85	74.07	55.56	59.26
Odienné B01	37.04	48.15	44.44	48.15	48.15	55.56	40.74	40.74	44.44	48.15	45.55
Odienné B02	44.44	44.44	40.74	44.44	44.44	40.74	40.74	37.04	48.15	37.04	42.22
Agbati B02	44.44	44.44	44.44	48.15	44.44	59.26	51.85	44.44	66.67	48.15	49.63
GamabiléB03	44.44	44.44	44.44	48.15	44.44	48.15	44.44	44.44	55.56	44.44	46.29
Kanina B03	55.56	51.85	51.85	51.85	70.37	85.19	70.37	62.96	66.67	55.56	62.22
Kanina B04	44.44	62.96	51.85	59.26	44.44	77.78	66.67	51.85	77.78	55.56	59.29
TantiégouB04	40.74	44.44	40.74	44.44	37.04	55.56	51.85	44.44	51.85	44.44	45.55
Timbo B04	48.15	44.44	48.15	48.15	44.44	70.44	44.44	44.44	59.26	48.15	49.99

DISCUSSION

The evaluation of the level of resistance of rice varieties to *Bipolaris oryzae* isolates resulted in their classification into four groups: Group 1 for the moderately sensitive varieties (WABC165), Group 2 for the sensitive varieties (IDSA10 and FKR45N), Group 3 for the moderately resistant variety (FKR 19) and Group 4 for the resistant varieties (Bouaké-AM, FKR60N, NIL 130, V10, FKR62N, and CY2). These results confirm those of Bouet *et al.* (2015), who found that Bouaké-AM, Nil 130 and V10 were resistant to helminthosporium in the Ivory Coast. Dembele (2014) showed also that FKR62N and FKR19 should present some resistance to *Bipolaris oryzae* isolates of many areas of Burkina Faso.

Resistance is the outcome interactions between genes (secondary genes for the host plant) or between molecules of the parasite and its host. These interactions lead to the synthesis of phytoalexins, proteins related to the development of the disease, by the host plant (Parlevliet, 2002).

The high level of resistance of observed in with varieties could be accounted for the presence of genes and/or QTL of interest that grant them this character of horizontal resistance to the disease. Sato *et al.* (2008) and Katara *et al.* (2010) identified many QTL involved in the resistance to helminthosporium.

Furthermore, the results herein reported showed that the isolates were virulent to all the tested rice varieties, with disease severity indexes (IS) varying between 37.77% to 62.22%. Ouédraogo (2008) described a variability among the populations of *Bipolaris oryzae*, classified them into three groups. The first group made of the most aggressive isolates with an IS greater than 50%. In this group are recorded isolates like Kanina B03, Odienné B01, and Kanina B04. The second group is characterized by non-aggressive isolates and the third group represents the moderately aggressive isolates with an IS between 25 and 50%. These results corroborate those of Ouédraogo (2008). In fact, the results from this researcher revealed the presence of pathogenic diversity among the population of *Bipolaris oryzae*. The results of this study as well as those of Ouédraogo (2008), confirm that the aggressivity of *Bipolaris oryzae* has nothing to do with its geographic origin (country of origin). Thus, two *Bipolaris oryzae* from Togo (Kanina B03, Kanina B04) and one from Ivory Coast (Odienné B01) showed a high aggressivity toward the studied rice varieties. The pathogenic

diversity would imply, for varietal selection, the implementation of many local trials so that to determine the varieties resistant to each virulent strain. The varieties Bouaké-AM, FKR60N, NIL 130, V10, FKR62N and CY2 showed some resistance during the period going from 7 days after transplanting (DAT) to 14 DAT. This seems to indicate that these varieties succeeded to reduce more or less the development of the attack during the same period. This would explain their resistance (or tolerance) to the disease. Results on the response of these varieties allowed to confirm their classification in 3 groups that are the resistant varieties (Bouake AM, Nil 130, FKR60N, V10, CY2, FKR62N and FKR19), the varieties moderately sensitive (FKR45N and IDSA10) and the sensitive variety (WABC165).

CONCLUSION

Investigating the response of the varieties to the isolates enabled to appreciate the level of resistance of those varieties. The varieties Bouaké- AM, FKR60N, NIL130, V10 FKR62N, and CY2 showed a good resistance to all the used isolates. They might have genes and /or interesting QTL that give them a certain horizontal resistance to many *Bipolaris oryzae* isolates. The study revealed the existence of a pathogenic diversity as well among the populations of *Bipolaris oryzae*, allowing to have many levels of aggressivity. All the isolates showed a virulence on all the tested rice varieties but at a different level of aggressivity. To well understand the level of resistance of these varieties to helminthosporium disease, it would be important to test them in field conditions so that to measure the combined effects of abiotic factors on their behavior.

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