



Current Research in Bacteriology

ISSN 1994-5426

science
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Rice Bacterial Blight in Togo: Importance of the Disease and Virulence of the Pathogen

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ABSTRACT

Bacterial blight, caused by *Xanthomonas oryzae* pv. *oryzae*, is new in Togo. A bacterial blight survey was carried out in the dry savanna and wet savanna zones in the Northern part of Togo revealed its distribution, incidence, severity and virulence of the bacterial strains. Presence of bacterial blight of rice in dry savanna and wet savanna zones was confirmed in 30% of the sites visited. The disease was more frequently found in the dry savanna zone than in the wet savanna zone. High incidence (45%) and severity (50%) of bacterial blight was especially found in the dry savanna zone closer to Burkina Faso. Eleven bacterial strains were isolated from the infected sites and were pathologically characterized by testing their virulence on the rice variety IR841 which is widely grown in Togo. Severity of between 63-90% obtained among strains revealed their highly virulent status and this first record the occurrence of bacterial blight, *Xanthomonas oryzae* pv. *oryzae*, in Togo.

Key words: *Xanthomonas oryzae* pv. *oryzae*, bacterial blight disease, dry and wet savanna zones, virulence, rice

INTRODUCTION

Pyricularia grisea (teleomorph: *Magnaporthe grisea*), Rice yellow mottle virus (RYMV) and *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) pathogens have been described as the major causative agents of rice diseases in Africa (Onasanya *et al.*, 2009). Bacterial blight disease, caused by *Xoo* is one of the most serious rice diseases with serious threat to rice production and is widespread throughout Asia, Australia and Latin America and is becoming important in several countries in Africa (Sere *et al.*, 2005).

Bacterial blight has become a major disease after the introduction of high yielding but susceptible rice cultivars. Consequently, comprehensive studies on pathogen diversity become important to provide useful information on the *Xoo* population structure and cultivar resistance (Singh *et al.*, 2001; Lee *et al.*, 2003). In West Africa bacterial blight is mainly found in irrigated rice ecosystems (Sere *et al.*, 2005). The characterization of *Xoo* virulent population structure towards

rice lines with a known gene of resistance will provide useful information for selection and deployment of cultivars with durable resistance (Onasanya *et al.*, 2009).

Previous studies in Asia using near isogenic lines are useful to study the bacterial blight population, structure and pathogenicity (Kihupi *et al.*, 2001; Singh *et al.*, 2001; Lee *et al.*, 2003). The knowledge provided by these studies was helpful to analyze *Xoo* pathogen population in Africa and its relationship with local and improved cultivars, to investigate sources of resistance to the African BLB population and to investigate the possibility of transferring the resistance genes identified into elite materials through marker-assisted and participatory varietal selections.

Recent surveys in Burkina Faso, Niger and Mali revealed the occurrence of bacterial blight causing significant crop damages (Basso *et al.*, 2011). The disease may cause damage at seedling stage resulting in complete wilting or death of affected tillers (Basso *et al.*, 2011).

Little information is available on the losses due to bacterial blight on farmers' fields. However, yield losses of 20-80% caused by the disease in severely infected fields have been reported during surveys on the status of bacterial blight (BB) in several West African countries (Onasanya *et al.*, 2009). The disease is new to Togo and information on the establishment and occurrence of BB in the country is lacking. However, since the disease was reported in Burkina Faso, neighbor country in the North of Togo, it might have become already established some years ago in some rice growing areas (Sere *et al.*, 2005). The present studies aimed to survey BB, to determine its incidence, severity and geographic distribution in the North of Togo and to characterize its strains as a prerequisite to develop a suitable control strategy to avoid epidemics.

MATERIALS AND METHODS

Research location: The study was jointly conducted at Institut Togolais de Recherche Agronomique (ITRA), Centre de Recherche Agronomique du Littoral, Lomé, Togo and at Plant Pathology Unit, Africa Rice Center, Cotonou, Benin from September 2008 to April 2009.

Study area: Rice is grown in four agro-ecological zones in Togo, however, this study focuses on the Northern part of the country which comprised of dry and wet savanna zones. The survey area was defined with regard to its geographic situation in relation to Burkina Faso, where BB was earlier reported to cause serious damages (Sere *et al.*, 2005). The dry and wet savanna zones are characterized by same tropical climate with a long rainy season from April to September and a long dry season from October to March.

Bacterial blight disease survey: A survey was carried out in the rainy season in September 2008. Forty-eight rice fields from ten localities were visited. The ten localities include Sinkassé, Tone, Tandjoare, Oti, Kéran, Binah and Kozah in the dry savanna zone and Bassar, Tchaoudjo and Sotouboua in the wet savanna zone. Fields of about 0.25 ha size minimum were selected from the rice-growing areas at a minimum of 5 km intervals along the practicable roads using the Global Position System (GPS) equipment. In each of the visited fields bacterial blight symptoms were evaluated on rice plants following two diagonals across the field. Hundred and twenty-five plants selected within two diagonals of the field were assessed for bacterial blight incidence and severity. The bacterial blight disease incidence was evaluated in 1 to 5 classes and severity was determined by scoring the expression of the symptom class severity 1 to 9 classes (Abbasi *et al.*, 2011; Sere *et al.*, 2005).

Samples collection and isolation of bacteria: During the field survey samples of rice leaves showing early symptoms of bacterial blight were collected from each field where the disease was observed in order to isolate the bacteria. All the collected leaf samples were kept in dried paper envelopes and stored in a cool box before bacterial isolation. Bacterial isolation was according to Sere *et al.* (2005).

Pathological characterization of bacteria strains: The pathological characterization was performed by testing the virulence of the isolated bacteria strains on cultivated rice variety using the method adopted by Sere *et al.* (2005). The test was conducted in an insect-proofed greenhouse at the Institut Togolais de Recherche Agronomique (ITRA) station in Lomé. The widely cultivated rice variety IR841 was used. IR841 is grown in irrigated as well in rain-fed lowland ecologies and was found to be highly susceptible to bacterial blight during the disease survey of the current study.

Statistical analysis: The Tukey test (Zhu and Kuljaca, 2005) at 5% was performed using Statistical Analysis Software (SAS) package to compare the means of severities scored by different strains (Onasanya *et al.*, 2009).

RESULTS

Distribution, incidence and severity of the disease: Rice bacterial blight was observed in 30% of the fields visited and occurred both in the wet and dry savanna zones but the highest disease severity class 9 was recorded in the locality of Djapiéni where the highest disease incidence was found. Among the contaminated sites, Djapiéni and Tonté localities recorded the highest incidence of class 5 with more than 75% of infected plants in the fields and were found in the extreme North of the dry savanna zone. The disease incidence was at the lowest in only two sites, Atalotè in the south of the dry savanna zone and Témèmè in the wet savanna zone, where the infected plants in the fields were less than 5%, while the disease incidence was found to decrease from the North in the dry savanna zone to the South in the wet savanna zone of the survey area (Table 1, Fig. 1).

Table 1: Rice bacterial blight disease incidence and severity in dry savanna and wet savanna zones in Northern Togo

Site code	Localities	Incidence classes	Severity classes	Ecozones
1	Djapiéni	5	9	Dry savanna
9	Nogyiog	2	5	Dry savanna
10	Tonté	5	5	Dry savanna
15	Tantiégou	3	7	Dry savanna
16	Tantiégou	2	7	Dry savanna
22	Koumbéloti	2	3	Dry savanna
26	Sagbiébou	2	3	Dry savanna
28	Atalotè	1	3	Dry savanna
29	Bounon	2	3	Dry savanna
32	Kawa	3	3	Wet savanna
33	Témèmè	1	3	Wet savanna

Incidence: Class 1: Infected plants lower than 5%; Class 2: Infected plants from 5 to 25%; Class 3: Infected plants from 26 to 50%; Class 4: Infected plants from 51 to 75%; Class 5: Infected plants from 76 to 100%. Severity: Class 1: Lesion/necrosis of the apex of the leaf; Class 3: Lesion/necrosis of lower than 25% of the leaf surface; Class 5: Lesion/necrosis of 25 to 50% of the leaf surface; Class 7: Lesion/necrosis of higher than 50% of the leaf surface; Class 9: Lesion/necrosis of the whole leaf surface

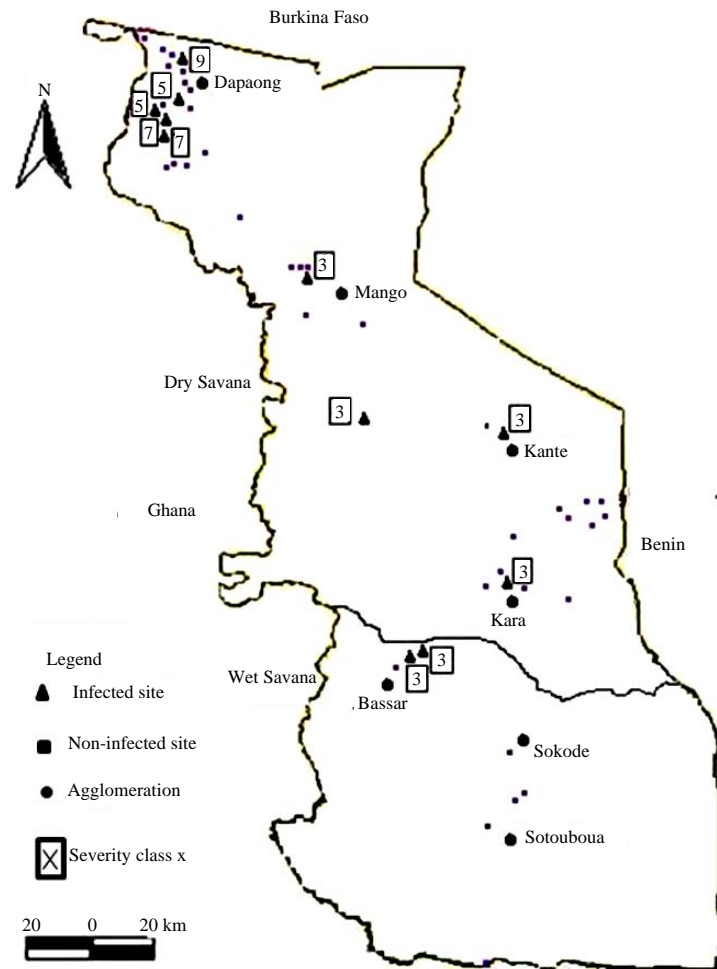


Fig. 1: Distribution of rice bacterial blight and severity classes in fields in dry savanna and wet savanna zones in Northern Togo. Severity: Class 1: Lesion/necrosis of the apex of the leaf; Class 3: Lesion/necrosis of lower than 25% of the leaf surface; Class 5: Lesion/necrosis of 25 to 50% of the leaf surface; Class 7: Lesion/necrosis of higher than 50% of the leaf surface; Class 9: Lesion/necrosis of the whole leaf surface

However, in all the contaminated sites, bacterial blight severity was moderate to high with the severity classes of 3 to 9 and the highly infected plants in the classes 5, 7 and 9 were found in the contaminated sites in the extreme North of the dry savanna zone while in the wet savanna zone the highest disease severity recorded was class 3 (Table 2, Fig. 1).

Isolation and characterization of bacterial strains: Eleven strains of *Xanthomonas oryzae* pv. *oryzae* were isolated from leaf samples from the dry and wet savanna zones and tested for virulence on the variety IR841. The disease symptoms appeared about three to five days after post inoculation (dpi). The pathological characterization revealed significant differences in virulence among *Xoo* strains. At 5 dpi, two strains (BB22-1; BB26-1) did not cause early symptoms on the inoculated leaves, but at 19 dpi 73% of the strains were scored severity class 3 while all other

Table 2: Percentage of rice bacterial blight contaminated sites in the incidence and severity classes

Incidence classes	% contaminated sites	Severity classes	% contaminated sites
1	18.2	1	0.0
2	45.5	3	54.5
3	18.2	5	18.2
4	0.0	7	18.2
5	18.2	9	9.1

Incidence: Class 1: Infected plants lower than 5%; Class 2: Infected plants from 5 to 25%; Class 3: Infected plants from 26 to 50%; Class 4: Infected plants from 51 to 75%; Class 5: Infected plants from 76 to 100%. Severity: Class 1: Lesion/necrosis of the apex of the leaf; Class 3: Lesion/necrosis of lower than 25% of the leaf surface; Class 5: Lesion/necrosis of 25 to 50% of the leaf surface; Class 7: Lesion/necrosis of higher than 50% of the leaf surface; Class 9: Lesion/necrosis of the whole leaf surface

Table 3: Virulence of *Xanthomonas. oryzae* pv. *oryzae* strains expressed as symptom severity classes induced on the rice variety IR841 after inoculation

Strain	Days post inoculation (dpi)			Tukey test* (at 33 dpi)
	5	19	33	
BB01-1	1	1	5	bc
BB09-1	1	3	7	ab
BB10-2	1	3	7	ab
BB15-3	1	3	7	ab
BB16-2	1	3	7	ab
BB22-1	0	1	5	bc
BB26-1	0	1	5	bc
BB28-1	1	3	7	ab
BB29-1	1	3	7	ab
BB32-1	1	3	9	a
BB33-4	1	3	7	ab

* $p \leq 0.05$. Severity: Class 1: Lesion/necrosis of the apex of the leaf; Class 3: Lesion/necrosis of lower than 25% of the leaf surface; Class 5: Lesion/necrosis of 25 to 50% of the leaf surface; Class 7: Lesion/necrosis of higher than 50% of the leaf surface; Class 9: Lesion/necrosis of the whole leaf surface

strains scored at least severity class 1. At 33 dpi all the strains severity were higher or equal to class 5 including strains from the wet savanna zone. Most of the strains (73%) at 33 dpi recorded severity higher or equal to class 7, of which one strain BB32-1 from the wet savanna zone has the highest note of class 9 (Table 3).

The statistical analysis revealed slight significant differences among strains ($p < 0.05$). Strain BB32-1 formed a virulence group while other strains formed ab and bc virulence groups (Table 3). The strain BB32-1 was significantly higher in virulence than those of groups bc and ab, while significant difference was found in virulence between the groups ab and bc with ab group having the higher virulence (Table 3). These results revealed that all the strains were highly virulent independent of their geographic origins.

DISCUSSION

A survey for rice bacterial blight in the dry and wet savanna zones in the North of Togo revealed the occurrence of the disease across the two eco-zones. The disease has never been reported before in Togo. Rice bacterial blight is widespread throughout Asia, Australia, United States and several rice-growing areas of Latin America and Africa (Sere *et al.*, 2005;

Onasanya *et al.*, 2009; Basso *et al.*, 2011). This study revealed the presence of rice bacterial blight in Togo and its occurrence in many rice growing areas in the Northern part of the country. The bacterial was frequently found in the fields and sites of the dry savanna zone closer to Burkina Faso and was rarely found in the wet savanna zone. The localities in the dry savanna might have become contaminated from the infected zones in Burkina Faso through movement of contaminated seeds and other means of bacterial disease dissemination (Onasanya *et al.*, 2009; Sere *et al.*, 2005). Higher incidence and severity of rice bacterial blight were observed in this study in neighboring sites in Burkina Faso, while the disease incidence and severity generally decreased from the dry to wet savanna zone in Northern Togo. This finding confirmed the earlier report on the bacterial blight distribution across Mali, Burkina Faso and Niger with possible spread prediction to Togo (Sere *et al.*, 2005).

The first ecozone could be easily contaminated from Burkina Faso, where the disease was observed some years ago and might be favored by environmental conditions and climate conditions (Onasanya *et al.*, 2009; Sere *et al.*, 2005). In previous studies conducted by Banito *et al.* (2007), cassava bacterial blight incidence and severity were higher in the herbaceous savanna without trees (dry savanna zone) than in the herbaceous savanna with few trees (wet savanna zone) which possibly could explain the results obtained in the present study. Similarly, survey studies revealed higher cassava bacterial blight incidence in the dry savanna zone than in the wet savanna and the forest zones in Cameroon (Banito *et al.*, 2007, 2010). Besides, higher temperature which is greater in the dry savanna zone than in the wet savanna zone tends to promote bacterial blight disease (Onasanya *et al.*, 2009).

The virulence test of *Xanthomonas oryzae* pv. *oryzae* strains on the rice variety IR841 revealed that most of the strains were highly virulent. However, differences in virulence among strains were statistically significant, indicating some potential differences in strain characteristics. Although, the survey revealed a decrease of the bacterial blight incidence and severity from the dry savanna to the wet savanna, all the strains collected in both ecozones were highly virulent. Also, the virulence test revealed that the highest severity note of class 9 was scored by a strain from the wet savanna zone, where the disease incidence and severity were generally low during the survey. The slight differences in virulence found among strains in this study might be due to the diversity in their genetic patterns and probably not related to geographical origin (Lee *et al.*, 2003; Liu *et al.*, 2007; Nino-Liu *et al.*, 2006; Onasanya *et al.*, 2010).

CONCLUSIONS

Our study was able to provide information about rice bacterial blight presence in Togo that has never been reported before in this country. The conducted rice bacterial blight disease survey revealed the presence of the bacterial in many rice growing areas in Togo and its distribution was established. The disease was found to occur with high incidence and severity and strains virulence status established. Although, pathogenic differences were found among strains using single rice variety, more research studies would be needed using various rice cultivars to investigate the presence of strain by genotype interactions in order to identify donors with durable resistance to rice bacterial blight disease in Togo.

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